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ROLE OF CTL MUTANTS IN DEMYELINATION INDUCED BY MOUSE HEPATITIS VIRUS, STRAIN JHM

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1. ABSTRACT

Mouse hepatitis virus, strain JHM (MHV-JHM) is a well described cause of demyelination. C57Bl/6 (B6) mice infected at the suckling stage in the presence of protective antibodies remain asymptomatic initially but later develop clinical disease (hindlimb paralysis). Infectious virus can be isolated from these mice. Recently, two MHV-specific target epitopes for cytotoxic CD8 T cells have been identified in B6 mice. Our results show that in all mice with hindlimb paralysis, mutations can be detected in the RNA encoding the immunodominant of the two epitopes. These mutations result in a loss of recognition by MHV-specific cytotoxic T cells. These changes are not detected, for the most part, in mice that remain asymptomatic nor in mice with acute encephalitis. These results suggest that the development of CTL escape mutants is necessary for hindlimb paralysis to develop in this model.

2. INTRODUCTION

MHV-JHM causes acute encephalitis and acute and chronic demyelination in susceptible strains of mice and rats. Most strains of mice are susceptible to the virus and mice of all ages develop an acute fatal encephalitis after intranasal or intracerebral inoculation. This disease can be prevented if mice are infected with attenuated strains of virus or if they are protected by infusions of antiviral antibodies or T cells. Under most circumstances, animals are most ill for a few days after inoculation of virus and the highest mor-

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tality is recorded in this period. Evidence of demyelination can be detected in mice that survive the acute infection (Kyuwa and Stohlman, 1990; Houtman and Fleming, 1996; Lane and Buchmeier, 1997).

Over the past few years, a different version of this basic model has been studied in our laboratory. In this model, suckling B6 mice are inoculated intranasally with MHV-JHM. In the absence of any therapeutic interventions, all mice succumb to an acute encephalitis. This fatal disease can be prevented however, if mice are nursed by dams that have been previously immunized to MHV-JHM. The mice remain asymptomatic for several weeks, but 40–90% then develop clinical disease manifested by hindlimb paralysis. Infectious virus can be isolated from mice with clinical disease but not from mice that remain asymptomatic whereas viral antigen can be detected in all mice. In marked contrast to these results, suckling BALB/c mice similarly treated remain asymptomatic once protected from acute encephalitis (Perlman, et al., 1987; Castro, et al., 1994).

Recently, MHV-specific CD8 T cell targets have been identified in B6 and BALB/c mice (Bergmann, et al., 1993; Castro and Perlman, 1995; Bergmann, et al., 1996). The majority of MHV-specific CD8 T cells recognize the nucleocapsid (N) protein in BALB/c mice whereas the surface (S) glycoprotein is recognized by CD8 T cells harvested from B6 mice. Further analyses in B6 mice revealed the presence of two epitopes encompassing residues 510 to 518 (S-510–518) and 598–605 (S-598–605). S-510–518 is the more immunodominant of the two epitopes. The S protein contains a hypervariable region that appears to be readily deleted without loss of viability (Parker, et al., 1989) and both epitopes are located in this region of the S protein. In contrast, the N protein is highly conserved among the various species of MHV and the epitope is located in a region not prone to variability.

Mutations in CD8 T cell epitopes that lead to a loss of recognition by virus-specific T cells have been identified in patients infected with hepatitis B virus, human immunodeficiency virus, Epstein-Barr virus and hepatitis C and in mice infected with lymphocytic choriomeningitis virus (LCMV) (Koup, 1994; Franco, et al., 1995; Zinkernagel, 1996). In all cases, CTL escape mutants appear to arise most commonly and to be most important when the CTL response is nearly monospecific and strong. Since B6 mice infected with MHV-JHM mount such a response and the response is directed at an epitope located in a region prone to sequence variability, we postulated that CTL mutations in epitope S-510–518 might develop during the course of the infection and contribute to the development of hindlimb paralysis in mice that were initially well and later developed disease.

3. MATERIALS AND METHODS

The materials and experimental methods used in these experiments were previously described (Perlman, et al., 1987; Pewe, et al., 1996).

4. RESULTS

To determine if CTL escape mutants could be detected in mice that developed hindlimb paralysis several weeks after infection, infectious virus was isolated from these mice and propagated a minimal number of times in tissue culture cells so that sufficient material was available for analysis. The results of these initial analyses showed that in every case, mutations were detected in epitope S-510–518. The sequence of this epitope is

CSLWNGPHL and mutations were detected in residues 2 to 7 of the epitope. In each mouse, a single mutation was identified in virus harvested from infected CNS tissue (Pewe, et al., 1996) and different muttions were detected in different mice.

In previous studies, passage through tissue culture cells was shown to select for a subpopulation of viruses initially present in the infected host (Meyerhans, et al., 1989). To determine if this type of selection occurred in our experiments, RNA was isolated from the spinal cords and brains of chronically infected mice and the sequence of epitope S-510–518 determined. As before, mutations in epitope S-510–518 were detected in all samples (Pewe, et al., 1996). Additionally, cDNA clonal analysis was performed to determine if there was any heterogeneity in the sequence of epitope S-510–518 not easily detected by bulk analysis. In some samples, more than one mutation was present. Occasionally, a subpopulation of wild type sequence could also be detected. As controls for these experiments, the following additional samples were analyzed:

1. In mice with acute encephalitis, no changes in epitope S-510-518 were detected (Pewe, et al., 1996).
2. Mice destined to develop hindlimb paralysis do so by 80 days p.i. and the vast majority of mice develop disease by 60 days. Analysis of RNA harvested from the spinal cords of these mice revealed changes in epitope S-510–518 in one case and large deletions which included the epitope in 3/14 samples. Only wild type sequence was detected in 10/14 samples which were analyzed (Pewe, et al., 1996; Pewe, et al., in press).
3. CD8 T cells are not present in SCID mice, but mice readily become persistently infected with MHV-JHM. No changes in epitope S-510–518 were detected in these mice (Pewe, et al., in press).
4. S-510–518 is the immunodominant CTL epitope (Castro and Perlman, 1995). No changes were detected in the subdominant T cell epitope S-598–605. In addition, a major CD4 T cell epitope was identified in the M protein (M-135–143) and at least three CD4 T cell epitopes are present in the S glycoprotein (Heemskerk, et al., 1995; Xue, et al., 1995, unpublished observations). Mutations were not detected in the M-specific epitope nor in two of the three S-specific epitopes. In the third S-specific epitope, located in the region encompassing amino acids 328–347, a change was noted in a minority of cDNA sequenced from mice with hindlimb paralysis. This mutation, resulting in a change from alanine to threonine at position 337 did not appear to affect recognition by MHV-specific CD4 T cells in proliferation assays.
5. If variants containing mutations in epitope S-510–518 contribute to the pathogenesis of the chronic demyelinating encephalomyelitis, they should arise at early times in the infectious cycle before virus would normally be cleared. MHV is usually cleared by 12–21 days p.i. (Kyuwa and Stohlman, 1990; Houtman and Fleming, 1996; Lane and Buchmeier, 1997). Changes in epitope S-510–518 can be detected as early as 10–12 days p.i. and comprise the majority of the viral RNA detected in some mice at 15 days p.i. These data are consistent with previous analyses performed in mice infected with LCMV (Pircher, et al., 1990; Weidt, et al., 1995).
6. To show that the changes that we detected affect recognition by MHV-specific CD8 T cells, variant peptides were analyzed in direct ex vivo cytotoxicity assays using lymphocytes harvested from the CNS of mice with acute encephalitis. Mutations in positions 3, 4, 5, 6 and 7 were analyzed in these experiments. In

each case, the mutations resulted in a complete or major loss in recognition by the CNS-derived lymphocytes consistent with their role in the process of virus persistence and resultant demyelination (Pewe, et al., 1996).

Of note, mice inoculated with virus containing mutated epitope S-510-518 are unable to clear the virus and usually die by 20–25 d p.i. Extensive demyelination and large amounts of viral antigen are detected in the CNS of these mice (unpublished observations).

A summary of the changes that we have detected thus far in epitope S-510–518 is shown in the Table 1.

5. DISCUSSION

Cytotoxic T cells play a major role in controlling viral infections. CTL escape mutants have been identified in some persistent human viral infections as well as in mice infected with LCMV. The role of CTL escape mutants has been questioned, however, since in general, the CTL response to a pathogen is polyclonal and polyspecific (Franco, et al., 1995). Changes in a single epitope would not be expected, under these circumstances, to result in a loss of CTL recognition. However, CTL escape mutants would be expected to be important if the CTL response is functionally monospecific and oligoclonal so that a change in the epitope would abrogate recognition by all or a majority of CTLs.

This scenario occurs in B6 mice infected with MHV-JHM. The CTL response in these mice is important for virus clearance and is directed primarily to a single epitope present in a region of the S glycoprotein previously determined to be hypervariable. Mutations in this epitope arise readily and early in the infection and do not appear to affect virulence. They affect recognition by lymphocytes harvested from the CNS, the site of inflammation and would be expected to contribute to virus persistence. An increase in virus load directly contributes to an increase in clinical disease and histological evidence of demyelination. Clinical disease may result from direct viral lysis of glial cells or more likely, from the host response to the virus. Our results suggest that CTL escape mutants are necessary, but not sufficient, for these processes to occur.

Table 1. Summary of changes identified in epitopes S-510-518 (number)

Wild type:	CSLWNGPHL	Position 5:	CSLW*S*GPHL (10)
			CSLW*T*GPHL (1)
Position 2:	C*Y*LWNGPHL (1)		CSLW*H*GPHL (1)
	C*F*LWNGPHL (2)		CSLW*Y*GPHL (1)
	C*P*LWNGPHL (1)		CSLW*D*GPHL (1)
Position 3:	CS*R*WNGPHL (2)	Position 6:	CSLWN*V*PHL (2)
	CS*P*WNGPHL (2)		CSLWN*R*PHL (1)
	CS*F*WNGPHL (1)		
		Position 7:	CSLWNG*L*HL (5)
Position 4:	CSL*R*NGPHL (3)		CSLWNG*H*HL (2)
Deletions:	C*F*WNGPHL (5)	Entire epitope deleted (7)	
	CSWNGPHL (1)		
	CSLNGPHL (2)		

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