Review

Antigenic diversity in pathogenic micro-living beings: semblance and contrasts

Raymond M.O

School of Life and Medical Sciences, University of Hertfordshire, Hertfordshire, England, United Kingdom. E-mail: raymond_m@yahoo.com.

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 perio-dic 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 (Barbour and Restrepo, 2000). Likewise, phase

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, PfEMP, 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.

variation (on/off-expression of one or more genes within a clonal population) as described in several bacteria is not considered as 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.

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.

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

† 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 bacteraemia.

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 proto-zoan 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 micro-organisms these molecules are glycoproteins that are anchored to the cell surface in different ways. The variant surface glycoproteins (VSGs) of *T. brucei* form homo-dimers 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 polype-ptides (Nash, 2002). All VSPs have a conserved hydro-phobic tail that spans the plasma membrane of the para-site. 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.

| Pathogen | Surface antigen | | Gene | | Switching rate $(\text{cell}^{-1}\ \text{generation}^{-1})$ | Mechanism of expression control |
	Name†	Size (kDa)	Name	Number		
T. brucei	VSG	55	*VSG*	~1000	$4 \times 20^{-2} - 2 \times 10^{-3}$	Gene replacement, transcriptional control, segmental gene conversion
P. falciparum	PfEMP1	200 – 350	*var*	~60	2×10^{-2}	Transcriptional control
B. bovis	VESA1	128	*ves*	~300	Unknown	Gene conversion¶
G. lamblia	VSP	20 – 200	*vsp*	~150	$2 \times 10^{-1} - 8 \times 10^{-2}$	Transcriptional control
P. carinii	MSG	120	*msg*	~100	10^{-2}	Gene replacement
B. hermsii	Vlp, Vsp	36, 20	*vlp, vsp*	59	$10^{-3} - 10^{-4}$	Gene replacement
A. marginale	MSP2,MSP3	36 – 44	*msp2, msp3*	10 – 20‡	Unknown	Segmental gene conversion
N. gonorrhoeae	Pilin	18 – 20	*pil*	19	4×10^{-3}	Segmental gene conversion
M. bovis	VSP	40 – 75	*vsp*	13	$10^{-2} - 10^{-3}$	Promoter rearrangement
C. fetus	SLP	97 – 149	*sapA*	5 – 9	$10^{-1} - 10^{-3}*$	Promoter rearrangement

† VSG, variant surface glycoprotein; PfEMP1, *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.
¶ Possible also transcriptional.
‡ 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* (Vlps 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 outermembrane 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 lipopoly-saccharides (Dworkin and Blaser, 1997). They form a capsule envelope of paracrystalline structure that func-tions 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 patho-gen 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 *Plas-modium falciparum* erythrocyte membrane protein-1 (PfEMP1) of the malaria parasite and the variant erythro-cyte 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 pre-dicted transmembrane domain and are involved in endo-thelial cytoadhesion with sequestration of parasi-tized 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 (Berriman 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 *ves1* gene encoding the VESA1a subunit is found together with a newly discovered *ves* multigene family, *ves 1*, 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 superfamily 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. gonorrhoeae*, 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).
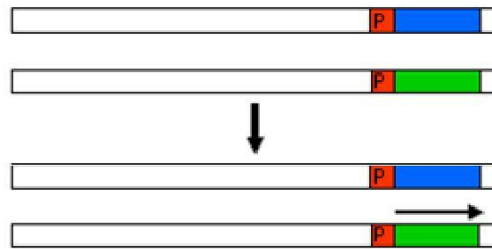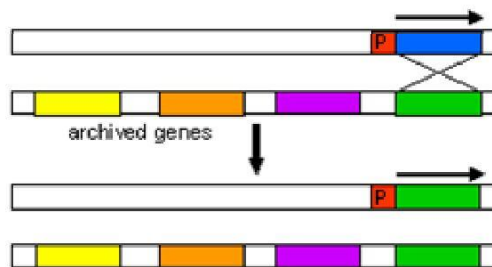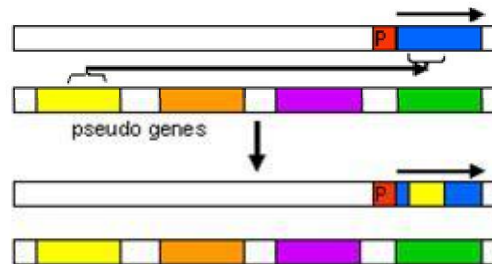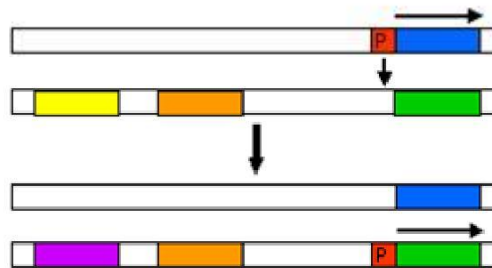
Molecular mechanisms

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 trans-cribed as polycistronic transcription units containing 8 – 12 expression site- associated genes (*ESAG*s) 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 ate-nuation, 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-

A: Transcriptional Control (*T. brucei, P. falciparum, G. lambila*)

B: Gene Replacement (*T. brucei, P. Carini, B. hermsii*)

C: Segmental Gene Conversion (*T. brucei, A. marginale, N. gonorrhoeae*)

D: Promoter Rearrangement (*M. bovis, C. fetus*)

Figure 1. Molecular mechanisms for antigenic variation in pathogens. See text for details.

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. lam-*

blia is also controlled by epigenetic mechanisms.

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; Stringer and Kelly, 2001). However, as the CRJE is too short to support homologous recombination, a site-specific recombinase may be involved in the process of *msg* gene exchanging (Stringer and Kelly, 2001).

The relapsing fever agent *B. hermsii* employs also a single expression site which is located at one end of one of the plasmids harbouring the *vlp* and *vsp* genes, lp28-1 (Kitten and Barbour, 1990; Barbour et al., 1991). Gene replacement occurs through homologous recombination within a ~60 nucleotide region, the upstream homology sequence (UHS), found in archived and expression site genes, and within a 214 nucleotide long downstream homology sequence (DHS) found periodically among the *vlp/vsp* archival gene and downstream of the expression site (Dai et al., 2006). For most recombination events, the upstream and downstream crossovers happen within the second half of the UHS and DHS, respectively (Dai et al., 2006).

Segmental gene conversion

Antigenic variation in *T. brucei*, *B. bovis*, *A. marginale* and *N. gonorrhoeae* is mediated by a third mechanism, segmental gene conversion (Table 2). In this process, in-

active genes or pseudogenes provide donor sequences to the actively transcribed gene (Figure 1C). Using segmental gene conversion, pathogens can generate a virtually limitless repertoire of surface antigens with the help of a few genes.

The above mentioned gene replacement of *VSG* genes in *T. brucei* is often a segmental gene conversion because the crossover between the donor and expressed *VSG* genes can also occur within the 3' ends of the coding region of both genes (Michels et al., 1983; Pays et al., 1985). Later in infection (by week 4), *VSG* genes are predominately modified by segmental gene conversion of smaller segments provided by *VSG* pseudogenes generating mosaic *VSG* genes (Marcello and Barry, 2007).

B. bovis uses a unique expression site for transcribing *ves* 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 *ves1* and *ves1* genes. In the course of segmental gene conversion, the actively transcribed *ves1* gene is gradually altered by repeated replacement of short stretches of sequences with equivalent sequence segments from inactive *ves* donor gene (Al-Khedery and Allred, 2006). Whereas the *ves1* at the LAT becomes quickly a mosaic gene, the donor *ves* genes remain unaltered. The donor sequence may come from *ves* 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 *pilS* 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 conversed 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 reassorts 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.

REFERENCES

Adam RD (2000) The *Giardia lamblia* genome. Int. J. Parasitol. 30: 475-484.

Al-Khedery B, Allred DR (2006) Antigenic variation in *Babesia bovis* occurs through segmental gene conversion of the *ves* multigene family, within a bidirectional locus of active transcription. Mol. Microbiol. 59: 402-414.

Allred DR, Al-Khedery B (2004) Antigenic variation and cytoadhesion in *Babesia bovis* and *Plasmodium falciparum*: different logics achieve the same goal. Mol. Biochem. Parasitol. 134: 27-35.

Ansorge I, Steverding D, Melville S, Hartmann C, Clayton C (1999) Transcription of 'inactive' expression sites in African trypanosomes leads to expression of multiple transferrin receptor RNAs in bloodstream forms. Mol. Biochem. Parasitol. 101: 81-94.

Barbet AF, Lundgren A, Yi J, Rurangirwa FR, Palmer GH (2000) Antigenic variation of *Anaplasma marginale* by expression of MSP2 mosaics. Infect. Immun. 68: 6133-6138.

Barbour AG, Restrepo BI (2000) Antigenic variation in vector-borne pathogens. Emerg. Infect. Dis. 6: 449-457.

Barbour AG, Burman N, Carter CJ, Kitten T, Bergstrom S (1991) Variable antigen genes of the relapsing fever agent *Borrelia hermsii* are activated by promoter addition. Mol Microbiol 5: 489-493.

Beale GH, Wilkinson JF (1961) Antigenic variation in unicellular organisms. Annu. Rev. Microbiol. 15: 263-296.

Becker M, Aitcheson N, Byles E, Wickstead B, Louis E, Rudenko G (2004) Isolation of the repertoire of *VSG* expression site containing telomeres of *Trypanosoma brucei* 427 using transformation-associated recombination in yeast. Gemone Res. 14: 2319-2329.

Berriman M, Ghedin E, Hertz-Fowler C, Blandin G, Renauld H, Bartholomeu DC, Lennard NJ, Caler E, Hamlin NE, Haas B, Böhme U, Hannick L, Aslett MA, Shallom J, Marcello L, Hou L, Wickstead B, Alsmark UC, Arrowsmith C, Atkin RJ, Barron AJ, Bringaud F, Brooks K, Carrington M, Cherevach I, Chillingworth TJ, Churcher C, Clark LN, Corton CH, Cronin A, Davies RM, Doggett J, Djikeng A, Feldblyum T, Field MC, Fraser A, Goodhead I, Hance Z, Harper D, Harris BR, Hauser H, Hostetler J, Ivens A, Jagels K, Johnson D, Johnson J, Jones K, Kerhornou AX, Koo H, Larke N, Landfear S, Larkin C, Leech V, Line A, Lord A, Macleod A, Mooney PJ, Moule S, Martin DM, Morgan GW, Mungall K, Norbertczak H, Ormond D, Pai G, Peacock CS, Peterson J, Quail MA, Rabbinowitsch E, Rajandream MA, Reitter C, Salzberg SL, Sanders M, Schobel S, Sharp S, Simmonds M, Simpson AJ, Tallon L, Turner CM, Tait A, Tivey AR, Van Aken S, Walker D, Wanless D, Wang S, White B, White O, Whitehead S, Woodward J, Wortman J, Adams MD, Embley TM, Gull K, Ullu E, Barry JD, Fairlamb AH, Opperdoes F, Barrell BG, Donelson JE, Hall N, Fraser CM, Melville SE, El-Sayed NM (2005) The genome of the African trypanosome *Trypanosoma brucei*. Sci. 309: 416-422.

Borst P (2002) Antigenic variation and allelic exclusion. Cell 109: 5-8.

Brayton KA, Knowles DP, McGuire TC, Palmer GH (2001) Efficient use of a small genome to generate antigenic diversity in tick-borne ehrlichial pathogens. Proc. Natl. Acad. Sci. USA 98: 4130-4135.

Brayton KA, Palmer GH, Lundgren A, Yi J, Barbet AF (2002) Antigenic variation of *Anaplasma marginale msp2* occurs by combinatorial gene conversion. Mol. Microbiol. 43: 1151-1159.

Brayton KA, Kappmeyer LS, Herndon DR, Dark MJ, Tibbals DL, Palmer GH, McGuire TC, Knowles DP Jr (2005) Complete genome sequencing of *Anaplasma marginale* reveals that the surface is skewed to two superfamilies of outer membrane proteins. Proc. Natl. Acad. Sci. USA 102: 844-849.

Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Ra-