

Generation of Antiviral CD11a^{high} T Cells in CD4⁺ T Cell-Depleted Mice and Adult Thymectomized Mice after Mouse Hepatitis Virus Infection

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ABSTRACT. The mechanism of CD11a^{high}CD8⁺ T cell induction after mouse hepatitis virus infection, which has been suggested to play a key role in the elimination of infectious virus from the spleen in C57BL/6 mice, was studied. In CD4⁺ T cell-depleted mice, CD11a^{high}CD8⁺ T cells were induced in the spleen and spleen cells showed virus-specific cytotoxic T lymphocyte activity after mouse hepatitis virus infection. The same results were obtained in adult thymectomized mice. These results indicate that CD11a^{high}CD8⁺ T cells can be generated after mouse hepatitis virus infection in the absence of either CD4⁺ T cells or the thymus. — **KEY WORDS:** CD11a, mouse hepatitis virus, thymus.

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T cell-mediated immunity has been suggested to play an important role in mouse hepatitis virus (MHV) infection in mice [4]. Recently, we characterized T cells expanded in the spleen in C57BL/6 (B6) mice during infection and suggest that CD11a^{high} T cells play a pivotal role in viral clearance *in vivo* and mediate virus-specific cytotoxic T lymphocyte (CTL) activity *in vitro* [6], but the mechanism of MHV-specific CTL generation *in vivo* has not been fully studied. In the present study, we examined the spleen viral titer, induction of CD11a^{high}CD8⁺ T cells in the spleen and virus-specific CTL activity after MHV infection in mice depleted of CD4⁺ T cells and adult thymectomized (ATx) mice.

Specific pathogen-free B6 mice at 6 to 8 weeks of age were purchased from SLC, Hamamatsu, Japan. The DL variant of JHM virus (JHMV) was propagated and plaque assayed on DBT cells as described previously [6]. Mice were infected intraperitoneally (ip) with 1×10^6 plaque-forming units (PFU) of JHMV. Depletion of CD4⁺ T cells *in vivo* was carried out as previously described [6]. Briefly, B6 mice were injected with 200 μ g of purified anti-CD4 (GK1.5) [2] monoclonal antibodies (mAb) three times per week from 2 days prior to infection during the experimental periods. Flow cytometric analysis was performed as previously described [6]. Virus-specific CTL activity was measured by using JHMV-infected IC-21 cells (H-2^b) as targets as previously described [6]. Six week-old B6 mice were thymectomized or sham-operated and used 3 weeks later.

Spleen viral titer, induction of CD11a^{high} T cells and virus-specific CTL activity in mice depleted of CD4⁺ T cells after JHMV infection was examined. Three B6 mice were injected with anti-CD4 mAb and infected ip with JHMV, and the induction of CD11a^{high}CD8⁺ T cells was examined by flow cytometry (Fig. 1). The same number of uninfected B6 mice injected with PBS or anti-CD4 mAb,

and those injected with PBS and infected ip with JHMV were used as controls. In anti-CD4 mAb-treated B6 mice, CD4⁺ T cells were barely detected in the spleen (<1%) (data not shown). Two-color analysis with anti-CD11a and anti-CD8 mAb showed that CD11a^{high}CD8⁺ T cells were induced in the mice after JHMV infection (Fig. 1a). B6 mice injected with anti-CD4 mAb were infected with JHMV and the spleen viral titer was determined. The viral clearance rate in the spleen was almost the same as that in mice which had not received anti-CD4 mAb (data not shown) [6]. Fresh spleen cells from anti-CD4 mAb-treated mice were collected at 7 days pi, and their cytotoxic activity against JHMV-infected and uninfected IC-21 cells was measured. A weak virus-specific CTL activity, but almost the same as that in untreated mice [6] was observed in CD4⁺ T cell-depleted mice (Fig. 1b).

The spleen viral titer, induction of CD11a^{high} T cells and virus-specific CTL activity after JHMV infection in ATx mice was examined. A total of nine ATx mice were infected ip with JHMV. The spleens were removed from three mice at 3, 5 and 7 days pi, respectively, and the viral titer was determined by plaque assay. Sham-operated mice were used as control. JHMV was cleared from the spleen in ATx mice similarly to in sham-operated mice (Fig. 2a). After JHMV infection, T cells were expanded in the spleen in both ATx and sham-operated mice (data not shown). Induction of CD11a^{high}CD8⁺ T cells in ATx mice was examined by flow cytometry as described above. Similar percentage of CD8⁺ T cells in the spleen from both ATx and sham-operated mice expressed higher amounts of CD11a at 7 days pi (Fig. 2b). Fresh spleen cells from ATx mice infected ip with JHMV 7 days before had a cytotoxic activity against JHMV-infected IC-21 cells but not uninfected cells (data not shown).

The requirement of CD4⁺ T cells in the generation of antigen-specific CD8⁺ CTL is intriguing. There are some reports that describe CD4⁺ T cell-dependent generation of virus-specific CD8⁺ CTL [3, 8], but others, including this report, show CD4⁺ T cell-independent generation of virus-specific CTL [7, 9]. The affinity between the T cell and the

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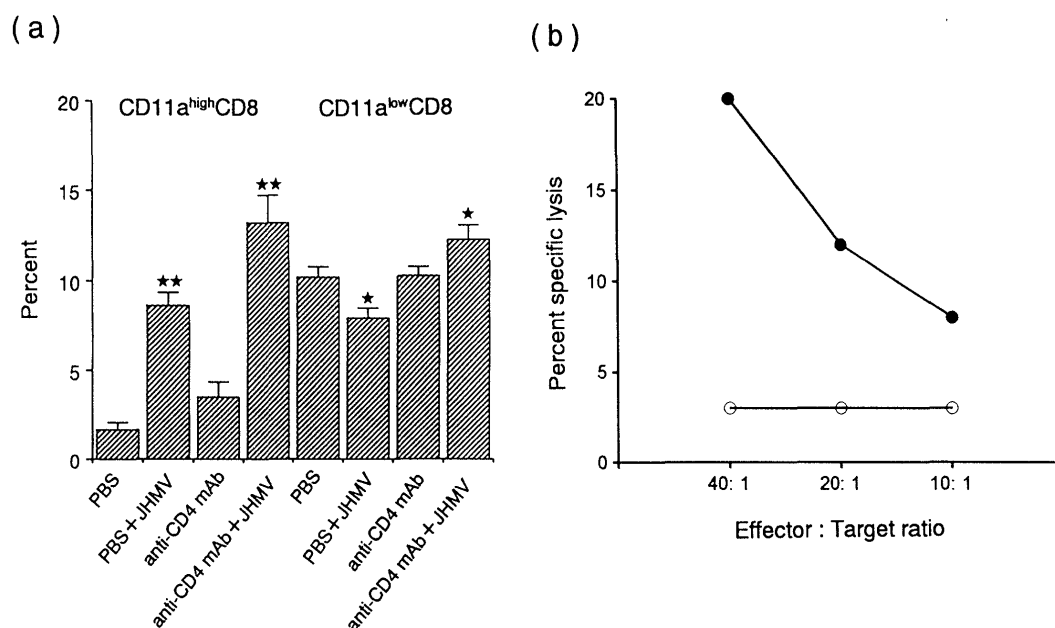


Fig. 1. Induction of CD11a^{high}CD8⁺ T cells and antiviral CTL activity in mice depleted of CD4⁺ T cells. B6 mice were injected with anti-CD4 mAb and infected ip with JHMV. One week later, spleen cells were incubated with FITC-conjugated anti-CD8 mAb and biotin-conjugated anti-CD11a mAb followed with R-phycoerythrin-conjugated streptavidin, and then analyzed by flow cytometry. Mean percentage of CD11a^{high}CD8⁺ and CD11a^{low}CD8⁺ T cells from three mice is depicted. Error bars indicate standard deviations. Values are significantly different from those in uninfected control mice are indicated by asterisks (★, $p < 0.05$; ★★, $p < 0.01$) (a). Cytotoxic activity of spleen cells from CD4⁺ T cell-depleted mice against JHMV-infected (●) and uninfected (○) IC-21 cells is shown. Similar results were obtained in two separate experiments (b).

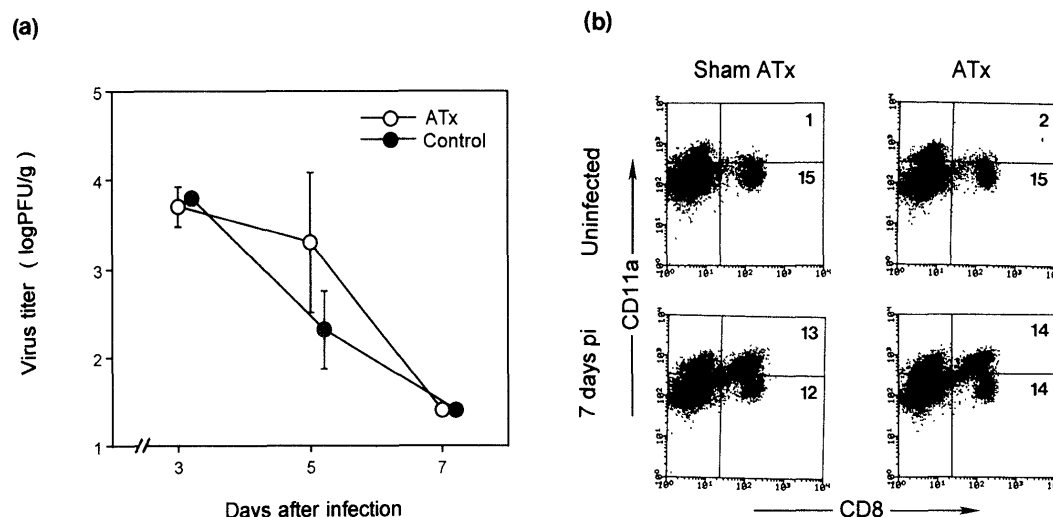


Fig. 2. Induction of CD11a^{high}CD8⁺ T cells and viral clearance after JHMV infection in ATx mice. ATx and sham-operated (control) mice were infected ip with JHMV. Viral growth in the spleens was determined by plaque assay. Mean \pm standard deviation for three spleens (a). Spleens were removed from uninfected ATx and sham-operated mice and from those infected with JHMV at 7 days pi. Spleen cells were incubated with FITC-conjugated anti-CD8 mAb and biotin-conjugated anti-CD11a mAb followed with R-phycoerythrin-conjugated streptavidin, and analyzed by flow cytometry. Representative dot plots of three mice are shown with the percentages for the second and fourth quadrants. Similar results were obtained in a separate experiment (b).

target cell may be critical, because it appears to depend on the antigen.

The thymus is the central lymphoid organ where T cells

differentiate and undergo negative and positive selection. It was reported in recent papers that antigen presentation in the thymus influences the T cell repertoire [1, 8]. Although

JHNV was replicated in the thymus after ip infection (data not shown), there was no obvious difference between the immune responses to JHNV in ATx and sham-operated mice. The results suggest that precursors of JHNV-specific T cells have been generated before viral infection, and expand and develop into effectors in the peripheral lymphoid tissues after viral infection. Alternatively, an insufficient antigen presentation in the thymus due to a decreased expression of the major histocompatibility complex antigens by JHNV infection [5] may result in malfunction of the thymus during JHNV infection.

In our previous study we suggested that CD11a^{high} T cells play a pivotal role in viral clearance *in vivo* and mediate virus-specific CTL activity *in vitro*, since they are temporally correlated [6]. In the present study we showed that CD11a^{high}CD8⁺ T cells was induced in mice depleted of CD4⁺ T cells and ATx mice after JHNV infection. In these mice functional viral clearance *in vivo* and virus-specific CTL activity *in vitro* were also induced. Although a more direct evidence is needed to prove the hypothesis described above, the present results also support it.

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