Resistance to human immunodeficiency virus 1 infection of SCID mice reconstituted with peripheral blood leukocytes from donors vaccinated with vaccinia gp160 and recombinant gp160

(human immunodeficiency virus 1 vaccination/immune response/animal model)

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ABSTRACT SCID mice reconstituted with adult human peripheral blood leukocytes (hu-PBL-SCID mice) make antigen-specific human antibody responses following secondary immunization and can be infected with human immunodeficiency virus 1 (HIV-1), suggesting that they might prove useful for evaluating protective immunity to HIV-1 following vaccination of PBL donors. HIV-seronegative volunteers were immunized with vaccinia expressing HIV-1gp160-1/Br and subsequently given booster injections of recombinant gp160 protein (rgp160). Their PBLs were used at intervals of 4–72 weeks after booster injections to construct hu-PBL-SCID mice, which were then challenged with 108–109 minimal animal infectious doses of highly homologous HIV-1mbl. Control hu-PBL-SCID mice were constructed from donors receiving vaccinia, alum, or hepatitis B vaccine. Protection against virus infection was defined as the absence of HIV-1 by culture and no detection of proviral genomes following PCR amplification. Control animals were highly susceptible to HIV infection. By contrast, hu-PBL-SCID mice reconstituted with cells from three of four donors immunized with vaccinia gp160 and recently injected with rgp160 showed no evidence of HIV-1 infection by culture or PCR assays. With increasing time after rgp160 injection, the ability of vaccinee-derived hu-PBL-SCID mice to resist HIV-1 infection diminished. These results demonstrate that a potentially protective human immune response was stimulated by this HIV gp160 immunization protocol and show the utility of the hu-PBL-SCID model in the rapid evaluation of candidate vaccines.

Development of a safe, effective vaccine represents the best approach to preventing the spread of AIDS. Evaluation of candidate vaccines to human immunodeficiency virus (HIV) has to date been limited to studies of protective immunity in chimpanzees (1–5) and phase I trials of the safety and immunogenicity of candidate subunit vaccines in humans (6–9). These studies have shown various degrees of immunogenicity in humans and limited protection of chimpanzees. Development of a model to assess the relative protective immunity achieved by these and future vaccines would greatly facilitate the evaluation of different vaccine approaches. Functional human lymphoid xenografts have been established by transferring mature lymphoid cells to mice with severe combined immunodeficiency (hu-PBL-SCID mice (PBL, peripheral blood leukocyte) (10–12)) or by grafting fetal lymphoid organs (SCID:hu mice (13)) and such mice are susceptible to infection with HIV-1 (14, 15). hu-PBL-SCID mice established by using tetanus-immune PBL donors produced a tetanus-specific secondary human antibody response (10), suggesting that hu-PBL-SCID mice derived from HIV-1 immune donors might mount a specific immune response to HIV-1. We have generated hu-PBL-SCID mice by using PBLs from donors immunized with 160-kDa glycoprotein (gp160) subunit vaccines and challenged the resulting mice with a high dose of HIV-1. hu-PBL-SCID mice derived from three of four vaccinees who had been primed with recombinant vaccinia virus expressing gp160 and booster injected with recombinant gp160 protein (rgp160) were resistant to challenge with homologous strains of HIV-1. The level of immunity adoptively transferred to hu-PBL-SCID mice (i.e., the fraction of challenged mice resistant to infection) diminished with increasing time after initial rgp160 booster injections of the PBL donors. A subsequent injection with rgp160 restimulated immune responses that transferred enhanced resistance to HIV-1 infection to hu-PBL-SCID mice. These data suggest that the level and nature of immunity stimulated by the combination of vaccinia gp160 priming and rgp160 injection may be sufficient to protect against primary HIV-1 infection.

MATERIALS AND METHODS

PBL Donors. Four HIV-uninfected volunteers were selected from participants in a phase I vaccine trial that consisted of priming and injecting with two distinct antigenic preparations of HIV-1 gp160 envelope protein. Donors were immunized initially by scarification with HIV-1gpl60-1/Br gp160 expressed in a vaccinia vector (vaccinia gp160) (ref. 16; HIVAC-1e, Oncogen/Bristol-Myers Squibb, Seattle). Vaccinia-naive donors were immunized with 107 plaque-forming units (pfu) and vaccinia-immune donors received 105 pfu of HIVAC-1e. These recipients were injected 8–12 months later with two doses of 160 μg of recombinant HIV-1mbl gp160 in alum. The rgp160 was derived from a baculovirus expression system (VacSyn, MicroGeneSys, West Haven, CT). Donor immune responses were determined in three assays. Neutralizing antibody titer was defined as the last dilution giving 50% inhibition of HIV-1 replication in PBL or CEM cells (both assays gave similar results) as described (17). T-cell proliferation was determined after stimulation with rgp160 (5 μg/ml), and data are expressed as the stimulation index; i.e., gp160-stimulated proliferation divided by the medium control. Antibody to gp160 was measured by ELISA, using rgp160 protein as the target antigen. All four donors had exhibited a primary T-cell proliferative response after gp160

Abbreviations: HIV, human immunodeficiency virus; PBL, peripheral blood leukocyte; rgp160, recombinant gp160; pfu, plaque-forming unit(s).

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vaccinia immunization, and all showed significantly enhanced T-cell proliferation after injecting with rgp160. Two of these subjects (078 and 033; see Table 1) were vaccinia naive and responded to rgp160 booster injections by producing neutralizing antibody and a strong T-cell proliferative response to gp160 (see Table 1) as well as to psoralen-inactivated HIV-1 (data not shown). The other two donors (065 and 053) were selected from vaccinia-immune subjects because of their strong T-cell proliferative response, but neither of these individuals produced neutralizing antibody. Control donors were volunteers who had received alum alone, vaccinia alone, or hepatitis B vaccine (Recombivax) at least 12 months prior to PBL reconstitution of control hu-PBL-SCID mice; these donors had no detectable antibody to gp160 (data not shown). Donors 065 and 078 were used in four independent experiments, and donors 053 and 033 were used in two experiments.

**PBL Reconstitution.** Heparinized peripheral blood was collected from each donor and 3–14 C.B-17 SCID mice per donor were injected intraperitoneally with 20 × 10⁶ PBLs within 18 hr of blood collection, generating a total of 113 mice for these experiments. All SCID mice used were prescreened to ensure a true SCID phenotype, and “leaky” animals with >5 µg of murine IgM per ml were excluded from these experiments. The animals were maintained in microisolator cages in a sterile environment both before and after PBL reconstitution. At 2 (Exps. 3 and 4) or 4 (Exps. 1 and 2) weeks after reconstitution, each hu-PBL-SCID mouse was challenged with 10⁴ (Exps. 3 and 4) or 10⁵ (Exps. 1 and 2) tissue culture ID₅₀ of HIV-1MB (18), the homologous strain of virus used for preparation of gp160 vaccines, by intraperitoneal injection while under halothane-induced anesthesia. This dose of virus represents 100–1000 minimal infectious doses in the hu-PBL-SCID model (15).

**Virus Isolation.** Fresh peritoneal lavage cells and spleen cell suspensions were prepared from virus-challenged hu-PBL-SCID mice. One milliliter containing 0.5–2 × 10⁶ cells was added to 1 ml containing 2 × 10⁶ human PBLs (from unrelated donors) previously stimulated for 48–72 h with 2.5 µg of phytohemagglutinin P (PHA-P) per ml and 5 units of recombinant human interleukin 2 (IL-2) per ml. These PBL cocultures were maintained in RPMI 1640 medium containing 20% fetal bovine serum, 2.5 µg of PHA-P per ml, and 5 units of IL-2 per ml in six-well 35-mm² plastic culture plates (Falcon 3046; Becton Dickinson Labware). Culture fluids were sampled for p24 antigen twice weekly, and cultures were replenished with 2 × 10⁶ PBLs in medium containing PHA and IL-2 at weekly intervals. The production of p24 viral core antigen was determined by using a commercial p24 capture ELISA (Coulter) according to the manufacturer’s instructions. Cultures were maintained for 4–5 weeks; if no p24 antigen (<30 pg/ml) was detected in the last medium sample, the tissue was designated as not infected. Positive cultures were designated as containing >500 pg of p24 antigen per ml and usually contained higher concentrations.

**Proviral DNA Detection.** HIV-1 proviral DNA was detected by PCR as described (19, 20); briefly, 1 µg of DNA from

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Table 1. Vaccination/experimental schedule and donor immune status

<table>
<thead>
<tr>
<th>Donor</th>
<th>Immunization</th>
<th>Date</th>
<th>hu-PBL-SCID experiment</th>
<th>Neutralizing antibody</th>
<th>gp160 antibody</th>
<th>T-cell proliferation</th>
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<td>10⁶ pfu of HIVAC-1e</td>
<td>09/28/88</td>
<td>09/28/88</td>
<td>&lt;1:10</td>
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<td></td>
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<td>07/28/88</td>
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<td>4. 05/01/91</td>
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</table>

Neutralizing antibody titer is defined as the last dilution of serum giving 50% inhibition of HIV-1 replication in human PBLs. Anti-gp160 titer is defined as the last dilution of serum giving an optical density 3 times background in a gp160 ELISA. T-cell proliferation is expressed as the stimulation index—i.e., cpm of [³H]thymidine incorporation in gp160-stimulated cultures divided by incorporation in medium alone control cultures. ND, not determined.
The heteroduplex product of the PCR mixture detected by liquid autoradiography was 32P-labeled SK19 probe. The heteroduplex product of the PCR mixture was detected by autoradiography after electrophoresis on 10% polyacrylamide gels. In reconstruction experiments, we detected 10 copies of the HIV genome, using as a standard the 8E5 T-cell line, which has a single integrated proviral copy (21).

**Data Analysis.** The effectiveness of vaccine protection was assessed by the ability to recover virus from hu-PBL-SCID mice 4 weeks postchallenge, a time at which peak virus titers are normally reached (15). Animals were scored as infected if any assay for HIV was positive—i.e., if HIV or proviral DNA was detected in any tissue. The rate of HIV-1 infection in 357 control hu-PBL-SCID mice challenged with similar infectious doses of HIV-11IB was 92% ± 8% (mean ± SE; see Table 2 and Fig. 1). Accordingly, experimental groups in which <16% of mice were protected were classified as not different from controls (see Fig. 1). In addition, data from individual experiments were analyzed for significant differences from pooled controls by Fisher’s exact test as indicated in Table 2.

**RESULTS**

**Status of HIV-1 Immunity in PBL Donors.** The immune status of the four vaccinated donors at the time of each hu-PBL-SCID experiment is summarized in Table 1. After priming with vaccinia gp160 and the first round of gp160 boosters, the two initially vaccinia-naive donors, 078 and 033, produced high titers of gp160-reactive antibody and detectable, although low, titers of neutralizing antibody. The two initially vaccinia-immune donors, 065 and 053, had lower levels of gp160-reactive antibody and no neutralizing antibody. Using the same microneutralization assay, 19/23 sera from naturally infected individuals showed neutralizing activity (range, 1:10–1:640; median, 1:30). Donor 078 thus developed a higher neutralizing titer against HIV-11IB than the median value for individuals infected with wild-type strains, although these strains were unlikely to be closely related to strain IIIB. In addition, PBLS from all donors showed high levels of T-cell proliferation when stimulated with rgp160 (Table 1) or whole inactivated virus (data not shown).

**HIV-1 Challenge of Vaccine-Derived hu-PBL-SCID Mice.** At the four intervals indicated in Table 1, hu-PBL-SCID mice were derived by using PBLS from two to four donors. The results of the four separate experiments, performed at various intervals of time after the first or second round of injecting donors with rgp160, are summarized in Table 2 and Fig. 1. hu-PBL-SCID mice derived from donor 065 4 weeks after the initial booster regimen and donor 078 10 weeks after the initial booster dose with rgp160 (Exp. 1) showed highly

Table 2. Numbers of hu-PBL-SCID mice susceptible to HIV-1 infection at different intervals after primary or secondary booster immunization with recombinant gp160

<table>
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<th>Weeks after vac-gp160 priming</th>
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<td>64-69; 101-107; 129-146; 134-153; 4-10</td>
<td>41-47; 48-72; 4-5; (Exp. 1) (Exp. 2) (Exp. 3) (Exp. 4)</td>
</tr>
</tbody>
</table>

Donor Mice infected/mice challenged

065 0/3 2/4 11/11 4/7
078 1/3 4/4 13/14 6/9
033 ND ND 4/4 4/6
053 ND ND 7/8 4/4
All donors combined 1/6 6/8 35/37 18/26

Pooled controls

10 donors* ND ND 32/36 ND
27 donors† 320/357 NA NA NA

All mice scored as virus infected had PBL coculture supernates containing >500 pg of p24 core antigen per ml by 2-3 weeks of culture, and mice were scored as negative if no p24 was detected after 4-5 weeks of culture and no proviral DNA was detected by PCR. Underlined results have a P value of <0.05 by Fisher’s exact test vs. either pooled vaccinated (32/36 infected) or unvaccinated controls (320/357 infected). ND, no data; NA, not applicable.

*Donors were immunized with either alum placebo, vaccinia alone, or a recombinant hepatitis B virus vaccine at AIDS vaccine evaluation unit sites, and their blood was transported to the Medical Biology Institute prior to generation of hu-PBL-SCID mice.
†Donors were normal, unvaccinated local volunteers used to generate hu-PBL-SCID mice, which were subsequently challenged with 103-104 tissue culture ID50 of HIV-11IB in separate but contemporaneous experiments.

**Fig. 1.** Percentage of hu-PBL-SCID mice protected against HIV-1 infection in four experiments involving four separate donors initially immunized with vaccinia gp160 and subsequently booster injected on two occasions with rgp160. The immunization schedule and dosages are presented in Table 1. Stippled area at 0-16% protection represents the level of protection that would not differ significantly from hu-PBL-SCID mice established from control donors. gp160 Ab, relative titer of antibody in a gp160 ELISA (see Table 1); neut Ab, presence of HIV-1 neutralizing antibody; T prol, relative level of donor PBL proliferation after stimulation with gp160.
significant ($P < 0.05$) resistance to infection after challenge with 10^6 tissue culture ID_{50} of HIV-1. After 41–47 weeks (Exp. 2), PBLs from donor 065 still retained the capacity to protect 50% of hu-PBL-SCID mice against viral challenge, but protective responses were no longer detected with PBLs from donor 078. Moreover, by 48–72 weeks (Exp. 3), no significant resistance to HIV-1 challenge was observed when PBLs from any of the four donors were transferred to SCID mice. PBLs obtained 5 weeks after a secondary gp160 booster injection of donors 065, 078, and 033 transferred an augmented protective response compared to the results obtained in Exp. 3, which serves as a prebooster control, but fewer animals were protected than immediately after the initial gp160 booster (Table 2). No protection was observed in hu-PBL-SCID mice derived from similarly immunized donor 053 in this experiment, but this does not rule out a protective response after the initial gp160 booster (not tested). Moreover, this donor had lower antibody and T-cell proliferative responses than other donors to gp160 (Table 1).

To groups of control hu-PBL-SCID mice were used in these experiments (Table 2). Mice derived from donors vaccinated with vaccinia alone, alum, or hepatitis vaccine (mock-vaccinated controls) were generated by the same protocol (including shipment of blood) as the hu-PBL-SCID mice derived from gp160 vaccinees and had the same high rate of infection as control hu-PBL-SCID mice reconstituted from local, unimmunized donors, or as mice derived from PBLs obtained over a year after injecting with gp160 (Exp. 3). In addition, no significant differences in human serum immunoglobulin levels were noted between protected (mean ± SE, 132 ± 28 μg/ml) and unprotected (181 ± 21 μg/ml) animals at the time of HIV-1 challenge. HIV-1-infected mice subsequently had lower immunoglobulin levels, as reported (15). The results thus appear to reflect adoptive transfer of a protective human immune response and not technical variation in protocols or levels of human cell reconstitution.

Evidence for Partial Protection of Vaccine-Derived hu-PBL-SCID Mice. The observed protection of some but not all HIV-1-challenged hu-PBL-SCID mice suggested that transferred immunity might be mediating some antiviral effect even in those animals from which HIV-1 was isolated. We therefore analyzed Exps. 3 (prebooster) and 4 (postbooster), which were identical in design except for the intervening secondary gp160 booster, in more detail. In PBL cocultures from hu-PBL-SCID mice in Exp. 3, high levels (>500 pg) of p24 antigen were detected in peritoneal lavage cells from 34 of 35 animals after 1 week of culture, whereas in Exp. 4, only 3 of 25 animals had virus detected in peritoneal lavage cells by 1 week of culture, and only 13 of 25 animals had detectable virus after 4 weeks of culture. No mice in Exp. 3 had virus isolated solely from spleen cells, while Exp. 4 had 4 mice in which virus was detected only in spleen cells and not in peritoneal cavity cells. These results suggest that adoptive transfer of PBLs from vaccinated donors to SCID mice had induced partial resistance to virus replication in mice that did become infected and that this resistance was more effective in the peritoneal cavity than in the spleen.

Correlation Between Donor Immune Status and Protection of hu-PBL-SCID Mice. We compared the percentage of each experimental group protected against HIV-1 challenge in all four experiments (Fig. 1) with the relative level of each of the donor immune responses measured (Table 1) at the time of each experiment. Two of three donors who protected hu-PBL-SCID mice had no detectable neutralizing antibody. The relationships between the T-cell proliferative responses, antibody levels to gp160, and protection are shown in Fig. 2. There is a positive correlation ($r = 0.772$) between the T-cell proliferative response to gp160 (expressed as log stimulation index) and percentage of HIV-1-resistant hu-PBL-SCID mice. By contrast, only a slight positive correlation ($r = 0.152$) is found between antibody levels to gp160 and protection. While these trends suggest the importance of T-cell immunity, it must be noted that the regression coefficients have large confidence intervals because of the small number of observations.

**DISCUSSION**

These results demonstrate that immunization of human volunteers by priming with vaccinia gp160 and injecting with gp160 induced an immune response in some donors that could be transferred by PBLs to SCID mice and that was sufficient to prevent infection by homologous virus in a highly significant fraction of challenged animals. This protective adoptively transferred immune response declined to nonprotective levels with increasing time after booster injection and was not restimulated by a second gp160 booster injection to levels achieved after the primary booster immunization (Fig. 1). These observations suggest that both the vaccinia gp160 priming and the gp160 booster dose can contribute to generation of effective immunity. These experiments were designed to evaluate protective immunity induced by gp160 vaccination of human PBL donors in the hu-PBL-SCID model. Our results suggest that the hu-PBL-SCID model should be of use both for evaluating the efficacy of future candidate vaccines and for defining the nature of protective immunity. While PBL transfer is likely to sample only a fraction of the donor immune response, and
the efficiency of the immune response in hu-PBL-SCID mice is likely to be less than that of the intact donor, we nonetheless were able to observe protection against relatively high viral challenges. It would thus seem likely that these results underestimate the level of donor immunity and would be unlikely to overestimate the effectiveness of this vaccination protocol.

These studies have not attempted to define the mechanism responsible for the adoptively transferred resistance to HIV-1 infection. The fact that donors 065, 078, and 033 gave rise to HIV-resistant hu-PBL-SCID mice, yet only donor 078 had demonstrable neutralizing antibody titers, suggests that neutralizing antibody is not essential for protection of HIV immunity in this model. The levels of total antibody reactive with gp160 also did not predict which SCID mice would resist infection (see Table 1 and Fig. 2), with the highest antibody titers being found at times when only partial protection was observed. The T-cell proliferative response to gp160 of each donor did show a positive correlation (r = 0.755) with the level of protection, although the small number of observations (n = 6) limits the significance of this analysis (see Fig. 2). This correlation between T-cell proliferative responses and protection and the poor correlation between antibody levels and resistance to infection (r = 0.152; Fig. 2) suggest that cellular immune responses to HIV-1 should be given more emphasis in future vaccine development. Recent reports (22–24) have indicated that either vaccinia gp160 or rgp160 immunization is sufficient to elicit virus-specific cytolytic T cells, which alone or in combination with other antigen-specific T-cell functions may have contributed to the protection observed in our experiments. Neutralizing antibody may correlate with the efficacy of candidate HIV-1 vaccines tested in other animal models but may not be sufficient for protection—e.g., macaques vaccinated with whole, inactivated simian immunodeficiency virus (SIV) developed neutralizing antibody titers of 1:8–1:128, yet none was able to resist challenge with live SIV (25). Finally, we cannot rule out a role for nonspecific murine effector cells (e.g., natural killer cells or macrophages) in the antiviral effects observed in our experiments, although any such effect would have to depend on the level of transferred human immune function. Future studies in the hu-PBL-SCID model in which purified human effector populations and antibodies are evaluated should clarify these issues.

These experiments provide two sources of encouragement for development of vaccines against HIV. First, a significant level of protective immunity was demonstrable after immunization with HIV envelope proteins despite the absence of neutralizing antibody in most of the PBL donors. Second, these results support use of the hu-PBL-SCID model as a step in evaluation of vaccine approaches, both in evaluating responses of vaccinated individuals and in dissecting the immunological mechanisms involved in generating protective immunity.

We acknowledge the efforts of Drs. Ann Collier, Robert W. Coombs, Mark Hoffmann, and David Berger in the vaccine trials at the University of Washington and the technical assistance of Jim Klanecki, Jan McClure, Kim von Sederholm, and Michael Dale for skilled handling of HIV-infected hu-PBL-SCID mice. We thank Dr. Lloyd Fishier of the University of Washington for statistical analysis of the data, Shiu-Lok Hu of Oncogene for vaccinia gp160 and analysis of antibody responses, and Gale Smith at MicroGeneSys for rgp160. Control donors were derived from studies coordinated by Dr. Bonnie Mathieson at the Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, and were from AIDS Vaccine Evaluation Units at the University of Rochester, Marshall University, and Vanderbilt University. This work was supported by National Institutes of Health Grants AI-29182 and AI-30238.