

Manipulation of dendritic cells for host defence against intracellular infections

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Abstract

Dendritic cells (DCs) are an important innate immune cell type which is the bridge between innate and adaptive immunity. Mounting experimental evidence suggests that manipulating DCs represents a powerful means to enhance host defence against intracellular infectious diseases. We have developed several strategies to manipulate DCs either *in vivo* or *in vitro* for the purpose of enhancing the effect of vaccination or immunotherapeutics. *In vivo* delivery of transgene encoding GM-CSF (granulocyte/macrophage colony-stimulating factor), a DC-activating cytokine, increases the number and activation status of DCs at various tissue sites and enhances antimicrobial immune responses in murine models. Co-expression or co-delivery of GM-CSF gene transfer vector with an antimicrobial vaccine enhances microbial antigen-specific T-cell responses and immune protection. Murine bone marrow-derived DCs are being manipulated *in vitro* and exploited as a vaccine delivery system. Transduction of DCs with a virus-vectored tuberculosis vaccine is a powerful way to activate T-cells *in vivo*. Such genetically modified DC vaccines can be administered either parenterally or mucosally via the respiratory tract.

Introduction

Many diseases for which there remains no effective vaccine are caused by intracellular pathogens, such as HIV, hepatitis, chlamydia and TB (tuberculosis). Current vaccine strategies have been successful in preventing infections by generating a strong humoral response leading to the generation of antibodies directed against the invading pathogen. However, these vaccines have failed to elicit a strong cellular immune response which is required to deal with intracellular infections. The failure of the current vaccine strategies has led to a great deal of research into the mechanism of generating an effective immune response against intracellular pathogens with the hopes of designing better vaccines. These efforts have discovered that a type 1 cellular immune response characterized by IFN- γ (interferon γ)-secreting CD4⁺ and CD8⁺ T-cells is required to combat intracellular infections. Generation of an efficient T-cell-mediated response relies on the antigen-presenting cell.

The most potent antigen-presenting cell is the DC (dendritic cell). DCs are found in a variety of bodily tissues, including lymphoid tissues such as the spleen and lymph nodes and the mucosal tissue of the lung, gut, genital tract and skin, where many pathogens gain entry to the body. DCs originate from bone marrow progenitors and mature with the aid of growth factors such as GM-CSF (granulocyte/

macrophage colony-stimulating factor), IL-4 (interleukin-4), Flt3 (Fms-like tyrosine kinase 3) ligand and IL-6. DCs differentiate along myeloid or lymphoid pathways into several populations of CD11c⁺ MHCII (MHC class II)⁺ CD11b[±] CD8[±] with discrete phenotypes and functions [1]. The most important function of the DC is to bridge innate and adaptive immune responses by processing scavenged pathogenic material into peptide fragments and presenting them to T-cells on MHCI and MHCII [2]. DCs are the most potent professional antigen-presenting cells, capable of 10–100-fold greater formation of peptide MHC complexes on their surfaces than other antigen-presenting cells [3], leading to greater T-cell activation, the hallmark of cellular immunity.

DCs act as innate immune cells, recognizing pathogen-associated molecular patterns through germline-encoded receptors such as Toll-like receptors and mannose receptors leading to release of alarm cytokines [IL-6 and TNF α (tumour necrosis factor α)] and chemokines [4,5], thereby generating a pro-inflammatory microenvironment suitable for further influx of immune cells. DCs are also capable of pathogen scavenging and degradation by a variety of mechanisms including receptor-mediated endocytosis, macropinocytosis or phagocytosis [2]. Upon antigen encounter, DCs migrate into lymphoid organs, usually the local draining lymph node. While migrating, DCs undergo a maturation process from an immature phenotype to a mature phenotype, whereby they express an enhanced level of MHCII, costimulatory molecules CD80 (B7-1), CD86 (B7-2) and CD40 and continue the secretion of cytokines, IL-12, IL-4 or IL-10 [2,5]. In the local draining lymph nodes, DCs activate T-cells, initiating an adaptive immune response. Many intracellular infections require strong type 1 immune responses characterized by activation of IFN- γ -secreting

Key words: cellular immunity, cytokine adjuvant, dendritic cell, host defence, intracellular infection, tuberculosis (TB).

Abbreviations used: AdAg85A, adenovirus expressing antigen 85A; AdI, empty adenovirus vector; AdGM, adenovirus expressing a murine granulocyte/macrophage colony-stimulating factor; Ag85A, antigen 85A; BCG, Bacille Calmette-Guérin; DC, dendritic cell; DCAg85A, DCs virally transduced with AdAg85A; Flt3, Fms-like tyrosine kinase 3; GM-CSF, granulocyte/macrophage colony-stimulating factor; IFN- γ , interferon γ ; IL, interleukin; MHCII, MHC class II; TB, tuberculosis; TNF α , tumour necrosis factor α .

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CD4⁺ and CD8⁺ T-cells. Since DCs are localized at all key mucosal surfaces where many intracellular pathogens enter the body, and have been shown to be the key in co-ordinating immune responses, one of the vaccine strategies has focused on targeting DC activation and expansion to generate long-lasting cellular immunity. The polarization of the cellular immune response is determined by the way the DC is activated during pathogen encounter and antigen processing, which is in turn determined by the nature of the pathogen. In order for a type 1 immune response to be initiated, the activated DC must secrete large amounts of IL-12 [6,7]. However, pathogen encounter does not always result in IL-12 secretion. For instance, when DCs encounter *Mycobacterium tuberculosis*, they secrete IL-10 and IL-6 [4,5].

Therefore generating a strong type 1 immune response by means of vaccination has been the goal of many vaccinologists who seek a safe and effective vaccine against such intracellular infectious diseases as pulmonary TB. TB remains a significant cause of illness in many countries around the world in spite of the introduction of BCG (Bacille Calmette–Guérin) vaccination during infancy 85 years ago (<http://www.who.int/tb/en/>). The failure of BCG in controlling adult TB appears to be due to a lack of long-lasting T-cell memory. In search for improved TB vaccines, vaccinologists have begun to target DCs both *in vivo* and *ex vivo* in order to generate potent T-cell activation leading to stable memory formation.

Expanding *in vivo* DC populations by GM-CSF cytokine adjuvant

Because DCs play such a pivotal role in the initiation of adaptive immune responses, targeting endogenous DC populations *in vivo* with cytokine adjuvants may amplify the level of immune activation. Moreover, the state of maturation of the DC is critical, as a DC that is not fully matured can lead to T-cell tolerance. Therefore a cytokine adjuvant that recruits and matures DCs could potentiate an immune response. We have taken a novel approach by using GM-CSF as a cytokine adjuvant to enhance the immunogenicity of a vaccine against intracellular infections.

We generated a replication-deficient AdGM (adenovirus expressing a murine GM-CSF transgene) which can be delivered to a variety of tissues, resulting in a high level but self-limited expression of GM-CSF [8]. When delivered intravenously, intramuscularly or subcutaneously, GM-CSF levels reach a peak in the serum at 3 days post-inoculation [9]. This systemic expression of the GM-CSF transgene leads to significant expansion of endogenous DC populations in the spleen, reaching a peak 200–260-fold increase at 1 week post-gene transfer [9]. Interestingly, while AdGM expands DCs in the spleen, AdGM does not appear to expand the T-cell, NK (natural killer) cell and macrophage population. The expansion of the DC population in the spleen is long lived, with DC numbers remaining increased 15-fold at day 75 post-AdGM [9]. In order to determine whether the DCs expanded by AdGM injection could result in increased

immune activation, the functionality of the recruited DCs was evaluated. It was found that these DCs produce large amounts of TNF α and IL-6, and have enhanced antigen capture and presentation capabilities. Most importantly, AdGM-expanded DCs were capable of far superior T-cell activation, resulting in potent cytotoxic T-lymphocyte generation [9]. These results suggest that AdGM expands a population of DCs that are functionally superior to the DCs that exist in the spleen in the absence of exogenous GM-CSF.

It has been postulated that one of the shortcomings of BCG as a vaccine is its poor ability to induce memory CD8⁺ T-cell responses. Since it has been demonstrated that AdGM enhances DC recruitment and subsequent T-cell activation following systemic or local administration, we next sought to determine whether GM-CSF could enhance the immunogenicity of BCG. When AdGM was co-administered subcutaneously with BCG, the immunogenicity of BCG was markedly enhanced up to 12 weeks post-immunization [10]. T-cells isolated from lymph nodes and spleen of BCG/AdGM-immunized mice secreted more IFN- γ than mice immunized with BCG alone or with BCG and Addl (the empty adenovirus) [10]. When AdGM was injected subcutaneously or intradermally, most of the GM-CSF transgene product was detected in the skin of the animal, leading to locally enhanced accumulation of cutaneous DCs and other MHCII-expressing antigen-presenting cells [11]. Such increased DCs at the site of vaccination and subsequently enhanced immune activation led to improved protection against secondary mycobacterial or *M. tuberculosis* challenge in the lung [10]. Similarly, intramuscular immunization with a plasmid DNA vaccine co-expressing both GM-CSF and *M. tuberculosis* Ag85A (antigen 85A) led to a much higher level of type 1 T-cell activation *in vivo* than the vaccine that expressed only Ag85A (X. Zhang, M. Divangahi, A. Zganiacz, J. Wang, J. Bramson and Z. Xing, unpublished work).

Since AdGM acts to expand endogenous DC populations, delivering AdGM to a naturally DC-rich area, such as a mucosal surface, may prove to be a far superior strategy to generate efficient immune protection against a pathogen that enters via the mucosa. To this end, we delivered AdGM intranasally (i.n.) to the airway of mice. Intranasal administration of AdGM resulted in an 8 times greater influx of immune cells into the airway lumen compared with Addl [12]. The predominant cell types infiltrating the airway lumen as a result of GM-CSF were monocytes/macrophages and lymphocytes which persisted for up to 19 days [12]. Of importance, AdGM delivery expanded and activated a myeloid DC population locally in the lung, which appeared to be macrophage-derived [12]. Upon *in vitro* re-stimulation with adenoviral antigens, both CD4⁺ and CD8⁺ T-cells from AdGM-treated mice produced significantly more IFN- γ than CD4⁺ and CD8⁺ T-cells from Addl-infected mice, suggesting that lung GM-CSF transgene expression enhanced viral antigen-specific type 1 immune responses via its effect on myeloid DCs [12]. It is important to note that the level of DC recruitment and activation demonstrated using the recombinant AdGM could not be duplicated by injection

of poly(ethylene glycol)-conjugated GM-CSF, unmodified GM-CSF or AdFlt3 (adenovirus expressing Flt3) [9,12].

AdGM can also be used as an immune adjuvant for an organism-based chlamydial vaccine to potentiate the immune activation in response to pulmonary *Chlamydia trachomatis* infection. Thus co-administration of inactivated chlamydial organisms with AdGM resulted in significantly enhanced numbers of DCs recruited to the airway and potent systemic type 1 immune activation which was accompanied by reduced *Chlamydia* bacterial burden upon secondary challenge [13]. Interestingly, not only did AdGM increase the cellular immunity, but it also significantly increased the amount of lung mucosal IgA [13].

Ex vivo manipulation of DCs

While targeting DCs *in vivo* by using adenoviral-mediated GM-CSF gene expression is an effective way to expand and activate endogenous DCs, its direct *in vivo* application may cause undesired inflammatory responses and trigger anti-adenoviral antibody responses which may limit the second administration. Since it is becoming increasingly difficult to treat TB infections caused by multidrug-resistant strains of *M. tuberculosis* and many patients who complete drug therapy are at increased risk of re-infection, there is now an urgent need to develop a therapeutic vaccine for these individuals. In this regard, DC-based TB vaccines possess the power of antigen presentation and do not trigger an overwhelming acute inflammatory response as a 'naked' viral vaccine does *in vivo*, thus serving as an ideal candidate for therapeutic TB vaccines.

DC-based TB vaccines have been examined to a limited extent in murine models. One of the first approaches to manipulating DCs *ex vivo* to induce immunity against pulmonary *M. tuberculosis* challenge was carried out by Demangel et al. [14]. They reported that *ex vivo*-generated DCs infected with BCG delivered intratracheally induced only a short-lived protective immune response against pulmonary *M. tuberculosis* challenge. However, it was also reported that the *ex vivo* DCs infected with BCG were able to harbour viable bacilli [14,15]. Delivering viable bacilli to the respiratory tract is not a safe approach to inducing TB immunity in TB patients, particularly those with immune-compromised conditions. Other strategies to using DC-based vaccines in more recent years have focused on pulsing *ex vivo*-derived DCs with whole proteins or immunodominant peptides [16,17]. Ag85A is a major secreted protein found in all clinically isolated strains of *M. tuberculosis*. DCs pulsed with whole Ag85A protein delivered intranasally gave rise to increased numbers of IFN- γ -secreting CD4⁺ and CD8⁺ T-cells in the lung [17]. However, these cells were not able to confer protection against pulmonary *M. tuberculosis* challenge [17]. We have demonstrated that DCs pulsed with Ag85A immunodominant CD4 and CD8 peptides induced slightly better immune responses than DCs pulsed with whole protein when delivered intramuscularly or intravenously [18].

We have developed a novel approach to using DC-based vaccines in anti-TB vaccinology. We infected bone marrow-derived DCs with a recombinant adenovirus expressing Ag85A (AdAg85A), which is capable of expressing Ag85A fused to a signal peptide sequence, so that Ag85A can be secreted from infected mammalian cells. We found that DCAdAg85A (DCs virally transduced with AdAg85A) induced significantly greater CD4⁺ and CD8⁺ IFN- γ ⁺ responses than peptide- or protein-pulsed DCs when delivered intramuscularly. This enhanced immune activation is probably due to the increased antigen presentation, by the infected DC on MHC I as well as antigen presentation of secreted Ag85A by endogenous DCs on MHC II. Upon infection with AdAg85A, DCs expressed enhanced co-stimulatory molecules CD80, CD86 and enhanced production of IL-6 and IL-12 [18], thus having greater type 1 immune-activating capabilities. In order to better target lung-specific immunity, DCAdAg85A was given intranasally. Mucosally administered DCAdAg85A resulted in recruitment of antigen-specific CD4⁺ IFN- γ ⁺ and CD8⁺ IFN- γ ⁺ T-cells to the airway lumen, which conferred protection against pulmonary *M. tuberculosis* infection (S. McCormick, M. Santosuosso, X. Zhang, Y. Wan and Z. Xing, unpublished work). Such respiratory mucosal immunization with DCAdAg85A may represent an effective way to trigger anti-TB memory T-cell responses without causing an unwanted inflammatory response. We are planning to use this strategy to treat pulmonary TB in mouse models.

To enhance further the antigen-presenting power of the DC, our preliminary results suggest that DCs transduced with AdAg85A co-expressing GM-CSF can further potentiate the antigen-specific T-cell responses. This is probably due to GM-CSF secreted by the virally transduced DCs recruiting endogenous DCs to the vaccination location and site of antigen deposition. The secreted GM-CSF may act on the infected DCs, causing full maturation, as well as acting on the endogenous DCs to provide a maturation signal to recruited DCs, which can then pick up the secreted Ag85A from the vaccine DCs to potentiate the number of DCs activated by a single immunization event. Thus DCs manipulated *ex vivo* to express an antigen of interest may recruit and expand the endogenous DCs, thus leading to a robust anti-TB immune response.

Conclusions

DCs are critical players in the initiation of an effective immune response against intracellular infections such as *M. tuberculosis*. Efforts that have focused on expanding endogenous populations of DCs *in vivo* have proven to be very effective in increasing the immunogenicity of vaccines and such cytokine adjuvants as GM-CSF would be prime candidates to be applied in conjunction with current BCG vaccination strategies. More recent efforts have focused on manipulating DCs *ex vivo* to generate cell-based vaccines that can be used *in vivo* to trigger type 1 immunity against intracellular infections. While *ex vivo*-derived DCs pulsed

with antigenic peptides or proteins have resulted in mediocre induction of protective immunity, virally transduced DCs are capable of much greater protective immunity. Such virally transduced DC vaccines may also serve as therapeutic vaccines to treat intracellular infections without causing tissue damage and virus-neutralizing antibodies.

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