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LONG-TERM LYMPHOID RECONSTITUTION OF SCID MICE SUGGESTS SELF-RENEWING B AND T CELL POPULATIONS IN PERIPHERAL AND MUCOSAL TISSUES

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Peyer's patch, peripheral lymph node, and mesenteric lymph node cells were transferred to immunodeficient SCID mice to assess the long-term (150–300 days) potential of these cells to repopulate the host's immune system. Results demonstrate that, irrespective of donor population, total serum Ig and isotype distribution appear normal within 4 weeks of reconstitution and remain at normal levels for up to one year following cell transfer. At the cellular level, each donor population reconstitutes splenic T and B cell compartments in a progressive and quantitatively indistinguishable manner. Immunohistological analyses of reconstituted mice indicate that, although some qualitative differences are evident, normal splenic composition and architecture are observed. In contrast, gut reconstitution varies significantly with donor population. Peyer's patch cells yield normal-appearing gut tissue with extensive infiltration of the lamina propria and intraepithelial compartments by T cells and IgA-secreting plasma cells. Peripheral lymph node cells give rise to T cells found almost exclusively in the lamina propria, while IgA secreting plasma cells are rarely detected. The course and extent of reconstitution further suggest that all donor populations contain long-lived T and B cells as well as self-renewing lymphocytes capable of extensive expansion. This latter observation has potentially important implications for both transplantation biology and gene therapy applications.

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The peripheral immune system consists of at least two relatively distinct elements represented, on the one hand, by mucosal lymphoid tissues (i.e., gut and lungs) and, on the other, by spleen and an extensive network of lymph nodes (LN).* Within the mucosal arm of the immune system, as represented by the gut, the lamina propria (LP), and Peyer's patches (PP) lining the gastrointestinal tract (*1–4*) form prototypic structures containing the majority of lymphocytes. The diversity of lymphocytes within mucosal tissues, as well as the microenvironments themselves, differ from those of peripheral lymphoid tissue in several ways. First, in short-term homing studies, cells isolated from LP or PP preferentially and rapidly return to gut-associated lymphoid tissue (GALT) following adoptive transfer to compatible hosts while LN cells preferentially home to LN and only poorly repopulate GALT (*5–8*). Second, immigration of circulating lymphocytes to PP appears restricted and may be dependent, at least in part, on expression of VLA4α/LPAM-1 determinants on the cell surface and the corresponding ligand on PP high endothelial venules (*9–12*). LN immigration, on the other hand, is suggested to be mediated by LN-specific homing receptors such as L-selectin and does not appear to be VLA4α/LPAM-1-dependent (*13–15*). Third, IgA-bearing B cells and their precursors are frequently found in the LP and PP, but only rarely among LN B cells (*5–7, 16, 17*). Finally, freshly isolated (*18*), or cloned T cells (*19–22*) obtained from PP preferentially enhance IgM to IgA isotype switching, while pe-

* Abbreviations: DAB, diaminobenzidine tetrahydrochloride; HRP, horseradish peroxidase; LN, lymph node; LP, lamina propria; MLN, mesenteric lymph node; OPD, o-phenylenediamine dihydrochloride; PLN, peripheral lymph nodes; PP, Peyer's patches.

ripheral T cells primarily support switching to Ig isotypes other than IgA.

Although these and other differences between LN and GALT have been well studied, it remains unclear whether the homing patterns observed shortly after cell transfer accurately reflect the long-term reconstituting potential of LN and PP–derived cells. In an effort to address this question, DBA/2 cells isolated from PP, mesenteric lymph node (MLN), or peripheral lymph node (PLN) were adoptively transferred to immunodeficient SCID mice and the long-term reconstituting potential of each donor population assessed by serum Ig levels, lymphoid organ composition, and immunohistological analyses. DBA/2 mice were chosen as the source of donor cells because they are MHC-compatible with SCID but their lymphocytes are readily distinguished by expression of the cell surface molecule Qa-2 (*23*), which is absent in the SCID mouse (C.B-17). Similarly, the Ig allotypic difference between DBA/2 (μ^a) and SCID (μ^b) distinguishes serum IgM of donor origin and the endogenous low levels normally found in SCID mice. SCID mice were selected as recipients because of an absence of mature T or B lymphocytes and of established immunoregulatory networks (*24, 25*). Using this system, we have assessed the long-term ability of PP, MLN, and PLN donor populations to reconstitute the peripheral and mucosal immune systems in SCID mice.

MATERIALS AND METHODS

Mice. Female DBA/2NCR mice were obtained from Frederick Cancer Research Facility (Fort Detrick, Frederick, MD). C.B-17ICR SCID/SCID breeding pairs were obtained from Dr. Nancy Jenkins (FCRF, Frederick, MD). All mice were housed in a NCI animal facility (Fort Detrick, Bldg. 571, Frederick, MD) under barrier conditions and maintained in a specific pathogen–free colony.

Cell and tissue preparations. PP and LN were removed from 7–10-week-old female DBA/2 mice and placed in RPMI 1640 containing 5% FCS, 2 mM L-glutamine and 40 μg/ml gentamycin. Tissues were teased apart and passed through a sterile stainless steel mesh. Cells were washed once and resuspended at 10^7 cells/ml in RPMI 1640 containing 5% FCS, 2 mM L-glutamine, 40 μg/ml gentamycin, 100 U/ml penicillin, 100 μg/ml streptomycin, and fungizone for 5 min, after which they were washed an additional three times and resuspended at 5×10^7 cells/ml in RPMI 1640 without FCS. SCID recipients received a single i.v. injection of 10^7 cells in a volume of 0.2 ml, which approximated the yield of lymphocytes obtained from a single DBA/2 donor mouse.

FACS analyses. The cell surface phenotype of lymphocytes from spleens of reconstituted SCID, untreated SCID, and normal DBA/2 mice were determined by flow cytometric analyses. Briefly, single cell suspensions were prepared in sorter medium (phenol red–free HBSS containing 0.1% sodium azide, 2 μg/ml BSA, 2 mM EDTA and 4 mM sodium bicarbonate). The resultant cells were resuspended at 2×10^7 cells/ml and 50 μl (10^6 cells) aliquots were stained with one or more of the following reagents: FITC monoclonal rat antimouse IgM (Zymed, San Francisco, CA), FITC-coupled goat antimouse IgG (Southern Biotechnology Associates, Birmingham, AL), FITC rat antimouse IgA (PharMingen, San Diego, CA), FITC monoclonal mouse antirat IgL kappa (PharMingen), FITC or PE monoclonal rat antimouse CD45(B220) clone RA3-6B2 (*26*), biotinylated monoclonal 1-9-9 mouse antimouse Qa-2 (*23*), biotin-MEL-14 (*13*), APC-avidin (Molecular Probes, Eugene, OR), FITC-avidin (Zymed), FITC antimouse CD8 (Becton Dickinson), PE-coupled rat antimouse CD4 clone GK1.5 (Becton Dickinson).(*27*), biotinylated monoclonal rat antimouse CD3 clone 500-A2 (*28*), FITC monoclonal antimouse α/β TCR (PharMingen). To prevent nonspecific FcR-mediated binding of antibodies, unlabeled mAb 2.4G2 specific for mouse FcγRII (*29*) was added prior to staining with labeled mAb. Once stained, cells were washed twice in sorter medium and resuspended for analyses on a Becton Dickinson FACS-star Plus equipped with argon and argon/dye lasers. Nonviable cells were excluded by forward-angle scatter and/or uptake of propidium iodide. All analyses were collected as list mode data and reanalyzed using Consort 40/VAX software (Becton Dickinson).

Tissue sections. At various times postreconstitution recipient mice were sacrificed and appropriate tissues frozen in OCT compound (Miles, Elkhart, IN) for sectioning and staining as previously described (*30*). Each block was sectioned at three levels to ensure representative sections. Immunohistologic staining of frozen sections was performed using biotinylated anti-CD4, anti-CD8 (Becton Dickinson), anti-IgM, and -IgA (Southern Biotechnology Associates). Sections were developed using horseradish peroxidase-biotin-avidin complexes (ABC Kit, Vector Laboratories), and the DAB reaction products were enhanced by addition of $NiCl_2$. Sections were viewed on an Olympus AH2 (Vanox) photomicrographic apparatus.

Quantitation of serum Ig levels. Serum IgA, IgG, and IgM levels were determined in reconstituted SCID, untreated SCID, and normal DBA/2 mice using a particle concentration fluorescence immunoassay on a Pandex machine (Pandex Division, Baxter Healthcare Corporation Round Lake, IL). Serial dilutions of purified monoclonal IgA, IgM, IgG1, IgG2a, IgG2b, IgG3 or sera from experimental animals were incubated for 30 min at room temperature with equal volumes of goat antimouse Ig coated polystyrene latex particles and either FITC labeled goat antimouse IgM-, IgG-, or IgA-specific antisera (Southern Biotechnology Associates, Birmingham, AL). Wells were washed twice with PBS and the relative fluorescence of each well determined as previously described (*31*).

ELISA analyses. To distinguish donor-derived serum IgM from the low levels of endogenous SCID antibody, an IgM allotype–specific, antigen capture ELISA was established. Individual wells of a 96-well plate were coated with 50 μl of isotype-specific goat antimouse IgM (1 μg/ml) (Southern Biotechnology Associates). Serially diluted serum samples from DBA/2, SCID, and reconstituted mice (20 μl/well) were incubated in the wells for 1 hr at room temperature. Wells were subsequently washed three times with PBS containing 1% BSA and 1% FCS, after which 50 μl of biotinylated IgM^a-specific mAb (1 μg/ml) (PharMingen) was added for an additional 1 hr. The presence of serum IgM^a in the wells was visualized by addition of avidin-biotin-HRP complex (ABC kit, Vector Labs, Burlingame, CA) for 15 min, followed by two washes with PBS and addition of o-phenyldiamine (OPD, Sigma Chemicals) at 0.34 mg/ml. The appearance of product was monitored at 490 nm in a Molecular Devices Vmax reader.

RESULTS

Phenotypic characterization of DBA/2 donor cells. The cell surface phenotype of DBA/2 cells used to reconstitute SCID mice is presented in Table 1. Although the ratio of T:B cells is quite different in each of the populations—1:2.5 in PP; 2:1 in

TABLE 1. FACS analysis of normal DBA/2 lymphoid populations

Phenotypic marker	Peyer's patch	Mesenteric LN	Peripheral LN
Qa-2	76[a]	100	91
CD45R (B220)	69	38	21
IgM	54	23	16
CD3	27	64	79
CD4	22	49	58
CD8	6	14	20
TCR α/β	25	61	78
L-selectin	52	57	79

[a] PP, MLN, and PLN were removed from 4–6 donors, pooled, stained with the indicated reagents and analyzed by flow cytometry. The numbers indicate the percentage of total cells expressing the indicated cell surface antigen.

MLN; and 4:1 in PLN—essentially all lymphoid cells in these organs express the Qa-2 antigen. The majority of T cells in all donor groups are CD4$^+$, and greater than 99% express the α,β form of the TCR characteristic of mature, peripheral T cells. No CD4$^+$/CD8$^+$ double-positive cells were detected. The majority of B cells in each population, as defined by expression of CD45(B220), co-express IgM. Cells scored as CD45(B220)$^+$ IgM$^-$ consist primarily of IgM dull cells and varying numbers of IgA and IgG–staining cells. The expression of L-selectin, a putative lymph node specific homing receptor (*13*), is highest on PLN cells (79%)—however, it should be noted that approximately half of the PP lymphocytes also express this marker.

Temporal appearance of serum Ig in reconstituted mice. The ability of the various donor populations to reconstitute SCID mice was assessed at various times following cell transfer by quantitation of serum Ig levels and splenic lymphocyte composition. At each time point untreated, age-matched SCID and DBA controls were examined along with two reconstituted SCID mice. PP reconstituted animals were monitored for approximately 300 days, whereas in the PLN and MLN studies sufficient mice were reconstituted for analysis to 150 days. Quantitation of serum IgM, IgG, and IgA immunoglobulin is described in Figure 1. Essentially normal levels of all isotypes are seen within 20–30 days postreconstitution, irrespective of the donor population. These levels are maintained throughout the course of the study. It is interesting that the rapid increase in serum Ig preceeds the appearance of donor-derived splenic B cells (see below), which remain low until approximately 100 days postreconstitution. It is also noteworthy that normal amounts of serum IgM, IgA, and IgG are observed with PP donor cells, which have previously been reported to contain a high frequency of IgA-committed precursors (*5–7, 16, 17*). Taken together, these data suggest that PP contain B cells that, upon transfer to SCID recipients, are capable of producing a sufficiently broad spectrum of Ig isotypes to yield normal serum Ig profiles. The donor origin of these immunoglobulins was demonstrated by an allotype-specific ELISA assay wherein virtually all of the serum IgM found in reconstituted mice bears the IgM^a allotypic determinant of the DBA/2 donor (data not shown).

Cellular composition of lymphoid organs in reconstituted mice. To assess the ability of PP, MLN, and PLN-derived cells to repopulate peripheral lymphoid tissues, the cellular composition of spleens from reconstituted mice was examined by FACS analyses. At each time point essentially all T and B cells recovered from recipient spleens expressed the MHC class I Qa-2 molecule (*23*) of the DBA/2 donor population. B cells recovered from these mice were enumerated by expression of Qa-2, CD45(B220), and IgM while T cells were identified by expression of Qa-2, CD4, and CD8. Examples from this analysis are presented in Figure 2. Normal DBA/2 spleen contains the expected proportions of T and B cells, all of which express Qa-2. In contrast, untreated SCID spleen contains few, if any, mature lymphocytes, all of which fail to express Qa-2 due to the absence of this gene in the SCID (C.B17) genome. The apparent expression of Qa-2 among some normal SCID splenocytes is due to a population of highly autofluorescent cells that are 10–20% of normal SCID spleen. These cells are clearly CD45(B220-), IgM-, CD4-, and CD8-negative, and therefore have been excluded from estimates of mature lymphocytes by the appropriate analysis

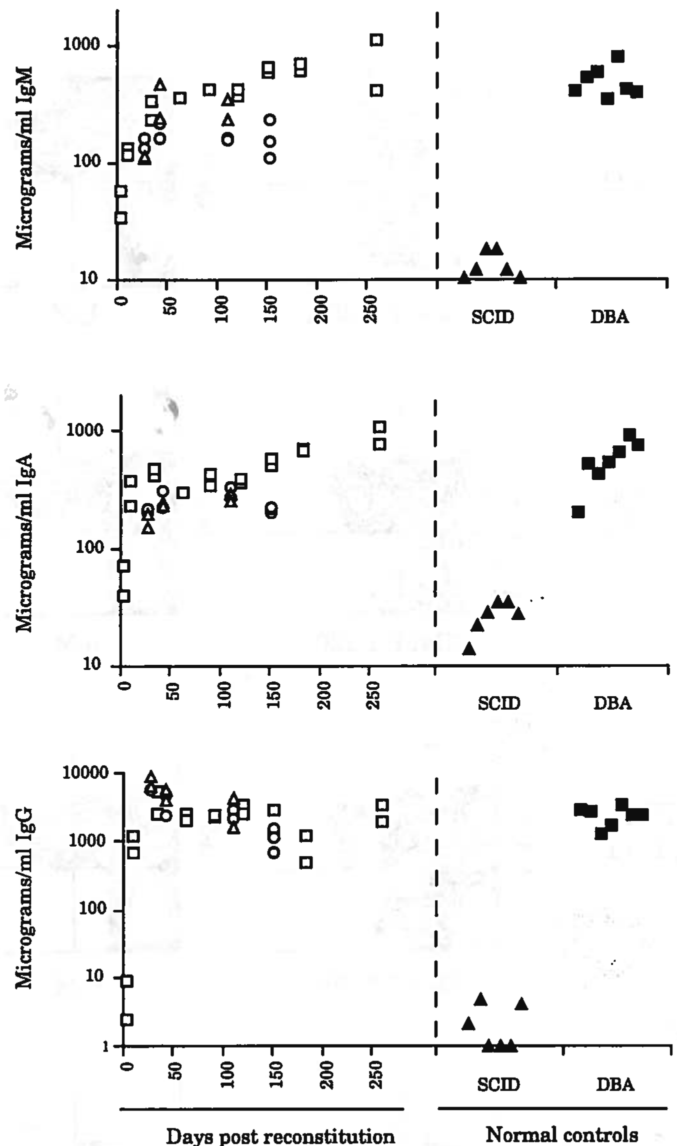

FIGURE 1. Temporal appearance of serum IgM, IgG, and IgA in PP (□), MLN (△), and PLN (○)–reconstituted SCID mice. At each time point two reconstituted mice were sacrificed and the serum IgM, IgG, and IgA quantitated. Each data point represents the concentration of the appropriate Ig isotype detected in an individual mouse.

gates. In PP reconstituted animals splenic B cells of donor phenotype increase gradually from 2% of total spleen cells (1.5% of injected B cells) on day 3, to approximately 35% on day 184, at which time the number of B cells plateaus (Fig. 3). This expansion represents a 130-fold increase (10^5 to 13.1×10^6) in the absolute number of splenic B cells. At approximately day 100 the number of B cells recovered from the spleen alone equals the number initially injected. By day 303 the expanded splenic B cell pool is 191% of the B cells injected. This expansion represents a minimal estimate of the extent to which transferred B cells expand as quantitative representation in all lymph nodes and other organs cannot be readily determined.

In contrast to the gradual increase of splenic B cells, T cells appear to accumulate more rapidly (Fig. 4). The increase in

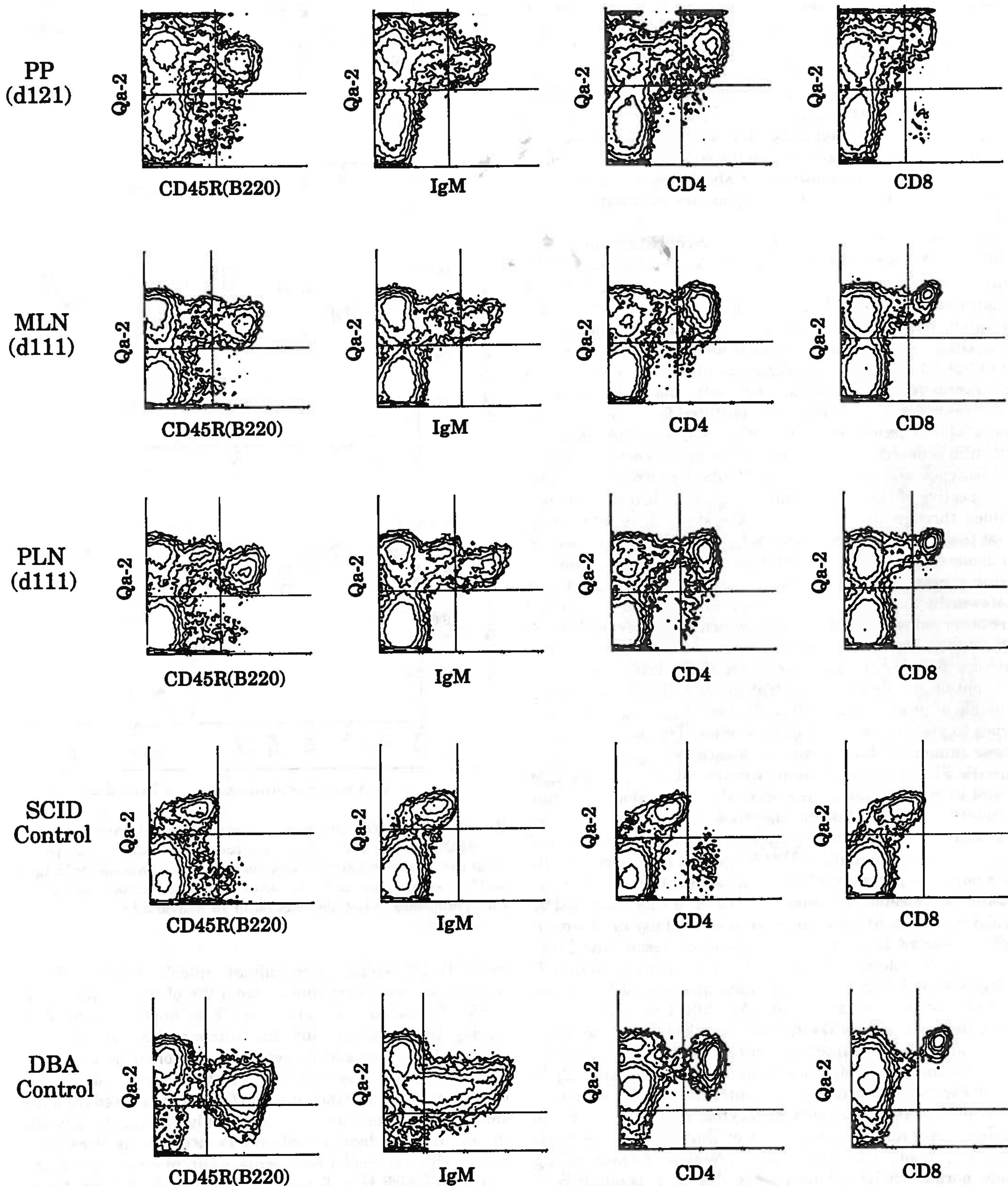

FIGURE 2. Flow cytometric analyses of PP, MLN, and PLN–reconstituted SCID mice. Individual reconstituted mice were sacrificed at the indicated times postreconstitution and splenic T and B cell populations analyzed for expression of CD45R(B220), IgM, CD4, CD8, and the donor cell–specific marker Qa-2. Results are compared with normal SCID and DBA/2 controls that were analyzed at the same time.

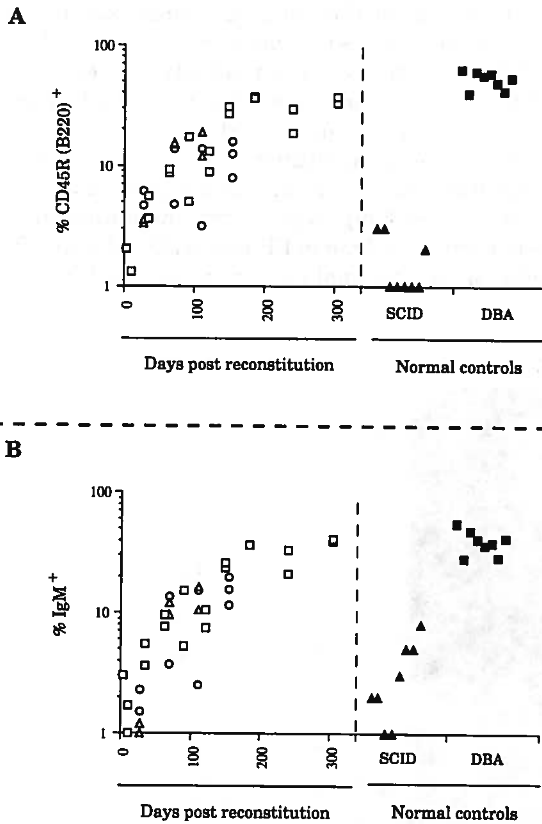

FIGURE 3. Temporal appearance of splenic B cells in reconstituted mice. The proportion of B cells in individual PP (□), MLN (△), and PLN (○)–reconstituted SCID mice, as well as normal SCID and DBA/2 mice was determined by flow cytometric analyses using CD45(B220) (panel A) and IgM (panel B) as lineage-specific markers. At each time point 2 reconstituted mice (the same mice used in serum Ig analyses [Fig. 1]) were analyzed. At day 150, 3 PLN-reconstituted mice were analyzed.

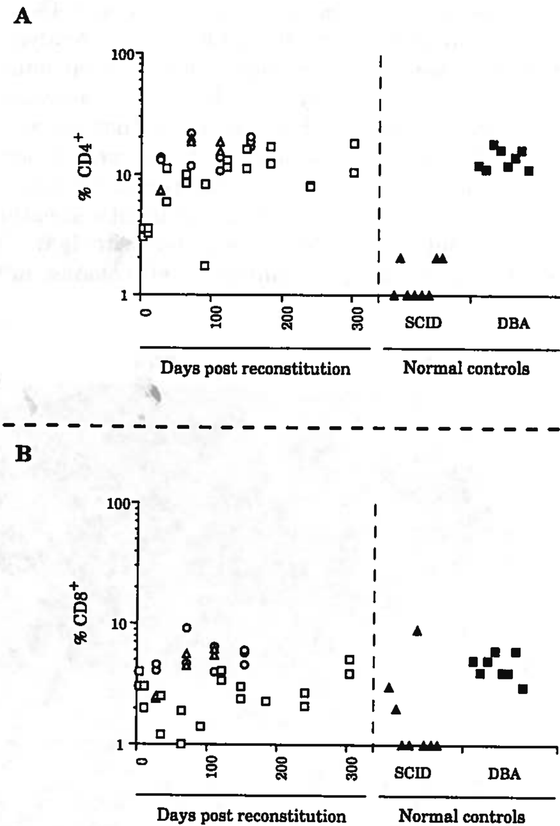

FIGURE 4. Temporal appearance of splenic T cells in reconstituted mice. The proportion of T cells in individual PP (□), MLN (△), and PLN (○)–reconstituted SCID mice, as well as normal SCID and DBA/2 was determined by flow cytometric analyses using CD4 (panel A) and CD8 (panel B) as T cell–specific markers. At each time point 2 reconstituted mice (the same mice used in serum Ig analyses [Fig. 1]) were analyzed. At day 150, 3 PLN-reconstituted mice were analyzed.

splenic T cells occurs primarily during the first 120 days posttransfer, during which time the number of CD4 and CD8 single-positive cells increases from 6% (3% $CD4^+$ and 3% $CD8^+$), representing 15% of the injected T cells, to 17% (15% $CD4^+$ and 2% $CD8^+$). This expansion constitutes a 12-fold increase (0.4×10^6 to 4.7×10^6) in absolute numbers and is due almost exclusively to $CD4^+$ cells. By day 10 the number of T cells recovered in the spleen alone already equals the total number initially injected. Expansion continues for an additional 100 days, at which time the splenic T cell pool is approximately 170% of the T cells injected, followed by a slower increase during the ensuing 180 days, such that by day 303 levels reach 240% of the initial T cell inoculum. The contrast with B cell repopulation is quite striking because the latter does not plateau until approximately day 200.

It is of further note that from day 121 through 303 the proportion of splenic T cells in reconstituted mice remains relatively constant with CD4 and CD8 single-positive cells accounting for 12.5 ± 3.6 and 3.1 ± 1.0 percent of the total cells in the spleen. These values are not significantly different from those observed in normal DBA/2 ($CD4^+$ $14.9\%\pm2.6$ and $CD8^+$ $4.47\%\pm0.9$) mice that were analyzed in parallel and housed in the same barrier facility. The lymphocyte profiles likely reflect minimal antigen stimulation due to the sterile conditions under which these animals are maintained. Similarly, from day 149 to 303 the percent of splenic IgM-staining cells in PP-reconstituted mice ($31.7\%\pm7.5$) does not differ from that found in normal DBA/2 mice ($41.8\%\pm10.6$). Although the representation of splenic IgM-bearing B cells appears normal, the percent total B cells in reconstituted mice ($30.9\%\pm6.2$), as defined by expression of CD45(B220), is only 50–60% of that found in normal DBA/2 spleen ($56.0\%\pm8.8$). The reason for this discrepancy remains unclear, however, one possibility is that a portion of the $B220^+$, IgM^- cells that are absent in reconstituted mice may consist of IgG^+ or IgA^+ B cells.

A similar pattern of B cell reconstitution is seen with either PLN or MLN as the donor source (Fig. 3). Initial B cell repopulation is relatively slow, with a plateau being reached between 70 and 111 days (somewhat earlier than PP-reconstituted animals). The percent of total B cells observed is the same as in PP animals for an equivalent period. T cells, again, repopulate much more rapidly with either donor population and are at nearly normal levels by day 27 for PLN and day 70 for MLN (Fig. 4). Again, $CD4^+$ cells constitute the bulk of T cell expansion.

To further analyze splenic composition, tissue sections from control and reconstituted SCID mice were compared

immunohistologically. Representative sections taken 45–63 days postreconstitution are presented in Figure 5A. Analysis of reconstituted mouse spleens, irrespective of donor population, reveals extensive lymphocytic infiltration as assessed by either immunohistology or H&E staining (not shown). Although this infiltration is less than that observed in normal DBA/2 spleen (Fig. 5B), tissue sections reveal formation of normal structures, with periarteriolar lymphatic sheaths containing $CD4^+$ and $CD8^+$ T cells associated with IgM^+ B cell follicles. Cytoplasmic IgM-staining B cell colonies are also observed throughout the red pulp regions. As might be expected, variation was seen from animal to animal, but MLN and PLN–reconstituted mice routinely showed greater numbers of $CD4^+$ T cells and somewhat fewer IgM^+ B cells than observed among PP-reconstituted mice.

To assess long-term reconstitution of GALT, similarly prepared sections of gut were examined, as it was not possible to quantitatively recover lymphocytes from the entire gut for flow cytometric analysis. Among PP-reconstituted mice, (Fig. 6A), repopulation of intestinal lamina propria and intraepi-

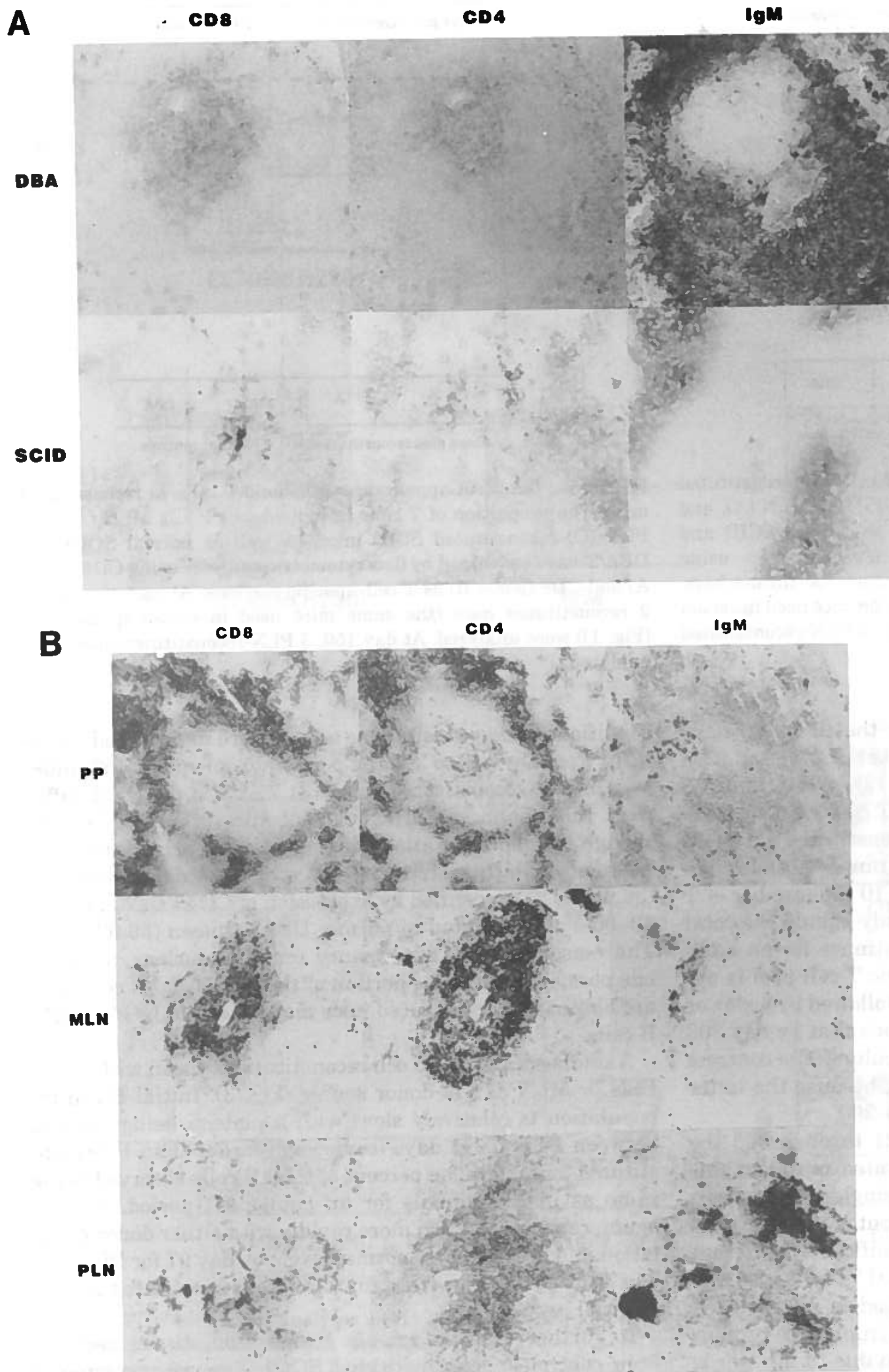

FIGURE 5. Immunohistologic analyses of T and B cell markers in splenic sections from PP, MLN, and PLN–reconstituted SCID mice. Serial sections from reconstituted (A) or normal controls (B) were stained with anti-CD8, anti-CD4, and anti-IgM reagents as shown. PP, MLN, and PLN reconstituted mice were sacrificed 63, 45, and 45 days postreconstitution, respectively.

thelial compartments is qualitatively comparable to that seen in normal controls (Fig. 6b). Extensive cellularity is observed, with a predominance of CD8+ T cells within the intraepithelial compartment and CD4+ cells in the lamina propria. Numerous IgA-secreting plasma cells are found located within the lamina propria. The presence of IgA in epi-

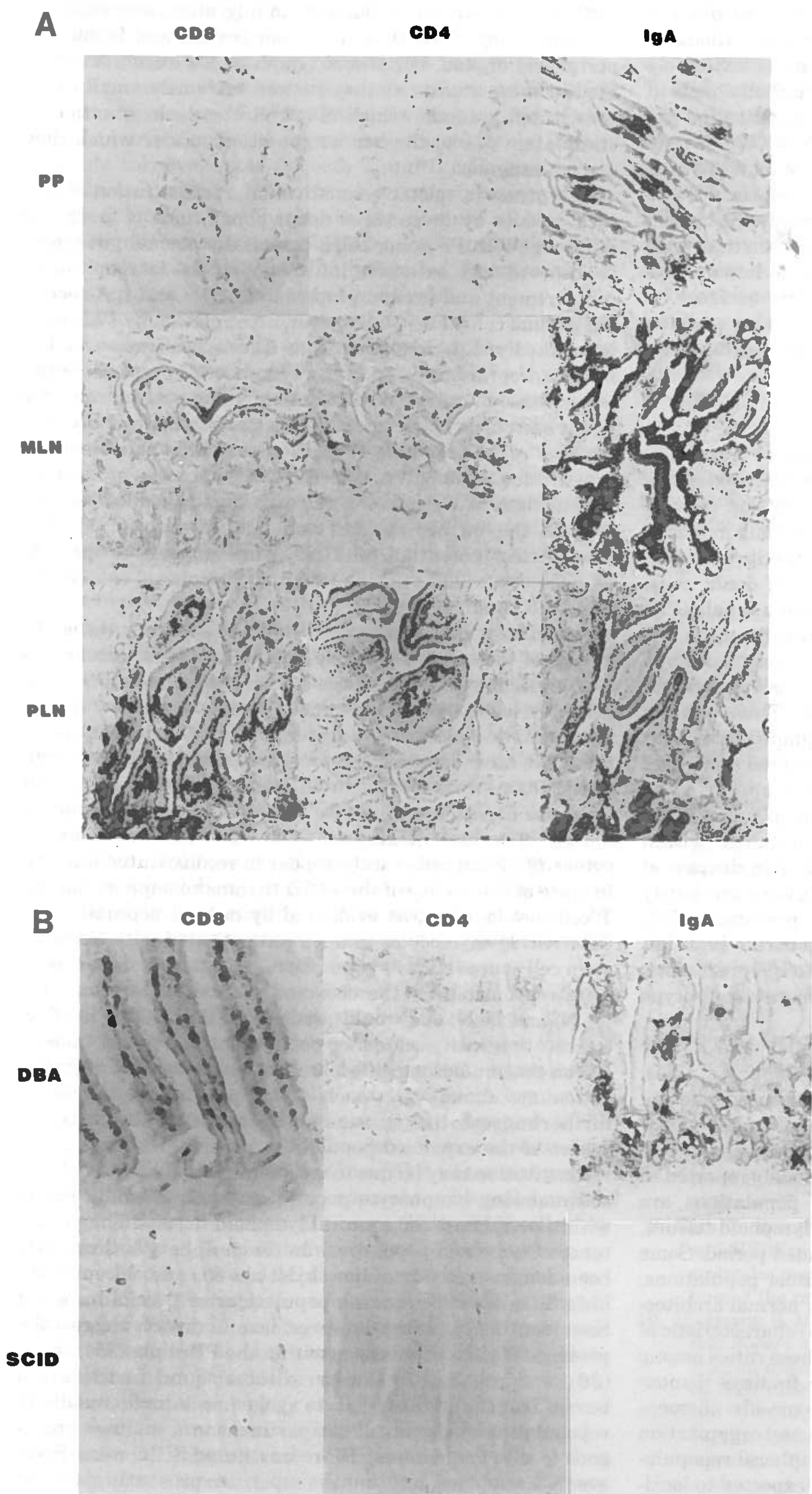

FIGURE 6. Immunohistologic analyses of T and B cell markers in gut sections taken from PP, MLN, and PLN–reconstituted SCID mice. Serial sections from reconstituted (A) or normal controls (B) were stained with anti-CD8, anti-CD4, and anti-IgM reagents as shown. PP, MLN, and PLN–reconstituted mice were sacrificed 63, 45, and 45 days postreconstitution, respectively.

thelial cells overlying plasma cells within the lamina propria is likely due to endosomal transport of secretory IgA. With PLN donor cells, extensive T cell infiltration is also seen, but localization is significantly different than with PP cells. Both $CD8^+$ and $CD4^+$ T cells are found primarily within the lamina propria and are only rarely seen intraepithelially. Reconstitution of IgA-secreting plasma cells is extremely poor although anti-IgA immunostaining of epithelial cells in crypt areas is seen. The pattern of MLN reconstitution appears intermediate to that seen with PP or PLN. $CD8^+$ T cells are fewer than with either PP or PLN, but are found in both the intraepithelial compartment and lamina propria. $CD4^+$ T cells are well reconstituted and found primarily in the lamina propria. Fewer IgA-secreting plasma cells are seen than with PP cells, and these are largely localized to the lamina propria. Particularly strong staining of the crypt surfaces by soluble IgA is also observed. Qualitatively similar staining was seen in sections taken at later time points (data not shown).

DISCUSSION

The present series of experiments demonstrate the ability of donor cells from PP, MLN, or PLN to facilitate extensive and long-term reconstitution of both serum Ig and cellular aspects of the immune system. Analysis of serum Ig indicates that levels and isotype profiles among reconstituted mice, irrespective of mucosal or peripheral origin of donor cells, rapidly become comparable to those of normal animals (Fig. 1) and remain indistinguishable from controls for up to one year posttransfer. The early realization of normal isotype levels is somewhat surprising in that there are very few B cells found in the spleen at this time (Fig. 3). Thus, either a small number of cells are producing large quantities of Ig or the majority of Ig-producing cells are sequestered elsewhere in the periphery. The various isotype levels appear to be subject to normal regulatory mechanisms, independent of the donor source, as they plateau and are maintained within normal ranges. This is particularly interesting in the case of PP-reconstituted mice since these donor cells have previously been shown to contain high numbers of IgA precursors (*5–7, 16, 17*), yet reconstituted animals do not generate high IgA serum levels. Thus, PP contain, in addition to IgA precursors, a population of cells capable of generating a normal Ig isotype profile.

In terms of cellular reconstitution, a number of earlier reports have indicated that, in short-term homing studies, cells from GALT and peripheral lymphoid tissues preferentially return to their tissue origin (*5–8*). Our results demonstrate that, although long-term GALT reconstitution by PP, MLN, and PLN is consistent with that previously reported in short-term homing studies, these donor populations are equally capable of repopulating peripheral lymphoid tissues, as represented by the spleen over an extended period. Some qualitative variation is seen between the donor populations, but in all cases repopulated spleens display normal architecture (Fig. 5), as well as T:B cell ratios (1:1.5) characteristic of normal spleen despite large differences in these ratios among the various donor cell populations. These findings demonstrate the phenomenal influence of organ-specific microenvironments in determining the composition and organization of resident lymphocytes. The extensive peripheral repopulation by PP cells, which might otherwise be expected to localize to mucosal surfaces, is somewhat surprising. In this context, it is noteworthy that 50% of PP donor lymphocytes express the cell surface molecule L-selectin. Although it is not known whether these cells express this marker before entering the PP or acquire it following immigration, expression of L-selectin may allow subsequent emigration and homing to peripheral organs. Peripheral lymph nodes were not evaluated in these studies as they remain extremely small in reconstituted animals, which may reflect a lack of antigenic stimulation due to the barrier conditions under which they are maintained.

In contrast to splenic reconstitution, reconstitution of mucosal tissues by these three donor populations is markedly different. With PP donor cells, essentially normal gut tissue is observed with extensive infiltration of the intraepithelial compartment and lamina propria by T cells and IgA-secreting plasma cells (Fig. 6). However, repopulation by PLN cells is markedly different leading to T cell infiltration of the lamina propria but little, if any, lymphocytic presence in the intraepithelial compartment. IgA-secreting plasma cells are rarely seen in any gut regions. Thus, gut homing by PLN cells remains restricted while PP cells appear fully capable of reconstituting either gut or spleen. MLN donor cells produce an intermediate pattern in the gut with T and B cells observed in both the intraepithelium and lamina propria. The reconstituting potential of MLN cells appear to provide an adequate cellular source for either peripheral or mucosal reconstitution.

The critical question raised by these experiments is the nature of the cells responsible for the long-term reconstitution. A number of possibilities can be considered. First, and probably least interesting, would be reconstitution due to contaminating pluripotential stem cells in the donor populations. We have assessed the presence of stem cells by monitoring thymi from reconstituted animals wherein stem cells would be expected to give rise to a predominant population (~80%) of normal intrathymic $CD4^+/CD8^+$ immature thymocytes (*32*). Such cells rarely appear in reconstituted animals in spite of the ability of the SCID thymus to support normal T cell development, as evidenced by normal populations of $CD4^+/CD8^+$ thymocytes in mice reconstituted with a known stem cell source such as bone marrow. Moreover, if stem cells were responsible for the observed reconstitutions with PP, MLN, and PLN, one would predict identical patterns of reconstitution with each donor population. The present observation that numerous differences in the patterns of reconstitution are found with each donor population, therefore, further suggests that classic stem cells are not likely to be the source of the expanded populations observed. Alternatively, reconstitution may be due to a combination of long-lived and self-renewing lymphocyte populations that normally reside within peripheral and mucosal lymphoid tissues. The persistence of lymphoid populations in the periphery has recently been demonstrated by Rajewsky et al. (*33*) and Sprent et al. (*34*). To date, self-renewing populations of B cells have not been identified in mice—however, several reports suggest the presence of such populations within the PP of pig (*35*), sheep (*36*), and rabbit (*37*) similar to those found in the avian bursa. Transfer of these cells to syngeneic animals results in repopulation of the B cell compartment in a manner analogous to that found among PP-reconstituted SCID mice. However, we would suggest that the expansion presently observed

is due to division of mature cells of established clonotypes rather than differentiation of uncommitted precursor cells. A third explanation for the reconstituting potential of donor cells is that the normal turnover rate of "antigen-naive" lymphocytes is altered by the availability of "space" and the absence of established immunoregulatory networks in the SCID recipient. In this way, lymphocytes that might be deleted by normal turnover events would continue proliferating without antigen-specific stimulation. As the lymphocytic content of a particular tissue approaches normal levels, the appropriate turnover rates for both T and B cells would, presumably, be restored. If correct, this scenario suggests that the life-span of a lymphocyte in the absence of antigenic stimulation is not solely controlled by preprogrammed intracellular events but may also be influenced by extracellular signals received from the microenvironment.

It is also formally possible that the observed repopulation is the result of antigen-driven lymphocyte expansion. Among the cells initially transferred would presumably be long-lived memory lymphocytes previously activated by exposure to antigen. These cells would seed the various lymphoid organs and be subsequently restimulated by antigens present in our colony. The extent to which this mode of reconstitution could limit the peripheral immune repertoire would depend upon the heterogeneity of the initial population. This explanation would seem unlikely, as reconstituted animals are kept under sterile conditions and receive minimal antigenic stimulation. An alternative source of antigen-driven expansion might be the minor histocompatibility differences that exist between DBA/2 and SCID (C.B-17) mice (*38*). This also seems unlikely as our mice have shown no signs of GVH-related disorders for up to one year postreconstitution. Furthermore, syngeneic reconstitution with CB.17 donor cells (unpublished data) results in an identical pattern of repopulation. Considering the phenotype of both donor and reconstituted T cell populations ($CD4^+$, $CD8^-$, α/β^+ or $CD8^+$, $CD4^-$, α/β^+), as well as the rapid T cell expansion, the most straightforward explanation would be a proliferation of mature, single-positive donor cells. The initial B cell repopulation would similarly arise from the division of existing IgM^+ donor cells, although the protracted appearance of these cells (Fig. 3) could allow for repopulation by only a subset of donor B cells.

The above studies indicate that—although PP, MLN, and PLN all contain self-renewing lymphocytic subsets—the reconstituting potentials of these donor populations are not equivalent. In particular, PP cells preferentially home to mucosal surfaces, but are also fully capable of restoring peripheral lymphocyte populations over an extended period. MLN-derived cells result in a similar long-term reconstitution while PLN-derived cells appear incapable of normal gut repopulation despite generating apparently normal lymphocyte populations in peripheral tissues. Thus, PLN cells may be "terminally" differentiated for organ-specific homing receptors, whereas PP and MLN cells can either alter or express multiple phenotypes, resulting in varied patterns of subsequent distribution. The self-renewing capacity as well as the differential homing properties of the various donor populations have potentially important implications for human transplantation biology and gene therapy applications. If lymphocytes from various human lymphoid tissues also exhibit similar homing and reconstitution potentials, then the long-term success of transplantation and gene therapy protocols may depend on the organ source of the donor cells. Clearly bone marrow represents the ideal donor cell source, but in cases where appropriate bone marrow is not available (i.e., certain breast cancer reconstitutions) significant reconstitution may be possible from mature lymphocytes found in PBL or other tissues. Recent gene therapy trials involving ADA-deficient patients suggest that, although ADA-corrected T cells persist in vivo for extended periods, not all immune functions return to normal (*39*). In light of the findings in the current report, this impaired immunoresponsiveness may be due to the restricted homing potential of transduced PBL. However, if transduced lymphocytes in peripheral blood are self-renewing, such cells may prove adequate for therapy regimens where systemic expression is required as long as expression of the transduced gene is maintained. It remains to be determined whether the use of alternative donor populations would avoid the limitations of immune function currently observed. Moreover, identifying donor populations that efficiently repopulate mucosal tissues will be critical in developing future gene therapy protocols that target the various disorders involving mucosal systems. Thus, the SCID mouse provides an ideal experimental tool for the evaluation of such attributes and the ability to now isolate phenotypically homogeneous subpopulations should provide the opportunity for definitive studies of such subsets.

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