

Peripheral Engraftment of Fetal Intestine into Athymic Mice Sponsors T Cell Development: Direct Evidence for Thymopoietic Function of Murine Small Intestine

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Summary

Adult athymic, lethally irradiated, $F_1 \rightarrow$ parent bone marrow-reconstituted (AT \times BM) mice were engrafted bilaterally with day 16–18 fetal intestine or fetal thymus into the kidney capsule and were studied for evidence of peripheral T cell repopulation of 1–12 wk postengraftment. Throughout that time period, both types of grafts were macroscopically and histologically characteristic of differentiated thymus or intestine tissues, respectively. Beginning at week 2 postengraftment, clusters of lymphocytes were present within intestine grafts, particularly in subepithelial regions and in areas below villus crypts. As determined by immunofluorescence staining and flow cytometric analyses, lymphocytes from spleen and lymph nodes of sham-engrafted mice (AT \times BM-SHAM) were essentially void of T cells, whereas in AT \times BM thymus-engrafted (AT \times BM-THG) mice, which served as a positive control for T cell repopulation, normal levels of T cells were present in spleen and lymph nodes by week 3 postengraftment, and at times thereafter. Most striking, however, was the finding that T cell repopulation of the spleen and lymph nodes occurred in AT \times BM fetal intestine-engrafted (AT \times BM-FIG) mice beginning 3 wk postengraftment. Based on H-2 expression, peripheral T cells in AT \times BM-FIG mice were of donor bone marrow origin, and consisted of $CD3^+$, T cell receptor (TCR)- α/β^+ T cells with both $CD4^+8^-$ and $CD4^+8^+$ subsets. Peripheral T cells in AT \times BM-FIG mice were functionally mature, as demonstrated by their capacity to proliferate after stimulation of $CD3\epsilon$. Moreover, alloreactive cytotoxic T lymphocytes were generated in primary in vitro cultures of spleen cells from AT \times BM-FIG and AT \times BM-THG mice, though not in spleen cell cultures from AT \times BM-SHAM mice. Histologic studies of engrafted tissues 3–4 wk postengraftment demonstrated that thymus leukemia (*Tl*) antigens were expressed on epithelial surfaces of intestine grafts, and that both TCR- α/β^+ and TCR- γ/δ^+ lymphocytes were present in intestine grafts. Collectively, these findings indicate that the murine small intestine has the capacity to initiate and regulate T cell development from bone marrow precursors, thus providing a mechanism by which extrathymic development of intestine lymphocytes occur.

25 years ago, Fichtelius et al. (1, 2) predicted that the murine small intestine is a first-level lymphoid organ, and that the intestine intraepithelial lymphocytes (IEL),¹ then called theliolymphocytes, are non-thymus-derived lymphocytes that arise directly from bone marrow precursors. Since then, evidence has come forth from a number of laboratories demonstrating that most, if not all, IEL in mice develop via an extrathymic pathway (3–13). Although the mechanisms

by which extrathymic maturation of IEL occurs have yet to be elucidated, it has been speculated that intestine tissues themselves may be important in the maturation process locally (2, 5, 6, 8–13). In the present study we reasoned that if the intestine is capable of regulating T cell development, T cell repopulation should occur in athymic mice engrafted into the periphery with intestine tissues. To directly test that possibility, athymic, lethally irradiated, bone marrow reconstituted (AT \times BM) mice were engrafted into the kidney capsule with either fetal intestine or fetal thymus, or left ungrafted, and were studied for subsequent T cell repopulation of peripheral immune compartments. Here, we report that functionally mature T cells expressing CD3 and TCR, including al-

¹ Abbreviations used in this paper: AT \times BM, athymic lethally irradiated, bone marrow reconstituted; FIG, fetal intestine engrafted; IEL, intraepithelial lymphocyte; THG, fetal thymus engrafted; *Tl*, thymus leukemia.

loreactive CTL, were seeded throughout peripheral immune compartments of athymic mice engrafted with fetal intestine or fetal thymus, thus formally demonstrating that murine small intestine can itself independently direct T cell development of bone marrow stem cells.

Materials and Methods

Mice. C57BL/6, (C57BL/6 × BALB/c)_{F1}, and CBA mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed at the University of Tulsa vivarium.

Radiation Chimeras and Tissue Engraftment. AT × BM radiation bone marrow chimeras [(BALB/c × C57BL/6)_{F1} → C57BL/6 thymectomized mice] were constructed as previously described (6). 2 wk postchimera construction, AT × BM mice were separated into three groups: one group (AT × BM fetal intestine engrafted [AT × BM-FIG]) received C57BL/6 fetal intestine grafts; a second group (AT × BM fetal thymus engrafted [AT × BM-THG]) received C57BL/6 fetal thymus grafts; a third group (AT × BM nonengrafted [AT × BM-SHAM]) was operated upon but received no grafts. Intestine and thymus tissues used for grafts were obtained from fetal mice of known gestational age and used between days 16 and 20 of fetal life. That time frame is similar to what has been used by others for fetal intestine grafts (14). AT × BM mice were engrafted by bilateral implantation under the kidney capsule as described elsewhere (14). Control animals consisted of AT × BM mice operated upon without graft implantation. In some experiments, mice were engrafted with fetal intestine after thymectomy but before irradiation and bone marrow reconstitution. This was done as an additional control to insure that hematopoietic cells generated in those mice had not been derived from engrafted tissues. Mice were killed 1–12 wk later, examined for absence of thymus and for presence of engrafted tissue, and lymphoid cells from spleen and lymph nodes were recovered.

Antibodies and Flow Cytometry. Antibodies used were: FITC-labeled mouse monoclonal anti-mouse H-2D^d (3-25.4) (PharMingen, San Diego, CA); rat anti-Tl (T3^b) antisera (HD168) (15) (generously provided by Drs. Obata, Stockert, and Old, Memorial Sloan-Kettering Cancer Center, New York); anti-CD45 (M1/9.3.4.HL.2) (16); PE-anti-Thy-1.2 (CalTag, South San Francisco, CA); anti-CD3 (KT3) (17) and biotin-anti-CD3 (YCD3-1) (Gibco Laboratories, Grand Island, NY); PE-anti-CD4 (YTS 191.1) (CalTag); FITC-anti-CD8 (YTS 169.4) (CalTag); biotin-anti-TCR- α/β (H57-597) (PharMingen); biotin-anti-TCR- γ/δ (GL3) (PharMingen); biotin-anti-TNP (PharMingen) used as control antibody; control rat antisera (Zymed Laboratories, San Francisco, CA); FITC anti-mouse Ig (Zymed Laboratories); PE-conjugated streptavidin (Jackson ImmunoResearch, West Grove, PA). For direct staining of lymphocytes (CD4, CD8, Thy-1, surface Ig, H-2^d), cells were reacted with a primary antibody (PE or FITC labeled), washed, and fixed in 2% formaldehyde. For indirect staining, cells were reacted with a biotin-labeled primary antibody (anti-CD3, anti-TCR- α/β , anti-TCR- γ/δ or control mAb), with unlabeled antibody (anti-CD45), or with isotype-matched control antibody, washed, reacted with PE-labeled anti-rat Ig or PE-labeled streptavidin as needed, washed, and fixed. Cells were analyzed on an EPICS 751 flow cytometer interfaced to an MDADS II computer (Coulter Electronics Inc., Hialeah, FL).

Histology. Engrafted fetal intestine and thymus tissues from AT × BM-FIG and AT × BM-THG mice were fixed in PBS-formalin (10%), and 7- μ m paraffin-embedded sections were cut and stained with hematoxylin and eosin. For identification of Tl or

TCR, 10- μ m frozen sections of intestine tissue grafts were fixed in acetone and pretreated with 0.3% H₂O₂ for 10 min at room temperature to remove endogenous tissue peroxidases. Sections were washed, blocked with normal hamster Ig (Accurate Chemical Co., Westbury, NY), and reacted at room temperature for 30 min with biotinylated anti-TCR- α/β , anti-TCR- γ/δ mAb, or control mAb, or with with rat anti-Tl antibody (15). For anti-TCR staining, tissues were washed and reacted for 30 min at room temperature with streptavidin-horseradish peroxidase (Zymed Laboratories), washed, and reacted with 3-3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO) containing 0.004% H₂O₂. Tissue sections were counter-stained for 1 min with 25% Delafield hematoxylin (Carolina Biological Supply, Burlington, NC). For staining of Tl antigens, tissues were reacted with anti-Tl antibody or control antibody, washed, and reacted with biotin-conjugated anti-rat antibody (Zymed Laboratories). Tissues were washed and reacted with streptavidin-horseradish peroxidase followed by substrate as described above.

Proliferation Assay. Proliferation assays were done as previously reported (13). 5×10^5 spleen cells were cultured at 37°C in 5% CO₂ in 200 μ l in 96-well microtiter plates in tissue culture medium consisting of RPMI 1640 (90%), FCS (10%), 2 mM L-glutamine, 25 mM Hepes, 100 U/ml penicillin, 100 μ g/ml streptomycin (Irvine Scientific, Santa Ana, CA) with anti-CD3 mAb (KT3), anti-CD45 mAb, or unstimulated (medium). After 48 h, cultures were pulsed with 1 μ Ci/well [³H]thymidine (ICN Radiochemicals, Irvine, CA), cultured for an additional 24 h, and harvested using a microanalytical cell harvester (M. A. Bioproducts, Walkersville, MD). Thymidine incorporation was measured by liquid scintillation (Beckman Instruments, Inc., Palo Alto, CA).

Microcytotoxicity Assay. Procedures of microcytotoxicity used in this laboratory have been previously reported (5, 6, 13). 5×10^6 responder lymphocytes (spleen or lymph node) from AT × BM-SHAM, AT × BM-THG, AT × BM-FIG, or normal C57BL/6 mice were cultured for 5 d in 2 ml of supplemented tissue culture medium with 5×10^6 mitomycin C; (Sigma Chemical Co.)-treated splenic allogeneic stimulator cells at 37°C in 5% CO₂. Responder cells were collected, diluted in 96-well microtiter plates, and cultured with ⁵¹Cr-labeled tumor target cells or with splenic lymphocyte blast target cells generated by a 2-d culture with 20 μ g/ml LPS (Sigma Chemical Co.). After a 4-h culture at 37°C, ⁵¹Cr was measured in the cell-free portion. Percent specific ⁵¹Cr release = $100 \times (\text{cpm experimental release}) - (\text{cpm spontaneous release}) / (\text{cpm detergent release}) - (\text{cpm spontaneous release})$.

Results

Histologic Characteristics of Thymus and Intestine Grafts. By 4–6 wk postengraftment, tissue grafts had grown to $\sim 4 \times 8$ mm for intestine and $\sim 3 \times 6$ mm for thymus grafts, and bore histologic characteristics and architecture of differentiated thymus or intestine, respectively (Fig. 1, A and B). Few lymphoid cells were evident in intestine grafts 1 wk postengraftment; however, beginning at week 2 postengraftment, clusters of lymphocytes were present in intestine grafts within villi and in subepithelial regions, particularly below villus crypts (Fig. 1, C and D). That finding closely paralleled what has been previously described for lymphocyte seeding into fetal intestine grafts in mice (14).

T Cells Expressing CD3 and TCR Are Present in Peripheral Immune Compartments of Athymic Mice after Engraftment with Fetal Intestine. Lymphocytes were recovered from lymph nodes

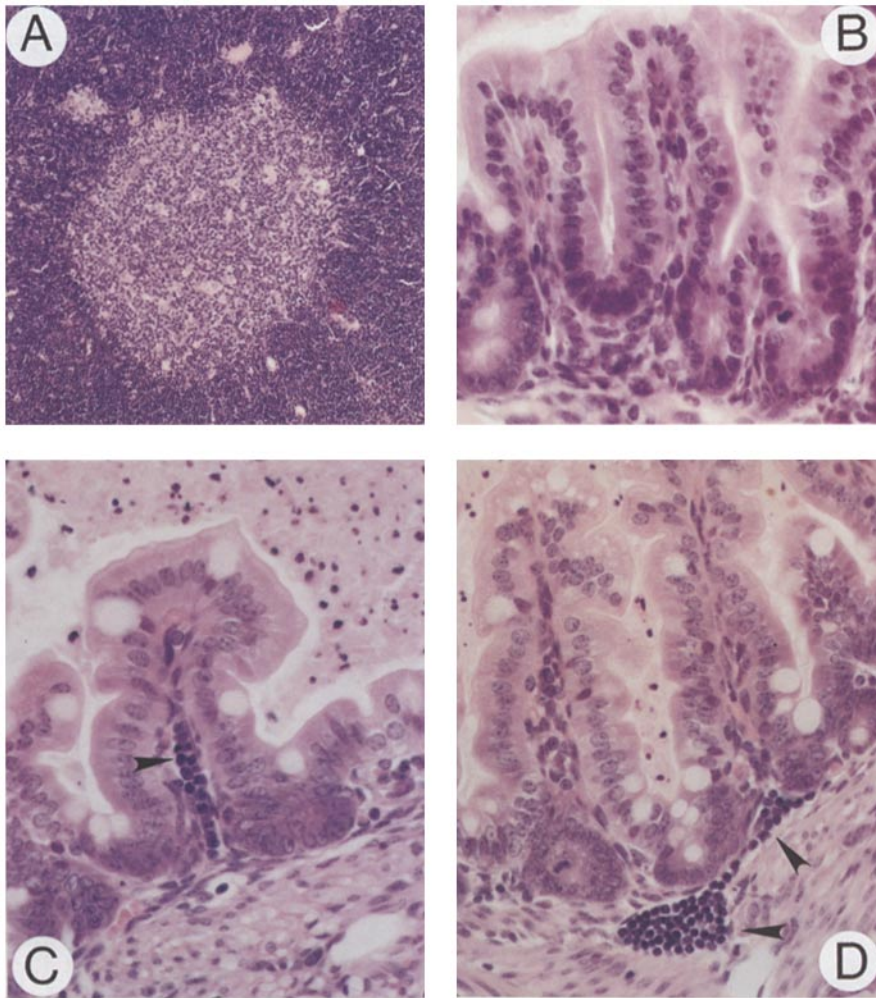


Figure 1. Hematoxylin and eosin-stained tissue sections of thymus and intestine grafts demonstrating the differentiated nature of those tissues. (A) Tissue section of thymus graft 1 wk postenugraftment showing medullary and cortical areas ($\times 100$). (B) Tissue section of intestine tissue graft 1 wk postenugraftment showing characteristic intestine villi ($\times 400$). Intestine tissues 3 wk postenugraftment showing clusters of lymphocytes (arrows) within villi (C) ($\times 400$) and extending into subepithelial regions (D) ($\times 400$). Photomicroscopy was done on a Nikon Microphot FX (Tokyo, Japan).

and spleen 1–12 wk postenugraftment, and were studied phenotypically by flow cytometric analyses and for the presence of functional T cells. Based on H-2^d expression, lymphocytes in all three types of AT \times BM mice were of donor bone marrow origin and thus were not lymphocytes derived from host or from engrafted tissues (Fig. 2). Throughout the study, spleen and lymph node cells from AT \times BM-SHAM mice consisted of B cells with essentially no T cells (Figs. 3–5). The lack of peripheral T cells in AT \times BM mice is a consistent finding, as previously reported by this laboratory (6, 12), and as subsequently determined from a large series of AT \times BM mice ($n = 19$) studied by flow cytometric analyses: $5 \pm 1\%$ Thy-1⁺, $6 \pm 1\%$ CD3⁺, $5 \pm 1\%$ CD4⁺, and $1 \pm 1\%$ CD8⁺ in spleen or lymph nodes (R. L. Mosley and J. R. Klein, unpublished results). AT \times BM-THG mice, which served as positive controls for the experiments at hand, had normal distributions of peripheral T and B cells by week 4 post-thymus engraftment (Figs. 3–5). In AT \times BM-FIG mice, however, the findings were striking in that Thy-1⁺, CD3⁺ T cells, consisting of both CD4⁺8⁻ and CD4⁻8⁺ subsets, were present in spleen and lymph nodes beginning 3 wk postenugraftment (Figs. 3 and 4); peripheral T cells were

not detected in AT \times BM-FIG mice before week 3 (Figs. 3–5). For a brief period at about week 4 post-intestine engraftment, in some AT \times BM-FIG mice as much as 90% of peripheral lymphocytes were T cells (data not shown), suggesting that a strong burst of T cell development had occurred, or possibly that there was a transient disruption of B cell development. After week 4, the proportion of peripheral T cells in AT \times BM-FIG mice (Figs. 3–5) was more typical of what occurs in thymus-bearing mice (18) and AT \times BM-THG mice (Figs. 3–5). Peripheral T cells in AT \times BM-FIG and AT \times BM-THG mice were primarily TCR- α/β ⁺, although some TCR- γ/δ ⁺ T cells also were present (Fig. 5). To determine whether peripheral maturation of T cells via intestine tissues was dependent upon, or influenced by, engraftment within the kidney, some mice were engrafted with fetal intestine subcutaneously in the shoulder region, as described by others (19). As seen in Fig. 6, peripheral T cells also were present in lymph nodes of those mice 3 wk postenugraftment, indicating that maturation of T cells was independent of site of location of engrafted intestine grafts.

It should be noted that peripheral T cell development did not occur in all intestine- or thymus-engrafted mice. Out

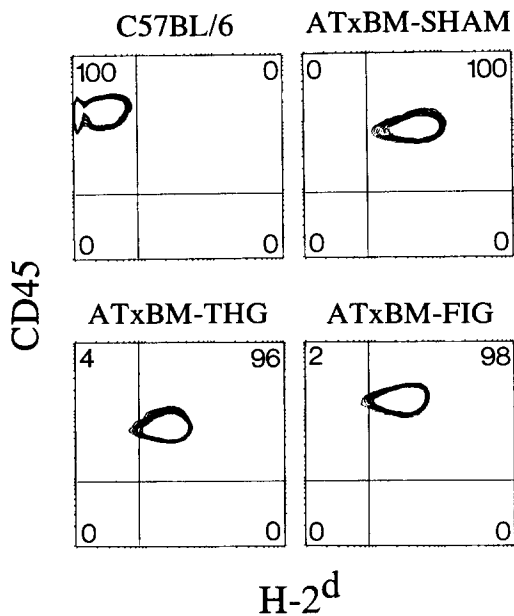


Figure 2. Expression of H-2D^d on peripheral lymphocytes. Lymphocytes from C57BL/6 (H-2^b) mice, the host strain of AT × BM mice, did not express H-2^d, whereas lymphocytes in all three AT × BM groups expressed H-2^d due to (H-2^{d/b})F₁ bone marrow reconstitution. Numbers in histograms indicate percentage reactivity above staining due to isotype control antibodies.

of 22 AT × BM-FIG mice studied in three independent experiments, 13 mice (59%) had evidence of extensive peripheral T cell development. Interestingly, that frequency is remarkably similar to the percentage (60%) of AT × BM mice repopulated with T cells in an earlier study of thymus engraftment (19). The reasons for the failure of repopulation

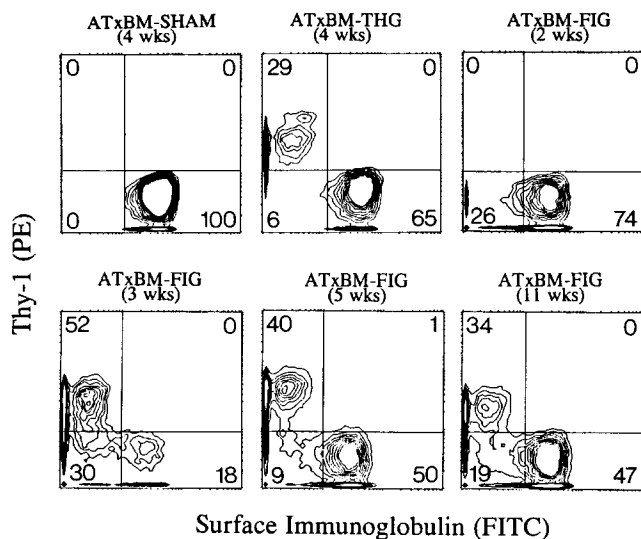


Figure 3. Expression of Thy-1 and surface Ig on peripheral lymphocytes from AT × BM-SHAM, AT × BM-THG, and AT × BM-FIG mice. Numbers in histograms indicate percentage reactivity above staining due to isotypic control antibodies.

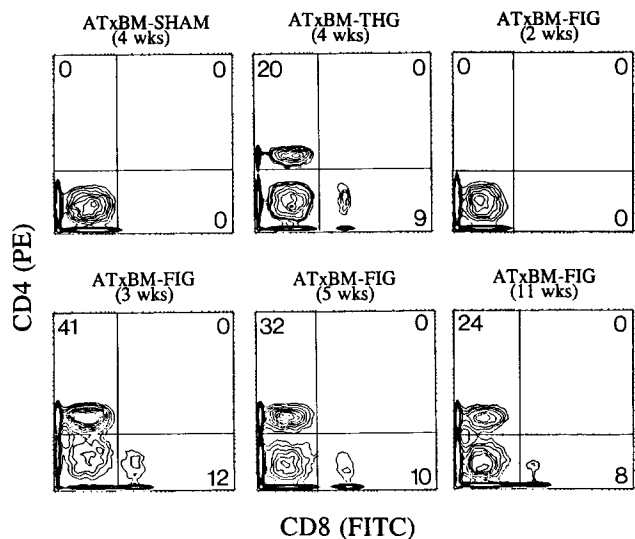


Figure 4. Expression of CD4 and CD8 on peripheral lymphocytes from AT × BM-SHAM, AT × BM-THG, and AT × BM-FIG mice. Numbers in histograms indicate percentage reactivity above staining due to isotype control antibodies.

in some mice are unclear. However, age of fetal intestine tissue used for grafts did not influence T cell development since peripheral T cell development occurred in animals engrafted with fetal intestine obtained from mice between days 16 and 20 of fetal life. It is possible that the nature and/or extent of graft revascularization is critical for T cell repopulation. Regardless, of mice in which T cell development occurred, the actual numbers of T cells were comparable between AT × BM-THG and AT × BM-FIG mice ($43.8\text{--}59.3 \times 10^6$ T cells/spleen for AT × BM-THG; $31.5\text{--}56.4 \times 10^6$ T cells/spleen for AT × BM-FIG).

Epithelial Cells Expressing Tl Antigens, and Lymphocytes Bearing TCR- α/β or TCR- γ/δ , Are Present in Intestine Grafts of AT × BM-FIG Mice. Some murine TCR- γ/δ^+ T cells have been shown to react with antigens encoded by genes in the *Tl* region (20, 21) located distal to the D locus of the MHC (22). It also has been demonstrated that murine small intestine express *Tl* antigens along luminal surfaces and extending into basilar portions of the epithelium (23, 24). That pattern can be seen in immunoperoxidase-stained intestine tissue sections from normal C57BL/6 mice using the anti-*Tl* antibody HD168 (Fig. 7 B), and by the lack of reactivity with rat control antibody for those tissues (Fig. 7 A). Intestine grafts from AT × BM-FIG mice 4 weeks post-engraftment also expressed *Tl* antigens (Fig. 7 C) in a pattern similar to that observed for normal murine intestine (23, 24) (Fig. 7 B), demonstrating that intestine tissues which grow in extra-intestinal sites retain characteristic features of normal gut epithelia.

To determine whether lymphocytes, either TCR- α/β or TCR- γ/δ were present within intestine grafts of AT × BM-FIG mice, histologic sections of intestine grafts were stained with anti-TCR- α/β or anti-TCR- γ/δ mAbs using the immunoperoxidase method. As shown in Fig. 8, both TCR-

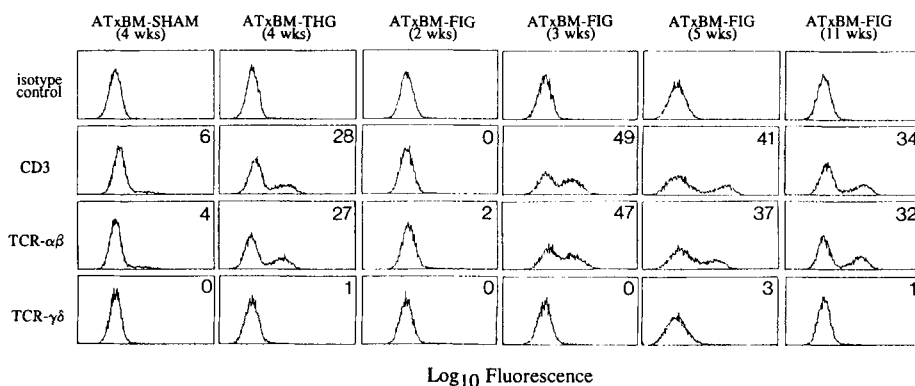


Figure 5. Expression of CD3, TCR- α/β and TCR- γ/δ on peripheral lymph node lymphocytes from AT \times BM-SHAM, AT \times BM-THG, and AT \times BM-FIG mice. Numbers in histograms indicate percentage reactivity above staining due to isotype control antibodies.

α/β^+ (Fig. 8, A and B) and TCR- γ/δ^+ (Fig. 8 C) T cells were present in intestine grafts 3 wk postengraftment. There was a notable difference in the distribution of those cells such that TCR- α/β^+ T cells were primarily found within the lamina propria, extending to the muscularis, and were less frequent within the epithelium (Fig. 8, A and B). Note the clustering of TCR- α/β^+ T cells in interior regions of transverse sections of villi compared with an occasional TCR- α/β^+ T cell in the epithelium (Fig. 8 B). In contrast, TCR- γ/δ^+ T cells were located in or near the epithelium, although some TCR- γ/δ^+ T cells also were present in portions of the lamina propria (Fig. 8 C). Overall, there were approximately threefold more TCR- α/β^+ T cells in intestine grafts than TCR- γ/δ^+ T cells, as estimated from sev-

eral tissue sections examined. Lymphocytes were not stained in intestine grafts using control mAb (data not shown). These findings strongly imply that the T cell development observed in AT \times BM-FIG mice occurred within the intestine grafts themselves.

Peripheral T Cells in Athymic Mice Engrafted with Fetal Intestine Are Responsive to CD3 Stimulation. To evaluate the functional status of peripheral T cells in AT \times BM-FIG mice, spleen cells from all three types of mice were studied for their ability to proliferate after CD3 stimulation, a T cell property normally acquired during intrathymic development. Shown in Fig. 9, cell proliferation was minimal in spleen cell cultures in the absence of stimulation, or upon culture with mAb to antigens that do not induce cell proliferation (anti-CD45). Stimulation of AT \times BM-SHAM spleen cells with anti-CD3 mAb also failed to cause proliferation due to a lack of T cells in those mice. However, CD3 stimulation of spleen cells from AT \times BM-FIG mice was comparable to that of AT \times BM-THG mice, demonstrating that splenic T cells in AT \times BM-FIG mice were responsive to CD3-mediated transmembrane signals.

Alloreactive, Antigen-specific CTL Are Generated in In Vitro Cultures of Spleen and Lymph Node Lymphocytes from Athymic Mice Engrafted with Fetal Intestine. T cell responses in AT \times BM-FIG mice were studied in cultures of peripheral lymphocytes stimulated in vitro with allogeneic spleen cells. CTL that lysed alloantigen-bearing tumor target cells, but did not lyse syngeneic target cells, were generated in primary antigen-stimulated spleen cell cultures of AT \times BM-THG (Fig. 10 A) and AT \times BM-FIG (Fig. 10 B) mice. That finding was confirmed using alloantigen-stimulated lymphocytes from AT \times BM-FIG mice assayed against syngeneic or allogeneic lymphocyte blasts, and was compared to alloreactive CTL responses generated from normal mice with intact thymus. No CTL activity was detected in alloantigen-primed lymphocyte cultures from AT \times BM-SHAM mice (Fig. 10 C), nor was CTL activity detected in syngeneic-primed lymphocyte cultures from AT \times BM-THG or AT \times BM-FIG mice (data not shown). However, alloreactive CTL, with lytic activity comparable to that generated in cultures of alloantigen-primed lymphocytes from normal mice with intact thymus (Fig. 10 F), were present in in vitro stimulated cultures of AT \times BM-FIG lymph node cells (Fig. 10 D) and spleen cells (Fig. 10

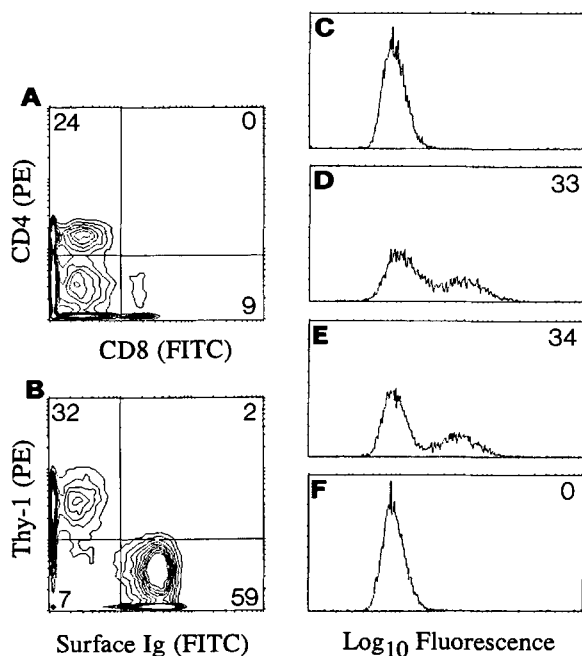


Figure 6. Expression of CD4 and CD8 (A), Thy-1 and surface Ig (B), CD3 (D), TCR- α/β (E), and TCR- γ/δ (F) on peripheral lymphocytes from AT \times BM-FIG mice 4 wk postengraftment with fetal intestine subcutaneously into the shoulder region. Staining due to control mAb is shown (C).

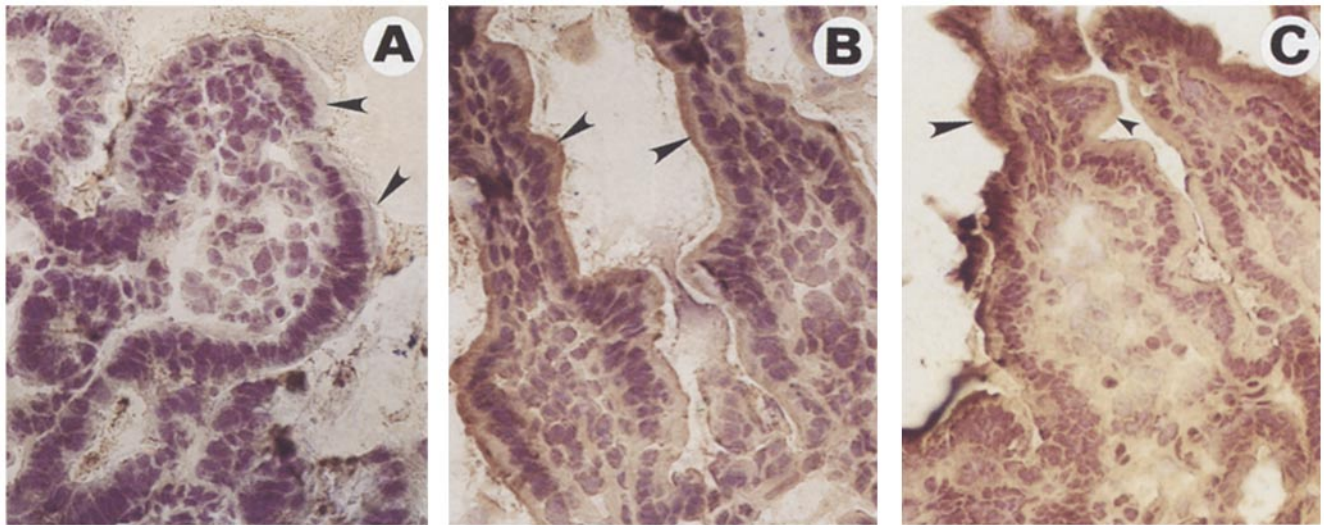


Figure 7. Expression of *Tl* antigens in intestine tissues by immunoperoxidase staining as described in Materials and Methods. No staining is evident in (A) normal C57BL/6 small intestine tissue sections stained with rat control antibody. (B) Staining of *Tl* antigens is evident in tissue sections of normal C57BL/6 small intestine and (C) fetal intestine grafts obtained from AT × BM-FIG mice 4 wk postgraftment when reacted with anti-*Tl* antibody HD168. Arrows indicate positions where *Tl* antigens would be located (×320).

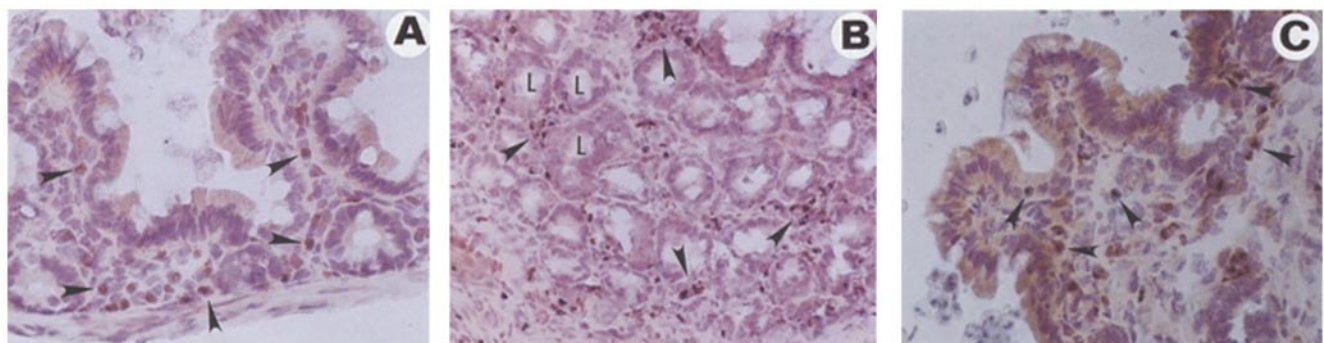


Figure 8. TCR- α/β^+ and TCR- γ/δ^+ T cells are present within intestine tissue grafts from AT × BM-FIG mice 3 wk postgraftment as determined by staining with TCR-specific mAbs as described in Materials and Methods. Clusters of TCR- α/β^+ T cells are primarily, though not exclusively, present in the lamina propria as shown in sagittal (A) (×245) and transverse sections (B) (×155) of villi. TCR- γ/δ^+ T cells are located near the epithelial region (C) (×245). (Arrows) locations of T cells; (L) intestine lumen.

E). Collectively, these data demonstrate that CTL precursors were present in AT × BM-FIG mice, and that those CTL were functionally indistinguishable from CTL in AT × BM-THG mice, or in mice with intact thymus.

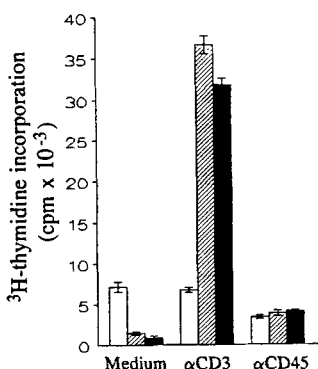


Figure 9. In vitro CD3-induced proliferation of spleen cells from AT × BM-SHAM (open bars), AT × BM-THG (hatched bars), and AT × BM-FIG (filled bars) mice 5 wk postgraftment.

Discussion

Based on studies in neonatally thymectomized mice, Fichtelius et al. predicted that the IEL are a thymus-independent lymphocyte population and postulated that the gut epithelium is a first-level lymphoid organ (1, 2). Although evidence has since come forth demonstrating the extrathymic nature of IEL T cells (3–13), the extent to which the intestine is itself responsible for that developmental process has yet to be elucidated. The present study provides formal evidence that the murine small intestine can directly mediate T cell development of bone marrow precursors. These findings are consistent with an early study involving peripheral engraftment of intestine tissues into mice in which lymphocytes were present in engrafted tissues in a manner similar to that reported here (14). In that study, however, detailed analyses of lymphocytes were not possible due to the unavailability of T cell-specific reagents at that time, nor were lym-

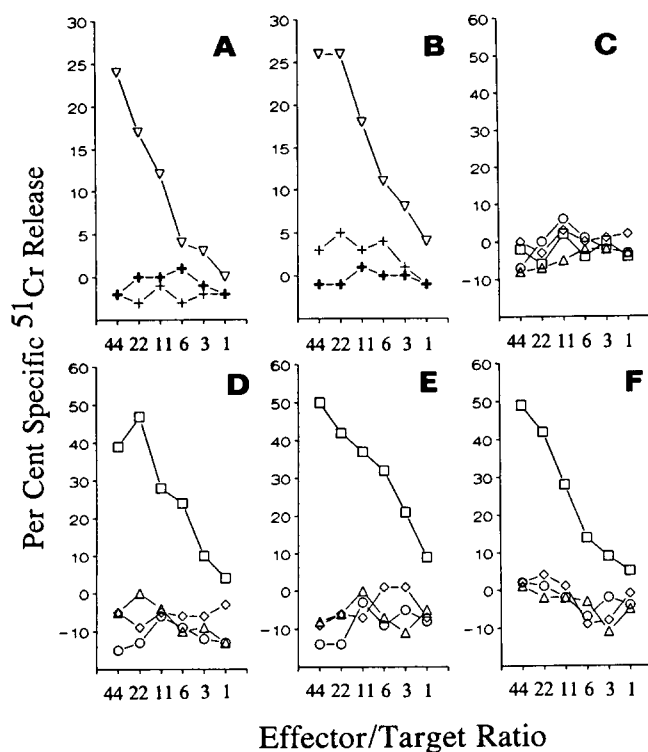


Figure 10. Alloreactive cytotoxic responses of splenic and lymph node lymphocytes after primary *in vitro* stimulation with allogeneic mitomycin C (M/C)-treated CBA (H-2^k) spleen cells. *In vitro* primed spleen cells from (A) AT × BM-THG and (B) AT × BM-FIG mice lysed H-2^k-bearing BW5147 tumor target cells, but did not lyse P815 (H-2^d) or EL4 (H-2^b) target cells. (C) Alloreactive CTL were not generated in primary *in vitro* cultures of AT × BM-SHAM mice. (D) Lymph node and (E) splenic lymphocytes from AT × BM-FIG mice, primed *in vitro* with M/C-treated CBA spleen cells, lysed CBA splenic blasts, but did not lyse non-H-2^k lymphocyte blasts (CB6F1, C57BL/6, BALB/c). (F) Alloreactive cytotoxic activity of C57BL/6 spleen cells primed *in vitro* with M/C-treated CBA spleen cells. Mice were studied 5–6 wk postgraftment. Target cells: BW5147 (▽), P815 (+), EL4 (+), CBA blasts (□), BALB/c blasts (○), C57BL/6 blasts (◇), CB6F1 blasts (△).

phocytes studied in peripheral compartments (14). More recently, it has been reported that early hematopoietic development occurs in fetal gut of mice (25–27), providing still further evidence that the small intestine is a site of lymphocyte development.

By all parameters studied, T cells that developed in AT × BM-FIG mice were similar to those in AT × BM-THG mice. In both types of animals, T cells were first detected 3 wk postgraftment and were phenotypically and functionally characteristic of T cells found in the spleen or lymph nodes of normal thymus-bearing mice (18). Peripheral T cells in AT × BM-FIG mice consisted of both CD4⁻8⁺ and CD4⁺8⁻ T cells in roughly equivalent proportions and were nearly all TCR- α/β ⁺ cells. Under natural conditions in mice the gut epithelium contains both TCR- α/β ⁺ and TCR- γ/δ ⁺ T

cells, most of which are CD4⁻8⁺ cells (6–13, 28–40), whereas the lamina propria principally consists of TCR- α/β ⁺ T cells and includes both CD4⁺8⁻ and CD4⁻8⁺ T cells (41, 42). Why so few TCR- γ/δ ⁺ T cells were present in the periphery of AT × BM-FIG mice is not clear, however, that difference could reflect the fact that more TCR- α/β ⁺ T cells were present in intestine grafts than TCR- γ/δ ⁺ T cells. That possibility, coupled with the recent finding that TCR- γ/δ ⁺, but not TCR- α/β ⁺, intestinal T cells selectively undergo apoptosis in culture (32), would allow longer-lived TCR- α/β ⁺ T cells to repopulate the spleen and lymph nodes over time. However, regardless of differences noted in T cell populations both inside and outside the intestine, the basic finding holds true, i.e., that the murine small intestine has the capacity to promote T cell development of bone marrow precursors.

It is interesting that under normal conditions few if any T cells migrate from the intestine epithelium to other peripheral tissues, as shown by the fact that T cells are abundant in the gut epithelium but not in the periphery of athymic mice (3–13), and as noted by the lack of crosscirculation between intestine and peripheral lymphocytes (42). Although it is not clear why peripheral T cells emigrated from intestine grafts in AT × BM-FIG mice, a number of factors could account for that. It is possible that revascularization of intestine grafts disrupts normal physical barriers that restrict or limit lymphocyte trafficking, thereby causing changes in migration patterns of lymphocytes into and out of intestine tissues. Second, given the influence of intestinal microflora and luminal antigens on IEL phenotype (30, 43) and function (43), the potential effects of the intestinal environment on lymphocyte migration cannot be overlooked. It is possible that sterile intestine tissues, such as those used for fetal intestine grafts in the present study, influence lymphocyte migration in a manner different from that of normal intestine tissues. Moreover, there is mounting evidence demonstrating that the intestinal immune compartment is a dynamic and highly pliable population of cells consisting of effector (5, 6, 13, 28) and regulatory subsets (37–39). Thus, intestine tissues transplanted to extraintestinal sites might receive signals that differ from those occurring within the gastrointestinal tract itself.

The data presented here, in conjunction with recent findings from other laboratories, suggest a detailed mechanism for extrathymic T cell development at the level of the murine small intestine. It has been previously demonstrated that the recombinase-activating gene (RAG-1) is expressed in murine small intestine tissues (10), and that both combinatorial and junctional diversity occur for the γ genes in IEL- γ/δ ⁺ T cells in athymic mice (12). Moreover, positive selection of TCR- γ/δ ⁺ IEL by MHC-encoded molecules (8) and clonal energy of potentially autoreactive T cell clones (39) now have been described for murine IEL. Taken together, those observations indicate that the murine small intestine is in many ways a functional thymus analogue locally.

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