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Cutting Edge: A Critical Role for IL-10 in Induction of Nasal Tolerance in Experimental Autoimmune Myocarditis

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Appropriate treatment of autoimmune myocarditis following virus infection remains a major clinical problem. Induction of nasal tolerance may provide a new approach to treatment. However, the exact mechanism of nasal tolerance is unknown. To assess the mechanism of nasal tolerance, we examined the role of IL-10 in the induction and suppression of autoimmune myocarditis. First we showed that blocking IL-10 concurrent with nasal administration of Ag abolished the disease-suppressing effect of nasal tolerization. It also led to increased cardiac myosin-specific IL-1 and TNF-α production. Then we demonstrated that blocking IL-10 during the effector phase increased not only the incidence and severity of disease but also Ag-specific IL-2, IL-4, and TNF-α production as well as cardiac myosin-specific IgG1 and IgG2b production, whereas blocking IL-10 during the induction phase had no effect. This study implicates IL-10 in the induction of nasal tolerance and in limiting inflammation later during the disease process. The Journal of Immunology, 2002, 168: 1552–1556.

Myocarditis is a frequent cause of cardiac disease among young adults and may be a precursor of heart failure due to dilated cardiomyopathy. The most common cause of myocarditis in the United States is infection with Coxsackievirus B3. Experimental autoimmune myocarditis (EAM) induced in mice by immunization with murine cardiac myosin (CM) is similar to human myocarditis (1).

Immunomodulatory therapies, especially immunosuppressive treatments, have been used in clinical trials for the treatment of myocarditis. Although immunosuppression is useful in down-regulating autoimmune damage in myocarditis, it may promote viral spread and myocardial cytolysis. In fact, clinical trials and experimental studies of murine models of viral myocarditis have shown that immunosuppressive treatments may actually increase disease severity and mortality (2). Consequently, studies are needed to find novel approaches to treatment designed to achieve Ag-specific immunosuppression without impairing the antiviral immunity. We obtained promising results by nasal administration of CM prior to the onset of the autoimmune disease (3).

Mucosal administration of Ag has been described as a method to induce Ag-specific tolerance and to suppress autoimmune diseases in several animal models, including experimental autoimmune encephalomyelitis (4), experimental autoimmune myasthenia gravis (5), and insulin-dependent diabetes mellitus (6). Depending on the amount of Ag given (7), the administration of Ag can either induce regulatory cells that suppress the immune response through the local production of cytokines or silence Ag-specific T cells by induction of clonal anergy or clonal deletion. However, the exact mechanism of inducing nasal tolerance by administration of Ag is still unclear.

IL-10, first recognized for its ability to inhibit activation and effector function of T cells, monocytes, and macrophages, is a multifunctional cytokine with diverse effects on most hemopoietic cell types (8). A principal function of IL-10 appears to limit and ultimately terminate inflammatory responses. In addition to these activities, IL-10 plays a key role in differentiation and function of a newly appreciated type of T cell, the T regulatory cell, which may figure prominently in control of immune responses and tolerance in vivo (9).

To assess the mechanism of nasal tolerance, we tested the effect of blocking IL-10 on the immunosuppression induced by nasal tolerance in EAM. Because the role of IL-10 in CM-induced EAM has never been examined, we also studied the effects of blocking IL-10 at different time points in the course of disease on the outcomes of disease, autoantibody levels, and cytokine production.

Materials and Methods

Mice

Female A/J mice (6–8 wk of age) were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained in the conventional animal facility at Johns Hopkins School of Medicine (Baltimore, MD) and were used in all experiments.

Ag preparation and induction of myocarditis

Murine CM was purified from pooled mouse hearts using a previously described procedure (9). The purified CM was emulsified with equal volume of CFA (Sigma-Aldrich, St. Louis, MO). Each mouse was injected s.c. with 100 μl of emulsion containing 200 μg of CM on days 0 and 7. To
induce a mild disease mice were immunized with a suboptimal immunization protocol (200 μg of CM in nonsupplemented CFA containing 1 mg/ml Mycobacterium tuberculosis H37Ra (Sigma-Aldrich)) rather than with our standard protocol (3, 9).

Nasal administration of Ag

As described previously (3), nasal administration of Ag was conducted by intranasal intubation 3 days before the first immunization with CM (day 0). Approximately 200 μg of CM in 30 μl of vehicle buffer (0.5 mol/L KCl, pH 6.8) was slowly instilled.

Purification of anti-IL-10

A rat IgG1 IL-10-specific mAb was obtained by culturing rat hybridoma JES 2A5 (kindly provided by Dr. G. Corfield, University of Bristol, Bristol, U.K.) in serum-free IMEM (Life Technologies, Grand Island, NY) and by purifying the mAb using a HiTrap Protein G Sepharose column (Amersham Pharmacia Biotech, Piscataway, NJ). An isotype-matched control Ab designated GL113 (IgG1) was used in control groups (the hybridoma cell line was kindly provided by F. Finkelman, University of Cincinnati, Cincinnati, OH).

Treatment protocols

Protocol 1: Effects of blocking IL-10 on the induction of nasal tolerance. Anti-IL-10 mAb or the isotype-matched control mAb (500 μg/mouse per time point, an effective dose (10)) were administered i.p. 7, 5, 3, and 1 days before the first immunization with CM (day 0). A total of 200 μg of CM in 30 μl of vehicle buffer (0.5 mol/L KCl, pH 6.8) was slowly instilled intranasally into mice on day −3. Mice were immunized with 200 μg of CM on days 0 and 7 and sacrificed on day 21.

Protocol 2: Effects of blocking IL-10 during different phases of disease. Three groups of mice (8–13 mice per group) were administered either anti-IL-10 mAbs or isotype-matched control mAbs during the early, initiation phase of disease, starting on day 0 until day 12 every other day. One group was sacrificed on day 21, the second group on day 28, and the third group on day 35. Another three groups of mice were administered anti-IL-10 mAbs or isotype-matched control mAbs during the late, effector phase of disease, starting on day 10 every other day until day of sacrifice. These mice were sacrificed on days 21, 28, or 35.

Measurement of serum levels of anti-CM Abs and cytokine production by splenocytes

Determination of serum levels of anti-CM Abs and cytokine production by splenocytes was performed as described previously (11). Ab endpoint titers for each individual mouse were calculated as the greatest positive dilution of Ab (11).

Histopathological evaluation

For the histological evaluation, serial sections were made through the heart. Every fifth section was stained with H&E. Evidence of myocarditis was evaluated independently and blindly by two pathologists using light microscopy according to a five-tier scoring system: grade 1, cardiac infiltrate up to 10% of the cardiac sections; grade 2, 11–30%; grade 3, 31–50%; grade 4, 51–90%; grade 5, >90%. The average score was taken for statistical analysis using a nonparametric test.

Statistical analysis

Differences in the disease severity and cytokine or Ab levels were analyzed using the Mann-Whitney test. Disease prevalence was compared using a χ² two-way analysis. Values of p < 0.05 were considered significant.

Results

IL-10 and induction of nasal tolerance

We first examined the role of IL-10, an immunoregulatory cytokine, in the induction of nasal tolerance and in the pathogenesis of EAM. Mice were administered 200 μg of CM intranasally 3 days before the first immunization with CM. The mice were injected with 500 μg of either anti-IL-10 mAb or isotype-matched control mAb. Blocking IL-10 abolished the disease-suppressing effect of nasal tolerance (Figs. 1 and 2). When mice were administered CM intranasally and treated with anti-IL-10, 8 of 11 mice had severe disease (Figs. 1A and 2–D), whereas only 2 of 10 mice in the isotype control group had even mild disease (Figs. 1A and 2A). In a control group of mice, which were only immunized with CM on days 0 and 7 and received buffer intranasally, comparable numbers of mice (8 of 12) developed disease (data not shown).

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To examine the effect of blocking IL-10 during the induction of nasal tolerance on the immune response to CM, CM-specific autoantibody production and cytokine production by CM-stimulated spleen cells were examined. TNF-α and IL-1 levels were significantly increased in mice treated with anti-IL-10 mAbs compared
with mice treated with isotype-matched mAbs. There was no significant difference in levels of TGF-β or other cytokines (Fig. 1B).

Total IgG, IgG1, and IgG2b autoantibody titers against CM at day 21 were also significantly increased in anti-IL-10 mAb-treated mice (Fig. 1C), but there were no significant differences in IgA autoantibody titers (data not shown).

Effect of blocking IL-10 during the induction phase of EAM

To further investigate the effect of IL-10 in the pathogenesis of EAM, we blocked IL-10 during the induction and the effector phases. We treated three separate groups of mice with either anti-IL-10 mAb or isotype-matched control mAb and sacrificed them at different time points, days 21, 28, and 35. A low incidence and mild severity in the control groups was desired to demonstrate a possible increase in severity of disease. Therefore, we immunized with a suboptimal protocol as described in Materials and Methods.

When mice were treated with anti-IL-10 mAb during the early, induction phase of disease, there was no significant change in the outcome of disease incidence or severity compared with the control mice regardless of the time of sacrifice (data not shown). There was also no significant difference in either the CM-specific cytokine production or the CM-specific Ab production (data not shown).

Effect of blocking IL-10 during the effector phase of EAM

When IL-10 was blocked during the late, effector phase of disease, the incidence and severity significantly increased. Four of nine mice treated with anti-IL-10 mAb starting on day 10 and sacrificed on day 21 had moderate to severe disease, whereas only one of nine mice in the control group had very mild disease (Fig. 3A). Five of nine mice sacrificed on day 28 and six of nine mice sacrificed on day 35 showed mild to moderate disease compared with only one mouse with inflammation in the heart in the control groups. The disease severity in the anti-IL-10 mAb-treated group of mice showed a tendency to decrease over time with a peak on day 21, but the incidence of disease remained constant over time.

Mice treated with anti-IL-10 mAb during the effector phase of disease also showed changes in the CM-specific cytokine production and in the CM-specific Ab production (Fig. 3B). TNF-α was significantly increased in all three groups of mice independent of the time of sacrifice. IL-4 was significantly increased in the mice sacrificed on day 28 or 35, and IL-2 was significantly increased in the mice sacrificed on day 21 (Fig. 3B). There was no significant difference in the levels of IL-1 or IFN-γ in any of the treatment groups (data not shown).

All groups of mice treated with anti-IL-10 mAbs had increased CM-specific total IgG, IgG1, and IgG2b titers (Fig. 3C).

Discussion

Blocking IL-10 abrogates nasal tolerance

Recently, nasal administration of Ag has been reported by us and others (3, 6, 12) as an alternative route to induce mucosal tolerance in some experimental autoimmune models, including EAM. The nasal route of administration appears to be more effective in inducing tolerance than oral administration (13). This difference can be explained by the fact that the Ag delivered orally is more likely to be degraded by acid and proteolytic enzymes that are present in the gastrointestinal environment. But the exact mechanism for mucosal tolerance still remains unclear.

The present study demonstrates that blocking IL-10 abolishes the disease-suppressing effect of nasal tolerization. Blocking IL-10 also led to an increase in Ag-specific production of proinflammatory cytokines, such as IL-1 and TNF-α, by splenocytes. Both cytokines are known to be crucial in the pathogenesis of EAM (14, 15) and to increase in CM-specific autoantibodies, especially of the IgG1 and IgG2b subclasses.

Tolerance following mucosal Ag delivery might be mediated by anergy or apoptosis of Ag-specific lymphocytes or induction of regulatory T cells, depending upon the dose of Ag administered. A single high dose of Ag causes deletion or anergy of both Th1 and Th2 autoreactive T cells (7). In contrast, repeated low doses of Ag favor the generation of regulatory cells that are thought to migrate to systemic sites and secrete cytokines, such as IL-4 and IL-10 (the prototypic Th2 cytokines) (16). These suppressive cytokines can then down-regulate production of proinflammatory cytokines.

The particular reasons why the mucosal environment may be especially conducive to the differentiation of T cells into Th2 pathway are unclear. One possibility is the presence of distinct APCs capable of Th2 induction in the mucosa. Recently, specialized dendritic cell (DC) populations have been identified (17). They differ in phenotype, localization, and function (17, 18). Iwasaki and colleagues (17, 18) reported lately that DCs from mucosal tissue
primed T cells for the production of IL-4 and IL-10, whereas the same stimulus induced no IL-10 production from splenic DCs.

The role of TGF-β in mediating mucosal tolerance remains controversial. Evidence shows that orally administered Ag can generate populations of cells secreting TGF-β in the gut-associated lymphoid tissue, and these cells are capable of regulating the development of Th1 responses (7). It has also been shown that in vivo blocking of TGF-β by anti-TGF-β Abs ablated the tolerance induction by nasal administration of acetylcholine receptors in a murine model of myasthenia gravis (19). However, TGF-β as mediator of mucosal tolerance has recently been challenged by a study of successful induction of mucosal tolerance in TGF-β-deficient mice, indicating an alternative mechanism of tolerance in these mice (20). In our previous study, we also found no evidence that TGF-β plays a significant role as a mechanism for nasal tolerance in our model of EAM (3). Although, in the recent study, mice treated with anti-IL-10 Abs had higher titers of IgG2b Abs against CM, there was no significant difference in Ag-specific TGF-β production by splenocytes or in CM-specific IgA Ab titers. Taken together, both our previous and our recent results do not support a major role for TGF-β in induction of nasal tolerance in our model of EAM.

IL-10 has not been investigated previously in the induction of nasal tolerance in EAM. IL-10 has been reported to play a key role in differentiation and function of a newly appreciated type of T cell, the T regulatory cell, which may figure prominently in control of immune responses and tolerance in vivo (21). Blocking IL-10 leads to increases in CM-specific autoantibody production, especially IgG1, and IgG2b subclasses and Ag-specific IL-1 and TNF-α production, both proinflammatory cytokines mainly produced by mononuclear cells. The study done by Akbari and colleagues (22) demonstrating a critical role for mucosal DCs in inducing tolerance by stimulating the development of CD4+ T regulatory cells and IL-10 production confirms our observation of IL-10 being important for nasal tolerance.

Proinflammatory cytokines are essential for the development of autoimmune myocarditis. Administration of either IL-1 or TNF-α promoted virus- and myosin-induced myocarditis in genetically resistant B10.A mice (14). The presence of myocarditis is associated with increased levels of TNF-α from CM-stimulated splenocytes in culture. Furthermore, when AJ mice are infected with CB3 and treated with an IL-1R antagonist, myocardial injury is diminished (23). Thus, IL-1 and TNF-α are clearly critical in the pathogenesis of autoimmune myocarditis. Both cytokines can up-regulate MHC class I and class II expression in the cardiac interstitium and on myocytes, possibly inducing or enhancing inflammation (15). However, the precise mechanisms involved in the release of proinflammatory cytokines from mononuclear cells are unknown. The results presented in this study implicate IL-10 production induced by nasal administration of Ag in suppression of the production of proinflammatory cytokines.

Blocking IL-10 during the effector phase enhances disease

IL-10, a cytokine with multiple immune regulatory functions, is known mostly for its anti-inflammatory, disease-limiting effects. In fact, it has been reported that administration or local expression of IL-10 can suppress and inhibit autoimmune diseases, such as experimental autoimmune encephalomyelitis (24) and collagen-induced arthritis (25). However, it has also been reported that IL-10 can aggravate or have no effect on autoimmune diseases, such as experimental autoimmune myasthenia gravis (26) or diabetes mellitus in nonobese diabetic mice (27). In addition, a beneficial effect of administration of IL-10 antagonist to humans in systemic lupus erythematosus has been reported (28). Because the role of IL-10 has never been investigated in myosin-induced EAM, we also designed a study to look into the effects of blocking IL-10 at different time points during the development of disease. While blocking IL-10 late during the disease enhanced disease severity and prevalence, blocking IL-10 during the induction phase of disease had no significant effect on severity, prevalence of disease, cytokine profile, or autoantibody titers. Early blocking of IL-10 might have no effect on the disease outcome, because early during the disease induction almost no IL-10 is detectable in the myocardium (29) or in the supernatants of CM-stimulated splenocytes (our unpublished observations). The peak of IL-10 production is after the maximum inflammatory stage (day 21) and persists into the recovery phase (29). In contrast, blocking IL-10 during the late phase of disease was accompanied by increased IL-2, a Th1 cytokine, IL-4, a Th2 cytokine, and with TNF-α, a proinflammatory cytokine produced mostly by mononuclear cells. This is consistent with our previous finding that high levels of IL-1 and TNF-α are strongly associated with the development of myocarditis. IL-10 has been reported to inhibit IL-1 and TNF-α production by mononuclear cells, to affect directly the function of T cells, and to inhibit IL-2 (Th1), IL-4, and IL-5 (Th2) production. Blocking IL-10 appears to abolish the suppressive effect of IL-10 on the production of IL-1, TNF-α, IL-2, and IL-4, which altogether contribute to increase in disease severity and incidence; we recently reported a critical role for IL-4 in CM-induced EAM in AJ mice (9). Thus, our results support the previous findings that IL-10 is mostly effective in limiting disease.

References


