

Maturation requirements for dendritic cells in T cell stimulation leading to tolerance versus immunity

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Abstract: The model that dendritic cell (DC) "maturation" describes the change from an immature, antigen-capturing cell to a mature, antigen-presenting cell is well-established. Classification of DCs in terms of function has been problematic previously. It is therefore proposed that mature and not immature DCs are responsible for antigen presentation and stimulation of T cells. Furthermore, DC antigen presentation to T cells can have two outcomes: tolerance or immunity. The particular outcomes appear to be determined by the activation state of the mature DC. DCs can be activated by a range of environmental stimuli or "danger signals". Here, the hypothesis is advanced that activated, mature DCs induce T cell immunity, and resting, nonactivated but fully differentiated mature antigen-presenting DCs can induce tolerance. This proposal extends to conventional DCs and plasmacytoid DCs. The paper also concentrates on the spleen as a site for DC maturation, in light of evidence from this laboratory for differentiation of DCs from splenic precursors in long-term, stroma-dependent cultures. The hypothesis advanced here serves to simplify many current issues regarding DC maturation and function. *J. Leukoc. Biol.* 78: 319–324; 2005.

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DENDRITIC CELLS (DCs) AND T CELL STIMULATION

DCs are well-recognized for their role in T cell activation and in initiation of antigen-specific immune responses [1–3]. Many DCs reside in and traffic through nonlymphoid peripheral tissues, continuously surveying the environment for invading microorganisms [4]. During infection, DCs in the periphery are triggered by exposure to microbial agents or inflammatory mediators to increase their expression of major histocompatibility complex (MHC) molecules and costimulatory molecules such as CD80 and CD86. This activates peripheral DCs into a state ready for T cell activation. Exposure to microbial products also modifies DC expression of chemokine receptors and adhesion molecules, causing migration from the periphery to the T cell zone of secondary lymphoid organs [5]. Activated DCs then display pathogen-encoded antigens to naïve antigen-specific T cells circulat-

ing through secondary lymphoid tissues, which respond and initiate primary T cell immune responses. For the purposes of this paper, reference is made mainly to conventional DCs, comprising myeloid-like CD11c⁺CD11b⁺CD8 α ⁻ DCs and lymphoid-like CD11c⁺CD11b⁻CD8 α ⁺ DCs, although the argument and hypotheses would appear to apply equally well to plasmacytoid precursor DCs (CD11c⁺CD11b⁻CD8 α ⁻).

More recently, DCs have been shown to play an important role in regulating peripheral tolerance to self-antigens. Under steady-state conditions and in the absence of microbial stimulation, apparently immature peripheral DCs or immature DCs in blood capture and transport antigens to secondary lymphoid organs [6, 7]. As sentinel cells of peripheral tissues, DCs continuously sample their environment for antigens. A major source of antigens during the steady-state, i.e., in the absence of infection, is apoptotic tissue cells that die during physiologic tissue turnover. Apoptosis occurs in numerous cells in the body on a daily basis [7]. Apoptotic cells represent a random source of self-antigens, critical for the maintenance of peripheral tolerance [8]. Cell death by apoptosis is not accompanied by inflammation, and DCs that internalize apoptotic cells do not become activated [9, 10]. In the steady-state, antigen-loaded DCs migrate spontaneously to secondary lymph nodes, mature en route, and acquire capacity to stimulate T cells. However, the outcome of T cell stimulation by steady-state DCs can be apoptosis [11], anergy [12], or the development of regulatory T (Treg) cells [13], depending on the state of maturation of the DCs. Each of these mechanisms can result in some form of T cell tolerance.

Tolerance is required to eliminate self-reactive T cells from the peripheral pool of lymphocytes. The majority of self-reactive T cells is deleted in the thymus via a process known as central tolerance. Developing thymocytes that bear high-affinity T cell receptors for self-antigens undergo apoptosis and are deleted by negative selection. However, central tolerance is not always complete. It is estimated that as many as 25–40% of T cells reactive to a self-peptide escape clonal deletion in the thymus [14]. These T cells include low-affinity, autoreactive T cells and T cells specific for self-antigens not presented in the thymus [14, 15]. Furthermore, T cells must remain tolerant to harmless environmental antigens found in the respiratory tract

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or intestines [16]. The existence of autoreactive T cells into the periphery necessitates the role for DCs in peripheral tolerance to prevent autoimmunity.

The existence of self-reactive T cells circulating in the periphery is not problematic if T cells are naïve and if they remain within lymphoid tissues and do not enter normal tissues to induce tissue damage. Self-reactive, naïve T cells therefore do not lead to disease, as long as they ignore or are separated from self-antigens [17]. However, naïve, self-reactive T cells are potentially a problem during infection or inflammation. DCs will capture and present pathogenic antigens to induce T cell immunity. However, they will also copresent numerous self-antigens captured during the steady-state. There is an inherent risk that DCs copresenting self-antigens during infection might activate self-reactive T cells that recognize an autoantigen leading to effector T cell formation and the initiation of autoimmunity. In establishing peripheral tolerance in the steady-state by filtering out autoreactive T cells before an acute infection, DCs can effectively focus adaptive immunity on the pathogen and so avoid autoimmunity [18].

CHARACTERISTICS OF IMMATURE VERSUS MATURE DCs

The two well-established maturation states for DCs include the “immature” and “mature” states. Immature, conventional DCs display a phenotype reflecting their specialized function as antigen-capturing cells. They are highly endocytic, able to acquire fluid-phase antigens by macropinocytosis, take up protein or antigen-antibody immune complexes by receptor-mediated endocytosis, and ingest entire cells by phagocytosis [1, 19]. They express relatively low levels of surface MHC-I and MHC-II gene products and costimulatory molecules such as CD80 and CD86 [20, 21]. Although immature DCs can capture antigens, they are unable to process and present them efficiently to T cells [22]. By comparison, freshly isolated, steady-state plasmacytoid DCs are more weakly endocytic than conventional DCs and poor T cell stimulators. They express MHC-I but not MHC-II and have a weak costimulator expression [23, 24]. However, conventional and plasmacytoid, immature DCs have been described as inducers of T cell tolerance [18, 25, 26].

Mature DCs are immunogenic in that they express cell surface molecules important for T cell activation. Maturation of DCs is associated with reduced antigen uptake through loss of antigen receptors and down-regulation of macropinocytosis and phagocytosis [27, 28]. However, maturation is also associated with increased efficiency in antigen processing and increased half-life of surface-expressed MHC-peptide complexes [20]. Higher cell surface expression and lower turnover of MHC-I and MHC-II molecules are required for a more effective presentation of antigen to CD8⁺ cytotoxic or CD4⁺ helper T cells (Th cells). Expression of costimulatory molecules by DCs is also required for productive T cell stimulation [29]. The phenotypic changes commonly associated with DC maturation make DCs potent activators of T cell immunity.

DC MATURATION AND TOLERANCE VERSUS IMMUNITY

The maturation state of DCs is considered a key determinant of the outcome of T cell activation leading to T cell tolerance or T cell immunity. One common model is that DCs with an immature phenotype are tolerogenic to T cells, and mature DCs are immunogenic [30, 31]. Other reports also claim that some level of maturation of DCs is required for tolerance induction, contradicting the favored model that only immature DCs induce tolerance, and mature DCs stimulate immunity [32, 33]. However, terminology used to define the state of maturation of DCs, including immature, mature, and “activated”, has been applied inconsistently in the literature. The terms “semimature” [34], “partially mature” [35], “mature but quiescent”, and “fully activated” [36] only add further uncertainty to the definition of DC maturation states. It is therefore important to define DC maturation states clearly and to clarify their relationship to tolerance versus immunity.

Definition of immature DCs

Immature DCs are generally described as highly endocytic, low expressors of MHC and costimulatory molecules and weak stimulators of T cells. DCs in this state of maturation play a predominant role in antigen capture from within their local environments. However, the proposal here is that resting, steady-state, immature DCs, by definition, would not be competent in T cell tolerance induction. A role for immature versus maturing DCs in tolerance induction comes from reports on the steady-state migration of immature DCs and the tolerization of T cells in lymph nodes. It has been assumed that immature DCs initiate T cell tolerance directly. However, studies on migratory, steady-state DCs have demonstrated that some maturation has occurred, allowing DCs to present antigens and stimulate T cells [16, 33]. Changes in the expression of chemokines and chemokine receptors such as CC chemokine receptor 7, which are known to accompany DC maturation induced by exposure to lipopolysaccharide (LPS), also occur in steady-state DCs, as they migrate to lymphoid organs [37]. Furthermore, migrating DCs have been shown to express MHC-II and costimulatory molecules at levels comparable with mature, immunogenic DCs [38]. These studies demonstrate that mature or maturing DCs and not immature DCs stimulate T cell tolerance and that this is facilitated by changes that convert the immature, antigen-capturing DC into mature, antigen-presenting cells (APCs). Immature DCs in the steady-state are thought to mature spontaneously and acquire the capacity to induce T cell tolerance. What is not yet known is the range of maturation signals available to immature DCs and whether the different lineages of maturing DCs contribute to functional diversity.

Defining mature DCs in terms of function

DCs undergoing maturation can differentiate to become exceptionally good APCs with the capacity to stimulate naïve T cells. In addition to activating T cell immunity, stimulation of T cells by mature, antigen-presenting DCs is also required for tolerance induction, and many reports have now demonstrated the

importance of mature DCs in mediating T cell tolerance dependent on the induction of Treg cells [13, 38]. The outcome of T cell stimulation as tolerance or immunity depends on whether mature DCs have been activated. Previous reports have used the terms “maturation” and “activation” interchangeably [39]. However, maturation and activation appear to be two distinct processes. Here, we refer to maturation as the process of DC differentiation from an immature to a mature state and activation as a process, dependent on additional stimuli including “danger signals”. Activation of DCs by infection or inflammation changes the capacity of mature DCs from tolerogenicity to immunogenicity. It is proposed therefore that resting or steady-state, mature DCs induce a state of tolerance, and activated, mature DCs induce a state of immunity. It is also feasible that mature, resting DCs, which are tolerogenic, can also be activated to form immunogenic DCs.

Under conditions of infection or inflammation, DCs encounter activating signals that may mature and activate DCs simultaneously, making them immunogenic. DC activators or danger signals include proinflammatory cytokines and bacterial or viral products such as LPS, CpG motifs, and double-stranded RNA [40–42]. These factors may induce the maturation and activation of DCs, allowing DCs to present antigens immunogenically. Activated DCs can be distinguished from resting, mature DCs by expression of higher levels of MHC and costimulatory molecules or by production of cytokines such as interleukin (IL)-12 and interferon- α , in the case of plasmacytoid DCs [35, 38, 43, 44]. The distinct functions of resting, steady-state DCs versus activated DCs in tolerance versus immunity have been demonstrated experimentally by Probst et al. [45]. They used a novel *in vivo* system involving inducible expression and presentation of a lymphocytic choriomeningitis virus-derived antigen by DCs. An *in vivo* comparison of DC function in the steady-state versus activation could be made for T cell stimulation without the need for adoptive transfer of DCs or T cells. The process of adoptive transfer can disturb steady-state cell function. In this study, antigen presentation by steady-state DCs resulted in antigen-specific tolerance, which could not be broken by subsequent rechallenge with antigen. By contrast, antigen presentation by activated DCs induced T cells to develop effector function and immunity.

DC SUBSETS IN SPLEEN

The spleen is a secondary lymphoid organ, which filters antigens from blood. It is divided broadly into the red and white pulp, separated by a diffuse marginal zone. Blood enters the spleen through the splenic artery that divides into progressively smaller arterioles, eventually emptying into the marginal zone [46]. This is an efficient antigen-trapping zone in the spleen. CD8 α^- DCs are located in the marginal zone and are ideally suited to sampling antigens from the blood that enters the spleen [47]. They have been reported to interact with B cells, which also reside in the marginal zones. Reports by Balazs et al. [6] have shown that a subset of CD4 $^-$ CD8 α^- CD11c lo DCs captures antigen from blood and activates B cells, initiating thymus-independent immune responses. CD8 α^- DCs may also interact with migrating T cells passing through the marginal

zone [48]. The white pulp surrounds splenic arterioles, forming the periarteriolar lymphoid sheath (PALS), which is populated mainly by T cells. CD8 α^+ DCs reside in the PALS and are ideally placed for T cell interaction.

Murine spleen contains three major endogenous populations of DCs. They are referred to as the CD4 $^-$ CD8 α^- , CD4 $^+$ CD8 α^- , and CD4 $^-$ CD8 α^+ subsets [49]. Most attention has been focused on the comparison of CD8 α^- (CD4 $^-$ CD8 α^- and CD4 $^+$ CD8 α^-) and CD8 α^+ (CD4 $^-$ CD8 α^+) DCs. CD8 α^- DCs are distinct from CD8 α^+ DCs by a number of criteria. They reside predominantly in the marginal zone of spleen [50] and primarily direct a Th2 response by activating T cells to secrete cytokines such as IL-4 [51–53]. CD8 α^- DCs are endocytic and can be strong stimulators of T cells [54–56]. By contrast, CD8 α^+ DCs, located in the T cell area of the PALS [50], produce IL-12 upon stimulation and induce a Th1 response [57]. CD8 α^+ DCs are also endocytic, with distinct capacity to internalize apoptotic cells [58, 59] and to cross-present MHC-I-restricted antigen [60]. In contrast to CD8 α^- DCs, CD8 α^+ DCs are implicated in the suppression of T cell responses, resulting in T cell tolerance [61, 62], although this has been challenged by reports that CD8 α^+ DCs can also be stimulators of CD8 $^+$ anti-viral cytotoxic T cells [55, 63].

FUNCTIONAL RELATIONSHIP BETWEEN SPLENIC DC SUBSETS

Although conventional CD8 α^- and CD8 α^+ DCs appear to be distinct populations of DCs, the functional relationship between subsets is not clear. One model proposes that each subset represents a different maturation stage of the same DC lineage [64]. By this model, it is hypothesized that immature CD8 α^- DCs are responsible for endocytosing antigens. Upon maturation, DCs migrate to the T cell area of the spleen and up-regulate CD8 α and CD205 expression, acquiring a CD8 α^+ DC phenotype [47]. In the mature state, CD8 α^+ DCs are responsible for antigen presentation and T cell stimulation. This model is supported by evidence that other DC subsets such as Langerhans cells [65] and plasmacytoid DCs [23] can up-regulate expression of CD8 α upon maturation. However, this model has been disputed by Naik et al. [66], who reported that CD8 α^- and CD8 α^+ DCs do not have a precursor-product relationship. A second model encompassing this finding proposes that the two subsets are developmentally independent. Studies have shown that all three subsets of splenic DCs are phenotypically and functionally immature but capable of being induced to mature [67] without any DC subset reverting to another [54]. They could therefore reflect separate DC lineages.

It is proposed that immature CD8 α^- DCs in the marginal zone sample antigens from the local spleen environment. Spleen-resident CD8 α^- DCs can internalize apoptotic splenocytes undergoing turnover in the spleen or capture particulates and circulating apoptotic cells that enter the spleen [47, 54]. In the steady-state, it is presumed that peripheral blood CD8 α^- DCs and marginal zone CD8 α^- DCs can mature and migrate to the T cell area of the PALS to present antigens to T cells in a tolerogenic context. Studies have shown that CD8 α^- DCs migrate into the central T cell areas of the PALS [42]. A model for

what is occurring must take into account the endocytic capacity of different subsets of DCs and the kinetics of cell turnover. For example, splenic DCs exhibit short half-lives of 2–3 days [54]. It has also been proposed that most migrating DCs die after arrival in lymphoid tissues [68]. It is hypothesized that after antigen capture, peripheral blood and splenic CD8 α ⁻ DCs mature and migrate to the T cell area of the spleen, where they present antigens directly to T cells for a short period of time before undergoing apoptosis [69]. Apoptotic DCs are then endocytosed by resident CD8 α ⁺ DCs, which phagocytose other cells (including CD8 α ⁻ DCs) and cross-present antigens derived by phagocytosis [58]. Endocytosis, reprocessing, and presentation of antigens by CD8 α ⁺ DCs could also provide greater stability and longer-term expression of MHC-peptide complexes. This DC subset has been shown to have a slower turnover rate and would be primarily responsible for T cell stimulation [48]. This model has not been widely accepted, and Kamath et al. [54] have disputed the estimation of turnover rates. However, such a model would be consistent with the functionally distinct capacities of CD8 α ⁻ and CD8 α ⁺ DCs, the hypothesis of separate DC lineages, and separate DC subset localizations in spleen.

DCs in the spleen would also be capable of responding to infection by maturation via activation from danger signals. Stimulation by danger signals synonymous with infection, such as bacterial LPS, would cause splenic CD8 α ⁻ DCs and any peripheral blood DCs to migrate to the T cell area of the spleen in a mature, activated state. T cells stimulated by activated DCs would be primed to form effector T cells. CD8 α ⁺ DCs may also become activated by inflammatory cytokines released by activated CD8 α ⁻ DCs and take an additional part in T cell priming. The basic model of antigen capture and presentation to T cells involving CD8 α ⁻ and CD8 α ⁺ DCs would be essentially similar during steady-state conditions and during infection. However, although the outcome of steady-state antigen presentation by mature DCs would be tolerance, antigen presentation by mature, activated DCs would lead to a state of immunity.

ENVIRONMENTAL SIGNALS FOR DC MATURATION

Problems associated with the isolation of a rare cell type have in part contributed to confusion over maturation states of DCs and their immunostimulatory capacity. The isolation of immature DCs is a particularly difficult task, which impacts on our capacity to clearly delineate the function of cells in different states of maturation. Another limitation of ex vivo DC studies is that cells are studied in isolation from the environment in which they develop. One model investigated in our laboratory is that different niches exist in spleen, which give rise to a diversity of DCs arising from a small number of precursors or progenitors [70, 71]. Evidence from studies on long-term, stroma-dependent cultures from spleen suggests that the splenic microenvironment supports development of immature and then mature DCs from progenitors maintained within the spleen [72–76]. In these cultures, DCs develop in the presence of a stromal cell environment comprising endothelial and fi-

broblast cells. Studies by others have now identified a specific role for spleen stroma in the production of regulatory DCs, which have the capacity to switch off T cell activation [77]. The development of function in these DCs is dependent on cell-cell contact and interaction with fibronectin and transforming growth factor- β produced by stromal cells. The main element of a niche model for DC hemopoiesis is the existence of a committed progenitor cell with the capacity to self-renew as well as differentiate into DCs [71]. One hypothesis to reconcile evidence for multiple DC lineages or subsets is compartmentalization and the existence of different microenvironments within organ sites. Different niches could contribute to the proliferation and differentiation of DCs of different types and in different stages of development. The maturation triggers provided by stromal cells are now under investigation using a series of cloned stromal lines with a different capacity to support DC development and maturation.

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