Low-dose interleukin 2 prevents the development of Epstein–Barr virus (EBV)-associated lymphoproliferative disease in scid/scid mice reconstituted i.p. with EBV-seropositive human peripheral blood lymphocytes

(immunodeficiency / latent viral infection / immunotherapy)

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Communicated by Marian E. Koshland, March 8, 1994

ABSTRACT When severe combined immune deficient (SCID) mice undergo i.p. injection with peripheral blood lymphocytes from normal human donors seropositive for EBV, a majority of these mice (hu-PBL-SCID mouse model) subsequently develop a fatal EBV+ lymphoproliferative disease (EBV-LPD) of human B-cell origin. Because T cells normally are critical in the control of EBV infection, we hypothesized that human T-cell dysfunction accounts for EBV-LPD in the hu-PBL-SCID mouse and that systemic administration of T-cell-derived cytokines would reestablish protective immunity against EBV-LPD. We show that the daily s.c. administration of a very low dose (500 international units) of polycyclic glycol-modified recombinant human interleukin 2 (PEG-IL-2) to hu-PBL-SCID mice can prevent the development of fatal EBV-LPD and significantly improves survival (78%), compared with the survival of hu-PBL-SCID mice treated with placebo (20%, P = 0.0008). Additional lymphocyte-depletion experiments showed that mouse natural killer cells and human CD8+ T cells provided cellular immunity necessary for the PEG-IL-2-mediated protective effect, whereas i.p. injection of human peripheral blood lymphocytes depleted of CD4+ T cells had no adverse effect when combined with PEG-IL-2 therapy and may have been beneficial. These data establish that very low-dose PEG-IL-2 therapy can overcome the immune deficiencies that lead to EBV-LPD in the hu-PBL-SCID mouse and point to the usefulness of this model for evaluating cytokine therapies in EBV-LPD. The use of low-dose IL-2 as a preventative therapy has potential application in immunocompromised individuals at high risk for EBV-LPD.

High-grade immunoblastic B-cell non-Hodgkin lymphomas and B-cell lymphoproliferative disease occur with increased frequency after the onset of an immunodeficient state (1). B-cell non-Hodgkin lymphoma and lymphoproliferative disease are well-described complications of secondary immunodeficiencies encountered in the postransplant setting and AIDS (2, 3) and are strongly associated with Epstein–Barr virus (EBV) (4, 5). As the use of aggressive immunosuppressive therapy for organ transplantation increases and the spread of AIDS continues, the incidence of EBV-associated B-cell lymphoproliferative disease (EBV-LPD) and EBV-associated non-Hodgkin lymphoma will continue to rise. Thus, development of a suitable animal model in which to study these disorders is essential.

The severe combined immune deficient (SCID) mouse lacks functional T and B lymphocytes (6). Lack of an effective immune response coupled with the intact hematopoietic microenvironment allows the SCID mouse to be reconstituted with human bone marrow or peripheral blood lymphocytes (PBL) (7). Intrapertitoneal (i.p.) transfer of high numbers of human PBL from individual donors seropositive for EBV gives rise to immunoblastic tumors of human B-cell origin in the SCID mouse, all of which contain EBV DNA (hu-PBL-SCID mouse model) (8, 9). These tumors may be monoclonal, oligoclonal, or polyclonal in nature, contain latent and lytic EBV gene products, and have a surface phenotype and karyotype that most closely resemble EBV-LPD and EBV-associated non-Hodgkin lymphoma in organ transplant recipients and AIDS patients (10, 11). Thus, the hu-PBL-SCID mouse model shows promise for understanding both the pathogenesis and treatment of EBV-induced lymphomagenesis in immunocompromised individuals (10, 12).

Multiple immunoregulatory T-cell events appear to limit the outgrowth of latently infected EBV+ B cells in the immunocompetent host (13). Although the exact mechanisms of immune dysfunction that lead to EBV-LPD are unclear, one factor could be deficient T-cell control over infected EBV+ B cells. As interleukin 2 (IL-2) is a cytokine produced by CD4+ T cells that is critical to the host’s normal immune response, we investigated whether therapy with exogenously administered low-dose human recombinant IL-2 (rIL-2) could replace the postulated T-cell deficiency and thus prevent the outgrowth of EBV-LPD in hu-PBL-SCID mice. We report that low-dose rIL-2 therapy prevents EBV-LPD in the hu-PBL-SCID mouse. The protective effect of rIL-2 requires the mouse natural killer (NK) and human CD8+ T cells but is maintained without human CD4+ T cells.

MATERIALS AND METHODS

Animals. C.B.17 scid/scid mice were obtained from the breeding colony of R. Bankert, (Roswell Park Cancer Institute, Buffalo, NY), and all breeder and experimental mice were housed in a specific pathogen-free environment provided by the Institute’s biocontainment facility. Food supplements and instruments were autoclaved, and all manipulations were performed in a laminar-flow hood. No evidence of leakiness (14), as determined by measuring mouse immunoglobulin in SCID mouse serum by inhibition ELISA (described below), was detected in the graft recipients.

Abbreviations: IL-2, interleukin 2; PBL, peripheral blood lymphocytes; EBV, Epstein–Barr virus; SCID, severe combined immune deficient; hu-PBL-SCID mouse model. SCID mouse reconstituted i.p. with EBV-seropositive PBL; EBV-LPD, EBV-associated B-cell lymphoproliferative disease; PEG-IL-2, polyethylene glycol-modified recombinant IL-2; NK, natural killer; rIL-2, recombinant IL-2; IU, international units; IL-2R, interleukin 2 receptor; hulg, human immunoglobulin; IFN-γ, interferon γ. To whom reprint requests should be addressed.

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Selection of Donors and Leukocyte Preparation. Human leukocytes were obtained from human immunodeficiency virus-seronegative healthy donors with an EBV-viral capsid antigen (VCA) titer >1.5, determined by an independent laboratory with an enzyme immunoassay to detect anti-VCA IgG levels (Roche Biomedical Laboratories, West Seneca, NY). Donors were "leukopheresed" after obtaining informed consent. Peripheral blood mononuclear cells were isolated after Ficoll/Hypaque (Sigma) separation. Monocytes were subsequently removed by overnight plastic adherence at 37°C. Nonadherent PBL were collected, enumerated, and prepared for injection.

Engraftment with Nondepleted and Depleted Human PBL. Four- to six-week-old SCID mice were injected i.p. with 5 \times 10^7 human PBL in 0.5 ml of phosphate-buffered saline. For depletion of specific human lymphocyte subsets before i.p. injection, 5 \times 10^7 human PBL were subjected to immunomagnetic bead depletion using a monoclonal antibody reactive against CD4 (helper T cells), CD8 (cytotoxic/suppressor T cells), or CD56 and CD16 (NK cells), as described (15). Efficiency of depletion was verified by flow cytometric analyses and was always >90%. For depletion of endogenous mouse NK cells, SCID mice were injected i.p. with anti-asialoaganglioside I (Wako Chemicals, Richmond, VA) antiserum, as described (16, 17), 1 day before human PBL injection and every 7 days thereafter for the study duration.

ELISA for Detection of Human-Cell Engraftment. Engraftment of human cells into SCID mice was documented by measuring human immunoglobulin (hulg) levels in SCID mouse serum by an inhibition ELISA (9). Microtiter plates (Nunc) were coated with human γ-globulin (0.5 mg/ml). Plates were blocked with 0.5% bovine serum albumin/1.0% normal goat serum (GIBCO/BRL)/bovine serum overnight. Known concentrations of human γ-globulin and experimental hu-PBL-SCID serum samples were plated in duplicate and diluted in serial 2-fold fashion from 1:100 to 1:800. Standard and experimental samples were used to inhibit the binding of peroxidase-conjugated goat anti-human IgG/A/M+ (Zymed). Optical density (OD_{490}) of o-phenylenediamine (Sigma) reaction products were measured on a Titertek Multiskan Plus MKII plate reader (Flow Laboratories). Standard curves and experimental SCID serum hulg concentrations were determined by using CRICKET GRAPH software (Cricket Software, Malvern, PA).

Pharmacokinetic Studies. After a single s.c. injection of 500 international units (IU) of proleukin-PEG (polyethylene glycol-modified human rIL-2), or PEG-IL-2, specific activity 3 \times 10^6 IU/mg, Chiron) (18-21) into six SCID mice, serum was collected by tail-vein incision (= 300 μl) from three SCID mice at 5 and 24 hr after PEG-IL-2 injection and from three SCID mice at 11 and 48 hr after PEG-IL-2 injection. Normal SCID mouse serum was used as a negative control, and SCID mouse serum supplemented with known amounts of PEG-IL-2 was used as a positive control and standard. All samples were diluted 1:20 and 1:40, and PEG-IL-2 concentrations were determined by using the CTL2 bioassay with a 20-hr incubation at 37°C followed by a 8-hr [H]thymidine pulse (22). The limit of detection in this bioassay was 1 pM or 0.2 IU/ml.

Description of Therapeutic Trial. All three human donors had been found of high-incidence type in their efficiency of tumor generation in hu-PBL-SCID mice, as described by Picchio et al. (23). Human PBL from each donor were injected into 12 SCID mice. Each mouse received an i.p. injection of 5 \times 10^7 PBL. After injection, mice were randomized to receive either 500 IU of PEG-IL-2 resuspended in 5% (wt/vol) dextrose water/0.1% human albumin (Armour Pharmaceuticals) or placebo (5% dextrose water/0.1% human albumin). Eighteen mice were entered in each arm of the study. Injections with PEG-IL-2 or placebo were given s.c. every 24 hr in a 200-μl vol until sacrifice. Evidence of human PBL engraftment was documented by hulg levels determined between weeks 6 and 9. Mice without measurable hulg levels were not monitored for response. When judged moribund, mice were anesthetized, sacrificed, and autopsied. All organs involved with tumor and other tissues of interest were collected for histology, flow cytometry, and protein analysis. All procedures were approved by the Institute Animal Care and Use Committee.

Flow Cytometric Analysis. Solid tumors (made into single-cell suspensions) and i.p. washes from hu-PBL-SCID mice were analyzed for cell-surface expression of two mouse antigens: CD45 (CD45-RED613, GIBCO/BRL) and NK 1.1 (Biotin anti-NK cell; Pharmingen). Cells were also analyzed for the following human antigens: CD3 (leu4-fluorescein isothiocyanate (FITC)), CD4 (leu3A-FITC), CD8 (leu2A-FITC), CD19 (leu12-FITC), from Becton Dickinson, and CD56 (NKH1-phycocerythrin) from Coulter Immunology. Samples were stained and analyzed on a FACScan using LYFII software (Becton Dickinson), as described (15).

Statistical Analysis. Experimental categories were statistically compared with the logarithm-rank test for analysis of mortality data and the two-sided t test analysis for other measured end points with P < 0.05 considered significant. Survival of engrafted animals was assessed on the basis of the week of sacrifice for each animal. Survival time for each group was then described by a Kaplan-Meyer plot.

RESULTS

The PEG-modified form of human rIL-2 was chosen for administration because this modification increases both the solubility and serum half-life while maintaining IL-2 bioactivity (18-21). Fig. 1A summarizes the kinetics of serum IL-2 concentration after a single s.c. injection of 500 IU of purified PEG-IL-2 into six SCID mice. The peak serum IL-2 concentration seen at 5 hr (17 ± 1.3 IU/ml) was ~100 pM, which results in (i) partial saturation of intermediate-affinity IL-2 receptors (IL-2R) constitutively expressed on human and mouse NK cells and mouse monocytes and (ii) complete saturation of high-affinity IL-2R expressed on activated human T and B cells (24-26). From 10 to 24 hr after injection, serum IL-2 concentration ranged from ~10 IU/ml (~40 pM) to 1.3 IU/ml (~7 pM), respectively, results shown in Table 1. The decrease in serum IL-2 concentration was largely immeasurable beyond 24 hr, mice were injected daily in the therapeutic trial described below.

Engraftment of human PBL into SCID mice, measured by the detection of hulg in serum (8), was assayed between 6 and 9 weeks after injection of human PBL and initiation of therapy. Twenty-nine of 36 mice (81%) demonstrated significant (>10 μg/ml) amounts of circulating hulg. These same mice were used in the randomized therapeutic trial described below. The seven SCID mice without evidence of engraftment all had undetectable hulg levels (<0.2 μg/ml). The incidence of engraftment did not vary among the three human donors or between PEG-IL-2- or placebo-treated SCID mice (Fig. 1B). Indeed, human PBL from each donor produced comparable hulg levels in both PEG-IL-2-treated and in placebo-treated hu-PBL-SCID mice.

To assess the protective effect of PEG-IL-2 in preventing EBV-LPD in the hu-PBL-SCID mouse, a randomized trial was performed. Thirty-six SCID mice were each injected i.p. with 5 \times 10^7 human PBL obtained from one of three donors and randomized to receive a daily s.c. injection of 500 IU of PEG-IL-2 or placebo for the study duration. Only the 29 mice with documented evidence of human engraftment were analyzed. Fig. 2 shows a significant difference in the development of fatal EBV-LPD between the two randomized groups. Hu-PBL-SCID mice treated with daily s.c. injections of PEG-IL-2 had a 78% survival at 16 weeks, whereas hu-PBL-SCID placebo-treated mice had a 20% survival at 16 weeks (P
Fig. 1. (A) Pharmacokinetics of PEG-IL-2 after a single s.c. injection of 500 IU in SCID mice. Samples were drawn at 5, 11, 24, and 48 hr after injection, and serum IL-2 concentration was determined by using a CTLL-2 bioassay (22). Each point represents the mean serum IL-2 concentration (±SE) from three mice. (B) Serum hulg (hIG) concentration in 29 hu-PBL-SCID mice measured between 6 and 9 weeks after each was injected with 5 × 10^7 human PBL from one of three EBV-seropositive donors. Immediately after injection of human PBL, mice were treated daily with PEG-IL-2 or placebo. Each point represents a single determination, done in duplicate. •, hu-PBL-SCID mice that eventually died of EBV-LPD.

= 0.0008). All deaths were attributable to massive tumor infiltration throughout the porta hepatitis, mesentery, spleen, liver, and occasionally the thymus. Tumors were shown to be immunoblastic in histologic appearance, to coexpress human CD45 and human CD19 by flow cytometric analyses, and to express EBV-latent membrane protein 1 by immunoblot analysis (27) (data not shown).

To elucidate which cell type(s) might account for the difference in survival between placebo- and PEG-IL-2-treated groups, a second randomized trial was done. Phenotypic characterization of i.p. washes undertaken 3.5 wk after the i.p. injection of human PBL and randomization to therapies (Fig. 3). Forward- and side-scatter flow cytometric analyses of i.p. washes revealed distinct differences in mononuclear cell populations between the two groups of mice. The PEG-IL-2-treated mice had a collection of cells in the monocyte/macrophage gate (R2, Fig. 3E) that were consistently absent in the placebo-treated group (Fig. 3A). These cells were identified phenotypically as being exclusively of mouse origin (data not shown). The mouse/human ratio within the lymphocyte gate was similar in the two groups (Fig. 3 B and F), and all mouse lymphocytes were found to be NK cells by surface expression of NK 1.1 (data not shown). No human CD19+ B cells were seen at this time point in either treatment group (Fig. 3 C and G). However, placebo-treated mice had a significantly greater proportion of human CD3+ T lymphocytes compared with PEG-IL-2-treated mice (Fig. 3 D and H, respectively). Despite this, the human CD4/CD8 ratio was similar in both groups (data not shown). The human non-B, non-T lymphocytes consistently seen in the PEG-IL-2-treated mice were subsequently identified as human CD56+ NK cells (Fig. 3I). The i.p. washes from both experimental and placebo groups of hu-PBL-SCID mice, when incubated in vitro with exogenous rIL-2, failed to show significant cytotoxic activity against endogenously generated human EBV+ B-cell targets (data not shown).

To further define which lymphocyte subsets are participating in the protective effect of PEG-IL-2 against EBV-LPD in vivo, lymphocyte-depletion experiments were undertaken before injection of PBL and therapy with PEG-IL-2 (Table 1). Ex vivo depletion of human lymphocyte subsets was always >90%, as verified by flow cytometry. Despite the abundance of human NK cells seen earlier in the i.p. washes of mice treated with PEG-IL-2, the use of human NK-depleted PBL with PEG-IL-2 therapy did not increase the incidence of EBV-LPD. As the efficiency of depletion was not 100%, the remaining human NK cells could have contributed to the persistent protection in the presence of PEG-IL-2. In contrast, in vivo depletion of mouse NK cells with weekly injections of anti-asialo ganglioside 1 resulted in the fulminant development of tumor in 100% of hu-PBL-SCID mice, despite daily s.c. PEG-IL-2. Likewise, injection of human CD8+ -depleted PBL also resulted in the development of fatal EBV-LPD in the majority (75%) of engrafted mice, despite daily s.c. PEG-IL-2. However, the latency period to death was significantly delayed in the human CD8+-depleted group when compared with the anti-asialo ganglioside 1-treated group (11.0 ± 1.2 wk vs 8.0 ± 0.3 wk, respectively, P = 0.01). Thus, in response to exogenous PEG-IL-2, both mouse NK cells and human CD8+ T cells appear to provide the requisite cellular components to prevent EBV-LPD in the hu-PBL-SCID mouse. As CD8 is also expressed in low density on ~30% of human NK cells, their contribution cannot be totally excluded. Injection of human CD4+-depleted PBL did not result in any tumor development when combined with daily s.c. PEG-IL-2, despite good efficiency of engraftment. Notably, although hulg levels in this group of hu-PBL-SCID mice (25 ± 3 μg/ml) were significantly higher than those seen in SCID mice failing to engraft (<0.2 μg/ml), these levels were between 35- and 70-fold lower than in all other groups of hu-PBL-SCID mice. Follow-up 2.5 mo later showed that hulg levels persisted in this range (28 ± 8 μg/ml).

To investigate whether PEG-IL-2 might interact directly with EBV+ B cells, we examined fresh tumor specimens from placebo-treated mice for IL-2R expression as well as for

Fig. 2. Overall survival of hu-PBL-SCID mice randomized to receive daily s.c. injections of 500 IU of PEG-IL-2 (c) or placebo (o), after i.p. injection of 5 × 10^7 human PBL. Thirty-six SCID mice were injected and randomized; 29 mice showed evidence of human engraftment and were included in this analysis.

Survival rate, %

Weeks after injection

p = 0.0008
Fig. 3. Flow cytometric analysis of i.p. washes taken from hu-PBL-SCID mice 3.5 wk after the initiation of daily s.c. injections with PEG-IL-2 or placebo. (Upper) (A–D) Mice treated with placebo. (Lower) (E–I) Mice treated with PEG-IL-2. A and E show forward scatter (size index) vs. side scatter (granularity index) and reveal a monocyte/macrophage population in the PEG-IL-2 group (E, R2) not seen in the placebo group (A, R2). These cells were all of mouse origin (data not shown). R1 indicates the lymphocyte gate, used for phenotypic analyses in A–I. Quadrants are identified in B, and percentage of positive cells is indicated in each quadrant. B and F show equivalent proportions of mouse (quadrant 1) and human (quadrant 4) lymphocytes. Quadrants 4 of C and G show an absence of CD19+ human B cells in both groups. The placebo-treated mice have a relatively greater percentage of human CD3+ T cells (D, quadrant 4), compared with PEG-IL-2-treated mice (H, quadrant 4). In a separate experiment, the abundance of human non-T, non-B cells consistently seen in the PEG-IL-2-treated mice are shown to be CD56+ human NK cells (J, quadrant 4).

functional responsiveness to rIL-2. By RNA analysis, EBV+ B cell tumors showed transcript for IL-2Rβ and IL-2Rγ but did not show transcript for IL-2Rα (data not shown). Thus the IL-2R protein subunits necessary for intermediate-affinity binding were present (28). However, when freshly extracted tumors were incubated for 72 hr in nanomolar concentrations of rIL-2, proliferation, cell numbers, or viability did not decrease (data not shown).

DISCUSSION

T cells normally play a critical role in the control of EBV infection (13). We hypothesized that human T-cell dysfunction contributes to the development of EBV-LPD in the hu-PBL-SCID mouse and that therapeutic replacement with T-cell-derived cytokines might reestablish immunocompetency. Here we show that the daily s.c. injection of low-dose PEG-IL-2 effectively prevents EBV-LPD development in the majority of hu-PBL-SCID mice inoculated with PBL from high-incidence EBV-seropositive human donors. Daily, low-dose PEG-IL-2 sustained serum IL-2 concentrations in vivo that saturate high-affinity IL-2R expressed on activated human T and B cells and partially saturate intermediate-affinity IL-2R expressed on human NK cells, mouse NK cells, and mouse monocytes (24–26), without systemic toxicity. Importantly, the dose used is ~1000-fold lower than doses of PEG-IL-2 or rIL-2 used in other therapeutic trials involving immunocompetent mice (21, 29).

The depletion experiments in this study clearly established that (i) mouse NK cells are a critical early cellular component of the IL-2-induced prevention of EBV-LPD in the hu-PBL-SCID mouse and (ii) human NK cells could not substitute for this effect in their absence. In vitro cytotoxicity assays with SCID mouse NK cells against endogenously derived EBV+ human B-cell targets did not provide evidence for a direct effect of rIL-2 on mouse NK-cell cytotoxic activity, suggesting that endogenous production of other factors might be important in the protective effect exerted by mouse NK cells. Flow cytometric analyses demonstrated the distinct presence of a mouse peritoneal monocyte/macrophage population in the PEG-IL-2-treated hu-PBL-SCID mice that was not seen in the placebo-treated mice. Tripp et al. (30) recently showed that mouse peritoneal macrophages can produce both tumor necrosis factor α and IL-12, which stimulate NK-cell production of interferon γ (IFN-γ) in SCID mice. Furthermore, the investigators found that exogenous rIL-2 maximized this macrophage-dependent IFN-γ response. Thus, one hypothesis for how both PEG-IL-2 and mouse NK effectors contribute to the IL-2-induced protection against

Table 1. Development of EBV-LPD in SCID mice after injection with different human PBL subpopulations and exogenous PEG-IL-2 therapy

<table>
<thead>
<tr>
<th>Injected population and exogenous therapy</th>
<th>Method of depletion*</th>
<th>Engrafted no./ injected no.</th>
<th>hulg., µg/ml ± SE</th>
<th>Death from LPD, no. (%)†</th>
<th>Latency, wk ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hu NK-depleted PBL + IL-2</td>
<td>Anti-CD56 and anti-CD16 purged</td>
<td>5/5</td>
<td>850 ± 291</td>
<td>1 (20)</td>
<td>9.0</td>
</tr>
<tr>
<td>Hu PBL + IL-2</td>
<td>Anti-asialo-GM1</td>
<td>5/5</td>
<td>1380 ± 198</td>
<td>5 (100)</td>
<td>8.0 ± 0.3</td>
</tr>
<tr>
<td>mouse NK-depleted</td>
<td>Anti-CD8</td>
<td>4/5</td>
<td>1750 ± 250</td>
<td>3 (75)</td>
<td>11.0 ± 1.2</td>
</tr>
<tr>
<td>Hu CD8-depleted PBL + IL-2</td>
<td>Anti-CD8 purified</td>
<td>4/5</td>
<td>25 ± 3</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Hu CD4-depleted PBL + IL-2</td>
<td>Anti-CD4 purified</td>
<td>5/6</td>
<td>1200 ± 328</td>
<td>1 (20)</td>
<td>8.0</td>
</tr>
<tr>
<td>Hu PBL + IL-2</td>
<td></td>
<td>5/6</td>
<td>1400 ± 352</td>
<td>4 (80)</td>
<td>7.8 ± 0.5</td>
</tr>
</tbody>
</table>

SCID mice were injected i.p. with 5 × 10⁷ human (Hu) PBL minus the depleted population and injected s.c. daily with 500 IU of PEG-IL-2 or placebo. Engraftment is defined as the detection of hulg (≥0.2 µg/ml) at week 8 after PBL injection.

*Ex vivo immunomagnetic bead depletion, except anti-asialo-ganglioside 1 (GM1) antisera (injected i.p. every 7 days). †Percentage represents the no. of mice that died per number of mice engrafted.
EBV-LPD is via a macrophage-dependent production of IFN-γ. It is of considerable interest that Tripp et al. (30) also found that IL-10 inhibits production of IL-12, tumor necrosis factor α, and IFN-γ in vitro because we have observed that nearly all tumor-bearing hu-PBL-SCID mice have extremely high circulating concentrations of human IL-10 (which cross-reacts in mouse), whereas those mice with tumor had low or undetectable levels (unpublished observations).

Human CD8+ T cells also appear critical in preventing EBV-LPD in the presence of exogenous PEG-IL-2 and yet provide a significantly delayed protective effect (=11 weeks), compared with mouse NK cells (=8 weeks). These findings are consistent with existing data, which suggest that NK cells may provide an early, less specific defense against viral infection or malignant transformation, while a more delayed, specific, and IL-2-dependent cytotoxic T-cell response is being mounted (for review, see ref. 31). Further, these results establish that in the presence of exogenous PEG-IL-2, human CD8+ T cells are operational in vivo in the hu-PBL-SCID mouse model.

Importantly, the absence of human CD4+ T cells did not adversely affect the PEG-IL-2-induced protection against EBV-LPD and yet significantly decreased the amount of hulg detected over several months, suggesting that chronic stimulation of human B-cell immortalization by IL-2 production was diminished. Veronesi et al. (32) recently reported that i.p. injection of CD4-depleted human peripheral blood mononuclear cells into SCID mice significantly delayed the onset of EBV-LPD (18.3 wk), when compared with nondepleted (8.3 wk), or CD8-depleted peripheral blood mononuclear cells (7.8 wk) (32). No exogenous cytokines were administered in that study. Our CD4-depleted hu-PBL-SCID mice treated with PEG-IL-2 showed no tumor development at 22 wk. Taken together, these observations suggest that the administration of low-dose PEG-IL-2 to hu-PBL-SCID mice engrafted with CD4-depleted PBL may have two advantages. (i) PEG-IL-2 can effectively replace the protectice effect mediated by the human CD4+ helper T-cell 1 (TH1) effector arm. (ii) Additional protection against the development of EBV-LPD may be gained by removing human CD4+ helper T-cell 2 (TH2) effector cells that produce such cytokines as IL-10, important for immunoglobulin production, and down-regulation of IFN-γ production, and outgrowth of EBV + B cells (for review, see ref. 33). Thus, in the hu-PBL-SCID mouse model of EBV-LPD, exogenous PEG-IL-2 may assist in limiting the amount or effect of counterregulatory cytokines that are endogenously produced, as shown in vitro (30). Additional studies with neutralizing monoclonal antibody will be necessary to further elucidate such possibilities.

In summary, we have demonstrated that the exogenous administration of low-dose IL-2, a cytokine normally produced by competent CD4+ cells, can exert a profound protective effect against the development of fatal EBV-LPD in the majority of hu-PBL-SCID mice. In humans, cytokine “replacement” therapy with rIL-2 has been effective in preventing life-threatening infections in an individual with SCID who was specifically deficient in IL-2 production (34). Low-dose rIL-2, comparable to the dose administered here, has been given to cancer patients without significant clinical toxicity (35, 36). As the hu-PBL-SCID mouse appears to be a useful model for studying EBV-LPD in humans (10, 12), the therapeutic approach described here may merit consideration in immunodeficient individuals who can be identified as being at high risk for EBV-LPD development, including patients with AIDS (37). Finally, this report describes a cytokine trial for the prevention of EBV-LPD in the hu-PBL-SCID mouse and suggests that this model may serve as a useful tool for evaluating additional cytokines in the prevention and treatment of EBV-LPD.