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IL-10-Dependent Suppression of Experimental Allergic Encephalomyelitis by Th2-Differentiated, Anti-TCR Redirected T Lymphocytes

Divya J. Mekala, Rajshekhar S. Alli, and Terrence L. Geiger

We previously showed that transgenically expressed chimeric Ag-MHC-ζ receptors can Ag-specifically redirect T cells against other T cells. When the receptor’s extracellular Ag-MHC domain engages cognate TCR on an Ag-specific T cell, its cytoplasmic ζ-chain stimulates the chimeric receptor-modified T cell (RMTC). This induces effector functions such as cytolsis and cytokine release. RMTC expressing a myelin basic protein (MBP) 89–101-IAα-ζ receptor can be used therapeutically, Ag-specifically treating murine experimental allergic encephalomyelitis (EAE) mediated by MBP89-101-specific T cells. In initial studies, isolated CD8⁺ RMTC were therapeutically effective whereas CD4⁺ RMTC were not. We re-examine here the therapeutic potential of CD4⁺ RMTC. We demonstrate that Th2-differentiated, though not Th1-differentiated, CD4⁺ MBP89–101-IAα-ζ RMTC prevent actively induced or adoptively transferred EAE, and treat EAE even after antigenic diversification of the pathologic T cell response. The Th2 RMTC both Th2-deviate autoreactive T cells and suppress autoantigen-specific T cell proliferation. IL-10 is critical for the suppressive effects. Anti-IL-10R blocks RMTC-mediated modulation of EAE and suppression of autoantigen proliferation, as well as the induction of IL-10 production by autoreactive T cells. In contrast to IL-10, IL-4 is required for IL-4 production by, and hence Th2 deviation of autoreactive T cells, but not the therapeutic activity of the RMTC. These results therefore demonstrate a novel immunotherapeutic approach for the Ag-specific treatment of autoimmune disease with RMTC. They further identify an essential role for IL-10, rather than Th2-deviation itself, in the therapeutic effectiveness of these redirected Th2 T cells. The Journal of Immunology, 2005, 174: 3789–3797.

T he development of therapies that Ag-specifically target autoreactive T lymphocytes represents a significant challenge. Because Ag-specific therapies are directed against only one or a limited number of antigenic epitopes, they often lack the potency of Ag-nonspecific immunomodulatory agents. Among Ag-specific therapies, cellular immunotherapy with T lymphocytes may be particularly adept at linking specificity and potency (1). Adoptively transferred T cells can home to sites of inflammation and may persist for >1 year (2, 3). T cells elaborate biologic response modifiers that may regulate autoimmune inflammation mediated by both Ag-specific and bystander T cells. Most importantly, adoptive immunotherapy with T cells has already shown clinical efficacy in infectious diseases and promise in cancer (3–5), suggesting similar potential in the treatment of autoimmune diseases.

We previously developed a novel candidate Ag-specific immunotherapy that uses chimeric receptors to redirect genetically modified therapeutic T cells against autoreactive T lymphocytes (6, 7). The chimeric receptors are composed of an extracellular domain that includes a covalently linked complex of autoantigenic peptide and its restricting MHC, and an intracellular domain composed of ITAM-rich signaling regions from the TCR. The extracellular autoantigen-MHC acts as a bait, luring pathologic T cells to the therapeutic cell (Fig. 1). The linked cytoplasmic signaling domain activates the therapeutic T cell after it engages the autoreactive target cell. Chimeric receptor-modified T cells (RMTC) therefore are stimulated in direct response to pathologic T cells. Engagement of the chimeric receptor with the target cell’s TCR induces regulatory functions native to the RMTC.

To test the clinical efficacy of RMTC, we synthesized a heterodimeric receptor that recognizes the TCR of myelin basic protein (MBP)-specific T cells that are pathologic in a murine model of multiple sclerosis and experimental allergic encephalomyelitis (EAE). One chain of this receptor consists of the MBP89–101 autoantigen peptide genetically linked to the extracellular and transmembrane domains of its restricting IAβ chain and the cytoplasmic domain of TCR-ζ. The second chain includes IAα, similarly linked to TCR-ζ. We previously showed that RMTC expressing this receptor are Ag-specifically stimulated by MBP89–101-reactive T cells in vitro and in vivo, and are effective in treating EAE induced by immunization with MBP89–101. Surprisingly, whereas CD8⁺ RMTC were therapeutically active, undifferentiated CD4⁺ RMTC showed no therapeutic effect (6). In this study, we reconsider the therapeutic activity of CD4⁺ RMTC and define mechanisms by which they may antagonize autoreactive effectors.

Murine EAE is orchestrated by Th1-polarized T cells (8). These T cells stimulate macrophage and other cell types that produce...
reactive oxygen species, NO, and other biologic response modifiers that locally damage CNS tissue. Adoptive transfer of myelin-Ag-specific Th2 T cells is protective in some, but not all, EAE systems and Th2 deviation correlates with disease remission (9–11). Systemic treatment with Th2 cytokines, such as IL-4 or IL-10, can ameliorate disease symptoms while their blockade enhances disease (12–15). We therefore hypothesized that despite the ineffectiveness of undifferentiated CD4+ RMTC, Th2-differentiated RMTC may be therapeutically active.

We also considered that Th1-differentiated RMTC might be therapeutic. Th1 cells secrete significant amounts of IFN-γ and TNF family members. These can promote immunopathology by cytokine production or cytoklysis, by the receptor-modified T lymphocyte.

**Materials and Methods**

**Mice**

Female SJL/J mice obtained from The Jackson Laboratory were age-matched within each experiment. 25S89P mice (6, 20) were backcrossed ≥7 generations with SJL/J mice before analysis.

**Peptides**

MBP89–101 (VHFFKNIVTFRTP) and proteolipid protein (PLP) 139–151 (HSLGKWLGHDPKF) were synthesized and purified to >90% purity by the St. Jude Hartwell Center for Biotechnology.

**Abs and flow cytometry**

Anti-CD4 (RM4-5) and anti-CD44 Abs were obtained from BD Pharmingen. Anti-class II MHC was obtained from Devaron (IA.B2) or BD Pharmingen (KH74). 2C11 (anti-CD3ε, gift from M. Blackman, Trudeau Institute, Saranac Lake, NY) was purified from hybridoma supernatants. Flow cytometry analysis was performed on a FACSCalibur (BD Biosciences) and flow cytometric sorting on a MoFlo high-speed cell sorter (DakoCytomation).

**Preparation of Th1 and Th2 cells and adoptive transfer**

Naïve (CD44lo) CD4 T cells from SJL or 25S89P mice were cultured (1 × 10^6 cells/ml) in the presence of anti-CD3 or 2 μg/ml Con A, 1 ng/ml recombinant murine (rm) IL-2, and irradiated spleen cells (1:10) either with 3.5 ng/ml rmIL-12 and 1 μg/ml anti-IL-4 Ab (clone 11B11; BD Pharmingen) to differentiate them into Th1 cells or with 400 U/ml rmIL-4 and 2 μg/ml anti-IFN-γ Ab (clone XMG1.2; BD Pharmingen) to differentiate them into Th2 cells. After 4 days, the cells were washed three times with PBS and then cultured with 1 ng/ml rmIL-2 for 3 more days. These differentiated cells were washed with PBS before use in vitro or adoptive transfer experiments. Adoptive transfers consisted of 5 × 10^6 cells in 100 μl of saline injected i.v.

**EAE induction and clinical evaluation**

EAE was induced with MBP89–101 as described previously (21). EAE was induced with PLP139–151 as with the MBP peptide except a single immunization of 100 μg of peptide was administered. Four to six animals were evaluated in each treatment arm. Data from one of two or more comparable experiments performed for each EAE study are shown. Clinical scoring was: 1, limp tail; 2, hind limb paresis or partial paralysis; 3, total hind limb paralysis; 4, hind limb paralysis and body/front limb paresis/paralysis; and 5, moribund.

**Adoptive transfer EAE**

SJL/J mice were immunized with 300 μg of MBP89–101 peptide in CFA containing 500 μg of heat-killed H37Ra strain Mycobacterium tuberculosis (Difco) on either side of the tail base on day 0. On day 7, the mice were reimmunized on either side of the flank. Ten days after the second immunization, draining lymph node (LN) cells were isolated and cultured with 15 μg/ml MBP89–101 peptide and 1 ng/ml rmIL-2 for 3 days. The cells were centrifuged and cultured with 1 ng/ml rmIL-2 for 4 more days. The cells (25 × 10^6 cells/mouse) were then transferred i.v. into naive SJL/J mice. Pertussis toxin (200 ng/mouse; List Biological Laboratories) was injected on days 0 and 2 after transfer. Disease was scored as described above.

**T cell proliferation**

Cells harvested from LN or spleen were cultured with MBP89–101 or PLP139–151 peptide. Cultures were pulsed with 1 μCi of [3H]TdR after 72 h and harvested −16 h later for scintillation counting. Samples were analyzed in triplicate.

**Cytokine analysis of draining LN cells and splenocytes**

LN cells or splenocytes were harvested, washed, and 2–3 × 10^5 cells stirred in 96-well plates with 50 μg/ml or the designated concentration of peptide. Culture supernatants were assayed at 48 h for IL-10, IFN-γ, and IL-4 by sandwich ELISA (BD Pharmingen) or Bio-Plex (Bio-Rad) using the manufacturers’ protocols.

**Analysis of in vitro cytokine response**

Th1 or Th2 cells from 25S89P or SJL/J mice were cocultured with irradiated 6F11 cells or control nonspecific T cells at a 1:5 ratio. After 48 h, culture supernatants were analyzed for IFN-γ by ELISA and IL-4 by Bio-Plex.
Evaluation of the effect of anti-IL-4 and anti-IL-10R Abs on EAE, proliferation, and cytokine production

Mice were immunized to induce EAE disease or draining LN proliferative responses. Therapeutic or control cells were administered i.v. at the time of immunization (day 0). The mice were further treated i.p. with 1 mg of purified blocking anti-mouse IL-4 (1B.11; National Cancer Institute Biophysical Resources Branch) and/or 0.5 mg of anti-mouse IL-10R (1B1.13a; BD Pharmingen) Abs or with control anti-rat IgG Ab (Jackson ImmunoResearch Laboratories) on days 0 and 7. Disease scoring and T cell proliferation and cytokine assays were performed as described above.

Statistics

Statistical significance in EAE studies was determined by calculating the mean daily score for individual animals during the time of observation. Experimental and control groups were compared by two-sided t tests at \( p < 0.05 \) using Excel spreadsheet software (Microsoft). Error bars show \( \pm 1 \) SD.

Results

Redirection of T cells with chimeric receptors

25SS9P transgenic (Tg) mice express an MBP89–101/I\(^\text{A}^{-\text{r}}\)/\I\(^\text{A}^{-\text{p}}\\) chimeric receptor on T cells using the hCD2 promoter and locus control region (6, 20). We previously demonstrated that this chimeric receptor functions as a substitute for the TCR, stimulating RMTC in vitro and in vivo in specific response to T cells expressing cognate MBP89–101-specific, I\(^\text{A}^{-\text{r}}\)-restricted TCR (6). To confirm that Th1 or Th2 functions in RMTC could be redirected by MBP89–101-specific T cells, we Th1- or Th2-differentiated flow cytometrically isolated naive CD4\(^{+}\) RMTC or non-Tg T cells. The Th1 or Th2 cells were then restimulated with irradiated MBP89–101/H-\(^\text{2}s\)-specific 6F11 T hybridoma cells, control MBP84–102/DR2-specific Ob T hybridoma cells, or control TCR-deficient 4G4 hybridomas. Production of the Th1 cytokine IFN-\(\gamma\) and the Th2 cytokine IL-4 were monitored as indicators of induced Th1 and Th2 activity, respectively (Fig. 2). The RMTC failed to produce either cytokine in the absence of stimulation or after coculture with the control nonspecific T cells. In contrast, Th1 RMTC produced IFN-\(\gamma\), but not IL-4, and Th2 RMTC produced IL-4, but not IFN-\(\gamma\), upon coculture with the MBP89–101/H-\(^\text{2}s\)-specific hybridoma. Quantities of each cytokine produced was comparable to that produced after stimulation with either Con A or anti-CD3 and anti-CD28 Abs. Unlike the Tg T cells, T cells lacking the chimeric receptor failed to respond to any of the irradiated cell lines. This confirms that the chimeric receptor can engage Ag-specific T cells and that this stimulates the RMTC to produce Th-appropriate cytokines.

Therapeutic effectiveness of Th1 and Th2 RMTC in EAE

To define the clinical efficacy of the RMTC, we analyzed their influence on EAE development in SJL mice. Mice were immunized with MBP89–101 peptide to induce disease. Th1 RMTC, Th2 RMTC, non-Tg Th1 or Th2 cells, or saline was administered at the time of initial immunization. Clinical disease progression was followed. Th2 RMTC proved highly effective in blocking disease development when compared with saline or non-Tg Th2 controls (\( p < 0.05 \), Fig. 3A). In contrast, disease progression in Th1 RMTC-treated mice, though moderately diminished relative to saline-treated mice, was not significantly different from that of non-Tg Th1 controls, indicating nonspecific effects of the adoptively transferred Th1 cells. This demonstrates that Th2 RMTC, in contrast to undifferentiated CD4\(^{+}\) RMTC or Th1 RMTC, specifically inhibit EAE development.

The inhibitory effect of the RMTC was Ag specific. Prophylactically administered Th2 RMTC failed to diminish disease induced with an alternative encephalitogenic Ag, PLP139–151 (Fig. 3B). These results confirm the specificity requirement for cognate TCR that we previously observed with unselected RMTC (6).

It was possible that the Th2 RMTC acted to subvert the priming of pathologic MBP89–101-specific Th1 cells and would not be effective against previously activated MBP-specific T cells. To test this, we treated animals with the RMTC at the time of first disease symptoms, ~11 days after immunization. As with prophylactic administration of RMTC, however, delayed treatment with Th2 but not Th1 RMTC suppressed the autoimmune response (Fig. 3C, \( p < 0.05 \)). This demonstrates that the RMTC were effective even after T cell priming.

To confirm that the Th2 RMTC were able to directly act on the MBP-specific effector T cells, we analyzed them in an adoptive transfer model of EAE. Draining LN cells from MBP89–101-immunized mice were briefly restimulated in vitro and then adoptively transferred into naive mice to induce disease. On the day of transfer, the mice additionally received Th2 RMTC, non-Tg Th2 cells, or saline. Th2 RMTC, but not control cells, were effective in preventing disease development (Fig. 3D, \( p < 0.05 \)), showing that Th2 RMTC can block EAE mediated by mature pathologic effector T cells.

Although the initial T cell response after MBP89–101 immunization is directed specifically against that epitope, with disease progression the epitope specificities of pathologic T lymphocytes broaden. Responses to the PLP139–151 and PLP178–191...
epitopes become prominent (22). This epitope spread mimics the complex epitope dynamics that is observed in human autoimmune diseases (23). If the activity of the Th2 RMTC was limited to the chimeric receptor-targeted MBP89–101-specific T cells, the RMTC would not be expected to be effective after diversification of the immune response. To test this, we treated animals 31 days after immunization with MBP89–101, at which time significant responses to both the PLP139–151 and PLP178–191 epitopes were detected (6). Delayed treatment with the Th2 RMTC, but not Th1 RMTC or control cells, largely ameliorated disease at this late time point (Fig. 3E, p < 0.05). Remission was sustained, lasting for >80 days. This demonstrates that Th2 RMTC effectively treat EAE after epitope diversification, implying that in addition to their direct effects on MBP89–101-specific T cells, the RMTC have beneficial bystander effects on other pathologic T cell responses.

Proliferation and cytokine deviation of targeted and spread responses by RMTC

To better define how the RMTC affected autoreactive T cells, we immunized mice with MBP89–101, treated them with RMTC, and analyzed the effect of the RMTC on autoantigen-specific proliferation of draining LN cells. We anticipated that autoantigen-induced proliferation measured after treatment with Th2 RMTC, which secrete the mitogenic cytokine IL-4, would be preserved or enhanced, whereas proliferation would be somewhat reduced after Th1 RMTC treatment due to the antiproliferative effects of IFN-γ. However, Th1 RMTC, as well as control non-Tg Th1 or Th2 T cells, did not significantly influence MBP89–101-specific proliferation. Unexpectedly, T cell response after treatment with the Th2-differentiated RMTC was significantly reduced (Fig. 4A). This did not appear to result from the development of anergy since the addition of IL-2 to the culture medium failed to revive the attenuated response (Fig. 4B). Response inhibition was also seen when treatment was delayed until 31 days after disease induction, implying that the Th2 RMTC could inhibit MBP-specific proliferation by effector T cells (Fig. 4C).

Although the T cell response to MBP89–101 was largely abrogated by the Th2 RMTC, a small residual response was consistently observed. To define the nature of this response, we analyzed the autoantigen-induced production of IFN-γ, IL-4, and IL-10 as representative Th1 and Th2 cytokines. Cells from mice immunized with MBP peptide and treated either with Th1 RMTC or control Th1 or Th2 T cells produced levels of IFN-γ comparable to those from control saline-treated mice, and minimal amounts of IL-4 or IL-10 (Fig. 5). This cytokine profile was reversed with the Th2 RMTC-treated animals. High levels of IL-4 and IL-10 and minimal quantities of IFN-γ were detected. This demonstrates that the Th2 RMTC divert the pathologic Th1 immune response to a protective Th2 response.

A similar although less pronounced Th2 deviation was observed in mice therapeutically treated with Th2 RMTC 31 days after disease induction (Fig. 6). Ten days after this late-time treatment...
significantly increased MBP-specific IL-4 production was observed, demonstrating a Th2 shift in the autoreactive T cell response. In contrast to treatment at the time of immunization, however, production of IFN-γ persisted, implying that the Th2 shift was incomplete. In summary, these results show that Th2 RMTC administered either prophylactically or therapeutically promote a Th2 deviation among autoreactive T cells. This deviation is associated with disease abatement and suppressed proliferative responses.

Th2 RMTC failed to prophylactically influence PLP139–151-induced disease, yet were strongly disease-suppressive when administered 31 days after MBP89–101 immunization. Because spread PLP139–151-specific responses were prominent at this time (6), we hypothesized that the Th2 RMTC suppressed, in a bystander manner, the PLP139–151-specific T cells. To test for this, we analyzed the T cell response to PLP139–151 in mice treated with RMTC 31 days after immunization and compared it with the response to MBP89–101. Significant bystander suppression was apparent (Fig. 7). PLP-specific proliferative responses of splenocytes isolated 10 days after treatment from Th2 RMTC-treated mice were significantly diminished when compared with splenocytes from control-treated mice (Fig. 7A). Furthermore, whereas IFN-γ production in response to stimulation with PLP139–151, as with MBP89–101, was not significantly altered at this time point, autoantigen-induced IL-4 production was increased (Fig. 7B and C). This demonstrates that delayed treatment with MBP89–101-specific Th2 RMTC not only promotes Th2 deviation and suppression of the targeted MBP-specific T cell response, but of the spread PLP-specific response as well.

**Role of IL-4 and IL-10 in RMTC effect**

Both IL-4 and IL-10 have down-modulatory activities in EAE and are produced by Th2 cells. We suspected that these cytokines played critical and potentially complementary roles in the action of the Th2 RMTC. To test for this, we immunized mice with MBP89–101 and treated them with Th2 RMTC as above, but also administered neutralizing anti-IL-4 or anti-IL-10R Abs. Blockade of the IL-4 signaling pathway in control saline-treated animals had minimal effects on ex vivo assayed MBP89–101-specific T cell responses of MBP89–101-specific T cells after Th2 RMTC treatment in vivo. A. The effect of RMTC prophylaxis on autoreactive T cell proliferation. Mice were treated i.v. with the designated cell type or saline at the time of s.c. immunization with MBP89–101. Draining LN were isolated 10 days later and autoantigen-induced proliferation was measured by [3H]Tdr incorporation after 72 h of culture with the designated concentration of MBP89–101. B. To determine whether the autoreactive T cells were anergic, draining LN were stimulated as in A with 0 or 50 μg/ml MBP89–101 peptide in the presence or absence of 2 ng/ml exogenous rmIL-2. C. The effect of therapeutic treatment with RMTC on T cell proliferation is shown. Mice were treated with RMTC 31 days after immunization and splenocytes were isolated 10 days later. Analysis was performed as in A. Similar results were obtained using mixed LN cells from treated animals. Plotted curves reflect average results from three independently analyzed animals per group, each assayed in triplicate. Error bars, ±1 SD.

**FIGURE 5.** Cytokine deviation after prophylactic Th2 RMTC treatment. Mice were treated with 5 × 10^6 RMTC or saline at the time of immunization with MBP89–101. Ten days later, draining LN cells were isolated and stimulated with the designated amount of MBP89–101 peptide. Production of IFN-γ (A), IL-4 (B), and IL-10 (C) in supernatant was determined after 48 h of culture. Plotted curves reflect average results from three independently analyzed animals per group, each assayed in triplicate. Error bars, ±1 SD.
proliferative responses, which remained robust (Fig. 8A). The suppressed proliferative responses to Ag in mice treated with Th2 RMTC also remained unchanged with anti-IL-4 treatment. Thus, blocking IL-4 did not have a significant influence on Th2 RMTC antiproliferative activity. In contrast, IL-10 was critical for proliferation suppression by Th2 RMTC (Fig. 8B). Anti-IL-10R treatment restored the proliferation of cells from Th2 RMTC-treated mice to levels observed in similarly anti-IL-10R-treated control animals. Thus, IL-10 and not IL-4 signaling is required for the proliferation inhibition mediated by the Th2 RMTC. Although IL-4 was not important for proliferation suppression by Th2 RMTC, analysis of cytokine production by the responding cells demonstrated that IL-4 was important for RMTC-mediated Th2 deviation (Fig. 9). When compared with mice receiving Th2 RMTC and control IgG, the MBP-specific T cells from immunized mice treated with Th2 RMTC and anti-IL-4 produced slightly increased amounts of IFN-γ, similar amounts of IL-10, yet no IL-4 when analyzed ex vivo. Thus, IL-4 is essential for IL-4 production by the self-specific T cells and hence their Th2 deviation. In contrast, IL-4 is not required for the induction of IL-10 production by the self-specific cells. Consistent with the well-documented role of IL-4 in Th2 development (24), these results suggest that the induction of IL-4 production by autoantigen-specific T cells is regulated in vivo by the presence of the same cytokine, potentially produced by Th2 RMTC, essentially forming a positive feedback circuit. A similar positive feedback circuit for IL-4 was observed in analyses blocking the IL-10 signaling pathway in vivo. Anti-IL-10R treatment in vivo blocked the Th2 RMTC-mediated induction of IL-10-producing cells. Significantly, anti-IL-10R Ab treatment of Th2 RMTC-treated mice also restored autoantigen-induced IFN-γ production to near control levels (Fig. 9A), demonstrating that IL-10 is also required for the Th2 RMTC-mediated block in Th1 differentiation.

Interestingly, blockade of IL-4 or IL-10 signaling was not neutral in control saline-treated mice. Anti-IL-10R induced low levels of IL-4 production and anti-IL-4 induced low levels of IL-10 production in cells from saline-treated mice (Fig. 9, B and C). This suggests that there is a natural cross-regulation between these cytokines after autoantigen priming. Importantly, these more subtle effects seemed not to carry over into Th2 RMTC-treated mice. The already high levels of IL-4 in Th2 RMTC-treated mice were not significantly increased in animals receiving anti-IL-10R vs control IgG. Likewise, levels of IL-10 in Th2 RMTC-treated mice were not significantly increased after IL-4 treatment. This suggests that this smaller perturbation in cytokine responses after Ab treatment is redundant with or superseded by the more profound changes mediated by the Th2 RMTC.

A synopsis of the effects of anti-IL-4 and anti-IL-10R on Th2 RMTC immunotherapy is shown in Table I. These results demonstrate that IL-10 and IL-4 act distinctly. IL-10 is needed for Th2 RMTC to suppress the Ag-mediated proliferative response of the MBP-specific T cells. It is also needed to suppress IFN-γ production and promote IL-10 production by MBP89–101-specific T
cells. However, IL-10 is not required for their IL-4 deviation. In a reciprocal manner, IL-4 does not influence the proliferative response of or IL-10 production by the MBP-specific T cells. However, it is required for IL-4 production.

Considering the distinct down-modulatory functions of IL-4 and IL-10 in EAE and their complementary actions during Th2 RMTC immunotherapy, it was conceivable that these cytokines acted synergistically to inhibit EAE. To test for this, we immunized mice with MBP89–101 and administered anti-IL-4, anti-IL-10R, or control Abs in conjunction with either Th2 RMTC or saline (Fig. 10). Anti-IL-4 treatment failed to influence disease course in mice treated with Th2 RMTC. This implies that IL-4 is not needed for the disease-suppressive activity of the Th2 RMTC. In contrast, blockade of IL-10 signaling abrogated the therapeutic effect of the RMTC. Clinical disease in the anti-IL-10R-treated animals was not significantly different from that of control-treated animals (p > 0.05). Therefore, although both IL-4 and IL-10 can modulate the proliferation/cytokine profiles of the MBP-specific T cells, only IL-10 is crucial for the immunotherapeutic effectiveness of the Th2 RMTC.

Discussion

Ag-specific therapies must be simultaneously potent and selective. To do this, they must be able to globally down-regulate the autoimmune response by targeting T cells specific for one or a few selected pathologic epitopes. Adoptive immunotherapy with Th2-differentiated RMTC fulfills these criteria. Th2 RMTC are specifically triggered by autoreactive MBP89–101-specific T cells, yet are nevertheless able to down-modulate EAE even after epitope spreading. In this study, remissions were sustained for >80 days after treatment. Because the chimeric receptor is stimulated only by self-specific T cells, the regulatory activity of the RMTC should mirror the activity of the autoimmune disease. This would be expected to provide homeostatic control, enhancing RMTC activity during periods of disease exacerbation and restricting it when disease is quiescent. RMTC themselves seem not to be inherently

Table 1. Synopsis of effects of treatments with Th2 RMTC, anti-IL-4, and anti-IL-10R on autoregmatic-specific T cell proliferation and cytokine production

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proliferation</th>
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a −, none or strongly diminished; +/−, low; +, moderate; ++, high.
hazardously. Genetically modified T cells expressing a CD4-ζ chimeric receptor, analogous to the IA′-ζ analyzed in these studies, have been successfully applied in Phase I clinical trials (2, 25).

Our studies lead to several conclusions. First, they demonstrate that Th1 RMTC, like undifferentiated CD4-+ RMTC, do not Ag-specifically modulate the autoimmune response. The interactions of Th1 cytokines with effector cells in EAE are complex and not fully elucidated. Thus, IFN-γ treatment can protect mice from EAE and IFN-γ−/− mice are susceptible to EAE (16, 17, 26), suggesting that down-regulatory functions of this Th1 cytokine are significant in EAE. TNF-α−/− mice likewise develop severe disease, whereas LTα−/− animals are protected (27, 28). Our data suggest that overall, the complex interplay of Th1-produced response modifiers, when focused on autoreactive T cells by RMTC, does not alter their immunopathologic potential.

In contrast, Th2 RMTC are highly efficient modulators of EAE. Since EAE is a Th1-mediated disease, the idea that Th2 diversion of the autoimmune response would quell immunopathology has long been advocated and is supported by a large body of data. For example, treatment with altered peptide ligands derived from encephalitogenic Ags or with tolerization regimens using self-Ags may alleviate EAE by diverting the Th1 response to a Th2 response (21, 29). Indeed, this diversion may be sufficiently strong that repeat peptide treatments may induce anaphylaxis (30). Treatment of mice with pharmacologic doses of IL-4 or with Th2-differentiated autoreactive T cells are also capable of suppressing disease (10, 14). Yet, Th2 diversion of the autoreactive response may not be wholly desirable. In some model systems Th2 cells are not able to antagonize the pathologic actions of Th1 effectors (9). Moreover in T cell-deficient mice, Th2-differentiated myelin-specific T cells are able to adoptively transfer a pathologically distinct form of EAE, suggesting that Th2 cells may also have some pathologic potential (31). This raises some concerns in treating multiple sclerosis or other autoimmune diseases solely through Th2-deviating mechanisms.

Although Th2 RMTC are highly effective in treating even advanced EAE, our data demonstrate that their efficacy cannot be explained by IL-4 or Th2 deviation alone. Treatment with IL-4-specific Abs blocks RMTC-induced IL-4 production by autoreactive T cells. This was expected because IL-4 plays an essential role in Th2 diversion, largely through the induction of GATA3 (32). However, blockade of IL-4 fails to inhibit proliferation-suppression or disease prevention mediated by the RMTC. Rather, IL-10, a cytokine common to both Th2 and other regulatory cell subsets, is necessary and seems primarily responsible for the immunotherapeutic effects of the RMTC.

The precise role IL-10 plays in EAE immunoregulation is not clear. One study of cytokine expression in the CNS of mice with relapsing EAE failed to correlate IL-10 expression with disease activity, suggesting it has a limited role in regulating the cyclical immunopathology (33). In contrast, IL-10−/− C57BL6 mice demonstrate increased susceptibility to severe EAE whereas Tg mice constitutively expressing IL-10 are protected (34–36), suggesting that IL-10 plays a significant role in restricting EAE severity. Although IL-4 administration can be immunotherapeutic in EAE and IL-4−/− mice develop more severe EAE than wild-type controls, comparison of IL-4−/− and IL-10−/− mice and mice transgenically expressing each cytokine suggests that IL-10 plays a more significant role in antagonizing disease (14, 34, 35, 37, 38).

Similarly, several studies have shown a significant role for IL-10 in both Ag-specific and -nonspecific immunotherapeutic applications. IL-10 would be expected to diminish the Th1-promoting actions of APC through its ability to down-regulate production of IL-12 and other proinflammatory cytokines (39). However, whereas IL-10 ameliorates autoimmunity in many models, the unregulated delivery of IL-10 may also exacerbate autoimmune disease (13, 40). Thus, pharmacologic intervention with IL-10 may induce effector or regulatory circuits that beneficially or adversely influence disease outcome. In contrast, studies using interventions that promote a more natural pattern of IL-10 production, as with the RMTC studied here, have consistently shown beneficial effects of this cytokine (41–45). For instance, IL-10 has been found to play important roles in the induction of intranasal and oral tolerance, and adoptive transfer of autoantigen-specific T cells constitutively expressing IL-10 can mollify autoimmune symptoms. Defining how the directed targeting of IL-10 with RMTC can act to modulate autoimmunity will therefore be important to establish in further studies.

In summary, we define a novel approach for the treatment of autoimmune disease through the directed targeting of pathologic T cells with TCR-specific RMTC. We further demonstrate that Th2 RMTC are potent and effective in treating even advanced EAE and identify IL-10 as critical to the therapeutic activity of these RMTC. Our results support the use of RMTC or other approaches capable of focusing IL-10 production directly at pathologic T cells as effective interventions against autoimmune diseases.

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References
