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CD40 Ligand Is Pivotal to Efficient Control of Virus Replication in Mice Infected with Lymphocytic Choriomeningitis Virus

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CD40 ligand (CD40L) is an important molecule that is known to be involved in T-B collaboration and certain aspects of cell-mediated immunity. However, its role in antiviral immunity has not been clearly defined as of yet. Therefore, mice with a targeted defect in the gene encoding this molecule were infected with one of two strains of lymphocytic choriomeningitis virus differing markedly in their capacity to spread in the host. Infection with lymphocytic choriomeningitis virus is initially controlled primarily by CD8\(^+\) effector cells, whereas long-term immune surveillance also depends upon CD4\(^+\) cells and B cells. Our results reveal that the primary activation, clonal expansion, and differentiation of CD8\(^+\) T cells does not require expression of CD40L. However, lack of expression results in rapid impairment of CTL responsiveness and failure to permanently control virus replication. This happens not only in mice infected with the rapidly spreading virus strain but also at a late stage in mice infected with the strain of more limited potential for spreading. In the latter mice, virus replication is initially controlled very efficiently, but high levels of virus can be detected in the blood and internal organs ~6 mo after virus inoculation. Since the impairment of immune function seems to be more pronounced in CD40L-deficient mice than in mice lacking either CD4\(^+\) cells or B cells, these results indicate that CD40L is pivotal to sustain efficient antiviral immune surveillance, including CD8\(^+\) T cells, and suggest that CD40L is critically involved in cellular interactions in addition to T-B cooperation.

M any viral infections take an acute course terminating in either rapid elimination of the infecting agent through the action of the induced host response or, rarely, in the death of the infected host. However, some viruses, including important human pathogens (e.g., HIV, CMV and hepatitis B), have the capacity to enter a state of prolonged dynamic interaction with the host resulting in a more chronic type of infection. The level of viral replication in the latter cases is determined to a large extent by the capability of the specific antiviral immune response to continually control the level of active infection (1). Therefore, the balance between virus and host may eventually tip to either side depending upon whether or not the involved effector systems can be sufficiently maintained over a relatively long period of time. This is clearly exemplified in the case of murine lymphocytic choriomeningitis virus (LCMV)\(^4\) infection, in which infection with certain isolates of virus that spread early and rapidly in the host will lead to exhaustion of the critical CTL precursor (CTLP) pool and to a complete breakdown of antiviral immune surveillance (2–5). Since knowledge of the factors involved in regulating long-term maintenance of an effective CTL effector pool in chronic viral infections is obviously of great importance, various gene knockout (GKO) mice have been analyzed to reveal the impact of critical cellular interactions on the long-term outcome of infection with LCMV. Previous studies have shown that CD4\(^+\) T cells, although not essential to the initial generation of CTL effectors, nevertheless play a critical role in maintaining CTL responsiveness and long-term control of virus replication (6–9). In this context, it should be emphasized that CD4\(^+\) T cells are not required for control of virus replication following infection with LCMV strains that spread slowly in the host (9–11), indicating that the importance of these cells does not reflect the possibility that virus-specific CD4\(^+\) cells contribute anything unique to the effector phase of the antiviral immune response; this finding is in marked contrast to CD8\(^+\) T cells (and perforin), which are mandatory for controlling infections even with slowly spreading LCMV isolates (7, 9, 11–13). Therefore, CD4\(^+\) cells seem to be required during the chronic phase of the infection either for maintenance of an expanded CTLp pool or for the production of antiviral Abs that may limit the viral load and thus reduce the need for effector CTL production. In a recent extension of these findings, both we and others (8, 14) have demonstrated that B cells are also important in the chronic phase of LCMV infection; in the absence of these cells, CTL responsiveness eventually disappears, probably as a result of gradual T cell exhaustion. In light of the similarity of the findings in CD4\(^+\) cell-deficient and B cell-deficient mice, it was natural to focus on the

\(^4\) Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; CTLp, CTL precursor; KO, knockout; CD40L, CD40 ligand; CD40L\(^{-/-}\), CD40L

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KO, wt, wild-type; ARM, Armstrong strain; i.c., intracerebral; PFU, plaque-forming units; p.i., postinfection; CSF, cerebrospinal fluid; PE, phycoerythrin; 7-AAD, 7-aminocoumarin D; GKO, gene KO.

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4 Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; CTLp, CTL precursor; KO, knockout; CD40L, CD40 ligand; CD40L\(^{-/-}\), CD40L

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role of T-B interaction in the permanent control of this infection. In this context, CD40 ligand (CD40L/CD40) interaction is known to be of crucial importance (15); therefore, we decided to study LCMV infection in CD40L−/− mice (16). Interestingly, three reports have been published during the last year, all of which demonstrate that these mice effectively control (acute) infection with LCMV despite lack of a substantial virus-specific IgG response (17–19). However, since our own recent studies and those of Planz et al. have revealed that the importance of B cells and Abs in LCMV infection varies considerably depending upon the virus strain studied (8, 14), we decided to reevaluate LCMV infection in CD40L−/− mice by primarily using a model system in which permanent control of virus replication has been found previously to critically depend upon B cells. Under these experimental conditions, we find a marked defect in the capacity of CD40L−/− mice to control LCMV infection as well as a rapid impairment of CTL responsiveness despite a strong initial CD8+ T cell response. However, even in mice infected with a slowly spreading LCMV strain, the ability to control virus replication eventually breaks down, indicating that maintenance of effective antiviral immune surveillance is critically dependent upon cellular interactions involving CD40L.

Materials and Methods

Mice

CD40L−/− mice (B6,129-Cd40l−/−) were bred from homozygous breeding pairs obtained from The Jackson Laboratory (Bar Harbor, ME). Wild-type (wt) 129/Sv and C57BL/6 mice were purchased from Bomholtgaard (Ry, Denmark). Mice from outside sources were always allowed to acclimate for at least 1 wk before use; at that time the animals were 7 to 10 wk old. B cell-deficient (μMT/μMT) mice were the progeny of breeding pairs obtained from National Institutes of Health (Bethesda, MD). These mice were bred using heterozygous female and homozygous male mice, and the offspring was selected by testing sera in a sandwich ELISA for the presence of IgM; heterozygous littermates were used as wt controls. Animals were housed under controlled (specific pathogen-free) conditions that included the testing of sentinels for unwanted infections according to Federation of European Laboratory Animal Science Association standards; no such infections were revealed.

Virus

LCMV of the Armstrong strain (LCMV-ARM, clone 53b) was kindly provided by M. B. A. Oldstone (Scripps Clinic and Research Foundation, La Jolla, CA) (20). LCMV of the Traub strain (LCMV-Traub) was produced and stored as described previously (21). Virus titrations were conducted by intracerebral (i.c.) inoculation of 10-fold dilutions into young adult Swiss mice. Titration endpoints were calculated by the Kärber method and expressed as mean LD50. For determination of organ titers, 10% organ homogenates were used as starting material.

Infection

Mice were infected i.v. in a volume of 0.3 ml. Unless otherwise specified, the virus dose was 103 LD50 of LCMV-Traub or 4800 plaque-forming units (PFU) of LCMV-ARM. In a few experiments, mice were infected i.c. with 0.83% NH4Cl treatment (Gey’s solution). CSF Spleens were removed from mice killed by ether anesthetization. Single-cell suspensions were obtained by pressing the organs through a fine steel mesh, and E were lysed by 0.83% NH4Cl treatment (Gey’s solution). CSF cells were obtained from the fourth ventricle of mice that had been ether-anesthetized and exsanguinated; background level in uninfected mice is <100 cells/μl (23).

Proliferation assay

Splenocytes from individual mice were plated at 0.5, 1, and 2 × 105 cells/well in 96-well flat-bottom microtiter plates, and IL-2 responsiveness was evaluated as proliferation for 24 h in the presence of 10 IU/ml of murine rIL-2 (Genzyme, Cambridge, MA) (9). Cultures were marked by adding 1 μCi of [3H]TdR per well (specific activity of 2 Ci/mmol) during the last 6 h of incubation.

Memory CTL restimulation

Splenocytes (50 × 106 cells) from mice primed with LCMV were transferred into histocompatible wt mice, irradiated (700 rad), and infected with 103 LD50 of LCMV on the day before cell transfer. At 4 days posttransplantation, the spleens of recipients were harvested, and LCMV-specific cytotoxicity of splenocytes was assayed (8, 9). This approach was chosen to mimic the conditions of natural stimulation as much as possible.

Cytotoxicity assay

Virus-specific CTL activity was assayed in a standard 51Cr release assay (24) using histocompatible MC57G cells infected with LCMV for 48 h as specific targets; uninfected MC57G cells served as control targets (9). The assay time was 5 h, and the percentage of specific release was calculated as described previously (21).

Serum IFN-γ

Blood was taken from mice infected i.v. 8 days earlier, and serum was isolated and frozen until analysis. Cytokine levels were quantitated using a sandwich ELISA (Endogen, Cambridge, MA); the limit of detection was 15 pg/ml.

mAbs

The following mAbs were purchased from PharMingen (San Diego, CA) as rat anti-mouse Ab: FITC-conjugated anti-CD49d (common α-chain of lymphocyte Peyer’s patch high endothelial venule adhesion molecule-1 and very late Ag-4) (R1-1, IgG2b); biotinylated anti-L-selectin (CD62L) (Mel-14, IgG2b); FITC-, phycoerythrin (PE)-, and Cy-Chrome-conjugated anti-CD8a (53-6.7, IgG2a); PE-conjugated anti-IFN-γ (XMG1.2, IgG1); and biotinylated anti-CD25 (IL-2Rα) (7D4, IgM).

Flow cytometric analysis

Cells were stained with directly labeled mAb and washed. In the case of the bioconjugated Ab, cells were additionally incubated with streptavidin-Tri-color (Caltag Laboratories, San Francisco, CA). Finally, cells were washed and fixed with 1% paraformaldehyde (25). The presence of intracellular IFN-γ was revealed as recently described (26). Briefly, splenocytes were stimulated in vitro for 6 h with anti-CD3ε in the presence of monensin, labeled with mAbs against appropriate cell surface markers, washed, fixed, permeabilized, and stained with anti-cytokine Ab. Samples were analyzed using a Becton Dickinson FACScan (Mountain View, CA), and 1 to 5 × 104 viable mononuclear cells were gated using a combination of low angle and side scatter to exclude dead cells and debris. Data analysis was conducted using the Lysis II program (Becton Dickinson), and results are presented as dot plots or histograms. Splenocytes from uninfected controls analyzed in parallel were used to set cutoffs for levels of expression. For analysis of DNA content, cells were stained with 7-aminoactinomycin D (7-AAD) (Sigma, St. Louis, MO) as follows (27): after staining for cell surface markers, cells were washed in FACS medium and then in PBS containing 0.03% saponin. The 7-AAD (4 μg/ml in PBS-saponin) was then added, and cells were incubated at room temperature and shielded from light for 30 min. Thereafter, samples were analyzed.

Results

Analysis of primary virus-specific CTL responses in CD40L−/− mice infected with different strains of LCMV

In recent studies, CD40L−/− mice have been found to mount a normal primary CTL response to LCMV (17, 19). To ascertain that this was also the case under our experimental settings, we first infected groups of CD40L−/− mice and wt B6 mice with a moderate dose of an LCMV strain that spreads slowly in the host (LCMV-ARM); no impairment of the primary LCMV-specific
CTL response in CD40L−/− mice was observed using this virus strain (Fig. 1). Furthermore, the generated CTLs are of biologic significance, as the generation of LCMV-specific CTLs was followed by severe meningeal inflammation (day 6 p.i.: 11 × 10^3 and 9 × 10^3 cells/μl CSF in GKO and wt mice, respectively) and mortality (100% mortality, median time to death 7 days for both strains) in i.c.-infected mice and by a marked reduction of virus levels in i.v.-infected animals (see Figs. 7 and 9). We subsequently infected similar groups of mice i.v. with a moderate dose of LCMV-Traub, which spreads much earlier and more rapidly than LCMV-ARM (27); many of the generated cells differentiate into an activated phenotype with a high capacity for the production of IFN-γ (25, 26, 28). Flow cytometric analysis (Figs. 3 and 4) of the CD8+ T cell subset on day 6 p.i. demonstrated that as many cells were actively cycling in CD40L−/− mice as in wt animals (Fig. 4). All of these cells had an activated phenotype (α4 integrinhi L-selectinlow) (Fig. 3), and most expressed high levels of CD25 (IL-2Rα chain) (Fig. 4). To ascertain that the expressed IL-2Rα represented functionally active receptors, we additionally evaluated the responsiveness of splenocytes to IL-2 in vitro. Corresponding to the phenotypic analysis, the in vitro proliferative response to low amounts of IL-2 was essentially identical in GKO and wt mice (Fig. 5); previous results have demonstrated that the majority of proliferating cells are CD8+ cells (9, 28). Higher proportions of activated CD8+ T cells were found on day 8 p.i., at which time 20 to 40% of these cells stained positively for intracellular IFN-γ regardless of the donor genotype (Figs. 3 and 4); levels of IFN-γ in serum tended to be slightly lower in infected CD40L−/− mice, but overlapping values were observed (611 [413–1200] pg/ml vs 1110 [880–2924] pg/ml, median (range) of three mice per group). On day 10 p.i., ~40% of the splenocytes from wt mice were CD8+ cells, most of which had an activated phenotype (Fig. 3). However, only ~25% of splenocytes in GKO were CD8+ cells, and fewer had an activated phenotype. This correlated with a diminished spleen cell number that was less than half of that in wt mice. Overall, the above results reveal that CD40L is not essential for the initial activation, expansion, and differentiation of the CD8+ subset in LCMV-infected mice. However, activated CD8+ cells disappear from the spleen prematurely, and CTL responsiveness is rapidly impaired in the absence of CD40L.

Absence of CTL memory in LCMV-infected CD40L−/− mice

To substantiate that a lack of CD40L results in permanent impairment of CTL responsiveness and not just a transient decrease in CTL activity, splenocytes from CD40L−/− mice and wt animals that had been infected 80 days earlier with LCMV-Traub were tested for their capacity to generate a secondary CTL response. As

FIGURE 1. Unimpaired primary CTL response in LCMV-ARM-infected CD40L−/− mice. GKO and wt mice were infected i.v. with 4800 PFU of LCMV-ARM, and splenic CTL activity was evaluated after 8 days. Results from individual mice are depicted.

FIGURE 2. Premature decay of primary CTL response in LCMV-Traub-infected CD40L−/− mice. GKO and wt mice were infected i.v. with 10^3 LD_{50} of LCMV-Traub, and splenic CTL activity was evaluated after 6, 8, and 10 days. Additionally, spleen virus titers were determined in those mice infected 10 days earlier. Results from individual mice are depicted; circles represent lysis of infected target (MC57G) cells, and triangles represent uninfected target cells.
seen in Figure 6, no recall response could be detected in any of four GKOs tested, whereas high CTL activity could be generated using splenocytes from all (five of five) wt mice. Since restimulation of CTL activity only marginally depends upon CD4+ cells (8), the complete failure to respond must reflect either a marked deletion of CTLp with specificity for LCMV or a substantially reduced capacity of these cells to undergo extensive clonal expansion.

FIGURE 3. Normal initial CD8+ cell expansion but premature disappearance of activated CD8+ cells in CD40L−/− mice infected with LCMV-Traub. GKO and wt mice were infected i.v. with 10^3 LD_{50} of LCMV-Traub, and splenocytes were analyzed by flow cytometry after 6, 8, and 10 days. Cells were stained with anti-CD8-PE, anti-α4 integrin-FITC, and biotinylated anti-L-selectin (plus streptavidin Tri-color). Results representing medians of groups of three mice are depicted. Only CD8+ and α4 integrin expression are depicted, but all α4 integrin^{high} cells were also L-selectin^{low}. Numbers in parentheses refer to size of subpopulations in uninfected mice.

FIGURE 4. Analysis of cell cycle progression, IL-2R expression, and intracellular IFN-γ in LCMV-Traub-infected CD40L−/− mice. GKO and wt mice were infected i.v. with 10^3 LD_{50} of LCMV-Traub, and splenocytes were analyzed by flow cytometry after 6 (cell cycle and IL-2R) and 8 (intracellular IFN-γ) days. Cells were stained with anti-CD8-PE in all cases. For cell cycle analysis, cells were subsequently permeabilized and stained with 7-AAD; CD8+ cells with ≥2 N of DNA content are indicated. IL-2R expression was analyzed using biotinylated-anti-CD25 (IL-2Rα chain); only CD8+ cells are depicted. Intracellular IFN-γ expression was evaluated after in vitro stimulation with anti-CD3 for 6 h followed by permeabilization and staining with anti-IFN-γ-PE. Cutoffs were set using either splenocytes from uninfected mice or irrelevant isotype-matched control mAb. Results representing the medians of groups of three mice are depicted.
Failure of virus control in CD40L<sup>−/−</sup> mice

To evaluate the relevance of the above findings with regard to the ability of CD40L<sup>−/−</sup> mice to exert long-term control of virus replication in vivo, we followed blood virus titers in GKO and wt animals infected with LCMV-Traub. As seen in Figure 7, some CD40L<sup>−/−</sup> mice could partially control the infection for a limited period, but invariably virus control broke down; at ~2 mo after virus inoculation, high titers of virus were found in the circulation of all GKO mice tested. Evaluation of virus levels in organs (Fig. 8) revealed that viremia was associated with high levels of virus in the internal organs of GKO, whereas little or no virus was detected in similarly infected wt mice. Reducing the inoculum by two logs tended to delay the breakdown of virus control but did not substantially change the final outcome of infection (Fig. 7). Notably, GKO mice were clinically more severely affected by infection with the lower virus dose, which is in keeping with the possibility that exhaustion of CTL responsiveness was delayed, thus resulting in a prolonged immunologic conflict in these permanently infected mice.

Since the above results appeared to be at variance with the findings of previous studies involving infection with LCMV-ARM (17, 19), we also decided to evaluate long-term virus control in CD40L-deficient mice infected with this LCMV strain. This evaluation was particularly relevant, as a previous study has indicated that LCMV-specific T cell memory at 2 mo p.i. is somewhat inferior in these mice (19). As can be seen in Figure 7, GKO initially control the LCMV-ARM infection well, and no virus is detected in the circulation for ~2 mo. However, virus eventually does appear in the circulation, and this appearance is associated with substantial virus replication in the internal organs (Fig. 9). Thus, CD40L is required for permanent control of virus replication even in mice infected with a strain of LCMV that only spreads slowly in the host and has a limited potential for causing persistent infection (4, 5).

FIGURE 5. Unimpaired IL-2 responsiveness in CD40L<sup>−/−</sup> mice infected with LCMV-Traub. GKO and wt mice were infected i.v. with 10<sup>3</sup> LD<sub>50</sub> of LCMV-Traub; after 6 days, splenocytes were cultured in vitro for 24 h in the presence or absence of IL-2 (10 IU/ml). The results depicted represent individual mice. Cells from uninfected mice do not respond to IL-2 and have been excluded for sake of clarity.

FIGURE 6. Lack of CTL memory in LCMV-Traub-infected CD40L<sup>−/−</sup> mice. GKO and wt mice were infected i.v. with 10<sup>3</sup> LD<sub>50</sub> of LCMV-Traub, and splenocytes were harvested and transferred to irradiated, infected recipients after 80 days. After 4 days, secondary LCMV-specific CTL activity was assayed and compared with the primary CTL response. Results from individual mice are depicted; a similar response pattern was obtained when two additional GKO mice and three wt mice were analyzed.

FIGURE 7. Time-course of blood virus titers in LCMV-infected CD40L<sup>−/−</sup> mice. GKO and wt mice were infected with either 10 (low) or 10<sup>5</sup> (moderate) LD<sub>50</sub> of LCMV-Traub or with 4800 PFU of LCMV-ARM; blood virus titers were assayed on the indicated days. Points represent individual mice; viremia was not observed in similarly infected wt mice.

FIGURE 7. Time-course of blood virus titers in LCMV-infected CD40L<sup>−/−</sup> mice. GKO and wt mice were infected with either 10 (low) or 10<sup>5</sup> (moderate) LD<sub>50</sub> of LCMV-Traub or with 4800 PFU of LCMV-ARM; blood virus titers were assayed on the indicated days. Points represent individual mice; viremia was not observed in similarly infected wt mice.
Long-term control of virus replication in B cell-deficient mice infected with LCMV-ARM

The fact that long-term control of virus replication is impaired even in CD40L$^{-/-}$ mice infected with LCMV-ARM was surprising, as previous analyses have indicated that LCMV-ARM, in contrast to LCMV-Traub, is well controlled in B cell-deficient mice (8, 29). To investigate whether this difference between CD40L$^{-/-}$ mice and B cell-deficient mice was real or merely reflected that capacity to control virus replication previously had not been evaluated sufficiently late in B cell-deficient mice, we followed virus titers in B cell-deficient mice infected with the same dose of LCMV-ARM used above to infect CD40L$^{-/-}$ mice. Evaluation of virus titers in lungs and liver (Fig. 10) on days 70 to 90 p.i. revealed little virus in B cell-deficient mice, confirming earlier findings. However, significant amounts of virus were detected in the lungs after ;150 days. Thus, even B cell-deficient mice have some reduced capacity for long-term control of virus replication following infection with a slowly spreading LCMV strain; however, antiviral immune surveillance is clearly less compromised than in CD40L$^{-/-}$ mice (cf Fig. 9).

Discussion

The present results reveal a mandatory role for CD40L in the maintenance of efficient immune surveillance in virus-infected mice. Our results further indicate that failure to control virus replication is associated with impairment of CTL responsiveness, but whether this is a primary or secondary event is not known with certainty. Notably, the speed with which the capacity to control virus replication collapses seems to vary with the initial virus load, and consequently is determined by a combination of inoculum size and the ability of the virus strain to spread in the host. The latter probably explains why no similar breakdown has been observed in the context of previous studies (17, 19), since only short-term evaluations were conducted in mice infected with a slowly spreading virus strain (LCMV-ARM). Furthermore, virus used in previous studies was inoculated i.p. rather than i.v., and the latter may accelerate the process of immune exhaustion due to a higher initial level of virus replication in critical organs.

At present, we do not know precisely how CD40L is involved in maintaining efficient antiviral immune surveillance. It is evident from these and previous findings (17, 19) that CD40L is not required for virus-induced activation, expansion, or differentiation of CD8$^+$ cells. Nor is this ligand needed for the targeting of effector T cells to infected areas and the formation of an inflammatory exudate (30), and we have preliminary data indicating that virus-induced macrophage activation is unimpaired in LCMV-infected CD40L$^{-/-}$ mice (31, 32). Furthermore, Oxenius et al. have recently presented data indicating that virus-induced Th cell activation and effector function is unimpaired except for T-B collaboration (18). Therefore, we originally initiated these studies based on the assumption that CD40L was required primarily for the appropriate interaction of CD4$^+$ cells and B cells. However, contrary to our expectations, the results obtained seem to indicate that the antiviral immune response is even more compromised in CD40L$^{-/-}$ mice than in class II-deficient mice (lacking CD4$^+$ cells) or B cell-deficient mice (8, 9). This finding could reflect a difference in the genetic background of the involved KO mice: both class II-deficient and B cell-deficient mice are on a C57BL
background, whereas CD40L \(^{-/-}\) mice are on a mixed C57BL/129 background, and earlier studies have clearly demonstrated that the non-MHC background may markedly influence T cell responsiveness and the outcome of infection with LCMV (24, 33, 34). However, we included both C57BL/6 and 129 wt controls in many of the experiments and never observed any difference between these strains in their responsiveness toward LCMV. Furthermore, low responsiveness to LCMV has always been found to be recessive (24, 33, 34). However, we cannot rule out the possibility that a minor difference in the capacity to cope with the infection, which is irrelevant in fully immunocompetent wt mice, may become exposed in mice with a defective Cd40L gene. Nevertheless, a more likely explanation is that the defect in CD40L \(^{-/-}\) mice is of a more complex nature than merely impaired T-B cooperation. Obviously, lack of CD40L markedly impairs Ab formation (17–19), and an increased virus load is known to contribute to exhaustion of the CTLp population (4, 24). However, the breakdown of CTL activity in LCMV-Traub-infected mice has already started around day 10 p.i., at which timepoint Abs contribute only marginally to virus elimination (8). Therefore, an increased virus load due to lack of antiviral Abs cannot explain this finding. Furthermore, the increased virus load due to lack of antiviral Abs cannot explain this finding. Therefore, an increased virus load due to lack of antiviral Abs cannot explain this finding. Furthermore, the breakdown of CTL activity in LCMV-Traub-infected mice has already started around day 10 p.i., at which timepoint Abs contribute only marginally to virus elimination (8). Therefore, an increased virus load due to lack of antiviral Abs cannot explain this finding. Furthermore, the breakdown of CTL activity in LCMV-Traub-infected mice has already started around day 10 p.i., at which timepoint Abs contribute only marginally to virus elimination (8). Therefore, an increased virus load due to lack of antiviral Abs cannot explain this finding.

The CD40L/CD40 interaction may provide a direct signal that CD40L and/or CD40 is expressed on cells belonging to this subset. Supporting this possibility, low levels of CD40L may be found on some activated CD8\(^+\) T cells (35–37). Using flow cytometry, we have not, however, been able to demonstrate expression of CD40L on LCMV-activated CD8\(^+\) cells, but this does not exclude the possibility that this molecule is expressed on the minority of virus-specific cells that escape activation-induced apoptosis and become the long-term precursors for the continued CTL surveillance in LCMV-infected mice. Also CD40 has been found on CD8\(^+\) cells (38), and we are presently investigating this possibility in LCMV-infected mice. However, even if CD40L/CD40 should be involved in preventing apoptosis of activated CD8\(^+\) cells, this is probably not the sole mechanism underlying the complete collapse of immune surveillance in mice lacking CD40L. Clearly Ab production is impaired (17–19), and this molecule may also be required for the maintenance of CD4\(^+\) T cell memory (30). Additionally, CD40L has been found to exert a direct antiviral activity against certain viruses (39). Indeed, the severity of the impairment and the speed with which it proceeds even in mice infected with a low dose of virus suggest that this molecule serves several critical functions in the complex network of cellular interactions that provide the basis for solid immunity.

In conclusion, our results clearly demonstrate that CD40L is a crucial molecule for the maintenance of effective long-term immune surveillance in virus-infected mice. Given that the understanding of the mechanisms underlying optimal memory function is critical to the designing of effective vaccines and probably also to meaningful manipulations of the immune system in chronically infected individuals (e.g., HIV-infected patients), our studies clearly prompt further analysis of the cellular and molecular interactions that are critical in this respect.

**References**


