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Spontaneously relapsing–remitting experimental autoimmune uveitis in rats allows successful therapeutic oral tolerance induction in ongoing disease

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ABSTRACT

Antigen-specific tolerance induction is a desired therapy for uveitis patients. Our relapsing–remitting rat model of experimental autoimmune uveitis (EAU) induced with IRBP peptide R14 enables us to test the effect of oral tolerance on the prevention of relapsing uveitis. We investigated several peptides overlapping the sequence of R14 for prevention and different doses of R14 for therapy to determine the tolerogenic epitope and the most effective therapeutic regimen for uveitis. Lewis rats were immunized with R14-CFA to induce EAU. Oral tolerance was induced prior to immunization (prevention) or after onset of EAU to prevent relapses (therapy). Therapeutic feeding was performed with high and/or low doses of oral antigen for clonal deletion of effector and induction of regulatory T cells. Uveitis was determined clinically and histologically; mesenteric lymph node (mLN) cells of tolerized rats were tested for surface markers, cytokines and Foxp3 expression. Preventive feeding of R14 and its major epitope R16, but none of the overlapping peptides significantly suppressed EAU and also prevented relapses, irrespective of their pathogenicity. Therapeutic feeding with R14 dramatically reduced relapses, while only the consecutive feeding of high and low-dose R14 had an ameliorating effect on the first course of disease. IL-10-producing T cells from mLN decreased after oral tolerization, and with R14-stimulation in vitro the TCRαβ+/Foxp3+ population increased in the low-dose fed group. No mLN population could be clearly assigned to successful oral tolerance induction during active autoimmune uveitis.

1. Introduction

Oral administration of soluble antigen can induce antigen-specific systemic tolerance and has been successfully applied in animal models of autoimmune diseases including experimental autoimmune uveitis (EAU) (Weiner et al., 1994; Nussenblatt et al., 1990; Thurau and Wildner, 2002; Weiner et al., 2011). Oral tolerance is a natural mechanism of the immune system to avoid adverse reactions to food antigens; it is antigen-specific and thus does not cause a general immunosuppression. However, oral tolerance is more efficient in preventing the activation of an immune response than in suppressing already ongoing immune reactions. Since most experimental animal models of autoimmune diseases have an acute, monophasic course they require preventive therapies for optimal efficiency (Torseth and Gregerson, 1998). This is in contrast to the situation in patients, who need intervention in an already primed immune response.

EAU in rats is an experimental model of autoimmune uveitis, which can be induced by various ocular autoantigens. Immunization with IRBP (interphotoreceptor retinoid-binding protein) peptide R14 (Diedrichs-Mohring et al., 2008; Kaufmann et al., 2012) can induce a relapsing course of disease with multiple non-synchronized relapses observed shortly after resolution of the first attack for an extended period of time. This model is highly suitable for testing the efficiency of oral tolerance induction in an already ongoing immune response, since tolerization can be initiated after onset of disease in order to prevent relapses.

The IRBP peptide R14 has a length of 23 amino acids and thus probably comprising several antigenic or tolerogenic epitopes. As previously published, pathogenic and tolerogenic peptides or epitopes, respectively, do not necessarily have to be identical. S-Antigen peptide PDSAg is highly pathogenic as well as orally tolerogenic (Wildner and Thurau, 1994), while two mimotopes,

Abbreviations: EAU, experimental autoimmune uveitis; CFA, complete Freund's adjuvant; PBS, phosphate-buffered saline; IRBP, interphotoreceptor retinoid-binding protein; mLN, mesenteric lymph node.

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Table 1
Amino acid sequences of peptides overlapping the sequence of R14.

Name	AA																										
R14	1169–1191							P	T	A	R	S	V	G	A	A	D	G	S	S	W	E	G	V	G	V	V	P	D	V
R4T	1163–1176	N	L	Y	L	T	I	P	T	A	R	S	V	G	A															
R4/R14	1169–1180							P	T	A	R	S	V	G	A	A	D	G	S											
PDIRBP	1174–1187												V	G	A	A	D	G	S	S	W	E	G	V	G	V				
R16	1177–1191															A	D	G	S	S	W	E	G	V	G	V	V	P	D	V

"AA" (amino acid) relates to the respective position within the IRBP protein.

peptides from milk casein and rotavirus, respectively, are only pathogenic, but not orally tolerogenic (Wildner and Diedrichs-Mohring, 2003, 2004). On the other hand, PDSAg-mimicking peptide B27PD is a strong oral tolerogen but an extremely poor pathogen and was thus used therapeutically for treating human uveitis (Wildner and Thurau, 1994; Thurau et al., 1999). Here we tested various peptides overlapping the sequence of peptide R14 for their uveitogenicity and their ability to induce oral tolerance to R14-induced EAU.

Oral tolerance can be induced by high and low dose of antigen (Torseth and Gregerson, 1998). High dose tolerance predominantly results in deletion of antigen-specific effector T cells, while low dose feeding of antigen induces regulatory T cells, which can transfer tolerance (Weiner et al., 1994; Gregerson et al., 1993). In autoimmune diseases the activation of autoreactive cells is not a onetime event, but happens consecutively, as shown by epitope spreading of the autoimmune response in relapsing diseases (Diedrichs-Mohring et al., 2008; Deeg et al., 2002). Thus the deletion of autoreactive T cells by a single or short-term high dose oral antigen application would probably not be sufficient to treat a long-lasting disease. Here we combined both regimens, trying to achieve a fast effect on the primary disease course by deleting the autoreactive cells via high-dose oral antigen and subsequently inducing regulatory cells using low doses to prevent relapses.

To further define the regulatory cell populations of the orally tolerized rats we investigated T cells from mesenteric lymph nodes for their type and cytokine expression. TCR-positive cells coexpressing CD4 and CD8 were increased after R14 stimulation in vitro. Very few T cells expressed IFN-γ or IL-17, while the T cell populations expressing IL-10 were decreased in all antigen-fed groups especially after antigen-stimulation. Only in the group that was solely fed with low-dose antigen Foxp3+ potential Treg cells were increased.

2. Materials and methods

2.1. Animals

Lewis rats (Lew/Orl Rj) were bred in our own colony or were purchased from Janvier (Le-Genest-St-Isle, France) and maintained under specific pathogen-free conditions. They had unlimited access to rat chow and water. Male and female animals were used for experiments at the age of 6–9 weeks. All experiments were approved by the Review Board of the local government (Regierung von Oberbayern) and conformed to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research.

2.2. Induction of EAU

Peptides R14, R16, PDIRBP, R4T and R4/R14 (for amino acid sequences see Table 1), derived from the sequence of human IRBP, were purchased from Polypeptide, Strasbourg, France or Biotrend, Cologne, Germany. Animals were immunized subcutaneously into both hind legs with a total volume of 100 μl of the respective peptides (R14: 15 μg, all other peptides were used at 50 μg/animal) emulsified in CFA, supplemented with *Mycobacterium tuberculosis* strain H37RA (BD, Heidelberg, Germany) to a final concentration of 2.5 mg/ml.

2.3. Induction of oral tolerance

Animals were fed with a gavage needle with peptide dissolved in a volume of 0.5 ml PBS or PBS only as indicated. To prevent EAU antigen was fed 3 times every other day prior to immunization, therapeutic feeding was initiated when the first rat showed clinical signs of uveitis and continued as indicated.

2.4. Grading of uveitis

Rats were examined daily with an ophthalmoscope for clinical signs of uveitis that were graded as described (de Smet et al., 1993). The clinical uveitis score only considers inflammation in the anterior part of the eye. Briefly, score 0.5 indicates dilated iris vessels, partial inflammatory infiltrates of iris rim and hazy anterior chamber; score 1: complete circular infiltration of iris rim; score 2: pupil area completely filled with cells and fibrin; score 3: formation of hypopyon; score 4: anterior chamber completely filled with cells, fibrin and blood. For histology eyes from sacrificed animals were immediately snap frozen in Tissue Tec OCT compound (Paesel and Lorey, Frankfurt/Main, Germany) in methyl butane at −80 °C. Cryosections of rat eyes were stained with hematoxilin and graded as described (de Smet et al., 1993). Briefly, score 0.5 describes destruction of less than 25% of the photoreceptor outer segments, while the maximum score of 4 represents the total destruction of the retina. Histology scores are restricted to retinal damage only and do not include anterior chamber involvement.

2.5. Immunofluorescence staining of mesenteric lymph nodes

Rats were sacrificed and mesenteric lymph nodes were collected. Single cell suspensions were prepared using a 70 μm nylon mesh (Falcon, Germany); the resulting cells were washed twice with RPMI. Cells were then either immediately used ex vivo or cultured for three days in RPMI1640/1% rat serum with 20 μg/ml peptide R14. Four hours prior to staining cells were incubated in 50 ng/ml PMA, 1 μg/ml ionomycin and 1 μg/ml brefeldin (all from Sigma–Aldrich, Germany). For surface staining mouse anti-rat TCR-αβ antibodies (clone R73, eBioscience, Germany) and mouse anti-rat TCR-γδ antibodies (clone V65, BioLegend/Biozol, Germany) both conjugated with FITC, anti-rat CD4-PE (Pharmingen, Germany) or anti-rat CD8-Alexa Fluor 647 (BioLegend/Biozol, Germany) were used. For intracellular staining cells were fixed and permeabilized according to the manufacturer's instructions for the rat anti-mouse/rat Foxp3 staining kit (eBioscience, Germany) and subsequently stained with mouse anti-rat IFN-γ antibodies (clone DB-1) conjugated with Alexa Fluor 647, rat anti-mouse IL-17 antibodies (clone TC11-18H10.1, crossreactive with rat IL-17) conjugated with PerCP/Cy5.5 (both BioLegend/Biozol, Germany) or mouse anti-rat IL-10-PE (clone A5-4, BD Biosciences, Germany). Foxp3-specific staining was performed with the rat anti-mouse/rat