IDENTIFICATION OF AN IMMUNODOMINANT AND HIGHLY IMMUNOPATHOGENIC DETERMINANT IN THE RETINAL INTERPHOTORECEPTOR RETINOID-BINDING PROTEIN (IRBP)

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Autoimmunity to ocular antigens is assumed to play a major role in the pathogenesis of numerous intraocular inflammatory conditions that are grouped under the term "uveitis" (1–3). This notion is supported by the finding that immunization with ocular components may cause in animals a disease that resembles in many aspects certain uveitic conditions in man (1–3). The animal disease has been designated experimental autoimmune uveoretinitis (EAU).[1] The antigen used in most studies for EAU induction has been the retinal S antigen (1–4), but we and other investigators have recently reported that another retinal component, the interphotoreceptor retinoid-binding protein (IRBP), is similarly uveitogenic in rats (3, 5–7), mice (8), rabbits (9), or monkeys (10). IRBP, a glycoprotein of ~140 kD, is the major protein in the interphotoreceptor matrix and is thought to function mainly in the transport of retinoids between the retina and the pigment epithelium (11). In addition to the eye, IRBP also localizes in the pineal gland (12), an organ that is related to light detection in many vertebrates (13). Consequently, animals immunized with IRBP often develop inflammation in the pineal gland (experimental autoimmune pinealitis [EAP]) as well (5, 6, 10).

In a recent study we have isolated several cyanogen bromide fragments of bovine IRBP and determined their capacity to induce EAU and EAP (14). Three of the fragments that exhibited considerable levels of immunopathogenicity were localized to regions within the known sequence of IRBP (15), thus enabling the selection of peptide sequences for synthesis and determination of immunopathogenicity. One criterion for peptide selection was the amphipathicity of the peptide sequence (16), as calculated by the algorithm of Margalit et al. (17). Two overlapping peptides, designated R4 and R9 (residues 1158–1180 and 1154–1180, respectively, according to the sequence reported by Borst et al. [15]), were found to produce EAU and EAP in

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

Lewis rats (18). No immunopathogenicity was detected in seven other synthetic peptides (18). It is noteworthy, however, that peptides R4 and R9 were immunopathogenic only at high doses (≥28 nmol/rat), which are approximately four orders of magnitude higher than that of whole IRBP (5, 7, 19). Significantly, R4 and R9, as well as the other seven peptides, were found in another study (Redmond, T. M., H. Sanui, L.-H. Hu, B. Wiggert, H. Margalit, J. A. Berzofsky, G. J. Chader, and I. Gery, manuscript submitted for publication) to be nonimmunodominant; namely, they were not recognized by lymphocytes sensitized against whole IRBP (see references 16 and 20 for detailed definition of immunodominance).

While testing additional synthetic peptides, derived from cyanogen bromide fragment CB71 (14), we have identified a determinant that is profoundly immunodominant in the Lewis rat. This peptide, which consists of residues 1169–1191, is predicted to be strongly amphipathic (17) and was found to be highly immunopathogenic. This work records the immunogenic and immunopathogenic capacities of peptide 1169–1191 and compares them with the capacities of two related but nondominant peptides, 1158–1180 and 1169–1188.

Materials and Methods

Antigens. Bovine IRBP was purified as described in detail elsewhere (21). The IRBP-derived peptides tested here were synthesized and purified by Applied Biosystems Inc., Foster City, CA, using the t-BOC chemistry, on a peptide synthesizer (430A; Applied Biosystems, Inc.). The peptide sequences were derived from the sequence of bovine IRBP, as determined and reported by Borst et al. (15). The peptides used in the present study included two previously tested sequences, 1158–1180 (HVDDTDLYLTIPTARSVGAADGS) and 1169–1188 (PTARSVGAADGSSWEGVGVV). These were compared with the novel sequence 1169–1191 (PTARSVGAADGSSWEGVGVVPDV). In addition, a series of truncated forms of peptide 1169–1191 were synthesized and used here.

Immunization of Rats. Male inbred Lewis rats, 8–12 wk old, were supplied by Charles River Breeding Laboratories, Inc., from the facility in Raleigh, NC. The rats were immunized by a single injection of IRBP or the peptides, emulsified in CFA, containing *Mycobacterium tuberculosis* H37Ra at 2.5 mg/ml. The emulsion was injected into one hind footpad in a volume of 0.1 ml containing various amounts of the antigens. An additional adjuvant, *Bordetella pertussis* bacteria (lot 91B; Michigan Department of Public Health, Lansing, MI), was injected intravenously, 10^{10} organisms/rat, concurrently with the immunization.

Disease Monitoring and Assessment. Immunized rats were examined daily for clinical ocular changes (5); disease occurrence and severity were verified by conventional histological examination (22). Pineal inflammation was determined by histological examination (22). The severity of the disease in the two organs was graded according to the intensity of the changes, using a scale of 0 to 4 (5).

Adoptive Transfer of Disease. Draining lymph node or spleen cells from immunized rats were collected 11–14 d after immunization and were used for adoptive transfer of EAU after their incubation with the homologous (immunizing) or heterologous antigens. The procedure was similar to that used in our previous studies (23, 24). Briefly, the lymphoid cells were incubated with IRBP or the peptides, as indicated, at 10 μg/ml, in aliquots of 4×10^6 cells/2 ml in 12-well cluster plates (Costar, Cambridge, MA). After incubation for 3 d the cells were collected, washed, and injected intraperitoneally into naive recipients. The development and severity of disease were assessed as described above.

Lymphocyte Proliferation Assay. Lymph node cell responses were measured as described in detail elsewhere (25). In brief, 3×10^5 lymphoid cells were cultured in triplicate in flat-bottomed microplates, with or without the tested antigen, in RPMI 1640 medium with 5% FCS, in a total volume of 0.2 ml. The cultures were incubated for a total period of 90 h, with a pulse of [^{3}H]thymidine (0.5 μCi/10 μl/well) given for the last 16 h. The data are

presented as stimulation index (SI) values (SI = mean cpm in cultures with stimulus/mean cpm in control cultures without stimulus). Control cultures incorporated [^{3}H]thymidine with levels of 500–2,000 cpm.

Results

Immunodominance of Peptide 1169–1191: Recognition by Lymphocytes Sensitized against Whole IRBP. The immunodominance of IRBP-derived peptides was determined according to their capacity to stimulate lymphocytes sensitized against whole IRBP. Fig. 1 summarizes a typical experiment in which lymph node cells from rats immunized with whole IRBP were tested for responsiveness in culture toward the protein, peptide 1169–1191, and two other peptides, 1158–1180, which overlaps partially with 1169–1191, and 1169–1188, which is a truncated form of 1169–1191. Vigorous responses were stimulated by a wide range of concentrations of 1169–1191, with proliferation levels similar to those stimulated by the whole protein. In contrast, no responses could be detected in cultures incubated with the other two peptides. These data, thus, show that peptide 1169–1191 is profoundly immunodominant while the other two peptides are nondominant.

Recognition of Whole IRBP by Lymphocytes Sensitized against 1169–1191. The immunodominance of peptide 1169–1191 was further depicted by the capacity of lymphocytes sensitized against it to recognize the whole IRBP molecule. As shown in Table I, lymphocytes sensitized against peptide 1169–1191 proliferated vigorously when incubated with either this peptide or whole IRBP. Conversely, no such dual crossrecognition with IRBP was found with peptide 1158–1180, which is immunogenic but nondominant. Thus, lymphocytes sensitized against peptide 1158–1180, which reacted well against the immunizing peptide, failed to respond against whole IRBP. Marginal or no response to either whole IRBP or the immunizing peptide was detected in cultures of lymphocytes from rats immunized against peptide 1169–1188, which is, therefore, essentially nonimmunogenic in Lewis rats.

Immunodominance of Peptide 1169–1191: Cross-stimulation of Lymphocytes for Adoptive Transfer of EAU/EAP. The disease induced by IRBP may be transferred to naive rats by lymphocytes sensitized against the protein, after a mandatory stimulation of these cells by the homologous or crossreacting antigens (19, 24). Peptides 1158–1180 and

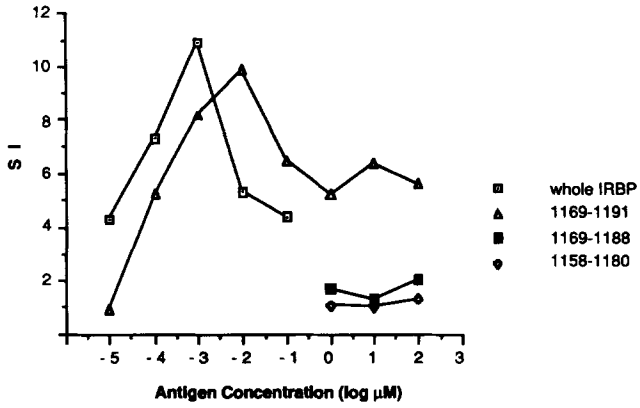

FIGURE 1. Lymphocytes sensitized against whole IRBP recognize the immunodominant peptide 1169–1191 but not the nondominant peptides. The lymphocytes were obtained from draining lymph nodes of Lewis rats, 14 d after immunization with 50 μg bovine IRBP.

TABLE I

Immunodominance of Peptides: Crossrecognition with Intact IRBP by Sensitized Lymphocytes

Immunizing antigen*	Antigen in culture‡			
	IRBP	1158-1180	1169-1188	1169-1191
IRBP	28.0§	0.8	1.1	10.3
1158-1180	1.6	22.8	ND	0.9
1169-1188	1.3	ND	2.4	1.5
1169-1191	8.2	0.6	1.8	20.7

* Lewis rats were injected with 0.5 nmol whole IRBP, or 100 nmol peptides.
‡ IRBP was tested at 0.6 μM and the peptides at 40 μM.
§ Stimulation index.

1169-1191 induce EAU and EAP in Lewis rats (reference 18 and data recorded below) and the diseases induced by these peptides were also found transferrable to naive recipients after activation in vitro (Table II). The system of disease transfer has provided, therefore, an additional assay to test the immunodominance of IRBP-derived peptides, according to their capacity to cross-stimulate lymphocytes in culture for disease induction in naive recipients. As shown in Table II, lymphocytes sensitized in vivo against whole IRBP were stimulated for disease induction by either IRBP or peptide 1169-1191, but not by peptide 1158-1180. The crossrecognition between whole IRBP and peptide 1169-1191 was also demonstrated by the stimulation with IRBP of lymphocytes sensitized against 1169-1191. In contrast, lymphocytes sensitized against 1158-1180, which produced disease after stimulation in vitro with this peptide, had no pathogenic activity after incubation with whole IRBP. Peptide 1169-1188 is not immunopathogenic in Lewis rats (see below) and it did not activate in this assay lymphocytes sensitized against IRBP or 1169-1191 (data not shown).

It is of note that in spite of the differences in immunodominance between peptides 1169-1191 and 1158-1180, lymphocytes sensitized against these two molecules exhibited a similarity in their capacity to transfer disease after stimulation in vitro with the

TABLE II

Immunodominance of Peptides: Cross-stimulation of Lymphocytes for Adoptive Transfer of EAU

Immunizing antigen	Antigen in culture	EAU		
		Incidence	Onset day (mean)	Severity (mean)
IRBP	IRBP	8/8	3.9	2.0
	1158-1180	0/4	-	0
	1169-1191	5/5	4.6	1.8
1158-1180	IRBP	0/3	-	0
	1158-1180	9/9	4.3	1.3
1169-1191	IRBP	6/7	4.0	1.2
	1169-1191	7/9	5.1	1.5

Data collected with recipient rats injected with 50-100 × 10^6 spleen or lymph node cells from immunized rats, after incubation in culture with the antigens as indicated.

homologous peptide. Furthermore, the disease-inducing capacity of lymphocytes sensitized against the two peptides resembled that of lymphocytes sensitized against whole IRBP (Table II).

Immunopathogenicity of Peptide 1169–1191. Lewis rats developed EAU and EAP after immunization with 1169–1191 at doses as low as 0.024 nmol (Table III). The minimal immunopathogenic dose of 1169–1191 is lower by far than that of the nondominant peptide, 1158–1180, which is ~28 nmol/rat (18). The high immunopathogenicity of 1169–1191 is also indicated by the early onset of EAU, as soon as 8 or 9 d after immunization (Table III and data not shown). The short onset time of EAU induced by 1169–1191 resembles that of the disease induced by whole IRBP (5, 7) and is remarkably shorter than that of EAU induced by peptide 1158–1180 (≥12 d) (18).

Fig. 2 demonstrates the typical histopathological changes in the eyes and pineal gland of rats immunized with peptide 1169–1191. Inflammatory infiltration is seen in most ocular tissues (Fig. 2, *b*, *c*, and *e*), as well as in the pineal gland (Fig. 2 *f*). Of particular interest are the changes in the posterior segment of the eye, which include retinal detachment with accumulation of fibrinous and cellular exudate in the sub-retinal space, as well as severe damage to the photoreceptor cell layer (Fig. 2, *b* and *c*). The changes observed in both the eyes and pineal glands of rats immunized with 1169–1191 closely resemble those routinely seen in rats immunized with whole IRBP (see references 5–7 for comparison). Another feature common to rats immunized with peptide 1169–1191 and whole IRBP is the "acute" kinetics of the ocular disease: after an early onset, the inflammation subsided rapidly and usually disappeared within 8–10 d later.

Immunogenicity of Peptide 1169–1191. In parallel with its high level of immunopathogenicity, peptide 1169–1191 was found to be a potent immunogen in Lewis rats, as demonstrated by its capacity to induce specific cellular immunity at very low doses. The immune response was measured by the lymphocyte proliferation assay and the results of a representative experiment are shown in Fig. 3. Positive responses were obtained with lymph node cells from rats immunized with 1169–1191 at doses as low as 0.1 nmol. It is also noteworthy that a good correlation was found between the minimal immunogenic and immunopathogenic doses of peptide 1169–1191. Thus, EAU was observed in this and in other experiments only in rats that developed detectable immune response to the peptide (data not shown).

TABLE III

Immunopathogenicity of Peptide 1169–1191 in Lewis Rats

	EAU			EAP	
Injected dose	Incidence	Onset day (mean)	Severity (mean)	Incidence	Severity (mean)
nmol/rat					
75	2/2	9.0	1.5	2/2	2.5
15	4/4	10.2	1.2	2/2	2.5
3	3/3	11.0	1.8	3/3	1.3
0.6	7/7	10.3	1.6	3/3	2.0
0.12	7/7	11.1	1.6	4/5	2.0
0.024	1/5	15.0	1.0	1/2	1.0
0.005	0/5	-	0	0/2	0

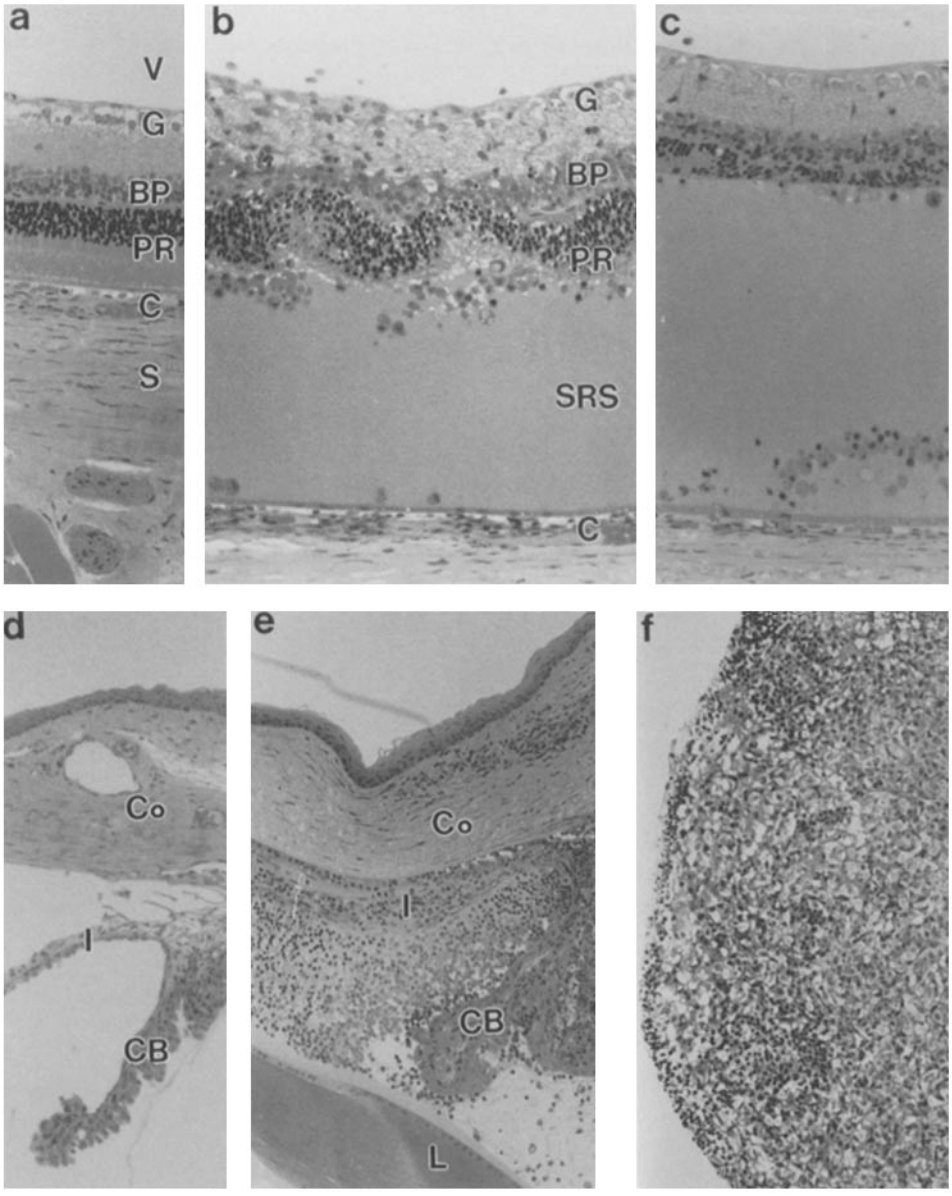

FIGURE 2. Histopathological changes in eyes and pineal gland of rats immunized with peptide 1169–1191. (*a*) Posterior segment of an eye of a normal rat, showing the typical stratiform morphology of this segment. *V*, vitreous; *G*, ganglion cell layer; *BP*, bipolar cell layer; *PR*, photoreceptor cell layer (which is composed of the nuclear and outer segment layers); *C*, choroid; *S*, sclera. (*b*) Posterior segment of a rat at the peak of EAU induced by peptide 1169–1191 (13 d after immunization with 1 nmol). The retina is detached, edematous, and infiltrated with inflammatory cells. The photoreceptor layer is severely damaged, with almost a complete loss of the outer segments. Accumulation of cellular and fibrinous exudate is seen in the sub-retinal space (*SRS*). Also, a few inflammatory cells are seen in the vitreous. (*c*) Posterior segment of a rat at a late stage of EAU induced by peptide 1169–1191 (16 d after immunization with 1 nmol). The retina is detached but contains fewer inflammatory cells. The photoreceptor cell layer is virtually destroyed. Cellular and fibrinous exudate is present in the sub-retinal space. (*d*) Anterior segment

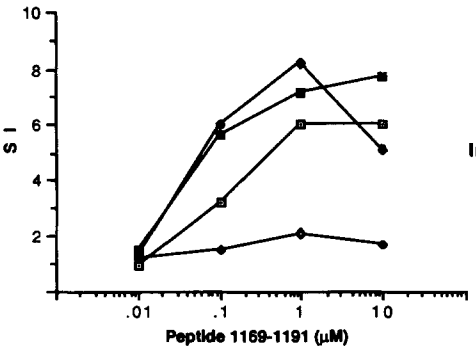

FIGURE 3. Proliferation responses of lymph node cells from rats immunized with different doses of peptide 1169–1191. The lymph nodes were collected 14 d after immunization.

Identification of the Active Epitope of Peptide 1169–1191. As mentioned above, marginal or no immunological capacities were found in peptide 1169–1188, which comprises the 20 residues at the NH_2 terminus of peptide 1169–1191. Peptide 1169–1188 was also found to be nonimmunopathogenic (see Table IV). These findings indicate, therefore, that the epitope responsible for the immunological activities of peptide 1169–1191 is localized at the COOH terminus of this peptide. To determine the minimal sequence carrying the immunological activities of peptide 1169–1191, a series of truncated forms of this peptide were synthesized and examined for their capacity to induce disease and to exhibit immunodominance, as shown by the capacity to stimulate in culture lymphocytes sensitized against whole IRBP.

Table IV summarizes the data of experiments in which the immunopathogenicity of the truncated peptides was tested in Lewis rats. Peptides 1173–1191 (19 residues) and 1177–1191 (15 residues) resembled peptide 1169–1191 in their immunopathogenicity, with the minimal disease-producing dose of 0.1 or even 0.01 nmol/rat. Decreased levels of immunopathogenicity were demonstrated by the shorter peptides, 1181–1191 (11 residues) and 1182–1191 (10 residues), with minimal immunopathogenic doses of $\sim$1 and $\sim$10 nmol/rat, respectively. On the other hand, no pathogenic activity was detected in peptide 1183–1191 (nine residues) or in any of the shorter peptides.

A complete correlation was found between the immunopathogenicity of the truncated peptides and their capacity to stimulate proliferation by lymphocytes sensitized against whole IRBP. A typical experiment is shown in Fig. 4. Peptides 1173–1191 and 1177–1191 resembled 1169–1191 in their stimulatory activity (not shown), while peptides 1181–1191 and 1182–1191 demonstrated gradually decreasing stimulatory capacities for the IRBP-sensitized lymphocytes. No response could be detected, however, in cultures incubated with peptides 1183–1191 or 1184–1191.

of an eye of a normal rat, showing the major structural components: *Co*, cornea; *I*, iris; *CB*, ciliary body. (*e*) Anterior segment of an eye of a rat at the peak of EAU induced by peptide 1169–1191 (13 d after immunization with 1 nmol). Inflammatory exudate occupies the chambers of this segment and inflammatory cells infiltrate the iris, the ciliary body, and even the cornea. The iris is attached to the cornea. No apparent changes are seen, however, in the lens (*L*). (*f*) Pineal gland of a rat, 16 d after immunization with 1 nmol of peptide 1169–1191. Inflammatory infiltration occurs throughout the pineal tissue. All sections are stained with hematoxylin and eosin (× 100).

TABLE IV

Immunopathogenicity of Truncated Forms of Peptide 1169–1191

Peptide	Dose	EAU		
		Incidence	Onset day (mean)	Severity (mean)
	nmol/rat			
1169–1191 (23)*	10	3/3	9.7	2.0
	1	3/3	11.0	1.7
	0.1	3/3	11.7	1.7
	0.01	0/3	-	0
1173–1191 (19)	10	3/3	10.3	1.7
	1	3/3	11.0	1.5
	0.1	3/3	11.7	1.3
	0.01	0/3	-	0
1177–1191 (15)	10	3/3	9.7	2.0
	1	3/3	11.0	1.7
	0.1	3/3	14.3	1.0
	0.01	1/3	14.0	1.0
1181–1191 (11)	10	3/3	9.5	1.5
	1	3/3	11.7	1.1
	0.1	0/3	-	0
	0.01	0/3	-	0
1181–1191 (10)	100	5/5	13.4	1.0
	10	2/3	17.5	1.0
	1	1‡/3	NA	0.5
	0.1	0/3	-	0
1183–1191 (9)	100	0/3	-	0
	10	0/4	-	0
1184–1191 (8)	100	0/3	-	0
	10	0/4	-	0
1185–1191 (7)	100	0/3	-	0
	10	0/4	-	0
1169–1188 (20)	100	0/3	-	0
	10	0/3	-	0

* Residues.

‡ Disease detected only by histological examination.

Discussion

This study has identified an immunodominant epitope of the IRBP molecule that is exceedingly immunogenic and immunopathogenic in the Lewis rat. In addition, data recorded here highlight the usefulness of the experimental system used in this and in previous studies. In particular, this experimental system allows for the first time an analysis of the relationships between immunodominance, immunogenicity, and immunopathogenicity of different peptide determinants of an organ-specific protein. This analysis became feasible when four types of peptides were identified among the IRBP-derived determinants, as summarized in Table V. In addition to the immunodominant peptide, 1169–1191, three types of nondominant peptides were identified, according to their capacity to induce immune response and EAU/EAP

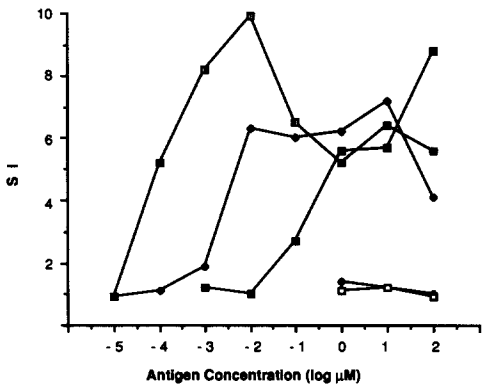

FIGURE 4. Recognition of truncated forms of peptide 1169–1191 by lymph node cells sensitized against whole IRBP. The 11- and 10-residue peptides reacted at higher concentrations than the parent peptide, whereas the nine- and eight-residue peptides were not recognized by the IRBP-sensitized lymphocytes. The lymph nodes were collected from rats 14 d after immunization with 50 μg bovine IRBP.

in Lewis rats. The data collected with the various peptides demonstrate the clear association between immunodominance and the level of immunological activities of the peptides. In particular, as recorded in this work, the immunodominant peptide 1169–1191 exhibits immunological capacities that are clearly superior to all of the nondominant peptides.

The feature of immunodominance was determined in the present study by the capacity of the peptides to stimulate lymphocytes sensitized against the whole IRBP protein. In addition to the conventional lymphocyte proliferation assay (Fig. 1), the immunodominance of peptides was determined here by their capacity to cross-stimulate IRBP-sensitized lymphocytes and make them capable to adoptively transfer disease to naive recipients (Table II). It is also noteworthy that the intact IRBP protein was recognized in culture only by lymphocytes sensitized against the immunodominant peptide 1169–1191, but not by lymphocytes sensitized against the nondominant peptides (Tables I and II). This finding is not clear and could be attributed to the processing of the native protein, which either does not produce the nondominant peptides, or produces them in association with hindering structures that interfere with binding to the TCR or the MHC molecule on APC. A similar finding has been recently reported with the myoglobin system (26).

The most striking difference observed here between the immunodominant (1169–1191) and nondominant (1158–1180) peptides has been the much smaller dose of the dominant peptide needed for induction of immune response and disease in

TABLE V

Features of the Four Types of IRBP-derived Peptides

Type	Peptide	Immunodominance	Immunogenicity	Immunopathogenicity
I	1169–1188	No	No	No
II	Majority of tested peptides*	No	Yes (low)	No
III	1158–1180	No	Yes (low)	Yes (low)
IV	1169–1191	Yes	Yes (high)	Yes (high)

Features relevant to the immune responses in Lewis rats.

* The sequences and immunological activities of these peptides are recorded (reference 18 and Redmond, T. M., H. Sanui, L.-H. Hu, B. Wiggert, H. Margalit, J. A. Berzofsky, G. J. Chader, and I. Gery, manuscript submitted for publication).

Lewis rats. Thus, the minimal dose of 1169–1191 for these activities (0.02–0.1 nmol/rat) (Fig. 3 and Table III) is ~1,000 times smaller than that of peptide 1158–1180 (≃28 nmol/rat) (reference 18 and Redmond, T. M., H. Sanui, L.-H. Hu, B. Wiggert, H. Margalit, J. A. Berzofsky, G. J. Chader, and I. Gery, manuscript submitted for publication). The superiority of the immunodominant peptide in initiating immune responses is not completely understood and may be attributed to at least two mechanisms: first, the immunodominant peptide may bind with greater affinity to the MHC antigen on the APC than does the nondominant peptide. Data supporting this notion have been provided by recent studies of Grey, Gefter, Unanue, and their coworkers (27–29). The second mechanism could involve the polyclonality of the response to the immunodominant peptide. Several reports have recently shown that immunodominant epitopes are the focus for a diversity of T cells with different fine specificities (20, 30, 31). These two hypothetical mechanisms are not mutually exclusive and both could facilitate the immune response toward the exceedingly low doses of the immunodominant epitope observed in our study.

Another difference between peptides 1169–1191 and 1158–1180, which could be attributed to the immunodominance of the former, is depicted by the kinetics and histopathological changes of the ocular diseases these peptides induce in Lewis rats. Thus, the disease induced by 1169–1191 is indistinguishable by both these features from the disease induced by whole IRBP (5–7). On the other hand, the EAU induced by 1158–1180 has a later onset and exhibits more "chronic" histopathological changes than the disease induced by intact IRBP (18).

In contrast to the striking differences between the immunogenicity and immunopathogenicity of peptides 1169–1191 and 1158–1180, lymphocytes sensitized against these two peptides were similar in their capacity to adoptively transfer EAU and EAP into naive recipients (Table II). This finding suggests, therefore, that the difference between immunodominant and nondominant epitopes lies mainly at the afferent limb (i.e., the sensitization phase) of the immune response.

The identification of immunodominant and nondominant determinants of IRBP in the present study made it possible to analyze these peptides for features common for immunodominant epitopes, i.e., amphipathicity (16, 17, 20) and the existence of "immunogenic motifs," as defined by Rothbard and Taylor (32). Amphipathicity has been a major criterion for selection of IRBP-derived peptides (18) and peptide 1169–1191 contains a 3_{10} helix, with a high amphipathic score of 24.2, using the algorithm of Margalit et al. (17). In addition, this peptide contains two sequences that meet the immunogenic motif criterion of Rothbard and Taylor (GAAD and GVVPD). Two such motifs (DLYLT and GAAD) are also located on peptide 1158–1180, but the amphipathicity of this nondominant peptide is quite low (it includes only one block of a predicted amphipathic segment: TARSVGAADGS). On the other hand, peptide 1169–1188, which is nondominant and essentially immunologically inactive in the Lewis rat, is part of the same amphipathic segment as 1169–1191, has an amphipathic score of 17.9, and contains one immunogenic motif (GAAD). It is conceivable, therefore, that in addition to the two aforementioned features, immunodominant peptides have other properties that promote their immunogenic capacities in certain hosts. As mentioned above, these properties could include the efficient binding to the specific MHC antigen on APC of that host and the polyclonality of the responding lymphocyte population. The additional three

residues, PDV, which distinguish 1169–1191 from 1169–1188, presumably participate in one or both of these properties.

The minimal immunogenic and immunopathogenic dose of peptide 1169–1191 in the Lewis rat (0.02–0.1 nmol/rat), is ~10 times higher than that of whole IRBP (≃0.002 nmol/rat) (5, 7, 19). This finding suggests that the large IRBP molecule carries more than one immunodominant epitope for Lewis rats. This notion is in line with the observation that the IRBP molecule consists of four repeats with high levels of sequence similarity (15). It is possible, therefore, that the homologues of the active site of 1169–1191 in the other repeats are also immunodominant and possess high immunological capacities. This possibility is currently under investigation in our laboratory.

The minimal immunopathogenic dose of 1169–1191 is comparable with that of peptide 68–88 of guinea pig myelin basic protein for induction of experimental allergic encephalomyelitis in Lewis rats (0.075 nmol/rat) (33). Peptide 68–88 has also been identified as the immunodominant epitope of myelin basic protein in the Lewis rat (34). On the other hand, remarkably higher doses than those of 1169–1191 were reported necessary for induction of EAU and EAP by peptides "M" and "N," which have been reported to be the immunopathogenic determinants of another retinal protein, S antigen (35, 36). The immunodominance of these two peptides has not been recorded, but it is conceivable that these determinants are not immunodominant epitopes of S antigen.

The active site of peptide 1169–1191 was localized to a 10-residue sequence at the COOH terminus of the peptide. Significantly, a complete correlation was observed between the level of immunodominance of the truncated peptides (Fig. 4) and their immunopathogenicity (Table IV). It is also noteworthy that on a molar basis, the 15-residue peptide (1177–1191) was as active as the 23-residue molecule (1169–1191), whereas the 10-residue peptide (1182–1191) was ~100 times less active (Fig. 4 and Table IV).

Further studies are currently underway to determine which amino acids of peptide 1169–1191, in particular at the COOH terminus, are pivotal for its immunological activities in the Lewis rat. In other experiments, the immunogenicity, immunopathogenicity, and immunodominance of peptide 1169–1191 and other IRBP-derived peptides are being examined in other rat strains as well as in other species.

Summary

Interphotoreceptor retinoid-binding protein (IRBP), a glycoprotein specific for the retina and pineal gland, induces inflammatory changes in these two organs in immunized animals. We report here on the identification of an immunodominant determinant of bovine IRBP that is highly immunogenic and immunopathogenic in the Lewis rat. The peptide, which comprises the sequence 1169–1191 of bovine IRBP, was shown to be immunodominant by its capacity to stimulate lymphocytes sensitized against whole IRBP. A comparison was made between peptide 1169–1191 and another peptide, 1158–1180, which is nondominant but is immunogenic and immunopathogenic in the Lewis rat. Peptide 1169–1191 was found to be superior in its immunological capacities; the minimal dose of 1169–1191 needed to induce cellular immune response or disease in Lewis rats (0.02–0.1 nmol/rat) is ≃1,000 times

smaller than that of 1158–1180. In addition, unlike the ocular disease induced by 1158–1180, the disease produced by 1169–1191 resembled that induced by whole IRBP in its kinetics and histopathological features. The immunological activity of 1169–1191 in the Lewis rat was localized to the 10 residues at the COOH terminus; no such activity was exhibited by the truncated peptide 1169–1188, which comprises the 20 residues at the NH_2 terminus of the full peptide. The usefulness of this unique experimental system in analyzing the role of immunodominance in peptide immunogenicity and immunopathogenicity is underscored.

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