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The role of dendritic cells in shaping the immune response

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Abstract
Dendritic cells are central to the initiation of primary immune responses. They are the only antigen-presenting cell capable of stimulating naive T cells, and hence they are pivotal in the generation of adaptive immunity. Dendritic cells also interact with and influence the response of cells of the innate immune system. The manner in which dendritic cells influence the responses in cells of both the innate and adaptive immune systems has consequences for the bias of the adaptive response that mediates immunity to infection after vaccination or infection. It also provides an opportunity to intervene and to influence the response, allowing ways of developing appropriate vaccination strategies. Mouse and human studies have identified myeloid, lymphoid and plasmacytoid dendritic cells. Studies in domesticated animals with agents of specific infectious diseases have confirmed the applicability of certain of the generic models developed from mice or from *in vitro* studies on human cells. *In vivo* and *ex vivo* studies in cattle have demonstrated the existence of a number of subpopulations of myeloid dendritic cells. These cells differ in their ability to stimulate T cells and in the cytokines that they produce, observations clearly having important implications for the bias of the T-cell response. Dendritic cells also interact with the innate immune system, inducing responses that potentially bias the subsequent adaptive response.

Keywords: dendritic cells; immune response

Introduction

The general concepts on the role and function of dendritic cells and how they interact with the innate immune system, and in so doing influence the bias of the adaptive immune response that is induced, hold true across all animal species. However, there are differences in the details of the way in which dendritic cells from different animal species interact with pathogens, or antigens derived from them, and in the details of the different dendritic cell subsets. This has implications for understanding the immune response in target species and also in how intervention is best done to protect domesticated animals from disease. Furthermore, investigations are possible in larger domesticated animals that are not possible in small rodents for technical reasons and in humans for ethical ones. A prime example is the use of surgical cannulation techniques to study regional immune responses or cells draining from the mucosa, and infections that are host-specific (Haig *et al.*, 1999; Hein and Griebel, 2003). Thus, we will aim to provide in this review details of how studies on cattle have provided novel data applicable to understanding the biology of the dendritic cell and how the initial interaction of dendritic cells with the innate immune system shapes the ensuing immune response.

Dendritic cells and the induction of immune responses

What makes dendritic cells special is that for the induction of immunity there is one control point. This is the

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dendritic cell. It is the only antigen-presenting cell (APC) recognized as being able to stimulate naive T cells and so stimulate primary immune responses *in vivo*. Dendritic cells can originate from cells of several lineages and are distributed in small numbers in most tissues. Of significance is that at the body surfaces, skin and mucosa they form a network of cells ideally situated to act as sentinels, taking up antigen or microbes that are transported by migration through the afferent lymphatics to the draining lymph nodes. This provides an efficient mechanism for contact with naive T cells that are present at a low frequency in blood and that migrate into the lymph node via the high endothelial venules. The dendritic cells' primary role in this situation is the uptake of antigen, which is processed and presented in the context of MHC molecules. However, antigen presentation alone is not enough. To stimulate a T-cell response, co-stimulation is required. This is provided by, amongst other molecules, CD80, CD86 and CD40 on the APC interacting with appropriate ligands: CD28 and CD40L.

The interaction of dendritic cells with cells of the innate immune system is now well recognized, as is the capacity of dendritic cells to respond to pathogen molecules via pathogen-associated molecular pattern receptors. These responses have a major impact on both the intensity of the response and its bias. As well as being effective in stimulating naive T cells, dendritic cells are the most efficient cell in stimulating resting memory T cells. Thus, these cells control both the intensity and the type of response and are, as a consequence, a target for immunization (Steinman and Pope, 2002).

Dendritic cells and the induction of tolerance

More recently evidence has accumulated that these cells are not only central to the induction of immune responses but are also involved in the induction of tolerance (Huang and MacPherson, 2001; Steinman *et al.*, 2003). Dendritic cells in the thymus, stromal interdigitating cells, have a key role in central tolerance, possibly as a consequence of their ability to stimulate T cells, but in an environment where this leads to clonal ablation. A role for antigen presentation by dendritic cells in the periphery and the induction of tolerance has also been proposed (Matzinger and Guerder, 1989). Why should dendritic cells sometimes produce tolerance? Evidence for a number of mechanisms being involved has been presented. These include, in mice, the presence of lytic CD8αα dendritic cells, the deletion of autoreactive CD8 cells by a Fas/FasL interaction and the induction of apoptosis by regulatory dendritic cells in the liver. Secretion of 'immunosuppressive' cytokines is another mechanism.

The concept that tolerance induction by dendritic cells depends on the delivery of antigen to immature dendritic cells in the absence of maturation signals has much support. If there are maturation signals, immunity develops. MacPherson and colleagues, in a rat model in which $CD4^{-}OX41^{-}$ (= $SIRP\alpha^{-}$; see below) and $OX41^{+}$ dendritic cells from afferent lymph draining the intestine were compared, reported the presence of remnants of apoptotic cells in the $SIRP\alpha^{-}$ population (Huang *et al.*, 2000). It was suggested that these cells might drain back to the lymph node and tolerize in the absence of 'danger' signals. Apoptosis unlike necrosis does not promote dendritic cell maturation. In a model in which allogeneic immature dendritic cells were used, the generation of immunoregulatory interleukin (IL)-10-producing, non-proliferating Tr1-like cells expressing high levels of cytotoxic T-lymphogate antigen 4 (CTLA-4) was reported (Jonuleit *et al.*, 2000). Studies using DEC205 to target dendritic cells indicate that in the absence of co-stimulation and maturation, which could be provided by CD40/CD40L, tolerance is induced (Hawiger *et al.*, 2001). Thus, it is suggested that in the steady state dendritic cells cause deletion of naive peripheral T cells and the induction of tolerance. Dendritic cells in lymphoid tissue present MHC–peptide complexes in the steady state, naive cells divide but are deleted and animals become tolerant. Infection and inflammation provide the signals that initiate immunity, which requires terminal differentiation and maturation and is mediated by two major families of molecules, toll-like receptor and tumor necrosis factor (TNF) (Steinman *et al.*, 2003).

Different types of dendritic cell and the polarization of the immune response

Dendritic cells may originate from cells of several lineages. The majority of studies involve myeloid dendritic cells. These are evident in humans, rodents and ruminants (as well as other animal species). They include Langerhans cells, dermal dendritic cells, afferent lymph veiled cells, interdigitating dendritic cells in lymph nodes and dendritic cells in lymph node follicles and Peyer's patches. They do not include the follicular dendritic cell. Plasmacytoid dendritic cells, which are also called interferon-producing cells, have been described in the lymph nodes of infected humans as well as blood in mice (Cella *et al.*, 2000; Colonna *et al.*, 2002), in the gut of pigs (Riffault *et al.*, 1996, 2001), and now in cattle lymph nodes (see below). Lymphoid dendritic cells ($CD8\alpha\alpha^{+}$ DC) appear to be primarily limited to the mouse spleen.

Polarization of the immune response by dendritic cells has been reported in a number of studies. Dendritic cells from the Peyer's patch and respiratory tract of rodents were noted to induce a Th2 bias, the responding cells synthesizing IL-4 and IL-10 and immunoglobulin G1 (Stumbles *et al.*, 1998; Iwasaki and Kelsall, 1999). Myeloid and lymphoid dendritic cells inoculated into the footpad of mice produced different Th responses (Maldonado-Lopez *et al.*, 1999). Early stud-

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ies of plasmacytoid dendritic cells in humans reported that precursors cultivated with IL-3 produced a Th2 response. However, later studies showed that exposing the same cells to influenza virus and CD40L produced a strong Th1 response (Cella *et al.*, 1999).

It is apparent that dendritic cells are not fixed with respect to the type of T-cell response that they induce. They are plastic. At any time cells *in vivo* have a certain characteristic phenotype and biology. But these cells respond to microbial products, infection by viruses or bacteria, cytokines in the microenvironment, and/or antigen dose. The maturation stage also appears to affect the type of T-cell response induced (Colonna *et al.*, 2002; Palucka and Banchereau, 2002; Manickasingham *et al.*, 2003). Thus, these cells change according to the signals and stimuli that they receive.

Sources of cells for experimental studies

One of the problems with studying *ex vivo* dendritic cells is the low numbers of cells in tissues and the prolonged incubation stages usually involved in their isolation. This yields low numbers of cells that have differentiated and potentially acquired different properties. Much work has been on cells cultured *in vitro* from precursors. These are commonly blood monocytes cultured with IL-4 and GM-CSF (granulocyte-macrophage colony-stimulating factor) (monocyte-derived dendritic cells, MoDC), although bone marrow cells are also used. These studies have led to the concept that the immature dendritic cells that are derived initially can be matured following exposure to appropriate cytokines or microbial products. Immature cells effectively take up antigen and are relatively poor at presentation, while mature dendritic cells have down-regulated antigen uptake and up-regulated ability to present to and stimulate T cells. Ruminants offer the advantage that dendritic cells can be isolated from afferent or pseudo-afferent lymph for direct *ex vivo* examination and study. Thus, the sources of dendritic cells include afferent lymph, lymphoid tissues including spleen, lymph node, Peyer's patch, tonsils, bone marrow precursors and monocytes.

Uptake of antigen can vary for the cells at various maturation stages. Routes of uptake include receptor mediated uptake via clathrin-coated pits, which delivers antigen primarily but not entirely to the endosomal compartment; and uptake via caveoli—these membrane invaginations contain caveolin rather than clathrin and have been reported to deliver antigen to the cytosol and endoplasmic reticulum as well as endosomes. Macropinocytosis is a feature of immature dendritic cells. This non-specific actin-dependent mechanism allows the non-specific sampling of the local microenvironment. Phagocytosis of bacteria is also down-regulated as dendritic cells mature. *In vitro*, MoDC lose this ability by 6 days of culture but it is evident in a proportion of dendritic cells in afferent lymph (afferent lymph dendritic cells, ALDC), indicating that although these cells are highly stimulatory for T cells they still retain this 'immature' feature and that *ex vivo* cells do not fit precisely into a simple model derived from *in vitro* studies (Howard and Hope, 2000).

Phenotypic and functional variation within the afferent lymph dendritic cell

We have used expression of the WC6 molecule to define the dendritic cells in afferent lymph for further studies of phenotypic and functional variation (McKeever *et al.*, 1991; Howard *et al.*, 1997). Recently we have shown that WC6 is the homologue of DEC205 in humans (D. R. Gliddon, J. C. Hope and C. J. Howard, unpublished). Within this DEC205$^+$ (WC6$^+$) population of dendritic cells draining from the skin, there are two major populations. The smaller is CD11a^{high}, CD26$^+$, CD13$^+$, CD5$^+$. The antigen recognized by monoclonal antibody CC81 is the homolog of human CD13 (D. R. Gliddon and C. J. Howard unpublished). The larger ALDC subset is SIRPα^+ (SIRPα is signal regulatory protein α), CD11a$^-$ or low, CD26$^-$, CD5$^-$, CD13$^-$, mannose receptor variable, CD21 variable, CD1b variable. SIRPα was originally called the bovine MyD1 antigen (Brooke *et al.*, 1998). This larger, heterogeneous population of ALDC appears to contain dendritic cells derived from Langerhans cells and dermal dendritic cells (Howard *et al.*, 2002).

SIRPα^+, CD26$^-$, CD13$^-$ and SIRPα^-, CD26$^+$, CD13$^+$ dendritic cells vary in capacity to stimulate CD4 and CD8 T cell responses (Howard *et al.*, 1997). The ability to stimulate CD8 T cells has been related to the capacity of SIRPα^+ cells to synthesize IL-1 (Hope *et al.*, 2001). Expression of other cytokines by the two populations also varies (Stephens *et al.* 2003). Most obvious, and potentially critical in ability of the different dendritic cells to stimulate different biased T-cell responses, is the higher level of expression of IL-12 by the SIRPα^- cells, which, taken with expression of CD26 and its effect on chemokines (Gliddon and Howard, 2002), would lead to the forecast that this dendritic cell population would promote a Th1 bias. The lower and variable levels of IL-12 and higher levels of IL-10 produced after culture by the heterologous SIRPα^+ ALDC suggests that these cells are likely to direct a more balanced T-cell response and that there are functionally distinct cells within this population.

A number of investigations of the ALDC have provided new information on how the molecules expressed on the surface of the cells that define them phenotypically affect the function of the cells. Although no differences in expression of CD80, CD86 and CD40 were noted, other differentially expressed molecules showed more potential for influencing cell function. The antigen that was originally called bovine MyD-1 was recognized

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by a panel of three monoclonal antibodies (IL-A24, CC149, CC156) that divided the ALDC into two major subsets. The molecule was identified as the bovine homolog of SIRPα. It was considered likely to be important in activation of dendritic cells as it contained an ITIM (immunoreceptor tyrosine-based inhibitory motif) (Brooke *et al.*, 1998). Subsequent studies of the molecule indicated that ligation of SIRPα stimulated a novel signalling pathway that inhibits TNF-α production by APC (Patel *et al.*, 2002; Smith *et al.*, 2003). The CD26 molecule that is expressed on the reciprocal ALDC population is known to affect NH_2 terminal truncation of various chemokines, which affects their ability to bind to receptors, potentially affecting the bias of the T-cell response that is induced (Gliddon and Howard, 2002).

Effect of bacteria and viruses or their antigens on dendritic cell function

It was shown that MoDC could phagocytose *Mycobacterium bovis* BCG and stimulate both CD4 and CD8 memory T cells present in the blood of vaccinated calves (Hope *et al.*, 2000). However, experiments in immunologically naive neonatal calves showed that there was a $CD8^+$ population in these animals that was stimulated by BCG-infected MoDC to proliferate and express interferon γ (IFNγ) (Hope *et al.*, 2002). These were considered to be a bovine NK cell and their production of IFNγ in the very early stages of the immune response is likely to contribute to a Th1 bias, providing evidence of an interaction between dendritic cells and the innate immune system and potential effects on the bias of the subsequent adaptive response.

Another way in which a Th1 bias could result from the infection of dendritic cells by *M. bovis* is through the secretion of cytokines by the infected APC and a bystander effect. Both IL-12 and IL-18 are transcribed following infection of MoDC by *M. bovis* (J. C. Hope and M. Thom, unpublished). In humans these two cytokines act synergistically on memory CD8 T cells, resulting in the synthesis of IFNγ (Lertmemongkolchai *et al.*, 2001). A proportion of cattle T cells also respond to IL-12 and IL-18 and synthesize IFNγ in the absence of any cognate APC:T-cell interaction. This provides another mechanism by which non-adaptive immune responses can influence the subsequent immune response.

Another dendritic cell that has more recently come under study is the plasmacytoid dendritic cell, which is also known as the interferon-producing cell. These cells have been identified in humans, pigs and mice and respond to virus and CpG motifs (via TLR9) by synthesizing large amounts of type 1 interferons (Cella *et al.*, 1999; Colonna *et al.*, 2002). Recent studies in cattle have shown the presence of what appears to be the homologous cell in the lymph nodes of calves inoculated with cytopathic bovine viral diarrhea virus (B. Charleston, L.S. Brackenbury and B.V. Carr, unpublished). Type 1 IFN has been reported to have adjuvant activity and the property of biasing a Th1 response (Le Bon and Tough, 2002). This provides a further example of the involvement of dendritic cells with the innate response and the potential to bias a later adaptive response.

Summary

These examples of bacteria and viruses causing a response in dendritic cells emphasize the plasticity of these APC. Dendritic cells are not homogeneous and the various phenotypes have different biological properties, the molecular basis of which is becoming evident. At any one point in time *in vivo* they have certain properties, e.g. the secretion of different cytokines, which will affect the T-cell response generated in the microenvironment in which the dendritic cells function. They can thus provide a target for antigen and the biasing of a particular response. However, the cells are plastic and will respond to microbial stimuli and potentially then induce a different response. Dendritic cells are the main control point for the induction of an adaptive immune response, but they also interact with the innate immune system and this in turn influences the shape or bias of the ensuing adaptive response.

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