Chapter C6

THE ROLE OF T CELL EPITOPES IN CORONAVIRUS INFECTION

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Abstract: Multiple MHV-specific CD4 and CD8 T cell epitopes have been identified in C57Bl/6 and BALB/c mice. In particular, at least two CD8 T cell epitopes are recognized in C57Bl/6 mice. In one model of MHV persistence, mutations are detected in the immunodominant CD8 T cell epitope recognized in this strain. These mutations contribute to virus persistence and to the development of more severe clinical disease.

Key words: Coronavirus, CTL escape, demyelination

1. INTRODUCTION

In most viral infections, host defense mechanisms are efficient and result in rapid clearance of the virus. Stable memory populations of T and B cells develop and protect the host from subsequent infections with the same agent. Viruses, in turn, have evolved mechanisms to evade the host immune response and are able to establish long-term persistent infections. Much has been learned over the past several about the strategies that viruses use to evade the immune response [reviewed in (1)]. This review will be focused on one strategy used by viruses to persist, i.e., the selection of cytotoxic CD8 T cell (CTL) escape variants. The first part of this article will discuss aspects of the T cell immune response to MHV, with a focus on CD4 and CD8 T cell epitopes recognized in rodents. Second, CTL escape in the context of mice infected with the neurotropic JHM strain of mouse hepatitis virus (MHV) will be described in detail.
2. CELL-MEDIATED IMMUNITY DURING MHV INFECTION OF RODENTS

The importance of CD4 and CD8 T cells in virus clearance in rodents infected with MHV is well documented, as described elsewhere in this volume (Chapter C4). In several studies, the CD8 and CD4 T cell epitopes recognized by MHV-specific lymphocytes have been identified. C57Bl/6 (B6) and BALB/c mice are used in most studies of MHV-induced pathogenesis and, as a consequence, most of the epitopes are H-2\(^b\)- or H-2\(^d\)-restricted. A summary of the epitopes identified thus far is shown in Table 1. These epitopes were identified using either splenocytes after \textit{in vitro} stimulation or, alternatively, lymphocytes harvested from the CNS of infected mice. In the latter case, cells were analyzed in direct \textit{ex vivo} cytotoxicity assays. In BALB/c mice, a CD8 T cell epitope encompassing residues 318-326 of the nucleocapsid (N) protein (epitope N318), was immunodominant (2) whereas, in B6 mice, two CD8 T cell epitopes encompassing residues 510-518 and 598-605 of the surface (S) protein (epitopes S510 and S598) were recognized (3, 4). Both are located within a hypervariable region of the protein that can tolerate deletion or mutation without loss of viability (5). CD4 T cell epitopes have also been identified in both strains of mice. In BALB/c mice, CD4 T cell epitopes were identified within the N protein whereas in C57BL/6 mice such epitopes were located within the S and transmembrane (M) proteins (6-8). These CD4 T cell epitopes are present outside the hypervariable region of the S protein.

Table 1. Summary of epitopes recognized by CD4 and CD8 T cells in MHV-infected rodents

| Cell type     | Host          | Protein | Residues       |
|---------------|---------------|---------|----------------|
| CD8 T cell\(^2\) | BALB/c mice    | N       | 318-326        |
| CD8 T cell\(^3,4\) | B6 mice       | S       | 510-518        |
| CD8 T cell\(^4\)  | B6 mice       | S       | 598-605        |
| CD4 T cell\(^9\)  | Lewis rat     | N       | 361-458        |
| CD4 T cell\(^7\)  | B6 mice       | M       | 128-147        |
| CD4 T cell\(^8,10\) | B6 mice      | S       | 329-343        |
| CD4 T cell\(^10\) | BALB/c mice   | S       | 329-343        |
| CD4 T cell\(^6\)  | BALB/c mice   | N       | 266-279        |
| CD4 T cell\(^5\)  | B6 mice       | S       | 358-372        |
|                |               | S       | 408-422        |
3. CTL ESCAPE IN MHV-INFECTED MICE

3.1 Overview of selection of CTL escape variants in viral encephalomyelitis

Infection with virulent variants of the JHM strain of MHV cause an acute fatal encephalitis in all susceptible rodents. Disease is largely confined to the central nervous system, with variable, albeit minor, infection of the liver. A persistent infection was noted in several studies when either mice were infected with attenuated strains of virus or when infection with virulent virus was modified by treatment with anti-viral antibodies or T cells (11). In one model, clinical disease manifested by hindlimb paralysis and histological evidence of demyelination developed several weeks after virus inoculation (12). In this model, suckling B6 mice (10 to 14 days old) were inoculated intranasally with a virulent variant of MHV. If nursed by naïve dams, they uniformly developed an acute encephalitis. However, when nursed by dams previously immunized

Table 2. Summary of mutations in epitope S510 identified in B6 mice with hindlimb paralysis.

| Position | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|----------|---|---|---|---|---|---|---|---|---|
| Contact  | *M| M | T | M | T | M/T| M |
| WT epitope | C | S | L | W | N | G | P | H | L |
| Variant epitopes | F(2)\(^A\) R(2) R(7) H(1) V(2) H(2) |
|                  | Y(1) F(3) G(1) D(2) R(1) L(6) |
|                  | P(2) P(2) C(1) S(12) E(1) |
|                  | T(1) |
| (untested)       | K(1) |
|                  | R(1) F(1) |

The immunogenicity of the mutated epitopes was tested in cytotoxicity assays. The amino acid changes listed above resulted in a greater than 20-fold decrement in ability to sensitize cells for CTL lysis, except for the L to P change noted at position 2 (4 fold decrement) (15). Some mutations were not analyzed in these assays (untested). *MHC (M) or TCR (T) contact residues based on published data (16). \(^A\) Numbers in parenthesis represent the number of animals from which the indicated mutations were detected.
to MHV, they were protected from the acute encephalitis. A variable fraction (40-90%) developed clinical disease and a demyelinating encephalomyelitis 3-8 weeks after inoculation. Infectious virus could be isolated from all mice with clinical disease but not from those that remained asymptomatic. Subsequent analysis of viral RNA harvested from the infected CNS revealed the presence of mutations in the immunodominant CD8 T cell epitope (epitope S510) in all samples of RNA and viruses isolated from symptomatic mice (Table 2) (13). These mutations abrogated recognition by epitope S510-specific CD8 T cells in direct \textit{in vivo} cytotoxicity assays using CNS-derived cells. These mutations were detected at early times and mutations were not detected in epitope S598 or in the regions flanking the epitopes (14). Mutations in epitope S510 were not detected in most mice that remained asymptomatic or in persistently infected mice with severe combined immunodeficiency (SCID) (14). These results suggest that CTL escape variants were selected by immune pressure and consequently contributed to the establishment of virus persistence and to the development of a demyelinating encephalomyelitis.

\section*{3.2 Requirements for selection of CTL escape variants in the CNS}

1. Immunodominance. It is conceivable that a CTL response directed against a single immunodominant epitope favored the emergence of virus mutants abrogating CTL recognition. In B6 mice infected with MHV, epitope S510 was immunodominant, with up to 50% of the CD8 T cell response in the CNS directed at this epitope, as determined by either direct \textit{ex vivo} intracellular interferon-\gamma assays or by staining with MHC class I/peptide tetramers (17, 18). The CD8 T cells were fully activated as measured by cytokine secretion, cytotoxic function and expression of activation markers, at least during the acute infection. This strong immune response to the immunodominant epitope is likely to be a major factor facilitating the selection of CTL escape variants. In support of this, mutations in the subdominant CD8 T cell epitope (epitope S598), an epitope with substantially less functional avidity have never been detected. This was true even when suckling mice were infected with virus mutated in epitope S510 so that epitope S598 became the dominant target for the CD8 T cell response (19). In other viral infections, epitopes with strong functional avidity were preferentially mutated at early times after inoculation (20). One apparent exception to this observation is that mutations were never identified in the immunodominant CD4 T cell epitope recognized in B6 mice (epitope
M133-147, Table 1), but mutations were detected in a subdominant CD4 T cell epitope (epitope S328-347, Table 1). However, the biological significance of this mutation is not known since CD4 T cell recognition to the mutated epitope was not affected by this mutation (8).

2. Hypervariable region. It is imperative that the CTL epitope mutations do not inactivate the virus. The two CTL epitopes (epitopes S510 and S598) recognized in B6 mice are located within a region of the protein that is highly permissive to both deletions and amino acid changes without the loss of infectivity (5). Presence of epitopes within this hypervariable region may provide an optimal genetic background for CTL escape, while keeping virus viable. Epitope S598 is also located in this hypervariable region, but mutations in this epitope were not detected. At present, it is not known whether the lack of mutations in epitope S598 reflect an effect on virus viability. It is now possible to address this issue, using recently described methods of reverse genetics (21). Alternatively, there may be insufficient immune pressure to select CTL escape mutations because the epitope has a low functional avidity (4) or because it is expressed relatively poorly on the cell surface. Mutations in epitopes with high functional avidity were detected at early times during the course of other persistent viral infections whereas mutations in epitopes with low functional avidity were not detected until disease had progressed (20, 22, 23).

3. Infection of suckling B6 mice. CTL escape variants were selected only in B6 mice (H-2^b background) inoculated at the suckling stage (10-14 days old) with virulent MHV-JHM and protected from acute encephalitis by nursing with dams previously immunized to MHV. In other models of MHV persistence, immunocompetent adult mice were inoculated with attenuated strains of MHV (11). Virus was cleared under these conditions, but viral RNA and ongoing demyelination were readily detected. CTL escape mutants were never detected in these mice. The host strain is also important in the selection of CTL escape mutants. Suckling BALB/c mice never develop a late onset demyelinating encephalomyelitis. The immunodominant CD8 T cell epitope recognized in BALB/c mice is located in a protein (nucleocapsid \[N\] protein) that is highly conserved among coronaviruses (2, 24). Consistent with the lack of clinical disease, mutations are never detected in this epitope (N318-326).

One explanation for the observation that CTL escape only occurred in suckling B6 mice relates to the role of anti-MHV antibody in pathogenesis. Vertical transmission of maternal neutralizing antibody plays a crucial role not only in providing protection from acute encephalitis but also for establishing a milieu for selection of CTL escape variants. Levels of neutralizing antibodies need to be sufficient to control the acute infection but
insufficient to achieve sterile immunity. Our recent data showed that, as maternal antibody waned, production of anti-MHV antibody by infected suckling B6 mice was substantially lower than by suckling BALB/c mice. Most striking was the lack of anti-MHV antibody producing cells in the CNS of B6 mice infected at the suckling stage (A. Dandekar and SP, unpublished data). Since anti-MHV antibodies provide a crucial role in preventing recrudescence of infectious virus in infected mice (25), lack of locally produced antibody may allow virus to continue to replicate and therefore predispose to the selection of CTL escape mutants. Interestingly, suckling BALB/c mice, which never show evidence of CTL escape, mount a robust humoral immune response in the CNS. Of note, Brown Norway, but not Lewis rats, mount a strong anti-MHV antibody response in the CNS. MHV causes a clinically silent demyelinating encephalomyelitis in Brown Norway rats, whereas Lewis rats develop symptomatic clinical disease, with elevated levels of infectious virus present. These results also suggest an important role for CNS-derived anti-MHV antibody in controlling the infection (26).

4. Monospecific polyclonal epitope S510-specific CTL response. As discussed above, CTL immune pressure focused on an immunodominant CTL epitope may favor selection of CTL escape variants by virtue of the significant loss of immune recognition when the epitope is mutated. However, this phenomenon may be less likely if CD8 T cells specific to the epitope are polyclonal (27). To determine the diversity of the epitope S510-specific T cell response, TCR Vβ element usage and the complexity of the complementary determining region 3 (CDR3) were determined. Epitope S510-specific CD8 T cells were obtained from the CNS of acutely and chronically infected mice by staining with peptide S510-loaded MHC class I tetramers and FACS analysis. A large number of different Vβ elements were detected within the epitope S510-specific population. Furthermore, analysis of CDR3 regions from this population revealed the presence of 400-600 different clonotypes per infected mouse, with many sequences common in all animals (17). This polyclonal response was not protective against the CTL escape, however, since it was functionally monospecific, with a strong focus on the center of the MHC class I/peptide complex (15).

5. Anatomical location of persistent virus. Since the CNS is a site of immune privilege, CTL escape mutants, once selected, may be able to replicate more efficiently in an environment with a less than optimal anti-MHV immune response. This may contribute to high level virus persistence, and eventually, to the development of clinical disease.
4. BIOLOGICAL SIGNIFICANCE OF CTL ESCAPE VARIANTS IN MHV-INFECTED MICE

Accumulating data suggest that evasion of the immune response by selection of CTL escape mutants occurs in several persistent infections, including humans infected with HIV, hepatitis B virus or hepatitis C virus and primates infected with SIV or hepatitis C virus (20, 28-30). A causal relationship between the presence of CTL escape variants and clinical outcomes is difficult to establish in infected humans or primates. Thus, the maternal antibody protection model described above is useful for determining the role of these variants in virus pathogenesis.

Pewe et al. (19) showed that escape from the CTL response was a major factor in disease progression in MHV-infected mice. Infection of suckling mice with CTL escape mutant virus resulted in higher mortality and morbidity than observed in mice infected with wild type virus. Furthermore, sequencing of infectious virus isolated from chronically infected mice revealed a lack of heterogeneity among isolates from a single animal. These data indicated that viral variants were selected during early infection and, once selected, they were fixed permanently, possibly due to continuous positive selection by CTL, ultimately leading to clinical disease.

5. CONCLUDING REMARKS

CTL escape has been demonstrated in several persistent infections. MHV-infected mice provide an excellent model for determining the role of these phenomena in viral pathogenesis. The results thus far show that CTL escape variants have a selective advantage and enhance clinical disease and demyelination. Although a specific virus has not been identified as an etiological agent in patients with MS, the development of CTL escape mutants provides a plausible mechanism for how a persistent, virus infection controlled by the immune system could progress to cause more significant disease. Further efforts will be directed at determining the mechanism of CTL escape and to determine why CTL escape has been identified in only a fraction of persistent viral infections.

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C6. THE ROLE OF T CELL EPITOPES IN CORONAVIRUS INFECTION

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