A Pathogenic Autoimmune Process Targeted at a Surrogate Epitope

By Alexander T. Kozhich,* Yoh-Ichi Kawano,* Charles E. Egwuagu,* Rachel R. Caspi,* Raj K. Maturi,* Jay A. Berzofsky,‡ and Igal Gery*

*From the *Laboratory of Immunology, National Eye Institute, and ‡Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892*

Summary

Immunization with the retinal interphotoreceptor retinoid-binding protein (IRBP) induces in a variety of animals an inflammatory eye disease, experimental autoimmune uveoretinitis (EAU). We have previously shown that sequence 1181-1191 of bovine IRBP (BOV 1181-1191) is immunodominant and highly uveitogenic and immunogenic in Lewis rats. Sequence 1181-1191 of the rat IRBP (RAT 1181-1191) differs from BOV 1181-1191 by two residues, at positions 1188 and 1190, that are pivotal for the immunological activity of the bovine epitope. Here we show that, unlike its bovine homologue, RAT 1181-1191 did not induce EAU or an immune response in Lewis rats. The immunological inactivity of RAT 1181-1191 in Lewis rats is due at least in part to its poor affinity toward the antigen-presenting cells of these rats, as shown by its failure to compete with binding of BOV 1181-1191 to Lewis adherent spleen cells. Moreover, unlike all other known autologous homologues of immunopathogenic epitopes, RAT 1181-1191 was not recognized by lymphocytes sensitized against BOV 1181-1191 and failed to stimulate proliferation, uveitogenic capacity or signal transduction in these cells. These findings thus suggest that RAT 1181-1191 is not a likely target for lymphocytes sensitized against BOV 1181-1191 in the process in which these cells recognize IRBP in the rat eye and trigger the inflammatory reaction of EAU. Our data further suggest that the target for the disease-inducing lymphocytes is sequence 273-283 of the rat IRBP: (*a*) sequence 273-283 is highly conserved and is identical in the bovine and rat proteins; (*b*) determinant 273-283 is a "repeat" of 1181-1191 in the fourfold structure of IRBP and shares seven residues with BOV 1181-1191; (*c*) rat peptide 273-283 is recognized by lymphocytes sensitized against BOV 1181-1191 and stimulates them for proliferation and for acquisition of uveitogenicity; and (*d*) moreover, sequence 273-283 is superior to BOV 1181-1191 in its capacity to generate uveitogenicity in lymphocytes sensitized against this bovine peptide. The present study thus describes for the first time a system in which an autologous homologue of an immunopathogenic epitope is inactive and a "surrogate" determinant apparently serves as the target for lymphocytes sensitized against the immunopathogenic peptide.

Xenogeneic organ-specific antigens are commonly used to induce experimental autoimmune diseases. In the majority of systems, the xenogeneic antigens are more active than their autologous homologues (1, 2), presumably due to the tolerance toward most of the latter antigens. It is noteworthy, however, that analysis of the known major immunopathogenic determinants of organ-specific antigens has shown close similarity or even identity between the sequence of xenogeneic and autologous epitopes and, as expected, strong antigenic cross-reactivity between them (1–5).

Experimental autoimmune uveoretinitis (EAU)[1] is an inflammatory eye disease that can be induced in a variety of species by immunization with ocular-specific antigens (6, 7). EAU resembles certain uveitic conditions in humans and is considered a model for these diseases (7–9). One of the uveitogenic antigens is interphotoreceptor retinoid-binding protein (IRBP), a glycoprotein of 140 kD that localizes in the retina and participates in the transport of retinoids between the neural retinal layers and the pigment epithelium (10). In previous studies we have identified sequence 1181-1191 of bovine IRBP (BOV 1181-1191) as the core of an epitope that is immunodominant in Lewis rats and is highly uveitogenic and immunogenic in these rats (11). Sequence analysis of IRBP revealed that this protein exhibits a fourfold repeat structure, with partial homology among the four repeats (12). One of the three repeats of BOV 1181-1191, at sequence

[1] *Abbreviations used in this paper:* EAU, experimental autoimmune uveoretinitis; IRBP, interphotoreceptor retinoid-binding protein; S.I., stimulation index.

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273-283, was found to cross-react with BOV 1181-1191 and to be uveitogenic and immunogenic in Lewis rats (13).

Recent analysis of the sequence of rat IRBP revealed that the rat peptide 1181-1191 (RAT 1181-1191) differs from its bovine homologue by two residues, at 1188 and 1190 (14). Both residues were shown in a previous study (15) to be pivotal for the immunological activities of BOV 1181-1191 in the Lewis rat. The present study examined the immunological activities of RAT 1181-1191 and analyzed the unique features of the immunopathogenic process initiated by BOV 1181-1191. Interestingly, T cells sensitized against BOV 1181-1191 did not cross-react with the RAT 1181-1191 in all tested in vitro assays, but rather, they recognized and were stimulated by another rat epitope, 273-283. These observations are interpreted to suggest that peptide 273-283 is the target for these lymphocytes in their initiation of the inflammation process in the eye.

Materials and Methods

Ag. Peptides were synthesized using the t-Boc chemistry (16), on a peptide synthesizer (model 430A; Applied Biosystems, Inc., Foster City, CA). Peptides were purified by preparative reverse phase HPLC and characterized by analytical HPLC, amino acid analysis, and plasma desorption ionization mass spectroscopy. The sequence of the different peptides used in this study and their designation are detailed in Table 1. Biotinylated peptide Biotin-GG-(BOV 1179-1191) was synthesized by solid phase method. Two glycine residues were added to serve as a spacer. Biotin was coupled using BOP/hydroxybenzotriazol in dimethylsulphoxide in manual mode. The peptide was purified by HPLC and characterized by mass spectroscopy. The biotinylated peptide is recognized by BOV 1181-1191–specific T cell line in in vitro proliferation assay (data not shown).

Isolation of Rat IRBP cDNA and DNA Sequence Analysis. RNA was isolated from Lewis rat retinas and reverse transcribed PCR was carried out essentially as described (17). PCR primers, 5'-ACAAGGATGTGGTGGTCCTCACCA-3' corresponding to mouse IRBP nucleotides 556-580 and 5'-TCAAAGCGCAGGTAGCC-CACATTGC-3', complementary to mouse IRBP nucleotides 1190-1214 (EMBL/GenBank/DDBJ accession no. Z11813) were used to amplify the rat cDNA fragment homologous to bovine IRBP amino acids 271-283. The DNA fragment was blunt-ended by addition of DNA polymerase (Klenow fragment) and dNTPs at 37°C for 30 min and was cloned into the SmaI site of Bluescript [KSII$^+$] sequencing vector (Stratagene, La Jolla, CA). DNA sequencing was performed by the dideoxynucleotide chain termination method using T3/T7 promoter primers and Sequenase sequencing system (United States Biochemical Corp., Cleveland, OH).

Table 1. *IRBP Synthetic Peptides Used in This Study*

Peptide	Sequence*
BOV 1179-1191	GSSWEGVGVVPDV
BOV 1181-1191	SWEGVGVVPDV
T1188	SWEGVGVTPDV
N1190	SWEGVGVVPNV
RAT 1179-1191‡	GSSWEGVGVTPNV
RAT 1181-1191‡	SWEGVGVTPNV
IRBP 271-283§	SQTWEGSGVLPCV
IRBP 273-283§	TWEGSGVLPCV

* The amino acid sequence numbering is according to Borst et al. (12). Residues in bold are different from bovine sequence 1179-1191.
‡ According to reference 14 and verified in our laboratory (our unpublished data).
§ Identical in the rat and bovine IRBP; the rat sequence was determined as described in Materials and Methods.

Animals. Male inbred Lewis rats, 8–11-wk-old, were supplied by Charles River Breeding Laboratories, Inc. (Raleigh, NC).

Immunization. The rats were immunized by a single injection of 1:1 emulsion of the peptides in PBS and CFA, containing *Mycobacterium tuberculosis* H37Ra at 2.5 mg/ml. The emulsion was injected into the left hind footpad in a volume of 0.1 ml containing various amounts of peptides. Killed *Bordetella pertussis* bacteria (lot 94; Michigan Department of Public Health, Lansing, MI) were injected intravenously, 10^{10} organisms/rat, concurrently with the immunization.

Disease Monitoring and Assessment. Immunized rats were examined daily for clinical ocular changes (18); occurrence and severity of the disease were verified by histology (18). The severity of the changes was graded on a scale of 0–4, based on the intensity of the inflammatory changes rather than on specific disease features, because diseased rats usually developed panuveitis that affected most ocular tissues.

Lymphocyte Proliferation Assay. Draining LN cells were obtained 12–16 d after immunization and their proliferative responses were measured as described in detail elsewhere (19). Briefly, lymphocytes were cultured in triplicate in flat-bottom microplates, with or without the tested Ag, 3×10^5/0.2 ml/well in RPMI 1640 medium supplemented with 1% normal rat serum, and 2-ME at 5×10^{-5} M. The total incubation time was 96 h, with the pulse of [^{3}H]thymidine (0.5 μCi/10 μl/well) given during the last 16 h. Data are presented as stimulation index (S.I.) values (mean cpm in cultures with stimulus/mean cpm in control cultures without stimulus).

Cell Line Responses. A T lymphocyte cell line specific for BOV 1181-1191 was established from sensitized lymph node cells, as described in detail elsewhere (19). The line cells were tested for proliferation after 7 d of incubation in IL-2–containing medium. Proliferation assay was performed as described in reference 19. In brief, 2×10^4 line cells and 5×10^5 irradiated thymus cells (being used as APCs) were incubated in 0.2 ml supplemented RPMI 1640 medium. Cells were incubated for 72 h and pulsed with [^{3}H]thymidine during the last 16 h.

Inositol Phosphates Accumulation. Generation of water-soluble inositol phosphates in the BOV 1181-1191–specific cell line was measured using the method of Jenkins et al. (20) with modifications. Briefly, line cells at 10^7/ml were incubated for 7 h in inositol-free DMEM (Biofluids, Inc., Rockville, MD), with 10% dialyzed FCS, 1% glutamine, and 50 μCi/ml *myo*-[2-^{3}H]inositol (17.8 Ci/mM; New England Nuclear, Boston, MA). Labeled cells were washed 1× with PBS and suspended at 10^6 cells/10 ml medium consisting of RPMI 1640 (Biofluids Inc.), 10% FCS, 10 mM LiCl, along with the antigens as indicated, at 20 μM, and APCs (irradiated thymus cells) at 20×10^6/10 ml. After a 20-min incubation the assay was terminated by the addition of a 750-μl solution of a 1:2 vol/vol chloroform/methanol, followed by a 500 μl 1:1 vol/vol mixture of chloroform/water. After a 5-s vortex, each sample was centrifuged at 500 *g* for 5 min. 650 μl of the upper aqueous phase was transferred to an anion exchange resin column (AG 1-X8; Bio-Rad

Laboratories, Inc., Hercules, CA) that was subsequently washed 3× with 1 ml H_2O. The columns were then washed 3× with 1 ml of 5 mM inositol solution. The inositol phosphates were eluted with a 1.5 ml solution of 1 M sodium formate and 0.1 M formic acid. Radioactivity was measured after addition of 10 ml of Hydrofluor (National Diagnostics Inc., Atlanta, GA), using a scintillation counter (Beckman Instruments, Inc., Fullerton, CA). Results were recorded as mean cpm values in duplicate.

Binding of Bovine 1179-1191 to Adherent Spleen Cells. Spleen cells were treated with ACK lysing buffer to lyse erythrocytes. After triple washing, cells were suspended in RPMI 1640 medium and incubated in petri dishes (100 × 15 mm) at 37°C for 1 h. After incubation, plates were washed three times with warm RPMI 1640 and adherent cells were collected using cell scraper and cold (4°C) RPMI 1640.

Adherent cells (2.5×10^6/ml) were incubated with biotinylated analog of BOV 1179-1191 (10 μM) at 37°C for 20 h. To inhibit binding nonbiotinylated peptides or rat MHC class II antibodies were coincubated at various concentrations with cells and labeled peptide. Cells were washed three times with cold (4°C) 1% BSA/PBS and were incubated with Fluorescein-UltraAvidin (Leinco Technologies, St. Louis, MO) at a concentration of 0.5 μg/100 μl of cells in 0.1% BSA/PBS for 45 min at 4°C. Cells were washed three times with the same buffer and analyzed by flow cytometry using a FACScan® (Becton Dickinson & Co., Mountain View, CA) and Lysys II software. To measure the relative binding, 10,000 propidium iodide–excluding viable cells were gated. Binding of labeled peptide was assessed as percent of cells having fluorescence higher than background (cells without biotinylated peptide). Specific binding of the unlabeled peptides was assessed by competition and expressed as percent inhibition of biotinylated peptide binding in the absence of competitors: Percent Inhibition = 100 − [(Binding with competitors − background)/(Binding without competitors − background) × 100]. Specificity of binding was demonstrated by competition with anti-MHC class II monoclonal antibodies. Binding of the biotinylated peptide is inhibited up to 60% by anti-I-A antibody (OX-6) but not influenced at all by anti I-E antibody (OX-17).

Adoptive Transfer of Disease. Draining lymph node cells from immunized rats were collected 14 d after immunization and were used for adoptive transfer of EAU after incubation with the peptides. The procedure was similar to that used in our previous studies (21, 22). Briefly, lymph node cells were incubated with the peptides (10 μM), in aliquots of 4×10^6 in 12-well cluster plates. After incubation for 3 d the cells were collected, washed, and injected intravenously into naive recipients. Development of disease was monitored and severity of changes was assessed as described above.

Results

RAT 1181-1191 Is Nonuveitogenic in Lewis Rats. Table 2 summarizes data of several experiments that determined the uveitogenicity of BOV 1181-1191, RAT 1181-1191, and the two peptide analogs, T1188 and N1190 (see Table 1 for the sequences of peptides used in this study). In accord with our previous studies (11, 15), BOV 1181-1191 was highly uveitogenic, producing severe ocular changes with early onset in all immunized rats. In contrast, no disease was detected in any of the rats immunized with RAT 1181-1191 at the high tested dose of 200 nmol/rat. A low level of uveitogenicity was exhibited by peptide T1188, that induced EAU of moderate severity in approximately two thirds of the rats, whereas the other peptide analog, N1190, was only slightly less uveitogenic than BOV 1181-1191.

RAT 1181-1191 Is Nonimmunogenic and Nonantigenic in Lewis Rats. Immunogenicity of the tested peptides was assessed by their capacity to induce cellular immunity, measured by the lymphocyte proliferation assay. As seen in Fig. 1, BOV 1181-1191 was highly immunogenic, inducing responses with S.I. levels of >20. In contrast, no detectable response was elicited by RAT 1181-1191. A moderate cellular response was also induced by analog T1188, whereas peptide N1190 stimulated a low-level response. LN cells of rats immunized with each of the four peptides were also tested for their response toward the other three tested peptides (Fig. 1). RAT 1181-1191 was not recognized by any of the LN cells, whereas various levels of cross-reactivity were observed among the other three peptides. LN cells sensitized against BOV 1181-1191 cross-reacted well with N1190 and marginally with T1188. Lymphocytes sensitized against T1188, on the other hand, did not cross-react with any other peptide, whereas cells sensitized against N1190 responded against BOV 1181-1191 with low levels, similar to those of the response to N1190 itself. It is of note that whenever reactive, BOV 1181-1191 stimu-

Table 2. *Uveitogenicity in Lewis Rats of the Bovine and Rat Peptides 1181-1191 and Their Analogs**

	EAU		
Peptide‡	Incidence	Onset day (mean)§	Severity (mean)§
BOV 1181-1191	12/12	10.0	2.8
RAT 1181-1191	0/6	–	0
T1188	11/16	13.7	1.5
N1190	3/3	11.0	2.5

* The peptides were injected at 200 nmol/rat, as described in Materials and Methods.
‡ See Table 1 for the sequences of the peptides.
§ The mean values in rats with disease.

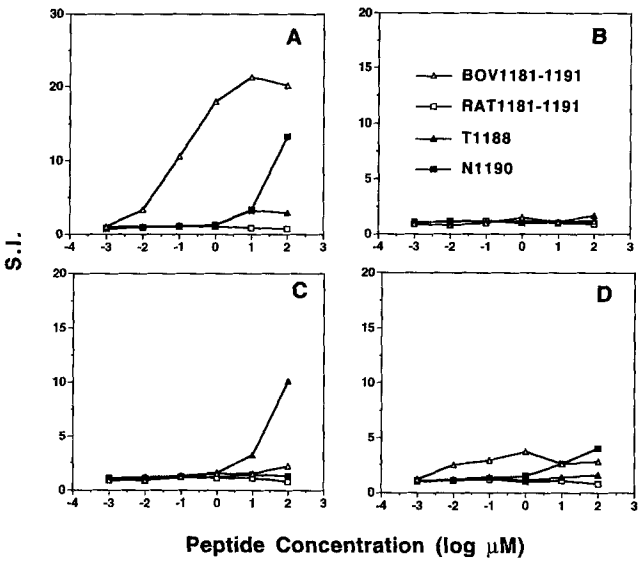

Figure 1. Immunogenicity of the bovine and rat peptides 1181-1191 and their analogs: proliferative responses of LN cells from rats immunized with 200 nm/rat of: (*A*) BOV 1181-1191; (*B*) RAT 1181-1191; (*C*) T1188; (*D*) N1190. The tested antigens include the immunizing peptides and their analogs. The data are presented as stimulation index values. The mean cpm ± SE values in the control cultures with no antigen were: (*A*) 1841 ± 106; (*B*) 1726 ± 399; (*C*) 1955 ± 351; (*D*) 1219 ± 176.

lated proliferation at concentrations lower by approximately three orders of magnitude than the active concentrations of the other two peptides. This observation is interpreted to show that BOV 1181-1191 interacts with the APCs with much higher affinity than the two analogs, although a higher affinity of the peptide–MHC complex for the TCR cannot be excluded.

RAT 1181-1191 Interacts Poorly with Lewis Rat Spleen Adherent Cells. The possibility that the inactivity of RAT 1181-1191 in Lewis rats is due at least in part to its poor interaction with the MHC on the APCs of these rats was tested by probing the capacity of this peptide to competitively inhibit the binding of biotin-labeled BOV 1179-1191 to adherent cells of Lewis rats. As shown in Fig. 2, RAT 1181-1191 was found to be inactive in this assay. In contrast, this binding was inhibited by ~70% in the presence of the same concentration of unlabeled BOV 1181-1191. A moderate inhibition of the binding of BOV 1181-1191 was achieved by analog T1188, whereas analog N1190 was essentially inactive in this assay. A low level of activity was also exhibited by peptide IRBP 273-283. This peptide, a "repeat" of determinant 1181-1191 (13), shares seven residues with BOV 1181-1191 (Table 1), and cross-reacts with it (reference 13 and data shown below).

RAT 1181-1191 Does Not Induce Generation of Inositol Phosphates in BOV 1181-1191–specific T Cells. To exclude the possibility that RAT 1181-1191 stimulates BOV 1181-1191–specific cells in ways other than proliferation we examined inositol phosphates production, which follows the antigen receptor occupancy in T cells (20, 23). As shown in Fig. 3, incubation of BOV 1181-1191–specific T cells with BOV 1181-1191 and accessory cells caused profound accumulation of inositol

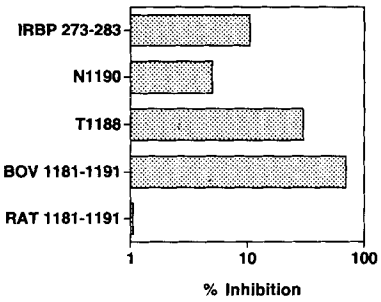

Figure 2. Inhibition of binding of biotinylated BOV 1179-1191 to adherent spleen cells by unlabeled peptides (see Table 1 for sequences). The assay was performed as described in Materials and Methods. Labeled BOV 1179-1191 was incubated with cells at the concentration of 10 μM, unlabeled peptides were added at 100 μM.

phosphates. In contrast, incubation of cells with RAT 1181-1191 did not generate any detectable increase in production of inositol phosphates over the background level. The inability of RAT 1181-1191 to induce generation of inositol phosphates practically rules out the possibility of its functional interaction with T cells specific to the BOV 1181-1191.

IRBP 273-283 Is a Potential Target for Pathogenic Lymphocytes, Sensitized Against BOV 1181-1191. The findings that RAT 1181-1191 is nonuveitogenic in Lewis rats (Table 2) and lymphocytes sensitized against BOV 1181-1191 do not recognize it (Fig. 1) make it unlikely that this rat determinant is the target for these lymphocytes in their initiation of the EAU process. It is conceivable, therefore, that the sensitized lymphocytes recognize another epitope in the rat eye. The following data provide evidence to support the hypothesis that IRBP 273-283 is the putative surrogate epitope.

Sequence 273-283 is highly conserved (24) and our analysis of the rat molecule revealed that, unlike 1181-1191, sequence 273-283 is identical in bovine and rat IRBP (Egwuagu, C. E., unpublished data). We have previously found a moderate level of cross-reactivity between bovine peptides 271-283 and 1179-1191 using LN cells from immunized rats (13). As shown in Fig. 4 lymphocytes of a cell line specific for BOV 1181-1191 recognized IRBP 273-283 and proliferated with a S.I. as high as 100 when stimulated with it in culture. In con-

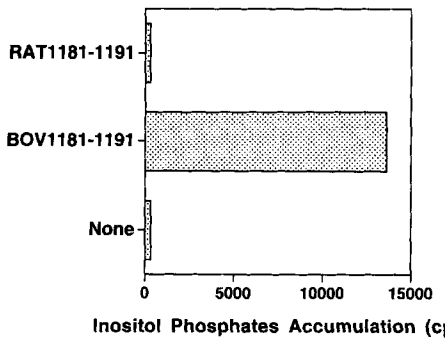

Figure 3. Inositol phosphates generation upon incubation of a BOV 1181-1191–specific cell line with RAT 1181-1191 or BOV 1181-1191 at 20 μM, in the presence of APCs. Total inositol phosphates were measured after a 20-min incubation, as detailed in Materials and Methods.

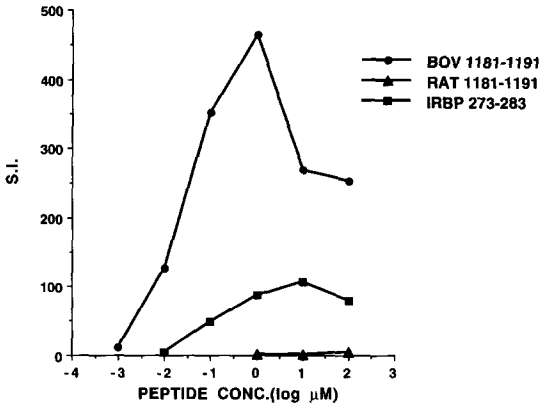

Figure 4. Recognition of the RAT 1181-1191 and IRBP 273-283 by a cell line-specific for BOV 1181-1191. The response against 1181-1191 is shown for comparison. The mean cpm ± SE value in unstimulated control cultures was 235 ± 22.

trast, RAT 1181-1191 did not stimulate any detectable response in these cells.

The notion that peptide 273-283 is the target for the BOV 1181-1191–specific EAU-inducing lymphocytes was supported in particular by data produced by the assay in which uveitogenicity is generated in lymphocytes by stimulation in culture with various antigens; only molecules recognized by the lymphocytes are stimulatory in this assay (21). Table 3 summarizes a series of experiments in which LN cells from rats immunized with BOV 1181-1191 were cultured with BOV 1181-1191, RAT 1181-1191, and IRBP 273-283. In addition, the corresponding 13-mer peptides of these determinants were tested, in order to examine the activity of peptides at a size optimal for the interaction with APCs (25). The system used here yielded lymphocytes with unusually strong uveitogenic activity; mild disease was produced even by cells cultured with no stimulant, when tested at the high number of 30×10^6 (for comparison with other systems, see references 13 and 15). Lymphocytes stimulated in culture with RAT 1181-1191 or RAT 1179-1191 were just marginally more uveitogenic than the controls cultured with no stimulant, while cells stimulated with the other peptides were profoundly more uveitogenic. Remarkably, peptides 273-283 or 271-283 were found superior to peptides BOV 1181-1191 or BOV 1179-1191 in their capacity to generate uveitogenicity in lymphocytes sensitized against BOV 1181-1191. Thus, at the high

Table 3. *Generation of Uveitogenicity in LN Cells from Rats Immunized with BOV 1181-1191**

Ag in culture	Cells injected/ recipient	EAU		
		Incidence	Day of onset (mean)‡	Severity (mean)‡
	$\times 10^{-6}$			
None	30	2/5	5.5	0.8
	5	0/6	–	–
RAT 1181-1191	30	3/5	5.0	1.0
	5	0/3	–	0
	2	0/2	–	0
RAT 1179-1191	30	5/5	4.0	1.5
	5	0/3	–	0
	2	0/3	–	0
BOV 1181-1191	30	9/9	4.0	1.2
	5	6/6	5.5	0.7
	2	2/4	–	0.5
BOV 1179-1191	30	5/5	4.0	1.4
	5	3/3	6.0	1.0
	2	2/2	–	0.8
IRBP 273-283	30	4/4	3.5	2.5
	5	6/6	5.2	1.3
	2	4/4	6.0	0.9
IRBP 271-283	30	8/8	2.4	2.7
	5	3/3	3.7	2.2
	2	3/3	4.0	1.7

* The primed LN cells were cultured with the different peptides, as described in Materials and Methods, and tested for uveitogenicity in naive recipients.
‡ The mean values of the rats with disease.

cell numbers, 30 and 5 × 10^6, lymphocytes stimulated by the repeat peptides 273-283 or 271-283 produced EAU that developed earlier and was much more severe than the disease induced by cells stimulated with BOV 1181-1191 or BOV 1179-1191. At the low cell number of 2 × 10^6, cells stimulated with the repeat peptides induced EAU in all recipients, detected both clinically and histologically, whereas the BOV peptides produced disease in only a portion of recipients and with changes detected only by histological examination.

Discussion

Data recorded here describe the unique features of the pathogenic autoimmune system initiated in Lewis rats by IRBP peptide 1181-1191. This sequence of bovine IRBP (BOV 1181-1191) is immunodominant in Lewis rats and is highly uveitogenic and immunogenic in these animals (11). The rat homologue of this sequence (RAT 1181-1191) on the other hand, was found to be completely immunologically inactive in Lewis rats (Table 2 and Fig. 1). The inactivity of RAT 1181-1191 is in contrast to observations in other known systems in which the autologous homologues of xenogeneic immunopathogenic peptides are pathogenic and immunogenic (1–5). The striking difference between RAT 1181-1191 and BOV 1181-1191 is attributable to different amino acids at positions 1188 and 1190 of the two peptides. We have previously shown that both residues are pivotal for the immunological potency of BOV 1181-1191 in Lewis rats, with the valine at position 1188 participating in the interaction of the peptide with the TCR on lymphocytes and the aspartic acid at 1190 interacting with the MHC on APCs (15). It is assumed, therefore, that substitution with threonine at 1188 and with asparagine at 1190 renders RAT 1181-1191 completely inactive in the Lewis rat. Indeed, substantial reduction in activity was observed even by single substitution at either residue 1188 or 1190. Thus, in line with their corresponding alanine analogs, tested in our previous study (15), peptide T1188 showed minimal or no cross-reactivity with BOV 1181-1191 (Fig. 1 *A*), whereas N1190 exhibited poor binding to Lewis rat APC (Fig. 2) and was recognized by lymphocytes sensitized against BOV 1181-1191 only at exceedingly high concentrations (Fig. 1). The current results can thus be explained by these different functional roles of residue 1188 and 1190. Substitution with threonine at 1188 alters the TCR recognition rather than MHC binding, so the analog competes (Fig. 2) and is immunogenic (Fig. 1 *C*), but the induced T cells do not crossreact with BOV 1181-1191 (Fig. 1 *C*). In contrast, substitution with asparagine at 1190, which affects binding to MHC (Fig. 2) but not TCR recognition, strongly reduces the immunogenicity, but the T cells that are elicited react to BOV 1181-1191 at concentrations lower than those of the immunogen N1190, presumably because BOV 1181-1191 binds with higher affinity to the MHC molecule but looks similar to the TCR (Fig. 1 *D*).

The uniqueness of the system studied here is further depicted by the failure of RAT 1181-1191 to be recognized by and to stimulate lymphocytes sensitized against BOV 1181-1191. Thus, lymphocytes sensitized against BOV 1181-1191 did not respond to RAT 1181-1191 as tested by proliferation (Fig. 1), stimulation of uveitogenicity (Table 3) and even failed to induce detectable signal transduction, assayed by generation of inositol phosphates (Fig. 3). It should be mentioned here, however, that despite the lack of response to RAT 1181-1191 by lymphocytes sensitized to BOV 1181-1191 in these in vitro assays, it cannot be ruled out that this rat peptide can be a target for these lymphocytes in vivo. In contrast to the observations with RAT 1181-1191, autologous homologues of immunopathogenic xenogeneic epitopes in other systems are recognized by lymphocytes sensitized against the latter peptides (1–5). Moreover, since lymphocytes sensitized against BOV 1181-1191 should recognize an antigenic epitope in the rat eye in order to initiate the EAU process, it is assumed that an IRBP epitope other than RAT 1181-1191 serves as the target for the lymphocytes sensitized against BOV 1181-1191. It is possible that there are more than one isoform of rat IRBP and sequence 1181-1191 of another isoform could cross-react with BOV 1181-1191. The data recorded here, however, provide evidence to suggest that IRBP peptide 273-283 is the putative target (surrogate) epitope.

Sequence 273-283 is a repeat of 1181-1191 in the fourfold structure of IRBP (12) and in the bovine molecule the two determinants share seven amino acids (Table 1). In addition, sequence 273-283 is highly conserved and we have found that it is identical in the rat and bovine proteins; previous studies showed the identity between the bovine and human determinants (24). Furthermore, a cross-reactivity was shown in our previous study between bovine peptides 271-283 and 1179-1191 (13) and the data recorded here now show that lymphocytes sensitized against BOV 1181-1191 are stimulated by the 273-283 determinant of rat IRBP to proliferate (Fig. 4) and to acquire strong uveitogenic capacity (Table 3). The data recorded in Table 3 are of particular interest by showing that IRBP 273-283 is superior to BOV 1181-1191 in generating uveitogenicity in lymphocytes sensitized against the latter peptide. This observation is interpreted to suggest that incubation in vitro with determinant 273-283 selectively stimulates the subpopulation of lymphocytes that recognize this epitope and these cells then effectively induce EAU, after their reexposure to peptide 273-283 in the eye. The population stimulated with BOV 1181-1191, on the other hand, is less uveitogenic presumably because a large portion of this population consists of cells that do not recognize determinant 273-283.

Data recorded here shed new light on the immunological profiles of bovine IRBP determinants 1181-1191 and 273-283 in Lewis rats. Our previous studies (13) with the corresponding 13-mer peptides have shown that 1179-1191 is more active than 271-283 in Lewis rats by several parameters: (*a*) peptide 1179-1191 is more immunodominant than 271-283, as depicted by the capacity of the former peptide to stimulate higher levels of proliferation in lymphocytes sensitized against whole IRBP; (*b*) peptide 1179-1191 was found to be more antigenic, stimulating proliferation of sensitized lymphocytes at much lower concentrations than peptide 271-283; (*c*) peptide 1179-1191 stimulates cellular immunity in vivo and induces EAU at doses lower than those of 271-283 by 2–3 orders of magni-

tude. These features of peptide 1179-1191 are assumed to be due to the high affinity of this determinant toward the MHC molecules of the Lewis rat (26), an assumption that is in line with the data of the binding in competition assay recorded here (Fig. 2). Peptide 271-283, on the other hand, was previously found to be more uveitogenic than 1179-1191 when tested at the high dose of 150 nmol/rat; at this dose the ocular changes induced by 271-283 were more severe and developed earlier than those induced by peptide 1179-1191 (13). This observation, as well as the finding recorded here, concerning the superiority of peptide 271-283 in generating uveitogenicity in culture (Table 3) can now be attributed to this epitope's presumed role as the target for the EAU-inducing lymphocytes.

The putative usage of a surrogate epitope in an immunopathogenic process, as described here, is presumably an uncommon phenomenon. It is conceivable, however, that future studies will reveal that surrogate epitopes are used in other systems, in particular those in which large molecules, with repeated sequences, serve as target antigens.

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Address correspondence to Dr. Alexander T. Kozhich, National Eye Institute, NIH, Bldg. 10, Rm. 10N208, Bethesda, MD 20892.

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