

NETHERLANDS SOCIETY FOR PARASITOLOGY

AUTUMN MEETING WITH INVITED SPEAKERS BECAUSE OF THE 25TH ANNIVERSARY
OF THE SOCIETY.

Bilthoven, October 24, 1986

PARASITISM: AN ANALYSIS OF SURVIVAL STRATEGIES OF HELMINTHS

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Genetics and immunity are important factors in the survival of helminths. Both parasite transmission and survival of parasites in host populations are influenced by a number of factors, including the genetically determined immune responsiveness of the host. The major histocompatibility complex (MHC) determines both the survival of the host species and of the individual. Survival of the host species is based on so called class I molecules regulating the effector phase of the immune response, whereas the individual is protected by class II molecules responsible for the central phase of the immune response, including antigen presentation, T-cells and immunoglobulin production. MHC polymorphism is important for the survival of the species, since one MHC type gives a survival advantage against one infectious agent, but increased susceptibility to another.

On the molecular level MHC polymorphism results in an immune response which is either effective, leading to low morbidity or mortality or if not effective to high morbidity or mortality.

These differences will probably result in variations in ability to regulate parasite production and survival and thus the transmission in the host population. In human, domestic animals and wild populations epidemiological studies are essential to assess the relevance of these aspects. Pertinent data may however, be obtained from well controlled model studies in mice (Wakelin 1985). The following systems will be presented (1) *Taenia taeniaeformis* (model for larval tapeworms of man and domestic animals), (2) *Trichuris muris* (model of *Trichuris trichiura* in man) and (3) *Trichinella spiralis* (model for *Trichinella* and other gastro-intestinal nematodes of man and domestic animals).

It will be concluded that genetically determined differences in protective immunity do exist, which may play a role in parasite transmission. These differences should be taken into account when (a) developing mathematical models for host-parasite relationships and (b) suggesting intervention strategies for helminth infections of human and veterinary importance. Of particular relevance is the qualitative difference between responder ('non wormy') and non-responder ('wormy') populations or individuals. If the non-response is genetically determined, the question becomes relevant whether immune intervention by vaccination is at all feasible.

From a general biological view point studies on immune responses and genetics will lead to a better understanding of the factors influencing host-parasite relationships, including behaviour, ecology and natural resistance.

References

Wakelin, D. Genetics, immunity and parasite survival (1985). In: Ecology and Genetics of Host-Parasite Interactions. The Linnean Society of London, pp. 39-54.

TRENDS IN SCHISTOSOMIASIS RESEARCH

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This summary will briefly outline the research on schistosomiasis carried out in our laboratory over the past decade in relation to "general trends" in schistosomiasis research.

Since highly effective and safe drugs like oxamniquine and praziquan-
tel have become available, a significant reduction of prevalence of
schistosomiasis or even an eradication by effective chemotherapy alone
has now theoretically become possible. Nevertheless, schistosomiasis
is in many areas of the world still rather increasing than decreasing,
and afflicts some 200 million people. Epidemiological research carried
out by our group in Zaïre and Burundi has been focussed on the effects
of mass chemotherapy, and on aspects of morbidity and transmission.
While epidemiological studies in the past have mainly dealt with basic
aspects of transmission, such studies are now being concentrated on
screening of populations to select for treatment those individuals or
groups at high risk, as long as complete eradication of the disease is
not feasible. For determination of the prevalence and intensity of in-
fections, the reliability of egg counts by parasitological techniques
has been studied, and recently serological techniques (determination
of circulating antigens) have also been implemented in this field of
research.

World-wide research on the analysis of the antigenic composition of
schistosomes has been intensified during the past decade. This re-
search is essential to obtain a better understanding of the immunolo-
gical aspects of the host-parasite relationship, to study the metabo-
lism of the parasite, and for developing improved immunodiagnostic
techniques. This research has been enormously stimulated by modern
biochemical and immunochemical techniques, including monoclonal anti-

body production and DNA-technology. Research in our group has been concentrated mainly on the analysis of excretory and secretory antigens, in particular on those antigens derived from the gut. From an immunodiagnostic point of view it appears that gut-associated antigens may be of interest both as target antigens for demonstration of specific antibodies and as candidates for demonstration as circulating antigens. With regard to the first aspect, several groups have now described a very pronounced antibody response against a 32 kDa gut-associated protein or glycoprotein in schistosome-infected individuals. The two best characterized circulating antigens, the proteoglycans CAA and CCA, are also derived from the gut. Using monoclonal antibodies, sensitive assays for the detection of these antigens have now been developed. A systematic comparison of these serological techniques and parasitological techniques for determining the worm burden is now being made.

The development of monoclonal antibodies against several gut-associated antigens has also stimulated further research on the involvement of such antigens in immunopathological processes in the host. Here again, a recently developed technique, application of colloidal gold labeled monoclonal antibodies in EM immunocytochemistry, has enabled us to study the precise localization of schistosome antigen in tissues (liver, kidney) of infected mice. Similar studies with human tissue material are now under way.

While gut-associated antigens have been found to be of particular interest for immunodiagnostic studies, and may possibly play a role in the immunomodulation of the host, several research groups have now conclusively shown that immunological effector mechanisms of the host are primarily directed against antigens of the tegument. It appears that in the rat model as in man, the antibody-dependent cellular cytotoxicity effector mechanisms directed against developing schistosomes are the main immunological components of an acquired resistance. Using a combination of monoclonal antibodies, DNA-technology and in vitro and in vivo cytotoxicity assays, several major antigens in protective immunity have been identified.

These include 22-26, 28 and 38 kDa antigens (Capron and co-workers), 24.5 and 27 kDa antigens (Butterworth and co-workers) and a 97 kDa antigen (Sher and co-workers). A significant degree of protection against a schistosome infection has been obtained in experimental animals either by immunization with purified antigen or anti-idiotypic antibody or by administration of specific monoclonal antibody.

It has been postulated that in man acquired immunity exists, but that the degree of immunity is dependent on the presence of blocking antibodies which interfere with protective antibodies competing for the same antigenic determinants.

Further research on protective antigens may well lead to the production of a vaccine which will, at least, give a significant degree of protection against developing a heavy infection.

VACCINE DEVELOPMENT RELATED MALARIA RESEARCH: AN INTERIM REVIEW

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The development of malaria vaccines, is one of the objectives of the malaria research programme which is coordinated in the Special Programme of the World Health Organization. The successful introduction of a method for the *in vitro* cultivation of *Plasmodium falciparum* combined with the advances made in the fields of immunology and recombinant DNA technology justify this effort. The general strategy for vaccine development is:

- a. to identify by the use of polyvalent and especially monoclonal antibodies, those parasite proteins that are expressed on the surface of extracellular developmental stages and that are directly involved in the induction of protective immunity.
- b. to identify in cDNA and DNA expression libraries those bacterial clones that express immunologically relevant polypeptides of the parasite.

c. to perform nucleotide sequencing of the cloned DNA in order to establish the amino-acid sequence of the parasitic proteins.

Such information would offer three different possibilities for vaccine production: large scale cultures of selected bacterial clones would be used for producing the immunogen, oligopeptides based on the DNA code and representing the antigenic determinant of the immunogenic proteins would be synthesized and coupled to a suitable carrier, or the identified genes would be used to transform an apathogenic virus and following its inoculation the infected cells would produce the immunogen.

In the cycle of malaria parasites there are three extracellular developmental stages: the sporozoites, the asexual erythrocytic stages and the gametes. Studies are underway to identify immunogenic proteins or their immunogenic determinants in all three of these stages. A sporozoite vaccine would prevent infection. A vaccine directed against the asexual erythrocytic stages would prevent disease and also block the development of gametocytes, and the transmission blocking vaccines would induce the production of antibodies which would interfere in the sporogonic development in the mosquito midgut.

Sporozoite vaccines

Sporozoites carry a species specific surface protein, the circumsporozoite (CS) protein, which has been identified by monoclonal antibodies, that interfere with the sporozoite infection of hepatocytes *in vivo* and *in vitro*. The gene of the CS protein has been sequenced. The immunogenic epitope of the CS protein of *Plasmodium falciparum* consists of repeats of a four amino acid oligopeptide. A sequence of three of the repeats coupled to tetanus toxoid has been successfully used as an immunogen in rabbits for the production of protective anti-sporozoite antibodies. However, in experimental studies in rodents strong indications have been obtained that antibodies alone might not be able to effect the complete neutralization of a sporozoite inoculum. Nevertheless complete protection appears possible by the additive effect of gamma-interferon which is produced by specifically stimula-

ted T-cells. This lymphokine is able to inhibit the intracellular development of exoerythrocytic stages in liver cells. As a result of these studies it might well be that effective vaccines require the presence of correctly expressed B-cell- as well as T-cell epitopes. It appears that a sporozoite vaccine based on the product of a bacterial clone, incorporating the complete gene of the CS protein has, however, not yet been successful in phase I trials in man.

Vaccines against the asexual erythrocytic stages

A relatively large number of proteins produced by the asexual erythrocytic stages of *Plasmodium falciparum*, has been identified by the use of immune sera collected from people living in endemic malarious areas and by stage specific monoclonal antibodies. The possible relationship of these proteins with the induction of protective immunity is based on the ability of antigen specific immunoglobulins to inhibit the growth of *in vitro* cultures.

It is remarkable that many of the identified proteins are characterized by the presence of oligopeptide repeats. The number of these repeats as well as their composition varies and some proteins contain more than one set of repeats. These repeats are also involved in immunologic crossreactivity between various parasite proteins.

Little is known about the biological function of the target proteins. Some are involved in interaction between merozoites and erythrocytes, and between parasitized erythrocytes and endothelial cells. Some of them are specific for a parasite clone, some are isolate specific, or specific for a geographical strain. In addition there are proteins that show the phenomenon of phenotypic variation. Conserved antigenic determinants, however, that relate to the induction of protective immunity have not yet been identified. It might well be that the repetitive epitopes do not serve as inducers of protective immunity but that they contribute to the masking of the true relevant immunogenic epitopes. Non-repetitive immunogenic determinants of the proteins of these developmental stages might have been missed due to the immunodominance of the repeats.

Progress in this field is further hampered by the fact that candidate immunogens of the human malaria parasite cannot be efficiently screened *in vivo*. The use of scarce Aotus and Saimiri monkeys for this purpose offers no reliable alternative for studying the effect of immunization in man.

Transmission blocking vaccines

In vitro produced gametocytes of *Plasmodium falciparum* become extracellular within ten minutes following exposure to a drop in temperature. Monoclonal anti macrogamete antibodies have been developed. They can be characterized as transmission blocking or non-blocking in a natural system, i.e. by the screening for oocyst development in mosquitoes fed with *in vitro* grown fertile gametocytes to which the purified immunoglobulins had been added. These monoclonal antibodies have been used for the identification of two macrogamete proteins each of which contain at least two non-repetitive epitopes. One of these interacts with specific blocking monoclonal antibodies and the other with non-blocking monoclonal antibodies. The first protein is expressed on the macrogamete surface immediately after activation; blocking antibodies against this protein interfere in the fertilization process. The second protein is produced primarily after activation; it replaces the first protein and its interaction with the blocking monoclonal antibody causes lysis of the developing ookinete. Both proteins are glycoproteins, but the sugar is not a part of the "protection" related epitope.

A complicating factor in these studies is that the epitopes have a conformational nature. Consequently denatured gamete proteins do not react with the monoclonal antibody, nor do the latter react with the parasitic polypeptides produced by bacterial clones of expression libraries.

The induction of transmission blocking immunity is not as artificial as has been assumed some years ago. Transmission blocking antibodies have been demonstrated in human sera from people infected with *P.falciparum* or *P.vivax*. Their epidemiological significance is still being studied.

Epicrisis

In the present stage of malaria vaccine research the main activities are concentrated at the laboratory bench. Candidate immunogens will have to be tested however, under field conditions. This will require a thorough understanding of the local epidemiological conditions. Such preparatory field studies should answer the question as to what age groups should be vaccinated and assess the potential consequences of vaccination. Reduction of malaria transmission resulting in a retardation of the natural development of antimalarial immunity might result in an unacceptable situation, where more adolescent and adults become sick than before the introduction of the vaccine. The timely initiation of quantitative epidemiologic studies carried out during the preparatory phase at the sites where vaccination might appear a welcome additional tool in malaria control, should be an essential part of the control strategy.

It is impossible to predict when malaria vaccines will become available for field trials and if the ultimate vaccine will be based on proteins, specific for one of the developmental stages or on a combination of parasite immunogens.

TRYPANOSOMIASIS - A CHALLENGE TO THE BIOLOGICAL SCIENCES

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Introduction

Trypanosomiasis, a disease of man and animals, and caused by different *Trypanosoma* spec., has a worldwide distribution.

This review will confine itself to the tsetse transmitted trypanosomiasis of man and animals, south of the Sahara. In this area, the incidence of reported cases in man is $\pm 20,000$ a year, whereas ± 50 million people are at risk. The FAO estimates a potential of 120 million cattle, against a present population of 20 million. Lack of milk, meat, draught power, manure can therefore be limiting factors in rural development projects.

Biology of tsetse and trypanosomes

The research on tsetse flies has concentrated on their occurrence in different habitats, their rearing in the laboratory with feeding on blood through artificial membranes and the role of attractants by which the tsetse fly finds a vertebrate host.

Morphological grounds are not sufficient to distinguish subspecies of the trypanosomes, but the following techniques have been developed in recent years.

- The blood infectivity incubation test to distinguish human pathogenic trypanosomes from non-pathogenic ones.
- The iso-enzyme pattern.
- DNA-hybridisation.

These techniques have clearly demonstrated that trypanosomiasis is a zoonose, and contributed greatly to our epidemiological knowledge.

A few characteristic cell organelles of the trypanosomes are the surface coat and the kinetoplast. The kinetoplast is a conglomeration of mini- and maxi DNA circles. The maxi-circles contain genes for mitochondrion enzymes, and in their absence no cyclic development can take place. If cyclic development takes place, the trypanosomes undergo profound morphological and biochemical changes, e.g. loss of surface coat and development of an active mitochondrion. At the end of the cycle they move to the mouthparts of the tsetse fly and are known as metacyclic trypanosomes. Some trypanosomes have an affinity to the tissues (e.g. *T. brucei*) others are living in the blood. These tissue forms can undergo morphological changes and may lodge in the ependymal cells of the choroid plexus.

The tissue forms may cause relapses, which must not be confused with

relapses due to drug resistance.

Before trypanosomes are taken up by the tsetse fly, they have undergone already some morphological changes (including the mitochondrion), the so called short stumpy (ss) forms of *T. brucei* are capable to survive and multiply in the tsetse fly.

It is only in recent years, that the different species of trypanosomes and their different stages can be cultivated *in vitro*.

Antigenic variation

Parasitologists estimate that from one cloned trypanosome, more than 100 antigenic different trypanosomes can be derived. Biochemists estimate that the DNA of one single trypanosome contains information for \pm 1000 antigenic variants.

The antigenic variation is due to the ability of the trypanosome to change its surface coat, which consists of a variant surface glycoprotein (VSG). It is anchored in the plasma membrane by a complex phosphor glycolipid tail. The variant part of the VSG is localised in the N terminal, whereas the common part cannot be reached by antibodies. The shedding of the coat, probably regulated by a phospholipase, only takes place by the short stumpy forms of the trypanosomes. The released VSG is responsible for the inducement of immunity.

The metacyclic trypanosomes have a constant limited mixture of VSG's of which some are also manifested in the vertebrate host after the bite of the tsetse fly. The ingested VSG is also present in the early parasitaemia's. Other VSG's appear in a semi-ordered fashion.

The vertebrate host will often resist a rechallenge with the same serodeme, but this depends upon the amount of antigen induced and the stability of the antigenic composition of the metacyclic trypanosomes. Duplicative transcription, whereby an expression linked copy (ELC) gene is inserted in a telomeric expression site forms for the majority of the VSG's, the basis of their synthesis at the DNA level. The trigger mechanism for the expression of the various VSG genes is unknown. The expression of VSG's in the animal is also dependant upon host factors, population dynamics and location of the trypanosomes in the various organs.

The great number of VSG's and the instability of the metacyclic VSG's makes the development of a vaccine very unlikely.

The inducement of SS forms may perhaps open an alternative to assist the host in overcoming a trypanosomal infection.

Clinical symptoms, pathology and pathogenesis

Clinical symptoms can vary greatly and depend upon factors linked with the host and the trypanosomes.

Several days after the tsetse transmitted infection a local skin reaction develops, the so called "chancre". This is followed by a swelling of the regional lymphnode.

Disseminated intravascular coagulation is found in acute cases of trypanosomiasis. Thrombocytopenia, embolus formation, hyaline membranes in the lungs, oedema in various organs, and profuse bleedings can cause acute death in man and animals. Anaemia is one of the early and most persistent symptoms. Immune complexes may play a role in the pathogenesis of the anaemia and the glomerulo-nephritis.

Disturbance in the metabolism of aminoacids may lead to products which have influence on the state of consciousness of the patient.

Inflammatory reactions can cause a myocarditis, encephalitis or orchitis.

Severe immunosuppression can occur in laboratory animals, but care should be taken to extrapolate these findings to man and ruminants. Susceptibility to secondly bacterial invasion can complicate trypanosomiasis. An insufficient immunological response to vaccinations may be another consequence of the immunosuppression.

Although the pathogenesis is poorly understood, it has contributed to a better use of drugs and supportive therapy, a start to understand the mechanism of trypanotolerance in cattle and game and diagnostic tests (e.g. rise of IgM in man). It has not yet produced effective measures to control the anaemia, immunosuppression etc. The most reliable way to diagnose trypanosomiasis is to find the trypanosomes. In the last years the anion-exchange and haematocrit centrifuge method has made it possible to find low number of parasites.

Antibodies can be found by various tests, of which the card agglutina-

tion and haemagglutination are the most promising for use under field conditions.

Trypanotolerant cattle

In areas of West-Africa, cattle breeds like the N'Dama and West African Shorthorn can exist under a certain tsetse challenge, although high challenges will also cause their death.

The mechanism by which they control trypanosomiasis is not well known, but probably a poly genetic trait, related with their immune apparatus. A research for genetic markers so far has not been very successful.

The small size of the animal, the relative small number of animals in West Africa and their susceptibility for rinderpest, has greatly hampered their distribution. Recent investigations have shown, however, that if the "productivity index" is taken as a parameter for production, the N'Dama compares very well with Zebu cattle, even under light tsetse challenge.

Drugs

The ideal drug for trypanosomiasis still does not exist. One constraint is that some drugs do not penetrate the brain and those that penetrate can give serious (lethal) side effects during treatment. Another constraint is the problem of drug resistance, which has been reported from all over Africa. It has become apparent that trypanosomes have a number of features unique in nature. These include the glycerol-3-phosphate-oxidase system, the transport of enzymes to glycosomes, the structure of the kinetoplast DNA, anchorage of the surface coat to the cell membrane, the insertion of the ELC etc. Each of them represent an excellent target for the development of a new rational chemotherapy.

Economic considerations have resulted that in the last years no new drugs have come on the market.

Tsetse control methods

Killing of game, and massive movement of the human population were methods of the past to control the tsetse flies.

The major advances have been made by the application of insecticides on the vegetation. Provided they are carefully used, e.g. low doses of endosulfan and their impact on the environment carefully monitored, there is still a place for insecticides in the future. Ecologists participate more and more in control schemes.

The so called trap and target method, whereby the flies are attracted by an a simple screen and an attractant like acetone, and subsequently killed by an insecticide, has been developed on an experimental scale in southern Africa and may be the method of choice for the future.

The sterile male technique has been used successfully in large field experiments in certain parts of Africa, provided that the original population was first reduced by insecticides.

The number of tsetse species involved in transmission, the difficulties in rearing these species in large numbers in the laboratory, the high ratio required between wild and sterile flies and the ability of the sterile males to transmit trypanosomiasis, are all obstacles for a wide spread use.

In conclusion it can be said that trypanosomiasis offers a great challenge to the biological sciences. It is very unlikely that one control method will evolve out of all these efforts.

Whether the control of trypanosomiasis will ultimately lead to prosperity or an ecological disaster is, however, not only decided by the biological sciences.