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Regulation of Extrathymic T Cell Development and Turnover by Oncostatin M

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

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.

Changes in T lymphocyte function underlie much of the progressive replacement of virgin T cells by memory cells that display decreased proliferative potential and a restricted repertoire (2–5). Numerous observations suggest that immune competence has a major influence on life span, and that disturbed T cell responses are implicated in the age-related increase in the incidence of infections, cancer, and autoimmune diseases (6–11). The mechanisms responsible for thymic involution are unknown (12). Its occurrence may reflect the fact that, from an evolutionary perspective, thymopoiesis can be considered an energy-expensive process, and there is no selective pressure for maintaining the same level of T cell repertoire diversity in aged as in young individuals (12). Importantly, thymic output and the size of peripheral T cell pools are independently regulated. Thus, an increase in thymic export (by thymic grafts) does not bring about a commensurate enlargement of peripheral T cell compartments (5, 13). The size of peripheral T cell compartments, rather, is determined by the number of available T cell niches. The term niche designates an environment that provides local conditions (such as expression of specific chemokines, cytokines, and MHC molecules) required for T cells to seed and survive long term in the peripheral compartment (14, 15). Furthermore, thymic output does not increase in the presence of peripheral T cell depletion (16). Hence, the consequences of the progressive age-associated decline in thymic function are magnified in individuals whose peripheral lymphoid compartments have been rendered hypoplastic by various factors, such as chemotherapy and HIV-1 infection (17–21).

In athymic subjects, continuous production of new T cells is afforded by proliferation of post-thymic T cells and by extrathymic T cell development (22–24). In various mouse models, extrathymic differentiation of hemopoietic stem cells has been detected in selected organs, such as bone marrow (25, 26), intestinal cryptopatches (27), and liver (28, 29). However, under normal circumstances, the ability of these organs to replenish and maintain lymph node (LN)3 and spleen T cell compartments is inferior to that of the thymus. Nevertheless, it was recently shown that expression of an oncostatin M (OM) (3) transgene, under the control of the proximal Lck promoter or the CD34 gene promoter, causes thymus atrophy and thymus-independent accumulation of immature and mature T cells in LNs (30–32). OM is a member of the IL-6 family of cytokines that acts as a growth regulator for many types of mammalian cells (33). In normal mice, this pleiotropic cytokine is produced late in the activation cycle of T cells and macrophages, and its best known activities in vivo are anti-inflammatory (34, 35). Breeding experiments with IL-6−/− and IL-7R−/− mice showed that induction of extrathymic development by the OM transgene occurs in the absence of IL-6, but is strictly dependent on IL-7R signaling (32). Intraperitoneal administration of recombinant human OM produced the same effect in nontransgenic mice (31).

The striking occurrence of extrathymic T cell development in LckOM transgenic mice provides unforeseen evidence for the existence of a lymphopoietic pathway whose regulation could be of therapeutic interest for individuals with senescence- or disease-associated thymic hypoplasia. Thus, the goal of this study was to

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3 Abbreviations used in this paper: LN, lymph node; B6, C57BL/6J; B6.PL, B6.PL-Thy-1(+/Cy); BrdU, 5-bromo-2′-deoxyuridine; CFSE, carboxy-fluorescein diacetate succinimidyl ester; OM, oncostatin M; CD62L, CD62 ligand.
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/6J (B6; Thy-1.2+) and B6.PL-Thy-1(Cy) (B6.PL; 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 p56

Lck

transgenic mice were initially provided by Bristol-Myers Squibb Pharmaceutical Research Institute that were used in this work had been bred in this manner for >13 generations. LckOM mice used in our experiments were heterozygous. As LckOM females develop ovarian failure at about 10 wk 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 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 rabbit serum (Low-Tox-M rabbit complement, Cedarlane) as a source of complement. The efficacy of depletion was assessed by flow cytometry. Timed pregnancies were established for B6.PL mice, and fetal liver cells were collected on day 13 postcoitum. Hemopoietic chimeras were created by injecting 4 × 106 LckOM bone marrow cells and 4 × 106 B6.PL fetal liver cells into irradiated (10 Gy) B6 recipients. 5-Bromo-2′-deoxyuridine (BrdU) labeling experiments were initiated in hemopoietic chimeras 75–90 days after transplantation.

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,κ), anti-CD8α (53-6.7; rat IgG2a,κ), biotinylated-anti-CD8α (53-6.7; rat IgG2a,κ) detected with Cy-Chrome-streptavidin or APC-streptavidin, biotinylated-anti-Thy-1.1 (OX-7; mouse IgG1,κ), biotinylated-anti-Vβ3 TCR (KJ25; hamster IgG) detected with FITC-streptavidin, FITC-conjugated anti-Thy-1.2 (53-2.1; rat IgG2a,κ), anti-Vβ5.1,2 TCR (MR9-4; mouse IgG1,κ), anti-Vβ6 TCR (RR4-7; rat IgG2b,λ), anti-Vβ7 TCR (BR310; rat IgG2b,κ), anti-Vβ8.1,2 TCR (MR5-2; mouse IgG2b,κ), anti-Vβ9 TCR (MR10-2; mouse IgG1,κ), anti-Vβ10 TCR (B21.5; rat IgG2a,λ), anti-Vβ11 TCR (RR3-15; rat IgG2b,κ), anti-Vβ13 TCR (MR12-3; mouse IgG1,κ), anti-Vβ14 TCR (14-2; rat IgM,κ), anti-Vβ17 + TCR (KJ23; mouse IgG2a,κ), PE-conjugated anti-Thy-1.1 (OX-7; mouse IgG2a,κ), PE-conjugated anti-Thy-1.2 (30-H12; rat IgG2b,κ), PE-conjugated anti-CD19 (ID3; rat IgG2b,κ), PE-conjugated anti-CD44 (IM7; rat IgG2b,κ), PE-conjugated anti-CD45RB (23G2; rat IgG2a,κ), PE-conjugated anti-CD62L (MEL-14; rat IgG2a,κ), PE-conjugated anti-CD122 (IL-2R β-chain; TM-β1; rat IgG2b,κ), and PE-conjugated anti-NK1.1 (PK136; mouse IgG2a,κ) 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 FACScan flow cytometer using CellQuest software or with a FACScan 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 (107) 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 × 106 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, showed a 30-fold increase in cellularity relative to controls (Table I). This was caused primarily by a massive accumulation of double-positive CD4+CD8+ lymphocytes that reached a maximum 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.
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 comparatively with 7 and 12 wk of age were 43 and 92 × 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 × 10^6 in the case of LckOM mice comparatively with 7 and 12 × 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

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

<table>
<thead>
<tr>
<th>Organ</th>
<th>Phenotype</th>
<th>LckOM (×10^6 ± SD)</th>
<th>C57BL/6</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>214.5 ± 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>16.41 ± 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-1.2+ CD4+ CD8+).
c DN, double negative T lymphocytes (Thy-1.2+ CD4− CD8−).

**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-1.2+ 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.
of LN CD4+ and CD8+ cells was strikingly different in LckOM mice relative to B6 controls. In LckOM mice, most CD4+ T cells were CD44high, CD45RBlow, CD62Llow, and IL-2Rβlow, 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 CD44high, CD45RBhigh, CD62Lhigh, and IL-2Rβhigh. The CD44highCD62Lhigh 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 that harbor a CD4+CD8” or CD4”CD8+ phenotype (45–47). However, their NK1.1− phenotype shows that LckOM T cells do not correspond to NK T cells (Fig. 4). Interestingly, while the aforementioned phenotypic analyses have been performed on LckOM LN cells, similar results were observed in LckOM spleen cells and in LNs and spleen of irradiated B6 mice transplanted with LckOM hemopoietic progenitors (data not shown).

**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− and CD62L+ subsets were analyzed separately, because CD62L− cells divide more rapidly than CD62L+ cells (38, 55) and because, similar to LckOM mice (Fig. 4), the proportion of CD4+CD62L− 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+ and CD62L− 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 into Thy-1.1+CD4+CD8+ thymocytes in the chimeras’ mesenteric LNs 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...
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...
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 Vβ 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 vivo 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^highCD45RB^-lowCD62L^-low for CD4^+ cells, and CD4^highCD45RB^-highIL2R^-beta^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, we observed the same nonnaive phenotype (depicted in Fig. 4), without conspicuous skewing of the Vβ 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^- cells that respond to non-self Ags can revert to a CD62L^- phenotype, a CD8^- compartment composed primarily of CD4^highCD62L^-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

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References

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