Meetings on Vaccine Studies towards the Control of Leishmaniasis

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Leishmaniasis is one of the major parasitic diseases targeted by the World Health Organization (WHO 1990 Tech Rep Ser 793). Recent epidemiologic studies indicate that the disease in the Americas is far more abundant and of greater public health importance than was previously recognized (G Grimaldi Jr et al. 1989 Am J Trop Med Hyg 41: 687). Control of leishmaniasis in this region is complicated by a variety of leishmanial parasites (at least 13 distinct New World Leishmania species are recognized as causing human illness) and by the fact that each of these parasites has a unique epidemiologic pattern (reviewed in G Grimaldi Jr, RB Tesh 1993 Clin Microbiol Rev 6: 230). These data explain the limited success of current control strategies (based on conventional measures such as vector reduction, elimination of infected reservoirs, personal protection, surveillance, and treatment) for American leishmaniasis. One obvious solution to the problem is the development of safe and effective vaccines against the disease. However, considering the genetic polymorphism and biological diversity of the parasites, development of effective vaccines may be a formidable task.

Clinical and experimental evidence indicate that vector, parasite, and host factors all influence the evolution and outcome of leishmanial infection. A number of Leishmania species are capable of producing a broad spectrum of disease in humans, ranging from asymptomatic infection to horribly disfiguring forms of mucosal leishmaniasis (ML) or the potentially fatal visceral leishmaniasis (VL). The more benign self-healing forms of leishmaniasis in humans usually result in protection against reinfection, and cell-mediated immunity, rather than humoral immunity, is considered of primary importance in acquired resistance. Immune protection to infection with L. major in resistant mice is associated with selective activation and differentiation of effective CD4+ helper T (Th) cells, the Th1 subset, which are characterized by a distinct cytokine secretion pattern (e.g., interleukine 2, IL-2, gamma interferon, IFN-γ, and lymphotoxin). In contrast, the progressive leishmanial infection in susceptible mouse strains is correlated with activation of the Th2 CD4+ cell response, which expresses IL-4, IL-5, IL-6, and IL-10 among other cytokines (RM Locksley et al. 1991 Res Immunol 142: 28). Several factors may affect the preferential induction and/or expansion of distinct Th cell subsets during leishmanial infection, including the cytokines (e.g., IL-12; IFN-γ) present during the initial events of cell differentiation, and the interaction with regional antigen-presenting cells (APC), which can preferentially present different classes of antigens (P Scott 1991 J Immunol 147: 3149, FP Heinzel 1994 Parasitol Today 10: 190). Production of IFN-γ by protective T cells has also been correlated with the ability of vaccinated and naive mice to control infection with L. donovani (KE Squires et al. 1989 J Immunol 143: 4244). However, the differential production of Th1- and Th2-derived cytokines does not seem to determine the genetically controlled or vaccine-induced rate of cure in murine visceral leishmaniasis (PM Kaye et al. 1991 J Immunol 146: 2763). In addition, contributions by CD8+ T cell to the mediation of protective immunity against leishmanial infections have been shown (JO Hill et al. 1989 J Exp Med 169: 1819).

Although an interplay between the host immune system and parasite pathogenic capabilities in the clinical expression of human leishmaniasis, the mechanisms have yet to be determined. T cell responses correlate with recovery from and resistance to human leishmaniasis. A lymphocytic response to leishmanial antigens usually develops during both CL and ML but is absent in diffuse cutaneous leishmaniasis (DCL) and VL. Conversely, anti-Leishmania antibody titers are generally low in the sera of patients with CL or ML but moderate to high in patients with DCL or VL (reviewed in Grimaldi, Tesh loc. cit.). When tested in vitro, peripheral lymphocytes from patients with CL or ML produced high levels of IFN-γ in response to parasite antigen (EM Carvalho et al. 1985 J Immunol 135: 4144, SC Mendonça et al. 1986 Clin Exp Immunol 64: 269, M Castés et al. 1988 J...

VACCINE PERSPECTIVES

The solid immunity observed following convalescence to CL or VL has suggested that vaccination may prove to be the most cost-effective intervention method for the prevention and control of the various leishmanial infections at a population level. Vaccines consisting of viable forms of the pathogen itself have been evaluated with CL in the Old World. Both cellular and humoral immune response to leishmanial antigens (MS Green et al. 1983 Parasite Immunol 5: 337) and resistance to reinfection usually develop in subjects vaccinated against CL using living L. major promastigotes (reviewed in CL Greenblatt 1985 Leishmaniasis. K-P Chang, RS Bray (eds). Elsevier. 163 pp.). However, large scale human trials have clearly indicated that protection requires prior controlled induction of disease with a virulent parasite (reviewed in AE Gunders 1987 The leishmaniasis in biology and medicine. W Peters, R Killlick-Kendrick (eds). Academic Press 928 pp.). As a consequence, this type of immunization can be used only with Leishmania species that produce benign self-healing lesions.

Prophylactic immunization using killed promastigote vaccine is currently only in experimental stages. Unlike the mouse studies (JGHoward et al. 1982 J Immunol 129: 2206), periodic boosting in humans using inactivated parasites promotes a delayed-type hypersensitivity (DTH) response to leishmanial antigen, which seems to increase the recipient’s chance of being protected (W Mayrink et al. 1979 Trans R Soc Trop Med Hyg 73: 385, E Nascimento et al. 1990 Infect Immun 58: 2198, M Castés et al. 1994 Vaccine 12: 1041). However, whether killed vaccine can achieve similar levels of immunity as living vaccine is at present unknown. Preliminary vaccination trials with killed organisms have given conflicting results. Although the efficacy of a phenol killed-promastigote vaccine showed an 82% protection rate against American CL (SB Pessoa, BR Pestana 1941 Arch Hig Saúde Públ 6: 141), a similar approach failed to vaccinate against Old World CL (DA Barberian 1944 Arch Dermatol Syphilol 50: 234). The field trial evaluating the efficacy of a polyvalent vaccine showed that more than 70% of the vaccinees became skin positive, but the study was inconclusive by low incidence rate in the control group (Mayrink et al. loc. cit.). In a similar trial, only 30% of vaccines showed skin test conversion; of those people that did convert, there was about a 70% decrease in the incidence of natural disease compared with the incidence in the control group (W Mayrink et al. 1985 Ann Trop Med Parasitol 79: 259). In a third trial in the Amazon region of Brazil, there were also reductions (67.3 and 85.7%) in the annual incidence of CL among vaccinees developing a positive leishmanin skin test. However, when the skin test-positive and skin test-negative vaccines in that study were combined, the difference between the vaccinated and control groups was not significant (CMP Antunes et al. 1986 Int J Epidemiol 15: 572). Other studies have shown that development of DTH response may not be necessarily accompanied by protection, following vaccination with attenuated promastigotes (PEC Manson-Bahr 1961 Trans R Soc Trop Med Hyg 55: 550, J Salazar 1965 Arch Venez Med Trop Parasitol Med 5: 365, D Heyneman 1971 Bull WHO 44: 499). DTH may also be a marker that is frequently linked with, while not itself being, the underlying mechanism of pathogenesis in L. braziliensis infection (NG Saravia et al. 1989 J Infect Dis 159: 725).

Over the last ten years much effort has been devoted to the development of standardized and safe vaccines that should be able to produce long-lasting immunity against all types of human leishmaniasis. Prospects for human immunoprophylaxis with a new generation of safe and effective subunit vaccines (using either recombinant or synthetic peptides or infectious recombinant vectors) is now within our reach (DM Yang et al. 1990 J Immunol 145: 2281, A Jardim et al. 1991 J Exp Med 172: 645, D McMahan-Pratt et al. 1993 Infect Immun 61: 3351). In order to review the current status of vaccine development towards the control of human leishmaniasis, the WHO Special Program for Research and Training on Tropical Diseases (TDR), the Pan American Health
Organization (PAHO), the National Health Foundation, Brazilian Ministry of Health (FNS/MS), and the Federal University of Bahia (UFBA), jointly organized two meetings in Salvador, Bahia (Brazil).

FIRST WORKSHOP

The first Workshop (on "Vaccine Efficacy Trials Against Leishmaniasis and Preparation of Phase III Protocols") aimed to review the progress made in various studies using crude killed promastigote antigens and plan future activities on field efficacy trials (Phase III) for candidate vaccines. The group reviewed general issues such as epidemiological aspects of the disease in several Latin American countries, as well as safety and regulatory procedures employed on vaccine studies [the WHO has prepared specific guidelines for evaluating vaccine, which include recommendations for field trials that address questions of design, selection of study populations, execution of the trials, data analysis, and ethical principles with regard to safety, protection, and benefits for individuals (WHO 1992 Tech Rep Ser, 822)]. The discussion was focused on (i) vaccine development program in the Old World with killed L. major produced by the Razi Institute, Iran (ii); previous South American human vaccination trials using an intramuscular injection of merthiolate-treated organisms made of a mixture of Leishmania strains; (iii) canine vaccination trials (using crude promastigote antigens) in Brazil and France; (iv) Phase I and II trials with the Brazilian Good Manufacturing Practice (GMP) produced one strain vaccine (merthiolate-treated promastigotes of L. amazonensis); and (v) other human leishmanial vaccine (killed promastigotes plus BCG) trials developed in Venezuela and Ecuador. Studies in Brazil (Dr SCF Mendonça and co-workers, this meeting) and Venezuela (Castés et al. loc. cit., M Castés, this meeting) have shown that a high proportion of healthy volunteers immunized with inactivated Leishmania promastigotes (with or without BCG) converted for T-cell responder phenotypes to parasite antigen, but vaccination consistently elicited low IFN-γ production from T lymphocytes of vaccinees. Whether the intensity or duration of the elicited responses and their roles in vaccine-induced immunity may vary according to the antigenic composition of the injected strain (or the quality control of the vaccine materials), or the genetic variability of the host remains to be established. In addition, field trials in Venezuela have shown that anti-leishmanial antibody responses were not enhanced in the two vaccine groups receiving killed promastigotes (with/without BCG) compared with the BCG alone and placebo groups. However, all vaccine groups showed a pattern of immune response consistent with either a response to the skin-test antigen or, more likely, seasonal endemic exposure to leishmanial infection (CE Sharpley et al. 1994 Vaccine 12: 1403). Future field studies (Phase III trials will be funded by WHO/TDR) are planned for testing killed Leishmania vaccine in several endemic foci in the Americas. Such studies are important since there is still much to be done for assessing the effectiveness of vaccination in the absence of natural challenge. The artificial induction of acquired resistance will also depend on detailed knowledge of the natural history of the human host-parasite relationship occurring in each endemic area. The group suggested that the WHO/PAHO should take an active part in the New World leishmaniasis vaccine program.

SECOND WORKSHOP

Participants at the second Workshop (on "Development of the Second Generation Vaccine Against Leishmaniasis") reviewed the latest strategies in vaccine design against leishmanial infections. Current research employing mouse models is providing the foundation for studies designed not only to identify leishmanial protective immunogens, but also to provide a better understanding of the immune mechanisms (by correlating specific immunologic responses with protection) responsible for solid immunity in vaccinated animals. Attention was focused on the induction of immunoprotection using (i) purified leishmanial antigenic components such as the major cell surface glycoprotein gp63 of Leishmania (DG Russell, J Alexander 1988 J Immunol 140: 1274, LP Kahl et al. 1989 J Immunol 142: 4441); the promastigote surface glycoprotein gp46 of L. amazonensis (J Champsì, D McMahon-Pratt 1988 Infect Immun 56: 3272) or related polyepitope antigens PSA-2 (JM Burns et al. 1991 J Immunol 146: 742, Dr E Handman and coworkers, this meeting), the pure protein dp72 of L. donovani (N Rachamin, CL Jaffe 1993 J Immunol 150: 2322); the glycolipid LPG (E Handman, GF Mitchell 1985 Proc Natl Acad Sci 82: 5910, MJ McConville et al. 1987 Proc Natl Acad Sci USA 84: 8941) or the lipophosphoglycan-associated membrane proteins LPG-AP/ KMP-11 of Leishmania (DM Russo et al. 1992 J Immunol 148: 202, A Jardim et al. 1995 Biochem J 305: 307), and the amastigote proteins P-2/A-2, P-4, and P-8 of L. pifanoi (Dr D McMahon-Pratt and coworkers, this meeting); or (ii) Leishmania antigens synthetized through gene cloning such as the rp63 (LL Button, WR McMester 1988 EMBO J 7: 93, DG Russel, J Alexander loc. cit.), rp24-LACK (Dr N Glaiichenhaus, this meeting) and rLeIF (Dr YAW Skeiey and co-workers, this meeting) leishmanial recombinant proteins; or (iii) Leishmania mimicked antigens by synthetic pep-
tides such as the gp63/pt-3 and gp63/pt-6 T cell epitopes (A Jardim et al. 1990 J Exp Med 172: 645). Complete or appreciable levels of protection against CL and/or VL have been achieved in genetically resistant and susceptible strains of mice by using these candidate vaccines, either in conjunction with adjuvants (Handman, Mitchell loc. cit., McConville et al. loc. cit., Champs, McMahon-Pratt loc. cit., Russel, Alexander loc. cit., Rachamim, Jaffe loc. cit.) or delivered in vivo by infectious multivaccine expression vectors such as the attenuated recombinant Salmonella/gp63 (Yang et al. loc. cit.), BCG/gp63 (Dr WR McMaster and co-workers, this meeting), or Vaccinia/gp63 (McMahon-Pratt et al. loc. cit.). Recent emphasis has also turned to the evaluation of defined leishmanial antigens in humans to identify epitopes that induce appropriate T-cell responses (MG Zallis et al. 1988 Mem Inst Oswaldo Cruz 83: 117, SG Reed et al. 1990 J Clin Invest 85: 690, JM Burns et al. 1991 J Immunol 146: 742, M Kemp et al. 1991 Scand J Immunol 33: 219, SCF Mendonça et al. 1991 Clin Exp Med 83: 472, DM Russo et al. 1991 J Immunol 147: 3575, DM Russo et al. 1992 J Immunol 148: 202). Either the recombinant or native form of gp63, for instance, is a strong T-cell immunogen (capable of inducing CD4+ T-cell proliferative response and IFN-γ production) in leishmaniasis patients (Mendonça et al. loc. cit., Russo et al. loc. cit.). Moreover, induction of protection using a potent vasodilator termed erythromycin-inducing factor (which can neutralize the immunosuppressive effect of saliva) present in sandfly saliva (RG Titus, JMC Ribeiro 1988 Science 239: 1306, JMC Ribeiro et al. 1990 Br J Pharmacol 101: 932, JMC Ribeiro et al. 1993 Science 260: 539), or the salivary immunosuppressive protein itself (Dr J David, this meeting) as vaccine is now within our reach. Alternatively, the recent successful immunization of susceptible strains of mice (RG Titus et al. 1995 Proc Natl Acad Sci USA, in press) using genetically avirulent [parasites lacking the dihydrofolate reductase-thymidylate synthetase, or dhfr-ts by gene replacement (A Cruz et al. 1991 Proc Natl Acad Sci USA 88: 7170)] L. major live vaccine has provided a revolutionary new approach in vaccinology. The ability of dhfr-ts' parasites to differentiate and persist briefly (for up to two months, declining with a half-life of about 2-3 days) may reflect a locus-specific advantage of dhfr-ts' knockouts in prolonging the period and diversity of antigen delivery by Leishmania (Titus et al. loc. cit.). Potentially, dhfr-ts' could be used as a delivery system for other antigen and/or adjuvant (e.g., IL-12).

CONCLUDING REMARKS

At the conclusion of the meeting the participants discussed about general problems encountered and future development. Attention was focused on (i) the value of killed promastigotes as vaccinating materials, and whether a single strain or defined antigen vaccine could induce heterologous protection; and (ii) how to measure vaccine effectiveness (the degree and duration of the protection) in humans without an actual challenge infection. Although many advances in understanding some of the cellular immune responses generated during leishmanial infections have been made, there is still many unanswered questions about the complex immunologic mechanisms involved in the control of human leishmaniasis. Furthermore, vaccine-induced immunity against leishmanial infection in murine models have the fundamental drawback of an uncertain correlation with human infection. However, as non-human primate host responses to Leishmania (VA Dennis et al. 1986 Exp Parasitol 61: 319, JI Githure et al. 1986 Trans R Soc Trop Med Hyg 80: 575, R Lujan et al. 1986 Am J Trop Med Hyg 35: 1103, TVouldoukis et al. 1986 J Parasitol 72: 472, OJ Pung, RE Kuhn 1987 J Med Primatol 16: 165, JO Olobo et al. 1992 Scand J Immunol 36: 48) are very similar to those observed in humans, primate models could provide an indication of the potential success and/or limitations for human vaccine against leishmaniasis. This will be especially important in the case of L. braziliensis complex parasites where the monkey models studied to date are susceptible to infection (R Lujan et al. 1986 Exp Parasitol 61: 348, R Lujan et al. 1990 J Parasitol 76: 594, FT Silveira et al. 1989 Rev Soc Bras Med Trop 22: 125, FT Silveira et al. 1990 Rev Inst Med Trop S Paulo 32: 387) and the murine model is limited. The parallels found between humans and other primates in susceptibility, clinicopathologic changes, and immunologic responses to leishmanial infections is not surprising, given their close phylogenetic and immunologic relationship (NL Letvin et al. 1983 Eur J Immunol 13: 345, HKishino, MHasegawa 1990 Methods Enzymol 183: 550, JKlein et al. 1993 Immunol Rev 11: 213). Success of a vaccine depends not only on the identification of protective antigens but also the adoption of a suitable vaccination protocol. For vaccination, the issues are route of immunization, doses, parasite stage, timing between vaccination and challenge. Of special interest will be studies using primates for delineating antigens or delivery systems (adjuvants/infectious agents, or a new construct/cocktail vaccine) relevant for protective immunity in humans.