Antigenic variation in African trypanosomiasis: a Memorandum*

After reviewing the present knowledge on antigenic variation of the trypanosomes of the Trypanosoma (Trypanozoon) brucei species, this Memorandum discusses the relevance of this phenomenon to the possible development of new tools for trypanosomiasis control.

As antigenic variation is related to protective immunity and immunopathology, it is of crucial importance for the feasibility of vaccine development and for treatment principles. It is also of interest as a model for understanding antigenic variation occurring during infections with Plasmodium knowlesi, Babesia, and others. Recent methods will permit in depth studies on the antigenic repertoire, the significance of basic antigens, and on the homogeneity of trypanosome populations. For epidemiological purposes, characteristic patterns of variation can be used for strain typing.

As regards the basic mechanism of variation, a better insight is required on the molecular structure of the variant antigens. Various methods have so far indicated that they are glycoproteins with a long polypeptide chain with 600 amino acids and 15-30 monosaccharide units.

The process of variation may be generated by pre-existing genetic information, recombination, or mutation.

The stimulus to change variants probably derives, directly or indirectly, from the host immune response, but may also be associated with other environmental factors.

The possible relation to acquired resistance, innate immunity, and host specificity, as well as the differences in severity of infection occurring amongst the same host species, are outlined. Histopathological and serological findings are considered in the light of the effect antigenic variation may have on the development of immunopathological lesions.

A series of recommendations is included.

Variation of antigens during the course of infection in the mammalian host is well recognized in the African trypanosomiasis. It probably protects parasites from the immune response of the host and so permits the maintenance of chronic infection. Taking into account that the transmission cycle of the Trypanosoma (Trypanozoon) brucei species is remarkably inefficient, the longevity of infections in mammals is assumed to be the essential mechanism for the continued existence of these parasites. At the same time, the repeated appearance of the antigens and the antibodies they induce may provide a basis for immunopathology and disease. Antigenic variation of trypanosomes has been reviewed in recent years by several authors (8, 16, 26, 35). The purpose of this memorandum is to review present knowledge, to identify significant gaps, and to define research priorities, particularly those where advantage could be gained from recent progress made in other branches of science.

There have recently been major advances in knowledge concerning cell surface membranes. Improved methods are now available for the study of membrane components, as, for example, by radiolabelling the surface macromolecules and by using immunofluorescence to demonstrate the location and movement of antigens in the membrane. There exist means to study the regulation of expression of surface antigens, including synthesis within the cell, and the genetic control of synthesis. These advances open up new possibilities for studies of African trypanosomiasis.

Such studies are relevant to improved control of disease for several reasons. Prevention of antigenic variation could permit the immune response to control the infection and/or obviate immunopathological damage. Associated with this is the question of whether immune protection can be

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acquired by natural infection or by vaccination. Experiments in animals have shown that protective immunity can be obtained against homologous challenge (7, 9, 10, 12, 31). Hence, antigenic variation is a crucial obstacle to further progress in vaccine development. Identification of variant types could also help to clarify the epidemiology of sleeping sickness in Africa. Finally, antigenic variation is not exclusive to T. brucei organisms, but is known to be a feature of other infectious agents, such as Plasmodium knowlesi and Babesia. Study of variation, at present most readily carried out in African trypanosomiasis, may therefore be relevant to the control of other infectious diseases.

THE PHENOMENON OF ANTIGENIC VARIATION

Methods for identification of surface antigens

Infections with salivarian trypanosomes are characterized by the appearance in the blood of the vertebrate host of a series of trypanosome populations that are antigenically different. Several serological methods have been used to distinguish between such populations, especially agglutination, lysis, neutralization, and gel diffusion tests (8, 16). While these tests are valuable for distinguishing between populations as a whole, they have the disadvantage that they fail to detect parasites with different surface variants when they are present in low proportions in a population.

Recent developments in immunofluorescence techniques now permit the demonstration of variants of individual trypanosomes. These studies can be carried out on trypanosomes in dried smears of blood (22), or on washed, intact trypanosomes in suspension (8). Variant-specific antisera for this work have been obtained either from rabbits after short-term infection with clones or from animals immunized with specific surface-coat glycoprotein.

Variants in relation to the life cycle

Shortly after infection of the tsetse fly, trypanosomes of the T. brucei complex lose their variant antigen. This is associated with loss of surface coat and infectivity to the mammalian host; similar changes occur when trypanosomes are put into culture in vitro. Surface coat and infectivity are re-acquired at the metacyclic stage of the developmental cycle in the fly (35). After cyclical transmission of any variant of the same strain by tsetse flies into a mammalian host, the first parasitaemias frequently comprise parasites that are identical with respect to surface variants, irrespective of the surface variant of the parasite infecting the fly. This surface variant of the initial parasitaemia is the so-called "basic antigen". It is not known whether metacyclic forms also represent a population uniform with respect to surface antigen, and whether this antigen is the same as the "basic antigen". It is also not known how many basic antigens occur within the T. brucei complex.

Studies with fly-transmitted trypanosomes have confirmed long-standing observations on infections with syringe-passaged trypanosomes that the sequence in which surface variants appear is ordered, i.e., early variants tend to appear in a similar sequence in different hosts. In the early stages of infection, new antigens are detectable at intervals of a few days, but it is possible that this rapid pace is not always maintained in the late stages of infection. It has been suggested (30) that the surface variants that appear in the blood originate in extravascular sites, but recent work has shown that organisms from tissue sites carry the surface antigen that had previously appeared on organisms in the blood (19). The host species may also influence this ordered sequence, and such an influence could be important when isolating populations of trypanosomes from naturally infected hosts and adapting them for maintenance in laboratory animals.

Antigenic variation has also been reported in T. congolense and T. vivax, and both these species in their bloodstream phase possess a surface coat that is lost on entering the fly. Metacyclic T. congolense re-acquires the surface coat, and reversion to a basic antigen has been reported, but these changes have not yet been demonstrated in T. vivax (35).

Diversity of the variant repertoire

The term "variant repertoire" is introduced to describe the different variants that may be generated from a single trypanosome. A total variant repertoire has never yet been determined; past assessments have been complicated by the fact that the populations studied may frequently have included organisms with more than one surface variant.

It has been shown that different isolates of trypanosomes of the subgenus Trypanozoon can produce similar surface variants even if they belong to different subspecies or originate from different hosts in different geographical regions. It has been reported that strains of T. brucei and T. gambiensese retain similar series of variants for periods of two years or more under field conditions. Other reports, how-
ever, have indicated major differences between the variant antigens of isolates of salivarian trypanosomes, even from the same locality. These topics have been reviewed by Gray & Luckins (16).

While such information is of considerable importance in relation to the situation in the field, it helps little in understanding the basic mechanisms involved in variation of surface antigens. The value of cloning trypanosome populations in studies of antigenic variation is widely recognized. However, cloned populations are liable to become antigenically heterogeneous even when passaged at 2–3 day intervals in normal mice, as has recently been shown by van Meirvenne et al. (22) and by Doyle (8). The development of new surface variants under such conditions may indicate an early immune response of the host since, according to Doyle, cloned populations maintained in irradiated mice can retain their original antigenic homogeneity during repeated passage for periods up to 35 days.

CELLULAR LOCATION AND MOLECULAR STRUCTURE OF VARIANT ANTIGENS

Identification and purification of variant-specific surface antigens of T. brucei

The demonstration of in vitro agglutination and immune lysis of bloodstream forms of trypanosomes by variant-specific antisera indicates the probable involvement of surface antigens in the phenomenon of antigenic variation. Electron microscopy has revealed a regular electron-dense layer (surface coat) overlying the trypanosome plasma membrane and covering the entire surface of the parasite (34), and ferritin-labelled antivariant antibodies can be shown to react specifically with this surface coat (36). Variant-specific antigenic determinants have been found to be associated with soluble glycoproteins extracted from bloodstream trypanosomes (2). Recently, the macromolecular constituents of the surface coat have been isolated and purified, and studies have begun of their detailed structure and its relationship to antigenic variation (6). Specific labelling of components was used to identify a class of soluble glycoproteins as constituents of the surface coat. These glycoproteins have been purified to homogeneity from several cloned populations derived from sequential variant populations in rabbits. In each instance, the purified glycoproteins consisted of a single polypeptide chain having a molecular weight of 65,000, consisting of about 600 amino acid residues and 15–30 monosaccharide units. The sugars found were D-mannose, D-galactose, D-glucose, and N-acetylglucosamine. The number of sugar residues varied slightly between glycoproteins purified from different clones. There were considerable differences in the isoelectric points of glycoproteins isolated from sequential clones. Amino acid analysis showed that these differences reflected extensive variation in amino acid composition, and peptide-mapping techniques of clone-specific glycoproteins have not so far shown multiple identical spots. These glycoproteins were immunologically distinct as demonstrated by immunodiffusion, cell-surface fluorescent-antibody labelling, and agglutination tests. Immunization with purified glycoprotein gave protection against challenge with the homologous but not the heterologous clones.

Quantitative studies suggest that the surface coat is composed largely or entirely of variant-specific glycoprotein, and a simple model has been proposed in which the glycoproteins are present as a surface monolayer (6). There is evidence that the glycoproteins are located in the carbohydrate-rich area lying close to the plasma membrane (Cross & Johnson, unpublished data), a hypothesis consistent with evidence of electron microscopic cytochemical studies (33, 38). The mode of linkage between the surface glycoproteins and the plasma membrane is not yet clear, but selective cleavage with trypsin has led to the tentative identification of a region of the glycoprotein molecule that may be involved in this attachment.

It has recently become apparent that spurious molecular heterogeneity can occur during antigen purification. Two sources of heterogeneity have been identified. Firstly, as noted above, previous methods may have been inadequate to ensure homogeneity of starting cell populations with respect to surface variants. Secondly, semipurified preparations from apparently homogeneous cell preparations can undergo degradation, probably as a result of contamination with endogenous proteases. This may lead to minor modifications sufficient to generate multiple components demonstrable by isoelectric focusing on gels or in columns. Degraded components may still react antigenically in the same manner as the intact molecule.

Immunoelectrophoretic analysis of trypanosome antigens

Another approach to the identification of trypanosome antigens is by immunoelectrophoresis in
agarose gel, using hyperimmune antisera raised in rabbits to soluble extracts of trypanosomes. Analysis of the soluble parasite extracts with corresponding antisera reveals some 30 different antigenic components. After absorption of such antisera with extracts of heterologous clones the variant-specific antibodies remain behind, as can be demonstrated by allowing the absorbed antisera to react with extracts of the homologous clones. A single variant-specific component in cloned variant populations of *T. brucei* has been shown by this technique (27). Similar experiments revealed several variant-specific components in extracts from cloned populations of *T. gambiense* (1).

**THE MECHANISM OF ANTIGENIC VARIATION**

In relation to variation in the surface antigens, discussion of the molecular and physiological events in both the host and the parasite involved must, owing to the lack of evidence, be largely restricted to theoretical considerations and to suggestions about how possible mechanisms might be investigated. It may be useful first to examine the possible ways in which antigenic variation could arise, and then to consider what sort of signal determines the time of expression and the identity of the variant type.

**Possible sources of variation**

There are two mechanisms by which variant types could arise: either a single antigen-determining locus could mutate in one or more individual parasites to generate a variant glycoprotein, or there could exist in all trypanosomes a series of genetic loci, any one of which could be expressed at any appropriate time.

**Mutation.** The appearance of a new trypanosome antigenic variant could be due to an error in replication of an antigen-determining gene. If, however, variants could be shown to arise regularly within a clone before sufficient cumulative cell fissions had occurred, then mutation would become an unlikely source of variation. In fact, mutation is highly unlikely to be the major factor in antigenic variation for several reasons:

1. There is a tendency for surface variants to appear in a preferred sequence during the course of parasitaemia.
2. Trypanosomes may revert to common basic antigenic types when they reach the metacyclic stage.
3. Serological cross-reactivity has not been detected between successive variant antigens.
4. There are a large number of amino acid substitutions between sequential variant antigens. A prohibitively large number of simultaneous point mutations would be required to produce the observed differences.

**Utilization of pre-existing genetic information.** The requirement here is for the existence in each parasite of different genetic loci, each coding for a separate protein or portion of a polypeptide chain, the number being indicated by the greatest number of variant antigens that can be formed by any one clone of trypanosomes. A control mechanism to ensure that only one antigen is synthesized by a parasite at any one time would also be required. This situation would be similar to that found in free-living ciliates such as *Tetrahymena* (23) and *Paramecium* (32).

There are three possible ways in which the alternative genes could be expressed:

1. According to a predetermined programme, always in the order A→B→C→D→...
2. In a favoured sequence dependent upon differential rates of derepression, with the locus most readily activated producing a component, perhaps the end-product antigen, that causes repression of all other loci. This implicates a complex genetic control mechanism, termed "mutual exclusion", which has been discussed with respect to phenomena reported in various protozoa (11, 23, 32).
3. Alternatively, new variant antigens may arise randomly. If variant types arose in a random fashion, those that had previously been expressed would be rapidly removed by the action of antibodies stimulated by a previous parasitaemic wave. Random variation does not at first sight appear to be consistent with the preferred sequence of variants characteristic of *T. brucei* (14) and *T. gambiense* (15), although difference in virulence among variants may be a regulating factor. (See below under "Selection ".)

**Recombination.** Either of the previously-mentioned mechanisms for generating antigenic variation could be affected by recombination. Although in other cell types this process is normally not sufficiently frequent to account for all of the observed molecular variation, it would give rise to increased diversity of
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antigenic type and hence should not be totally dismissed. This consideration would be particularly relevant if sexual processes were found to occur in trypanosomes. If trypanosomes are found to be heterozygous at an antigen-determining locus, possibilities of allelic exclusion as well as intragenic recombination would arise. Further examination for sexual stages, particularly in the vector, should therefore be undertaken, and such work would also be valuable from the point of view of using genetic markers to identify strains.

**Influence of extranuclear information.** The significance of the substantial amounts of DNA in the kinetoplast has not been established, and formal evidence is so far lacking to show that the structured genes coding for variants are located in the nucleus. Any influence of the kinetoplast on nuclear organization, changes in metabolism, and the development of different morphological stages might also include an effect on the process of antigenic variation.

In conclusion, the most reasonable explanation for antigenic variation is the presence in the trypanosome genome of a number of different antigen-determining loci. Although mutation and recombination can be ruled out as major contributory factors to antigenic variation, they may be important at certain stages in the life cycle of the trypanosome, for example, if a pre-existing repertoire of variant antigens were exhausted following a long sequence of parasitaemias.

**Production of variants**

The expression of variant antigen, irrespective of how it is determined, may be effected either by induction of by selection.

**Induction.** One factor capable of inducing variant antigen production in trypanosome blood stages could be the host's immune response. Antibody-induced movement of variant antigens, analogous to capping of antigens on lymphocytes and other cells, has been demonstrated in *T. brucei* (8). If antigen–antibody complexes subsequently entered the body by endocytosis, they might initiate an intracellular change that could influence gene expression. It is also possible that changes in genetic activity may be stimulated by the mere removal of the surface antigen coat, allowing an influx of ions into the cell.

Other host environmental factors cannot be excluded. Temperature, ionic composition, enzymes, and hormones are examples of factors that are known to be important in the control of gene expression in other cells. There are known to be temperature differences in different parts of the host body, and body temperature is raised during high parasitaemia, a time when new variants are known to arise. When the trypanosomes invade other tissues, the new local environment is quite different from that of the vascular system. In myocardial tissue, for example, *T. brucei* expresses variant antigens that tend to lag behind the sequential series of those trypanosomes present at the same time in the bloodstream (19), although the reason for this is unknown. The potential effects of variant-specific antibody and of other features of the environment in eliciting new variants are not mutually exclusive, and at different times either or both may be effective. For example, it may be necessary that surface antigen be removed by complexing with specific antibody before other environmental factors can induce variations.

**Selection.** The host's immune response may play a role in the selection of variants. Predominant variants that are present in adequate numbers to induce an antibody response would thereby be eliminated, while minor variant populations would survive. This mechanism could select not only for regularly-occurring phenotypic variants, but could also favour the appearance of variants resulting from probably rare phenomena such as mutation and genetic recombination.

Alternatively, selection could depend on virulence. Differences in virulence have been observed between variants of different antigenic types, and competition between variants may determine the sequence of their appearance (17, 26, 28).

**Conclusions**

The mechanisms of antigenic variation in trypanosomes are poorly understood. It is most likely that different antigens are coded for by individual genes and that these may be expressed in a sequential manner throughout the course of parasitaemia. The stimulus to change probably derives from the action of variant-specific antibodies, although other aspects of the environment within the host may be significant.

Although we have considered only mechanisms that may be operative in the mammalian host, similar mechanisms, excluding immune selection, could function in the invertebrate host. Environmental factors may be particularly important at certain stages during the life cycle, such as at transfer from host to host. It is known that many parasites die during these stages.
VARIATION IN RELATION TO HOST DEFENCE SYSTEMS

Innate resistance

Innate resistance is here defined as host resistance independent of the specific immune response.

Host specificity. It is in general characteristic of the salivarian trypanosomes that they are relatively non-specific as regards host, most mammalian species being susceptible to experimental infection. There are, however, some well established examples of host species that are completely refractory to infection with certain trypanosome species or subspecies. For example, many T. brucei strains will not infect man and this has been used as a taxonomic characteristic, those strains that can infect belonging to the subspecies T. (T.) b. gambiense, including the nosodeme T. rhodesiense, and those that cannot belonging to the subspecies T. (T.) b. brucei. T. vivax, a common pathogen of cattle, will not infect many rodent species or any primates. T. simiae, a pathogen of pigs, will not infect cattle, and early studies reported that the baboon (Papio) is not susceptible to infection by any salivarian species (3).

Mechanisms. The mechanisms of innate resistance have been studied in very few cases, and even in these are poorly defined. In the case of the T. brucei subspecies in man, resistance is associated with the presence in human serum of high molecular weight proteins that have not yet been characterized. These substances act by lysing and neutralizing parasitic strains that are not infective to man. Susceptibility to their effects appears to bear no relationship to variants, since a single variant type can include forms that are either susceptible or resistant to lysis. It has also been shown that different variants derived from the same clone can differ markedly in their susceptibility to lysis. Hence, there appears to be no association between antigenic type and lysis.

Acquired resistance

The only protective response that has been clearly demonstrated is protection against challenge by individual variant types in animals specifically immunized against these variants. Evidence of other forms of protection must be sought by considering the outcome of natural and experimental infections. The extremes range from death of the host during the first parasitaemic wave to complete suppression of parasitaemia and elimination of all organisms, with intermediates in which the host initially survives a series of parasitaemic waves, leading to chronic low-level parasitaemias. In some instances, chronic infection may occur without manifest disease. Since reports of natural cure are scarce and poorly documented, most practical interest attaches to the intermediate effects. It is clear that many wild and domestic animal hosts survive in the face of repeated challenge, with low parasitaemia and little detriment to health. Possible mechanisms of acquired resistance will be considered in terms of parasite factors and host factors.

Parasite factors. Differences in “virulence” of trypanosome strains are obviously important. It is well known that certain strains are capable of producing only low-grade infection in several different host species. This may be due to a slow rate of reproduction of all the variant types within the repertoire of the strain, but there is very little information at present available on the dynamics of trypanosome reproduction.

Interactions between parasite populations at different stages in the antigenic type sequence have been noted (17); for example, the progression of the infection with a virulent type has been found to be delayed by the presence of another infection with a different type. Whether this effect is due to interaction between the types or between the responses to them, or to other factors, is as yet unknown.

In the infections that have been most studied, i.e., laboratory animals and hosts in which pathogenic effects are usual, antigenic variation is considered to continue throughout the life of the animal. However, there are, according to van Meirvenne (personal communication) examples of great interest in which the same antigenic type can be isolated over extended periods from the same host. Whether antigenic variation occurs in trypanosome populations in wild animals and in healthy carriers has not been investigated. In field conditions, superinfection with several trypanosome “species” may further complicate the issue.

Host factors. There is clear evidence that resistance to infection is related to the taxonomic position of the host. Some animal species are obviously resistant (4), for example carnivora (17), the Ndama cattle of the Fouta Djallon, and West African shorthorn cattle. Chronic infections have also occasionally been observed in animals that are usually susceptible to the lethal effects of infection, e.g., in Zebu cattle under light challenge, or with exceptionally good husbandry.

Considerable differences in the severity of disease caused by the same strain within a normally sus-
ceptible species have been noted. The following immunological explanations can be offered:

(1) It is possible that the resistant animals are those that are genetically capable of producing highly avid antibodies to the antigens of the strain with which they are infected.

(2) The immune response of resistant animals may become more rapid or more efficient as the infection progresses. The efficiency of production of antibody could be increased by a T cell helper effect in respect of the supposedly constant portions of the variant antigen molecule, as has been suggested by Brown in malaria (4, and unpublished data).

(3) Prolonged infections may generate antibodies that are no longer strictly variant-specific but are cross-reactive.

Possible models for the study of nonpathogenic infections include those trypanosome strains that produce chronic infections in small laboratory animals, healthy human carriers, wild game animal species, some laboratory models such as infections in rodents maintained at high ambient temperatures (25), and salivarian trypanosome infections in the domestic fowl.

Two kinds of so-called healthy human carriers may be distinguished. Individuals have been recognized in the Zambesi region who show high parasitaemia for periods of many months with no obvious ill health, and West Africans have been described in whom parasitaemia is low and may be discovered only by extensive microscopical examination following some other indication of infection, such as a positive immunofluorescence test or elevation of serum IgM.

Many species of wild animal, particularly *T. gelaphus* spp., members of the Reduncini, and the giraffes, may show a 30% prevalence of trypanosomes in the peripheral blood on a single microscopical examination, without evidence of pathogenic effect (20).

In addition to the use of strains naturally capable of producing chronic infections in laboratory animals, it has also been shown that *T. brucei* infections normally lethal to mice during the first parasitaemic wave may be converted into chronic relapsing infections by maintaining the animals at 37°C (25).

Early studies directed to investigating the possible importance of guinea fowl and francolin as reservoirs of *T. rhodesiense* (3) showed that these inoculations produced infections that were rapidly controlled to submicroscopic levels. Nevertheless, trypanosomes could be demonstrated in the peripheral blood by subinoculation into laboratory mice, and the parasites persisted at this low level for periods of a year or more, leaving the birds clinically undisturbed. This effect has been confirmed recently using both day-old chicks and adult fowl as hosts (Hicks & Herbert, unpublished data).

Generally, it is essential that such studies are carried out with precisely defined populations of hosts and parasites. Laboratory models have obvious attractions, but conclusions derived from such models may not be applicable to human diseases. With the accumulation of an extensive reference collection of antigenic types and antisera, human carriers, and game animals, it will become possible to study more directly some aspects of resistance to trypanosomiasis in man and cattle.

**Immunopathology**

Immunopathological mechanisms are likely to be responsible for a large part of the pathology characteristic of African trypanosomiasis. Antigenic variation implies repeated stimulation by new antigens, as well as the continuous release of common antigens. Toxic factors affecting the immune system may also be important. The pathological effects seen in the disease may be produced in the following ways:

(a) In the vascular spaces, variant antigen present on the surface of the parasite or released in plasma may react with corresponding antibodies. This type of reaction leads to the formation of circulating immune complexes, which can become localized in vessel walls or in filtering membranes and cause various lesions, for example glomerulonephritis. Immune complexes have also been shown to interfere with the induction and effector mechanisms of the immune response. Such an effect in trypanosomiasis could help the parasite to escape from the mechanisms of immune resistance.

The fact that antigenic variation leads to successive waves of antigen and antibody excess should favour the development of manifestations of immune-complex disease.

(b) Similar immune complexes, formed locally in the extravascular spaces, may participate in the development of local inflammatory foci. The accumulation of lymphocytes and of other mononuclear cells in tissue lesions characterizing trypanosomiasis may also reflect the involvement of cellular mechanisms in these lesions. These may include
cytotoxic lymphocytes and direct antibody-dependent cell-mediated effects. The respective participation of common antigens and of variant antigens is unclear.

(c) The Jarisch-Herxheimer reaction seen following the administration of trypanocidal drugs (21, 29) may be mediated by complement fragments released following massive antigen–antibody combination. A similar mechanism may be responsible for many of the symptoms of untreated infections.

(d) The high levels of serum IgM that have been frequently observed in trypanosomiasis may be related to repeated antigenic stimulation with different variant antigens. The release of mitogenic factors from the trypanosome may be a possible additional cause.

(e) It is known that some immune complexes can bind to erythrocytes and cause haemolytic anaemia. In laboratory animals, there is evidence that haemolytic toxins are directly responsible for the anaemia (24).

(f) It has also been shown that the variant antigen itself can be absorbed to erythrocyte surfaces, which suggests that erythrocytes as well as circulating parasites may be removed by the antibody response to each antigen (18).

Development of vaccines

Antigenic variation provides the parasite with a powerful means of evading host immune responses. Nevertheless, it is possible to consider two ways by which protective immunity to salivarian trypanosomes might be induced in man and cattle. These are by producing an immune response that destroys parasites immediately upon inoculation or very soon afterwards, before antigenic variation can occur, or by inducing a protective immune response that transcends variant-specificity.

Prevention of infection. Metacyclic trypanosomes, at the time of inoculation and before they assume the full characteristics associated with survival in the vertebrate host, may have special surface antigens in place of, or in addition to, the variable surface glycoproteins. Antibodies raised against such antigens could be protective. Alternatively, if all parasites revert to a basic variant type as a result of tsetse transmission, an artificially-induced immune response to this basic antigen might destroy all parasites before variation was initiated. Investigations of both these possibilities would be greatly helped by development of tsetse tissue culture or other techniques for producing metacyclic trypanosomes.

The availability of vaccine of this type would be a great step forward, but it should be recognized that any breakthrough infection that occurred from mechanical transmission (37), or due to a waning of anti-metacyclic immunity, would find the host completely unprotected against subsequent parasite multiplication.

Induction of non-variant-specific immunity. Circumstantial evidence described above suggests that an immune response can sometimes develop that is capable of restricting parasite multiplication irrespective of antigenic variation. In experimental malaria, there already exist precedents for supposing that this immunity can occur in the presence of continuous antigenic variation, for example by the helper T cell effect to common determinants (Brown et al., unpublished data). These studies suggest that it may become possible to manipulate the immune response to produce non-variant-specific protection.

It is not possible, at this stage, to say whether or not any of these approaches are likely to be successful. Techniques do exist, however, for exploring their feasibility under critical laboratory conditions, so that much more accurate predictions about immunological aspects of vaccination should be possible within a few years. These studies, though necessarily carried out on defined laboratory materials, must also take into account strain and host differences, which are likely to be very significant in the field.

RECOMMENDATIONS

1. Immunofluorescence methods for identifying antigenically different trypanosomes should be readily applicable to the examination of material collected under field conditions. The increasing availability of purified variant-specific trypanosome antigens and of homologous antisera also raises the possibility of applying radioimmunoassay and immunoenzyme techniques to the antigenic analysis of trypanosome populations.

2. Hitherto, studies on the surface antigens of metacyclic T. brucei have been limited by the low infection rates in tsetse flies. Improved infection rates can be obtained if Glossina morsitans is fed initially on infected animals at first peak of parasitaemia, and subsequently on sterilized skin surfaces (Jenni, unpublished data). Examination of trypanosomes developing in local reactions at the
site of the tsetse bite might also be helpful in assessing homogeneity as regards the surface antigens of the initial infecting stages and their relationship to their metacyclic antecedents.

3. Synthesis, secretion, and control of variant antigens. The intracellular site of synthesis of variant antigens and the mode of transport to the surface membrane is unknown. Study of variant synthesis would be particularly important both during the process of epimastigote to metacyclic transformation in the fly and the period of antigenic modulation in the mammalian host. Such studies could also provide information on the question of (continuous) turnover and release of variant antigen from the parasite surface.

Techniques are available which could be applied to these problems, but in many cases they may require the cultivation of bloodstream trypanosomes in vitro, at least for short periods.

4. Detailed structural analysis of variant specific antigens is now clearly possible. The development of this approach should throw light on several aspects of antigenic variation. Amino acid sequencing could reveal the genetic basis of antigenic variation and may indicate how variation has evolved. Extension of antigen purification and structural studies to trypanosome species other than T. brucei might show variations in antigen structure that correlate with speciation, host specificity, virulence, and pathogenicity.

Structural studies should be combined with immunological techniques designed to recognize the antigenic sites in the molecule and their relation to the induction of protective immunity. Attention should also be focused on the regions of attachment of the variant-specific antigens to the plasma membrane. It is possible that this area could be exploited by drug action, for example, to interfere with variant-antigen function.

Emphasis should be given to developing suitable in vitro systems for bloodstream trypanosome maintenance or cultivation sufficient to permit controlled experiments on the molecular mechanism of antigen synthesis secretion and control.

5. Infection of the host may involve more than one variant, i.e., the inoculum may comprise a mixed population. For the purpose of establishing how variants arise, an essential prerequisite is that the initial injected trypanosomes are, as near as possible, genetically homogeneous, preferably derived from a single trypanosome.

6. Further examination for sexual stages, particularly in the vector, should therefore be encouraged (also from the point of view of using genetic markers to identify strains).

7. In order to demonstrate conclusively the stimulatory effect of variant-specific antibody and/or environmental perturbation in inducing antigen change, trypanosomes would have to be cultured under stringently controlled in vitro conditions so that the effect of added antibody, or of a changed condition in the medium, could be monitored in the absence of any other changes.

8. The following suggestions would facilitate a study of the mechanisms of antigen variation and its possible manipulation:

(a) The development of an in vitro culture system in order to study basic mechanisms under stringently controlled conditions.

(b) An investigation to determine the extent of genetic variation, possibly by isoenzyme studies or by measuring DNA homologies by means of molecular hybridization to characterize so-called clones, strains, and species. Only when working with genetically identified clones can many basic problems be studied. Also, once characterized, clones containing genetic markers could be used to reinvestigate the possibility of sexual processes in the life cycle.

(c) A study on chemotherapy specifically interfering with the process of antigenic variation. The mechanism controlling antigenic variation might provide a specific target either by affecting the parasite directly or by preventing the appearance of new variants and so allowing the immune response to eliminate the existing infection. Although a wide variety of metabolic inhibitors such as actinomycin D, cordycepin, and α-amanatin are currently used to study the effect of blocking RNA synthesis in experimental systems, and may be of use in investigating the basic mechanisms involved in antigenic variation, these drugs would be highly toxic to the host.

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REFERENCES


