The Role of Donor CD4+ T Cells in the Reconstitution of Oral Immunity by Herpes Simplex Virus Type 1 in Severe Combined Immunodeficiency Mice

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Severe combined immunodeficiency (SCID) mice with ill-developed Peyer's patches develop neither antibodies nor protection against lethal herpes simplex virus type 1 (HSV-1) infection by oral immunization. However, SCID mice carrying spleen cells from immunocompetent BALB/c mice had serum anti–HSV-1 antibody; anti–HSV-1 IgA antibody was detected in eye wash samples, and the mice were protected against lethal HSV-1 infection (88% survival rate). Western blotting showed that antibodies in SCID mice carrying spleen cells from BALB/c mice recognized 60-kDa HSV-1. The effector cells in transferred spleen cells were CD4+, not CD8+, T cells. Donor T cells were detected in the submucosal layer of the gut in SCID mice 1 day after transfer. Rapid movement of donor T cells to the gut may have a role in mucosal immunity to HSV-1. Thus, the normal environment for mucosal immunity develops in SCID mice without prior presence of CD4+ T cells.

Oral administration of vaccine is critical because it results in secretory immune responses in the local area and in distant mucosal and glandular tissue and provides systemic immunity [1, 2]. The morphologic components of the intestinal immune system are referred to collectively as gut-associated lymphoid tissue, which includes both inductive organized lymphoid tissue and diffuse effector lymphoid tissue. The Peyer's patch and lymphoid follicles are organized lymphoid organs of the intestine. Peyer's patches have various roles in mucosal immunity, including antigen uptake and antigen presentation [3–7]. Luminal antigens are passed by membranous cells (M cells) of the follicle-associated epithelium of Peyer's patches to dendritic cells and are presented to T cells by subepithelial dendritic cells. These T cells induce or suppress B cell differentiation, but the role of $CD4^+$ and $CD8^+$ T cells in mucosal immunity remains controversial [8, 9]. Lymphocytes activated by luminal antigen in Peyer's patches leave the mucosa and return to diffuse effector sites. Effector cells have a role in regional immune response by cytotoxic activity, by cytokine regulation, and by controlling the production and secretion of immunoglobulins, especially IgA.

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Immunocompetent mice with Peyer's patches develop mucosal immunity and, if they receive live herpes simplex virus type 1 (HSV-1) orally, can be protected against lethal challenge by HSV-1 [10–12]. Intragastric administration of HSV-1 induces systemic immunity and mucosal response, as measured by the presence of antibody-secreting cells in the Peyer's patches of the gut [11]. Immunocompromised mice, such as nude, SCID, and alymphoplasia mice, develop Peyer's patches poorly or lack them altogether [13–17]. SCID mice have ill-developed Peyer's patches that cannot be identified grossly or microscopically [13, 14, 16, 17] and do not develop mucosal immunity [8–10]. SCID mice also lack mature functioning T and B cells, although pre-T and pre-B cells are present. Thus, SCID mice can serve as a murine model of AIDS. Only low levels of immunoglobulins are present in SCID mice [18], but the reconstitution of lymphoid tissues can be achieved after intravenous injection of histocompatible bone marrow cells [13, 19].

To study the role of Peyer's patches in mucosal immunity, we examined the development of immunity in SCID mice carrying spleen cells from congenic BALB/c mice and challenged them with HSV-1. We further investigated the role of each lymphocyte subset in the process of inducing immunity by oral immunization.

Materials and Methods

Animals. Male BALB/c mice and CB-17 SCID mice (7–8 weeks old) were purchased from Charles River Japan. Mice were maintained in sterile cages in the animal facility of Teikyo University. Sterile food and water were provided.

Virus. HSV-1 (Miyama +GC strain) [20] was supplied by K. Kumagai (Tohoku University School of Dentistry, Sendai, Japan). Virulent virus was obtained by passage in green monkey kidney

cells and was stored at −80°C. Before use, the virus was diluted to the appropriate inoculum dose with 2% fetal bovine serum (FBS)/RPMI 1640.

Oral immunization. HSV-1 was adjusted to 10^7 pfu/mL. HSV-1 (0.2 mL, 2×10^6 virus particles) was administered orally to SCID mice via a stainless steel cannula inserted directly into the stomach. This intragastric immunization was done in SCID mice 1 day after adoptive cells transfer from BALB/c mice. Control mice were prepared by intragastric inoculation with PBS (0.2 mL).

Challenge infection. At 20 days after oral immunization, mice were challenged by infection with HSV-1 (Miyama +GC strain) by intraperitoneal (ip) inoculation of 0.2 mL of medium containing 5×10^5 pfu of virus [10] or by scarification of the left cornea with a 26-gauge needle and addition of a 5-μL drop of medium containing 10^5 pfu of virus [21]. Survival times were noted after challenge. Surviving mice were subjected to deep anesthesia.

Adoptive transfer of spleen cells. Splenic mononuclear cells were prepared as described elsewhere [10]. The spleens were removed from BALB/c mice and were minced in 2% FBS/RPMI 1640. The cell suspension was washed twice, was resuspended in serum-free RPMI 1640, and was adjusted to a final concentration of 2×10^8 cells/mL. Splenic cell suspension (0.5 mL) was injected into each recipient SCID mouse through the tail vein. Cell numbers were approximated as the yield of lymphocytes obtained from a single mouse.

Thy 1.2^+ cells, $B220^+$ cells, $L3T4^+$ T cells, and $Ly2^+$ T cells from the spleen cells of BALB/c mice were separated by magnetic cell sorter (MACS; Miltenyi Biotec) [22]. In brief, a single-cell suspension of spleen was prepared as described above for splenic mononuclear cells. In total, 1 mL of PBS, 0.5% bovine serum albumin (BSA), 2 m*M* EDTA (PBS/BSA/EDTA) buffer contained 10^8 total cells. We added 100 μL of anti–Thy 1.2, and anti-CD45R (B220) microbeads (Miltenyi Biotec) per 10^8 cells, mixed the solution well, and incubated the cells for 15 min at 10°C. Cells were washed with buffer, were centrifuged at 300 *g* for 10 min, and were resuspended in 500 μL of buffer per 10^8 total cells. Cells were passed through a separation column and were sorted with a MACS. Cells trapped by the magnets were infused with PBS/BSA/EDTA buffer. To sort $CD4^+$ and $CD8^+$ T cells from BALB/c splenocytes, we added 100 μL of anti-CD4 (L3T4) or anti-Ly 2 microbeads to 10^8 T cells after the BALB/c splenocytes were passed through a nylon wool column (Wako).

We followed the procedure described above for magnetic sorting of Thy 1.2^+ or $B220^+$ cells. Results were checked by flow cytometer (FACScan; Becton Dickinson) with fluorescein isothiocyanate (FITC)–labeled anti–Thy 1.2 monoclonal antibody (MAb), phycoerythrin (PE)–labeled anti-CD45R (B220) MAb, FITC-labeled anti-CD4 MAb, or PE-labeled anti–CD-8 MAb (all MAbs from PharMingen). Data for 10,000 cells/sample were stored and then were analyzed with FACScan software. $B220^+$ cells comprised <5% of the population of spleen cells when Thy 1.2^+ cells were obtained, and Thy 1.2^+ cells comprised <5% of the population of spleen cells after sorting for $B220^+$ cells. B cells made up <4% of the cells after passing through the nylon wool column. After sorting for $CD4^+$ or $CD8^+$ cells, each cell type comprised <1% of the T cell population after magnetic sorting of T cells.

In the adoptive transfer procedure, SCID mice received 5×10^7 Thy 1.2^+ cells, 5×10^7 $B220^+$ cells, 2×10^7 $CD4^+$ cells, or 2×10^7 $CD8^+$ cells in volumes of 0.5 mL. Cell numbers were approximated as the yield of lymphocytes obtained from a single mouse.

Spleen weights and gross and microscopic examinations of the gut in SCID mice. We weighed the spleens of 10 mice in each of the 5 groups: untreated SCID mice, SCID mice given BALB/c whole spleen cells or BALB/c $CD4^+$ T cells and treated by oral immunization with live HSV-1 21 days after cell transfer, untreated BALB/c mice, and BALB/c mice orally immunized with live HSV-1. After removal, each spleen was immediately placed in RPMI 1640 to prevent excessive drying and was weighed on a microbalance (model AC100; Mettler). Spleens samples were fixed and embedded in paraffin. The gut was removed simultaneously and was observed grossly and microscopically after preparation.

Collection of serum and eye wash samples for antibody assay. Blood was collected from the retro-orbital sinuses of 4 or 5 mice from each group 20 days after intragastric inoculation with live HSV-1 or PBS. Blood samples from each mouse were kept separately. Twenty days after inoculation, eye wash samples from the corneas of both eyes of each mouse were collected daily for 7 days. In brief, the cornea of each eye was irrigated with 20 μL of PBS containing 1% BSA, and the solution then was aspirated; this was done 10 times for each eye. Eye wash samples were pooled for each mouse.

ELISA of serum and eye wash samples. HSV-1 antibody titers were measured by sandwich ELISA [23]. We coated Falcon microtest plates (Becton Dickinson) with 100 μL of anti–HSV-1 rabbit serum 1:1000 (Dako Laboratories) and incubated the plates overnight at 4°C with a crude HSV-1 antigen prepared by the method described by Jennings et al. [24]. The serum and eye wash samples were diluted in PBS-BSA. The samples (100 μL) then were added to the wells in triplicate and were incubated at 37°C for 1 h. After plates were washed with 0.05% Tween-20/PBS (PBS-T), peroxidase-conjugated anti–mouse immunoglobulin (Dako) diluted 1:1000 and goat anti–mouse IgG (γ-chain specific), IgM (μ-chain specific), and IgA (α-chain specific)—all diluted 1:500 and all from Binding Site—were added. After the plates were incubated at 37°C for 1 h, we added 100 μL of substrate: 10 mg of *o*-phenylenediamine dihydrochloride in 25 mL of 0.1 *M* citrate phosphate buffer and 75 μL of 6% H_2O_2. The reaction was stopped after 5 min by addition of 2*N* H_2SO_4, and substrate conversion was measured spectrophotometrically at 492 nm. Concentrations of antibodies were calculated from the ELISA data by interpolation of the sample curve onto the standard curve by weighted probit analysis. Results were expressed as the reciprocal value of the dilution at 50% reduction of the positive control value [25]. Positive control serum was obtained by pooling serum samples collected from 10 mice 32 days after ip inoculation with 10^4 pfu of live HSV-1.

ELISPOT assay. The numbers of spleen cells producing different anti–HSV-1 isotypes were measured by ELISPOT assay, as elsewhere [26]. In brief, 96-well nitrocellulose bottom plates (Millipore) were coated with HSV-1 antigen by overnight incubation at 4°C. After blocking with RPMI 1640/10% FBS, spleen or Peyer's patch cells in 100 μL were added and incubated for 6 h at 4°C. Plates then were washed with PBS and PBS containing 0.05% PBS-T and were incubated overnight with goat anti–mouse IgG (1:1000), anti-IgM (1:500), or anti-IgA (1:500) peroxidase conjugate. After another wash with PBS, spots were developed

with the use of 3-amino-9-ethylcarbazole substrate (Sigma) in citrate buffer (pH 5).

Western blots. Lysis buffer (0.1 *M* Tris, 1% 2-mercaptoethanol, 5% SDS, and 0.002% bromophenol blue) was added to virus-infected cell lysate, and the mixture was boiled for 5 min at 100°C. Proteins were separated by electrophoresis on polyacrylamide gels (Mini-Protean II Ready Gels; Bio-Rad Laboratories) in the presence of 0.1% SDS. Separated proteins were transferred electrophoretically to nitrocellulose membranes (Bio-Rad Laboratories). Nonspecific protein binding was blocked by treating the membrane with Tris-buffered saline (TBS; 25 m*M* Tris-HCl and 150 m*M* NaCl [pH 7.4]) containing 0.05% Tween 20 (TBS-T) and 3% skim milk (Wako) at 37°C for 1 h. Next, we incubated overnight 100 μL of mouse serum at 4°C with the membrane in 5 mL of TBS-T containing 1% skim milk and 0.05% Tween-20. After a wash with TBS-T, the membrane was incubated for 1 h at 37°C with peroxidase-conjugated anti–mouse immunoglobulins at the same dilutions described for ELISA. The membrane was washed 3 times with TBS-T and once with TBS. Diaminobenzidine and H_2O_2 were added to the TBS-T for staining. To determine the size of HSV glycoprotein D (gD), crude HSV-1 crude antigen transferred to a nitrocellulose membrane was incubated overnight at 4°C with anti–HSV-1 gD MAb (Biogenesis) diluted 1:500. After a wash with TBS-T and TBS, the membrane was stained with diaminobenzidine and H_2O_2.

Migration of transferred splenocytes. In vivo 5-bromo-2-deoxyuridine (BrdU) labeling was used to assess migration of the transferred donor T cells. A cell proliferation kit (Amersham International) was used to detect BrdU uptake. In brief, we incubated 3×10^8 Thy 1.2^+ cells obtained from 3 BALB/c mice by magnetic sorting with 1 mL of BrdU labeling solution by diluting labeling regent 1:1000 with 2% FBS/RPMI 1640 at 37°C for 1 h. After a wash, cells were suspended in RPMI 1640 without fetal FBS, and 0.5 mL of cell suspension was injected into 3 SCID mice through the tail vein. Mice were killed by deep anesthesia 24 h after cell transfer, and organs were removed, fixed in 10% buffered formalin, and embedded in paraffin. Specimens were covered with sufficient mouse anti-BrdU antibody. After specimens were washed, peroxidase anti–mouse IgG was added and incubated for 30 min at room temperature; diaminobenzidine solution was added for detection of T cells. Control organs of 3 untreated SCID mice were stained by the method described above.

Statistical analysis. Data are shown as mean ± SD. We used the Student's *t* test for analysis of differences between spleen weights of untreated SCID mice, SCID mice given BALB/c whole spleen cells or $CD4^+$ T cells and orally immunized with live HSV-1, BALB/c mice orally immunized with live HSV-1, and untreated BALB/c mice. $P < .05$ was considered to be significant.

Results

Oral immunity in SCID mice given whole spleen cells from BALB/c mice. All control BALB/c and SCID mice died when challenged ip with HSV-1 (5×10^5 pfu): mean survival times were 7.2 ± 1.2 and 6.5 ± 0.8 days, respectively. Mean survival times of BALB/c and SCID mice after ocular challenge with 10^5 pfu of HSV-1 were 8.6 ± 0.8 and 7.6 ± 0.7 days, respectively. At the time of death, HSV-1 was recovered from the brain, especially the brain stem of both mouse strains, regardless of the challenge route. Immunocompetent BALB/c mice orally immunized with live HSV-1 had antibodies against HSV-1 detected by ELISA in their serum 20 days after immunization (table 1); these immunized BALB/c mice survived >20 days after ip challenge, and 80% survived after ocular challenge (figure 1). No SCID mouse showed anti–HSV-1 antibodies after intragastric immunization with live HSV-1 (table 1), and all immunized SCID mice died within 9 days of lethal ip or ocular challenge (figure 1). However, high titers of antibodies, primarily IgG, were detected 20 days after intragastric immunization in the serum samples of SCID mice given whole spleen cells from BALB/c mice (table 1).

At 20 days after intragastric inoculation with live HSV-1, a significant number of antibody-forming cells were detected by ELISPOT in the spleens of SCID mice carrying spleen cells from BALB/c mice (figure 2). The number of anti–HSV-1 immunoglobulin-producing cells in the spleens of SCID mice with BALB/c spleen cells was ~30% that of antibody-forming cells in the positive control mice.

Detection of antibody in eye wash samples. Table 2 shows the ELISA antibody titers of eye wash specimens by mouse

Table 1. Anti–herpes simplex virus type 1 (HSV-1) antibody levels in the serum of BALB/c mice, SCID mice, and SCID mice with spleen cells from BALB/c mice and immunized orally or intraperitoneally with live HSV-1.

Mouse strain	Immunogen	Inoculation route	ELISA titers[a]		
			Immunoglobulin	IgG	IgM
BALB/c	HSV-1	Intraperitoneal	230 ± 12	245 ± 10	35 ± 6.1
BALB/c	HSV-1	Oral	38 ± 2.3	32 ± 1.0	12 ± 4.2
SCID	HSV-1	Oral	1.3 ± 0.5	ND	ND
SCID-BALB/c[b]	HSV-1	Oral	56 ± 3.6	42 ± 3.2	ND
SCID	PBS	Oral	2.5 ± 1.5	ND	ND
BALB/c	PBS	Oral	7.2 ± 2.2	ND	ND

NOTE. Data are mean ± SD ELISA titers, expressed as the reciprocal value of the dilution at 50% reduction of the positive control serum samples.

[a] IgA titers were not detected (ND).

[b] SCID mice received 10^8 spleen cells/mouse from immunocompetent BALB/c mice and then intragastric immunization from live HSV-1.

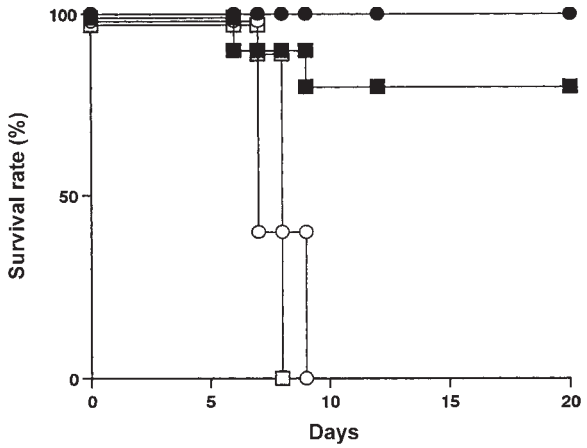

Figure 1. Survival rates for SCID and BALB/c mice after intraperitoneal challenge with herpes simplex virus type 1 (HSV-1; 5×10^5 pfu/mouse) after intragastric immunization with live HSV-1 (2×10^6 pfu/mouse: ●, BALB/c mice; ○, SCID mice) or ocular challenge with live HSV-1 (10^5 pfu/mouse: ■, BALB/c mice; □, SCID mice). There were 10 mice/group.

strain. Low levels of anti–HSV-1 IgA antibodies were found in the eye wash samples of BALB/c mice orally immunized with HSV-1. Anti–HSV-1 IgA antibodies also were detected in the samples from SCID mice carrying spleen cells from immunocompetent BALB/c mice, similar to samples from immunocompetent BALB/c mice. Figure 3 shows the results of Western blotting for antibodies in eye wash samples from BALB/c mice immunized orally with live HSV-1 and from SCID mice carrying spleen cells from immunocompetent BALB/c mice. The bands were detected at ~60 and 40 kDa for immunocompetent BALB/c mice (figure 3, *lane c*); a band was detected at ~60 kDa for SCID mice carrying spleen cells from immunocompetent BALB/c mice (figure 3, *lane e*). The molecular weight of this band was consistent with HSV-1 gD (figure 3, *lane f, insert*).

Oral immunity induced in SCID mice bearing donor CD4⁺ T cells. Figure 4 shows Western blots of serum samples from SCID mice given whole spleen cells, B220⁺ cells, Thy 1.2⁺ cells, CD4⁺ T cells, or CD8⁺ T cells after oral immunization and blots of BALB/c mice immunized orally. Several bands were observed in serum samples of BALB/c mice immunized orally with live HSV-1 (figure 4, *lane b*). In SCID mice carrying whole BALB/c spleen cells, 1 band was detected at ~60 KDa (figure 4, *lane c*). The same band was observed for SCID mice bearing T cells or CD4⁺ T cells (figure 4, *lanes d* and *f*), but this band was not seen in SCID mice carrying B cells (B220⁺ cells; figure 4, *lane e*) or CD8⁺ cells (figure 4, *lane g*). Thus, oral immunity was induced in SCID mice reconstituted by adoptive transfer of T cells only, especially CD4⁺ cells, revealing that adoptive transfer of B cells was not required for immunity. The experiment was repeated 3 times, and an identical pattern emerged each time.

Lethal challenge. Twenty days after intragastric inoculation, mice were challenged by ip inoculation with HSV-1 (5×10^5

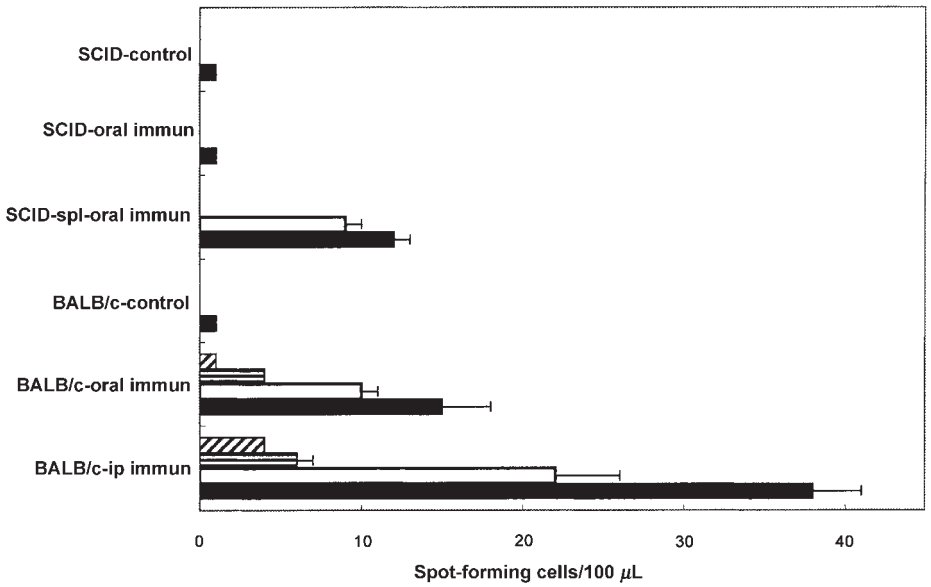

Figure 2. No. of spleen cells producing different herpes simplex virus type 1 (HSV-1) antibody isotypes: immunoglobulin *(black bars),* IgG *(white bars),* IgM *(striped bars),* and IgA *(hatched bars)* for each group of mice ($n = 3$/group). SCID-control, negative control, nontreated SCID mice given PBS orally; SCID-oral immun, nontreated mice immunized orally with live HSV-1; SCID-spl-oral immun, SCID mice carrying whole spleen cells (10^8 cells/mouse) from immunocompetent BALB/c mice immunized orally with live HSV-1; BALB/c-control, negative control, nontreated BALB/c mice given PBS orally; BALB/c-oral immun, nontreated BALB/c mice immunized orally with live HSV-1; BALB/c-ip immun, positive control, nontreated BALB/c mice inoculated intraperitoneally (ip) 3 times with live HSV-1 (killed 60 days after initial ip inoculum of 10^2 pfu of HSV-1 and booster inoculations of 10^4 pfu 50 and 7 days before the assay).

Table 2. Anti–herpes simplex virus type 1 (HSV-1) antibody levels in the eye wash SCID mice carrying spleen cells from BALB/c mice inoculated orally with live HSV-1.

	ELISA titers			
Mouse strain	Immunoglobulin	IgG	IgM	IgA
BALB/c	4.8 ± 1.0	4.4 ± 0.5	3.5 ± 0.6	3.3 ± 1.2
SCID	ND	ND	ND	ND
SCID-BALB/c[a]	4.3 ± 3.0	3.4 ± 1.2	ND	2.4 ± 3.3

NOTE. Data are mean ± SD ELISA titers, expressed as the reciprocal value of the dilution at 50% reduction of the positive control serum samples. ND, not detected.

[a] SCID mice received 10^8 spleen cells/mouse from immunocompetent BALB/c mice and then intragastric immunization with live HSV-1.

pfu/mouse). Of the SCID mice that received BALB/c spleen cells, 88% survived (table 3). Survival in the SCID mice that received Thy 1.2^+ cells and $CD4^+$ cells was 80% and 75%, respectively, but mice given $B220^+$ or $CD8^+$ BALB/c spleen cells did not survive lethal challenge of HSV-1. The SCID mice given whole spleen cells or $CD4^+$ cells from BALB/c mice and challenged by corneal inoculation with 10^5 pfu had survival rates of 78% and 80%, respectively. All SCID mice that received $CD8^+$ cells died after ocular challenge. BALB/c mice without oral immunization and SCID mice carrying spleen cells, Thy 1.2^+ cells and $CD4^+$ T cells without immunization by HSV-1 were not protected against lethal ip or ocular HSV-1 challenge.

Migration of splenocytes from donor BALB/c mice into systemic and intestinal lymphoid tissues. When various tissues from SCID mice that received BALB/c Thy 1.2^+ cells were examined 24 h after adoptive transfer, donor T cells were found predominantly in the spleen (figure 5*B*), small intestine (figure 5*D*), and liver (not shown).

Spleen weights and gross and microscopic findings in the gut. Mean spleen weight of BALB/c mice immunized with HSV-1 was greater than of untreated BALB/c mice (mean weight, 2.15), and the mean spleen weight in each SCID mice group was significantly less than that of untreated BALB/c mice (table 4). The average weight, however, of spleens of SCID mice that underwent adoptive transfer of whole BALB/c spleen cells or $CD4^+$ T cells followed by oral immunization with live HSV-1 was not statistically different from untreated SCID mice. The definite lymph follicle or lymph follicle with a germ center was not observed microscopically in SCID mice even after oral immunization. Peyer's patches also were not detected grossly or microscopically in SCID mice bearing BALB/c whole spleen cells or $CD4^+$ T cells with oral immunization of live HSV-1.

Discussion

Transfer of congenic bone marrow cells into SCID mice reconstitutes both T cell and B cell function of various organs (e.g., thymus, spleen, and lymph node) [13, 19]. The present study clarified that the oral immunity is reconstituted in SCID mice with ill-developed Peyer's patches by transfer of spleen cells from immunocompetent BALB/c mice. These SCID mice have antibodies against HSV-1 and are protected against lethal infection by HSV-1.

Anti–HSV-1 IgA antibody was detected in eye wash samples from SCID mice carrying spleen cells from BALB/c mice (table 2). Furthermore, SCID mice that received BALB/c spleen cells, Thy 1.2^+ cells, and $CD4^+$ T cells were protected against both ocular and ip challenge. Clearly, the common mucosal immune system that is characteristic of mucosal immunity acts in reconstituted SCID mice carrying spleen cells from BALB/c mice orally immunized with live HSV-1. Donor-transferred cells may have proliferated in SCID mice, and SCID mice, in essence, may have become BALB/c mice.

In orally immunized BALB/c mice, several bands were detected by Western blotting at ~130 (data not shown), 60, and 40 kDa. Only 1 60-kDa band was found in SCID mice that received Thy 1.2^+ or $CD4^+$ cells and whole spleen cells, and this band differed from bands in BALB/c mice. However, when one considers the antigenic site used by anti–HSV-1 antibody in SCID mice carrying donor spleen cells, Thy 1.2^+ cells, or $CD4^+$ T cells, it is reasonable to think that mucosal immunity can be reconstituted in SCID mice by transfer of $CD4^+$ T cells from BALB/c mice.

The 60-kDa band we observed is consistent with that of gD, as shown in figure 3 *(lane f)* [27, 28], and does not differ in size from glycoproteins B and C (data not shown), and it is possible that it is gD. gD is essential for entry of virus into host cells, acts

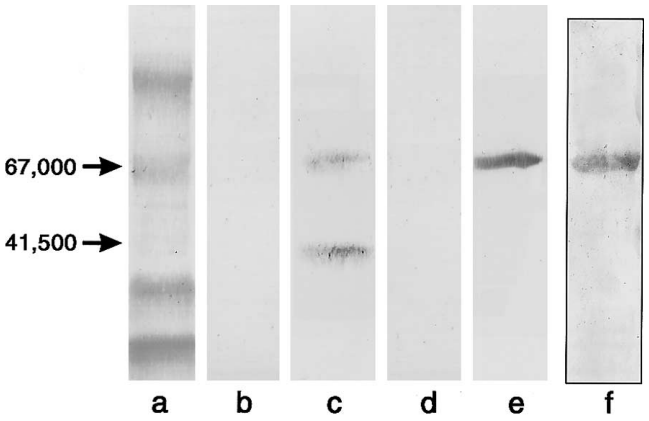

Figure 3. Herpes simplex virus type 1 (HSV-1) IgA antibodies in eye wash samples determined by Western blotting. Lanes: *a,* kaleidoscope prestained standard (*upper arrow,* 67-kDa bovine serum albumin; *lower arrow,* 41.5-kDa carbonic anhydrase); *b,* eye wash samples of BALB/c mice given PBS orally; *c,* eye wash samples of BALB/c mice inoculated orally with live HSV-1 (2×10^6 pfu/mouse); *d,* eye wash samples of nontreated SCID mice given PBS orally; *e,* eye wash samples of SCID mice carrying whole spleen cells (10^8 cells/mouse) from BALB/c mice orally inoculated with live HSV-1 (2×10^6 pfu/mouse); and *f,* HSV-1 glycoprotein.

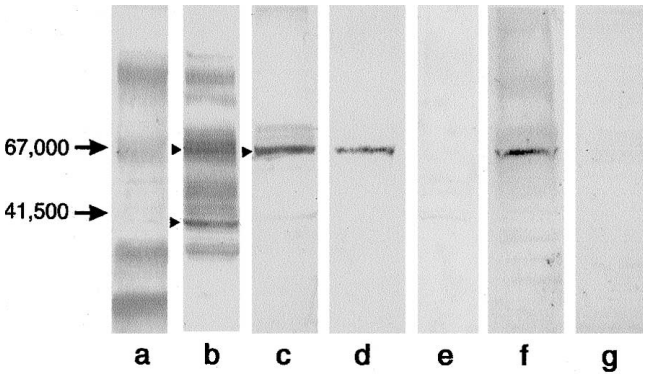

Figure 4. Herpes simplex virus type 1 (HSV-1) antibodies in serum determined by Western blotting. Lanes: *a,* kaleidoscope prestained standard (*upper arrow,* 67-kDa bovine serum albumin; *lower arrow,* 41.5-kDa carbonic anhydrase); *b,* anti–HSV-1 immunoglobulins *(arrowheads)* of BALB/c mice immunized with live HSV-1; *c,* antibody *(arrowhead)* of SCID mouse bearing whole spleen cells (10^8 cells/mouse) from BALB/c mice immunized orally with live HSV-1; *d,* antibody of SCID mice bearing Thy 1.2^+ cells (5×10^7 cells/mouse) of BALB/c mice immunized orally with live HSV-1; *e,* antibody of SCID mice bearing $B220^+$ cells (5×10^7 cells/mouse) of BALB/c mice orally immunized with live HSV-1; *f,* antibody of SCID mouse with $CD4^+$ T cells (2×10^7 cells/mouse) of BALB/c mice immunized orally with live HSV-1; and *g,* antibody of SCID mouse bearing $CD8^+$ T cells (2×10^7 cells/mouse) from BALB/c mice immunized orally with live HSV-1. Orally inoculated HSV-1, 2×10^6 pfu/mouse.

as a protective immunogen, and stimulates protective humoral and cellular immunity as needed. Because of the high survival rate of our SCID mice given spleen cells from BALB/c mice, we hypothesize that SCID mice have this antibody. Cellular immunity of SCID mice carrying spleen cells from BALB/c mice was not examined. There is a possibility that cellular immunity also has a role in this protection. However, spleens of SCID mice carrying BALB/c mice spleen cells or $CD4^+$ T cells and immunized with live HSV-1 did not become larger than those of untreated SCID mice. Lymph follicles or lymph follicle with germ centers were not observed in the spleens of SCID mice given spleen cells or $CD4^+$ T cells from BALB/c mice and orally immunized with live HSV-1, nor were such follicles observed in untreated control BALB/c mice. Thus, no morphologic evidence was found for the activation of cellular immunity. Further investigation into cellular immunity of recipient SCID mice is needed.

In general, orally administered antigenic material is passed through the M cells in Peyer's patches. M cells have the highly specialized role of antigen uptake and presentation to dendritic cells, which then present antigen to $CD4^+$ T cells. $CD4^+$ T cells prime the precursors of antibody-secreting cells [4]. One characteristic of parenteral immunization is that these antibody-secreting cells migrate through lymphoid tissue. Anti–HSV-1 antibody-forming cells were present in the spleen and in the same numbers as in immunocompetent BALB/c mice (figure 2). The effector cells among the spleen cells transferred from BALB/c mice were $CD4^+$ T cells. It is possible that the transfer of $CD4^+$ donor T cells may restore B cell function in SCID mice and that B cells produce anti–HSV-1 immunoglobulins. However, it remains unclear whether transferred $CD4^+$ T cells act directly on precursors of B cells or whether cytokine factors such as interleukin (IL)–2 act on these precursor cells. Further studies are needed.

Three stages are described in the formation of Peyer's patches [16, 17]. The first is the appearance of vascular cell adhesion molecule 1 (VCAM-1) spots in the intestine, which probably represent an initial anlage stage Peyer's patch. Accumulation of cells bearing IL-7 receptor α (IL-7Rα), CD4, or I-associated antigen (Ia) in this region follows, and mature lymphocytes appear just before birth. In SCID mice, formation of VCAM-1 spots and cell clusters bearing IL-7Rα, CD4, or Ia clusters is observed, but mature lymphocytes do not appear. Therefore, it is likely that $CD4^+$ T cells transferred from BALB/c enter the anlage Peyer's patch and fulfill its function. There is a possibility that the passively transferred $CD4^+$ cells include $CD4^+$ antigen-presenting cells (APCs) [29], but it is reasonable to think that the $CD4^+$ T cell is critical because APCs exist naturally in SCID mice [30]. Hence, the critical cells among transferred cells are the $CD4^+$ T cells.

It is controversial whether $CD4^+$ or $CD8^+$ T cells are the most critical for mucosal immunity. $CD4^+$ T cells are thought to be important in the function of Peyer's patches because the patches contain more $CD4^+$ T cells than are found in distant villi [31]. Even a study of oral tolerance found evidence that $CD4^+$ T cells were more critical than $CD8^+$ cells in the induction phase [8]. This is similar to thymus reconstitution in SCID mice. The thymus develops normally in SCID mice, providing an adequate environment for thymocytic maturation, but functional T cell are not present, especially $CD4^+$ T cells [19, 32].

Table 3. Survival rates of SCID mice carrying spleen cells, Thy 1.2^+ cells, and $CD4^+$ T cells from BALB/c mice after lethal challenge with herpes simplex virus type 1 (HSV-1) after oral immunization by live HSV-1.

Mouse strain	Cells transferred from BALB/c	Challenge route	Survival rate[a]
BALB/c	None	Intraperitoneal	10/10 (100)
SCID	Spleen cells[b]	Intraperitoneal	7/8 (88)
SCID	Thy-1.2 cells[c]	Intraperitoneal	4/5 (80)
SCID	CD4 T cells[d]	Intraperitoneal	3/4 (75)
SCID	CD8 T cells[d]	Intraperitoneal	0/4 (0)
BALB/c	None	Corneal	8/10 (80)
SCID	Spleen cells[b]	Corneal	7/9 (78)
SCID	CD4 T cells[d]	Corneal	4/5 (80)
SCID	CD8 T cells[d]	Corneal	0/5 (0)

[a] Data are dead mice/total mice (%).
[b] Each mouse received 10^8 cells.
[c] Each mouse received 5×10^7 cells.
[d] Each mouse received 2×10^7 cells.

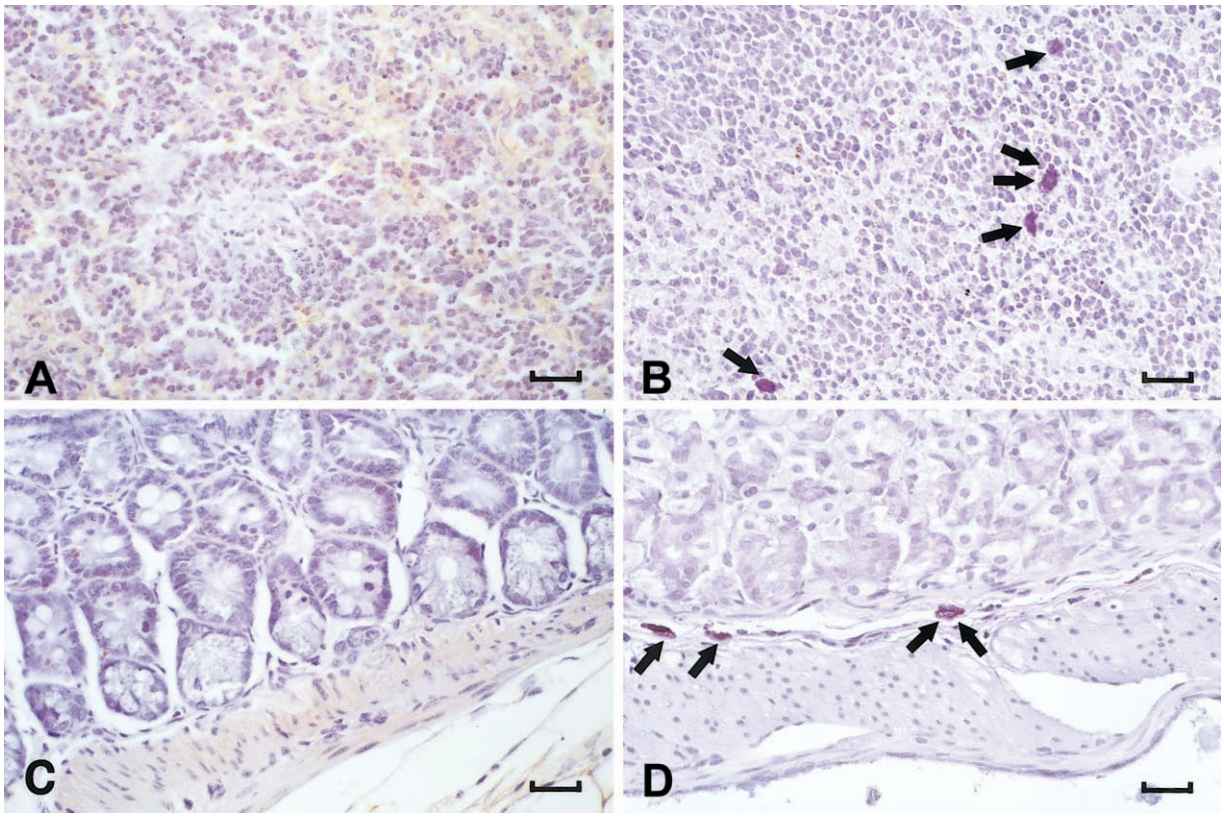

Figure 5. Localization of 5-bromo-2-deoxy-uridine (BrdU)–labeled BALB/c T cells in SCID mice 1 day after intravenous adoptive transfer. *A* and *C,* Control spleen and intestine of nontreated SCID mice with BrdU immunostaining, respectively. *B,* Donor T cells observed in spleen *(arrows). D,* Submucosal layer of intestine *(arrows).* Hematoxylin counterstaining. Original magnification, ×100; all bars, 100 μm.

There are reports of successful immunoreconstitution in SCID mice with the use of congenic lymphocytes isolated from age- and sex-matched mice in which donor cells were harvested from Peyer's patches and mesenteric lymph node tissues. Rapid homing and formation of novel Peyer's patch tissues in the small intestines of these animals were observed [8, 33, 34]. Our results demonstrate that T lymphocytes in spleen cells preferentially migrate to submucosa of the gut, spleen (figure 5*A* and 5*B*), and liver (data not shown). It is possible that these lymphocytes have a major role in reconstitution of the mucosal immunity.

Custer et al. [13] observed Peyer's patches or solitary follicles in SCID mice 20 weeks after the transfer of bone marrow cells from congenic mice. However, we did not observe Peyer's patches or Peyer's patch–like structures in the present study grossly or microscopically, and we believe that the observation period was too short for their appearance. Peyer's patches were not formed, but the function was reconstructed. We conclude that the antigen-presenting function of the intestinal mucosa in SCID mice develops normally but that the mucosa lacks functional $CD4^+$ T cells.

Our findings are of interest because the role of $CD4^+$ cells in mucosal immunization is critical. This is likely to be important in the design of human immunodeficiency virus (HIV) vaccines because the intestinal tract is a major port of entry for HIV and may be a major site of primary viral replication. There have been many recent attempts to create an oral vaccine against HIV because the mucosa is the primary infective site [35–37]. As shown in the present study, $CD4^+$ T cells have a major role in

Table 4. Mean spleen weights of untreated SCID mice, SCID mice given whole spleen cells , or $CD4^+$ T cells from BALB/c mice 20 days after oral immunization with live herpes simplex virus type 1 (HSV-1), untreated BALB/c mice, and BALB/c mice 20 days after oral immunization with live HSV-1.

Mouse strain	Cells transferred from BALB/c	Immunization	Spleen weight, mean g ± SD	*P*
BALB/c	None	PBS[a]	0.123 ± 0.041	—
BALB/c	None	HSV-1[b]	0.127 ± 0.027	<.05[c]
SCID	None	PBS[a]	0.052 ± 0.016	<.01
SCID	Spleen cells[d]	HSV-1[b]	0.055 ± 0.012	<.01
SCID	CD4 T cells[e]	HSV-1[b]	0.055 ± 0.01	<.01

[a] Mouse orally immunized with PBS.
[b] Mouse orally immunized with 2×10^6 pfu or live HSV-1.
[c] *P* vs. untreated BALB/c spleen weight.
[d] Each mouse received 10^8 cells.
[e] Each mouse received 2×10^7 cells.

mucosal immunity, but, in AIDS patients, the $CD4^+$ T cells are reduced in number. This presents a major hurdle to developing any oral vaccine, especially a therapeutic vaccine for treatment of HIV/AIDS.

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