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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Genetic basis of antigenic variation

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Abstract. Antigenic variation in trypanosomiasis is the sequential expression of a series of antigens on the surface of the parasites. How many variants a single cell can express and how variation occurs are at present unknown. Nucleic acid hybridization studies have revealed that the messenger RNA (mRNA) coding for the variant-specific surface antigen is an abundant mRNA species. They also indicate that the abundance of the antigen mRNA is unique to each trypanosome clone but that the sequence is not unique and is found at low levels in unrelated cell clones. Possibly, antigenic variation is a selective increase of transcriptional activity of different antigen genes, and other mechanisms, such as the DNA/gene arrangement, may contribute to the highly mutable character of trypanosomes.

Antigenic variation in trypanosomiasis (reviewed by Barry and Hajduk p. 51) is the sequential expression of a series of antigens on the surface of the parasites. In a normal host, the initial population of parasites derived from a single cell uniformly expresses a single membrane surface antigen. Subsequent parasitemias rise and fall in waves, each new parasite population carrying a new membrane surface antigen immunologically distinct to previous antigens. Only recently has work been devoted to the biochemical characterization of the antigens involved in the antigenic variant (see Cross p. 32; Barbet et al. p. 38). The trypanosomes carry on their external membrane surface a single predominant glycoprotein that carries the immunospecificity for each variant trypanosome population. The surface glycopeptide constitutes 90% of the total membrane protein and as much as 10% of the cell protein by weight. The pure variant-specific surface antigens have similar molecular weights, ranging from 57,000 to 65,000 daltons. The antigens differ greatly in isoelectric points and are rapidly synthesized by intact cells in vitro. Amino acid sequence analysis of four variant antigens shows that the 30 amino acid residues on N-terminus bear no relationship to each other. My colleagues and I have begun to investigate how antigenic variation occurs. We purified the messenger RNAs coding for a variety of variant antigens and studied the mechanisms of expression of the variant antigen structural genes. We then used the pure RNAs in hybridization studies to analyze the genetic basis of antigenic variation.

In our original hybridization study on T. brucei, we used complementary DNA probes synthesized from purified mRNAs to analyze the sequence complexities of the populations (Williams et al. 1978). The complexity measurements of the total messenger RNA population of cloned trypanosomes indicated the presence of abundant and scarce RNA sequences. We determined that approximately 20% of the total cytoplasmic messenger RNA population has a sequence complexity of 9.6 kilobases. This would be equivalent to three or four messenger RNAs averaging 2 kilobases long. This quantity of abundant RNA sequences in the cytoplasm of the trypanosome is unexpected and compares with such unusual cell populations as myeloma cells where the predominant protein synthetic activity in the cell cytoplasm is producing one cell protein, namely, immunoglobulin.

To analyze the sequences further, we compared two unrelated cell clones of trypanosomes. We synthesized complementary DNA from messenger RNA of a clone of cells isolated from strain LUMP 227 and hybridized it to RNA purified from a clone of trypanosomes isolated from strain S427. The object of this experiment was to determine whether the two unrelated clones had any sequences in common. Our data indicated that the abundant RNA sequences were clone specific. That is to say,
the abundant sequences were unique to each clone that we tested. We reasoned that the specificity in abundant RNA sequences was due to the variant antigen messenger RNA because the variant antigens are the only readily observable difference between cell clones of T. brucei. The presence of variant antigen mRNAs in the population is suggested also by our finding that one of the major proteins synthesized in a cell-free-protein synthesizing system was the variant antigen.

All the complementary DNA hybridized with the RNA from the unrelated clone of the trypanosomes, indicating that although the abundance of the messenger RNA sequences is clone specific, the sequences are present in both clones. Because the RNA was isolated from intact polysomes, it appears that the RNAs for different variant antigens are not only transcribed but also translated in each cell clone. If this is true, the translated variant antigen proteins will not be detectable either by purification techniques to separate contaminants in the variant antigens or by immunofluorescent techniques to measure trypanosome clone integrity. At any rate, these techniques are not as sensitive as the hybridization techniques used in this study. The result suggests that at least some of the genes coding for the low complexity or abundant RNA sequences in independently isolated T. brucei clones are not clone specific but that their mode of expression is clone specific.

In the antigenically unrelated T. brucei clones, the specificity of expression may be the result of factors that modulate gene activity and define mRNA function. Our result indicates that the genes for several, or possibly many, variant antigens are transcriptionally and translationally active at the same time in the same cell and that antigenic variation is perhaps a selective increase of transcriptional activity of different trypanosome antigen genes. In addition to transcription, a change in the rate of RNA processing or a change in mRNA half-life may contribute to the change in mRNA abundance and to the appearance of new variant antigens.

In view of the large number of variant antigens observed in the field and in the laboratory, we believe there are other mechanisms contributing to the polyphenotypic character of the trypanosome surface antigens. Because the variant antigens are all restricted to a relatively small molecular weight range (57,000–65,000 daltons) and all are located on the exterior of the cell membrane, they may be encoded in the genome DNA as a multigene family. Nucleotide sequence homology between the variant antigens has yet to be demonstrated, but the proteins seem to exhibit the other three properties of a multigene family, i.e., multiplicity, close linkage, and related or overlapping phenotypic function (Hood 1976). If our data are correct, i.e., if unrelated T. brucei strains have several or many variant antigens in common, a multigene family seems possible.

As there is no conclusive evidence for any specific mechanism, a look at several other highly mutable or polyphenotypic gene systems may be worthwhile. One of the most obvious systems that produces large numbers of variant proteins is the immunoglobulin gene family. This system comprises a gene family that is limited in numbers but is able to produce extremely large numbers of different proteins. Differentiation of antibody-producing cells results in DNA rearrangement such that the constant and variable genes of immunoglobulin molecules are moved closer together in the genome. The rearrangement of different constant and variable genes can contribute to the production of immunoglobulins with totally different immunospecificities. Such a system is possible also in the trypanosome, although some data refute it. Recent data on immunologic cross-reactivity of different variant antigens (see Barbet et al. p. 38) suggest that the different variant antigen polypeptides do not share any large region of amino acids. Nevertheless, DNA/gene rearrangement may contribute to the production of a large spectrum of antigens.

Another mechanism responsible for genetic instability and an apparent high rate of mutation is the transposition of genetic elements (reviewed by Nevers and Saedler 1977). Insertion sequences (IS) in bacteria and controlling elements in Zea mays (Fincham and Sastry 1974) are small pieces of DNA that are capable of inserting themselves into genomic DNA and disrupting the expression of the gene. The IS DNA sequences are capable of inserting and excising themselves and producing varied frequencies of mutant expression and recombination. Depending on how and where the sequence is inserted, the inserted genetic elements may cause different gene products from a single gene, a total shutdown of the gene transcription, the production of modified gene products or entirely new and unrelated gene products if the insertion sequence disrupts a controlling or organizer gene. Similar types of DNA insertions are thought to be responsible for chromosomal rearrangements and mutations of the highly variable white eye locus in Drosophila melanogaster (Green 1975). The gene inversions, deletions, and transpositions may create new nucleotide sequences or even new genes at their fusion points.
The importance of large-scale DNA/gene rearrangements in explaining the creation of a diverse array of antibodies and the observation of very highly mutable or unstable genes may have direct relevance to an understanding of the large number of variant antigens produced from a single trypanosome or from a strain of trypanosomes. The only way to elucidate variant antigen diversity is with the recent technique of DNA cloning in bacteria.