Commentary

The Induction of Tolerance by Dendritic Cells That Have Captured Apoptotic Cells

By Ralph M. Steinman,[§] Shannon Turley,[‡] Ira Mellman,[‡] and Kayo Inaba[*]

From the [*]Laboratory of Immunobiology, Graduate School of Biostudies, Kyoto University, Kyoto 606-8502, Japan; the [‡]Department of Cell Biology, Yale University Medical School, New Haven, Connecticut 06520-8002; and the [§]Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, New York 10021-6399

What makes a protein immunogenic, particularly for strong T cell–mediated immunity? To a first approximation, this determination seems to be made by dendritic cells (DCs). Immature DCs, as in skin (1–4), lung (5), blood (6, 7), and spleen (7, 8), take up proteins, immune complexes, microbes, and dying cells. However, in order to use these antigens to stimulate a T cell response, the DCs must undergo a characteristic process of terminal differentiation called "maturation." The known stimuli for DC maturation are numerous and include inflammatory cytokines, CD40 ligand (CD40L), viral and microbial constituents such as double-stranded RNA and LPS, and certain CpG oligonucleotides.

DC Maturation as a Control Point in the Initiation of Immunity

Maturation changes DCs in many ways that help explain their potent immunogenicity. Examples include de novo expression of T cell costimulatory molecules like CD86 (9, 10); the capacity to produce IL-12 (11, 12) and resist immunosuppression by IL-10 (13); the development of a new repertoire of chemokine receptors, especially CCR7 (14–17), that guide entry into lymphatics and migration to the T cell areas (18); the production of DC survival and stimulatory molecules like CD40 and TNF-related activation-induced cytokine receptor (TRANCE-R) (19, 20); and a redistribution of MHC class II molecules from lysosomes to the cell surface (21, 22). Recently, it has been found (Inaba, K., S. Turley, T. Iyoda, F. Yamaide, S. Shimoyama, C. Reis e Sousa, R.N. Germain, J. Mellman, and R.M. Steinman, manuscript submitted for publication) that immature DCs endocytose proteins into MHC class II–rich intracellular compartments, but the cells must also mature to form MHC–peptide complexes there and to export the complexes to the cell surface together with B7 costimulators. DC maturation is understandably a key control point in converting an antigen into an immunogen.

The role for DCs in determining immunogenicity seems well established, but many are now pursuing their role in other contexts: immune deviation, i.e., skewing T cells to the Th2 phenotype; immune regulation, i.e., inducing Tr1 cells that make IL-10; and bona fide tolerance, i.e., deletion and anergy. We will comment on the idea that the uptake of dying cells by immature DCs is critical for the maintenance of peripheral tolerance, including the findings in two papers in this issue. Huang et al. demonstrate that DCs in afferent lymph carry apoptotic bodies derived from the intestinal epithelium (23). They present evidence, using an isoform of intestinal nonspecific esterase, that DCs continually deliver samples of this tissue to the lymph node. Sauter et al. find that DCs phagocytose apoptotic and necrotic cell lines, but only the latter cause DCs to mature into strong stimulators of T cell immunity (24). Both papers suggest that the uptake of apoptotic cells allows DCs to induce peripheral tolerance to self. We will first outline why it makes sense for DCs, such potent agents of immunity, to also ensure tolerance to cell-associated self-antigens that unavoidably are present at sites of foreign antigen deposition.

The Value of Peripheral Tolerance Induction by DCs

Central or thymic tolerance is likely to be mediated by thymic DCs (25, 26), but these DCs may not be able to delete T cells that react with many self-antigens in peripheral tissues. Many proteins may not have access to the thymus during development, especially antigens that are expressed after the thymus has generated a T cell repertoire, e.g., breast constituents that are first expressed at puberty (27). Therefore, autoreactive T cells that are not deleted in the thymus need to be silenced in the periphery to prevent immune responses to self-tissues.

We would argue that it is essential for DCs to play a role in the induction of peripheral tolerance. The reasoning is as follows. Maturing DCs have the capacity to process and present peptides from dying cells to CD4 and CD8 T lymphocytes (28–31). In fact, DCs may be the principal cells that present antigens from dying cells ("cross presentation"; see below). In this light, consider what might occur during influenza infection of the airway (Fig. 1). During infection, there is extensive death of virus-infected, airway epithelial cells or "self." How do DCs focus immunity on the virus, when they also should be presenting self-antigens from the infected, apoptotic, airway epithelial cells (29, 32; Fig. 1)? Because cell death is a feature of many infections, the dan-

Address correspondence to Ralph M. Steinman, The Laboratory of Cellular Physiology and Immunology, The Rockefeller University, 1230 York Ave., New York, NY 10021-6399. Phone: 212-327-8106; Fax: 212-327-8875; E-mail: steinma@rockvax.rockefeller.edu
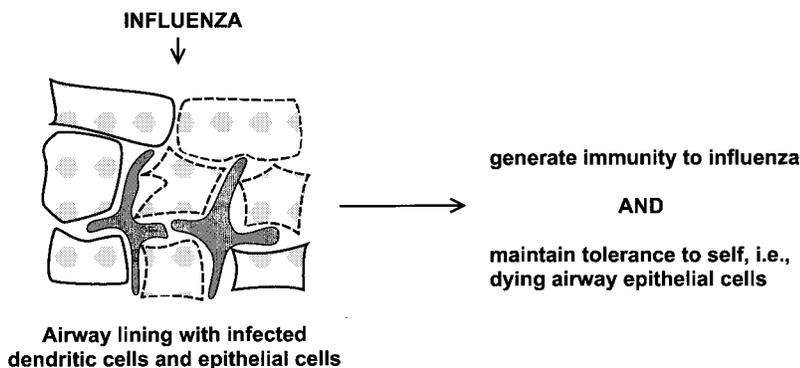
Figure 1. An illustration of the self-nonself problem from the perspective of DCs. During influenza infection of the airway, a site rich in DCs (reference 59), there is extensive apoptotic death of infected airway epithelial cells, which the DCs would likely process simultaneously with the influenza virus (references 29, 30).

ger of what Ehrlich rightly termed "horror autotoxicus" is hardly limited to influenza.

Thus, peripheral tolerance to those peptides that can be processed from dying cells seems critical for preventing autoreactivity, but when and how does this occur? It would be valuable for DCs to induce peripheral tolerance to dying noninfected tissues, since this would inactivate the key self-reactive T cells before the DCs are called upon to initiate immunity to microbial antigens. In effect, peripheral tolerance should share with central thymic tolerance the capacity to self-tolerize before foreign antigen exposure and to use the same APC that will later be called upon to initiate immunity.

Peripheral Tolerance to Tissue Antigens Via Bone Marrow–derived Cells in Draining Lymph Nodes

Precise tools have been developed to study peripheral tolerance. Neo self-antigens are expressed as transgenes in peripheral tissues, and then the animal is injected with the corresponding antigen-reactive, TCR transgenic T cells. Adler et al. (33) expressed the influenza hemagglutinin (HA) in many tissues. When HA-reactive CD4+ T cells were injected, the T cells were anergized, and when bone marrow chimeras were examined, the marrow-derived cells had to express the MHC that was recognized by the anergized, HA-reactive, TCR transgenic T cells (33). Anergy did not develop if only nonhematopoietic tissue cells expressed the appropriate MHC. In similarly elegant studies, Kurts et al. (34, 35) expressed OVA sequences in insulin-producing β cells of pancreatic islets (Fig. 2). The OVA antigen in tissue cells was again presented to T cells by marrow-derived cells

(34), and the TCR transgenic CD8+ T cells seemed to be tolerized by deletion after a series of cell divisions (35). Kurts et al. showed that the tolerizing, marrow-derived cells were confined to the draining lymph nodes (Fig. 2), i.e., the nodes that received afferent lymphatics from the tissue expressing the OVA antigen (the pancreas or kidney in their studies). Analogous results have been reported by others (36–38).

Somehow then, self-antigens in peripheral tissues are transferred to marrow-derived cells in a lymph node, and this can tolerize adult T cells. Although the marrow-derived cells have yet to be pinpointed, DCs are a likely candidate since they comprise a link between the peripheral tissues and the lymph node, the latter being the site where the tolerizing self-signals appear to be presented.

The Capture of Tissue Cells by DCs

Phagocytic inclusions have been described previously in DCs that traffic from tissues to lymph nodes in afferent lymph (39–41). Huang et al. now show that these inclusions are apoptotic bodies (23). Furthermore, their new data indicate that the apoptotic bodies derive from intestinal epithelium, presumably picked up from epithelial cells undergoing normal cell turnover.

The only comparable description of phagocytic inclusions in DCs in situ is a report involving NK cell–mediated clearance of allogeneic leukocytes (42). DCs may also take up apoptotic bodies during negative selection in the thymic medulla (43), but one cannot visualize this, presumably because the digestion of apoptotic cells is so rapid (28). Likewise, it is difficult to identify macrophages with phagocy-
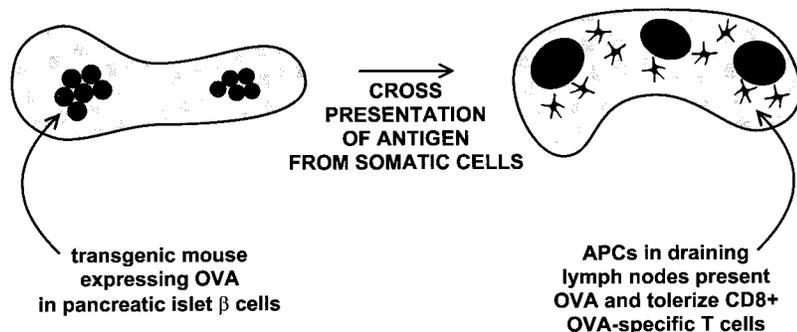

Figure 2. Diagram of the model of Kurts et al. (references 34, 35). Self-peptides in tissue cells, here the insulin-producing β cells of the pancreatic islets, are presented in a tolerogenic way by bone marrow–derived cells in the draining pancreatic lymph nodes.

tosed dying cells in situ. For example, many developing thymocytes undergo apoptosis if they fail to be positively selected. The thymocytes are likely to be scavenged by macrophages in the cortex, but this is only evident histologically if massive thymocyte death is induced with steroids or irradiation (43).

Therefore, the sighting by Huang et al. (23) of apoptotic epithelial cells in mesenteric lymph DCs suggests a major flux of tissue antigens via DCs that are heading to lymph nodes. Immature DCs or their precursors may always be trafficking through tissues (44, 45), picking up apoptotic material from cells undergoing the turnover that is characteristic of most tissues. If the events described by Huang et al. (23) silence reactivity to the intestinal peptides that are processed from dying cells, then DCs maturing during a subsequent intestinal infection would only stimulate a response to foreign antigen, thus alleviating the problem posed in Fig. 1.

Processing of Apoptotic Cells onto MHC Class I and II Products

Formation of MHC class I–peptide complexes from antigens in endocytosed dying cells (29, 30) illustrates phenomena termed the "exogenous pathway" and the "cross-presentation" of antigens. In the exogenous pathway, MHC class I molecules present peptides derived from endocytosed proteins, rather than newly synthesized ("endogenous") proteins in the cytoplasm. One example of the exogenous pathway is cross-presentation, since exogenous peptides from cells of one MHC, or even xenogeneic MHC, are presented by DCs of a different MHC.

DCs efficiently carry out the exogenous pathway for MHC class I. This applies to peptides derived from immune complexes (46), bacteria (47), and apoptotic cells dying because of viral (29, 30) or bacterial (31) infection. Rodriguez et al. have shown that molecules with molecular masses of 3–20 kD somehow can escape the endocytic system of DCs into the cytoplasm (48). They postulate that the endocytic vacuoles of DCs have a transporter or pore whereby substrates gain access to TAP molecules in the endoplasmic reticulum, followed by presentation on MHC class I. The recent results from the Bhardwaj and Amigorena laboratories also provide evidence that the exogenous pathway is expressed much more efficiently in DCs than in macrophages and B cells (30, 46, 48).

Effects of Apoptotic Cells on DC Maturation

The paper by Sauter et al. in this issue introduces the critical events of DC maturation to this topic. The uptake of apoptotic cells in the steady state must not mature the DCs if these cells are to induce tolerance rather than immunity, and indeed this is what Sauter et al. (24) and Gallucci et al. (49) now report. Immature DCs selectively carry out phagocytosis of apoptotic cells (28, 30), as is also the case for the uptake of microbes, latex, and immune complexes (4, 46, 50). For one thing, relevant phagocytic receptors are better expressed on immature DCs, e.g., $\alpha_V\beta_5$ integrin for apoptotic bodies and FcγR for immune complexes (30, 46).

If DCs only take up apoptotic cells when immature (28, 30), if apoptotic cells do not mature the DCs (24), and if immature DCs are poor stimulators of immunity (1), then what are the immunological consequences to the carriage of large numbers of dying somatic cells by DCs in lymph (23)? Is uptake immunologically "null," like the clearance of apoptotic bodies by macrophages, or might peripheral tolerance ensue?

Hypothesis: Immature DCs Phagocytose Tissue Cells Undergoing Normal Cell Turnover by Apoptosis; This Leads to Tolerance or Regulation of Self-reactive, Adult T Cells in the Draining Lymph Node

In the steady state, i.e., in the absence of inflammation, infection, and necrosis, DCs are always found in afferent lymph, where they are also called "veiled cells." Veiled cells might derive from precursors in the blood (6, 51) including monocytes (44, 45). The idea is that circulating immature DCs and monocytes can traffic through tissues, picking up cells that die by apoptosis (28, 30), and then enter the afferent lymph (Fig. 3). In the steady state, these DCs will not receive maturation stimuli and therefore will be unable to stimulate immunity to the self-antigens they have captured.

How might immature DCs induce tolerance to self-antigens in phagocytosed apoptotic cells? One view is that migratory immature DCs tolerize T cells directly because of a lack of costimulators (Fig. 3). There are potential difficulties
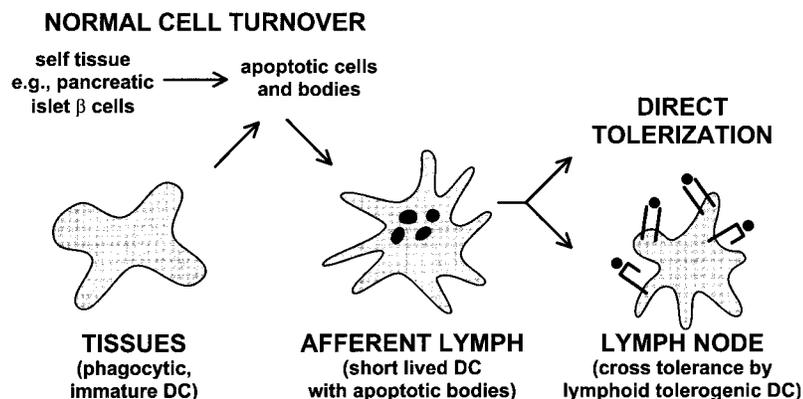
NORMAL CELL TURNOVER

Figure 3. Pathways whereby DCs might induce peripheral tolerance to antigens within tissue cells undergoing normal cell turnover by apoptosis. DC precursors traffic through tissues, phagocytose dying cells or apoptotic bodies, and then enter the lymph. Upon reaching the lymph node, the DCs tolerize naive, self-reactive T cells either directly, or indirectly (as diagrammed here) after reprocessing by different regulatory or tolerogenic DCs in the lymph node.

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with this idea. For example, we have just found that immature DCs do not process endocytosed antigens well to form MHC–peptide complexes (Inaba, K., S. Turley, T. Iyoda, F. Yamaide, S. Shimoyama, C. Reis e Sousa, R.N. Germain, J. Mellman, and R.M. Steinman, manuscript submitted for publication). Therefore, self-reactive T cells would not be able to recognize their ligand on immature DCs. Also, the immature DCs may lack the CD40 and TRANCE-R that sustain DC viability for the 3–4 d needed before T cell tolerance becomes evident (35, 37). In contrast, as summarized above, mature DCs express high levels of MHC–peptide, as well as the CD40 and TRANCE-R that sustain DC viability during the interaction with activated T cells (52). Possibly the migratory immature DCs overcome some of these potential shortcomings and develop their tolerizing function upon encountering T cells or other stimuli after reaching the node.

A second mechanism is that there will be subsets of DCs, as first proposed by Suss and Shortman (53), that are somehow specialized to regulate immunity or to induce tolerance. Direct evidence for this DC subset remains elusive. However, the idea is that immature DCs capture apoptotic bodies peripherally and transfer tissue-derived peptides to tolerogenic DCs upon reaching the lymph node. We are intrigued by this possibility because of the information that migratory DCs in lymph are short lived and appear to be processed by longer-lived, resident DCs in lymph node (28). When migratory DCs are injected into mice, the cells leave the injection site (54), presumably via the afferent lymph, but <1% of the injected cells can be recovered 2 d later in a lymph node according to new data from Josien et al. (55). The dying, injected DCs are not totally destroyed by some "big Mac," but instead can be processed and presented by DCs in the lymph node (28). The lymph node DCs can express high levels of MHC–peptide and interestingly, relatively low levels of surface CD86 costimulator (56). If these lymph node DCs efficiently form MHC–peptide complexes from incoming DCs and their contents, the former subset may be the best candidate to present self-antigens from apoptotic cells in a tolerogenic way. We postulate that there are resident, lymph node DCs which in the steady state induce tolerance to antigens in apoptotic bodies carried by migratory lymph DCs (Fig. 3).

Tolerogenic DCs may constitute a separate differentiation pathway, as suggested by Suss and Shortman (53). Perhaps these DCs derive from the distinct plasmacytoid precursor termed DC2 by Liu and colleagues (57, 58). The tolerizing function of DCs may be quite sophisticated. For example, tolerance could ensue by deletion or anergy of the self-reactive T cell, as suggested by the work of Adler et al. (33) and Kurts et al. (34, 35), discussed above. Alternatively, DCs might expand regulatory T cells. The latter may be needed for self-antigens at body surfaces like the intestine and airway, which are unlikely to be devoid of DC maturation stimuli.

Conclusion

DCs are specialized to control immunity, to trigger immune responses, and also, it appears, to maintain tolerance.

These two spheres become intimately linked when one appreciates that cell death often accompanies infection and that DCs can present self-antigens from dying cells. The maturation of peripheral DCs, which is often triggered by infectious agents, should allow at least some phagocytosed self-antigens to become immunogenic. We develop the hypothesis that immature DCs in the steady state are inducing tolerance to self-antigens within phagocytosed apoptotic bodies, derived from the normal turnover of tissues. This occurs well before the entry of a foreign antigen, so when infection and DC maturation take place, the immune system can focus on the foreign peptides that the DCs have processed.

Sauter et al. (24) report that the uptake of apoptotic cells does not directly mature DCs. Also Huang et al. (23) find that intestinal lymph DCs normally carry phagocytosed, apoptotic, intestinal epithelial cells towards the lymph node, presumably without inducing intestinal autoimmunity. It is known that marrow-derived cells within lymph nodes can tolerize T cells to peptides synthesized in other tissues. Thus, DCs may traffic through tissues, pick up apoptotic cells arising from normal cell turnover, and then, upon migration to lymph nodes in afferent lymph, silence T cells to self-antigens in the phagocytosed apoptotic bodies. Tolerance to self-antigens in the steady state need not be direct. It may instead involve transport of apoptotic bodies in short-lived migratory DCs to longer-lived, tolerizing DCs in the lymph node. The latter are able in the steady state to form high levels of MHC–peptide complexes but either lack key costimulators for immunity or have unique products for inducing tolerance.

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