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*J Immunol* 2007; 178:1662-1670; doi: 10.4049/jimmunol.178.3.1662
http://www.jimmunol.org/content/178/3/1662

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Agonistic Anti-CD40 Antibody Profoundly Suppresses the Immune Response to Infection with Lymphocytic Choriomeningitis Virus

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Previous work has shown that agonistic Abs to CD40 (anti-CD40) can boost weak CD8 T cell responses as well as substitute for CD4 T cell function during chronic gammaherpes virus infection. Agonistic anti-CD40 treatment has, therefore, been suggested as a potential therapeutic strategy in immunocompromised patients. In this study, we investigated whether agonistic anti-CD40 could substitute for CD4 T cell help in generating a sustained CD8 T cell response and prevent viral recrudescence following infection with lymphocytic choriomeningitis virus (LCMV). Contrary to expectations, we found that anti-CD40 treatment of MHC class II-deficient mice infected with a moderate dose of LCMV resulted in severe suppression of the antiviral CD8 T cell response and uncontrolled virus spread, rather than improved CD8 T cell immune surveillance. In Ab-treated wild-type mice, the antiviral CD8 T cell response also collapsed prematurely, and virus clearance was delayed. Additional analysis revealed that, following anti-CD40 treatment, the virus-specific CD8 T cells initially proliferated normally, but an increased cell loss compared with that in untreated mice was observed. The anti-CD40-induced abortion of virus-specific CD8 T cells during LCMV infection was IL-12 independent, but depended partly on Fas expression. Notably, similar anti-CD40 treatment of vesicular stomatitis virus-infected mice resulted in an improved antiviral CD8 T cell response, demonstrating that the effect of anti-CD40 treatment varies with the virus infection studied. For this reason, we recommend further evaluation of the safety of this regimen before being applied to human patients. The Journal of Immunology, 2007, 178: 1662–1670.

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This work was supported in part by the Danish Medical Research Council, Lundbeck Foundation, and Novo Nordisk Foundation. C.B. was the recipient of a postdoctoral fellowship from the Danish Medical Research Council.

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Abbreviations used in this paper: DC, dendritic cell; LCMV, lymphocytic choriomeningitis virus; MHC II, MHC class II; WT, wild type; ICCS, intracellular cytokine staining assay; p.i., postinfection; MFI, mean fluorescence intensity; VSV, vesicular stomatitis virus; L, ligand.

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permanent deletion or transient anergy of the antiviral T cells (5, 36–39). The role of CD4 T help and costimulation in the antiviral CD8 T cell response and viral clearance has been intensively studied in this model. Acute infection of mice that lack CD4 T cells or CD40L initially results in viral clearance from most organs, reflecting a virtually intact primary CD8 effector T cell response (5, 33, 36). However, 2–4 mo postinfection (p.i.), recrudescence of virus was observed, and CD8 memory T cells were impaired in number and function (generation of cytotoxic effector cells and cytokine production) (4, 12, 13, 36, 40). In the present study, we investigated whether agonistic anti-CD40 could substitute for CD4 T cells in generating a sustained CD8 T cell response and prevent recrudescence of viral replication following LCMV infection.

Much to our surprise, we found that treatment with agonistic anti-CD40 in MHC II-deficient mice infected with a moderate dose of LCMV resulted in severe immune exhaustion and uncontrolled virus spread, rather than improved CD8 T cell memory. In wild-type (WT) mice, the antiviral CD8 T cell response collapsed prematurely, and virus clearance was delayed following treatment with anti-CD40. Based on these results, we recommend that extreme caution be exerted with regard to the therapeutic use of agonistic anti-CD40 during ongoing viral infection.

Materials and Methods

**Mice**

WT C57BL/6 mice were purchased from Taconic Farms. B6.SJL mice (B6.SJL-Pipp/iBoaAtac, CD45.1), MHC II−/− mice, and matched WT littermates, backcrossed five to six times onto a C57BL/6 background, were obtained from Taconic Farms. Transgenic mice (Tcr318) were expressed as PFU/g of tissue.

**Virus**

LCMV of the visceroctrotic Traub strain was produced, stored, and quantified as previously described (35). Mice to be infected received 200 PFU of the virus in an i.v. injection of 0.3 ml inoculation by this route results in a generalized and immunizing infection. Vascular stomatitis virus (VSV) of the Indiana strain (41) was used in a dose of 105 PFU i.v. This dose induces the progeny of breeding pairs provided by H. Pircher and R. M. Zinker-nagel (University of Zurich, Zurich, Switzerland), Fas-deficient mice (Fas−/−) and IL-12-deficient (IL-12−/−) mice, both on a C57BL/6 back-
ground, were obtained from The Jackson Laboratory. Mice were always allowed to rest for at least 1 wk before entering into experiments, at which time the animals usually were 7–9 wk old. All animals were housed under controlled (specific pathogen-free) conditions that included testing of sen-
tinels for unwanted infections according to the Federation of European Laboratory Animal Science Association standards. No such infections were detected.

**Flow cytometric analysis**

A total of 1 x 10^6 cells was stained with directly labeled mAbs in FACS medium (PBS containing 10% rat serum, 1% BSA, and 0.1% NaN3) for 20 min in the dark at 4°C. After washing twice, cells were fixed with 1% paraformaldehyde. For BrdU staining, cells were stained for surface markers as described above, resuspended in FACS medium (PBS containing 1% BSA, 0.1% NaN3, and 3 μM monen) and subsequently incubated with relevant surface Abs in the dark for 20 min at 4°C. Cells were washed twice in PBS before adding the anti-BrdU Ab. For BrdU staining, cells were stained for surface markers as described above, resuspended in FACS medium (PBS containing 1% BSA, 0.1% NaN3, and 3 μM monen) and subsequently incubated with relevant surface Abs in the dark for 20 min at 4°C. Cells were washed twice in PBS before adding the anti-BrdU Ab. After incubation for 30 min on ice, cells were washed with PBS and resuspended in PBS with 0.1% NaN3, and 0.5 μg/ml DNase I (Sigma-Aldrich). Following incubation for 15 min at 37°C, cells were washed once in FACS medium and resuspended in PBS with 0.5% saponin. After 10 min of incubation in the dark at 37°C, cells were pelleted and resuspended in PBS with 0.5% saponin and relevant Abs. For BrdU staining, cells were stained for surface markers as described above, resuspended in PBS plus 1% NaNO_2_ transferred to ice-cold 0.1 M NaCl solution, and fixed by adding ice-cold 96% ethanol drop by drop. After incubation for 30 min on ice, cells were washed with PBS and resuspended in PBS with 0.01% Tween 20 and 1% paraformaldehyde. After incubation for 1 h at room temperature, cells were pelleted and resuspended in PBS with 0.15 M NaCl and 4.2 mM MgCl_2, (pH 5) containing 50 Kunitz units/ml DNase I (Sigma-Aldrich). Following incubation for 15 min at 37°C, cells were washed once in PBS before adding the anti-BrdU Ab. After incubation with Ab for 30 min at room temperature, cells were washed in PBS and analyzed. Samples were acquired on a FACSCalibur (BD Biosciences), and at least 10^5 mononuclear cells were gated using
a combination of forward angle and side scatter to exclude dead cells and debris. Data were analyzed using CellQuest software (BD Biosciences).

Statistical evaluation

Results were compared using the Mann-Whitney U test. A value of $p < 0.05$ was considered as evidence of statistical significance.

Results

Agonistic anti-CD40 treatment leads to uncontrolled virus spread and rapid immune exhaustion in LCMV-infected MHC II$^{-/-}$ and WT mice

To investigate whether anti-CD40 could substitute for CD4 T cell help in preventing recrudescence of virus and impairment of CD8 T cell memory during LCMV infection, adult MHC II-deficient mice were infected with LCMV and treated 1 day later with either agonistic anti-CD40 or rat IgG for control. Lung virus titers were determined 28 and 80 days p.i. (Fig. 1A). In agreement with previous findings (4, 5, 40), virus was present in the lungs of LCMV-infected MHC II$^{-/-}$ mice treated with control Abs on days 28 and 80 p.i., whereas no virus could be detected in LCMV-infected WT mice. Contrary to expectations, treatment with agonistic anti-CD40 did not induce virus clearance in MHC II$^{-/-}$ mice. Instead, lung virus titers were increased by 3–4 logs in
these mice compared with control-treated, LCMV-infected MHC II mice.

Analysis of spleen cells from the same mice revealed a complete collapse of the LCMV-specific CD8 T cell response in infected, anti-CD40-treated MHC II−/− mice. Thus, in contrast to WT and control-treated MHC II−/− mice, CD8 T cells from α-CD40-treated MHC II-deficient mice failed to produce IFN-γ, TNF-α, and IL-2 following stimulation with GP33 (Fig. 1B), and the same pattern was observed for NP396-specific CD8 T cells (data not shown). Consistent with previous findings (40), the functional capacity of virus-specific CD8 T cells waned with time in control-treated MHC II−/− mice with respect to IL-2 production as well as the production of IFN-γ on a per cell basis (lower mean fluorescence intensity (MFI)).

Subsequent analysis disclosed that anti-CD40-induced impairment of the LCMV-specific CD8 T cell response occurred rapidly after infection. Already 10 days p.i., the number of GP33 and NP396 dextramer-binding CD8 T cells was reduced by 1 log (Fig. 2). Moreover, the virus-specific cells remaining in anti-CD40-treated mice produced reduced amounts of cytokine or no cytokine at all (data not shown). In contrast, the number and quality of LCMV-specific CD8 T cells was not affected in control-treated and untreated MHC II−/− mice at this early time point. Thus, in CD4 T cell-deficient mice, LCMV infection followed by anti-CD40 treatment caused an early collapse of the virus-specific CD8 T cell response and uncontrolled virus growth, rather than preventing recrudescence of virus following an initially controlled infection.

To investigate whether the negative effect of anti-CD40 treatment during LCMV infection was a phenomenon related to the deficiency of CD4 T cells in MHC II−/− mice, anti-CD40-treated, LCMV-infected WT mice were analyzed with regard to the antiviral CD8 T cell response and virus clearance. The total number of Ag-specific CD8 T cells was estimated on days 6, 8, 10, and 60 p.i. As seen in Fig. 3A, virus-specific CD8 T cell response and virus clearance. The total number of Ag-specific CD8 T cells was estimated on days 6, 8, 10, and 60 p.i. As seen in Fig. 3A, virus-specific CD8 T cell response followed by anti-CD40 treatment caused an early collapse of the virus-specific CD8 T cell response and uncontrolled virus growth, rather than preventing recrudescence of virus following an initially controlled infection.
Regarding quality, however, the negative effect of anti-CD40 treatment was marked also on days 8 and 60 p.i. (Fig. 3B). Correlating with the impairment of the virus-specific CD8 T cell response, organ virus titers on day 10 p.i. were 3–4 logs higher in anti-CD40-treated mice compared with matched controls (Fig. 3C).

In contrast to the findings in anti-CD40-treated, LCMV-infected MHC II-deficient mice, the viral infection in WT mice was almost completely controlled after 2 mo (Fig. 3C), despite the persistence of a markedly reduced and dysfunctional CD8 T cell subset. Thus, LCMV infection, combined with anti-CD40 treatment, resulted in inhibition of antiviral CD8 T cell responses and uncontrolled virus spread in both MHC II/−/− and WT mice. Whereas this situation seems irreversible in MHC II/−/− mice, WT mice eventually control the infection, despite long-standing impairment of the antiviral CD8 T cells.

Anti-CD40 treatment improves the antiviral CD8 T cell response during VSV infection

LCMV is a vicerotropic, highly replicating virus that induces a massive virus-specific CD8 T cell response. The profound activation of innate defense mechanism with production of many inflammatory signals (45) in combination with a high viral load might render LCMV-specific CD8 T cells particularly susceptible to exhaustive differentiation. To address this possibility, we asked whether the effect of anti-CD40 would be different in a viral model where activation of the innate immune system is less pronounced, viral replication is limited, and, consequently, the antigenic load is low (46, 47). For that purpose, VSV-infected mice treated with anti-CD40 or rat IgG were analyzed with respect to the number of virus-specific CD8 T cells identified as CD8+ VLA-4+ IFN-γ+ cells. anti-CD40 treatment negatively influences T cell expansion and proliferation in WT mice. C57BL/6 mice were infected i.v. with 200 PFU of LCMV and treated with 100 μg of anti-CD40 or rat IgG on day 1 p.i. Mice were given BrdU at 0.4 mg/ml in their drinking water the last 3 days before analysis. On the days indicated, spleens were analyzed for total cell numbers, B220, CD8, CD4, and BrdU expression. Averages ± SD are shown for four mice per group, *, p < 0.05 relative to matched controls.

Anti-CD40 treatment improves the antiviral CD8 T cell response during VSV infection

FIGURE 5. Monophasic, dose-dependent inhibition of the LCMV-specific CD8 T cell response in anti-CD40-treated, LCMV-infected mice. C57BL/6 mice were infected with 200 PFU of LCMV i.v. and treated with graded doses of anti-CD40 (100, 10, and 1 μg, respectively) on day 1 p.i. Control mice received 100 μg of rat IgG (RlG) instead of anti-CD40. Ten days p.i., spleens were harvested, incubated with viral peptide (GP33–41 and NP396–404) for 5 h, and analyzed for IFN-γ production by ICCS. The totals of GP33–41 (GP33)- and NP396–404 (NP396)-specific CD8 T cells identified as CD8+ VLA-4+ IFN-γ+ cells are shown as averages ± SD for three mice.

FIGURE 6. Anti-CD40 treatment negatively influences T cell expansion and proliferation in WT mice. C57BL/6 mice were infected i.v. with 200 PFU of LCMV and treated with 100 μg of anti-CD40 or rat IgG day 1 p.i. Mice were given BrdU at 0.4 mg/ml in their drinking water the last 3 days before analysis. A, Frequency of CD8 T cells labeled with BrdU on days 6, 8, and 10 p.i. Mean ± SD is depicted. B and C, Splenocytes were incubated for 5 h with GP33–41 peptide and analyzed for IFN-γ production by ICCS. B, Representative forward scatter (FSC)/side scatter (SSC) plots of gated CD8+ IFN-γ+ cells. The proportion of live and dead cells is defined in gates R1 and R2, respectively. C, The ratio between R1 and R2 is depicted. Points represent individual mice, *, p < 0.05 relative to matched controls.
cells at different time points p.i. Numbers refer to the percentage of proliferating donor (CD45.2
antigen-presenting cells (APC)).

The day after cell transfer, mice were infected with 200 PFU of LCMV and treated with anti-CD40 or rat IgG. A, Representative plots of gated CD8 T cells are shown. B, Averages ± SD for four mice are presented. *p < 0.05 relative to matched controls.

FIGURE 8. Normal cell cycle progression, but reduced CD8 T cell recovery in LCMV-infected, anti-CD40-treated mice. Spleen cells from TCR318 (CD45.2+) mice expressing a GP33-specific TCR were labeled with CFSE, and 2 × 10^7 cells were adoptively transferred into B6.SJL (CD45.1+) mice. The mice were infected with LCMV and treated with anti-CD40 or rat IgG. Representative plots of gated CD8 T cells at different time points p.i. Numbers refer to the percentage of proliferating donor (CD45.2+) CD8 T cells out of all donor CD8 T cells.

Anti-CD40-mediated inhibition of the LCMV-specific CD8 T cell response is dose dependent but monophasic

Even though 100 μg of anti-CD40 is the standard dose used for treatment of mice (17, 28, 31), we wanted to ascertain that our findings do not reflect a toxic overdose phenomenon. We therefore treated LCMV-infected B6 mice with graded amounts of anti-CD40 in an attempt to find a more optimal dose. As before, the antiviral CD8 T cell response was analyzed 10 days p.i. (Fig. 5).

Lowering the dose of anti-CD40 10- or 100-fold reduced the observed inhibition, but did not augment the CD8 T cell response relative to untreated mice, demonstrating that the dose-dependent effect of anti-CD40 is monophasic.

Normal initial proliferation, but increased cell loss, among virus-specific CD8 T cells following anti-CD40 treatment of LCMV-infected mice

To better understand what happens to the immune response following anti-CD40 treatment of LCMV-infected hosts, we first conducted a kinetics analysis of the virus-induced changes in cell numbers, cell subset distribution, and proliferative patterns (Fig. 6). Although the total of spleen cells in control mice increased with time, the number of splenocytes in anti-CD40-treated mice initially increased faster than in control mice, but then decreased markedly between days 8 and 10 p.i. Not surprisingly, the total number of B cells was increased in anti-CD40-treated mice, demonstrating the importance of CD40 ligation in activation of this cell subset. The anti-CD40 treatment also affected the number of CD4 T cells, although later and to a lesser extent than for CD8 T cells, which were significantly decreased already 8 days p.i. Likewise, as validated by BrdU incorporation, anti-CD40 treatment led to a reduction in the total number of proliferating CD8 T cells earlier and more markedly than in the case of proliferating CD4 T cells. Notably, the percentages of proliferating CD8 T cells were similar in anti-CD40-treated and rat IgG-treated animals until day 10 p.i. (Fig. 7). This discrepancy between the relative fraction of proliferating cells and total cell numbers strongly suggests that a higher proportion of activated CD8 T cells from anti-CD40 treated mice die as compared with the situation in control mice. This assumption was supported by an analysis of the ratio of live to dead cells (defined by forward scatter/side scatter) in the GP33-specific CD8 T cell pool on day 6 p.i. Thus, a significantly lower ratio of live to dead cells was found in the Ag-specific CD8 T cell pool from anti-CD40-treated mice compared with matched control mice (Fig. 7, B and C).

To further investigate the discrepancy between a high frequency of proliferating cells and poor T cell expansion in anti-CD40-treated mice, these parameters were analyzed using adoptive transfer of TCR-transgenic cells. Spleen cells from TCR318 (CD45.2+) mice expressing a GP33-specific TCR on approximately two-thirds of their CD8 T cells were labeled with CFSE and adoptively transferred into B6.SJL mice, which express CD45.1 on their lymphocytes. The day after cell transfer, mice were infected with LCMV and treated with anti-CD40 or rat IgG. Proliferation and total number of donor (CD45.2+) CD8 T cells were analyzed 60, 72, and 96 h p.i. As can be seen from Fig. 8, the donor cells in both cases gradually went through several rounds of cell division, and at 96 h the majority of donor CD8 T cells had divided several times, irrespective of host treatment. However, despite a similar proliferative pattern, significantly fewer donor CD8 T cells were recovered from anti-CD40-treated hosts at 72 and, particularly, 96 h p.i., again indicating that part of the Ag-specific CD8 T cells were lost as a consequence of anti-CD40 treatment (Fig. 8B).

Anti-CD40-mediated deletion of virus-specific CD8 T cells during LCMV infection occurs independently of IL-12, but partly through Fas-FasL interactions

Conditioning of DCs through CD40-CD40L interactions leads to the production of IL-12, which has been described to be needed as a third signal for CD8 T cell differentiation (48). However, high doses of IL-12 led to inhibition of CD8 T cell expansion and cytolytic capacity in LCMV-infected mice (49, 50). We therefore speculated that the anti-CD40-induced inhibition of virus-specific CD8 T cells during LCMV infection might be mediated through the induction of endogenous production of IL-12. To test this hypothesis, IL12β2−/− mice were infected with LCMV and treated with anti-CD40 or rat IgG the next day. Because apoptosis in many circumstances is initiated by Fas-FasL interactions (51), congenic Fas−/− mice were analyzed in parallel. Although the number of virus-specific CD8 T cells in untreated, LCMV-infected mice was
FIGURE 9. Anti-CD40-mediated exhaustion of virus-specific CD8 T cells during LCMV infection occurs independently of IL-12, but partly through Fas-FasL interactions. C57BL/6 (WT), IL-12−/− (IL-12), and Fas−/− (Fas) mice were infected with 200 PFU of Ld10, LCMV i.v. and treated with 100 μg of anti-CD40 or rat IgG on day 1 p.i. On day 10 p.i., splenocytes were incubated with viral peptide (GP33-41 or NP396-404) and analyzed for IFN-γ production by ICCS. A, Total CD8 T cell response. B, Representative histograms of gated CD8 T cells depicting the percentage (above the line) and MFI (below the line) of IFN-γ-stained cells following stimulation with GP33-41.

not influenced by the host genotype, significantly more GP33-specific CD8 T cells were recovered from Fas−/− compared with WT mice following anti-CD40 treatment, and the same trend was seen for NP396-specific CD8 T cells, although in this case the difference did not reach statistical significance. In contrast, the lack of IL-12 did not ameliorate the anti-CD40-induced impairment of the LCMV-specific CD8 T cell response (Fig. 9), indicating that this cytokine is not a decisive factor in the anti-CD40-mediated inhibition of effector T cell generation during LCMV infection. Thus, anti-CD40 treatment of LCMV-infected mice seems to trigger apoptosis in an IL-12-independent manner, involving the Fas-FasL pathway.

Discussion

Despite the diverse array of costimulatory molecules present on CD4 T cells, CD40 ligation on the APCs seems sufficient to provide the necessary signals required for optimal CD8 T cell stimulation (52). Thus, agonistic Abs to CD40 have successfully been used to boost weak CD8 T cell responses to tumors, vaccine-encoded Ags, and some infections in mice. Moreover, these Abs have experimentally been able to substitute for CD4 T cells in preventing recrudescence of latent MHV-68 infection. Together, these findings have raised hope that anti-CD40 therapy may be applied in the treatment of cancer patients and immunocompromised individuals such as HIV-infected patients.

In the present report, we demonstrate that treatment with agonistic Abs to CD40 in the experimental murine LCMV model causes CD8 T cell suppression and uncontrolled virus growth in MHC II−/− mice, whereas WT mice eventually controlled the infection, despite a chronically impaired CD8 T cell response. As early as 10 days p.i., the number of LCMV-specific CD8 T cells were significantly reduced in Ab-treated mice when analyzed by ICCS for IFN-γ as well as MHC/peptide dextramer binding, indicating that a great proportion of the virus-specific CD8 T cells were physically deleted. The few remaining Ag-specific CD8 T cells were severely impaired in cytokine production and seemed to remain so even after elimination of the infection. To our knowledge, this is the first report showing that CD40 ligation with a well-defined agonistic Ab may also induce severe immunosuppression.

We do not believe that our findings reflect a nonspecific, toxic effect of the anti-CD40 Abs used, since 100 μg of the FGK45 Ab clone is the standard dose used in mice (17, 28, 31). Moreover, although lowering the amount of Ab reduced CD8 T cell exhaustion, we did not observe an augmented response at any dose, indicating that the anti-CD40-mediated effect, although dose dependent, is monophasic. Finally, treating VSV-infected mice with the same dose of anti-CD40 significantly improves the virus-specific CD8 T cell response, demonstrating that 100 μg of agonistic anti-CD40 can be beneficial in another viral model.

In addition to our findings in the VSV model, anti-CD40 treatment was previously shown to have a positive effect in MHV-68-infected MHC II−/− mice by preventing the reactivation of latent virus normally observed under CD4 T cell-deficient conditions (31). In this case however, anti-CD40 treatment did not improve the antiviral CD8 T cell response, in contrast to most findings using agonistic CD40 Abs in CD4 T cell-deficient mice.

The opposing effect of anti-CD40 treatment observed in the LCMV vs the VSV and MHV-68 models most likely reflects differences in viral tropism and inflammatory signals. Thus, LCMV Traub is a viscerotropic virus that replicates extensively in the lymphoid organs and induces a very robust CD8 T cell response. In contrast, VSV replicates poorly in mice and MHV-68 replicates slowly, leading to a lower antigenic load and a more modest CD8 T cell response in both cases. That LCMV induces a more potent costimulatory environment than VSV has previously been documented by several studies (13, 45, 53), and we have recently shown that concurrent infection with LCMV improves the CD8 T cell response to VSV, directly demonstrating the inferior character of the costimulatory signals delivered during the latter infection compared with LCMV (54).

Even though a high antigenic load can induce tolerance in terms of either anergy or deletion of relevant T cells (34), this is hardly what triggers T cell exhaustion during combined LCMV infection and anti-CD40 treatment. Thus, the virus dose used causes acute transient infection and protective immunity in the absence of anti-CD40 and therefore does not have the potential by itself to induce tolerance. Moreover, Roth et al. (28) showed that agonistic CD40 Abs could break peripheral tolerance induction of self-reactive CD8 T cells in an adoptive transfer model. In this study, CD8 T cells expressing a transgenic TCR for the immunodominant GP33 LCMV epitope were tolerized when adoptively transferred into H8-transgenic mice expressing the GP33 epitope under the control of the MHC I promoter. However, if these recipients were subsequently treated with anti-CD40 Abs, the TCR-transgenic cells were activated and mediated severe immunopathology (28). Thus, fully functional Ag-specific effector CD8 T cells were induced upon anti-CD40 treatment despite a high antigenic load.

What then is the mechanism of the anti-CD40-mediated abor- tion of the antiviral CD8 T cell response during LCMV infection? Based on the literature, it was conceivable that IL-12 might be involved in the anti-CD40-induced suppression of LCMV-specific
CD8 T cells. Thus, CD40 ligation of DCs induces production of IL-12, and high levels of IL-12 during LCMV infection have been shown to inhibit antiviral CD8 T cell expansion and effector functions (49). Our results using IL-12β−/− mice do not, however, provide any support for a central role of this cytokine in the anti-CD40-mediated suppression of the LCMV-specific CD8 T cell response.

The increased cell death observed among the antiviral CD8 T cells from anti-CD40-treated mice, combined with the fact that T cell exhaustion is partially prevented in Fas−/− mice, strongly suggests that the majority of LCMV-specific CD8 T cells are driven to activation-induced apoptosis in Ab-treated mice (51). Because the proliferation of antiviral CD8 T cells is comparable in mice receiving anti-CD40 and mice receiving rat IgG early after LCMV infection, primed cells do not immediately undergo apoptosis. Interestingly, a recent article showed that proliferative potential does not necessarily correlate with a gain of effector function (55): therefore, it is possible that the LCMV-specific T cells may never reach a functionally mature state before they are silenced.

Our findings, with combined LCMV infection and anti-CD40 treatment in MHC II−/− and WT mice, very much resemble the situation seen after high-dose LCMV clone 13 infection alone (37). In the latter case, the antiviral CD8 T cell response was rapidly silenced after infection, and a chronic infection was established in both mouse strains. Depending on epitope specificity, the antiviral CD8 T cells were either anergized or physically deleted, and cell deletion was significantly delayed in FasL−/− mice (56). The chronic viral infection eventually resolved in WT mice, correlating with recovery of adequate, but still impaired effector activity in GP33-specific CD8 T cells. In contrast, in MHC II−/− mice, virus-specific CD8 T cells remained dysfunctional and virus persisted (37).

One way in which LCMV clone 13 mediates immunosuppression is believed to be through direct infection of DCs and subsequent changes in the maturational state of these cells (57, 58). This strategy may likely apply to other viremicotropic LCMV strains, including the LCMV-Docile and LCMV-Traub strains. It is possible, therefore, that direct infection combined with CD40 ligation simply overstimulates the DCs. The consequence of this may be hyperactivated T cells, which are prone to undergo premature activation-induced cell death (59). Because neither VSV nor MHV-68 provide the same strong and generalized activation of the innate immune system (13, 28, 45), this hypothesis also offers a plausible explanation for the different effects of anti-CD40 treatment during infection with these viruses vs LCMV. Interestingly, a recent in vitro study showed that agonistic Abs to CD40 induced maturation of immature human DCs, but apoptosis of mature human DCs, which subsequently failed to generate specific CD4 T cell responses (60). It is possible that anti-CD40 in our situation induces apoptosis of LCMV-infected DCs. However, the fact that LCMV-specific CD8 T cells initially expand faster in anti-CD40-treated mice compared with controls, exclude that the impairment of the CD8 T cell response is solely due to lack of initial stimulation. Rather, the LCMV-specific CD8 T cells receive sufficient stimuli by the DCs to go through several rounds of cell division before undergoing apoptosis.

A final possibility not considered above is that the Ab interacts with CD40 expressed on the LCMV-activated CD8 T cells themselves. Although we cannot rule out that possibility, we believe it is unlikely, because we have not been able to demonstrate CD40 expression on these cells with confidence (unpublished observation). The exact interplay among LCMV, anti-CD40, DCs, and antiviral CD8 T cells leading to apoptosis of the latter remains to be elucidated, but lies beyond the scope of this article.

The primary aim of the present report was to investigate whether agonistic Abs to CD40 could generally substitute for CD4 T cells and thus would prevent viral recrudescence in LCMV-infected MHC II−/− mice. In contrast to the observation in MHV-68-infected MHC II−/− mice (31), we found that treatment with agonistic CD40 Abs in combination with LCMV infection leads to early exhaustive differentiation of the antiviral CD8 T cells and uncontrolled virus spread in both MHC II−/− mice and WT mice. Hence, agonistic Abs to CD40 may sometimes, quite unexpectedly, inhibit the antiviral immune response. At present we do not know how often this might occur, but the phenomenon described in this study potentially applies to other viruses with the capacity to cause immunosuppression and/or persistent infection. Because immunocompromised persons, such as AIDS patients and cancer patients receiving chemotherapy, are highly susceptible to opportunistic infections with this type of virus, we call upon caution for the general use of CD40 mAb-based immunotherapy in these patient groups.

Acknowledgments

We thank Jörgen Scholler (DakoCytomation) for providing MHC/peptide dextramers and Sally Sarawar and Stephen Schoenberger (La Jolla Institute for Allergy and Immunology) for providing the anti-CD40 Ab.

Disclosures

The authors have no financial conflict of interest.

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