

## VACCINE STRATEGIES AGAINST SCHISTOSOMIASIS

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*In this review the authors analyze the effector and regulatory mechanisms in the immune response to schistosomiasis. To study these mechanisms two animal models were used, mouse and rat. The mouse totally permissive host like human, show prominent-T cell control in the acquisition of resistance. But other mechanisms like antibody mediated cytotoxicity (ADCC) involving eosinophils and IgG antibodies described in humans, are observed in rats. Also in this animal, it is observed specific IgE antibody high production and blood and tissue eosinophilia.*

*Using the rat model and schistosomula as target, some ADCC features have emerged: the cellular population involved are bone marrow derived inflammatory cell (mononuclear phagocytes, eosinophils and platelets), interacting with IgE through IgE Fc receptors.*

*Immunization has been attempted using the recombinant protein Sm28/GST. Protection has been observed in rodents with significant decrease of parasite fecundity and egg viability affecting the number, size and volume of liver egg granulomas. The association of praziquantel and immunization with Sm28/GST increases the resistance to infection and decreases egg viability. The authors suggest the possibility of the establishment of a future vaccine against Schistosoma mansoni.*

Key words: schistosomiasis – immunity regulation – vaccine strategies

Schistosomiasis is most extensively explored, not only because of the high prevalence of the infection in endemic areas and its health consequences, but also because the fascinating biological features of the life cycle and the characteristics of the infection, associating permanently resistance and pathology, constitute a particularly attractive model of a chronic transmissible disease. Considered in terms of biological features the most relevant to immunology, the life cycle of schistosomes indeed offers a series of events that promote a close interaction between the parasite and the immune system: active cutaneous penetration of infective larvae and their transformation into schistosomula, complex migratory cycle in the vertebrate host, intravascular localization and blood feeding habits of adult worms, egg deposition in mucosae, liver, and various tissues, and prolonged survival in immune vertebrate hosts. These prominent characters of a vertebrate in-

fection have elicited numerous immunological research in three major areas: (a) effector mechanisms of resistance; (b) mechanisms of parasite survival and regulation of host immune system; (c) immune mechanisms of pathogenesis. In each of these research areas, novel mechanisms have been uncovered, leading to more general concepts bearing implications far beyond the specific field of schistosomiasis. From rodent models to man, these studies have allowed promising approaches, from bench to bedside, towards the possible immunological control of one of the major human parasitic diseases, by pointing to which parasite antigen may induce protection and which component of the immune response should be elicited by the immunization schedule.

### RELEVANCE OF EFFECTOR MECHANISMS IN EXPERIMENTAL MODELS TO IMMUNITY IN HUMANS

The study of effector mechanisms of acquired resistance in experimental schistosomiasis has been marked for many years by a

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debate regarding the relevance of the two major models used, the mouse and the rat. The mouse, a totally permissive host like humans, has initially appeared as the most suitable model and an animal of choice for immunological studies. Extensive studies have clearly established the prominence of T cell-mediated immunity in the acquisition of resistance. In this model indeed, macrophage activation through its classical lymphokine-dependent pathway appears as the essential component of protective immunity, while the role played by the antibody response has remained equivocal and controversial (James, 1986). The existence of antibody-dependent cell-mediated cytotoxicity (ADCC) involving eosinophils and IgG antibodies described in humans by Butterworth et al. in 1975, which was not observed in the mouse, led some groups to consider the use of the rat as a model in which ADCC could be easily characterized. Although this animal model has an important drawback, since schistosomes do not reach their complete sexual maturity and are spontaneously eliminated three weeks after the infection, the strong and prolonged resistance acquired after worm rejection constitutes an alternative situation for an analysis of the effector mechanisms underlying resistance, and this approach is conformed by the demonstration in the rat model of three immunologic hallmarks of human response to schistosomes: high production of specific IgE antibodies, blood and tissue eosinophilia, and existence of ADCC mechanisms (Capron & Capron, 1986).

Extensive studies performed in our laboratory have attempted to a detailed analysis of the humoral components and the cellular partners involved in *in vitro* killing of target schistosomula. Two major features of these cytotoxicity mechanisms have emerged. One, contrary to other immunological situations where ADCC has been described, the cellular partners involved are not lymphoid cells but rather bone marrow-derived inflammatory cell populations: mononuclear phagocytes, eosinophils, and platelets. Second, the major antibody isotypes cooperating with the effector cell are identified either as IgE for each of the three cell populations and also in the particular case of eosinophils as a subclass of IgG with anaphylactic properties, i.e. rat IgG2a (Capron et al., 1978, 1981; Capron et al., 1975; Joseph et al., 1983).

The interaction of IgE with these inflammatory cells led our laboratory and other groups

to demonstrate the expression by a subset of each of these cell populations of a Fc receptor of low affinity for IgE, which we named IgE Fc Receptor, type II (FcεR<sub>II</sub>) (Capron et al., 1986), a denomination now extended to IgE Fc receptors borne by subsets of B and T cells with the alternative name of CD23 (Delespesse et al., 1988). Genes encoding the FcεR<sub>II</sub>/CD23 receptor have been cloned for B cells, and characterized in macrophages and an eosinophil cell line (Kikutani et al., 1986; Yokota et al., 1988). The precise molecular structure of FcεR<sub>II</sub> on eosinophils and platelets is still under investigation. These studies besides bringing a new conceptual approach of the participation of these cells in IgE-dependent allergic situations (Dessaint et al., 1990), indicated that effector mechanisms unravelled in rat schistosomiasis could also be considered in man. Numerous convergent studies indeed indicated that IgE immune complexes can induce, both in rats and men, cell activation and trigger the release of many cytotoxic mediators, including reactive oxygen intermediates, PAF and, in the case of eosinophils, major basic protein and eosinophil peroxidase, all of which could be implied in schistosomula killing (Capron & Dessaint, 1985). Accordingly, IgE antibody-dependent cell-mediated cytotoxicity could be confirmed in human schistosomiasis (Joseph et al., 1978, 1983; Capron et al., 1984). The essential question brought by these novel *in vitro* ADCC mechanisms was related to the implication, so far unsuspected, of anaphylactic antibody isotypes, and specially IgE, in mechanisms of protection against metazoan parasites. The production of monoclonal anti-schistosome antibodies of the rat IgE and IgG2a isotypes led to the demonstration of their high protective capacity by passive transfer (Grzych et al., 1982; Verwaerde et al., 1987). Together with diminished protection passively conferred by IgE-depleted immune rat serum and abrogation of immunity after anti-μ and anti-ε-antibody treatment of neonate rats (Bazin et al., 1980; Kigoni et al., 1986), evidence was then accumulated that IgE, at least in rats, could have a more beneficial function than being mainly involved in deleterious allergic manifestations.

Although, as discussed above, many *in vitro* evidence in human schistosomiasis and *in vivo* experiments in the rat model argue for a role of IgE in protective immunity to schistosomes, it was difficult to extrapolate from experimentally demonstrated mechanisms in rats that



harbour schistosome infection for a few weeks to the situation in endemic areas where human subjects may be infected for decades. It is only recently that three sets of epidemiological studies have brought convergent evidence for a protective role of IgE antibody in human infection. Studying the rate of reinfection after treatment in a community exposed to *Schistosoma haematobium* infection in the Gambia, Hagan et al. (1991) have demonstrated a positive correlation between the specific IgE antibody response to worm antigen and the acquired resistance to reinfection. Multiple regression analyses show in particular that the risk of reinfection is ten times more likely when IgE antibodies are absent or in the lowest quintile. Similar findings have been made in a study by A. E. Butterworth and ourselves in Kenya in a community exposed to *S. mansoni* infection as well as by A. Dessein in Brazil (personal communication).

Without excluding the participation of additional mechanisms, specific IgE antibody response appears therefore as a strong correlate of protective immunity in humans, confirming the views we have expressed for many years regarding the novel function of this class of antibody. The function of IgE antibodies in triggering clearance of invading schistosomula may be related to their ability to activate Fc $\epsilon$ R<sub>II</sub>-positive effector cells in ADCC reactions as observed *in vitro*, but other mechanisms cannot be excluded. Through its classical interaction with Fc $\epsilon$ R<sub>I</sub>-positive mast cells, IgE can induce the discharge of potent mediators with strong vascular effects, and some of these such as PAF can recruit and/or activate eosinophils, mononuclear phagocytes, and platelets. Besides, in response to Fc $\epsilon$ R<sub>I</sub>-mediated activation, mast cells have recently been found to release various cytokines that can stimulate IgE and eosinophil production (Gordon et al., 1990). In this respect, IgE antibodies may be involved both in the initiation and effector arms of the protective response to invading schistosome larvae.

Two even more general concepts emerge from these studies of a parasitic model. One regards the evolutionary significance of IgE and the reasons of its maintenance at hormone-like levels in normal subjects, which is hardly understandable if only to induce explosive pathological reactions, but may be related to its role in defence against metazoan parasites poorly susceptible to other immune effectors.

This points also to the general significance of isotopic selection, which is obviously one of the key issues in our understanding of the regulation of the functional expression of antibody responses. This mechanism of isotype selection is indeed a prominent feature of the antibody response to helminth parasites, and now appears as one of the major guidelines in the definition of a vaccine strategy (Capron et al., 1987; Capron & Dessaint, 1988). Recent findings indicate that specific cytokines control isotype-specific antibody responses, switch and selection of the IgE isotype being dictated by the preferential production of interleukin-4 (IL-4) and suppressed by gamma-interferon (IFN- $\gamma$ ). Thus, the basic question of the induction and regulation of protective immunity to schistosomes has now to be addressed in terms of how the parasites can stimulate or prevent T cell responses that lead to the secretion of specific cytokines.

By using T cell subset-specific monoclonal antibodies in mice and rats, Phillips et al. (1987a, b) have brought evidence that depletion of CD4<sup>+</sup> T cells decreases the level of resistance to challenge as well as antibody, delayed-type hypersensitivity, and eosinophilic responses, but increases morbidity in mice. Conversely, depletion of CD8<sup>+</sup> T cells reduced susceptibility, indicating that this lymphocyte subset had a suppressive effect on CD4<sup>+</sup> cells. In the mouse where the division of CD4<sup>+</sup> T cell clones into IL-2 and IFN- $\gamma$  producing Th1 and IL-4 and IL-5 producing Th2 subsets is advocated, it has been shown that CD4<sup>+</sup> T lymphocytes from *S. mansoni*-infected animals secrete high levels of IL-4 and IL-5 but no IL-2 or IFN- $\gamma$  in response to parasite antigens (Scott et al., 1989). Although IL-5 production, which is particularly involved in the regulation of eosinophil production, continues after the peak in IL-4 responses (Grzych et al., 1991). It should be stressed that we do not fully understand the stimuli that control the production of a particular subset of cytokines by T cells. It is moreover difficult to extrapolate observations on the preferential induction of a precise T cell subset from mice to men, since most human T cell clones do not appear to segregate into specific profiles of interleukin secretion (Paliard et al., 1988). It is nevertheless already clear that the net ratios of cytokines produced may play complex roles in promoting or blocking protective immunity and immunopathological aggression of the infected host.

## REGULATORY MECHANISMS THAT CONTROL THE EXPRESSION OF HOST-PROTECT IMMUNITY

Immune responses to schistosomes are characterized by their marked complexity and intertwining of host-protective and parasite-protective components. The very fact that schistosomiasis in a chronic infection indicates that the worms have the possibility to suppress or evade host-protective immune response, and many experimental observations have unravelled the diversity of their evasion tactics (Capron & Dessaint, 1989). From an immunological standpoint, the regulation of ongoing responses can be considered at three levels.

A first series of mechanisms responsible for the partial immunity observed in schistosomiasis is regulation of the protective response at the level of immune recognition. Natural infection and immunization of mice lead to the production of anti-idiotypic antibody cascades that result in a down-regulation of the expression of cellular and humoral immune responses to the parasite. Auto-anti-idiotypic responses have been shown to suppress responses to discrete antigenic epitopes and the expression of specific antibody idiotypes or T cell clonotypes (Phillips et al., 1990). Contrariwise, anti-idiotypic immunization by protective antibodies has been demonstrated to induce protective immunity (Grzych et al., 1985). Thus, idiotype regulation can represent an efficient regulatory mechanism that, progressively during the course of a chronic infection, tunes on or off the lymphocyte clones involved in the development of protection. Likewise in chronic human schistosomiasis, antibodies to egg antigens have been found to trigger anti-idiotypic specific suppressor T cells: differences in antibody idiotypes among patients suggest that idiotype regulation of responsiveness to egg antigens plays a role in determining the severity of the disease (Montesano et al., 1989).

Again at the level of specific recognition by T cells, induction of suppressor T cells in mice infected by *S. japonicum* appears to depend on the presentation of parasite antigens by *I-E* major histocompatibility complex molecules (Stavitsky, 1987). The role played by antigen presentation in determining T cell responsiveness is also shown in humans; HLA-DQ and DR allotypes being linked to resistance and susceptibility (Hirayama et al., 1987).

A second series of mechanisms that controls the expression of protective immunity is regulation of T cell proliferation. Disturbed T cell function in mice infected by *S. japonicum* has been related to decreased responsiveness to IL-1 (Yamashita et al., 1989). Parasite-derived factors may be directly involved in such unresponsiveness. In particular, adult schistosomes have been shown to release a low molecular weight inhibitor capable of selectively blocking T cell proliferation (Mazingue et al., 1987). Besides, schistosome eggs injected to infected or vaccinated mice do suppress their IFN- $\gamma$  response attributed to Th1 cells (Pearch et al., 1991). Since IFN- $\gamma$  inhibits proliferation of mouse Th2 cells and IL-10 reciprocally inhibits Th2 cells, the expression of resistance in mice might reflect the balance in CD4<sup>+</sup> Th1 and Th2 cell clones, and that this balance could be modified not only by their reciprocal regulatory influences, but also directly by the parasite itself (Scott et al., 1989).

Finally, another mechanism that regulates the expression of protective immunity stems from isotypic regulation itself. Evidence for the selective production of defined antibody classes during the course of experimental schistosome infection in rats raised questions about the function of other isotypes shown not to be directly implied in killing pathways. The decrease in immunity observed at certain periods of the infection in rats indeed is not related to a sharp decrease in antibody production, but is concomitant with the appearance of non-anaphylactic IgG subclasses. A representative IgG2c monoclonal antibody was shown to inhibit the capacity of an IgG2a monoclonal antibody both to induce eosinophil-dependent killing of schistosomula *in vitro* and to confer passive protection *in vivo*. The concept of blocking antibody was supported by the observation that this IgG2c antibody can inhibit the recognition by the protective IgG2a antibody of the carbohydrate moiety of a major surface glycoprotein of schistosomula described as gp38 (Grzych et al., 1984). Blocking antibodies could also be characterized in murine schistosomiasis (Omer-Ali et al., 1988).

The possibility that a similar phenomenon might be important in humans infected by *S. mansoni* was first indicated by the observation that susceptibility to reinfection after treatment of schoolchildren is significantly correlated with the presence of high levels of antibodies that inhibit the binding to the major gp38 schistoso-



mulum surface antigen of the protective monoclonal IgG2a antibody. In addition, IgM and IgG2 antibodies isolated from the sera of various individuals directly block the eosinophil-dependent killing of schistosomula mediated by IgG antibodies from the same sera, and IgM antibodies with specificity for schistosomulum surface antigens are present in higher levels in the young, susceptible children than in the older, resistant subjects (Khalife et al., 1986; Gordon et al., 1990). More recently, analyzing the isotypes of the antibody response to a recombinant protective protein (Sm28/GST) and its derived synthetic peptides, we found a significant correlation between susceptibility to reinfection to *S. mansoni* in humans and the increased production of IgG4 antibodies to this protective schistosome antigen (Sm28) and its defined B cell epitopes (Auriault et al., 1990). Consistently, in the framework of his studies on resistance to *S. haematobium* infection, Hagan et al. (1991) have also shown a clear correlation between IgG4 antibody response to schistosome antigens and increased susceptibility to reinfection.

Collectively, the main message from these studies is that in human schistosomiasis blocking antibodies are important components of the clinical expression of acquired resistance at its early stages, and afterwards, clinical expression of immunity is positively correlated to the presence of detectable IgE antibody responses to schistosomes. Such indications are obviously in the heart of the design of defined anti-schistosome vaccine.

#### CONCEPTUAL BASIS OF A VACCINE STRATEGY

Considered as a whole, these observations and the confrontation of studies in experimental models, particularly the rat, and in infected humans point to three major messages.

The first relates to the selection of potentially protective antigens. A major emphasis has been given in the past to the identification in various parasites of stage-specific antigens, but as far as schistosomiasis is concerned, increasing evidence arising from experimental studies in laboratory animals or epidemiological surveys in human populations supports to the original views of Smithers & Terry (1969) and their description of concomitant immunity. The molecular basis of concomitant immunity obviously implies that identical or cross-reactive epitopes are expressed at various stages of the parasite life cycle. Common

antigens or common epitopes on both the adult stage of schistosomes, as the major stimulus of the protective immune response, and on invading schistosomula as the privileged targets of its expression appear thus as essential components for the induction of an optimal protection. In this respect, the selection of antigens or cross-reactive epitopes common to several stages, rather than stage-specific molecules, has to be favoured (Capron & Dessaint, 1988).

The second message concerns the respective roles played by surface versus excretory-secretory molecules in the induction of a protective immune response. Although the efficiencies of effector mechanisms, either cellular or humoral or both, implies the accessibility of surface-associated molecules. Considered in terms of their immunogenicity in experimental infection or in natural human infection, schistosomula integral membrane proteins do not necessarily represent the optimal vaccine candidates, contrary to excreted-secreted proteins. It is striking in this respect that by using antibody probes from immunised or infected animals and humans, the majority of over thirty different schistosome genes so far cloned, encode proteins which are either enzymes or non surface structural proteins which are transiently expressed on the worm surface.

Finally, the concept of isotype selection which we have developed since 1976 appears as an essential factor for the expression of optimal resistance to reinfection both in experimental models and in humans. As mentioned above, the dynamic balance in chronic schistosomiasis between effector and blocking antibody isotypes against the same molecule appears to account for the variable expression of immunity. Among effector antibody isotypes, IgE and IgA appear to act on different functional targets of the parasite. The optimal induction through immunisation of the appropriate antibody isotype is therefore of primary importance to the success of any vaccine strategy. Our approach towards this goal is however presently hampered by our limited knowledge of the basic immunological mechanisms driving isotype selection, and by empirical criteria in the choice of appropriate adjuvants.

#### ADVANCES TOWARDS VACCINE STRATEGIES AGAINST SCHISTOSOMIASIS

On the basis of these concepts, we have developed during the last five years exten-

sive investigations aiming at the identification and molecular characterization of potentially protective molecules against schistosomiasis. By using rat monoclonal or polyclonal antibody probes of defined isotypes, selected for their protective activity by passive transfer experiments in rats, several molecules have now been selected.

The first schistosome protective molecule to be identified was a glycoconjugate described as gp38, the target antigen of a rat anti-*S. mansoni* monoclonal IgG2a antibody isotype shown to be strongly cytotoxic *in vitro* in an ADCC system involving eosinophils, and highly protective by passive transfer (Grzych et al., 1982). Studies of the glycoprotein indicated that the protective epitope was expressed on its carbohydrate moiety. An anti-idiotypic vaccine, based on the production of an Ab<sub>2</sub> monoclonal antibody to the IgG2a monoclonal, bearing the internal image of its epitope, conferred a significant degree of protection on immunized rats (Grzych et al., 1985). The glycanic epitope could be characterized, besides schistosome surface gp38 molecule, in adults on a 115 kDa non-surface glycoprotein that is excreted by the worm, as well as in cercariae (at 130 kDa), miracidia (30-300 kDa) and even in the intermediate snail host *Biomphalaria glabrata* (Dissous & Capron, 1983; Dissous, et al., 1986). This glycanic structure was also expressed in various fresh water snails and in the hemocyanin of an ancestral marine mollusc, *Megathura crenulata*, well known by immunologists as keyhole limpet hemocyanin (KLH). The KLH oligosaccharide has been purified and shown to exert a strong protective activity against schistosome infection in rats (Grzych et al., 1987).

Following these initial studies, the genes encoding several schistosome proteins have been cloned in our laboratory, among which one of them, initially named P28 (Balloul et al., 1987), appears as a very promising vaccine candidate. After its successful cloning in collaboration with Transgene, P28 was identified as a glutathione-S-transferase (GST) (Taylor et al., 1988), and distinct in its molecular structure of a GST recently cloned from a *S. japonicum* cDNA library (Davern et al., 1987). Sm28/GST has been expressed in various vectors, including *E. coli*, *Saccharomyces cerevisiae*, and the vaccinia virus. Vaccination experiments performed with the highly purified native protein indicated a level of protec-

tion close to 70% in rats, 50% in the mouse and in hamsters. Immunization performed with the recombinant protein in the presence of aluminium hydroxyde in hundreds of rodents confirmed the initial results and led to a mean of 60% protection in rats and 45% in mice (Balloul et al., 1987; Capron et al., 1987). On this basis, several vaccination experiments were undertaken in baboons and a very significant protection, up to 80%, could be obtained in some animals. However, a large degree of individual variation was noticed and the mean protection observed was 42% (Capron et al., 1987; Boulanger et al., 1991).

During these experiments, our attention was drawn to the existence, even in the very partially protected animals, of a significant decrease in the size and volume of egg granulomas in the liver, whereas a mean reduction of 68% of fecal egg output per female worm and per day was noticed. Similar observations were made in the monkey *Patas patas* immunized against *S. haematobium* infection, and a marked difference was shown between immunized and control animals in terms of urinary bladder lesions using ultrasound tomography.

The optimization of our vaccine strategy can now be summarized as follows: (a) Considering the operational importance of a single shot vaccine we have demonstrated in rats that a single injection of 25 micrograms of Sm28/GST in the presence of alum or BCG could induce levels of protection equivalent to the multidose schedule. (b) We have attached particular importance to the comparison of B and T cell epitopes of Sm28/GST recognized after immunization and by schistosomiasis patients. Extensive studies have allowed the identification of several major epitopes common to rats, mice and men. The identification of a particular sequence, 115-131, as a major helper T cell epitope involved in the induction of the IgE antibody response has led to the construction of an "octopus" (8 copies of the sequence coupled to a lysine core) which is highly immunogenic and protective (Auriault et al., 1990). (c) In order to understand the role played by the enzymatic function (GST) of Sm28/GST, we have produced a monoclonal antibody that inhibits the enzymatic activity. Results obtained both *in vitro* and *in vivo* indicate that, together with a significant protection against challenge (60%), a dramatic reduction in egg laying and egg viability is conferred by this neutralizing antibody. In con-



trast, another protective monoclonal antibody, but which does not inhibit the enzymatic activity, had no effect on egg production and viability. (d) On the basis of this information, a major emphasis has now been given to the effect of immunization with Sm28/GST on egg laying and viability. We have now established that besides its protective activity against challenge infection, vaccination with Sm28/GST strongly affects parasite fecundity and egg viability. In order to optimize this effect, the possible synergy between immunization and Praziquantel treatment has been explored, since chemotherapy and host-immunity appear to synergize in the destruction of schistosomes (Brindley & Sher, 1987). The immunization followed by Praziquantel treatment leads to both an increase in resistance to reinfection and to a dramatic decrease (94%) in egg viability, whereas a 70% reduction in the number and surface density of liver granulomas. (e) Immune effector mechanisms underlying the effect of immunization on worm fecundity have been explored. Whereas protection against challenge infection is mainly IgE-mediated, evidence has been obtained that IgA antibodies play a significant role in reducing worm fecundity and egg viability. These findings have been recently substantiated by the observation in human populations of a significant correlation between the IgA response to Sm28/GST and its derived peptides and the acquisition of immunity, as assessed by the decrease in parasite egg output. Recent results obtained in cattle schistosomiasis confirm that a major effect of immunisation with schistosome GST is to very significantly reduce egg output and egg viability. In the framework of immunization experiments performed in Sudan in collaboration with Martin Taylor (LSHTM) and Ahmed Bushara (Veterinary Faculty Khartoum), it was indeed shown that the vaccination of calves with *S. bovis* GST led to a dramatic reduction in egg production (83%) and acquisition of resistance to a lethal infection.

We have attempted, in this review mostly based on the work performed in our laboratory during the last 15 years, to show how extensive analysis of effector and regulatory mechanisms of the immune response to schistosomes in a model, the rat, relevant to human schistosomiasis, might lead in a near future to a promising approach towards a vaccine against one of the major human parasitic diseases.

In a disease where the humoral compo-

nents of the immune response appear to play a significant role, the understanding of the distinct functions played by the various antibody isotypes that are produced is crucial. If one accepts the view that driving appropriate isotypic selection or preferential isotype induction is a major goal to achieve, appropriate ways of optimal immunogen presentation by the rational use of adapted adjuvants obviously represent key issues. On the basis of all these experiments and of the recent epidemiological informations confirming the views which we have expressed for many years regarding the protective role of IgE and the relevance to man of the rat model in which these mechanisms have been established *in vitro* and *in vivo*, we are now encouraged to move to phase I human trials, which will be undertaken during the next few months.

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