Available online at www.sciencedirect.com
SCIENCE DIRECT®
Microbes and Infection 5 (2003) 311–319
Microbes and Infection
www.elsevier.com/locate/micinf

Review

Dendritic cells, new tools for vaccination

Jesus Colino, Clifford M. Snapper *

Department of Pathology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, USA

Abstract

Our rapidly expanding knowledge of the biology of the dendritic cell (DC), a major antigen-presenting cell connecting innate and adaptive immunity, suggests new possibilities for the development of vaccines and therapeutic strategies against pathogens, through the manipulation of their function in vivo, or the injection of the DC itself, once properly instructed ex vivo.

Keywords: Vaccines; Immunotherapy; Dendritic cells

1. Introduction

During the last decade, intensive research has focused on understanding dendritic cell (DC) biology [1,2]. As a result, we now have a significant understanding of the plasticity of DC function and its major role in maintaining homeostasis through the induction of protective immune responses against pathogens and tumors, and in the maintaining of peripheral tolerance. In this review, we will discuss the major checkpoints involved in DC function (Fig. 1), focusing on their role in anti-bacterial immunity, and discuss how DCs or their products may be used or altered to prevent and manage invasive infections.

2. Overview of DC function

DCs show a unique functional duality during their development, designed to ultimately provide secondary lymphoid tissues with useful information about the antigenic composition in the periphery. At the immature stages of development, DCs resident in peripheral tissues are specialized in antigen-capture, acting as *sentinel cells* (Fig. 1). They are strategically located, among macrophages, at epithelial barriers that often serve as major portals of pathogen entry. Thus, in the gastrointestinal tract, DCs located in intraepithelial pockets below M cells are well equipped to detect and sample infectious agents for the transport of microbial antigen to the subepithelial lymphoid tissue [3]. DCs avidly internalize non-opsonized pathogens by macropinocytosis, phagocytosis or through mannose receptors (C-type lectins), or complexes of antibody and microbial antigen via Fcγ receptor types I and III. By virtue of their high phagocytic and endocytic capacities, DCs constitutively internalize samples of their antigenic microenvironment, which in the event of infection, will also include microbial antigens. In addition, DCs become activated and mobilized in response to infection (i) by direct recognition of pathogen-associated molecular patterns (PAMPS); e.g. LPS or cell-wall components, by Toll-like receptors (TLRs), or (ii) indirectly through receptors for inflammatory cytokines and chemokines.

Immature DCs upon activation with PAMPS secrete inflammatory cytokines (e.g. IL-1β, TNF-α, IL-6 and IL-12) and chemokines (e.g. MIP-1α and MIP-1β; RANTES and MCP-1) which serve in part to *recruit circulating DC precursors and other immune cells* to the site of infection and participate in their activation (Fig. 1, point 1). Later on, anti-inflammatory cytokines, chiefly IL-10, are secreted by DCs to counterbalance the pathogenic stimulus, thus preventing the induction of exaggerated immune and inflammatory responses.

Pathogen-mediated activation induces DCs to undergo maturation. This is a complex process with the dual goal of transforming immature DCs in the peripheral tissue into cells that will migrate to the secondary lymphoid organs and then act as professional antigen-presenting cells (APCs) for the priming of naive T cells (Fig. 1, points 2–4). Toward accomplishing the first goal, activated DCs lose their phagocytic capacity and tissue adhesive structures, increase their expression of receptors for lymphoid chemokines (i.e. CCR7), and

* Corresponding author. Tel.: +1-301-295-3490; fax: +1-301-295-1640.
E-mail address: csnapper@usuhs.mil (C.M. Snapper).

DOI: 10.1016/S1286-4579(03)00033-9

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
2003		**00-00-2003 to 00-00-2003**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Dendritic cells, new tools for vaccination	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Uniformed Services University of the Health Sciences,Department of Pathology,Bethesda,MD,20814**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Our rapidly expanding knowledge of the biology of the dendritic cell (DC), a major antigen-presenting cell connecting innate and adaptive immunity, suggests new possibilities for the development of vaccines and therapeutic strategies against pathogens, through the manipulation of their function in vivo, or the injection of the DC itself, once properly instructed ex vivo.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**9**	

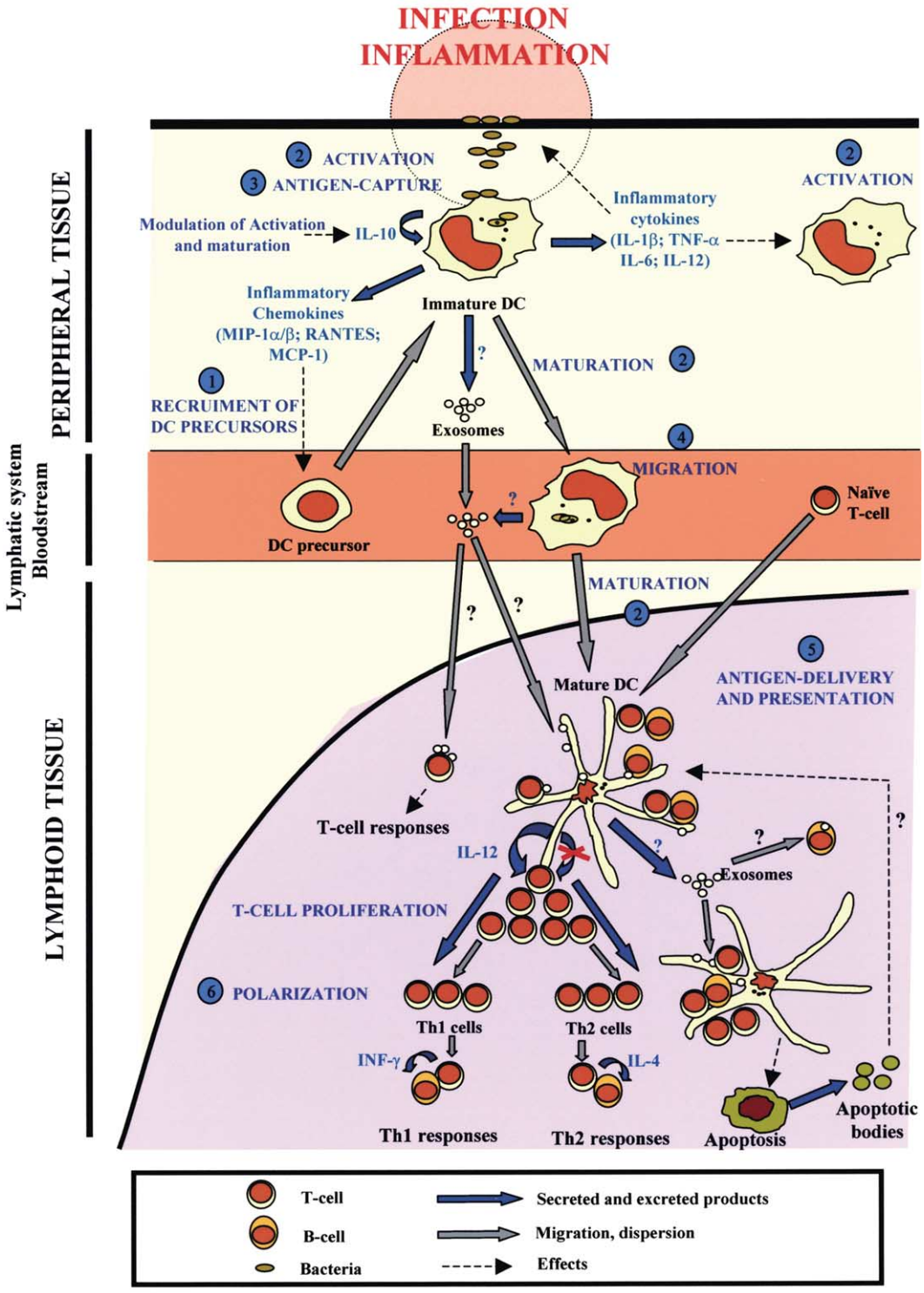

Fig. 1. Overview of DC function during a primary infection. Numbers on the figure refer to the major steps of DC function which could be altered in order to design vaccines and immunotherapeutic approaches against pathogen infections, as indicated in Table 1. Upon inflammation, immature DCs located at the major portals of pathogen entry, internalize the pathogen (3, antigen-capture), becoming activated by direct recognition of PAMPS (2, activation). Activated DCs release inflammatory chemokines to recruit DC precursors (1, recruitment of precursors) and inflammatory cytokines that reinforce inflammation and bystander DC activation (2). DCs activated by the pathogen undergo maturation, a process that occurs during DC migration from the peripheral tissues to the secondary lymphoid organs (4, migration). Maturation transforms the DC from an antigen-capture cell (3) into a professional APC (5, antigen-delivery and -presentation). Migrating DCs entering a secondary lymphoid organ, interact and present the processed antigenic profile of the pathogen to T cells, and likely B cells, to initiate a primary immune response (5). Depending on the pattern of cytokines secreted during the interaction, the immune response will polarize towards either a Th1 or Th2 response (6, polarization). DCs upon completing their APC function, likely, die by apoptosis. Reprocessing by resident DCs of apoptotic bodies containing processed antigen could be a secondary, but unlikely, source for antigen presentation. Alternatively, DCs could deliver antigen locally or at a distance through secreted exosomes. The physiological role of DC exosomes is a matter of debate.

reorganize their cytoskeleton for the acquisition of high cellular mobility [4]. Towards the second goal, DCs strongly upregulate their expression of co-stimulatory molecules (i.e. CD40, CD80 and CD86) and upregulate synthesis and translocation to the surface of MHC molecules complexed to processed microbial antigens. Concomitantly, MHC class II degradation is reduced, resulting in enhanced retention (several days) of peptide-MHC complexes on the cell surface. Thus, DCs, unlike macrophages and other professional "scavengers", respond to the microbial challenge by *migrating* to secondary lymphoid organs while *generating a processed antigenic profile of the pathogen* associated with MHC molecules. Another unique feature of DCs relative to macrophages, is the ability of DCs to attenuate their proteolytic potential. Thus, DC lysosomes can sequester antigen for extended periods and still efficiently use the antigen for generating peptide-MHC class II complexes [5].

Once in the secondary lymphoid organ, mature DCs (effector state) instruct T cells, and likely B cells and NK cells, to *induce an effective primary immune response*, and establish immunological memory (Fig. 1, point 5). This ability of DCs to induce a primary immune response is also a unique property among APCs. We recently demonstrated that bone-marrow-derived dendritic cells (BMDCs) loaded in vitro with *Streptococcus pneumoniae* and then transferred into naive mice were able to induce primary Ig isotype responses not only for bacterial proteins, but also for capsular and cell-wall polysaccharide antigens [6]. The Ig isotype responses to the bacterial proteins uniformly required DC expression of major histocompatibility complex class II, CD40 and B7 and the production of IL-6, strongly suggesting a requirement for cognate interactions with T cells and likely with B cells. Similar requirements for anti-polysaccharide antigens were observed only for the IgG1 isotype, suggesting that for polysaccharide-specific responses classical cognate interactions may affect the quality of the Ig isotype response, but are not critical for DC induction of primary anti-polysaccharide Ig. An unresolved question has been whether DCs migrating to the secondary lymphoid organs acted either directly as APCs or transferred their captured antigens to resident DCs for antigen presentation. Two mechanisms have been proposed to explain how DCs might transfer antigen in an indirect pathway of antigen presentation (Fig. 1, point 5): (i) antigen-loaded DCs that migrate to the secondary lymphoid organ could undergo apoptosis or necrosis and be phagocytosed by resident DCs (ii) migrating DCs could actively release antigen-bearing vesicles (exosomes) derived from the DC lysosomal compartment, which could then be captured by resident DCs. The relative importance of the direct and indirect pathways in anti-microbial immunity has not yet been resolved, but recent data from our laboratory strongly argue against the indirect pathway hypothesis. Thus, we showed that viable, but not necrotic, BMDCs loaded with whole *S. pneumoniae* induced significant antigen-specific Ig isotype responses in vivo [6]. Furthermore, BMDCs undergoing apoptosis induced after contact with bacteria demonstrated markedly impaired polysaccharide- and protein-specific IgG responses upon transfer into naive mice (submitted manuscript). Collectively, our data argue for an active, direct role for the antigen-loaded migrating DCs in this system. However, we hypothesize that for anti-polysaccharide responses, the active release of exosomes by antigen-loaded DCs could play an important inductive role both through antigen transfer between DCs themselves, and between DCs and B cells, that are in tight contacts or synapse.

DCs and T cells can also interact through the formation of an immunological synapse [7]. In this synapse, T-cell receptors and co-stimulatory molecules congregate in a central area surrounded by a ring of adhesion molecules. Sustained signaling via these synaptic interactions is required in order for the T cell to enter the first cell division cycle. Specifically, naive $CD4^+$ T helper cells require more than 20 h of continuous stimulation to become committed to cell division, whereas shorter periods did not result in effector T-cell differentiation. Thus, the stability and duration of the synapse will determine T-cell function. Factors disrupting the synapse, such as DC death, will significantly affect T-cell function. In this regard, we recently observed a strong association between the progression of *S. pneumoniae* induced DC apoptosis and a decreasing ability of such DCs to induce T-cell-dependent primary Ig isotype responses in vivo. On the other hand, factors that prevented DC apoptosis, such as IL-10, served to prolong the APC function of DCs for induction of in vivo anti-bacterial humoral immunity (submitted manuscript).

DCs induce antigen-specific T cells not only to proliferate, but also determine the development of T-cell regulatory or effector functions, including $CD4^+$ helper *T-cell polarization* (i.e. Th1 vs. Th2) *to secrete different patterns of cytokines* (Fig. 1, point 6). IL-12 is the prototypic Th1-polarizing cytokine produced by DCs, whereas other factors, including IL-4, or the absence of IL-12 production lead to Th2 polarization. The ability of DCs to influence the pattern of cytokines secreted by T cells represents a critical function which can profoundly influence the final outcome of the immune response to a pathogen. Thus, cytokines secreted by Th1 cells (IFN-γ, TNF-β and IL-2), are typically considered necessary for protection against infections with viruses and with intracellular bacteria and protozoans, whereas secreted Th2 cytokines (IL-4, IL-5, IL-6 and IL-13) are important for protective responses against multicellular nematode parasites. Several factors appear to influence the ability of DCs to polarize T-cell cytokine responses. (i) *DC subsets*: the subset of DC used for T-cell stimulation may effect a bias for either a Th1 or Th2 response. (ii) *Dynamics of DC migration to the lymphoid organ*: early after initiation of the immune response, large numbers of recently stimulated DCs actively secreting IL-12 and entering into the T-cell areas may preferentially induce Th1 cell priming. In contrast, at a later time, when the DC influx decreases, and the surviving DCs in T-cell areas have downregulated their secretion of IL-12,

preferential priming for Th2 and other regulatory T cells may occur [8]. (iii) *Nature of the maturation stimuli*: bacterial PAMPs induce strong production of IL-12 by DCs, which can be potently boosted by activated T cells through CD40L, whereas other DC stimuli such as cholera toxin, TNF-α or IL-1 do not induce IL-12 (iv) *Microenvironmental factors*: IL-12 production by DCs can also be enhanced by mediators released in the DC microenvironment. For example, IFN-γ and IL-4 can enhance IL-12 production by activated DCs, while prostaglandin E2 and IL-10 exert an inhibitory effect. Results from our group indicate a role for autocrine IL-10 in modulating DC IL-12 production early after activation by *S. pneumoniae* in order to adjust the maturation response to the magnitude of the stimuli (submitted manuscript). Thus, in the absence of autocrine or paracrine IL-10, the level of IL-12 production was sustained for long periods of time (>8 h).

Thus, *DCs represent the critical link between innate and adaptive immunity*, upon which, appropriate concerted action is required for a successful host defense against an invading pathogen.

3. Therapeutic and prophylactic uses of dendritic cells

Significant progress in our understanding of DC biology and their critical function in immunity have prompted many to explore their potential use in immunotherapy and prophylaxis. Most of these pioneering strategies have been developed for anti-tumor therapy, and some are currently being tested in human trials, with variable success. A comprehensive list of the safety requirements of DC preparations used for cancer immunotherapy has been published [9]. Below, we discuss various strategies to prevent or treat infectious diseases for optimizing the use of DCs for mediating their functional effects (Fig. 1) upon transfer into the host as summarized in Table 1.

Table 1
Summary of the potential therapeutic and prophylactic uses of DC

Step of DC function	Potential therapeutic approach
Recruitment of DC precursors	Treatment of patients with DC-poietins: Flt3L, GM-CSF
	Increasing DC-poietin deposition and antigen co-delivery:
	DC-poietin conjugates or fusion proteins with the antigen
	Encapsulation
	Viral vectors encoding GM-CSF
	Transfer of autologous DC expanded ex vivo with DC-poietins
DC activation and maturation	Treatment with proinflammatory cytokines: IL-1β, IFN-α
	Microbial-derived products:
	Mucosal adjuvants: Cholera toxoid mutants
	PAMPS: CpG-oligos, viral dsRNA
Antigen-capture	DC loaded ex vivo with inactivated pathogen before transfer
	DC transfected ex vivo with pathogen DNA plasmids or RNA
	DC loaded with immunologically relevant peptides
	Targeting DCs in vivo with sensor molecules:
	Ligands of MHC and co-stimulatory molecules (CD40)
	Antibodies specific for endocytosis receptors (CD205)
	PAMPs: CpG-oligos, OmpA
	Heat-shock proteins
	Engineered bacteria and virus
Migration and homing	Route of DC administration: subcutaneous vs. intravenous
	Chemokine and chemokine receptors engineered DC: CXCR5, CCR7
	DC subset
Antigen delivery and presentation	Migratory pathways
	Exosomes
Polarization	DC subset
	Nature of the DC maturation or activation stimuli (e.g. bacterial PAMPS vs. inflammatory cytokines)
	DC-poietin (e.g. Flt3 *vs.* GM-CSF)
	Route of DC administration (e.g. i.v. vs. s.c.) and DC homing
	DC treatment with anti-inflammatory cytokines: IL-10

3.1. Expanding DC numbers

One approach to optimizing DC function is to increase their numbers, usually, prior to the administration of an antigen-based vaccine. It will favor and reinforce the natural recruitment of DC precursors (Fig. 1, point 1) Two major strategies have been explored to achieve this goal: (i) the transfer of autologous DCs expanded in vitro in the presence of cytokine cocktails and (ii) the in vivo mobilization of stem-cell precursors of DCs and expansion of immature DCs by treatment with the DC-poietins Flt3L or granulocyte-macrophage colony-stimulating factor (GM-CSF).

Flt3L, the ligand of the FMS-like tyrosine kinase 3, strongly promotes the proliferation, differentiation and survival of early hematopoietic progenitor/stem cells toward DCs and to a lesser extent, NK cells. Noticeably, Flt3L induces increases in the numbers of all DC subsets. In contrast, GM-CSF is selective in its induction of proliferation of cells of the monocyte-macrophage lineage leading to myeloid DC expansion. The relative expansion of different DC subsets could influence the nature of the induced response. Thus, Pulendran et al. [10] observed that pretreatment of mice with Flt3L selectively enhanced the IgG2a response to soluble ovalbumin which resulted from the induction of a polarized Th1 response, whereas GM-CSF pretreatment increased IgG1 antigen-specific responses, indicating polarization of the cytokine response to Th2. Therefore, the selection between GM-CSF or Flt3L, in theory, might allow for modulation of the response that may favor host defense, depending on the nature of the infectious disease.

The use of Flt3L for augmenting host resistance to pathogens has met so far with limited success. Thus, Flt3L pretreatment has been shown to promote resistance against secondary infection with *Listeria monocytogenes* [11] and partial protection against progressive cutaneous Leishmaniasis [12] in mice, but in both the cases, it is not clear that the protective effect was directly due to DC expansion as opposed to the expansion of other cell lineages. Moreover, to be

effective, repetitive injections of high doses of Flt3L were required. Further, the treatment did not resolve an ongoing *Leishmania* infection. In light of these studies, the future application of Flt3L to human population appears uncertain.

GM-CSF is also a very poor adjuvant when delivered systemically. This drawback has been partially resolved through strategies focused on effecting efficient and prolonged deposition of GM-CSF at the vaccination site and its co-delivery with the antigen (i) by coupling the GM-CSF with carrier molecules such as polyethylene-glycol, (ii) as fusion proteins with the antigen, (iii) by encapsulation inside liposomes in combination with the antigen, (iv) or encoded into viral vectors. Some of these strategies have proven to be effective in mouse models of bacterial infection. Thus, vaccination with a fusion protein of a pneumococcal surface protein (PspA) and GM-CSF protected mice against a lethal challenge with virulent *S. pneumoniae* [13]. Most recently, co-immunization of mice with transgenic adenoviral GM-CSF and BCG vaccine, strongly enhanced Th1 antigen-specific immunity, and the protective efficacy of the vaccine against a challenge with *Mycobacterium tuberculosis* [14].

3.2. Activating DCs

DCs that capture antigen, in the absence of concurrent activation, may induce antigen-specific tolerance, as opposed to immunity. Thus, when the antigen does not activate the DCs by itself, an adjuvant might be required in combination with the DC-poietin in order to induce proper immunity. In this regard, it has been shown that DCs can play an active role in the induction of mucosal tolerance to food or orally administered soluble antigens when a concurrent adjuvant is not present. Thus, induction of tolerance to orally administered soluble ovalbumin, is further enhanced in mice pretreated with Flt3L to expand DCs, but this can be reversed by additional administration of the inflammatory cytokine IL-1 (Fig. 1, point 2), a cytokine which can activate DCs [15]. IFN-α can also promote DC activation and hence act as an adjuvant. Thus, IFN-α markedly enhances an antigen-specific humoral response in mice when co-administered with Flt3L [16].

A second group of molecules that can activate DCs are microbial-derived products (Fig. 1, point 2). The most broadly used products in experimental models are LPS a systemic adjuvant and TLR4 ligand, and cholera toxin (CT), a mucosal adjuvant. Although both are highly toxic, many attempts have been made to detoxify them while retaining their adjuvant properties. One promising strategy is the use of a mutant of the CT subunit A, which is non-toxic and induces a strong and polarized Th2 response when used as a mucosal adjuvant [17]. Alternatively, DCs may be activated through TLR9 by oligodeoxynucleotides containing unmethylated CpG motifs or through TLR3 by viral dsRNA or polyI-:polyC. Stimulatory oligodeoxynucleotides in combination with Flt3L have been shown to be effective in the enhancement of humoral and cellular responses to soluble proteins and anti-tumor immunity in mice [18]. Polynucleotides have two major advantages as adjuvants for human use: (i) low toxicity and (ii) the ability to synthesize them in a pure and standardized form.

3.3. Improving antigen-capture: learning to use DCs

A next level of intervention is the design of strategies to improve the delivery of the antigen to the DCs by ex vivo loading, or in vivo targeting in order to reproduce and reinforce the physiologic antigen-capture step of DC function (Fig. 1, point 3). An invaluable amount of information has been obtained using DCs loaded ex vivo with bacteria prior to transfer to the recipient animal. This experimental approach proved for the first time that transfer of DCs loaded with the inactivated pathogen induces protective immune responses in naive recipients later subjected to experimental infection. Immunity has been obtained against pathogens that have so far proved difficult to prevent using vaccine approaches: *M. tuberculosis* [19], *Borrelia burgdorferi* [20] *Chlamydia trachomatis* [21] and *Candida albicans* [22]. *C. albicans* provides a paradigmatic example of how this approach can also help to design vaccine strategies. DCs loaded with the yeast form, but not with the hyphal form, generate protective anti-fungal immunity. This was based on the ability of the DCs to discriminate between the two forms of the pathogen, responding to the yeast by producing IL-12 and stimulating a protective Th1 response, but secreting the Th2 cytokine IL-4 in response to hyphae, which was not protective. However, suppression of IL-4 production by hyphae-pulsed DCs allowed for a protective response to occur.

The success of this approach is based on two factors. (i) Pathogens can directly activate the DCs. (ii) A single DC after pathogen internalization, is able to competently present an array of T-cell pathogen-derived peptides covering the complete T-cell antigenic repertoire of the bacteria, thus inducing a protective, multi-specific immune response. This approach allows for use of hidden or unknown T-cell protective epitopes, hard to detect in screening tests. Furthermore, our results demonstrating that DCs loaded with inactivated *S. pneumoniae* are able to stimulate a polysaccharide-specific, as well as protein-specific, humoral responses in naive recipients [6] expand the potential prophylactic use of DCs, since protection against extracellular bacteria is typically mediated by capsular polysaccharide-specific antibody responses.

For most infectious diseases, the use of DCs pulsed with the whole pathogen, whether inactivated or attenuated, may not represent an ideal approach for human vaccination because of safety concerns. We thus need to determine which pathogen components are both safe and useful when used in combination with DCs. The consequent reduction of antigenic variety inherent in this latter approach might have unexpected pitfalls. For instance, adoptive transfer of DCs pulsed with inactivated *C. trachomatis* has proven successful in experimental models for promoting anti-*Chlamydial* immunity through stimulation of a Th1 response. However, a similar adoptive transfer of DCs pulsed with the recombinant chlamydial major outer membrane protein, which has been

regarded as a promising candidate for the development of conventional vaccines, did not induce protection, and this was associated with a polarized Th2 response [21]. One alternative is the use of DCs transfected ex vivo with DNA plasmids or RNA derived from the pathogen, in order to endogenously produce the microbial antigens after transfer. This strategy has been used with success in experimental tumors, and recently has shown promise in anti-infectious immunity. Thus, DCs transfected with RNA from yeast, but not hyphal, *C. albicans* induced a protective immune response similar to that observed upon transfer of DCs pulsed with the intact yeast [22].

Another approach is the use of DCs loaded with relevant immunogenic peptides in an attempt to increase the safety and reproducibility of the vaccine. Nevertheless, this approach may introduce more problems than it resolves. (i) Loading with peptides drastically restricts the repertoire of potential T-cell clones stimulated, and therefore, the ability to induce a protective response. (ii) It is difficult to envisage standardized mixes of peptides able to stimulate CD4, CD8 and regulatory T cells. DCs, instead, are specialized to produce these peptides from the whole pathogen. (iii) Efficient MHC class I and class II presentation occurs only when the peptides are generated within the DC itself (iv) Only in mature DCs are the cell surface MHC-peptide complexes stable; in immature DCs, they are constantly renewed. This approach would require the concurrent use of a DC activator if the DCs are immature, or alternatively, an already mature DC. The use of a fully matured DC immediately prior to transfer, could prove less effective since the in vivo half-life of the mature DC is likely less than the activated immature DC.

Vaccination of the general human population with ex vivo generated DCs, a procedure non-exempt of risk, costly and difficult to reproduce, does not appear to be a feasible first-line prophylactic approach, and probably will be restricted to instances in which conventional therapies for treating malignancy or chronic infection have failed, to focal vaccination or to improve immunity in immunodeficient patients (e.g. bone marrow transplant recipients). A more feasible approach is to efficiently deliver the antigen in vivo to the *endogenous* DC by cell-targeting. Several approaches currently being evaluated employ fusion proteins or chemical complexes of the immunogenic antigen or the viral vector, with a "sensor molecule" which bind surface receptors expressed on DCs, and in many cases, able to deliver an activation signal. The current sensor molecules tested include:

1. *Ligands of MHC and co-stimulatory molecules*, which are expressed mostly by APCs: many studies have demonstrated the ability of CD40-ligand and anti-CD40 antibodies to activate APCs which selectively express CD40, suggesting that these ligands would be effective carriers for antigen targeting to and subsequent presentation by DCs. Recently, a new approach has been tested using MHC class II, as target, and recombinant antibodies specific to MHC, containing genetically introduced class II-restricted T-cell epitopes, as sensors [23]. These antibodies, termed "troybodies", strongly enhanced epitope delivery to the APC for efficient T-cell stimulation, and thus could be a promising strategy for targeted peptide delivery. However, caution must be taken when targeting DCs through MHC, since DCs are very sensitive to the induction of apoptosis via surface MHC cross-linking, and the level of expression of MHC will vary with the activation and functional maturation stage of the DC.
2. *Antibodies and their fragments specific for endocytic receptors*: An analogous approach involved the use of fusion proteins of a peptide and one antibody specific to DEC-205 (CD205), an endocytic receptor and member of the mannose receptor family. DEC-205 expression is restricted to DCs within the T-cell areas of lymphoid tissues and particularly expressed by lymphoid DCs. It is well known that endocytosis of microbial antigens through DEC-205 binding results in efficient antigen-processing and -presentation to T cells. DCs targeted in vivo with the fusion antibodies for DEC-205 indeed, efficiently stimulated the proliferation of T cells specific for the peptide. Nevertheless, since the endocytosis of the fusion antibodies did not induce DC maturation, the treatment resulted in the induction of peptide-specific tolerance [24]. This study was among the first to demonstrate that DCs have a key role in the maintenance of peripheral tolerance, and that this is related to the state of activation/maturation of the DCs. These results stressed the importance of the use of concurrent DC activation signals or vaccine designs able to induce DC maturation in order to elicit protective immunity. Nevertheless, this approach could be used to induce antigen-specific tolerance for the treatment of autoimmune diseases.
3. *PAMPs*, (e.g. CpG oligonucleotides that signal via TLR9, or peptidoglycan and the outer membrane protein A of *Klebsiella pneumoniae* (OmpA), both utilizing TLR2 for signaling): the use of PAMPs as sensor molecules allows a further manipulation of the outcome of the immune response to induce protective immune responses. Since TLR molecules are differentially expressed on DC subsets, it is possible to selectively target functionally distinct DCs through the selection of the PAMP. Thus, TLR9 and TLR7 are selectively expressed by plasmacytoid human DCs and produce IFN-α after TLR9 binding with CpG motifs, whereas myeloid human DCs express all TLRs except TLR7 and TLR9 and produce high levels of IL-12 after TLR2 or TLR4-mediated activation with peptidoglycan or LPS, respectively [25]. Furthermore, OmpA and the antigens coupled to OmpA, are delivered into the MHC class I presentation pathway after DC uptake, through cross-presentation [26]. This ability to present exogenous antigens for presentation to $CD8^+$ T cells makes OmpA a promising carrier to develop CTL-inducer vaccines.

4. *Heat-shock proteins* (*Hsps*): Although some controversy exists, host Hsps appear to activate DCs via TLR2 and TLR4 in a similar manner as PAMPs. This property, in combination with the recognized ability of Hsp to bind peptides and direct them into the MHC class I presentation pathway, makes Hsp, an ideal sensor molecule for peptide-based vaccines against tumors and intracellular pathogens. Indeed, Hsps appear to play an important role in autologous resistance to tumors [27]. The use of Hsp has additional advantages which are reminiscent of those offered by the use of ex vivo pulsed DCs. Due to their promiscuity in peptide binding, the Hsp strategy is, in theory, applicable to a wide variety of antigens. The immunization with Hsp peptide complexes derived from infected cells is potentially directed against the whole antigenic repertoire and, therefore, the specific identification of the immunogenic epitopes should not be required. Thus, Hsp-peptide complex vaccines could be "blindly" prepared against a newly emergent pathogen or variant, once isolated, without further characterization of its antigenic or immunogenic profile. Furthermore, since hsp are self-antigens, they should not elicit immune responses to themselves. Bacterial Hsp proteins, such as mycobacterial hsp70, have similar properties as syngenic Hsp and could be exploited for possible novel properties. Thus, syngenic Hsp and peptide complexes induce restricted $CD8^+$ T-cell responses, but the complexes of mycobacterial hsp70 also elicited $CD4^+$ T-cell responses, and as fusion proteins with ovalbumin, induce specific antibody responses.
5. *Engineered bacteria and virus*: Due to the ability of the DC to specifically recognize and internalize pathogens, attenuated virus or bacteria could be used as carriers for specific antigens or genetic information. Interestingly, this approach was largely explored before our understanding that DCs would be a major target for these vaccines.

3.4. Reinforcing DC antigen-delivery and presentation

DCs process the microbial antigens obtained in the periphery, and present them to T cells in the secondary lymphoid organs. This involves the proteolysis of proteins into peptides of appropriate sizes for binding to MHC class II and class I molecules. To optimize T-cell priming during an ongoing infection, DCs must home from the periphery to the appropriate microenvironments within the secondary lymphoid organs. Therefore, another level of intervention could be to facilitate and direct DC homing.

3.4.1. Route of administration and migration

It is well known that the route of antigen administration can affect the quantity and quality of an immune response. This is due, in part, to the particular subset and migration pathways of the DC capturing the antigen (Fig. 1, point 4), and in part due to the particular lymphoid organ into which the stimulated DC migrates. Intradermally (i.d.) and subcutaneously (s.c.) injected DCs preferentially home to the T-cell areas of the draining lymph nodes, not to the spleen, whereas intravenously (i.v.) injected DCs home first to the lung, and then, preferentially to the spleen, and, to some extent, to the kidney and liver, but not to lymph nodes [28].

Intraperitoneal, and probably intralymphatic, injected DCs show an intermediate biodistribution. DCs injected i.v., as we observed in mice, may remain in the spleen for relatively longer periods of time (>5 d). The specific lymphoid site to which the DC homes could have a major impact on the particular cytokine polarization of the response, due to architectural and functional differences intrinsic to the different lymphoid organs (Fig. 1, point 6). Thus, the same preparation of DCs when injected i.v. are prone to induce non-polarized responses, whereas DCs injected i.d. or s.c. are prone to induce Th1 polarized responses.

The preferential homing of the DC to a particular lymphoid organ, based on the route of administration, could be used not only to affect cytokine polarization, but also to enhance the immune response to antigens of low immunogenicity, as well as thymus-independent (TI) antigens. It is well known that the spleen plays a major role in the induction of anti-capsular polysaccharide antibody responses which are critical for host defense against encapsulated bacteria. In this regard, we demonstrated that the i.v. injection of BMDCs pulsed ex vivo with *S. pneumoniae* induced antibody responses specific for the bacterial capsular polysaccharide and for the phosphorylcholine determinant of the cell-wall C-polysaccharide [6]. However, these polysaccharide-specific antibody responses were impaired when DCs were injected i.p. Recently, new data implicating myeloid BMDCs in the initiation of antibody responses specific for TI antigens has been obtained, with the identification of a subset of blood CD11c-low immature mouse DCs in vivo, likely counterparts of the ex vivo generated BMDCs, as being the primary cells capturing and transporting hematogenous bacteria to the spleen [29]. This CD11c-low DC provided critical signals to TI antigen-specific B cells resident in the splenic marginal zone, allowing them to survive, and promoting their differentiation into IgM-secreting plasmablasts. Thus, our observation that DCs can induce polysaccharide-specific Ig responses in vivo may open a new window for designing polysaccharide-based vaccines against encapsulated bacteria. DC homing will also depend on the subset and activation stage of the DCs, as they express different chemokine receptors and have different adhesion properties. Thus, alteration of these properties in antigen-pulsed ex vivo DCs may represent a further means of optimizing immune responses. Potential chemokine receptors of interest include (i) CCR7, which is necessary for lymphatic entry and migration to the T-cell areas of secondary lymphoid organ, (ii) CCR6, which may direct DC migration into the epithelial layer of the Peyer's patch or possibly the marginal zone of the spleen or, (iii) CXCR5, which can direct the migration of DCs into B-cell follicles. Thus, CXCR5-specific gene transduction of BM-

DCs led to their redirection to B-cell follicles, as opposed to T-cell areas of the draining lymph node after s.c. injection, with concurrent enhancement of the elicited antibody response [30]. We additionally observed a strong decrease in the ability of i.v. injected CXCR5$^{-/-}$ BMDCs to induce polysaccharide-specific antibody responses in vivo (unpublished data).

3.4.2. Exosomes

Ideal vaccines, as we comment, should be cell-free. In this regard, the question arises as to how we might utilize the essential properties of the DC without using the DC itself. One potential solution involves the use of exosomes. Exosomes are vesicles of endosomal origin that are secreted by DCs, and express high levels of functional MHC class I and class II-peptide complexes, co-stimulatory molecules, such as CD86, and chaperons, such as hsp, molecules that play key roles in elicitation of T-cell-dependent immune responses [31]. DC-secreted exosomes may serve as carriers of cytosolic and membrane proteins to distant cells, as well as participate in local interactions with lymphocytes (Fig. 1). Thus, exosomes containing loaded peptides can stimulate, by themselves, antigen-specific CD4^{+} T-cell and CTL responses both in vitro and in vivo.

To date, exosomes have been implicated just in the induction of T-cell priming and therefore, have been explored for their potential use in anti-cancer therapy. The first trials in human patients have been carried out with some very promising success seen in the regression of tumors at skin and lymph node sites [31].

4. Concluding remarks

Since the onset of the vaccination and antibiotic eras, many pathogens which were major health problems have either been eradicated or their impact on the population has been reduced to a minimum, at least in developed countries. Nevertheless, practical approaches for inducing immunity against many remaining human pathogens, both in developed and underdeveloped countries, are still urgently required. Due to their key position in connecting innate and adaptive immune responses, DCs represent a logical target for such interventions. Continued research on the complexities of DC biology, as well as experience obtained in clinical trials, will greatly help in this effort.

References

[1] J. Banchereau, F. Briere, C. Caux, J. Davoust, S. Lebecque, Y. Liu, B. Pulendran, K. Palucka, Immunobiology of dendritic cells, Annu. Rev. Immunol. 18 (2000) 767–811.

[2] P. Guermonprez, J. Valladeau, L. Zitvogel, C. Thery, S. Amigorena, Antigen presentation and T cell stimulation by dendritic cells, Annu. Rev. Immunol. 20 (2002) 621–667.

[3] M.R. Neutra, N.J. Mantis, A. Frey, P.J. Giannasca, The composition and function of M cell apical membranes: implications for microbial pathogenesis, Semin. Immunol. 11 (1999) 171–181.

[4] C. Winzler, P. Rovere, M. Rescigno, F. Granucci, G. Penna, L. Adorini, V.S. Zimmermann, J. Davoust, P. Ricciardi-Castagnoli, Maturation stages of mouse dendritic cells in growth factor-dependent long-term cultures, J. Exp. Med. 185 (1997) 317–328.

[5] S.J. Turley, K. Inaba, W.S. Garrett, M. Ebersold, J. Unternaehrer, R.M. Steinman, I. Mellman, Transport of peptide-MHC class II complexes in developing dendritic cells, Science 288 (2000) 522–527.

[6] J. Colino, Y. Shen, C.M. Snapper, Dendritic cells pulsed with Intact *Streptococcus pneumoniae* elicit both protein- and polysaccharide-specific immunoglobulin isotype responses in vivo through distinct mechanisms, J. Exp. Med. 195 (2002) 1–14.

[7] S. Stoll, J. Delon, T.M. Brotz, R.N. Germain, Dynamic imaging of T cell-dendritic cell interactions in lymph nodes, Science 296 (2002) 1873–1876.

[8] A. Lanzavecchia, F. Sallusto, Regulation of T cell immunity by dendritic cells, Cell 106 (2001) 263–266.

[9] F.O. Nestle, J. Banchereau, D. Hart, Dendritic cells: on the move from bench to bedside, Nat. Med. 7 (2001) 761–765.

[10] B. Pulendran, J.L. Smith, G. Caspary, K. Brasel, D. Pettit, E. Maraskovsky, C.R. Maliszewski, Distinct dendritic cell subsets differentially regulate the class of immune response in vivo, Proc. Natl. Acad. Sci. USA 96 (1999) 1036–1041.

[11] S.H. Gregory, A.J. Sagnimeni, N.B. Zurowski, A.W. Thomson, Flt3 ligand pretreatment promotes protective immunity to *Listeria monocytogenes*, Cytokine 13 (2001) 202–208.

[12] I.B. Kremer, M.P. Gould, K.D. Cooper, F.P. Heinzel, Pretreatment with recombinant Flt3 ligand partially protects against progressive cutaneous leishmaniasis in susceptible BALB/c mice, Infect. Immun. 69 (2001) 673–680.

[13] C. Wortham, L. Grinberg, D.C. Kaslow, D.E. Briles, L.S. McDaniel, A. Lees, M. Flora, C.M. Snapper, J.J. Mond, Enhanced protective antibody responses to PspA after intranasal or subcutaneous injections of PspA genetically fused to granulocyte-macrophage colony-stimulating factor or interleukin-2, Infect. Immun. 66 (1998) 1513–1520.

[14] J. Wang, A. Zganiacz, Z. Xing, Enhanced immunogenicity of BCG vaccine by using a viral-based GM-CSF transgene adjuvant formulation, Vaccine 20 (2002) 2887–2898.

[15] E. Williamson, G.M. Westrich, J.L. Viney, Modulating dendritic cells to optimize mucosal immunization protocols, J. Immunol. 163 (1999) 3668–3675.

[16] A. Le Bon, G. Schiavoni, G. D'Agostino, I. Gresser, F. Belardelli, D.F. Tough, Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo, Immunity 14 (2001) 461–470.

[17] S. Yamamoto, H. Kiyono, M. Yamamoto, K. Imaoka, K. Fujihashi, F.W. Van Ginkel, M. Noda, Y. Takeda, J.R. McGhee, A nontoxic mutant of cholera toxin elicits Th2-type responses for enhanced mucosal immunity, Proc. Natl. Acad. Sci. USA 94 (1997) 5267–5272.

[18] M. Merad, T. Sugie, E.G. Engleman, L. Fong, In vivo manipulation of dendritic cells to induce therapeutic immunity, Blood 99 (2002) 1676–1682.

[19] C. Demangel, W.J. Britton, Interaction of dendritic cells with mycobacteria: where the action starts, Immunol. Cell Biol. 78 (2000) 318–324.

[20] M.L. Mbow, N. Zeidner, N. Panella, R.G. Titus, J. Piesman, *Borrelia burgdorferi*-pulsed dendritic cells induce a protective immune response against tick-transmitted spirochetes, Infect. Immun. 65 (1997) 3386–3390.

[21] J. Shaw, V. Grund, L. Durling, D. Crane, H.D. Caldwell, Dendritic cells pulsed with a recombinant chlamydial major outer membrane protein antigen elicit a CD4(+) type 2 rather than type 1 immune response that is not protective, Infect. Immun. 70 (2002) 1097–1105.
[22] A. Bacci, C. Montagnoli, K. Perruccio, S. Bozza, R. Gaziano, L. Pitzurra, A. Velardi, C.F. d'Ostiani, J.E. Cutler, L. Romani, Dendritic cells pulsed with fungal RNA induce protective immunity to *Candida albicans* in hematopoietic transplantation, J. Immunol. 168 (2002) 2904–2913.
[23] E. Lunde, K.H. Western, I.B. Rasmussen, I. Sandlie, B. Bogen, Efficient delivery of T cell epitopes to APC by use of MHC class II-specific Troybodies, J. Immunol. 168 (2002) 2154–2162.
[24] D. Hawiger, K. Inaba, Y. Dorsett, M. Guo, K. Mahnke, M. Rivera, J.V. Ravetch, R.M. Steinman, M.C. Nussenzweig, Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo, J. Exp. Med. 194 (2001) 769–779.
[25] D. Jarrossay, G. Napolitani, M. Colonna, F. Sallusto, A. Lanzavecchia, Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells, Eur. J. Immunol. 31 (2001) 3388–3393.
[26] P. Jeannin, T. Renno, L. Goetsch, I. Miconnet, J.P. Aubry, Y. Delneste, N. Herbault, T. Baussant, G. Magistrelli, C. Soulas, P. Romero, J.C. Cerottini, J.Y. Bonnefoy, OmpA targets dendritic cells, induces their maturation and delivers antigen into the MHC class I presentation pathway, Nat. Immunol. 1 (2000) 502–509.
[27] P.K. Srivastava, R.J. Amato, Heat shock proteins: the 'Swiss Army Knife' vaccines against cancers and infectious agents, Vaccine 19 (2001) 2590–2597.
[28] A.A. Eggert, M.W. Schreurs, O.C. Boerman, W.J. Oyen, A.J. de Boer, C.J. Punt, C.G. Figdor, G.J. Adema, Biodistribution and vaccine efficiency of murine dendritic cells are dependent on the route of administration, Cancer Res. 59 (1999) 3340–3345.
[29] M. Balazs, F. Martin, T. Zhou, J. Kearney, Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses, Immunity 17 (2002) 341–352.
[30] M.T. Wu, S.T. Hwang, CXCR5-transduced bone marrow-derived dendritic cells traffic to B-cell zones of lymph nodes and modify antigen-specific immune responses, J. Immunol. 168 (2002) 5096–5102.
[31] C. Thery, L. Zitvogel, S. Amigorena, Exosomes: composition, biogenesis and function, Nat. Rev. Immunol. 2 (2002) 569–579.