

Supplement Review

The instructive role of dendritic cells on T-cell responses

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Chapter summary

Immune responses are initiated in the T-cell areas of secondary lymphoid organs where naïve T lymphocytes encounter dendritic cells (DCs) that present antigens taken up in peripheral tissues. DCs represent the interface between the universe of foreign and tissue-specific antigens and T lymphocytes, and they are the key players in the regulation of cell-mediated immunity. We discuss how the nature of the DC maturation stimuli and the density and quality of DCs present in the T-cell areas of secondary lymphoid organs determine the magnitude and class of the T-cell response.

Keywords: dendritic cells, effector and memory T cells, T-cell activation, T-cell tolerance

Introduction

DCs possess specialized features, such as pathogen recognition, antigen capturing and processing machinery, migratory capacity and costimulatory molecules, that allow them to act as the professional antigen presenting cells (APC) [1]. DCs represent the interface between the universe of foreign and tissue-specific antigens and T lymphocytes. They also play a role in all aspects of T-cell responses, from the deletion of self-reactive thymocytes to the generation of effector and memory cells, as well as the induction of peripheral tolerance.

In this review, we shall discuss how DCs provide a qualitative and quantitative framework for T-cell antigen recognition. We shall first summarize the requirements for T-cell activation and differentiation in terms of concentration of peptide-MHC complexes, costimulatory molecules and cytokines. We shall then consider how DCs assemble these components and deliver them, as discrete short-lived packets, to the T-cell areas. Finally, we shall discuss how the nature of the DC maturation stimuli determines

the density and quality of antigen-carrying DCs and, consequently, the magnitude and class of T-cell responses.

Activation and differentiation of naïve T lymphocytes

The signals that lead to T-cell activation are generated at the level of the immunological synapse, a specialized area of contact between T cells and APC where adhesion molecules and TCRs segregate into distinct supramolecular complexes [2,3]. At the synapse, the TCRs are sequentially triggered by peptide-MHC complexes, a process that allows the signal to be sustained for as long as the synapse is in place [4,5]. Synapses are stable in the absence of disturbing influences, but they can be disrupted by cell division, by death of APC or by external influences, such as collagen or chemokines. T cells continuously search for antigen and can rapidly shift from one APC to another offering a higher level of stimulation. While the duration of TCR stimulation depends on the duration of the synapse, the intensity of signal that T cells receive is dependent both on the level of peptide-MHC complexes

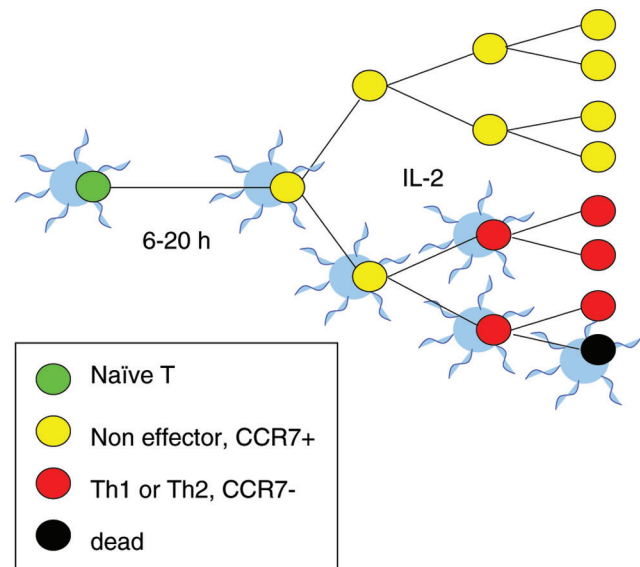
that trigger TCRs and the level of costimulatory molecules that amplify the signalling process [6].

The efficiency of signal transduction varies with the nature of the APC and the T cell's developmental stage [6,7]. In activated, effector and memory T cells, TCR triggering is efficiently coupled to signal transduction pathways so that the cells can respond to low doses of antigens even in the absence of costimulation. In contrast, TCRs are inefficiently coupled in naïve T cells. Engagement of CD28 by B7 molecules expressed by professional APC recruits membrane rafts containing kinases and adapters to the synapse, and amplifies up to 100-fold the signalling process initiated by the TCRs. In the absence of costimulation, naïve T cells can thus be activated only by extremely high (nonphysiologic) doses of antigens and they require a prolonged stimulation, while in the presence of costimulation they can respond to ~100-fold lower doses of antigen and can also respond more rapidly. Depending on the antigen dose and the level of costimulation, naïve T cells require between 6 and >30 hours of TCR stimulation to become committed to cell division, while memory/effector T cells respond within 0.5–2 hours [7].

Once committed to division, T cells proliferate rapidly in response to IL-2, which is produced in an autocrine or paracrine fashion by activated T cells. We have shown that the duration of TCR stimulation together with polarizing cytokines determines the progressive differentiation of CD4⁺ and CD8⁺ T cells, leading to the generation of terminally differentiated effector cells as well as intermediates [8,9]. T cells that receive a short TCR stimulation proliferate, but fail to differentiate to effector cells and retain the lymph-node homing capacity characteristic of naïve T cells. In contrast, T cells that receive a prolonged TCR stimulation in the presence of IL-12 or IL-4 differentiate to Th1 or Th2 cells. As part of their differentiation programme, Th1 and Th2 cells lose the lymph-node homing receptors and acquire receptors that control their migration to inflamed nonlymphoid tissues where they can execute effector functions.

T cells interact with DCs in a highly dynamic environment where they have to compete to achieve a level of TCR stimulation sufficient to drive their activation and differentiation processes. We suggest that the progressive process of T-cell differentiation combined with the stochastic stimulation of proliferating T cells by random T cell–DC interactions leads to the generation of both intermediates and terminally differentiated cells within the same responding clone (Fig. 1) (F Sallusto, unpublished data). We consider this intraclonal diversification as a fundamental property of the immune system since, on the one hand, it prevents clonal exhaustion and, on the other, it allows the generation of distinct T-cell subsets that play a role in effector and memory responses [5]. The intraclonal

Figure 1



Stochastic stimulation of proliferating T cells leads to intraclonal functional diversification. By establishing immunological synapses with dendritic cells (DCs), naïve T cells (green) achieve stimulation and become committed to proliferate in response to autocrine or paracrine IL-2. T-cell receptor stimulation is sustained by serial encounters with DCs and, in the presence of polarizing cytokines (IL-12 and IL-4, not shown), drives T-cell differentiation to Th1 or Th2 effector cells that have lost CCR7 expression (red). T cells receiving a shorter stimulation do not acquire effector function and do retain lymph-node homing capacity (yellow). An excessive stimulation leads to activation-induced cell death (black).

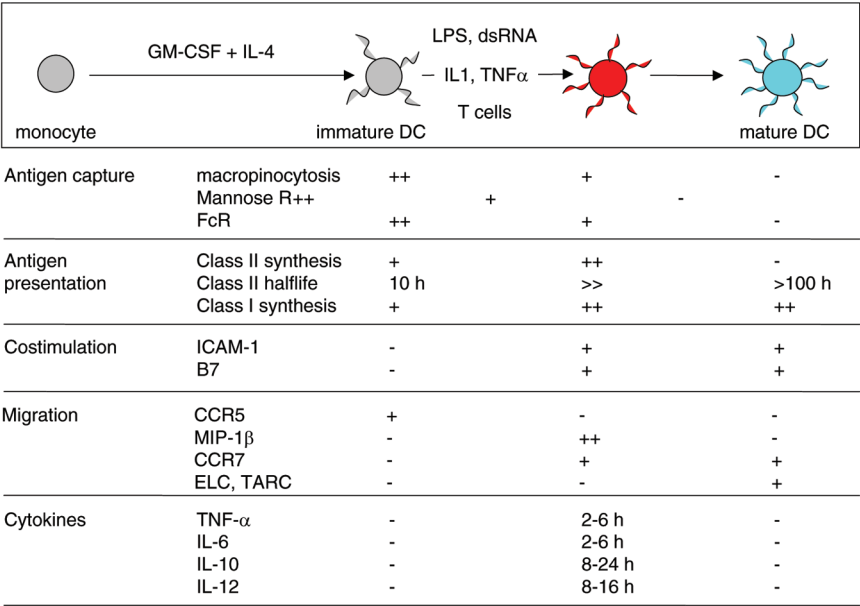
differentiation model is supported by the existence of distinct subsets of memory cells: 'central memory' cells that represent intermediates, and 'effector memory' cells that represent terminally differentiated cells [10].

The DC maturation process

DCs that migrate from tissues to lymph nodes have a life expectancy of only a few days and can therefore be viewed as disposable packets, each carrying a given amount of peptide–MHC complexes, costimulatory molecules and cytokines. These packets are assembled during DC maturation, a process that is initiated by pathogens and/or inflammatory stimuli. The production of homogeneous populations of human immature DCs from human peripheral blood monocytes cultured with granulocyte/macrophage-colony-stimulating factor and IL-4 [11] has been instrumental in identifying the maturation stimuli and in dissecting the DC maturation process (Fig. 2).

DC maturation is triggered and modulated by a variety of receptors for microbial products, cytokines and T cells [12,13]. Human monocyte derived and myeloid DCs express several Toll-like receptors such as TLR2 and TLR4 that trigger maturation in response to bacterial peptidoglycan

Figure 2



The maturation programme studied in monocyte-derived dendritic cells. DC, dendritic cells; ELC, endothelial-like cells; FcR, receptors for crystallizable fragment [of antibody]; GM-CSF, granulocyte/macrophage-colony-stimulating factor; ICAM-1, intracellular adhesion molecule-1; LPS, lipopolysaccharide; MIP, macrophage inflammatory protein; TARC, thymus- and activation-regulated chemokine.

and lipopolysaccharide, respectively. Interestingly, these receptors are absent in plasmacytoid DCs (also known as interferon-producing cells [IPC]) that instead express TLR9, which mediates the response to CpG DNA [14,15]. The differential responsiveness of myeloid and plasmacytoid DCs to pathogens underlines a division of labour between human DC subsets. DC maturation can be also triggered by tumour necrosis factor (TNF)-α and IL-1, and can be inhibited by IL-10. Finally, all DC types are exquisitely sensitive to T-cell feedback signals delivered by activated T cells through CD40 ligand (CD40L).

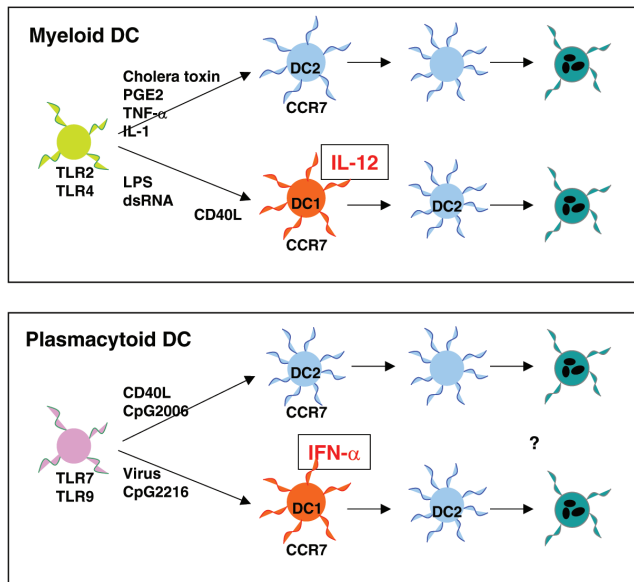
The maturation process coordinately regulates antigen capturing, processing and presentation, expression of costimulatory molecules, cytokine production and lifespan. Immature DCs are extremely efficient in antigen capture since they possess high levels of constitutive macropinocytosis and express endocytic receptors for microbial patterns, such as the mannose receptor [16]. Maturation increases synthesis of MHC class II molecules, while decreasing their degradation, thus favouring the rapid accumulation of long-lived peptide-MHC complexes, which are retained for several days, while class II synthesis is shut off [17]. Presentation on MHC class I molecules is also enhanced by an approximately 10-fold increase in the rate of synthesis, which is sustained in mature DCs [18]. DCs are capable of transporting phagocytosed antigens from the endocytic compartment to the cytosol, leading to their 'cross-presentation' to CD8⁺

T cells [19,20]. Finally, maturation stimuli upregulate the expression of B7.1 and B7.2, thus enhancing the T-cell stimulatory capacity of DCs. While the upregulation of MHC molecules ensures higher capacity for antigen presentation, upregulation of costimulatory molecules ensures an efficient amplification of signalling in naïve T cells.

Cytokine production by DCs is subject to a tight regulation, which is particularly relevant in the case of IL-12 and IFN-γ, the major Th1-polarizing cytokines [21]. IL-12 production is elicited by most pathogens and is boosted by activated T cells through CD40L [22]. In contrast, IL-12 is not induced by other maturation stimuli such as TNF-α, IL-1, and cholera toxin. IL-12 production can be modulated by cytokines and mediators present during induction of maturation: IFN-γ and IL-4 enhance IL-12 production induced by appropriate stimuli, while prostaglandin E₂ and IL-10 exert an inhibitory effect. In addition, one has to consider that IL-12 production is restricted to a narrow temporal window, 8–16 hours after induction of DC maturation [9]. In summary, the Th1-polarizing capacity of DCs is contingent on a number of variables that include the lineage of DCs, the microenvironment in which they are stimulated, the maturation stimuli, and the kinetics of maturation (Fig. 3).

Dynamic changes in DC type and concentration impact on T-cell responses

Because the half-life of mature DCs is short and because cytokine production is transient, the number and type of

Figure 3

Reactivity, flexibility, kinetics and exhaustion in myeloid and plasmacytoid dendritic cells (DCs). The figure summarizes the properties of immature myeloid and plasmacytoid DCs. Indicated are the most relevant Toll-like receptors (TLR) and chemokine receptors, and the response to various maturation stimuli. DC1 and DC2 refer to the capacity of the cells to induce Th1 and Th2 responses, respectively. CD40L, CD40 ligand; LPS, lipopolysaccharide; PGE2, prostaglandin E₂.

DCs present in the T-cell areas will reflect, in highly dynamic fashion, the conditions of the tissues from which the lymph is drained. Under steady-state conditions, only a few tissue-resident DCs 'spontaneously' mature and migrate to the draining lymph nodes, carrying antigens and apoptotic bodies taken up in peripheral tissues [23]. These migrating DCs do not induce effector responses, but rather trigger an abortive T-cell proliferation that leads to immunological tolerance [24,25]. It is possible that spontaneously matured DCs deliver to T cells a qualitatively distinct tolerizing signal. Alternatively, according to the progressive differentiation model, we suggest that the low frequency and short lifespan of these DCs, together with the low level of antigen and B7, may deliver a weak stimulus to T cells, which is not sufficient to sustain proliferation and to promote differentiation.

When pathogens (or adjuvants) are present in peripheral tissues, resident DCs are activated *en masse* and migrate to the draining lymph nodes. At the same time monocytes are recruited from peripheral blood into inflamed tissues, where they rapidly differentiate to DCs that capture antigens and, on maturation, migrate to the draining lymph nodes. This mechanism sustains antigen sampling and presentation for extended periods of time. Maturing DCs

produce large amounts of inflammatory cytokines and chemokines that promote monocyte recruitment [26]. The relative role of tissue-resident DCs, such as Langerhans cells and dermal DCs, versus recruited DCs, such as monocyte-derived DCs and IPC, remains to be established. Production of IFN-I by IPC may be important to promote maturation of monocytes and to protect them from the cytopathic effects of viruses [27,28].

In summary, under inflammatory conditions, the T-cell areas of draining lymph nodes receive large numbers of highly stimulatory DCs for a sustained period of time. The high DC density and the high levels of antigen and B7 molecules deliver a strong and sustained stimulation to specific T cells, leading to their rapid proliferation and differentiation. High levels of IL-2 are produced under these conditions and drive clonal expansion of committed T cells irrespective of whether they continue to receive TCR stimulation. One should also consider that DC-T cell interaction results in a reciprocal stimulation. Activated T cells trigger DCs via CD40L or TNF-related activation-induced cytokine, improving their T-cell stimulatory capacity, boosting IL-12 production, and prolonging their lifespan [29]. It is possible that regulatory T cells may suppress antigen presentation by DCs via production of inhibitory cytokines or by direct contact [30].

There is growing evidence that the capacity of DCs to induce Th1 or Th2 responses is contingent on appropriate stimulation and timing (Fig. 3). As already discussed, myeloid DCs produce IL-12 only in response to some pathogens or CD40L, and within a narrow time window. In addition, IPC produce large amounts of IFN-I, another Th1-polarizing cytokine, in response to viruses but not in response to CD40L; again, only within a narrow time window. In contrast, Th2 responses may be induced by DCs that do not produce Th1-polarizing cytokines, either because they have been conditioned by nonpermissive stimuli or because they have exhausted their IL-12 or IFN-I-producing capacity. In this case, Th2 polarization is driven by IL-4 produced by T cells themselves or derived from exogenous sources, such as natural killer T cells or mast cells. It is worth considering that the dynamics of DC migration to the draining lymph nodes may lead to preferential generation of Th1 cells during the early phases of the immune response, when active DCs enter the T-cell areas in large numbers. This is followed by induction of Th2 and nonpolarized T cells at later time points when the influx of DCs ceases and the DCs surviving in the T-cell area exhaust their IL-12-producing capacity [31].

Competition for DC shaping T-cell responses

The availability of antigen-presenting DCs and of antigen-specific T-cell precursors represents the limiting factors in the immune responses. There is growing evidence that responding T cells compete *in vivo* for access to DCs and

that this competition can be relieved by providing more DCs [32]. At the initial phase of a primary response, the low frequency of naïve T cells specific for a given antigen makes competition among responding cells unlikely. However, as the responding cells proliferate, competition for sustained TCR stimulation will increase, particularly among cells of the same clone, which have the same avidity and occupy the same niche. This intraclonal competition contributes to functional diversification: T cells achieving a sustained stimulation differentiate to effector cells, while those receiving a short stimulation remain in an intermediate state giving rise to central memory T cells. In contrast, interclonal competition may take place preferentially in secondary responses due to the larger numbers of antigen-specific cells present, and may therefore explain the selection of high-avidity T cells under these circumstances.

Conclusions

It is becoming increasingly clear that DCs provide the adaptive immune system with the essential function of context discrimination. DCs can integrate multiple stimuli from pathogens, inflammatory cytokines and T cells, and can provide distinct outputs in terms of antigen presentation, costimulation and cytokine production. Like other cells involved in the innate immune response, DCs produce large amounts of inflammatory chemokines that contribute to the recruitment of DC precursors in inflamed tissues, thus sustaining antigen sampling in peripheral tissue and presentation to T cells in lymph nodes. Finally, the T-cell activation and differentiation programme translates antigen concentration, cytokine and costimulatory molecule composition, and DC density into distinct cell fates ranging from tolerance to inflammation, cytotoxicity and memory.

Glossary of terms

APC = antigen presenting cells; CD40L = CD40 ligand; DC = dendritic cell; IPC = interferon-producing cells.

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