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Antigenic Variation in Malaria

Minireview

Piet Borst, Wilbert Bitter, Richard McCulloch, Fred Van Leeuwen, and Gloria Rudenko
The Netherlands Cancer Institute
Division of Molecular Biology
Plesmanlaan 121
1066 CX Amsterdam
The Netherlands

Parasite variability is a major factor in parasite survival strategies. Some parasites rely on random mutations to create variation, and if the mutation rate is high, as in HIV, this may suffice to create unsurmountable difficulties for the host. Other parasites augment variability by regularly replacing the antigens exposed to the host immune system. African trypanosomes (sleeping sickness) and Neisseria gonorrhoeae (gonorrhea) are prime examples of this antigenic variation in the strict sense (Borst, 1991; Borst and Rudenko, 1994; Dybrig, 1993).

Since 1965, insiders have been aware of indications (Brown and Brown, 1965) that malaria parasites also use antigenic variation in the strict sense to escape host destruction. As the proteins involved had never been fully characterized and as the genes coding for these variant antigens escaped cloning, the evidence for antigenic variation did not seem compelling to outsiders or even to the insiders who write textbooks (Cox, 1993). The three papers published in this issue of *Cell* (Baruch et al., 1995; Smith et al., 1995; Su et al., 1995) should serve to dispel this doubt. Together, these papers establish that malaria parasites contain a large family of genes for variant antigens and that the differential expression of these genes helps the parasite to escape the host immune response.

The variant antigens of malaria parasites discussed here are not on the surface of the parasite itself, but on the surface of the cell in which Plasmodium falciparum multiplies, the erythrocyte (Miller et al., 1994; Pasloske and Howard, 1994). The antigens reside in large proteins (200–350 kDa) collectively known as PfEMP1 (for P. falciparum–infected erythrocyte membrane protein 1). These proteins are secreted by the parasite and find their way into the erythrocyte membrane, where they are concentrated in structures known as knobs (Figure 1). These knobs are only found in infected erythrocytes, and they are mainly composed of P. falciparum proteins other than PfEMP1 (Berendt et al., 1994).

Why would a parasite hiding safely in an erythrocyte, a cell unable to present antigen, reveal its presence to the host immune system by inserting its proteins into the host cell membrane? This seemingly suicidal action appears to be required to avoid another death trap, the host spleen. The reticuloendothelial system of the spleen is able to spot damaged erythrocytes and remove them from circulation. When malaria parasites divide rapidly in the erythrocyte, they damage their host cell. The spleen can sense the damage, and the parasites perish in the endocytosed erythrocyte, together with their sickly host cell. To avoid splenic death, P. falciparum modifies the host cell by inserting PfEMP1. These proteins make the erythrocyte attach to vascular endothelium, resulting in their retention in vascular beds. Only erythrocytes infected with immature parasites circulate in the bloodstream. Although this trick allows the parasite to avoid splenic death, it now becomes vulnerable to immune attack. Plasmodium uses antigenic variation of PfEMP1 to mitigate the consequences of this attack.

Evidence that PfEMP1 is remarkably variable has been accumulating for 30 years (Miller et al., 1994; Pasloske and Howard, 1994). This antigenic diversity is correlated with functional diversity. Different populations of infected erythrocytes may adhere to a variety of endothelial receptors, including vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), CD36, E-selectin (also known as endothelial leukocyte adhesion molecule 1, or ELAM-1), and the extracellular matrix protein thrombospondin (TSP), all proteins involved in adhesion of host leukocytes or platelets to endothelium. Obviously, the structures allowing different PfEMP1 proteins to adhere to these receptors must also be quite diverse.

The two teams that found the gene family encoding PfEMP1 used different approaches that proved to be in part complementary. The Wellems–Miller–Newbold–Ravetch team (Smith et al., 1995; Su et al., 1995) stumbled on the gene family during a chromosome walk that was started by Wellems and coworkers to find the genetic locus involved in resistance of P. falciparum to chloroquine. By genetic methods, this locus had been positioned in a segment of chromosome 7. When Su et al. (1995) tried to find the drug resistance gene, they fortuitously discovered a number of adjacent genes that looked like genes encoding members of the PfEMP1 family. They call these genes *var* genes (see Table 1 for nomenclature). The other team,

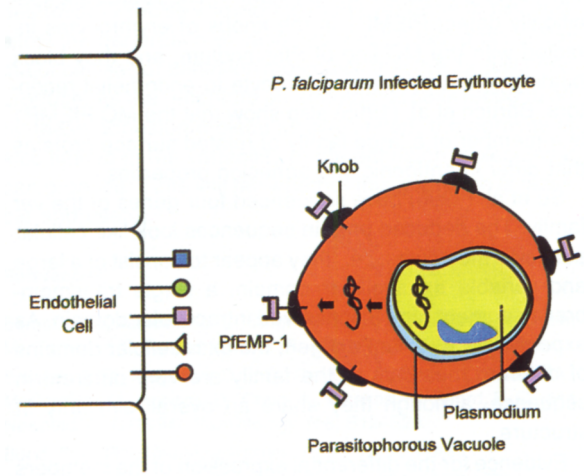

Figure 1. P. falciparum–Infected Erythrocyte

P. falciparum–infected erythrocytes express on the surface of the erythrocyte a variant antigen from the PfEMP1 family, which plays an important role in survival of the infected cell by mediating attachment to the vascular endothelium. See text for details.

Table 1. Plasmodium DBL Superfamily

Name	Definition	Description
PfEMP1	P. falciparum–infected erythrocyte membrane protein	A family of proteins secreted by P. falciparum within an infected erythrocyte that migrate to knob structures on the erythrocyte surface. These function as erythrocyte receptors for endothelial cells and are encoded by *var* genes.
var genes	Large family of highly variant genes containing conserved DBL domains	Genes encoding proteins such as PfEMP-1 that function as erythrocyte receptors for various cell surface molecules present on endothelial cells. Differential expression results in antigenic variation.
DABP	Duffy antigen–binding protein	Proteins of P. vivax and P. knowlesi that bind an erythrocyte surface receptor (Duffy antigen) during parasite invasion.
EBA-175	Erythrocyte-binding protein	Protein of P. falciparum (analogous to DABP) that binds an erythrocyte surface molecule (sialic acid) during parasite invasion.

led by R. Howard (Baruch et al., 1995), used an antiserum directed against one member of the PfEMP1 family to identify a fragment of the corresponding gene in a DNA expression library. Getting a full-length cDNA clone proved difficult, however. Plasmodium switches at a high rate between members of the PfEMP1 family, and populations of infected erythrocytes are therefore always heterogeneous in PfEMP1 mRNA. They finally identified overlapping cDNA sequences from one parasite strain that encode two versions of PfEMP1.

What is the evidence that the *var* genes cloned by these two groups code for PfEMP1 and that PfEMP1 undergoes antigenic variation in the strict sense? The results presented by Baruch et al. (1995) are relatively straightforward, as they focused primarily on one member of the PfEMP1 family, produced by the Malayan Camp (MC) line of Plasmodium. This MC PfEMP1 mediates binding of infected erythrocytes to a surface receptor of endothelial cells, CD36, and to TSP, but not to other receptors, such as ICAM-1. Baruch et al. (1995) have used fusion proteins made with fragments of their cDNA to make antisera against segments of the MC PfEMP1. These antisera specifically detect PfEMP1 in the knobs of erythrocytes infected with the MC line of Plasmodium, and they block binding of the infected erythrocyte to endothelial receptors. Baruch et al. (1995) also show that the MC PfEMP1 is a member of a large family of related surface proteins that differ in antigenic and adhesion properties.

Su et al. (1995) have sequenced four genes of the *var* family. The deduced protein sequences look like cellular adhesion molecules; i.e., they appear to consist of a large and variable extracellular domain, a single transmembrane segment, and a conserved intracellular domain. As expected for a variant antigen, the extracellular domains of different members of the family are very different in sequence, although they share an overall similarity in structure.

Evidence for the differential expression of the members of the *var* family in different parasite lines can be found in all three papers (Baruch et al., 1995; Smith et al., 1995; Su et al., 1995), but the most extensive analyses are presented by Smith et al. (1995). As the rate of switching is as high as 2.4% per generation (Smith et al., 1995), it is impossible to obtain large populations of parasites only expressing a single *var* gene. The problem is compounded by the extensive homology among different *var* genes, making gene-specific probes hard to obtain. Smith et al. (1995) analyzed a series of Plasmodium clones that differ in antigenic and cytoadhesive properties and find that in some cases, specific *var* transcripts were virtually unique to the clones from which they were derived. This shows that the differential control of *var* gene expression must be tight, implying that a single infected erythrocyte may only express one or at most a few *var* genes. This is in agreement with the previous work on the adhesive and antigenic properties of infected erythrocytes. Su et al. (1995) studied the transcription of three *var* genes in different Plasmodium clones. No transcripts were found for two of the genes in any of the clones, whereas one gene was expressed in one parasite clone, but not in two others. Taken together, these analyses establish that *var* genes are differentially expressed and that differential expression is linked to antigenic variation of the adhesive protein PfEMP1 on the erythrocyte surface.

In large families, it is often difficult to get a head count, especially when some heads look rather different. The *var* gene family has at least 50 members, and although some of the genes are clustered (Su et al., 1995), the members are scattered over most if not all malaria chromosomes (Peterson et al., 1995; Su et al., 1995). An upper limit estimate (Su et al., 1995) gives 150 members. Since the genes are large, this would correspond to 6% of the malaria genome. Which fraction of these genes is functional is not known. As the available evidence suggests that the family evolves at a very high rate, one can expect a substantial fraction of the family members to be nonfunctional.

The adhesion function of the PfEMP1 proteins may have evolved from other adhesion proteins required for Plasmodium to get into erythrocytes. Su et al. (1995) stress that the *var* gene products are related to the erythrocyte-binding protein EBA-175 of P. falciparum (Sim et al., 1994) and to the Duffy antigen–binding proteins (DABPs) of P. vivax and P. knowlesi. The single-copy genes for EBA-175 and DABP are conserved and present in all Plasmodium lines. The common motifs in *eba-175* and *var* gene products may indicate a common origin and have led Su et al. (1995)

to group the proteins in one superfamily of Duffy binding–like (DBL) proteins.

With the *var* genes in hand, the interesting biological questions can now be tackled. How does Plasmodium switch the antigen produced? How do the PfEMP1 genes evolve so rapidly? How does PfEMP1 get from the place of synthesis in the parasite to its correct position in the knobs of the erythrocyte membrane? Thus far, there are only some data bearing on the first question, the mechanism of switching.

The analysis of antigenic variation in other organisms has delineated three basic mechanisms for switching in large families of surface antigens (Borst, 1991). The first mechanism is the pretranscriptional, e.g., the replacement of the antigen gene transcribed. This can be done by gene conversion or by reciprocal recombination. The promoter remains unaltered. The second mechanism is transcriptional, e.g., the activation of the promoter of a silent gene and the switching off of the promoter of the previously active antigen gene. This is one of the switching modes used by African trypanosomes, but the mechanism remains unresolved (Borst and Rudenko, 1994). The third mechanism is posttranscriptional, e.g., changing the reading frame of antigen mRNAs. An example is the Opa proteins, minor surface proteins of Neisseria species. The *opa* genes contain CTCTT repeats in their signal sequence. Depending on the copy number of these repeats, the mRNA will either be translated into full-length protein or into a truncated N-terminal fragment. Variations in copy number probably result from slipped mispairing during DNA replication (Borst, 1991).

The limited data now available suggest that Plasmodium does not simply imitate African trypanosomes in its switching mechanism. Expressed PfEMP1 genes are not invariably located in telomeric expression sites, as are the *VSG* (for variant-specific surface glycoprotein) genes of trypanosomes (Borst, 1991; Borst and Rudenko, 1994). There are also no indications for activation of silent *var* genes by duplicative transposition into an expression site. This indicates that Plasmodium must either transfer *var* genes into expression sites by a nonduplicative mechanism (e.g., reciprocal recombination) or control the large *var* gene family by transcriptional or posttranscriptional mechanisms. An intriguing finding is the presence of a large amount of a very heterogeneous transcript of about 2 kb that is related to the 3′ half of the *var* genes. The authors speculate that this transcript is involved in recombination processes required for switching. Another speculative possibility is that Plasmodium transcribes all *var* genes all the time, but controls expression at the level of RNA processing by partial degradation of all mRNAs but one. Nuclear run-on experiments should allow the Wellems group to test this. Differential RNA degradation would be a novel mechanism for switching the protein made in antigenic variation. Admittedly this would be wasteful, but antigenic variation is an intrinsically wasteful survival strategy anyhow, and degrading excess mRNAs is less wasteful than the posttranscriptional mechanism used by Neisseria to control its *opa* genes (Borst, 1991).

Although antigenic variation looks like a perfect way for a parasite to elude the murderous immune system of its host, the strategy is far from simple in practice (Borst, 1991). Obviously, the parasite has to maintain a sizeable repertoire of variant antigens and the ability to switch at appropriate rates the variant antigen produced. Less obvious, but equally important, the parasite also has to avoid gross population heterogeneity during a chronic infection. If each parasite in the population were to express a different *var* gene, then the complete repertoire would be expressed at all times, and antigenic variation would not work. In African trypanosomes, the order of expression of variant antigen genes seems to be determined by a complex combination of genetic and epigenetic factors, and it will be of interest to see how Plasmodium solves this vexing problem.

Is the *var* gene family the only source of antigenic variation in Plasmodium? Once an organism starts on this strategy, it may become a specialist. In Neisseria bacteria, there are at least two types of surface protein independently undergoing antigenic variation (Borst, 1991). In trypanosomes, there is suggestive evidence for antigenic variation of receptors involved in food uptake, in addition to antigenic variation of the main component of the surface coat, VSG (Borst and Rudenko, 1994). It will be interesting to test whether antigenic variation in Plasmodium will also extend to proteins exposed on the surface of the parasite itself.

One always hopes that a breakthrough in the understanding of parasite biochemistry will be translatable into better control of parasitic disease, and there is some basis for this hope in the case of the *var* genes. The ability of P. falciparum to make PfEMP1 correlates with virulence. Sequestration of infected erythrocytes in brain capillaries is considered a prerequisite for cerebral malaria, a lethal complication of P. falciparum infection. This sequestration is dependent on PfEMP1 in the knobs on the erythrocyte surface. If one could prevent PfEMP1 from gluing the erythrocytes onto the vessel endothelium, the infected erythrocytes would be flushed out toward the spleen and destroyed. Precise knowledge of the *var* gene family may allow the generation of vaccines that protect against the major classes of adhesion molecules that the parasite can produce. As the receptors on endothelial cells recognized by these adhesion molecules are limited, the parasite is also limited in the variability of the adhesion molecules it can produce. Time will tell whether this approach is feasible and useful.

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