Treatment of Autoimmune Anterior Uveitis with Recombinant TCR Ligands

Grazyna Adamus,1,2 Gregory G. Burrows,3,4 Arthur A. Vandenbark,4 and Halina Offner4

PURPOSE. To determine protective properties of recombinant TCR ligands (RTLs) as a new treatment for experimental autoimmune anterior uveitis (AU). RTLs comprise the rat RT1.B β1/1 domains, linked either to the guinea pig MBP69-89 peptide (RTL201), to the corresponding rat MBP69-89 peptide (RTL200), or to the cardiac myosin peptide CM-2 (RTL203).

METHODS. AU associated with experimental autoimmune encephalomyelitis (EAE) was actively induced in Lewis rats by injection of myelin basic protein emulsified in complete Freund’s adjuvant (CFA) or passively by the transfer of pathogenic T cells. Rats received five daily doses each of 300 µg RTL201 in saline, intravenously. Control rats received the same dose of RTL203 or an “empty” β1/1 protein (no peptide). The rats were evaluated for the suppression of clinical and histologic signs of AU.

RESULTS. RTL201 prevented active and passive AU and reduced the clinical symptoms of established AU. RTL201 completely prevented clinical and histologic AU in the treated rats, compared with disease progression in the untreated rats or those treated with an “empty” construct. The suppression of clinical AU correlated with a significant reduction in inflammatory cells infiltrating the eyes of the RTL201-treated rats. Furthermore, RTL201 inhibited T cell proliferation, DTH responses, and cytokine mRNA expression in the eye, in contrast to the untreated rats. In comparison with RTL201, RTL200 was less effective in protecting the eye from AU. RTL203 also significantly inhibited clinical AU, but not EAE.

CONCLUSIONS. RTL constructs suppressed clinical and histologic AU by inhibiting the systemic activation of specific T cells and preventing the recruitment of inflammatory cells into the eye. These findings suggest a possible clinical application of this novel class of peptide/MHC class II constructs in patients with AU that is mediated by T-cell responses to known antigenic peptides. (Invest Ophthalmol Vis Sci. 2006;47:2555–2561) DOI:10.1167/iovs.05-1242

Uveitis, a sight-threatening inflammatory disease of the eye, can result from immune-mediated responses against ocular antigens. In autoimmune uveitis, T cells appear to play an important role in the pathogenicity of disease. For the disease to develop, autoreactive T cells must be activated outside the eye and then must pass the blood-ocular barrier, enter the eye, and cross-react with ocular autoantigens.

Despite significant research efforts and advances in diagnosis and therapy, ocular autoimmune diseases, which cover a variety of ocular diseases with different clinical symptoms and pathogenicity, remain significant causes of visual impairment in humans. The most common form of uveitis is anterior uveitis (AU). Approximately 50% of patients will have uveitis with an associated systemic disease. Vogt-Koyanagi-Harada disease, sympathetic ophthalmia, and birdshot retinochoroidopathy are conditions that demonstrate a significant genetic HLA association with both HLA class II and class I molecules, suggesting that uveitis has an autoimmune predisposition.

Animal models of autoimmune uveitis have been useful in testing new therapeutic approaches to treat intraocular inflammation. Similar to uveitis in humans, experimental uveitis in animals is genetically controlled. Therapies for experimental uveitis include the induction of oral tolerance, treatment with antibodies against cytokines, and treatment with antibodies against surface T-cell molecules and major histocompatibility complex (MHC) class II molecules. Blocking the antigen-presentation function of MHC molecules by competitor peptides has been proposed as a possible immunotherapy for MHC-associated autoimmune diseases. In experimental autoimmune encephalomyelitis (EAE), the MHC blockade by competitor peptides with high MHC class II binding affinity could prevent development of EAE in Lewis rats induced with guinea pig myelin basic protein (MBP). Several MHC class II binding competitor peptides were used for such treatment, including nonimmunogenic disease-related peptides, immunogenic peptides, and EAE- and non-EAE-related peptides. The efficacy of these competitor peptides to inhibit MBP-induced proliferation of an encephalitogenic T-cell line in vitro correlated with their respective MHC binding affinities. During disease induction with the entire MBP, coimunization of the competitor peptides resulted in the concentration-dependent inhibition of clinical signs of EAE, although polyclonal T-cell responses to MBP were not completely inhibited. The coadministration of immunogenic or nonimmunogenic EAE- or non-EAE-related MHC class II-binding competitor peptides inhibited the development of EAE induced with an entire MBP.

In previous studies, we used an experimental model of AU in association with EAE, to examine the molecular mechanism of uveitis in association with systemic inflammatory disease. In Lewis rats, AU and EAE developed simultaneously when the rats were injected with MBP. In this model, the target autoantigens in EAE and AU included myelinated neurons found within the central nervous system and the iris, respectively. AU, as well as EAE, can be transferred to naive animals by activated T-cell lines specific for MBP. MBP-induced AU onset usually coincides with the onset of EAE but has a longer duration, persisting after clinical signs of EAE subside. Encephalitogenic cells that recognize the immunodominant MBP epitope at residues 69-89 appear to play a role in the development of AU. T cells infiltrating the iris and ciliary body are found to share similar characteristics with the T cells that cause EAE. Because we found that these eye-infiltrating T cells

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proliferate in the presence of MBP, we concluded that in Lewis rats, MBP-specific cells are encephalitogenic as well as uveitogenic.20

In the present study, we tested a novel class of constructs, recombinant TCR ligands (RTLs), to determine whether they can prevent the development of AU associated with EAE. We studied the clinical effects of RTLs, which consist of rat RT1.B β1α1 domains noncovalently or covalently linked to the 69-89 peptide of guinea pig MBP (RTL201), to the corresponding 69-89 peptide from rat MBP (RTL200), or to the cardiac myosin peptide CM-2 (RTL203). Initially, the β1α1 molecule was loaded with the soluble antigenic peptide 69-89, the major antigenic MBP epitope in Lewis rats. Earlier studies have shown that the β1α1/MBP69-89 peptide complex (RTL201) is highly specific in its ability to bind to and inhibit the function of MBP69-89-specific T cells and to suppress clinical and histologic signs of EAE, delayed-type hypersensitivity (DTH) responses to MBP, and proliferation responses of lymph node T cells.21 In the present study, we found that RTL201 was also able to prevent and to treat active and passive AU associated with EAE by blocking the activation of pathogenic cells, which prevented the uveitogenic T cells from entering the eye.

METHODS

Induction and Clinical Assessment of AU and EAE

Female Lewis rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN), 8 to 12 weeks old, were used in the studies. The rats were housed in germ-free conditions at the Animal Care Facility of Veterans Affairs Medical Center (Portland, OR), according to institutional and federal guidelines. All animal experimentation procedures adhered to the ARVO Statement for the Use of Animals Ophthalmic and Vision Research and were approved by the institutional animal committee.

AU associated with EAE was induced by subcutaneous injection of 25 μg guinea pig MBP in Freund’s complete adjuvant (CFA), supplemented with 150 μg Mycobacterium tuberculosis strain H37Ra (Difco, Detroit, MI). The rats were assessed daily for signs of clinical EAE and histologic changes in the eye and brain. The eyes were removed at the peak of the disease and fixed in 10% neutral buffered formaldehyde solution. They were then embedded in paraffin and stained with hematoxylin and eosin. The eyes were scored for histologic signs of ocular inflammation by microscopy, according to the following scale: 0, normal; 1, slight iris vessel dilation and thickened iris stroma, a few scattered inflammatory cells, or both; 2, engorged blood vessels in the iris, abnormal pupil contraction, and occasional vitreous cells; 3, hazy anterior chamber and decreased red reflex; and 4, marked presence of vitreous cells.

Recombinant TCR Ligands

Recombinant protein containing a single polypeptide chain of the α1 domain linked to the β1 domain (in this study, termed “β1α1”) was prepared as described previously.21 RTLs comprise the rat RT1.B β1α1 domains that are linked noncovalently or covalently to the guinea pig MBP69-89 (RTL201) PQKQRSQDENPVHVIF and to the corresponding rat MBP69-89 (RTL200) PQKQRSQDENVVIF. There is only one single amino acid’s difference at position 80 of the MBP sequence where threonine in RTL200 (rat) is substituted by serine in RTL201 (guinea pig). RTL203 contains an irrelevant peptide, covalently coupled cardiac myosin-derived peptide, CM-2 (KLELQSALEEASLEH).

Noncovalent Binding. Rat MBP69-89 or the cardiac myosin peptide CM-2 was incubated with a β1α1 molecule at a 1:10 protein-to-peptide molar ratio at room temperature for 24 hours. All subsequent manipulations were performed at 4°C. The free peptide was then removed by centrifugal ultrafiltration (Centricon-10 membranes; Millipore, Bedford, MA) and serially diluted, which concentrated the retained solution until the concentration of free peptides was less than 2 μM.

Covalent Binding. The β1α1 molecule with covalently coupled rat and guinea pig antigenic peptides: β1α1-Rt-MBP69-89 (RTL200) and β1α1-Gp-MBP69-89 (RTL203) constructs were prepared and carefully characterized, as in earlier studies.22 Briefly, the covalently coupled β1α1, a peptide molecule complex, was produced by an insertion sequence that encoded a covalently coupled antigenic peptide and a thrombin cleavage site embedded within a flexible linker. Within this linker, a unique Spel restrictionendonuclease site allowed the production of β1α1 molecules with different covalently coupled peptides by cutting the β1α1 peptide construct and directionally cloning the DNA fragment of interest.

Treatment of Active AU and EAE

Rats received five doses of 300 μg RTL200, RTL201, or RTL203 in saline intravenously on days 3, 7, 9, 11, and 14, after an injection of 25 μg MBP in CFA. In control experiments, the control group of rats remained untreated or received 300 μg free MBP69-89 or 300 μg ‘empty’ β1α1 protein (no peptide). The rats were evaluated daily for clinical signs of AU or EAE.

Treatment of Passive AU

Lewis rats received an injection of 1 × 107 Gp-MBP69-89-specific T cells. T lymphocytes were obtained from lymph nodes of rats immunized 10 days earlier with Gp-MBP69-89 in CFA. The T cells were stimulated with the Gp-MBP69-89 peptide for 72 hours, and then 1 × 107 blast T cells were transferred intravenously into the tail vein of a recipient rat. The treatments started with injections of 300 μg RTL201 in saline on days 1, 3, and 5. The rats were assessed daily for changes in clinical signs of AU and EAE.

Histologic Grading of Experimental Uveitis

The eyes were removed at the peak of the disease and fixed in 10% neutral buffered formaldehyde solution. They were then embedded in paraffin and stained with hematoxylin and eosin. The eyes were scored for histologic signs of ocular inflammation by microscopy, according to the following scale: 0, normal; 1, slight iris vessel dilation and thickened iris stroma, a few scattered inflammatory cells, or both; 2, engorged blood vessels in the iris, abnormal pupil contraction, and occasional vitreous cells; 3, hazy anterior chamber and decreased red reflex; and 4, marked presence of vitreous cells.

RNA Isolation and cDNA Synthesis

Total RNA was isolated from the iris/ciliary body or lumbar spinal cord tissues that were dissected from two rats, from each of the experimental and control groups. The total RNA was then pooled for each group. Extraction was performed according to the manufacturer’s procedures (TRIzol; Invitrogen, Inc., Carlsbad, CA). RNA concentration was determined by spectrophotometry. One microgram total RNA from each sample was annealed with oligo(dT)12-18 (300 ng/50 μL reaction) and reverse-transcribed to cDNA, using 80 U of MmLV reverse transcriptase per reaction for 1 hour at 37°C. The reaction was stopped by heating the sample for 5 minutes at 90°C.

PCR Amplification

PCR amplification was performed with a thermocycler (Ericomp, Inc., San Diego, CA). The 50-μL reaction mixture consisted of 5 μL cDNA, 1 μL (0.3 μM) sense and antisense primer, 200 μM each of deoxynucleotide, 1.3 U Taq polymerase, 1.5 mM MgCl2, and 5 μL 10× Taq polymerase buffer. For cytokine amplification, conditions were as follows: denaturation for 1 minute at 94°C and elongation for 3 minutes at 72°C. The annealing temperature from 62°C to 46°C was decreased in 1°C increments, followed by 20 cycles at 50°C. The annealing temperature for IL-2 and -4 was from 67°C to 50°C, decreasing in 1°C increments, followed by 21
cycles at 55°C. At the end of amplification, the reaction mixture was heated 10 minutes at 72°C and cooled to 4°C. Fifteen microliters of each PCR product was separated by gel electrophoresis on 2% agarose containing ethidium bromide and then analyzed under UV light against the DNA molecular markers.

**Delayed-type Hypersensitivity**

DTH reactions were measured by an ear-swelling assay 48 hours after injection of 20 µg antigen into the ear pinna. The specific response was calculated as the difference between ear thicknesses after MBP injection minus ear thickness before MBP injection.

**T-Lymphocyte Proliferation Assay**

T lymphocytes recovered from the lymph nodes of the control and RTL-treated Lewis rats were seeded at 2 × 10^5 cells in 200 µL/well and cultured with 1 × 10^5 irradiated antigen-presenting cells (APCs) and Gp-MBP_{69-89} peptide. The cultures were incubated for 3 days before 0.5 µCi/10 µL/well [³H]thymidine was added for overnight incubation. The cells were harvested onto glass fiber filters, and [³H]thymidine uptake was assessed in a β scintillation counter (Wallac model 1200; PerkinElmer, Boston, MA). The data are expressed as a stimulation index (SI), which was calculated by dividing the proliferation (counts per minute incorporated) measured in the presence of the antigen by the proliferation measured with the medium alone. Stimulation was considered positive if the SI of immunized rats was equal to or greater than twice the background (SI = 2).

**Statistics**

Data from individual experiments are expressed as the mean ± SD. Statistical significance was performed for the control and treated groups using paired and unpaired Student’s t-tests. Differences in the peak score were assessed by Mann-Whitney test and/or ANOVA. P ≤ 0.05 was considered significant.

**RESULTS**

**Effect of RTL201 (β1α1/MBP_{69-89}) on Development of AU and EAE**

We tested RTLs as preventative treatment for AU. Because T cells specific for the major uveitogenic/encephalitogenic epitope MBP_{69-89} play a role in AU development, we examined whether treatment with RTL constructs could inhibit eye inflammation as well as EAE. Three different MHC class II RT1.B-derived RTLs were used: RTL200 (β1α1-Rt-MBP_{69-89}), RTL201 (β1α1-Gp-MBP_{69-89}), and RTL203 (β1α1-CM-2). RTL203, which contains the coupled cardiac myosin-derived peptide CM-2, was used as an irrelevant peptide control. The RTLs were prepared by noncovalent and covalent binding of α1β1 to the pathogenic peptide MBP_{69-89}.

In the untreated control group, there was clinical evidence of AU and EAE, as assessed by clinical scores starting at disease onset on days 10 to 11. Figure 1 shows that RTL201, prepared by noncovalent binding, completely suppressed AU and EAE development that had been induced by immunization with MBP. In the group treated with RTL203, the expression of clinical AU was also considerably suppressed, with the AU scores lower (0.65 ± 0.57) than those of the control group (2.05 ± 0.47) and reaching statistical significance (ANOVA; P < 0.01). RTL201 inhibited the proliferation of T cells (Fig. 1C), which was consistent with the inhibition of AU in vivo. The proliferative response of T cells was measured in draining lymph node cells obtained from both treated and control rats at the peak of EA and AU, which were stimulated for 72 hours in vitro with Gp-MBP_{69-89} (50 µg/mL) or whole Gp-MBP (10 µg/mL). The T cells from the RTL201-treated rats showed a two- to threefold decrease in their proliferative response to Gp-MBP_{69-89}, or to whole Gp-MBP, compared with T cells from the control rats. The T cells from the RTL203-treated rats also showed a mild response to Gp-MBP_{69-89}. In addition, as shown in Figure 1D, RTL201 treatment significantly suppressed DTH responses to MBP_{69-89} (P = 0.01).

**Effect of RTL201 on Cytokine Expression**

Cytokine mRNA expression in the iris/ciliary body at the peak of MBP-induced AU was examined to confirm that the Th1-type response was suppressed. In the control group, significant production of mRNA for IL-2 and IFN-γ was detected in the iris/ciliary body and spinal cord (Fig. 1E). Either no IL-4 mRNA or a very low amount was detected in the iris/ciliary body, as opposed to the preparation from the spinal cord, where the upregulation of IL-4 mRNA was observed. In the RTL201-treated group with suppressed AU, there was no expression of IL-2 and IFN-γ RNA in the iris/ciliary body at the peak of AU. These findings correlated with our histopathological findings, which showed a complete lack of infiltrating cells in the iris (Fig. 2). In contrast, the expression of Th1-specific cytokines in the spinal cord was reduced but not abolished. These data were consistent with the finding that clinical EAE was not totally suppressed by RTL201 (Fig. 1B).

**Effect of RTLs on Cell Infiltration into the Eye**

There was a significant reduction in the number of uveitogenic and inflammatory cells infiltrating the eye and spinal cord. Figure 2 shows a representative histologic staining of the iris for rats treated with RTL201, RTL203, or empty β1α1. The untreated (control) and β1α1-treated rat eyes showed a large number of inflammatory cells infiltrating the iris. Mononuclear cell infiltration was found in and around the iris and ciliary body. Nodular cell infiltration of the iris was common. In contrast, all rats treated with RTL201 showed only a few cells on the surface of iris. In addition, treatment with RTL203 (the irrelevant peptide) almost completely suppressed the entry of inflammatory cells into the iris, which was consistent with the clinical assessment (Fig. 1). In the RTL203-treated group, we also found that in two of five rats, the architecture of the retina was disrupted, showing evidence of leakage without cellular infiltrate (data not shown). Histologic severity of AU in treated rats correlated with clinical severity (Table 1).

**Effectiveness of Guinea Pig RTLs versus Rat RTLs**

We also examined the effect of a single amino acid substitution in the MBP_{69-89} as exists in rat and guinea pig MBP peptides. Two RTLs prepared by the covalent binding of RTL200 and RTL201 differed at a single amino acid position: threonine (RTL200) for serine (RTL201) at position 80 of the MBP sequence. Lewis rats were treated with 300 µg RTL200 or RTL201 on days 3, 7, 9, and 11. RTLs were prepared by the covalent linking of MBP_{69-89} peptide to the β1α1 protein. Figure 3 shows the clinical scores at the peak of symptoms after treatment with RTL200 and RTL201. AU developed in the control rats, whereas only mild disease developed in rats treated with RTL200. RTL201 and RTL200 were both effective in suppressing EAE (ANOVA P < 0.0001) in comparison to the control group. AU of significantly lesser severity and shorter duration was characteristic of the group treated with RTL201 (ANOVA: P = 0.0426), but RTL200 had a lesser effect on suppressing AU. Although overall protection at the peak of disease was lower, the duration of AU in the RTL200-treated rats lasted only 7 to 8 days, compared with 19 to 21 days in the control rats (data not shown).
Inhibitory Effect of RTL201 on Established Disease

In the experiments described thus far, we observed the inhibitory effect of RTL201 on active AU induction. In the next experiment, we asked whether RTL201 also effectively treats established AU. Lewis rats were treated with 300 μg RTL201 on the first day of disease onset, followed by injections of RTL201 at the same dose (300 μg) on days 11, 13, 15, and 17. Figure 4 shows that in the RTL201-treated group, the severity of clinical AU was significantly lowered (P = 0.0109) but not abolished, suggesting only partial protection from AU at this dosage regimen. EAE did not develop in any of the RTL201-treated rats (P = 0.0005), but in the control rats, EAE progressed to complete hind limb paralysis.

Inhibitory Effect of RTL201 on Passively Transferred Disease

The effect of RTL201 on passively transferred AU was evaluated in recipient rats after intravenous injection of 1 × 10⁷ Gp-MBP69-89-specific T cells. In a typical passive transfer EAE/AU experiment, the onset of AU and EAE was found to occur between days 3 and 5.⁹ The injection of 300 μg RTL201 in saline on days 1, 3, and 5, after the encephalitogenic/uvectogenic T-cell transfer, completely blocked the induction of clinical signs of AU (P = 0.0015; Fig. 5) and EAE (data not shown).

DISCUSSION

In this study, we explored a novel methodology for targeting T cells to treat AU-associated with EAE. We constructed a single-chain molecule derived from an antigen-binding β1 domain and an α1 domain of the rat RT1.B class II, with or without the major pathogenic peptide MBP69-89. These complexes inhibited clinical and histologic AU. Our studies demonstrated that RTL201 (β1α1-Gp-MBP69-89) could suppress and treat clinical signs of AU by directly inhibiting the systemic activation of MBP69-89-specific T cells and preventing the recruitment of inflammatory cells into the eye. Further, AU presented a remarkable reduction of inflammatory cells infiltrating the eye. RTL201 was effective in suppressing both actively and passively induced AU, as well inhibiting disease after the onset of clinical symptoms.

In addition to the successful suppression of AU, RTL201 inhibited the activation of MBP69-89-reactive T cells and DTH.
responses. This inhibition of the systemic activation of specific pathogenic cells led to a significant reduction of clinical symptoms of EAE and the complete suppression of AU signs, which suggests that AU is easier to suppress than is EAE. A possible explanation for this difference in suppression may be due to the different number of pathogenic T cells in the periphery, which are critical for the development of inflammation in the eye and spinal cord. The presence of residual EAE suggests that, other than the 69-89 peptide, epitopes may participate in the disease, and these epitopes may not be I-A restricted (e.g., MBP_87-99 peptide, which is I-E restricted). Although this encephalitogenic epitope is also uveitogenic, it appears to play a lesser role in inducing AU. In general, RTLs, in inhibiting eye inflammation, appear to be specific in their ability to inhibit pathogenic T-cell activities. Therefore, RTLs may represent a new class of reagents for treating human uveitis. Most cases of human uveitis of unknown etiology are considered to have an autoimmune basis,TABLE 1. Clinical and Histological Scores in Treatment of AU and EAE

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incidence</th>
<th>AU</th>
<th>Clinical Peak Score</th>
<th>Histology Peak Score</th>
<th>EAE</th>
<th>Clinical Peak Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (control)</td>
<td>5/5</td>
<td>10/10</td>
<td>2.3 ± 0.33</td>
<td>2.05 ± 0.47</td>
<td>5/5</td>
<td>2.9 ± 0.65</td>
</tr>
<tr>
<td>RTL201</td>
<td>0/5</td>
<td>0/10</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0/5</td>
<td>1.0 ± 0.87</td>
</tr>
<tr>
<td>RTL203</td>
<td>5/5</td>
<td>7/10</td>
<td>0.5 ± 0.3</td>
<td>0.65 ± 0.57</td>
<td>5/5</td>
<td>1.9 ± 1.75</td>
</tr>
<tr>
<td>Empty β1α1</td>
<td>5/5</td>
<td>8/10</td>
<td>1.1 ± 0.96</td>
<td>1.2 ± 0.92</td>
<td>5/5</td>
<td>2.90 ± 0.42</td>
</tr>
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</table>

To understand the mechanism of RTL protection, one has to evaluate pro- and anti-inflammatory cytokine expression during AU and EAE. During AU and EAE, there was no significant difference in the expression of the proinflammatory cytokines TNF-α, IL-1β, IL-6, IFN-γ in the iris/ciliary body and spinal cord. Our studies showed a marked reduction in RNA transcripts for IL-2 and IFN-γ in the organs of RTL201-treated rats, a reduction that may be related to the low number of infiltrating cells in the target organs. In our study, other cytokines were not evaluated in rats, but there is new evidence from in vitro studies of EAE mice treated with RTLs showing that RTLs induce IL-10 in an antigen-dependent fashion. IL-10 was secreted on restimulation of the RTL-pretreated T-cell clones with APCs and the antigen, suggesting that TCR interaction with the RTL results in a default production of IL-10 that persists even on re-exposure to specific antigen. In the treatment of EAE in SJL mice, after only three daily injections of RTL401, PLP_139-151-specific splenocytes showed enhanced secretion of TNF-α, IFN-γ, IL-6, and IL-10 only in mice that showed clinical improvement. In mice with passive EAE, treatment with RTL-401 (PLP_139-151) also strongly enhanced the production of the Th2 cytokine IL-13 in the spleen, blood,
and spinal cord tissue, with variable effects on other Th1 and Th2 cytokines. Moreover, pretreatment of PLP_{139-151}-specific T cells with RTL401 in vitro induced high levels of secreted IL-13. The role of these and other cytokines during RTL treatment is under further investigation in our laboratory, to gain a better understanding of the therapeutic mechanism of RTLs on the induction and prevention of disease.

Recent studies examining the mechanism of RTL inhibition have shown that RTLs linked to the Gp-MBP_{69-89} peptide bind the TCR on the T cells, which triggers specific downstream signaling events that, in effect, deplete intracellular calcium stores without fully activating the T cells. The resultant antigen-specific activation of the transcription factor NFAT, uncoupled from the activation of NF-kB or extracellular signal-regulated kinases, constitutes a unique downstream foundation for the inhibitory effects of RTLs on pathogenic CD4\(^+\) T cells.

In our studies, RTL200 and RTL203 also protected rats from AU, but to a lesser extent. The findings from our RTL200 (\(\beta1\alpha1\)-RT-MBP_{69-89}) experiments suggest that small differences in the amino acid sequence of the pathogenic epitope might produce considerable differences in clinical outcomes. At the same time, the substitution of the MBP peptide with an unrelated peptide in RTL203 (\(\beta1\alpha1\)-CM-2) also significantly reduced the severity of AU. This ability of the unrelated to the MBP peptide, CM-2 (RTL203) to suppress AU implies that the protein \(\beta1\alpha1\), loaded with any antigenic peptide, may bind weakly to any rat I-A (RT1.B)-restricted T cell, resulting in at least some inhibitory activity. Other peptides should be tested for their effects in treating ocular inflammation, to elucidate further the nonspecific suppressive effect of RTLs on uveitis and to design clinical trials.

Uveitis is a heterogeneous group of diseases affecting different parts of the eye and showing an association with various HLA class I or class II molecules. Experimental autoimmune disease models have targeted factors that appear to play a crucial role in the disease process, such as pathogenic T cells, autoantigenic peptides, and MHC molecules, as well as regulatory T cells. Methods targeting T-cell receptors have also been successfully used to suppress EAE, but have met with lesser success in preventing uveitis. Because susceptibility to autoimmune disease is linked with expressions of certain MHC genes, MHC molecules were targeted for treatment. Treatment of rats with MHC class I and II blocking antibodies resulted in the downregulation of experimental autoimmune uveoretinitis (EAU) induced by S-antigen. In addition, peptides with a high affinity to MHC class II molecules also inhibited EAU (Kozhich A, et al. IOVS 1992;33:ARVO Abstract 1672) and EAE. Recently, Pennesi et al. developed a humanized EAU model in HLA transgenic mice, in which disease-relevant epitopes appear to be largely restricted by human class II molecules. These investigators reported that severe EAU, induced with an S-antigen (arrestin), developed in HLA-DR3 transgenic mice, to which wild-type B6 mice are known to be highly resistant. In the HLA-DR3 transgenic mice, anti-HLA antibodies blocked the proliferation of T lymphocytes in response to retinal antigens. Of note, an antigen-specific T-cell repertoire for S-antigen or interphotoreceptor retinoid-binding protein (IRBP) differed from that of wild-type mice, suggesting differences in the selected antigen-specific repertoire. The HLA-DR3 transgenic mouse model corroborates the involvement of class II molecules in human uveitis and supports an etiologic role for retinal antigens in human disease. Targeting T cells in treatment with RTLs opens a new avenue for treating autoimmune uveitis.

In conclusion, we have shown that RTL constructs inhibited clinical and histologic AU by decreasing the systemic activation of specific T cells and preventing the recruitment of inflammatory cells into the eye. These findings suggest a possible clinical application of this novel class of peptide/MHC class II con-
structures for patients with uveitis, which is mediated by T-cell responses to known antigenic peptides.

References


