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Depletion of CD4⁺ T Cells Precipitates Immunopathology in Immunodeficient Mice Infected with a Noncytoidal Virus

Jan Pravsgaard Christensen,* Christina Bartholdy,* Dominik Wodarz,† and Allan Randrup Thomsen²*

IFN-γ-deficient (IFN-γ⁻/⁻) mice inoculated with intermediate doses of a slowly replicating strain of lymphocytic choriomeningitis virus become chronically infected. In such mice a hypercompensated CTL response is observed that partially controls virus replication. Here we have investigated whether CD4⁺ Th cells are required to establish and maintain this new equilibrium. The absence of IFN-γ does not impair the generation of IL-2-producing CD4⁺ cells, and depletion of these cells precipitates severe CD8⁺ T cell-mediated immunopathology in IFN-γ⁻/⁻ mice, indicating an important role of CD4⁺ T cells in preventing this syndrome. Analysis of organ virus levels revealed a further impairment of virus control in IFN-γ⁻/⁻ mice following CD4⁺ cell depletion. Initially the antiviral CTL response did not require CD4⁺ cells, but with time an impaired reactivity toward especially the glycoprotein 33–41 epitope was noted. Enumeration of epitope-specific (glycoprotein 33–41 and nucleoprotein 396–404) CD8⁺ T cells by use of tetramers gave similar results. Finally, limiting dilution analysis of CTL precursors reveal an impaired capacity to sustain this population in CD4⁺-depleted mice, especially in mice also deficient in IFN-γ. Thus, our findings disclose that T cell help is required to sustain the expanded CTL precursor pool required in IFN-γ⁻/⁻ mice. This interpretation is supported by mathematical modeling that predicts an increased requirement for help in IFN-γ⁻/⁻ hosts similar to what is found with fast replicating virus strains in normal hosts. Thus, the functional integrity of CD8⁺ effector T cells is one important factor influencing the requirement for T cell help during viral infection. The Journal of Immunology, 2001, 166: 3384–3391.

The outcome of any viral infection is the net result of a balance between, on the one hand, the ability of the virus to replicate inside the host and, on the other, the capacity of the host to mount and maintain an effective immune response. This fact has perhaps been most clearly illustrated in murine models using noncytoidal viruses (1, 2). Under these conditions, the infection will run its course free from constraints imposed by virus-induced tissue destruction. Therefore, analysis of noncytoidal infections has been extensively used to study the dynamics of virus/host interactions, and the theoretical spectrum of this interplay has been revealed together with a high sensitivity to variation depending on both host and virus parameters. The two extremes of the observed spectrum are 1) a sustained CTL response resulting in virus elimination/control and establishment of solid memory, and 2) exhaustion of virus-specific CTL precursors (CTLps)² leading to virus persistence (3). Both these outcomes are of limited pathological consequence to the host. However, an unfortunate intermediate is extensive T cell-mediated immunopathology and chronic tissue damage. This situation occurs when the immune response is insufficient to completely control the infection, but still sufficient to inflict substantial cell damage in infected organs (4).

The kinetics of initial virus multiplication and subsequent virus distribution seem to play a critical role in determining the outcome of a noncytoidal virus infection. Parameters such as cell tropism and the replication rate of the virus as well as the dose of infection are important determinants in this context. Thus, rapidly replicating, invasive strains are more likely to exhaust the immune response and cause persistence than are slowly replicating ones, and the same is true for a high dose vs a low dose infection (1, 5–9).

With regard to the host, T cell responder status and thus initial T cell precursor frequencies are important in influencing the outcome of infection, as is the extent of the subsequent clonal expansion (1, 3, 4, 9–11). The CD8⁺ T cell population constitutes an important part of the antiviral immune response. Virus-specific CD8⁺ CTLs are crucial for elimination of infected cells (12, 13), and in addition, CD8⁺ cell-mediated production of antiviral cytokines such as IFN-γ may further reduce viral replication (14). In contrast, the role of CD4⁺ T cells is less clearly defined (15). CD4⁺ T cells are important in regulating the immune response, and besides providing help to B cells, they may exert direct effector function by generating an abundance of cytokines (16). Additionally, they are believed to be important for the conditioning of APCs, making these capable of delivering costimulatory signals to the Ag-specific CD8⁺ cells (17–19). Thus, CD4⁺ T cells probably contribute to the antiviral CD8⁺ T cell response in two ways: 1) CD4⁺ T cell/APC interaction secures an optimally stimulating APC, and 2) CD4⁺ cells provide most of the IL-2 involved in driving the essential clonal expansion (20).

Infection of mice with lymphocytic choriomeningitis virus (LCMV) is a typical example of an infection with a noncytoidal virus, and this model highlights the crucial role for CD8⁺ T cells in resolution of infection (12, 13, 21, 22). However, studies...
concerning the contribution of CD4+ cells to resolution of this infection are ambiguous. By use of either CD4-depleted mice, CD4 knockouts, or MHC class II-deficient mice, it has been shown that CD4+ cells are dispensable for the initiation of the primary CTL response, but are pivotal for the long term maintenance of CD8+ effector cell activity and an expanded CTLp pool (21, 23–26). Furthermore, under conditions of high dose infection or infection with rapidly replicating and disseminating LCMV strains, CD4+ cells are required to prevent rapid exhaustion of the CTL pool (27, 28). In contrast, CD4+ cells do not seem to be required for clearance of less invasive strains of LCMV, e.g., LCMV Armstrong (23, 28, 29).

As mentioned above, IFN-γ may be important in reducing viral spreading and is thus likely to be an additional parameter important in the virus-host balance. However, the role of IFN-γ in the outcome of an LCMV infection has, like that of CD4+ cells, been somewhat elusive. Whereas this cytokine in general is said to be primarily important for resolution of infections with cytolytic viruses (30), we have recently shown that absence of IFN-γ during LCMV infection may have serious consequences for the outcome of infection with this noncytolytic virus. Thus, while wild-type mice control an infection with the vicerotropic and rapidly invasive LCMV Traub strain, IFN-γ−/− mice develop a severe CD8+ T cell-mediated wasting disease and succumb to the infection (31). In contrast, challenge with the less invasive LCMV Armstrong strain does not lead to wasting disease in IFN-γ−/− mice. However, a chronic state of infection is induced in the majority of these mice. Notably, virus persistence is not the result of CTL exhaustion; instead, virus coexists with augmented ex vivo CTL activity (32), and analysis of cell cycle status and activation markers indicates that the CD8+ T cell population is subject to permanent stimulation and increased cellular turnover. Whether CD4+ cells play a role in maintaining this delicate new balance, e.g., by delivering help to the CD8+ cell-mediated immune response in these mice, is not known. Therefore, in the present study we examined the importance of CD4+ cells for CD8+ T cell-mediated virus control under these conditions. Our study reveals that CD4+ cells are crucial for maintaining the CD8+ T cell-mediated control of LCMV Armstrong infection in IFN-γ−/− mice. Most striking, the clinical effect of CD4+ T cell depletion is severe wasting and death. Thus, our findings underscore that CD4+ T cells are pivotal for the host to adapt in a situation where the functional integrity of the generated effector T cells is perturbed.

Materials and Methods

**Mice**

C57BL/6 (B6) wild-type mice were obtained either from Bornholmgaard (Ry, Denmark) or The Jackson Laboratory (Bar Harbor, ME). IFN-γ-deficient (IFN-γ−/−) mice (C57BL6-ifg<tm1>) were also derived from The Jackson Laboratory either directly or as the progeny of breeder pairs. Seven- to 10-wk-old mice were used in all experiments, and animals were always allowed to acclimatize to the local environment for at least 1 wk before use. All animals were housed under specific pathogen-free conditions as validated by screening of sentinels. All animal experiments were conducted according to national guidelines.

**Virus**

LCMV of the Armstrong strain (clone 53b) was used in all experiments. Mice to be infected received a dose of 4800 PFU in an i.v. injection of 0.3 ml.

**Depletion of CD4+ T cells**

The oCD4 mAb GK1.5 was used. Mice to be depleted received a dose of 0.1 ml of clarified ascitic fluid in 0.5 ml of PBS i.p. on days −1, 0, 2, 5, 9, and 16 postinfection (p.i.). Flow cytometric analysis consistently revealed <1% CD4+ T cells in such treated mice.

**Virus titration**

Organ virus titers were assayed by intracerebral inoculation of 10-fold dilutions of a 10% organ suspension into young adult Swiss mice. Titration end points were calculated by the Kärber method and are expressed as the mean lethal dose (LD_{50}).

**Cell preparations**

Spleens from mice were aseptically removed and transferred to HBSS. Splenocyte suspensions were obtained by pressing the organs through a fine sterile steel mesh, and erythrocytes were lysed by 0.83% NH4Cl treatment. The cells were washed twice with HBSS, and cell concentration was adjusted in RPMI 1640 containing 10% FCS supplemented with 2-ME, 1-glutamine, and penicillin-streptomycin solution.

**Limiting dilution analysis**

CTLp frequencies were determined as previously described (33). Briefly, 3-fold dilutions of responder cells were added in 100 µl of medium to round-bottom 96-well microtiter plates. Replicates (24 wells) were plated for each responding cell dilution and cocultured with 100 µl (3 × 10^5 cells) of 2500 rad gamma-irradiated, T cell-depleted syngeneic splenocytes pulsed with either glycoprotein 33–41 (gp33–41) or nucleoprotein 396–404 (np396–404) the two immunodominant MHC class I-restricted peptides of LCMV in H-2b mice (33–35) or unpulsed splenocytes. The medium contained 10 U/ml of human rIL-2. Three identical sets of cultures were initiated with different stimulators and incubated for 7 days at 37°C in a humidified atmosphere. On day 4, 20 µl of medium with IL-2 (100 U/ml) was added to the cultures. The contents of individual wells were tested for cytoxicity at the end of the culture period by incubating each well with 5000 ^{59}Cr-labeled, peptide-pulsed or unpulsed EL-4 cells (H-2b, MHC-I”) for 6 h. Wells were considered positive if the cytotoxic activity exceeded the average + 3 × SD of the spontaneous release of target cells incubated with medium alone. Minimal estimates of pCTL frequencies were obtained according to the Poisson distribution.

**Cytotoxicity assays**

Virus-specific CTL activity was assayed in a standard ^{51}Cr release assay using EL-4 cells pulsed with 1 h at 37°C with LCMV gp33–41 or np396–404 peptide; unpulsed EL-4 cells served as a control target. The assay time was 5 h, and the percent specific release was calculated as described previously (36).

**mAb for flow cytometry**

The following mAbs were all purchased from Pharmingen (San Diego, CA) as rat anti-mouse Abs: FITC-conjugated anti-CD49d (common Ag-4), PE- and Cy-Chrome-conjugated anti-CD8a, biotinylated anti-CD44, FITC-conjugated anti-CD4 (for control of cell depletion, RM4-4 not cross-reacting to GK1.5 was used), PE-conjugated anti-IFN-γ, and PE-conjugated anti-IL-2.

**MHC/peptide tetramers for flow cytometry**

H-2D^b-gp33–41 and H-2D^b-np396–404 tetramers were obtained through the National Institutes of Allergy and Infectious Disease tetramer facility and the National Institutes of Health AIDS Research and Reference Reagent Program.

**Flow cytometric analysis**

Cells (1 × 10^6) were stained with directly labeled mAb in staining buffer (10% rat serum, 1% BSA, and 0.1% NaN3 in PBS) for 20 min in the dark at 4°C and subsequently washed. If biotin-conjugated Ab was used, cells were additionally incubated with streptavidin-Tricolor or streptavidin-Cy-Chrome (Caltag, San Francisco, CA), washed, and fixed with 1% paraformaldehyde (37–40). For visualization of LCMV-specific cytokine-producing CD4+ cells, splenocytes were incubated with MHC class II (A<sup>α</sup>)-restricted gp33–41 peptide at 1 µg/ml for 5 h in the presence of IL-2 (50 U/ml) and monensin (3 µM) (41). After incubation cells were surface stained as described, washed, permeabilized, and stained with cytokine-specific mAbs. Finally, cells were washed and fixed as previously described. For tetramer staining, cells were incubated with the tetramers at pretitrated optimal concentrations for 1 h at room temperature, followed by surface labeling as described above.

Cells were analyzed using a FACSCalibur (Becton Dickinson, San Jose, CA), and at least 10^6 cells were gated using a combination of low angle and side scatter to exclude dead cells and debris. Data analysis was conducted using CellQuest software (Becton Dickinson).
We describe a mathematical model that takes into account the dynamics between LCMV replication and an LCMV-specific CTL response. The model includes four variables: susceptible uninfected host cells (s), infected cells (i), LCMV-specific CTL 

\[ \frac{ds}{dt} = a_s - (a_f + b_s)i \]

\[ \frac{di}{dt} = b_s i - (d_i + e_i)i \]

\[ \frac{dc}{dt} = b_c (s - i) - d_c c \]

\[ \frac{dx}{dt} = d_f x - d_c c \]

Thus, the model describes how susceptible host cells become infected, how infected cells decay, how virus-specific CTL are generated, and how CTL decay. The equations are used to simulate the infection process and to predict the outcome under different conditions.

The results show that depletion of CD4 cells is critical for the control of the acute infection in IFN-γ−/− mice. CD4+ T cells play a role in stabilizing CD8+ T cell responses, and their absence leads to severe wasting disease and chronic immune response.

Depletion of CD4+ T cells leads to severe CD8+ T cell-mediated disease in IFN-γ−/− mice

Infection of immune-competent mice with the slowly replicating LCMV Armstrong strain induces a potent CD8+ T cell-mediated immune response. However, depletion of CD4+ T cells results in a chronic wasting disease characterized by long-standing coexistence of virus and significant ex vivo virus-specific CTL activity. The depletion of CD4+ T cells leads to an impaired CD8+ T cell response, which is critical for virus control in IFN-γ−/− mice.
gradually disappearing in wild-type mice as the infection is controlled (32). The responsiveness of splenic effector cells from infected IFN-\(\gamma^{-/-}\) mice was assayed and compared with that of their CD4\(^+\) cell-competent counterparts. The responses to two immunodominant MHC class I-restricted peptide epitopes (gp33–41 and np 396–404, respectively) were determined on days 7 and 14 p.i. (Fig. 4). Although both groups of animals were clearly able to raise a CTL response that killed target cells pulsed with either peptide, analysis of per cell cytolytic activity on day 14 p.i. revealed that depletion of CD4\(^+\) cells significantly impaired the response to gp 33–41-pulsed target cells in IFN-\(\gamma^{-/-}\) mice (Fig. 4C). Indeed, in two independent experiments only two of seven CD4-depleted mice exhibited gp33–41-specific killing within the range of the response in CD4-intact animals. A trend toward higher numbers of virus-specific CD8\(^+\) cells was observed in IFN-\(\gamma^{-/-}\) mice at both time points. With regard to the effects of CD4\(^+\) cell depletion, marginal differences were detected when the mice were analyzed on day 7 p.i. However, when analyzed on day 14 p.i. (Fig. 5C), CD4 depletion was associated with lower numbers of both gp33–41- and np396–404-specific cells in IFN-\(\gamma^{-/-}\) mice. In relative terms the difference was most pronounced for gp33–41-specific cells (~3-fold) consistent with the result of the

Depletion of CD4\(^+\) cells affects the number of virus-specific cells in IFN-\(\gamma^{-/-}\) mice

To directly enumerate virus-specific CD8\(^+\) T cells, tetramer staining was performed in infected CD4-depleted and CD4-competent, IFN-\(\gamma^{-/-}\) and wild-type mice. Spleen cells harvested from mice infected 7 and 14 days previously were allowed to bind to fluorescence-stained tetramer complexes containing MHC class I (H-2D\(^b\)) expressing either gp33–41 or np396–404 and were subsequently analyzed by flow cytometry (Fig. 5, A and C). Generally, a trend toward higher numbers of virus-specific CD8\(^+\) cells was observed in IFN-\(\gamma^{-/-}\) mice at both time points. With regard to the effects of CD4\(^+\) cell depletion, marginal differences were detected when the mice were analyzed on day 7 p.i. However, when analyzed on day 14 p.i. (Fig. 5C), CD4 depletion was associated with lower numbers of both gp33–41- and np396–404-specific cells in IFN-\(\gamma^{-/-}\) mice. In relative terms the difference was most pronounced for gp33–41-specific cells (~3-fold) consistent with the result of the
CTL analysis. In contrast, CD4 depletion had little if any effect on the number of virus-specific cells in wild-type mice infected 14 days previously. This pattern suggests that IFN-γ−/− mice, due to their genetic defect, compensate by generating more virus-specific CD8+ cells during the acute infection, and that the ability to maintain this compensation requires CD4+ cells.

The CTLp level declines in infected IFN-γ−/− mice in the absence of CD4+ cells

Finally, we investigated whether the absence of CD4+ cells affected the ability to generate and maintain an expanded CTLp population in infected IFN-γ−/− mice. Limited dilution assays were performed with splenic cells from infected IFN-γ−/− and wild-type mice either depleted of CD4+ cells or left untreated (Fig. 5). Particularly on day 14 p.i. (in nine of nine mice tested), but also in some (three of nine) animals analyzed on day 7 p.i. (in nine of nine mice tested), but also in some (three of nine) animals analyzed on day 7 p.i., CTLp frequencies for gp33-41- and np396-404-specific CTLs in CD4-depleted knockout mice were below the ranges observed in their CD4+ cell-competent counterparts, a tendency that was not nearly as marked when comparing CD4-depleted and CD4-competent wild-type mice. Interestingly, the relative differences between CD4-depleted and CD4-competent IFN-γ−/− mice were more pronounced at the CTLp level than at the level of tetramer-positive cells. This is consistent with the interpretation that CTLp, as detected by limiting dilution analysis, constitutes a minor subset of less differentiated cells, giving rise to the CD8+ effectors eventually detected by tetramer (and CTL) analysis. Taken together, the above results suggest that CD4+ cells are pivotal to maintain the more extensive expansion of virus-specific CTLp required to control LCMV Armstrong strain infection in IFN-γ−/− mice, and that elimination of CD4+ cells shifts the balance toward exhaustive differentiation of virus-specific CD8+ T cells.

Mathematical modeling of the consequences of CD4+ cell deficiency in the presence or the absence of IFN-γ

In parallel to experimental testing, we also used a mathematical model to investigate the effect of CD4 depletion in IFN-γ−/− mice. The present mathematical model represent an extended version of the model recently used to successfully describe the course of infection in IFN-γ−/− mice. The model was modified as described in Materials and Methods to accommodate the experimental observation that CTL activation and expansion in wild-type mice initially is CD4 independent, but requires CD4+ T cells for a sustained response. We also assume that infection occurs with a relatively slowly replicating strain. Simulations of the dynamics of CTL and virus load are shown in Fig. 6. In wild-type mice a sustained CTL memory response is generated that resolves the primary infection and ensures long term immunological control and clearance. IFN-γ−/− mice are characterized by low level persistent virus replication. However, the CTL response is still sustained and controls the infection over the long term. The higher virus load maintains a higher number ofCTL in the memory phase compared with the wild type, and the small bursts of virus drives the CTL
number up, which can keep viral replication in check. The validity of this prediction has previously been documented experimentally (32). CD4+ helper cell deficiency also results in only a small loss of virus control. However, the simulation suggests that over time the CTL response slowly decays that can lead to reduced levels of virus control over the long term. Consistent with this prediction, a slow decline in the number of CTLp has previously been observed in CD4- deficient mice infected with LCMV Armstrong strain (26). Hosts deficient in both CD4+ cell help and IFN-γ production show a rapid loss of virus control and a fast loss of the LCMV-specific CTL response.

These patterns are also reflected in Fig. 7, which shows the effect of CD4+ helper cell deficiency in IFN-γ−/− hosts on the levels of CTL-induced pathology (characterized by a low overall number of host cells). In the presence of CD4+ cell help, the model suggests that the host experiences transient immunopathology followed by relatively efficient virus control. In contrast, in the absence of help, severe and sustained immunopathology is predicted, which, in practical terms, corresponds to wasting and death of the host.

Discussion

Both CD4+ cells and IFN-γ have previously been found to contribute to resolution of infections with LCMV, and the absence of either tends to shift the balance toward a more chronic infection. The importance of IFN-γ critically depends on the tropism and dose of infection. Thus, while infection of IFN-γ−/− mice with an intermediate dose of the viscerotropic LCMV Traub strain is associated with severe immunopathology and death (31), infection of these mice with even higher doses of the less invasive LCMV Armstrong strain develops into a chronic infection characterized by coexistence of virus and hypercompensated CTL effector function (32). The impact of CD4+ cells on the outcome of infection also varies with tropism and dose of the virus strain. Thus following infection with high doses of rapidly invasive LCMV strains, CD4+ cells are required to prevent rapid exhaustion of the CD8+ T cell response and associated chronic infection (27, 28). Similarly, CD4+ cells are important during long term control of infection with lesser doses of rapidly invasive LCMV strains even if the primary virus control may not require this subset (2). In contrast, CD4+ cells play only a minor role during acute infection as well as in the long term control of slowly replicating LCMV strains (27, 28). However, if the role of CD4+ cells depends upon the rate of virus spread, it might be expected that these cells would also be needed for the control of infections even with slowly replicating viruses in hosts partially impaired in antiviral effector capacity. In line with this intuitive deduction, the outcome of mathematical simulations suggests that in IFN-γ−/− mice, CD4+ cells are required for a sustained CTL response and permanent control of infection even with a slowly replicating virus. Therefore, this study was undertaken to test whether, as predicted, CD4+ T cells contribute to the CD8+ cell-mediated virus control under the conditions of the chronic infection previously observed in IFN-γ−/− mice infected with the slowly replicating LCMV Armstrong strain.

The present findings disclose that CD4+ T cells are pivotal for the control of an LCMV Armstrong strain infection in IFN-γ−/− mice. Thus, in the absence of CD4+ cells, LCMV Armstrong-infected IFN-γ−/− mice develop severe CD8+ cell-mediated wasting and die about 3 wk after infection. Elimination of CD4+ cells is also accompanied by an impaired ability to limit the virus spread in these mice; thus, at 2 wk p.i. virus levels in spleen, liver, and lungs are higher in IFN-γ−/− mice following CD4 depletion. This together with a partially impaired CTL response readily explains the occurrence of fatal immunopathology following CD4 depletion (4).

As to the mechanism(s) through which CD4+ cells could influence the outcome of LCMV infection in IFN-γ−/− mice, several possibilities have to be considered. First, CD4+ cells might be antiviral effectors in their own right. However, there is no precedence in the entire LCMV literature for such a scenario. Therefore, the possibility that this should be the case in animals in which the capacity to produce IFN-γ is abolished seems extremely unlikely. Second, CD4+ cells could function through help to B cells. Even though neutralizing Abs may contribute to the long term control of
infection with certain LCMV strains (2, 43, 44). Abs have never been found to play a role during the acute phase of the infection. Furthermore, the evidence for generation of neutralizing Abs in the context of LCMV Armstrong infection is entirely negative. Therefore, it seems most appropriate to focus on the role of CD4+ cell help in the generation and maintenance of the antiviral CTL response.

Previous results have clearly established that CD4+ T cells are not required for generation of primary LCMV-specific CTL effectors (21, 23–25). The present data confirm that this holds true in IFN-γ−/− mice. However, it has also previously been documented that CD4+ cells are required to maintain an expanded virus-specific CTLp pool (25, 26). This is normally of little practical consequence to CD4-deficient mice infected with a slowly replicating LCMV strain such as LCMV Armstrong, probably because the initial burst of CTL activity suffices to drive the virus load almost to the level of extinction. However, if the antiviral capacity of the generated effectors is reduced, as in IFN-γ−/− mice, virus replication is not as efficiently curtailed, and a high level of CTL activity needs to be sustained for a longer period. Under these conditions the role of CD4+ cells in sustaining an expanded CTLp pool becomes exposed even in mice infected with a slowly replicating LCMV strain. What seems to be happening is that a higher antigenic load gradually becomes established in IFN-γ−/− mice, and this leads to augmented CTL differentiation and a higher number of CD8+ effector cells. Because the CTLp pool is less efficiently sustained in the absence of CD4+ cells, the increased usage slowly depletes this population in CD4-deficient mice, and a downward spiral is initiated. Fewer CTLp gradually leads to fewer CTL effectors further impairing the capacity to control the infection. The consequence is a sustained Ag load, which, in turn, further drives the available CTLp toward terminal differentiation. Eventually this may result in complete depletion (exhaustion) of the CTLp pool (8). However, if this process is not rapid, the host is likely to succumb to the immunopathology that is induced in the futile attempt to control the infection (4). The above interpretation is supported by mathematical simulation. The chosen model is based on relatively simple assumptions already experimentally substantiated in the literature. Although the theoretical results do not prove anything by themselves, the fact that there is consistency between theoretical and experimental data provides important support for the feasibility of the suggested scenario. In this context it may be relevant to point out that this model not only provides predictions that fit the present experimental observations, but it also appropriately predicts a number of other outcomes already experimentally documented.

In many respects our present results match those previously obtained by infection of CD4-deficient, but otherwise immunocompetent, mice with high doses of rapidly invasive LCMV strains (27, 28). However, there are two important differences. First, in the present study the failure to control the infection relates to an impaired host response, not to a factor intrinsic to the virus. Thus, we show here that CD4+ T cells are pivotal for the host to adapt in a situation where the functional capacity of the generated effector T cells is impaired. In other words, a qualitative (functional) defect within the effector subset substantially shifts the threshold for CD8+ cell exhaustion. Second, following infection with high doses of invasive viruses, exhaustion occurs so rapidly that most animals survive. Although this may be considered an advantage in certain respects, the rapid exhaustion also blurs the picture, while the more gradual depletion we observed allows a better separation of different phases. Thus initially a higher CD8+ T cell response is noted, and it is only with time that the depletion becomes obvious at all levels, first and most pronounced at the CTLp level and later also at the CD8+ effector level. Therefore, the present data confirm and extend the idea that CD4+ T cells are required to protect against exhaustive differentiation of the generated CTLp following an increased/chronic demand. Precisely how this effect is obtained is not certain, but production of IL-2 may be a critical factor (20).

The present study underscores the interdependence of CD4+ and CD8+ T cells in antiviral immunity. Thus, CD4+ cells are pivotal for CD8+ T cell-mediated control not only when high doses of fast replicating virus are introduced, but also following challenge with “normal” virus, provided that the functional capacity of the generated virus-specific effector T cells is impaired, i.e., a deficient CD4+ T cell response markedly reduces the capacity of the host to provide a flexible immune response. This finding is clearly of importance when trying to understand the complex cellular interactions that may take place during chronic viral infections in humans, e.g., hepatitis B and HIV infection. In this context it may be relevant to note that a good and sustained CD4+ T cell response is generally required for slow progression in AIDS patients (45), who are also noted for functional defects within their HIV-specific CD8+ T cells (46). Thus, declining CD4+ T cell numbers in individuals already impaired in effector cell capability may be what precipitates the complete immunological collapse in AIDS patients.

References


