PART I Recognition of bacteria

CHAPTER 1

The dendritic cell in bacterial infection: Sentinel or Trojan horse?

Benjamin M. Chain and Janusz Marcinkiewicz

1.1 INTRODUCTION

Dendritic cells play a key role in the initiation and regulation of T-cell dependent immune responses. Much of their significance lies in their role as a cell linking the evolutionarily ancient innate immune system to the more complex and sophisticated adaptive immune system. Understanding their function in the context of bacterial infection, therefore, where the strands of innate and adaptive immunity are so closely interwoven, is likely to be particularly significant.

The cell biology of the dendritic cell poses a number of specific questions relating to bacterial physiology and pathophysiology. In particular, much of the literature in the field has been concerned either with understanding how dendritic cells process and present bacterial proteins in the context of a "particulate" as opposed to a "soluble" form, or with mapping the interactions between dendritic cells and bacterial cell wall components. This chapter first provides a brief overview of present understanding of the dendritic cell system and its role in immune responses, and then addresses questions relating more specifically to the interaction between dendritic cells and bacteria.

1.2 DENDRITIC CELLS AND THE IMMUNE RESPONSE

1.2.1 The dendritic cell family

T-cell recognition of antigen has a requirement for the antigen to be first *processed* and then *presented* by another cell, termed the "antigen presenting cell." This requirement, first determined empirically, can now be understood in terms of the well-established model of T-cell recognition, involving the tripartite molecular interaction between T-cell antigen receptor, antigen peptide

fragment, and MHC molecule (see Chapter 2 for more details). The requirement for multiple other ligand/receptor interactions between T cell and antigen presenting cell in order to achieve full T-cell activation ("co-stimulation") adds further molecular detail to this overall recognition process. The nature of the antigen presenting cell, which is responsible for T cell activation *in vivo*, therefore becomes a question central to the understanding of T-cell immunity.

The dendritic antigen presenting cell was first identified by Steinman (Steinman, 1991), as a rare cell type found in the T-cell areas of spleen and lymph nodes of mice, which serves as a potent activator of T cells. These T-cell–associated dendritic cells must be clearly distinguished from follicular dendritic cells, found within B-cell follicles and concerned with the trapping and storage of antigen/antibody complexes for B-cell recognition. This latter cell type will not be discussed in this chapter.

The principle characteristics of the T-cell associated dendritic cells are the ability to activate both naïve and memory T cells (associated with high surface expression of both class I and class II MHC molecules), an unusual morphology showing extensive thin cytoplasmic processes or dendrites (*in vivo* these cells were sometimes described as "interdigitating cells" for the same reason), and an absence of Fc receptors and phagocytic activity. The latter features were of particular importance in distinguishing these cells from the macrophage, which had previously been believed to be the main cell type involved in the presentation of antigen to T cells. The inability of dendritic cells to phagocytose immediately raised the question of how such cells would process and present bacteria or other particulate antigens, a question which was indeed addressed in a number of early studies (Kaye et al., 1985; Guidos et al., 1984).

Although dendritic cells, as originally defined, are cells localised within the T cell areas of secondary lymphoid tissue, it is now generally accepted that this cell is closely related to antigen presenting cells found within most other tissues of the body. This relationship has been explored most thoroughly in relation to skin (Macatonia et al., 1987; Larsen et al., 1990b) where there is compelling evidence for a differentiation pathway that links skin Langerhans' cells to dendritic cells within the draining lymph nodes (Hill et al., 1990). In this model, Langerhans' cells respond to local inflammatory stimuli by migrating out of the skin, via afferent lymphatics (where they were previously identified as veiled cells, because of their extensive membrane ruffling), and then into the lymph node where they transform into interdigitating dendritic cells. These dendritic cells are quite short lived and disappear from the T-cell areas (perhaps by apoptosis) within a few days of arrival (Garside et al., 1998).

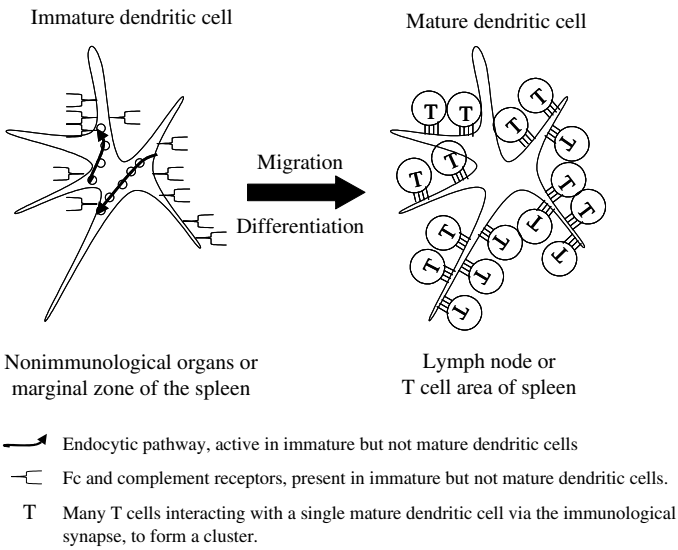

Figure 1.1. Dendritic cells exist in immature and mature forms, distinguished both by function and anatomical location.

A key feature of this model is that dendritic cells exist in two quite distinct differentiation stages, a more immature ("precursor") form found primarily outside lymphoid tissue and a mature form identical to the interdigitating cells of secondary lymphoid tissue (see Fig. 1.1). Immature dendritic cells have now been identified in many organs, including heart, liver, kidney, etc. (e.g., Larsen et al., 1990a). An immature dendritic cell type has also been described in the spleen, within the marginal zone surrounding the white pulp (Leenen et al., 1998). Appropriate stimulation induces migration and differentiation of these cells into interdigitating cells of the T-cell areas (Sousa and Germain, 1999). In addition, many *in vitro* models that mimic this two-step dendritic cell differentiation have been described (Sallusto and Lanzavecchia, 1994; Romani et al., 1989). Dendritic cell precursors differ from their mature counterparts in both quantitative and qualitative respects. In general, immature forms have higher endocytic capacity, express several Fc and complement receptors (see Chapter 4), and are phagocytic (albeit rather weakly). They are less efficient in activating resting naïve T cells and express lower levels of the various molecular determinants of antigen presentation (see below). Immature dendritic cells, therefore, may represent the "antigen capture" arm of the antigen presentation system, whereas mature dendritic

cells represent the "antigen presentation" arm. The anatomical separation of antigen presentation (which takes place in lymph nodes or spleen) from the site of infection is a fundamental feature of the immune system. Indeed, the presence of differentiated dendritic cells outside lymphoid tissue is almost invariably associated with chronic inflammation and pathology.

The view of the dendritic cell system presented above has rapidly won widespread acceptance. Its most influential implication is the idea that antigen presentation is an inducible rather than a constitutive process. Under resting conditions, the flow of maturing dendritic cells from tissue to lymph node is small (although not absent; Anderson et al., 2001), and the extent of antigen presentation is limited. In the face of immune challenge, this flow dramatically increases and antigen presentation therefore also increases. The molecular signals that drive dendritic cell migration and differentiation are still being elucidated and include microbial receptors on the dendritic cells (see below), inflammatory cytokines (Cumberbatch et al., 2001), chemokines (Caux et al., 2000), and reactive oxygen species (Rutault et al., 1999) and their products (Alderman et al., manuscript in preparation). Many of these mediators are often produced by components of "innate" immunity (e.g., macrophages, neutrophils), leading some to suggest that an innate immune response is a necessary determinant of antigen presentation (Janeway, 1992). It seems more likely, however, that tissue response to injury (Ibrahim et al., 1992) rather than immune recognition is the underlying cause of dendritic cell migration/differentiation. Delivery of sterile gold beads (Porgador et al., 1998), topical sensitisers (Hill et al., 1993), and sterile allogeneic transplants (Larsen et al., 1990b) are all potent activators of dendritic cell migration and maturation.

1.2.2 Relationship between dendritic cells and macrophages

The relationship between the dendritic cell and the macrophage has been a much debated issue. The consensus is that most dendritic cells share a common precursor with the circulating monocyte and hence with the tissue macrophage. Immature dendritic cells are recruited from a bone marrow-derived blood precursor, via specific adhesion molecules on the precursor surface (Strunk et al., 1997). Recruitment is increased during an ongoing immune response, via the release of specific chemokines. Interaction with the extracellular matrix may also be important in regulating dendritic cell differentiation (Randolph et al., 1998). *In vitro*, dendritic cells can also be derived from blood monocytes, but there is little evidence that this process is important *in vivo*. There have been persistent, but often contradictory, reports that one or more other populations of nonmyeloid dendritic cells exist (e.g.,

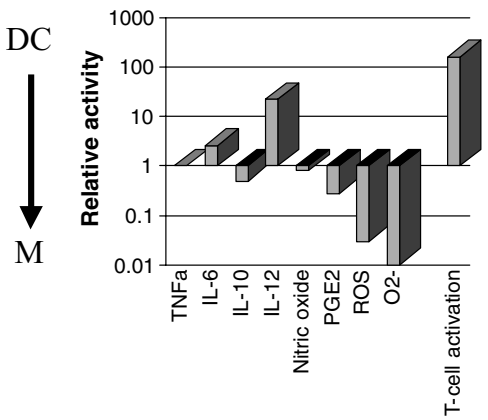

Figure 1.2. Comparative functional analysis of dendritic cells and macrophages. Bone marrow culture murine dendritic cells, and mouse peritoneal macrophages were stimulated with LPS and IFN-γ, and the production of a variety of mediators measured. T-cell activation was measured using unstimulated cells, in allogeneic antigen presentation assays. For each parameter, the ratio between dendritic cells and macrophages is given. Note that the DC are much more efficient at T cell stimulation and at producing IL-12, a T-cell regulatory cytokine. In contrast, macrophages show greater production of prostaglandins, reactive oxygen species, and nitric oxide. Experimental details are given in Marcinkiewicz et al. (1999).

lymphoid dendritic cells or plasmacytoid dendritic cells); the lineage relationships of these various other populations remain very unclear, as do their physiological importance, and they are not discussed further in this chapter.

Once within tissues, immature dendritic cells can be clearly distinguished from macrophages in terms both of morphology and cell surface phenotype. Nevertheless, immature dendritic cells do share many properties with macrophages, and differences are quantitative rather than qualitative. Immature dendritic cells, both in lymphoid tissue and outside it, share many surface markers with macrophages, including many of the myeloid lineage markers, various isotypes of Fc receptors, and complement receptors (Leenen et al., 1997; Woodhead et al., 1998; King and Katz, 1989). As discussed further below, immature dendritic cells, including skin Langerhans cells (Sousa et al., 1993), have been shown to be phagocytic, have high rates of fluid phase endocytosis, and have a well-developed phagolysosome system. Nevertheless, when compared directly to macrophages, even immature dendritic cells are only weakly phagocytic and have a much reduced lysosomal function (see Fig. 1.2).

It is best to regard dendritic cells as a distinct member of the myeloid family, sharing some molecular and functional properties with the other

members of the family (both macrophages and granulocytes), but characterised by extreme specialisations that maximise the efficiency of antigen presentation.

1.2.3 The molecular cell biology of the dendritic cells

The dendritic cell is the only cell able to simulate a primary T-cell immune response (at least in the normal physiological situation). In contrast, effector T cells (both CD4 helpers and CD8 cytotoxic cells) have less stringent requirements and are activated by their targets, whether these be B cells (leading to T-cell dependent antibody production), macrophages (in T-cell dependent macrophage activation), or any cell expressing class I MHC and the antigen peptide, which is the target of the cytotoxic CD8 T cells. Memory T cells lie in between naïve T cells and effector cells, in terms of their requirement for dendritic cell presentation. Even with memory cells, however, dendritic cells provide the most efficient presentation.

The molecular features of dendritic cells responsible for their potent antigen presenting cell activities are not fully understood. The expression of high levels of MHC molecules (both class I and class II) and the expression of a panoply of "co-stimulatory" molecules involved in optimising T-cell activation are two important features. Dendritic cells are also able to interact with many T cells simultaneously, both *in vitro* and *in vivo*, to form clusters. This interaction is mediated principally by ICAM/β_2 integrin interactions (DCs express all three ICAM molecules at high level; King and Katz, 1989). Cluster formation allows T cells of different specificities to interact with each other and also stabilises T cell/dendritic cell interactions independently of antigen recognition, to allow sufficient time for the formation of the "immunological synapse," which is essential for T cell triggering. The long dendritic cell processes, which are so characteristic of this cell type, also presumably maximise opportunities of T-cell–antigen interaction (Al Alwan et al., 2001). Finally, dendritic cells within the lymph node are the major producers of IL-12, a cytokine that initiates the T helper 1 type of response that leads to macrophage activation and bacterial clearance. IL-12 production is principally regulated by the interaction of CD40 on the dendritic cell surface with CD40 ligand on activated T cells. The dendritic cell therefore acts as a bridge transmitting paracrine signals between helper and effector T cells within a cluster.

1.3 DENDRITIC CELLS AND BACTERIAL IMMUNE RESPONSES

This brief outline of the workings of the dendritic cell system provides a framework for specific questions regarding the role of the dendritic cell

system in bacterial infection. It is worth noting, however, that remarkably few studies have focused specifically on this interaction, and much of what follows remains, therefore, speculative.

1.3.1 What activates dendritic cell migration/differentiation in response to bacterial infection?

Bacteria, and several bacterial components such as endotoxin, are potent activators of dendritic cell migration and differentiation (Sallusto and Lanzavecchia, 1994; Sousa and Germain, 1999). This response is primarily activated by engagement of receptor complexes (sometimes called pattern recognition receptors to distinguish them from the antigen-specific receptors of T and B cells) that recognise bacterial components. The molecular details of pattern recognition receptors, and how they transduce signals within the cell, is an area of very active research (Triantafilou et al., 2001). The family of Toll-like receptors now believed to be very important in this process are briefly discussed below, but other families of receptors may well exist such as TREM (triggering receptor expressed on myeloid cells)-1 (Bouchon et al., 2001). Immature dendritic cells, at least *in vitro*, express many Toll receptors, allowing them to respond directly to bacterial challenge. However, dendritic cell response may also be indirectly mediated by cytokines such as TNF-α and IL-1 produced by other cell types in response to bacterial invasion. Not all pattern recognition receptors on the dendritic cell stimulate migration, however. The DEC 205 lectin and the mannose receptor, for example, serve to facilitate binding and uptake of mannose-containing structures into processing compartments, but do not induce migration or differentiation (Mahnke et al., 2000).

Engagement of Toll receptors also induces IL-12 and other pro-inflammatory cytokines, suggesting that most bacterial responses are directed toward a Th1, rather than Th2, type of response. However, some bacterial toxins may interfere with Th1 priming and deviate the response toward a Th2 response (Boirivant et al., 2001; Cong et al., 2001). Such deviation is discussed in more detail in Chapter 11.

1.3.2 Toll-like receptors (TLRs) and bacterial recognition

It is now rapidly becoming established that the TLRs, cell surface proteins with a intracellular domain homologous to that of the IL-1 receptor (so called Toll/IL-1 receptor homology – TIR domain), are crucial for the recognition and discrimination of microbes. There are at least ten *tlr* genes in mammals and they can form homo-dimers (and possibly also hetero-dimers), suggesting that the range of bacterial components that can be recognised by these

Table 1.1. *Specificity of the TLRs*

TLR	Bacterial ligands binding
TLR2 and TLR6	peptidoglycan, Mycoplasma lipoprotein
TLR2 and TLR?[a]	lipoproteins, lipoarabinomannan, certain LPS molecules
TLR3	double stranded RNA (viral)
TLR4	enteric and other bacterial LPS molecules
TLR5	flagellin
TLR9	CpG DNA

[a] Nature of TLR2 binding partner not defined.

cell surface proteins may be large (Kimbrell and Beutler, 2001). The known ligands for the various TLRs is shown in Table 1.1. It is believed that the TLRs require additional proteins to form a recognition complex at the surface of myeloid cells. Among these proteins are CD14, MD2, and the β_2-integrin, Mac-1, all of which can confer increased cellular responsiveness to LPS and certain other agonists. In addition to controlling innate responses to microorganisms, by activating NF-κB through the intracellular adapter protein MyD88, it has been reported that MyD88-deficient mice have a major defect in activation of antigen-specific Th1 lymphocytes. This suggests that the TLRs may play a role in controlling adaptive immune responses (Schnare et al., 2001). The role of TLRs in the activation of NF-κB is described in Chapter 6.

1.3.3 How do dendritic cells process bacterial antigens?

The extent to which dendritic cells take up and process bacteria directly remains debatable. Many studies show that immature dendritic cells, at least *in vitro*, phagocytose bacteria and other particulates and then process and present bacterial antigens. There is also limited evidence for bacterial phagocytosis *in vivo* (Inaba et al., 1993; Paglia et al., 1998). Phagocytosis in these experiments is often measured in the presence of an enormous excess of free bacteria, which does not reflect the normal physiological situation. Even under these conditions, the phagocytic index of dendritic cells is often much smaller than that of macrophages. Furthermore, dendritic cells are ill-equipped to kill any bacterium that is internalised, because their ability to produce an oxygen burst or to synthesise nitric oxide is much less than that shown by macrophages (Fig. 1.2) (Yu et al., 1996; Marcinkiewicz et al., 1999; Bryniarski et al., 2000).

A more likely general scenario, therefore, is that dendritic cells normally act in concert with components of the innate immune system in first killing

and then processing bacteria (Bryniarski et al., 2000). In early stages of infection, the neutrophil is the major phagocyte present at sites of infection. Neutrophil phagocytosis is extremely efficient and is likely to remove rapidly most free bacteria from the dendritic cell microenvironment. Both phagocytosed bacteria and any remaining extracellular bacteria can be efficiently killed by the combination of oxygen radical production and hypochlorous acid formed by neutrophil myeloperoxidase (Marcinkiewicz et al., 2000). Dendritic cell processing of dead bacteria can then occur by the action of cell surface proteinases on the dendritic cell, by the uptake of bacterial fragments via lectin or scavenger receptors, or perhaps by the uptake of apoptotic neutrophils containing internalised bacteria. The latter would be particularly important in stimulating a bacterial CD8 T-cell response (believed to be important for intracellular bacterial infection), since uptake of cell associated antigen seems to load preferentially class I MHC via the ill-defined "cross-priming" pathway (Albert et al., 1998). Bacterial fragments may alternatively enter afferent lympatics and be carried down to the draining lymph nodes to be processed and presented *in situ*.

1.3.4 Dendritic cells as a means of bacterial invasion – sentinels or Trojan horses?

A specialised case of dendritic cell phagocytosis concerns those bacteria that normally propagate within the cell. Many such bacteria target macrophages, raising the question of whether invasion of dendritic cells can also occur. These bacteria include the most important pathogenic species, such as *Mycobacteria*, *Listeria*, and *Salmonella*. This ability to survive within the killing machine of the macrophage is a key bacterial immune evasion strategy. Several recent studies have addressed this problem directly, although most have used *in vitro* models of dendritic cell function, which may not reflect the situation *in vivo*. *In vitro* internalisation of live bacteria into dendritic cells has been demonstrated for *Mycobacterium tuberculosis* (Gonzalez-Juarrero and Orme, 2001), *M. avium* (Mohagheghpour et al., 2000), *Listeria monocytogenes* (Kolb-Maurer et al., 2000), and *Salmonella typhimurium* (Niedergang et al., 2000). In some cases, processing and presentation of bacterial antigens by infected dendritic cells has been demonstrated (Svensson et al., 1997; Tascon et al., 2000; Paschen et al., 2000).

In many cases, the ability of dendritic cells to take up antigen is linked to the ability of the host to mount an effective immune response. Bacterial invasion of dendritic cells, however, may be neither necessary nor desirable. Even attenuated Salmonella, for example, that were unable to survive within host macrophages could survive within dendritic cells, illustrating the very