Correlation Between Interleukin-1 Production and Engraftment of Transplanted Bone Marrow Stem Cells in Patients With Lethal Immunodeficiencies

By Indira Sahdev, Richard O'Reilly, and Michael K. Hoffman

Interleukin-1 (IL-1) production by endotoxin-stimulated, cultured monocytes from 19 patients with lethal congenital immune disorders were studied and compared with normal controls. Lipopolysaccharide (LPS) stimulated IL-1 production was normal in three of three patients with Wiskott Aldrich syndrome (WAS), two of three combined immunodeficiency with T-cell predominance (CIDTP) and nine of 13 with severe combined immunodeficiency (SCID). Monocytes deficient in IL-1 production could be restored to normal production after incubation with indomethacin in three of five deficient patients. Monocytes from the other two patients could not be induced to generate IL-1, suggesting either an intrinsic deficiency or an alternate inhibitory mechanism as the basis for the IL-1 deficiency observed. In patients with SCID who were transplanted with HLA-haplotype disparate, T-cell depleted marrow without preparative chemotherapy, deficiency of monocyte IL-1 production was correlated with graft failure. Immune reconstitution was achieved in IL-1 deficient patients only when donor monocytes were also engrafted. We hypothesize that deficiencies of IL-1 production may contribute to the heterogeneous expression of combined immunodeficiencies, and may also restrict the engraftment and functional development of allogeneic lymphoid progenitors from a T-cell depleted marrow graft.

INTERLEUKIN-1 (IL-1), a cytokine produced by macrophages/monocytes, has been shown to be an essential stimulant for the proliferation of T and B lymphocytes. Because of the importance of IL-1 to the development of immunological function, we questioned whether deficiencies of IL-1 production might contribute to the pathogenesis of specific genetic variants of the congenital immune disorders associated with profound deficiencies of antigen-specific humoral and cell-mediated immunity. Accordingly, we have investigated the capacity of monocytes derived from patients with severe combined immunodeficiency (SCID), combined immunodeficiencies with T cell predominance (CIDTP), and Wiskott Aldrich Syndrome (WAS) to generate IL-1 in response to endotoxin. In a minority of patients with SCID and CIDTP, we have observed deficiencies of IL-1 production. Since patients with SCID are usually not treated with cytotoxic drugs before transplantation, and normally achieve a chimeric state in which donor cells are only detected in lymphoid populations, we further investigated whether abnormalities of IL-1 production detected pretransplant persisted posttransplant and further, whether such abnormalities were associated with any alterations in the normal course of engraftment or immunologic reconstitution.

MATERIAL AND METHODS

Patients. This prospective study includes all but one (parents refused test) SCID patients transplanted between April 1982 and September 1986. In addition, we studied three of six CIDTP patients diagnosed during this time. The patient population included 13 patients with SCID, three patients with CIDTP, and three patients with WAS. All patients were advised of procedures and attendant risks, in accordance with institutional guidelines, and gave informed consent. The distinctive features of the immune deficiency observed in each patient are summarized in Table 1.

The diagnosis of SCID was based on the absence of clinically detectable lymphoid tissue, or thymus shadow, severe hypogammaglobulinemia, and the failure of circulating lymphocytes to respond by in vitro transformation to stimulation with mitogens, antigens, or allogeneic cells. Of the patients with SCID, three patients had the classical form and were profoundly deficient in both T and B lymphocytes, five had B cells, two patients had SCID associated with the abnormal capping defect described by Gelfand et al., two patients had adenosine deaminase (ADA) deficiency and one patient had SCID associated with short limbed dwarfism. Six of these 13 patients demonstrated low numbers of mitogen and alloantigen unresponsive phenotypically dysmature maternal T lymphocytes in the circulation by HLA typing of separated E-rosettes, or HLA and karyotypic analyses of IL-2 expanded cells from the peripheral blood.

The patients with CIDTP presented with a profound deficiency of both cell-mediated and humoral immunity. However, each of these patients exhibited low but detectable responses to mitogens and allogeneic cells on in vitro stimulation, differentiating them from patients with SCID. Each of the patients with WAS presented with a history of recurrent sinopulmonary infections and thrombocytopenia, abnormally small platelets, and the inability to produce antibodies to polysaccharide antigens characteristic of this disease.

Transplants. Each patient studied in this series was a recipient of one or more allogeneic marrow grafts. Six patients (two SCID, one CIDTP, three WAS) received unfractionated marrow grafts from HLA-identical siblings. Of these, three with WAS were prepared with busulfan (2 mg/kg/d for four days) and cyclophosphamide (40 to 50 mg/kg/d for four days). Thirteen patients received HLA haplotype mismatched marrow grafts depleted of T cells by soybean agglutination and E-rosette depletion (SBA E-) as described by Reisner et al. For 12 of 13 patients, their HLA haploidentical mothers were used as donors. One patient, unique patient no. (UPN) 267, received a graft from the father, who shared more HLA alleles with the patient. Of these patients, three patients with CIDTP and two with SCID with the capping abnormality were prepared for transplantation with cytotoxic arabinoside (3 g/m² every 12 hours for eight doses) or busulfan (2 mg/kg/d for four days) followed by cyclophosphamide (40 to 50 mg/kg/d for four days).
SBAE grafts or matched conventional grafts did not receive cyclo-

phosphamide before their initial transplant; however, four of these
disease (GVHD) as described by Storb et al9 from day 1 to day 29

patients with WAS who received matched conventional transplants
posttransplant treatment with immunosuppressive agents. The
preparative regimens and donors used are included in Table 2.

Patients with SCID or CIDTP who received haplotype disparate
SBA E- grafts or matched conventional grafts did not receive posttransplant treatment with immunosuppressive agents. The patients with WAS who received matched conventional transplants also received standard methotrexate prophylaxis for graft vs host disease (GVHD) as described by Storb et al9 from day 1 to day 29 posttransplant.

Evaluation of engraftment and immune reconstitution. Following transplantation, patients were monitored for engraftment by

Table 1. Distinctive Features of Immunodeficiency Patients Receiving Bone Marrow Transplant

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (mo)</th>
<th>Disease</th>
<th>Maternal Lymphocytes</th>
<th>Absolute Lymphocyte Count (mm$^{-3}$)</th>
<th>T Cells (%)</th>
<th>B Cells (%)</th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
<th>% of Normal Controls</th>
<th>MLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>511</td>
<td>6</td>
<td>Classical SCID</td>
<td>+</td>
<td>600</td>
<td>18 NA</td>
<td>42</td>
<td>9</td>
<td>5</td>
<td>0.6</td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>A1</td>
<td>6</td>
<td>Classical SCID</td>
<td>+</td>
<td>1,650</td>
<td>8 12</td>
<td>150</td>
<td>10</td>
<td>8</td>
<td>4</td>
<td>0.3</td>
<td>2.9</td>
</tr>
<tr>
<td>431</td>
<td>12</td>
<td>Classical SCID</td>
<td>+</td>
<td>700</td>
<td>17.5 4.9</td>
<td>445</td>
<td>64</td>
<td>10</td>
<td>1.5</td>
<td>1.9</td>
<td>0.7</td>
</tr>
<tr>
<td>369</td>
<td>2.5</td>
<td>SCID c B cells</td>
<td>+</td>
<td>768</td>
<td>17 87.5</td>
<td>21</td>
<td>18</td>
<td>6</td>
<td>9.4</td>
<td>13.5</td>
<td>4</td>
</tr>
<tr>
<td>345</td>
<td>1</td>
<td>SCID c B cells</td>
<td>+</td>
<td>900</td>
<td>25 4.69</td>
<td>12</td>
<td>5</td>
<td>4</td>
<td>0.6</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>396</td>
<td>22</td>
<td>SCID c B cells</td>
<td>–</td>
<td>1,680</td>
<td>2 95</td>
<td>7</td>
<td>22</td>
<td>1</td>
<td>0.6</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>664</td>
<td>9</td>
<td>SCID c B cells</td>
<td>–</td>
<td>680</td>
<td>4 95</td>
<td>190</td>
<td>12</td>
<td>6</td>
<td>0.5</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>534</td>
<td>8</td>
<td>SCID c B cells</td>
<td>+</td>
<td>1,600</td>
<td>25 36</td>
<td>7.5</td>
<td>16</td>
<td>NA</td>
<td>0.6</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>324</td>
<td>1.6</td>
<td>SCID c ADA defic</td>
<td>–</td>
<td>729</td>
<td>5 47.8</td>
<td>12</td>
<td>16</td>
<td>6</td>
<td>4.3</td>
<td>1.3</td>
<td>0.9</td>
</tr>
<tr>
<td>285</td>
<td>10</td>
<td>SCID c ADA defic</td>
<td>–</td>
<td>780</td>
<td>12 23</td>
<td>22</td>
<td>5</td>
<td>15</td>
<td>0.5</td>
<td>1.4</td>
<td>1.5</td>
</tr>
<tr>
<td>441</td>
<td>10</td>
<td>SCID c abnormal capping</td>
<td>–</td>
<td>2,132</td>
<td>76 30.1</td>
<td>430</td>
<td>18</td>
<td>10</td>
<td>4.6</td>
<td>0.6</td>
<td>2.3</td>
</tr>
<tr>
<td>442</td>
<td>3</td>
<td>SCID c abnormal capping</td>
<td>–</td>
<td>3,864</td>
<td>64 21.8</td>
<td>465</td>
<td>28</td>
<td>15</td>
<td>3.3</td>
<td>13</td>
<td>0.9</td>
</tr>
<tr>
<td>267</td>
<td>9</td>
<td>SCID c short limb dwarfism</td>
<td>–</td>
<td>1,706</td>
<td>36 4.9</td>
<td>7</td>
<td>18</td>
<td>14</td>
<td>8.4</td>
<td>1.4</td>
<td>1.5</td>
</tr>
<tr>
<td>360</td>
<td>13</td>
<td>CIDTP</td>
<td>–</td>
<td>986</td>
<td>75 11.2</td>
<td>8</td>
<td>10</td>
<td>2</td>
<td>2.7</td>
<td>4</td>
<td>5.4</td>
</tr>
<tr>
<td>410</td>
<td>36</td>
<td>CIDTP</td>
<td>–</td>
<td>7,168</td>
<td>60.7 12</td>
<td>23</td>
<td>81</td>
<td>12</td>
<td>15</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>557</td>
<td>3.3</td>
<td>CIDTP</td>
<td>–</td>
<td>NA</td>
<td>53 0.9</td>
<td>196</td>
<td>13</td>
<td>11</td>
<td>11.2</td>
<td>1.9 5</td>
<td></td>
</tr>
<tr>
<td>406</td>
<td>22</td>
<td>Wiskott Aldrich syndrome</td>
<td>–</td>
<td>2,772</td>
<td>73 13.4</td>
<td>542</td>
<td>52</td>
<td>47.5</td>
<td>52</td>
<td>91</td>
<td>16</td>
</tr>
<tr>
<td>498</td>
<td>24</td>
<td>Wiskott Aldrich syndrome</td>
<td>–</td>
<td>3,556</td>
<td>66 12.7</td>
<td>1,061</td>
<td>86</td>
<td>173</td>
<td>36</td>
<td>19</td>
<td>16.5</td>
</tr>
<tr>
<td>204</td>
<td>9</td>
<td>Wiskott Aldrich syndrome</td>
<td>–</td>
<td>2,918</td>
<td>NA NA</td>
<td>829</td>
<td>72</td>
<td>53</td>
<td>48</td>
<td>52</td>
<td>13</td>
</tr>
</tbody>
</table>

Abbreviations: SI, stimulation index; NA, not available.

respectively. The other patients with SCID received no preparative
cytoreduction before their initial transplant; however, four of these
patients required second SBAE grafts after preparation with
antithymocyte globulin or cytotoxic arabinoside followed by cyclo-

phosphamide before durable engraftment and/or functional recon-
stititution was achieved. Details regarding the transplant procedures,
preparative regimens and donors used are included in Table 2.

IL-1 activity was determined by measuring the proliferative effect
of the supernatant on mouse thymocytes. Thymocytes from 6- to
8-week-old C3H/HeJ mice were cultured in 0.5 mL of complete
Mishell Dutton culture medium containing 2-mercaptoethanol 5 x
10^{-6} mol/L (Sigma, St Louis). The supernatant was centrifuged,
passed through a millipore filter (0.45 μm) and stored at −70°C,
until tested. This assay has been successfully used for years in
various laboratories. It produces highly reproducible results despite
the fact that the number of monocytes tested is estimated from the
number of monocytes in the PBL seeded in the culture vessel. In
establishing this assay, we conducted extensive studies to confirm the
receptor between seeded cells and IL-1 production, and in the
course of the experiments reported here, we sporadically eluted
surface adherent cells by treatment with 0.2% EDTA to verify the
number of seeded macrophages by direct cell count of nonspecific
esterase positive cells. Variations of 10% were observed but never
exceeded 20%.

ML-1 cytokines were measured by incubating 10^6 monocytes from 11 healthy donors.

µg/mL lipopolysaccharide (LPS) (prepared from Salmonella abortus by C. Galanos, Feinburg, FRG) with or without Indomethacin 10^{-6} mol/L (Sigma, St Louis). The supernatant was centrifuged, passed through a millipore filter (0.45 μm) and stored at −70°C, until tested. This assay has been successfully used for years in various laboratories. It produces highly reproducible results despite the fact that the number of monocytes tested is estimated from the number of monocytes in the PBL seeded in the culture vessel. In establishing this assay, we conducted extensive studies to confirm the receptor between seeded cells and IL-1 production, and in the course of the experiments reported here, we sporadically eluted surface adherent cells by treatment with 0.2% EDTA to verify the number of seeded macrophages by direct cell count of nonspecific esterase positive cells. Variations of 10% were observed but never exceeded 20%.

IL-1 activity was determined by measuring the proliferative effect
of the supernatant on mouse thymocytes. Thymocytes from 6- to
8-week-old C3H/HeJ mice were cultured in 0.5 mL of complete
Mishell Dutton culture medium containing 2-mercaptoethanol 5 x
10^{-6} mol/L, to which 0.05 mL of serially diluted (1:1, 1:5, 1:25,
1:125) monocyte supernatant was added. In modifying the original
Mishell Dutton medium we replaced fetal calf serum with 1% human serum. The final concentration of thymocytes was 1 x 10^6 cells/0.1 mL of medium. Flat bottomed Falcon 11 microtest tissue culture plates (No. 3040; Falcon Plastics, Oxnard CA) were used as culture vessels. Cells were cultured for three days. For the last four hours of incubation, 0.2 μCi 3H-thymidine was added. The cultures were harvested on glass fiber filters with an automatic harvester and the radioactivity of filters was measured in a scintillation counter.

IL-1 was quantitated and is expressed in units. A standard IL-1
preparation obtained from a healthy donor (E.W.) was tested in the
thymocyte proliferation assay in four different dilutions and a
regression curve established in each experiment. An arbitrary value
of 100 units was chosen for this IL-1 preparation and the activity of a
given test sample was calculated against this standard curve. An
example is shown in Fig 1, which shows the standard curve (A) and
IL-1 containing culture supernatants of monocytes obtained from 11
healthy donors.
RESULTS

Initial experiments were designed to measure the quantities of IL-1 generated by defined numbers of macrophages from 11 normal donors under the standard conditions of the assay. Figure 1 shows IL-1 values in the supernatants of macrophages cultured from these normal donors under standard assay conditions. IL-1 production, as measured by proliferation of mouse thymocytes in response to different dilutions of these supernatants, was remarkably uniform. Variations that could be ascribed either to age or sex of the macrophage donor were not observed. From these normal donors, we chose one normal donor supernatant as a standard IL-1 preparation.

IL-1 production was also found to be constant over time. As shown in Fig 2, supernatants generated from macrophages from the same individual on three separate occasions spaced four days apart exhibited striking uniformity in results.

Studies with macrophages from patients with different variants of SCID, CIDTP, and WAS are presented in Fig 3 and compared with macrophages from normal controls. Of the 13 patients with the different variants of SCID, five were found to have macrophages deficient in the production of IL-1. Patients with IL-1 deficiency were observed in each variant of SCID, except SCID with abnormal lymphocyte capping. One of three patients with CIDTP was also found to have macrophages deficient in IL-1 production. Thus, in this limited series, deficiencies of IL-1 production were not characteristic of any specific variant of lethal combined immunodeficiency. Macrophages from each of the three patients with WAS generated IL-1 in quantities equal to those generated by normal controls.

We have previously reported apparent deficiencies of IL-1 production in cancer patients. In contrast to IL-1 deficiency at early stages of malignancy, an IL-1 deficiency at advanced cancer stages could be attributed to the production of prostaglandin (PGE) by cultured macrophages from these patients. PGE has recently been shown to suppress the production of IL-1. When the macrophages were cultured in the presence of indomethacin, an inhibitor of prostaglandin E production, IL-1 production was found to be normal (our unpublished data).

A similar test of monocytes derived from our primary immune deficiency patients revealed that of the five patients who scored as nonresponders in the IL-1 assay, three...
1 1 control individuals and endosomes LPS for 24 hours and the supernatant IL-1 activity was determined in a LAF assay in four concentrations. This preparation was used as a standard IL-1 in subsequent experiments and arbitrarily assigned 100 units. Responses of monocytes were measured in a LAF assay in four concentrations. Responses of monocytes were determined in a LAF assay for 4 days and the supernatant IL-1 activity was measured in a LAF assay for 4 days. The first group consists of patients whose monocytes respond normally, while in the second group IL-1 function is apparently obscured by PGE E. Group III shows the two patients with SCID whose macrophages failed to generate IL-1 in the presence as well as in the absence of indomethacin.

Of the 19 patients in this series, unfractionated bone marrow from HLA-matched siblings was transplanted into six patients including each of the three with WAS, one with CIDTP, and two with SCID. The patients with WAS and CIDTP who were prepared for transplantation with busulfan and cyclophosphamide, achieved a full engraftment of both lymphoid and hematopoietic cells of donor type, as demonstrated by karyotypic analysis of marrow cells and separated T and B lymphocytes. IL-1 production at one month post-transplant was normal in each case and equal to that measured before transplant. The two patients with SCID who were transplanted without preparative cytoreduction and they both achieved a durable engraftment of donor lymphoid cells; myeloid and erythroid elements have remained exclusively of host origin, as determined by serological and karyotypic analyses. Macrophages in one of these patients produced IL-1 normally before transplant, and continued to produce this cytokine throughout the posttransplant period (Fig 3). The other patient had an indomethacin reversible mild deficiency of IL-1 pretransplant that turned to normal values within 1 month posttransplant. The origin of the IL-1 producing macrophages in this patient cannot be determined since donor and host are matched for both sex and HLA.

Thirteen patients (11 SCID, two CIDTP) received SBA- E T-cell depleted marrow grafts from an HLA haplo-type disparate parental donor. Four patients (UPN 441, 442, 360, 557) were cytoreduced with cyclophosphamide and either busulfan or cytosine arabinoside before their first transplant. Of these four, two patients with SCID (UPN 441, 442) achieved engraftment of both lymphoid and hematopoietic cells of donor origin and are immunologically reconstituted 26 months posttransplant. One of the two patients with CIDTP (UPN 557) achieved T lymphocyte engraftment of donor type but is fully reconstituted 3 months posttransplant. Each of these patients produced IL-1 normally pretransplant. IL-1 production by macrophages tested 1 month posttransplant and thereafter is also normal (Fig 4). The fourth patient (UPN 360) in this group, a child with CIDTP, failed to engraft. A deficiency of macrophage IL-1 production was detected both before and after transplantation (Fig 4). At each time tested, this deficiency could be reversed by the addition of indomethacin to the culture medium.

The remaining nine patients with SCID received a primary SBA- E T-cell depleted parental marrow grafts without preparatory cytoreduction. Of these nine patients, six patients were durably engrafted with donor lymphoid elements; five achieved reconstitution of T-cell function. In each of these six cases, T cells have been repeatedly shown to be of donor origin by HLA phenotype and karyotype; marrow cells, circulating monocytes, and B lymphocytes remain of host origin. Macrophages from each of the five patients who achieved early full reconstitution of T-cell function produced IL-1 normally pretransplant, and at each testing posttransplant (Fig 4). The one patient (UPN 267) of the six who remained immunodeficient despite demonstration of engraftment of paternal donor T cells exhibited an indomethacin

Fig 1. IL-1 production by LPS-stimulated human monocytes. Peripheral monocytes (10^6 cells/mL) from one donor were treated with 1 μg LPS for 24 hours and the supernatant IL-1 activity was determined in a LAF assay in four concentrations. This preparation was used as a standard IL-1 in subsequent experiments and arbitrarily assigned 100 units. Responses of monocytes from 11 control individuals are shown.

Fig 2. Repeated tests of IL-1 production by monocytes from a single donor on several different days. For experimental details see legend to Fig 1.
reversible deficiency of IL-1 production pretransplant that persisted posttransplant. Three patients (UPN 431, 324, 664) failed to engraft. Each of these nonengrafted patients was also found to have macrophages deficient in IL-1 production both before and after transplant.

Second transplants were administered to patients no. 267 and 431 without cytotherapy and to patients no. 324 and 664 after immunosuppression with cyclophosphamide and either antithymocyte globulin (ATG) (UPN 324) or cytosine arabinoside. Patient no. 267 continued to be chimeric with paternal cells but failed to achieve functional reconstitution. Immune reconstitution was achieved only after a fourth SBA+ E- graft from a HLA haplodesparate sibling donor was administered after treatment with busulfan and cyclophosphamide.

Fig 3. IL-1 production by monocytes from immune deficient patients before bone marrow transplantation. Control; △, SCID; ■, WAS; x, CIDTP.

Fig 4. IL-1 production by monocytes from immune deficient patients after bone marrow transplantation. Control; △, SCID; ■, WAS; x, CIDTP.
phamide, which resulted in full engraftment of both lymphoid and hematopoietic cells from her HLA mismatched sibling donor. After this fourth transplant IL-1 deficiency was reversed. Patients no. 431 and 324 failed to achieve engraftment of lymphoid cells and remained IL-1 deficient. These patients died shortly after third transplants were administered following preparation with cyclophosphamide and either busulfan (UPN 431) or total body irradiation (UPN 324). In each of these three cases, death was caused by a process antedating the final transplant: cytomegalovirus (CMV) hepatitis in patient no. 431, an Epstein-Barr virus (EBV) associated lymphoproliferative disorder developing in host type cells in UPN 324, and an interstitial pneumonia and complicating infections with respiratory syncytial virus (RSV) and CMV in UPN 664.

Table 3 correlates the success of HLA haplotype disparate SBA E- marrow grafts for SCID administered without pretransplant cytoreduction with the capacity of monocytes to generate IL-1. As can be seen, five of five patients with host macrophages capable of normal IL-1 production achieved engraftment and functional reconstitution. In contrast, the four IL-1 deficient patients failed to achieve either durable engraftment (UPN 324, 431, 664) or reconstitution (UPN 267) (P < .02).

In prospective analyses, our group has been investigating other mechanisms that might contribute to graft resistance in patients with SCID, in particular, the potential of transplacentally derived maternal T cells, host natural killer cells, and metabolically rescued ADA host T cells to inhibit engraftment or reconstitution. Accordingly, we have evaluated correlations between IL-1 production and these variables. In this series, six of 13 patients with SCID were found to be engrafted with transplacentally derived nonfunctional maternal T cells before initial transplantation; five engrafted following a primary transplant without cytoreduction. Macrophages from five of the six patients with maternal cells and from four of seven patients without maternal cell engraftment produced normal levels of IL-1. Thus, no difference was detected between patients with or without maternal engraftment.

Natural killer (NK) cells have been proposed as a major contributor to marrow graft resistance in several murine models of marrow transplantation, including a murine model of SCID. In preliminary evaluations, we and others have found a correlation between NK cell function and graft resistance in patients with SCID not exhibiting ADA deficiency. In the present study, each of the four patients who failed to achieve reconstitution following initial transplants of SBA E- HLA haploidentical marrow administered without cytoreduction had normal to high NK function, as assessed by cytotoxicity for K562 targets, while for the five patients who initially engrafted and achieved reconstitution, each of the three who were tested were deficient in NK function. We were, therefore, interested to determine whether a correlation existed between NK cell function and the IL-1 deficiencies observed.

Analysis of 11 patients with SCID or CIDTP studied for both activities before transplant is presented in Table 4. As can be seen, of five patients tested who exhibited a deficiency of IL-1 production, (which was reversible in the presence of indomethacin in three patients), each exhibited normal to high levels of NK cell function. Of six patients with normal endotoxin induced IL-1 production, NK function was deficient in four patients and normal in two (UPN 441, 442).

DISCUSSION

IL-1 is a multifaceted lymphokine with profound effects on immune and other functions of body. It is essential for the maturation and clonal expansion of T and B lymphocytes. The expansion of T cells is under the control of interleukin 2 (IL-2), a molecule whose synthesis and release by T cells requires IL-1. The response of resting T cells to IL-2 is also facilitated by IL-1. B cell responses to antigen in tissue culture are exquisitely IL-1 dependent, both for their differ-

<table>
<thead>
<tr>
<th>Patient (UPN)</th>
<th>Disease</th>
<th>% Cytotoxicity</th>
<th>IL-1 Production (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>511</td>
<td>Classical SCID</td>
<td>3</td>
<td>98</td>
</tr>
<tr>
<td>369</td>
<td>SCID c B cells</td>
<td>17</td>
<td>95</td>
</tr>
<tr>
<td>534</td>
<td>SCID c B cells</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>557</td>
<td>CIDTP</td>
<td>11</td>
<td>98</td>
</tr>
<tr>
<td>441</td>
<td>SCID c capping</td>
<td>43</td>
<td>92</td>
</tr>
<tr>
<td>442</td>
<td>SCID c capping</td>
<td>41</td>
<td>98</td>
</tr>
<tr>
<td>431</td>
<td>Classical SCID</td>
<td>80</td>
<td>7</td>
</tr>
<tr>
<td>324</td>
<td>SCID c ADA</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>267</td>
<td>SCID short limbed Dwarfism</td>
<td>65</td>
<td>5</td>
</tr>
<tr>
<td>664</td>
<td>SCID c B cells</td>
<td>45</td>
<td>9</td>
</tr>
<tr>
<td>360</td>
<td>CIDTP</td>
<td>63</td>
<td>12</td>
</tr>
</tbody>
</table>

Normal controls: 25-45 (90-100) (Zar, JH. Biostatistical Analysis, 1984.)
entiation and their clonal expansion. Recent evidence also suggests that IL-1 is identical to hematopoietin, a molecule that has been shown to stimulate early stages of hematopoiesis.

Given the important role of IL-1 in the early development of hematopoietic progenitors and in the generation of antigen reactive T and B lymphocytes, we were interested to determine whether patients with lethal genetic immune deficiencies exhibited abnormalities correlated either with pheno-
typic expression of the underlying immune deficiency or the response of afflicted patients to marrow transplantation. Our findings indicate that patients with WAS, and the majority of patients with SCID (nine of 13) or CIDTP (two of three) possess monocytes capable of generating IL-1 normally in response to endotoxin. However, five patients (four SCID, one CIDTP) were detected whose monocytes failed to generate IL-1 in response to this stimulus. This deficiency persisted into the posttransplant period, reflecting the persistence of host monocytes in noncytoreduced patients. IL-1 deficiency was reversed only in one patient who was engrafted with donor monocytes following cytoreduction with busulfan and cyclophosphamide.

The basis for the IL-1 deficiency observed in these cases is not clear. The deficiency was not characteristic of any specific variant of SCID or CIDTP, nor was it correlated with the presence or absence of paternal transplacentally derived lymphocytes in the circulation. For three of the five patients with IL-1 deficiency, culture of their monocytes with indomethacin led to the generation of normal quantities of IL-1, suggesting an indomethacin-sensitive suppressor mechanism similar to the one detected in patients with advanced cancer. However, two patients failed to generate IL-1, in the presence or absence of indomethacin, suggesting either that IL-1 production was inhibited by alternative mechanisms or that their monocytes were intrinsically deficient in their capacity to generate this monokine. If, indeed, the cells of these two patients were incapable of generating IL-1, this defect might significantly contribute to the SCID syndrome observed. In addition, patient no. 267, whose IL-1 deficiency was indomethacin sensitive in vitro, nevertheless, remained immunodeficient, despite documented engraftment of paternally derived T cells, until the patient was subsequently engrafted (following cytoreduction) with donor derived IL-1 producing monocytes. Chu et al have also described a patient with a severe deficiency of both T and B cell immunity in a child whose lymphocytes were found to lack a functional receptor for IL-1.

Our understanding of the cells or factors that induce or regulate IL-1 production by macrophages is still fragmentary. T cells may cause the release of IL-1. However, as demonstrated by the normal production of IL-1 by most patients with SCID, functional T cells are not required to induce this cytokine. IL-1 production may also be inhibited by mediators, particularly tumor necrosis factor (TNF) produced by NK cells (unpublished data), B cells, or macrophages themselves. Thus, the strong correlation between deficiency of IL-1 production and normal to high NK cell function observed in this series of SCID patients may reflect a functional link between these two activities.

Indeed, having observed a striking correlation between IL-1 dysfunction and elevated C-reactive protein in SCID patients, we have subsequently tested whether SCID patient NK cells secrete TNF and found that this is so (unpublished data). In addition to TNF, SCID patient NK cells produce significant amounts of gamma interferon (unpublished data), a lympho-

ticin that enhances the cytotoxic activity of TNF and its ability to influence IL-1 production by macrophages. NK cell lines originating from SCID patients have been established at this center and in vitro interactions between these NK cells and normal human monocytes are currently being studied in our laboratory.

The correlation between IL-1 deficiency and a failure to achieve durable engraftment or immune reconstitution in recipients of SBA E-haplo tolerant T cell-depleted grafts was striking. It is possible that deficiencies of IL-1 directly limit the engraftment or expansion of a T-cell depleting, SBA E marrow transplant, particularly since such transplants are also relatively depleted of mature mono-
cytes capable of providing an initial IL-1 stimulus. It is also possible that the IL-1 deficiencies observed in these cases do not directly contribute to graft failures, but reflect the activity of other cells capable of inhibiting engraftment such as NK cells, which may also influence IL-1 production. In previous studies, our group and Peter et al have reported a correlation between the presence of NK cells with normal to high cytotoxic activity and a failure to engraft haplo tolerant SBA E parental marrow cells in patients with ADA forms of SCID. Murphy et al22 have also demonstrated that SCID mice can resist engraftment of parental or alogenic marrow cells through the action of NK cells. While these reports strongly support a role of NK cells in the graft resistance observed in patients with SCID, other transplant groups have not found such correlations. In part, this may reflect differences in case distribution, T cell depletion techniques used, the additional conditioning with drugs used by these groups or analyses that do not take into account alternative mechanisms of resistance, particularly alloreactive T cells, which may be active in patients with ADA-, SCID, or CIDTP. The strong, but not perfect correlation between deficiencies of IL-1 production and normal to high NK cell functions in our patients with SCID, however, could indicate that other accessory cell functions such as IL-1 production, which can be modulated by NK cells but may be intrinsically abnormal in some patients, may be a more consistent prognosticator of engraftment and more closely linked to the etiology of the graft failures observed. Indeed, IL-1 may be important not only for the expansion of differentiated antigen-reactive T cells, but also for the develop-

ACKNOWLEDGMENT

The authors wish to thank Dr M. Weinblatt for his editorial assistance in preparing this manuscript.
REFERENCES

Correlation between interleukin-1 production and engraftment of transplanted bone marrow stem cells in patients with lethal immunodeficiencies

I Sahdev, R O'Reilly and MK Hoffman

Updated information and services can be found at:
http://www.bloodjournal.org/content/73/6/1712.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml