



Positive and negative regulation of pathogen induced dendritic cell function by G-protein coupled receptors

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Abstract

The induction of IL-12 from dendritic cells (DC) is a major initiating step in host resistance to intracellular pathogens. We have studied the regulation of this response using an in vivo model in which IL-12 production by splenic CD8 α^+ DC is followed after injection of a soluble extract (STAg) of the protozoan parasite *Toxoplasma gondii*. Our findings indicate that the potent IL-12 response observed is highly dependent on both the chemokine receptor CCR5 and G $_i$ -protein coupled signaling. In addition, we have examined the basis of the unresponsiveness of DC to secondary STAg injection which occurs following primary exposure to this parasite stimulus. Our results demonstrate that this refractory state correlates with the down-regulation of CCR5 expression on DC which, in turn, appears to depend on the induction of endogenous lipoxin A $_4$ (LXA $_4$), a product of arachidonic acid metabolism. Since LXA $_4$ is known to also signal through a G-protein coupled receptor pathway, these findings taken together support a major role for G-protein signaling in the regulation of microbial-induced DC function. Published by Elsevier Science Ltd.

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1. Introduction

IL-12 is the major cytokine responsible for IFN- γ induction in parasite-infected mice. The intracellular protozoan parasite *Toxoplasma gondii* has provided a powerful model for studying the induction and regulation of IL-12 synthesis and its role in host resistance. Previous work established that deficiencies in IL-12 (Gazzinelli et al., 1993), IL-12 receptor signaling (Fukao et al., 2001) or the transcription factor ICSBP (Scharton-Kersten et al., 1997) involved in its induction, all render mice extremely susceptible to acute infection, with survival kinetics similar to that observed in IFN- γ deficient animals. Thus, the IL-12/IFN- γ immune response axis plays a crucial role in the cell-mediated control of *T. gondii* infection.

A number of cell types have been studied for their capacity to produce IL-12 in response to in vitro infection with *T. gondii* or exposure to tachyzoite extract (STAg). The observation that spleen cells from uninfected mice stimulated with *T. gondii* can produce IL-12 even in the absence of T

lymphocytes or IFN- γ led to the discovery of a cell population that can act as a source of the former cytokine. To identify these, cells mice were injected with STAg or live tachyzoites and IL-12 immunolocalized in spleen sections. All of the IL-12 positive cells were found in T cell areas of spleen and were shown by FACS analysis to be CD11c $^+$ DEC 205 $^+$ dendritic cells (DC). Further analysis in vivo and in vitro revealed that STAg-stimulated IL-12p40 production is confined to the CD8 α^+ subset of murine DC (Sousa et al., 1997). The IL-12 response of DC to *T. gondii* is extremely rapid peaking within a few hours and exceeds that observed with most other known microbial stimulators of IL-12 production. These observations make CD8 α^+ DC a strong candidate for the cell population responsible for initiating the IL-12/IFN- γ cascade during early *T. gondii* infection.

Concomitant to IL-12 induction by STAg, resident splenic DC move from the red pulp and marginal zone and cluster around the central arteriole within the T cell areas of the spleen (Sousa et al., 1997). Since the events following STAg injection provide a striking example of the scenario expected during microbial-induced DC activation and T cell priming, we decided to analyze this model in greater detail. We began by investigating the signals required for the dramatic migration of DC into splenic T cell areas observed after in vivo stimulation with STAg and focused on the likely role of

Abbreviations: CCR, CC-chemokine receptor; DC, dendritic cell; IL, interleukin

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chemokine/chemokine receptor interactions in this process. In situ analysis revealed endothelial cells and DC themselves as major sources for the MIP-1 α and MIP-1 β observed. Interestingly, the endothelial cell produced chemokine was localized to the central arteriole where it was detected for at least 6 h following STAg stimulation. This observation may be important in explaining the centripetal movement of DC occurring within the T cell areas (Aliberti et al., 2000).

Since CCR5 is a major chemokine receptor expressed on DC, we investigated its possible role in STAg-induced DC migration. CCR5-deficient animals showed a defective pattern of splenic DC mobilization following STAg injection, in which DC move into white pulp but fail to cluster in T cell areas (Aliberti et al., 2000). Unexpectedly, CCR5-deficient mice also demonstrated greatly impaired DC IL-12 production in response to this parasite stimulus in vivo (Aliberti et al., 2000). More importantly, DC from CCR5 deficient mice were found to produce significantly less IL-12p40 than wild-type DC. These findings indicate that in addition to its function in cell migration, CCR5 signaling plays an important role in the activation of DC by STAg as judged by IL-12 induction.

Mice deficient in CCR5 have previously been shown to be less resistant to infection with *Leishmania donovani* (Sato et al., 1999), as well as *Cryptococcus neoformans* (Huffnagle et al., 1999). In agreement with our observation of decreased IL-12 production in STAg-stimulated CCR5-deficient DC, CCR5 knockout mice showed increased susceptibility to *T. gondii* succumbing within 2 weeks of infection (Aliberti and Sher, 2002). These animals also showed higher parasite cyst counts that correlated with lower serum levels of both IL-12 and IFN- γ (Aliberti et al., 2000).

Interestingly, once the DC IL-12 response induced by STAg injection fades, it cannot be recalled by re-injection of the *T. gondii* extract for a period of 5–7 days. This phenomenon, termed “DC paralysis,” was shown to be independent of suppression by IL-10 or T cells or DC apoptosis. Importantly, the induction of DC paralysis was shown to result in decreased systemic IL-12 and IFN- γ productions, as well as immunopathology following infection with live *T. gondii* (Reis e Sousa et al., 1999). By measuring CCR5 expression on freshly isolated DC following in vivo STAg stimulation, we observed a correlation between the levels of this chemokine receptor and the kinetics of IL-12 paralysis suggesting that the latter phenomenon is due to the down-regulation of CCR5 on DC (Aliberti et al., 2002). One mechanism that could account for this down-regulation in CCR5 expression involves ligation of another set of G-protein coupled receptors that have been previously shown to bind bacterial-derived formyl-peptides (Deng et al., 1999; Le et al., 2001; Li et al., 2001). One of these, FPRL-1, is also known to bind with high affinity members of a newly described family of eicosanoid mediators, the lipoxins. These arachidonic acid derivatives are products of multiple transcellular pathways catalyzed by lipoxygenases (LO), including 5-, 8- and 15-lipoxygenases

(Serhan et al., 1986). Previous studies had demonstrated that ligation of FPRL-1 by an HIV envelope-derived peptide results in down-regulation of CCR5 on human monocytes (Deng et al., 1999; Le et al., 2001; Li et al., 2001). Because of FPRL-1s reported binding of lipoxin and in particular lipoxin A₄ (LXA₄), we asked whether LXA₄ is involved in the loss of CCR5 function observed during the induction of paralysis by STAg injection.

We found that in vivo administration of STAg up-regulates LXA₄ secretion in mouse spleen within 6 h following injection of the extract (Aliberti et al., 2002). Interestingly, mice genetically deficient for an LXA₄-generating enzyme, 5-LO failed to secrete this eicosanoid (Aliberti et al., 2002). Importantly, we were unable to induce paralysis in the same 5-LO knockout animals in contrast to wild-type mice. In parallel, DC from these 5-LO deficient mice displayed faster recovery of CCR5 expression than did the normal controls. In support of these observations, injection of a stable LXA₄ analog markedly suppressed the induction of IL-12 from DC by STAg in vivo. Interestingly, the LXA₄ analog treated mice also showed defective DC mobilization in the spleen suggesting that ligation of FPRL-1 has broad inhibitory effects on chemokine induced DC function in vivo (Aliberti et al., 2002).

The above findings led us to propose the following model for the induction of DC paralysis. This model argues that, in addition to triggering CCR5 signaling and IL-12 expression, molecules in STAg induces the production of LXA₄. This eicosanoid, presumably by binding to FPRL-1 on DC, down-modulates CCR5, thereby suppressing IL-12 responsiveness and also down-regulates other chemokine receptor activities involved in DC migration. We propose that the above pathway functioning together with the normal process of ligand mediated chemokine receptor endocytosis maintains long-term suppression of CCR5 activity and STAg-induced IL-12 production.

2. Conclusions

The findings described in this review emphasize the concept that microbial-induced DC responses are highly regulated not only by the pathogen-derived molecules that trigger them but also by immunosuppressive mediators of host origin. The explanation for this tight regulation of DC function may lie in the need to respond rapidly and potently to invading microbes without inducing immunopathology. Our work, in agreement with the findings of Braun and Kelsall (2001) stresses the importance of G-protein coupled receptors in this control process and identifies CCR5 as one receptor that positively regulates IL-12 production by pathogen-stimulated DC. It will be important to extend these findings to other microbial-induced DC responses. In the case of the *T. gondii* system, many of the details of the pathway we have proposed remain to be elucidated. These concern the nature of the parasite ligands involved in both

CCR5 and LXA₄ triggering as well as the formal identification of FPRL-1 as the LXA₄ binding receptor mediating paralysis induction. Nevertheless, it is already clear from the many in vivo correlates observed in our studies that exogenous manipulation of this pathway may offer a powerful strategy for regulating DC function with the goal of enhancing host resistance or preventing immunopathology.

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