PROGRESS TOWARD THE DEVELOPMENT OF VACCINES AGAINST PARASITIC DISEASES : A REVIEW BASED ON STUDIES PERFORMED BY PARTICIPANTS OF THE LAVERAN SEMINAR

LIANCE M.

Summary :

Although significant advances have been made during the past 20 years, a better understanding of the orchestration of the immune response against a parasite candidate vaccine is still required for preparing vaccines which can induce the acquisition and the persistence of sterile immunity, without producing side effects in humans. This was, among others, one conclusion of the discussions between Thesis students and scientists. Only some aspects of the different steps of immune responses (recognition, protective effector mechanisms, cell regulation, cytokine activities, immunopathology, parasite escape mechanisms) are here reviewed. They are mainly related to malaria and schistosomiasis, but also to other parasitic diseases, for which several experimental models have been developed.

KEY WORDS : vaccination. peptides. immunogenicity. parasitic diseases. cell-mediated immunity. immunopathology. parasite escape mechanisms.

Résumé : ÉTAT ACTUEL DE L'IMMUNOPROPHYLAXIE CONTRE LA PATHOLOGIE INDUITE PAR LES PARASITES : UNE REVUE BASÉE SUR LES TRAVAUX DES PARTICIPANTS DU SÉMINAIRE LAVERAN

Malgré les progrès réalisés au cours des 20 dernières années, la compréhension des différentes composantes de la réponse immunitaire dirigée contre un antigène parasitaire à potentiel vaccinant demeure incomplète. Elle est pourtant un prérequis pour espérer pouvoir traiter une population humaine par une molécule qui induirait une immunité stérilisante de longue durée, et qui n'aurait pas d'effet délétère. C'est l'une des conclusions retenue après les débats qui ont eu lieu entre de jeunes chercheurs et des scientifiques confirmés. Certains aspects seulement des étapes de la réponse immunitaire (reconnaissance, mécanismes protecteurs, régulation cellulaire, activités des cytokines, processus pathologiques, évasion parasitaire) sont ici passés en revue, à propos de divers modèles parasitaires, en particulier à propos du paludisme et de la bilharziose, et en fonction des hôtes, pour la plupart encore expérimentaux.

MOTS CLÉS : vaccination. peptides. immunogénicité. parasitoses. immunité cellulaire. immunopathologie. évasion parasitaire.

INTRODUCTION

The second Laveran seminar entitled "Cellular immunity to parasites and fungi : effector mechanisms, immunoregulation, immunopathology", was held in "Les Pensières", Annecy (Conferences Center of Fondation Marcel Mérieux, the property of Charles Mérieux), under a giant photograph of Pasteur and colleagues, in 1-4 March 1994. It was attended by 25 invited participants, bringing together Thesis students and scientists to examine the progress and the difficulties met in this field. This paper outlines some of the multiple strategies undertaken in order to either reduce the pathology induced by parasites, or to optimize an anti-parasite vaccine (for partially, at least, preventing infections). In the past, the quest for parasite-vaccinating molecules was essentially directed toward the cloning of potentially protective polypeptides, and expression in multiple vectors. Effectively, it is agreed that a non living, defined vaccine would be easy to produce on a large scale by recombinant DNA technology, as well as being ethically acceptable in human populations.

Laboratoire de Parasitologie, Faculté de Médecine, 6 Rue du Général Sarraill, 94000 Créteil, France – Téléphone: 49 81 36 11 – Téléfax: 49 81 36 01

However, it is also necessary to examine the degree of discrimination between self and nonself vaccinating structures of the immune system, and to subsequently analyse the potential protective and deleterious activities of these candidate vaccines. Thus, a broad range of topics related to different steps of immune responses during various parasitic diseases was addressed.

DATA ON MOLECULE VACCINES AND THEIR RECOGNITION

Several structural and biological characteristics of an ideal molecule vaccine were described.

In the first instance, such a molecule must impair the parasite life cycle. Immunization with the whole molecule of the 28 kDa *Schistosoma mansoni* glutathione S-transferase (SM28 GST) not only reduces the worm burden independently of its enzymatic activity (implied in the detoxification of free radicals), but also reduces female fecundity and egg viability, these results being linked to the inactivation of the enzymatic site (Capron, 1992). Numerous studies have

concerned the antigens of *Plasmodium falciparum*. Irradiated-sporozoite-induced protection may depend more on antigens appearing when the parasites enter the hepatocyte, than on antigens present on the sporozoite. The liver stage antigen 3, specific to both liver stages and sporozoites, induces the production of antibodies, both in natural human malaria and in immunized mice. These antibodies exert an inhibitory effect on the entry of the sporozoite in cultured human hepatocytes, as they inhibit the entry of *P. yoelii* in murine hepatocytes (Pasquetto, pers. comm.). The erythrocyte-binding antigen (EBA-175) may be a receptor acting as a "bridge" between erythrocytes and merozoites (Camus & Hadley, 1985), and some of its specific antibodies obtained from immunized mice inhibit erythrocyte invasion by this parasite stage.

Exogenous proteins require degradation to linear peptide sequences and an effective binding of these peptides to the polymorphic major histocompatibility complex (MHC) class II molecules on the surface of antigen presenting cells (APC) is requisite for activating T helper (Th) cells. Thus, molecule vaccines must have a functional agretope i.e., the site of contact between a peptide antigen and the MHC, in a wide range of population. In this way, the SM28 GST appears to be a promising molecule vaccine as it induces protective immunity in rodents as well as in cattle and in primates (Boulanger *et al.*, 1991; Bushara *et al.*, 1993). MHC restriction of immune recognition has been described for *Plasmodium* derived peptides injected to mice and an association of low antibody responders to HLA-DR4 antigens during human malaria vaccine trials also indicate the need to abolish or reduce the MHC restriction of recognition (Banic *et al.*, 1994). According to C. Leclerc, we must do better than nature (e.g., the parasites ?) does. One strategy for increasing the diversity of peptide sequences that can be presented without affecting the corresponding functionally independent epitope is the creation of "mixagretopes" (pool of an epitope analogues). Thus, considering that the central parts of 2 out of 8 derived synthetic peptides of SM28 GST serve as epitopes, amino acids located at the C- and N-terminus of the peptides have been substituted by others. Preliminary results show that spleen cells from mice immunized with the mixagretope derived from the peptide 190-211 can be stimulated by the initial synthetic peptide (Ferru, pers. comm.). Other chemical modifications can be used to increase the antigenicity of peptides. Artificial constructions of antigens could theoretically avoid the requirement of a carrier molecule : in this way, a multiple construction of 8 copies of the peptide 115-131 of the SM28 GST was successfully realized for *in vitro* proliferation tests (Auriault *et al.*, 1991). It has also been suggested that

lipid chains participate in stabilizing the antigen-MHC-T cell receptor complex at the APC surface during presentation, and coupling of palmitic acid to peptides of SM28 GST increases the duration of the T cell sensitization. Immunization of rabbits with such a coupling to EBA 175-peptide 4 is, in some cases, followed by an early, intense and long-lasting antibody response (Gilardeau, pers. comm.). But the level of protection against a challenge infection can also be improved using adjuvants, some of them (BCG, aluminium hydroxyde) being authorized for human use.

Responses toward epitopes i.e., sites for contact between a peptide antigen and the T cell receptor, could depend on the processing by distinct APC, because of their potential functional heterogeneity for Th subsets (release of different stimulatory signals). The respective functional importance of dermal dendritic or Langerhans cells and macrophages during the infection with *Leishmania major* is currently under study (Belkaïd, pers. comm.). Responses toward epitopes are also related to genetic factors : as Th cells are MHC class II restricted in their interactions with B cells, and as cytotoxic T cells are MHC class I restricted, their ability to induce T and/or B cell immunity would be analysed with regards to the effector mechanisms involved in each disease. It was reported that the SM28 GST and some of its synthetic peptides induce both T and B cell responses in infected rats, mice, as well as in humans (Auriault *et al.*, 1991). The genetic status can also influence T cell responses after immunization, as was shown or suggested, using congenic strains of mice, regarding to the SM28 GST (Auriault *et al.*, 1991) and the EBA 175-peptide 4 (Gilardeau, pers. comm.), respectively.

In order to protect the majority of individuals, it also appears suitable to encourage the development of molecule vaccines expressed i) on the different stages of a parasite : such is the case for the SM28 GST, an excretory/secretory molecule brought to the adult and the larval forms, where it is transiently expressed (Balloul *et al.*, 1987) ii) detected in most parasite isolates of a same species, as is the p126 *P. falciparum* antigen (Banic *et al.*, 1994) iii) and expressed on different parasite species, as is the SM28 GST, present in all species of schistosomes so far examined (Bushara *et al.*, 1993).

EFFECTOR MECHANISMS OF HOST-PROTECTIVE IMMUNITY

The immune system uses many mechanisms for attacking pathogens, but not all of these are activated after either infection or immuni-

zation. This finding, partially at least, explains the necessity of long-term research to optimize vaccine development. Moreover, contrasting results are often obtained according to the experimental model used, and these models were considerably discussed during all the seminar time. For instance, how can *in vitro* studies concerning the phagocytosis of yeasts by macrophages be conducted? One methodology was described, using heat-killed yeasts, and a colorimetric technique that allows the discrimination between ingested and adsorbed yeasts by host cells (Giannimis *et al.*, 1993; Giannimis, pers. comm.).

During schistosomiasis, antibody-dependent cell-mediated cytotoxicity (ADCC) appears to be the main mechanism of killing parasites (cytotoxic destruction of schistosomulum targets) both in rat and human. These ADCC systems involve macrophages, eosinophils, and platelets as cellular partners and IgE, anaphylactic subclasses of IgG, and IgA as humoral components, while in humans, anti-SM28 GST IgG4 correlates with the lack of immunity and appears as blocking antibodies (Auriault *et al.*, 1991; Capron, 1992). Anti-SM28 GST IgA *in vitro* inhibit worm fecundity, and the importance of human IgA antibodies was underlined in Kenyan patients in whom high IgA serum levels are associated with a reduced number of faecal eggs (Renom, pers. comm.). Mice provide an accurate model for studying liver granulomas and fibrosis due to schistosomiasis. In these hosts, the protective immunity mainly involves antibody independent cellular effector mechanisms (Auriault *et al.*, 1991). As reported by C. Auriault, after immunization with the SM28 GST, an infection is characterized by a reduced parasite burden, a decreased amount of liver collagen, and no change in the humoral responses. This protection would be, at least partially, mediated by IFN- γ produced by CD8⁺ cells.

During toxoplasmosis, cell-mediated immunity is also believed to be the major protective mechanism. Cells of the intestinal epithelium are the initial targets for parasites released from cysts or oocysts. In rats with an acute infection, enterocytes not only may act as APC, but also as effector cells, when activated by IFN- γ , in preventing tachyzoite replication (Dimier et Bout, 1993). Preliminary *in vitro* experiments suggest that this enterocyte toxicity is also depending on sensitized intestinal CD8⁺ T cells (Lepage, pers. comm.).

In malaria, it was initially demonstrated that antibodies directed against a major component of the surface of the sporozoite inhibit the parasite development, but it appears now that liver-stage parasites are an important target of cell effector mechanisms (Renia *et al.*, 1991). Immunization of mice with the Py1 peptide of *P. yoelii* led to an activation of lymph node CD4⁺ and CD8⁺ T cells which

are able to eliminate liver-stage parasites *in vitro*. Immunization does not protect mice against a challenge with sporozoites, but some Th1 and Th2 clones specific for the peptide confer a significant degree of protection in BALB/c mice. The mechanism(s) by which T lymphocytes eliminate their targets remains not well understood. Nevertheless, it is suggested that CD4⁺ cells exert a direct antiplasmodial activity (without the participation of IL-6 and IFN- γ), mediated through antigen presentation within MHC class II molecules. Indeed, the parasite elimination from cultured hepatocytes is reversed when protective cell clones are analyzed in the presence of some anti-class II antibodies (Renia *et al.*, 1991, 1994). Other antigens, heat shock-like proteins, are expressed on the infected hepatocyte surface. They are the target of ADCC, involving as cell killers the non-parenchymal liver cells (Mazier *et al.*, 1990). It has also been suggested that macrophages and specific IgG1 participate in such mechanisms (Marussig, pers. comm.). Non-specific factors are also involved in protective immunity, and it has been shown that, among others, C-reactive protein protects against pre-erythrocytic stages of malaria (Mazier *et al.*, 1990).

IMMUNOREGULATION OF HOST EFFECTOR MECHANISMS

The identification by Mosmann and colleagues of CD4⁺ Th1 and Th2 cell subsets a few years ago can explain partially, at least, the lack of efficient vaccines so far developed against the major parasitic diseases. They are characterized by the secretion of different lymphokines and by their functional behaviour: Th1 cells promote the synthesis of IgG2a, can mediate delayed-type hypersensitivity and activate macrophages whereas Th2 cells promote the synthesis of IgG1, IgE, and IgA antibodies (Germann *et al.*, 1993). It is now established that a dysregulated expansion of one or the other subset may be one reason for the development of protective immunity or pathology. Among factors involved in the differentiation of T cells, genetic factors can determine the development of humoral or cell-mediated immunity. This was demonstrated during infection with *L. major*, using mice with two distinct backgrounds. Although the situation appears more complex at an early stage, T CD4⁺ cells from the resistant C57BL/6 mice produce IFN- γ and IL-2 (secreted by Th1 cell clones), whereas cells from susceptible BALB/c mice produce IL-4 and IL-10 (secreted by Th2 cell clones) at 5 weeks p.i. It appears that IFN- γ has a central role in the activation of macrophages to kill the intracellular amastigotes (Heinzel *et al.*, 1991). Conversely, Th2

responses are associated with resistance against the infection with *Trichuris muris* (Hermanek *et al.*, 1994). Although diseases are often associated with a dominant Th cell phenotype, in alveolar echinococcosis, for example, Th1 and Th2 responses appear associated throughout the infection (Emery, pers. comm.). The site of antigenic challenge, the adjuvants used, and the genetics of the host, are among many factors that can bias Th cell responses in experimental models (Bancroft, 1993). For instance, Th1 or Th2 cell clones can be preferentially obtained from mice immunized with the Py1 peptide according to their background (Renia *et al.*, 1991, 1993). It is obvious that parasite influences are also involved in the T cell differentiation toward the one or the other Th phenotypes. During murine infection with *S. mansoni*, worms stimulate Th1 cell responses (as it is the case after vaccination with irradiated cercariae) whereas eggs would induce Th2 cell responses, as suggested by a low IL-4 and IL-5 production during single sex infections (Capron, 1992). These observations highlight the importance of a better understanding of this aspect of host-parasite relationships if effective immunoprophylaxis with synthetic peptides is likely to require reversal of a dominant pattern of Th cell response. Regarding the host, the regulation of the type of an immune response is mediated by two major signals. IL-12, mainly produced by monocytes, has a major role in CD4⁺ T cell differentiation toward a Th1 phenotype and induces IFN- γ production by natural killer cells (NK) and T cells. On the contrary, IL-4, mainly produced by basophils and mastocytes, not only promotes Th2 cell development but also inhibits the differentiation of Th1 cells (Scott, 1993). What can be done in order to polarize a response specific to a parasite (or to a protective peptide derived parasite) toward Th1 or Th2 cells? According to C. Leclerc, several strategies for inducing Th1 responses may be used. Administration of IL-12 or of recombinant bacteria inducing IL-12 (such as BCG) may stimulate specific Th1 T cells. Administration of neutralizing anti-IL-4 antibodies may also be helpful to abrogate Th2 cells development. But because cells producing different cytokines are not uniformly located, the results of such immunomanipulations must be examined in different organs, or at least in the spleen, the most important organ in lymphocyte recirculation. Preliminary results of *in vivo* treatments with anti-IL-4 antibodies were reported. In toxoplasmosis, the survival of mice is increased, despite an unmodified parasite burden (Villard, pers. comm.). During the early stage of cutaneous leishmaniasis, the number of dermal macrophages is reduced, but not that of Langerhans cells (Belkaid, pers. comm.).

Other cell types than T lymphocytes are involved in immunoregulation during the course of several parasitic

diseases. NK cells are triggered by *Toxoplasma gondii* and *L. major* (among other pathogens) to produce significant levels of IFN- γ (Bancroft, 1993). There is no evidence for a NK-mediated cytotoxicity in mice infected with *S. mansoni* (Abbe *et al.*, 1983), whereas IFN- γ appears to have an important role in the resistance of immunized mice. It could be postulated that NK cell cytokine release is involved in the regulation of the formation of the hepatic granulomas in this model, and preliminary results seem to indicate that a non specific *in vivo* NK activation increases the survival of infected mice, and that *in vitro* specifically activated NK produce IFN- γ (Asseman, pers. comm.).

DELETERIOUS EFFECTS OF IMMUNE RESPONSES

The effector molecules induced by the immune response can be very dangerous in contributing to the pathology associated with various parasitic infections. This data indicates the necessity of the analysis of immunodominant sites on antigens in order to avoid the use of those inducing deleterious host reactions.

In man as in mouse, an over-production of TNF is a crucial event in the pathogenesis of cerebral malaria. It experimentally appears to be the result of a cascade of CD4⁺ T cell derived cytokines, IL-3, GM-CSF and IFN- γ (Grau *et al.*, 1990). TNF could induce nitric oxide (NO) from endothelial cells, smooth muscle cells, macrophages, and subsequently, by neurons. NO, a potent effector molecule of macrophage activity against parasites in mice, also mediates neuro-transmission, and it could contribute to increase intracranial pressure, through vasodilatation of cerebral vessels. Moreover, the coma reversibility of human cerebral malaria could be explained by a reduced generation of NO in the central nervous system (Clark *et al.*, 1991). The involvement of NO in murine cerebral malaria pathogenesis is under study and, at present, it is not confirmed by the measurement of nitrate and nitrite in the urine of *Plasmodium* infected mice. Treatment with competitive inhibitors of nitric oxide synthase (the enzyme that generates NO from L-arginine) also fails to demonstrate its role (Asensio *et al.*, 1993). Conversely, in humans, preliminary results suggest that an over-production of NO is associated with the severity of the disease (Asensio, pers. comm.).

Because vaccines against tetanus and diphtheria toxins are among the most effective, because non living defined vaccines are ethically more acceptable in human population, and because a solid immunity against *Plasmodium* sp. is difficult to induce (Edelman *et al.*, 1993), J.H.L. Playfair concentrates his work on

the pathological mechanisms responsible for several clinical forms of the disease and on the development of an anti-toxic vaccine. Parasitised blood supernatants induce TNF release from mouse macrophages or human blood monocytes, and the factors involved in this release are phospholipid molecules. Mice immunized with sub-toxic doses of these "toxic exoantigens" produce antibodies which block the TNF induction. 60 % of mice immunized and challenged with the lethal *P. yoelii* recover with very high parasitaemias and without appearing ill. But malaria is a multifactorial disease, as the malarial hypoglycaemia itself is probably due to several cytokines and possibly to the direct actions of insulinomimetic malarial antigens. Mice, although "protected against early mortality" die later of anaemia. Other problems are encountered in developing vaccines from these antigens. Firstly, they induce predominantly IgM antibodies and there is no evidence for a memory response. So, an attempt is made to confer T-dependence. Secondly, their similarity to host molecules suggests the possibility of inducing autoimmunity (Bate *et al.*, 1994).

PARASITE REGULATORY PROCESSES OF HOST IMMUNE EFFECTOR MECHANISMS

All parasites have evolved numerous ways of evading the consequences of immune attack. These escape mechanisms could explain the loss of protective immunity after immunization. P.H. David described numerous survival mechanisms falling into five broad categories.

Some parasites hide from their host. Nematodes defy immune responses to survive for long periods in otherwise immunocompetent hosts with their disposable surface coat. Protozoa choose to enter into a favourable host cell type. So, *T. gondii* can survive in macrophages because of the inhibition of lysosome symbiosome fusion, and *Leishmania* sp. resides in the parasitophorous vacuole of the same cell, because it highly appreciates the acidity of this intracellular compartment. Sequestration of *P. falciparum* in the postcapillary venules of various organs, although responsible for the severe forms of cerebral malaria and thus for the host death, would also possibly be a way of evasion.

Parasites may deceive their host. They disguise themselves to look like the host (uptake of host DNA or surface acquisition of host proteins). They exhibit diverse forms of the same antigen found in various natural isolates (*P. falciparum*), and in modifying or deleting a gene for an antigen after a vaccination trial (*P. knowlesi*). The presence of many crossreacting epitopes among *Plasmodium* antigens may result in a

low-affinity antibody production and may delay the emergence of protective immunity. Antigenic variation in *Plasmodium* and *Trypanosoma* appears to be a primary mechanism of immune evasion that allows the parasite to maintain a chronic infection, and that may have evolved to ensure that the parasite survives between transmission seasons. That many candidate vaccines are genetically diverse is one other reason for suggesting, as emphasized by C. Auriault *et al.* (1991), that an ideal future vaccine will probably require the combination of several peptidic epitopes of parasite antigens.

Parasites destroy or inactivate the host response. Schistosomes release different neuropeptides which exert suppressive activities on cell populations (Capron, 1992). Such an immunosuppressive phenomenon was described during the course of the cattle infection with *Hypoderma* sp. A serine protease is secreted, which not only induces an anti-inflammatory reaction around larvae, but also impairs the proliferative responses of peripheral blood mononuclear cells (Chabaudie et Boulard, 1992). It is not yet known if this enzyme directly reduces the T cells IL-2 production, but it increases the monocytes production of PGE₂, a prostaglandine known to induce immunosuppression (Nicolas-Gaulard, pers. comm.).

Parasites also take advantage of the host. As in mice with the severe combined immunodeficiency phenotype, female schistosome egg production is promoted by injections of TNF, it can be postulated that schistosomes exploit the host pro-inflammatory cytokine for their own survival. Moreover, J.H.L. Playfair has suggested that cytokines possibly could act as growth factors for parasites. *Plasmodium* parasite multiplication in the salivary glands of mosquitoes could be enhanced when insects are fed with blood containing antibodies, and immunization with a malaria heat shock like protein could enhance transmission to the mosquito by increasing the number of circulating sexual stages in mice (Marussig, pers. comm.).

Parasites sometimes divert the host responses, inducing different cytokine profiles from stage to stage of the infection, as *S. mansoni* does, and they lastly take flight, as added by P. Ambroise-Thomas, some of them changing their host, some other free living, the last changing their reproductive processes.

CONCLUSION

Significant advances have been reported. They mainly concerned 2 major worldwide parasitic diseases: malaria, the most lethal, and schistosomiasis (chronic and debilitating disease), although contrasting results were shown according to the expe-

rimental model used. The seminar was not intended as an overview of cellular immunity during infections with long-lived parasites, but rather to highlight specific areas of current interest, said P. Ambroise-Thomas. He pointed out that the seminar provided a clear illustration of the enthusiasm of trainees for their respective fascinating study models, whereas numerous investigations made during more than 20 years were only partially successful. He also acknowledged them for their modesty, seriousness, and humour. The seminar also provided an environment where individuals could meet from various topics, and a rare opportunity for trainees to discuss their results at length, and debate on their projects with eminent scientists. Finally, D. Camus emphasized the training and scientific interests of this second Laveran seminar, and hoped by this time next year some definite progress will be reported.

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REFERENCES

- ABBE T., FORBES J.T. and COLLEY D.G. : Natural killer cell activity during murine *Schistosomiasis mansoni*. *Journal of Parasitology*, 1983, 69, 1001-1005.
- ASENSIO V.C., OSHIMA H. and FALANGA. : *Plasmodium berghei* : Is Nitric Oxide Involved in the Pathogenesis of Mouse Cerebral Malaria ? *Experimental Parasitology*, 1993, 77, 111-117.
- ASENSIO V. : Le neuroludisme: analyse des mécanismes immunitaires pathogènes dans un modèle expérimental. Personal communication, 1994.
- ASSEMAN C. : Rôle des cellules NK dans la pathologie à *Schistosoma mansoni*. Personal communication, 1994
- AURIAULT C., WOLOWCZUK I., GRAS-MASSE H., MARGUERITE M., BOULANGER D., CAPRON A. and TARTAR A. : Epitopic characterization and vaccinal potential of peptides derived from a major antigen of *Schistosoma mansoni* (Sm28 GST). *Peptid Research*, 1991, 4, 6-11.
- BALLOUL J.M., GRZYCH J.M., PIERCE R.J. and CAPRON A. : A purified 28,000 dalton protein from *Schistosoma mansoni* adult worms protects rats and mice against experimental schistosomiasis. *Journal of Immunology*, 1987, 138, 3448-3453.
- BANCROFT G.J. : The role of natural killer cells in innate resistance to infection. *Current Opinion in Immunology*, 1993, 5, 503-510.
- BANIC D.M., DELPLACE P., MAZINGUE C. and CAMUS D. H-2b restriction of the immune response to the p126 *Plasmodium falciparum* antigen. *Clinical and Experimental Immunology*, 1994, 95, 472-478.
- BATE C., KWIATKOWSKI D.P., TAVERNE J. and PLAYFAIR J.H.L. : Immunizing Against Toxic Malarial Antigens : Reply. *Parasitology Today*, 1994, 10, 24.
- BELKAID Y. : Définition d'un modèle expérimental pour analyser les mécanismes précoces d'induction différentielle de réponses T-dépendantes de type 1 ou 2 à un agent pathogène intra-cellulaire strict *Leishmania major*. Personal communication, 1994.
- BOULANGER D., FRIED G.D.F., STURROCK R.F., WOLOWCZUK I., BALLOUL J.M., GREZEL D., PIERCE R.J., OTIENO M.F., GUERRET S., GRIMAUD J.A., BUTTERWORTH A.E. and CAPRON A. : Immunization of mice and baboons with the recombinant Sm28GST affects both worm viability and fecundity after experimental infection with *Schistosoma mansoni*. *Parasite Immunology*, 1991, 13, 473-490.
- BUSHARA H.O., BASHIR M.E.N., MALIK K.H.E., MUKHTAR M.M., TROTTEIN F., CAPRON A. and TAYLOR M.G. : Suppression of *Schistosoma bovis* egg production in cattle by vaccination with either glutathione S-transferase or keyhole limpet haemocyanin. *Parasite Immunology*, 1993, 15, 383-390.
- CAMUS D. and HADLEY T.J. : A *Plasmodium falciparum* antigen that binds to host erythrocytes and merozoites. *Science*, 1985, 230, 553-556.
- CAPRON A. : Immunity to schistosomes. *Current Opinion in Immunology*, 1992, 4, 419-424.
- CHABAUDIE N. and BOULARD C. : Effect of hypodermin A, an enzyme secreted by *Hypoderma* (Insect Oestridae), on the bovine immune system. *Veterinary Immunology and Immunopathology*, 1992, 31, 167-177

ABBE T., FORBES J.T. and COLLEY D.G. : Natural killer cell

- CLARK I.A., ROCKETT K.A. and COWDEN. : Proposed Link Between Cytokines, Nitric Oxide and Human Cerebral Malaria. *Parasitology Today*, 1991, 7, 205-207.
- DIMIER I.H. and BOUT D.T. : Rat intestinal epithelial cell line IEC-6 is activated by recombinant interferon- γ to inhibit replication of the coccidian *Toxoplasma gondii*. *European Journal of Immunology*, 1993, 23, 981-983.
- EDELMAN R., HOFFMAN S.L., DAVIS J.R., BEIER M., SZTEIN M.B., LOSONSKY G., HERRINGTON D.A., EDDY H.A., HOLLINGDALE M.R., GORDON D.R. and CLYDE D.F. : Long-term persistence of sterile immunity in a volunteer immunized with X-irradiated *Plasmodium falciparum* sporozoites. *Journal of Infectious Diseases*, 1993, 168, 1066-1070.
- EMERY I. : Analyse séquentielle des réponses des lymphocytes spléniques au cours de l'échinococcose alvéolaire hépatique expérimentale. Personal communication, 1994.
- FERRU I. : Etude des mécanismes de protection à médiation cellulaire induits par des constructions peptidiques de l'antigène Sm28GST de *Schistosoma mansoni*. Personal communication, 1994.
- GERMANN T., GATELY M.K., SCHOENHAUT D.S., LOHOFF M., MATTNER F., FISCHER S., JIN S.C., SCHMITT E. and RÜDE E. : Interleukine-12/T cell stimulating factor, a cytokine with multiple effects on T helper type1 (Th1) but not on Th2 cells. *European Journal of Immunology*, 1993, 23, 1762-1770.
- GAIMIS J., LOMBARD Y., FONTENEAU P., MULLER C.D., LEVY R., MAKAYA-KUMBA M., LZADINS J. and POINDRON P. : Both mannose and β -glucan receptors are involved in phagocytosis of unopsonized, heat-killed *Saccharomyces cerevisiae* by murine macrophages. *Journal of Leucocyte Biology*, 1993, 54, 564-571.
- GAIMIS J. : Contribution à l'Étude de la phagocytose de *Saccharomyces cerevisiae* par le macrophage en l'absence d'opsonines. Personal communication, 1994.
- GILARDEAU M. : Contrôle génétique de la réponse immune vis-à-vis de l'antigène EBA 175 de *Plasmodium falciparum*. Personal communication, 1994.
- GRAU G.E., PIGUET P.F., VASSALLI P. et LAMBERT P.H. : Immunopathologie des lésions cérébrales du paludisme. *Médecine/Sciences*, 1990, n° spécial, 52-58.
- HEINZEL F.P., SADICK M.D., MUTHA S.S. and LOCKSLEY R.M. : Production of interferon-gamma, interleukine 2, interleukine 4, and interleukine 10 by CD4⁺ lymphocytes *in vivo* during healing and progressive murine leishmaniasis. *Proceedings of the National Academy of Sciences, USA*, 1991, 88, 7011-7015.
- HERMANEK J., GOYAL P.K. and WAKELIN D. : Lymphocyte, antibody and cytokine responses during concurrent infections between helminths that selectively promote T-helper-1 or T-helper-2 activity. *Parasite Immunology*, 1994, 16, 111-117.
- LEPAGE A. : Prévention de la toxoplasmose humaine et animale. Etude des mécanismes immunitaires effecteurs de la protection anti-*Toxoplasma gondii* après infection par voie orale. Personal communication, 1994.
- MARUSSIG M. : Etude des mécanismes effecteurs interférant avec le développement hépatique des plasmodies. Personal communication, 1994.
- MAZIER D., GOMA J., PIED S., RENIA L., NUSSLER A., MILTGEN F., MATTEI D. and GRAU G. : Hepatic phase of malaria : a crucial role as "go-between" with other stages. *WHO bulletin*, 1990, 68, supplement, 126-131.
- NICOLAS-GAULARD I. : Etudes des mécanismes moléculaires au cours de l'immuno-dépression induite par *Hypoderma* spp. (Varon). Personal communication, 1994.
- OGASAWARA K., NARUSE H., ITOH Y., GOTOHDA T. and ARIKAWA J. : A strategy for making synthetic vaccines. *Proceedings of the National Academy of Sciences, USA*, 1992, 89, 8995-8999.
- PASQUETTO V. : Etude *in vitro* des mécanismes effecteurs de l'immunité contre les stades pré-érythrocytaires de *Plasmodium falciparum*. Personal communication, 1994.
- RENIA L., GRILLOT D., MARUSSIG M., CORRADIN G., MILTGEN F., LAMBERT P.H., MAZIER D. and DEL GIUDICE G. : Effector functions of circumsporozoite peptide-primed CD4⁺ cell clones against *Plasmodium yoelii* liver stages. *Journal of Immunology*, 1993, 150, 1471-1478.
- RENIA L., SALONE MARUSSIG M., GRILLOT D., PIED S., CORRADIN G., MILTGEN F., DEL GIUDICE G. and MAZIER D. : *In vitro* activity of CD4⁺ and CD8⁺ T lymphocytes from mice immunized with a synthetic malaria peptide. *Proceedings of the National Academy of Sciences, USA*, 1991, 88, 7963-7967.
- RENOM G. : Approche épitopique fonctionnelle de la réponse IgA spécifique au cours de l'infection humaine par *Schistosoma mansoni*. Personal communication, 1994.
- SCOTT P. : IL-12 : initiation cytokine for cell-mediated immunity. *Science*, 1993, 260, 496-497.
- VILLARD O. : Rôle de l'interleukine 4 endogène chez la souris C57BL/6 au cours de la toxoplasmose aiguë. Personal communication, 1994.