Pathogenicity of Trypanosomes

Proceedings of a workshop held at Nairobi, Kenya, 20-23 November 1978

editors: George Losos and Amy Chouinard
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/IDRC publication/. Compilation of workshop papers on /trypanosomiasis/ particularly in /Africa south of Sahara/ - discusses the /metabolism/ of the trypanosome /parasite/s, mechanisms of /disease transmission/, effects on /blood/ and /serum/ /protein/ levels in /cattle/, /immunology/cal aspects, /disease resistance/.


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Mechanisms of antigenic variation in salivarian trypanosomes

J.J. Doyle, H. Hirumi, and A.L.W. de Gee

Abstract. Since the early 1900s there has been controversy on whether trypanosomes require stimulation from a host's antibodies to vary their surface antigens. Our studies in vitro and in vivo indicate that elimination of variant types is hastened by the host's antibodies but antigenic variation is not dependent upon them. We cultured and maintained in vitro a total of 14 clones of variable antigen type 052 of *T. brucei* and, using immunofluorescent techniques, detected antigenic variation in 9 of them. Variant type 221 appeared along with other types that were infective to mice but were not recognized by antisera against 052 and 221. Although 052 and 221 have equal growing times in vivo; in vitro 221 outgrows 052 sufficiently to be detected by present techniques. Despite different growing times in vitro, the direction of variation is the same in vivo as in vitro.

As early as 1909, some scientists postulated that during trypanosomiasis the host's antibodies against surface antigens induced the trypanosome to undergo antigenic variation (Ehrlich 1909; Ehrlich, Roehlant, and Gulblausen 1909); others (Levaditi and McIntosh 1909) believed that the antigenic variation was the result of mutational events independent of environment. The controversy is still largely unresolved, but Beale (1954) and Sommerville (1970) have clearly shown that, under in vitro culture conditions, environmental stimuli including antibodies induce change in the surface antigens of several free living protozoa.

Now that it is possible to clone and maintain animal-infective bloodstream trypanosomes in vitro (Hirumi, Doyle, and Hirumi 1977; Hirumi, Hirumi, and Doyle 1978), the processes underlying antigenic variation can be investigated under defined conditions. Previously, the parasite had to be maintained in normal or immunosuppressed laboratory animals. Recently, Cross (1975) isolated and characterized the variant-specific surface glycoproteins of bloodstream trypanosomes, permitting the production of highly specific antisera for use in the antigenic analysis of trypanosome populations. In the past, antisera were derived by infection of a suitable host. These advances together with immunofluorescence techniques that allow analysis of the antigenic type of individual living trypanosomes have enabled us to observe the process of antigenic variation in vitro in the absence of host antibodies (Doyle et al. submitted for publication).

We cultured and maintained in vitro a total of 14 clones of variable antigen type clone 052 of *T. brucei* stock S427 (Cross and Manning 1973; Cross 1975) for up to 60 days and detected antigenic variation in 9 of them. A new variant type (221) appeared in all clones. Also appearing were populations of trypanosomes that were infective for mice but not recognized by antiserum to 052 or 221 type trypanosomes. They probably are a mixture of variant populations to which we do not, as yet, have specific antisera. This phenomenon is similar to antigenic variation in vivo in that variable antigen type 221 consistently appears in the first relapse of mice infected with clones of variable antigen type 052. Again, variable antigen type 221 occurs together with trypanosomes against which we do not have antisera. Thus, antigenic variation can occur in vitro in the same direction as in vivo in the absence of antivariant antibodies.
In vivo, mice inoculated with a single trypanosome exhibit new variants in the first relapse, generally 10–12 days after infection, whereas, in vitro, new variants are first detected 18–46 days after initiation of the clones. In the normal host, antibodies eventually remove the original variant population, facilitating detection of new variants: in vitro, where there are no antibodies, the population doubling time (PDT) of trypanosomes is the key to detection. Both 052 and 221 variable antigen types have PDTs of approximately 6 h in vivo, whereas they are 14 h and 8 h respectively in vitro. The marked difference in the PDTs in vitro allows detection of 221 type trypanosomes, which inevitably overgrow the original 052 population to the point of detection. Present techniques are not sensitive enough to detect any new variant type that has a PDT similar to or longer than the original variant.

The difference in PDTs is most intriguing in that both populations originally derived from a single trypanosome and, thus, have the same genotype but different surface antigens.

Differential growth rates of variant populations of *T. brucei* in vivo have been recorded (McNeillage and Herbert 1968; Van Meirvenne et al. 1975), and my colleagues and I have observed the phenomenon in *T. vivax*-infected mice and goats (de Gee, Shah, and Doyle submitted for publication). We examined two variant populations of the same genotype that were poorly infective to mice (at most two parasitemic waves). We found that goats infected with either of the two populations suffered from a relapsing infection and that the trypanosomes appearing in the goats 10–12 days after infection could cause lethal relapsing infections in mice. Immune lysis analysis indicated that the original and the goat-derived populations were antigenically different.

The physiological differences of the variable antigens are poorly understood but may be of great importance in elucidating the genetic mechanisms underlying antigenic variation. It may be that *T. vivax* infections in mice are close to *T. brucei-rhodesiense* infections in which preliminary evidence suggests a correlation between a clone’s acquisition of a given variable antigen type and its ability to infect humans (Van Meirvenne, Magnus, and Janssens 1976).

Another area of parasite physiology and host-parasite relationships may prove to be relevant to our understanding of the phenomenon of antigenic variation: the ability of *T. brucei* to undergo a complex series of physiological changes during the course of a parasitemic wave. Whereas the changes apparently adapt the trypanosomes for onward transmission to the tsetse fly, they preclude further multiplication in the mammalian host (Vickerman 1971; Vickerman and Tetley p. 23). While the majority of parasites in a parasitemic wave are undergoing this physiological shift, the trypanosomes carrying new variable antigen types are able to continue multiplying in the mammalian hosts. How this phenomenon relates to the process of antigenic variation is at present under study.

Although the process of antigenic variation is complex, it does not require the action of host antibodies to induce it. Whether or not other physiological stimuli are involved is uncertain, but the occurrence of other complex physiological changes in bloodstream trypanosomes suggests that the switch to display a new variant surface antigen is only part of a far larger process.