Antigenic Variation in Relapsing Fever *Borrelia*.
ABSTRACT

Antigenic Variation in Relapsing Fever *Borrelia*.

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The spirochete *Borrelia hermsii* avoids the immune response of its mammalian host through multiphasic antigenic variation. Serotype specificity is determined by Variable major proteins (Vmp), in the outer membrane. Through a non reciprocal recombination between linear plasmids, a formerly silent vmp gene replaces another vmp gene at a telomeric expression locus downstream from a common expression site. *B. hermsii* before and after the switch from serotype 7 to serotype 21, was examined in detail.

The nucleotide sequence of the vmp7 and vmp21 genes and flanking regions was determined. The vmp7 and vmp21 are 77% identical in their coding sequence, and the deduced translation products are 63% identical. No antigenic cross reactivity is observed between Vmp7 and Vmp21. This suggests a folding of the proteins in which the similar regions are buried, and not exposed when it is presented at the bacterial surface. Vmp7 and Vmp21 have consensus sequences of prokaryotic lipoproteins and are processed as such when expressed in *E. coli*.

The 5' regions of silent and expressed vmp7 and vmp21 were compared. Silent and active vmp7 and vmp21 genes shared a block of homologous sequence at their 5' ends. Sequences upstream of silent vmp7 and vmp21 genes lacked a promoter and differed substantially from each other. In this antigenic switch a vmp gene was activated by a recombination event which placed it downstream of a promoter.

The vmp gene promoter is preceded by a poly(dT-dA) run and three imperfectly-repeated elements of 2 kb. Each of the 2 kb repeats contains inverted repeats of approximately 0.2 kb at their termini. There is no evidence of the presence of similar elements elsewhere in the genome of *B. hermsii*. One or more of these elements may stimulate vmp gene switch or expression.

The African relapsing fever species *Borrelia crocidurae* and the American species *B. hermsii* display many similarities. In both species the vmp genes are localised to linear plasmids, and the vmp genes are activated on the transcriptional level. The nucleotide sequence of their expression sites, however, are not related. Still, the possibility that the switch is mechanistically similar in *B. crocidurae* and *B. hermsii*, cannot be ruled out.

The binding of *B. crocidurae* causes aggregation of erythrocytes around the spirochete. The aggregation is reminiscent of the erythrocyte rosetting seen in malarial infections. The erythrocytes at the *B. crocidurae* surface may protect them from clearance by the host. Thus, the rosetting may constitute an additional mechanism in *B. crocidurae* for the evasion of the immune reaction.

Key words: *Borrelia*, Relapsing fever, Antigenic variation, Vmp lipoprotein, Rosetting.

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by

Nils Burman
Front Cover: *Borrelia*, a vertebrate host and a soft-bodied tick vector.
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The spirochete *Borrelia hermsii* avoids the immune response of its mammalian host through multiphasic antigenic variation. Serotype specificity is determined by Variable major proteins (Vmp), in the outer membrane. Through a non reciprocal recombination between linear plasmids, a formerly silent *vmp* gene replaces another *vmp* gene at a telomeric expression locus downstream from a common expression site. *B. hermsii* before and after the switch from serotype 7 to serotype 21, was examined in detail.

The nucleotide sequence of the *vmp7* and *vmp21* genes and flanking regions was determined. The *vmp7* and *vmp21* are 77% identical in their coding sequence, and the deduced translation products are 63% identical. No antigenic crossreactivity is observed between Vmp7 and Vmp21. This suggests a folding of the proteins in which the similar regions are buried, and not exposed when it is presented at the bacterial surface. Vmp7 and Vmp21 have consensus sequences of prokaryotic lipoproteins and are processed as such when expressed in *E. coli*.

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Key words: *Borrelia*, Relapsing fever, Antigenic variation, Vmp lipoprotein, Rosetting.
This thesis is based on the following articles which are referred to in the text by their roman numerals I-V.


GENERAL BACKGROUND.

1. Pathogenicity.

1.1 An overview.

Pathogenicity is the capability of a microorganism to infect a host. The infection may or may not cause disease in the host. Some microorganisms cause disease in all non immune hosts, whereas others cause disease only in hosts with impaired immune defences (Finlay and Falkow, 1989). The first step of infection is to enter the host. Once inside of that host, the pathogen has to persist or multiply, and subsequently spread to new hosts. Persistence and multiplication will expose the microorganism to the hosts immune mechanisms. Living successfully within a host, therefore, requires some means to evade these immune defences (Falkow, 1993). Spreading of microorganisms to new hosts may be accomplished in a variety of ways. A common feature of many routes of transmission is that they are rarely open at all times. This limits the microorganism so that it must be accessible at the right place at the right time to be able to transfer into a new host. As a consequence, a successful pathogen must enter its host, multiply and stay out of reach of the immune system for a sufficient period of time that it may transfer as many of its descendants as possible into new hosts (Mims, 1988).

1.2 Entry into the host.

The best protection against microbial infection is to deny the microbes access into the body. The skin of animals is an effective barrier against microbial entry. However, animals have to breathe, eat, and eliminate excess heat and waste products. These points of contact with the environment are protected by special mechanisms; mucosal layers with unfavourable pH or abundant in phagocytic cells, and flushing or peristaltic movement. Microorganisms have evolved mechanisms which overcome these barriers allowing entry by most of these routes. *Escherichia coli* may enter by the oral route in contaminated food, *Mycoplasma pneumoniae* enters through the mucosa of the respiratory tract, *Neisseria gonorrhoeae* through the urogenital tract, and staphylococci through cuts in the skin. Other microorganisms are injected into the host animal during the blood meal of biting...
arthropods (Finlay and Falkow, 1989). The tsetse fly injects Trypanosoma parasites which cause sleeping sickness, and ticks inject Borrelia which causes relapsing fever (Felsenfeld, 1971; Donelson, 1989). In some cases, the insect merely cut the skin of the animal host allowing the microbe to enter. This is the case for louse-borne relapsing fever, where the spirochete only enters the host if the louse is crushed, and its haemolymph is rubbed into the wound of the bite (Felsenfeld, 1971).

1.3 Avoiding host defence-systems.

Once inside of the host, the microorganism is exposed to the immune system of the animal. There exist numerous ways in which the immune system may be avoided. One obvious way is to stay out of reach of the actions of the immune system by invading and living inside of a host cell. This is done by enteroinvasive E. coli by induced endocytosis, a process that requires active participation of both the host and the bacterial cell. Neisseria meningitidis covers its surface with a capsule which protects it from complement fixation, and the subsequent engulfment by macrophages. The strategy of covering the surface may also be used to evade the antibody response. This may be achieved by a capsule made up of carbohydrates which resemble host tissue carbohydrates. Serum resistance, the prevention of lysis by complement, is another way to avoid the host defences. This may be accomplished in a variety of ways, the most common of which is to sterically prevent the binding of complement complexes at the surface of the microorganism (Finlay and Falkow, 1989).

1.4 Antigenic variation.

The antigens which evoke immune reactions in the host are mainly the surface molecules of the microorganism. Thus a good way to escape the immune reaction is to change the molecules on the surface. This strategy is used by many microorganisms and may be accomplished in a number of ways, most of which involve DNA rearrangements (Finlay and Falkow, 1989). One method is phase variation, or site specific DNA inversion. This variation causes an on/off regulation when an entire gene is inverted and moved away from its promoter, as in the
flagellar phase variation of *Salmonella typhimurium* (Heichman and Johnson, 1990). The inversion may also take place within a gene, as in the pilV gene of the enteric bacterial plasmids of the incI incompatibility group. The rearrangement of three to four invertible segments generates six to seven variant open reading frames leading to the variation in the pilV gene (Kim and Komano, 1992; Dybvig, 1993).

True antigenic variation involves a mechanism in which a fraction of the parasite population starts to express a new dominating surface molecule, which is immunologically unrelated to the previous. When the immune system attacks the main antigenic variant (serotype) the subpopulation expressing the new surface molecule will pass unnoticed and will survive. The switch in expression to a new surface molecule must occur at such a rate that the new variant is present before the entire old population has been killed. The rate of switching must not be too high, since this would lead to massive population heterogeneity and the selection of antibodies against the entire surface antigen repertoire at once. The three main examples of species undergoing antigenic variation are the African trypanosomes, *Neisseria*, and *Borrelia*. A common feature of all these species is that there are several genes encoding outer surface proteins, but only one is expressed at any given time (Borst, 1991).

The activation of a new variable surface glycoprotein (VSG) in *Trypanosoma* can be achieved in any of three ways. 1) Activation of a nontelomeric silent gene by duplicative transposition, or gene conversion, to a telomeric expression site, replacing the resident gene. 2) Activation of a silent telomeric gene by duplicative transposition into a telomeric expression site. This process resembles a gene conversion and is called telomere conversion because it involves two telomeres. 3) Activation of a silent telomeric gene and the simultaneous inactivation of the previous expression site. The mechanism by which the last type of variation is achieved is not known (Borst and Greaves, 1987; Donelson, 1989).

The antigenic variation of *Neisseria* pili also involves silent and expressed genes. In *Neisseria* however, the silent genes are incomplete pseudogenes, lacking the first part of the pilin gene. The switch is accomplished by the replacement of part of the previously expressed pilin gene with sequence from a silent pseudogene. A high rate of recombination is maintained by the uptake of homologous *Neisseria* DNA by
transformation. The switch is maintained in the absence of transformation but the rate of recombination is lower (Borst and Greaves, 1987; Borst, 1991).

The variation of the variable major proteins (Vmp) of *Borrelia hermsii* is very similar to the variation of the VSGs of the African trypanosomes. In fact, much of the pathogenesis and symptoms produced by the two microorganisms are similar. In *B. hermsii* the only apparent mechanism of Vmp gene activation is gene conversion, however, the number of examples of serotype switches that have been studied are few (Borst, 1991).

1.5 Homologous recombination.

There are two primary requirements for homologous recombination. The first is a region of homology between the interacting DNA molecules. Approximately 50 bp of homology is sufficient for recombination to take place. The other requirement is the introduction of a break in the DNA strand or strands. The break is needed to produce the single strand which may base pair with homologous DNA sequences in another DNA molecule. A protein has been shown to be important for recombination. The RecA protein specifically binds to single stranded DNA and participates in its annealing to homologous duplex DNA, displacing the resident strand. Nicking and ligation of invading and resident strands result in a crossover junction involving four DNA strands. The cross connection can easily move in a zipper-like fashion, creating regions with heteroduplexes in both DNA strands. A cut by a nuclease followed by ligation separates the crossover junction into two separate DNA molecules with heteroduplex regions. DNA replication will separate the heterologous strands into two unequal daughter molecules that will eventually segregate into different cells. The heteroduplexes may also be resolved by the replacement of unpaired bases by mismatch repair. The recombination process is not always reciprocal. The process in which gene B is replacing gene A at its locus while gene B is retained in its original locus, is called gene conversion. It is difficult to distinguish gene conversion from a reciprocal recombination where one of the recombination products has been lost. Both the parents and the progeny of the exchange must be analysed, which is difficult in organisms other than spore forming fungi. DNA repair mechanisms seem to be important in recombination. The most
probable mechanism for gene conversion involves double strand break repair. The initial event is thought to be a double strand break in the region that will receive new DNA. A 5' to 3' exonuclease removes one strand from each end. The remaining single strand searches the genome for homologous DNA that may act as template for strand elongation (Szostak et al., 1983; Borst and Greaves, 1987; Watson et al., 1988; Barbour, 1989; Brock and Madigan, 1991; Plasterk, 1992).

1.6 Spread of microorganisms to new hosts.

Microorganisms may spread by most routes that are used to gain entry into the host. Thus, microorganisms may spread in aerosol drops in the air, from sneezing or coughing, or they may contaminate water or food that is ingested by new hosts. Some microorganisms will spread to new hosts only as a result of close contact or via the exchange of bodily fluids between hosts. The spread of other microorganisms relies on the same biting arthropod that injected it into its present host. Since the probability of a microorganism to be transferred to a new host increases with time, this is an important factor in the pathogenesis of a microorganism. The longer the microorganism can reside in a host, the higher the probability that it may be spread to a new host. This is especially true for vector transmitted microorganisms such as the relapsing fever Borrelia (Finlay and Falkow, 1989; Brunham et al., 1993)

2. Relapsing fever.

2.1 History.

Diseases with symptoms resembling relapsing fever were described in ancient Greece. Epidemics of yellow fever, sweating sickness or famine fever, all probably relapsing fever, have been recorded since the 6th century AD. The first well documented epidemic of relapsing fever was described in Ireland between 1739 and 1741. The disease was first named relapsing fever by Craigie in 1843. The causative agent of relapsing fever was discovered by Obermeier in 1868, and the agent was called Spirochaeta recurrentis until Swellengrebel named the genus Borrelia in 1907 (Felsenfeld, 1971; Saint Girons and Barbour, 1991). The genus designation Borrelia was not widely adopted until the 1930 edition of Bergey’s
Manual (Barbour, 1987). During the first half of the 20th century relapsing fever was actively studied. There were two main reasons for this interest. First, there was the seriousness of the disease, especially the epidemic form, which has high mortality rates. The second reason was that early immunologists found relapsing fever to be a useful model for the study of the immune system. Most of the knowledge concerning relapsing fever \textit{Borrelia} was acquired in the late 19th and the first half of the 20th century. The examination of relapsing fever \textit{Borrelia} has declined since 1950 (Barbour and Hayes, 1986). However, modern recombinant DNA technology has resulted in a resurgence of studies of relapsing fever \textit{Borrelia}.

2.2 The genus \textit{Borrelia}.

The \textit{Borrelia} have a helical shape, a property they share with the other spirochetes. The outermost layer of a borrelial cell is an outer cell membrane. This membrane is relatively fluid, allowing outer membrane proteins to move quickly and freely across the surface. The outer cell membrane surrounds a protoplasmic cylinder consisting of peptidoglycan, the inner cell membrane, and the cytoplasm. In the periplasmic space the flagella, similar to other bacterial flagella, are situated. The flagella are inserted at the termini of the protoplasmic cylinder. The number of flagella varies with borrelial species, and between individuals of a single strain. \textit{Borrelia} are genetically divergent, but they share some characteristics apart from their helical shape. All borrelial species are transmitted to vertebrates by hematophagous arthropods. The guanosine-cytosine content in genomic DNA is between 27 and 32%. The relationship between a given \textit{Borrelia} species and its vector is specific, and may be used to determine the borrelial species (see below) (Barbour and Hayes, 1986). Species classification may be further defined by the difference in host susceptibility between \textit{Borrelia} species (Felsenfeld, 1971). When the \textit{Borrelia} have entered the bloodstream they divide by binary fission approximately once every 6 h. Once the immune reaction is provoked and antibodies bind, the borreliae are cleared from the bloodstream by either complement induced lysis or phagocytosis via C3b receptors (Barbour and Hayes, 1986). Antibodies are critical for the clearance of borreliae from the blood. Irradiated mice with reconstituted B-cell defence rid themselves of the bacteria, whereas mice with reconstituted T-cell defence do not
One of the limiting factors for the study of *Borrelia* is the difficulty of growing the bacteria outside of an animal or human body. For a long time, the best way of growing *Borrelia* was to inject them into fertilised eggs (Felsenfeld, 1971). A property of *Borrelia* that encouraged the search for a culture medium was that relapsing fever borreliae grow extracellularly in vertebrates as well as in their tick vectors. By combining the accumulated knowledge and his own observations, Kelly was able to formulate a defined medium for the growth of *Borrelia* in the mid 1970s. A modified form of Kelly's medium is currently used for the growth of most *Borrelia* species. Gelatine is added to the medium, not because it is necessary for growth, but because *Borrelia* thrives in a higher viscosity. With gelatine in the broth medium higher cell densities are obtained (Barbour and Hayes, 1986).

Another important feature of *Borrelia* is that they have linear plasmids (Plasterk et al., 1985.). Bacterial linear plasmids were first found in *Streptomyces rochei* and have been detected in several *Streptomyces* species and in related bacteria, as well as in *Borrelia*. In 1989 the agent causing Lyme disease, *Borrelia burgdorferi*, was shown to harbour a linear chromosome as well as linear plasmids. Whether the relapsing fever *Borrelia* species have a linear chromosome has not yet been fully determined, although, it is very likely. The ends, or telomeres of the borrelial linear plasmids analysed to date have similar features. The two strands are covalently closed at the end and form a perfectly palindromic AT rich terminal hairpin loop. The only example of this type of telomere structure in prokaryotes is found in an unusual temperate coliphage. All other examples are found in eukaryotic cells and their viruses (Hinnebusch and Tilly, 1993). The origin of these linear replicons in *Borrelia* is not clear. They may have been acquired through a horizontal transfer from the African swine fever virus (ASFV). This animal virus has a telomere structure similar to the borrelial linear plasmids, and it is transmitted by *Ornithodoros moubata*, the vector of *Borrelia duttoni* (Barbour, 1993; Hinnebusch and Tilly, 1993). The copy number of the linear plasmids and the chromosome of *B. hermsii* was determined to approximately 14 for an expression plasmid, 8 for a silent plasmid and 16 copies of the chromosome per cell during growth in mice (Kitten and Barbour, 1992). Considering the similarity in copy number between the
linear plasmids and the chromosome it has been suggested that the term minichromosomes be used rather than plasmids (Bergström et al., 1992).

2.3 Vectors and reservoirs.

The epidemic relapsing fever caused by *Borrelia recurrentis* is transmitted by the human body louse, *Pediculus humanus humanus*. After the blood meal, the borreliae pass from the gut to the haemocele of the louse where they multiply. Since the borreliae are not present in the gut or salivary glands they will not be transmitted by the bite of the louse. Transmission from the louse to a human depends on contamination of the bite wound by rubbing it with the contents of a crushed louse during scratching (Felsenfeld, 1971). The endemic tick borne relapsing fever has more than one causative agent. The tick vectors for relapsing fever all belong to the genus *Ornithodoros*, of the family Argasidae (Fig. 1). The *Ornithodoros* are softbodied ticks that live in nests and burrows of wild animals as well as in the stables of domestic animals (Saint Girons and Barbour, 1991.) The ticks are nocturnal feeders that find their blood meals within a radius of 30 meters (Gobeau, 1984.). The *Ornithodorus* ticks attach to the animal for the blood meal for a short time, usually 10 to 30 minutes. The bite is rarely painful (Felsenfeld, 1971). The *Borrelia* enter with the blood meal into the midgut. In the tick the borreliae are not exposed to the acidity and proteases they may encounter in insects, since the blood is digested intracellularly by epithelial cells in the gut. The borreliae penetrate the gut wall and enter the hemolymph where they multiply. The bacteria then accumulate in different tissues, especially in the ganglia, coxal organs, salivary glands and reproductive organs. The coxal organs and salivary glands are critical for the transmission of borreliae into the vertebrate host. The *Ornithodoros* ticks use the coxal organ or the salivary glands to rid themselves of excess fluid during feeding and *Borrelia* in the coxal fluid or saliva can be injected into the host (Barbour and Hayes, 1986). The relapsing fever borreliae are passed to the next tick generation through the eggs at a low frequency.

A specificity exists between the tick vector and the *Borrelia* species transmitted, but it is not absolute, at least under experimental conditions (Felsenfeld, 1971). *Ornithodoros* ticks with its *Borrelia* may be found world-wide except for in
Australia and New Zealand. In Africa three main *Ornithodoros-Borrelia* complexes may be found: *O. moubata* harbouring *B. duttoni*, *O. erraticus erraticus* with *B. hispanica*, and *O. erraticus sonrai* with *B. crocidurae*. *B. hispanica* is the only species that is present in Europe, where it is found mainly in the southernmost countries; Spain, Portugal, Cyprus and Greece. Several *Ornithodoros-Borrelia* complexes may be found in Asia, the most important being *O. tholozani* harbouring *B. persica*.

In North America *B. hermsii*, transmitted by *O. hermsii*, is the most important cause of sporadic cases of relapsing fever. Other North American *Ornithodoros-Borrelia* complexes are *O. parkeri-B. parkeri* and *O. turicata-B. turicatae*. In South and Central America *B. venezuelensis* transmitted by *O. venezuelensis* is the best known complex. Knowledge of both *Borrelia* and its tick vectors in South America is poor and awaits further investigation (Gobeau, 1984). For most *Borrelia* species the
vertebrate host is a rodent. Examples of exceptions from this are *B. recurrentis* and *B. duttoni* that apparently only infect humans (Barbour and Hayes, 1986).

### 2.4 Symptoms of relapsing fever.

There is a large variation in the severity of relapsing fever, depending on the susceptibility and overall condition of the affected individual and which *Borrelia* species causes the infection. The overall course of the infection starts with the bite by an infected tick. After an incubation period, the first fever attack sets in. This fever attack ends in a crisis that is followed by an interval relatively free of symptoms. Then one or more relapses may follow. The incubation period is usually five to eight days in louse borne cases. In tick borne relapsing fever the incubation period varies extensively with the infecting species. In artificial experiments the average incubation time is six to seven days. The incubation time probably reflects the number of infecting borreliae, and their growth rate. Occasionally early signs of the disease, like headache, weakness, and malaise, may be present. The onset of the first attack is usually abrupt with a sudden rise in body temperature close to 40°C. The fever lasts five to seven days in louse borne, and three to five days in tick borne relapsing fever. The fever is often accompanied by a violent headache, pain, tenderness of the muscles and backache. Frequently, more or less severe bleeding is observed. A petechial skin rash may be seen at the end of the attack. Neurological symptoms spanning from signs of meningitis to severe depressions or hallucinations have been reported, but these are often reversible. During the febrile period, borreliae are measured in growing numbers in the blood. The attack ends in a crisis lasting one to two hours. The cause of this crisis is the partial or total disintegration of the borreliae in the circulation. The crisis involves an abrupt drop in temperature, often to subnormal values, a drop in blood pressure and intense sweating. After the crisis, the symptoms essentially disappear. Some milder forms of headaches and nausea may persist in the interval before the relapse. The interval usually lasts five to six days in louse borne and seven days in tick borne relapsing fever. The relapse is usually less severe than the original attack, and the relapses have a tendency to become shorter and milder as the disease proceeds (Felsenfeld, 1971; Gobeau, 1984). The lower number of spirochetes in the blood during
2.5 Experimental studies.

In 1918 Jancso reported that spirochetes of the first attack differed serologically from the relapse. He also noted that the change was reversible. Ten years later Brussin, Rogowa and Meleney revealed that the antigenic variation was multiphasic rather than biphasic. In 1951 Schuhardt and Wilkerson isolated single cells and were able to show that serological variants still appeared. Thus, they showed that the variants appeared during the infection and were not necessarily present in the inoculum (Barbour, 1991). Coffey and Eveland (1967) presented the first major experimental evidence for antigenic variation in *B. hermsii*. Using immunofluorescence to identify major serotypes infecting rats, they found that the attack serotype O was followed by the sequential appearance of serotypes A, B and C, generally in that order. They found that when a relapse serotype was injected into a rat it tended to revert to the serotype generally preceding it. Since Coffey and Eveland had used mixed populations they could not completely rule out the possibility that the relapse phenomenon was caused by a stable mixed population (Coffey and Eveland, 1967).

Stoenner *et al.* (1982) started an infection in a mouse with a clonal population. Isolating relapse serotypes, obtaining clonal populations from them, and then raising antiserum against every new serotype, they generated a collection of 25 different serotypes each with a corresponding antiserum. In the studies of this collection little or no crossreactivity was observed between any of the serotypes. Stoenner *et al.* (1982) also showed the antigenic switches to be reversible. Among the many findings made by this group was the observation that the change of serotype could occur spontaneously during growth in broth, without the direct binding of a specific antibody to the borreliae. They found the change to be $10^{-4}$ to $10^{-3}$ per cell per generation. The order of appearance was examined, and any of the 25 serotypes
could appear in the first relapse but some serotypes dominated (Stoenner et al., 1982). The existence of serotypes appearing only in broth culture, that had lost the capacity for switching was reported. These serotypes were designated serotype C and serotype Y (Stoenner et al., 1982; Barbour and Stoenner, 1984). Populations from Stoenner's serotype collection were further studied by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The serotypes could be distinguished by abundant proteins, located on the bacterial surface, which differed in apparent molecular mass between the serotypes. The serotype specific proteins, Vmp, reacted only with homologous polyclonal and monoclonal antiserum in western blots (Barbour et al. 1982). The Vmp7 and Vmp21 proteins were cleaved with proteases and cyanogen bromide (CNBr). Analysis of the peptides revealed that the Vmp proteins were extensively different in amino acid sequence and that they contained no long invariable regions (Barbour et al., 1983). However, N-terminal amino acid sequences of two CNBr peptides from Vmp7 and Vmp21 were 80% homologous. This led to the suggestion that the vmp genes were members of a polygene family (Barstad et al., 1985). The logical extension of the study of the variation of the Vmp proteins of *B. hermsii* was the encoding genetic material. The genes *vmp7* and *vmp21*, coding for Vmp 7 and Vmp21, respectively, were cloned (Meier et al., 1985; Plasterk et al., 1985). The DNA from serotype 7 cells was shown to carry *vmp7* specific DNA in two loci, one of which was present in serotype 21 DNA as well. The regulation of Vmp expression was shown to be at the transcriptional level, since the *vmp7* transcript was present only in serotype 7 cells (Meier et al., 1985). The cloned vmp genes were shown to be of two types, one variant expressed its Vmp in *E. coli* cells, while the other one did not. The expression linked copies of *vmp7* and *vmp21* have the same sequence for several kilobases upstream, as seen by restriction enzyme analysis. This common sequence was designated the upstream expression region. The silent copies showed low or no similarity to each other, and the upstream expression region did not resemble their 5' region. Plasterk *et al.* were also able to show that the vmp genes were situated on linear plasmids, 24 kb and 28 kb in size. The observation was made in the works of Meier *et al.* (1985) and Plasterk *et al.* (1985) that the switching mechanism in *Borrelia* is similar to that of *Trypanosoma* and *Neisseria*. They concluded that during the switch, the *vmp7* gene in the expression site is removed and replaced by a copy of the *vmp21* gene.
The silent \textit{vmp7} and \textit{vmp21} appears to be unaffected by the switch (Fig. 2). They suggest three possible mechanisms for the switch from serotype 7 to serotype 21.

![Diagram of plasmids](image)

**Fig. 2.** Schematic representation of the plasmids taking part in the serotype switch from serotype 7 to serotype 21 in \textit{B. hermsii}. The white surface of the serotype 7 cell (top) represents Vmp7 protein expressed from the \textit{vmp7} gene (also depicted in white) at the expression site. The grey of the serotype 21 cell (bottom) represents the Vmp21 protein and \textit{vmp21} genes. The black arrowhead in the expression plasmid depicts the promoter where \textit{vmp7} transcript (white arrow) or \textit{vmp21} transcript (grey arrow) is initiated. Different patterns in the regions flanking the \textit{vmp} genes reflect differences in sequence. The black boxes indicate the upstream and downstream homology blocks.
1) A single site specific recombination, with the subsequent loss of one of the recombination products. 2) Genes on cassettes activated by gene conversion, with crossover regions on either end of the genes. 3) Silent \textit{vmp} genes activated by the insertion of a transposable DNA element upstream of the genes. From their observations the single site-specific recombination model is the most likely. The other two models were discarded on the basis of missing second recombination sites downstream of the \textit{vmp} genes in model 2) or upstream of the transposable element in model 3) (Plasterk \textit{et al.}, 1985).

Much information concerning the antigenic variation in \textit{B. hermsii} was gathered during the 1980s However, the data regarding the switch mechanism raised new questions. What is the structure of the linear plasmid ends? What is their role in the switching mechanism? Are there sites involved in recombination that are missed by restriction enzyme analysis? How is the \textit{vmp} gene activated when it is inserted at the expression site? Many of these questions could be answered by gaining a more detailed knowledge of the structures involved. The determination of the DNA sequence of the \textit{vmp} genes and flanking regions was an obvious starting point.
AIMS OF THIS THESIS

To identify and characterise important components involved in the antigenic switch in relapsing fever *Borrelia*.

To compare the antigenic variation in relapsing fever *Borrelia* species of different geographical origin and determine if the switching mechanisms employed are governed by similar genetic elements.

To explain additional pathogenic mechanisms that may be responsible for the diverse symptoms displayed during relapsing fever infections.
3. The *B. hermsii* vmp genes and their products. (Paper I)

3.1 Genetic organisation of vmp7 and vmp21.

In an attempt to characterise the serotype specific proteins of *B. hermsii*, the nucleotide sequence of the genes for Vmp7 and Vmp21 in their expressed loci, was determined. The vmp7 and vmp21 genes were isolated before and after a switch from serotype 7 to serotype 21. The cloned genes expressed full-length Vmp proteins when introduced into *E. coli* cells (Plasterk et al., 1985). A sequence comparison of the entire control region revealed that this region of the two genes is identical, and only 5 bases differ between vmp7 and vmp21 in the first 100 bases of the open reading frames. The control region (the upper sequence in Fig. 3) consists of a near consensus sigma 70 prokaryotic promoter (Scherer et al., 1987; Hawley and McClure, 1983). The transcriptional start site from this promoter was determined on mRNA produced during expression in *E. coli*. The transcription was shown to begin at a G residue 29 bp upstream of the adenine of the starting AUG codon. Within this 29 bp there is a consensus ribosomal binding site (Gold et al., 1981). The coding sequence of vmp7 is terminated at a UGA stop codon and consists of 1107 bp. The vmp21 open reading frame consists of 1092 bp and terminates at a UAA stop codon. In both genes these codons are followed by several more stop codons in the same reading frame. Partially palindromic sequences after the genes may serve as terminators of transcription.

3.2 Vmp similarity.

Despite the strict serotype specificity of vmp7 and vmp21, the genes are highly homologous. The overall nucleotide sequence identity between vmp7 and vmp21 is 77%. The nucleotide sequence analysis of four additional vmp structural genes has revealed identities ranging between 43 and 78% (Restrepo et al., 1992). The differences between vmp7 and vmp21 are, however, not evenly distributed throughout the genes. The genes are most similar in their 5' part, being 95% identical in the first 100 bp. The lowest homology over a 100 bp region is 53%, which is found in the middle part of the genes. Near the 3' end the genes are more
similar and the homology in the last 100 bp is 80%. The high degree of similarity between the \textit{vmp7} and \textit{vmp21} suggests that the genes may be descendants of a common ancestral gene and that they have evolved by genetic drift (Kimura, 1981). The observation that the mismatches appear as single, double, and triple mismatches and so on, in a descending order of frequency, suggests that this may be the case. The presence of insertions or deletions in multiples of three bases, which maintain the reading frame over the homologous regions, further strengthens the possibility of genetic drift as a means to generate \textit{vmp} polymorphism. The identical sequence of the control region suggests that the DNA rearrangement between the silent \textit{vmp7} and \textit{vmp21} occurs close to, or within the \textit{vmp} genes. Thus the switch in expression from \textit{vmp7} to \textit{vmp21} would involve the transposition of the entire \textit{vmp21} gene into the expression site.

The \textit{vmp7} and \textit{vmp21} code for polypeptides of 369 and 364 amino acids, respectively. The Vmp7 protein has a predicted molecular mass of 37.2 kilodalton (kDa) and the molecular mass of Vmp21 is predicted to be 37.1 kDa. This is in agreement with the apparent molecular mass determined by SDS-PAGE, which are approximately 38 kDa for Vmp7 and 36 kDa for Vmp21 (Barbour, 1985). The deduced Vmp7 and Vmp21 amino acid sequences are 63% identical. As for the DNA sequence, the greatest divergence is seen in the central parts of the proteins. The low content of guanosine and cytosine in the borrelial DNA influences the codon usage in the \textit{vmp} genes. Several of the codons used frequently in the \textit{vmp} genes are rarely used in highly expressed genes in \textit{E. coli}. The \textit{vmp} genes are expressed in detectable amounts in \textit{E. coli} despite these differences in codon usage. The Vmp proteins are not similar to any proteins in the protein databases. Some relatedness is, however, observed with the analogous outer surface proteins OspA and OspB of \textit{B. burgdorferi}, which causes Lyme disease in humans. The amino acid identity is, however, only 20% and the similarity is approximately 40 to 45%. The Vmp proteins have a high content of charged amino acids except in the immediate N-terminal region. The Vmp proteins have no regions of sustained hydrophobicity or any obvious membrane spanning regions that could serve to anchor the protein in the borrelial outer membrane.
3.3 The Vmp proteins are lipoproteins.

Despite the low similarity between the Osp proteins of *B. burgdorferi* and the Vmp proteins of *B. hermsii*, they are analogous in being borrelial outer surface proteins with a similar genetic localisation. From the sequence of OspA and OspB, Bergström *et al.* (1989) suggested that they were lipoproteins. The lipoprotein nature of OspA and OspB was later confirmed by Brandt *et al.* (1990). This led us to examine the sequence of Vmp7 and Vmp21 for characteristic features of prokaryotic signal sequences (Wu and Tokunaga, 1986). The first amino acids of Vmp7 and Vmp21 are basic and the middle part of this region is hydrophobic. The Vmps also contain an amino acid sequence resembling the consensus for prokaryotic lipoproteins (L-X-X-C). The 'X' represents small uncharged amino acids and the cysteine is the attachment site for the fatty acid residues. Vmp7 and Vmp21 have the sequence L-M-I-G-C, with the cysteine at amino acid position 27.

Since the expression of the Vmp proteins in *E. coli* yields protein with the same molecular mass as in *Borrelia*, we assumed that the processing of the Vmp proteins is the same in *E. coli* and *B. hermsii*. In a series of experiments we collected evidence that Vmp7 is processed as a lipoprotein during expression in *E. coli*. During growth in the presence of $^{14}$C-palmitate the Vmp7 protein was radiolabelled. This indicates that a lipid moiety is added post-translationally to the Vmp7 protein. In the next experiment the cleavage of the signal peptide was blocked using the inhibitor of signal peptidase II, Globomycin (Hussain *et al.*, 1980). The Globomycin treatment resulted in the appearance of a larger protein, presumably the unprocessed Vmp7. The unprocessed Vmp7 had an apparent molecular mass of 36.5 kDa compared to 34 kDa for the normal Vmp7. Further evidence for the post translational modification of Vmp7 was obtained in an *in vitro* coupled transcription-translation assay. In this *in vitro* assay only the larger 36.5 kDa protein was produced from the *vmp7* DNA, and not the native form seen in *B. hermsii*. The difference in molecular mass between the unprocessed and processed Vmp7 is accounted for by the removal of the 26 amino acids of the signal peptide. In the absence of lipophilic regions or other membrane spanning regions in the Vmp7 and Vmp21 proteins, we believe that the lipid moiety is the anchor which positions and/or holds the Vmp proteins in the outer membrane of *B. hermsii*. 
3.4 Hypothesis on Vmp structure.

The Vmp proteins of *B. hermsii* are serotype specific, and under normal conditions no crossreactivity is seen between different Vmps. It is clear that the retention of an epitope from a previously displayed Vmp in a new Vmp would lead to a direct selection against that serotype by the host. The infection would then be terminated and the strategy of antigenic variation would be futile. In this light, the high degree of similarity seen between the Vmp7 and Vmp21 proteins is intriguing. The conjecture is that the identical regions of the two proteins are buried in the interior of the protein when it is presented on the bacterial surface. Thus, only the regions that are most different are exposed to the host, and elicit the immune response. Lacking X-ray crystallographic data for Vmp7 or Vmp21 only vague predictions can be made of the structure of the proteins. Computer generated predictions of probable sites of antibody binding reveals that the antigenic sites are situated predominantly in regions where the similarities in amino acid sequence are particularly low. The sites are also distributed throughout the proteins which is in accordance with the findings of Barstad *et al.* (1985) for monoclonal antibodies to Vmp7 and Vmp21. The secondary structure prediction suggests that the Vmp proteins may be folded into compact molecules where the serotype-specific regions are exposed on the surface.

3.5 Similarities between VSG and Vmp proteins.

Other pathogens that use programmed gene rearrangements to change their surface molecules are the gonococci and the African trypanosomes. The gonococcal antigenic variation involves the replacement of less than half of the chromosomally located pilin gene (Bergström *et al*., 1986; Swanson *et al*., 1986; Meyer, 1987). In *B. hermsii* however, a complete or near complete copy of a *vmp* gene is replaced at the expression locus by the transposition between different linear plasmids. Thus, the switching mechanism of *B. hermsii* more closely resembles the gene conversion observed in the African trypanosomes. This is interesting since the trypanosomes are eukaryotes and the borreliae are prokaryotes. In addition to the similarities in transmission and switching mechanisms between *Trypanosoma* and *Borrelia*, analogies in their surface proteins may be found. Despite the lack of appreciable
similarity in amino acid sequence between the trypanosomal VSG and the borrelial Vmp proteins, there are structural similarities between the two types of proteins. Both surface proteins are anchored in the outer membrane by a post-translationally attached hydrophobic molecule. The VSG glycoproteins are anchored to the cell membrane by a glycolipid at the C-terminal end of the VSG proteins (Turner, 1985; Ferguson et al., 1988; Menon et al., 1988). In *B. hermsii* we believe that the Vmp proteins are anchored in the membrane by an N-terminally attached lipid molecule. Another similarity is the predicted folding of the proteins. The VSG proteins are made up of numerous helical bundles that align side by side, forming a compact molecule that predominantly exposes the interhelical loops at the surface (Freymann et al., 1984). In the *B. hermsii* Vmp proteins, the predicted alpha helices are situated in regions where Vmp7 and Vmp21 are similar and the interhelical regions have the highest potential for antigenicity. A folding of the Vmp proteins like the trypanosomal VSG proteins, with similar helices in compact bundles, would explain why no crossreactivity is observed despite the 63% identity between the Vmp7 and Vmp21 proteins.

4. *vmp* gene activation. (Paper II)

4.1 Activation by promoter addition.

Previous studies identified an upstream homology region believed to be important for the replacement of the *vmp7* gene by *vmp21* in the expression plasmid (Plasterk et al., 1985; Paper I). By comparing the sequences downstream of the expressed and silent *vmp7* and *vmp21* genes, the presence of a downstream recombination region was revealed. Approximately 200 bp of complete homology was found about 1 kb downstream of the silent and expressed *vmp7* and *vmp21* (Kitten and Barbour, 1990). These findings shed more light on the possible genetic mechanisms of the serotype switch. How the newly inserted *vmp* gene is activated remains unexplained. Two modes of *vmp* activation were anticipated. The gene could be activated by its placement downstream of a promoter at the expression site. The other possible mode of activation could be by removing the *vmp* gene from repression at the silent site. By studying how the DNA sequences upstream of the silent and expressed forms of *vmp7* and *vmp21* differed, we hoped to determine the
RESULTS AND DISCUSSION

When the DNA sequences upstream of silent vmp7, silent vmp21, expressed vmp7 and expressed vmp21 are aligned it is clear that the expression site for vmp7 and vmp21 is identical. The identical expression site includes a run of 16 T residues, the sigma 70 promoter and the putative ribosome binding site. Upstream of the promoter the silent vmp7 and vmp21 show no homology to either each other or to the expression site. Thus, the similarity between the silent and expressed vmp genes starts a few bases after the promoter sequence. The silent and expressed genes are highly homologous over a region of approximately 100 bp. After this 100 bp region the homology between vmp7 and vmp21 is lower. This suggests that the silent vmp genes are silent due to the lack of a promoter. To rule out the use of a potential transcriptional start site present within the homology region, the transcriptional start site was determined on mRNA extracted from B. hermsii. Transcription of the vmp gene was shown, by primer extension analysis, to start from the upstream promoter, present only in the expression site. These data led us to the conclusion that vmp21 is activated by being placed downstream of the promoter at the expression site. The strongest argument against the repression of silent vmp genes is the lack of homology between the silent upstream sequences.

4.2 Suggested mechanism for a serotype switch.

During the switch in expression of Vmp7 to Vmp21 the vmp7 gene is replaced by all or most of the vmp21 gene. The vmp21 gene is activated because transcription of the gene is initiated at the promoter immediately preceding the vmp21 gene. The presence of an upstream homology region and a downstream homology region suggests that the vmp gene switch takes place by gene conversion. One cannot, however, completely rule out the possibility that the switch is brought about by reciprocal recombination with a double crossover. This would generate two products, in the first the new vmp is fused to the expression site and in the second the formerly active vmp is present in a new silent locus. The second recombination product has not been observed, but if it was lost after recombination the outcome would be indistinguishable from a gene conversion (Plasterk et al., 1985). In both mechanisms the homology blocks are important in aligning the silent and expressed
vmp genes for recombination. The upstream and downstream homology blocks are sufficiently long to allow for stable base-pairing between plasmids (Singer et al., 1982; Watt et al., 1985). In the reciprocal recombination model the upstream and downstream homology blocks would serve as the upstream and downstream crossover points. In the favoured gene conversion model, single or double strand breaks introduced in the expression plasmid are repaired using the silent vmp DNA as template. The template vmp DNA is aligned by the upstream and downstream homology blocks. The mechanism by which strand breaks are introduced in the expression plasmid is not yet fully understood.

5. Elements in the expression plasmid. (Paper III)

5.1 Repeats of insertion-sequence-like structures.

Due to the site specific activation of vmp genes in the expression plasmid we were interested in studying the DNA 5' of the control region. The nucleotide sequence 6.9 kb upstream of the expressed vmp7 was determined. Several features that potentially explain the non reciprocal nature of the genetic switch were found. Three imprecisely repeated 2 kb elements were found. Each of the repeats contained an element with terminal inverted repeats resembling an insertion sequence or a transposable element (Galas and Chandler, 1989). One element was shown to form stem and loop structures in vitro. The repeated elements were only found upstream of the expressed vmp gene. This suggests a role for the elements in expression or in recombination. The inverted repeats may stimulate recombination by creating breaks in the DNA, like the transposable elements of maize (Federoff, 1989). In transposons the enzyme creating the strand break, the transposase, is coded for within the transposable element itself. If such an enzyme is involved in the switching mechanism in B. hermsii it must be encoded elsewhere in the genome, since no obvious candidate gene could be located in the 7 kb investigated.

5.2 A Poly(dT-dA) preceding the vmp promoter.

The other interesting feature observed was the presence of runs of 16 T residues preceding the vmp promoter. Long runs of T residues are rare in prokaryotic organisms and appear at only this one location in the B. hermsii genome. However,
the region preceding the promoter of the *B. burgdorferi* outer surface protein (Osp) genes includes a 20 bp segment with 16 non-contiguous T residues (Bergström *et al.*, 1989). The role of the T rich regions in *B. hermsii* and *B. burgdorferi* may be to enhance transcription, like the runs of Ts that act as positive regulators in yeast (Struhl *et al.*, 1985). The role may also be to introduce breaks in the DNA to stimulate recombination. Poly(dT·dA) exhibits a different structure than random DNA, and may strain the T strand, eventually leading to a break (Wohlrab *et al.*, 1987; Peck and Wang, 1981; Rhodes and Klug, 1981). The different DNA structure, or the opening of the double strand in a bubble may also be a signal for an endonuclease. This is seen in the 16 dG residues of the nuclease hypersensitive region upstream of the chicken β-globulin gene (Nickol and Felsenfeld, 1982). Considering the presence of T-rich regions upstream of the outer surface protein genes of *B. burgdorferi* and *B. hermsii*, a role as a transcriptional activator appears most likely. The role of stimulating recombination by the introduction of strand breaks seems unlikely since *B. burgdorferi* lacks antigenic variation. *B. burgdorferi* does, however, cause persistent infections in humans. Recombination stimulated by breaks in the T-rich region can not be entirely ruled out simply because the switch in expression of Osp types has not been demonstrated in *B. burgdorferi* (Wilske *et al.*, 1992).
Fig. 3. Alignment between the control regions of vmp7 of B. hertnsii and vmpC2 of B. crociirdiae. The -35 and -10 elements of the promoters, start of transcription and ribosome binding sites are marked on the sequences. The +1 position is the first nucleotide of the open reading frames. The first amino acids of the Vmp proteins are given above and below the DNA sequences, respectively. T-rich sequences are marked in bold and italicized letters.

RESULTS AND DISCUSSION

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6. Antigenic variation in \textit{Borrelia crocidurae}. (Paper IV)

6.1 Similarities between \textit{B. crocidurae} and \textit{B. hermsii}.

The African relapsing fever species \textit{Borrelia crocidurae} causes spirochetemia when injected into mice. The course of the infection is similar to that of \textit{B. hermsii}. The first attack of spirochetemia is followed by the apparent clearance of spirochetes from the blood, and subsequently a relapse. \textit{B. crocidurae} cells isolated from two subsequent relapses were serologically different. Antisera raised against serotype C1 and C2 cells reacted strongly only with the homologous VmpC1 or VmpC2 protein and no crossreactivity was observed. The amino acid sequences of VmpC1 and VmpC2, deduced from the nucleotide sequence of the cloned genes, are substantially different. The difference may in part be due to the large difference in size, 37 kDa for VmpC1 and 18 kDa for VmpC2. The VmpC1 and VmpC2 proteins are similar to the Vmp proteins of \textit{B. hermsii}. The similarities fit well with the proposed grouping into Vmp families based on sequence similarities and Vmp size (Restrepo \textit{et al.}, 1992). The \textit{vmpC2} gene is activated at the transcriptional level. Whether this activation is achieved by promoter addition, as in \textit{B. hermsii} (Paper II), remains to be clarified.

6.2 Differences between \textit{B. crocidurae} and \textit{B. hermsii}.

The \textit{vmpC2} is transcribed from an expression site that is different (Fig. 3) from the shared \textit{vmp} expression site used by \textit{B. hermsii} (Paper I). An alternative expression site, also different from the \textit{vmpC2} expression site, has been shown for \textit{vmp33} (formerly \textit{vmpC}) in the culture derived serotype C of \textit{B. hermsii} (Carter \textit{et al.}, 1994). This expression site is used in cells unable to perform serotype switching (Stoenner \textit{et al.}, 1982). However, the \textit{B. crocidurae} serotype C2 cells undergo serotype switching when injected in mice. This makes the \textit{vmpC2} expression site the first example of an alternative to the shared expression site of \textit{B. hermsii}, in a switching-competent borrelial cell.

Many similarities are observed in the relapsing fever induced by the American species \textit{B. hermsii} and the African species \textit{B. crocidurae}. The similarities of the Vmp proteins, the localisation of the genes on linear plasmids, and the activation of \textit{vmpC2} on the transcriptional level, indicate a high degree of similarity. Despite the
lack of a reliable mechanism for serotype switching in *B. crocidurae*, the conclusion can be made that the genetic elements involved in serotype switching are different than in *B. hermsii*. However, the possibility still exists that the switch is mechanistically similar.

The genetic content of *B. crocidurae* is larger than that of *B. hermsii*, thus the coding capacity should be greater. We have found one possible use of this larger coding capacity in the additional ability of *B. crocidurae* to bind to mammalian erythrocytes (Paper V).

7. *Borrelia crocidurae* causes rosetting of red blood cells. (Paper V)

7.1 Erythrocyte binding correlates with duration of spirochetemia.

During the growth of *B. crocidurae* in mice we observed that erythrocytes were aggregated around the spirochetes during the spirochetemia. This has never been observed for *B. hermsii*. The aggregates are reminiscent of the rosettes which form around erythrocytes infected with *Plasmodium* parasites (David *et al.*, 1988). Binding between erythrocytes and borreliae has been reported previously for *B. crocidurae* and *B. duttoni* (Moser, 1958). Further experiments showed that the duration of the attack, and relapse spirochetemias were longer in *B. crocidurae* infections than in *B. hermsii* infections. It has previously been reported that two relapsing fever *Borrelia* species, *B. hermsii* and *B. turicatae*, are eliminated from the mouse by a T-cell independent immune response mechanism (Stoenner *et al.*, 1982; Newman and Johnson, 1984). It is possible that by surrounding itself with erythrocytes *B. crocidurae* may delay the time of clearance by sterically hindering B-cell interactions with the spirochete surface. If this were true, *B. crocidurae* may be using erythrocytes as a disguise in addition to antigenic variation for the evasion of the host immune system.

7.2 Aggregation is influenced by temperature *in vitro*.

The aggregation between *B. crocidurae* and erythrocytes could be reconstituted *in vitro*, by mixing broth cultured borreliae with fresh blood. Aggregate formation was observed *in vitro* between *B. crocidurae* and erythrocytes from mice, guinea pigs and humans. No aggregation was observed for *B. hermsii*. The aggregation was
observed at 37°C but no erythrocytes were bound by *B. crocidurae* at 25°C. This indicates that the binding between *B. crocidurae* and erythrocytes is temperature regulated. The requirement for a temperature regulation is not clear. Since the receptor and ligand for the erythrocyte binding are unknown, the nature of the regulation is as well. Possible mechanisms include a temperature dependent regulation of the receptor expression, and a temperature optimum for the association between the receptor and the ligand. It is possible that this temperature allows the bacteria to dissociate from the erythrocyte in the tick vector, where the temperature is lower than 37°C.

7.3 Aggregates may cause damage in the host.

The erythrocyte aggregation around *B. crocidurae* is similar to the rosette formation of erythrocytes infected by *Plasmodium* parasites (David *et al.*, 1988). The rosetting of erythrocytes in malaria has been suggested to be important in the development of cerebral malaria (David *et al.*, 1988; Carlson and Wahlgren, 1992). A potential correlation was observed between the small and unstable rosettes formed around infected erythrocytes of blood group 0 and the lower probability of developing cerebral malaria in humans of blood group 0 (Carlson and Wahlgren, 1992). It is tempting to speculate that the aggregation of erythrocytes by *B. crocidurae* causes symptoms by obstructing microvessels. Blocked capillaries could explain the presence of petechie and other bleedings and perhaps also the neurological symptoms observed in some relapsing fever infections.
CONCLUSIONS.

Vmp7 and Vmp21 of *B. hermsii* are remarkably similar. The lack of antigenic crossreactivity suggests that these proteins fold into compact molecules that only expose the non-homologous regions on their surfaces.

The Vmp proteins of *B. hermsii* are lipoproteins. The lipid moiety presumably anchors the proteins in the borrelial membrane.

The *vmp* genes are activated by promoter addition rather than by the removal of a repressor.

Elements are present upstream of the control region that may stimulate recombination or enhance expression.

The serotype switches of *B. crocidurae* and *B. hermsii* are similar, but transcription of *vmpC2* in *B. crocidurae* is initiated from a novel expression site.

The binding of erythrocytes by *B. crocidurae* may be an additional means of immune evasion in relapsing fever.
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