Regulation of Extrathymic T Cell Development and Turnover by Oncostatin M

Catherine Boileau, Magali Houde, Gaël Dulude, Christopher H. Clegg and Claude Perreault

J Immunol 2000; 164:5713-5720; doi: 10.4049/jimmunol.164.11.5713
http://www.jimmunol.org/content/164/11/5713

References  This article cites 79 articles, 37 of which you can access for free at:
http://www.jimmunol.org/content/164/11/5713.full#ref-list-1

Subscription  Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions  Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts  Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Regulation of Extrathymic T Cell Development and Turnover by Oncostatin M

Catherine Boileau,* Magali Houde,* Gaël Dulude,* Christopher H. Clegg, † and Claude Perreault²*¹

Chronic exposure to oncostatin M (OM) has been shown to stimulate extrathymic T cell development. The present work shows that in OM transgenic mice, 1) massive extrathymic T cell development takes place exclusively the lymph nodes (LNs) and not in the bone marrow, liver, intestines, or spleen; and 2) LNs are the sole site where the size of the mature CD4⁺ and CD8⁺ T cell pool is increased (6- to 7-fold). Moreover, when injected into OM transgenic mice, both transgenic and nontransgenic CD4⁺ and CD8⁺ T cells preferentially migrated to the LNs rather than the spleen. Studies of athymic recipients of fetal liver grafts showed that lymphopoietic pathway modulated by OM was truly thymus independent, and that nontransgenic progenitors could generate extrathymic CD4⁺CD8⁺ cells as well as mature T cells under the paracrine influence of OM. The progeny of the thymic-independent differentiation pathway regulated by OM was polyclonal in terms of Vβ usage, exhibited a phenotype associated with previous TCR ligation, and displayed a rapid turnover rate (5-bromo-2'-deoxyuridine pulse-chase assays). This work suggests that chronic exposure to OM 1) discloses a unique ability of LNs to sustain extrathymic T cell development, and 2) increases the number and/or function of LN niches able to support seeding of recirculating mature T cells. Regulation of the lymphopoietic pathway discovered in OM transgenic mice could be of therapeutic interest for individuals with thymic hypoplasia or deficient peripheral T cell niches. The Journal of Immunology, 2000, 164: 5713–5720.
evaluate the development and turnover of extrathymic T cells produced under the influence of OM. We found that chronic production of OM endowed LNs with the unique ability to sustain T cell development and attract mature T cells. These extrathymically produced T cells had a diversified TCR Vβ repertoire, showed a rapid turnover rate, and expressed differentiation markers associated with previous TCR ligation.

Materials and Methods

Mice
C57BL/6 J (B6; Thy-1.2−) and B6.PL-Thy-1.1 (B6; Thy-1.1+) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). LckOM transgenic mice were initially provided by Bristol-Myers Squibb Pharmaceutical Research Institute (Seattle, WA). In LckOM mice, the p561Lck promoter targets expression of the bovine OM gene to thymocytes of age, heterozygous transgenic mice were obtained by breeding heterozygous LckOM males with B6 females. The LckOM genotype was confirmed in each animal by visual inspection at the time of sacrifice. Cell transplants were performed at least 2 wk after surgery.

Thymectomy
At 4–5 wk of age, mice were anesthetized by i.p. injection of 75 mg/kg sodium pentobarbital (Somnotol, MTC Pharmaceuticals, Cambridge, Ontario, Canada), and the thymus was removed with a suction cannula introduced over the suprasternal notch. Completeness of thymectomy was verified in each animal by visual inspection at the time of sacrifice. Cell transplantation was performed at least 2 wk after surgery.

Bone marrow and fetal liver cell transplantation
Bone marrow collected from the femurs and tibias of LckOM donors was T cell depleted with a specific anti-Thy-1.2 mAb (Cedarlane, Hornby, Canada) and washed twice in cold PBS. Then, uniradiated recipients were injected via the lateral tail vein with a spleen cell suspension containing 4 × 10^6 CFSE-labeled T lymphocytes, and the spleen and mesenteric LNs were removed 36 h later for flow cytometric analysis.

Isolation of hepatic and intestinal lymphocytes
Isolation of hepatic and intestinal intraepithelial lymphocytes was performed using density centrifugation as previously described (29, 36).

FIGURE 1. Proportion of lymphocyte subsets in the thymus, mesenteric LNs, and spleen of LckOM mice and B6 controls. Based on three-color staining, cells were defined as double-negative T cells (Thy-1−CD4−CD8−), double-positive T cells (Thy-1+CD4+CD8+), single-positive T cells (Thy-1−CD4+CD8− or Thy-1−CD4−CD8+), or B lymphocytes (Thy-1−CD19+). Results represent the mean of three or four mice per group.

Monoclonal Abs
The following Abs were obtained from PharMingen (Mississauga, Canada): Cy-Chrome-conjugated anti-CD4 (RM4-5; rat IgG2a, anti-CD8α (53-6.7; rat IgG2a, anti-CD19 (53-6.7; rat IgG2a, anti-TCR-β (B21.5; rat IgG2a, anti-TCR-α (KJ25; hamster IgG) detected with FITC-streptavidin, FITC-conjugated anti-Thy-1.2 (53.2-1; rat IgG2a, anti-Vβ5.1,2 TCR (M90-4; mouse IgG1, anti-Vβ6 TCR (RR4-7; rat IgG2b, anti-Vβ7 TCR (TR310; rat IgG2b, anti-Vβ8.1.2 TCR (MR5-2; mouse IgG2a, anti-Vβ9 TCR (MR10-2; mouse IgG1, anti-Vβ10 TCR (B21.5; rat IgG2a, anti-Vβ11 TCR (RR3-15; rat IgG2b, anti-Vβ13 TCR (MR12-3; mouse IgG1, anti-Vβ14 TCR (14-2; rat IgM, anti-Vβ17 TCR (KJ23; mouse IgG2a, PE-conjugated anti-Thy-1.1 (OX-7; mouse IgG2a, anti-conjugated anti-Thy-1.2 (30-9; rat IgG2b, PE-conjugated anti-CD19 (ID3; rat IgG2a, PE-conjugated anti-CD4 (IM7; rat IgG2b, PE-conjugated anti-CD45RB (23G2; rat IgG2a, PE-conjugated anti-CD62L (MEL-14; rat IgG2a, PE-conjugated anti-CD122 (IL-2R β-chain; TM-β1; rat IgG2b, and PE-conjugated anti-NK1.1 (PK136; mouse IgG2a, Abs and their isotypic controls. PE-conjugated anti-CD8α was purchased from Cedarlane, FITC-conjugated anti-BrdU was obtained from Becton Dickinson (Mountain View, CA), and Cy5-streptavidin was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA).

Flow cytometry and BrdU labeling
Cell surface staining and BrdU labeling were performed as previously described (37, 38). Analyses were performed with a FACS Calibur flow cytometer using CellQuest software or with a FACS Scan flow cytometer using LYSIS II software (all from Becton Dickinson).

In vivo cell trafficking
Spleen cells from 12- to 20-wk-old B6 or LckOM donors were labeled with carboxy-fluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Eugene, OR) as previously described (39). Splenocytes (10^7) were incubated at 37°C for 15 min in PBS (2 ml) supplemented with CFSE (0.5 μM) and washed twice in cold PBS. Then, uniradiated recipients were injected via the lateral tail vein with a spleen cell suspension containing 4 × 10^6 CFSE-labeled T lymphocytes, and the spleen and mesenteric LNs were removed 36 h later for flow cytometric analysis.

Results
LNs represent the sole site of massive extrathymic T cell development in LckOM mice
The relative and absolute numbers of lymphocyte subsets found in the thymus, LNs, and spleen of LckOM mice and normal B6 controls, aged 4–20 wk, are depicted in Figs. 1 and 2, respectively. The most dramatic findings were observed in the LNs, which, at 12 wk and to a lesser extent by a more progressive increase in the numbers of B cells and single-positive CD4+ and CD8−...
lymphocytes that rose progressively from 4–20 wk. Data depicted in Figs. 1 and 2 concern mesenteric LNs; other LNs (axillar and cervical) showed the same proportions of various lymphocyte subsets, but were slightly less hypercellular than mesenteric nodes (data not shown). LckOM spleens were also hypercellular. In the spleen, however, increased cellularity was due essentially to an accumulation of B lymphocytes; there was a minimal accumulation of immature T cells and no significant increase in the number of CD4+ or CD8+ T cells. Young (4-wk-old) LckOM mice presented severe thymic hypoplasia with very low numbers of immature thymocytes. Thymic cellularity increased with age in LckOM mice, but this was due mainly to a major accumulation of B cells and, to a lesser extent, to increasing numbers of single-positive CD4+ and CD8+ T cells. Immature thymocytes were virtually absent from the thymus of old (20-wk-old) LckOM mice.

Because extrathymic T cell development can take place in the liver (28, 29), intestine (27, 40), and bone marrow (25, 26), we assessed the number of CD4+CD8− thymocytes as well as single-positive CD4+ and CD8+ T cells in these organs in LckOM mice (Fig. 3). We found no notable increase in the number of CD4+CD8−, CD4+, or CD8+ T cells in the bone marrow and intestines compared with B6 mice. A minimal, but statistically significant, accumulation of CD4+CD8+ cells was evident in the liver. Together, these results indicate that LckOM LNs are remarkable in at least two ways. First, assuming that developing thymocytes must go through a CD4+CD8− stage, we can conclude that the LNs constitute the sole site where massive extrathymic thymopoiesis occurs in LckOM mice. As judged by the number of CD4+CD8+ T cells, the level of T cell production in the LNs of LckOM mice is considerable. Thus, in the mesenteric LNs alone, it reaches a level of $214 \times 10^6$ at 12 wk of age (Table I). Second, LNs of OM transgenic mice also present a conspicuous increase in the pool size of mature CD4+ and CD8+ T cells (Fig. 2). Hence, the mean numbers of single-positive T cells in the mesenteric LNs at 12 and 20 wk of age were 43 and $92 \times 10^6$ in the case of LckOM mice comparatively with 7 and $12 \times 10^6$ for B6 mice (Table I and data not shown).

**CD4+ and CD8+ T lymphocytes are CD44high in LckOM mice**

Analysis of expression of CD44, CD45RA or RB, CD62L, and IL-2Rβ gives important information regarding previous Ag encounter by T cell populations. As depicted in Fig. 4, the phenotype

![FIGURE 2. Absolute numbers of lymphocyte subsets in the thymus, mesenteric LNs, and spleen of LckOM mice and B6 controls. Populations are defined in Fig. 1. Results represent the mean of three or four mice per group.](http://www.jimmunol.org/content/jimmunol/151/12/5715.full.pdf)

**FIGURE 3.** T lymphocyte subsets in the bone marrow (tibiae plus femurs), liver, and intestine of LckOM and B6 mice. Populations are defined in Fig. 1. DP, Thy-1+CD4+CD8− cells. The number (mean ± SD) of DP cells in the various organs is shown above the bars. There were three or four mice per group. *p < 0.05, by Student's t test.

### Table I. Absolute number of lymphocytes in the thymus, mesenteric LNs, and spleen of 12-wk-old LckOM and C57BL/6 mice

<table>
<thead>
<tr>
<th>Organ</th>
<th>Phenotype</th>
<th>LckOM (×10⁶ ± SD)</th>
<th>C57BL/6 (×10⁶ ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>CD19+</td>
<td>24.32 ± 12.4</td>
<td>0.39 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>CD4+</td>
<td>8.53 ± 0.96</td>
<td>5.59 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>CD8+</td>
<td>8.83 ± 3.31</td>
<td>3.52 ± 1.83</td>
</tr>
<tr>
<td></td>
<td>DP</td>
<td>0.5 ± 0.32</td>
<td>63.57 ± 7.7</td>
</tr>
<tr>
<td></td>
<td>DN</td>
<td>3.49 ± 0.12</td>
<td>1.63 ± 0.2</td>
</tr>
<tr>
<td>Mes LNs</td>
<td>CD19+</td>
<td>95.62 ± 83.8</td>
<td>3.87 ± 1.91</td>
</tr>
<tr>
<td></td>
<td>CD4+</td>
<td>24.34 ± 20.8</td>
<td>4.23 ± 0.65</td>
</tr>
<tr>
<td></td>
<td>CD8+</td>
<td>18.18 ± 6.63</td>
<td>2.82 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>DP</td>
<td>214.8 ± 121.9</td>
<td>0.11 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>DN</td>
<td>17.41 ± 13.1</td>
<td>0.32 ± 0.17</td>
</tr>
<tr>
<td>Spleen</td>
<td>CD19+</td>
<td>147.3 ± 143.5</td>
<td>29.07 ± 13.6</td>
</tr>
<tr>
<td></td>
<td>CD4+</td>
<td>14.61 ± 12.9</td>
<td>17.85 ± 9.72</td>
</tr>
<tr>
<td></td>
<td>CD8+</td>
<td>12.57 ± 12.6</td>
<td>9.46 ± 4.18</td>
</tr>
<tr>
<td></td>
<td>DP</td>
<td>2.25 ± 3.23</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>DN</td>
<td>4.84 ± 3.03</td>
<td>2.44 ± 1.0</td>
</tr>
</tbody>
</table>

*a* Four to five mice per group.  
*b* DP, double positive T lymphocytes (Thy-1+CD4+CD8−).  
*c* DN, double negative T lymphocytes (Thy-1+CD4−CD8−).
of LN CD4\(^+\) and CD8\(^+\) cells was strikingly different in LckOM mice relative to B6 controls. In LckOM mice, most CD4\(^+\) T cells were CD44\(^{high}\), CD45RB\(^{low}\), CD62L\(^{low}\), and IL-2R\(^{low}\), a phenotype found following TCR engagement by either non-self Ags or self ligands (41–43). In addition, the vast majority of CD8\(^+\) T cells were CD44\(^{high}\), CD45RB\(^{high}\), CD62L\(^{high}\), and IL-2R\(^{high}\). The CD44\(^{high}\)CD62L\(^{high}\) phenotype is found in two types of CD8\(^+\) cells: revertants and class I-restricted T cells triggered by self ligands (42–44). Thus, the phenotype of both CD4\(^+\) and CD8\(^+\) T cells of LckOM mice does not correspond to that of resting cells, but, rather, suggests that these cells have sustained significant levels of TCR signaling by heretofore undetermined ligands. Parenthetically, an activated phenotype can also be found in NK T cells or CD4\(^+\)CD8\(^-\) mesenteric LN T lymphocytes from 6-wk-old LckOM (bold line) and B6 mice (dotted line). Three-color staining was performed with anti-CD4, anti-CD8, and anti-CD69. The percentage of LckOM cells on right side of the marker is indicated. These results are representative of three such experiments.

Extrathymic T cells have a polyclonal Vβ repertoire and a rapid turnover rate

In LckOM mice, aged 12–20 wk, the total numbers of single-positive CD4\(^+\) and CD8\(^+\) T cells was significantly increased relative to that in normal mice (Fig. 2). Therefore, we asked whether these mature T cells had a polyclonal origin and how their expansion could be explained in kinetic terms. Functional in vitro studies of cytokines of the IL-6 family suggest that OM could have pleiotropic effects on T cell development in vivo. Thus, OM has been shown to support the differentiation of CD34\(^+\) cells into CD3\(^+\) T cells (48). In addition, IL-6, which shares the gp130 receptor subunit with OM (49), can prolong T cell survival (15) and provide costimulation for naive T cells (50, 51) by preventing apoptosis (52). Therefore, to address these questions, we created hemopoietic chimeras by injecting a 1:1 mixture of B6.PL fetal liver cells and T cell-depleted LckOM bone marrow cells into lethally irradiated thymectomized B6 mice and performed studies specifically on Thy-1.1\(^+\) cells (of B6.PL origin). Under these experimental conditions, Thy-1.1\(^+\) cells were 100% of extrathymic origin, as they were derived from the differentiation of fetal liver cells in athymic hosts. Furthermore, Thy-1.1\(^+\) cells were not transgenic themselves, but, rather, developed under the paracrine influence of OM (Fig. 5). Among spleen Thy-1.1\(^+\) cells, both CD4\(^+\) and CD8\(^-\) T cells expressed a TCR Vβ repertoire that was as diverse as that of age-matched B6 and LckOM controls when assessed by flow cytometric analysis (Fig. 5). Although analyses based on size heterogeneity or on sequence of the CDR3 region will be required to assess more precisely the diversity of extrathymic T cells (53, 54), our results indicate that CD4\(^+\) and CD8\(^+\) extrathymic T cells have a polyclonal origin.

BrdU pulse-chase experiments were performed to evaluate the turnover of extrathymic T cells in chimeras. Specifically, we sought to determine whether OM-dependent expansion of extrathymic T cell compartments was due to prolonged survival of resting cells or to an increased proliferation rate. During the pulse period, chimeras and control mice were given BrdU-supplemented water for 20 days (38, 55). Again, analyses in chimeras were performed specifically on Thy-1.1\(^+\) cells. Results for CD62L\(^{low}\) and CD62L\(^{high}\) subsets were analyzed separately, because CD62L\(^{low}\) cells divide more rapidly than CD62L\(^{high}\) cells (38, 55) and because, similar to LckOM mice (Fig. 4), the proportion of CD4\(^+\)CD62L\(^{low}\) cells was much increased in chimeras relative to that in B6 controls. The key finding was that BrdU-labeled CD4\(^+\) and CD8\(^+\) cells accumulated more rapidly among extrathymic T cells than in controls. Thus, when CD62L\(^{low}\) and CD62L\(^{high}\) subsets in chimeras were compared with their normal counterparts in euthymic controls, the rate of appearance of BrdU-labeled cells was more rapid for extrathymic T cells than for classic T cells (Fig. 6). In contrast, the kinetics of BrdU incorporation by Thy-1.1\(^+\)CD4\(^+\)CD8\(^-\) thymocytes in the chimeras’ mesenteric LNcs were similar to those of CD4\(^+\)CD8\(^-\) cells in the thymus of B6 mice (data not shown). After being given BrdU water for 20 days, mice were transferred to normal water to examine the rate of decay of BrdU-labeled cells up to day 70. The disappearance of BrdU-labeled T cells was swifter for extrathymic T cells than for classic T cells (Fig. 6). This was conspicuous in the first 10 days after BrdU withdrawal, when the proportion of BrdU\(^+\) elements was relatively stable in B6 controls but was sharply decreased in extrathymic T cells. Collectively, these results indicate that extrathymic T cells proliferate actively and have a high turnover rate.

**LNs of LckOM attract CD4\(^+\) and CD8\(^-\) T cells**

In LckOM mice LNs differ from the spleen as well as other organs not only in that they are the sole site of extrathymic T cell development, but also because the numbers of LN CD4\(^+\) and CD8\(^-\) T cells are increased ~6- to 7-fold relative to those in age-matched B6 mice (Fig. 2). The selective expansion of the LN single-positive T cell compartment is probably due at least to a minimal extent to the accumulation of T cells produced in situ. However, another explanation would be the preferential homing of recirculating extrathymic T cells to the LNs. To evaluate the latter possibility, we assessed the in vivo distribution of CFSE-labeled splenocytes from B6 and LckOM donors 36 h after injection into
FIGURE 5. TCR repertoire of extrathymic T cells. Hemopoietic chimeras were created by injecting a 1/1 mixture of B6.PL fetal liver cells and T cell-depleted LckOM bone marrow cells into lethally irradiated/thymectomized B6 mice. A. Presence of CD4⁺CD8⁻ cells in the mesenteric LNs of hemopoietic chimeras, 75 days after transplantation. B. A large proportion of CD4⁺CD8⁻ cells originate from nontransgenic fetal liver cells (i.e., are Thy-1.1⁺). A dot-plot histogram gated on CD4⁺CD8⁻ cells is shown. C. Vβ expression patterns in CD4⁺ and CD8⁺ splenocytes from euthymic B6 mice (thymic T cells), LckOM mice, and Thy-1.1⁺ cells (derived from B6.PL fetal liver cells) of hemopoietic chimeras (extrathymic T cells). These results represent the mean of five to seven individuals per group. Error bars represent the SD.

B6 and LckOM hosts. Fig. 7A depicts the results from these studies in the form of mesenteric LN/spleen ratios calculated from the absolute numbers of injected CD4⁺ and CD8⁺ T cells that were recovered from these two sites. The notable finding was that, whatever their source (B6 or LckOM) or their type (CD4⁺ or CD8⁺), the proportion of T cells that home to the LNs was greatly increased in LckOM recipients. Increased mesenteric LN/spleen ratios in OM transgenic recipients were due to both an increased accumulation of T cells in the LN and decreased homing to the spleen (Fig. 7B). It was also observed that the propensity to home to the LN rather than the spleen was greater for B6 than for LckOM T cells. The latter characteristic was T cell autonomous, because when B6 and LckOM splenocytes were coinjected, their respective recovery from the mesenteric LNs and spleen was exactly the same as that shown in Fig. 7 (data not shown). The preferential LN homing of T cells injected into LckOM hosts was quite remarkable considering that the size of the T cell pool in LckOM LNs was already increased and that, in a variety of experimental models, the recovery of injected T cells was inversely related to the number of host T cells already present in lymphoid organs (22, 43, 56, 57).

Discussion

Extrathymic T cell development in LckOM mice points to the existence of a novel pathway of T cell maturation whose unique characteristics raise fundamental issues concerning the regulation of T cell production and homeostasis. From a topographical point of view, the LNs of these mice are most peculiar. They are the sole site of a massive extrathymic T cell production, and they display an unusual propensity to attract recirculating CD4⁺ and CD8⁺ T cells. The single-positive progeny of this extrathymic pathway is polyclonal, shows a phenotype associated with earlier Ag encounter, and displays a rapid turnover rate.

Why T cell development normally takes place in the thymus is not known yet. No adhesion molecule-ligand pair has been identified on T cell precursors or thymic stroma that explains convincingly a selective entry or a preferential survival of T cell precursors in the thymic microenvironment (58–61). Accordingly, the reason why extrathymic T cell production induced by OM is limited to the LNs, particularly the mesenteric LNs, is not inherently obvious. The fact that we found no evidence of extrathymic T cell development in other sites reported to have some ability to support T cell production (namely the liver, bone marrow, and intestines) suggests that chronic exposure to OM induces changes that uniquely affect LN stromal (nonlymphoid) cells. An alternative possibility would be that the LN stroma normally expresses a
The influence of OM.

Cipher the molecular interactions responsible for the striking ability of OM to alter lymphoid organ structure, size, and function. Clearly, further investigations must be pursued to determine the mechanisms by which OM affects these properties.

**FIGURE 7.** Migration of CFSE-labeled LckOM and B6 T cells. Spleen cell suspensions containing $43 \pm 5 \times 10^6$ CFSE-labeled T lymphocytes derived from LckOM or B6 mice were injected through the tail vein of LckOM or B6 recipients. Recipients were sacrificed after 36 h to assess the numbers of CFSE-labeled T cells in the spleen and mesenteric LNs. A. The mesenteric LN/spleen ratio was calculated from the absolute number of CFSE$^+$ T cells recovered from these two sites. Each dot represents one individual. The bar indicates the mean of the group. MLN/spleen ratio differences in LckOM vs B6 recipients were significant ($p < 0.05$, by Student’s t test) for CD4$^+$ and CD8$^+$ T cells from B6 as well as LckOM donors. B. Absolute number (mean $\times 10^6$ $\pm$ SD) of CD4$^+$ and CD8$^+$ B6-derived T cells recovered from the spleen and mesenteric LN of B6 and LckOM recipients. There were five to seven mice per group.

Unique structure/molecule that is essential for the homing and development of OM-conditioned prethymic cells. The absence of immature thymocytes in the spleen of LckOM mice discloses unanticipated heterogeneity in the ability of secondary lymphoid organs to sustain T cell development. The latter observation is consistent with recent evidence that the rules governing the development of organized structure in the spleen and LNs are different. Thus, mice deficient either in osteoprotegerin ligand (a TNF family molecule) or in transcription factor Id2 lack LNs but have a normal spleen, while the reverse is observed in Hox11-deficient mice (62–64). Likewise, B cell/T cell segregation is differentially affected in the spleen vs LNs of LT$\alpha^{−/−}$ and TNF receptor type I $\alpha^{−/−}$ mice (65).

Moreover, some CD4$^+$ CD8$^+$ intrathymic thymocytes (but not prethymic progenitors present in fetal liver) can, when injected into thymectomized nontransgenic mice, develop into both CD4$^+$ CD8$^+$ and single-positive T cells in the LNs but not in the spleen (66). Clearly, further investigations must be pursued to decipher the molecular interactions responsible for the striking ability of LNs to support extrathymic T cell development under the influence of OM.

When transplanted into thymectomized hosts together with OM transgenic bone marrow, nontransgenic fetal liver cells yielded a major accumulation of CD4$^+$ CD8$^+$ T cells in the LNs and generated mature T cells with a polyclonal V$\beta$ repertoire. This suggests that significant levels of thymus-independent positive selection takes place extrathymically (presumably in the LNs) under the paracrine influence of OM; otherwise, CD4$^+$ CD8$^+$ would die by neglect (67, 68). This observation is consistent with evidence that thymic epithelial cells are not the only cells that can support positive selection, and that in vivo positive selection can be mediated by hemopoietic cells (69, 70).

Nevertheless, it remains to be determined whether the extrathymic pathway modulated by OM follows the same rules regarding positive and negative repertoire selection as the classical thymic pathway. Other important questions that must be addressed concern the immunocompetence of extrathymic T cells and whether they are self tolerant. Because reconstitution of nu/nu mice with LckOM bone marrow restored immune responsiveness to allogeneic mouse melanoma cells, the progeny of the OM-dependent pathway shows at least some level of immunocompetence (31). However, it remains to be determined whether T cells that have differentiated in the LNs can generate protective immune responses against microbial pathogens as efficiently as conventional T cells do.

When injected into 12- to 20-wk-old LckOM mice, T cells harvested from the spleen of normal or LckOM donors preferentially homed to the LNs rather than the spleen. This was somewhat unexpected, because 1) in LckOM recipients the size of the T cell pool was normal in the spleen but was increased 6- to 7-fold in the LNs; and 2) injected T cells usually home preferentially to lymphoid organs that contain less T cells (22, 43, 56, 57). This bias is attributed to the higher number of available (or empty) T cell niches in T-depleted as opposed to T-replete lymphoid organs. Thus, one logical extension of our findings is that the number of T cell niches increases under the influence of sustained OM production. Recently, a number of indications have been presented suggesting that resident dendritic cells represent fundamental constituents of the peripheral T cell niches (71–74). Because of their abundant expression of MHC class I and class II molecules and their specific chemokine and cytokine expression profile, dendritic cells seem to have a unique ability to control the homing of postthymic T cells and to provide the continuous TCR ligation required for the survival of naive and memory T cells in the periphery (72, 75–77). Interestingly, OM and Flt3 ligand act synergistically to enhance the in vitro proliferation of hemopoietic stem cells committed to macrophage/dendritic cell formation (78). Therefore, it will be of great interest to evaluate the influence of OM on the number, phenotype, and function of dendritic cells in vivo. The postulated ability of OM to increase the number of functional T cell niches would be, to our knowledge, unprecedented and could be of medical interest in circumstances where the number of such niches is deficient (38).

T cells that have developed extrathymically under the influence of OM display two striking features that are perhaps related: these T cells have a rapid turnover rate and the phenotype of Ag-experienced cells (CD4$^{high}$CD45RB$^{low}$CD62L$^{lo}$ for CD4$^+$ cells, and CD4$^{high}$CD45RB$^{high}$IL2R$\gamma^{high}$ for CD8$^+$ cells). As stated above, a CD4$^{high}$ activated phenotype is indicative of previous TCR interaction either with conventional non-self Ag or with peripheral self ligands (42–44). Two findings argue against the possibility that CD4$^+$ and CD8$^+$ extrathymic T cells have been primed en masse by environmental Ags. First, they show the same nonnaive phenotype (depicted in Fig. 4), without conspicuous skewing of the V$\beta$ repertoire, in LckOM mice 4–18 wk of age (data not shown). The second argument is based on the CD62L phenotype of CD8$^+$ elements. Indeed, although some CD8$^+$ T cells that respond to non-self Ags can revert to a CD62L high phenotype, a CD8$^+$ compartment composed primarily of CD4$^{high}$CD62L$^{high}$ elements has been found, to our knowledge, in only one situation: following expansion driven by self-ligands in lymphopenic hosts (44). In the latter situation, it has been proposed that, consecutive to lymphopenia, the increased level of available (empty) T cell...
niches may allow greater accessibility to niche APCs presenting self ligands or growth factors that promote T cell division (43, 44).

LckOM are certainly not lymphopenic. Thus, we surmise that the activated phenotype of LckOM T cells supports the concept that LckOM mice show a major increase in the number and/or function of T cell niches. This strengthens the need to study the effects of OM on the numbers, phenotype, and function of dendritic cells. In this regard, it is noteworthy that IL-6, which belongs to the same family as OM, has been reported to modify the processing of self ligands by dendritic cells and to increase the presentation of otherwise cryptic epitopes (79). Such a mechanism could be instrumental in expanding the size of the peripheral T cell compartment by increasing the reactivity of T cells toward self ligands.

Acknowledgments

We are indebted to Nathalie Beaudoin and the animal caretakers of the Guy Bermin Research Center for their invaluable help during the course of these studies.

References
