

The role of dendritic cells in the immune response to *Salmonella*

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Abstract

Dendritic cells (DC) are an important link between the innate and adaptive immune response and are key antigen presenting cells in triggering specific immunity. This review summarizes the role of DC and the DC subsets during infection with the facultative intracellular bacterium *Salmonella*. The capacity of DC to stimulate *Salmonella*-specific T cells by direct and indirect presentation of *Salmonella* antigens as well as the cytokine production capacity of DC upon *Salmonella* encounter are discussed. In addition, changes in the number, localization and cytokine production by splenic DC subsets during infection are reviewed. Studying the function of DC during *Salmonella* infection provides insight into the capacity of this phagocytic antigen presenting cell to initiate and modulate an immune response during bacterial infection.

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1. Introduction

Dendritic cells (DC) are phagocytic antigen presenting cells important in triggering adaptive immunity to protein and particulate antigens including bacteria. DC are located at the sites of antigen exposure such as mucosal surfaces and underlying the skin. After encountering antigen at such peripheral sites, they migrate to secondary lymphoid organs where they interact with T cells. DC residing in peripheral tissues are in an immature state. Immature DC can capture antigens, including bacteria, but are not yet efficient stimulators of T cells [1,2]. This capacity is acquired in a process called maturation where immature DC undergo specific alterations that change them into effective activators of naïve T cells [1,3]. DC maturation includes down-regulating their capacity to capture antigens, up-regulating MHC and costimulatory molecule expression, and altering chemokine production and chemokine receptor expression [1,3]. Microbial products such as LPS, as well as proinflammatory cytokines including TNF- α or IL-1 β , trigger DC maturation. Thus, DC maturation induced by antigen and inflammatory sti-

muli optimizes their capacity to present antigens to and activate naïve T cells in secondary lymphoid organs.

DC are identified based on several aspects of their phenotype and function. For example, murine DC are often identified by expression of surface molecules including CD11c and MHC-II. While the integrin CD11c is constitutively expressed on immature as well as mature murine DC, mature DC express higher surface levels of MHC-II, CD86, CD80 and CD40 compared to immature DC [1,3]; up-regulation of these molecules is a feature of DC maturation. In addition, the bulk population of murine splenic DC (CD11c⁺ MHC-II⁺ cells) can be further divided into subsets based on surface expression of other molecules including CD8 α , CD4, CD11b and DEC-205 [4]. Thus, CD8 α ⁺CD4⁻DEC-205⁺CD11b⁻, CD8 α ⁻CD4⁺DEC-205⁻CD11b⁺ and CD8 α ⁻CD4⁻DEC-205⁻CD11b⁺ subsets are present among mouse splenic DC. Additional DC subsets have also been characterized in the peripheral lymph nodes and Peyer's patches of mice [4,5].

Different functions, particularly cytokine secretion upon microbial stimulation, have been attributed to the DC subsets [5–11]. For example, CD8 α ⁺ DC are more prone to produce IL-12 and influence CD4⁺ T cells to produce Th1 cytokines compared to CD8 α ⁻ DC [5,6,8–14]. The DC subsets also localize to distinct regions in secondary lymphoid organs, with CD8 α ⁺CD11b⁻ DC

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preferentially localizing to T cell areas while $CD8\alpha^-CD11b^+$ DC are within the splenic marginal zone [15]. In Peyer's patches, $CD8\alpha^-CD11b^+$ and $CD8\alpha^-CD11b^-$ are localized in the subepithelial dome while $CD8\alpha^-CD11b^-$ and $CD8\alpha^+CD11b^-$ are found in the interfollicular region [5,16]. The differential cytokine production capacity and localization within lymphoid organs suggest that the DC subsets may have different roles during an immune response.

2. Infection with *Salmonella*

Salmonella are Gram negative, facultative intracellular bacteria that are naturally acquired by the oral route. *Salmonella* causes two general types of infections. One is a fairly mild gastroenteritis often associated with *Salmonella*-contaminated foods, particularly poultry products. The other is a more severe, penetrating disease, Typhoid fever, that is caused in humans by *S. typhi* and in mice by *S. typhimurium*. Orally-acquired bacteria must penetrate the gut epithelial barrier. Exactly how *Salmonella* does this is not entirely clear and may depend on the invasiveness of the strain. For example, invasive *Salmonella* use M cells scattered in the epithelium overlying Peyer's patches to cross the intestinal barrier while non-invasive strains can penetrate the intestine in an M cell-independent manner [17–19]. Recent data suggest that DC may be involved in M cell-independent transport of non-invasive *Salmonella* from the intestine [19]. A potential role of DC in mediating bacterial transit across the intestinal epithelium is supported by the observation that $CD8\alpha^-CD11b^-$ DC in Peyer's patches are present in follicle-associated epithelium and can be in close contact with M cells [5]. Together these data suggest a role of intestinal DC in sampling intestinal bacteria and facilitating their penetration across the gut. Once *Salmonella* penetrates the intestine, other organs infected include the mesenteric lymph nodes, spleen and liver.

3. Presentation of *Salmonella* antigens by DC

Salmonella has two features that make it an interesting bacteria to use for studying the interaction between this bacterium and DC. First, *Salmonella* can survive and replicate in phagocytic cells including macrophages and DC [20,21], the cells which should kill the bacteria and present bacterial antigens to initiate a specific immune response. Second, *Salmonella* remain confined in vacuolar compartments and thus differ from other intracellular bacteria such as *Listeria* and *Shigella*.

In vitro experiments have shown that immature DC, such as freshly isolated splenic DC or those derived from murine bone marrow, can process *Salmonella* for

peptide presentation on MHC-II as well as MHC-I [11,21,22]. The capacity of DC to process *Salmonella* for MHC-I presentation is one example of many demonstrating that exogenous antigens can be processed for presentation on MHC-I, molecules better known for their capacity to present peptides derived from endogenous proteins [23]. The pathway used for presentation of *Salmonella*- or *E. coli*-encoded antigens on MHC-I depends on components of the cytosolic antigen presentation pathway such as the TAP transporter, newly synthesized MHC-I molecules and the proteasome [24,25]. Presentation of *Salmonella* antigens on MHC-I or MHC-II by infected DC requires active internalization and processing of the bacteria [11,25]. Furthermore, all three splenic DC subsets ($CD8\alpha^+$, $CD8\alpha^-CD4^+$ and $CD8\alpha^-CD4^-$) internalize *Salmonella* following a brief co-culture, and both $CD8\alpha^+$ and $CD8\alpha^-$ DC process *Salmonella* for peptide presentation on MHC-I and MHC-II [11]. Thus, DC and splenic DC subsets can process *Salmonella* and directly present peptides derived from *Salmonella*-encoded antigens to $CD4^+$ and $CD8^+$ T cells.

Importantly, however, direct presentation of *Salmonella* antigens to T cells by infected DC occurs when the DC are infected with *Salmonella* that do not induce death in the cells. In contrast, cells infected with *Salmonella* that induce apoptotic death, which occurs when DC or macrophages (MΦ) are infected with *S. typhimurium* expressing the type III secretion system, can not directly present *Salmonella* antigens [25,26]. However, *Salmonella*-induced apoptosis of MΦ provides a reservoir of *Salmonella* antigens that can be presented by bystander DC [26]. That is, neighbouring DC ingest apoptotic material from MΦ induced to undergo apoptosis by *Salmonella* infection and present peptides from *Salmonella*-encoded antigens on MHC-I and MHC-II [26]. Interestingly, the capacity to act as bystander APC appears to be a unique feature of DC, as bystander MΦ ingest *Salmonella*-induced apoptotic cells but do not present peptides from *Salmonella* antigens [26]. MΦ may degrade apoptotic material to an extent that precludes presentation of peptides for T cell recognition. Indeed, data from experiments where bystander DC and MΦ are added simultaneously to *Salmonella*-induced apoptotic cells suggest that MΦ compete for apoptotic material and limit the availability of antigens for presentation by bystander DC (Yrlid and Wick, unpublished data). Thus, depending on the type of *Salmonella* DC encounter (apoptosis-inducing or not), *Salmonella*-infected DC can process *Salmonella* for direct presentation of *Salmonella* antigens to T cells or can act as bystander APC that engulf antigenic material from neighboring cells that have undergone *Salmonella*-induced apoptotic death. Thus, the capacity of *Salmonella* to induce apoptotic death in infected

antigen presenting cells does not necessarily mean avoidance of induction of specific immunity.

4. Cytokine production by *Salmonella*-infected DC

The cytokines TNF- α , IL-12, and IFN- γ are important for host survival of *Salmonella* infection [27], and the capacity of DC to produce these cytokines upon encounter with *Salmonella* has been assessed. Following a brief pulse of freshly isolated splenic DC with *S. typhimurium*, IL-12p40 was produced primarily by CD8 α^+ DC while relatively few CD8 α^- CD4 $^+$ and CD8 α^- CD4 $^-$ DC produced this cytokine [11]. Despite IL-12p40 production, however, little of the bioactive form of IL-12, IL-12p70, was detected when DC were infected with *Salmonella* [11,21]. The low level of IL-12p70 production may reflect a lack of accessory signals or cytokines [9,10] when isolated DC are infected with *Salmonella*.

In contrast to IL-12p40 production, CD8 α^- DC were the predominant splenic DC subset producing TNF- α in response to *Salmonella* infection, while much fewer CD8 α^+ DC producing this cytokine were detected [11]. For both TNF- α and IL-12p40, bacterial contact with DC was sufficient to stimulate cytokine production and a significant fraction of cells not containing bacteria were cytokine positive. Although it has been shown that DC can produce IFN- γ in an IL-12-dependent manner [6], IFN- γ does not appear to be produced by DC infected with *Salmonella*, at least in the culture conditions examined [11]. Thus, DC encounter with *Salmonella* results in production of cytokines known to be important in host survival to infection with this bacterium.

5. Changes in DC subsets in response to oral *Salmonella* infection

In vivo studies also examined features of splenic DC and the DC subsets in response to *Salmonella*. These data revealed a doubling in the absolute number of total splenic DC in mice orally infected with *Salmonella* compared to control animals. Specifically, a significant increase in the number of CD8 α^- CD4 $^-$ and CD8 α^+ but not CD8 α^- CD4 $^+$ DC was apparent starting at 5 days post infection [7]. Subset-specific in situ redistribution of splenic DC accompanied the observed quantitative changes in DC number, and increases in CD8 α^+ and CD8 α^- CD4 $^-$ DC associated with the red pulp were apparent. Ex vivo intracellular cytokine analysis showed significant increases in the frequency of CD8 α^+ DC producing TNF- α beginning at day 2 post infection, while an increase in the number of CD4 $^+$ DC producing this cytokine was detected only transiently. No signifi-

cant increase in splenic DC producing IL-12p40, IFN- γ or IL-10 was detected during the early stages of *S. typhimurium* infection [7].

Splenic DC also harbor *Salmonella* during infection, and a similar percentage of *Salmonella*-containing DC were present in the three splenic DC subsets of infected mice [11,28]. This suggests that the splenic DC subsets have a similar capacity to internalize *Salmonella* during infection. DC also become activated during *Salmonella* infection, and splenic DC with increased surface expression of CD86 and CD40 are apparent beginning approximately 1 week post-infection [28]. Finally, splenic DC isolated from *Salmonella*-infected mice present bacterial antigens to CD4 $^+$ and CD8 $^+$ T cells upon ex vivo coculture with primary, antigen-specific T cells [11]. Together these data suggest that splenic DC have a role in triggering *Salmonella*-specific T cells during infection. Furthermore, the differential modulation of splenic DC subsets with regard to organization, number and cytokine production during the early stages of acute *Salmonella* infection may function to fine-tune the immune response to *Salmonella*.

Thus, emerging data are beginning to elucidate the role of DC and the DC subsets to anti-*Salmonella* immunity. However, further studies are needed to understand the role of these antigen presenting cells and the contribution of other phagocytic antigen presenting cells in the immune response to *Salmonella*.

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