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PRIMARY MURINE CORONAVIRUS INFECTION IN MICE

A Flow Cytometric Analysis

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T cell- mediated immune responses play a pivotal role in both viral clearance and immunopathology in mice infected with murine coronavirus, strain JHM (JHMV)[1,2]. In the present study, we attempted to characterize T cells induced during primary JHMV infection by flow cytometric analysis.

Female, 6 to 8 week old C57BL/6 (B6) mice were infected intraperitonealy with 10^6 PFU of JHMV. Although JHMV replicated for the first 3 days but was eliminated from spleens of B6 mice at 7 days postinfection (pi). Flow cytometric analysis was caried out to characterize spleen cells from JHMV-infected B6 mice.[3] Most drastic changes were noted as an increased number of $CD8^+$ T cells and their decreased CD8 intensity at 7 days pi. Time course study showed that intensity of αβ T cell receptors declined with the CD8 intensity, while intensity of the lymphocyte function antigen-1 (LFA-1) and CD43 on $CD8^+$ T cells increased. Two-color analysis demonstrated that $CD8^{dull}LFA\text{-}1^{bright}$ T cells were induced transiently in both C57BL/6 and BALB/c mice following JHMV infection (Figure 1). At 7 days pi a half of $CD8^+$ T cells were partitioned into $CD8^{dull}LFA\text{-}1^{bright}$ T cells. Forward and side scatter profiles of $CD8^{dull}LFA\text{-}1^{bright}$ T cells indicated that the population appeared to be activated T cells. Although $CD45RB^{dull}CD8^+$ and $CD44^{bright}CD8^+$ T cells were observed in JHMV-infected mice, expansion of $CD25^+CD8^+$ and $CD11b^+CD8^+$ T cells, which were reported as markers of cytotoxic T lymphocytes in choriomeningitis virus infection in mice[4,5], was not observed. Since the kinetics of the expansion of $CD8^{dull}LFA\text{-}1^{bright}$ T cells was correlated with the viral elimination *in vivo*, we measured fresh cytotoxic activities of spleen cells from JHMV-infected B6 mice against syngeneic JHMV-infected macrophage-like cell line (IC-21 cells). The Ig^- splenocytes from mice 7 days pi but neither those from uninfected or 14 days pi showed a weak, but significant cytotoxic activity against JHMV-infected H-2-matched cells *in vitro*. Therefore, these results suggest that the T cell population

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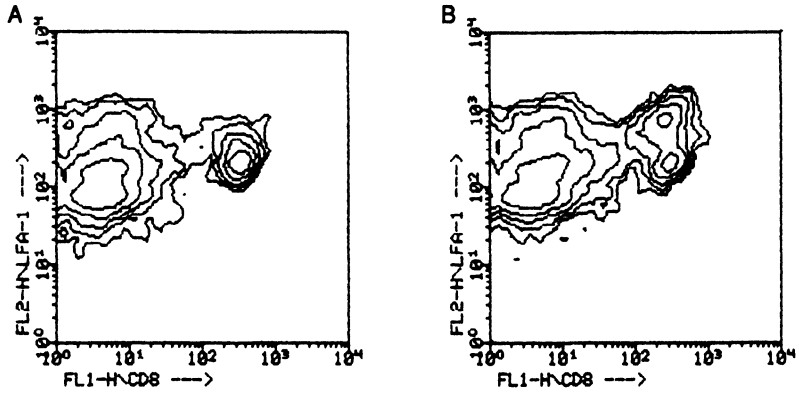

Figure 1. Two- color analysis of spleen cells from uninfected B6 mouse (A) and that at 7 days pi (B). Spleen cells were stained with anti-CD8 (FL1) and anti-LFA-1 (FL2) (antibodies, and analyzed by a FACScan^{TM} flow cytometer.

may mediate the cytotoxicity against virus- infected cells *in vivo*, and thus the flow cytometric analysis is applicable to monitor coronavirus- induced primary cytotoxic T lymphocytes population *in vivo*.

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