Review

Antigenic variation in pathogenic micro-organisms: similarities and differences

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Antigenic variation is a process by which pathogenic micro-organisms escape the immune response of their mammalian hosts. By convergent evolution, protozoal, fungal and bacterial pathogens have developed similar genetic mechanisms for true antigenic variation. In this review article, the biology, the surface antigens and their encoding genes, and the molecular mechanisms of antigenic variation of the protozoa Trypanosoma brucei, Plasmodium falciparum, Babesia bovis, Giardia lamblia, the fungus Pneumocystis carinii, and the bacteria Borrelia hermsii, Anaplasma marginale, Neisseria gonorrhoeae, Mycoplasma bovis and Campylobacter fetus are compared.

Key words: Antigenic variation, pathogens, micro-organisms, molecular mechanisms, immune evasion.

INTRODUCTION

Antigenic variation is a strategy used by different pathogenic micro-organisms to evade the immune response of their mammalian hosts and to establish a chronic infection. True antigenic variation is defined as the periodic expression of homologous, but antigenically distinct, surface molecules within a clonal population (Beale and Wilkinson, 1961). The different surface antigen variants are encoding by a multimember family of related genes, normally expressed one at a time. Consequently, based on this definition, antigenic drift (the accumulation of point mutations over time in a known strain) and antigenic shift (recombination of two different strains infecting the same host) as demonstrated for some viruses, are not regarded as examples of antigenic variation (van der Woude and Bäumler, 2004). True antigenic variation has been described for the protozoan parasites Trypanosoma brucei, Plasmodium falciparum, Babesia bovis and Giardia lamblia, for the fungus Pneumocystis carinii, and for the bacteria Borrelia hermsii, Anaplasma marginale, Neisseria gonorrhoeae, Campylobacter fetus and Mycoplasma bovis. In addition, the complete genome sequences of the bacteria Helicobacter pylori, Mycobacterium tuberculosis and Treponema pallidum revealed large families of related genes that may be involved in antigenic variation (Tomb et al., 1997; Cole et al., 1998; Fraser et al., 1998). In this review, the biology, the antigens, and the genes of micro-organisms that undergo true antigenic variation will be summarized. Furthermore, the molecular mechanisms of antigenic variation employed by these pathogens, that facilitate evasion of the host immune response, will be reviewed.

ABBREVIATION

B-ES, bloodstream-form VSG expression site, CRJE, conserved recombination junction element, DHS, downstream homology sequence, ESAG, expression site-associated gene, ESB, expression site body, LAT, locus of active transcription, MSG, major surface glycoprotein, MSP, major surface protein, PIEMP, Plasmodium falciparum erythrocyte membrane protein, SLP, surface layer protein, UCS, upstream conserved sequence, UHS, upstream homology sequence, VESA, variant erythrocyte surface antigen, Vlp, variable large protein, VSG, variant surface glycoprotein, VSP (G. lamblia), variant surface protein, VSP (M. bovis), variable surface lipoprotein, Vsp, variable small protein.

Biology

Although pathogens that undergo antigenic variation live in different environments within their mammalian hosts, recurring patterns of life styles and transmissions can be identified (Table 1). Most of these pathogens live extracellularly and can be found in similar tissues. For in-
Table 1. Pathogens in which antigenic variation occur.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Disease</th>
<th>Habitat</th>
<th>Transmission</th>
<th>Persistence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. brucei</em></td>
<td>African trypanosomiasis</td>
<td>Extracellular</td>
<td>Vector-borne (tsetse flies)</td>
<td>Months – years</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>Malaria</td>
<td>Intracellular</td>
<td>Vector-borne (mosquitoes)</td>
<td>Weeks – months</td>
</tr>
<tr>
<td><em>B. bovis</em></td>
<td>Redwater fever</td>
<td>Intracellular</td>
<td>Vector-borne (hard ticks)</td>
<td>Years</td>
</tr>
<tr>
<td><em>G. lamblia</em></td>
<td>Giardiasis</td>
<td>Extracellular</td>
<td>Water-borne</td>
<td>Weeks – months</td>
</tr>
<tr>
<td><em>P. carinii</em></td>
<td>Pneumocystis pneumonia</td>
<td>Extracellular</td>
<td>Air-borne</td>
<td>Years</td>
</tr>
<tr>
<td><em>B. hermsii</em></td>
<td>Relapsing fever</td>
<td>Extracellular</td>
<td>Vector-borne (soft ticks)</td>
<td>Weeks – years</td>
</tr>
<tr>
<td><em>A. marginale</em></td>
<td>Anaplasmosis</td>
<td>Intracellular</td>
<td>Vector-borne (hard ticks)</td>
<td>Years</td>
</tr>
<tr>
<td><em>N. gonorrhoeae</em></td>
<td>Gonorrhoea</td>
<td>Extracellular†</td>
<td>Sexual</td>
<td>Months</td>
</tr>
<tr>
<td><em>M. bovis</em></td>
<td>Mycoplasmosis</td>
<td>Extracellular</td>
<td>Air-borne, direct contact, oral</td>
<td>Months – years</td>
</tr>
<tr>
<td><em>C. fetus</em></td>
<td>Campylobacteriosis</td>
<td>Extracellular</td>
<td>Food-borne, water-borne, sexual, oral</td>
<td>Weeks – months</td>
</tr>
</tbody>
</table>

† Occurs also intracellularly.

tance, the protozoan parasite *T. brucei* and the spirochete bacterium *B. hermsii* multiply in the blood. The gram-negative bacterium *C. fetus* (ssp. *fetus*) can enter the bloodstream and can cause bacteremia.

Other extracellular pathogens reside in mucous membranes of certain body cavities: the gram-negative bacterium *N. gonorrhoeae* in the mucosa of the urogenital and oropharyngeal tract; the wall-less bacterium *M. bovis* in the mucosa of the urogenital and respiratory tract; *C. fetus* in the mucosa of the genital (ssp. *venerealis*) and gastrointestinal (ssp. *fetus*) tract; and the fungus *P. carinii* in the mucosa lining the alveoli.

The protozoan parasite *G. lamblia* is somewhat exceptional as it lives in the lumen of the small intestine. Interestingly, all three intracellular pathogens, the protozoan parasites *P. falciparum* and *B. bovis*, and the rickettsia *A. marginale*, reside inside the same cell type, namely erythrocytes.

As all these pathogens in the different bodily environments are confronted to the humoral immune response of the host in one or the other way, they have to establish mechanisms to evade the host’s immune defence in order to establish chronic infections.

The development of antigenic variation of surface coat proteins reflects the evolutionary driving force for the persistence of these pathogens in their immunocompetent mammalian host as part of their infrequent transmission.

### Surface antigens

At first glance it seems that the antigens involved in antigenic variation have nothing in common; they vary considerably in size with molecular masses ranging between 18 and 350 kDa (Table 2). Even within one species the antigens can substantially differ in size (e.g. 20 – 200 kDa for the VSP of *G. lamblia*). However, on closer inspection, similarities among these antigens can be identified. Generally, these antigens are highly immunogenic but poorly immunologically cross reactive (Kyes et al., 2001).

In extracellular pathogens, these antigens are attached to the outer surface of the cell. In several microorganisms these molecules are glycoproteins that are anchored to the cell surface in different ways. The variant surface glycoproteins (VSGs) of *T. brucei* form homodimers that are attached to the outside of the plasma membrane via a glycosylphosphatidylinositol anchor per subunit (Cross, 1990). The VSG molecules compose a densely packed monolayer that shields other proteins from antibodies. The variant surface proteins (VSPs) of *G. lamblia* are sparsely glycosylated, cysteine-rich polypeptides (Nash, 2002). All VSPs have a conserved hydrophobic tail that spans the plasma membrane of the parasite. In addition, the VSPs are modified with a palmitate residue which may also be involved in anchoring the protein to the plasma membrane.
### Table 2. Features of antigenic variation in pathogenic micro-organisms.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Surface antigen</th>
<th>Gene</th>
<th>Switching rate (cell⁻¹ generation⁻¹)</th>
<th>Mechanism of expression control</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. brucei</td>
<td>VSG</td>
<td>VSG</td>
<td>~1000</td>
<td>Gene replacement, transcriptional control, segmental gene conversion</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>PIEMP1</td>
<td>var</td>
<td>~60</td>
<td>Transcriptional control</td>
</tr>
<tr>
<td>B. bovis</td>
<td>VESA1</td>
<td>ves</td>
<td>~300</td>
<td>Gene conversion¶</td>
</tr>
<tr>
<td>G. lamblia</td>
<td>VSP</td>
<td>vsp</td>
<td>~150</td>
<td>Transcriptional control</td>
</tr>
<tr>
<td>P. carinii</td>
<td>MSG</td>
<td>msg</td>
<td>~100</td>
<td>Gene replacement</td>
</tr>
<tr>
<td>B. hermsii</td>
<td>Vlp, Vsp</td>
<td>vlp, vsp</td>
<td>59</td>
<td>Gene replacement</td>
</tr>
<tr>
<td>A. marginale</td>
<td>MSP2, MSP3</td>
<td>msp2, msp3</td>
<td>10 – 20‡</td>
<td>Segmental gene conversion</td>
</tr>
<tr>
<td>N. gonorrhoeae</td>
<td>Pilin</td>
<td>pil</td>
<td>19</td>
<td>Segmental gene conversion</td>
</tr>
<tr>
<td>M. bovis</td>
<td>VSP</td>
<td>vsp</td>
<td>13</td>
<td>Promoter rearrangement</td>
</tr>
<tr>
<td>C. fetus</td>
<td>SLP</td>
<td>sapA</td>
<td>5 – 9</td>
<td>Promoter rearrangement</td>
</tr>
</tbody>
</table>

† VSG, variant surface glycoprotein; PIEMP1, *Plasmodium falciparum* erythrocyte membrane protein 1; VESA1, variant erythrocyte surface antigen 1; VSP (G. lamblia), variant surface protein; MSG, major surface glycoprotein; Vlp, variable large protein; Vsp, variable small protein; MSP2/3, major surface protein 2/3; VSP (M. bovis), variable surface lipoprotein; SLP, surface layer protein.

‡ Pseudogenes.

* Frequency for gene rearrangement.

The major surface antigens (MSGs) of *P. carinii* are glycoproteins containing mannose-rich oligosaccharides (Nakamura, 1998). A hydrophobic domain at the C-terminus of the MSGs has been implicated in membrane anchorage of these surface antigens. Likewise, the pilin proteins of *N. gonorrhoeae* are glycopolypeptides (Deitsch et al., 1997). Pilins are the major subunits of the neisserial type IV pili and are involved in the epithelial cell binding of the bacterium. The surface antigens of *B. hermsii* (Vips and Vsps) and of *M. bovis* (VSPs) are lipoproteins (Barbour and Restrepo, 2000; Lysnyansky et al., 1999). Whereas the Vlp/Vsp proteins are attached to the outer membrane of *B. hermsii* by their lipid moieties, the acylated VSP proteins with their long-chain fatty acids are anchored in the plasma membrane of the wall-less *M. bovis*. The surface layer proteins (SLPs) of *C. fetus* are noncovalently attached to the outer cell wall of lipopolysaccharides (Dworkin and Blaser, 1997). They form a capsule envelope of paracrystalline structure that functions like the densely packed VSG coat of *T. brucei* as a protective environmental barrier.

The variant antigens of intracellular micro-organisms can be attached to the outer surface either of the pathogen or the host cell. The major surface proteins 2 and 3 (MSP2, MSP3) of *A. marginale* are outer membrane proteins with surface-exposed hypervariable regions (Barbour and Restrepo, 2000). In contrast, the *Plasmodium falciparum* erythrocyte membrane protein-1 (PIEMP1) of the malaria parasite and the variant erythrocyte surface antigen 1 (VESA1) of *B. bovis* are inserted on the external tip of so-called membrane knobs of infected red blood cells (Kyes et al., 2001; O’Connor and Allred, 2000). Both surface antigens have a single predicted transmembrane domain and are involved in endothelial cytoadhesion with sequestration of parasitized red blood cells (Kyes et al., 2001; Barbour and Restrepo, 2000).

**Genes**

The surface antigens involved in antigenic variation are encoded by large families of nonallelic genes. Only one gene is usually expressed at any one time and antigenic switching in the mammalian host occurs at high rates of $10^{-4}$ to $10^{-1}$ per cell per generation (Turner, 1997; Roberts et al., 1992; Nash et al., 1990; Keely et al., 2003; Stoenner et al., 1982; Criss et al., 2005; Lysnyansky et al., 1996; Tu et al., 2003) (Table 2). The number of sur-
face antigen genes in the different pathogens varies considerably and ranges between 5 and 1000 (Van der Ploeg et al., 1982; Gardner et al., 2002; Allred and Al-Khedery, 2004; Nash et al., 1990; Kutty et al., 2001; Dai et al., 2006; Brayton et al., 2002; Hamrick et al., 2001; Lysnyansky et al., 1999; Tu et al., 2001) (Table 2). Interestingly, the repertoire of surface antigen genes appears to be significantly larger in the eukaryotic pathogens than in the prokaryotic micro-organisms.

The genomic organisation of surface antigen genes differs in the various pathogens. In *T. brucei*, most of the VSG genes are clustered in large subtelomeric arrays on the 11 megabase chromosomes (Berriiman et al., 2005). Most of the subtelomeric VSG genes are pseudogenes of which only 33 and 13% have intact N-terminal and C-terminal domains, respectively, indicating that silent VSG gene require to obtain functional N-terminal and/or C-terminal domains to be expressed (Marcello and Barry, 2007). Other VSG genes are located at the termini of around 100 minichromosomes (Borst, 2002). In addition, each of the 20 telomeric expression sites carries a VSG gene (Cross, 1990). In *P. falciparum*, var genes are scattered on all 14 chromosomes except one (Gardner et al., 2002). Two-third of the var genes are located near the end of chromosomes singly or in groups of twos or threes with two genes frequently arranged in tail-to-tail orientation (Gardner et al, 2002). The rest of the var genes is found alone or in clusters of three to seven genes in tandem repeats (head-to-tail orientation) in chromosome-internal regions (Gardner et al., 2002). A unique organisation has been recently described for the ves genes in *B. bovis*. The previously identified ves1a gene encoding the VESA1a subunit is found together with a newly discovered ves multigene family, vesβ1, in a head-to-head orientation (Al-Khedery and Allred, 2006). The vsp genes of *G. lamblia* that have been cloned and characterised so far and that can be divided into different families are mostly not telomeric and reside on one or several regions of chromosomes 4 and 5 (Adam, 2000). Although there are indications that vsp genes are somewhat clustered, the different vsp families appear not to be linked (Adam, 2000). In *P. carinii*, msg genes can be found on all chromosomes in clusters of 2 – 4 genes (Stringer and Kelly, 2001). As far as it is known, all msg genes are located at the end of chromosomes (Stringer and Kelly, 2001). In *B. hermsii*, vsp and vlp genes are arrayed in 10 clusters of 2 – 14 genes on linear plasmids of 28 – 32 kb (Dai et al., 2006). The msp2 and msp3 genes of *A. marginale* belong to a superfamly of surface antigen genes (Brayton et al., 2005). The genome of *A. marginale* contains one full-length expression site gene for each msp2 and msp3 (Brayton et al., 2005). In addition, there are seven functional pseudogenes for msp2 and for msp3, four of which in each case are closely linked in a tail-to-tail arrangement (Brayton et al., 2001). In *N. gonorrohoeae*, most pil genes are truncated at the 5’ end and are arranged in 5 pilS loci while one full-length and functional pil gene in located in 1 pilE locus (Haas et al., 2001). Four of the pilS loci and the pilE locus are clustered in a 35 kb region of the chromosome (Hamrick et al., 2001). The vsp genes of *M. bovis* are arranged in a chromosomal cluster of about 23 kb (Lysnyansky et al., 1999). Each vsp gene is also linked to highly homologous upstream regions composed of two cassettes (Lysnyansky et al., 1999). In *C. fetus*, sap genes are tightly clustered in a 53.8 kb chromosomal region (Tu et al., 2003).

**Molecular mechanisms**

Pathogenic micro-organisms use one or more of four genetic mechanisms for antigenic variation: transcriptional control, gene replacement, segmental gene conversion and promoter rearrangement (Table 2).

**Transcriptional control**

The first mechanism, transcriptional control, also known as *in situ* switch, is used by the protozoa *T. brucei*, *P. falciparum* and *G. lamblia* (Table 2). In this process, the expression of the old variant antigen gene at one locus is silenced while the expression of a new variant antigen gene at another locus is activated (Figure 1A). The *in situ* switch occurs without any DNA rearrangements at the loci themselves.

*T. brucei* has ~20 bloodstream-form VSG expression sites (B-ES) which are located at the ends of chromosomes (Becker et al., 2004). B-ESs are transcribed as polycistronic transcription units containing 8 – 12 expression site-associated genes (ESAGs) in addition to the telomeric VSG gene. Only one of the 20 B-ESs is fully active while the ‘silent’ B-ESs were found to be actively transcribed in their promoter-proximal part generating truncated transcripts that are inefficiently processed and released from the nucleus (Ansorge et al., 1999; Vanhamme et al., 2000). To avoid transcriptional attenuation, it was discovered that B-ESs have to be present in a specialised region in the nucleus termed expression site body (ESB) (Navarro and Gull, 2001). It appears that the ESB recruits the full transcriptional machinery for RNA synthesis, elongation and processing. In this model, transcriptional switching would be due to replacement of the active B-ES by an inactive B-ES in the ESB.

In ring stages of *P. falciparum*, it appears that many, if not all, var genes are capable of expression but that only one var gene is transcribed into a full-length functional mRNA (Taylor et al., 2000; Kyes et al., 2001). It seems that the silencing of var genes is determined by several epigenetic factors including reversible chromatin modification (acetylation of histones), interaction between the var 5’ promoter and var intron promoter, and perinuclear gene movement (Ralph and Scherf, 2005; Kyes et al., 2007). In fact, recent evidence indicates the existence of a single var gene transcription site at the nu-
nuclear periphery harbouring the dominantly expressed var gene (Ralph et al., 2005; Marty et al., 2006; Voss et al., 2006). Thus, the regulation of var gene expression in *P. falciparum* bears some physical similarity to the expression of VSG genes by B-ESs in *T. brucei*. However, whereas var genes are transcribed by RNA polymerase II (Kyes et al., 2007), transcription of VSG genes is mediated by RNA polymerase I (Günzl et al., 2003).

In *G. lamblia*, expression of vsp genes is not associated with gene movement or DNA rearrangement, but with acetylation of histones (Kulakova et al., 2006). These findings indicate that transcription of vsp genes in *G. lamblia*,...
Gene replacement

The second mechanism, gene replacement (sometimes also referred to as gene conversion) is utilised by T. brucei, P. carinii, and B. hermsii (Table 2). This process involves the replacement of the gene at the expression site by a copy of an archived gene from a location somewhere else in the genome (Figure 1B). This results in the loss of the expression site copy of the old gene, but the original archived copy of the gene is usually retained.

In T. brucei, VSG gene switching by gene replacement relies on non-reciprocal homologous recombination. This event is usually mediated between a few copies of a characteristic 70-bp repeat upstream of the VSG donor gene and a large array of the same repeat in the active expression site on the 5’ side, and between conserved sequences in the untranslated region at the 3’ ends of the VSG genes (Cross, 1990; Taylor and Rudenko, 2006).

The fungus P. carinii uses a single expression site, termed the upstream conserved sequence (UCS), for expression of msg genes (Stringer and Keely, 2001). Interestingly, the UCS is attached to the msg transcript and encodes for the sequence found at the 5’ end of most msg mRNAs (Wada et al., 1995). The presence of different msg genes attached to the UCS implies that msg gene switching occurs by recombination (Wada et al., 1995; Sunkin and Stringer, 1997). A common 23-bp sequence termed conserved recombination junction element (CRJE) which is found at the 5’ termini of all silent msg genes could be the crossover point (Wada et al., 1995; Sunkin and Stringer, 1997). The fungus P. carinii uses a single expression site for transcribing vsg genes, the locus of active transcription (LAT) (Al-Khedery and Allred, 2006). The LAT is found on chromosome 1 and includes a bidirectional promoter which drives the co-expression of juxtaposed and divergently oriented vesi1α and vesi1β genes. In the course of segmental gene conversion, the actively transcribed vesi1α gene is gradually altered by repeated replacement of short stretches of sequences with equivalent sequence segments from inactive vesi donor gene (Al-Khedery and Allred, 2006). Whereas the vesi1α at the LAT becomes quickly a mosaic gene, the donor vesi genes remain unaltered. The donor sequence may come from vesi genes located on the same or different chromosomes as the LAT (Al-Khedery and Allred, 2006).

In A. marginale, full-length msp2 and msp3 genes are transcribed from a single expression site (Barbet et al., 2000; Brayton et al., 2001; Meeus et al., 2003). For variation of the expressed msp2 and msp3 genes, A. marginale uses two modes of segmental gene conversion. At first, a truncated msp2 and msp3 pseudogene recombines into the expression site generating diversity in the hypervariable region of the full-length expressed msp2 and msp3 genes, respectively (Brayton et al., 2001; Meeus et al., 2003). The crossover points for the recombination event are probably located in short stretches of the 5’ and 3’ conserved regions flanking the hypervariable regions of the expressed msp2/msp3 genes and msp2/msp3 pseudogenes (Brayton et al., 2002; Meeus et al., 2003). In a second level of variation, the new expression site msp2 and msp3 gene can be altered by segmental gene conversion of small stretches of pseudogenes (Brayton et al., 2002 Meeus et al., 2003). This recombination event involves short segments in the hypervariable region of msp2 and msp3 sequences (Brayton et al., 2002; Meeus et al., 2003). Whereas the msp2 and msp3 genes of the expression site are altered by these recombination mechanisms, the involved pseudogenes remain unchanged.

N. gonorrhoeae carries a single pil gene expression locus, pilE (Hamrick et al., 2001). During antigenic variation, part or all of a silent pilE copy is recombined into the pilE gene by segmental gene conversion (Haas and
Mayer, 1986). The crossovers occur at conserved regions bordering six variable regions found in the silent pilS gene and in corresponding regions of the pilE gene (Haas and Meyer, 1986; Hamrick et al., 2001). The pil gene finally expressed may thus be a chimera of sequences originating from several pilS loci.

Promoter rearrangement

The last mechanism for antigenic variation is promoter rearrangement which is use by M. bovis and C. fetus (Table 2). In this mechanism a promoter sequence located upstream of the expressed variable surface antigen gene is rearranged in front of an open reading frame of a silent variable surface antigen gene by DNA inversion (Figure 1D).

In M. bovis, a specific cassette designated A2 is the active promoter for expression of vsp genes (Lysnayansky et al., 2001). During antigenic variation, this regulatory element is juxtaposed to a silent vsp gene by DNA inversion and allows transcription of the recipient gene (Lysnayansky et al., 2001). The site-specific DNA inversion occurs between specific 35-bp sequences, designated vis (vsp inversion sequence), present within converged region 37-bp upstream of all known vsp genes (Lysnayansky et al., 2001).

For expression of sapA genes, C. fetus utilizes a single promoter (sapA promoter) (Dworkin and Blaser, 1996). In the process of antigenic variation, the bacterium reassigns the sapA promoter and one, or more, complete open reading frames of sapA genes by DNA inversion (Dworkin and Blaser, 1997). The reciprocal DNA rearrangement at sapA locus is mediated by 5' but not by 3' conserved regions present in each sapA gene (Tu et al., 2003). DNA recombination between two sapA genes mediated by central conserved regions allows the formation of new sapA genes (Tu et al., 2003).

Conclusion

Despite belonging to different kingdoms (Eubacteria, Protista, and Fungi), pathogenic micro-organisms that undergo true antigenic variation have developed similar strategies to escape the immune response of their mammalian host. This does not only apply to the adopted life style and environment within the host but also to the underlying genetic mechanisms for successful antigenic variation of the involved surface antigens. It can be assumed that each of the four mechanisms for antigenic variation use by two to four different pathogens have been generated through convergent evolution driven by the adaptive immune system of the host. A consequent of antigenic variation is that the development of vaccines against these pathogens may prove impractical. In fact, no vaccines providing sterile immunity against infection by any of these pathogens have been produced to date.

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