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 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 to show that APCs can process and present self-Ags to T cells 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 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 CD8α−, but not CD8α+ DCs were the cross-presenting APCs in vivo. Also in this issue, Scheinacker 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 the H+/K+-ATPase–derived peptide bound to I-Ak 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...
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 CD8α− 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 pri mate cells for an endogenous 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 CD8α+ DCs when injected intravenously into mice (14). In a perhaps more physiological model, Krets 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 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−CD8α+), whereas peripheral LN, Peyer’s patches, and mesenteric LN contain two additional populations: CD11b+CD4−CD8α− and mesenteric LN (20). 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 CD8α+ DCs could derive from CD8α− 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 CD8α+ DCs were the only cells that presented gB to CD8+ T cells in the pancreatic LN of their transgenic mice. Likewise, splenic CD8α+ but not CD8α− DCs, presented OVA to CD8+ T cells in mice which had been injected with OVA-loaded splenocytes (21). On the other hand, both CD8α+ and CD8α− 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 Ags–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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References

1. Hawiger, D., K. Inaba, Y. Dorsett, M. Guo, K. Mahnke, M. Rivera, J.V. Ravetch, R.M. Steinman, and M.C. Nussenzweig. 2001. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. *J. Exp. Med.* 194:769–780.
2. Steinman, R.M., and M.C. Nussenzweig. 2002. Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. *Proc. Natl. Acad. Sci. USA.* 99:351–358.
3. Matzinger, P., and S. Guerder. 1989. Does T cell tolerance require a dedicated antigen-presenting cell? *Nature.* 338:74–76.
4. Kurts, C., W.R. Heath, F.R. Carbone, J. Allison, J.F. Miller, and H. Kosaka. 1996. Constitutive class I-restricted exogenous presentation of self-antigens in vivo. *J. Exp. Med.* 184:923–930.
5. Kurts, C., H. Kosaka, F.R. Carbone, J.F.A.P. Miller, and W.R. Heath. 1997. Class I-restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive CD8+ T cells. *J. Exp. Med.* 186:239–245.
6. Forster, I., and I. Lieberam. 1996. Peripheral tolerance of CD4+ T cells following local activation in adolescent mice. *Eur. J. Immunol.* 26:3194–3202.
7. Adler, A.J., D.W. Marsh, G.S. Yochum, J.L. Guzzo, A. Nigam, W.G. Nelson, and D.M. Pardoll. 1998. CD4+ T cell tolerance to parenchymal self-antigens requires presentation by bone marrow-derived antigen-presenting cells. *J. Exp. Med.* 187:1555–1564.
8. Kurts, C., M. Cannanile, I. Klebba, and T. Brocker. 2001. Dendritic cells are sufficient to cross-present self-antigens to CD8+ T cells in vivo. *J. Immunol.* 166:1439–1442.
9. Belz, G., G. Behrens, C. Smith, J. Miller, C. Jones, K. Lejon, C. Fathman, S. Mueller, and K. Shortman, F.R. Carbone, and W.R. Heath. 2002. The CD8α+ dendritic cell is responsible for inducing peripheral self tolerance to tissue-associated antigens. *J. Exp. Med.* 196:1099–1104.
10. Scheinecker, C., R. McHugh, E. Shevach, and R. Germain. 2002. Constitutive presentation of a natural tissue autoantigen exclusively by dendritic cells in the draining lymph node. *J. Exp. Med.* 196:1079–1090.
11. Harshyne, L.A., S.C. Watkins, A. Gambotto, and S.M. Barratt-Boyes. 2001. Dendritic cells acquire antigens from live cells for cross-presentation to CTL. *J. Immunol.* 166:3717–3723.
12. Heath, W.R., and F.R. Carbone. 2001. Cross-presentation in viral immunity and self-tolerance. *Nat. Rev. Immunol.* 1:126–134.
13. den Haan, J.M., S.M. Lehr, and M.J. Bevan. 2000. CD8+ but not CD8- dendritic cells cross-prime cytotoxic T cells in vivo. *J. Exp. Med.* 192:1685–1696.
14. Iyoda, T., S. Shimoyama, K. Liu, Y. Omatsu, Y. Akiyama, Y. Maeda, K. Takahara, R.M. Steinman, and K. Inaba. 2002. The CD8α+ dendritic cell subset selectively endocytoses dying cells in culture and in vivo. *J. Exp. Med.* 195:1289–1302.
15. Huang, F.P., N. Platt, M. Wykes, J.R. Major, T.J. Powell, C.D. Jenkins, and G.G. MacPherson. 2000. A discrete sub-population of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. *J. Exp. Med.* 191:435–444.
16. Valdez, Y., W. Mah, M.M. Winslow, L. Xu, P. Ling, and S.E. Townsend. 2002. Major histocompatibility complex class II presentation of cell-associated antigen is mediated by CD8α+ dendritic cells in vivo. *J. Exp. Med.* 195:683–694.
17. Kurts, C., J.F.A.P. Miller, R.M. Subramaniam, F.R. Carbone, and W.R. Heath. 1998. Major histocompatibility complex class I-restricted cross-presentation is biased towards high dose antigens and those released during cellular destruction. *J. Exp. Med.* 188:409–414.
18. Kurts, C., R.M. Sutherland, G. Davey, M. Li, A.M. Lew, E. Blanas, F.R. Carbone, J.F. Miller, and W.R. Heath. 1999. CD8 T cell ignorance or tolerance to islet antigens depends on antigen dose. *Proc. Natl. Acad. Sci. USA.* 96:12703–12707.
19. Hugues, S., E. Mougneau, W. Ferlin, D. Jeske, P. Hofman, D. Homann, L. Beaudoin, C. Schrake, M. Von Herrath, A.
Lehuen, and N. Glaiichenhaus. 2002. Tolerance to islet antigens and prevention from diabetes induced by limited apoptosis of pancreatic beta cells. *Immunity.* 16:169–181.

20. Hernandez, J., S. Aung, W.L. Redmond, and L.A. Sherman. 2001. Phenotypic and functional analysis of CD8+ T cells undergoing peripheral deletion in response to cross-presentation of self-antigen. *J. Exp. Med.* 194:707–717.

21. Liu, K., T. Iyoda, M. Saternus, Y. Kimura, K. Inaba, and R. Steinman. 2002. Immune tolerance following delivery of dying cells to dendritic cells in situ. *J. Exp. Med.* 196:1091–1097.

22. Ferguson, T.A., J. Herndon, B. Elzey, T.S. Griffith, S. Schoenberger, and D.R. Green. 2002. Uptake of apoptotic antigen-coupled cells by lymphoid dendritic cells and cross-priming of CD8+ T cells produce active immune unresponsiveness. *J. Immunol.* 168:5589–5595.

23. Shortman, K., and Y.J. Liu. 2002. Mouse and human dendritic cell subtypes. *Nat. Rev. Immunol.* 2:151–161.

24. Murphy, D.B., S. Rath, E. Pizzo, A.Y. Rudensky, A. George, J.K. Larson, and C.A. Janeway. 1992. Monoclonal antibody detection of a major self peptide MHC class II complex. *J. Immunol.* 148:3483–3491.

25. Zhong, G., C. Reis e Sousa, and R.N. Germain. 1997. Production, specificity, and functionality of monoclonal antibodies to specific peptide-major histocompatibility complex class II complexes formed by processing of exogenous protein. *Proc. Natl. Acad. Sci. USA.* 94:13856–13861.

26. Porgador, A., J.W. Yewdell, Y. Deng, J.R. Bennink, and R.N. Germain. 1997. Localization, quantitation, and in situ detection of specific peptide-MHC class I complexes using a monoclonal antibody. *Immunity.* 6:715–726.

27. Dadaglio, G., C.A. Nelson, M.B. Deck, S.J. Petzold, and E.R. Unanue. 1997. Characterization and quantitation of peptide-MHC complexes produced from hen egg lysozyme using a monoclonal antibody. *Immunity.* 6:727–738.

28. Inaba, K., M. Pack, M. Inaba, H. Sakuta, F. Isdell, and R.M. Steinman. 1997. High levels of a major histocompatibility complex II-self peptide complex on dendritic cells from the T cell areas of lymph nodes. *J. Exp. Med.* 186:665–672.

29. Velazquez, C., R. DiPaolo, and E.R. Unanue. 2001. Quantitation of lysozyme peptides bound to class II MHC molecules indicates very large differences in levels of presentation. *J. Immunol.* 166:5488–5494.

30. Green, E.A., E.E. Eynon, and R.A. Flavell. 1998. Local expression of TNF-α in neonatal NOD mice promotes diabetes by enhancing presentation of islet antigens. *Immunity.* 9:733–743.

31. Larsen, C.P., R.M. Steinman, M. Witmer-Pack, D.F. Hanskin, P.J. Morris, and J.M. Austyn. 1990. Migration and maturation of Langerhans cells in skin transplants and explants. *J. Exp. Med.* 172:1483–1493.

32. Resnick, M., N. Roguel, H. Bercovier, C. Enk, S. Frankenburg, and E. Kedar. 1988. Lysis of murine macrophages infected with intracellular pathogens by interleukin 2-activated killer (LAK) cells *in vitro.* *Cell. Immunol.* 113:214–219.

33. Abrams, J.R., S.L. Kelley, E. Hayes, T. Kikuchi, M.J. Brown, S. Kang, M.G. Lebwohl, C.A. Guzzo, B.V. Jegasothy, P.S. Linsley, and J.G. Krueger. 2000. Blockade of T lymphocyte costimulation with cytotoxic T lymphocyte-associated antigen 4-immunoglobulin (CTLA4-Ig) reverses the cellular pathology of psoriatic plaques, including the activation of keratinocytes, dendritic cells, and endothelial cells. *J. Exp. Med.* 192:681–694.

34. Jung, S., D. Unutmaz, P. Wong, G. Sano, K. De los Santos, T. Sparwasser, S. Wu, S. Vuthoori, K. Ko, F. Zavala, et al. 2002. *In vivo* depletion of CD11c+ dendritic cells abrogates priming of CD8+ T cells by exogenous cell-associated antigens. *Immunol. 17:211–220.

35. Miller, M.J., S.H. Wei, I. Parker, and M.D. Cahalan. 2002. Two-photon imaging of lymphocyte motility and antigen response in intact lymph node. *Science.* 296:1869–1873.

36. Stoll, S., J. Delon, T.M. Brotz, and R.N. Germain. 2002. Dynamic imaging of T cell-dendritic cell interactions in lymph nodes. *Science.* 296:1873–1876.