[The widespread prevalence and spread of tropical diseases in developing countries, despite the conventional measures adopted to control them, warrant a search for new tools against the diseases. The revolutionary advances in the fields of immunology and molecular biology in recent years, particularly in the hybridoma and recombinant DNA technology, offer promise to provide such tools which could supplement the current methods of control. The author describes in the following short review, the current advances in immunology of some of these diseases and their possible application for early diagnosis, for a greater understanding of host-parasite interactions and for induction of resistance to the infections.—Ed.]

TRENDS IN IMMUNOLOGY AND PARASITIC DISEASES

D. SUBRAHMANYAM
CIBA-GEIGY Research Centre, Goregaon, Bombay 400 063, *India*

PARASITIC diseases comprise a large group caused by heterogeneous pathogens, among which protozoa and helminths are the most prominent. This article attempts to present an overview of some of the current approaches on the application of recent advances in immunology in the control of these diseases.

Over a billion people of the world are at the risk[1] of exposure to parasitic diseases such as malaria, filaria, schistosomiasis, trypanosomiasis and leishmaniasis. Several more are exposed to other parasitic infections such as amoebiasis, ancylostomiasis, ascariasis, strongyloidiasis, dracontiasis, toxoplasmosis, trichuriasis, cysticercosis and hydatidosis. Many of these diseases are vector-borne and are prevalent largely in the developing countries of the tropical and subtropical regions of Asia, Africa and Latin America. In India alone, recent estimates indicate that 36 million people suffer from filariasis and another 4 million from malaria.

The battle against vector-borne diseases is mainly waged on two fronts, namely, controlling the vectors by chemical agents and the parasites by drugs. In recent years, it is realised that these measures are inadequate largely due to the development of resistance in insects to insecticides[2] and the emergence of resistance in parasites to chemotherapeutic agents[3]. Even early diagnosis of these diseases presents considerable problems. Additional tools are, therefore, essential for rapid diagnosis of these infections and for a greater understanding on the host-parasite interactions in order to provide effective means of their control. With this in view, the UNDP/World Bank/WHO Special Programme on Tropical Diseases Research was launched in 1976 which intensified goal-oriented research to produce new control measures against 6 major tropical diseases. The recent advances in the field of immunology in general, and of certain parasitic diseases in particular, offer hope of providing such tools to combat these diseases.

IMMUNODIAGNOSIS

Early diagnosis of the parasitic infection is essential for a detailed study of its epidemio-

logy, identification of infecting species, differentiation of past and current infections, evaluation of the immunity status of the individual and for taking prophylactic measures against the infection. Intensive efforts are being made in several laboratories to exploit the early immune response to the infection, in evolving at rapid serodiagnostic tests. There are several immunological tests to detect antibodies to antigens of the invading parasites such as by skin test and by indirect haemagglutination and fluorescent antibody techniques[4]. However, due to sharing of antigens between different phylogenetically related helminths, these tests lack specificity[5]. Recently, attempts are being made to improve on the specificity, using enzyme-linked immunosorbent assay[6] with purified species-specific antigens.

A more recent introduction to this field is the application of hybridoma technology[7] The method involves fusion of an antibody forming cell with a mutant myeloma cell. By cloning the hybrid, it is possible to produce continuously growing cell lines secreting monoclonal antibodies of desired specificity. The availability of these antibodies directed against species-specific parasite antigens, in almost unlimited quantities, facilitates development of sensitive and absolutely specific immunoassays. Mitchell *et al.*[8] developed a specific solid-phase competitive radioimmunoassay (RIA) for diagnosis of *Mesocestoides corti* infection in mice using hybridoma antibodies.

Immunodiagnostic methods based on detection of antibody may not distinguish clearly between exposure and active infection. It seems more appropriate to detect the circulating parasite antigens rather than the antibodies in order to detect current infections. Mackey *et al.*[9] and Avraham *et al.*[10] reported successful solid-phase RIA based on identification of circulating antigens of animals infected with malaria using ^{125}I-labelled specific IgG or ^{125}I-Protein A respectively. The test is highly sensitive and it detected[3] infection even at as low a level of 8 parasites in a million red blood cells. The solid-phase RIA using hybridoma antibody, described above, could also be modified to detect circulating antigens by a sandwich immunoassay.

Monoclonal antibodies have also been shown to be capable of mediating a variety of parasiticidal effects[11] and to transfer resistance to parasitic infections such as schistosomiasis and malaria. They are also used for the detection of functional antigens of the parasites and in parasite taxonomy[12] such as in identification of leishmania organisms.

Immunology of Host-Parasite Interactions

The immune response of the host to a parasitic infection fluctuates rather widely. A variety of factors such as the genetics of the host (immune response genes) and of nutritional, immunological and parasitological origin determine the outcome of the infection. Much information is now available on the nature of these factors which influence the interplay between the host and the parasite.

Immune Responses

There is a heightened immune response seen in the host to several infections. In malaria infection, for instance, a 7-fold increase in the synthesis of immunoglobulins is evident of which only a third is directed against the malaria parasite[13]. Available evidence suggests that only 5% of this antibody may be functional in terms of protection. The protective effect is often stage-specific. For example, protective antibodies directed to merozoites do not protect the animal against infection with sporozoite stage of the parasite.

Effective immune responses to most parasitic infections seem to be dependent on an interplay between T and B cells. In malaria, for example, neonatal thymectomy and treatment of animals with antithymocyte serum[14], lead, in general, to a greater susceptibility to the parasitic infection. Here, T cell, possibly, is serving a "helper" function.

Recent studies in a variety of host-parasite systems reveal the importance of antibody-dependent cell-mediated adhesion and cytotoxicity to the parasite as a primary defence mechanism against metazoan parasites in the host. These reactions were studied in greater detail in schistosomal[15] and filarial infections[16,17] in rodents and primates. IgG and IgE antibodies have been demonstrated *in vitro* to interact with the larval stages of parasites and with a variety of cells including neutrophils, eosinophils and macrophages. Eosinophil-mediated effects may also require mast cells in the schistosomal system. Certain lymphokines liberated by T cell mitogens seem to augment the antibody-dependent neutrophil mediated damage to the filarial parasites[18].

The above observations do not in any way imply that cellular immunity mediated by T cells has a secondary role as antiparasite effector mechanism in many situations.

Evasion of Immune Responses of the Host by the Parasites

The parasites seem to evade the immune responses of the host by a variety of mechanisms[19] such as (i) immunosupp ession, (ii) induction of immune unresponsiveness, (iii) release of factors that block immune-mediated rejection of the parasite, (iv) antigenic variation, and (v) antigen mimicry.

The response of lymphocytes to nonspecific mitogens such as phytohemagglutinin and conconavalin A has been shown to be dep essed in malaria[20], trichinosis[21], schistosomiasis[22], leishmaniasis[23], trypanosomiasis[24] and filariasis[25,26]. Ottesen *et al.*[27] demonstrated that chronic filarial infection in humans with *Wuchereria bancrofti* is characterised by a state of specific cellular immune unresponsiveness to filarial antigens derived from adult *Brugia malayi*. Certain parasites such as trypanosomes[28], plasmodia[29] and babesia[29] adapt to the host's immune response by antigenic variation. Schistosomes seem to evade immune response by acquiring host-like antigens[30].

Deleterious Effects of Immune Responses to the Host

Immune responses against the invading parasites are not always advantageous to the host[31]. In malarial infections, antibodies generated against normal and parasitic red blood cells may cause increased haemolysis and anaemia[32]. Tropical eosinophilia seen in areas endemic to filarial infections seems to be due to hypersensitivity of certain individuals to homologous parasite antigens[33]. Furthermore, the increased blastogenic response seen in lymphocytes of patients with chronic filarial disease to adult worm antigens suggests its involvement in the etiology of elephantiasis[34].

Immune complexes formed between antigens and antibodies may localize in filtering membranes of target organs and cause considerable pathologic lesions. This is amply demonstrated in malaria, trypanosomiasis and schistosomiasis[31].

Induction of Resistance to Parasitic Infections

Several advances have taken place in recent years in inducing resistance to parasitic-infections. Cohen and his colleagues have demonstrated development of high degree of resistance in monkeys to malaria infection by immunization with merozoites of *Plasmodium knowlesi* in Freund's complete adjuvant[35] (FCA). This observation together with development of elegant techniques for continuous cultivation[36] of malaria parasites *in vitro* offer hopes of arriving at an effective vaccine against malaria. Vigorous search is underway for finding more acceptable adjuvants[37,38] to replace FCA.

The ability of radiation-attenuated live parasite stages in inducing stage-specific resistance has been demonstrated in several protozoal[39] and helminthic infections[40]. However, for any practical and successful application of an immunization programme against

helminthic infections, it is desirable to have purified soluble antigens that confer resistance to the infection. Evidence is forthcoming, in recent years, that non-living parasite antigens could confer high degree of protection against parasite infection. Kilejian[41] reported development of a very effective immunity in ducklings against *P. lophurae* with a histidine-rich protein of the parasite. The existence of a similar protein was observed by the investigator in *P. falciparum*. Rajasekariah *et al.*[42] isolated a protein fraction from oncospheres of *Taenia taeniaformis* which when injected parenterally with or without adjuvants stimulated a high degree of protective immunity in mice against challenge infection. Scott and Snary[43] successfully induced protection in mice against acute lethal *Trypanosoma cruzi* infection by vaccination with a cell surface glycoprotein of the parasite in combination with adjuvants. Mehta *et al.*[44] found that homogenates of larval stages of rodent filarial parasite *Litomosoides carinii* in FCA confer resistance to the infection.

The role of secretory and excretory products of parasites in induction of immunity is virtually unexplored, particularly in the case of human parasitic infections. Development of suitable methods for *in vitro* maintenance and perhaps even cultivation of parasites would facilitate such studies. Nevertheless, the cytotoxicity and death of the parasite consequent to the antibody-dependent cellular adhesion strongly suggests the presence of antigens, possibly protective in nature, on the parasite surface. Once the relevant parasite antigens are defined, the respective genes can be identified and inserted into bacterial plasmid vectors[45] for cloning and subsequent production of the antigens in large quantities. This recombinant DNA research has already found great application in the hormone field[46].

With the recent developments in analytical methodology in identification of surface antigens of parasites[47] by radio-labelling techniques, in production of hybridomas that detect specific antigens and in genetic engineering technology, rapid advances are expected in this area which should provide us the much needed additional measures of controlling at least a few of the parasitic infections.

ACKNOWLEDGEMENT

The investigations from the author's laboratory were supported in part by grants from UNDP/World Bank/WHO Special Programme for Research in Tropical Diseases, The Indian Council of Medical Research and the International Atomic Energy Agency.

1. "Report of Director General," *WHO, WHO Chronicle*, 1977, **31**, 207.
2. "22nd WHO Expert Committee Report," *Tech. Rept. Ser.*, 1976, No. 585.
3. Steck, E. A., *The Chemotherapy of Protozoan Diseases*, U.S. Government Publication, Washington, DC, USA, 1972.
4. Kagan, I. G., *Exp. Parasitol.*, 1972, **31**, 126.
5. Sadun, E. H., In Cohen, S. and Sadun, E., Eds. *Immunology of Parasitic Infections*, Blackwell Scientific Publication, Oxford, 1976, p. 47.
6. Voller, A., Huldt, G., Thors, G. and Engvall, E., *Brit. Med. J.*, 1975, **1**, 659.
7. Kohler, G. and Milstein, C., *Eur. J. Immunol.*, 1976, **6**, 511.
8. Mitchell, G. F., Cruise, K. M., Chapman, C. B., Anders, R. F. and Howard, M. C., *Aus. J. Exp. Biol. Med. Sci.*, 1979, **57**, 287.
9. Mackey, L., McGregor, I. A. and Lambert, P. H., *Bull. Wld. Hlth. Org.*, 1980, **58**, 439.
10. Avraham, H., Spira, D. T., Gorsky, Y. and Sulitzeanu, D., *J. Immunol. Methods*, 1980, **32**, 151.
11. Yoshida, R., Nussenzweig, R. S., Potocnjak, P., Nussenzweig, V. and Aikawa, M., *Science*, 1980, **71**, 207.
12. Pratt, D. M. and David, J. R., *Nature*, 1981, **291**, 581.
13. Cohen, S., *Proc. Roy. Soc. B*, 1979, **203**, 323.
14. WHO Report on "Development in Malaria Immunology," *WHO Tech. Rept. Ser.*, 1975, p. 579.
15. Capron, A., Capron, M. and Dessaint, J. P., In *Progress in Immunology IV*, M. Fongereau and J. Dausset, Eds., Acad. Press, 1980, p. 782.
16. Mehta, K., Sindhu, R. K. and Subrahmanyam, D. and Nelson, D. S., *Clin. Exp. Immunol.*, 1980, **41**, 107.
17. —, —, —, Hopper, K., Nelson, D. S. and Rao, C. K., *Immunology*, 1981, **43**, 117.

18. Hopper, K., Mehta, K., Subrahmanyam, D. and Nelson, D. S., *Clin. Exp, Immunol.*, 1981, (in press).
19. Ogilvie, B. M. and Wilson, R. J. M., *Brit. Med. Bull.*, 1976, **32**, 177.
20. Spira, D. T., Golenser, J. and Gery, I., *Clin. Exp. Immunol.*, 1976, **24**, 139.
21. Faubert, G. M. and Tanner, C. E., *Immunology*, 1975, **28**, 1041.
22. Pelley, P. R., Ruffier, J. J. and Warren, K. S., *Infect. Imm.*, 1976, **13**, 1176.
23. Farah, F. S., Lazary, S. and DeWeck, A. L., *Immunology*, 1976, **30**, 629.
24. Mansfield, J. M. and Wallace, J. H., *Infect. Imm.*, 1974, **10**, 335.
25. Portaro, J. K., Britton, S. and Ash, L. R., *Exp. Parasitol.*, 1976, **40**, 438.
26. Mehta, K., Sindhu, R. K., Subrahmanyam, D. and Rao, C. K., *Indian J. Med. Res.*, 1980, **72**, 38.
27. Ottesen, E. A., Weller, P. F. and Heck, L., *Immunology*, 1977, **33**, 413.
28. Gray, A. R., *Trans. R. Soc. Trop. Med. Hyg.*, 1975, **69**, 131.
29. Brown, K. N., *Parasites in the Immunized Host, Mechanisms of Survival*, Porter, R. and Knight, J., Eds., Elsevier–North Holland, 1974, p. 33.
30. Smithers, S. R., Terry, R. J. and Hockley, D. J., *Proc. R. Soc.*, 1969, **171**, 483.
31. Houba, V. and Lambert, P. H., *Adv. Biosci.*, 1974, **12**, 617.
32. Zuckerman, A. and Spira, D. T., *J. Inf. Dis.*, 1961, **108**, 339.
33. Ottesen, E. A., Neva, F. A., Paranjape, R. S., Tripathy, S. P., Thiruvengadem, K. V. and Beaven, M. A., *The Lancet*, 1979, p. 1158.
34. Piessens, W. F., Ratiwayanto, S., Tuti, S., Palmieri, J. H., Piessens, P. W., Koiman, I. and Dennis, D. T., *New Engl. J. Med.*, 1980, **302**, 833.
35. Mitchell, G. H., Butcher, G. A. and Cohen, S., *Immunology*, 1975, **29**, 397.
36. Trager, W. and Jensen, J. B., *Science*, 1976, **193**, 673.
37. Siddiqui, W. A., Taylor, D. W., Kan, S., Kramer, K., Richmand-Crum, S. M., Kotani, S., Shiba, T. and Kusumoto, S., *Ibid.*, 1978, **201**, 1237.
38. —, Kan, S. C., Kramer, K., Case, S., Palmer, K. and Niblack, J. F., *Nature*, 1981, **289**, 64.
39. Miller, L. H., *J. Inf. Dis.*, 1977, **135**, 855.
40. Subrahmanyam, D., In *International Symposium on 'Nuclear Techniques in the Study and Control of Parasitic Diseases of Man and Animals'*, International Atomic Energy Publication, Vienna, 1981.
41. Kilejian, A., *Science*, 1978, **201**, 922.
42. Rajasekariah, G. R., Mitchell, G. F. and Rickard, M. D., *Int. J. Parasitol.*, 1980, **10**, 155.
43. Scott, M. T. and Snary, D., *Nature*, 1979, **282**, 73.
44. Mehts, K., Sindhu, R. K. and Subrahmanyam, D., *Acta Tropica*, 1981, (in press).
45. Singer, M. F., In *Genetic Engineering*, Setlow, J. K. and Hollander, A., Eds., Plenum, New York, 1979.
46. Gait, M. J., *Nature*, 1979, **277**, 429.
47. Philipp, M., Parkhouse, R. M. E. and Ogilvie, B. M., *Ibid.*, 1980, **287**, 538.

THE CHARLES A. LINDBERGH FUND INC—LINDBERGH GRANTS

The fund annually presents grants of up to $ 10,580 (cost of Charles Lindbergh's "Spirit of St. Louis" in 1927) to individuals whose research strives to create a better balance between technology and the environment. All disciplines of study are represented. The deadline for making application for a 1982 grant is November 16th 1981. Grant recipients will be announced in late January.

The fund welcomes candidates who are affiliated with an academic or non-profit institution, as well as individuals having a record of practical accomplishment. Citizens of all countries are eligible.

Proposals will be evaluated on a competitive basis with emphasis on the research's practical application in resolving conflicts between technological growth and the preservation of our environment. An independent grants review panel screens the applications and make recommendations to the Lindbergh Fund's board of directors. The board makes final selections.

1982 Lindbergh Grant announcements may be obtained by contacting Gloria S. Parkins, Administrator, The Charles A. Lindbergh Fund, Inc, 99, Park Avenue, New York, N.Y. 10016 (212) 688-3451.