JOURNAL OF VIROLOGY, Jan. 1997, p. 797–799 Vol. 71, No. 1
0022-538X/97/$04.00+0

In Vivo Administration of Interleukin-2 Protects Susceptible Mice from Theiler's Virus Persistence

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Received 12 July 1996/Accepted 17 September 1996

In vivo administration of interleukin-2 (IL-2)-secreting tumor cells results in complete protection against persistent infection by Theiler's murine encephalomyelitis virus (TMEV) in susceptible DBA/2 mice. The IL-2-mediated protection was found to depend on the inoculum size as well as the timing of IL-2 administration. IL-2-treated and TMEV-infected mice displayed a three- to fourfold relative increase in virus-specific cytotoxic T-lymphocyte (CTL) precursors. Thus, we postulate that the persistence of TMEV infection in susceptible mice reflects limited numbers of relevant CTL precursors and their time course of induction and activation.

Theiler's murine encephalomyelitis virus (TMEV), a picornavirus, causes in susceptible mouse strains a chronic demyelinating disease that closely resembles multiple sclerosis. The DA strain of TMEV is responsible for a biphasic disease of the central nervous system (13). Upon intracranial injection, the DA virus initially infects the neurons and causes an acute grey-matter encephalomyelitis. After a few weeks, the virus migrates to the white matter of the spinal cord, where it causes chronic inflammation and primary demyelination. The development of demyelinating lesions is thought to be the result of both direct viral damage and an immunopathological virus-specific delayed-type hypersensitivity reaction mediated by $CD4^+$ Th-1 cells (3, 4). In the early phase of the disease, both humoral and cell-mediated immunity have been reported to be required for virus clearance. Thus, the humoral response is supposed to play a role in limiting early viral infection, although significantly lower concentrations of anti-TMEV antibodies have been found in resistant strains compared with susceptible strains of mice (7, 16). $CD4^+$ T cells also contribute to protection at the early stage by providing help for induction of anti-TMEV antibody production (14).

The crucial role of class I restricted $CD8^+$ T cells in clearing TMEV infection in resistant mice is well established. Thus, β_2-microglobulin-deficient mice on a resistant *H-2*^{b} background, lacking stable major histocompatibility complex class I molecules and functional $CD8^+$ T cells, are susceptible to TMEV infection (5, 17, 19). The fact that susceptible FVB (*H-2*^{q}) mice transgenic for the *H-2D*^{b} gene (1) or B10.Q (*H-2*^{q})and B10.S (*H-2*^{S}) mice transgenic for the *H-2D*^{d} gene (18) are able to clear TMEV infection also emphasizes the importance of a class I-restricted response for controlling the virus. It has been reported that $CD8^+$ cytotoxic T lymphocytes (CTLs) are present in both susceptible and resistant mouse strains (12, 15, 20).

One possible explanation for viral persistence in susceptible mice is that they possess insufficient numbers of TMEV-specific CTL precursors and are therefore unable to mount an early, efficient CTL response for clearing the virus. The aim of the present study was to determine whether in vivo administration of interleukin-2 (IL-2)-secreting tumor cells, known to efficiently induce and increase the frequencies of specific CTL precursors in vivo (10), would prevent the persistence of TMEV infection.

Therefore, susceptible DBA/2 mice were inoculated intracerebrally with 10^5 PFU of the persistent DA strain of TMEV. In addition to DA virus, some mice received either 5×10^6 to 10×10^6 irradiated (10,000 rads) IL-2-transfected P815 cells (P815-IL-2) or P815 wild-type cells (P815wt) 7 days prior to the inoculation, on the day of inoculation, and 7 days postinoculation. The mice were sacrificed 45 days postinoculation. The spinal cords were isolated, and a previously described quantitative dot blot hybridization assay (2) was used to determine the levels of viral RNA. As shown in Fig. 1, the DA virus persisted in DBA/2 mice receiving virus alone or virus and P815wt cells, while mice infected with DA virus and treated with P815-IL-2 cells completely eliminated the virus.

We also tested whether the IL-2-mediated protection was dependent on the viral dose. Thus, DBA/2 mice were treated with P815-IL-2 as described above and infected with 1×10^5 or 5×10^5 PFU of DA virus. All mice infected with the lower dose of DA virus were protected (i.e., nine of nine mice), while only one of five mice infected with the higher dose was able to clear the virus (data not shown). Thus, by increasing the viral load, the protective effect of in vivo administration of IL-2 is abolished.

If the IL-2-mediated protection against persistent infection is due to induction of specific CTL precursors, one might expect that the timing of P815-IL-2 administration would also be important for eliminating the virus. The timing of IL-2 treatment did play a role, since IL-2 administration 14 days after infection with DA did not result in elimination of the virus (Fig. 2), suggesting that an early recruitment of sufficient numbers of specific CTLs is necessary for clearing the DA virus in susceptible mice.

As noted above, injection of P815-IL-2 cells into syngeneic recipients efficiently increased the number of specific CTL precursors in vivo (9). Spleen cells from DBA/2 mice infected with DA only, from mice infected with DA and treated with P815wt, and from mice infected with DA and treated with P815-IL-2 were isolated 45 days postinfection and restimulated at 2×10^6 cells/ml for 5 days in vitro with syngeneic DA-infected (3 PFU/ml) and irradiated (2,000 rads) spleen cells at

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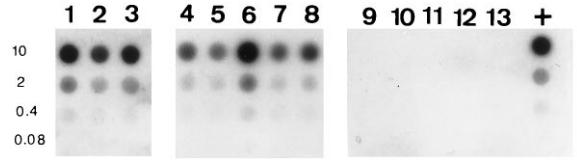

FIG. 1. Dot blot hybridization analyses of spinal cord RNA (10 to 0.8 μg) obtained 45 days postinfection from DBA/2 mice inoculated with 10^5 PFU of DA and treated with P815wt cells (lanes 4 to 8), P815-IL-2 cells (lanes 9 to 13), or untreated (lanes 1 to 3). +, result obtained with RNA from a control susceptible SJL/J mouse. The integrity of RNA was demonstrated by hybridizing the same RNA samples with a β-actin probe (data not shown).

1×10^6/ml. Proliferating cells were subsequently used as effector cells in a ^{51}Cr-release assay. Prior to assay, the targets were labeled with 200 μCi of $Na^{51}CrO_4$ for 90 min, washed three times, distributed in 96-well flat-bottom plates, and incubated overnight at 37°C. Two to 4 h before addition of the various effector cells, the target cells were infected with 20 PFU of DA virus. After a 4-h incubation of effectors and targets, the specific ^{51}Cr release was determined. As shown in Fig. 3, an increased CTL response, on DA-infected D 2.1 (*H-2*^{d}) fibroblasts (22) was observed in DBA/2 mice infected with DA and treated with P815-IL-2 compared with DBA/2 mice receiving DA only or DA and P815wt cells, although the latter two groups displayed a certain degree of anti-DA-specific CTL activity. One of the loci controlling viral persistence is the *H-2D*^{b} gene (1). Because the *D*^{b} and *L*^{d} molecules are structurally very similar (21), we also assayed for CTL activity on DA-infected *L*^{d}-expressing and *D*^{d}-expressing target cells (6). An increased DA-specific, *L*^{d}-restricted CTL response had indeed been generated in the DA- and P815-IL-2-treated group of mice (Fig. 3). No increase in DA-specific *H-2D*^{d}-restricted CTLs was observed. This pattern of CTL response was highly reproducible. The different target cells expressed similar levels of major histocompatibility complex class I molecules (data not shown).

To determine whether increased numbers of DA-specific CTL precursors had been induced by IL-2 administration, limiting dilution analyses were performed as previously described in detail (8). In brief, the indicated numbers of spleen cells from the various groups of DBA/2 mice were cultured in 24 replicates with syngeneic, DA-infected spleen cells (3×10^5/ml), which had been irradiated 3 h after infection. Seven days later, each well was assayed for cytolytic activity as described above. Cultures were considered positive when ^{51}Cr release was 3 standard deviations above the mean value of ^{51}Cr release

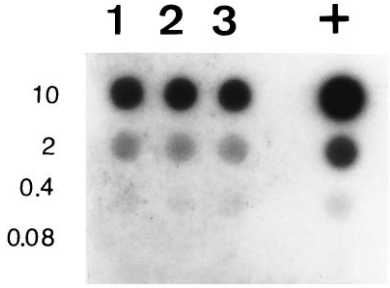

FIG. 2. Dot blot hybridization analyses of spinal cord RNA (10 to 0.8 μg) obtained 45 days postinfection from DBA/2 mice infected with 10^5 PFU of DA and treated with P815-IL-2 cells 14 days later (lanes 1 to 3). +, result obtained with RNA from a control susceptible SJL/J mouse. RNA integrity was controlled as described in the legend to Fig. 1.

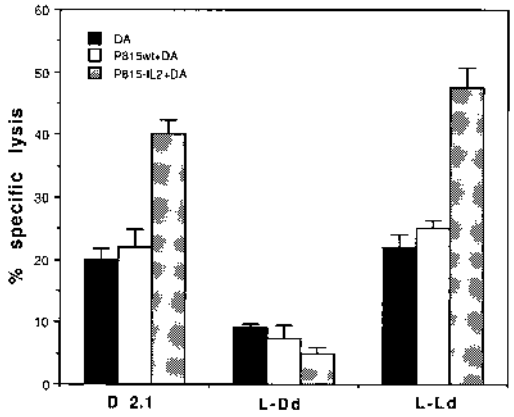

FIG. 3. Anti-DA-specific CTL responses from DBA/2 mice infected with 10^5 PFU of DA, 10^5 PFU of DA and P815wt cells, or 10^5 PFU of DA and P815-IL-2 cells. Spleen cells from the different groups of mice were isolated 45 days postinfection and stimulated for 5 days in vitro with syngeneic DA-infected and irradiated spleen cells. The various effector cells were used in a ^{51}Cr-release assay on *H-2*-compatible (D 2.1) targets and on *L-D*^{d}- and *L-L*^{d}-transfected fibroblasts infected with DA. Specific lysis at an effector/target ratio of 30:1 is presented.

obtained from cultures without responder cells. The frequencies were determined by the zero-order term of the Poisson distribution, and the lines were fitted by the least-squares method as described previously (8). A representative experiment is shown in Fig. 4. Thus, DBA/2 mice infected with DA virus had virtually undetectable numbers of DA-specific CTLs. In DA-infected and P815wt-treated mice, a low number of specific CTL precursors was observed (around 1 in 20,000), while DA-infected and P815-IL-2-treated DBA/2 mice showed a frequency of about 1 in 6,000 anti-DA-specific CTLs. Although the actual frequencies varied somewhat between different experiments, the relative increase in CTL precursors in the latter group remained stable. The amount of viral RNA in the spinal cord of mice (three mice per group) was determined in parallel. Similar levels of virus were present in the DA-infected and DA-infected plus P815wt-treated groups of mice, whereas the DA-infected plus P815-IL-2-treated group had completely eliminated the virus, as exemplified in Fig. 1 (data not shown). Thus, a three- to fourfold relative increase in DA-specific CTL precursors correlates with a complete eradication of the normally persistent DA infection in DBA/2 mice.

Taken together, our results suggest that early induction and

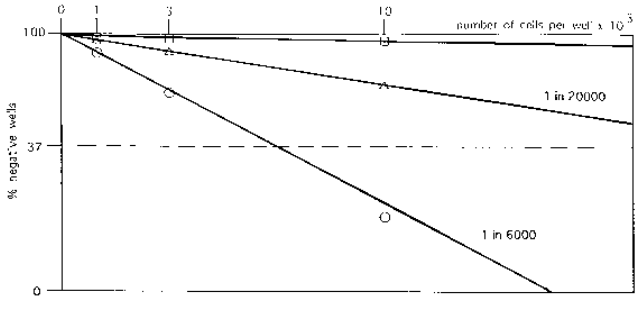

FIG. 4. Frequency determination of DA-specific CTL precursors from DBA/2 mice infected with 10^5 PFU of DA (□), 10^5 PFU of DA and P815wt cells (△), or 10^5 PFU of DA and P815-IL-2 cells (○). The indicated numbers of responder cells were cultured in 24 replicates with syngeneic, DA-infected and irradiated spleen cells for 7 days and subsequently assayed for cytolytic activity. DA-infected and ^{51}Cr-labeled D 2.1 fibroblasts were used as target cells.

recruitment of sufficient numbers of DA-specific CTLs are necessary for clearing the virus before it reaches immunoprivileged sites and causes chronic infection. The observation that $CD8^+$ T cells are detected earlier in the central nervous system of resistant mice (11) supports the idea that the magnitude of the early CTL response is important for disease evolution. The fact that DBA/2 mice can be protected also indicates that the class I-restricted viral epitopes with a protective potential are presented in these mice and that the T-cell repertoire contains the relevant specificities.

We thank M. Tuneskog for excellent technical help and M. Gau for preparing the manuscript.

This work was supported by grants from the Centre National de la Recherche Scientifique, the Institut Pasteur Fondation, and the National Multiple Sclerosis Society. S. Dethlefs was supported by a fellowship from the Verband der Chemischen Industrie/Frankfurt.

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