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Variability in Immune Response to Pathogens: Using Measles Vaccine to Probe Immunogenetic Determinants of Response

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> *"The measles had not prevailed on the Faroes since 1781; they broke out early in April, 1846. … of the 7,782 inhabitants, about 6,000 were taken with measles … 225 persons in all died … of the many old people still living on the Faroes who had had the measles in 1781, not one … was attacked the second time."*
> [P. L. Panum, *Observations Made during the Epidemic of Measles on the Faroe Islands in the Year 1846*]

The Faroe Islands measles outbreak described above is of interest to geneticists in several regards: Why did some people survive and some die? Why was the case fatality rate so high? Why was the protective effect of prior exposure so high? Understanding the genetic influences on the phenotypes of protective and nonprotective antibody responses provides a unique window to understand the variability in host response to pathogens.

Infectious diseases have been postulated to be one of the main forces of natural selection, shaping the genetic constitution of man (Haldane 1949). Disease susceptibility, resistance, and progression are influenced by HLA genes (Bodmer 1996). For example, in Brazilian Amazon tribes, Black et. al. (1977) explored reasons for the pronounced susceptibility of these peoples to measles. The major finding was a high degree of HLA homozygosity in these virgin populations. Black et al. speculated that, because of the high rate of homozygosity, HLA polymorphisms were restricted, and hence the range of immune-response genes might be similarly restricted. The deficiency of HLA homozygotes in other populations studied has also been hypothesized to be caused by selection against homozygotes by infectious diseases (Hedrick 1990).

It now appears that the HLA haplotype is one crucial determinant of immune response, but other factors that participate in the processing of antigens and their display on antigen-processing cells (APCs) also contribute to susceptibility to viral infections. Although studies of immune response to wild-virus infection are difficult and depend on the ability to study subjects in proximity to their infection, this limitation can be overcome by using vaccines to probe the immunogenetic determinants of immune responsiveness. Measles immunization results in the delivery of a live, attenuated virus that undergoes limited in vivo replication causing attenuated "infection," inducing immune responses without disease. Hence, live-measles immunization is an excellent model with which to study the spectrum of host immune responses to the most transmissible disease known to man.

We have used the antibody response to live-measles vaccine as a probe with which to examine systematically the influence of specific HLA and other genes on antibody response. The advantage of this model is that subjects can be immunized at any time, with little risk, and under defined conditions. Knowledge of the immunogenetic factors related to vaccine response may provide clues about the mechanisms of host defense to pathogens, leading to the design of novel vaccines, as well as helping to identify individuals at risk for aberrant responses to infection or vaccines. This brief review will highlight current research and directions for future investigation into the immunogenetic determinants of host-pathogen/host-vaccine response.

Vaccine Response as a Marker of Disease Susceptibility

Postimmunization antibody response can be used as a marker of disease susceptibility. For example, the level

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of antibody response after hepatitis B immunization predicts susceptibility to disease on exposure (Ellis 1993). In studies of measles, postimmunization measles antibody in the "low positive" range did not protect against clinical measles when subjects were exposed to the wild measles virus, whereas high levels were protective (Chen et al. 1990). Furthermore, nonresponders to a single dose of measles vaccine who demonstrated an antibody response only after a second immunization were still six times more likely than were responders to a single dose of measles vaccine to develop measles on exposure to wild virus (Mathias et al. 1989). Others examined "poor responders," who were reimmunized and developed poor or low-level antibody responses only to lose detectable antibody and develop measles on exposure 2–5 years later. They concluded that there is a strong correlation between low antibody levels after a single dose of vaccine and high susceptibility to infection with exposure (Deseda-Tous et al. 1978).

The Role of the HLA Genes in Vaccine Immune Response

Genetic factors, such as the HLA genes, and their interaction with other factors (host, environmental, etc.) are increasingly recognized as playing a significant role in disease susceptibility, resistance, and progression (Bodmer 1996; Detels et al. 1996; Singh et al. 1997). The HLA genes encode the HLA antigens expressed on antigen-presenting cells. These glycoproteins provide the context in which processed antigenic peptides are presented to T cells and, hence, determine the ability of the individual to respond immunologically to endogenous and exogenous antigens. Therefore, the ability of an individual to respond to viral infection (or vaccination) by antibody production is, in part, under the control of the immune-response genes within the HLA complex on the short arm of human chromosome 6.

Once in the cell, measles proteins are processed into smaller antigenic peptides. Both the endogenous and exogenous antigen-processing pathways are important in the development of antibody to measles (Malnati et al. 1992; Leopardi et al. 1993). Classically, in the endogenous pathway, cytosolic proteins are processed into smaller antigenic peptides that are translocated, via the transporters associated with antigen processing (TAP proteins), into the endoplasmic reticulum (ER; Androlewicz et al. 1993; Townsend and Trowsdale 1993). There, peptides bind to class I HLA and the stable antigen: class I HLA complexes transit the secretory pathway and reach the cell surface. Because they serve as selective peptide transporters, the TAP proteins regulate which antigens are available for presentation to $CD8^+$ T cells and function as mediators of cellular immune responses. In contrast, the exogenous pathway involves endocytosis of antigens; the proteolytic processing of these antigens and their binding to class II HLA molecules occur within an acidic endosomal compartment. Hence, the exogenous pathway, as classically defined, does not require the peptide to cross a cellular lipid bilayer and so is TAP independent. Once formed, the antigen–class II HLA complex is transported to the cell surface for presentation to $CD4^+$ T cells.

The recognition that the host immune response is related to antigen processing and presentation is crucial to the design of vaccines with adequate epitope formation, and it suggests several possible mechanisms by which genetic variations could lead to poor antibody responses in some individuals. For instance, differences in the expression (quantitative or otherwise) of major histocompatibility complex (MHC) molecules on cell membranes could influence immune response. Alternatively, as discussed below, antigen-processing cells (APCs) might be unable to form functional associations between specific foreign antigens and HLA molecules.

TAP Genes: Function and Variation

The TAP genes were discovered in 1990 (Deverson et al. 1990; Spies et al. 1990; Trowsdale et al. 1990), and the genomic sequence of both TAP genes has been determined. The two TAP genes identified thus far, TAP1 and TAP2, are physically situated within the class II HLA gene cluster on the short arm of human chromosome 6, within the DP–DQ interval. The TAP genes encode a heterodimeric complex within the ER membrane and show homology to the ATP-binding cassette superfamily of transporters.

The TAP genes are polymorphic, with variant amino acids in the membrane-spanning and ATP-binding cassette domains (Quadri et al. 1995). Four alleles of the TAP1 gene (two polymorphic sites at positions 333 and 637) and eight alleles of the TAP2 gene (four polymorphic sites at amino acid positions 379, 565, 665, and 687) have been identified, to date (Powis et al. 1993; Szafer et al. 1994), with evidence for other rare TAP2 alleles (Aoki et al. 1993). Even with this moderate degree of polymorphism (as compared with the extreme variability seen in the class I and II HLA loci), TAP genes may contribute to a much greater degree of variation in selective antigen transport at the level of the actual functional transporter. In part, this may be due to the very early and crucial step in the antigen-processing pathway in which TAP gene products act, "magnifying" the impact of the polymorphism of these genes. We and others hypothesize that polymorphisms of TAP genes can restrict the peptides that are bound and then presented (Neefjes et al. 1993, 1995; Shepherd et al. 1993; Hill and Ploegh 1995) and that this restriction can occur independent of linkage with other class II molecules

(Heemels and Ploegh 1994; Barron et al. 1995; Momburg et al. 1995; Savage et al. 1995).

Measles-Antigen Processing

The measles virus carries a spherical, single-stranded RNA-based genome that encodes at least six structural proteins (hemagglutinin protein [H], phosphoprotein, nucleoprotein [N], fusion glycoprotein [F], matrix protein, and large protein). When measles virus binds to the CD46 receptor of cell surfaces, measles envelope H and F are endocytosed into the host cell, whereas viral N is injected directly into the cytosol. Thus, antigens are incorporated into the host cell by endogenous and exogenous mechanisms and fit with a biological model whereby measles-virus antigens are presented by both the endogenous pathway, for the N protein, and the exogenous pathway, for the H and F proteins.

Exceptions to these segregated classic pathways of antigen processing and presentation have been observed in the cellular response to measles and other antigens. Endogenous antigens, classically presented by class I mechanisms, can be presented by a class II mechanism, and class II recognition of certain cytosolic peptides may require TAP1 function. Furthermore, both class I and II molecules can complex with antigenic peptides in a pre-Golgi compartment, into which class II–restricted antigens are delivered by the TAP proteins (Nuchtern et al. 1990; Weiss and Bogen 1991; Malnati et al. 1992). Stimulation of some class II–restricted T-cell clones depends on a functional TAP transporter and on antigen presentation of soluble measles-virus antigens (Jacobson et al. 1989; Long and Jacobson 1989; Nuchtern et al. 1990). This may, in part, account for the influence of specific TAP alleles on phenotypic variations in antibody titers in response to vaccination.

Measles virus may also directly affect expression of class II antigens. HLA class II–restricted immune responses are required for normal immune handling of the measles virus (Jacobson et al. 1984, 1988) and for vaccine-induced antibody response (Nuchtern et al. 1990; Leopardi et al. 1993). The interplay of multiple genes is likely to be similar to that seen in infections with other viruses, such as HIV, where the cumulative contributions of class I, class II, and TAP genes are important in regulating the response to infection (Kaslow et al. 1996).

The Influence of TAP and Other HLA Genes on Antibody Response to Measles Vaccine

We use the response to live-measles vaccine as a model with which to determine associations between the extreme phenotypes of antibody nonresponse/hyperresponse and specific HLA alleles, as well as associations with HLA homozygosity. Our ongoing studies suggest that seronegativity after vaccination clusters among related family members, that genetic polymorphisms within the HLA significantly influence antibody levels, and that HLA homozygosity is significantly associated with nonresponse to immunization (Hayney et al. 1994, 1996, 1997; Poland et al. 1995).

We have examined measles-antibody levels as a function of different TAP alleles among schoolchildren who had received measles vaccine 5–11 years earlier and who were highly unlikely to have ever been exposed to the wild virus (Hayney et al. 1995). We found a significant association between antibody response and the variation in amino acids found at position 665 of the TAP2 locus, with hyperresponders dramatically more likely to be heterozygous at position 665 than nonresponders ($P = .016$; Hayney et al. 1997). Hyperresponse was associated with an excess of TAP2 A/B genotypes, and nonresponse with an excess of TAP2 A/C genotypes. These data support the concepts that TAP-determined restriction affects antibody response to measles-vaccine antigens (Dimanlig et al. 1994; Hayney et al. 1997) and that homozygosity, in and of itself, may impair antibody response by restricting the diversity of peptides that can be presented to T cells (Black et al. 1977; Black and Salzano 1981; Hedrick 1990).

The linkage of TAP genes within the class II HLA cluster raises the concern of the independent contribution of TAP and class II HLA genes to vaccine response. Accordingly, we have used logistic regression analysis to determine the independent effects of TAP2 position 665 and DRB1 homozygosity. Both TAP2- and DR-locus homozygosity were significantly more frequent among nonresponders than among hyperresponders ($P = .015$ for TAP2; $P = .011$ for DR). Nonresponders were five times more likely to be homozygous at TAP2 position 665 than were hyperresponders and 6.4 times more likely to be DR homozygous. There were no significant interactions between TAP2 and DR homozygosity, suggesting an independent, additive effect of TAP2 homozygosity at position 665 on antibody levels following measles vaccination.

We have also examined the association between measles-antibody level and other class I and II HLA genes. We found that the difference in distribution of class II HLA-DR alleles among nonresponders and hyperresponders was statistically significant (Poland et al. 1995; Hayney et al. 1996, and in press). Measles-vaccine nonresponders lacked HLA DRB1*13 alleles significantly more often than did hyperresponders or normal controls, and nonresponders had an excess of DRB1*07 alleles. In addition, nonresponders had a significantly increased frequency of DR homozygosity ($P = .0001$) and an excess of DQA1*05 alleles ($P = .017$), whereas hyperresponders had an excess of DQA1*01 alleles ($P = .016$). The homozygosity rate for any DQA1 allele

was noted to be significantly higher among nonresponders than among hyperresponders (23.9% vs. 9.4%; $P = .037$), and homozygous nonresponders were more likely to be DQA1*05 than was any other homozygote genotype.

Similar analyses of the overall distribution of class I HLA-A, -B, and -C alleles revealed significant differences for the HLA-B alleles and HLA-C alleles (Poland et al., in press) but not for the HLA-A locus. In particular, HLA-B7 and -B51 alleles were associated with hyperresponse, and B8, B13, B44, and C5 alleles with nonresponse. We also found evidence of an allele dose response of the B7 allele, with a B7 allele frequency of 6% for nonresponders, 15% for normal responders, and 22% for hyperresponders ($P = .0001$). Finally, we also found that, for the HLA-B locus, nonresponders were 2.1 times more likely to be homozygous than were normal responders and 3.7 times more likely to be homozygous than were hyperresponders ($P = .031$).

Future Prospects

The use of live viral vaccines (such as measles) as a model for understanding host response to pathogens has deepened our understanding of the role and importance of HLA genes in influencing individual variability to antigens. Moreover, this model has drawn our attention to the role of the non-HLA genes influencing the antigen-processing pathway genes. Our work has demonstrated that HLA polymorphisms do not explain all of the individual variation in vaccine responsiveness. Rather, the genetics implicates the antigen-processing pathway, particularly the TAP genes, as an important and independent contributor to individual variability in vaccine responsiveness. For this reason, the role of other genes influencing antigen processing, such as the LMP and DM genes, and genes producing chaperone proteins, heat shock proteins, proteasomes, tapasins, and others (G_m and K_m allotypes) should be studied by models such as this in order to determine their relative contributions to individual host response to pathogens.

Vaccines may be used as a probe to understand the observed variability in phenotypic responses such as antibody response. Prospective studies of subjects with known HLA haplotypes have proven to be valuable tools for identifying genetic influences on human immune responses. Additional studies exploring potential differences in antigen processing and presentation in subjects with extreme phenotypes (i.e., nonresponse and hyperresponse) and differing HLA backgrounds will offer uniquely important information. Fundamentally, such studies should allow us to understand more fully why some hosts apparently resist infection, some survive infection, and some die. This work may allow the prospective design of nearly universally immunogenic vaccines for a variety of pathogens and may lead to strategies for circumventing nonresponse by designing vaccines specifically for class I, class II, or combined pathways of antigen processing. In principle, it should be possible to design vaccines that take advantage of individual differences in HLA haplotypes, antigen presentation and processing, and other downstream events in the host immune response.

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