# The Cross-Reacting Determinant of the Variable Surface Glycoprotein of Metacyclic *Trypanosoma congolense*

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References

The International Laboratory for Research on Animal Diseases was set up to address the problems of important animal diseases that affect livestock production in Africa, with its initial targets being trypanosomiasis and East Coast fever. The major emphasis of ILRAD's programme is in immunological approaches to the control of these two diseases. However, in order for such an approach to be successful, it is necessary that a parasite antigen, or antigens, be found that are accessible to antibody attack *in vivo.* In the case of African trypanosomes, the major surface proteins, which are called variable surface glycoproteins (VSGs), elicit a substantial immune response. However, these parasites ensure the successful continuation of succeeding waves of parasitaemia by changing their surface VSGs in a process called antigenic variation.[1]

Although the surface coats of the African trypanosomes undergo continuous waves of change during the course of an infection, the different VSGs all contain similar structures at the carboxy-terminal (C-terminal) end of the molecule. This moiety is believed to function as an anchor by which the VSG is attached to the surface membrane of the parasite.[2] This putative anchor has been shown to comprise a complex glycosylphosphatidylinositol (GPI) moiety. Also found in the C-terminal portion of the VSG is an immunologically cross-reactive determinant (CRD).[3] Antibodies directed against the CRD epitopes react with VSGs from different species of African trypanosomes, as well as a variety of proteins from many lower and higher eukaryotes.[2] Galactose, glucosamine, inositol 1,2-cyclic phosphate, and 1,2-dimyristoylglycerol (1,2-DMG) have all been shown to influence the binding of anti-CRD antibodies.[4,5] Interestingly, however, in the case of African trypanosomes, this cross-reactivity was initially believed to be observable only in VSGs released from the surface of the parasite* by the action of a GPI-specific phospholipase-C (GPI-PLC).[5,6] GPI-PLC acts by cleaving the 1,2-DMG from the GPI moiety and results in the formation of sVSG (a soluble form VSG), which, when analysed by sodium dodecylsulphatepolyacrilamide electrophoresis (SDS-PAGE),[6] has a slightly higher apparent molecular mass (Mr) than does the GPI-containing, membrane form VSG (mfVSG). The GPI-PLC activity is reported to be activated by thiol-reducing reagents and inhibited by a variety of metal ions and detergents.[7,8,9,10] However, in some cases the effect of these reagents has not been distinguished from a possible effect of the reagent upon the substrate rather than the enzyme itself. Clearly, this is of significant importance in the understanding of the process involved in the exposure of the CRD and release of the VSG from its membrane anchor. We have studied this using bloodstream and procyclic forms of several species of African trypanosomes and, in more detail, bloodstream forms and metacyclic forms of *T. congolense*. (The metacyclic forms are the infective forms of the parasite that are transmitted by the tsetse fly when it takes a blood meal from a

mammalian host.) Like the bloodstream forms, the metacyclic forms have a surface coat that undergoes antigenic variation. The procyclic forms are culture-adapted forms that resemble the parasites that are found in the midgut of the tsetse fly; they have no surface coat and show no binding of anti-CRD antibodies.

> (* Strictly speaking this may not be the case since, by using immunoelectronmicroscopy, anti-CRD reactivity can be observed in fixed parasites. The reactivity is observed both on the surface of the parasite and in internal organelles such as the endocytotic network and *trans*-Golgi apparatus.[12,13] However, because the anti-CRD antibodies do not recognize the CRD epitope in healthy, living trypanosomes, the CRD is unlikely to be useful as a potential antigen for vaccination purposes.)

Bloodstream forms of *T. congolense*, when analysed by SDS-PAGE and western blotting techniques using affinity purified anti-CRD reactive antibody (IgG), gave a single reactive band with an Mr of approximately 55,000. In contrast, metacyclic forms of the parasites gave several more or less discrete bands in the Mr range between 49,000 and 57,000. In the latter case we were surprised to observe several phenomena that did not accord with the dogma of increased CRD exposure (i.e., increased anti-CRD antibody binding to VSG) with the mfVSG to sVSG transition.

We observed that the metacyclic forms of *T. congolense* have, as expected, a CRD epitope on their VSGs. However, the 1,2-DMG does not need to be removed from these VSGs in order to cause increased binding of the anti-CRD antibodies to VSG. Furthermore, the exposure of the CRD is exquisitely sensitive to the method of sample preparation. For example, in contrast to reduced samples, sonicated samples that were not reduced with dithiothreitol showed no reactivity with the anti-CRD antibody. A variety of metal ions or detergents were tested for their effects on the exposure of the CRD. The results varied according to the reagent added and whether or not the reagent was added before or after sonication. Different results were also obtained when samples were prepared by the freezing and thawing of hypotonic lysates rather than by sonication.

While our results do not necessarily negate previous conclusions regarding the relationship between the exposure of CRD and the removal of 1,2-DMG, they clearly show that the exposure of CRD does not necessarily mean that the 1,2-DMG has been removed, as has been suggested by some workers.[5,11] Indeed, these workers suggested that the exposure of CRD could be used as an assay for the release of the myristate anchor by the GPI-PLC. Furthermore, our results show that the pathway to the exposure of the CRD is clearly a complex one that can be halted at various intermediate steps by appropriate handling of the samples and that the reduction of disulphide bonds plays an important and, perhaps, crucial role in these events. An unravelling of these "unfolding" steps may help us to elucidate the various steps involved in the synthesis, packaging, transport and breakdown of the molecules during the intracellular processing of VSG.

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