Commentary

Antigen Presentation by Dendritic Cells In Vivo

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Induction of Peripheral Tolerance by Dendritic Cells. Although dendritic cells (DCs) are known to be critical for inducing T cell immunity in immunized or infected individuals, it was recently proposed that DCs are also essential to silence potentially pathogenic self-reactive T cells that have escaped negative selection in the thymus (1, 2). One important prediction of such a model would be that DCs ingest, process, and present self-Ags in vivo under steady state conditions. Three papers published in this issue examine the role of DCs in maintaining tolerance to self-Ags.

Self-Ags Are Captured and Processed by DCs in Healthy Individuals. Presentation of self-Ags by professional APCs was first demonstrated in the thymus for Ags expressed by cortical bone marrow–derived cells and for abundant soluble Ags that could gain access to this organ by trafficking through the blood (3). A number of studies have also shown that APCs can process and present self-Ags to T cells in the peripheral lymphoid organs. In healthy animals, this often results in T cell tolerance. In pioneering studies, Heath and colleagues have used RIP-OVA transgenic mice in which OVA was selectively expressed in pancreatic β cells and proximal kidney (4). To assess whether OVA was processed and presented to T cells in vivo, these authors injected RIP-OVA mice with OVA-specific TCR transgenic $CD8^+$ T cells. Within 3 d after transfer, transgenic T cells proliferated in the pancreatic and kidney draining LN and were eventually deleted (5). T cell proliferation was not observed in any other lymphoid organs further suggesting that Ag presentation was occurring exclusively in the draining LN. Somewhat similar findings were reported for MHC class II–restricted epitopes in transgenic mice that expressed either the SV40 large T Ag or the influenza hemagglutinin (HA) in pancreatic β cells (6, 7).

Although these studies have shown that self-Ags could be processed and presented to T cells in the periphery, the nature of the APCs that are involved remains unresolved. Construction of bone marrow chimeras demonstrated that self-Ags were processed and presented by bone marrow–derived APCs. This was first shown for MHC class I–restricted epitopes in RIP-OVA transgenic mice (4) and later on for MHC class II–restricted epitopes (7). More recently, Kurts and colleagues have created transgenic mice in which MHC class I K^b molecules were selectively expressed in $CD11c^+$ cells (8). Elegant experiments using bone marrow from these CD11c-K^b mice and RIP-OVA recipients showed that $CD11c^+$ cells are responsible for cross-presentation of OVA in this animal model. In this issue, Belz and colleagues have gone a step further by directly showing that self-Ags can be processed and presented to $CD8^+$ T cells by a restricted subset of DCs (9). They have constructed transgenic mice, RIP-YSS, in which the yellow fluorescent protein was fused to a class I epitope from Herpes simplex virus-1 glycoprotein B (gB) and expressed under the control of the rat insulin promoter. Similar to other transgenic strains, gB was readily processed and presented to $CD8^+$ T cells in pancreatic draining LN. To identify the nature of the cross-presenting APCs, Belz et al. used a very sensitive T cell hybridoma that produced β-galactosidase in response to stimulation with the MHC class I–restricted gB epitope. Incubating this hybridoma with purified DC subsets prepared from the pancreatic LN of RIP-YSS transgenic mice revealed that $CD8\alpha^+$, but not $CD8\alpha^-$ DCs were the cross-presenting APCs in vivo. Also in this issue, Scheinecker et al. provide another major break through by showing that DCs are responsible for the processing of a self-Ag in the stomach of healthy unmanipulated animals (10). These authors have studied the gastric proton pump H^+/K^+-ATPase whose expression is restricted to gastric parietal cells. Using an mAb against the H^+/K^+-ATPase β subunit, they could detect this self-Ag inside rare $CD11c^+$ cells in the gastric draining LN. Furthermore, $CD11c^+$ cells purified from the gastric LN induced the activation of a T cell clone which was specific for a H^+/K^+-ATPase–derived peptide bound to I-A^d molecules. T cell activation was not inhibited by chloroquine, further suggesting that DCs constitutively processed and presented H^+/K^+ ATPase in vivo.

Tissue Damage Facilitates Capture and Presentation of Self-Ags by DCs. Although the new data from Heath and Germain's laboratories clearly show that DCs can process and present self-Ags to $CD4^+$ and $CD8^+$ T cells in vivo, the mechanisms by which Ags are captured in vivo have not been elucidated. DCs may acquire self-Ags locally in

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peripheral tissues and subsequently migrate to the draining LN. Alternatively, self-Ags may reach afferent lymphatic vessels and be taken up by resident DCs in the draining LN. This issue is even more complicated because soluble and cell-associated Ags are likely to be captured by different mechanisms. Scheinecker et al. (10) have also observed physical contacts between $CD8\alpha^-$ DCs and H^+/K^+-ATPase-expressing parietal cells in the stomach of healthy mice. Thus, there is at least some indirect evidence that DCs constitutively residing in the stomach can acquire cell-associated self-Ags in situ and subsequently transport them to the gastric LN where they can be visualized in intracellular vesicles within DCs. The question remains as to how cell-associated Ags are taken up by DCs? Physiological interactions between hematopoietic cells in the absence of cell death can result in transfer of membrane components between cells. DCs are highly interactive cells with extensive membrane processes that facilitate cell clustering interactions with other cells. Thus, it is theoretically possible that DCs acquire Ags in the absence of apoptosis or necrosis of the donor cells. Indeed, experimental evidence supporting this hypothesis has been reported in the case of primate cells for an endogeneous melanoma Ag (11). On the other hand, apoptotic cell death facilitates the entry of self-Ags into the cross-presentation pathway. This was first demonstrated in vitro for both MHC class I– and MHC class II–restricted epitopes (12) and has now been shown in vivo (13–16). For example, cells that had been induced to undergo apoptosis by exposure to osmotic shock were efficiently taken up by splenic $CD8\alpha^+$ DCs when injected intravenously into mice (14). In a perhaps more physiological model, Kurts et al. have shown that peripheral Ags expressed at low levels did not enter the cross-presentation pathway unless released by CTL-mediated tissue destruction (17, 18). Furthermore, the induction of pancreatic β cell apoptosis promoted the presentation of islet Ags by DCs (19).

What Is the Fate of Self-reactive T Cells That Have Encountered Self-Ags In Vivo? Although DCs can readily capture and present self-Ags to T cells, the immunological outcome depends on different parameters including the phenotype of the DCs and the nature of the cytokine environment in which DC–T cell interactions occur. In the steady state, that is in the absence of pathological or experimentally induced tissue injury, presentation of self-Ags to $CD4^+$ and $CD8^+$ T cell induces complete or partial T cell tolerization through either deletion (5, 20) or induction of unresponsiveness (1, 7). But what happens when parenchymal cells are dying such as would happen during physiological cell turnover (2, 15, 19) or as the result of tissue injury? In this issue, Liu et al. have addressed this question by injecting syngeneic animals with OVA-loaded splenocytes that have been induced to die (21). In agreement with other reports (13, 14, 22), dying cells were taken up by $CD8^+$ DCs in situ. In addition, ingestion and presentation of cell-associated OVA resulted in the deletion of OVA-specific $CD8^+$ T cells and eventually in the induction of immune tolerance. Thus, the capture of dying cells by DCs in the steady state can lead to profound tolerance to cell-associated Ags at least in the case of $CD8^+$ self-reactive T cells. Whether this also leads to the tolerization of $CD4^+$ T cells remains to be determined, but a recent study from our laboratory suggests that this may well be the case (19).

What Is the Phenotype of the DCs That Present Self-Ags to T Cells In Vivo? Although DC subtypes have been defined by multiple markers, the functional properties of these various DC populations remain a matter of controversy. At least five phenotypically defined populations of DCs have been reported in healthy mice, all of them express CD11c. Three exist in the spleen ($CD11b^+CD4^-$, $CD11b^+CD4^+$, $CD11b^-CD8\alpha^+$), whereas peripheral LN, Peyer's patches, and mesenteric LN contain two additional populations: $CD11b^{low}CD4^-CD8\alpha^-$ and $langerin^+$ (23). Although experiments performed under steady state conditions in healthy animals have shown that all subpopulations arise from independent precursors, it has also been reported that $CD8\alpha^+$ DCs could derive from $CD8\alpha^-$ DCs by a maturation process (23). With such a complicated situation, it is not surprising that the nature of the DCs which present self-Ags in vivo has remained controversial. In this respect, the three papers published in this issue report different results, but this is not too surprising considering differences in the experimental systems which have been studied. Thus, Belz et al. found that $CD8\alpha^+$ DCs were the only cells that presented gB to $CD8^+$ T cells in the pancreatic LN of their transgenic mice. Likewise, splenic $CD8\alpha^+$ but not $CD8\alpha^-$ DCs, presented OVA to $CD8^+$ T cells in mice which had been injected with OVA-loaded dying splenocytes (21). On the other hand, both $CD8\alpha^+$ and $CD8\alpha^-$ DCs presented H^+/K^+-ATPase–derived MHC class II–restricted epitopes in the gastric LN of BALB/c mice (10). Thus, although the three papers being discussed here provide new insights into the nature of the APCs which present self-Ags to T cells, more experimental models will have to be studied to draw any definitive conclusions on the role of each DC subset in the processing and the presentation of self-Ags. Furthermore, because these three studies all relied on ex vivo Ag presentation assays using sorted populations of DCs, it was not possible to determine which cells within each sorted population actually presented Ags and what was their phenotype. This could possibly be done by using mAbs reacting to specific peptide/MHC complexes (24–27). Unfortunately, generating such reagents is not an easy task. Furthermore, those which have been generated so far often exhibit a relatively low avidity for their ligand, eventually precluding their use for identifying the APCs that process and present Ags in vivo. Despite these limitations, mAbs reacting to specific peptide–MHC complexes have successfully been used to show that DCs from the T cell areas of LN express high levels of self-peptides bound to MHC class II molecules (28, 29). The same mAbs have been used to show that local expression of TNF-α in pancreatic β cells promoted the presentation of self-Ags by DCs in the islets (30). Molecular probes reacting to peptide/MHC complexes may be used to identify and characterize the APCs that present Ags to T cells. This is an

important issue because the outcome of DC–T cell interactions is likely to be influenced by the phenotype of the DCs. In this respect, recent experiments have provided direct evidence that Ag-loaded immature DCs silence T cells either by deleting them or by expanding regulatory T cells (2). Such a phenomenon is likely to occur under steady state conditions (1) and/or when cells undergo apoptosis and are captured by DCs in the absence of inflammatory cytokines (19, 21). In contrast to immature DCs, DCs expressing a more mature phenotype induce the development of a functional immune response. This may occur in infectious diseases, during powerful T cell immune responses such as those observed in transplantation (31), contact allergy (32), and autoimmunity (33), and upon immunological manipulations such as the administration of agonistic anti-CD40 mAbs (21).

What Next? Further analyzing the role of DCs in the maintenance of T cell tolerance to self-Ags may benefit from the generation of new animal models. In this respect, Brocker and colleagues have used the DC-specific CD11c promoter to selectively direct the expression of specific MHC molecules in DCs (8). Jung et al. have recently reported a novel diphteria toxin-based system that allows the inducible, short-term ablation of DCs in vivo (34). While this latter experimental model has shown that DCs are required for the development of a cytotoxic T cell response against *Listeria monocytogenes* and *Plasmodium yoelii*, it may also be useful for analyzing the role of DCs in the development of T cell tolerance to self-Ags. Such studies may also greatly benefit from the advent of new imaging techniques such as two-photon laser scanning microscopy (TPLSM). TPLSM provides the ability to track fluorescently labeled cells over time deep within light-scattering tissue, while largely avoiding the problems of bleaching and phototoxicity associated with conventional confocal microscopy. Indeed, TPLSM has recently been used to study the dynamic of DC–T cell interactions in the LN of mice which had been injected with Ag-loaded DCs (35, 36). There is little doubt that TPLSM will soon be used to study interactions between self-reactive T cells and DCs which present self-Ags in vivo.

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