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DENDRITIC CELL-BASED VACCINATION AGAINST OPPORTUNISTIC FUNGI*

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SUMMARY

Efficient responses to the different forms of fungi require different mechanisms of immunity. Dendritic cells (DCs) are uniquely able to decode the fungus-associated information and translate it in qualitatively different T helper (Th) immune responses, *in vitro* and *in vivo*. DCs sense fungi in a morphotype-specific manner, through the engagement of distinct recognition receptors ultimately affecting cytokine production and co-stimulation. Adoptive transfer of different types of DCs activates protective and non-protective Th cells as well as regulatory T cells and affects the outcome of the infections. DCs transfected with fungal RNA also restore antifungal resistance in haematopoietic transplantation. Thus, the remarkable functional plasticity of DCs in response to fungi can be exploited for the deliberate targeting of cells and pathways of cell-mediated immunity in response to fungal vaccines.

INTRODUCTION

Infections caused by systemic fungal pathogens are a significant health problem in immunocompetent and immunocompromised host. Opportunistic fungal pathogens, which more typically require immunosuppression to infect the host, include *Candida albicans*, which is a normal inhabitant of the human gut, and *Aspergillus fumigatus*, which is ubiquitous in the environment. As a pathogen *C. albicans* is associated with a wide spectrum of diseases in humans, ranging from allergy, severe intractable muco-cutaneous diseases to life-threatening bloodstream infections (*Calderone*, 2002). Aspergilli are respiratory pathogens, and pulmonary infections are usually acquired through the inhalation of conidia able to reach small airways and the alveolar space, where the impaired host defence mechanisms allow hyphal germination and subsequent tissue invasion. *A. fumigatus* is associated with a wide spectrum of diseases ranging from benign colonisation of the lung and allergy to life-threatening diseases such as invasive pulmonary aspergillosis or allergic broncho-pulmonary aspergillosis (*Latgé*, 2001). The delicate balance between the host and these otherwise harmless fungi may turn into a parasitic relationship, resulting in the

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development of severe infections. However, fungi are not mere passive participants in the infectious process and a hypothetical set of virulence factors has been attributed to them (*Denning*, 2000; *Rooney* and *Klein*, 2002). Among these, the ability to form hyphae from budding yeasts or from swelling conidia and the subsequent filamentous growth are thought to be important for virulence (*Hogan* et al., 1996).

Host defence mechanisms against fungi are numerous and range from relatively primitive and constitutively expressed non-specific defences to sophisticated adaptive mechanisms that are specifically induced during infection (*Romani* and *Kaufmann*, 1998). Although the role of innate immunity was originally considered to be a process for defence of the host early in infection, it is now clear that there is an important reciprocal relationship between innate and adaptive immune responses. Through the involvement of a set of germline-encoded pattern recognition receptors (PRRs) and Toll-like receptors (TLRs) that recognise and are triggered by evolutionarily conserved molecules essential to pathogen function (PAMPs, pathogen -associated molecular patterns), cells of the innate immune system not only discriminate between different pathogens, but also contribute to discrimination between self and pathogens at the level of the adaptive T helper (Th) immunity (*Medzhitov* and *Janeway*, 1997; *Schnare* et al., 2001). Cytokines and other mediators play an essential role in the process and, indeed, may ultimately determine the type of effector response that is generated towards the pathogens (*Romani*, 1996). The recognition of fungi at sites of infection leads to the production of chemokines and cytokines that not only activate the innate cell population but also drive the adaptive immune response down different pathways of differentiation. As the different Th cell subsets are endowed with the ability to release a distinct panel of cytokines, capable of delivering the activating and deactivating feedback signals to effector phagocytes, the activation of an appropriate Th subset may be instrumental in the generation of a successful immune response to the fungal pathogens (*Puccetti* et al., 1995; *Romani*, 1997). To limit the pathologic consequences of excessive inflammatory cell-mediated immune reactions, the immune system resorts to a number of protective mechanisms including the reciprocal cross-regulatory effects of Th1 and Th2-type effector cytokines, such as interferon (IFN)-γ and interleukin (IL)-4, and the generation of regulatory T cells (Treg). Thus, innate and adaptive immune responses are intimately linked and controlled by sets of molecules and receptors that act to generate the most effective form of immunity for protection against fungal pathogens.

It has became apparent that understanding how immune responses are activated will enable the construction of better vaccines and vaccine strategies that are effective at eliciting acquired protective immunity to pathogens. The model has brought DCs to centre stage as promising targets for intervention for immunotherapy and vaccine development (*Steinman* and *Pope*, 2002) and has shifted the emphasis from the "antigen" towards the "adjuvant" (*Gallucci* et al., 1999). Thus, the promise of a fungal vaccine will demand for an adjuvant capable of both stimulating the appropriate type of response best tailored to combating the infection and being effective in conditions of immunosuppression.

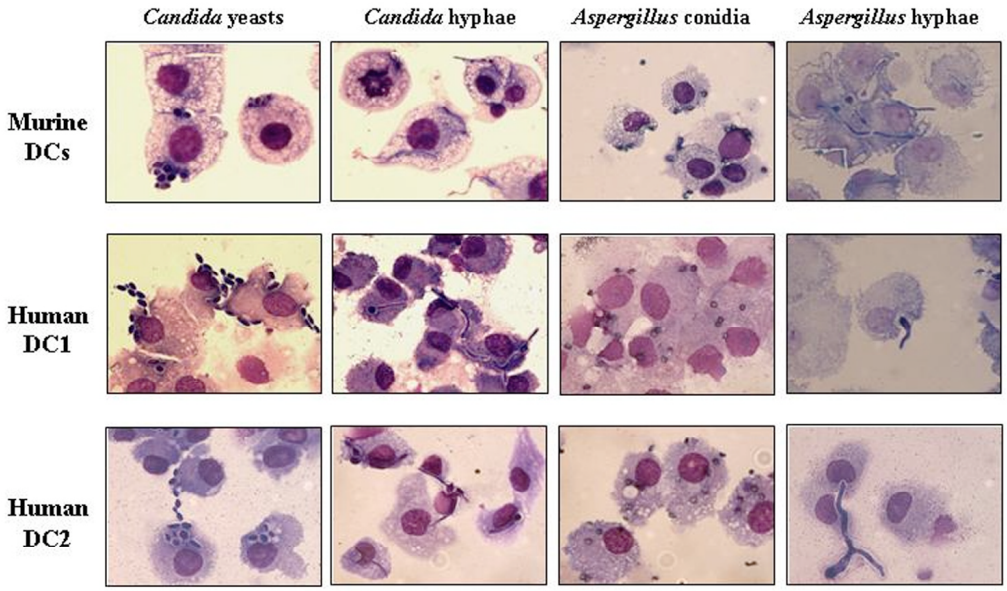

Figure 1: Dendritic cells internalise yeasts and hyphae of *Candida albicans* and conidia and hyphae of *Aspergillus fumigatus*. Murine DC1 were purified from spleens and immature human DC1 and DC2 from $CD11c^+$ blood mononuclear cells, as described (*Bozza* et al., 2003). DCs were exposed to un-opsonised yeasts for 30 min or un-opsonised conidia and hyphae for 60 min before the assessment of phagocytosis, as described (*Bozza* et al., 2003). After a Diff Quik staining, aliquots of cells were spun down on slides on a cytocentrifuge and mounted in buffered glycerol to be examined for conidia internalisation by light microscopy. For each experiment, at least 5 fields in each slide were counted, and at least 200 DCs were analyzed in each well. All conditions were tested in triplicates.

DCs AS NATURAL ADJUVANTS

Since their original discovery in 1973, DCs have assumed centre stage as the key initiator of adaptive immunity (*Lanzavecchia* and *Sallusto*, 2001). In infections, they are central in the balancing act between immunopathology and protective immunity generated by host-microbe interactions. DCs are strategically located at the interface of potential pathogen entry sites and take up antigen, move into secondary lymphoid tissues and activate both helper and cytotoxic T cells. Pathogen-mediated activation induces DCs to undergo maturation consisting in antigen acquisition down-regulation, increased expression of the Major Histocompatibility Antigen Complex (MHC) and co-stimulatory molecules, IL-12 production, and altered expression of chemokine receptors (*Lanzavecchia* and *Sallusto*, 2001). As they mature, DCs migrate to the T cell areas of lymphoid organs, where they translate the tissue-derived information into the language of Th cells, providing them with an antigen-specific "signal 1", a co-stimulatory "signal 2" and a "signal 3" which determines the polarisation of naive Th cells into Th1 or Th2 cells. In addition to DCs initiating immunity, certain subpopulations of DCs are able to down-regulate immune responses (*Shortman* and *Heath*, 2001). The ability of DCs to influence the pattern of cytokine secreted by T cells represents a critical

function, which can profoundly influence the final outcome of the immune response to pathogens. Several factors appear to influence the ability of DCs to polarise T-cell cytokine responses, including the DC subsets, the nature of the maturation stimuli and the host microenvironment (*Shortman* and *Liu*, 2002). At the end, DCs represent the critical link between innate and adaptive immunity, upon which, appropriate concerted action is required for a successful host defence against an invading pathogen. Progress in our understanding of DC biology and their critical function in immunity have prompted investigations to explore their potential use in immunotherapy and prophylaxis.

INTERACTIONS BETWEEN FUNGI AND DCs

In vitro

Efficient responses to the different forms of fungi require different mechanisms of immunity (*Romani*, 1997; *Romani* and *Kaufmann*, 1998). DCs showed a remarkable functional plasticity in response to the different forms of fungi, being able to discriminate between the different forms in terms of maturation, cytokine production and induction of Th cell reactivity, *in vitro* and *in vivo* (*Fè d'Ostiani* et al., 2000; *Huang* et al., 2001; *Bacci* et al., 2002; *Bozza* et al., 2002a,2003; *Claudia* et al., 2002; *Garlanda* et al., 2002). Both murine and human DCs were able to phagocytose *Candida* yeasts, *Aspergillus* conidia, and hyphae from both (Figure 1). The uptake of the different fungal elements occurred through different forms of phagocytosis. Transmission electronic microscopy indicated that internalisation of yeasts and conidia occurred predominantly by coiling phagocytosis, characterised by the presence of overlapping bilateral pseudopods that led to a pseudopodal stack before transforming into a phagosome wall. In contrast, entry of hyphae occurred by a more conventional zipper-type phagocytosis, characterised by the presence of symmetrical pseudopods which strictly followed the contour of the hyphae before fusion. However, the fate of the different forms of the fungi inside cells appeared to be quite different. Two and four hours later, numerous yeast cells were found partially degraded inside phagosomes. In contrast, as early as one hour after infection, *Candida* hyphae appeared to escape the phagosome and were found lying free in the cytoplasm of cells. For *Aspergillus*, two hours after the exposure, numerous conidia were found inside DCs with no evidence of conidia destruction, as opposed to hyphae, that were rapidly degraded once inside cells. As killing of conidia would seem to be a necessary prerequisite to obtain efficient antigen presentation, it can be postulated that either a small number of conidia are actually degraded by mature DCs thus allowing their antigen processing and presentation or, alternatively, antigens could be processed and regurgitated by other infected phagocytes and then transferred to DCs for presentation.

Multiple receptors on phagocytes and DCs participate in the microbial recognition event either independently or through receptor cooperativity (*Mosser* and *Karp*, 1999). Receptors that have been identified on immature DCs include PRRs, lectins such as the mannose receptors (MR), DEC-205 and DC-SIGN as well as Fc receptors (FcεRI and FcγR) and receptors for a number of components of the complement system (CR). Work on innate recognition of pathogens has defined a number of PAMPs and their cognate

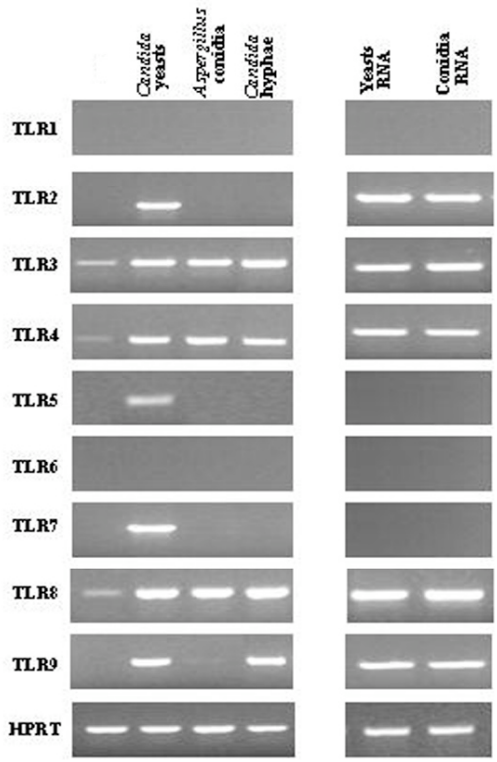

Figure 2: Fungi activate Toll-like receptor expression on dendritic cells. Murine CD11c$^+$ DCs were purified from spleens (for *Candida*) or from lungs (for *Aspergillus*) and exposed to un-opsonised *Candida* yeasts, *Candida* hyphae and *Aspergillus* conidia or fungal RNA, for 60 or 120 min, respectively, as described (*Bacci* et al., 2002; *Bozza* et al., 2003). TLR expression was assessed by RT-PCR. cDNA levels were normalised against the HPRT gene. None, cells exposed to the diluent alone.

PRRs on phagocytes (*Medzhitov* and *Janeway*, 1997; *Romani*, 1996). For fungi, PAMPs include cell-wall components such as glucans, mannans, mannoproteins and phospholipomannan (*Calderone*, 2002) capable of mediating phagocytosis and activation of pro-inflammatory pathways upon recognition by MR and β-glucan receptors, mannose-binding lectins (MBL) and CR3 through the involvement of TLRs 2 and 4 (*Ezekowitz* et al., 1990; *Brown* et al., 2003; *Cambi* et al., 2003; *Wang* et al., 2001; *Mambula* et al., 2002; *Netea* et al., 2002).

Internalisation of yeasts, conidia or hyphae involved different receptors (*Claudia* et al., 2002; *Romani* et al., 2002). Live un-opsonised yeasts, conidia or hyphae were mainly internalised through a phagocytic process. Internalisation of yeasts and conidia occurred through the lectin-like receptors, including MR, DC-SIGN and dectin-1. For hyphae, the internalisation by DCs mainly occurred through CR3 and FcgR II and III. The results are consistent with the view that fungi have exploited common pathways for entry into DCs, which may include a lectin-like pathway for unicellular forms and opsono-dependent pathways for filamentous forms. In terms of sugar specificity, this may vary among fungi, as DCs recognise *Candida* yeasts through a mannose-fucose receptor (*Newman* and

Holly, 2001) and *Aspergillus* conidia through a lectin receptor of galactomannan specificity (*Bozza* et al., 2002a). It also appears that unicellular fungal forms may exploit the CR3 receptor on DCs as a niche to avoid degradation through the multi-lectin pathway while allowing their own persistence. In doing so, fungi share with pathogenic bacteria the ability to avert activation of phagocytes by entry through complement receptors that are not accompanied by phagocyte activation (*Ehlers* and *Daffè*, 1998). Interestingly, the entry of heat-inactivated fungi may occur through different pathways, as inactivated *Candida* yeasts were mainly internalised through CR3 (*Claudia* et al., 2002), a finding that may have important implications in terms of vaccination strategies against fungi.

TLR2 and 4 have been implicated in the activation of phagocytes by fungi (*Wang* et al., ,2001; *Mambula* et al., 2002; *Netea* et al., 2002). It is believed that microbial detection by DCs trough TLRs is responsible for pathogen discrimination and the initiation of the appropriate effector response accordingly (*Schnare* et al., 2001). Distinct patterns of TLR expression were observed on splenic and pulmonary DCs upon exposure to *Candida* and *Aspergillus*, respectively. Both yeasts and conidia up-regulated the expression of TLR3, TLR4 and TLR8, but only yeasts up-regulated the expression of TLR2, TLR5, TLR7 and TLR9. The exposure to *Candida* hyphae was followed by the up-regulated expression of TLR3, TLR4, TLR8 and TLR9 (Figure 2). Similar results were obtained upon exposure to *Aspergillus* hyphae (data not shown). The extent to which TLR expression on DCs implicates the functional activity of TLRs in response to fungi is far from being understood. Nevertheless, it is intriguing that the TLR9 agonist CpG-ODN could convert an *Aspergillus* allergen to a potential protective antigen, suggesting the potential for TLR agonists to act upon the degree of flexibility of the immune recognition pathways to antigens and allergens (*Bozza* et al., 2002b).

It has recently been shown that fungal RNA acts as potent DC activator (*Bacci* et al., 2002; *Claudia* et al., 2002; *Bozza* et al., 2003). Others have shown that pulsing DCs with antigen-encoded mRNA resulted in the loading of both MHC class I and II antigen presentation pathways and the delivery of an activation signal (*Ni* et al., 2002). Although extracellular mRNA induced DC activation by signalling through a nucleotide receptor (*Ni* et al., 2002), fungal RNA also activated TLR expression on DCs (Figure 2). The expression of TLR2, TLR3, TLR4, TLR8 and TLR9 was upregulated upon exposure to fungal RNA from both yeasts and conidia. As DCs efficiently took up extracellular fungal RNA (Figure 3), this indicates that DCs are allowed to orchestrate the immune response against both intracellular and extracellular fungi.

Upon exposure to fungi or fungal RNA, DCs underwent functional maturation, as indicated by the upregulated expression of co-stimulatory molecules and MHC class II antigens and cytokine production (*Bacci* et al., 2002; *Claudia* et al., 2002; *Bozza* et al., 2003). The production of cytokines occurred differently in response to un-opsonised yeasts, conidia and hyphae and the pattern of cytokine production correlated with the pattern of receptor entry and/or the levels of opsonisation. Upon phagocytosis of yeasts or conidia, high and sustained levels of IL-12 were observed. However, DCs produced IL-4 and IL-10 in response to hyphae. It was also found that the receptor exploitation on DCs and fungal opsonisation dramatically affected the pattern of cytokine production.

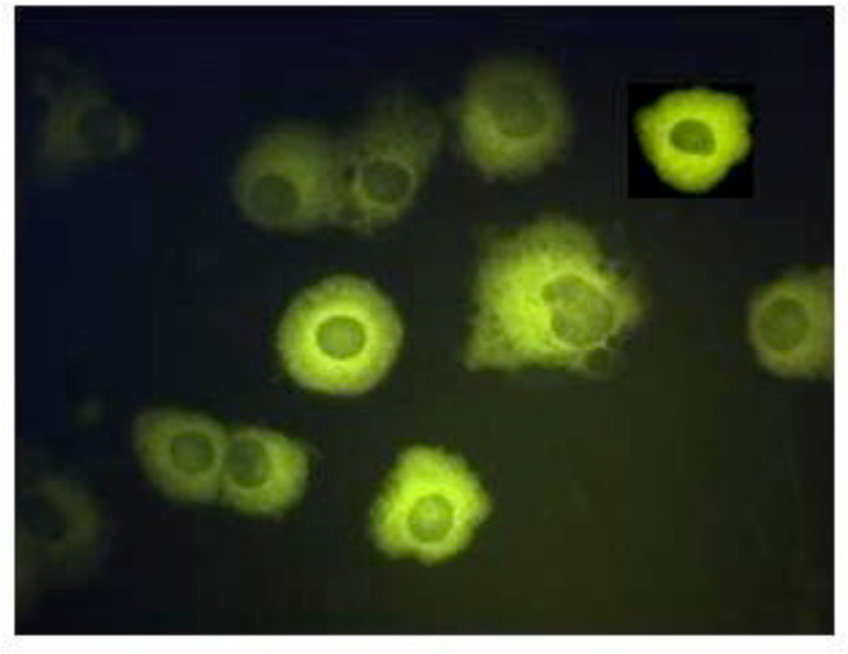

Figure 3: Uptake of fungal RNA by dendritic cells. Fluorescent probe syto17 was added to fungal total RNA (25μg) at the concentration 100μM and incubated for 2 h in dark. After removal of unbound dye, cells were transfected with labelled RNA and the cationic lipid N-[1-(2,3-dioleoyloxypropyl]-*N,N,N*,-trimethylammonium methylsulfate (DOTAP) for 120 min at 37°C (*Bacci* et al., 2002; *Bozza* et al., 2003). Cells were washed with PBS and fixed with paraformaldehyde 4% for 10 min. Photographs were taken using a high Resolution Microscopy Colour Camera AxioCam Colour, using the AuxioVision Software Rel. 3.0.

In vivo

DCs have a primary role in pathogens surveillance at the mucosal surfaces (*Huang* et al., 2001). Studies *in vivo* suggested that DCs had the ability to internalise *Aspergillus* conidia and *Candida* yeasts at the sites of the infection (*Bozza* et al., 2002a; *Montagnoli* et al., 2003). Soon after the infection, *Candida* yeasts were found inside DCs from the gut and *Aspergillus* conidia inside pulmonary DCs. In the case of *Candida*, the fungus appeared to translocate across the epithelial layers and to be subsequently phagocytosed by DCs (unpublished observations). In the case of *Aspergillus*, it should be considered that, in normal circumstances, a state of tolerance to inhaled antigens is achieved through several mechanisms, including IL-10 production by local DCs (*Akbari* et al., 2001). It is known that DCs of the respiratory tract are specialised for uptake/processing but not for antigen presentation, the latter requiring cytokine maturation signals that are encountered after migration to regional lymph nodes. We found that DCs present in the alveolar spaces phagocytosed conidia, translocated to the space below, within the alveolar septal wall, and reached the draining lymph nodes where fungus-pulsed DCs instructed local development of antifungal Th reactivity (see below).

DCs TRANSLATE FUNGUS-ASSOCIATED INFORMATION TO Th LYMPHOCYTES

Upon exposure to *Candida* or *Aspergillus*, DCs activated different types of naive $CD4^+$ Th cells *in vitro* and *in vivo* (*Fè d'Ostiani* et al., 2000; *Bacci* et al., 2002; *Bozza* et al., 2002a,2003; *Claudia* et al., 2002; *Romani* et al., 2002; *Garlanda* et al., 2002). *In vitro*, $CD4^+$ T splenocytes co-cultured with yeast-pulsed DCs produced high levels of IFN-γ, but not IL-4 or IL-10. In contrast, DCs exposed to *Candida* hyphae induced low levels of IFN-γ, but high levels of IL-4 and IL-10. *Candida*-pulsed DCs were also capable of priming antigen-specific $CD4^+$ Th responses *in vivo*. Adoptive transfer of purified DCs, pulsed with yeasts or hyphae, resulted in priming of $CD4^+$ T cells for Th1 or Th2 cytokine production, respectively (see below). *In vivo* studies confirmed that the opsonic phagocytosis of fungi is responsible for type 2 cytokine production and Th2 cell activation, an effect counteracted by the Th1-promoting activity of the non-opsonic entry through MR (*Claudia* et al., 2002). In the case of *Aspergillus*, the migration and maturation of pulmonary DCs in mice with aspergillosis correlated with their ability to induce T cell priming in the lymph nodes and spleens. The number of IFN-γ-producing $CD4^+$ T cells greatly increased in both the lymph nodes and spleens of mice injected with *Aspergillus* conidia, while IL-4-producing cells were increased in mice exposed to hyphae (*Romani* et al., 2002).

There is compelling evidence that Treg specialised in the attenuation of immune responses play a critical role in immune regulation (*Read* and *Powrie*, 2001). Immune responses driven by Th1 and Th2 cells are also influenced by Treg whose main function is counter-regulation or suppression of immune responses mediated by Th1 and Th2. Different types of Treg have been found to be implicated in the control of organ-specific autoimmunity, transplantation tolerance and inflammatory responses evoked by enteric organisms. Pathogen-specific Treg, with immunosuppressive activity, have also been described (*McGuirk* et al., 2002). Although protective immunity to *C. albicans* is mediated by antigen-specific Th1 cells, paradoxically, some Th2 cytokines are required for the maintenance of the antifungal immune resistance (*Romani*, 1997). Therefore, in addition to the Th1/Th2 balance, other mechanisms seem to be involved in the regulation of Th1 immunity to the fungus. A role for DCs in the induction of Treg has been described (*Roncarolo* et al., 2001). DCs from Payer's patches induced the activation of $CD4^+CD25^+$ T cells negatively regulating antifungal Th1 reactivity in mice with gastrointestinal candidiasis (*Montagnoli* et al., 2002). Activation of Treg required DCs expressing co-stimulatory molecules and producing IL-10, the last activity being strictly dependent on local levels of opsonising antibodies (*Montagnoli* eet al., 2003). Adoptive transfer of IL-10-producing *Candida*-pulsed DCs induced the activation of $CD4^+CD25^+$ T cells in the mesenteric lymph nodes, decreased the inflammatory response at sites of infection and contributed to the occurrence of memory protective immunity to the fungus. As hyphae, more than yeasts, are endowed with the ability to activate IL-10-producing DCs, at least *in vitro* (*Montagnoli* et al., 2002), it appears that DCs orchestrate the overall immune response to *C. albicans*, including active priming to the yeasts and tolerance to the hyphae. Whether these apparently contradictory roles could be attributed to distinct DC lineages or to a

single DC type, which are instructed by environmental stimuli to perform different functions is still a matter of debate (*Shortman* and *Heath*, 2001). Nevertheless, our data point out an extreme functional plasticity of DCs in response to the different forms of fungi.

All together, these data indicate that DCs fulfil the requirement of a cell uniquely capable of discriminating between the different forms of fungi in terms of the type of immune response elicited. The emerging paradigm calls for the exploitation of distinct receptors on DCs by the different forms of unopsonised or opsonised fungi and the dependency of the DC activation program and ensuing Th cell response on the receptor choice and mode of entry. Indeed, i) the non-opsonic phagocytosis through MR results in the production of pro-inflammatory cytokines, including IL-12, and expression of co-stimulatory molecules and MHC class II antigens; ii) up-regulation of co-stimulatory molecules also occurs along with the production of IL-4/IL-10 upon the opsonic entry through CR3 and FcgR; iii) both the expression of co-stimulatory molecules and class II antigens and the production of IL-12 are inhibited by entry through CR3. *In vivo* studies confirmed that the opsonic phagocytosis of fungi is responsible for type 2 cytokine production and Th2 cell activation, an effect counteracted by the Th1-promoting activity of the non-opsonic entry through MR. It is conceivable that the balance between the two types of phagocytosis at different body sites very likely determines the type of immune response elicited, which may help to explain the longstanding notion of compartmentalisation in antifungal Th immunity (*Romani* and *Kaufmann*, 1998). The results are also in line with evidences in humans showing an increased susceptibility to fungal infection in patients with defective MBL but not antibody or complement deficiency (*Calderone*, 2002; *Latgé*, 2001).

EXPLOITING DCs AS FUNGAL VACCINES

Fungus-pulsed DCs activated $CD4^+$ Th cell responses upon adoptive transfer into immunocompetent mice (*Bacci* et al., 2002; *Bozza* et al., 2002a,2003). The analysis of antigen specific proliferation and cytokine production by $CD4^+$ T cells from draining lymph nodes and spleens revealed that levels of IFN-γ were higher, and those of IL-4 lower, in mice immunised with yeast- or conidia-pulsed DCs as compared to mice receiving unpulsed or hypha-pulsed DCs. The ability of fungus-pulsed DCs to prime for Th1 and Th2 cell activation upon adoptive transfer *in vivo* correlated with the occurrence of resistance and susceptibility to the infections. Resistance to either *C. albicans* or *A. fumigatus* infection was greatly increased upon transfer of yeast-pulsed or conidia-pulsed DCs, respectively, as indicated by the decreased fungal burden in the target organs. The fungal burden was not reduced upon transfer of unpulsed or hypha-pulsed DCs, being actually increased upon transfer of the latter. Therefore, adoptively transferred fungus-pulsed DCs are able to prime specific antifungal Th responses *in vivo*, the quality of which depends on forms of the fungus and nature of cytokines. Indeed, the ability to induce anti-candidal protective Th1 immunity *in vivo* was impaired upon transfer of DCs exposed to the yeasts in the absence of IL-12, and potentiated upon transfer of DCs exposed to the hyphae in the absence of IL-4 (*Fè d'Ostiani* et al., 2000). These

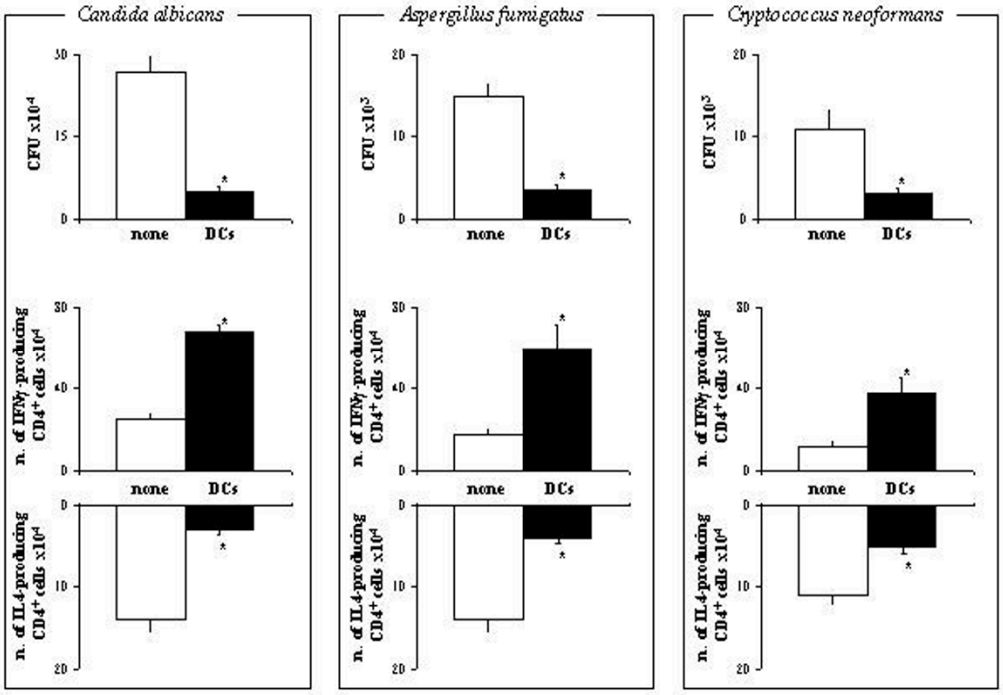

Figure 4: Adoptively transferred fungus RNA-transfected-dendritic cells induce Th1-mediated resistance to fungal infections. Splenic DCs were transfected with RNA from *Candida* or *Cryptococcus* yeasts, or *Aspergillus* conidia as described (*Bacci* et al., 2002; *Bozza* et al., 2003). DCs (5 x 10^5) were administered into recipient mice subcutaneously, 2 and 1 week before the intravenous injection of 5 x 10^5 *C. albicans* or *A. fumigatus* conidia or the intranasal injection of 10^4 *C. neoformans*. Resistance to infection was assessed in terms of colony forming units (CFU, mean ± SE) and number of cytokine producing $CD4^+$ T cells (ELISPOT assay) a week after the infection in the kidneys (candidiasis and aspergillosis) or in the lungs (cryptococcosis) (*Bacci* et al., 2002; *Bozza* et al., 2003). *Indicates $p<0.05$ (mice receiving pulsed DCs versus mice not receiving DCs).

results suggest that production of IL-12 or IL-4 by DCs may crucially contribute to the induction of protective and non-protective immune responses in fungal infection. Interestingly, inactivated yeasts failed to induce DCs maturation *in vitro* and DCs pulsed with inactivated yeasts failed to promote Th1 immunity upon adoptive transfer *in vivo* (data not shown). Therefore, these data may account for the long-standing observation of the inability of inactivated *Candida* to induce memory anti-candidal protective immune responses (*Romani* and *Kaufmann*, 1998).

Antifungal protective immunity *in vivo* was also observed upon adoptive transfer of DCs transfected with fungal RNA (*Bacci* et al., 2002; *Bozza* et al., 2003). The efficacy was restricted to DCs transfected with RNA from yeasts or conidia but not with hyphal RNA. *Ex vivo* DCs, transfected with yeast RNA or conidial RNA, adoptively transferred into otherwise susceptible recipients, conferred protection against *C. albicans* or *A. fumigatus* infection, respectively (Figure 4). The effect was fungus-specific, as no cross-protection was observed upon adoptive transfer of DCs

pulsed with either fungal species (*Bozza* et al., 2003). It is of interest that DCs transfected with RNA from *Cryptococcus neoformans*, an opportunistic fungus on occasion, also induced protection in a murine model of pulmonary cryptococcosis (Figure 4), a finding expanding upon the vaccinating potential of DCs in fungal infections. The frequency of IFN-γ-producing Th1 cells was increased and that of IL-4-producing cells decreased in protected mice (Figure 4), a finding suggesting the occurrence of a Th1-dependent antifungal resistance.

The infusion of fungus-pulsed or RNA-transfected DCs accelerated the recovery of functional antifungal Th1 responses in mice with allogeneic haematopoietic stem cell transplantation (HSCT), an experimental model in which autologous reconstitution of host stem cells is greatly reduced to the benefit of a long-term, donor type chimerism in more than 95% of the mice and low incidence of graft-versus-host disease (*Mencacci* ete al., 2001). Patients receiving T cell-depleted HSCT are unable to develop antigen-specific T cell responses soon after transplantation (*Velardi* et al., 1988). However, functional recovery of the T cell system after T cell-depleted allogeneic HSCT has been demonstrated (*Verfuerth* et al., 2000) and both donor and recipient DCs may participate to the reconstitution of the T cell repertoire in transplantation through distinct pathways of antigen presentation (*Lechler* et al., 2001). We have demonstrated that an imbalanced production of Th1 and Th2 cytokines was responsible for the susceptibility to fungal infections in our HSCT model. However, readdressing the balance between Th1 and Th2 subsets, as by treatment with Th2 cytokine antagonists, accelerated the recovery of Th1-mediated antifungal resistance (*Mencacci* et al., 2001). The recovery of functional Th1 cells producing IFN-γ was accelerated by the infusion of fungus-pulsed or RNA-transfected DCs, a finding suggesting that DCs may contribute to the educational program of T cells in HSCT during reconstitution, as already suggested (*Lechler* et al., 2001).

All together, our studies will suggest that DCs could act as effective vaccines against fungal infections and that RNA-transfected DCs could be of vaccinating potential in conditions that negate the use of attenuated microorganisms, such as immunosuppression, or in the case of poor availability of protective antigens.

CONCLUSIONS AND PERSPECTIVES

DCs have a unique role in infections, as they are regarded as both sentinel for innate recognition and initiator of Th cell differentiation and functional commitment. Through the use of distinct recognition receptors, murine DCs showed a remarkable functional plasticity in the recognition of fungi. It appears that the DC/fungi interaction dynamics, more than fungal dimorphism, could be responsible for fungal virulence. The implications of these findings are manifold. First, as cytokines are known to modulate the expression of opsonic and non-opsonic receptors (*Raveh* et al., 1998), and antibodies differently opsonise fungi (*Casadevall*, 1995), it is likely that the levels of cytokines may influence the DC/fungi interaction *in vivo* and that the different ability of antibodies to opsonise fungi may contribute to the protective and non-protective activity of antibodies in fungal infections (*Casadevall*, 1995). Second, as clinical

resistance represents a significant component of the overall drug resistance of the anti-fungals (*Alexander* and *Perfect*, 1997), one major strategy to prevent antifungal drug resistance is to improve the immune functions of the immunocompromised host. A variety of cytokines, including chemokines and growth factors proved to be beneficial in experimental and clinical fungal infections (*Romani* and *Kaufmann*, 1998). However, establishing the clinical utility of cytokines as therapy for fungal infections in patients has been difficult. The Th1/Th2 balance itself was also found to be the target of immunotherapy. Thus, the deliberate targeting of cells and pathways of cell-mediated immunity to the fungus may represent a useful strategy in developing effective strategies of vaccination to fungi. The ultimate challenge will be to design fungal vaccines capable of inducing optimally effective immunities by targeting specific receptors on DCs *in vivo*. This implicates that we have to learn from pathogens how to manipulate DCs for immunotherapy.

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