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Dendritic cells as a tool to combat infectious diseases

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Abstract

Dendritic cells (DCs) form a network of potent antigen-presenting cells that initiate and amplify immune responses. The detection and capture of microorganisms by DCs trigger stimulus-specific maturation programs that enable DCs to convey pathogen-associated signals to the adaptive branch of the immune system. The appropriate activation of DCs is critical for their ability to direct the development of either a Th1 or a Th2 response, thereby determining the outcome of microbial infections. Advances in the understanding of DC interactions with microbes provide new concepts for immune interventions. In different models of infectious disease, it has been demonstrated that DCs can serve as vaccine carriers, mediating protection against various types of pathogens. The studies of the requirements of ex vivo manipulations of DCs may lead to the design of vaccines that induce protective immunity to infections by appropriate targeting of DCs in vivo.

Keywords: Infection; Vaccination; Dendritic cells; Th1/Th2 cells; *Leishmania* parasites

1. Introduction

The many advances in our understanding of the determinants of protective immunity to infections, of the relevance of adjuvants to the quality and magnitude of immune responses and of the mechanisms maintaining immunological memory offer the promise for the development of new strategies of vaccination and immunotherapy. It has been found that the administration of the antigen of interest is often not sufficient to elicit an effective immune response unless an adjuvant is added. This requirement for adjuvants can be explained by the molecular and cellular bases of immune regulation. The stimulation of T cells, key components of the adaptive immunity, and the characteristics of a T-cell response to antigen depend on the signals that the T cell receives from cells of the innate branch of the immune system, the antigen-presenting cell (APC). APCs first need to differentiate from a resting to an activated state, in which they can present the antigen in the context of MHC molecules and mediate co-stimulatory signals. The activation of APCs is supposed to be a major mechanism by which adjuvants augment immune responses.

2. Dendritic cells

Dendritic cells (DCs) are the most potent type of APCs and are responsible for the initiation of immune responses. Situated in peripheral tissues and in lymphoid organs, DCs are uniquely suited to detect and capture pathogens. They express members of the recently identified Toll-like receptor (TLR) family, which bind common chemical moieties associated with microbial organisms. TLR ligands include bacterial lipopolysaccharide, lipopeptides, hypomethylated CpG DNA motifs, ds RNA and flagellin [1]. TLR signaling triggers a maturation program in DCs that leads to upregulation of MHC and costimulatory molecules and expression of pro-inflammatory cytokines. As a result, DC acquire the unique ability to prime naive T cells [2]. Because of their pivotal functions, DCs have begun to be appreciated as a mandatory target in the creation of new adjuvants. Most importantly, DCs can be used to circumvent the need for exogenous adjuvants, by loading them with the antigen of interest ex vivo and injecting them back into animals or humans to manipulate the immune response. Indeed, several studies have documented the therapeutic

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potential of DC-based immune interventions in a variety of murine tumor models and, more recently, in clinical trials [3]. However, only few studies so far have explored the in vivo efficacy of DC-mediated vaccination in infectious diseases (for review see Ref. [4]).

3. Interaction of DCs with pathogens: lessons learnt from the leishmaniasis model

Infection of inbred mice with *Leishmania* parasites is perhaps the disease model that has been used most widely to define the cell populations and cytokines controlling host resistance or susceptibility to a microbial pathogen. In this experimental system, the immunological mechanisms leading to the restriction or the facilitation of parasite growth are well characterized. A plethora of data demonstrate that protective immunity is associated with the development of a Th1 response, while a Th2 cytokine pattern prevails in mice that succumb to infection [5]. IL-12 is considered to have a central regulatory function in directing the development of protective Th1 cells [6,7]. These findings were paradigmatic for many infectious diseases.

DCs have been shown to play a decisive role in the initiation, regulation and maintenance of *Leishmania*-specific T-cell responses [8–12]. Langerhans cells (LCs), members of the DC lineage residing in the epidermis, internalize *Leishmania major* parasites and this process results in the expression of increased cell surface levels of MHC and costimulatory molecules, and the release of IL-12 [11,13]. In vivo tracking experiments suggested that LCs transport the ingested parasites from the site of infection in the skin to the T-cell areas of the proximal lymph nodes [14]. This migration is guided by chemokines and correlates with the maturation of DCs into potent APCs, enabling them to effectively stimulate a primary T-cell response. It is important to note that this scenario, which is summarized in Fig. 1, mediates the principal sensitization of *Leishmania*-specific T cells in both disease-resistant and disease-susceptible hosts. After healing of the original skin lesion in resistant mice that have become immune to re-infection, DCs may also contribute to the sustained stimulation of parasite-specific T cells that maintain protective immunity to leishmaniasis [15]. This extraordinary efficiency in antigen presentation by DCs may be explained by the unusual stability of MHC class II molecules loaded with immunogenic parasite peptides [16].

Interestingly, the exposure to *Leishmania* parasites has differential effects on DCs from disease-resistant and disease-susceptible mice. Expression of the costimulatory molecule B7.1 (CD80) was reported to be down-regulated on epidermal LCs from susceptible mice but not on those from resistant animals [17]. It has also been shown that CD40 engagement, which enhances IL-12 production by *Leishmania*-exposed DCs from resistant mice, fails to induce IL-12 but rather potentiates the release of IL-4 by *Leishmania*-exposed DCs from susceptible mice [18]. This correlated with the finding that infected DCs from susceptible mice primed stronger Th2 responses [18]. Importantly, IL-4 was shown to have contrasting effects on DC development, on the one hand, and T-cell differentiation, on the other, in *Leishmania*-susceptible mice [19]. When available exclusively during the initial activation of DCs that precedes T-cell priming, IL-4 instructed the development of IL-12-producing DCs, Th1 responses and resistance to cutaneous leishmaniasis. When also present later, during T-cell priming, IL-4 induced Th2 differentiation and susceptibility to disease.

In conclusion, these findings suggest that an appropriate instruction of DCs is critical for their differentiation into qualitatively distinct APCs directing the development of naive T cells toward either a Th1 or a Th2 phenotype. For the induction of protective immunity to an intracellular pathogen such as *Leishmania*, it is essential to condition DCs to produce IL-12, a potent Th1-promoting factor. This notion is supported by the finding that a mere expansion of the number of mature DCs, which can be achieved by treatment of mice with Flt3 ligand, is not sufficient to mediate complete protection against cutaneous leishmaniasis [20]. DCs need to be educated in a specific manner to acquire the ability to drive an efficient immune response to a given pathogen. A recent report shed light on the molecular mechanisms contributing to this plasticity of DC functions by showing that exposure of DCs to microorganisms or their components induces stimulus-specific programs of gene expression [21]. Understanding these processes in more detail will be a key to the development of strategies for DC-based vaccination and immunotherapy.

4. Arming DCs for the induction of antimicrobial immunity

The knowledge of the crucial role of DCs in the tuning of antimicrobial immune responses and the ability to culture DCs ex vivo has led to their use for manipulations of the immune system. The ultimate aim is the design of vaccines that induce protective immunity to infections by appropriate targeting of DCs in tissues and modulation of their functions in vivo.

In various models of infectious disease, DCs have been examined for their capacity to serve as adjuvants and vaccine carriers mediating protection against bacterial, viral, parasitic or fungal pathogens [4]. For example, DC-based vaccination improved immunity to *Borrelia burgdorferi*, the cause of Lyme disease [22], *Chlamydia trachomatis* [23], lymphocytic choriomenin-

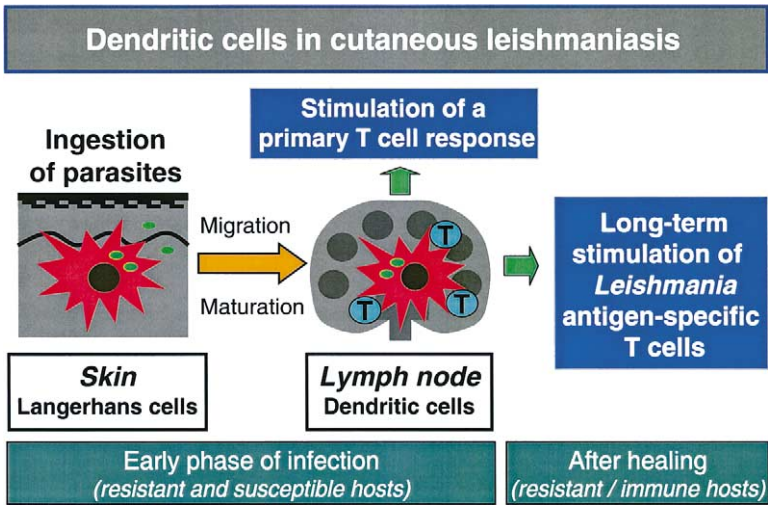

Fig. 1. The role of DCs in cutaneous leishmaniasis. *Leishmania* parasites are transmitted by sandflies and their natural site of entry into the mammalian host is the skin. After infection, LCs leave the epidermis and take up parasites in the dermal compartment. Subsequently, they transport the ingested parasites to the T-cell areas of the draining lymph node. This migration occurs during the first 48 h of infection and correlates with the differentiation of LCs into potent APC that are capable of stimulating resting T cells with specificity for *Leishmania* antigens. DCs mediate the initiation of the T-cell response to *Leishmania* in both resistant and susceptible hosts. After healing of the original skin lesion in resistant mice that have become immune to re-infection, lymph node DCs have the unique ability to present persistent *Leishmania* antigen to T cells and may thus be responsible for the sustained stimulation of specific T cells that maintain protective immunity to leishmaniasis.

gitis virus [24], *Candida albicans* [25] and *Leishmania major* [26]. In experimental leishmaniasis it was demonstrated that a single treatment with parasite lysate-pulsed DCs was sufficient to induce maximal levels of protection. Importantly, protective immunity correlated with the ability of antigen-pulsed DCs to express IL-12 and was hallmarked by the development of a Th1-polarized immune response [26]. DC-based immunization led to the establishment of solid immunity that completely protected the mice against rechallenges with *Leishmania* parasites [26]. Furthermore, vaccination with antigen-pulsed DCs was shown to confer protection even after extended time intervals between immunization and infection with *Leishmania*. The course of lesion development in mice that had been infected 6 weeks after prophylactic treatment with parasite antigen-pulsed DCs was comparable to that observed after a time interval of only 1 week (Fig. 2). Thus, DCs delivering microbial antigen are able to induce solid and long-lasting protection against infectious disease.

In the studies listed above, live organisms or crude preparations of killed pathogens were used as the source of antigens for DC loading. However, crude microbial extracts may vary in their quality and, moreover, may also contain antigen(s) that stimulate disease-promoting rather than host-protective immune responses. Thus, the choice of microbial antigen, or combinations of antigens, with which to charge DCs is going to have a profound influence on the efficacy of DC-based vaccination. Significant levels of protection against experimental leishmaniasis could be induced by DCs that had been pulsed with a defined cocktail of recombinant parasite antigens (Berberich and Moll, unpublished observations), demonstrating that the development of a DC-based subunit vaccine is feasible. On the other hand, DCs pulsed with chlamydial outer membrane protein, although secreting IL-12 and stimulating IFN-γ secretion by T cells in vitro, were reported to elicit a non-protective Th2-type immune response in vivo [27]. These findings emphasize the importance of the nature of the antigen used for DC loading.

Another critical aspect is the route of DC-associated antigen delivery. It has been shown that the type of Th response induced by DCs is influenced by the microenvironment of T-cell priming. This may be explained by the local cytokine milieu determining the mode of DC function. The significance of this parameter was documented by the observation that the induction of a Th1 response and resistance against leishmaniasis required the intravenous treatment of mice with antigen-pulsed DCs, whereas intradermal or intraperitoneal administration of pulsed DCs was not protective [26]. In contrast, protective immunity to *Candida albicans* was seen only after subcutaneous but not intravenous administration of yeast-pulsed DCs [28]. Thus, the optimal route of injection is likely to depend on the type of pathogen and the quality of the immune response required for effective protection.

Finally, the stage of DC maturation is of major relevance for the homing and function of antigen-loaded DCs following adoptive transfer. Regarding this issue, it needs to be considered that the induction of full DC maturation ex vivo may impair the ability of DCs to migrate to relevant lymphoid organs in vivo. Immuniza-

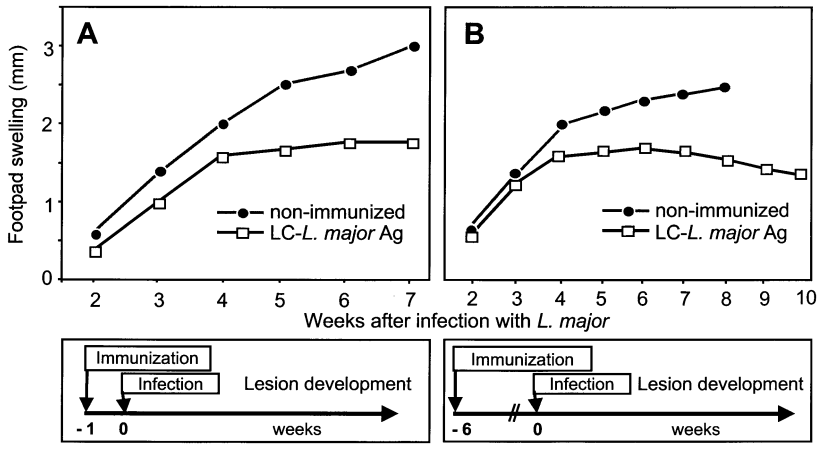

Fig. 2. Treatment with antigen-loaded DCs confers long-lasting protection against leishmaniasis. Genetically susceptible BALB/c mice were immunized i.v. with $2–3 \times 10^5$ epidermal LCs that had been pulsed in vitro with *Leishmania major* lysate (□). Control animals were treated with PBS (●). One week (A) or 6 weeks (B) thereafter, the mice were infected intradermally with 2×10^5 *L. major* promastigotes into a foodpad. The increase in size of the infected compared to the non-infected contralateral footpad was measured weekly. Control mice were sacrificed at 8 weeks after infection because of severe footpad necrosis. Data represent the mean ± S.D. of five mice per group.

tion with immature DCs, on the other hand, may result in the induction of tolerance.

5. Future considerations

The potent immunoregulatory functions of DCs provide new approaches for immune interventions. The exploration of strategies for the ex vivo manipulation of DCs enabling them to induce antimicrobial immunity will help to define the factors that may allow the instruction of DC functions in vivo. In particular, specific molecules that induce the activation of DCs and influence their mode of action, such as CD40 ligand, CpG oligonucleotides or cytokines/chemokines, may be valuable tools for the engineering of vaccines.

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References

[1] S. Akira, K. Takeda, T. Kaisho, Nat. Immunol. 2 (2001) 675–680.
[2] M. Cella, E. Sallusto, A. Lanzavecchia, Curr. Opin. Immunol. 9 (1997) 10–16.
[3] J. Banchereau, B. Schuler-Thurner, A.K. Palucka, G. Schuler, Cell 106 (2001) 271–274.
[4] H. Moll, C. Berberich, Int. J. Med. Microbiol. 291 (2001) 323–329.
[5] S.L. Reiner, R.M. Locksley, Annu. Rev. Immunol. 13 (1995) 151–177.
[6] F.P. Heinzel, D.S. Schoenhaut, R.M. Rerko, L.E. Rosser, M.K. Gately, J. Exp. Med. 177 (1993) 1505–1509.
[7] F. Mattner, J. Magram, J. Ferrante, P. Launois, K. Di Padova, R. Behin, M.K. Gately, J.A. Louis, G. Alber, Eur. J. Immunol. 26 (1996) 1553–1559.
[8] H. Moll, Immunol. Today 14 (1993) 383–387.
[9] H. Moll, S. Flohé, M. Röllinghoff, Eur. J. Immunol. 25 (1995) 693–699.
[10] P.M. Gorak, C.R. Engwerda, P.M. Kaye, Eur. J. Immunol. 28 (1998) 687–695.
[11] E. von Stebut, Y. Belkaid, T. Jakob, D.L. Sacks, M.C. Udey, J. Exp. Med. 188 (1998) 1547–1552.
[12] P. Konecny, A.J. Stagg, H. Jebbari, N. English, R.N. Davidson, S.C. Knight, Eur. J. Immunol. 29 (1999) 1803–1811.
[13] C. Blank, H. Fuchs, K. Rappersberger, M. Röllinghoff, H. Moll, J. Infect. Dis. 167 (1993) 418–425.
[14] H. Moll, H. Fuchs, C. Blank, M. Röllinghoff, Eur. J. Immunol. 23 (1993) 1595–1601.
[15] H. Moll, S. Flohé, M. Röllinghoff, Eur. J. Immunol. 25 (1995) 693–699.
[16] S. Flohé, T. Lang, H. Moll, Infect. Immun. 65 (1997) 3444–3450.
[17] M.L. Mbow, G.K. DeKrey, R.G. Titus, Eur. J. Immunol. 31 (2001) 1400–1409.
[18] H. Qi, V. Popov, L. Soong, J. Immunol. 167 (2001) 4534–4542.
[19] T. Biedermann, S. Zimmermann, H. Himmelrich, A. Gumy, O. Egeter, A.K. Sakrauski, I. Seegmüller, H. Voigt, P. Launois, A.D. Levine, H. Wagner, K. Heeg, J.A. Louis, M. Röcken, Nat. Immunol. 2 (2001) 1054–1060.
[20] I.B. Kremer, M.P. Gould, K.D. Cooper, F.P. Heinzel, Infect. Immun. 69 (2001) 673–680.
[21] Q. Huang, D. Liu, P. Majewski, L.C. Schulte, J.M. Korn, R.A. Young, E.S. Lander, N. Hacohen, Science 294 (2001) 870–875.
[22] M.L. Mbow, N. Zeidner, N. Panella, R.G. Titus, J. Piesman, Infect. Immun. 65 (1997) 3386–3390.
[23] H. Su, R. Messer, W. Whitmire, E. Fischer, J.C. Portis, H.D. Caldwell, J. Exp. Med. 188 (1998) 809–818.
[24] B. Ludewig, S. Ehl, U. Karrer, B. Odermatt, H. Hengartner, R.M. Zinkernagel, J. Virol. 72 (1998) 3812–3818.
[25] C. Fè d'Ostiani, G. Del Sero, A. Bacci, C. Montagnoli, A. Spreca, A. Mencacci, P. Ricciardi-Castagnoli, L. Romani, J. Exp. Med. 191 (2000) 1661–1673.

[26] S.B. Flohé, C. Bauer, S. Flohé, H. Moll, Eur. J. Immunol. 28 (1998) 3800–3811.
[27] J. Shaw, V. Grund, L. Durling, D. Crane, H.D. Caldwell, Infect. Immun. 70 (2002) 1097–1105.
[28] A. Bacci, C. Montagnoli, K. Perruccio, S. Bozza, R. Gaziano, L. Pitzurra, A. Velardi, C. Fè d'Ostiani, J.E. Cutler, L. Romani, J. Immunol. 168 (2002) 2904–2913.