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Review

Within-host dynamics of antigenic variation

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Abstract

Genomes of some parasites contain dozens of alternative and highly diverged surface antigens, of which only a single one is expressed in any cell. Individual cells occasionally change expression of their surface antigen, allowing them to escape immune surveillance. These switches appear to occur in a partly random way, creating a diverse set of antigenic variants. In spite of this diversity, the parasitemia develops as a series of outbreaks, in which each outbreak is dominated by relatively few antigenic types. Host-specific immunity eventually clears the dominant antigenic types, and a new outbreak follows from antigenic types that have apparently been present all along at low frequency. This pattern of sequential dominance by different antigenic types remains unexplained. We review the five most prominent theories, which have developed mainly from studies of the protozoans *Trypanosoma* and *Plasmodium*, and the bacterial spirochete *Borrelia*. The most promising theories depend on some combination of mechanisms to create favored connectivity pathways through the matrix of transitions between variants. Favored pathways may arise from biased switches at the molecular level of gene expression or from biases imposed by immune selection. We illustrate the concept of connectivity pathways by reanalysis of data on transitions between variants from *Borrelia hermsii*.

Keywords: *Borrelia*; Immune escape; Parasites; *Trypanosoma*

Contents

1. Introduction

Some parasites store alternative genes for antigenic surface molecules. Each individual parasite usually expresses only one of the alternatives (Deitsch et al., 1997; Fussenegger, 1997; Frank, 2002). Parasite lineages change expression from one stored gene to another at a low rate. In *Trypanosoma brucei*, the switch rate is about 10^{-3} or 10^{-2} per cell division (Turner, 1997).

Antigenic switches affect the dynamics of the parasite population within the host. For example, the blood-borne bacterial spirochete *Borrelia hermsii* causes a sequence of relapsing fevers in an individual (Barbour, 1987, 1993, 2003). Each relapse and recovery follows from a spike in bacterial density. The bacteria rise in abundance when new antigenic variants escape immune recognition and fall in abundance when the host generates a specific antibody response to clear the dominant variants. The sequential expression of antigenic

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variants benefits the parasite by extending the total time of infection and allowing greater opportunity for transmission.

Several parasites change their surface antigens by altering expression between variant genes in an archival library (Deitsch et al., 1997; Fussenegger, 1997; Frank, 2002). This active switching raises interesting problems for the population dynamics and evolution of antigenic variation within individual hosts. Note that we use the word "parasite" for both prokaryotic and eukaryotic infectious agents.

The main question concerning the dynamics of antigenic variation is: How does the parasite express a sequence of variants in a way that extends the length of infection? The population of parasites in the host can be large, so even rarely expressed variants can be in significant abundance. If the parasite population expresses all of the variants in significant numbers early during infection, then the host may quickly raise an immune response to all variants and clear the parasite. If the parasite population switches to new variants too slowly, the host may clear all parasites before they can change expression and escape host immunity. At some intermediate rate of antigenic change, dynamical interactions between antigenic switching and host immunity can extend infection through sequential dominance of different parasite variants.

The first section begins with evidence that, in each parasite cell division, switching between expression of different antigenic variants happens stochastically. We follow by showing evidence that, in spite of stochastic switching of expression, the population of parasites tends to be dominated by a loosely arranged sequence of different variants.

The second section reviews various theories to explain how sequential dominance of antigenic variants extends the time of infection. We also mention another potential benefit of antigenic variation—such variation may allow a parasite to reinfect a previously exposed host that has developed immunity against some of the antigenic variants in the archival library.

The final section of the paper reanalyses some old data on *B. hermsii*, one of the best studied cases of antigenic switching between archival variants. The data come from Stoenner et al. (1982), an early attempt to measure the nonrandom pattern of switching between different variants. If we knew the rate at which each variant switched to other variants, then we could calculate the consequences of variable switch rates for causing sequential dominance and extended infection. Stoenner et al.'s (1982) data provide only very crude estimates of switch rates, not sufficient to draw firm conclusions. However, analysis of these data provide some hypotheses about sequential dominance in *B. hermsii*, and highlight what could be learned from more precise estimates of switch rates.

2. Stochastic switching versus ordered parasitemias

In *T. brucei*, lineages switch stochastically between variants. Turner and Barry (1989) measured the switch probability per cell per generation for changes between particular antigenic types. Each entry in Table 1 shows $\log_{10}$ of the probability that a cell expressing a particular variant, designated by a number in the left column, switches to another variant designated by a number in the column headings.

Table 1
$\log_{10}$ of probability of switching between antigenic variants in *Trypanosoma brucei*

	1.22	1.3	1.61	1.62	1.63	1.64
1.63	−2.7		−2.7			−2.3
1.64	−3.4		−3.4		−3.4	
1.64	−3.0		−3.8		−3.1	
1.64	−3.4	−2.6		−4.0		
1.64	−2.7	−2.2		−5.7		

Values are from Turner and Barry (1989). The numbers in the column headings and row labels are names for particular antigenic variants. Table entries show $\log_{10}$ of the switch probability per cell per generation. Variants were identified by antibodies raised to particular types.

The different rows in Table 1 summarize data from five separate experiments. Overall, it appears that each type can potentially switch to several other types, with the probability of any transition typically of the order of 10^{-4} to 10^{-2}. *T. brucei* stores and uses many different antigenic variants, perhaps hundreds (Vickerman, 1989; Barry, 1997). Thus, the limited sample in Table 1 does not provide a comprehensive analysis of switch probabilities between all types.

Switches between types within a cellular lineage occur stochastically. But the sequence of variants that dominate sequential waves of parasitemia tends to follow a repeatable order (Gray, 1965; Wilson and Cunningham, 1972; Capbern et al., 1977; Barry, 1986). For example, Fig. 1 shows the date at which different variants first appeared in *Trypanosoma vivax* infections of rabbits. Some separation occurs between variants that arise early versus late.

Temporal separation in the rise of different antigenic variants allows trypanosomes to continue an infection for a longer period of time (Vickerman, 1989). If all variants rose in abundance early in the infection, they would all stimulate specific immune responses and be cleared, ending the infection. If the rise in different variants can be spread over time, then the infection can be prolonged.

Data from *B. hermsii* in Fig. 2 show the host immune system's role in structuring the sequential dominance of antigenic variants. Each peak in the density of bacteria in the host's blood causes a rise in specific antibodies to the dominant bacterial variant. Those antibodies quickly clear the specific matching variant, leading to another round with a new bacterial variant rising followed by specific antibodies to the new variants.

Similar data from trypanosomes demonstrate how host antibody titers drive the clearance of particular variants (Gray, 1965). Each round of clearance establishes an opportunity for a new expansion of the parasite population from a previously rare antigenic variant.

3. Theories of sequential dominance

The puzzle is how stochastic changes in the surface antigens of individual parasites can lead to an ordered temporal pattern

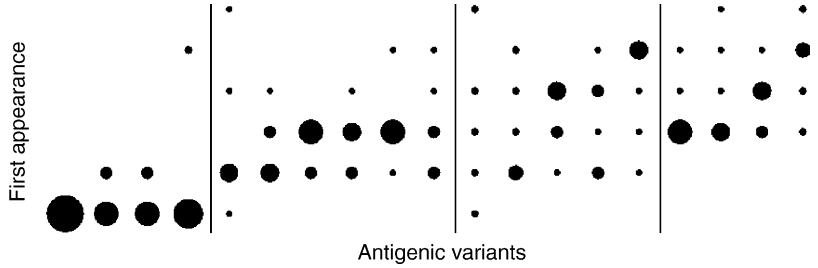

Fig. 1. The sequence of appearance for 19 antigenic variants of *Trypanosoma vivax* in rabbits. Each column shows a different antigenic variant. The rows are the day since inoculation at which a variant was first detected during an infection. The days of measurement are, from bottom to top, 12, 19, 26, 33, 40, and 47/55, where data from Days 47 and 55 are combined in the top row. Barry (1986) collected data from six rabbits. The diameter of each circle shows, for each variant, the frequency of rabbits in which a variant first appeared on a particular day following inoculation. We discarded variants for which there were observations from fewer than five of the six rabbits. We have arbitrarily ordered the variants from those on the left that appear early to those on the right that appear late. The vertical bars crudely group the variants into categories defined by time of appearance. From Frank (2002).

at the level of the population of parasites within the host (Agur et al., 1989; Frank, 1999; Turner, 1999, 2002; Barry and McCulloch, 2001). Five hypotheses have been developed, none of which has strong empirical support at present. We briefly describe each idea.

First, the antigenic variants may differ in growth rate. Those that divide more quickly could dominate the early phases of infection, and those that divide more slowly could increase and be cleared later in the infection (Seed, 1978). Computer studies and mathematical models show that variable growth rates alone cannot easily explain wide separation in the times of appearance of different variants (Kosinski, 1980; Agur et al., 1989). Only with a very large spread in growth rates would the slowest variant be able to avoid an immune response long enough to develop an extended duration of total infection. Aslam and Turner (1992) measured the growth rates of different variants of *T. brucei* and found little difference between the variants.

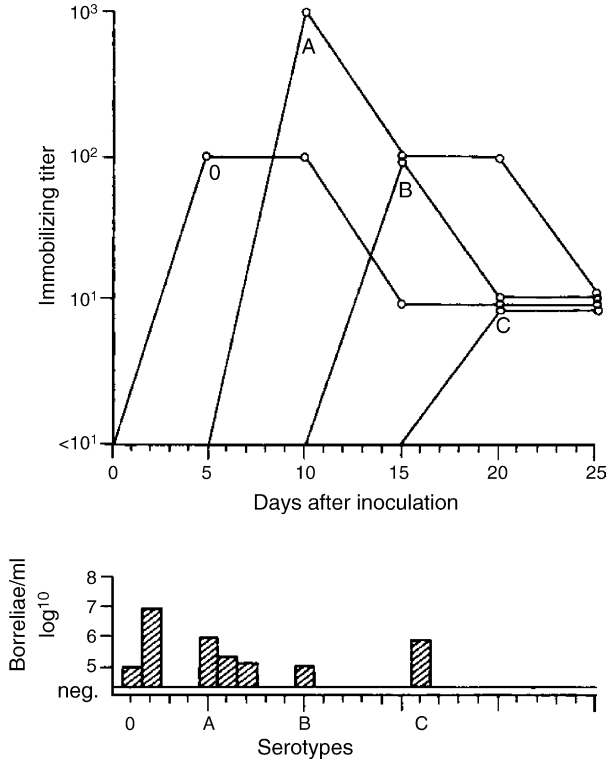

Fig. 2. Sequential dominance of *Borrelia hermsii* antigenic variants driven by host immune response. The bottom plot shows four successive waves of bacterial population growth, each wave dominated by a different antigenic variant (serotype). The height of the bars measures the $\log_{10}$ density of bacteria per millilitre of host blood. The top plot shows the rise of matching antibody titers, each curve labeled for the specificity of the antibodies to a given antigenic variant (serotype). Data from Coffey and Eveland (1967), plot redrawn from Barbour (1987).

Second, parasite cells may temporarily express both the old and new antigens in the transition period after a molecular switch in antigenic type (Agur et al., 1989). The double expressers could experience varying immune pressure depending on the time for complete antigenic replacement or aspects of cross-reactivity. This would favor some transitions to occur more easily than others, leading to temporal separation in the order of appearance for different antigenic variants. This model is rather complex and has gained little empirical or popular support, as discussed in several papers (Barry and Turner, 1991, 1992; Agur, 1992; Munoz-Jordan et al., 1996; Borst et al., 1997).

Third, the switch probabilities between antigenic variants may be structured in a way to provide sequential dominance and extended infection (Frank, 1999). If the transition probabilities from each variant to the other variants are chosen randomly, then an extended sequence of expression does not develop because the transition pathways are too highly connected. The first antigenic types would generate several variants that develop a second parasitemia. Those second-order variants would generate nearly all other variants in a random switch matrix.

The variants may arise in an extended sequence if the parasite structures the transition probabilities into separate sets of variants, with only rare transitions between sets. The first set of variants switches to a limited second set of variants, the second set connects to a limited third set, and so on. Longer infections enhance the probability of transmission to other hosts. Thus, natural selection favors the parasites to structure their switch probabilities in a hierarchical way in order to extend the length of infection. Paget-McNicol et al. (2002) also developed a model in which switch rates vary, but did not consider how natural selection might modulate switch rates.

Fourth, Recker et al. (2004) noted that hosts with stronger cross-reactive immune responses against *Plasmodium falciparum* variants are more likely to sustain chronic infections. Presumably, chronic infections mean that the parasite's repertoire of antigenic surface molecules can be structured into a pattern of sequential dominance. Based on these points, Recker et al. developed a model in which host immunity develops against two distinct components of the variable surface antigens. One part of the immune response develops lasting immunity against a unique component of each antigen. Another part of the host response develops short-lived immunity against a component of the antigenic molecule that is shared by other antigenic types.

With these points in mind, imagine how a malarial infection would play out. One or a few antigenic variants dominate the initial parasitemia. The host develops specific immunity against each variant. One part of the immune response is specific for each variant and is long-lived, clearing each variant and preventing another dominant wave of parasitemia by that variant. Another part of the immune response against a particular variant cross reacts with many other variants—this cross-reactive component lasts only for a short while. As the initial parasitemia develops, some cells will have switched expression to other antigenic surface variants. As the first parasitemia clears, the next wave of parasitemia will develop from those rare variants that are least affected by the cross-reactive part of the host immune response. As those favored types develop into strong parasitemia, the process repeats, favoring in the subsequent wave those variants that cross-react least with the previous wave.

Molineaux et al. (2001) developed a more complex model of *P. falciparum* parasitemia dynamics and host immunity. Their model includes fitted values for how the various components of immunity clear parasites and variation in growth rate of different variants. This is an interesting analysis, but with so many parameters, it is difficult to determine whether the good fit with data arises from so many degrees of freedom or from a model that properly highlights the essential features of antigenic variation.

Turner (1999) proposed a fifth explanation for high switch rates and ordered expression of variants. The parasite faces a trade-off between two requirements. On the one hand, competition between parasite genotypes favors high rates of switching and stochastic expression of multiple variants early in an infection. On the other hand, lower effective rates of switching later in an infection express variants sequentially and extend the total length of infection.

Many *T. brucei* infections in the field probably begin with inoculation by multiple parasite genotypes transmitted by a single tsetse fly vector (MacLeod et al., 1999). This creates competition between the multiple genotypes. According to Turner (1999), competition intensifies the selective pressure on parasites to express many variants—variation allows escape from specific immunity by prior infections and helps to avoid cross-reactivity between variants expressed by different genotypes. These factors favor high rates of stochastic switching.

The effective rate of switching drops as the infection progresses because the host develops immunity to many variants. Effective switches occur when they produce novel variants, and the rate at which novel variants arise declines over the course of infection. Those novel variants, when they do occur, can produce new waves of parasitemia, promoting parasite transmission.

Turner's idea brings out many interesting issues, particularly the role of competition between genotypes within a host. But his verbal model is not fully specified. For example, delayed expression of some variants and extended infection depend on the connectivity of transition pathways between variants, an issue he does not discuss. The problem calls for mathematical analysis coupled with empirical study.

Connectivity of transition pathways between variants plays an important role in most theories. In Agur et al.'s (1989) model, host immunity acting differentially on double expressers during the switch process favors some transitions over others. In Frank's (1999) model, the different rates of molecular switching between variants provides structure to transition pathways. In Recker et al.'s (2004) model, short-lived and cross-reactive host immunity favors particulars sequences of antigenic dominance. Turner's (1999) model is not fully specified, but to work it must also provide a tendency for some transitions to be favored over others—this may occur by chance with random and rare switching or perhaps may favor common switches early and rare switches later in the sequence, more or less as in Frank's (1999) model.

Connectivity of transition pathways has not been studied empirically. The next section provides some preliminary hints at what an empirical analysis could reveal.

4. Connectivity of transition pathways

A mouse infected with *B. hermsii* develops a dense infection of bacteria in the blood within a few days. Host immunity clears most of the first wave of infection, but some bacterial cells switch to different antigenic variants. Those variants rise in density to form the first relapse.

Stoenner et al. (1982) infected a mouse with a particular antigenic variant and then measured which antigenic variants were present in the first relapse (see also Barbour and Stoenner, 1985). Variants were classified by reactivity with 24 different and specific sera (polyclonal antibodies from hosts infected with a single variant). If a bacterial variant reacted with a particular serum, it did not cross react with other sera. Some bacteria did not react with any of the 24 sera. We described those nonreactive bacteria as nontyped.

For each of the 24 variants that could be distinguished, between 8 and 12 mice were used. Each mouse was inoculated with about 25 bacteria of a particular variant. The initial inocula had contamination of less than 1% of other variants. Each mouse was scored for the presence or absence of each type during the first relapse. For example, if among the 10 mice inoculated initially with a particular variant, a particular type was detected during the first relapse in three cases, then the frequency of that variant in the first relapse was recorded as 0.3.

All of the data can be summarized in a transition matrix, showing the probability of variant *i* occurring in the first relapse

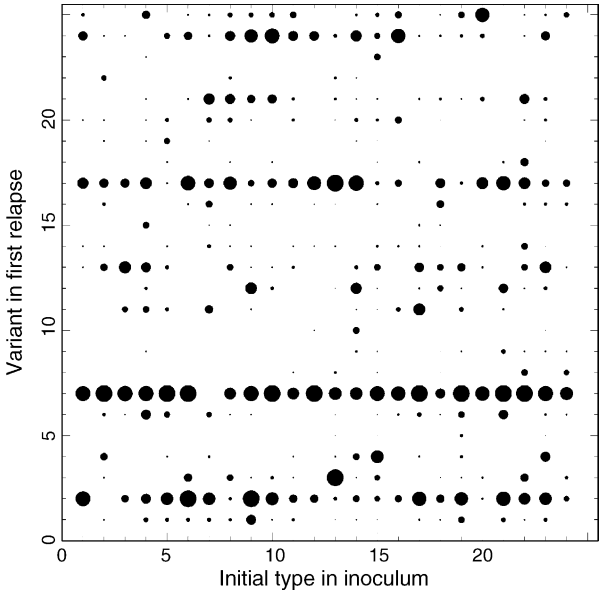

Fig. 3. Frequency of antigenic variants in first relapse of infections by *Borrelia hermsii*. The area of the circle for each x–y pair is proportional to the probability that the variant on the y-axis was detected in the first relapse of an infection initiated with the variant on the x-axis. The largest circles have an area that corresponds to a probability of one. Variants in the 25th position on the y-axis are the nontyped aggregate class. Data from Stoenner et al. (1982).

in a mouse inoculated with variant j. Fig. 3 shows this transition matrix. The area of each circle is proportional to the transition probability, with the jth column giving the initial type in the inoculum and the ith row describing the types in the first relapse.

The data do not distinguish between the processes of switching gene expression at the molecular level, the relative growth rates of the different variants, and the interactions with host immunity. Rather, this is simply a description of the resulting transitions between types. The data do provide a sense of the essential feature of any process that favors a particular order in sequential dominance—a bias in transition from one type to other types.

Whatever the underlying process, the differential transition probabilities could be the key to understanding the dynamics of sequential dominance. As an exercise, consider the predictions that follow from the relative transition probabilities in the matrix shown in Fig. 3. We start with the initial type in the inoculum. Those variants that were observed in the first relapse at a frequency of greater than a threshold z comprise the predicted variants for the first relapse. For each variant in the first relapse, any variant that follows with threshold greater than z in the matrix occurs in the predicted variants in the second relapse. And so on for following relapses, until all variants have been used.

Fig. 4 shows the predicted sequence of variants for different starting inocula. This illustrates how differential transition probabilities create connectivity pathways through sequences of variants. From simple presence data in the first relapse, we have predictions about the ordering of variants in sequential dominance. Most theories, differing in the underlying mechanism that determines the transition probabilities, share this common feature of transition probabilities and consequent sequence pathways. Empirically, it may be easier to work as illustrated here, that is, from the end results of transition rates or probabilities, to predicted sequences, and then eventually to the underlying mechanism that shapes the transition matrix and connectivity pathways.

Note in Fig. 4 that a few common types almost always arise in the first relapse. This tends to funnel the connectivity pathways through these common types no matter which variant

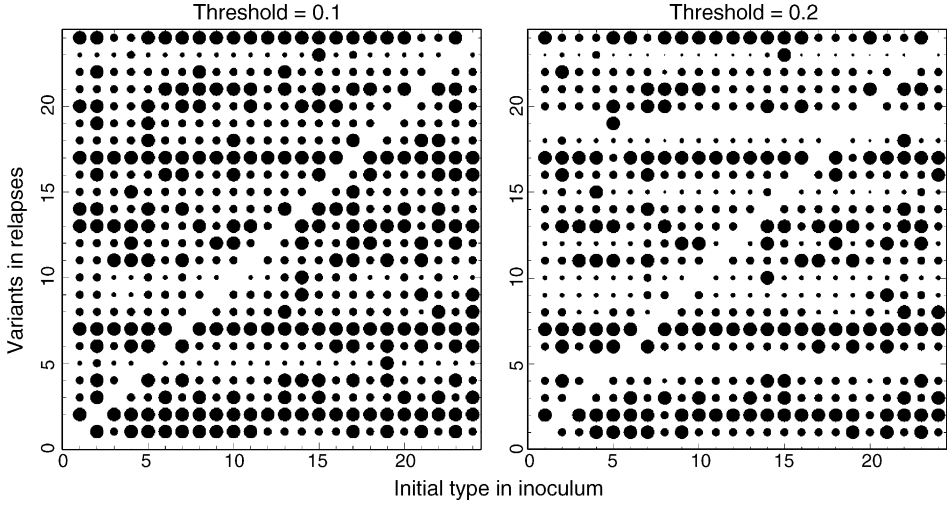

Fig. 4. Predicted sequence of antigenic types in *Borrelia hermsii* based on the transition matrix in Fig. 3. Each column predicts the sequence of relapses starting with inoculum that contains the variant numbered on the x-axis. The largest circles show the variants predicted to be in the first relapse, the next largest circles predict the variants in the second relapse, and so on. For example, in the right plot, starting with variant 1, the analysis predicts the presence of variants 2, 7, 17, and nontyped variants in the first relapse (nontyped variants are in position 25). No prediction is made with regard to dominance among those four types, the analysis predicts only presence above a threshold. Note that last type is an aggregate class for those variants that do not react with the 24 test sera. The analysis predicts the presence of those types with the second largest circle in the second relapse—again, it is likely that the relapse would be dominated by only a small number of these variants. Third and fourth relapses follow with decreasing circle sizes. Borrrelia infections typically have three or so strong relapses, so these predictions provide a reasonable qualitative match to bacteremia dynamics.

starts the infection, allowing the parasite to take advantage of the structure in the transition matrix. Without these common points of departure early in the sequence, some variants would tend to fail in tracing a good path through the variants, with a relatively even spread of variants arising over time. Such funneling through key variants may be a common feature of complex antigenic variation.

5. Conclusions

How does sequential dominance arise from stochastic changes in gene expression? The main theories depend on some combination of mechanisms to create favored connectivity pathways through the matrix of transitions between variants. Favored pathways may arise from biased switches at the molecular level of gene expression or from biases imposed by immune selection.

Empirically, it may be most productive to measure the relative tendency for certain transitions, building up the matrix of transitions between variants as suggested by Fig. 4. That matrix can be used to predict sequences independently of the particular mechanisms that cause biased transitions. Once a match has been achieved between the predictions from the transition matrix and the sequential pattern of dominance, then one may be in a better position to sort between alternative mechanisms that generate the biases in transition.

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