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REVIEW

Re-entry of mature T cells to the thymus: an epiphenomenon?

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In certain situations mature post-thymic T cells are able to leave their residence in the secondary lymphoid tissues and re-enter the thymus. The physiological significance of this phenomenon is discussed.
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THE POOL OF MATURE T CELLS

Typical mature T cells expressing αβ-T-cell receptor molecules arise in the thymus and are released into the periphery as a mixture of mature CD4 and CD8 cells.[1] These cells are immunologically naive and, in mice, are marked by low (lo) expression of CD44 and high (hi) levels of CD62L (L-selectin) and chemokine receptor CCR7.[2–7] Naive T cells live for prolonged periods in interphase and recirculate continuously between blood and lymph through defined regions of the secondary lymphoid tissues, that is, spleen, lymph nodes (LNs) and Peyers patches. T-cell entry to these organs is highly specific and, for LN, involves sequential contact between CD62L and CCR7 on T cells interacting with vascular addressins and CCL19, 21, respectively, expressed on the surface of high endothelial venules. Homing of naive T cells to nonlymphoid tissues is generally thought to be minimal, although this view has recently been contested.[8]

Contact with foreign antigen in the spleen and LN drives naive T cells to proliferate extensively and differentiate into activated effector cells[4,9,10] These cells tend to downregulate CD62L and CCR7 expression but synthesize a new range of homing molecules, which enables the cells to migrate into nonlymphoid tissues. The receptor/ligand interactions involved in migration to nonlymphoid tissues are highly complex.[6,7] Expression of CLA and CCR4 is important for activated T-cell entry to skin, whereas several other homing molecules, including α4β7, α4β1 (VLA-4) and CCR9, control migration to the gut. CD44 is involved in T-cell entry to the liver, whereas CCR5 is important for migration to a number of nonlymphoid sites, including sites of inflammation involved in autoimmune disease.

At the end of the immune response, elimination of the pathogen concerned causes most effector cells to die rapidly, leaving only a few cells to survive and become long-lived memory cells.[4,9,10] Most of these cells revert to resting cells and re-acquire expression of CD62L and CCR7, thus allowing the cells to re-join the recirculating lymphocyte pool and cease to migrate through nonlymphoid sites. Nevertheless, memory cells differ from naive cells in retaining the $CD44^{hi}$ phenotype of effector cells. T cells with the same phenotype as memory cells are found naturally in normal unimmunized mice. These memory–phenotype cells comprise a small proportion (10–20%) of T cells in young mice but become a dominant population in old age. Memory–phenotype cells are presumed to be the descendents of cells responding to a variety of foreign and self-antigens.

T-CELL MIGRATION TO THE THYMUS

As mentioned above, normal resting naive and memory cells reside within the confines of the recirculating lymphocyte pool, migration to nonlymphoid sites being minimal. In the case of the thymus, early studies showed that injection of mice with mature lymphocytes caused almost none (<0.1%) of the donor cells to enter the thymus,[11–14] thus showing that this organ is not part of the recirculating lymphocyte pool.[15] These and other findings have consolidated the view that the thymus is a 'primary' lymphoid organ: once released from the thymus as newly formed cells, mature T cells rarely if ever return to the thymus. Subsequent studies have confirmed that 'back-migration' of normal T cells to the thymus is extremely limited. Nevertheless, parabiosis experiments have shown that some normal T cells do re-enter the thymus. Thus, after establishing parabiosis, around 0.3% of total cells in the thymus were partner derived at day 14 and were almost entirely situated in the medulla;[16] partner-derived cells were not seen in the cortex until day 21, indicating that the cells in the medulla at day 14 were mature T cells derived from the circulation rather than from cortical stem cells.

Significant migration of mature T cells to the thymus is also detectable when very large numbers of T cells are injected into normal mice. Thus, studies in which allotype-marked T cells were detected in

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tissue sections after intravenous (i.v.) injection of 1×10^8 total LN cells showed that about 0.2% of cells in the thymic medulla were of donor origin;[17] in the cortex, by contrast, donor cells were barely detectable, that is, 0.01% or less. Significantly, the cells in the medulla remained at constant levels for at least 24 weeks. As the proportion of donor cells in LN declined fourfold during this time, the data implied that the cells reaching the host thymus stayed there and were not part of the recirculating pool.

In the latter study, it is of interest that most of the donor cells in the medulla had a $CD62L^{lo}$ phenotype, suggesting that migration to the thymus was restricted to a small subset of cells with an activated phenotype. In fact, earlier experiments with rat T-cell lines had shown that, after i.v. injection, a surprisingly high proportion, about 1%, of the injected cells reached the host thymus within 3 days and remained there for at least 2 months;[18] migration to the thymus depended on antigen activation of the T-cell lines just before injection. These findings were supported by studies in mice on T-cell responses to transplantation antigens.[19] Here, it was shown that some of the responding T cells were activated in the spleen or LN and then migrated into the thymus.

Quantitative evidence on this issue came from a comparison of thymic migration by normal T cells versus activated T cells after i.v. injection.[20] To enhance detection of allotype-marked donor cells in the host thymus, thymic suspensions were depleted of immature HSA^{hi} cells before fluorescence-activated cell sorting analysis, thus enriching for the small subset (5%) of mature medullary thymocytes. When normal small resting T cells were injected, the % of donor cells in the mature component of host thymocytes was undetectable ($<0.01\%$) even with injection of large numbers of cells, that is, 4×10^7. However, significant migration to the thymus occurred following injection of activated T cells (which were obtained as T-blast cells from thoracic duct lymph of irradiated H-2-different recipients of naive T cells). With injection of large numbers of syngeneic activated T cells, about 0.5% of HSA^- thymocytes were of donor origin; when the donor blasts were labeled with ^{125}I-5-iodo-2′deoxyuridine, a DNA precursor, the % of donor cells in thymus suspensions reached 5%, suggesting that thymic homing was skewed to cycling cells. In sections, the cells reaching the thymus were largely restricted to the medulla and remained there for at least 1 month.

Additional evidence that thymic homing is largely restricted to activated T cells came from studies in rats. Thus, homing of mature T cells to the rat thymus was very low with $CD45RC^+$ cells, that is, cells with the phenotype of resting naive and memory cells, but clearly detectable with $CD45RC^-$ cells, which include activated effector cells as well as resting cells.[21] In a follow-up study, however, appreciable thymic homing was observed with $CD45RC^+$ cells, leading the authors to conclude that thymic homing can also apply to small resting T cells.[22] In this study, it is notable that the medulla of the rat strain used contained a high proportion of non-T cells, including 12% B cells, implying that …'the thymic medulla shares many features of peripheral lymphoid organs such as LNs'.[22] This is not the case in the mouse where the proportion of B cells in the thymus is normally very low. However, certain mouse strains prone to autoimmune disease, for example, $(NZB\times SJL)F_1$ mice, do have features of secondary lymphoid tissues, and mature T and B cells readily enter the thymus of these mice.[23] This situation also applies to the preneoplastic thymus of AKR mice—see below.

THE IMMUNODEFICIENT THYMUS

All of the above data refer to thymic homing in normal adult animals. When immunodeficient hosts are used, the results are somewhat different. If the cellularity of the thymus is reduced by $>95\%$ following heavy irradiation or treatment with cortisone, migration of naive LN T cells into the host thymus after adoptive transfer is very low,[20,24] indicating that the acute depletion of cells does not make the thymus more permeable to circulating T cells. When the thymus is congenitally acellular, by contrast, peripheral T cells readily enter the thymus. Thus, after i.v. injection of 4×10^7 LN T cells into SCID mice, 1–2% of cells in thymocyte suspensions were of donor origin.[25] Although the SCID thymus contains very few lymphoid cells, total yields of donor cells in the thymus after i.v. injection were about 30-fold higher for SCID hosts than for normal hosts. Nearly, all of the donor cells recovered from the SCID thymus had the phenotype of typical naive T cells and comprised a mixture of CD4 and CD8 cells. These findings indicate, therefore, that naive T cells are excluded from entering the normal thymus but do enter the atrophic thymus of congenitally T-deficient SCID mice. Similar findings were subsequently reported for other congenitally-deficient mice, namely RAG-$2^{-/-}$ mice, following parabiosis to normal mice.[26]

Whereas the thymus of SCID- and RAG-deficient mice show a drastic ($>98\%$) reduction in total numbers of lymphoid cells, the thymus of mice deficient in pre-Tα—which guides early thymocyte differentiation into $CD4^+CD8^+$ cells—contains significant numbers of T cells (10% of normal), mostly of the γδ-lineage.[27] When pre-T$\alpha^{-/-}$ mice were parabiosed to normal mice for 1 week, the remarkable finding was that around 50% of mature CD4 and CD8 T cells in the pre-T$\alpha^{-/-}$ thymus were derived from the normal partner strain;[28] most of the immigrant cells were naive $CD44^{lo}$ cells. In a follow-up study, i.v. injection of 2×10^7 LN T cells led to the appearance of 2% donor cells in thymocyte suspensions from pre-T$\alpha^{-/-}$ mice but only 0.001% donor cells in thymocytes from normal mice.[26] Taking into consideration the differing size of the thymuses, it was calculated that the total number of immigrant mature T cells was about 1×10^5 cells per thymus and was no higher in the lymphopenic pre-T$\alpha^{-/-}$ thymus than in the normal thymus. The implication therefore is that, in contrast to the SCID thymus (see above), the lymphopenic thymus of pre-T$\alpha^{-/-}$ mice is not more permeable to circulating mature T cells than the normal thymus. In this study, it should be mentioned that, based on the results of injecting a dye, CFSE, into the spleen and then searching for labeled cells in the thymus, about 50% of the immigrant T cells in the normal thymus were found to be naive $CD44^{lo}$ cells. This finding is surprising and seemingly at odds with the conclusion from the earlier studies mentioned above that the normal thymus is impermeable to naive resting T cells.

THE NEONATAL AND AGED THYMUS

The evidence that circulating naive T cells are generally excluded from re-entering the thymus applies to normal adult animals. The situation in neonates is quite different. Thus, studies in which newborn mice were injected i.v. with LN T cells enriched for resting cells showed that donor cells comprised a high proportion, 1–5%, of the component of HSA^- cells in the host thymus, compared with only 0.02% for donor cells reaching the thymus of adult control mice.[29] In neonatal hosts, the donor cells comprised a mixture of naive $CD44^{lo}$ CD4 and CD8 cells and localized largely in the medulla where they remained for at least 2 months.

As in the neonate, T cells readily enter the thymus of aged mice.[30] After i.v. injection of naive T cells (T-cell receptor transgenic CD8 cells), localization of the donor cells in the host thymus was about 10-fold higher for old (1.5 years) mice as hosts than for young mice. These findings applied to aged C57BL/6 mice. For AKR mice, that is,

mice prone to developing thymic lymphomas, the preneoplastic hyperplastic thymus becomes permeable to circulating T and B cells, and these cells lodge in the medulla in substantial numbers after i.v. injection[31,32] Migration of mature lymphocytes into the AKR thymus increases considerably with age until the mice eventually develop lymphoma.

DISCUSSION

As a whole, the above data indicate that mature T-cell traffic in the normal adult thymus is unidirectional: after export from the thymus, naive T cells circulate in the peripheral lymphoid tissues for prolonged periods but rarely if ever return to the thymus. This scenario is in keeping with the view that the thymus is a primary lymphoid organ. As discussed above, however, the 'gate' to the thymus is partly ajar in the neonatal period and old age, and also in certain pathological states in adults, especially in mice with congenital lymphopenia. Why naive T cells return to the thymus in these situations is unclear. In the case of the normal adult thymus, the failure of naive T cells to re-enter the thymus presumably reflects that the small blood vessels of this organ do not express sufficient levels of the 'homing' ligands such as vascular addressins (PNAd and MadCAM-1) and/or certain chemokines that naive T cells use for their recirculation through secondary lymphoid tissues. It would seem more likely, therefore, that permeability of the thymus to circulating mature T cells reflects increased expression of these homing ligands.

Although direct information on this issue is sparse, it is of interest that the increased permeability of the preneoplastic AKR thymus for mature T cells with age correlates with a progressive increase in the expression of PNAd and MadCAM-1 on small blood vessels in the thymic medulla.[32] Similar increased expression of these vascular addressins is seen in the peri-islet lymphocyte infiltrations of pre-diabetic NOD mice;[33] although enriched for memory–phenotype cells, the islets of NOD mice contain substantial numbers of typical naive T cells, implying that the influx of these cells reflects *de novo* synthesis of vascular addressins. Whether this scenario is also applicable to T-cell homing to the thymus of normal neonates and mice with congenital lymphopenia has yet to be resolved. For neonates, it is of interest that, in mice, the expression of vascular addressins is different at birth, at least in LN;[34] moreover, both for sheep[35] and humans,[36] the pattern of blood-to-lymph recirculation of naive T cells during the perinatal period differs from adults in showing prominent migration through the skin and other nonlymphoid tissues.

For mice, the permeability of the neonatal thymus to circulating T cells correlates with the medulla being relatively immature at birth and occupied largely by γδ-cells rather than αβ-cells.[34,37] The medulla is also poorly formed in congenitally lymphopenic SCID mice, but is rapidly reconstituted after an influx of mature T cells following i.v. injection.[25] It is conceivable, therefore, that a poorly formed medulla somehow makes the thymus leaky and allows entry of naive T cells. Direct evidence on this issue is lacking.

For the normal thymus, re-entry of mature T cells from the circulation is largely restricted to activated cells and certain subsets of memory cells. As discussed earlier, these cells express high levels of a variety of selectins, selectin ligands, integrins and chemokine receptors, but which of these newly expressed homing molecules allow the cells to localize in the thymus is uncertain. However, there could be parallels with the recruitment of thymic stem cells. In this respect, the normal thymus is known to express low but significant levels of several vascular addressins and selectins,[38,39] and interaction with these ligands is considered to guide stem cell entry into the thymus. Such entry involves a number of counter-receptors on stem cells, including CD44,[40,41] PSGL-1,[39] α4-integrins,[41] as well as chemokine receptors such as CXCR4 and CCR5.[42] These and other homing/adhesion molecules are highly expressed on subsets of activated T cells and are probably largely responsible for controlling the entry of these cells into the normal thymus. Direct evidence on this issue is sparse, however, although T-cell homing to the rat thymus is reported to involve α4-integrin–VCAM-1 interaction.[21]

The physiological significance of T-cell re-entry to the thymus is a matter for speculation. One possibility is that the antigens on immigrant T cells contribute to tolerance induction. We became interested in this possibility when injection of newborn mice with allogeneic naive CD8 T cells was found to cause profound intrathymic tolerance to the foreign Mtv antigens on the T cells.[43] The implication was that the newborn thymus is directly permeable to naive T cells, which we then proved in later experiments—see above. But the tolerance model used in these experiments was highly contrived and of dubious relevance to tolerance induction to self-antigens. Self-tolerance is thought to be controlled through intrathymic contact with epithelial cells and resident dendritic cells, plus immigrant dendritic cells from the periphery, and it is difficult to envisage how tolerance could be further improved by exposure to antigens expressed on immigrant mature T cells. Nevertheless, as for Mtv antigens, it is possible that migration of antigen-bearing T cells into the thymus could contribute to acquired tolerance to foreign antigens, especially alloantigens.[44,45]

Another suggestion for T cells returning to the thymus is that these cells may contribute to positive selection. In fact, direct evidence in favour of this idea has come from studies in which the ligand controlling positive selection in the thymus was expressed selectively on immigrant mature T cells.[26] Under normal conditions, however, positive selection is known to be controlled by immature T-cell contact with the rich network of epithelial cells in the cortex, whereas most immigrant T cells settle in the medulla with only very small numbers reaching the cortex. Hence, it seems doubtful whether positive selection mediated by T cells in experimental models is physiologically relevant.

A third possibility is that migration of mature T cells into the thymus plays a role in the development of $CD4^+CD25^+Foxp3^+$ T-regulatory cells (Tregs). These cells are heavily dependent on interleukin (IL)-2 for their survival in the periphery and also seem to require contact with IL-2 for their formation in the thymus[46–49] Thus, numbers of Tregs in the thymus are significantly reduced in the absence of IL-2. What then is the source of IL-2 in the thymus? In considering this question, it is notable that Tregs are undetectable in the mouse thymus until a few days after birth.[50,51] As T cells released from the neonatal thymus undergo substantial proliferation and activation in the periphery, presumably to foreign antigens,[52] one can envisage a scenario where some of the activated T cells rapidly re-enter the thymus; here, the immigrant cells release IL-2 locally, thus promoting the development of Tregs. In addition to IL-2, these migrant cells might be needed to release other γc-cytokines in the thymus. Thus, Treg development is only partly reduced (by twofold) in the absence of IL-2 but is undetectable for $\gamma c^{-/-}$ thymocytes.[46] There is also evidence that Treg formation requires intrathymic contact with thymic stromal-derived lymphopoietin made by medullary epithelial cells.[50] As the medulla is poorly formed at birth (see above), re-entry of activated T cells to the thymus during this period might also be needed to promote formation of the medulla and induce synthesis of thymic stromal-derived lymphopoietin. This scenario might not be applicable to humans because Treg development is evident in the fetus at the end of the first trimester and presumably occurs in a relatively antigen-free environment.[53,54]

The least interesting explanation for back-migration of T cells to the thymus is that such migration is merely an epiphenomenon: after appropriate activation by specific antigen and expression of new homing molecules, activated T cells can migrate to almost any site in the body, including the thymus, to mediate their effector function. Precisely where the cells localize, the thymus or elsewhere, is largely an accident. Hopefully, future experiments will come up with an interesting new idea to dispel this gloomy scenario.

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