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Self-encounters of the third kind: lymph node stroma promotes tolerance to peripheral tissue antigens

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Multiple mechanisms have evolved to maintain tolerance among CD8$^+$ T cells to innocuous antigens that arise in cutaneous and mucosal tissues. In the thymus, medullary thymic epithelial cells directly present peripheral tissue antigens (PTAs) and incite the deletion of self-reactive thymocytes. Cross-presentation of PTAs by functionally immature, CD8α^+ dendritic cells can lead to the deletion of self-reactive CD8$^+$ T cells in secondary lymphoid organs. A third mechanism of deletional tolerance has recently been uncovered in which lymph node-resident stromal cells of non-hematopoietic origin present endogenously expressed PTAs to circulating CD8$^+$ T cells. Emerging data suggest that lymph node stroma is a unique niche for controlling self-reactive T cells.

BACKGROUND

An important component of T-cell development is the removal of self-reactive thymocytes before they leave the thymus called central tolerance. Elimination of self-reactive thymocytes is coordinated by medullary thymic epithelial cells (mTECs) and dendritic cells (DCs).[1] Thymic antigen-presenting cells impose self-tolerance upon developing T cells by presenting peptides derived from self-antigens on major histocompatibility complex (MHC) molecules. Thymocytes that recognize self-peptide–MHC complexes with high affinity are eliminated or deleted. Genes encoding peripheral tissue antigens (PTAs) or proteins that are typically restricted to organs such as the pancreas, eye, thyroid, liver, or central nervous system[2] are expressed in the thymic stroma. Promiscuous gene expression occurs primarily in mTECs and provides these rare stromal cells with a panoply of tissue-restricted self-antigens.[3] The autoimmune regulator (Aire) gene governs the expression of a battery of PTAs, antigen presentation, and the induction of tolerance by mTECs.[4–6] Although mTECs appear to be largely responsible for PTA expression in the thymus, these self-antigens can be transferred to neighboring DCs that process and present endocytosed antigens to thymocytes.[7] Self-reactive thymocytes that encounter their cognate antigen in this context are then deleted.[5,6,8]

Peripheral induction of T-cell tolerance occurs primarily in lymph nodes (LNs) and, to a lesser extent, in the spleen and liver. DCs are considered key mediators of peripheral tolerance to self and also innocuous non-self antigens. According to the dominant model of this process, tissue-resident DCs capture antigens from their native microenvironment and then transport their endocytic cargo along afferent lymphatics to draining LNs. On arrival in the LNs, the migratory DCs transfer antigen by an ill-defined mechanism to LN-resident CD8α^+ DCs, which then present peptide–MHC complexes to naive T cells. The presentation of tissue-derived self-antigens by non-activated or immature DCs can lead to the deletion or the paralysis of self-reactive T cells and to the formation of T regulatory cells.[9,10] Recent studies have uncovered a peripheral mechanism of tolerance induction wherein lymph node stromal cells (LNSCs) of non-hematopoietic origin directly present PTAs to circulating CD8$^+$ T cells.

NEW FINDINGS

In early 2007, a population of radioresistant LN cells was described that could promote T-cell tolerance to an intestinal antigen using the iFABP-tOVA transgenic mouse model.[11] In these mice, soluble truncated hen ovalbumin (OVA) is expressed under the control of the intestinal fatty acid-binding protein (iFABP) promoter resulting in OVA expression in the small intestine.[12] Unexpectedly,

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a low level of OVA expression was also observed in CD45$^-$ stromal cells of LNs but not spleen or other extraintestinal tissues. Thus, OVA was cross-presented by CD8α^+ DCs in mesenteric LNs and directly presented by LNSCs throughout the body, resulting in the activation of naive OT-I (OVA-specific T-cell receptor transgenic CD8$^+$ T cells) CD8$^+$ T cells in all LNs of the body but not spleen or intestine. Using bone marrow chimeras in which naive OT-I T cells encountered their cognate antigen exclusively in the context of radio-resistant stroma, the direct presentation of OVA by LNSCs was found to be sufficient to incite the activation and subsequent deletion of OVA-specific CD8$^+$ T cells. This discovery established that promiscuous expression and display of a gut self-antigen by the LN stroma can incite tolerance among CD8$^+$ T cells.

The cellular components of LN stroma (CD45$^-$ cells) can be classified into three major subsets: blood endothelial cells, lymphatic endothelial cells, and T-zone fibroblastic reticular cells.[13] The T-zone fibroblastic reticular cell subset, which forms the reticular network of the LN and interacts closely with T cells as they scan the paracortical microenvironment, is CD45$^-$ CD31$^-$ gp38$^+$. LNSCs are also localized in the T zone, interact with T cells, and have a CD45$^-$ CD31$^-$ gp38$^+$ surface phenotype, suggesting that they may be related to the T-zone fibroblastic reticular cell subset. Yet other properties of LNSCs align this novel cell population with mTECs. For example, like mTECs, LNSCs are UEA-I$^+$ B7-1$^+$ MHC class II$^+$, express keratins, and contain mRNAs corresponding with PTAs from various organs including the retina, pancreas, thyroid, liver, skin, central nervous system, and intestine. Aire has also detected in CD45$^-$ cells in LNs at the RNA and protein levels (ref. 11 and J Gardner and M Anderson; personal communication); however, conflicting results on Aire protein detection suggest that the overall expression level in these cells may be relatively low compared to mTECs.[14] Future studies are thus needed to clarify the developmental origin of this tolerogenic stromal cell.

Additional evidence for this new mechanism of tolerance came soon after from a study of CD8$^+$ T-cell tolerance to the melanocyte-associated antigen, tyrosinase.[14] When FH T-cell receptor transgenic mice (FH T cells recognize tyrosinase$_{369-377}$ in the context of the MHC class I molecule, AAD) were crossed with AAD mice, the percentage of FH T cells in wild-type (tyrosinase$^+$) lymphoid compartments was diminished compared to albino AAD$^+$ mice (lacking endogenous tyrosinase expression). This suggested that FH CD8$^+$ T cells were being deleted by an antigen-dependent mechanism. Despite intrathymic expression of tyrosinase, transplantation of tyrosinase$^+$ thymi into albino mice did not lead to deletion of FH T cells, suggesting that FH T-cell deletion in AAD mice was being mediated by peripheral rather than central mechanisms. Adoptive transfer of FH cells into tyrosinase$^+$ hosts resulted in T-cell activation in all LNs followed by their rapid elimination. Elegant experiments with bone-marrow chimeras and conditional ablation of Langerhans cells revealed that peripheral presentation of tyrosinase to circulating FH T cells was mediated by a unique radio-resistant cell population of LNs rather than DCs. Importantly, they provided evidence that tyrosinase was expressed and presented by a stromal population that is present in all LNs of the body, whereas spleen lacked tyrosinase display. These findings suggest a principal role for LNSCs in deleting self-reactive CD8$^+$ T cells by presenting an endogenously expressed melanocyte antigen.

More recent findings have corroborated the notion that direct presentation of self-antigen by LNSCs is sufficient to incite tolerance among intestine-reactive CD8$^+$ T cells.[15] While studying the pathogenesis of inflammatory bowel disease in the GFAP-HA mouse, a transgenic mouse model in which the glial fibrillary acidic protein (GFAP) promoter drives the expression of the influenza virus protein, hemagglutinin (HA), in enteric glial cells, it was discovered that the intestine was protected from destruction by transferred HA-specific CD8$^+$ T cells (CL4). At early time points after transfer into GFAP-HA hosts, CL4 T cells proliferated in all mesenteric and extraintestinal LNs and did not preferentially accumulate in antigen-draining lymphoid tissues. One week later, most of the CL4 T cells had been eliminated in an antigen-specific manner. The CD8$^+$ T-cell tolerance was so profound in the GFAP-HA mice that transfer of CL4 cells failed to induce inflammatory bowel disease even in a lymphopenic Rag2$^{-/-}$ host. To ascertain the cells that were presenting HA-MHC class I complexes, sorted CD8α^+ and CD8α^- DCs from mesenteric LNs and CD45$^-$ UEA-I$^+$ cells (LNSCs) from various LNs of GFAP-HA transgenic mice were co-cultured with naive CL4 T cells. The CL4 cells responded to LNSCs and CD8α^+ DCs but not to CD8α^- DCs. Notably, the HA presentation by CD8α^+ DCs occurred via cross-presentation, whereas the LNSCs expressed and presented HA directly. Using bone marrow chimeras to exclude the contribution of cross-presenting CD8α^+ DCs, it was then shown that direct presentation by LNSCs was indeed sufficient to trigger the primary activation and later deletion of CL4 T cells.

Yet another group has now confirmed that direct presentation of self-antigen by radio-resistant CD45$^-$ cells of LNs can promote deletional tolerance among CD8$^+$ T cells. In studies with a new transgenic mouse model in which the Aire promoter was used to drive expression of the pancreatic islet antigen islet-specific glucose-6-phosphatase-related protein (IGRP) and green fluorescent protein,[16] it was shown that IGRP was presented by extra-thymic Aire-expressing stromal cells leading to the proliferation and subsequent deletion of IGRP-specific CD8$^+$ T cells (J. Gardner and M. Anderson; personal communication). Intravital imaging combined with immunostaining with an Aire-specific antibody revealed that IGRP-reactive CD8$^+$ T cells interact with Aire-expressing stromal cells in the LN paracortex, in close proximity to the T–B junction. Taken together, these new studies suggest that direct presentation of PTAs by LNSCs may be a mechanism of broad import for inciting self-tolerance among CD8$^+$ T cells.

NEW DIRECTIONS

Though it now seems certain that CD8$^+$ T cells scanning LNs encounter PTAs

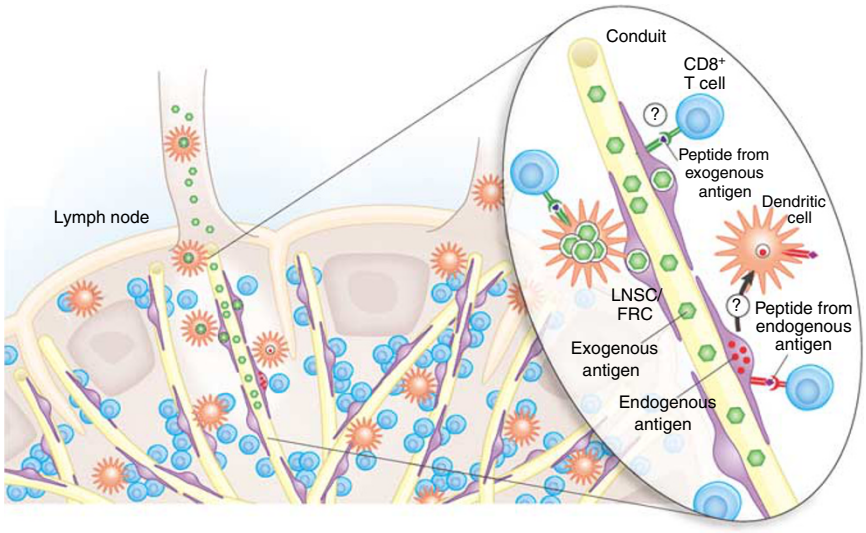

Figure 1 In the lymph node, CD8$^+$ T cells encounter PTAs presented by DCs and LNSCs. PTAs are transported to the lymph node by tissue-resident DCs or by diffusion in afferent lymph. Free antigen can drain from afferent lymphatics into the subcapsular sinus and eventually into the conduit system. Lymph node-resident DCs can acquire PTAs from conduits or from tissue-resident DCs that migrate into the lymph node via afferent lymphatic vessels. Cross-presentation of PTAs by DCs under steady-state conditions can incite T-cell tolerance. LNSCs are non-hematopoietic cells that share many characteristics with FRCs, including their surface phenotype and association with the reticular network in the paracortex. Promiscuous expression of PTAs by LNSCs allows these cells to directly present self-peptide/MHC class I complexes to circulating CD8$^+$ T cells and promote tolerance. LNSCs may also cross-present antigen acquired from conduits. DCs acquiring endogenous self-antigen from LNSCs may comprise yet another mechanism for self-antigen display to CD8$^+$ T cells. PTA, peripheral tissue antigen; DC, dendritic cell; LNSC, lymph node stromal cell; FRC, fibroblastic reticular cell.

being cross-presented by DCs and directly presented by LNSCs (**Figure 1**), the studies described above beget many new questions. First, to what extent do LNSCs contribute to peripheral tolerance induction compared with DCs? Self-antigen presentation by LNSCs is sufficient to promote tolerance in the scenarios described above, but whether it is necessary is currently under investigation. The work of Nichols and colleagues clearly points to CD45$^-$ stromal cells as primary mediators of deletional tolerance to tyrosinase$_{369–377}$, but the role of these cells in the tolerance to other self-antigens will depend on the range of antigens that are expressed and presented by LNSCs. Thus, it will be important in future studies to evaluate the repertoire of antigens displayed by LNSCs and to identify the factors that regulate their expression of self-antigen.

The T-zone localization of LNSCs is cause to wonder whether neighboring DCs can cross-present PTAs acquired from the stroma (**Figure 1**), as occurs in the thymus between mTECs and thymus-resident DCs.[5] Although our studies in the iFABP-tOVA system suggested that this does not occur with cytoplasmic OVA, it remains possible that proteins with different subcellular localization or longer-lived proteins can be transferred to DCs.[11] Another interesting question is whether LNSCs are capable of capturing and presenting lymph-borne antigens that percolate through the conduits (**Figure 1**). We look forward to future studies that will shed light on the potential versatility of LNSCs in antigen presentation.

Numerous mechanisms have been implicated in tolerance induction by DCs, but the molecular underpinnings of tolerance induction by LNSCs remain enigmatic. Most recently, the role of transforming growth factor-β1 signaling in CD8$^+$ T-cell tolerance was examined using the GFAP-HA mouse model of inflammatory bowel disease described above. This work provided evidence that although transforming growth factor-βRII signaling on transferred CD8$^+$ T cells is required for tolerance induction by cross-presenting DCs, LNSC-mediated CD8$^+$ T-cell tolerance develops independently of transforming growth factor-βRII signaling (F. Magnusson and K. Khazaie; personal communication). Additional efforts will be needed to illuminate the specific mechanisms by which LNSCs promote tolerance.

Developing T cells first encounter self-antigen in the thymic stroma where mTECs display endogenously expressed PTAs. In the periphery, naive T cells have another opportunity for tolerogenic encounter with DCs that acquire PTAs from tissues. CD45$^-$ radio-resistant LN stroma endowed with endogenously expressed PTAs represents a self-encounter of a third kind. By continuously exposing circulating T cells to PTAs, the LN stroma may constitute a novel niche for eliminating potentially hazardous self-reactive CD8$^+$ T cells from the peripheral lymphocyte repertoire.

DISCLOSURE

The authors declared no conflict of interests.

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