Immunosuppression by CD4$^+$ regulatory T cells induced by chronic retroviral infection

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Normal levels of CD4$^+$ regulatory T cells are critical for the maintenance of immunological homeostasis and the prevention of autoimmune diseases. However, we now show that the expansion of CD4$^+$ regulatory T cells in response to a chronic viral infection can lead to immunosuppression. Mice persistently infected with Friend retrovirus develop approximately twice the normal percentage of splenic CD4$^+$ regulatory T cells and lose their ability to reject certain tumor transplants. The role of CD4$^+$ regulatory T cells was demonstrated by the transmission of immunosuppression to uninfected mice by adoptive transfers of CD4$^+$ T cells. CD4$^+$ T cells from chronically infected mice were also immunosuppressive *in vitro*, inhibiting the generation of cytolytic T lymphocytes in mixed lymphocyte cultures. Inhibition occurred at the level of blast-cell formation through a mechanism or mechanisms involving transforming growth factor-β and the cell surface molecule CTLA-4 (CD152). These results suggest a possible explanation for HIV- and human T cell leukemia virus-I-induced immunosuppression in the absence of T cell depletion.

Long-term infections with retroviruses such as HIV and human T cell leukemia virus-I are associated with an increased risk for Kaposi's sarcoma (1, 2), non-Hodgkin's lymphoma (3, 4), anogenital cancers (5), and liver cancer (6). Although the depletion of HIV-infected CD4$^+$ T cells plays a major role in the immunosuppression associated with AIDS, it has also been shown that immunosuppression occurs before the onset of AIDS, and even before CD4$^+$ T cells become depleted (7). Little is currently known about the mechanisms of this HIV-induced immunosuppression *in vivo*.

We have used mice infected with Friend virus (FV) as an experimental model to study *in vivo* mechanisms of retrovirus-induced immunosuppression (8). We have been particularly interested in chronic or persistent FV infections because much of the damage caused by human retroviral infections is a result of chronic infection. Chronic infections with FV occur in strains of mice that are able to resolve acute infection by mounting strong virus-specific cytotoxic T lymphocyte (CTL), T helper, and B cell responses (9). Despite these immune responses, the virus is never completely eradicated, and the mice harbor lifelong but low level persistent infections. Persistent virus is primarily localized in a small fraction of splenic B cells, with very little infection in T cells (10). No clinical signs have previously been associated with chronic FV infections, and 95% of the animals live a normal lifespan. For unknown reasons, the remaining 5% relapse with FV-induced erythroleukemia (11).

Studies have shown that CD4$^+$ T cells are critical for the long-term control of persistent FV infections. Mice that are experimentally depleted of CD4$^+$ T cells reactivate FV and progress to erythroleukemia (10). One mechanism of CD4$^+$ T cell-mediated control of persistent FV is the production of IFN-γ, which has both direct and indirect antiviral activities (12). The current studies further probe the role of CD4$^+$ T cells in chronic FV infection. Paradoxically, we found that CD4$^+$ T cells in chronically infected mice were associated not only with protection from relapse of FV-induced erythroleukemia but also with a previously unknown form of FV-induced immunosuppression.

Materials and Methods

Mice. C57BL/10 (H-2^b), A.BY (H-2^b), and DBA/2 (H-2^d) mice were purchased from The Jackson Laboratory. (C57BL/10 $\times$ A.BY)F$_1$ mice were bred in the Rocky Mountain Laboratories and were used for *in vivo* experiments. All mice were females of 12–24 weeks of age at the beginning of the experiments and were treated in accordance with the regulations and guidelines of the National Institutes of Health and the Animal Care and Use Committee of Rocky Mountain Laboratories.

Viruses and Virus Infection. The FV stock was a complex of B-tropic Friend murine leukemia helper virus and polycythemia-inducing spleen focus-forming virus (13). Chronically infected mice were mice that had been rested for 2 months after i.v. infection with 1,500 spleen focus-forming units of virus.

Cell Lines. FBL-3 is an FV-induced tumor line from the C57BL/6 (B6) mouse (14). E♂G2 is an AKV/Gross' virus-induced tumor cell line from a B6 mouse (15). EL4 is a dimethylbenzanthracene-induced tumor cell line from a B6 mouse (16). EL4/HER2 is an EL4 cell clone transfected with the human oncogene HER2/NEU from SY-BR-3, a human breast-cancer cell line (American Type Culture Collection). P815 is a chemically induced mastocytoma from a DBA/2 mouse (17). LB27.4 is an H-2$^{b/d}$ class II$^+$ B cell hybridoma (18).

Tumor Inoculation and Measurement of Tumor Size. (B10 $\times$ A.BY)F$_1$ mice were injected intradermally on the dorsal region with 1×10^7 FBL-3 or E♂G2 cells, or 2×10^7 EL4/HER2 cells in 0.1 ml of RPMI medium 1640. Mean tumor diameter was measured with a micrometer two times a week.

Flow Cytometric Analyses. The following antibodies were used for surface labeling of cells: phycoerythrin-conjugated anti-CD4 (RM4–5), anti-CD8a (53–6.7), anti-CD25 (PC61), and anti-CD38 (90); FITC-conjugated anti-CD8a (53–6.7), anti-CD69 (H1.2F3), and anti-CD25 (7D4); biotin-conjugated anti-CD4 (RM4–5); and streptavidin-conjugated allophycocyanin (PharMingen). Dead cells were stained with propidium iodide and gated out of analyses. Samples of 10,000 cells each were analyzed on a FACSCalibur flow

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Abbreviations: FV, Friend virus; CTL, cytotoxic T lymphocyte; MLC, mixed lymphocyte culture; TGF-β, transforming growth factor-β.

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cytometry system (Becton Dickinson) with CELLQUEST software (Version 3.1, Becton Dickinson).

Cell Purification. Thy-1.2$^+$, CD4$^+$, and CD8$^+$ T cell subsets were positively purified from spleens of mice by using the MidiMACS separation system (Miltenyi Biotec, Auburn, CA). For the CD4$^+$CD69$^+$ and CD4$^+$CD69$^-$ subsets, CD4$^+$ cells were purified from pooled spleen cells by using mouse CD4$^+$ T cell enrichment columns (R & D Systems) and were stained with phycoerythrin (PE)-conjugated anti-CD69 mAb and washed three times. The negative and positive subpopulations then were purified by using anti-PE magnetic beads (Miltenyi Biotec).

Mixed Lymphocyte Cultures and Cytotoxicity Assay. A total of 1.5×10^6 purified Thy-1.2$^+$ T cells, 5×10^5 CD8$^+$ T cells, or 1×10^6 CD4$^+$ T cells (responder cells) were cultured in 24-well plates for 5 days with 5×10^6 irradiated (1,000 rad) allogeneic DBA/2 (H-2^d) spleen cells (stimulator cells) and 3.5×10^6 irradiated (1,000 rad) (B10 $\times$ A.BY)F$_1$ spleen cells (feeder cells) in 2.5 ml of RPMI medium 1640 containing 10% (vol/vol) FBS and 5×10^{-5} M 2-mercaptoethanol. Cultured cells were counted and tested for cytotoxicity. For the antibody blocking experiments, 25 μg/ml anti-IL10 receptor rat mAb (1B1.3a; ref. 19), 100 μg/ml anti-CD152 hamster mAb (UC10–4F10–11, PharMingen; refs. 20 and 21), or 20 μg/ml anti-TGF-β mouse mAb (1D11, R & D Systems; refs. 22 and 23) were added to the allogeneic mixed lymphocyte cultures (MLCs). All wells contained 25 μg/ml anti-Fc receptor mAb (24G.2) (PharMingen) to inhibit Fc-mediated antibody crosslinking. This concentration of anti-Fc receptor mAb did not change the generation of CTL activity. All mAbs were dialyzed overnight with RPMI medium 1640. Cytotoxic activity was measured with a ^{51}Cr-release assay as described (24).

Adoptive Transfers. Uninfected mice were adoptively transferred intravenously with 1×10^7 purified CD8$^+$ cells or 2×10^7 CD4$^+$ cells from spleens of chronically infected mice with growing FBL-3 tumors (14–25 days after tumor cell inoculation). These numbers of cells approximated the number of each cell subset in one spleen equivalent. The recipient mice were injected intradermally with 1×10^7 FBL-3 on the same day.

Results

Although it has been shown that infection with FV caused suppression of various host immune responses, immunosuppression was demonstrated only during the acute phase of infection and was restricted to mice that were highly susceptible to FV-induced erythroleukemia (25–27). To determine whether mice harboring chronic FV infections were also immunosuppressed, they were challenged with antigenic tumor transplants. Three types of rejectable mouse-cell tumors were studied: (*i*) E♂G2, a tumor induced by AKV, a murine leukemia virus that is antigenically distinct from Friend murine leukemia virus; (*ii*) EL4/HER2, a chemically induced tumor expressing the HER2 human oncogene; and (*iii*) FBL-3, a Friend murine leukemia virus-induced tumor that expressed FV antigens but did not produce live virus. When naive mice were transplanted with E♂G2, the tumors were rejected within 4 weeks (Fig. 1). Interestingly, only two of nine mice chronically infected with FV were able to reject these same tumors. There was not a complete loss of antitumor immunity, as most chronically infected mice were still able to reject EL4/HER2 tumors. Unexpectedly, the suppression of anti-tumor responsiveness extended to FV-induced FBL-3 tumors. Because FBL-3 expresses numerous FV antigens (28, 29) and can be used to immunize mice against acute FV-induced disease, we expected an anamnestic response rather than a suppressed one. The rejection of FBL-3 tumors is mediated predominantly by CD8$^+$ T cells (14), so the results suggested that the CD8$^+$ T cell response in these mice was suppressed.

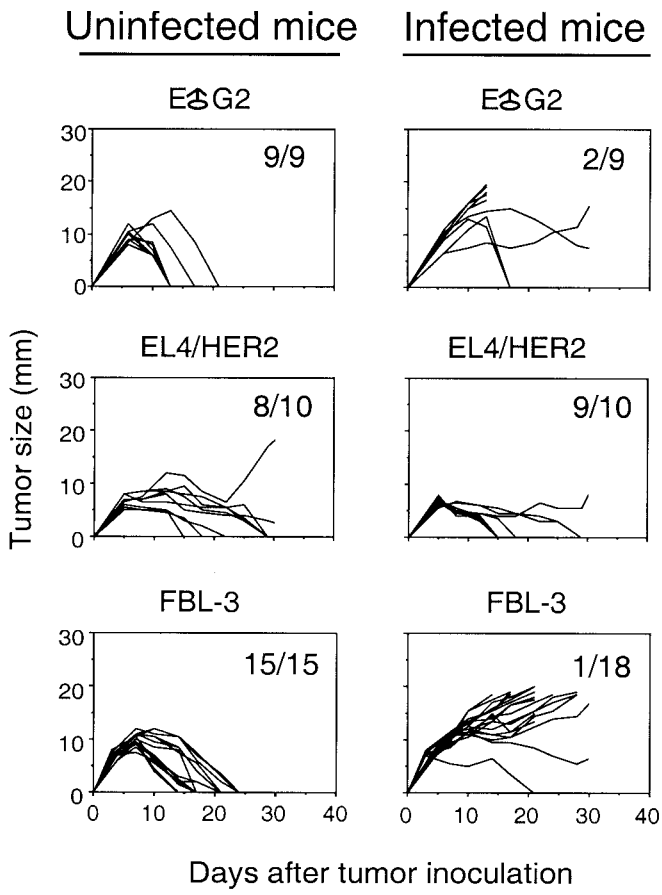

Fig. 1. Growth curves of tumors in uninfected mice and FV-infected mice. Each line represents tumor growth in a single animal transplanted with AKV-induced E♂G2 tumor cells, human oncogene-transfected EL4/HER2 tumor cells, or FV-induced FBL-3 tumor cells, as indicated. Numbers in the boxes indicate the number of mice that rejected their tumors over the total number of mice tested. Mice with complications caused by tumor metastasis or tumor size greater than 20-mm diameter were euthanized for humanitarian reasons.

To establish an *in vitro* method to analyze the immunosuppression of chronically infected mice further, allogeneic MLCs were tested for the generation of CTL responses. MLCs using T cells purified from chronically infected mice consistently generated 20–30% less cytolysis than uninfected mice, representing a 4-fold decrease in lytic units (Fig. 2*A*). The targets in this assay expressed MHC class I, but not class II, molecules, indicating that the generation of class I restricted CTL activity was suppressed. Furthermore, the suppression of allogeneic reactivity in MLCs demonstrated a broad immunosuppression not confined to FV-specific antigens.

To examine more thoroughly whether suppression was confined to MHC class I restricted CD8$^+$ T cells, MLCs using purified CD8$^+$ or CD4$^+$ T cells were tested for cytolytic activity against targets that expressed only MHC class I molecules or both class I and class II molecules, respectively. Surprisingly, when purified CD8$^+$ T cells were used for the MLCs, high cytotoxic reactivity was obtained from infected mice as well as uninfected mice (Fig. 2*B*). However, when purified CD4$^+$ T cells from infected mice were used for the MLCs, significantly less cytotoxic reactivity developed than when CD4$^+$ T cells from uninfected mice were used (Fig. 2*C*). Thus, in contrast to the results with whole T cells, the data from purified T cell subsets indicated that suppression was isolated to the CD4$^+$ T cell compartment.

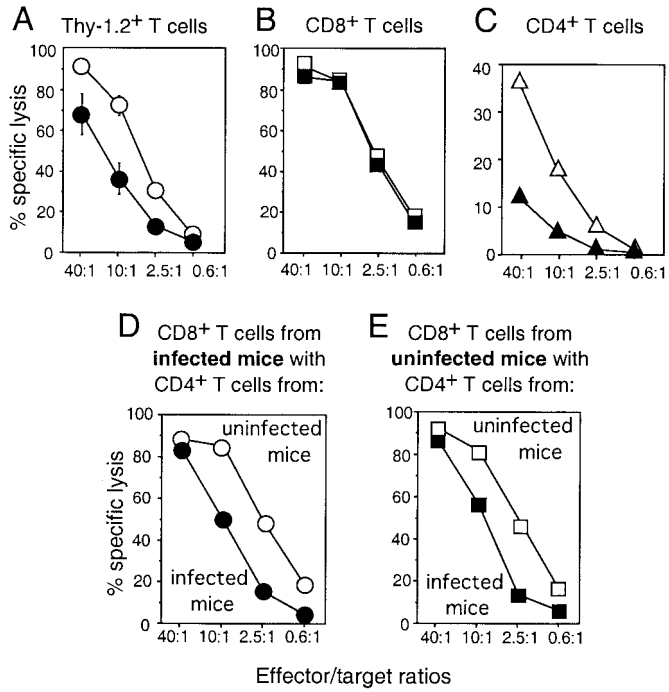

Fig. 2. Alloantigen-specific cytotoxic reactivity by MLC cells. T cells from spleens of either uninfected mice or chronically infected (B10 × A.BY)F_1 (H-2^b) mice were pooled, purified, cultured in MLC for 5 days, and tested for alloantigen-specific cytotoxic reactivity. (*A*) Thy-1.2$^+$ T cells from uninfected (○) or infected mice (●) were cultured and tested against P815 (H-2^d) target cells. Means and standard errors of five mice are shown. Two independent experiments showed similar results with statistically significant differences at the 40:1, 10:1, and 2.5:1 ratios ($P < 0.05$ by Student's *t* test). (*B*) Purified CD8$^+$ cells from pooled spleen cells of either uninfected (□) or infected (■) mice were cultured and tested against P815 target cells. Results shown for *B* and *C* are means from duplicate samples; two independent experiments gave similar results. (*C*) Purified CD4$^+$ cells from pooled spleen cells of either uninfected (△) or infected (▲) mice were cultured and tested against LB27.4 (H-2^d) MHC class II$^+$ target cells. (*D* and *E*) Pooled and purified CD8$^+$ cells from either infected (*D*) or uninfected (*E*) mice were cultured with purified CD4$^+$ cells from either uninfected mice (○, □) or infected mice (●, ■) in MLC and tested for cytotoxic reactivity against P815 targets. Results shown in *D* and *E* are means from duplicate samples; three independent experiments gave similar results.

One explanation that could reconcile these results is that the CD4$^+$ T cells from chronically infected mice were suppressing the generation of CD8$^+$ CTLs when the T cell populations were mixed. To test this hypothesis, MLCs were set up by using mixed or matched CD4$^+$ and CD8$^+$ T cells from infected or uninfected mice. CD4$^+$ T cells from infected mice inhibited the generation of CD8$^+$ CTLs in MLCs regardless of whether the CD8$^+$ T cells came from infected or uninfected mice (Fig. 2 *D* and *E*). In contrast, there was no inhibition by CD4$^+$ T cells from uninfected mice. Thus, FV infection had induced or expanded a population of inhibitory CD4$^+$ T cells that could suppress the generation of both CD8$^+$ and CD4$^+$ CTLs *in vitro*.

To determine whether CD4$^+$ T cells were also involved in the suppression of antitumor responses *in vivo*, CD4$^+$ and CD8$^+$ T cells were purified from the spleens of chronically infected mice and adoptively transferred to uninfected mice. The recipient mice were transplanted with FBL-3 tumors on the same day. Mice adoptively transferred with CD8$^+$ T cells rejected tumor transplants in a normal manner (Fig. 3 *Left*). In contrast, all mice adoptively transferred with CD4$^+$ T cells failed to reject FBL-3 tumors (Fig. 3 *Right*).

Flow cytometry was used to determine whether chronically infected mice had altered subpopulations of CD4$^+$ T cells

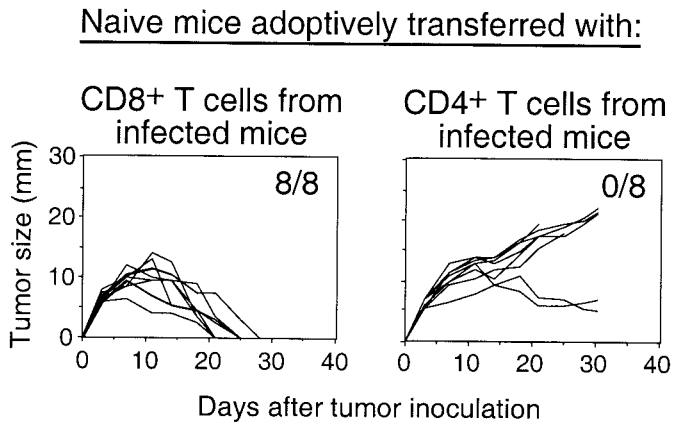

Fig. 3. Adoptive transfer of suppression. Purified CD8$^+$ T cells (*Left*) or purified CD4$^+$ cells (*Right*) from spleens of chronically infected/FBL-3 transplanted mice were transferred into uninfected mice. The recipients were inoculated intradermally with 1×10^7 FBL-3 tumors on the same day of the cell transfer. Numbers in the boxes indicate the number of mice that rejected their tumors over the total number of mice tested.

expressing CD25 or CD38, cell surface molecules associated with regulatory cells (30, 31). We also analyzed the CD69 early activation marker because previously we had found significantly increased levels of CD4$^+$CD69$^+$ cells in chronically infected mice. The subset of CD4$^+$ T cells expressing CD38 and the subset expressing CD69 were increased in chronically infected mice, as was the subpopulation that stained positive for both these markers (Table 1). In addition, the subpopulation of CD25$^+$ cells coexpressing CD69 was also increased in the chronically infected mice. In terms of real cell numbers rather than percentages, the persistently infected mice never fully recover from the FV-induced splenomegaly that occurs during acute infection, and their spleens contain an average of twice as many cells as naive mice (3.8×10^8 cells per spleen vs. 1.8×10^8 cells per spleen). Thus, the increase in the percentage of CD4$^+$ T cells expressing markers associated with regulatory T cells also reflects an increase in absolute number.

Table 1. Comparative expression of cell surface markers on T cells in persistently infected versus uninfected mice

Cell groups	Uninfected, % Mean	Uninfected, % SD	Infected, % Mean	Infected, % SD	*P* value (Student's *t* test)
T cell subsets in the spleen					
CD8$^+$	7.8	0.4	7.3	3.0	0.8585
CD4$^+$	14.1	1.3	16.1	1.8	0.4530
Various subsets of CD4$^+$ cells					
CD25$^+$	15.7	1.1	18.6	2.1	0.0505
CD38$^+$	**14.3**	**1.9**	**22.6**	**3.0**	**0.0034**
CD69$^+$	**14.3**	**3.2**	**26.4**	**3.2**	**0.0018**
CD25$^+$ CD69$^-$	9.9	0.8	8.7	0.7	0.0827
CD25$^+$ CD69$^+$	**5.9**	**0.7**	**9.9**	**2.1**	**0.0113**
CD25$^-$ CD69$^+$	**9.7**	**0.9**	**17.3**	**1.8**	**0.0003**
CD38$^+$ CD69$^-$	6.8	1.4	8.8	0.9	0.0531
CD38$^+$ CD69$^+$	**7.3**	**1.1**	**13.8**	**2.4**	**0.0026**
CD38$^-$ CD69$^+$	**7.0**	**1.0**	**11.4**	**1.9**	**0.0058**
CD38$^+$ CD25$^-$	**9.9**	**2.2**	**16.0**	**1.2**	**0.0029**
CD38$^+$ CD25$^+$	**2.6**	**0.6**	**4.3**	**1.0**	**0.0280**
CD38$^-$ CD25$^+$	8.0	0.9	7.5	1.4	0.6325

Bold indicates statistically significant differences between infected and uninfected mice. $n = 4$ for both infected and uninfected groups.

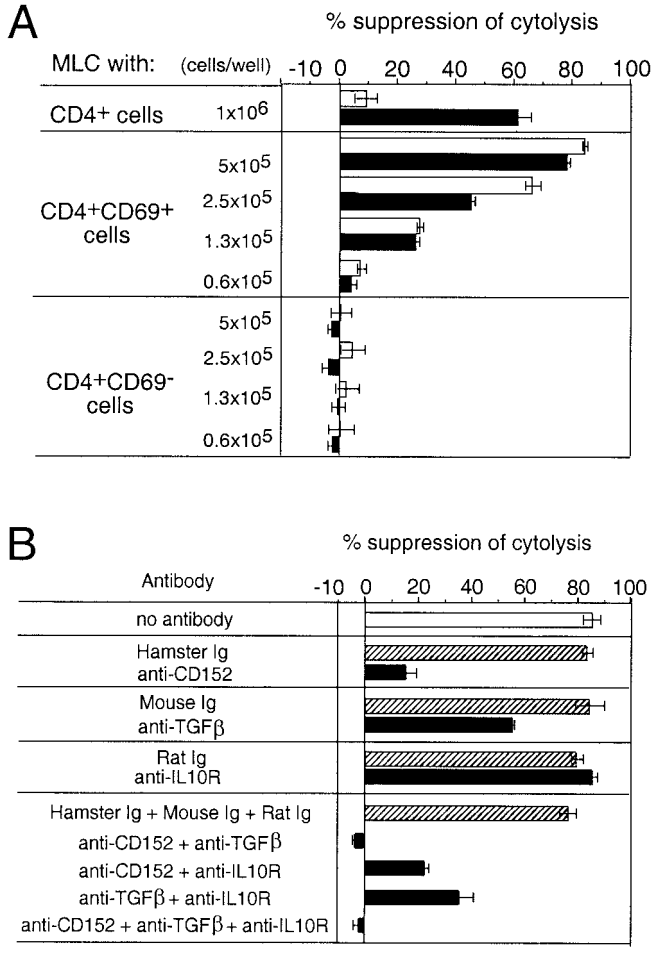

Fig. 4. $CD4^+CD69^+$ T cell-mediated suppression of alloantigen-specific CTL generation. (*A*) Purified $CD8^+$ T cells from uninfected (B10 × A.BY)F_1 mice and the indicated numbers of purified $CD4^+$ T cell subsets from either chronically infected (black bars) or uninfected (B10 × A.BY)F_1 mice (white bars) were cultured in MLC. Cultured cells were tested for cytolytic activity against P815 target cells. The bars show percent suppression of specific cytolysis at a 40:1 effector-to-target ratio at the specific lysis of 92.7%. Error bars were determined from triplicate wells of a single assay. Similar results were obtained from this experiment at the 10:1 effector-to-target ratio and also from multiple target-to-effector ratios in an independent experiment. $CD4^+$ T cells from spleens of the infected mice in this assay were 31.8% $CD69^+$, whereas the $CD4^+$ T cells from spleens of the uninfected mice were 17.5% $CD69^+$. The purity of all purified T cell subsets was greater than 92%. (*B*) Blocking of $CD4^+CD69^+$ cell-mediated suppression with mAbs. Anti-CD152 mAb, anti-TGF-β mAb, or anti-IL-10 (IL10R) receptor mAb were added to MLC containing $CD8^+$ T cells from uninfected mice and 3×10^5 $CD4^+CD69^+$ cells from chronically infected (B10 × A.BY)F_1 mice. The same concentrations of control antibodies were added as indicated. Cultured MLC cells were harvested and tested for cytotoxicity against P815 cells, and the percentage of suppression of specific lysis was calculated for the 40:1 effector-to-target ratio. Error bars were determined from triplicate wells of cytotoxic assay; similar results were obtained from the 10:1 effector-to-target ratio as well as from an independent experiment.

The high expression levels of CD69 on the $CD4^+$ T cells allowed us to use this marker to do an immunomagnetic bead purification and test whether this subset could suppress the generation of $CD8^+$ CTLs in allogeneic MLCs. Interestingly, the $CD4^+CD69^+$ cells from both infected and uninfected mice showed similar dose-dependent suppressive effects (Fig. 4*A*). In contrast, the $CD4^+CD69^-$ cells were not suppressive. Thus, CD69 marked a subpopulation of splenic $CD4^+$ T cells that were enriched for immunosuppressive activity regardless of FV infection. The reason for the difference between infected and uninfected mice when using total $CD4^+$ cells seems to be the quantitative increase in the subpopulation of $CD4^+CD69^+$ regulatory cells in the infected mice.

To analyze possible mechanisms of $CD4^+CD69^+$ T cell-mediated suppression, we added antibodies to the MLC to block previously described mediators of suppression: transforming growth factor-β (TGF-β), a cytokine with potent immunosuppressive capacity; IL-10R, the receptor for the immunosuppressive cytokine, interleukin 10 (32–34); and CTLA-4 (CD152), a cell surface molecule that transduces suppressive regulatory signals (21, 35, 36). The $CD4^+CD69^+$ T cell-mediated suppression of allogeneic CTL generation was partially reversed by the addition of anti-CD152 mAb or anti-TGF-β mAb (Fig. 4*B*). Furthermore, an additive effect was obtained when both CD152 and TGF-β were blocked; anti-IL-10 receptor mAb did not block. Flow cytometric analysis of the cultures showed that the $CD8^+$ cells did not undergo blast cell formation when $CD4^+CD69^+$ regulatory cells were present, although the levels of IL-2 at 12 and 24 hr were roughly equivalent to the cultures containing $CD4^+CD69^-$ cells (data not shown). Thus, chronic FV infection induced the expansion of $CD4^+CD69^+$ regulatory T cells that suppressed the proliferation of $CD8^+$ T cells *in vitro* by means of mechanisms involving CD152 and TGF-β, but not IL-2 or IL-10.

Discussion

These results demonstrate a mechanism of virus-induced immunosuppression by means of the generation or expansion of $CD4^+$ regulatory T cells. Such cells compose a normal subset of cells that actively suppress immune responses directed against self antigens (30, 37). Suppressive activity by regulatory T cells has been well documented by experiments in which adoptive transfers of these cells have protected mice and/or rats against experimental autoimmune encephalitis (23, 38, 39), autoimmune colitis (32, 33), and autoimmune diabetes (40–44). There is also extensive evidence that regulatory T cells can mediate tolerance to transplantation with foreign tissues (45). However, our study provides clear evidence that an overabundance of $CD4^+$ regulatory cells can lead to immunosuppression.

The transfer of suppression to uninfected mice by adoptive transfers of $CD4^+$ T cells obtained from chronically infected mice (Fig. 3) is reminiscent of studies showing that T cells could transfer tolerance from one animal to another in a process termed "infectious tolerance" (46). We also found that FBL-3 tumor rejection in chronically infected mice could be partially restored by *in vivo* depletions of $CD4^+$ T cells (data not shown). It makes little sense to improve immune responsiveness by depleting $CD4^+$ T cells unless that population contains cells with suppressor activity. Because FBL-3 tumor rejection *in vivo* is mediated by $CD8^+$ T cells (14), it seems that the $CD4^+$ regulatory cells act on $CD8^+$ T cells to inhibit their function. We found a similar situation *in vitro* where $CD4^+$ regulatory T cells acted to reduce generation of $CD8^+$ CTLs in MLCs. Furthermore, the subset of $CD4^+$ T cells that coexpressed CD69 and that was increased in the chronically infected mice was immunosuppressive *in vitro*. Taken as a whole, the results provide strong evidence that $CD4^+$ regulatory T cells mediate the immunosuppression of chronically infected mice. Several different mechanisms of suppression by $CD4^+$ T cells have been described, including TGF-β secretion stimulated by cell-to-cell signaling through CD152 (CTLA-4; refs. 47–49). Our antibody blocking data are consistent with such a mechanism; in contrast, we found no evidence of involvement for IL-10 (33, 50, 51).

CD69 has been described as an early marker of activation on lymphocytes (52), but our data show that it is also expressed on many $CD4^+$ regulatory cells as well. We do not propose that CD69 is a specific marker for $CD4^+$ regulatory cells, but rather

that $CD4^+$ regulatory cells can be enriched by sorting them on the basis of CD69 expression. Interestingly, both CD25, the cell surface marker most commonly associated with immunoregulatory cells, and CD38, another marker described on immunoregulatory cells, are described also as activation markers (31, 53, 54). Presently, it is unclear why molecules associated with activation also are associated with suppression. However, once specifically activated, $CD4^+$ regulatory T cells have been demonstrated to suppress immune responses in a nonspecific manner (41, 55), which may possibly explain the broad nature of the immunosuppression in our studies.

It will be of great interest to determine whether specific viral gene products, such as the highly conserved immunosuppressive peptide found in the envelope protein of many retroviruses (56), are responsible for generating regulatory T cells, or whether it is simply the persistence of the infection that induces peripheral tolerance. Also, it will be interesting to determine whether these findings have any relevance to human retroviral infections. In that regard, it has been shown that patients with HIV have a significant increase in $CD4^+$ T cells expressing CD38 (57–59), but those cells have been considered ''activated,'' and their possible role in immunosuppression has not been studied. Interestingly, another cell surface marker on $CD4^+$ T cells that is up-regulated in patients with HIV is a molecule known as regeneration and tolerance factor (RTF; ref. 60). Although cells expressing RTF also have been considered activated, the association between RTF and the generation of peripheral tolerance (61, 62) suggests that $CD4^+$ T cells expressing RTF may be involved in immunosuppression. It is possible that the ability of HIV and human T cell leukemia virus-I to escape immunological destruction and establish persistent infections is related to their ability to suppress the immune system through the generation of $CD4^+$ regulatory cells. If human retrovirus-induced immunosuppression is even partly caused by regulatory cells, then this aspect of the disease may be amenable to treatment with immunomodulatory therapies.

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