NOTES

Control of Simian Immunodeficiency Virus SIVmac239 Is Not Predicted by Inheritance of Mamu-B*17-Containing Haplotypes

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It is well established that host genetics, especially major histocompatibility complex (MHC) genes, are important determinants of human immunodeficiency virus disease progression. Studies with simian immunodeficiency virus (SIV)-infected Indian rhesus macaques have associated Mamu-B*17 with control of virus replication. Using microsatellite haplotyping of the 5-Mb MHC region, we compared disease progression among SIVmac239-infected Indian rhesus macaques that possess Mamu-B*17-containing MHC haplotypes that are identical by descent. We discovered that SIV-infected animals possessing identical Mamu-B*17-containing haplotypes had widely divergent disease courses. Our results demonstrate that the inheritance of a particular Mamu-B*17-containing haplotype is not sufficient to predict SIV disease outcome.

Host genetics are a dominant force in determining AIDS disease progression, viral evolution, and viral burden. Numerous studies with human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV), the macaque model for HIV, report strong associations with major histocompatibility complex (MHC) genes and disease progression (4, 28). The human MHC alleles HLA-B*27 and HLA-B*57 and the Indian rhesus macaque alleles Mamu-B*17 and Mamu-A*01 have been linked to decreased plasma virus concentrations and thus slowed disease progression (12, 21, 26, 31). While the precise mechanism of protection is unclear, mounting evidence from CD8 depletion (17, 27), viral transmission (11), and viral escape (8, 23) studies implicates cytotoxic T lymphocytes (CTL) as critical.

Studying correlations between MHC genetics and disease progression in HIV-infected humans is problematic because of undefined HIV inocula and the inherent genetic variability between unrelated individuals. MHC-defined Indian rhesus macaques provide an excellent resource for elucidating the influence of particular MHC alleles in SIV disease progression. Both the human allele HLA-B*57 and the rhesus allele Mamu-B*17 are overrepresented among long-term nonprogressors (21, 31), and both alleles present immunologically relevant HIV- or SIV-derived peptides to CD8+ T cells (15, 16, 22, 23). However, not all HLA-B*57-positive infected individuals or Mamu-B*17-positive infected rhesus suppress viral replication (21, 31). It is currently unknown whether genes tightly linked to HLA-B*57 or Mamu-B*17 influence HIV or SIV disease progression.

The human MHC contains one of the most gene-dense regions in the genome, and it is estimated that of the purported 128 expressed genes, about 40% have immune function (19). When subjects are matched for multiple MHC class I alleles, the genetic backgrounds of other immune response genes located within the MHC region are typically undefined. Using microsatellites, we compared disease progression in SIVmac239-infected Indian rhesus macaques that possess identical Mamu-B*17-containing haplotypes by descent. A previous study with an MHC-defined family of SIV-infected Indian rhesus macaques demonstrated that the inheritance of putative MHC haplotypes influenced disease progression (7). While that study noted trends in survivorship within one family, we sought to determine the global influence of several Mamu-B*17-containing haplotypes on disease progression with Indian rhesus macaques infected with the clonal SIV isolate SIVmac239.

MHC haplotypes of SIV-infected Indian rhesus macaque families. A total of 187 SIVmac239-infected Indian rhesus macaques were screened for the presence of Mamu-B*17 by sequence-specific PCR (31). Of the 44 Mamu-B*17-positive animals, 16 were chosen for analysis based on availability of archived tissue. We performed microsatellite analysis as previously described (24, 30) (Fig. 1 and data not shown). Analysis of the 16 Mamu-B*17-positive animals with respective sires and dams revealed eight distinct Mamu-B*17-containing haplotypes (Fig. 2). Five of the eight haplotypes were inherited by multiple SIV-infected animals from common sibships (Table 1). In all cases, Mamu-B*17 was inherited on a single chromosome, and no recombination events occurred between any of the microsatellites spanning the MHC (data not shown). Moreover, polymorphism within a highly informative microsatellite locus outside the MHC confirmed that none of the animals are genetically identical (data not shown).

Since chronic-phase plasma virus concentration is the best...
FIG. 1. Genomic localization of microsatellites in the MHC region of Indian rhesus macaques. Positions of microsatellites are based on the rhesus MHC genomic sequence (6). Shaded boxes represent each MHC gene cluster, and the class IB cluster is enlarged to the right for clarity.

predictor of disease progression (18), we defined disease progression based on the geometric mean of all measurements of plasma virus concentration from week 10 onward. We designated animals whose chronic geometric mean plasma virus concentration was under $10^3$ viral RNA copies/ml as “elite controllers.” Although this retrospective study used animals that were administered various vaccines, there was no correlation of vaccination with elite control (31). When the entire 5-Mb MHC region was considered, none of the eight Mamu-B*17-containing haplotypes were identical to one another. This supports a previous observation that Indian rhesus MHC microsatellite haplotypes are identical only in related animals (24), underscoring the MHC diversity inherent even when animals share one or more MHC class I alleles.

To more closely examine the class IB region microsatellite haplotypes among Mamu-B*17-positive animals, we identified additional polymorphic microsatellites in the class IB region (Fig. 1). Shared between all Mamu-B*17 haplotypes was a region defined by microsatellites L13-1494 and G09-164763 (Fig. 1 and 2). One microsatellite in this region, H11-9268, showed exceptional linkage to Mamu-B*17. As supported by a microsatellite analysis of 75 Indian rhesus macaques, the microsatellite allele value of 235 for H11-9268 was found only in Mamu-B*17-positive animals ($n = 25$) and not in Mamu-B*17-negative animals ($n = 50$) (data not shown). This suggests a putative location for Mamu-B*17 near the Mamu-B4 gene defined by Daza-Vamenta et al. (6) and provides a robust and simple technology for genotyping Indian rhesus macaques for Mamu-B*17. Additionally, this technology can greatly improve breeding for Mamu-B*17-positive macaques by identifying sires and dams homozygous for Mamu-B*17.

Elite control is not predicted by inherited MHC haplotype. We observed dramatic variation of chronic-phase viral loads in animals possessing identical Mamu-B*17 haplotypes (Table 1). In those haplotypes inherited by at least one SIV controller (haplotypes A through D), the same Mamu-B*17 haplotype was associated with control in some but not all animals, with the exception of haplotype D, where only one animal was available (Table 1). For example, geometric mean plasma virus concentrations from animals possessing haplotype A ranged from 361 to 1,430,000 viral RNA copies/ml (Table 1). When plasma virus concentrations from haplotype A are plotted versus time, dramatic variation is evident (Fig. 3). In animals possessing haplotypes A through C and E, we observed a variation of chronic-phase plasma virus concentrations of at least 2 log units (Table 1 and Fig. 3). None of the five Mamu-B*17-containing haplotypes found in more than one animal were unambiguously associated with SIV control, even though animals were infected with identical virus. Therefore, we conclude that the inheritance of a particular Mamu-B*17-containing 5-Mb MHC haplotype alone is not sufficient to predict SIV disease outcome.

Since entire 5-Mb MHC haplotypes were controlled for, any effect from genes linked to Mamu-B*17 or sequence variants in Mamu-B*17 regulatory elements should be apparent. Our results show that these factors do not explain variation in SIV disease progression in Mamu-B*17-positive animals. Although MHC class I allele differences between animals can influence the CTL repertoire (5, 29), we were able to completely match animals for half of their MHC genes.

We cannot, however, exclude the possibility that genes on the other MHC haplotype modulate the effect of Mamu-B*17. Ideally, this could be examined by studying full siblings who inherit the Mamu-B*17-containing haplotype from one parent and an identical haplotype from their other parent. Using our panel of microsatellite markers, we discovered one full sibling pair that met these criteria. Animal 97035 is completely MHC identical to animal 95096 by descent (data not shown). Animal 97035 was infected with an SIVmac239 variant mutated in the
Mamu-B*17-restricted Nef IW9 epitope and was thus excluded from this study (10). Nonetheless, both 95096 and 97035 controlled SIV for more than 3 years. Expanded studies of MHC-identical full siblings will be required to determine the extent to which sharing of both haplotypes predicts SIV control.

An unresolved question is whether differences in Mamu-B*17-restricted T-cell responses or immune escape from these responses account for the differences in viral load among animals inheriting the same Mamu-B*17 haplotype. In HIV-infected people, the breadth and magnitude of CTL responses

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**TABLE 1.** Animal family, treatment, and outcome details

<table>
<thead>
<tr>
<th>Animal</th>
<th>Haplotype</th>
<th>Potentially confounding treatment*</th>
<th>Survivorship (wk)</th>
<th>Chronic-phase plasma virus Concn (geometric mean)</th>
<th>Nef IW9 CTLd</th>
<th>IW9 plasma sequence (WT, IRYPKTFGW)</th>
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<tr>
<td>90092</td>
<td>A</td>
<td></td>
<td>26a</td>
<td>1,430,000</td>
<td>3,070</td>
<td>...m... (29)</td>
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<tr>
<td>92080</td>
<td>A</td>
<td>MVA intradermally (Gag, Env, Nef, Rev, Tat)</td>
<td>61</td>
<td>108,000</td>
<td>ND</td>
<td>t........ (52)</td>
</tr>
<tr>
<td>95096</td>
<td>A</td>
<td>Lipopeptide GagCM9 epitope</td>
<td>266c</td>
<td>361</td>
<td>105</td>
<td>ND</td>
</tr>
<tr>
<td>98014</td>
<td>A</td>
<td>Irradiated rhesus PBMC and CpG</td>
<td>134</td>
<td>500</td>
<td>966</td>
<td>t........ (23)</td>
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<tr>
<td>96078</td>
<td>B</td>
<td>Whole Nef and Gag</td>
<td>152</td>
<td>85,300</td>
<td>510</td>
<td>P........ (111)</td>
</tr>
<tr>
<td>98016</td>
<td>B</td>
<td>PBS in IFA</td>
<td>116</td>
<td>177</td>
<td>740</td>
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<tr>
<td>95061</td>
<td>C</td>
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<td>208</td>
<td>219</td>
<td>ND</td>
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<tr>
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<td>C</td>
<td></td>
<td>53c</td>
<td>116</td>
<td>1,765</td>
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<tr>
<td>96112</td>
<td>D</td>
<td>CpG in IFA</td>
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<tr>
<td>96072</td>
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<td>67</td>
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<tr>
<td>99026</td>
<td>E</td>
<td>CD4 IgG2</td>
<td>61</td>
<td>44,800</td>
<td>845</td>
<td>P........ (53)</td>
</tr>
<tr>
<td>97044</td>
<td>F</td>
<td>CpG in IFA</td>
<td>128</td>
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<tr>
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<td>G</td>
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<td>71,300</td>
<td>ND</td>
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<tr>
<td>99067</td>
<td>H</td>
<td>Nonspecific IgG2</td>
<td>79</td>
<td>506,000</td>
<td>680</td>
<td>...I... (79)</td>
</tr>
</tbody>
</table>

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* MVA, modified vaccinia virus Ankara; PBMC, peripheral blood mononuclear cells; IFA, incomplete Freund’s adjuvant; PBS, phosphate-buffered saline; IgG, immunoglobulin G.

* Alive as of publication.

* This study was terminated prematurely for reasons unrelated to SIV status.

* Expressed as the number of spot-forming cells per million PBMC. ND, assay not performed.

* WT, wild type. The number of weeks postinfection is given in parentheses. Sequence variation is scored as in reference 10.
restricted by HLA-B*5701 are not markedly different between controllers and progressors, even though HLA-B*5701, like Mamu-B*17, is highly overrepresented among HIV controllers (20). We examined CTL activity against the immunodominant, Mamu-B*17-restricted Nef165-173IW9 epitope (22) by gamma interferon enzyme-linked immunospot in all but four of the animals shown in Table 1. All animals except 97044 mounted Nef IW9 responses, but there was no consistent difference in response magnitude between the progressors and controllers. More subtle quantitative differences in CTL activity might be revealed by studying additional subdominant Mamu-B*17-restricted epitopes (9) and by testing cells obtained at the same time point following infection. The sporadic availability of samples from animals in the current study required assessment of Nef IW9-specific CTL from between 6 and 324 weeks postinfection.

We also analyzed viral escape from Nef IW9 CTL responses in these animals. Though many groups have examined proviral SIV (23) and HIV (1, 20) sequences from individuals who control virus, the relationship between these sequences and replicating virus is questionable (2). We coupled ultrasensitive viral RNA extraction with reverse transcription-PCR using amplification primers flanking the Nef IW9 epitope sequence to extract viral sequence from plasma virus. This approach successfully generated sequence from samples containing fewer than 100 copies of virus per ml of plasma. Chronic-phase sequence variation was pervasive within the Nef IW9 epitope in both SIV controllers and progressors (Table 1). Therefore, SIV control is not likely to be attributable to the unusual preservation of wild-type Nef IW9 epitope sequences.

While plasma virus concentrations from Mamu-B*17-positive infected animals are generally lower than those from Mamu-B*17-negative animals (31), we speculate that loci outside the MHC may explain the stark difference in SIV disease progression among animals possessing the same Mamu-B*17 haplotype. Surveying the genome for additional loci involved in SIV and HIV control is thus an urgent priority. Though resources such as rhesus macaque linkage maps (25) will further the search for other quantitative trait loci associated with SIV resistance, the haplotype diversity among Mamu-B*17-positive controllers suggests that such analyses will not be simple or straightforward. Indeed, comprehensive identification of genes involved in SIV control using functional genomics would likely require hundreds of animals with defined SIV pathogenesis and unprecedented cooperation among the many investigators possessing samples from these animals. Inspiration for this ambitious undertaking can be drawn from other large-scale collaborative efforts recently initiated to answer fundamental questions about HIV and SIV pathogenesis (3, 13, 14).

In all, our results suggest that inheritance of haplotypes containing “protective” MHC class I alleles alone does not predict SIV disease outcome and thus is unlikely the sole factor conferring protection during SIV infection.

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REFERENCES