M. Har-Noy, et al. [2008] Med. Hypotheses Res. **4**: 85–91.

REVIEW

Immunotherapy for Invasive Aspergillosis in Immunocompromised Post-Engraftment Allogeneic Bone Marrow Transplant Patients

M. Har-Noy[1,3], L. Weiss[1], E. Sioonov[2], I. Polacheck[2] and R. Or[1]

Hadassah-Hebrew University Medical Center, [1]Department of Bone Marrow Transplant and Cancer Immunotherapy; [2]Dep of Clinical Microbiology and Infectious Diseases, Jerusalem, Israel 91120; and [3]Immunovative Therapies, Ltd., POB 974, Sh Israel 60850.

Abstract. Invasive aspergillosis (IA) is a dangerous infection that is common in immunocompromised patients. IA is a major cause of mortality in bone marrow transplant (BMT) patients due to steroid-induced immunosuppression and chemotherapy-induced neutropenia. In normal individuals, *Aspergillus* is controlled by a Type 1 immune response. However, immunocompromised patients have a decreased ability to mount a Type 1 immune response. BMT patients are treated with glucocorticoids to suppress the Type 1 immune response which is associated with graft versus host disease (GVHD) toxicity. Therefore it is a complex problem to develop strategies to enhance Type 1 immunity without also causing GVHD. To overcome this problem, we propose that multiple intradermal injections of activated allogeneic Th1 memory cells will create a pool of alloantigen-specific Th1 memory cells in the circulation. Intradermal allogeneic injections are expected to be rejected and thus not cause GVHD. Additional intradermal allogeneic Th1 cell injections should activate the anti-alloantigen memory cells in circulation causing them to migrate to the sites of fungal infection and produce Type 1 cytokines. This Type 1 cytokine production in the microenvironment of the fungal infection should serve as an adjuvant to the stimulation of innate immune responses against the fungus and the development of Type 1 anti-fungal adaptive immunity.

Correspondence: Dr. M. Har-Noy, Hadassah-Hebrew University Medical Center, Department of Bone Marrow Transplant and Cancer Immunotherapy, POB 12000, Jerusalem, Israel 91120.

Abbreviations used: IA, invasive aspergillosis; BMT, bone marrow transplant; GVHD, graft versus host disease

Received on 01-20-2008; accepted on 04-01-2008.

1. Introduction

The creation of a high titer of alloantigen-specific Th1 immune cells in the circulation of immunosuppressed patients and the subsequent reactivation of this memory pool is proposed to provide a novel strategy to eliminate and protect these patients from opportunistic infections. The proposed mechanism of this protection is based on the concept of "heterologous immunity". Heterologous immunity is an immunological mechanism that occurs when the immune system is biased by a high frequency of memory T cells specific for a first infection; and, whereby the subsequent reactivation of these memory cells alters the host's primary immune response to a second unrelated infection [1,2].

The focus of our hypothesis is the development of a treatment for *Aspergillus* infection in immunocompromised patients, especially patients post-allogeneic bone marrow transplant (BMT). However, we feel the same approach may also potentially benefit patients with viral infections, such as hepatitis B and C, as well as patients with advanced cancers.

Aspergillus spp is a ubiquitous fungus found in nature. It is commonly isolated from soil, plant debris, and the indoor air environment. Among all filamentous fungi, *Aspergillus* is the one most commonly isolated in invasive infections and *Aspergillus fumigatus* is the most common cause of invasive aspergillosis (IA) in the EU and USA [3].

IA is a fulminant and highly lethal infection that is common in immunocompromised patients [4,5]. The incidence of IA is increasing despite recent advances in therapy and IA remains a major cause of mortality in immunosuppressed patients [6]. IA is an especially serious problem following BMT procedures due to steroid-induced immunosuppression and chemotherapy-induced neutropenia. IA is the leading cause of both nosocomial pneumonia and death in recipients of allogeneic BMT [7,8].

IA infection is initiated upon inhalation of conidia (fungal spores) by immunocompromised patients. Conidia are efficiently cleared from the lungs in healthy individuals, but in immunocompromised patients they can germinate to form hyphae that invade the surrounding tissues, resulting in a severe and progressive pneumonia that can subsequently disseminate to other organs.

The antifungal agents approved for the treatment of IA have clinical response rates ranging from 33% to 52% [9,10]. Current therapies for IA are inadequate and include: voriconazole [11]; amphotericin B, which causes nephrotoxicity in 80% of patients [12]; liposomal amphotericin B which is a less nephrotoxic formulation [13], but can be hepatotoxic and is expensive; itraconazole which has many drug interactions [14]; surgical excision of infarcted tissue [15]; and caspofungin [16], recently approved by the US Food and Drug Administration as salvage therapy for IA patients refractory or intolerant to other therapies. Despite aggressive anti-fungal therapy with these agents, the prognosis for IA in BMT patients remains extremely poor with mortality rates of 90% or more [17,18].

Evidence from normal individuals, as well as in patients surviving IA demonstrate that protection from disease is correlated with a Type 1 immune response [19]. Immunosuppressed patients lack the ability to mount a Type 1 immune response making these patients particularly susceptible to opportunistic infections with these organisms.

In immunosuppressed patients refractory to standard treatments for IA, immunotherapy strategies that can stimulate Type 1 immunity are being developed for treatment of this opportunistic infection [20,21]. However, vaccination or adoptive transfer of immune cells to create Type 1 immunity is an especially difficult challenge in the post allogeneic BMT setting where patients are immunosuppressed to prevent lethal graft vs. host disease (GVHD).

GVHD is a lethal side effect of allogeneic BMT and is correlated with a "cytokine storm" of Type 1 cytokines [22]. GVHD is prevented by treatment with glucocorticoids which directly enhance Type 2 cytokine production and inhibit Type 1 cytokine production [23]. Therefore, the challenge in this setting is to elicit a Type 1 cytokine response to eliminate the lethal *Aspergillus* infection without also eliciting lethal

GVHD.

2. Hypothesis

Post-BMT patients with opportunistic *Aspergillus* infection can be treated with multiple intradermal inoculations with activated Th1 memory cells that are allogeneic to both the host and the graft. It is predicted that this treatment will elicit an anti-alloantigen Type 1 memory immune response that when reactivated by subsequent alloantigen injections will serve as an adjuvant for activation of both innate and adaptive anti-fungal immunity to clear *Aspergillus* infection. The allogeneic cell injections will be rejected by the host without causing GVHD.

3. Discussion

We have previously hypothesized that ex-vivo differentiated and expanded Th1 memory cells activated prior to infusion could elicit anti-tumor immunity in immunocompetent patients without pre-conditioning or GVHD toxicity; and without the requirement for a matched donor by a mechanism called the "Mirror Effect" [24]. We now extend this hypothesis to apply to immuno-suppressed patients with opportunistic infection.

We suggest that administration of fully allogeneic cells in an immunocompromised host will not cause GVHD toxicity. The allogeneic rejection response remains intact in immuno-suppressed patients, and therefore fully mis-matched allogeneic cells would be expected to be rejected in this setting and not cause GVHD. For example, severely injured burn patients have profound immune dysfunction yet universally reject allogeneic skin grafts [25]. Immuno-compromised HIV+ patients will also reject allografts [26,27]. Further, intradermal injection rather than intravenous infusion of the allogeneic should reduce the risk of engraftment and thus GVHD toxicity.

Intradermal injection of allogeneic cells (antigen) producing Th1 cytokines (adjuvant) is proposed to create anti-alloantigen Th1 immunity. Skin is a highly immunogenic organ populated by dendritic cells (DC), including epidermal Langerhans cells (LC) [28]. LC cells that reside in the skin play a key role in the initiation and regulation of the immune response throughout the body. LC will readily respond to an allogeneic cell inoculation and capture and process alloantigens resulting from the rejection response. The activated allogeneic Th1 memory cells that are inoculated will express Type 1 cytokines and express CD40L. These "danger" stimuli are expected induce the LC to mature and migrate through the afferent lymph vessels toward the T-cell areas of secondary lymphoid organs [29]. CD40 ligation of LC by CD40L-expressing allogeneic Th1 memory cells is known to trigger enhanced LC IL-12 production [30,31] which is known to result in IL-12-dependent priming of allo-specific Th1 cells [31].

Multiple allogeneic cell injections of activated Th1 memory cells is expected to cause the development of an increasing pool of circulating alloantigen-specific Type 1 memory cells in the circulation that will become activated upon each allogeneic injection. One consequence of reactivating this memory T cell pool is the synthesis and secretion of large concentrations of the cytokines that they have been pre-programmed to synthesize [32]. We propose that reactivation of memory T cells that are programmed to produce type 1 cytokines, such as interferon-◎, are capable of priming for a Type 1 anti-fungal immune response via the "heterologous immunity" mechanism [33,34].

Heterologous immunity is the term used to describe the phenomenon by which memory T cells that were generated during an earlier infection are reactivated in response to a second, unrelated infection. When the immune system is biased by a high frequency of memory cells specific for a given pathogenic antigen, the activation of these cells during an unrelated pathogen infection can significantly enhance clearance of the unrelated infection [33]. The pathogenesis of viral infections in the lung has been shown to be related to the host experience with unrelated pathogens [34]. We propose that injection of allogeneic cells in a patient with a high titer of Th1 memory cells specific for the alloantigens will cause increased Type 1 cytokine release which will in turn serve to stimulate immunity against an opportunistic infection

Activated memory cells are known to express chemokine receptors CCR5, CCR2 or CCR3 that stimulate the upregulation of adhesion receptors in the lung endothelium. [35]. This non-specific infiltration of activated Th1 memory cells producing Type 1 cytokines at the sites of fungal infection in the lungs are expected to have a potent stimulatory effect on local innate and adaptive immune cells responding to the fungus.

Activation of both innate and adaptive immune mechanisms is essential for host control of fungal infection. Effector mechanisms of the innate immune system are a major defense against IA [36]. Resistance to infection requires unimpaired innate anti-fungal activity of pulmonary phagocytic cells operating in a cytokine environment rich in Type 1 cytokines [37] as would be provided by the activated alloantigen-specific Th1 memory cells infiltrating the sites of fungal infection.

In normal individuals, resident alveolar macrophages ingest and kill resting conidia, while neutrophils attack hyphae germinating from conidia that escape macrophage surveillance [38]. The effectiveness of this immune response is evident from the observation that challenge, even with a large number of conidia, fails to cause disease in immunocompetent animals [39].

However, in immunosuppressed patients reduced numbers or impaired function of neutrophils are by far the best-characterized risk factors for IA [40]. Type 1 cytokine production as a result of the activation of alloantigen-specific Th1 memory cells infiltrating pulmonary lesions should serve to activate alternative anti-fungal innate effector cells. The Type 1 cytokines (predominantly IFN-γ, TNF-α, IL-1, IL-2, IL-12 and IL-18) produced as a result of the activation of alloantigen-specific Th1 memory cells should activate alternative innate immune effector cells such as NK cells and dendritic cells (DC), as well activate T-cells [41]. In turn, these cells should produce Type 1 cytokines which will create an autocrine and paracrine cytokine network serving to both maintain and enhance the production of Type 1 cytokines [42].

In immunocompromised hosts, recruitment of NK cells to the lungs has been shown to be an effective defense mechanism against IA [43]. DCs orchestrate the overall antifungal immune resistance in the lungs and were also found to be essential for the activation of Type 1 immune responses to *Aspergillus* [44].

Activated innate immune cells produce IL-12 and IL-18, which synergistically act in autocrine feedback loop to enhance the production of IFN-◎ [45, 46]. The production of IFN-◎ by activated NK cells functions in the priming process of Th1 cells, which in turn supports the expansion and effector function of CD8+ CTLs in the Type 1 adaptive immune response [47]. This cascade of immunological events triggered by activation of alloantigen specific Th1 immunity is expected to enhance cellular immune function against opportunistic infection in immunocompromised hosts.

DC are the innate immune cells recognized as initiators of the immune response to pathogens, including *Aspergillus,* and serve as a bridge between innate and adaptive immunity. DC have a primary role in surveillance for pathogens at the mucosal surfaces [48]. A dense network of DC has been described in the respiratory tracts [49].

Immature DC in the respiratory track recognize and phagocytose fungus. Upon phagocytosis and signaling from Type 1 cytokines, such as TNF-α, DC become activated and then migrate as mature DC to the lymph nodes [50,51]. Mature DC produce IL-12 and in turn activate naïve T-cells in the lymph nodes via presentation of fungal antigen in the context of MHC I and MHC II molecules, concurrent with the expression of co-stimulatory molecules. Type 1 cytokine production by DC promotes the development of a Type 1 adaptive immune response [52]. Type 1 immune responses have been shown to successfully control IA in patients with hematological malignancies [19].

The proposed mechanism of heterologous immunity for treatment of opportunistic infection by activation of resident allospecific Th1 memory cells causing a switch in existing Type 2 immunity to a resident infection to Type 1 immunity is supported by several observations. For example, the opposite shift occurs in in-

fection with Schistosoma mansoni which induces a Type 2 immune response. This response causes a down-regulation of existing Type 1 responses and elevation of Type 2 responses to unrelated foreign immunogens [53]. Type 1-mediated pathology in mouse models of disease can be ameliorated by concurrent infection with an unrelated parasite which elicits Type 2 immunity [54]. Adoptive immunotherapy can induce anti-tumor activity through the production of Type 1 cytokines, even though the transferred cells are not able to recognize tumor antigens. For example, polyclonal Th1 cells administered to mice with non-immunogenic tumors resulted in rejection of 60-90% of the tumors. Cured animals developed a tumor-specific memory and were capable of rejecting rechallenges with the same tumor [55]. Similarly, co-injection of a PPD-specific Th1 clone and PPD antigen in a murine metastatic tumor model produced anti-metastatic effects and anti-tumor activity [56].

That Type 1 immunity to fungus can be induced in an immunosuppressed host is supported by the observation that immunization of cortisone immunosuppressed mice with multiple injections of A. fumigatus confers protection to rechallenge with a lethal dose of conidia in the context of increased production of Type 1 cytokines [57]. In addition, Type 1 immunity can be preserved [58] and also be induced in chimeric hosts [17,59,60].

In conclusion, multiple intradermal infusions of activated Th1 memory cells, allogeneic to both the host and donor, will create a pool of Type 1 alloantigen-specific memory cells that when activated will traffic to the lungs and sites of active fungal infection. At the site of fungal infection, the activated memory cells will release Type 1 cytokines which will activate local NK cells and DC to eliminate fungal spores. The Type 1 inflammatory environment will serve as an adjuvant to the development of Type 1 adaptive immunity to clear the fungal infection and protect against recurrence. Since this approach is not specific for the fungal antigens, it could also be used to elicit protective immunity in immunocompromised patients to any pathogen susceptible to a Type 1 immune response.

References

1. **Yang HY, Dundon PL, Nahill SR, Welsh RM** [1989] Virus-induced polyclonal cytotoxic T lymphocyte stimulation. J Immunol 142: 1710-1718.
2. **Selin LK, Nahill SR, Welsh RM** [1994] Cross-reactivities in memory cytotoxic T lymphocyte recognition of heterologous viruses. J Exp Med 179: 1933-1943.
3. **Maschmeyer G, Haas A, Cornely OA** [2007] Invasive aspergillosis: epidemiology, diagnosis and management in immunocompromised patients. Drugs 67: 1567-1601.
4. **Denning DW, Stevens DA** [1990] Antifungal and surgical treatment of invasive aspergillosis: review of 2,121 published cases. Rev Infect Dis 12: 1147-1201.
5. **Denning DW** [1998] Invasive aspergillosis. Clin Infect Dis 26: 781-803; quiz 804-805.
6. **Bodey GP, Vartivarian S** [1989] Aspergillosis. Eur J Clin Microbiol Infect Dis 8: 413-437.
7. **Zmeili OS, Soubani AO** [2007] Pulmonary aspergillosis: a clinical update. QJM 100: 317-334.
8. **Meyers JD** [1990] Fungal infections in bone marrow transplant patients. Semin Oncol 17: 10-13.
9. **Peterson PK, McGlave P, Ramsay NK, Rhame F, Cohen E, Perry GS, 3rd, Goldman AI, Kersey J** [1983] A prospective study of infectious diseases following bone marrow transplantation: emergence of Aspergillus and Cytomegalovirus as the major causes of mortality. Infect Control 4: 81-89.
10. **Patterson TF** [2002] Fungal susceptibility testing: where are we now? Transpl Infect Dis 4 (Suppl 3): 38-45.
11. **Shao PL, Huang LM, Hsueh PR** [2007] Recent advances and challenges in the treatment of invasive fungal infections. Int J Antimicrob Agents 30: 487-495.
12. **Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW, Kern WV, Marr KA, Ribaud P, Lortholary O, Sylvester R, Rubin RH, Wingard JR, Stark P, Durand C, Caillot D, Thiel E, Chandrasekar PH, Hodges MR, Schlamm HT, Troke PF, de Pauw B** [2002] Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med 347: 408-415.
13. **Wingard JR, Kubilis P, Lee L, Yee G, White M, Walshe L, Bowden R, Anaissie E, Hiemenz J, Lister J** [1999] Clinical significance of nephrotoxicity in patients treated with amphotericin B for suspected or proven aspergillosis. Clin Infect Dis 29: 1402-1407.
14. **Walsh TJ, Finberg RW, Arndt C, Hiemenz J, Schwartz C, Bodensteiner D, Pappas P, Seibel N, Greenberg RN, Dummer S, Schuster M, Holcenberg JS** [1999] Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia. National Institute of Allergy and Infectious Diseases Mycoses Study Group. N Engl J Med 340: 764-771.
15. **Caillot D** [2003] Intravenous itraconazole followed by oral itraconazole for the treatment of amphotericin-B-refractory invasive pulmonary aspergillosis. Acta Haematol 109: 111-118.
16. **Habicht JM, Passweg J, Kuhne T, Leibundgut K, Zerkowski HR** [2000] Successful local excision and long-term survival for invasive pulmonary aspergillosis during neutropenia after bone marrow transplantation. J

Thorac Cardiovasc Surg 119: 1286-1287.
17. **Gonzalez GM, Gonzalez G, Najvar LK, Graybill JR** [2007] Therapeutic efficacy of caspofungin alone and in combination with amphotericin B deoxycholate for coccidioidomycosis in a mouse model. J Antimicrob Chemother 60: 1341-1346.
18. **Denning DW** [1996] Therapeutic outcome in invasive aspergillosis. Clin Infect Dis 23: 608-615.
19. **Hebart H, Bollinger C, Fisch P, Sarfati J, Meisner C, Baur M, Loeffler J, Monod M, Latge JP, Einsele H** [2002] Analysis of T-cell responses to Aspergillus fumigatus antigens in healthy individuals and patients with hematologic malignancies. Blood 100: 4521-4528.
20. **Perruccio K, Bozza S, Montagnoli C, Bellocchio S, Aversa F, Martelli M, Bistoni F, Velardi A, Romani L** [2004] Prospects for dendritic cell vaccination against fungal infections in hematopoietic transplantation. Blood Cells Mol Dis 33: 248-255.
21. **Beck O, Topp MS, Koehl U, Roilides E, Simitsopoulou M, Hanisch M, Sarfati J, Latge JP, Klingebiel T, Einsele H, Lehrnbecher T** [2006] Generation of highly purified and functionally active human TH1 cells against Aspergillus fumigatus. Blood 107: 2562-2569.
22. **Blazar BR, Korngold R, Vallera DA** [1997] Recent advances in graft-versus-host disease (GVHD) prevention. Immunol Rev 157: 79-109.
23. **Elenkov IJ** [2004] Glucocorticoids and the Th1/Th2 balance. Ann N Y Acad Sci 1024: 138-146.
24. **Har-Noy M, Slavin, S** [2008] The Anti-Tumor Effect of Allogeneic Bone Marrow/Stem Cell Transplant Without Graft vs. Host Disease Toxicity and Without a Matched Donor Requirement? Med Hypotheses 70: 1186-1192.
25. **Maile R, Barnes CM, Nielsen AI, Meyer AA, Frelinger JA, Cairns BA** [2006] Lymphopenia-induced homeostatic proliferation of $CD8^+$ T cells is a mechanism for effective allogeneic skin graft rejection following burn injury. J Immunol 176: 6717-6726.
26. **Mzezewa S, Jonsson K, Sibanda E, Aberg M, Salemark L** [2003] HIV infection reduces skin graft survival in burn injuries: a prospective study. Br J Plast Surg 56: 740-745.
27. **Stock PG, Roland ME, Carlson L, Freise CE, Roberts JP, Hirose R, Terrault NA, Frassetto LA, Palefsky JM, Tomlanovich SJ, Ascher NL** [2003] Kidney and liver transplantation in human immunodeficiency virus-infected patients: a pilot safety and efficacy study. Transplantation 76: 370-375.
28. **Larregina AT, Falo LD, Jr.** [2005] Changing paradigms in cutaneous immunology: adapting with dendritic cells. J Invest Dermatol 124: 1-12.
29. **Laurin D, Kanitakis J, Bienvenu J, Bardin C, Bernaud J, Lebecque S, Gebuhrer L, Rigal D, Eljaafari A** [2004] Allogeneic reaction induces dendritic cell maturation through proinflammatory cytokine secretion. Transplantation 77: 267-275.
30. **Cella M, Scheidegger D, Palmer-Lehmann K, Lane P, Lanzavecchia A, Alber G** [1996] Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. J Exp Med 184: 747-752.
31. **Kelsall BL, Stuber E, Neurath M, Strober W** [1996] Interleukin-12 production by dendritic cells. The role of CD40-CD40L interactions in Th1 T-cell responses. Ann NY Acad Sci 795: 116-126.
32. **Chen HD, Fraire AE, Joris I, Brehm MA, Welsh RM, Selin LK** [2001] Memory CD8+ T cells in heterologous antiviral immunity and immunopathology in the lung. Nat Immunol 2: 1067-1076.
33. **Selin LK, Varga SM, Wong IC, Welsh RM** [1998] Protective heterologous antiviral immunity and enhanced immunopathogenesis mediated by memory T cell populations. J Exp Med 188: 1705-1715.
34. **Chen HD, Fraire AE, Joris I, Welsh RM, Selin LK** [2003] Specific history of heterologous virus infections determines anti-viral immunity and immunopathology in the lung. Am J Pathol 163: 1341-1355.
35. **Sallusto F, Lenig D, Mackay CR, Lanzavecchia A** [1998] Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med 187: 875-883.
36. **Roilides E, Katsifa H, Walsh TJ** [1998] Pulmonary host defences against Aspergillus fumigatus. Res Immunol 149: 454-524.
37. **Cenci E, Mencacci A, Fe d'Ostiani C, Del Sero G, Mosci P, Montagnoli C, Bacci A, Romani L** [1998] Cytokine- and T helper-dependent lung mucosal immunity in mice with invasive pulmonary aspergillosis. J Infect Dis 178: 1750-1760.
38. **Schaffner A, Douglas H, Braude A** [1982] Selective protection against conidia by mononuclear and against mycelia by polymorphonuclear phagocytes in resistance to Aspergillus. Observations on these two lines of defense in vivo and in vitro with human and mouse phagocytes. J Clin Invest 69: 617-631.
39. **Dixon DM, Polak A, Walsh TJ** [1989] Fungus dose-dependent primary pulmonary aspergillosis in immunosuppressed mice. Infect Immun 57: 1452-1456.
40. **Wald A, Leisenring W, van Burik JA, Bowden RA** [1997] Epidemiology of Aspergillus infections in a large cohort of patients undergoing bone marrow transplantation. J Infect Dis 175: 1459-1466.
41. **Antin JH, Ferrara JL** [1992] Cytokine dysregulation and acute graft-versus-host disease. Blood 80: 2964-2968.
42. **Mailliard RB, Son YI, Redlinger R, Coates PT, Giermasz A, Morel PA, Storkus WJ, Kalinski P** [2003] Dendritic cells mediate NK cell help for Th1 and CTL responses: two-signal requirement for the induction of NK cell helper function. J Immunol 171: 2366-2373.
43. **Morrison BE, Park SJ, Mooney JM, Mehrad B** [2003] Chemokine-mediated recruitment of NK cells is a critical host defense mechanism in invasive aspergillosis. J Clin Invest 112: 1862-1870.
44. **Bozza S, Gaziano R, Lipford GB, Montagnoli C, Bacci A, Di Francesco P, Kurup VP, Wagner H, Romani L** [2002] Vaccination of mice against invasive aspergillosis with recombinant Aspergillus proteins and CpG oligodeoxynucleotides as adjuvants. Microbes Infect 4: 1281-1290.
45. **Okamura H, Kashiwamura S, Tsutsui H, Yoshimoto T, Nakanishi K** [1998] Regulation of interferon-gamma production by IL-12 and IL-18. Curr Opin Immunol 10: 259-264.
46. **Micallef MJ, Tanimoto T, Kohno K, Ikeda M, Kurimoto**

M [1997] Interleukin 18 induces the sequential activation of natural killer cells and cytotoxic T lymphocytes to protect syngeneic mice from transplantation with Meth A sarcoma. Cancer Res 57: 4557-4563.
47. **Trinchieri G, Scott P** [1995] Interleukin-12: a proinflammatory cytokine with immunoregulatory functions. Res Immunol 146: 423-431.
48. **Banchereau J, Steinman RM** [1998] Dendritic cells and the control of immunity. Nature 392: 245-252.
49. **Pollard AM, Lipscomb MF** [1990] Characterization of murine lung dendritic cells: similarities to Langerhans cells and thymic dendritic cells. J Exp Med 172: 159-167.
50. **Bozza S, Gaziano R, Spreca A, Bacci A, Montagnoli C, di Francesco P, Romani L** [2002] Dendritic cells transport conidia and hyphae of Aspergillus fumigatus from the airways to the draining lymph nodes and initiate disparate Th responses to the fungus. J Immunol 168: 1362-1371.
51. **Bauman SK, Huffnagle GB, Murphy JW** [2003] Effects of tumor necrosis factor alpha on dendritic cell accumulation in lymph nodes draining the immunization site and the impact on the anticryptococcal cell-mediated immune response. Infect Immun 71: 68-74.
52. **Huffnagle GB, Deepe GS** [2003] Innate and adaptive determinants of host susceptibility to medically important fungi. Curr Opin Microbiol 6: 344-350.
53. **Kullberg MC, Pearce EJ, Hieny SE, Sher A, Berzofsky JA** [1992] Infection with Schistosoma mansoni alters Th1/Th2 cytokine responses to a non-parasite antigen. J Immunol 148: 3264-3270.
54. **Whary MT, Fox JG** [2004] Th1-mediated pathology in mouse models of human disease is ameliorated by concurrent Th2 responses to parasite antigens. Curr Top Med Chem 4: 531-538.
55. **Saxton ML, Longo DL, Wetzel HE, Tribble H, Alvord WG, Kwak LW, Leonard AS, Ullmann CD, Curti BD, Ochoa AC** [1997] Adoptive transfer of anti-CD3-activated $CD4^+$ T cells plus cyclophosphamide and liposome-encapsulated interleukin-2 cure murine MC-38 and 3LL tumors and establish tumor-specific immunity. Blood 89: 2529-2536.
56. **Shinomiya Y, Harada M, Kurosawa S, Okamoto T, Terao H, Matsuzaki G, Shirakusa T, Nomoto K** [1995] Anti-metastatic activity induced by the in vivo activation of purified protein derivative (PPD)-recognizing Th1 type CD4+ T cells. Immunobiology 193: 439-455.
57. **Centeno-Lima S, Silveira H, Casimiro C, Aguiar P, do Rosario VE** [2002] Kinetics of cytokine expression in mice with invasive aspergillosis: lethal infection and protection. FEMS Immunol Med Microbiol 32: 167-173.
58. **Williams MA, Adams AB, Walsh MB, Shirasugi N, Onami TM, Pearson TC, Ahmed R, Larsen CP** [2003] Primary and secondary immunocompetence in mixed allogeneic chimeras. J Immunol 170: 2382-2389.
59. **Ruedi E, Sykes M, Ildstad ST, Chester CH, Althage A, Hengartner H, Sachs DH, Zinkernagel RM** [1989] Antiviral T cell competence and restriction specificity of mixed allogeneic (P1 + P2 → P1) irradiation chimeras. Cell Immunol 121: 185-195.
60. **Ildstad ST, Wren SM, Bluestone JA, Barbieri SA, Sachs DH** [1985] Characterization of mixed allogeneic chimeras. Immunocompetence, in vitro reactivity, and genetic specificity of tolerance. J Exp Med 162: 231-244.