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Bacterial Antigenic Variation, Host Immune Response, and Pathogen-Host Coevolution

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INTRODUCTION

Many bacterial pathogens are exquisitely adapted to host parasitization. Their niche is primarily determined by the biochemical milieu of the host. As such, pathogens are selected to exhibit environmentally responsive and adaptive molecular traits which allow adherence, entrance, and replication within the host (7, 26). One major set of selective forces which operates to shape the phenotype of the bacterial pathogen is the host immune system. The host immune response following contact with the pathogen is itself adaptive and seeks to eliminate or restrict bacterial replication. Thus, successful bacterial pathogens must be able to avoid or adapt to evolving host defenses. However, in order to persist within a niche over long periods of time, the pathogen not only must be able to survive within an individual host but also must be able to infect other hosts. To achieve ecologic success (i.e., persistence in its niche within the host population), pathogens require mechanisms both for survival within hosts and transmission between hosts. The set of immune responses in the host population, which are determined by major histocompatibility complex (MHC) polymorphism and attendant antigen-specific T- and B-cell responses, thus are a set of selective forces which act on the population of bacteria.

The evolutionary important quantity on which natural selection acts to shape the genotype-phenotype of a pathogen is its basic reproductive rate, Ro (20). Ro is an epidemiologic concept and defines the average number of secondary infections an infected host produces in a fully susceptible population. Ro must equal or exceed 1 if the pathogen is to invade and persist in a host community. Natural selection will tend to maximize Ro for a given pathogen, and microbial attributes which enhance Ro are selected. This includes genes which encode attributes which enhance transmission or extend the duration of host infectivity. Because infectious discharges and other pathophysiologic changes in the infected host can substantially enhance transmission, pathogen-induced disease may be viewed as one of the important mechanisms for between-host survival (19).

Pathogens which reduce the life span and/or affect the fertility of individual hosts may also exert selection on host populations (9). Over evolutionary time, pathogens can select host traits that reduce the impact of microbial pathogens on host life span and fertility (1, 12, 18). Thus, successful pathogens can be seen to be engaged in a dynamic coevolutionary interaction with their host population. Two time scales set the boundaries for the types of adaptive traits used by pathogens and hosts. One time scale is rapid and set by the replication rate of the pathogen, which is substantially less than the life span of the host. The genetic basis for adaptive pathogen traits on this time scale often involve DNA rearrangement; likewise, host responses to pathogens also involve similar mechanisms. The average host life span determines the second, much longer time scale. The genetic basis for adaptive traits on this time scale characteristically involve allelic polymorphism for both the host and the pathogen.

Bacterial antigenic variation and host antibody selection of escape variants constitute an interesting model system to contemplate pathogen-host coevolution. It is a Darwinian process that involves both the generation of genetic diversity in the pathogen and the operation of immune selection at the molecular level by the host. In this article, we review the molecular mechanisms for antigenic variation that bacterial pathogens display and that result in within-host and between-host survival. We also note parallels between host and pathogen mechanisms. Although the paradigm of antigenic variants arising through immune selection is emphasized in this review, it is also becoming apparent that not all protein polymorphism in a pathogen arises from immune selection (16). Pathogen protein polymorphisms may also permit modulation of its host range.

WITHIN-HOST MECHANISMS

During infection of an individual host, pathogen mechanisms which prolong the duration of infectivity and hence the opportunity for transmission between hosts result in an increased Ro. Since the host's immune system rapidly develops specific responses to an invading pathogen, microbial mechanisms for phase and antigenic variation can reduce the restrictive effect of host immune responses. This is most often accomplished by genetic mechanisms which involve DNA rearrangement (2). Table 1 (upper portion) shows four bacteria for which the genetic mechanisms are at least partially understood.

DNA rearrangement alters gene expression, can be reversible or irreversible, and depends upon DNA inversion or gene conversion/recombination. Examples of mechanisms which involve DNA inversion are flagellar antigen expression in salmonellae (28) and pilin expression in *Moraxella bovis* (17). These are reversible in nature, occur as relatively low-frequency events (10^{-5} to 10^{-3} per cell per generation), and permit transcriptional expression of only one of a limited set of genes at a time. Examples of mechanisms which

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TABLE 1. Coevolution of bacterial antigen variation and host immune adaptation

Survival	Pathogen						Host				
	System	Example (reference)	Mechanism	No. of genes/cell	No. of alleles	Generation rate[a]	System	Mechanism	No. of genes/cell	No. of alleles	Generation rate
Within host	DNA inversion	H1 and H2 flagella, salmonellae (28)	Recombination	2	?	10^{-5} to 10^{-3}	IgV genes	Gene conversion	>1,000	?	?
		α- and β-pilins, *M. bovis* (17)	Recombination	2	?	?	TCRαβ	Gene conversion	>100	?	?
	DNA transposition	Pilin variation, *N. gonorrhoeae* (8)	Transformation, recombination	≈40	?	10^{-4}	TCRδ	Gene conversion	>10	?	?
		Variable major protein variation, *B. hermsii* (22)	Recombination	26	?	10^{-4} to 10^{-3}	IgV hypermutation	Error-prone repair	1	1	10^{-3}
		Opacity protein variation, *N. gonorrhoeae* (31)	CTCTT insertion/deletion, recombination	11	>50	10^{-3} to 10^{-2}					
Between host	Allelic polymorphism	MOMP, *C. trachomatis* (30)	Point mutation, recombination	1	>15	?	MHC allelic polymorphism	Gene conversion, point mutation	6	>100	10^{-6} to 10^{-5}
		Por protein, *N. gonorrhoeae* (4)	Point mutation, recombination	1	>20	?					
		M protein, *S. pyogenes* (13)	Recombination	1	>70	10^{-4} to 10^{-3}					

[a] Generation rate refers to the rate at which genetic variants arise per cell division or generation.

involve gene conversion/recombination include pilin variation in *Neisseria gonorrhoeae* (23) and variable major protein variation in *Borrelia hermsii* (22). In these mechanisms, an antigen-specifying gene is present in a storage form and expression results from duplicative transposition to an expression site. This can occur intrachromosomally as gene conversion or by transformation-mediated recombination (8, 27). Gene conversion is a relatively high-frequency event (10^{-4} to 10^{-2} per cell per generation), is irreversible, and allows the cell to express a single gene product, which is often assembled from a very large set of gene fragments. Antigen-specifying gene segments which utilize gene conversion generally number between 10 and 100 copies per chromosome. Because gonococci (as well as several other human bacterial pathogens such as *Haemophilus influenzae* and *Streptococcus pneumoniae*) are naturally competent for DNA-mediated transformation and also frequently undergo spontaneous autolysis, transformation-mediated recombination, rather than gene conversion, may be the important mechanism for the generation of variant sequences of gonococcal pilin in vivo (8, 21, 27).

Gene rearrangement as a within-host pathogen survival strategy runs the risk of recombinational error. However, it is advantageous to the pathogen because it allows for preadaptation of a small fraction of the population to sudden changes in the host that would arise too quickly for repressor-activator systems (which are based on sensor-regulatory proteins) to respond (24). The within-host survival strategy of phase and antigenic variation is correctly viewed as molecular immune selection. Immune responses do not drive the process but merely select viable progeny with new characteristics. The mechanisms underlying DNA rearrangement provide the genetic variation on which selection acts.

BETWEEN-HOST MECHANISMS

Between-host microbial strategies are driven by different epidemiologic forces than are within-host strategies. The between-host strategies act to promote survival of the pathogen in a transmitter pool of the host population (rather than in the infected individual). Transmitter pools are composed of individual hosts who have often had prior exposure to one or more strains of the pathogen because only they have the social or behavioral attributes which ensure pathogen transmission. The transmitter pool concept is explicitly seen with sexually transmitted pathogens. Only hosts who exceed a critical threshold in sexual behavior participate in the maintenance and transmission of the sexually transmitted disease pathogen within the population (3). Heterogeneity in dominant surface antigens permits reinfection of hosts within the transmitter pool despite prior immune experience with other strains of the pathogen.

The primary genetic mechanism which results in between-host survival for the pathogen is allelic polymorphism of the major surface antigen gene. Each pathogen clone contains a single copy of the antigen gene, but within the pathogen species, multiple alleles occur. The size of the pathogen allelic repertoire may be related to the size of the host transmitter population (the smaller the transmitter pool, the larger the allelic repertoire) and will be affected by the transmission rate within the reservoir population, the entry and exit of hosts from the transmitter pool, and the time to decay of protective antibodies. The waxing and waning of herd immunity result in cyclic variation in the frequency of pathogen-specific alleles in the host population, based on time scales determined by the decay of type-specific protec-

tive immune responses and by the demographic and social forces determining entry and exit from the host transmitter pool.

Antigenic variation based on allelic polymorphism is selected by immune mechanisms in hosts that have been previously exposed to the pathogen. The stable coexistence of multiple pathogen strains critically depends on the degree of cross-immunity induced by each strain. Allelic polymorphism may arise by either mutation or recombination as outlined in the bottom portion of Table 1. Allelic variation resulting from single-site mutation has been observed in the *Chlamydia trachomatis* major outer membrane protein (MOMP) and occurs with serovariant surface antigens of many other pathogens (30). Antibodies with type specificity are required to select differential survival of pathogens with such mutations. The genetic mechanism(s) and kinetics underlying point mutation are uncertain but, in principle, could resemble the somatic hypermutation mechanism of B lymphocytes (15).

The second mechanism for allelic variation involves recombination. This produces a novel mosaic antigen gene whose protein product can evade prevailing immune selective pressures by generating large blocks of antigenic change. Large-scale antigenic change permits pathogen persistence in the face of either more broadly cross-reactive immune responses or an expanding repertoire of type-specific immune responses in the transmitter population. Recombination may have generated blocks of sequence variation in the major porin protein (Por) of *N. gonorrhoeae* and in the MOMP of *C. trachomatis* (Table 1). Transformation is likely the dominant mechanism by which mosaic antigens originate in transformation-competent bacterial species such as *Neisseria spp.*, *Haemophilus spp.*, and *S. pneumoniae* (21). Mosaic genes for penicillin-binding proteins in *N. gonorrhoeae* (29) and *S. pneumoniae* (6) and the IgA protease gene of *N. gonorrhoeae* (11) have all been reported, and recently, we have observed mosaicism for the MOMP antigen gene of *C. trachomatis* (unpublished data). Presumably, mosaic antigens are produced spontaneously during concomitant infection of an individual host with multiple strains of the bacterial pathogen. Bacterial strains which express the mosaic antigens may be selected by partial immunity in the host.

Allelic polymorphism of dominant surface antigens of bacterial pathogens is preserved by immune selection by the human host population (10). In the situation where there are allelic variants, each capable of infecting only certain hosts (because of strain-specific immunity), frequency-dependent selection ensues. At any one time, the most frequent allelic variants will be at a selective disadvantage (because of herd immunity) while rarer variants will enjoy a selective advantage (capable of larger Ro because of the absence of herd immunity). Thus, frequency-dependent selection of pathogen genotypes based on strain-specific immunity in the host population will act to maintain allelic polymorphism in the pathogen. This can result in a constant prevalence of the pathogen in the host population but one that is generated by successive and cyclical waves of allelic variants. Experience with gonococcal Por allelic variants over time is an empiric example of this phenomenon (25).

HOST RESPONSE TO PATHOGEN ANTIGENIC VARIATION

The genetic mechanisms employed by human hosts to respond to antigenically variant pathogens are remarkably similar to the microbial strategies. These include allelic polymorphism of HLA antigens involved in presenting pathogen-derived peptides to immune surveillance; HLA polymorphism results in between-host resistance (12, 14). Gene conversion events for assemblage of T- and B-lymphocyte antigen recognition molecules (5, 32) and site-specific somatic mutation to improve pathogen recognition by B lymphocytes are involved in within-host resistance to the pathogen (15) (Table 1).

The genetic mechanisms for all host traits except somatic hypermutation are known to also occur in bacterial pathogens, although hypermutation may ultimately prove to be one genetic mechanism for rapidly generating new alleles of major surface antigens in pathogens. It is remarkable how the genetic strategies that involve DNA rearrangement, which permit within-host survival of the pathogen, are very similar to the within-host responses used by the host in dealing with the pathogen. Undoubtedly, this relates to the time scale of replication events exhibited by bacterial pathogens and the need for hosts to contend with antigen-specific lymphocytes that replicate on an order of magnitude closer to that of the pathogen. Without a rapidly replicating defense system, hosts would quickly be outevolved by replicating pathogens. As pathogens use allelic variation of dominant surface antigens as a longer-term strategy for persisting and cycling in partially resistant host populations, the host itself uses allelic variation to engender partial resistance to microbes by MHC polymorphism among individuals in the population (12).

CONCLUSIONS

Major bacterial pathogens are engaged in long-standing and highly successful interactions with their hosts. Strategies which permit both within-host and between-host survival are essential to the ecologic success of the pathogen, and natural selection acts to maximize the basic reproductive rate, Ro, of the pathogen. In general, pathogen genetic mechanisms for invading and persisting within a single host involve DNA rearrangement and for persisting in host populations they involve allelic polymorphism of a major surface antigen gene. The rearrangement of genes prolongs the duration of infectivity of a single host, and allelic polymorphism enhances transmission in a partially immune host population; both result in increased Ro for the pathogen. Both MHC polymorphism and strain-specific immunity create diversity in host population susceptibility to infection. Allelic polymorphism of the dominant antigen gene of a pathogen maintains diversity because of host strain-specific immunity. To the extent that pathogens differentially affect host survival and reproduction, they drive host population MHC polymorphism (12). Both types of selection (pathogen and immune) sustain allelic polymorphism through frequency-dependent selection.

A remarkable and surprisingly symmetrical coevolutionary arms race between the pathogen and host is apparent. Within-host pathogen phenotypes are strongly affected by immune selection pressures, and two related genetic mechanisms (gene conversion/recombination and DNA inversion) result in the creation of pathogen diversity, with new genotypes composed of rearranged genes which permit survival in the face of an evolving immune response in the host. Lymphocytes, like pathogens, use gene conversion as the major adaptive mechanism to identify microbes. These strategies involve a short time cycle set by pathogen and lymphocyte replication rates. Pathogen selection drives diversity within the host population primarily through MHC allelic polymorphism, and host population herd immunity

drives allelic polymorphism of dominant surface antigens of pathogens. These strategies involve a much longer time cycle set by the replication rate of the host population and the duration of herd immunity. These insights reveal how closely linked are the biological and evolutionary trajectories of bacterial pathogens and their host populations.

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REFERENCES

1. **Anderson, R. M., and R. M. May.** 1979. Population biology of infectious disease: part I. Nature (London) **280**:361–367.
2. **Borst, P., and D. R. Greaves.** 1987. Programmed gene rearrangements altering gene expression. Science **235**:658–667.
3. **Brunham, R. C., and F. A. Plummer.** 1990. A general model of sexually transmitted disease epidemiology and its implication for control. Med. Clin. North Am. **74**:1339–1352.
4. **Carbonetti, N. H., V. I. Simnad, H. S. Seifert, M. So, and P. F. Sparling.** 1988. Genetics and protein I of *Neisseria gonorrhoeae*: construction of hybrid porins. Proc. Natl. Acad. Sci. USA **85**:6841–6845.
5. **Davis, M. M., and P. J. Bjorkman.** 1988. T-cell antigen receptor genes and T-cell recognition. Nature (London) **334**:395–401.
6. **Dowson, C. G., A. Hutchison, J. A. Brannigan, R. C. George, D. Hansman, J. Linares, A. Tomosz, J. Maynard Smith, and B. G. Spratt.** 1989. Horizontal transfer of penicillin-binding protein genes in penicillin-resistant clinical isolates of *Streptococcus pneumoniae*. Proc. Natl. Acad. Sci. USA **86**:8842–8846.
7. **Finlay, B. B., and S. Falkow.** 1989. Common themes in microbial pathogenicity. Microbiol. Rev. **53**:210–230.
8. **Gibbs, C. P., B. Y. Reimann, E. Schultz, A. Kaufmann, R. Haas, and T. F. Meyer.** 1989. Reassortment of pilin genes in *Neisseria gonorrhoeae* occurs by two distinct mechanisms. Nature (London) **338**:651–652.
9. **Haldane, J. B. S.** 1949. Disease and evolution. Ricerca Sci. **19**(Suppl):68–76.
10. **Haldane, J. B. S., and S. D. Jayakar.** 1963. Polymorphism due to selection depending on the composition of a population. J. Genet. **58**:318–323.
11. **Halter, R., J. Pohler, and T. F. Meyer.** 1989. Mosaic-like organization of IgA protease genes in *Neisseria gonorrhoeae* generated by horizontal genetic exchange in vivo. EMBO J. **8**:2737–2744.
12. **Hill, A. V. S., C. E. M. Allsopp, D. Kiviatkowski, N. M. Anstey, P. Twumasi, P. A. Rowe, S. Bennett, D. Brewster, A. J. McMichael, and B. M. Greenwood.** 1991. Common West African HLA antigens are associated with protection from severe malaria. Nature (London) **352**:595–600.
13. **Hollingshead, S. K., V. A. Fischetti, and J. R. Scott.** 1986. Complete nucleotide sequence of type 6 M protein of the group A streptococcus. J. Biol. Chem. **261**:1677–1686.
14. **Howard, J. C.** 1991. Disease and evolution. Nature (London) **352**:565–567.
15. **Lebecque, S. G., and P. J. Bearhart.** 1990. Boundaries of somatic mutation in rearranged immunoglobulin genes: 5′ boundary is near the promoter and 3′ boundary is ≈1 Kb from V(D)J gene. J. Exp. Med. **172**:1717–1727.
16. **Lund, B., F. Lindberg, B. I. Marklund, and S. Normark.** 1987. The Pap G protein is the α-D-galactopyranosyl-(1→4)-β-D-galactopyranose binding adhesin of uropathogenic *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:5898–5902.
17. **Marrs, C. F., W. W. Ruehl, G. K. Schoolnik, and S. Falkow.** 1988. Pilin gene phase variation of *Moraxella bovis* is caused by an inversion of the pilin gene. J. Bacteriol. **170**:3032–3039.
18. **May, R. M., and R. M. Anderson.** 1979. Population biology of infectious disease: part II. Nature (London) **280**:455–461.
19. **May, R. M., and R. M. Anderson.** 1983. Epidemiology and genetics in the coevolution of parasites and hosts. Proc. R. Soc. Sect. B **219**:281–313.
20. **May, R. M., and R. M. Anderson.** 1990. Parasite-host coevolution. Parasitology **100**(Suppl):S89–S101.
21. **Maynard Smith, J., C. G. Dowson, and B. G. Spratt.** 1991. Localized sex in bacteria. Nature (London) **349**:29–31.
22. **Meier, J. T., M. I. Simon, and A. G. Barbour.** 1985. Antigenic variation is associated with DNA rearrangements in a relapsing fever Borrelia. Cell **41**:403–409.
23. **Meyer, T. F., N. Mlawer, and M. So.** 1982. Pilus expression in *Neisseria gonorrhoeae* involves chromosomal rearrangement. Cell **30**:45–52.
24. **Miller, J. F., J. J. Mekalanos, and S. Falkow.** 1989. Coordinate regulation and sensory transduction in the control of bacterial virulence. Science **243**:916–922.
25. **Plummer, F. A., J. N. Simonsen, H. Chubb, L. Slaney, J. Kimata, M. Bosire, J. O. Ndinya-Achola, and E. N. Ngugi.** 1989. Epidemiologic evidence for the development of serovar-specific immunity after gonococcal infection. J. Clin. Invest. **83**:1472–1476.
26. **Relman, D. A., and S. Falkow.** 1990. A molecular perspective of microbial pathogenicity, p. 25–32. *In* G. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practice of infectious diseases, 3rd ed. Churchill Livingstone Press, New York.
27. **Seifert, H. S., R. S. Ajioka, C. Marchal, P. F. Sparling, and M. So.** 1988. DNA transformation leads to pilin antigenic variation in *Neisseria gonorrhoeae*. Nature (London) **336**:392–395.
28. **Simon, M., J. Zieg, M. Silverman, G. Mandel, and R. Doolittle.** 1980. Phase variation: evolution of a controlling element. Science **209**:1370–1374.
29. **Spratt, B. G.** 1988. Hybrid penicillin-binding proteins in penicillin-resistant strains of *Neisseria gonorrhoeae*. Nature (London) **332**:173–176.
30. **Stephens, R. S., R. Sanchez-Pescador, E. Wagar, C. Inouye, and M. Urdea.** 1987. Diversity of the major outer membrane proteins of *Chlamydia trachomatis*. J. Bacteriol. **169**:3875–3885.
31. **Stern, A., M. Brown, P. Nickel, and T. F. Meyer.** 1986. Opacity genes in *Neisseria gonorrhoeae*: control of phase and antigenic variation. Cell **47**:61–71.
32. **Tonegawa, S.** 1983. Somatic generation of antibody diversity. Nature (London) **302**:575–581.