Severe Combined Immunodeficiency among the Navajo. I. Characterization of Phenotypes, Epidemiology, and Population Genetics

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Abstract Previous studies have identified a high incidence of severe combined immunodeficiency (SCID) among the Navajo Native American population. To determine the incidence and population genetics of this condition, we reviewed the death certificates of all children who died between 1969 and 1982, established the cases that met criteria identified in previously investigated cases, and interviewed the selected children's families. SCID cases were distributed spatially and temporally. Segregation parameter estimates of 0.27–0.38 were obtained from data from 24 interviewed families, suggesting an estimated gene frequency of 2.1% (arguing against a multifactorial inheritance). SCID cases referred to specialty centers lacked T and B cells in their blood, and their serum immunoglobulins ranged from absent to near normal.

Severe combined immunodeficiency (SCID) describes a group of conditions in which there is a failure of antibody responses and of cell-mediated immunity (WHO Scientific Group 1986). Most cases are congenital, and approximately 25% of cases are the consequence of adenosine deaminase deficiency. The etiology of the remaining 75% of cases is poorly understood, and for clinical purposes these cases are divided into those with X-linked inheritance (incidence estimated at 1 in 1,000,000 live births) (Gatti and O'Reilly 1979) and those with autosomal recessive inheritance (incidence estimated at 1 in 1,000,000 births) (O'Reilly 1979). Nothing is known of the underlying biochemical abnormality in these cases, and immunologic tests point to further heterogeneity. For example, approximately 60% of cases have circulating B cells and no T cells and 20% have neither T cells nor B cells in the blood. The
occasional engraftment of maternal lymphocytes in scid patients (Pollack et al. 1982; Flomenberg, Dupont et al. 1983) confuses the classification of scid by lymphocyte phenotype, and the rarity of the condition makes further investigation difficult.

In 1980 we reported five cases of scid in a population of Athapaskan-speaking Navajo (Murphy et al. 1980). The incidence of scid at that time was estimated to be 50 per 100,000 live births. There were no obvious etiologic factors, although we found that serum from affected children contained sufficient interferon to inhibit proliferative responses by mitogen-stimulated lymphocytes (Jones et al. 1983).

We now report the results of an epidemiologic study undertaken to determine as completely as possible the distribution among the Navajo of scid cases in space, time, and pedigree. We also report the results of immunologic tests undertaken on affected infants who were diagnosed in time for referral to tertiary care centers to define the phenotypic heterogeneity of scid among the Navajo.

Materials and Methods

Population and Geographic Distribution. The Navajo are a relatively endogamous population of American Indians of Athapaskan descent who have a restricted repertoire in the histocompatibility (HLA) (Troup et al. 1982) and GM (Williams et al. 1985) systems and whose HLA antigens resemble those of other native North American populations (Williams et al. 1981; Kostyu and Amos 1981). The majority live in scattered rural settlements on the Navajo Reservation, which covers approximately 25,000 square miles of northern Arizona and New Mexico and southern Utah. Although the availability of health care has improved in recent decades (Broudy and May 1981), infectious disease continues to be a major health problem.

Clinical and Immunologic Studies. The World Health Organization (WHO) classification (WHO Scientific Group 1986) of scid states that this category of disease should be restricted to infants with severe and potentially fatal defects in cell-mediated immunity and antibody production. Subcategorization is based on enzymatic abnormalities, mode of inheritance, or level of faulty cellular development. Some children have low levels of T cells and B cells, whereas others have a low level of T cells and a normal amount of B cells. Thus the overall clinical and laboratory patterns allow assignment of this diagnostic label only to severely ill children.

Laboratory criteria for the diagnosis of scid in this study at a tertiary care center were (1) lack of normal levels of T cells in blood and (2) immunoglobulin deficiency or, where immunoglobulins were present,
abnormalities in their electrophoretic mobility together with antibody deficiency. Supportive clinical criteria were absence of the thymus (determined by chest X-ray), opportunistic infections, and diarrhea. Criteria at autopsy were absence of the thymus and small lymph nodes with abnormal architecture.

The following case study is illustrative. Patient 16 weighed 8 lbs., 15 oz. at birth. He was the first child of healthy unrelated Navajo parents from the Cameron area. At 1 month of age he was admitted to the hospital with a cough and cyanosis and was treated for chlamydia pneumonia for 8 days. When he was discharged, he had an oral ulcer (noma), which progressed to a 1 × 2 cm lesion over the next 4 days. He was admitted to the hospital again and treated with Pediazole for 10 days, during which time he developed oral candidiasis, diarrhea, and fever. No thymus was seen on the chest X-ray. The oral ulcer healed slowly over the next 6 weeks. At age 2 months he developed fever and cyanosis. His blood count showed 9800 white blood cells per square millimeter, 94% segmented neutrophils, 4% lymphocytes, and 2% metamyelocytes, and he had pneumococci and *Klebsiella* species in a tracheal aspirate. The pneumonia worsened despite antibiotic treatment, and tests for respiratory syncytial virus pneumonia were positive. Lymphocyte phenotyping of blood mononuclear cells (MNC) showed 0% T3, 0% T4, 0% T8, 57% Leu 11, 0% B, and 7% Ia; there were no proliferative responses to phytohemagglutinin (PHA) or in mixed lymphocyte culture. He died before he could be given a bone marrow graft.

**Immunologic Methods.** Blood T lymphocytes were stained with monoclonal antibodies (OKT3, OKT4, OKT8, Ortho Reagents, Raritan, NJ), either directly conjugated or unconjugated, and then with fluorescein conjugated goat-anti-mouse IgG. B cells with identified by surface IgM, and pre-B cells by cytoplasmic u chains and lack of surface IgM (Hayward 1978). Natural killer cells were identified with anti-CD16 monoclonal antibodies (Becton-Dickinson, Mountain View, CA) by indirect immunofluorescence. Serum immunoglobulins were measured by nephelometry. Lymphocytes were cultured in RPMI 1640 with 10% human serum; PHA cultures were stimulated for 3 days with a 4-hour 3H thymidine pulse, and mixed lymphocyte cultures for 6 days with an 8-hour 3H thymidine pulse [methods are further described by Hayward and Harbeck (1983)]. Blood mononuclear cells were HLA typed with NIH extended or Terasaki trays by standard microcytotoxicity tests.

**Epidemiologic and Population Genetic Studies**

*Death Records.* Initially, the death records of all Native Americans who had died in Arizona between the years 1969 and 1978 were reviewed. Children who died between the ages of 1 and 24 months were catego-
rized as having a death associated with an infectious disease or for other reasons (i.e., trauma or congenital abnormalities). Analysis of this review suggested differences between Navajo and non-Navajo children (see Figure 1). Subsequently, additional death records were obtained and the 1969–1982 death records of all Navajo children who died in Arizona and New Mexico between 1 and 24 months of age were evaluated in further detail. Criteria for possible immunodeficiency and thus inclusion in further record searches included (1) definitive diagnosis of SCID, (2) infectious disease (including pneumonia, diarrhea, and encephalitis), (3) failure to thrive or malnutrition, and (4) unknown causes (including sudden infant death syndrome). Excluded were cases of trauma, congenital malformation, and death within 2 weeks of birth. Three hundred deaths that fit these criteria were chosen for further investigation.

Hospital Chart Review. Attempts were made to locate all hospital and clinic charts on all 300 cases: charts for 248 cases were found and reviewed. Charts were initially sought at the hospital or clinic where the death was certified. Additional records were obtained from the homes of the parents, the birth site, or clinics mentioned in other charts. All retrievable charts were examined at each hospital and clinic identified. For many children it became possible to follow in some detail the entire illness history. Information recorded from each chart included nature and duration of illness; X-ray or autopsy data on presence of thymus; white blood cell count, differential, absolute number, and appearance of leukocytes; serum immunoglobulin levels; skin rashes, candidiasis, and mucocutaneous ulcers; presence or absence of lymphoid tissue; failure to thrive; comments on liver or spleen size and consistency; and unusual infectious agents or those associated with depressed immune function (such as pseudomonas species).

Abnormal objective values included length and weight less than the 10th percentile for age, less than 1500 lymphocytes/mm³, serum IgG levels below 2 standard deviations below age mean, absent thymic shadow on chest X-ray, and absence of lymphoid tissue on physical examination.

Cases were assigned on the basis of these data to one of four patient groups:

1. Known immunodeficiency: definitive diagnosis from a tertiary care center (see Table 1).
2. Probable immunodeficiency: (a) recurrent infections, failure to thrive, and abnormal laboratory results; (b) recurrent infections, including skin lesions and failure to thrive, with no available laboratory data; (c) recurrent infections, including skin lesions and failure to thrive, with normal laboratory findings.
3. Possible immunodeficiency: death at 2 months of age or less with first respiratory or gastro-intestinal (GI) infection and abnormal laboratory findings.
4. Questionable immunodeficiency: death at 24 months or less with first major GI or respiratory infection but no abnormal laboratory findings or evidence of failure to thrive.

**Demographic Information.** Families of known and probable cases were located with the help of local public health nurses and asked for interviews. Informed consent and interview data were collected with the assistance of a Navajo interpreter who had already lost two affected SCID children. Interview data included places of residence and work during the life of each parent; type of work; fertility history of the mother, including offspring of all partners; health status of all offspring; and details of each parental sibship (including members who had died, their age at death, and the cause if known; and the number, sex, and health status of offspring of each sibling). For families initially identified in the chart review process, additional information on the course of the illness of the affected child was obtained by interview. This additional information was included in the final designation of status. Children for whom the natural history of the disease was consistent with SCID were designated “clinical SCID” and were included with the cases in all subsequent analyses. (For example, several children in this category had other siblings who died of similar conditions before our study period; still others were siblings of children who developed SCID as determined in a tertiary care center after 1982.) Children with medical data suggesting SCID but whose families could not be located were not included as cases.

**Segregation Analysis.** Segregation analysis for single-gene Mendelian inheritance was performed on the family data using standard methods (Morton 1962). Maximum likelihood estimates of the ascertainment probability (π), the segregation ratio (p), and the proportion of sporadic cases (X) were obtained under a model of segregation with incomplete ascertainment, a mixture of sporadic cases, and a uniform segregation frequency in a sample of nuclear families. An alternative model is the multifactorial threshold model described for birth defects and other apparently non-Mendelian traits (Carter et al. 1982). Under a multifactorial threshold model, expression of a trait depends on the additive effects of several minor genes and environmental factors. That model was tested for goodness of fit (Gladstien et al. 1978). The test is applicable to disorders showing different incidence rates in males and females and involves a comparison of the observed number of nuclear families having at least two affected offspring with the expected distribution for such families under the multifactorial threshold model. The computation of the ex-
Table 1. Clinical Findings and Outcomes

<table>
<thead>
<tr>
<th>Year of Case Birth</th>
<th>Age at Presentation</th>
<th>Main Symptoms</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 1972</td>
<td>birth</td>
<td>Diarrhea, pneumonia</td>
<td>Marrow</td>
<td>Died, GVH</td>
</tr>
<tr>
<td>2 1975</td>
<td>birth</td>
<td>None</td>
<td>TEG</td>
<td>Died, Candida sepsis</td>
</tr>
<tr>
<td>3 1975</td>
<td>2 wk</td>
<td>Diarrhea, pneumonia</td>
<td>None</td>
<td>Died, RSV</td>
</tr>
<tr>
<td>4 1975</td>
<td>2 mos.</td>
<td>Diarrhea, noma</td>
<td>TEG</td>
<td>Died, renal failure</td>
</tr>
<tr>
<td>5 1978</td>
<td>2 mos.</td>
<td>CMV, pneumonia</td>
<td>None</td>
<td>Died, CMV</td>
</tr>
<tr>
<td>6 1978</td>
<td>3 mos.</td>
<td>Diarrhea, pneumonia</td>
<td>TEG</td>
<td>Died, sepsis</td>
</tr>
<tr>
<td>7 1979</td>
<td>15 mos.</td>
<td>Staph skin sepsis</td>
<td>Grafted</td>
<td>Died, bronchiectasis</td>
</tr>
<tr>
<td>8 1979</td>
<td>8 mos.</td>
<td>Diarrhea, thrush</td>
<td>Grafted</td>
<td>Reconstituted</td>
</tr>
<tr>
<td>9 1979</td>
<td>4 mos.</td>
<td>Diarrhea, GVH</td>
<td>Grafted</td>
<td>Died, antibody deficiency</td>
</tr>
<tr>
<td>10 1982</td>
<td>1 mo.</td>
<td>Diarrhea, ulcers</td>
<td>Grafted</td>
<td>Reconstituted</td>
</tr>
<tr>
<td>11 1982</td>
<td>13 mos.</td>
<td>Oral ulcers</td>
<td>Grafted</td>
<td>Died, noma</td>
</tr>
<tr>
<td>12 1984</td>
<td>8 mos.</td>
<td>URTI, ulcers</td>
<td>None</td>
<td>Died, meningitis</td>
</tr>
<tr>
<td>13 1981</td>
<td>5 mos.</td>
<td>Diarrhea</td>
<td>None</td>
<td>Died, GVH</td>
</tr>
<tr>
<td>14 1985</td>
<td>4 mos.</td>
<td>Diarrhea, pneumonia</td>
<td>Grafted</td>
<td>Died, EBV hepatitis</td>
</tr>
<tr>
<td>15 1984</td>
<td>4 mos.</td>
<td>Pneumonia, ulcers</td>
<td>None</td>
<td>Died, CMV hepatitis</td>
</tr>
<tr>
<td>16 1985</td>
<td>4 mos.</td>
<td>Pneumonia, ulcers</td>
<td>None</td>
<td>Died, RSV pneumonia</td>
</tr>
<tr>
<td>17 1985</td>
<td>2 mos.</td>
<td>Diarrhea, ulcers</td>
<td>Grafted</td>
<td>Died, EBV hepatitis</td>
</tr>
<tr>
<td>18 1985</td>
<td>9 mos.</td>
<td>Meningitis, UTI</td>
<td>Grafted</td>
<td>Died, interstitial pneumonia</td>
</tr>
</tbody>
</table>

a. URTI, upper respiratory tract infections; GVH, graft versus host disease; EBV, Epstein-Barr virus; CMV, cytomegalovirus; RSV, respiratory syncytial virus; TEG, thymic epithelial graft.
b. Age at presentation is the subjects age when the principal symptoms first appeared.
c. From nonirradiated blood transfusion in neonatal period.

The expected distribution is conditioned on several family-dependent variables, including parental status, numbers of sons and daughters, and whether each family was ascertained through only affected males or through at least one affected female. The probabilities of observing the data are derived for a range of heritabilities and ascertainment probabilities.

Results

Clinical Findings. Clinical data on the cases referred to tertiary centers are summarized in Table 1. Cases identified after 1982 and therefore after the study period are included to demonstrate the narrow range of clinical findings. Significant findings include the occurrence of oral mucosal ulceration in four of the nine cases seen since 1982; in three of these, the lesions were severe enough to be described as noma or cancrum oris. The high frequency of diarrhea is expected.
Table 2. Immunologic Findingsa

<table>
<thead>
<tr>
<th>Lymphocytes (1600–5480 mm³)b</th>
<th>T cells (45–78)</th>
<th>CD4 (29–63)</th>
<th>CD8 (18–44)</th>
<th>CD16 (4–16)</th>
<th>B (3–12)</th>
<th>IgGc</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>Age</td>
<td>CD5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Birth</td>
<td>720</td>
<td>ND</td>
<td>0</td>
<td>1000b</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Birth</td>
<td>450</td>
<td>20% ER</td>
<td>ND</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2 wk</td>
<td>700</td>
<td>5% ER</td>
<td>0</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>2 mos.</td>
<td>700</td>
<td>7% ER</td>
<td>0</td>
<td>135</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>2 mos.</td>
<td>1000</td>
<td>55% ER</td>
<td>ND</td>
<td>158</td>
<td>91</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3 mos.</td>
<td>1500</td>
<td>17% ER</td>
<td>0</td>
<td>143</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>15 mos.</td>
<td>2210</td>
<td>3% ER</td>
<td>0</td>
<td>48</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>8 mos.</td>
<td>3840</td>
<td>2% ER</td>
<td>52</td>
<td>561</td>
<td>10</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>4 mos.</td>
<td>4320</td>
<td>7% ER</td>
<td>0</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>1 mo.</td>
<td>1606</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>0</td>
<td>1050</td>
</tr>
<tr>
<td>11</td>
<td>13 mos.</td>
<td>2600</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>22</td>
<td>0</td>
<td>248</td>
</tr>
<tr>
<td>12</td>
<td>8 mos.</td>
<td>4320</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>0</td>
<td>59</td>
</tr>
<tr>
<td>13</td>
<td>5 mos.</td>
<td>740</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>14</td>
<td>4 mos.</td>
<td>1410</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>4 mos.</td>
<td>1960</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>0</td>
<td>131</td>
</tr>
<tr>
<td>16</td>
<td>4 mos.</td>
<td>392</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>57</td>
<td>0</td>
<td>364</td>
</tr>
<tr>
<td>17</td>
<td>2 mos.</td>
<td>376</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>409</td>
</tr>
<tr>
<td>18</td>
<td>9 mos.</td>
<td>330</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

a. ER, E-rosette-forming cells; B, B cells, IgG, IgA, and IgM are serum level (mg/dl).
Patients 1–9 were evaluated before the availability of monoclonal antibodies; therefore only ER data were available. Patients 10–18 did not have ER studies.
b. Range of values in healthy siblings (n = 8) of Navajo patients (percent); no Ig isotype values for Navajos are available.
c. When IgG is present alone, it is probably mostly maternal. Subjects 10 and 16 had received intravenous IgG before levels were measured. Serum samples from subjects 16, 17, and 18 were measured by high-resolution electrophoresis; in each case one or more monoclonal bands were seen in IgG and IgM.

Immunologic Studies. In the earlier cases (pre-1982), T cells were identified only by the sheep red blood cell rosette test because monoclonal antibodies were not yet available. The findings on the later cases (numbers 10–18), described in Table 1, indicate a consistent lack of T cells with the CD3 antigen in all the subjects except case 14, who had active graft versus host disease when examined (Table 2).

PHA did not stimulate cells from any of the patients. Lymphocytes from an occasional patient did respond in mixed lymphocyte culture (MLC) reaction with parent or sibling stimulator cells.

The HLA typing data shows that the A2, A24, B15, B27, and B35 antigens were often found, as is generally the case with the Navajo (Troup et al. 1982).
Epidemiology. Figure 1 illustrates the distribution of early childhood deaths among Navajo and non–Navajo Native Americans in Arizona born between 1969 and 1978. Noninfectious deaths vary from year to year in both groups, with no clear trend over time. In contrast, the frequency of infectious deaths dropped precipitously among the non–Navajo tribes, whereas it remained high among the Navajo. This overall decrease in infectious diseases among Native Americans correlates with the increased availability of medical care and improved nutrition (Broudy and May 1981). The distribution by sex of cases and noncases was calculated from the 248 individuals whose charts were located. Cases were those with definitive diagnoses and those classified as clinical cases by the criteria given earlier. Among the cases 52% were male; among noncases 58% were male. SCID among the Navajo appears to show no differential distribution by sex.

The geographic distribution of on-reservation clinical cases from 1968 to 1981 (Arizona and New Mexico) was analyzed based on the service unit in which the parents resided at the time of the child’s death. In all but two cases this was the same service unit in which the child was born. The distribution of cases was found to be homogeneous throughout the reservation, not including the Tuba City Service Unit (chi-square value for non–Tuba City service units = 3.645, d.f. = 5, p = 0.6041). SCID represented 5.56% of all deaths to children under 24 months in 6 of the 7 service units; in Tuba City SCID was responsible for 14.5% of deaths (difference of Tuba City from the rest of the reservation, one-sided binomial test, p = 0.0171). The risk ratio of Tuba City to the remainder of the reservation was 2.60 (90% confidence interval 1.46–4.66).

The minimum incidence rate was calculated from these data. From 1969 to 1981 there were approximately 54,000 live births on the entire Navajo Reservation. The 28 on-reservation cases represents an incidence of 52 per 100,000. The year-to-year variation in total number of cases of SCID was compared to the total number of deaths for each year. The greatest number of deaths from SCID was in 1976: almost 10% of all deaths of Navajo children below 24 months of age. Over the entire period SCID deaths represented 7% of all Navajo deaths in the 1–24 months age group.

Population Genetics. Segregation analysis was performed on data from 24 interviewed families, using only data on the full sibships of the probands. Four separate analyses were run, omitting or including as cases those apparently affected children not found in our chart reviews (e.g., those born outside the study period). Segregation parameter estimates ranged from 0.27 to 0.38. In this maximum likelihood estimation procedure, with the small number of families, neither recessive nor dominant models of inheritance could be rejected with significant chi-square values. It was possible, however, to reject the multifactorial threshold model.
Figure 1. Number of deaths in Native American children, age 1-24 months, in Arizona 1969-1978. (A) Navajo; (B) non-Navajo. Deaths attributed to infectious diseases are depicted in dotted columns and deaths attributed to noninfectious causes are in hatched columns. Data for 1978 is only partial.
Figure 2. Pedigree of three interrelated families of infants with SCID from the western Navajo Reservation. ●, female with SCID; ■, male with SCID; ○, normal female; □, normal male. Note the relationships among parents of affected children.

for a wide range of heritabilities and ascertainment probabilities because there were statistically too many affected individuals in the families.

An evaluation of pedigrees was possible among families in the western region of the reservation. Figure 2 presents a pedigree from that area, which accounted for a large proportion of the excess cases in the Tuba City Service Unit; it includes several cases outside the 1969–1982 study period for statistical analysis. Affected families are related to each other horizontally; a potential common ancestor has been determined for only two families, relating affected children as third cousins. In one family both the mother and the father of the probands are related to other parents of probands; in three families both members of a grandparent pair are related to other parents or grandparents of probands.

These results are all consistent with an autosomal recessive disease. Given the incidence of SCID of 52 per 100,000, the estimated gene frequency would be 22.5 per 1000 (2.25%). Families (both affected and unaffected) are represented multiple times in these pooled data. Therefore we also calculated the average of the gene frequency estimates for each year in the period 1968–1981, because any particular family is represented only once in a calendar year. The average gene frequency estimate is 21 per 1000 (2.1%). Because of the high likelihood under ascertainment of SCID, this estimate is probably conservative.

Discussion

Immunologic Characteristics. Our subjects differ from classical descriptions of SCID in several ways. Only 3 cases were clearly lymphopenic when symptoms appeared (cases 16, 17, and 18), although cases 10 and
14 were low in comparison with age-matched controls. Many of the subjects had substantial amounts of serum IgG when diagnosed. In most cases these subjects were young enough for this IgG to be derived from their mothers. Six of the 18 cases had readily measurable amounts of IgM (values of <9 mg/dL are disregarded because the rate nephelometry method is generally unable to distinguish between these low levels and complete absence of IgM). In patient 14 the presence of near-normal amounts of IgM led to considerable delay in diagnosis, and it was only in subsequent patients that serum immunoglobulins, when present, were analyzed by high-resolution electrophoresis. Both the subsequent cases with detectable IgM (17 and 18) had monoclonal IgM bands. None of the subjects had B lymphocytes detected in the blood by immunofluorescence for surface immunoglobulin, even when serum immunoglobulin was present. This finding suggests that the immunoglobulin that was made did not arise following the normal development of the B cell series.

Monoclonal antibody phenotyping of blood MNC for T cells gave negative results except for one patient (case 14) who had a mild skin rash 2 months following an unirradiated blood transfusion. When tested by us or at referring hospitals, many of the patients had a low level (<10%) of E-rosette-forming cells. The E rosette receptor (CD2) is expressed on natural killer cells and on T cells. Because all the tested patients had normal or high numbers of natural killer cells with the CD16 (Leu 11a) antigen (Lanier et al. 1983), it seems likely that these cells were responsible for the rosetting. Patients 2, 3, and 4 had numerous cell-containing parallel tubular arrays (Payne et al. 1977). This organelle was subsequently found to be a marker of NK cells (Payne and Glasser 1981). The lack of cells with T cell antigens is consistent with the uniform lack of response to PHA we observed and supports a severe T cell deficiency.

Proliferation in MLC was detected with several patients' lymphocytes, as has previously been reported in other SCID patients. In each case the subjects' lymphocytes failed to proliferate in PHA stimulated cultures, although they did proliferate in the presence of IL2, whether PHA was added or not. The phenotype of the cells that proliferated in the presence of IL2 is unknown, although natural killer cells would seem possible candidates. These cells are known to be capable of expressing IL2 receptors, they are present in the subjects' MNC preparations, and they proliferate in cultures containing IL2 (Flomenberg, Welte et al. 1983). The similarity among HLA types in patients and North American Indians in general and the occurrence in several families of HLA matched but healthy siblings argue against the presence of the Navajo SCID gene on chromosome 6.

Epidemiology and Genetics. Because oral ulceration is uncommon in infants, its presence was useful in facilitating epidemiologic studies, such
as this one, that rely on retrospective data collection. The mother of one child identified by chart review as having SCID reported that she had had four children die of the same conditions, with the skin lesions being, for her, the most characteristic feature (Rotbart et al. 1986).

With high rates of infectious disease and malnutrition common among the Navajo into the 1970s, the presence of SCID was unlikely to be detected. Deaths from SCID have been occurring at least since the 1950s according to our interviews, but such deaths were routinely attributed to infectious diseases or malnutrition. It has only been with the substantial improvements in the availability of medical care that such children have become identifiable.

The most likely genetic model is a recessive one. None of the Navajo children evaluated at tertiary care centers had abnormalities in the purine nucleotide enzyme pathway, as seen in some cases of SCID with autosomal recessive inheritance patterns. The estimated segregation parameters are higher than the expected 0.25, but not significantly so, and may be due to sampling error in this small sample. The gene frequency estimated for Navajo SCID as a genetic recessive is comparable to that for cystic fibrosis in the US white population or slightly higher than the prevalence of Tay-Sachs disease among the Ashkenazim (1 per 1500). However, it is clear for the clusters in the Tuba City area that the gene frequency in the western reservation is higher.

In researching Navajo pedigrees in the later phases of data collection, it became clear that much of the information becomes blurred at the time of the Long Walk to Fort Sumner, New Mexico, in 1868. This historical event resulted in major population reduction, an event commonly associated with increased frequencies of recessive alleles. The pedigree data are remarkable for the lack of common ancestors identified. This is consistent with Navajo marriage rules, which tend to maximize nonconsanguineous matings in the face of tribal and regional endogamy through rules prohibiting marriage into either the mother’s or the father’s clan or to members of related clans. In the one pedigree presented, it is notable that the linkage requires kinship through males and thus is consistent with the marriage rules. The pedigrees suggest that, rather than the increased homozygosity expected in association with inbreeding, there may be excess heterozygosity associated with maximized outbreeding. In that case the gene frequency estimates based on random mating at genetic equilibrium would underestimate the true frequency.

Two cases of SCID in Native Americans in this geographic area have now been diagnosed outside the Navajo population. One was among the Jicarilla Apache in northern New Mexico and the other among the San Carlos Apache in central Arizona. Both tribes are Athapaskan speakers, closely related genetically to the Navajo (Williams et al. 1985; Kostyu and
Amos 1981). The Jicarilla Apache were interred at Fort Sumner with the Navajo. The other Apache patient has a Navajo father.

With continuing improvements in health status for Navajo children, SCID will probably come to account for an increasing share of early mortality in that population. Successful treatment by bone marrow transplants continues to depend on the speed of diagnosis and referral and the availability of a suitable donor. Although we allude to differences in immunologic function between Navajo children and the classic presentation of SCID in other populations and although there is remarkable similarity among the Navajo cases, it is premature to consider Navajo SCID as a separate entity. With the rapid rate of advancement of understanding of the molecular genetics of human immunodeficiency diseases (Puck et al. 1989), however, additional information regarding this population will be forthcoming in the near future.

Acknowledgments The research for this article was supported in part by the Division of Research Resources, National Institutes of Health, under Clinical Research Center grant RR 69 and by a grant from the US Public Health Service, Indian Health Service. Our special appreciation goes to our Navajo interpreters, James Redsteer and Rita Canyon, without whose substantial help and energy the epidemiology and family interviews could not have been completed, and to David Marsh, who participated in the chart reviews at the Gallup, New Mexico, Indian Health Service Hospital.

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Literature Cited


Applying Public Health Strategies to Primary Immunodeficiency Diseases
A Potential Approach to Genetic Disorders

INSIDE: Continuing Education Examination
Applying Public Health Strategies to Primary Immunodeficiency Diseases: A Potential Approach to Genetic Disorders

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Summary

Primary immunodeficiency (PI) diseases are a group of primarily single-gene disorders of the immune system. Approximately 100 separate PI diseases have been described, but <20 probably account for >90% of cases. Although diverse, PI diseases share the common feature of susceptibility to infection and result in substantial morbidity and shortened life spans. Most important, prompt diagnosis and treatment can now lead to life-saving treatment and result in marked improvements in the quality and length of life for persons with PI diseases.

In November 2001, a workshop was convened by CDC in Atlanta, Georgia, to discuss ways to improve health outcomes among persons with PI disease. A multidisciplinary panel of persons knowledgeable in PI diseases and public health met to identify and discuss public health strategies that can be applied to PI diseases and possibly for other genetic disorders. A systematic assessment based on the established public health framework was applied to the growing group of PI diseases, whose diverse genetic mutations span multiple components of the immune system but all lead to increased incidence and severity of infections.

During the meeting, specialists in clinical immunology, public health, genetics, pediatrics, health communication, and ethics from state and federal agencies, academic centers, professional organizations, and advocacy foundations discussed the four components of the public health framework as they relate to PI diseases. These four components include 1) public health assessment (application of traditional public health methods to assess the occurrence and impact of PI diseases on communities); 2) population-based interventions (development, implementation, and evaluation of screening tests administered to newborns and clinical algorithms for early recognition of symptomatic persons to facilitate the earliest possible diagnosis and treatment for PI diseases); 3) evaluation of screening and diagnostic tools (to ensure their quality and appropriateness for identification of patients with PI diseases); and 4) communication (communication with and information dissemination to health-care providers and the public to facilitate prompt and appropriate diagnosis and intervention). The working group's deliberations focused on challenges and opportunities, priority research questions, and recommendations for future action for these four components. These recommendations, developed by workshop participants, will be useful to medical and public health professionals who are evaluating methods to increase recognition of PI diseases and other genetic disorders.

Introduction

Advances in human genetics and the evolution of the Human Genome Project will play a central role in the practice of medicine and public health in the 21st century. However, gene discovery is only the beginning. For the majority of diseases, a gap exists between discovering or sequencing genes and using human genomic information to improve health outcomes (1). Public health research and policy have a crucial role in closing that gap. Moving from gene discovery to clinical and public health application requires full engagement of public health to 1) quantify the effect of genetic discoveries
on population health, 2) develop policies regarding and guidelines for the appropriate use of genetic tests and services, 3) develop interventions to improve health outcomes, 4) initiate and maintain behavior change among patients and healthcare providers, and 5) address the quality of and access to services. Genomic breakthroughs have been identified as major challenges for public health in the 21st century (2). However, the usefulness of these breakthroughs in clinical practice depends on the availability of population-based data to determine the prevalence of gene variants among different populations, the population-based risk for disease associated with gene variants, gene-environment interactions, and the effectiveness of genetic tests and services (3–5).

As part of efforts to highlight the emerging role of human genomics in the practice of public health in the United States, CDC, in collaboration with research, academic, clinical, and foundation partners, evaluated public health strategies that can be used to close the gap between gene discoveries and clinical practice for primary immunodeficiency (PI) diseases — approximately 100 primarily single-gene disorders of the immune system. Identification of the genes responsible for these conditions is progressing rapidly; therefore, a population-based framework is needed that can be applied also to other genetic disorders and gene discoveries. This report describes the concerns, challenges, and opportunities and provides recommendations for public health action regarding such a framework.

Background

With completion of the Human Genome Project, 30,000–35,000 genes have been mapped (6–9), each of which contains the code for a specific product, typically a protein. Through the proteins they encode, genes determine and regulate all human body processes. Human genomics includes a continuum from the study of single-gene disorders with high penetrance to common genetic variants or polymorphisms at multiple loci, with low penetrance, and that have complex gene-environment interactions (10). Genetic disorders are caused by mutations, or alterations, in a gene or set of genes. Mutations can be inherited or occur de novo. The effect of a mutation on a gene depends on how it alters the expression or function of the gene product and the role of that protein in the body. Mutations in certain genes have severe effects, whereas mutations in others do not.

The majority of genetic disorders result from a complex interplay of multiple genetic changes and environmental factors. However, certain disorders result when a mutation alters or causes an absence of the product of only one gene. Examples of such single-gene disorders are cystic fibrosis (CF) and phenylketonuria (PKU). Single-gene disorders can be either X-linked (i.e., caused by a defect in a gene on the X chromosome) or autosomal (i.e., caused by a defect in a gene on an autosomal or nonsex chromosome). Single-gene disorders can result from either dominant or recessive patterns of inheritance or expression. Selected chromosomal disorders, which might be inherited, involve microdeletions of multiple genes at closely linked loci. Although single-gene disorders are individually rare, they collectively contribute to a substantial proportion of pediatric morbidity and mortality (1).

PI diseases are a group of primarily single-gene disorders of the immune system (11–13). Primary denotes the genetic nature of the defects, differentiating them from secondary, or acquired, immunodeficiencies caused by malnutrition, infection (e.g., human immunodeficiency virus [HIV] infection), chemotherapy, or other external agents. Approximately 100 separate PI diseases have been described, but <20 probably account for >90% of cases. The disorders vary in the severity and spectrum of symptoms, but without effective and early treatment, they can be fatal. A high index of suspicion and prompt diagnosis can lead to lifesaving treatment and substantial improvement in quality of life for persons with PI diseases. Causes of PI diseases vary, but single-gene defects can lead to a missing enzyme, a missing structural component, developmental arrest at a specific differential stage of immune development, or a nonfunctional protein. As with all single-gene disorders, selected PI diseases are known to be X-linked or autosomal, with both dominant and recessive patterns of inheritance or de novo mutations; others might have more complex modes of inheritance not yet understood. Approximately 80% of affected persons are aged <20 years, and because certain PI diseases are inherited in X-linked recessive fashion, 70% of cases occur among males (13).

Advances in human genomics have led to identification of the gene defects responsible for >60 PI diseases and have prompted development of new diagnostic and therapeutic tools and potential gene therapies (14–20). New molecular techniques have facilitated identification of different types of mutations underlying PI diseases. Single-nucleotide substitutions, or point mutations, involve an alteration in the sequence of nucleotides in a gene. These include missense mutations, which alter the amino acids in the protein product of a gene; nonsense mutations, which generate premature stop codons in the genetic code; RNA (ribonucleic acid) splice-site mutations, which can lead to frameshift mutations; and regulatory mutations, which affect aspects of gene expression. Mutations also can involve insertions or deletions of DNA (deoxyribonucleic acid) sequences. Progress in the delineation of the
mechanisms by which these genetic mutations cause PI diseases has added to the understanding of the normal immune system and the processes that underlie conditions that occur with far greater frequency than PI disease (21).

**Clinical Characteristics and Effect of PI Diseases**

The clinical hallmark of PI diseases is an increased susceptibility to infection, the severity of which varies by defect (13,22). In certain cases, the body fails to produce any or sufficient antibodies to fight infection. In other cases, the cellular (e.g., T-cell) defenses against infection fail to work properly. Shared features of the disorders are an unusual rate or severity of infection, infection with unusual or opportunistic organisms, and infection associated with specific syndromes (13). PI diseases also are associated with other immunologic disorders (e.g., autoimmune diseases) and carry an increased risk for cancer, particularly lymphoid malignancies (22). PI diseases often are classified according to the affected components of the immune system (Table 1).

**Antibody Deficiencies**

Approximately half of the diseases are associated with inadequate or defective antibody production, caused by too few antibody-producing B cells or B cells that do not function properly, resulting in inadequate production of antigen-specific antibodies (23). These disorders are characterized by recurrent sinus and pulmonary infections and septicemias with bacteria (13,24). The most severe defect in this category is X-linked agammaglobulinemia (XLA), typified by a limited number or no mature B cells or antibody-secreting plasma cells. Affected persons develop severe, recurrent bacterial infections, usually during the first year of life.

Other antibody defects are common variable immunodeficiency (CVID) and immunoglobulin A (IgA) deficiency. CVID is characterized by variably low levels of immunoglobulin G (IgG), immunoglobulin M (IgM), and IgA, and sub-optimal antibody responses after vaccination. CVID patients usually experience recurrent bouts of pneumonia and infections of the joints, bones, and skin. These persistent infections lead to organ damage, often resulting in disability or death from chronic lung disease (25). Moreover, affected females with CVID had a >400-fold increased risk for lymphomas in their fourth and fifth decades of life compared with age-matched general population risks in one U.S. study (25). IgA deficiency, similar to other PI diseases, has a wide clinical spectrum. Although all affected persons lack IgA in the mucous membranes lining the airways and digestive tract, certain persons are asymptomatic whereas other have recurrent infections. For reasons not completely understood, the incidence of allergy or autoimmune disease is increased among patients with selective IgA deficiency. Certain IgA-deficient persons might have severe or fatal anaphylactic reactions to blood or blood-products containing IgA.

**Combined B- and T-Cell Deficiencies**

Combined B-cell and T-cell immunodeficiencies constitute approximately 20% of PI diseases (23). In the most serious forms (e.g., severe combined immunodeficiency [SCID] disorders), survival beyond the first year of life is rare without prompt immune reconstitution through hematopoietic stem cell transplantation (15,16,19,26,27). Immune reconstitution with gene therapy has been achieved for forms of SCID (14,20). Early diagnosis of SCID is critical because the chances for successful treatment are highest for infants who have not yet experienced severe opportunistic infections (19). Mutations in eight different genes cause SCID (19,28). Approximately half of all cases are linked to the X chromosome. X-linked SCID results from a mutation in the interleukin 2 receptor gamma (IL2RG) gene that produces the common gamma chain subunit, a component of multiple IL receptors. The product of the IL2RG gene activates a key signaling molecule, Janus-associated kinase 3 (JAK3 gene product). A mutation in JAK3 also can result in SCID. Other forms of SCID are associated with deficient activity in the enzyme adenosine deaminase (ADA gene product) or a defect in the recombination-activating gene (RAG). The genetic defect has not been identified for certain forms of SCID. Other combined immunodeficiencies are part of well-defined immunodeficiency syndromes (e.g., Wiskott-Aldrich syndrome [WAS], ataxia telangiectasia, and hyper-IgE syndrome), all of which are associated with recurrent infections and decreased life expectancy (Table 1).

Cellular immune deficiencies, resulting from defects in T-cell maturation or function, contribute an estimated 10% of PI cases (23). One example is DiGeorge syndrome, which is typified by aberrant development of the heart, parathyroid glands, or thymus. The absence of a thymus gland in patients with DiGeorge syndrome leads to low T-cell numbers and decreased function, but the degree of immunologic impairment varies considerably (29,30). Approximately 90% of these patients have a microdeletion in chromosome 22q11.2, such that multiple genes from this region are absent (additional information is available at http://www.genetests.org).

**Defective Phagocytes**

An estimated 18% of PI cases result from defective phagocytes (23). Phagocytic defects result in the inability of cells that normally engulf and kill invaders to remove pathogens or
# TABLE 1. Examples of primary immunodeficiency diseases, by affected component of the immune system

<table>
<thead>
<tr>
<th>Designation</th>
<th>Gene</th>
<th>Genetic locus</th>
<th>Mode of inheritance</th>
<th>Description/Pathogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibody deficiencies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-linked agammaglobulinemia (XLA)</td>
<td>BTK</td>
<td>Xq21.3–q22</td>
<td>X-linked recessive</td>
<td>Mutations in the gene encoding Bruton’s tyrosine kinase (BTK), a regulator of B-cell development; absence of mature circulating B cells and undetectable or substantially low serum immunoglobulin (Ig) levels lead to recurrent bacterial infections during the first year of life</td>
</tr>
<tr>
<td>Common variable immunodeficiency (CVID)</td>
<td>Unknown</td>
<td></td>
<td>Complex</td>
<td>Unknown variable defects in B- and T-cell function and regulation result in recurrent bacterial infections, usually during the second or third decade of life</td>
</tr>
<tr>
<td>ICOS deficiency</td>
<td>ICOS</td>
<td>2q33</td>
<td></td>
<td>One subset of CVID is ICOS deficiency, inducible host stimulator defect</td>
</tr>
<tr>
<td>Immunoglobulin A (IgA) deficiency</td>
<td>IGAD1</td>
<td>6p21.3</td>
<td>Autosomal dominant</td>
<td>Absent or marked reduction of serum IgA; majority of patients are asymptomatic; others have recurring respiratory infections, chronic diarrhea, allergies, or autoimmune disease</td>
</tr>
<tr>
<td>Hyper-IgM syndrome type 2 (AID deficiency)</td>
<td>AID</td>
<td>12p13</td>
<td>Autosomal recessive</td>
<td>Defect in the activation-induced cytidine deaminase (AICDA) required for Ig isotope switching and somatic hypermutation in B cells; low IgG and IgA, normal or increased IgM</td>
</tr>
<tr>
<td>Hyper-IgM syndrome type 3</td>
<td>CD40</td>
<td>20q12–q13.2</td>
<td>Autosomal recessive</td>
<td>Low IgG, IgA; normal or increased IgM; bacterial and opportunistic infections</td>
</tr>
<tr>
<td><strong>Cellular deficiencies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DiGeorge syndrome</td>
<td>DGCR</td>
<td>22q11.2</td>
<td>Autosomal dominant</td>
<td>Hemizygous chromosomal deletion results in developmental defect of the thymus; also can cause congenital heart disease, hypoparathyroidism, and other congenital defects</td>
</tr>
<tr>
<td>Interferon gamma receptor deficiency</td>
<td>IFNGR1</td>
<td>6q23–q24</td>
<td>Autosomal recessive</td>
<td>Autoimmune endocrinopathies; increased susceptibility to mycobacterial disease</td>
</tr>
<tr>
<td>IL-12 receptor deficiency</td>
<td>IL12B</td>
<td>5q31.1–q33.1</td>
<td>Autosomal recessive</td>
<td>Defect in the receptor for interleukin-12; increased susceptibility to mycobacterial disease</td>
</tr>
<tr>
<td>IL12RB1</td>
<td></td>
<td>19p13.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Combined B- and T-cell deficiencies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-negative, B-positive — severe combined immunodeficiency (SCID): T cells are missing, but B cells can be present</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-linked SCID</td>
<td>IL2RG</td>
<td>Xq13.1</td>
<td>X-linked recessive</td>
<td>Most common form of SCID; caused by a mutation in the IL-2 receptor gene on the X chromosome needed for the normal growth and function of T cells and B cells; lymphopenia occurs primarily from the absence or near absence of T cells and natural killer cells; B cells are immature and defective</td>
</tr>
<tr>
<td>Jak3 deficiency</td>
<td>JAK3</td>
<td>19p13.1</td>
<td>Autosomal recessive</td>
<td>Mutation in the gene that encodes Janus-associated kinase 3 (JAK3) needed for differentiation of hematopoietic cells; Jak3 deficiency is a rare cause of T-cell lymphopenia</td>
</tr>
<tr>
<td>IL7R deficiency</td>
<td>IL7R</td>
<td>5p13</td>
<td>Autosomal recessive</td>
<td>Defect in the IL7 receptor needed for T-cell development; T-cell numbers are low; B cells are present but nonfunctional</td>
</tr>
<tr>
<td>CD45 deficiency</td>
<td>PTPRC</td>
<td>1q31–q32</td>
<td>Autosomal recessive/</td>
<td>Mutation in the protein tyrosine phosphatase receptor type C CD45 gene results in a lack of expression of CD45; T-cell numbers are low; B cells are present but defective</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Autosomal dominant</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 1. (Continued) Examples of primary immunodeficiency diseases, by affected component of the immune system

<table>
<thead>
<tr>
<th>Designation</th>
<th>Gene</th>
<th>Genetic locus</th>
<th>Mode of inheritance</th>
<th>Description/Pathogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-negative, B-negative — SCID: both T cells and B cells are missing</td>
<td>RAG1 deficiency</td>
<td><strong>RAG1</strong></td>
<td>11p13</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td></td>
<td>RAG2 deficiency</td>
<td><strong>RAG2</strong></td>
<td>11p13</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td></td>
<td>Artemis deficiency</td>
<td><strong>Artemis</strong></td>
<td>10p13</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td></td>
<td>ADA deficiency</td>
<td><strong>ADA</strong></td>
<td>20q13.11</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Other combined deficiencies</td>
<td>ZAP 70 deficiency</td>
<td><strong>ZAP70</strong></td>
<td>2q12</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td></td>
<td>CD3 deficiency</td>
<td><strong>CD3E/G</strong></td>
<td>11q23</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td></td>
<td>PNP deficiency</td>
<td><strong>PNP</strong></td>
<td>14q13</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Other combined immunodeficiency syndromes</td>
<td>X-linked hyper-IgM syndrome</td>
<td><strong>CD40L</strong></td>
<td>Xq26.3-q27.1</td>
<td>X-linked recessive</td>
</tr>
<tr>
<td></td>
<td>Wiskott-Aldrich syndrome (WAS)</td>
<td><strong>WASP</strong></td>
<td>Xp11.22- Xp11.23</td>
<td>X-linked recessive</td>
</tr>
<tr>
<td></td>
<td>Ataxia-telangiectasia</td>
<td><strong>ATM</strong></td>
<td>11q22.3</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td></td>
<td>Chronic mucocutaneous candidiasis</td>
<td>Unknown</td>
<td>Autosomal recessive</td>
<td>Chronic mucocutaneous candidal infections and autoimmune endocrinopathies</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>CMCT</strong></td>
<td>2p</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td></td>
<td>Autoimmune polyendocrinopathy with candidiasis and ectodermal dysplasia (APECED)</td>
<td><strong>AIRE-1</strong></td>
<td>21q22.3</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td></td>
<td>X-linked lymphoproliferative syndrome</td>
<td><strong>SH2D1A</strong> (SAP)</td>
<td>Xq25</td>
<td>X-linked recessive</td>
</tr>
<tr>
<td></td>
<td>Hyper-IgE syndrome</td>
<td>Unknown</td>
<td>4q21</td>
<td>Autosomal dominant with variable penetrance</td>
</tr>
<tr>
<td></td>
<td>Ectodermal dysplasia associated with immune deficiency (EDA-ID)</td>
<td><strong>IKBKG</strong> (NEMO)</td>
<td>Xq28</td>
<td>X-linked recessive</td>
</tr>
</tbody>
</table>
TABLE 1. (Continued) Examples of primary immunodeficiency diseases, by affected component of the immune system

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<th>Mode of inheritance</th>
<th>Description/Pathogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phagocytic defects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congenital neutropenia</td>
<td>Unknown</td>
<td></td>
<td>Autosomal recessive</td>
<td>Persistent neutropenia from birth</td>
</tr>
<tr>
<td>Cyclic neutropenia</td>
<td></td>
<td>19p13.3</td>
<td>Autosomal recessive</td>
<td>Neutropenia in 3–4-week cycles</td>
</tr>
<tr>
<td><strong>Leukocyte adhesion defect (LAD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD1</td>
<td></td>
<td>21q22.3</td>
<td>Autosomal recessive</td>
<td>Disorder of neutrophil adhesion caused by lack of CD18; characterized by recurrent or progressive necrotic soft-tissue infection, periodontitis, poor wound healing, leukocytosis, and delayed umbilical cord detachment</td>
</tr>
<tr>
<td>LAD2</td>
<td></td>
<td>11</td>
<td>Autosomal recessive</td>
<td>Defect in GDP fucose transporter 1; associated with mental retardation, soft-tissue infection, and delayed healing</td>
</tr>
<tr>
<td><strong>Chronic granulomatous disease (CGD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-linked CGD</td>
<td>CYBB</td>
<td>Xp21.1</td>
<td>X-linked recessive</td>
<td>Disorder of white blood cell bactericidal function characterized by granulomatous lesions of the skin, lungs, and lymph nodes; hypergammaglobulinemia; anemia; defective killing of certain bacteria and fungi</td>
</tr>
<tr>
<td>Autosomal recessive CGD</td>
<td>CYBA</td>
<td>16q24</td>
<td>Autosomal recessive</td>
<td>All defects result in defective nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase</td>
</tr>
<tr>
<td></td>
<td>NCF1</td>
<td>7q11.23</td>
<td>Autosomal recessive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NCF2</td>
<td>1q25</td>
<td>Autosomal recessive</td>
<td></td>
</tr>
<tr>
<td><strong>Complement deficiencies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chédiak-Higashi syndrome</td>
<td>CHS1 (LYST)</td>
<td>1q42.1-42.2</td>
<td>Autosomal recessive</td>
<td>Defect in the lysosomal-trafficking regulator gene; results in partial albinism, bleeding tendency, and fatal lymphoproliferation from EBV; treatment is by bone-marrow transplantation</td>
</tr>
<tr>
<td>Deficiency of individual complement components</td>
<td>CIQA, C1OB, C1QG, C1R, C15, C2, C3, C4A, C4B, C5, C6, C7, C8A, C8B, C8G, C9</td>
<td>Various</td>
<td>Autosomal recessive</td>
<td>Absence of complement components; results in increased infections and lupus-like diseases; C1, C2, C3, C4 associated with autoimmunity and pyogenic infections</td>
</tr>
<tr>
<td>Factor B, Factor H1</td>
<td>BF, HF1</td>
<td></td>
<td></td>
<td>C5-C9 and properdin deficiencies associated with neisserial infections</td>
</tr>
</tbody>
</table>

**Sources:**
infected cells from the body. Chronic granulomatous disease (CGD), caused by a defect in intracellular killing of bacteria by phagocytes, usually appears in childhood, but milder forms can appear in the second or third decade of life. It can be inherited as an X-linked or autosomal-recessive defect; affected persons experience frequent and severe infections of the skin, lungs, and bones and tumor-like masses called granulomas. In leukocyte adhesion defect (LAD), phagocytes lack an essential adhesion molecule, preventing them from migrating to sites of infection. The result is recurrent, life-threatening infections, especially of the soft tissues. Chédiak-Higashi syndrome is a rare and usually fatal disorder caused by granule defects in phagocytes, platelets, and melanocytes. Patients have partial oculocutaneous albinism and often experience overwhelming and fatal infections with Epstein-Barr virus. Both LAD and Chédiak-Higashi syndrome are inherited as autosomal-recessive defects.

Complement System Defects

Defects in the complement system occur less frequently than other PI diseases. They are associated with a nonfunctional protein or the absence of a complete complement molecule capable of attaching to antibody-coated foreign invaders and opsonizing bacteria. The most common defect, C2 deficiency, is an autosomal-recessive inherited defect in the gene for the complement protein C2. Affected persons have recurrent and severe infections with encapsulated bacteria, frequently meningitis, and a susceptibility to autoimmune diseases. Terminal complement protein (C6-8) deficiencies are associated with severe infections with Neisseria meningitidis and N. gonorrhoeae.

Prognoses for Patients with PI Diseases

Although PI diseases share selected clinical manifestations, both the timing of the onset of symptoms and the prognosis vary considerably. Patients with antibody or complement deficiencies can have near-normal life spans, if their deficiencies are diagnosed early, managed appropriately, and are not affected by concurrent chronic diseases. Persons with phagocytic disorders, combined immunodeficiency disorders, and antibody disorders with chronic infections have guarded prognoses; the majority are chronically ill and require intensive treatment. Certain severe PI diseases (e.g., SCID) become apparent early in life, with only a short asymptomatic period after birth. Without an effective early intervention, the majority result in death during the first years of life.

Incidence and Birth Prevalence Estimates

The true frequency of PI diseases in the general population, either individually or in the aggregate, has not been ascertained, but estimates have been reported. Certain countries have developed registries to collect information regarding cases of PI diseases (31–36). The minimum prevalence of PI has been estimated by using data collected from these registries. At least five factors cause these registries to underestimate the true prevalence of PI diseases: 1) lack of clinical recognition, 2) lack of reporting to the registries, 3) overrepresentation of certain referral centers, 4) lack of a standardized case definition, and 5) death before recognition. Population-based data related to incidence and prevalence are critically needed.

The reported minimal estimate of birth prevalence of SCID based on recognized cases is 1/100,000, but this underestimates the prevalence because of infant deaths occurring before diagnosis (15). In contrast, selective IgA deficiency, the most common immunodeficiency, was found in as many as 1/328 healthy blood donors (37). In aggregate, the estimated incidence of diagnosed PI diseases has been reported as 1/10,000 persons (22,38,39). As a comparison, incidence estimates for CF are 1/2,500 among whites and for PKU are 1/16,000 persons (40,41).

Diagnosis

Early detection is possible for the majority of PI diseases, is critical for the success of certain therapies, and can be lifesaving. Genetic diseases (e.g., single-gene disorders with high penetrance) can be detected along a continuum of symptomatic expression by using 1) screening tests to evaluate asymptomatic newborns for conditions that require early intervention and 2) clinical algorithms for early recognition of symptomatic persons before the onset of clinical morbidity, with confirmatory laboratory diagnosis (including genetic testing) (Figure 1). Effective treatment regimens can be initiated early in the course of disease to reduce morbidity, disability, and mortality.

The first clinical clue in diagnosis of a PI disease is usually a history of infections that are persistent, recurrent, difficult to treat, or caused by unusual microbes. Because PIs are frequently inherited, a positive family history is also a key diagnostic tool (42); in a series of 70 PI patients identified in an immunology clinic, 18.6% (N = 13) had family histories of immunodeficiency (43). The type of infection identified in either the
patient or the family history also might indicate the nature of an immunodeficiency. Infections with bacterial organisms are frequently observed among patients with antibody deficiencies; severe infections from viruses, fungi, and other opportunistic organisms characterize T-cell immunodeficiencies. Recurrent infections with staphylococcal and other catalase-positive organisms indicate phagocytic defects, and recurrent *Streptococcus pneumoniae* or *Neisseria* infections characterize patients with complement deficiencies.

Physical examination can identify characteristic physical findings and anatomic changes secondary to infections. Patients with PI diseases often appear chronically ill, with pallor, malaise, and a distended abdomen caused by hepatosplenomegaly. Patients with XLA typically lack peripheral lymph nodes, adenoids, and tonsils. Lymphadenopathy is observed frequently among patients with CGD. In WAS, the genetic mutation causes thrombocytopenia as well as immune defects; children have bruising, petechiae, and eczematous rash (44). However, clinical symptoms can vary from patient to patient, even for identical mutations of the same gene (45). Typical radiographic findings include an absent thymus, which is the hallmark of DiGeorge syndrome and multiple types of SCID. Children with infant-onset ADA deficiency often have characteristic skeletal abnormalities of the ribs and hips readily apparent on radiograph.

Laboratory tests are required to diagnose a PI disease (46). No single testing modality is appropriate for all situations. Given that certain PI diseases have overlapping features and that selected ones can be caused by combined immune defects, clinicians advocate a stepwise approach to screening the immune system (Figure 2). The majority of initial tests are available through commercial or hospital laboratories and include tests to assess humoral immunity (i.e., Ig proteins and specific antibodies), cellular immunity (e.g., lymphocyte/mononuclear cell quantitation or functional assays), phagocytic cell function, and complement components and function.

Genetic testing involves "analysis of human DNA, RNA, chromosomes, proteins, and certain metabolites to detect heritable disease-related genotypes, mutations, phenotypes or karyotypes for clinical purposes" (47). In cases for which the location of the genetic defect is known, testing involves direct testing of the patient’s DNA to identify specific mutations. In certain cases, an assay to measure mRNA (messenger RNA) (e.g., polymerase chain reaction [PCR]) or the protein product (e.g., immunoblotting or flow cytometry) can confirm a diagnosis when the gene product is absent; however, this method cannot detect disease associated with a nonfunctional protein. A simple, reliable way to evaluate function for T cells is delayed type hypersensitivity skin tests and for B cells, antibody responses after vaccination.

**Treatment**

Interventions for PI diseases are aimed at preventing infection, prolonging life, and improving quality of life (48). Use of antibiotics to treat and prevent infections is a key element in patient management. In certain cases, prophylactic antibiotics help to prevent infections (e.g., trimethoprim-sulfamethoxazole to prevent *Pneumocystis carinii* pneumonia among patients with T-cell defects and prevent recurrent infections among patients with CGD). Research has demonstrated the safety and efficacy of replacement therapy with intravenous Ig (IVIG) among patients with defects in antibody production (49). Enzyme replacement therapy for ADA deficiency also is effective (50). Curative interventions, primarily bone-marrow and stem-cell transplantation, have been used with varying degrees of success for an expanding array of PI diseases (15,16,19,26,27,51,52). Clinical trials also have demonstrated that gene therapy can restore near-normal immune function among patients with SCID caused by mutations in *IL2RG*, and similar types of therapy are promising for other immunodeficiencies (14,17,20). However, recently, the occurrence of T-cell leukemia in two of 10 children administered gene therapy for *IL2RG* SCID (mutant gamma-chain IL-2 receptor) has prompted a halt to all gene therapy using retroviral vectors for immunodeficiency. In these cases, the retroviral gene construct of the *IL2RG* gene inserted itself on the oncogene *LMO2* that is aberrantly expressed in acute lymphocytic leukemia of childhood. Thus, insertional oncogenesis was the probable cause of the T-cell leukemia in these two cases (53–55).
Public Health Framework

The defining characteristics of PI diseases make them candidates for a public health intervention approach. Although the clinical manifestations and underlying genetic defects are diverse, PI diseases share the common feature of increased susceptibility to infection and collectively result in substantial morbidity and shortened life spans. Most important, prompt diagnosis and treatment can be life-saving and result in marked improvements in the quality and length of life.

The foundation for a public health intervention to improve the health status of persons with PI diseases is population-based information regarding the incidence, prevalence, and natural history of the diseases; the accuracy of diagnostic methods; and the efficacy of early interventions. However, the majority of these data are lacking. The heterogeneity of PI diseases and the limited understanding of the relation between genotype and phenotype also hinder intervention efforts. Additional obstacles include the difficulty of diagnosis in the absence of a high index of suspicion and the lack of awareness among health-care providers and the public, which impedes the timely recognition of affected persons by using a combination of clinical suspicion and diagnostic testing.

To address these impediments and improve health outcomes among persons with PI diseases, CDC and partners have adapted a population-based public health framework developed as part of CDC’s strategic plan for genomics and public health, to the problem of PI diseases (56). The framework has four components as follows:

* Lymphocyte phenotyping includes enumeration of B, T, and NK cells.
• public health assessment — application of traditional public health methods to assess the impact of PI diseases on community health;
• population-based interventions — development, implementation, and evaluation of screening tests administered to newborns and clinical algorithms for early recognition of symptomatic persons to facilitate the earliest possible diagnosis and treatment for PI diseases;
• evaluation of screening and diagnostic tools — evaluation of screening and diagnostic tools to ensure their quality and appropriateness for identification of patients with PI diseases; and
• communication — communication with health-care providers and the public to facilitate prompt and appropriate diagnosis and intervention.

CDC has begun to apply this framework in the context of ethical, legal, and social considerations to different conditions, most recently to hereditary hemachromatosis, a treatable, adult-onset, single-gene disorder of iron metabolism (57–61). For example, gaps in data related to the natural history of the disease, penetrance, optimal treatment for asymptomatic persons, and the psychosocial effect of genetic testing precluded recommendations for population screening for mutations in HFE, the associated gene (62–64). However, educational efforts are under way to facilitate early diagnosis (e.g., iron overload and HFE mutation testing). Lessons learned from applying the framework to hemochromatosis is being applied to other conditions, including PI diseases.

In November 2001, CDC convened a multidisciplinary panel of specialists to identify and discuss public health strategies that can be applied to PI diseases and also used as an approach for other genetic disorders (65). A systematic assessment based on the established public health framework was applied to the growing group of recognized PI diseases, for which diverse genetic mutations span multiple components of the immune system but all lead to the increased incidence and severity of infections. During the meeting, specialists in clinical immunology, public health, genetics, pediatrics, health communication, and ethics from state and federal agencies, academic centers, professional organizations, and advocacy foundations discussed the public health framework as it relates to PI diseases. The working group’s deliberations were organized around the four components of the framework and centered on challenges and opportunities, priority research questions, and recommendations for public health action. The remainder of this report reflects their analysis of the problem, their conclusions and recommendations, and subsequent deliberations and findings.

Public Health Assessment

Assessment Tools

The majority of what is understood regarding PI diseases derives from accumulation of data from clinical case reports, case series, and case registries. This approach has advantages but has not provided a complete understanding of the incidence, prevalence, and natural history of PI diseases. A public health assessment of the magnitude and characteristics of the problem in the United States, using population-based data, is needed. Quantitative public health methods can be used to assess the effect of gene variants on the risk for disease, disability, and death and to determine the impact of population-based interventions on improved health outcomes. The traditional tools of public health assessment are 1) surveillance, 2) epidemiology, and 3) laboratory science.

Surveillance Systems. Surveillance is the systematic collection, analysis, and interpretation of data related to health outcomes and other health-care events for use in planning, implementation, and evaluation of population-based health activities (66,67). Surveillance data can be derived from traditional data sets (e.g., vital records and health surveys) or obtained proactively from health-care providers, health-care institutions with electronic patient records, or laboratories. Effective surveillance requires standardized case definitions for each disorder of interest.

A surveillance system for PI diseases should be used to determine the incidence and prevalence of these conditions. Assuming routine performance of genotyping, a laboratory-based surveillance component should facilitate the calculation of the prevalence of gene variants among cases. The ability to link cases with other data sets will help determine the morbidity, mortality, disability, and health-care costs associated with PI diseases and help set priorities based on public health impact. The availability of outcomes data will allow evaluation of the effect of changes in health-care policy and practice.

Epidemiologic Research. Epidemiology is the study of the distribution and determinants of disease in specified populations, including assessment of the causal effect of preventive interventions on health outcomes. Although clinical research can identify gene variants and other risk factors for PI diseases, population-based analytic epidemiologic studies are needed to quantify the effect of gene variants on the risk for disease, death, and disability and to determine the relations between genotype and phenotype in the population (7). Epidemiologic studies that contribute to the understanding of the natural history and clinical course of PI diseases and the benefits of early detection and intervention can improve individual outcomes and reduce the public health burden of this group of diseases. Epidemiologic research methods also are
needed to assess the determinants and uses of genetic testing and other promising interventions and health-care practices.

**Laboratory Science.** Both surveillance and epidemiologic research are conducted in conjunction with laboratory efforts. These center on diagnostics, phenotypic characterization, genetic analysis, studies of genotype-phenotype relations, and development and evaluation of screening and diagnostic tests.

**Existing Data-Collection Systems**

Existing population-based data from which to derive a public health assessment of PI diseases are limited. Available data are derived from case-based disease registries that collect patient-specific information from multiple sources.

**Disease and Mutation Registries.** Case-based registries usually are designed to improve patient care but can be helpful for studying rare diseases. In 1992, the Immune Deficiency Foundation (IDF) initiated a registry of U.S. patients with CGD and 5 years later expanded the project to include seven other disorders — hyper-IgM syndrome, XLA, CVID, WAS, SCID, LAD, and DiGeorge syndrome (36). The most reliable data from these registries are for CGD, for which IDF has calculated a minimum estimated U.S. incidence of 1/200,000 live-born infants (36). The registry also is used to collect data related to natural history and clinical course, including the response to treatment. In 1995, IDF conducted a national, cross-sectional survey of approximately 17,000 immunologists and medical school faculty to estimate the burden of PI diseases in the United States, to describe characteristics of persons with these disorders, and to identify problems related to access to treatment. Approximately 1,500 physicians reported caring for an estimated 21,000 patients with PI disease (68).

Other countries have developed their own registry-based estimates of the frequency of PI diseases, ranging from an estimated prevalence of 2.1/100,000 in Australia (31) to 6.8/100,000 in Norway (32–34). A registry maintained by the European Society for Immunodeficiencies (ESID) collects data regarding patients from approximately 25 countries in Europe (69). As of July 2000, the ESID registry contained clinical data for approximately 8,900 patients from 26 countries (70). An example of a registry for another genetic disorder that might be a model for PI diseases is the CF registry, which is based on case ascertainment at comprehensive treatment centers. The Cystic Fibrosis Foundation (CFF) sponsors the National Cystic Fibrosis Patient Registry to collect data regarding all patients examined at CFF-supported and accredited care centers (71). Data are used to support epidemiologic studies, direct research, and design clinical trials, all with the goal of improving the survival of persons with CF (72).

Other sources of case-based information are the Internet-based, locus-specific immunodeficiency mutation databases established by ESID and expanded by other investigators (73,74). These databases contain information regarding specific mutations and certain clinical features of affected persons. The first Internet-based immunodeficiency mutation database, BTKBase, was initiated in 1995 to collect information related to mutations in the BTK gene (Bruton’s tyrosine kinase), which causes XLA (75). Similar locus-specific mutation databases have been developed since then (69,73). Mutation databases can be used to analyze the types of mutations and their distribution in exons and introns, including their location in protein domains. Mutation databases that contain clinical information can be helpful in assessing genotype-phenotype relations and determining the presence of gene variants in asymptomatic family members (76).

Data from disease and mutation registries can be used to estimate the minimal incidence of a disorder, characterize epidemiologic features, and define a range of clinical characteristics in a cohort of patients (36). However, although each has its applications, current registries provide incomplete population-based data regarding the burden of PI diseases. Continued growth of disease and mutation registries relies on the submission of case reports by physicians, resulting in overrepresentation of certain clinical centers in the sample collection (59). Incomplete ascertainment limits the representativeness of the data. Moreover, the lack of standardized case definitions precludes the calculation of sound population-based rates from these sources. The value of mutation databases for public health assessment also is limited by the rarity of genetic laboratory confirmation of PI diagnoses. In other cases, the mutated sequence might be known but not submitted to the database.

**Population-Based Morbidity and Mortality Data.** To contribute to the study of the impact of single-gene disorders, existing population-based data sources were reviewed. Surveillance databases already have been used to evaluate the impact of hereditary hemochromatosis (59). Hospital discharge data provide information concerning short-stay hospitalizations for specific conditions and have been used, for example, to document the substantial morbidity rate and hospitalization charges associated with birth defects and genetic diseases among children (57,77,78). However, the national hospital discharge survey enumerates hospital discharges rather than individual patients, and for rare or underdiagnosed diseases might provide more limited information because of potential inaccuracy of coding and duplication caused by multiple hospitalizations for the same patient. Managed care organizations maintain substantial, linked, computerized inpatient and
outpatient databases that can be helpful in determining incidence rates (79). One example is the Vaccine Safety Datalink (VSD), a partnership between CDC and four health-maintenance organizations designed to evaluate vaccine safety among children. Computerized data concerning vaccinations, medical outcomes, and health services usage are provided for a well-defined population of approximately 1 million children (1993–1996). In addition to determining vaccine-related adverse events, this database could be examined for other relatively infrequent events, including PI diseases (78–80).

Mortality data can provide population-based information concerning survival and cause-specific mortality regarding genetic disorders (60,81–83). Since 1968, CDC’s National Center for Health Statistics has compiled data from all death certificates filed in the United States and made these data available in Multiple-Cause Mortality Files (82,84). The files include demographic and geographic information regarding the decedent and International Classification of Disease (ICD) codes for the underlying cause of death and ≥20 conditions listed on the death certificate (85,86). Methodologic limitations include reliance on coding systems that are not unique or specific enough for birth defects and genetic diseases; delay between death and availability of data; and limited information regarding risk factors. Despite these limitations, mortality files and other population-based data sources will be critical for planning interventions for PI diseases, especially as the causes and treatments of these disorders are further elucidated by epidemiologic studies and human genome research (75,87,88).

Population-Based Disease Surveillance. Efforts to collect population-based epidemiologic and surveillance data related to patients with other genetic diseases also might be helpful models for assessment of PI diseases. Population-based birth-defects surveillance systems also hold promise for collection of data regarding PI diseases (87). Each state has a different approach to birth-defects surveillance. Data sources include vital records, hospital and clinic records, and administrative databases. The diversity of approaches — particularly methodologies used to generate timely data, applications to monitor prevention activities, and projects to improve access to health services and early intervention — provides useful resources for developing surveillance systems for other childhood diseases.

CDC’s program to prevent complications from hemophilia and other bleeding and clotting disorders includes a national surveillance system, prevention interventions conducted through a nationwide network of hemophilia treatment centers (HTCs), and epidemiologic and prevention research. CDC’s first state-based surveillance effort was designed to identify all patients with hemophilia in six states, characterize the patient population, and identify risk factors and outcomes of care (89,90). Through this effort, CDC derived the first population-based estimate of hemophilia prevalence in the United States and demonstrated the effectiveness of the HTC model. In 1996, to address gaps in this system (e.g., lack of patient follow-up and specimen collection), CDC and the HTCs initiated a prospective universal data collection (UDC) system. The UDC system is designed to guide clinical practice, monitor blood safety, develop a specimen repository, and monitor the clinical extent and progression of joint disease (91). Although the UDC system is more comprehensive than the initial surveillance effort, the requirement for informed consent might affect its population-based representativeness.

Workshop Recommendations for Action. The goal of public health assessment for PI diseases is to collect population-based data to define the incidence and prevalence of the disorders. Recommendations from the workshop for public health assessment for PI diseases include the following:

- Collect population-based data regarding the incidence, prevalence, and natural history of PI diseases.
- Collect population-based data regarding the relations between genotype and phenotype for these diseases.
- Collect population-based data regarding the effect of early recognition and effective therapies on morbidity and mortality.
- Target three subsets of PI diseases as priorities for a systematic public health assessment; possibilities include — profound T-cell defects, because of their resulting high mortality in the absence of interventions; — antibody deficiencies, because of the substantial number of persons affected and the high burden of morbidity; and — CGD, because of the existence of an established IDF data set.
- Conduct pilot activities to improve the collection, use, and quality of surveillance and epidemiologic data. These might include — convening a working group of clinical immunologists and scientists to provide guidance regarding case definitions for registry and surveillance activities; — developing collaborations between public and private advocacy groups to expand data collection and completeness of disease registries and to conduct further analyses; — exploring use of existing population-based databases for their potential in yielding useful information regarding the incidence, prevalence, and natural history of PI diseases; and
Population-Based Interventions

Two major areas were discussed at the workshop, 1) early clinical recognition of PI diseases and 2) newborn screening.

Early Clinical Recognition

Background and Rationale. Timely and effective population-based interventions can reduce morbidity and mortality from genetic diseases (Figure 1). For PI diseases, these interventions center on early diagnosis and implementation of effective therapy (e.g., hematopoietic stem cell transplantation, Ig replacement, and administration of antibiotics). The intervention component of the public health framework for PI diseases therefore involves development of strategies for early diagnosis, implementation of pilot demonstration projects, and evaluation of the effect of these interventions on morbidity, disability, health-care costs, and mortality.

When evidence indicates that early diagnosis and treatment will avert the late stages of disease and prevent morbidity, disability, and premature mortality, increased early clinical recognition is one component of a public health response. The goal is to identify persons who have early symptoms indicative of a PI disease so they can receive diagnostic testing to confirm the presence or absence of disease and receive appropriate interventions to prevent adverse outcomes. Although data regarding the benefits of early symptomatic screening are limited, information from clinical centers supports improved outcomes for certain PI diseases through early intervention (25,92–94). The effect might vary, depending on the genetic defect, the age at diagnosis, presence of prior infections, and history of vaccination and blood transfusion (93).

Symptom-Based Screening — Clinical Algorithm.

Increasing early symptomatic screening for PI diseases requires concerted efforts to increase awareness of these conditions among physicians and health-care systems. Primary-care clinicians, particularly pediatricians and family practice physicians, provide the first point of contact for persons with PI diseases by recognizing the possibility of an immunologic problem and the need for appropriate evaluation. Clinicians need to be aware of the estimated prevalence of PI diseases, the natural history of the disorders, the availability and efficacy of treatment, and most importantly, the common early symptoms. Early recognition of PI diseases in the clinical setting can be facilitated by development and evaluation of a symptom-based screening algorithm. Such an algorithm can be designed to 1) identify persons with a frequency of infections who fall outside the normal range of infections; 2) increase physicians’ awareness of the types, frequency, and appearances of PI diseases; 3) facilitate physicians’ understanding of useful screening approaches (e.g., family history); and 4) trigger appropriate action without overburdening the medical care system.

The enhanced early clinical recognition approach has multiple advantages. Symptom-based screening occurs in the usual health-care setting and requires no additional screening infrastructure. Although certain children and adults seen in primary-care settings might have clinical symptoms suggesting PI disease, the number tested still will be considerably lower than that required for universal screening. Finally, including a PI disease as a suspected diagnosis will occur in a clinical setting that offers options for follow-up and referral.

However, the benefits of the symptom-based approach will be limited if diagnostic testing and treatment are unavailable or delayed. For example, researchers at Mt. Sinai School of Medicine are studying whether PI diseases are underrecognized among minority and economically disadvantaged persons. The percentage of white non-Hispanic patients among whom PI diseases are diagnosed and treated at Mt. Sinai is disproportionately high (92%), compared with the population of the hospital’s catchment area of East Harlem, which is predominantly Hispanic (52%) and black non-Hispanic (37%). Possible reasons for the disparity include receipt of care in emergency departments and clinics with multiple providers, lack of regular contact with a primary-care physician, and lack of continuity of care. Investigators are evaluating use of profiles of diagnostic codes that might indicate probable PI diseases and help providers identify patients earlier. Improvements in the specificity and accuracy of coding have been identified.
as needs. Mt. Sinai also is undertaking outreach and educational efforts directed toward providers serving minority populations to increase their awareness and improve the timely diagnosis of PI diseases (65). Such efforts at other centers and in a population-based approach might substantially affect the care of patients with PI diseases.

**Assessment and Evaluation of Impact.** Initiation of treatment after identification of a PI disease and early in the course of disease might be sufficient to prevent premature mortality, but a patient’s quality of life will not improve if the sequelae are not reversible or the disease progression cannot be halted. Thus, systematic studies of the natural history of disease and the effectiveness of interventions in modifying health outcomes are critical. In addition, if the clinical validity of an early recognition algorithm is not sufficiently sensitive, cases will be missed; if the algorithm is not specific enough, too many persons will be referred for testing. Proposed algorithms therefore need to be assessed for analytic validity (e.g., comparing the number and type of infections reported by patients to the documentation in the medical record), clinical validity (e.g., determining the proportion of persons with specific symptoms who have or do not have a PI disease), and clinical utility (e.g., determining whether early detection of a specific disorder affects long-term outcomes and is cost-effective).

The limited experience with symptom-based screening methods for a group of diverse disorders demonstrates the challenges in establishing clinical algorithms that can be applied readily in busy clinical practices with accuracy and efficiency (43,95). Findings indicate that clinical algorithms vary in their analytic and clinical validity, especially depending on the age of the population. Therefore, algorithms must be refined to improve sensitivity and avoid missed cases and to increase specificity to reduce costs associated with the immunologic workup of unaffected children and adults. New practice parameters, including information related to diagnosis and treatment, are in development, and physicians need to be made aware of these to assist in the early identification and management of these patients (L. Kobrynski, M.D., Emory University, Atlanta, Georgia, personal communication, 2003).

**Workshop Recommendations for Action.** Different approaches for early clinical recognition have been used in clinical settings, but none have been systematically evaluated. Workshop recommendations for early clinical recognition are as follows:

- Collect data related to the effect of early interventions on morbidity and mortality associated with PI diseases.
- Identify a group of diseases that can benefit from using an early clinical recognition algorithm. Possibilities include SCID, XLA, CVID, CGD, and WAS.
- Establish a working group to create a system of clinical algorithms for early clinical recognition of PI diseases. The working group should include primary-care physicians. Possible early-recognition tools are scoring systems, lists of warning signs, questionnaires, or alert bulletins.
- Select target audiences and adjust the early-recognition tools for each audience.
- Before widespread application of the algorithms, evaluate the usefulness and accuracy of early clinical signs and symptoms and initial laboratory tests for early recognition of PI diseases. Explore existing databases to test proposed algorithms.
- Report on the effectiveness of the tools among the original target audiences and amend the tools as indicated.
- Evaluate the usefulness of family history in recognizing single-gene disorders early.
- Conduct collaborative studies among clinical centers to examine the natural history of selected PI diseases.
- Conduct research regarding impediments to access to treatment and case management.
- Conduct needs assessments related to timely diagnosis, access to treatment, and ongoing care.

**Newborn Screening**

Certain severe PI diseases become apparent early in life, with only a short asymptomatic period after birth. Without an effective intervention, the majority result in irreversible complications and death before the end of the first year of life. The most useful method for improving the outcomes of diseases with such a narrow window for detection and intervention might be population-based newborn screening (NBS).

**Existing Newborn Screening Programs.** NBS programs began in the 1960s with the development of an accurate and sensitive test for PKU, an inherited disorder of metabolism (96). Children affected with PKU are unable to metabolize the amino acid phenylalanine. If untreated, affected children will be severely mentally retarded and experience other neurologic symptoms. However, dietary therapy started soon after birth will reduce symptoms and allow affected children to develop normally. The average incidence of PKU is approximately 1/16,000 births.

The PKU assay uses a dried blood spot (DBS) specimen. Blood is collected from the heel of an infant 1–2 days after birth. The heel is pricked, and a few drops of blood are spotted onto a filter paper card, dried, and sent to a state or regional public health laboratory. Small filter-paper disks containing dried blood are punched from the specimens and used to test the newborn for PKU and other disorders. This simple, easily transported, and inexpensive specimen-collection method has led to development of population-based...
screening of newborns throughout the world (41,97–99). Babies in the United States are screened for 4–30 different metabolic, hematologic, and endocrinologic disorders within a few days of birth. All of these tests are performed by using DBS specimens. As a population-based public health activity, NBS programs are the responsibility of state public health agencies and operate under policies determined at the state level, although laboratory screening might be contracted to other states or to academic or private laboratories (97,100).

Newborn Screening Quality Assurance Program. CDC’s Newborn Screening Quality Assurance Program (NSQAP) produces, certifies, and distributes DBS materials for external quality control and performance surveillance to help NBS laboratories evaluate and improve the quality of their testing and to foster standardization of NBS services (101). Approximately 250 national and international screening laboratories from 45 countries participate in the quality assurance program. NSQAP recently added quality assurance materials for disorders detected by tandem mass spectrometry (102,103) and CF (101).

Principles for Evaluating Evidence for Newborn Screening. Guidelines for NBS programs are linked to ethical, legal, and social considerations and are based on the premise that screening should be conducted only when science and technology can serve both the individual person and the public good. Certain landmark reports (47,98,104) identify criteria for population-based NBS programs. The criteria typically follow standard principles of population screening developed in 1968 (105). These principles emphasize the

- importance of a specific condition to public health;
- availability of an effective screening test;
- availability of diagnosis and treatment;
- existence of a recognizable latent or early symptomatic phase for the condition and an adequately understood natural history;
- an agreed upon policy regarding whom to treat;
- a balance between screening costs and health expenditures; and
- availability of case-finding capabilities.

These criteria have been discussed and modified multiple times (64,100). With the advent of new testing technologies, the criteria and corresponding evidence and ethical problems are being revisited at the state and national levels (64).

Newborn Screening and SCID. Among PI diseases, SCID is a candidate for development of an NBS protocol. SCID is characterized by profound deficiencies of T- and B-cell function and is usually lethal during infancy without successful immune reconstitution, ideally during the first months of life (15,16,19).

Efficacy of Early Identification and Treatment. Research indicates that infants with SCID who receive hematopoietic stem-cell transplants from related donors in the first 3.5 months of life have approximately 95% chance of survival, compared with a survival rate of 76% for infants receiving this treatment after 3.5 months (27). Infants who received stem cell transplants during the first 28 days of life demonstrated higher levels of T-cell reconstitution and thymic output than did those who received a transplant later; updated survival estimates were 95% (N = 21/22) for infants receiving transplants during the first 28 days, compared with 74% (N = 71/96) for infants receiving transplants after the neonatal period (19). An analysis of registry data for 475 SCID patients from 37 centers in 18 European countries reported that long-term survival among patients who received stem-cell transplants has improved, probably because of more effective prevention of complications (106). Differences were identified by SCID phenotype, with poorer outcomes occurring among SCID patients without B cells than among those with B cells. Immune reconstitution using gene therapy in clinical trials has also been achieved for forms of SCID (14,17,18,20,52); however, as discussed previously, the unexpected complication of T-cell leukemia occurred among 2 of 10 children receiving therapy for IL2RG SCID (53–55). Similar types of therapy are promising for other immunodeficiencies (26).

The need to identify at birth children with SCID, as evidenced from clinical studies, permits time to institute therapies for immune reconstitution before the onset of opportunistic and other infections associated with negative outcomes. SCID meets certain traditional criteria for NBS, as follows (105):

- SCID is fatal during infancy without immune reconstitution.
- A short asymptomatic period exists after birth.
- Effective treatments are available.
- Early intervention improves outcome.
- Profound deficiencies of cellular and humoral immunity might be detectable with screening tests.

Development and Evaluation of Screening Tests. Data regarding the analytic and clinical validity of the screening tests are critical in considering an NBS program. One study, which was conducted in New York state in the 1970s, assessed the effectiveness of a DBS screening test for ADA deficiency based on ADA enzyme activity (107,108). This led to the detection of 12 partially ADA-deficient patients (i.e., persons whose erythrocytes lacked ADA but who had substantial ADA in other cell types and who were clinically and immunologically normal) (109), but no cases of ADA SCID were detected.
However, because of variability in the tests used, two patients with ADA SCID were missed at one hospital. Data regarding genotype-phenotype correlation are now accumulating for ADA deficiency and is important to consider in NBS (110). The majority of ADA-deficient patients have SCID, but in 15%–20% of these, the condition is diagnosed late in childhood or in adulthood with more variable immunodeficiency; normal persons with partial ADA deficiency also have been identified (111).

Identification of SCID at birth will require developing a high-throughput screening test. Data indicate that a T-cell count might be an effective screening tool. The phenotypic hallmark of SCID is profound T-cell lymphopenia, with counts substantially below the first percentile of normal; transplacental maternal T-cell engraftment might cause this number to be higher only in a limited number of cases. Compared with healthy infants, whose total lymphocyte counts at birth are 2,000–11,000 cells/µL (112), counts in SCID patients are usually <1,500–2,000 cells/µL (Figure 3). CD3+ T-cell counts in infants with SCID are typically <500 cells/µL (normal: 3,000–6,500 cells/µL) (15,16,28,113). In a study of a large urban, primarily minority cohort of 800 healthy children, median total lymphocyte counts at ages 0–3 months were 5,400 cells/µL (10th–90th percentile, 3,400–7,600 cells/

**FIGURE 3. Absolute lymphocyte count distributions in severe combined immunodeficiency (SCID) — 25 newborns with SCID and 14 healthy newborns at birth evaluated at Duke University**

![Absolute lymphocyte count](image)

- **Absolute lymphocyte count**
  - SCID
  - Control

<table>
<thead>
<tr>
<th>Total lymphocytes/mm³</th>
<th>SCID</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2,000</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>4,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6,000</td>
<td></td>
<td></td>
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<tr>
<td>8,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10,000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Range of absolute lymphocyte counts (cells/mm³) at birth**
- 25 SCID newborns (age 0–16 days): 114–2,210
- 14 normal infants (age 0–8 days): 1,670–8,910

**Range of T-cell counts (cells/mm³) at birth**
- 25 SCID infants (age 0–16 days): 0–84
- 14 normal infants (age 0–8 days): 903–7,226

**Normal number of lymphocytes (percentage of total leukocytes) at different ages**
- **Birth**: 5,500 (2,000–11,000) cells/mm³ (31%)
- **6 months**: 7,300 (4,000–13,500) cells/mm³ (61%)
- **21 years**: 2,500 (1,000–4,800) cells/mm³ (34%)

**Distribution of total lymphocytes and T-cell subsets in normal healthy children at ages 0–3 months**
- **N = 800** Median total lymphocyte counts: 5,400 cells/µL (10th–90th percentile 3,400–7,600 cells/µL)
- **N = 699** Median CD3 T-cell counts: 3,680 cells/µL (10th–90th percentile 2,500–5,500 cells/µL)
- **N = 699** Median CD4 T-cell counts: 2,610 cells/µL (10th–90th percentile 1,600–4,000 cells/µL)


These include routine screening of newborns for SCID can be considered. The genetic basis of the detection of one or a limited number of specific mutations, the number and wide spectrum of molecular defects and lack of data regarding genotype-phenotype relations that can cause SCID currently precludes development of a standardized case definition of the disorder; determining the analytical validity of the proposed assay; developing a standardized case definition of the disorder; determining cost-benefit; and assessing ethical, legal, and social implications.

The possibility of detecting lymphopenia caused by other genetic causes or HIV infection also needs to be considered. Although children with these conditions do not have SCID, any child identified with severe lymphopenia requires further evaluation. By testing all infants, children with a fatal but treatable disease can be identified and treated, and valuable information can be obtained regarding the incidence of these disorders in the population and the frequency of different mutations among affected persons and in the population.

In considering SCID as a possible addition to state newborn screening, evidence-based criteria should be used but might require re-examination in terms of weighting of different criteria. For example, the question of whether a condition is a key public health problem often is decided on the basis of prevalence. Such disorders as SCID with a prevalence of perhaps 1/100,000 might not be considered a critical public health concern by everyone. Cost concerns (i.e., cost-effectiveness or cost-benefit of proposed screening tests) are also important and need to be considered systematically. Detection of a disorder with a low prevalence might be more cost-effective than detection of a much more common disorder, depending on the severity of the health outcomes, effectiveness of interventions, and cost of screening and treatment. Economic analysis is a way of systematically integrating and evaluating multiple screening criteria. State newborn screening advisory committees should consider this more objective process.

Workshop Recommendations for Action. Workshop recommendations for NBS are as follows:

- Determine the feasibility of NBS for SCID.
- Establish partnerships among investigators and CDC laboratory personnel to develop assays to measure T-cell lymphocytes from DBS.
- Establish partnerships among investigators and CDC laboratory personnel to validate methods to measure T-cell lymphocytes or TREC from DBS. Validation methods can include blinded comparisons of T-cell counts by using the proposed assays from DBS, with a manual differential count from cord blood samples as the benchmark.
- Collaborate with partners to review data regarding population-based normal ranges of T-cells, CD4+ cells, and TREC at birth.
• Pilot test a validated assay. Integrate the proposed assays into an existing NBS panel on an investigational basis with Institutional Review Board (IRB) approval. Demonstrate adequate follow-up capacity and ability to ensure access to treatment without financial barriers. After pilot testing has demonstrated that NBS for T-cell lymphopenia can be performed with an extremely high degree of accuracy at acceptable cost and that follow-up services and treatment can be provided to all affected children identified through screening, a national-level body might recommend that states include this test in the standard NBS panel. Each state should have an advisory committee to consider such a recommendation.

Evaluation of Screening and Diagnostic Tests

Genetic Tests and PI Diseases. Advances in molecular biology and genetic technology have facilitated localization of disease genes and identification of disease-causing mutations, allowing for more rapid development of new genetic tests. PI diseases are among the approximately 800 health conditions for which genetic tests are available in clinical practice (120,121). As the genetic defects associated with PI diseases continue to be discovered, more genetic tests will become available for clinical diagnosis, carrier detection, prenatal diagnosis, and disease management (13,45,122).

The genetic aspects of PI diseases and their implications for diagnosis and patient management have been extensively reviewed (22). Mutation detection is the most reliable diagnostic method (45). However, because of the substantial number of mutations across the spectrum of genes that characterize immunodeficiency, targeting one or a limited number of mutations is inappropriate. Methodologically, DNA-based detection involves different molecular techniques, although DNA sequencing is the usual diagnostic method. Evaluation of mRNA or protein also can be used because absent or low levels of specific mRNA or protein are diagnostic for certain PI diseases. Finally, in conjunction with a family history, clinical and laboratory findings in certain X-linked disorders can also provide a diagnosis.

As tools for the diagnosis and screening of PI diseases evolve, defining and pursuing measures that will ensure their safe and effective use become increasingly critical. Genetic testing in the United States has developed successfully, providing options for avoiding, preventing, and treating inherited disorders. Nonetheless, application of genetic tests is increasing in clinical and public health practice. Concerns related to rapid commercialization of genetic tests are complex and controversial. Appropriate use of tests, quality of laboratory testing, direct-to-consumer marketing, and the potential for discrimination and stigmatization call for public health leadership. Such leadership is needed to protect the public from inappropriate testing and to ensure that tests are properly evaluated and integrated into medical and public health practice (47,56).

Evaluation of Genetic Tests. In 1999, the National Institutes of Health (NIH)-U.S. Department of Energy Task Force on Genetic Testing published recommendations to promote safe and effective genetic testing (47). The Task Force recognized the need to evaluate genetic tests in population-based settings before their use in clinical practice. To ensure the appropriate level of review, the panel recommended that genetic tests be evaluated according to three criteria, analytic validity, clinical validity, and clinical utility. Systematic assessment based on these measures provides data to determine whether a genetic test being considered for use in population-based screening or clinical diagnosis is safe and effective as the technology moves from research to clinical settings (123,124). The criteria also can be applied to screening tests and clinical algorithms.

Analytic validity is the ability of a test to measure the analyte of interest. In the case of a genetic test, analytic validity refers to the ability of the test to classify the genotype or analyte related to the genotype (125). The four main elements of analytic validity are analytic sensitivity, analytic specificity, laboratory quality control, and assay robustness. However, an analytically valid test is useful only if it helps to diagnose or predict disease (i.e., the test must also be clinically valid) (125). Clinical validity is the accuracy with which a test predicts a particular clinical outcome. It reflects both the sensitivity of the test — the proportion of affected persons with a positive test — and specificity of the test, penetrance of the mutations identified by the test, and the prevalence of disease (123,124). Penetrance is the proportion of persons with the mutation who develop the disease. Clinical utility is the usefulness of the test and the value of the information to the person being tested. Clinical utility is assessed according to the benefits and risks associated with the test and the ensuing result or interventions. Clinical utility focuses on health outcomes associated with testing and requires an understanding of the natural history of the disorder.

The Foundation for Blood Research, in collaboration with CDC, has developed a framework for assessing the availability, quality, and usefulness of data related to genetic tests and testing protocols (126). This approach, called ACCE (analytic validity; clinical validity; clinical utility; and ethical, legal, and social implications), derives from the three evaluation criteria described previously, in addition to a fourth that addresses the safeguards and impediments that should be considered in the context of the others (126,127). The evaluation process begins only after the clinical disorder and the test setting (e.g., diagnosis or population screening) have been established. Specific questions (Table 2) help to define the disorder,
the setting, and the type of testing and to address ACCE. The first disorder to undergo an ACCE review was CF (61). Others in progress include hereditary hemochromatosis and breast cancer.

**Development and Availability of Genetic Tests.** The Task Force has addressed the need to encourage development and maintenance of tests for rare genetic diseases, establish a comprehensive system to collect data related to rare diseases, and assess the validity of genetic tests for these conditions (47). Evaluation of genetic tests involves collection and analysis of data regarding analytic validity, clinical validity, clinical utility, and other aspects from laboratories and users. However, for selected PI diseases, genetic testing is available from only a limited number of laboratories, or even only one laboratory, worldwide. Immunodeficiency diseases for which clinical genetic tests or research testing are available, based on information from the GeneTests Laboratory Directory (121), are provided in this report (Table 3). The directory lists 11 PI diseases for which clinical genetic tests are offered in only one laboratory; three diseases for which testing is available only

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**TABLE 2. Targeted questions for evaluating genetic tests, considering analytic validity; clinical validity; clinical utility; and ethical, legal, and social considerations**

<table>
<thead>
<tr>
<th>Element/Setting</th>
<th>Disorder/Setting</th>
<th>Component</th>
<th>Question</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Analytic validity</td>
<td>Is the test qualitative or quantitative?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specificity</td>
<td>How often is the test positive when a mutation is present?</td>
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<tr>
<td></td>
<td></td>
<td>Quality control</td>
<td>Have repeated measurements been made on specimens?</td>
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<td></td>
<td></td>
<td>Robustness</td>
<td>Is an internal quality-control program defined and externally monitored?</td>
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<tr>
<td></td>
<td></td>
<td>Clinical validity</td>
<td>How often is the test positive when the disorder is present?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specificity</td>
<td>How often is the test negative when the disorder is not present?</td>
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<tr>
<td></td>
<td></td>
<td>Prevalence</td>
<td>What is the prevalence of the disorder in this setting?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Predictive value</td>
<td>What are the positive and negative predictive values?</td>
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<tr>
<td></td>
<td></td>
<td>Penetrance</td>
<td>What are the genotype/phenotype relations?</td>
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<tr>
<td></td>
<td></td>
<td>Clinical utility</td>
<td>What is the natural history of the disorder?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Natural history</td>
<td>What is the effect of a positive (or negative) test on patient care?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intervention</td>
<td>Do methods exist to resolve false-positive results in a timely manner?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quality assurance</td>
<td>If appropriate, how is confirmatory testing performed to resolve false-positive results in a timely manner?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pilot trials</td>
<td>Has the test been adequately validated on populations to which it might be offered?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Economics</td>
<td>What are the economic benefits associated with actions resulting from testing?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Facilities</td>
<td>What are the financial costs associated with testing?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Education</td>
<td>What are the economic benefits associated with actions resulting from testing?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monitoring</td>
<td>Is informed consent required?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethical, legal, and social considerations</td>
<td>What are the genetic, environmental, or other modifiers?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Impediments</td>
<td>What is known regarding stigmatization; discrimination; privacy/confidentiality; or personal, family, and social concerns?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Safeguards</td>
<td>What safeguards have been described and are these safeguards in place and effective?</td>
</tr>
</tbody>
</table>

**Sources:**


<table>
<thead>
<tr>
<th>Disorder</th>
<th>Genes and loci</th>
<th>Clinical genetic testing</th>
<th>Research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine deaminase deficiency</td>
<td>Adenosine deaminase (ADA), 20q13.11</td>
<td>1 laboratory in United States (biochemical)</td>
<td>1 laboratory in United States</td>
</tr>
<tr>
<td>Ataxia-telangiectasia</td>
<td>Serine-protein kinase ATM, 11q22.3</td>
<td></td>
<td>1 laboratory in United States</td>
</tr>
<tr>
<td>Autoimmune polyendocrinopathy syndrome type 1 (APECED)</td>
<td>Autoimmune regulator (AIRE-1) 21q22.3</td>
<td>1 laboratory in Italy</td>
<td>1 laboratory in Italy†</td>
</tr>
<tr>
<td>Bloom syndrome</td>
<td>Bloom syndrome protein (BLM), 15q26</td>
<td>1 laboratory in United States</td>
<td>11 laboratories in United States; 2 laboratories in Israel (DNA-based)</td>
</tr>
<tr>
<td>Cartilage-hair hypoplasia</td>
<td>RMRP, 9p21-p12</td>
<td>1 laboratory in Switzerland (DNA-based)</td>
<td>1 laboratory in Switzerland; 2 laboratories in United States</td>
</tr>
<tr>
<td>Chronic granulomatous disease</td>
<td>Cytochrome B-245 light chain (CYBA), 16q24 Cytochrome B-245 heavy chain (CYBB), Xp21.1 Neutrophil cytosol factor 1 (NCF1), 7q11.23 Neutrophil cytosol factor 2 (NCF2), 1q25</td>
<td>1 laboratory in Switzerland (DNA-based and biochemical)</td>
<td>1 laboratory in Switzerland</td>
</tr>
<tr>
<td>Familial atypical mycobacteriosis</td>
<td>Interferon-gamma receptor alpha chain (IFNGR1), 6q23-q24 Interferon-gamma receptor beta chain (IFNGR2), 21q22 Interleukin-12 beta chain (IL12B), 5q31-q33 Interleukin-12 receptor beta-1 chain (IL12RB1), 19p13</td>
<td>1 laboratory in France (DNA-based and biochemical)</td>
<td>1 laboratory in France†</td>
</tr>
<tr>
<td>Hyper Immunoglobulin D syndrome</td>
<td>Mevalonate kinase (MVK), 12q24</td>
<td>1 laboratory in United States (DNA-based)</td>
<td></td>
</tr>
<tr>
<td>Lymphoproliferative disease, X-linked</td>
<td>SH2D1A (SAP), Xq25</td>
<td>1 laboratory in United States (DNA-based)</td>
<td></td>
</tr>
<tr>
<td>Nijmegen breakage syndrome</td>
<td>Nibrin (NBS), 8q21</td>
<td>1 laboratory in Russia</td>
<td>1 laboratory in Japan; 2 laboratories in United States</td>
</tr>
<tr>
<td>Properdin deficiency, X-linked</td>
<td>PFC, PFDS Xp11.4-p11.23</td>
<td>1 laboratory in the Netherlands (DNA-based)</td>
<td></td>
</tr>
<tr>
<td>Purine nucleoside phosphorylase deficiency</td>
<td>Purine nucleoside phosphorylase (PNP), 14q13</td>
<td>1 laboratory in United States (biochemical)</td>
<td></td>
</tr>
<tr>
<td>Wiskott-Aldrich syndrome</td>
<td>Wiskott-Aldrich syndrome protein (WASP), Xp11</td>
<td>1 laboratory in United States; 1 laboratory in Israel; 1 laboratory in the Netherlands; 2 laboratories in Canada (DNA-based)</td>
<td>3 laboratories in United States; 1 laboratory in Israel; 1 laboratory in Canada†</td>
</tr>
<tr>
<td>X-linked agammaglobulinemia</td>
<td>Bruton’s tyrosine kinase (BTK), Xq21.3-q22</td>
<td>1 laboratory in United States (DNA-based)</td>
<td>2 laboratories in United States</td>
</tr>
<tr>
<td>X-linked severe combined immunodeficiency</td>
<td>Interleukin-2 receptor gamma chain (IL2RG), Xq13.1</td>
<td>2 laboratories in United States (DNA-based and biochemical)</td>
<td>2 laboratories in United States</td>
</tr>
</tbody>
</table>

*Additional information is available at http://www.genetests.org.
†Indicates the same laboratory performing both clinical testing and research for the disorder.

Guidance and criteria for transferring genetic tests from the research and development phase to clinical and public health practice also are needed. Certain genetic tests were developed in research laboratories and then made available for patient testing. For such rare diseases as PI, a laboratory that primarily conducts research might be the only clinical testing site available. A mechanism needs to be established to enable these laboratories to participate in and contribute to the continu-
ous test evaluation and validation process. Concurrently, criteria need to be developed to guide the transition of genetic testing from research into clinical and public health use.

For certain PI diseases, genetic tests are available only from non-U.S. laboratories (Table 3). The Clinical Laboratory Improvement Amendments (CLIA) require that U.S. laboratories refer a specimen for testing only to a CLIA-certified laboratory or a laboratory meeting equivalent requirements as determined by the Center for Medicare and Medicaid Services.* To ensure access to quality genetic testing, a process is needed to evaluate the tests and practices of non-U.S. laboratories that receive test referrals from the United States, determine performance equivalence to CLIA standards, and ensure access to and availability of testing for rare disorders.

Additional needs include 1) collection of population-based data regarding analytic validity, clinical validity, and clinical utility for immunologic tests used to diagnose PI diseases (Figure 2); 2) development of algorithms for use of laboratory tests and clinical information to increase the likelihood of early clinical diagnosis of PI diseases; and 3) population-based research to evaluate the utility of genetic tests as early diagnostic tools for PI diseases, both as part of NBS programs and for confirmatory or follow-up diagnosis.

**Workshop Recommendations for Action.** Recommendations from the workshop include the following:

- Evaluate potential genetic tests for their validity, utility, and feasibility as both screening tests and confirmatory or follow-up diagnostics in combination with other tests.
- Ensure that CLIA-compliant laboratory testing is accessible, available, and valid for diagnosing rare genetic diseases, including suspected PI diseases, in collaboration with agencies providing oversight for CLIA, NIH Office of Rare Diseases, CDC, and others.
- Support the formation of treatment networks and referral centers to ensure access to diagnosis and care for persons with PI diseases.
- Collect data regarding the analytic and clinical validity of molecular tests used for diagnosis and any proposed screening tests.
- Review gene databases in the United States and Europe to highlight the availability and possible sources of data regarding the validity and quality of tests.
- Identify centers for pilot testing of any proposed screening assays to determine clinical validity, in collaboration with states, CDC, other federal agencies, and other partners. Integrate any proposed validated assay into an existing NBS panel on an investigational basis with IRB approval. Demonstrate adequate follow-up capacity and ability to ensure access to treatment without financial barriers.

**Education and Communication**

To encourage early recognition of PI diseases, followed by appropriate referral and treatment, primary-care providers, parents, and other caregivers must be educated regarding the symptoms of PI diseases, resources for referral, and treatment options. The effectiveness of a health communication and education campaign depends on the consistency of the messages and the coordination of communication strategies to reach targeted audiences among the groups involved in PI research and education.

**Existing Efforts.** Multiple agencies and organizations sponsor outreach and educational efforts designed to increase awareness of PI diseases. NIH, Mt. Sinai Hospital, the Jeffrey Modell Foundation (128), and IDF (129) have all targeted proactive outreach efforts to a range of audiences (e.g., health-care providers, patients, families, and teachers), although health-care providers have been the primary focus. Outreach activities and resources include conferences and workshops, Internet-based training and resources, community-based training, distribution of awareness posters, media briefings and news releases, consulting networks, and a visiting professor program. The National Organization for Rare Disorders (NORD) also provides print and online resources for health-care providers on multiple rare diseases, including PI diseases (130).

Although these educational efforts have been ongoing for years, outcomes have not been formally evaluated. In addition, various educational activities or messages have not been coordinated, and consensus has not been developed among the organizations or scientists involved in educational research related to PI diseases. Because the diseases vary in severity, symptoms, etiology, and outcomes, coherent messages regarding groups of PI diseases are difficult to create, and no agreement exists concerning which disorders should be the focus of a health communication campaign. Although educational efforts should highlight PI diseases that can benefit from and be targeted for early recognition and that have established criteria for early clinical recognition, priorities for educational efforts have yet to be established.

**Components of Effective Programs.** Effective health communication and education programs should be preceded by consensus in the scientific community regarding which PI diseases to include in an educational program, the associated symptoms, and the recommended screening and management steps. To encourage early recognition, education regarding PI diseases also will need to reach multiple audiences, including the general public, parents, physicians, school nurses, child care providers, and policy makers. Reaching each audience

*42 CFR§493.1242(c).*
with consistent but targeted messages will require careful coordination among different agencies.

Attempts to reach primary-care providers, recognized as the front line in the fight against PI diseases, must overcome multiple barriers. Other diseases with higher prevalence command the attention of physicians. Primary-care providers with heavy caseloads and limited time for continuing education activities probably focus their continuing education efforts on problems encountered most frequently among primary-care providers. Health-care providers are most likely to attend to the most prevalent health problems among their patients. The prevalence of PI diseases (individually or collectively) has not been established, although estimates classify them as rare to extremely rare. With such high-prevalence diseases as asthma claiming high priority for providers’ attention and concern, focusing on less prevalent health problems might be difficult.

Development of a broad health communication campaign for providers and the public is premature. Research to determine the prevalence and etiology of PI diseases and the efficacy of early treatment must be completed before effective messages and educational materials for the public and providers can be developed. However, pending delineation of defined symptoms, disease groups, and treatment recommendations, health communication efforts still can be useful. Although research has not yet yielded a defined set of educational goals related to PI diseases, health communication efforts can be used in the interim to increase awareness among scientists and clinicians. Certain health-care providers might be unaware of PI diseases and research, and researchers might be unaware of opportunities for funding and participation in PI investigations.

**Workshop Recommendations for Action.** Workshop recommendations include the following:

- Target health-care providers and scientists for early-stage communication activities. Increase their awareness of studies under way, questions motivating research programs, opportunities for participation and funding, and resources.
- Use research concerning the outcomes of previous and ongoing educational programs to determine how best to reach target audiences with information related to PI diseases. Systematically analyze the range of outreach efforts to determine 1) information reach, 2) frequency of message contact, and 3) interaction of messages from different organizations. Use evidence-based outcome assessments to determine awareness, knowledge, and uses of information from previous education and communication programs.
- Convene a working group of health communication specialists to establish campaign goals, audiences, and strategies, even as research continues and consensus is reached regarding disorders to include in a health communication campaign and case definitions and clinical recommendations are developed. The working group should
  - determine additional formative research needed to assess target audiences’ awareness, knowledge, and behaviors related to PI;
  - develop or revise materials that are consistent with campaign goals;
  - develop additional materials as needed to achieve campaign goals;
  - pretest materials with target audiences;
  - disseminate messages that are consistent with recommendations from pretesting, and
  - include process and evidence-based outcome evaluations as part of campaign planning.

**Conclusion**

This report presents a framework for stakeholders and policy makers who will collaborate to define the future of an emerging and promising field of study that can markedly improve health in persons with PI diseases. The recommended interventions encompass multiple goals—helping children, educating clinicians, developing and maintaining awareness of PI diseases, and providing information for policy development and change. Additional efforts are needed to define priorities in future public health actions and associated costs and benefits. The proposed public health framework is critical for PI diseases and serves as a model for other genetic disorders that can benefit from early diagnosis and opportunities for interventions to improve health outcomes.

**References**


Terms and Abbreviations Used in This Report*

ACCE analytic validity; clinical validity; clinical utility; and ethical, legal, and social implications

ADA adenosine deaminase gene

AICDA activation-induced cytidine deaminase gene

allele alternative form of a gene that exists at a specific gene location (locus) on a chromosome

analyte substance measured by a laboratory test

APECED autoimmune polyendocrinopathy with candidiasis and ectodermal dysplasia

autosome nuclear chromosomes other than sex chromosomes; the diploid human genome consists of 46 chromosomes: 22 pairs of autosomes, and one pair of sex chromosomes (the X and Y chromosomes)

autosomal dominant abnormal gene on one of the autosomal chromosomes from either parent, transmission of which can cause a particular trait or disorder

autosomal recessive abnormal gene on one of the autosomal chromosomes from each parent, transmission of both abnormal genes is required to cause a particular trait or disorder

B cell antibody-producing lymphocyte; a type of white blood cell

birth defect defect present at birth, whether caused by mutant genes or by prenatal events that are not genetic

BTK Bruton's tyrosine kinase gene

CF cystic fibrosis

CFF Cystic Fibrosis Foundation

CGD chronic granulomatous disease

chromosome one of the thread-like structures in the cell nucleus; consists of chromatin and carries genetic information (DNA); human cells normally contain 46 chromosomes (23 pairs)

CLIA Clinical Laboratory Improvement Amendments

* Additional definitions are available at http://www.genome.gov/glossary.cfm.
codon
three-base sequence of DNA or RNA that specifies an amino acid
complement
a set of serum proteins that binds antigen-antibody complexes to kill microorganisms
CVID
common variable immunodeficiency
DBS
dried blood spot
deletion
particular kind of mutation; loss of a piece of DNA from a chromosome
DNA
deoxyribonucleic acid
EDA-ID
ectodermal dysplasia associated with immune deficiency
enzyme
protein that facilitates a specific biochemical reaction
ESID
European Society for Immunodeficiencies
exon
protein-coding DNA sequence of a gene
gene
functional and physical unit of heredity, consisting of a segment of DNA arranged linearly along a chromosome; the majority of genes contain the information for making a specific protein leading to a particular characteristic or function
gene product
biochemical material, either RNA or protein, resulting from expression of a gene
gene therapy
treatment of a genetic disorder by replacing, supplementing, or manipulating nonfunctional genes with normal genes
genetic marker
landmark for a target gene, either a detectable trait that is inherited with the gene or a distinctive segment of DNA
genetic testing
examining a sample of blood or other body fluid or tissue for biochemical, chromosomal, or genetic markers that indicate the presence or absence of genetic disease
genome
complete DNA sequence, containing all genetic information and supporting proteins, in the chromosomes of a person or species
genomics
study of the functions and interactions of all the genes in the genome, including their interactions with environmental factors

genotype
a person’s genetic makeup, specifically the alleles present at specific gene loci

genotype/phenotype
correlation
association between the presence of a certain mutation or mutations (genotype) and the resulting physical trait, abnormality, or pattern of abnormalities (phenotype)
HIV
human immunodeficiency virus
HTCs
hemophilia treatment centers
Human Genome Project
international research project to map each human gene and to completely sequence human DNA
IDF
Immune Deficiency Foundation
Ig
immunoglobulin
ILR2G
interleukin 2 receptor gamma gene
incidence
number or proportion of new cases of a specified condition among a population during a specified period
inherited
transmitted through genes from parents to offspring
insertion
type of mutation in which a DNA sequence is inserted into a gene, disrupting the normal structure and function of that gene
IRB
Institutional Review Board
IVIG
intravenous immunoglobulin
JAK3
Janus-associated kinase 3 gene
LAD
leukocyte adhesion defect
locus
position on a chromosome where a specific gene is located
microarray technology
methods for measuring expression of multiple genes simultaneously under specific conditions relative to baseline (i.e., up regulation or down regulation)
missense
a genetic mutation that alters the amino acids in the protein product of a gene
mRNA
messenger RNA
mutation
permanent heritable change in the molecular sequence of a gene
NADPH
nicotinamide-adenine dinucleotide phosphate
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>NBS</td>
<td>newborn screening</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>likelihood that a person with a negative test result is actually not affected by the disease</td>
</tr>
<tr>
<td>NHGRI</td>
<td>National Human Genome Research Institute</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>nonsense</td>
<td>a genetic mutation in single base-pair substitution in DNA resulting in premature stop codons in the genetic code</td>
</tr>
<tr>
<td>NORD</td>
<td>National Organization for Rare Disorders</td>
</tr>
<tr>
<td>NSQAP</td>
<td>Newborn Screening Quality Assurance Program</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>penetrance</td>
<td>frequency with which a genotype manifests itself in a specific phenotype</td>
</tr>
<tr>
<td>phenotype</td>
<td>clinical presentation or expression of a specific gene or genes, environmental factors, or both</td>
</tr>
<tr>
<td>PI</td>
<td>primary immunodeficiency</td>
</tr>
<tr>
<td>PKU</td>
<td>phenylketonuria</td>
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<tr>
<td>positive predictive value</td>
<td>likelihood that a person with a positive test result is actually affected by the disease</td>
</tr>
<tr>
<td>prevalence</td>
<td>number or proportion of existing cases of a specified condition in a population</td>
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<tr>
<td>RAG</td>
<td>recombination-activating gene</td>
</tr>
<tr>
<td>regulatory (gene)</td>
<td>a genetic mutation that affects aspects of gene expression</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>SCID</td>
<td>severe combined immunodeficiency</td>
</tr>
<tr>
<td>screening</td>
<td>testing on a population basis to identify persons at risk for developing specific disorders</td>
</tr>
<tr>
<td>sensitivity</td>
<td>frequency with which a test yields a positive result when the abnormality or disease in question is actually present in the person being tested</td>
</tr>
<tr>
<td>sequencing</td>
<td>process by which the nucleotide sequence is determined for a segment of DNA</td>
</tr>
<tr>
<td>sex chromosome</td>
<td>the X and Y chromosomes</td>
</tr>
<tr>
<td>single-gene disorder</td>
<td>a disorder caused by one or a pair of mutant alleles at a single locus</td>
</tr>
<tr>
<td>specificity</td>
<td>frequency with which a test yields a negative result when the abnormality or disease in question is not present in the person being tested</td>
</tr>
<tr>
<td>splice site</td>
<td>a genetic mutation that can lead to frameshift mutations</td>
</tr>
<tr>
<td>T cell</td>
<td>a white blood cell or lymphocyte that develops in the thymus and mediates cellular immune responses</td>
</tr>
<tr>
<td>TREC</td>
<td>T-cell antigen receptor excision circles</td>
</tr>
<tr>
<td>UDC</td>
<td>universal data collection</td>
</tr>
<tr>
<td>WAS</td>
<td>Wiskott-Aldrich syndrome</td>
</tr>
<tr>
<td>X-linked recessive</td>
<td>genes transmitted on the X chromosome</td>
</tr>
<tr>
<td>XLA</td>
<td>X-linked agammaglobulinemia</td>
</tr>
</tbody>
</table>
Applying Genetic and Public Health Strategies to Primary Immunodeficiency

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Continuing Education Activity Sponsored by CDC
Applying Public Health Strategies to Primary Immunodeficiency Diseases
A Potential Approach to Genetic Disorders

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1. Read this MMWR (Vol. 53, RR-1), which contains the correct answers to the questions beginning on the next page.
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3. Indicate whether you are registering for CME, CEU, or CNE credit.
4. Select your answers to the questions, and mark the corresponding letters on the response form. To receive continuing education credit, you must answer all of the questions. Questions with more than one correct answer will instruct you to “Indicate all that apply.”
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Continuing Medical Education (CME). CDC is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians. CDC designates this educational activity for a maximum of 1.5 hours in category 1 credit toward the AMA Physician’s Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

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Goal and Objectives

This MMWR provides recommendations regarding public health strategies for primary immunodeficiency (PI) diseases. These recommendations were prepared by CDC staff and other specialists in PI diseases after consultation with a multidisciplinary panel. The goal of this report is to familiarize readers with a public health framework for addressing health problems resulting from a group of primarily single-gene disorders. Upon completion of this continuing education activity, the reader should be able to describe 1) the four components of a public health framework; 2) how public health assessment can be applied to PI and other genetic diseases; 3) the framework for evaluating genetic tests, including analytic validity, clinical validity, clinical utility, and ethical, legal, and social considerations; 4) two public health interventions to increase early diagnosis and treatment for genetic diseases (i.e., newborn screening and early clinical recognition); and 5) the key components of an effective health education program for PI diseases.

To receive continuing education credit, please answer all of the following questions.

1. Primary immunodeficiency diseases are usually . . .
   A. single-gene disorders of the immune system.
   B. fatal without early treatment.
   C. disorders characterized by recurrent bacterial/viral infections.
   D. all of the above.

2. Common variable immunodeficiency (CVID) is characterized by all of the following features, except . . .
   A. low levels of immunoglobulin G, M, or A.
   B. recurrent infection of the respiratory or gastrointestinal tract.
   C. an increased incidence of lung cancer.
   D. death from chronic lung disease.

3. Population-based surveillance for PI diseases should involve . . .
   A. active assessment of inpatient hospitalization records, outpatient clinic records, and vital records in a defined geographic area.
   B. evaluation of population-based mortality data from death certificates.
   C. computerized inpatient databases from hospital discharge or managed care organizations.
   D. prevalence estimates based on case-based disease registries.
   E. all of the above.
   F. A, B, and C.

4. The goal of early clinical recognition is to . . .
   A. reduce disability and premature mortality from PI disease.
   B. identify newborns with PI disease.
   C. identify those persons with symptoms of PI diseases for referral to an immunologist.
   D. A and C.
   E. all of the above.

5. Which of the following is not true regarding newborn screening?
   A. Newborn screening programs were first begun to identify infants with phenylketonuria.
   B. Severe combined immunodeficiency (SCID) is a candidate for newborn screening because it is fatal during infancy without treatment and because intervention before appearance of clinical symptoms can improve outcomes.
   C. Newborn screening for SCID can be performed by screening for B-cell lymphopenia.
   D. Detecting specific DNA sequences for disease-causing alleles for SCID is possible by using dried blood spots.

6. The clinical validity of a genetic test is . . .
   A. the ability of a test to measure the gene of interest.
   B. dependent on the penetrance of the genetic mutation.
   C. focuses on the health outcomes associated with testing.
   D. reflective of the proportion of affected persons with a positive test.
   E. B and D.

7. Educational efforts for PI diseases need to . . .
   A. tailor messages to targeted groups of health-care providers.
   B. use outcome assessments to determine knowledge and uses of education messages.
   C. provide consistent messages regarding symptoms, screening, and management.
   D. include different strategies for disseminating information.
   E. be evidence-based.
   F. all of the above.

8. Diagnostic testing for T-cell disorders involves all of the following, except . . .
   A. complete blood count (CBC) with differential.
   B. lymphocyte phenotyping.
   C. mitogen stimulation.
   D. nitroblue tetrazolium (NBT).

9. Which of the following statements is true?
   A. Defects in Bruton’s tyrosine kinase (BTK) gene results in defects of B-cell function.
   B. SCID caused by defects in the adenosine deaminase (ADA) gene is inherited as an X-linked disorder.
   C. Lack of CD18 results in a disorder known as Chédiak-Higashi.
   D. Patients with Wiskott-Aldrich syndrome have defective platelets caused by antiplatelet antibodies.

10. Indicate your work setting.
    A. State/local health department.
    B. Other public health setting.
    C. Hospital clinic/private practice.
    D. Managed care organization.
    E. Academic institution.
    F. Other.

11. Which best describes your professional activities?
    A. Physician.
    B. Nurse.
    C. Health educator.
    D. Office staff.
    E. Other.

12. I plan to use these recommendations as the basis for . . . (Indicate all that apply.)
    A. health education materials.
    B. insurance reimbursement policies.
    C. local practice guidelines.
    D. public policy.
    E. other.
13. Each month, approximately how many patients do you treat?
   A. None.
   B. 1–5.
   C. 6–20.
   D. 21–50.
   E. 51–100.
   F. >100.

14. How much time did you spend reading this report and completing the exam?
   A. <2.0 hours.
   B. >2.0 hours but <3.0 hours.
   C. >3.0 hours but <4.0.
   D. >4.0 hours.

15. After reading this report, I am confident I can describe the four components of a public health framework.
   A. Strongly agree.
   B. Agree.
   C. Neither agree nor disagree.
   D. Disagree.
   E. Strongly disagree.

16. After reading this report, I am confident I can describe how public health assessment can be applied to PI and other genetic diseases.
   A. Strongly agree.
   B. Agree.
   C. Neither agree nor disagree.
   D. Disagree.
   E. Strongly disagree.

17. After reading this report, I am confident I can describe the framework for evaluating genetic tests, including analytic validity, clinical validity, clinical utility, and ethical, legal, and social considerations.
   A. Strongly agree.
   B. Agree.
   C. Neither agree nor disagree.
   D. Disagree.
   E. Strongly disagree.

18. After reading this report, I am confident I can describe two public health interventions to increase early diagnosis and treatment for genetic diseases (i.e., newborn screening and early clinical recognition).
   A. Strongly agree.
   B. Agree.
   C. Neither agree nor disagree.
   D. Disagree.
   E. Strongly disagree.

19. After reading this report, I am confident I can describe the key components of an effective health education program for PI diseases.
   A. Strongly agree.
   B. Agree.
   C. Neither agree nor disagree.
   D. Disagree.
   E. Strongly disagree.

(Continued on pg CE-4)
Correct answers for questions 1–9.


20. The objectives are relevant to the goal of this report.
   A. Strongly agree.
   B. Agree.
   C. Neither agree nor disagree.
   D. Disagree.
   E. Strongly disagree.

21. The teaching strategies used in this report (text, figures, and tables) were useful.
   A. Strongly agree.
   B. Agree.
   C. Neither agree nor disagree.
   D. Disagree.
   E. Strongly disagree.

22. Overall, the presentation of the report enhanced my ability to understand the material.
   A. Strongly agree.
   B. Agree.
   C. Neither agree nor disagree.
   D. Disagree.
   E. Strongly disagree.

23. These recommendations will affect my practice.
   A. Strongly agree.
   B. Agree.
   C. Neither agree nor disagree.
   D. Disagree.
   E. Strongly disagree.

24. The content of this activity was appropriate for my educational needs.
   A. Strongly agree.
   B. Agree.
   C. Neither agree nor disagree.
   D. Disagree.
   E. Strongly disagree.

25. The availability of continuing education credit influenced my decision to read this report.
   A. Strongly agree.
   B. Agree.
   C. Neither agree nor disagree.
   D. Disagree.
   E. Strongly disagree.

26. How did you learn about this continuing education activity?
   A. Internet.
   B. Advertisement (e.g., fact sheet, MMWR cover, newsletter, or journal).
   C. Coworker/supervisor.
   D. Conference presentation.
   E. MMWR subscription.
   F. Other.
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Workshop summaries

Improving cellular therapy for primary immune deficiency diseases: Recognition, diagnosis, and management

Linda M. Griffith, MD, PhD,a Morton J. Cowan, MD,b Luigi D. Notarangelo, MD,c Jennifer M. Puck, MD,d Rebecca H. Buckley, MD,e Fabio Candotti, MD,f Mary Ellen Conley, MD,g Thomas A. Fleisher, MD,h H. Bobby Gaspar, MD, PhD,i Donald B. Kohn, MD,j Hans D. Ochs, MD,k Richard J. O’Reilly, MD,l J. Douglas Rizzo, MD, MS,m Chaim M. Roifman, MD, FRCP, FCACB,n Trudy N. Small, MD,l and William T. Shearer, MD, PhD,o on behalf of the workshop participants*

Bethesda, Md, San Francisco and Los Angeles, Calif, Boston, Mass, Durham, NC, Memphis, Tenn, London, United Kingdom, Seattle, Wash, New York, NY, Milwaukee, Wis, Toronto, Ontario, Canada, and Houston, Tex

More than 20 North American academic centers account for the majority of hematopoietic stem cell transplantation (HCT) procedures for primary immunodeficiency diseases (PIDs), with smaller numbers performed at additional sites. Given the importance of a timely diagnosis of these rare diseases and the diversity of practice sites, there is a need for guidance as to best practices in management of patients with PIDs before, during, and in follow-up for definitive treatment. In this conference report of immune deficiency experts and HCT physicians who care for patients with PIDs, we present expert guidance for (1) PID diagnoses that are indications for HCT, including severe combined immunodeficiency disease (SCID), combined immunodeficiency disease, and other non-SCID diseases; (2) the critical importance of a high degree of suspicion of the primary care physician and timeliness of diagnosis for PIDs; (3) the need for rapid referral to an immune deficiency expert, center with experience in HCT, or both for patients with PIDs; (4) medical management of a child with suspicion of SCID/combined immunodeficiency disease while confirming the diagnosis, including infectious disease management and workup; (5) the posttransplantation follow-up visit schedule; (6) antimicrobial prophylaxis after transplantation, including gamma globulin administration; and (7) important indications for return to the transplantation center after discharge. Finally, we discuss the role of high-quality databases in treatment of PIDs and HCT as an element of the infrastructure that will be needed for productive multicenter clinical trials in these rare diseases. (J Allergy Clin Immunol 2009;124:1152-60.)

Key words: Allogeneic hematopoietic stem cell transplantation, gene therapy, primary immunodeficiency, clinical trial

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*See Appendix E1 in this article’s Online Repository at www.jacionline.org for a list of expert opinion and workshop participants.

*This workshop was supported by the Division of Allergy, Immunology and Transplantation, National Institute of Allergy and Infectious Diseases, and the Office of Rare Diseases Research, National Institutes of Health, Bethesda, Md.

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The opinions expressed are those of the authors and do not represent the position of the National Institute of Allergy and Infectious Diseases, the Office of Rare Diseases Research, the National Institutes of Health, or the US Government.

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A collaborative network of North American investigators who care for patients with primary immunodeficiency diseases (PIDs), the Primary Immune Deficiency Treatment Consortium (PIDTC), will be launched in the near future. Representatives of this group met previously to identify critical needs and propose and prioritize future clinical studies in hematopoietic stem cell transplantation (HCT) for PIDs. At that time, to assess the feasibility of such studies, we surveyed a large number of centers as to previous experience with allogeneic HCT for PIDs. We discovered, in contrast to our expectation of referral to specialty centers, that many patients had in fact received HCT for PIDs in programs with experience of only a few cases of this type. The objectives of the present 1.5-day workshop were to summarize current expert opinion for the early detection and diagnosis and clinical management in anticipation of, during, and after definitive treatment for PIDs. Therapeutic options for these patients include allogeneic HCT, enzyme replacement, and gene therapy (GT). Our goal is to provide guidance for health care staff, including those having limited opportunity to care for patients with PIDs.

We have previously reviewed the many distinct variants of severe combined immunodeficiency (SCID; see Table I of our previous publication). In addition, there are a number of combined immunodeficiency diseases (CIDs; eg, NEMO, ZAP-70 deficiency, and IL-12R/IFN-γR deficiency) and non-SCID PID diseases (eg, Wiskott-Aldrich syndrome [WAS], chronic granulomatous disease [CGD], and cartilage hair hypoplasia) that are of sufficient immunologic severity that consideration of HCT also is appropriate. CIDs might be partially permissive for T-cell development because they affect later stages in T-cell development or are due to hypomorphic mutations. Because even experts in PIDs have difficulty in defining the spectrum of CIDs, we present in Table I the group expert opinion of diagnostic criteria and findings supportive of CID. In preparation for the workshop and development of this article, 5 working groups were established to focus on diagnosis of SCID, pre-HCT management, management of non-SCID PIDs, post-HCT management, and the role of databases in future clinical trials. To identify factors affecting the diagnosis and treatment of children with SCID and to determine current practices for managing children with SCID/CID before and after transplantation, 3 separate surveys were done of immunologists and transplantation physicians from the United States and Canada. The discussions of the working groups, as well as results from the surveys, helped to form the basis of this report.

EARLY SUSPICION FOR A DIAGNOSIS OF PID
Recognition of the features of congenital immunodeficiency

Early diagnosis (Fig 1) makes possible early definitive therapy and avoids the complications of pretreatment infections that damage the lungs, liver, kidneys, and other vital organs. Timely diagnosis critically depends on the ascertainment of family history, if positive, and the awareness of primary caregivers of the early signs, symptoms, and laboratory features that indicate a potentially serious underlying problem of cellular immunodeficiency. Once a diagnosis is made, powerful reconstitution measures must be put into place without delay to provide an immune system sufficiently robust to enable survival and good health.

Role of the primary care physician in diagnosis of SCID

The key to a timely diagnosis of PID is suspicion by the primary care provider (Fig 2). At the same time, a timely diagnosis has the potential to be life-saving for the patient, with enormous benefit for the child involved, the family, and society in general. Infants undergoing transplantation in the first 3.5 months of life have a much higher rate of survival than those undergoing transplantation later. If not the primary care pediatrician, it is usually the allergist, pulmonologist, and/or intensive care physician or gastroenterologist who first comes in contact with a child with SCID/CID.

It is possible to identify children with a cellular immune defect with a few relatively simple tests and procedures. Many cases of T-cell immunodeficiency can be predicted on the basis of obtaining an absolute lymphocyte count (ALC) either at birth or in the first 3 months of life. T cells normally comprise 70% of circulating lymphocytes. Because most infants with SCID lack T cells, they are often (but not always) lymphopenic. Correct interpretation of the ALC requires evaluation in the context of age-specific reference intervals. The ALC reference interval is 3,400 to 7,600 cells/mm3 for healthy newborns. A low lymphocyte count for age and low/absent quantitative IgA and IgM levels, although not always present in patients with SCID/CID, should raise the possibility of a severe immunodeficiency. IgG is not useful in young infants because it is largely maternal. In addition, a chest radiograph is indicated to look for a thymic shadow, which is often absent in patients with SCID. Not to be forgotten is the value of performing a physical examination of the patient, noting growth and development, abnormal physical findings (eg, DiGeorge facies), and the presence or absence of tonsils or lymph nodes. With the X-linked form representing nearly 45% of cases of SCID, occurrence in maternal male relatives is particularly important. A history of recurrent or persistent infections is important. Patients with opportunistic infections, such as Pneumocystis jiroveci (previously Pneumocystis carinii) pneumonia (PCP), or viral pneumonias with, for example, cytomegalovirus (CMV), respiratory syncytial virus, or parainfluenzae that do not resolve

**Abbreviations used**

ADA: Adenosine deaminase
ALC: Absolute lymphocyte count
CGD: Chronic granulomatous disease
CID: Combined immunodeficiency disease
CMV: Cytomegalovirus
CT: Computed tomography
Cy: Cyclophosphamide
GT: Gene therapy
GVHD: Graft-versus-host disease
HCT: Hematopoietic stem cell transplantation
HLH: Hemophagocytic lymphohistiocytosis
NK: Natural killer
PCP: Pneumocystis jiroveci (previously Pneumocystis carinii) pneumonia
PE: Physical examination
PID: Primary immunodeficiency disease
PIDTC: Primary Immune Deficiency Treatment Consortium
SCID: Severe combined immune deficiency
TMP/SMX: Trimethoprim-sulfamethoxazole
USIDNET: United States Immunodeficiency Network
WAS: Wiskott-Aldrich syndrome

**Role of the primary care physician in diagnosis of SCID**

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TABLE I. Combined immunodeficiency: Working definition

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<tr>
<td>CID</td>
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<tr>
<td>“Leaky” SCID</td>
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<td>Profound immunodeficiency associated with various multisystem syndromes</td>
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CID: Criteria and supportive findings

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<td>After excluding other secondary causes of immunodeficiency, such as HIV and drugs, CID might be suspected by fulfilling at least 2 of the following criteria:</td>
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<tr>
<td>1. presentation with typical infections (eg, PCP, CMV-induced pneumonitis, oral thrush, and recurrent invasive infections) and/or lymphoid malignancy and/or &lt;2 y of age with granuloma/autoimmunity;</td>
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<tr>
<td>2. confirmed reduced numbers, decreased function, or both of circulating T cells;</td>
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<tr>
<td>3. low T-cell receptor excision circle numbers, restricted diversity of the T-cell repertoire, or both;</td>
</tr>
<tr>
<td>4. significant mutation in a gene involved in T-cell function, evidence of defective expression/function of the encoded protein, or both.</td>
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<th>Supportive findings</th>
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<tr>
<td>1. Family history of profound T-cell deficiency</td>
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<tr>
<td>2. Signs and symptoms relevant to syndromes that might be associated with profound T-cell deficiency, such as: short stature (eg, cartilage hair hypoplasia), microcephaly (eg, DNA ligase 4), mental retardation (eg, ADA deficiency), and progressive neurodegeneration (eg, purine nucleoside phosphorylase) deficiency</td>
</tr>
<tr>
<td>3. Abnormal thymus morphology (dysplastic changes, such as lack of Hassal corpuscles and abnormal architecture)</td>
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Note: It was recognized by the group that this “definition” of CID has been developed based on experience with immunodeficient patients and that it is a useful working diagnosis that will require validation in future studies.

*Onset of thrush, chronic diarrhea and failure to thrive in the first months of life*

*Recurrent infections*

- Bacterial pathogens, but also opportunistic organisms such as *Pneumocystis jiroveci*, *Candida albicans*, and viruses such as varicella, adenovirus, cytomegalovirus, Epstein-Barr virus (EBV), parainfluenza 3.

*Pneumonitis that does not clear*

- PCP, RSV, CMV, and parainfluenza

*Rashes, with erythroderma, or eczema that doesn’t resolve with therapy*

*Other physical findings: hepatosplenomegaly, lymphadenopathy*

*Family history of children dying < 6 months of age*

*Lymphopenia, particularly absence of functional T-cells. B-cells may be present, but do not make specific antibodies*

- ALC < 3400 (may be normal); IgM < 20 (may be normal or elevated in some SCID / CID); IgA < 5; lymphocyte proliferation to mitogens < 10% of normal

**FIG 1.** Features of congenital cellular immunodeficiency.

must raise the possible diagnosis of SCID/CID. These patients often present with failure to thrive associated with chronic diarrhea and persistent skin rashes.

If there appears a red flag of suspicion of immunodeficiency, the crucial next step by the primary care provider is referral of the patient to an immune deficiency specialist who is expert in the diagnosis, treatment, and management of cellular immunodeficiencies. This individual will have the knowledge to quickly perform definitive sophisticated tests to rule in or out the presence of SCID and to arrange for HCT if the diagnosis is confirmed.

**Role of the immune deficiency expert in diagnosis of SCID**

With referral to the immune deficiency specialist (Fig 3), the patient will have a more extensive evaluation of his or her lymphocytes by means of flow cytometry using antibodies to CD3,
CD4, CD8, CD19, and CD16/CD56 to determine whether the infant has normal percentages of T cells and subsets, B cells, and natural killer (NK) cells and to determine whether there are naive (CD45RA^hi^) T cells present. The function of T cells will be determined by means of lymphoproliferation in vitro in response to stimulation with mitogens, such as PHA, concanavalin A, and pokeweed mitogen, and to antigens, such as Candida species and tetanus (the latter only if the infant has been immunized). Infants with severe T-cell defects will have low numbers of all T-cell subsets; either high, normal, or low numbers of B cells; absent, normal, or high numbers of NK cells; and very low (usually < 10% of normal) or no in vitro lymphocyte proliferation. Although transplacentally transferred maternal T cells can rarely result in a normal ALC, the transferred cells do not afford immune reconstitution for the infant. General classes of potential gene defects can be predicted based on the immunophenotypic pattern observed in the patients with SCID, although exceptions are well described. Although not required for HCT, genomic DNA sequencing of specific SCID genes can enable the immunologist to better inform parents of the potential future outcomes of their child once treated with HCT and to provide genetic counseling. A subgroup of patients with SCID (Artemis and ligase IV deficiency) who lack both T and B cells (T^−^B^−^NK^+^ SCID) will have a defect that makes them more susceptible to alkylating agents and ionizing radiation, which are often used as conditioning agents for HCT.

Newborn screening for PIDs

Universal newborn screening for SCID is not yet available, although pilot trials are in progress. It has been suggested that every newborn should have a complete blood count with differential performed to detect those who might have a serious cellular immunodeficiency. If lymphopenia is found, then flow cytometry should be performed to determine whether T cells are missing. Another method of gaining similar information by using heel-stick blood already obtained in the newborn nursery on every child would be to assay for T-cell receptor excision circles, which are byproducts of thymocyte antigen receptor gene rearrangement. Low or absent T-cell receptor excision circles indicate impaired production of new naive T cells. There are currently 3 newborn screening pilot studies ongoing in Wisconsin, Massachusetts, and the Navajo Indian Reservation using the latter technology.
Referral to a center with experience in PIDs

Because children with a high suspicion of having SCID/CID need to be managed carefully and quickly, referral to an immune deficiency specialist and transplant center with experience in diagnosing and treating PIDs should be made as soon as possible. When a diagnosis of SCID is made, the immune deficiency specialist, transplantation physician, or both proceeds rapidly to prepare the patient for HCT or, in certain settings, enzyme replacement or GT (see Fig E1 in this article’s Online Repository at www.jacionline.org). It is recognized that transplantation options for patients with SCID vary considerably, ranging from infusion of HLA-matched sibling bone marrow or T-cell–depleted, HLA-mismatched, related stem cells without prior conditioning or post-HCT immunosuppression, to infusion of T-cell–replete unrelated cord blood or adult stem cells after immunosuppressive or myeloablative conditioning. Pretransplantation conditioning regimens, method and use of T-cell depletion, and/or graft-versus-host disease (GVHD) prophylaxis vary widely among transplantation centers. Transplantation regimens are often influenced by the genetic cause of the immunodeficiency, the degree of HLA matching between donor and host, the clinical status of the patient, and the technology and experience available for hematopoietic stem cell processing. Tissue typing of the patient and close relatives or the search for matched related donors, as well as using national resources to search for a matched unrelated donor or cord blood, will enable determination of the best donor of hematopoietic stem cells. In addition, haplidentical donors (typically a parent) are an important source for HCT after removal of mature T cells by means of negative selection with soy lectin and sheep erythrocytes or anti–T-cell mAbs or by means of positive selection with anti-CD34 mAbs. Different centers have individual approaches to the type of donor cells to use and whether to use no conditioning or to provide myeloablative or nonmyeloablative conditioning, prophylactic GVHD treatments, or both to the patient.

Similarly, if a therapeutic alternative to HCT is considered preferable after evaluation of the patient with a PID, then referral to the appropriate treatment specialists should be made. For example, options available for treatment of SCID caused by adenosine deaminase (ADA) deficiency include enzyme replacement therapy with pegylated, bovine polyethylene glycol adenosine deaminase or autologous hematopoietic stem cell GT. GT for ADA SCID has been shown to be effective for a cohort of 10 children without the complications of insertional mutagenesis that were seen after GT in patients with X-linked SCID. GT for ADA was effective for ADA SCID, even after treatment failure with pegylated ADA.

MANAGEMENT OF SCID/CID BEFORE HCT

Management of the child with suspected SCID/CID while confirming diagnosis

Supportive care of a patient with SCID/CID should begin at the time of initial contact, when the suspicion of severe immunodeficiency first arises (Table II). Meanwhile, certain prophylactic measures both in terms of isolation and pharmacologic therapy should be instituted. The child should be placed in protective isolation with good handwashing procedures to minimize exposure to hospital-acquired infections. Prophylaxis for PCP and bacterial infections should be started as soon as possible, and other fungal and viral prophylaxis should also be considered. Avoidance of breast-feeding is probably one of the more difficult measures to institute, given the importance of this to both the infant and mother. The likelihood of transmission of CMV to babies with SCID from breast milk is sufficiently significant that many immunologists/transplantation centers recommend stopping breast-feeding pending determination of the mother’s CMV serologic status. Live attenuated viral vaccines, such as rotavirus and varicella, should be avoided, including vaccination of siblings with varicella. Every effort should be made to identify infections that might be relatively asymptomatic in patients with PIDs because of a lack of immune response in this highly susceptible patient population. Treatment should be instituted when infectious organisms are identified while efforts are underway to select a donor for corrective cellular therapy. Previous therapies, such as blood transfusions, are also important factors in patient morbidity. All blood products (platelets and erythrocytes) should be CMV seronegative, leukodepleted, or both to prevent transmission of CMV and irradiated to eliminate the risk of fatal transfusion-associated GVHD.

Management of infectious diseases in the child with possible SCID/CID

All children, even if asymptomatic, should have screening studies for a variety of viral agents (Table III). Depending on laboratory studies and/or clinical indicators, such as increased transaminase values or respiratory symptoms, various progressively invasive procedures are recommended. Importantly, because of their T- and B-cell defects and absent immune responses, these patients are often relatively less symptomatic for any given degree of infection. Chest radiographic results might be normal, whereas high-resolution chest computed tomographic scans will show significant parenchymal disease. Bronchoalveolar lavage (and sometimes lung biopsy) might be the only way to diagnose PCP and viral pneumonias because sputum is not very sensitive or practical for detecting these organisms in infants and young children. Identification and treatment for respiratory syncytial virus, adenovirus, CMV, and EBV infections is critical.

DIAGNOSIS AND MANAGEMENT OF PATIENTS WITH NON-SCID PIDs

Non-SCID PIDs that are correctable by means of HCT, including WAS, CGD, familial hemophagocytic lymphohistiocytosis (HLH), and other diseases, are presented in Table E1 in this article’s Online Repository at www.jacionline.org. The risks of HCT must be compared with the expected long-term clinical outcome without HCT. For example, in patients with familial HLH, the prognosis without HCT is extremely poor. Unfortunately, the risk/benefit ratio over the short-term for WAS and CGD can be difficult to predict in the less severe phenotypes because of insufficient natural history information and known variability in clinical course for these disorders. In general, for these HCT procedures, an HLA-matched family donor or matched unrelated donor and myeloablative conditioning have been used. Clinical investigation of the efficacy and safety of reduced-intensity regimens compared with standard myeloablative regimens is needed. GT clinical trials have been and/or are being performed for CGD, leukocyte adhesion deficiency type 1, and WAS and have generated results that encourage further development of gene transfer as an alternative therapeutic option for non-SCID PID (see Table E1).
TABLE II. Management of a child with suspicion for SCID/CID while confirming diagnosis

<table>
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<th>Management</th>
<th>Details</th>
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| Refer to transplantation center as soon as possible. | 1. Protect against ill contacts.  
2. Place in protective isolation with mandatory good hand washing to minimize exposure to hospital-acquired infections.  
3. Manage as outpatient if clinically indicated.  
4. A high degree of suspicion for infection is important, because infections can be clinically relatively asymptomatic due to immunity which is defective, in this highly susceptible patient population.  
Notes: The additional use of gowns, gloves and masks varies from center to center, with more than half using all of these isolation approaches with admittedly little to no documented proof of their efficacy. |
| Start PCP prophylaxis. | 1. Can start trimethoprim-sulfamethoxazole at 1 to 4 wk of age if total bilirubin level is not increased and monitor LFT results.  
2. At 4-6 wk old, start trimethoprim-sulfamethoxazole or can use atovaquone, dapsone, or intravenous pentamidine (the latter is given every 2 wk).  
3. At >6 wk old, trimethoprim-sulfamethoxazole is given orally 2-3 d/wk.  
Notes: Although trimethoprim-sulfamethoxazole is not recommended for children <6 wk of age because of possible hepatic toxicity, some experienced centers start it as early as 1 wk of age if the total bilirubin level is not increased and with careful monitoring of liver transaminase levels. The standard PCP prophylactic agent is trimethoprim-sulfamethoxazole administered orally 2-3 d/wk. Alternative therapies are available if trimethoprim-sulfamethoxazole cannot be given. |
| Consider fungal prophylaxis, especially for Candida species. | 1. Diflucan (fluconazole)  
2. Monitor LFTs.  
3. If liver inflammation is present, caspofungin is an alternative.  
Notes: Fluconazole should be considered to prevent primarily Candida species infection, again with careful monitoring of LFT results. |
| Start bacterial prophylaxis. | 1. IVIG: monitor IgG trough level and maintain >500-800; or fixed dose of 400-500 mg/kg per dose every 3 to 4 wks.  
2. Subcutaneous gamma globulin is an option. |
| Consider viral prophylaxis. | 1. Acyclovir  
2. Maintain adequate hydration.  
Notes: In terms of viral prophylaxis, there appears to be no consensus among immunologists/transplantation centers, with about half starting acyclovir at the time of diagnosis. |
| Breast-feeding | 1. Not an issue in terms of GVHD risk  
2. Stop nursing until CMV status of mother is known; if seronegative, then it is okay to breast-feed.  
Notes: The likelihood of transmission of CMV to babies with SCID from breast milk is sufficiently significant that many immunologists/transplantation centers recommend stopping breast-feeding until the mother’s CMV serologic status can be determined; if negative, then breast-feeding can be resumed. Development of GVHD from maternal lymphocytes known to be present in breast milk does not appear to be the case in human subjects. There are no documented cases of maternal-infant GVHD in babies with SCID as a result of breast milk, although it is obviously difficult, if not impossible, to differentiate from maternal-fetal GVHD, a known presenting feature of SCID. |
| Immunizations | 1. Avoid live vaccines, including rotavirus, MMR, Flu-mist, and BCG.  
2. Siblings should NOT get varicella vaccine.  
Notes: Unfortunately, the early application of the live attenuated rotavirus vaccine has resulted in symptomatic infections in patients given subsequent diagnoses of SCID (personal communication, M. Cowan). Vaccination of healthy siblings with the varicella vaccine should be avoided. |
| Blood products | 1. Red blood cells and platelets should be CMV negative, if possible; leukodepleted; and irradiated. If CMV-negative blood is not available, then leukodepletion is essential.  
2. Fresh frozen plasma does not require irradiation.  
Notes: A previous blood transfusion with nonirradiated blood has potential to transfer T lymphocytes capable of producing fatal transfusion-associated GVHD. |

*IVIG*, intravenous immunoglobulin; *LFT*, liver function test; *MMR*, measles-mumps-rubella vaccine.

**MANAGEMENT OF CHILDREN WITH PIDs AFTER HCT OR OTHER DEFINITIVE TREATMENT**

Posttreatment antimicrobial prophylaxis, reimmunization, and long-term follow-up of children with SCID and other PIDs after HCT or GT

It is expected that patients undergoing transplantation for severe immunodeficiencies will be closely monitored by the transplantation center for at least the first year after HCT or GT (see Table E2 in this article’s Online Repository at www.jacionline.org). After this time, much of the patient’s care might shift to the referring immune deficiency specialist or primary care physician because of proximity to the patient’s home or insurance constraints. The posttransplantation guidelines suggested for years 1 to 5 and beyond are intended to provide information about potential problems that might arise during those time periods that are unique to patients with PIDs.28 If possible, during years 1 to 5, the patient should return to the transplantation center at least once a year for an evaluation of the immune system. Patients can experience autoimmune disease29,30 or atypical GVHD. The presentation of atypical GVHD includes cytopenias, steroid-responsive pneumonitis,
and kidney disease, as well as the more typical skin, liver, and gastrointestinal disease. If the patient has recurrent or unusual infections or any manifestation of GVHD, including diarrhea, they should be referred back to the transplantation center. After 5 years, the patient should return to the transplantation center at least once every 2 years. The transplantation center will continue to monitor immune reconstitution and chimerism and medical problems particular to this later time period after HCT.

The duration and intensity of antimicrobial prophylaxis for patients with PIDs after HCT depends on the expected time course of immunologic reconstitution and the patient’s pre- and post-HCT infectious disease history. Table E3 (available in this article’s Online Repository at www.jacionline.org) provides expert opinion regarding antimicrobial prophylaxis of PCP, HSV, and EBV; yeast and mold; and CMV after HCT for PIDs. As described in Table E4 (available in this article’s Online Repository at www.jacionline.org), gamma globulin supplementation is provided for antibacterial prophylaxis in the early posttransplantation period. To determine when to discontinue gamma globulin, a variety of criteria are considered, including duration of therapy; trough levels of serum IgM, IgG, or both; and/or the ability to make antigen-specific antibody after immunization. More stringent criteria for discontinuation are used for patients having pre-existing or ongoing infection. Also, for patients receiving immunosuppressive therapy for GVHD or autoimmune conditions, gamma globulin replacement should be

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<th>Symptoms/organisms</th>
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<td>Screening studies are mandatory, regardless of symptoms.</td>
<td>1. Quantitative PCR on blood for HIV, CMV, EBV, adenovirus, and human herpes virus-6 2. Nasal wash for respiratory viruses</td>
</tr>
<tr>
<td>Increased transaminase levels</td>
<td>1. Hepatitis B surface antigen and quantitative PCR for hepatitis C virus</td>
</tr>
<tr>
<td>Any respiratory symptoms</td>
<td>1. Arrange to transfer to transplantation center. 2. Nasal wash for respiratory viruses 3. Note polymicrobial infection is common. 4. Chest radiography, O₂ saturation</td>
</tr>
<tr>
<td>Cough and/or O₂ requirement and/or tachypnea and/or retractions</td>
<td>1. Consider chest computed tomography because chest radiography might not reveal infiltrates. 2. If clinically stable, consider bronchoalveolar lavage, even if radiologic results are negative. 3. If no improvement on empiric therapy, consider lung biopsy, depending on clinical condition. 4. Start treatment for bacterial, fungal (Candida species), PCP, and CMV infections while waiting for results of cultures.</td>
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Notes: Chest radiographic results might be normal while high-resolution chest computed tomographic scans will show significant parenchymal disease. Bronchoalveolar lavage (and sometimes lung biopsy) might be the only way to diagnose PCP and viral pneumonias because sputum is not very sensitive for detecting these organisms in infants and young children.

RSV | 1. Aerosolized ribavirin should be given until symptoms improve. 2. Preferred administration is for 2 h (every 8 h). 3. Consider giving intravenous Synagis (palivizumab).  |
Notes: RSV infection should be treated in children with possible SCID/CID, even if it appears to be in the upper airway only. The efficacy of these approaches has not been documented in this patient population, and it is clear that the most important therapy is cellular correction of the underlying immune deficiency. However, the life-threatening nature of RSV and its potential for long-term effects on lung function result in many centers using these drugs sometimes until immunity can be restored and the RSV infection is cleared.

Adenovirus | 1. Cidofovir is the treatment of choice.  |
Notes: Use cidofovir until the adenovirus has cleared.

CMV | 1. Ganciclovir is first-line therapy. 2. Foscarnet/cidofovir is second-line therapy. 3. If the patient is neutropenic/thrombocytopenic, foscarnet can be given. 4. Foscarnet can be added to ganciclovir/cidofovir if poor response to single agent.  |
Notes: The combination of foscarnet and ganciclovir should be considered if there appears to be a poor response to the single agent.

EBV (infection of B cells) | 1. Rituximab is first-line therapy but is contraindicated with HBV infection and possibly with PCP infection. 2. Ganciclovir is second-line therapy with questionable efficacy; avoid, if possible, because of marrow suppression and renal toxicity.  |
Notes: Rituximab should be avoided in patients with HBV infections and possibly those with PCP. The use of ganciclovir is controversial in that although it is effective in vitro, there is little if any documented evidence that it is useful in vivo. Given the marrow suppression and possible renal toxicity of ganciclovir, it should be used with caution.

RSV, Respiratory syncytial virus
continued. The majority of patients lacking donor B cells will require immunoglobulin replacement for life. For patients with PIDs receiving T-replete HCT and preparative conditioning, many of the guidelines developed for allogeneic HCT for malignant indications apply. Much of this guidance, including medications and dosages, has been recently updated. How- ever, many patients with SCID who receive HLA-matched sibling grafts will not have B-cell reconstitution because they are not conditioned. Also, for children with SCID who receive T cell–depleted, HLA-mismatched, related family donor transplants with or without prior cytoablation, a significantly different strategy of management is needed due in part to the lesser extent of B-cell chimerism achieved.

General principles of management after HCT for PIDs

Critical issues in post-HCT management for PIDs include the antimicrobial regimens used and duration of prophylaxis, which might vary depending on the type of HCT regimen received and the standard practices of the center. In general, CD4 cell counts of greater than 200/μL and PHA proliferative responses of greater than 50% of normal control values are used as cellular immunity parameters to discontinue prophylaxis (see Fig E2 in this article’s Online Repository at www.jacionline.org). For patients with acute or chronic GVHD, antimicrobial prophylaxis is continued until these milestones have been met and immunosuppressive therapy has been discontinued. Patients having pre-existing infections will require specific treatment until clinical, imaging, and laboratory assessments of disease are negative. Growth and development should be monitored carefully. If the patient received a preparative regimen, endocrine problems, neurocognitive delays, osteopenia, and dental problems can arise.

When to return to the transplantation center after discharge

Importantly, we list problems that should elicit immediate return to the transplantation center after HCT for evaluation and recommendations as to appropriate care (see Fig E3 in this article’s Online Repository at www.jacionline.org). These include any need for hospitalization, management of GVHD or critical infection, and indicators of reduced numbers or function of cellular immunity, humoral immunity, or both after HCT.

Long-term outcomes after HCT for SCID

Multiple advances in the diagnosis and treatment of SCID and other PIDs have occurred in the last decade, resulting in growing numbers of affected children surviving long-term. Observation of the long-term outcomes of HCT-treated patients with SCID is now receiving special emphasis. Complications of HCT or pre-existing infections, such as chronic GVHD, incomplete immune reconstitution, or neurodevelopmental delay, require careful assessment by the immunologist and related subspecialists. There are recently recognized concerns for the mental health, quality of life, and well-being of patients rescued by heroic measures and their families. In the long-term follow-up reports now appearing in the literature, the encouraging news is that immunodeficient children treated with HCT have achieved educational goals, and some have produced normal offspring. Genetic counseling of the parents before and after transplantation is important for family expectations of their child’s overall health and what clinical problems to expect, including neurodevelopmental problems, and long-term immune function.

ROLE OF DATABASES

It is anticipated that a close interaction between 2 databases that collect information about patients with immune deficiency, the Center for Blood and Marrow Transplant Research (CIBMTR) and the United States Immunodeficiency Network (USIDNET), will facilitate operations of the PIDTC in the study of outcomes for patients with PIDs who receive HCT as primary therapy. Although the CIBMTR operates under a Department of Health and Human Services mandate to collect outcomes for all patients who receive either related or unrelated allogeneic HCT therapy in the United States, the USIDNET is a voluntary registry of patients given diagnoses of PIDs. The 2 databases collaborated in 2008 to harmonize data collection forms for pretransplantation and posttransplantation evaluation of patients with immune deficiency, including SCID, CGD, and WAS. As of the time of publication of this report, these revised forms are available online at the CIBMTR Web site and for electronic submission through CIBMTR’s FormsNet application. In addition, USIDNET and CIBMTR have agreed to generate new and/or harmonized data forms for 4 additional types of PID, in particular familial HLH, X-linked lymphoproliferative syndrome, DiGeorge syndrome, and pigmentary dilution disorders. A strategy similar to that used for the SCID, WAS, and CGD forms will be followed to develop harmonized forms for these disorders. A team of content experts and data collection specialists representing USIDNET and CIBMTR will work together to revise current data collection forms or develop new forms using standard formats and incorporate data elements essential for understanding outcomes of treatment for these rare diseases. The purpose of this new series of disease-specific forms will be to collect data to perform cross-sectional and longitudinal studies and to compare outcomes in patients who receive HCT or thymic transplantation (for DiGeorge syndrome) versus alternative treatments. The patients’ data will be collected by USIDNET and CIBMTR, and they will be shared in an agreed standard format to create final unified datasets for analytic purposes. Data will be shared for single studies that might include both patients undergoing transplantation and those not undergoing transplantation.

Longer term, it is anticipated that USIDNET and CIBMTR will endeavor to develop a uniform strategy for data sharing. In addition, in discussion at the current workshop, it became apparent that enhancement of USIDNET as a resource would also benefit collaborative studies of patients with PIDs who receive other primary therapy. These cases are sufficiently rare that multicenter and international collaborations are essential to develop a foundation of knowledge about current practice that can be used to develop the prospective clinical research protocols of the future. Availability of efficient resource databases will be critical to the success of such collaborative projects.

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

The expert opinion provided here for diagnosis and management of PIDs before, during, and after HCT represents the career
experience of more than 30 immunologists and transplantation physician investigators. Although it would be desirable to provide more formal guidelines, PIDs are rare, and this level of evidence-based recommendation is not yet available. We are hopeful that the collaborative studies of the PIDTC will contribute to the development of a database sufficiently robust that evidence-based guidelines can be provided in the future.

REFERENCES


APPENDIX E1

EXPERT OPINION AND WORKSHOP PARTICIPANTS

Expert opinion

Early suspicion for a diagnosis of PID. Rebecca H. Buckley, Thomas A. Fleisher, Jennifer M. Puck (Co-Chairs), Jacob J. H. Blessing, Marcia Boyle, Kimberly Risma, John M. Routes, William T. Shearer, Troy R. Torgerson

Management of SCID/CID before HCT. Morton J. Cowan, Chaim M. Roifman (Co-Chairs), Laura Burroughs, Charlotte Cunningham-Rundles, Suk See DeRavin, Christopher C. Dvorak, H. Bobby Gaspar, Naynesh Kamani, Neena Kapoor, Donald B. Kohn, Joshua D. Milner, Luigi D. Notarangelo, Richard J. O’Reilly, Jennifer M. Puck, Paul Szabolcs

Diagnosis and management of patients with non-SCID PIDs. Fabio Candotti, Elizabeth M. Kang, Kimberly E. Nichols (Co-Chairs), Michael H. Albert, Elie Haddad, Hans D. Ochs, Jordan Orange, David J. Rawlings

Management of children with PIDs after HCT or other definitive treatment. Mary Ellen Conley, Trudy N. Small (Co-Chairs), Javier Chinen, Louise M. Markert, Sung-Yun Pai, Kirk R. Schultz, Paul Szabolcs

Role of databases. Luigi D. Notarangelo, J. Douglas Rizzo (Co-Chairs), Morton J. Cowan, Linda M. Griffith, Josiah F. Wedgwood

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REFERENCES


• Transfer of patient to a facility with experience in HCT treatment of SCID

• Assessment for maternally transferred or transfusion acquired T cells

• Selection of donor
  • HLA matched sibling; haplotype disparate
  • Related or unrelated adult
  • Unrelated cord blood

• Type of transplant and residual T cell contamination
  • T-cell depleted using lectin or T-cell antibodies
  • CD34+ hematopoietic stem cell enriched
  • Unmodified

• Pretransplantation conditioning
  • None
  • Myeloablative
  • Reduced intensity
  • Immunosuppressive

• Post-transplantation prophylaxis against GVHD (Yes or No)

• Prophylaxis and treatments of infection
  • Gammaglobulin, IV or SC
  • Antibiotics, antivirals
  • Isolation at home or in hospital

FIG E1. Preparation of patients with SCID for HCT. IV, Intravenous; SC, subcutaneous.
For SCID patients receiving T-cell depleted HCT without conditioning
  - Antimicrobial regimens and duration of prophylaxis vary widely between centers, from IVIG and only PCP prophylaxis, to IVIG plus prophylaxis against PCP, HSV, and fungus
  - CD4 counts > 200 / ul and PHA proliferative responses > 50% of normal control are used as cellular immunity parameters to discontinue prophylaxis.

For patients receiving T replete (unmodified) HCT from alternative donors
  - There is more consensus among centers as to prophylaxis guidelines for infants and children receiving unmodified HCT for PID
  - Guidelines for PID are similar to those of HCT for other diseases, with some important exceptions (ie, duration of IVIG and revaccination schedules).

Patients with pre-existing infections will be monitored and treated differently
  - Patients with pre-existing viral (HSV, CMV, adenovirus, RSV), PCP, toxoplasmosis, other fungal, or other infections, need targeted antimicrobial treatment until clinical symptoms resolve, microbial tests and scans are negative and immune phenotype and function are restored.

FIG E2. Management after HCT for PIDs. HSV, Herpes simplex virus; IVIG, Intravenous immunoglobulin.
- Hospitalization required
- Development of rash (include consideration of GVHD), pneumonia, muscle weakness, warts, cytopenias, (ANC < 1000, platelets < 100k, anemia, evidence of hemolysis), joint pain, oral ulcers, poor weight gain, delayed growth and/or delayed tooth development
- Increased frequency of infections, need for > 2 courses of antibiotics, hospitalization for infection.
- Development of thrush or any opportunistic infection
- Changes in lymphocyte phenotype especially drop in CD4 numbers by 20% or to < 200/mm3.
- Patient is exposed to varicella, measles, mumps, or rubella

**FIG E3.** When to call the transplantation center after discharge. ANC, absolute neutrophil count.
<table>
<thead>
<tr>
<th>Disease and confirmatory diagnostic criteria</th>
<th>Indication to HCT</th>
<th>Recommended conditioning</th>
<th>Chimerism</th>
<th>Non-HCT and/or pre-HCT management</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cartilage hair hypoplasia</strong></td>
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<tr>
<td>Mutation analysis of the <em>RMRP</em> gene</td>
<td>HCT is recommended in patients with severe T-cell deficiency, especially if MFD or MUD is available. Haploidentical transplants might also have a role in the management of this disease, when clinically appropriate. Importantly, HCT will not improve skeletal abnormalities.</td>
<td>Limited published experience for HCT is based on conditioning with Bu+Cy.\textsuperscript{1,8} Studies are needed to evaluate the efficacy of the RIC regimen that would be preferred in cases with significant pretreatment risk factors.</td>
<td>Mixed donor chimerism is not expected to have negative consequences in this disease.</td>
<td>Supportive and/or pretreatment management (eg, TMP/SMX or IVIG/SCIG) for numeric or functional T-cell deficiency and antibody defects. GT is not available.</td>
</tr>
</tbody>
</table>

| **CD40 ligand deficiency**                  |                   |                          |           |                                  |
| Mutation analysis of the *CD40LG* gene      | HCT is recommended if MFD is available. Transplants from other donor sources (MUD or haploidentical donors) should be strongly considered in the presence of severe disease complications. | Published experience for HCT is based on conditioning with Bu+Cy.\textsuperscript{5} Studies are needed to evaluate the efficacy of the RIC regimen that would be preferred in cases with significant pretreatment risk factors. | Mixed donor chimerism is likely to be beneficial. | General: TMP/SMX, Supportive and/or pretreatment management (eg, TMP/SMX or IVIG/SCIG) should be implemented together with careful avoidance of and surveillance for *Cryptosporidium* species infection. If present, *Cryptosporidium* species infection should be aggressively treated to eradicate this pathogen. GT is not available. |

| **Chediak-Higashi syndrome**                |                   |                          |           |                                  |
| Mutation analysis of the *LYST* gene        | HCT is recommended if MFD or MUD is available. Haploidentical transplants might also have a role in the management of this disease when clinically appropriate. | Published experience for HCT is mostly based on conditioning with Bu+Cy or TBI+Cy. Late neurologic complications are not prevented despite successful HCT. Studies are needed to evaluate the efficacy of the RIC regimen that would be preferred in cases with significant pretreatment risk factors. | Mixed donor chimerism is likely to be beneficial. | Induction of remission of accelerated phase before HCT seems to improve disease-free survival.\textsuperscript{13,16} GT is not available. |

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<table>
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<tr>
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<th>Recommended conditioning</th>
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<th>Non-HCT and/or pre-HCT management</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CGD</strong></td>
<td>HCT is recommended for gp91phox-deficient patients (X-CGD) if MFD is available. Transplants of X-CGD from MUD or of other genetic variants from MFD or MUD are considered in the presence of severe disease complications or poor compliance to medical management. Haploidentical transplants might also have a role in the management of this disease, when clinically appropriate.</td>
<td>Published experience for HCT is mostly based on conditioning with Bu + Cy, although the use of nonmyeloablative conditioning has also been reported as an essential option in cases with significant pre-HCT risk factors.</td>
<td>Mixed donor chimerism is likely to be beneficial.</td>
<td>Supportive and/or pretreatment management with antibiotic and antifungal prophylaxis should be implemented together with regular imaging and medical follow-up. Aggressive treatment of infections with antibiotics, antymycotics, leukocyte transfusions, and G-CSF is warranted. Steroid treatment is useful for cases of severe inflammation (ie, pulmonary disease and colitis). Administration of IFN-α can be recommended, although there is no consensus on its long-term efficacy in diminishing the risk of severe infections. GT is available and has been used both as a curative attempt and as a bridge to HCT in patients with severe infections.</td>
</tr>
<tr>
<td><strong>Griscelli syndrome type 2</strong></td>
<td>HCT from any available donor source is recommended for all patients who have not experienced severe neurologic involvement. Haploidentical transplants might also have a role in the management of this disease, when clinically appropriate.</td>
<td>Published experience for HCT is mostly based on conditioning with Bu + Cy, although the use of nonmyeloablative conditioning can be considered.</td>
<td>Mixed chimerism is sufficient to stabilize disease.</td>
<td>Dexamethasone, VP16 and cyclosporine. Remission of hemophagocytic syndrome/accelerated phase should be attempted before performing HCT. Transplantation should not be postponed because of only partial remission. GT is not available.</td>
</tr>
<tr>
<td><strong>HLH</strong></td>
<td>HCT from any available donor source is recommended as soon as the hemophagocytic syndrome is controlled. Neurologic disease is associated with a poor outcome.</td>
<td>Published experience for HCT is mostly based on conditioning with Bu + Cy, although the use of nonmyeloablative conditioning can be considered.</td>
<td>Mixed chimerism with &gt;20% of donor leukocytes is associated with sustained remission of the disease.</td>
<td>Dexamethasone, VP16 and cyclosporine. Complete remission of hemophagocytic syndrome/accelerated phase should be attempted before performing HCT, but transplantation should not be postponed because of only partial remission. GT is not available.</td>
</tr>
<tr>
<td><strong>IPEX:</strong> Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome</td>
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### Table E1 (Continued)

<table>
<thead>
<tr>
<th>Disease and confirmatory diagnostic criteria</th>
<th>Indication to HCT</th>
<th>Recommended conditioning</th>
<th>Chimerism</th>
<th>Non-HCT and/or pre-HCT management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis can be confirmed with mutation analysis of the <em>FOXP3</em> gene. Clinical and laboratory diagnostic criteria are available on the Web site of GeneReviews (<a href="http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&amp;part=ipex">http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&amp;part=ipex</a>).</td>
<td>HCT from MFD or MUD is recommended and should preferably be performed early before onset of diabetes.</td>
<td>Published experience for HCT is limited and is based on RIC.</td>
<td>Partial donor chimerism can result in sustained remission of the disease.</td>
<td>Pre-HCT management with T-cell inhibitors, such as tacrolimus, rapamycin, and cyclosporine A, possibly combined with other immunosuppressive drugs (eg, azathioprine, methotrexate, or steroids) can be useful. Rituximab can be beneficial for autoantibody-mediated autoimmunity. GT is not available.</td>
</tr>
<tr>
<td><strong>Leukocyte adhesion deficiency type I</strong></td>
<td>HCT from MSD or MUD is recommended because of long-term disease risks. Haploidentical transplants might also have a role in the management of this disease, when clinically appropriate.</td>
<td>The majority of published HCT experience is based on myeloablative regimens, although recent use of RIC has suggested an improved safety profile.</td>
<td>Mixed donor chimerism at even relatively low levels is likely beneficial for infection control and can result in lack of significant symptoms.</td>
<td>Initial GT attempts have been unsuccessful. However, with optimization of vector choice and transduction conditions, gene transfer is likely to be beneficial in the future.</td>
</tr>
<tr>
<td>Diagnosis is aided by flow cytometric analysis of WAS protein expression in leukocytes and can be confirmed with mutation analysis of the WAS gene. Clinical and laboratory diagnostic criteria have been published and are available on the Web site of the European Society for Immunodeficiency (<a href="http://www.esid.org/workingparty.php?party=3&amp;sub=2&amp;id=73">http://www.esid.org/workingparty.php?party=3&amp;sub=2&amp;id=73</a>).</td>
<td>HCT from MFD or MUD is recommended. The preferred donors are MFD/MUD (70% to 80% survival) versus haploidentical donors (40% survival).</td>
<td>Because long-term mixed chimerism is associated with autoimmune complications, it is generally accepted that myeloablative conditioning should be used.</td>
<td>Long-term mixed chimerism is undesirable because it is associated with autoimmune complications.</td>
<td>Supportive and/or pretreatment management with antibiotic prophylaxis (eg, TMP/SMX) and IVIG/SCIG should be implemented in severe cases. Topical emollients and steroids are useful for treatment of eczema. Exacerbations of thrombocytopenia can be managed with systemic steroids and high-dose IVIG. Acute bleeding with platelet numbers &lt;10,000/μL requires platelet transfusions. For autoimmune processes, danazol and rituximab are available options. GT trials are at the early stages.</td>
</tr>
<tr>
<td><strong>WAS–X-linked thrombocytopenia</strong></td>
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<thead>
<tr>
<th>Disease and confirmatory diagnostic criteria</th>
<th>Indication to HCT</th>
<th>HCT</th>
<th>Non-HCT and/or pre-HCT management</th>
</tr>
</thead>
<tbody>
<tr>
<td>See above.</td>
<td>The decision to perform HCT might be made based on biomarkers (WAS protein expression levels, response to vaccination, and immune laboratory values) or case-specific clinical reasons.</td>
<td>Likely myeloablative regimens.</td>
<td>There is lack of consensus on how to manage patients with WAS who present with thrombocytopenia with or without eczema in the absence of recurrent infections, autoimmunity, and malignancy. In addition to measures described above for the thrombocytopenia seen in severe WAS cases, splenectomy might be considered for intractable bleeding complications but can result in higher rate of infectious complications. Antibiotic prophylaxis after splenectomy is mandatory. Thrombopoietic agents (eg, eltrombopag) are under study. Current and planned GT trials exclude patients with mild WAS from enrollment.</td>
</tr>
</tbody>
</table>

**X-linked lymphoproliferative syndrome**

Diagnosis can be confirmed with mutation analysis of the *SH2D1A* or *XIAP* genes. Clinical and laboratory diagnostic criteria have been published and are available on the Web site of the European Society for Immunodeficiency (http://www.esid.org/workingparty.php?party=3&sub=2&id=73).

HCT from MSD or MUD is recommended, preferably before development of lymphoma, hemophagocytic syndrome, or other disease complications. Haploidentical transplants might also have a role in the management of this disease, when clinically appropriate. Studies are needed to evaluate the efficacy of the RIC regimen that would be preferred in cases with significant pretreatment risk factors. Mixed donor chimerism is likely to be beneficial and is not expected to have negative consequences in this disease. GT is not available.

**Severe congenital neutropenia**

Acquired truncation mutations of the G-CSFR gene and neutrophil elastase accumulation in cytoplasm are believed to be the basis of this disorder.

G-CSF resistance leaves no alternative therapy. MFD or MUD has proved successful in the European experience. High-grade myeloablative pretransplantation conditioning is essential because of normal T-cell responses. Mixed chimerism might be beneficial. Be supportive care with prophylactic antimicrobial agents and white blood cell transfusions might offer some temporary relief. GT is not available.

**MHC class II deficiency**

Defects in promoters occupying DNA-binding protein, such as CIITA transactivators, are responsible for the “bare lymphocyte” syndrome.

Early demise without HCT prompts treatment but poor survival (54% with MFD and 32% with haploidentical donors) at 1 y after HCT. Nonmyeloablative conditioning is frequently unsuccessful. Lack of expression of HLA class II antigens on thymic epithelium prevents normal CD4⁺ T-cell production. Mixed chimerism is beneficial. General supportive care with TMP/SMX, IVIG, or SCIG before HCT is helpful. Chronic diarrhea is a serious problem that requires eradication of intestinal pathogens.

*Bu, Busulfan; CIITA, MHC (major histocompatibility complex) Class II Trans-Activator; Cy, cyclophosphamide; G-CSF, granulocyte colony-stimulating factor; G-CSFR, granulocyte colony stimulating factor receptor; IVIG, intravenous immunoglobulin; LYST, lysosomal trafficking regulator; MFD, matched family donor; MUD, matched unrelated donor; phox, phagocyte oxidase protein; RIC, reduced-intensity conditioning; RMRP, mitochondrial RNA processing endoribonuclease; SCIG, subcutaneous immunoglobulin; TBI, total body irradiation; TMP/SMX, trimethoprim-sulfamethoxazole; VP16, VePesid (etoposide).*
### TABLE E2. Follow-up schedule after transplantation

<table>
<thead>
<tr>
<th>Years</th>
<th>Follow-up schedule</th>
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<tbody>
<tr>
<td>1-5</td>
<td>If possible, the patient should return to the HCT treatment center at least once a year for an evaluation of the patient’s immune system. Patients can have autoimmune disease or atypical GVHD during this period. This includes cytopenias, steroid-responsive pneumonitis, and kidney disease, as well as the more typical skin, liver, and gastrointestinal disease. If the patient has recurrent or unusual infections or any manifestation of GVHD, including diarrhea, he or she should be referred back to the treatment center. Growth and development should be monitored carefully. If the patients received a preparative regimen, endocrine problems, neurocognitive delays, osteopenia, and dental problems can arise. In general, lineage-specific chimerism should be evaluated approximately every 6 to 12 mo after HCT. First year: Monthly PE, including blood pressure, and assessment of growth (height, weight, and head circumference) with a growth chart Every 3 mo: complete blood count with differential, lymphocyte phenotype (minimum: T, B, NK, CD4⁺CD45RA⁺, CD8) and function (minimum PHA), and serum immunoglobulin measurement Six and 12 mo: LFTs, chemistry 7 metabolic test panel, clinical pulmonary assessment, urinalysis, and PFTs (if too young, pulse oximetry with 6-minute walk). If any chronic pulmonary symptoms, abnormal PFTs, or abnormal chest computed tomographic scan, refer to pediatric pulmonologist, if possible. 12 mo: Dental, ophthalmologic, chest radiograph (if abnormal, computed tomographic scan), thyroid function, and echocardiogram Second year: Every 3 mo: PE, assessment of growth, and lymphocyte phenotype and function 24 mo: dental, ophthalmology, chest radiograph (if abnormal, computed tomographic scan of the chest and sinuses), thyroid function, and neurocognitive testing Years &gt;2: If off immunoglobulin replacement and normal T-cell function, yearly PE, assessment of growth, lymphocyte phenotype and function, and immunoglobulin measurement If on IVIG, PHA &lt;50% of normal value, or both, same as above, but every 6 mo; yearly chest radiograph if with respiratory tract infections (if abnormal, computed tomographic scan of the chest and sinus) All patients: yearly dental, ophthalmology, endocrine, PFTs (if too young, pulse oximetry with 6-minute walk) Follow-up after 5 y: If possible, the patient should return to the treatment center at least once every 2 years. The treatment center will continue to monitor immune reconstitution and chimerism. The medical problems that are described above, growth failure, endocrine dysfunction, neurocognitive problems, and dental problems, can persist or develop. In addition, some patients have intractable warts. Secondary malignancies should be considered. When the patient reaches adolescence, genetic counseling should be offered.</td>
</tr>
</tbody>
</table>

**IVIG**, Intravenous immunoglobulin; **LFT**, liver function test; **PE**, physical examination; **PFT**, pulmonary function test.
### TABLE E3. Antimicrobial prophylaxis after transplantation

<table>
<thead>
<tr>
<th>Prophylaxis</th>
<th>Details</th>
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<tbody>
<tr>
<td><strong>PCP</strong></td>
<td>Although all patients with SCID should receive PCP prophylaxis, the duration of this prophylaxis varies from center to center. In patients undergoing continued immunosuppression, PCP prophylaxis is continued after HCT until patients are no longer undergoing GVHD prophylaxis or treatment and demonstrate return of immunologic function. PCP prophylaxis is continued in some centers until the CD4 T-cell count exceeds a threshold ($\geq 200/\mu L$ or $\geq 500/\mu L$) and/or in combination with T-cell proliferative responses to PHA of at least 50% of the lower limit of normal.</td>
</tr>
<tr>
<td><strong>HSV and EBV</strong></td>
<td>Acyclovir prophylaxis is generally given to all patients with an HSV-seropositive donor, particularly those who have recurrent cold sores. Some centers also use acyclovir for EBV prophylaxis. For patients receiving conditioning followed by a T-replete transplant, most centers recommend acyclovir at the start of conditioning and continued until at least 30 d after HCT. Practices vary for recipients for an HLA-matched sibling HCT or T cell–depleted, mismatched related HCT in the absence of conditioning, from no prophylaxis to prophylaxis continued until the CD4 cell count is $\geq 200$ cells/µL, or $\geq 500$ cells/µL, $\geq$ PHA response of at least 50% of the lower limit of normal. In HLA-mismatched, T-replete cord blood transplantation, it is common practice to continue prophylaxis until all immunosuppressive agents are discontinued, typically not before a year after UCBT. In many centers, particularly those in which T cell–replete alternative donor HCT for PID is performed, PCR for EBV viral DNA is monitored at least weekly for the first 3 mo after HCT.</td>
</tr>
<tr>
<td>Yeast and mold</td>
<td>Risk factors for invasive fungal infections include neutropenia, mucositis, an indwelling catheter, and prolonged antibiotic and corticosteroid use. Therefore the risk for a patient with a PID will depend on how many of these factors exist in an individual patient. For patients who do not receive conditioning and have no evidence of GVHD, some centers do not provide systemic prophylaxis unless the patient has evidence of thrush. Other centers start patients on fluconazole after diagnosis, continuing until milestones of immune reconstitution are met. For patients with PIDs at risk for invasive <em>Aspergillus</em> species infection (ie, those with GVHD), prophylaxis against <em>Aspergillus</em> species is warranted, similar to patients undergoing transplantations for malignant disorders.</td>
</tr>
<tr>
<td>CMV</td>
<td>CMV infection is a major risk factor of mortality in patients with SCID after HCT, particularly in recipients of unmodified alternative donor HCTs. All patients should receive CMV-seronegative and/or filtered blood products. Patients, particularly those with CMV-seropositive donors, should be monitored closely for CMV reactivation, similar to patients undergoing transplantation for malignant disorders. Patients with evidence of CMV viremia should receive pre-emptive therapy with ganciclovir or foscarnet. Patients undergoing immunosuppressive therapy and/or those with acute or chronic GVHD should be monitored closely until CD4 cell counts are $&gt;200/\mu L$, PHA responses are $&gt;50%$ of the lower limit of normal, and immunosuppressive therapy is discontinued.</td>
</tr>
</tbody>
</table>

*HSV*, Herpes simplex virus; *UCBT*, umbilical cord blood transplantation.
TABLE E4. Intravenous gamma globulin after transplantation

<table>
<thead>
<tr>
<th>IVIG administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>All centers administer gamma globulin supplementation in the early posttransplantation period to achieve a trough IgG level of approximately 500 to 700 mg/dL, the higher level for patients with pre-existing or ongoing pulmonary complications, such as bronchiectasis. A variety of criteria are used to discontinue gamma globulin. Some centers taper or discontinue the gamma globulin after 6-12 mo. Some wait until the serum IgM level has reached a near-normal concentration. Others immunize the patient to an antigen for which there is no antibody in the therapeutic gamma globulin preparations. There is general agreement that it is important to immunize the patient after the gamma globulin therapy has been discontinued and verify antigen-specific antibody levels. If the patient is unable to make antigen-specific antibody after immunization, gamma globulin therapy should be restarted. Patients lacking donor B cells continue to receive gamma globulin unless there is evidence of host antibody responses, such as increasing trough serum IgG levels while on stable gamma globulin doses and antigen-specific antibody titers to immunizations. Gamma globulin replacement should be continued in patients undergoing immunosuppressive therapy for GVHD or autoimmune conditions. The following should be noted: 1. The majority of patients lacking donor B cells will require IVIG for life. 2. The presence of normal serum IgM or IgA levels, the presence of isohemagglutinins, or both DO NOT always indicate an ability to make antigen-specific IgG.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>When to stop IVIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>For patients off immunosuppressive therapy, without GVHD, immunoglobulin replacement can be stopped when the trough serum IgG level is &gt;600 mg/dL on stable immunoglobulin replacement doses. Some centers prefer to continue IVIG until there is evidence of donor B cells, preferably immunoglobulin-switched B cells. Consider increasing the interval between IVIG doses by 2 weeks. If the serum IgG level remains &gt;600 mg/dL, the replacement can be discontinued. Monitoring IgG levels and specific antibody responses after stopping IVIG is essential because reinstitution of IVIG might be needed for adequate protection.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vaccine administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>After discontinuation of the immunoglobulin therapy, administration of vaccines can begin on a schedule similar to that given to normal infants, with the exception of live vaccines. Prevaccine and postvaccine titers must be measured to determine responses. Vaccinations with toxoid, such as DTaP, or protein-conjugated polysaccharide vaccines (HIB and 7-valent pneumococcal conjugate vaccine) can be initiated immediately after criteria for discontinuation of IVIG have been met. In contrast, immunization with live vaccines (chicken pox, MMR, and rotavirus) should not be considered until at least 2 y after HCT AND should not be initiated until at least 12-15 mo after cessation of IVIG. In addition, for live vaccines, the patient should be off of all immunosuppressive therapy without evidence of GVHD. Some groups also recommend evidence of response to at least 2 posttransplantation killed vaccines before administering any live vaccines.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Example of potential revaccination schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTaP × 3, IPV × 3, HIB × 3, Prevnar × 3, hepatitis B × 3</td>
</tr>
<tr>
<td>Killed influenza vaccine, yearly starting 6 mo after HCT</td>
</tr>
<tr>
<td>Booster: DTaP, HIB, Prevnar, IPV 12 mo after primary series completed</td>
</tr>
</tbody>
</table>

**DTaP**, Diphtheria, tetanus, and pertussis vaccine; **HIB**, *Haemophilus influenzae* type b vaccine; **IPV**, inactivated (Salk) polio vaccine; **IVIG**, intravenous immunoglobulin; **MMR**, measles-mumps-rubella.
Rotavirus vaccine induced diarrhea in a child with severe combined immune deficiency

To the Editor:

Rotavirus is the most common cause of acute gastroenteritis in young children worldwide, and accounts for more than 2.5 million deaths annually.1 Two live oral vaccines for rotavirus are currently licensed: RotaTeq (CSL Limited, Parkville, Victoria, Australia), a pentavalent bovine-human reassortant vaccine, and Rotarix (GlaxoSmithKline [GSK] Australia Pty Ltd, Boronia, Victoria, Australia), a human monovalent vaccine. Both vaccines became available on the Australian National Immunization Program in July 2007 with RotaTeq given on the National Immunization Program schedule at 2, 4, and 6 months of age. Live vaccines such as measles mumps rubella (MMR) are generally contraindicated in immunosuppressed populations because of potential morbidity and mortality.2 This has not been applied to rotavirus vaccines, in which risk of vaccine-associated disease is felt to be less than the risk from being exposed to natural infection.2 Current guidelines support the administration of rotavirus vaccine to children infected with HIV, the largest immunosuppressed population studied to date.3 The side effect profile is likely to involve gastrointestinal symptoms (vomiting and diarrhea).

A 9-month-old girl born to nonconsanguinous parents presented to the hospital with a history of faltering growth and chronic diarrhea. She was fully immunized according to the National Immunization Program schedule, including oral RotaTeq at 2, 4, and 6 months of age. She had mild diarrhea after the first dose of RotaTeq and remained well until 4 months of age (weight, 6 kg, 50th percentile), at which time she developed persistent vomiting and diarrhea with poor weight gain, worsening at 6 months.

At 9 months of age, her weight was 5.8 kg (<3rd percentile), and assessment of her faltering growth and chronic diarrhea revealed rotavirus in her stool, lymphopenia (lymphocyte count, 2.08 × 10^9/L; range, 4.0-10.0 × 10^9/L) and undetectable IgG, IgA, and IgM. Lymphocyte subsets confirmed absent T cells with absent lymphocyte function and normal levels of B and natural killer cells.

A diagnosis of severe combined immune deficiency (SCID) was made (genotype unspecified; IL7RA [interleukin 7 receptor alpha], ADA [adenosine deaminase], and PNP [purine nucleoside phosphorylase]-negative).

Serial stool samples (n = 14) were collected from admission (at age 9 months) to assess for the presence of rotavirus. RNA was extracted from each sample and subjected to a VP6 [viral protein 6]-specific RT-PCR assay.4 Each PCR product was sequenced to characterize the origin of the VP6 gene. All VP6 genes exhibited 100% identity to the RotaTeq vaccine VP6. Successful cord blood transplantation was performed at 11 months of age from a matched unrelated donor. Vaccine rotavirus was cleared post-transplant and last detected at 13.5 months of age, thus excreted for at least 7.5 months.

Severe combined immunodeficiency is the most severe type of primary immune deficiency, occurring in 1 in 50,000 to 100,000 live births. It often presents within the first year of life with severe infections and faltering growth. Without stem cell transplantation, SCID is fatal. There are no previous reported cases of prolonged rotavirus shedding after vaccine administration in a patient with SCID. In this case, 3 doses of RotaTeq were administered before this diagnosis was made. It is likely that rotavirus vaccine excretion persisted from dose 1, with exacerbation of symptoms after doses 2 and 3. Studies of RotaTeq have shown that viral shedding occurred in 9% of 360 recipients after dose 1, 0% after dose 2, and 0.3% after dose 3, usually between days 1 and 15 after the dose.5

This is the first reported case of persistent rotavirus vaccine excretion and chronic diarrhea in a severely immunocompromised patient. Because the diagnosis of a primary immune deficiency, such as SCID, is often made in the first year of life, it is important to consider this diagnosis when treating children with prolonged diarrhea and faltering growth, especially as we enter the universal rotavirus vaccination era.

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Role of omalizumab and steroids in Churg-Strauss syndrome

To the Editor:

The first case report on a patient who developed Churg-Strauss syndrome (CSS) after therapy with omalizumab, the anti-IgE recombinant humanized mAb, was described by Winchester et al1 in 2006. Later, several similar cases were reported in the medical literature; the authors suggested a possible role for anti-IgE treatment in the emergence of CSS and recommended careful monitoring of emerging symptoms of this vasculitis in patients treated with omalizumab.2-4

In contrast, Giavina Bianchi et al5 presented the case of a patient with CSS with uncontrolled asthma that improved with omalizumab treatment without increase of CSS clinical severity. The authors hypothesized that steroid tapering during omalizumab therapy could have caused CSS clinical appearance.

Here we describe the case of a 42-year-old Caucasian man with a 5 year history of asthma. One year after asthma onset, blood hypereosinophilia became evident (1600/μL), and the patient

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Here we describe the case of a 42-year-old Caucasian man with a 5 year history of asthma. One year after asthma onset, blood hypereosinophilia became evident (1600/μL), and the patient
Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968–99

Corinne Antoine, Susanna Müller, Andrew Cant, Marina Cavazzana-Calvo, Paul Veys, Jak Vossen, Anders Fasth, Carsten Hellmann, Nicole Wulfhekel, Reinhard Segel, Stéphane Blanché, Wilhelm Friedrich, Mario Abrun, Graham Davies, Robert Bredius, Ansgar Schulz, Paul Landais, Alain Fischer, for the European Group for Blood and Marrow Transplantation and the European Society for Immunodeficiencies

Summary

Background Transplantation of allogeneic haemopoietic stem cells can cure several primary immunodeficiencies. This European report focuses on the long-term results of such procedures done between 1968 and December, 1999, for primary immunodeficiencies.

Methods The report includes data from 37 centres in 18 countries, which participated in a European registry for stem-cell transplantation in severe combined immunodeficiencies (SCID) and in other immunodeficiency disorders (non-SCID). 1082 transplants in 919 patients were studied (556 in 475 SCID patients; 512 in 444 non-SCID patients; four procedures excluded owing to insufficient data). Minimum follow-up of 6 months was required.

Findings In SCID, 3-year survival with sustained engraftment was significantly better after HLA-identical than after mismatched transplantation (77% vs 54%; p<0.002) and survival improved over time. In HLA-mismatched stem-cell transplantation, B(+) SCID had poorer prognosis than B(-) SCID. However, improvement with time occurred in both SCID phenotypes. In non-SCID, 3-year survival after genotypically HLA-matched, phenotypically HLA-matched, HLA-mismatched related, and unrelated-donor transplantation was 71%, 42%, 42%, and 59%, respectively (p<0.0006). Acute graft versus host disease predicted poor prognosis whatever the donor origin except in related HLA-identical transplantation in SCID.

Interpretation The improvement in survival over time indicates more effective prevention and treatment of disease-related and procedure-related complications—eg, infections and graft versus host disease. An important factor is better prevention of graft versus host disease in the HLA-non-identical setting by use of more efficient methods of T-cell depletion. For non-SCID, stem-cell transplantation can provide a cure, and grafts from unrelated donors are almost as beneficial as those from genetically HLA-identical relatives.

Lancet 2003; 362: 553–60
See Commentary page 541

Introduction

Primary immunodeficiencies are inherited disorders characterised by impairment of innate or adaptive immunity, commonly leading to lethal complications. Transplantation of allogeneic haemopoietic human stem cells can cure most of the lethal forms of immunodeficiencies, including severe combined immunodeficiencies (SCID), several T-cell immunodeficiencies, Wiskott–Aldrich syndrome, phagocyte disorders such as leucocyte adhesion deficiency and chronic granulomatous disease, haemophagocytic syndromes such as familial lymphohistiocytosis, Chediak-Higashi syndrome, Griscelli’s disease, and X-linked lymphoproliferative syndrome. At first, only HLA-identical relatives were used as donors, but the introduction of T-cell depletion of bone marrow in 1981 allowed efficient prevention of graft versus host disease and thus the successful treatment of SCID by transplantation of HLA-mismatched stem cells. The greater availability of unrelated donors has led to more transplants from unrelated donors.

In this study, data gathered in the SCETIDE (Stem Cell Transplantation for Immunodeficiencies) registry were analysed to give the long-term results of human haemopoietic stem-cell transplantsations in primary immunodeficiencies in Europe since 1968. The outcome of transplants from donors of various origins—HLA-identical siblings, phenotypically compatible parents, HLA-matched unrelated donors, and HLA-haploidentical family donors—has been assessed. Previous analyses in 1986, in 1990 for SCID (n=185), and in 1994 for other forms of immunodeficiencies (n=149) helped to define prognostic factors related to disease conditions, patients’ status before stem-cell transplantation, donor origin, and the transplant procedure. This report, including the previous cases (SCID and non-SCID), is based on the analysis in the long-term of a total of 919 patients treated in 37 European centres between 1968 and 1999. The large number of cases registered in the database gives sufficient statistical power for assessment of changing trends in outcome over different periods.

Methods

Patients

The European Group derived all data from the SCETIDE registry, established for the European Group for Blood
and Marrow Transplantation and the European Society for Immunodeficiency. The electronic database was developed to register haematopoietic stem-cell transplantation for primary immunodeficiency. All the centres affiliated to the European Working Party currently undertaking such procedures for SCID and inborn errors were enrolled. Between 1988 and December, 1999, 37 centres in 18 European countries recorded relevant data. Centres included data on children presenting with an immunodeficiency and undergoing haematopoietic stem-cell transplantation. Data collection was continuous and systematic in each centre. Information was gathered on the basis of a questionnaire built up and validated by the European Working Party. Each centre was in charge of the quality control of its own data, undertaken by data managers in the largest centres. The data were then transmitted to the Department of Biostatistics, Hôpital Necker Enfants Malades, Paris, which did an additional assessment of coherence of the data before the analysis.

Of the 1082 transplantations, 556 were done in 475 SCID patients and 512 in 444 non-SCID patients. During the inclusion period, insufficient data were retrieved for four procedures (two in SCID, two in non-SCID), which were therefore excluded from this report. The median follow-up after transplantation was 9 years for SCID and 7 years for non-SCID.

**Procedures**

Marrow was used as the source of haematopoietic stem cells in 88% of transplants, peripheral stem cells in 12%, and cord blood in 0-7%. Of the SCID patients, 107 with HLA-identical related donors, 87 with HLA-mismatched donors, and 11 with unrelated donors did not receive any conditioning regimen. For the other SCID patients, the conditioning regimen consisted of busulphan (8 mg/kg) and cyclophosphamide (200 mg/kg) in most cases, in accordance with the recommendations of the European Group for Blood and Marrow Transplantation and European Society for Immunodeficiency working group.

For non-SCID patients, all but ten received a conditioning regimen consisting of busulphan (16-20 mg/kg) and cyclophosphamide (200 mg/kg). T-cell depletion was used in 91% of HLA-mismatched cases and in 41% of unrelated marrow samples. Methods of T-cell depletion included E-rosetting, soybean agglutination, monoclonal antibodies, or (since 1996) positive selection of CD34-positive cells. In 1986, the group agreed that in non-HLA-identical transplantation, the graft should contain no more than $5 \times 10^7$ T cells/kg after T-cell depletion. This threshold was changed to $1 \times 10^7$ T cells/kg in 1998. In vivo immunosuppression (anti-CD45R (LFA-1) with or without CD2, Campath Ig, monoclonal antibodies, or antithymocyte globulin) was given to most non-SCID patients who received an unrelated or an HLA-mismatched graft.

In non-SCID patients, prophylaxis against graft versus host disease after HLA-identical stem-cell transplantation consisted of methotrexate (before 1983) or ciclosporin with or without a short course of methotrexate. No prophylaxis against graft versus host disease was used in SCID patients after an HLA-identical transplantation. All patients who received a graft of haematopoietic stem cells that was depleted of T cells by either monoclonal antibodies or rosetting were treated with ciclosporin A for at least 2 months. Recipients of CD34-selected cells were not given any prophylaxis. Graft versus host disease was graded according to standard criteria. Various techniques were used to study chimerism, including karyotyping, assessment of red-blood-cell antigens, immunoglobulin allootypes, HLA typing, and more recently Southern-blot hybridisation or PCR analysis with microsatellite probes as well as FISH, and allele-specific antibodies to HLA.

The development of T and B lymphocytes and their function were analysed by standard methods (T-cell and B-cell markers, in-vitro T-cell proliferation induced by lectin or antigen, serum immunoglobulin concentrations, and serum antibodies after immunisation).

<table>
<thead>
<tr>
<th>Number (of category)</th>
<th>Related donor</th>
<th>Unrelated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genotypically HLA identical</td>
<td>Phenotypically HLA identical</td>
</tr>
<tr>
<td><strong>SCID</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>475</td>
<td>104</td>
</tr>
<tr>
<td>B-cell dysgenesis</td>
<td>12 (3%)</td>
<td>2</td>
</tr>
<tr>
<td>ADA deficiency</td>
<td>52 (11%)</td>
<td>19</td>
</tr>
<tr>
<td>Low T and low B</td>
<td>327 (25%)</td>
<td>22</td>
</tr>
<tr>
<td>Low T</td>
<td>237 (48%)</td>
<td>39</td>
</tr>
<tr>
<td>Other</td>
<td>58 (13%)</td>
<td>12</td>
</tr>
<tr>
<td><strong>Non SCID</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>444</td>
<td>148</td>
</tr>
<tr>
<td>Without-AIDS syndrome</td>
<td>303 (23%)</td>
<td>33</td>
</tr>
<tr>
<td>T-cell deficiencies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omits syndrome</td>
<td>43 (10%)</td>
<td>9</td>
</tr>
<tr>
<td>PNP deficiency</td>
<td>4 (1%)</td>
<td>2</td>
</tr>
<tr>
<td>HLA class II deficiency</td>
<td>52 (12%)</td>
<td>17</td>
</tr>
<tr>
<td>CD200 ligand deficiency</td>
<td>11 (2%)</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>76 (17%)</td>
<td>19</td>
</tr>
<tr>
<td>Phagocyte-cell disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agammaglobulin</td>
<td>5 (1%)</td>
<td>3</td>
</tr>
<tr>
<td>Chronic granulomatous disorders</td>
<td>17 (4%)</td>
<td>13</td>
</tr>
<tr>
<td>Leucocyte schizophrenia</td>
<td>28 (6%)</td>
<td>9</td>
</tr>
<tr>
<td>Haemophagocytic syndromes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial lymphohistiocytosis</td>
<td>82 (14%)</td>
<td>19</td>
</tr>
<tr>
<td>Cheeke-Heggs syndrome</td>
<td>20 (4%)</td>
<td>10</td>
</tr>
<tr>
<td>XLP (Puttico)</td>
<td>2 (1%)</td>
<td>0</td>
</tr>
<tr>
<td>Griscelli's disease</td>
<td>6 (1%)</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>17 (4%)</td>
<td>8</td>
</tr>
</tbody>
</table>

*Table 1: Type of immunodeficiency, according to donor origin and HLA matching.*
Statistical analysis

Data available as of Dec 1, 1999, with a minimum follow-up of 6 months, were retained for analysis. Engraftment was examined only in patients alive 1 month after transplantation, and haemopoietic stem cells. Analysis of acute graft versus host disease was restricted to patients who showed engraftment and were alive 1 month after transplantation. For chronic graft versus host disease, only patients who showed engraftment and were alive 3 months after transplantation were included. Survival times started from the date of the last procedure. The number of previous transplants was introduced as a covariate in multivariate analyses. A centre effect was explored in terms of the number of transplants (50 or more, large centre; less than 50, small centre).

Differences in observed distributions were analysed by χ² test. Variables affecting the development of T-cell and B-cell function 6 months after transplantation and acute graft versus host disease were sought with a logistic regression model. Survival was considered when evidence for sustained engraftment was present associated with an improvement of the immunodeficiency condition (labeled as survival in the text). The cumulative survival was estimated by the product-limit method. The log-rank test and Wilcoxon's rank-sum test were used to compare cumulative survival between groups. A Cox's proportional-hazard model was used to assess the effect of independent predictors (demographics, comorbidity, transplant characteristics, and therapy before transplantation) on survival of patients. ASCET TID database was developed by use of Access software (version 2000). Statistical analyses were done with SAS (version 6.12) and R software for multivariate analyses. GLM and Survival 3 libraries were used.

Role of the funding source

The study had European community support of the database, data collection, and meetings, and support from the European Group for Blood and Marrow Transplantation for meetings.

### Results

Details of diagnosis are shown in table 1 as well as the origin of stem-cell donors. Information on recipient's age at transplantation, number of procedures per patient, and number of procedures undertaken as a function of time is presented in table 2.

**SCID patients**

3-year survival with evidence of sustained engraftment and improvement of the immunodeficiency disorder was significantly better for HLA-identical than for HLA-mismatched transplantation (77% vs 54%; p=0.002; figure 1). Within the HLA-identical group, 3-year survival after transplantation from genotypically or phenotypically identical related or unrelated donors did not differ significantly (81%, 72%, and 63%, respectively). Significant improvements have occurred over time in survival after both HLA-identical (p=0.04) and non-identical (p=0.0007) stem-cell transplantation (figure 1). SCID phenotype also had an effect on survival after non-HLA-identical stem-cell transplantation (table 3); B(-) SCID had a poorer prognosis than B(+) SCID, confirming and extending a previous observation. Survival rates in both SCID phenotypes (data not shown). In the non-HLA-identical setting, use of a myeloablative conditioning regimen had a positive effect on survival in the B(-) SCID group, but it did not reach significance compared with the other SCID groups. For patients with aseptic meningeal failure, 3-year survival was 81% for HLA-matched and 29% for HLA-mismatched transplantation. For reticular dysgenesis, 3-year survival was 75% and 29%, respectively.

In Cox's regression multivariate analysis, only age at transplantation and the use of trimethoprim-sulphamethoxazole prophylaxis had a significant effect on survival after related HLA-identical transplantation (table 3). Independent predictors of mortality for SCID patients after a related HLA-mismatched graft are given in table 3. The most powerful predictors of death were B(-) SCID phenotype, the absence of protected environment,
and the presence of pulmonary infection before transplantation. The probability of survival for the subgroups stratified according to these criteria varied from 74% (all positive criteria) to zero (all negative criteria). A center size effect was explored by segregation of data from experienced centers (30 or more procedures). In these centers, survival was better for non-HLA-identical transplantation (57% vs 43%; p=0.009) and no differences were evident in other types of transplant. The occurrence of acute graft versus host disease (grade 2 or higher) led to poorer survival in related HLA-mismatched transplantation (52% vs 77%; p=0.004). No effect was apparent for chronic graft versus host disease, possibly because of lack of statistical power. The frequency of acute graft versus host disease increased over time after haploidentical transplantation, from 35-40% before 1996 to 22% thereafter (p<0.001), possibly because more stringent methods of T-cell depletion were used. This factor could partly account for the observed better survival with time. The main reported causes of death were infections (56%), graft versus host disease (25%), and B-cell lymphoproliferative syndrome (5%).

The rate of sustained engraftment after HLA-identical stem-cell transplantation was 96% compared with 90% after HLA-non-identical transplantation (odds ratio 2.7, 95% CI 1.2–7.4). In the latter group, only age at transplantation affected the engraftment rate, with a cut-off at 6 months (97% vs 86%, patients younger or older than 6 months; odds ratio 5.0 95% CI 1.4–16.8). No significant differences were noted in relation to the use of a conditioning regimen or the SCID phenotype. Engraftment was 88% in SCID B-(-), 93% in SCID B(+), and 91% in adenosine deaminase deficiency.

Detailed analysis of the quality of immune reconstitution was beyond the scope of this study and the information provided by the database. Nevertheless, data were analysed at last follow-up. Positive T-cell function was defined as a T-cell count of 1 × 10^9/L and positive T-cell response to antigens. Positive B-cell function was defined by the absence of intravenous immunoglobulin replacement therapy. There were differences between B(+) and B(−) SCID patients (table 2). T-cell reconstitution arose in most recipients of HLA-identical stem-cell transplantation, but after non-HLA-identical transplantation it arose in a smaller proportion of B(+) SCID than B(−) SCID patients. Similarly, reconstitution of B-cell function was more common both after HLA-identical transplantation and after non-identical transplantation in B(+) SCID than in B(−) SCID.

Non-SCID patients
Figure 2 shows the probability of survival with evidence for sustained engraftment after non-SCID transplantation in the immunodeficiency condition, according to donor origin, in non-SCID patients. Survival was significantly better after HLA-matched than after HLA-mismatched transplantation. 3-year survival after genotypically HLA-matched, phenotypically HLA-matched, HLA-mismatched related, or unrelated-donor transplantation was 71%, 42%, 42%, and 59%, respectively (p=0.0006). No difference in survival between genotypically HLA-identical and unrelated-donor transplantation was noted. 75% of patients with unrelated donors were HLA identical to the donors. An increased risk of death was associated with phenotypically HLA-identical donor transplantation (hazard ratio 2.23, 95% CI 1.3–2.8) and related HLA-mismatched transplantation (3.16, 1.5–3.2) compared with genotypically HLA-identical transplantation. By contrast with SCID, there was no evidence...
of improvement in survival in non-SCID patients since 1983, whatever the donor origin or the HLA compatibility (figure 3).

In univariate analysis, a moderate effect of the type of immunodeficiency on survival was noted, 3-year survival for phagocytic-cell disorders, haemophagocytic syndromes, Wiskott-Aldrich syndrome, and T-cell deficiencies was 70%, 59%, 62%, and 43% (p=0.02). The cumulative probability of survival did not differ significantly among the patients with immunodeficiencies in separate analyses of genotypically HLA-identical and HLA-mismatched transplants (table 5). There was a tendency toward a poorer outcome for T-cell deficiencies after both types of transplants. HLA class II immunodeficiency within the T-cell-deficiency group seemed to carry a poor prognosis after HLA non-identical transplantation; only 32% of patients survived to 1 year. There was a significant difference in survival between identical and haploidentical transplantation only for patients with Wiskott-Aldrich syndrome (table 5).

By the Cox's proportional-hazards model, no risk factor was identified after genotypically HLA-identical transplantation or after non-HLA-identical transplantation. No centre effect was apparent in non-SCID patients. Acute graft versus host disease (grade 2 or higher) increased risk of death for patients with non-SCID. Chronic graft versus host disease was not associated with a poor outcome; moreover, the frequency of this complication was low overall (14–32% according to subgroup). The main causes of death were infections (70%), pneumonia in a third of cases), graft versus host disease (9%), toxic effects of the conditioning regimen (9%), B-lymphocyte proliferative syndrome (5%), and rejection (3%).

The rate of sustained engraftment after related genotypically HLA-identical transplantation was 99% compared with 81%, 79%, and 73%, respectively, after related phenotypically HLA-identical, unrelated-donor, and related HLA-mismatched transplantation (p=0.001). In the latter group, the engraftment rate was significantly affected by the period during which transplantation was done (53%, 77%, 77%, and 85% in 1968–85, 1986–90, 1991–96, and 1997–99, respectively) from the earlier to the more recent period, indicating improvement in the effect of the immunosuppressive conditioning used to achieve engraftment. No significant difference was noted according to the type of immunodeficiency. Altogether, with improved survival, disease was classified as cured or improved in 94% of patients for whom engraftment was present 6 months or longer after transplantation.

![Cumulative probability of survival](image)

Table 3: Factors affecting outcome in SCID patients after HLA-identical or after related HLA-mismatched stem-cell transplantation.

<table>
<thead>
<tr>
<th>After HLA-identical transplantation</th>
<th>Patients</th>
<th>Deaths</th>
<th>3-year survival, % (95% CI)</th>
<th>p</th>
<th>Hazard ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at transplantation (months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤9</td>
<td>92</td>
<td>12</td>
<td>85 (77–93)</td>
<td>0.0004</td>
<td>1</td>
<td>0.12</td>
</tr>
<tr>
<td>&gt;9–12</td>
<td>50</td>
<td>12</td>
<td>73 (59–86)</td>
<td>0.0004</td>
<td>2.2 (1.9–5.6)</td>
<td>0.0002</td>
</tr>
<tr>
<td>&gt;12</td>
<td>31</td>
<td>14</td>
<td>58 (35–72)</td>
<td>0.0004</td>
<td>3.3 (2.7–39.4)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Prephenotypes*</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>yes</td>
<td>93</td>
<td>14</td>
<td>79 (72–87)</td>
<td>0.0024</td>
<td>1</td>
<td>0.002</td>
</tr>
<tr>
<td>no</td>
<td>38</td>
<td>12</td>
<td>62 (47–78)</td>
<td>0.0024</td>
<td>1</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table 4: Immunological reconstitution, according to SCID phenotype.

<table>
<thead>
<tr>
<th>% with reconstitution</th>
<th>B&lt;sub&gt;1&lt;/sub&gt;</th>
<th>B&lt;sub&gt;2&lt;/sub&gt;</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-identical transplants</td>
<td>(n=30)</td>
<td>(n=34)</td>
<td>0.017</td>
</tr>
<tr>
<td>T-cell function</td>
<td>85</td>
<td>92</td>
<td>0.51</td>
</tr>
<tr>
<td>B-cell function</td>
<td>83</td>
<td>88</td>
<td>0.017</td>
</tr>
<tr>
<td>Non-HLA-identical transplants</td>
<td>(n=30)</td>
<td>(n=70)</td>
<td>0.002</td>
</tr>
<tr>
<td>T-cell function</td>
<td>67</td>
<td>90</td>
<td>0.002</td>
</tr>
<tr>
<td>B-cell function</td>
<td>44</td>
<td>99</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Figure 2: Cumulative probability of survival in non-SCID patients, according to donor source (related or unrelated donor) and HLA matching.
**Discussion**

This study on the outcome of haemopoietic stem-cell transplantation in patients with primary immunodeficiencies is based on the largest cohort of immunodeficient patients treated by transplantation reported so far. This type of analysis is complex because of the array of different immunodeficiency diseases presenting distinct problems both in terms of transplantation procedures and specific risks of complications. For simplicity, patients were classified as having SCID or non-SCID disorders.

The analysis shows that the results of transplantation for SCID have improved over time, as reported by Buckley and colleagues from a single centre. Which factors might account for this improvement? Earlier diagnosis, resulting in healthier patients at the time of transplantation, does not seem to account for better survival in the later years because frequencies of pretransplantation complications and age at transplantation have not changed over time. An improvement in engraftment rate is not the cause, since it remained constant and fairly high over time. Therefore, the observed better event-free survival most likely indicates more effective prevention or treatment of disease-related and transplantation-procedure complications, notably infections and graft versus host disease. An important factor for improved survival is better prevention of graft versus host disease in the non-HLA-identical setting by use of more efficient methods of T-cell depletion (combination of E-rosetting and soybean agglutination and, more recently, positive selection of CD34-positive cells).

Despite incomplete or possibly declining immune function observed late after transplantation in a proportion of SCID patients, very few late deaths were observed. A careful analysis of long-term T-cell and B-cell function is required to assess the risk of late complications. Overall, the outcome of B(+) SCID was significantly better than that of B(-) SCID. Both survival and quality of immune reconstitution were better in B(+) SCID patients, particularly after non-HLA-identical transplantation. These findings extend conclusions drawn from a previous analysis. The likely scenario is that several factors account for the survival difference, including lower engraftment rates in B(-) SCID possibly caused by the residual natural-killer-cell activity detectable in most patients with B(-) SCID as well as a higher rate of severe post-transplantation complications.

Future analysis should focus on the possible relation between SCID variants as defined by mutation analysis and outcome of transplantation, because the genetic causes of most SCID phenotypes have now been identified. For instance, B(-) SCID, characterised by increased cell sensitivity to radiation secondary to mutations of the DCLRE1C (Artemis) gene, could carry a poorer prognosis because of defective repair of DNA breaks, occurring around the time of
Clinical picture

Persistent left superior vena cava

Hans-Ulrich Schulz, Rene Mantke,
Jutta Freitag, Hans Lipperott

A 69-year-old woman was scheduled to undergo cardiac pacemaker implantation because of a sick sinus syndrome. A cardiac stimulation electrode was inserted via the left subclavian vein. Although the intervention was performed under fluoroscopic control, passage to the right in the direction of the superior vena cava was not possible. Instead, the pacemaker electrode took an unusual left-sided downward course crossing to the right at the level of the coronary sinus. This was most likely due to a persistent left superior vena cava that was confirmed by phlebography with injection of contrast medium simultaneously via the right and left subclavian veins done after successful cardiac pacemaker electrode placement via the right subclavian vein. Clinicians should be aware of the possibility of a persistent left superior vena cava when placing a central venous line or a cardiac pacemaker electrode. In general, this rare condition does not prevent successful placement of central venous lines or cardiac pacemaker leads.
Assessment of thymic output in adults after haematopoietic stem-cell transplantation and prediction of T-cell reconstitution

Daniel C Douek, Robert A Vescio, Michael R Betts, Jason M Brenchley, Brenna J Hill, Lan Zhang, James R Berenson, Robert H Collins, Richard A Koup

Summary
Background The potential benefits of haematopoietic stem-cell transplantation are tempered by the depletion of T-cells accompanying this procedure. We used a new technique which quantifies the excisional DNA products of T-cell receptor (TCR) gene rearrangement to measure thymic output directly in patients with multiple myeloma, and thus assessed the contribution of the thymus to immune recovery after transplantation.

Methods We studied 40 patients, 34-68 years of age, who had been randomly assigned myeloablative chemotherapy and autologous peripheral-blood haematopoietic stem-cell transplantation with unmanipulated grafts or grafts enriched for CD34 stem cells. CD4 and CD8 T-cell counts were measured, thymic output was estimated serially until 2 years after transplantation, and percentages of naive T-cells were measured.

Findings The production of substantial numbers of new naive T cells by the thymus could be detected by 100 days post-transplant; there was a significant inverse relation between age and recovery of new T cells. In the CD34-unselected group, numbers of TCR-rearrangement excision circles returned to baseline after 2 years, whereas in the CD34-selected group, numbers at 2 years were significantly higher than both baseline numbers (p=0.004), and 2-year numbers in the unselected group (p=0.004). Increased thymic output correlated with, and was predictive of, increased naive T-cell numbers and broader T-cell-receptor repertoires.

Interpretation Our results provide evidence that the adult thymus contributes more substantially to immune reconstitution after haematopoietic stem-cell transplantation than was previously thought, and therefore could be a target for therapeutic intervention.

Lancet 2000; 355: 1875-81

Introduction
Myeloablative chemotherapy followed by bone-marrow transplantation or peripheral-blood haematopoietic stem-cell transplantation is associated with substantial T-cell immunodeficiency.1-3 Although the thymus is the primary site of T lymphopoiesis during fetal and early postnatal life, its role in reconstitution of the adult immune system after bone-marrow or haematopoietic stem-cell transplantation remains controversial.1-3 T-cell reconstitution can involve both a thymus-dependent pathway (the generation of new naive T cells from haematopoietic stem cells), and a thymus-independent pathway (peripheral expansion of pre-existing memory T cells).4-6 The thymus-dependent pathway has the potential advantage of reconstituting antigen-specific responses which were not present within the mature T cells that routinely accompany bone-marrow and haematopoietic stem-cell grafts. However, studies suggest that the thymus-dependent pathway is age-dependent and becomes severely limited after childhood,4-6 presumably due to thymic involution. These studies relied on cell-surface molecules, such as isoforms of CD45, to differentiate naive recent thymic emigrants from memory or effector T cells. However, T cells expressing a naive phenotype are not necessarily accurate surrogate markers of thymic function. After thymic emigration, CD45RA-positive naive T cells can have a long, quiescent lifespan,7,8 may proliferate in an antigen-independent manner,9,10 or may rapidly convert to CD45RO-positive memory or effector T cells.10 Furthermore, naive T-cell markers may be acquired by memory T cells.10

During thymocyte development, rearrangement of the T-cell-receptor (TCR) gene leads to the excision of circular DNA fragments from genomic DNA.11 We have developed an assay which can be used to estimate thymic output more accurately by measuring the numbers of TCR-rearrangement excision circles (TREC) in peripheral-blood T cells.11 These products are stable, unique to T cells, and are not duplicated during mitosis, which means that they are diluted out with each cell division.12-17 Thus TREC are markers of developmental proximity to the thymus, and their concentrations in peripheral blood can be used to estimate thymic output.17 To accomplish this estimation, we used a TREC derived from a unique recombination event which is common to about 70% of thymocytes destined to become mature TCR-αβ T cells.18 We previously reported that although thymic function declines with age, substantial output is maintained into late adulthood. Furthermore, the adult thymus can contribute to immune reconstitution in HIV-infected individuals after treatment with antiretroviral drugs.19
In this study, we aimed to find out whether the adult thymus can make a contribution to immune recovery by quantification of numbers of TREC in the peripheral-blood mononuclear cells of patients with multiple myeloma, who received myeloablative chemotherapy and autologous peripheral-blood haematopoietic stem-cell transplantation with unmanipulated grafts or grafts enriched for CD34-positive haematopoietic stem cells.

**Methods**

**Patients**

Samples from 40 patients aged 34–66 were available from a group of 134 patients with multiple myeloma enrolled in a multicentre phase III trial. The subgroup of patients we studied had received haematopoietic stem-cell transplantation and had stable or responsive disease after a minimum of three chemotherapy cycles. Patients who had disease progression within 1 year of transplantation were excluded. No DNA samples remained available for patients who had undergone immunoglobulin gene analysis, so these patients were also excluded from this study.

Patients were eligible for the phase III trial if they were 18–70 years old, and if they had one of the following features at diagnosis or any time thereafter: IgG concentrations greater than 50 g/L, IgA concentrations greater than 10 g/L, or serum M-component concentrations greater than 4 g/L; more than one osteolytic bone lesion or radiographic evidence of diffuse osteoporosis; β2-microglobulin concentrations greater than 3 mg/L; and non-secretory myeloma if bone marrow plasmacytosis was greater than 30%. Ineligibility was defined by more than 3 months of alkylator-based therapy or 6 months of any other prior chemotherapy, or by disease progression. The baseline was the day the patient was registered.

**Methods**

Within 5 weeks of assessment, patients received mobilisation chemotherapy with 2 g/m² cyclophosphamide and prednisolone (100 mg/day) on the first 4 days. Granulocyte colony-stimulating factor (G-CSF, 10 μg/kg/day) was started the day after cyclophosphamide and was continued until haematopoietic stem cells were harvested upon neutrophil recovery. Patients were randomly assigned to receive selection of CD34-positive haematopoietic stem cells or no selection. Patients received a myeloablative chemotherapy regimen of busulfan (14 mg/kg) and cyclophosphamide (120 mg/kg), and thawed CD34-selected or unselected products 2 days after the last infusion of cyclophosphamide. The median number of CD34 cells infused was 5·4×10⁶ and 8·6×10⁶ for the selected and unselected groups, respectively (p=0.012, Wilcoxon’s test).

Granulocyte-macrophage-CSF was given daily until neutrophil counts were at least 1000/mL. Median time to neutrophil engraftment was 12 days.

Quantification of TREC in DNA of peripheral-blood mononuclear cells was done by quantitative-competitive PCR. DNA was extracted from the cells, and 1 μg was added to each PCR reaction, which also contained 10³, 10⁴, or 5×10⁴ molecules of an internal competitor standard. The reactions were run for 35 cycles and incorporated phosphorus-32-labelled dCTP. PCR products were separated on non-denaturing 6% polyacrylamide gels. Bands were imaged and analysed with a Cyclone phosphorimager (Packard, Meriden, CT, USA).

We measured percentages of naïve T cells and TREC in nine patients from whom viably cryopreserved peripheral-blood mononuclear cells were available. Such cells were stained with fluorochrome-conjugated antibodies against CD4, CD8, CD45RO, and CD27 (Becton-Dickinson, San Jose, CA, USA) and analysed with four-colour flow cytometry (FACScalibur, Becton-Dickinson). Proportions of naïve T cells in each CD4 or CD8 subset were measured by gating on CD45RO-negative, CD27-negative cells (FACSort software, Becton-Dickinson). Quantification of TREC in sorted CD4 and CD8 T cells was done by real-time quantitative PCR with the 5'-nuclease (TaqMan) assay and an ABI7700 system (Perkin-Elmer, Norwalk, CT, USA). Such cells were sorted into CD4 and CD8 subsets by use of MACS magnetic microbeads (Miltenyi-Biocore, Auburn, CA, USA). Cells were lysed in 100 μg/mL proteinase K (Boehringer, Indianapolis, IN, USA) for 1 h at 56°C, then 10 min at 95°C, at 10 cells/mL. Real-time quantitative PCR was done on 5 μL of cell lysate (50,000 cells) with the primers: ACTGCCCTTTTAAACCATGCT and GGGCGCCTGGA GGTTTATGG, and probe FAM-5'-ACACCTCTCAG TTTTGTTAAGTGCCTCCACT-TAMRA (Megabases, Chicago, IL, USA). PCR reactions contained 1× U Platinum taq polymerase (Gibco, Grand Island, NY, USA), 3·5 mmol/L MgCl₂, 0·2 mmol/L dNTPs, 500 mmol/L each primer, 150 mmol/L probe, and Blue-638 reference (Megabases). Conditions were 95°C for 5 s, then 95°C for 30 s, 60°C for 1 min, for 40 cycles. A standard curve was plotted, and numbers of TREC in samples were calculated by the ABI7700 software. Absolute CD4 and CD8 T-cell counts were measured as described previously.

**Immunological Analyses**

Immunosuppression recovery from a state of T-cell depletion poisons skewing TCR repertoires. To assess the effect of increases in concentrations of TCR-rearrangement excision circles on the breadth of the TCR repertoire, we compared the representation of TCRBV gene families (those encoding the TCR β-chain variable region) in CD4 T cells of three patients who had increases in numbers of TREC with those of three patients who had no increases. cDNA was synthesized from 24 μL of sorted CD4 T-cell lysate described above with oligo-dT and Thermoscript reverse transcriptase (Gibco). 23 separate 35-cycle PCR reactions were set up with a common TCRBC 3’-primer and 23 5’-primers specific for each of the 25 TCRBV families, excluding the pseudogenes TCRBV 10 and TCRBV 19. Heminested PCR reactions were then done with the same TCRBV 5’-primers, but with a nested 5’-labelled internal

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CD4 selection</th>
<th>No selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) (%)</td>
<td>48 (8)</td>
<td>71 (14)</td>
</tr>
<tr>
<td>Mean (SD) (%)</td>
<td>49 (14)</td>
<td>71 (28)</td>
</tr>
<tr>
<td>Number with blast</td>
<td>10 (5%)</td>
<td>15 (25%)</td>
</tr>
<tr>
<td>Disease status</td>
<td>Stable</td>
<td>Stable</td>
</tr>
<tr>
<td>Partial remission</td>
<td>10 (5%)</td>
<td>15 (25%)</td>
</tr>
<tr>
<td>Complete remission</td>
<td>2 (10%)</td>
<td>6 (25%)</td>
</tr>
</tbody>
</table>

Table 1: Baseline characteristics

Figure 1: Recovery of CD4 and CD8 T cells after haematopoietic stem-cell transplantation

Mean values for absolute CD4 (filled symbols) and CD8 (open symbols) T-cell counts at CD4-selected (circles) and unselected (squares) transplantation. B=baseline, E=neutrophil engraftment.
Figure 2: Increases in numbers of TREC in peripheral-blood mononuclear cells post-transplantation

Exxonline, Enzyme-linked immunosorbent assay. Solid line = mean for CD34-selected, dashed line = mean for CD34-unselected.

TCRBV V-primer. PCR products were run on non-denaturing 6% polyacrylamide gels. Band intensities were analysed with a Cyclone phosphorimager and Optiquant software (Faccard Instruments). The lytic volumes all contained a similar original number of cells, and all the cDNA was included in the PCR master mix which was distributed evenly between all the reactions. Therefore band intensities of the TCRBV products from each sample could be compared relative to each other without the necessity of adjusting for cDNA content. Thus the data for each sample are expressed as the band intensity of each TCRBV product relative to the sum of intensities of all TCRBV products. Complete primer sequences and PCR conditions are available from the authors by request.

Statistical analyses

Statistical analyses (two-sample t tests, Wilcoxon's rank sum test, and Pearson's and Spearman's rank correlation coefficients) were calculated by means of Excel and SAS software. Spearman's rank correlation coefficients are given throughout the text. For analysis of peripheral-blood mononuclear cells in the 40 patients, an r of more than 0·3 or less than -0·3, and p of less than 0·05 were regarded as significant. For analysis of the sorted T cells in nine patients, an r of more than 0·6 or less than -0·6, and p less than 0·05 were regarded as significant.

Results

Patients

19 of the 40 patients received granulocyte-CSF-mobilised peripheral-blood mononuclear cells enriched for CD34-positive haematopoietic stem cells, and 21 received unmanipulated autografts. CD34 enrichment, which was used clinically in this case to diminish tumour load, decreases autograft T-cell burden by three log factors, but does not abolish it. There were no significant baseline differences between the two groups with respect to age, T-cell counts, numbers of TREC, or previous chemotherapy (p>0·24 for all, \( t \) test; table 1).

Recovery of CD4 and CD8 T cells

CD4 and CD8 T-cell numbers in peripheral blood were calculated before and after haematopoietic stem-cell transplantation. There was no significant difference in the mean or median CD4 or CD8 T-cell counts between the groups that received CD34-selected or unselected autografts at any time up to 1 year post-transplant (p>0·1, \( t \) test and Wilcoxon's test; figure 1). For both groups and both T-cell subsets, there was an initial rapid increase in cell counts over the first 100 days post-transplantation, which was greater in the CD8 than in the CD4 T-cell subset. This increase was followed by a slower rise in CD4 T-cell numbers, but a slight decline in CD8 T-cell numbers over the subsequent 9 months. Unlike in children and adolescents, there was no significant correlation between age and increases in total T-cell numbers (r=0·2 at one year post-transplantation), a finding confirmed in another study.

Quantification of thymic output

Figure 2 shows the changes in numbers of TREC from baseline to 2 years post-transplantation. Myeloablative chemotherapy substantially decreased the number of TREC such that by neutrophil engraftment they were close to, or at, undetectable levels, indicating the depletion of naive T cells. By day 100 post-transplant, there was a significant increase in CD4 and CD8 T cells, with a peak at 3 months. The CD4 T-cell counts remained high for up to 2 years, whereas CD8 cells decreased by 1 year post-transplantation and continued to fall to undetectable levels by 2 years post-transplantation.

Table 2: Spearman's rank correlation coefficients between naive T cells and numbers of TREC

<table>
<thead>
<tr>
<th></th>
<th>CD4 TREC</th>
<th>CD8 TREC</th>
<th>PBMC TREC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 days</td>
<td>6 months</td>
<td>1 year</td>
</tr>
<tr>
<td>Prop. of naive CD4 cells</td>
<td>0·85</td>
<td>0·78</td>
<td>0·83</td>
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<td>Naive CD4-cell count</td>
<td>0·82</td>
<td>0·76</td>
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</tr>
<tr>
<td>Prop. of naive CD8 cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naive CD8-cell count</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PBMC, peripheral-blood mononuclear cells.

*Non-significant correlation for sample size (F<0·05).

Table 2: Spearman's rank correlation coefficients between naive T cells and numbers of TREC.
increases in numbers of TREC were seen in most patients, and these increases continued until 1 year post-transplant, after which numbers remained constant or decreased slightly. In the CD34-unselected group, numbers at 2 years post-transplant returned to baseline levels. However, in the CD34-selected group, such numbers were significantly higher than at baseline (p=0.004, t test; p=0.003, Wilcoxon’s test), and were also significantly higher than in the unselected group at 2 years (p=0.046, t test; p=0.03, Wilcoxon’s test). There were no significant correlations between numbers of TREC and either the number of cycles of chemotherapy before transplantation (r=−0.20, p<0.23), autograft CD34-cell dose per kg (r=−0.14, p<0.28), or remission post-transplant (r=−0.17, p=0.12), at all time points measured.

Thymic output and age

We have previously shown an age-dependent decrease in numbers of TREC in the peripheral blood of healthy individuals,11 which is indicative of an age-dependent decrease in thymic function. Similarly, we found in this study that at baseline there was a significant negative correlation between age and the number of TREC (r=−0.56 [95% CI −0.58 to −0.52]). Furthermore, by 6 months and 1 year post-transplant, there was a significant negative correlation between age and absolute numbers of TREC (−0.39 [−0.68 to −0.11] and −0.49 [−0.70 to −0.21], at the two time points, respectively), and between age and change in numbers of TREC from engraftment (−0.33 [−0.58 to −0.03] and −0.50 [−0.71 to −0.22] at the two time points, respectively). Figure 3 shows the relation between age and change in numbers from engraftment to 1 year post-transplant. Median values for TREC were 3393 per µg DNA for patients younger than 50 years, and 814 per µg DNA for those 50 years or older. Concentrations in many of the patients were greater than those found in normal individuals of similar age.12 However, these data from whole peripheral-blood mononuclear cells are not directly comparable, since there are likely to be lower T-cell concentrations in the peripheral blood of patients recovering from haematopoietic stem-cell transplantation than in that of normal controls.

Relation between numbers of TREC and naive T cells

For both CD4 and CD8 T cells, there was a significant positive correlation between numbers of TREC 100 days, 6 months, and 1 year post-transplant, and the percentage or absolute number of naive T cells at 1 year. Spearman’s rank correlation coefficients are given in table 2. Although numbers of TREC correlated with naive T cells, there was no significant correlation between such numbers and total CD4 or CD8 T-cell count at any time point (r=−0.16, p=0.2). In six of the nine patients, numbers of TREC rose above the normal value for their age. Figure 4 shows, as an example, results for one patient who made no TREC response and one who made a large TREC response.

Relation between numbers of TREC and TCR repertoire

Figure 5 shows that even though the CD4 T-cell TCRBV repertoires were skewed in all six patients when compared with a normal control, the number of TCRBV families represented in those patients who had increases in numbers of TREC post-transplant was substantially higher than in those who did not; even with only three patients per group, the difference at 1 and 2 years post-transplant is quite obvious. Indeed, patient 39, who had only 26% of TCRBV families represented on the day of neutrophil engraftment, rapidly developed a broad TCRBV repertoire with 91% of all TCRBV families represented; this development was concomitant with an increase in numbers of TREC.

Discussion

There is a substantial, and potentially detrimental, period of T-cell immunodeficiency after bone-marrow transplantation and haematopoietic stem-cell transplantation.12 Rapid but transient recovery of limited T-cell immunity is mediated by the expansion of a small population of pre-existing T-cell clones—the thymus-independent pathway.13,14,15,16 However, complete reconstitution of comprehensive immunity with a broad TCR repertoire would require the generation of new naive T-cells from a source such as the thymus.17,18 Because the adult thymus is progressively replaced by fat with age, thymus-dependent T-cell recovery is assumed to be severely limited in adults. Indeed, previous studies have...
indicated that thymus-dependent T-cell recovery after intensive chemotherapy is virtually absent by 25 years of age. However, we have shown in this and previous studies that there is a quantitative, rather than qualitative, change in thymic function with age, and although involution of the thymus limits the thymus-dependent pathway, it does not totally eliminate it. Thus, the remaining thymic tissue may be called upon in adults to replenish damaged immune systems with a potentially broader repertoire of naïve T cells.

The quantification of TREC in peripheral blood as a measure of thymic function overcomes the disadvantages associated with the use of T-cell surface molecules such as CD45RA as markers for recent thymic emigrants. Indeed, such analyses may underestimate residual thymic activity in adults due to conversion of naïve CD45RA recent thymic emigrants to memory CD45RO recent thymic emigrants. Our data suggest that there is a substantial contribution from the thymus to immune reconstitution after myeloablative chemotherapy and haematopoietic...
stem-cell transplantation in adults. Our results support previous studies which indicate that the rise in T-cell numbers, particularly CD8 cells, early after engraftment is mediated by the rapid expansion of limited subsets of pre-existing mature T-cells, which contributes substantially to repopulation of the total T-cell pool, and that naive T-cell increases occur later.\textsuperscript{5,6,18}\ We observed an initial rapid rate of increase in CD4 and CD8 T-cells which was not sustained further than 100 days post-transplant. However, numbers of TREC, which measure the production of new naive T-cells from haematopoietic stem-cells (or a more committed progenitor cell), continued to increase steadily until 1 year post-transplant. Indeed, increases in numbers of TREC correlated with naive T-cell counts and percentages, but not total T-cell counts, suggesting that such increases in naive T-cells in adults were due to their de novo generation from haematopoietic stem-cells, rather than from the expansion of pre-existing T-cells.\textsuperscript{10}\ Furthermore, increases in numbers of TREC as early as 100 days post-transplant were predictive of increases in both CD4 and CD8 naive T-cells by 1 year post-transplant.

We and others found that, unlike in children and adolescents, there was no correlation between age and increases in CD4 T-cell counts in adults after chemotherapy and haematopoietic stem-cell transplantation. A previous study\textsuperscript{1} also found no relation between the small increases in CD4 CD45RA naive T-cells and age. However, our data show both a marked rise in the numbers of TREC and a significant negative correlation between age and TREC concentrations post-transplant. The correlation with age is not unexpected considering the substantial decrease in thymic tissue with age, and suggests indirectly that the origin of such new naive T-cells post-transplant is indeed the thymus. However, whether the substantial de novo generation of naive T-cells could have an extrathymic origin in the absence of exogenous cytokines or growth factors is unclear. Although some specialised T-cell subsets may develop in local sites such as the gut,\textsuperscript{19}\ liver,\textsuperscript{20}\ and bone marrow (in mice),\textsuperscript{21}\ there is no direct evidence that any extrathymic site can serve the function of the thymus in producing conventional, diverse TCR-\beta T cells in humans. We have reported that the adult thymus, although having less corticomedullary tissue, displays a normal range of thymocyte subsets and is capable of generating functional thymocytes with a wide TCR repertoire.\textsuperscript{10}\ Other studies have shown that thymic size, determined radiographically, in HIV-infected adults correlates with naive CD4 T-cell increases on treatment,\textsuperscript{22}\ and that naive CD4 T-cell increases after chemotherapy in children are associated with enlargement of the thymus above baseline—a phenomenon termed thymic rebound.\textsuperscript{23}\ We quantified TREC in T-cells from adult liver and bone marrow and found very low concentrations compared with those of peripheral blood mononuclear cells, which argue against these organs as sites for significant TCR-\beta T-cell development (DCD, RAK, unpublished observations). More definitively, we have reported that individuals with complete thymic aplasia (DiGeorge syndrome) have no normal TCR-\beta T-cells and undetectable numbers of TREC until successful thymic transplantation, at which point such concentrations and naive T-cell counts increase.\textsuperscript{10}\ Together, these data strongly suggest that residual thymic tissue is the most likely source for the new TREC-positive naive T-cells, which reconstitute the immune system after haematopoietic stem-cell transplantation in adults. Analysis of TREC in thymectomised patients receiving chemotherapy and transplantation could further define the origins of T cells generated de novo.

There have been concerns that purging procedures which further eliminate mature T cells from grafts may exacerbate post-transplant immunodeficiency, especially after allogeneic transplant.\textsuperscript{24}\ The use of CD34-positive haematopoietic stem-cell selection in this group of patients has been shown to purge tumour cells efficiently from the autograft, but not to affect immune recovery as measured by number of T cells, or the occurrence or type of intercurrent infections.\textsuperscript{25}\ Indeed, we found that, although there were no significant differences in CD4 and CD8 T-cell counts between the CD34-selected and unselected groups, there were significantly higher numbers of TREC 2 years post-transplant in the CD34-selected group, and that these numbers were above normal for age in many of the patients. This finding suggests that, at least in autologous transplants, the thymus-dependent pathway of T-cell reconstitution can generate substantial numbers of mature T cells. Furthermore, the supranormal numbers of TREC may be a manifestation of the thymic rebound described after chemotherapy in children.\textsuperscript{1}\ Complete recovery of broad T-cell immunity after haematopoietic stem-cell transplantation or bone-marrow transplantation may require the generation of new naive T-cells from the thymus.\textsuperscript{26}\ We found that increases in concentrations of TREC post-transplant were associated with the development of broader CD4 T-cell TCR repertoires, and that patients with no increases in TREC (and no naive T-cell increases) had limited and highly skewed repertoires. However, although a broader TCR repertoire would be theoretically more desirable, there is as yet little clinical evidence to indicate a direct causal relation between broader TCR repertoires and decreased morbidity.

The ability to quantify thymic output allows us to measure directly the relation between the thymus-dependent and thymus-independent pathways in a recovering immune system. The development of interventions that increase thymic output after chemotherapy, haematopoietic stem-cell transplantation, and bone-marrow transplantation may limit the period of T-cell immunodeficiency associated with these procedures. Our data suggest that the aged thymus contributes substantially to immune reconstitution early after haematopoietic stem-cell transplantation, and that this contribution results in increased naive T-cell reconstitution and a broader TCR repertoire. Furthermore, prediction of who will have poor T-cell reconstitution should be possible, and this prediction may be of greatest importance in allogeneic transplants where opportunistic infections secondary to T-cell immunodeficiency are a major source of morbidity and mortality.\textsuperscript{27}\ A future goal, therefore, may be to identify such individuals as candidates for early intervention to increase thymic output. Although no therapies are currently available, animal studies suggest that interleukin-7 may be able to fulfill this function.\textsuperscript{28}\ Thus, such therapeutic interventions may now be a realistic goal in adults, and the measurement of numbers of TREC in peripheral blood may serve as a clinical indicator of the potential for recovery of a damaged immune system.
Contributors

The project was conceived and planned by Robert Collins, Robert Vescio, Richard Keup, and Daniel Drexler. Robert Vescio and James Berenson recruited and managed patients and their treatment, and identified clinical events. Lin Zhang coordinated the collection of samples and measurement of T-cell counts. Michael Berti analyzed naive T cells. Brenda Hill and Jason Brechbiller did TCRβ analysis. Daniel Drexler developed, carried out, and interpreted TRBC assays. Richard Keup and Daniel Drexler interpreted all data, and all investigators contributed to the writing of the paper.

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