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Blockade of CTLA-4 Signals Inhibits Th2-Mediated Murine Chronic Graft-Versus-Host Disease by an Enhanced Expansion of Regulatory CD8\(^+\) T Cells\(^1\)

Jinkyo Sakurai,*,† Junko Ohata,*, Kiyoshi Saito,*, Hiroaki Miyajima,‡ Takao Hirano,§ Takao Kohsaka,*, Shoji Enomoto,† Ko Okumura,¶ and Miyuki Azuma\(^2,*\)

CTLA-4 (CD152) is thought to be a negative regulator of T cell activation. Little is known about the function of CTLA-4 in Th2-type immune responses. We have investigated the effect of initial treatment with anti-CTLA-4 mAb on murine chronic graft-vs-host disease. Transfer of parental BALB/c splenocytes into C57BL/6 \(\times\) BALB/c F\(_1\) mice induced serum IgE production, IL-4 expression by donor CD4\(^+\) T cells, and host allo-Ag-specific IgG1 production at 6–9 wk after transfer. Treatment with anti-CTLA-4 mAb for the initial 2 wk significantly reduced IgE and IgG1 production and IL-4 expression. Analysis of the splenic phenotype revealed the enhancement of donor T cell expansion, especially within the CD8 subset, and the elimination of host cells early after anti-CTLA-4 mAb treatment. This treatment did not affect early IFN-\(\gamma\) expression by CD4\(^+\) and CD8\(^+\) T cells and anti-host cytolytic activity. Thus, blockade of CTLA-4 greatly enhanced CD8\(^+\) T cell expansion, and this may result in the regulation of consequent Th2-mediated humoral immune responses. These findings suggest a new approach for regulating IgE-mediated allergic immune responses by blockade of CTLA-4 during a critical period of Ag sensitization. *The Journal of Immunology, 2000, 164: 664–669.

Cytotoxic T lymphocyte-associated Ag-4 (CD152), a homologue of CD28, is a glycoprotein expressed on the surface of activated T cells and shares its ligands, CD80 and CD86 (CD80/86),\(^3\) with CD28. CTLA-4 is hardly detectable on resting T cells and is transiently induced only after activation. The amount of CTLA-4 expressed on activated T cells is only 3–5% of the amount of cell-surface CD28 (1). Cell-surface expression of CTLA-4 is regulated by its rapid clearance from the surface of activated T cells through clathrin-mediated endocytosis (2–4). Despite low surface expression, CTLA-4 has a much higher avidity for CD80/86 as compared with CD28 (5, 6). Cross-linking of CTLA-4 has a negative regulatory effect on T cells by down-regulating IL-2 production, IL-2R expression, and cell cycle progression in vitro (7–9). In murine experimental disease models, blockade of CTLA-4 with anti-CTLA-4 mAb enhances T cell responses against nominal Ag (10), established tumors (11), and autoantigens (12–14). Recent results further suggest a critical role of CTLA-4 for induction and maintenance of peripheral tolerance in autoimmunity (15, 16) and allograft survival (17, 18). In Th2-dominant immune responses, CTLA-4 blockade accelerated responses against the nematode, *Nippostrongylus brasiliensis*, which induced a typical Th2-type immune response characterized by IL-4 and IL-5 cytokine production with resulting eosinophilia, mastocytosis, and IgE production (19).

A graft-vs-host disease (GVHD) can be caused in inbred F\(_1\) mice by the injection of T cells of parental origin. The injection of C57BL/6 (B6) splenocytes into F\(_1\) mice (referred as B6 GVH) results in an “immunosuppressive” acute GVHD characterized by an anti-host cell-mediated Th1 cytokine-driven disease (20–22). By contrast, the injection of splenocytes from the BALB/c or DBA/2 parent into F\(_1\) mice (referred as BALB/c GVH or DBA GVH) results in an “immunostimulatory” chronic GVHD characterized by an autoantibody-mediated Th2 cytokine-driven disease (23–25). Blockade of both CD28 and CTLA-4 signals by a combination of anti-CD80/CD86 mAbs or CTLA-4Ig fusion protein efficiently inhibited both types of GVHD (26–29). We recently demonstrated the regulatory function of the CTLA-4–CD80/86 pathway in the development of acute GVHD (30). Treatment with anti-CD80/86 mAbs in the CD8-dependent B6 GVH model induced by donor T cells lacking CD28 expression exacerbated the clinical manifestations of acute GVHD and increased T cell responses against host alloantigen. However, the function of CTLA-4 in the development of chronic GVHD induced by Th2-dominant immune responses has not been investigated. In this study, we have examined the effects of initial blockade of CTLA-4 signals after donor T cell transfer in a parent into F\(_1\) BALB/c GVH model.

Materials and Methods

**Mice**

Female BALB/c (H-2\(^b\)) donor mice and (BALB/c \(\times\) C57BL/6)F\(_1\) (CBF\(_b\), H-2\(^b\)) recipient mice were purchased from SLC (Hamamatsu, Shizuoka, Japan). Donors were 6–8 wk old and recipients were 8–10 wk old at the time of transfer.

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Monoclonal Abs

A hybridoma-producing anti-mouse CTLA-4 mAb (UC10.4F10.11, hamster IgG) was generously provided by Dr. Jeffrey A. Bluestone (University of Chicago, Chicago, IL). This mAb was purified from ascites as described (31). Fab of anti-CTLA-4 mAb were prepared as described (7), the purity was confirmed by SDS-PAGE analysis, and the similar enhancing activities between intact mAb and Fab were confirmed in allogenic MLR responses in vitro as described (7). The pyrogen level was <0.01 ng/μg protein as determined by a Limulus amebocyte lysate assay. Hybridomas producing mAbs against I-Aβ, I-Ad, I-Aq (M5/114, rat IgG2b), CD24 (HSA, J11d, rat IgM), and CD8 (3.155, rat IgG) were obtained from American Type Culture Collection (Manassas, VA) and were used as culture supernatants. Monoclonal Abs against the following Ags were used for immunofluorescence analysis: CD3 (145-2C11, hamster IgG), CD4 (RM4-5, rat IgG2a), CD8a (53-6.7, rat IgG2a), CD45R/B220 (RA3-6B2, rat IgG2a), CTLA-4 (UC10-4F10, hamster IgG), H-2Kb (AF6-89.5, mouse IgG2a), IFN-γ (XMG1.2, rat IgG1), and IL-4 (BVD4-1D11, rat IgG2b). All FITC-, PE-, and peridinin chlorophyll-a protein (PerCP)-conjugated mAbs were obtained from PharMingen (San Diego, CA). Immunofluorescence, flow cytometry, and data analysis were performed using FACSsort and CellQuest software (Becton Dickinson Immunocytometry Systems, San Jose, CA), as described previously (32).

Induction of chronic GVHD and in vivo treatment with anti-CTLA-4 mAb

Single-cell suspensions of splenocytes from BALB/c mice were used as the source of GVHD-causing T cells. Unirradiated CBF1 recipients received 1 × 10^8 splenocytes diluted in 0.5 ml PBS by i.v. injection. Recipients receiving splenocytes were randomly divided into two groups of 6–12 mice and treated with either control hamster Ig (PharMingen) or anti-CTLA-4 (4F10) mAb. One hundred micromgams of mAb or control Ig per mouse were injected i.p. on day 0, 1, and 2 and then every other day until day 12 posttransfer. In certain experiments, BALB/c splenocytes were treated with anti-I-A, anti-CD24, and anti-CD8 mAbs and rabbit complement to deplete APC and CD8+ T cells. As assessed by flow cytometry, this procedure resulted in <3% contaminating CD8+ T cells and >90% purity of CD4+T cells. CBF1 recipients received 3 × 10^7 CD8-depleted T cells and were treated with mAb as described above.

Measurement of serum IgE

Mice were bled from the retro-orbital plexus under ether anesthesia every 3 wk, and serum samples were individually aliquoted and stored at –80°C. The quantitation of total IgE and IgE allotypes was determined by ELISA as described previously (33–36).

Determination of IgG1 and IgG2a Ab against host alloantigen

EL4 T lymphoma cells (H-2b, 1 × 10^6 cells/100 μl) were incubated with the serially diluted (×40, ×100, ×200, ×1000) serum samples (100 μl each) from either normal or recipient CBF1 mice for 45 min at 20°C, washed once, and then incubated with either FITC-conjugated goat anti-mouse IgG1 (Southern Biotechnology Associates, Birmingham, AL) or biotinylated-rabbit anti-mouse IgG2a Ab (Zymed, San Francisco, CA) for 30 min at 20°C in the dark. For detection of biotinylated Ab, fluorescence was visualized by FITC-streptavidin (Dako-Japan, Kyoto, Japan). Samples were analyzed by flow cytometry. Mean fluorescence intensity (MFI) was calculated by subtracting the MFI of cells stained with the same dilution of sera from normal CBF1 and the second Ab or FITC-streptavidin from the MFI of cells stained with recipient sera.

Multicolor staining for intracellular cytokines and cell-surface Ags

Single-cell suspensions of splenocytes from recipient mice were stained with PMA (50 ng/ml; Sigma, St. Louis, MO) and ionomycin (250 ng/ml; Sigma) in the presence of brefeldin A (5 μg/ml) for 4 h. After washing with staining buffer (PBS with 1% FCS and 0.1% sodium azide), cells were incubated with FITC-, PE-, and/or PerCP-conjugated mAb against cell-surface Ags as usual. Cells were fixed with 100 μl of fixation buffer (4% paraformaldehyde in PBS) at 4°C for 20 min and then washed twice with staining buffer. The fixed cells were resuspended in 50 μl of permeabilization buffer (PBS with 1% FCS, 0.1% sodium azide, and 0.1% saponin) with appropriate fluorochrome-conjugated anti-cytokine mAb at 4°C for 30 min. Stained cells were washed twice with permeabilization buffer, resuspended in 200 μl of staining buffer, and then analyzed by flow cytometry.

Results

An initial treatment with anti-CTLA-4 mAb inhibits Th2-mediated chronic GVHD

To investigate the effect of anti-CTLA-4 mAb treatment, GVHD was induced by injecting parent BALB/c spleen cells into CBF1 mice. Recipient mice were injected i.p. with 100 μg of anti-CTLA-4 (4F10) mAb eight times until day 12. The elevation of serum IgE, which is a characteristic feature for chronic GVHD, was examined every 3 wk after splenocyte transfer. In the control Ig-treated mice, the levels of total serum IgE started to increase at 3 wk posttransfer, reached a maximum at 9 wk, and remained high until 15 wk (Fig. 1). In contrast, the group of mice treated with anti-CTLA-4 mAb showed an elevation of total IgE at 6 wk, but an additional enhancement was not observed at later time points. The serum IgE was decreased to half of that in the control mice from 9 to 15 wk. Both IgEa and IgEb allotypes were detected and responded in a similar manner. IgEa was produced from both donor BALB/c and host CBF1 cells, while IgEb was produced by host CBF1 cells alone. These results suggest that both donor and host B cells were activated. Autoantibodies against dsDNA in both groups of mice were increased in parallel with serum IgE (data not shown). To verify the antagonistic activity of anti-CTLA-4 mAb, Fab of anti-CTLA-4 mAb were also injected. The use of Fab also inhibited total serum IgE, although the inhibitory effect was not as effective as the intact anti-CTLA-4 mAb (Table I). This may come from less binding abilities or a rapid clearance of Fab.

We next examined Ab production against host alloantigen. Either IgG1 or IgG2a Ab against host alloantigen at 3 and 12 wk was measured by reactivity with EL-4 lymphoma cells (H-2b) using isotype-specific Abs by flow cytometry. The anti-host IgG1 titer
Table I.  Anti-CTLA-4 Fab also inhibit serum IgEx

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum IgE (µg/ml)</th>
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<tbody>
<tr>
<td>Control Ig</td>
<td>9.8 ± 8.8</td>
</tr>
<tr>
<td>Anti-CTLA-4 Fab</td>
<td>7.9 ± 4.7</td>
</tr>
<tr>
<td>Anti-CTLA-4 mAb</td>
<td>0.1 ± 0.0</td>
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x Chronic GVHD was induced as described in Materials and Methods. Three groups of six mice were treated with either control hamster Ig, intact anti-CTLA-4 mAb, or Fab of anti-CTLA-4 mAb as described in Fig. 1. Total serum IgE were measured by ELISA. Values are the mean ± SD, and the data are representative of two separate experiments.

was elevated in the GVHD mice at 3 wk, and this was further increased at 12 wk. This enhancement was significantly reduced by anti-CTLA-4 treatment (Fig. 2). In contrast, the anti-host IgG2a titer was not elevated at 3 wk, and this was not affected by anti-CTLA-4 treatment. The IgG2a titer at 12 wk was slightly enhanced, and the treatment with anti-CTLA-4 mAb minimally reduced this response. These results suggest that Th2 cytokine-dependent IgE and IgG1 production during the late phase of GVHD was inhibited by the anti-CTLA-4 treatment.

To investigate the cytokine profile in GVHD mice, intracellular expression of IL-4 and IFN-γ in splenic CD4+ or CD8+ T cells at 2 and 8 wk posttransfer was analyzed by flow cytometry. Although IL-4 was not detected in CD4+ T cells from normal CBF1 mice, a clear induction of IL-4 production was observed in CD4+ T cells in the GVHD mice at 8 wk, but not at 2 wk (Fig. 3). IL-4 was preferentially observed in donor cells. Treatment with anti-CTLA-4 mAb significantly reduced the percentage of IL-4-producing CD4+ T cells. IFN-γ expression in CD4+ T cells from mice with GVHD was slightly enhanced at 2 and 8 wk compared with control CBF1 mice. However, the anti-CTLA-4 treatment did not significantly affect IFN-γ production at either early or late time points. In contrast with CD4+ T cells, IFN-γ expression in CD8+ T cells was clearly augmented at 2 wk posttransfer in both control and anti-CTLA-4-treated mice. Interestingly, a clear reduction of IFN-γ-producing CD8+ T cells was observed in control mouse, but not in the anti-CTLA-4-treated mice at 8 wk. Our results demonstrated that the anti-CTLA-4 treatment suppressed the generation of IL-4-producing Th2 cells but increased IFN-γ expression in CD8+ T cells during the late phase of disease.

An initial treatment with anti-CTLA-4 mAb enhances CD8+ T cell expansion

To investigate how Th2-mediated immune responses are inhibited by anti-CTLA-4 treatment, we examined the early events during or just after mAb treatment. The number of total splenocytes in control GVHD mice was increased 3-fold with a peak at 3 wk, whereas splenocyte expansion was less in the anti-CTLA-4-treated mice (Fig. 4A). Anti-CTLA-4 treatment seems to eliminate host B cells (Fig. 4B). The percentage of donor cells (H-2b+) rapidly increased with the peak at 2 wk and was maintained at 12–15% of total splenocytes (Fig. 4C). The anti-CTLA-4 mAb treatment clearly enhanced donor cell expansion to ~2-fold at 2–3 wk. The donor cells consist of >90% CD3+ T cells in both anti-CTLA-4-treated and control GVHD mice. An increase in the absolute
number of donor T cells was observed during the first 2 wk (Fig. 4D), and this was preferentially observed in the CD8 subset (Fig. 4D, E and F). Furthermore, the anti-CTLA-4 treatment strikingly augmented CD8+ T cell expansion and persistently maintained an elevated number of CD8+ donor T cells throughout the experimental period (Fig. 4F). This was consistent with the maintained IFN-γ expression at 8 wk (Fig. 3). We next investigated whether the donor CD8+ T cells possessed cytolytic activity against host cells. We measured cytotoxicity of whole splenocytes against EL-4 (H-2b) target cells at 2 and 3 wk posttransfer. Splenocytes treated with anti-CTLA-4 mAb exhibited higher cytotoxicity at 2 and 3 wk posttransfer compared with untreated GVHD mice (Fig. 5). However, the percentages of donor CD8+ T cells in the anti-CTLA-4-treated mice were 2- to 3-fold higher in the control mice. Therefore, the higher cytolytic activity was a consequence of the presence of more CTL rather than a functional change in CD8+ T cells at the early phase. This conclusion was also supported by the results of IFN-γ production as shown in Fig. 3. These results indicate that the functional ability of CD8+ T cells was not affected by the anti-CTLA-4 treatment at the early events, but the anti-CTLA-4 treatment caused a significant increase in the expansion of CD8+ T cells in these GVHD mice.

Suppression of Th2 cell activation by CTLA-4 blockade is dependent on donor CD8+ T cells

To examine the involvement of donor CD8+ T cell expansion in the inhibition of Th2 cell responses, we tested the effect of CTLA-4 blockade in a chronic GVHD model induced by transfer of splenocytes depleted of CD8+ T cells. The mice receiving CD8-depleted T cells showed a elevation of serum IgE at 3 wk (Fig. 6). As compared with the transfer of whole splenocytes presenting in Fig. 1, the elevation of IgE was rapidly induced. Interestingly, the anti-CTLA-4 treatment did not affect IgE production at 3 wk but seemed to slightly augment IgE production at late time points. The rapid elevation of IgE may result from the absence of CD8+ T cells, which regulate Th2 cell activation. This result indicates that suppression of Th2 cell activation by anti-CTLA-4 treatment requires donor CD8+ T cells.

Discussion

In this study, we demonstrate that the in vivo administration of anti-CTLA-4 mAb in the parent into F1 GVHD model reduces the manifestations of Th2-mediated chronic GVHD. Previous reports showed that intact anti-CTLA-4 mAb can mediate either agonist

![FIGURE 4](image_url)

**FIGURE 4.** Anti-CTLA-4 mAb treatment enhances donor CD8+ T cell expansion and eliminates host cells. Splenocytes from control (○) and anti-CTLA-4-treated (□) mice at the indicated week after transfer were removed, and splenocytes were stained with either PerCP-anti-CD3 mAb, FITC-anti-H-2b mAb, and PE-anti-CD4 mAb or PE-anti-CD8 mAb, or with appropriate fluorochrome-conjugated control Ig. Samples were analyzed by flow cytometry. The total cell number of splenocytes (A), the percentage of H-2b+ donor cells in total lymphocytes (donor cells) (B), the absolute cell number of CD3+ H-2b+ lymphocytes (host non-T cells) (C), H-2b+CD3+ lymphocytes (donor T cells) (D), CD4+CD3+ H-2b+ (donor CD4+ T cells) (E), and CD8+CD3+ H-2b+ (donor CD8+ T cells) (F) are presented. The absolute cell number was calculated from the percentage for the indicated fraction and total splenocyte count after erythrocyte depletion. Values are the mean ± SD from three mice in each group. Data are representative of two experiments with similar results.

![FIGURE 5](image_url)

**FIGURE 5.** Splenocytes treated with anti-CTLA-4 mAb exhibit cytotoxicity against host alloantigen. Splenocytes from untreated (○) and anti-CTLA-4-treated (□) mice at 2 and 3 wk were used as effector cells. Cytotoxicity against EL-4 cells (H-2b) was measured. The mean percentages of donor CD8+ T cells in control and anti-CTLA-4-treated mice were 8.0% and 19.0% at 2 wk and 4.0% and 15.7% at 3 wk, respectively. Values are the mean ± SD from each group of three mice. Data are representative of three experiments with similar results.

![FIGURE 6](image_url)

**FIGURE 6.** Elevated serum IgE induced by CD8-depleted T cells is not affected by anti-CTLA-4 mAb treatment. Chronic GVHD was induced by the transfer of CD8-depleted T cells (3 × 10^7) from BALB/c mice into unirradiated CBF1 mice. Mice were divided into two groups of six mice and were treated with either anti-CTLA-4 mAb (○) or control hamster Ig (●) as described in Fig. 1. Each plot represents the mean ± SD, and the data are representative of two similar experiments.
and antagonist activities in vitro (7, 37), while the in vivo administration of intact mAb usually functions as an antagonist in various T cell-mediated immune responses (10, 13, 14, 19). Our results in a mouse GVHD model are consistent with the function of anti-CTLA-4 mAb as an antagonist. Inoculation of DBA/2 spleen cells into (C57B6 x DBA/2)F1 mice resulted in lymphoid hyperplasia, autoantibody production, elevated levels of IgE, and an immune glomerulonephritis, i.e., a disease that resembles systemic lupus erythematosus in humans (20, 23, 38). These features suggested a central role for Th2 cell activation in the pathogenesis. In fact, recent reports have shown the elevation of IL-4 mRNA levels in DBA GVH mice (25, 39). Disease was inhibited by injection of anti-IL-4 mAb (40, 41), suggesting a critical role of IL-4. Similar to DBA GVH mice, elevation of serum IgE and anti-host IgG1 Ab production and the enhancement of IL-4 expression in donor CD4+ T cells were demonstrated in BALB/c GVH mice. Injection of anti-CTLA-4 mAb consistently inhibited the Th2 features in this chronic GVHD model.

It has been reported that blockade of CTLA-4 in our GVHD model suppresses Th2-mediated immune responses in several experimental autoimmune disease models, including DO11.10 TCR transgenic mice (10, 16), nonobese diabetic mice (12), and experimental allergic encephalomyelitis (13, 14). Similarly, Th2-mediated immune responses to nematode parasite infection can be augmented by anti-CTLA-4 treatment (19). Furthermore, CTLA-4-deficient mice have a fatal lymphoproliferative disorder (42), and blockade of CTLA-4 efficiently induced antitumor responses (11, 43). Altogether, these results indicate that CTLA-4 signals limit T cell activation and expansion.

How does blockade of CTLA-4 in our GVHD model suppress Th2-mediated immune responses? We observed a moderate but substantial donor CD8+ T cell expansion at 2–3 wk. CTLA-4 blockade strikingly augmented the expansion of donor CD8+ T cells, but the effector function of CD8+ T cells at a single-cell level was not affected. The importance of CD8+ T cells in the inhibition of IgE was directly demonstrated by transfer of splenocytes depleted of CD8+ T cells. This suggests that donor CD8+ T cells are essential for the effect of anti-CTLA-4 mAb. Both acute and chronic GVHD share common initiating events, the recognition of allogeneic MHC class II molecules by donor CD4+ T cells, which results in increased IL-2 production during the first 2 days after parental cell transfer (44, 45). However, the most striking differences, which determines the direction toward acute or chronic GVHD, are the engagement of donor CD8+ T cells, which terminates B cell hyperactivity (46). Previously, Via et al. (47) compared T cell chimerism between DBA GVH and B6 GVH mice at 2 wk after transfer and reported the dominant expansion of CD4+ donor T cells in DBA GVH mice, but, on the contrary, CD8+ donor T cells in B6 GVH mice. Thus, the level of engagement of donor CD8+ T cells at an early phase plays an important role for regulation of the consequent development of chronic GVHD. Prior reports have suggested a regulatory role for CD8+ T cells in the development of chronic GVHD (46, 48, 49). The CD8+ T cell expansion caused a reduction in the number of host cells, especially B cells at the early phase after transfer, and a suppression of IL-4 producing Th2 cells at the late phase after transfer. These results suggest two major roles for donor CD8+ T cells; the first is their capacity to make IFN-γ, which inhibits the differentiation of IL-4-producing Th2 cells, and the second is their cytolytic potential, which eliminates host cells. A regulatory role of CD8+ T cells has been demonstrated in other Th2-mediated immune responses, which resulted in reduced IgE Ab production (50–53). Depletion of CD8+ T cells in vivo during a critical period after immunization produced high and persistent IgE responses and led to decreased production of IFN-γ, resulting in a net decrease in the ratio of IFN-γ to IL-4 (51, 53). Therefore, manipulation of CD8+ T cells may be beneficial for regulation of Th2-mediated allergic immune responses. Expansion of donor CD8+ T cells and augmentation of IFN-γ expression at late time points were not observed in control GVHD mice. Cytokines secreted from Th2 cells may inhibit activation of CD8+ T cells.

In this BALB/c GVH model, we also observed a mild enhancement of CD4+ donor T cell expansion at 1–2 wk as well as CD8+ donor T cells. However, CTLA-4 blockade seems to preferentially affect CD8+ T cells rather than CD4+ T cells. Why does CTLA-4 blockade preferentially affect CD8+ T cells? Differential expression of CTLA-4 by CD4 vs CD8 T cells is unlikely, because both cell types express similar amounts of CTLA-4 after stimulation (7, 8, 54). In contrast with our result, two recent studies using CTLA-4-deficient mice have suggested that CTLA-4 is less important in regulating CD8+ T cells compared with CD4+ T cells (55, 56). In these experiments, highly antigenic viral Ag or peptide-Ag were used for inducing primary CD8+ T cell responses. Varying the priming conditions, such as peptide concentration, antigenic capacity of Ags and costimulatory signals may affect the dependency of CTLA-4-negative signals in primary responses of CD8+ T cells. In addition, the contribution of initial activation of CD4+ T cells in the subsequent CD8+ T cell activation may be another possible explanation. Blockade of CTLA-4 by in vivo administration of anti-CTLA-4 mAb in the early stages of tumor growth enhanced the capacity to generate antitumor T cell responses in which final effector T cells should be CD8+ T cells (11, 43). In both tumor-transplantation and GVHD models, it is possible that an initial enhancement of CD4+ T cell expansion by prevention of CTLA-4 signals may further augment subsequent CD8+ T cell responses. In agreement with this notion, Chambers et al. (57) suggested the possibility that the initial expansion of CD4+ T cells may determine a skewing the CD4/CD8 T cell ratio based on the results of the phenotype in neonatally CD4- or CD8-depleted CTLA-4-deficient mice. CD8+ T cell depletion did not alter the onset of severity of lymphoproliferative disorder, whereas CD4+ T cell depletion completely prevented. Although further studies will be required, we cannot negate a possibility that the initial expansion of CD4+ T cells by CTLA-4 blockade may be responsible for the sequential CD8+ T cell expansion.

In this study, we demonstrate the immunosuppressive effect of CTLA-4 blockade in a murine chronic GVHD model. CTLA-4 blockade at the initial Ag sensitization enhanced expansion of CD8+ T cells in the early response and resulted in the regulation of the consequent Th2-mediated humoral immune response. Our results imply a new approach for regulating IgE-mediated allergic diseases by blockade of CTLA-4 signals during a critical period of Ag sensitization.

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