Uveoretinitis and Pinealitis Induced by Immunization With Interphotoreceptor Retinoid-Binding Protein

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Rats immunized with microgram amounts of interphotoreceptor retinoid-binding protein (IRBP), a glycoprotein which localizes specifically in the eye and pineal gland, developed uveoretinitis and pinealitis. The severity and onset of changes were found to be dose-related and to be enhanced by Bordetella pertussis bacteria. In general, the inflammatory changes induced by IRBP resembled those provoked by S-antigen (S-Ag), but significant differences were noted between the two diseases. The possible usefulness of the new experimental autoimmune disease is discussed.

Investigation of uveitic conditions in man has been considerably facilitated by animal disease models, in particular the entity termed “experimental autoimmune uveoretinitis” (EAU).1,2 The antigen used in most studies to induce EAU has been S-antigen (S-Ag),3 although a low level of uveitogenity was detected in preparations of rhodopsin.4-6 S-Ag is also present in the pineal gland,7,8 an organ closely related to the visual system, and this organ is thus often affected by inflammation in animals immunized with S-Ag.4,7,8 Recent studies have established the organ specificity of another retinal protein, interphotoreceptor retinoid-binding protein (IRBP). This glycoprotein, with an apparent molecular weight of approximately 140,000, is thought to mediate the transport of retinoids between the photoreceptor cells and the pigment epithelium, and is the major soluble protein of the interphotoreceptor matrix.9 In addition to the retina, IRBP is also found in the pineal gland.10 The present study shows that immunization with IRBP provokes pathogenic processes which selectively affect the eye and pineal gland.

Materials and Methods. Immunization of rats: Male Lewis rats, approximately 10 weeks old, were supplied by Harlan-Sprague Dawley, Walkersville, MD. All procedures involving animals were performed in adherence to the ARVO Resolution on the Use of Animals in Research. Purified bovine IRBP was prepared as described elsewhere,9 emulsified (1:1) in complete Freund’s adjuvant containing 2 mg/ml Mycobacterium tuberculosis (H37 Ra) (Difco, Detroit, MI), and injected into one hind footpad in a volume of 0.1 ml. A suspension of Bordetella pertussis bacteria (Michigan Dept. Public Health, Lansing, MI, lot 91) was injected intravenously, as indicated, 1010 organisms per rat, concomitantly with the antigen emulsion. Control rats were similarly immunized with bovine serum albumin.

Evaluation of disease: The rats were observed daily for clinical ocular changes, and disease occurrence and severity were verified by histological examination. Enucleated eyes and pineals were fixed, and tissue sections were prepared and stained with hematoxylin and eosin as described elsewhere.8 As with rats immunized with S-Ag, all rats with IRBP-induced EAU had panuveitic changes with inflammation affecting numerous tissues in both the anterior and posterior segments (see below). Therefore, the grading of disease...
Table 1. Induction of EAU by IRBP

<table>
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<tr>
<th>Exp #</th>
<th>IRBP (µg/Rat)</th>
<th>B. pertussis</th>
<th>Affected rats/total</th>
<th>Day of onset (mean)</th>
<th>Severity (mean)</th>
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<td>3/3</td>
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<td>Yes</td>
<td>1/4*</td>
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<td>1.0†</td>
</tr>
</tbody>
</table>

* Lack of disease in the negative rats was verified by histologic examination.
† Values obtained in the positive rat of this group.

Results. Uveitogenic capacity of IRBP: The development of EAU was monitored in Lewis rats, immunized with varying doses of IRBP, with or without the additional treatment with B. pertussis. The data of three experiments are recorded in Table 1 and show that IRBP is highly uveitogenic, producing EAU in all tested rats at the doses of 0.8 µg/rat or higher. A lower dose, 0.3 µg, produced disease in one out of the four immunized rats. The high capacity of IRBP to induce EAU was also demonstrated by the early onset of disease; clinical changes became apparent as soon as 8 days after immunization with IRBP at the high doses in rats treated simultaneously with B. pertussis. The disease onset was slower in rats immunized with low doses of IRBP, and was always more delayed in animals not treated with B. pertussis. Treatment with B. pertussis also markedly increased the severity of EAU.

Clinical features of IRBP-induced EAU: All affected rats in the present study developed bilateral disease. The main clinical changes included protrusion, blood vessel dilation, and infiltration with inflammatory cells throughout the eyeball (Fig. 1B). Although these changes generally resemble those seen in rats with EAU induced by S-Ag, it is of note that the two diseases varied in certain aspects. The changes at high doses of ≥20 µg/rat were found to be milder in rats immunized with IRBP as compared to those produced by S-Ag (our repeated observations; rats immunized with the high doses of S-Ag often show the maximal severity graded as “4”). In addition, the two diseases differ in their course. The IRBP-induced EAU was found to have earlier onset, but to subside more rapidly than severity, on a scale of 0–4, was based on the intensity of these changes, rather than on certain specific changes, as is done for EAU in guinea pigs. It is also of note that a complete correlation was found in the present study between the clinical and histological findings.

Fig. 1. Changes in the anterior segment of rat eyes with IRBP-induced EAU. A, A normal rat eye, focused at the iris. B, Eleven days after immunization with 20 µg bovine IRBP and treatment with B. pertussis. The dilation of iridic blood vessels and the haze throughout the anterior segment, with a focal accumulation of exudate at the nasal side of the center. The focal accumulation of exudate was found to be a characteristic feature of IRBP-induced EAU. C, The anterior segment, at the angle, of a control rat, immunized with bovine serum albumin and treated with B. pertussis (X150). D, Ten days after immunization with 16 µg IRBP and treatment with B. pertussis. Severe inflammation occupying the anterior and posterior chambers. The episcleral tissue is also infiltrated (X150). E, The infiltrating cells, mostly PMNs, at a high magnification (X700). Co = cornea.
the disease induced by S-Ag. While the clinical changes of IRBP-induced EAU disappeared within <7 days, those of S-Ag induced disease are often visible for up to 15 days.

Histopathological changes in rats immunized with IRBP: The histopathology in eyes with EAU induced by IRBP generally resembled that of S-Ag induced EAU. The major changes included panuveitic infiltration, with accumulation of inflammatory cells mainly in the anterior and posterior chambers (Fig. 1D) and in the edematous retina and expanded subretinal space (Fig. 2B). The infiltrating cells were both polymorphonuclear (PMN) and mononuclear leukocytes (MNL), with PMNs being the majority in the anterior segment (Fig. 1E) and with similar proportions of the two cell types accumulating in the posterior segment of the eye. The inflammatory reaction subsided rapidly, with no residual apparent pathological changes noticed in the anterior segment (not shown), but with severe changes in the retina, where the photoreceptor cell layer was found to disappear focally in moderately affected eyes, or completely in the severely inflamed eyes (Fig. 2C).

As with rats immunized with S-Ag, animals injected with IRBP also developed pinealitis (Fig. 3). The histological changes in the pineal were found in all rats developing EAU following immunization with IRBP and, unlike in the eye, consisted exclusively of MNL infiltration (Fig. 3B). At early stages, the inflammation localized mainly at the stalk (St) and subcapsular areas of the pineal gland, while, at later changes, more central areas became affected (Fig. 3A). The changes in the pineal gland of rats immunized with IRBP resembled those in animals immunized with S-Ag. However, the pinealitis was generally more severe in rats immunized with IRBP than in those immunized with S-Ag, and the infiltration in the former rats was found more often to affect the adjacent meninges (Me) as well (Fig. 3A).

Discussion. Our data show that IRBP is a potent uveitogenic molecule. Immunization with IRBP produced EAU in all tested rats at the dose of $\geq 0.8 \mu g$ and in one out of four at the low dose of 0.3 $\mu g$. These uveitogenic levels are lower by far than the ones reported for another retinal specific protein, rhodopsin, and even lower than the uveitogenic dose of S-Ag ($\sim 5 \mu g$/rat). The preparations of IRBP used in the present study were purified to homogeneity and showed no cross antigenicity with S-Ag by various humoral and cellular immunological tests (data not shown).

EAU induced by IRBP differs from the disease induced by S-Ag mainly by two features: (a) EAU induced by IRBP has a markedly shorter course than the S-Ag induced disease and (b) in spite of its being highly uveitogenic at low doses, IRBP does not produce panuveitic changes as severe as those often observed in rats.
Fig. 3. The pineal gland, 17 days after immunization with IRBP (20 \( \mu \)g) and treatment with \( B. \) pertussis. A, Intense infiltration throughout the pineal gland as well as the adjacent meninges (\( \times 100 \)). B, The infiltrating cells, essentially MNLs only, from a central area of the gland, at a high magnification (\( \times 700 \)). St = stalk; Me = meninges.

immunized with S-Ag at high doses (\( \geq 20 \mu g/\text{rat} \)). Despite the aforementioned differences between the diseases induced by IRBP and S-Ag, the similarity between the two diseases is perhaps of more interest. Rats immunized with either one of the antigens developed inflammatory changes in both their eyes and pineal glands, and a close similarity was noted between the type of inflammation in the affected tissues. In both diseases, a heavy involvement of PMNs was found in the inflammation sites of the eyes, whereas the inflammation of the pineal gland consisted of mononuclear cells only. (Observations on the S-Ag induced disease in rats are recorded\(^{2,8,11}\).) The finding that two different antigens produce this dissociation in the type of inflammation in the eye and pineal organ thus provides new support for our assumption\(^8,12\) that the PMN involvement in the rat eye is mediated by local factors, and is not necessarily due to an Arthus-like immune response.

The results reported here thus describe a new experimental disease which is induced by immunization with IRBP, and affects the eye and pineal gland. Further, the newly described disease and the one induced by S-Ag provide a unique system in which two separate tissue-specific antigens (IRBP and S-Ag) are capable of provoking pathogenic autoimmune processes that affect the same tissues by a similar mechanism. This system may be useful for investigating the specificity of processes of disease induction and suppression in the affected tissues. Another aspect derived from the present study is the possible participation of autoimmune response to IRBP in uveitic conditions in man. Indeed, monkeys immunized with IRBP were found to develop ocular inflammatory changes which closely resemble those in sympathetic ophthalmia and the Vogt-Koyanagi-Harada disease (S. Hirose et al, in preparation). The possible involvement of IRBP in immunopathogenic processes in humans is under investigation.

Key words: interphotoreceptor retinoid-binding protein (IRBP), experimental autoimmune uveoretinitis (EAU), pinealitis, retinal S-antigen

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References
The kinetics and pathways of hydrolysis of methionine enkephalin (TGGPM), leucine enkephalin (TGGPL), and [D-Ala²]met-enkephalinamide (TAGPM) in homogenates of anterior segment tissues of the albino rabbit eye were studied using reversed phase HPLC. Both TGGPM and TGGPL were equally susceptible to hydrolysis with a half-life ranging from 11–50 min and were 11–23 times more susceptible to hydrolysis than TAGPM. All three peptides were hydrolyzed most rapidly in the corneal epithelium, followed by the ir- scillary body, conjunctiva, corneal stroma, lens, and tears. Aminopeptidases were responsible for over 90% of the hydrolysis of TGGPM and TGGPL, while dipeptidyl peptide and dipeptidyl carboxypeptidase were responsible for the remainder. In contrast, dipeptidyl carboxypeptidase was principally responsible for the hydrolysis of TAGPM, which by design is resistant to aminopeptidase action. Overall, these findings suggest that, in order to deliver short chain biologically active peptides, aminopeptidases play a principal role in degrading short chain biologically active peptides. This study was undertaken to test this hypothesis using three enkephalins, all pentapeptides, as model compounds. These were methionine enkephalin (Tyr-Gly-Gly-Phe-Met), leucine enkephalin (Tyr-Gly-Gly-Phe-Leu), and [D-Ala²]-met-enkephalinamide (Tyr-D-Ala-Gly-Phe-Met-NH₂), hereafter to be abbreviated as TGGPM, TGGPL, and TAGPM, respectively. These peptides were chosen for study primarily because their pathways of degradation in the brain and elsewhere in the body have been well characterized. In this study, the kinetics and pathway of enkephalin degradation in tears and aqueous humor, as well as homogenates of the conjunctiva, corneal epithelium, corneal stroma, irisciliary body, and lens of the albino rabbit were monitored over 180 min. This information would facilitate the understanding of the susceptibility of both endogenous and topically applied enkephalins to hydrolysis by aminopeptidases in anterior segment tissues.

Materials and Methods. Enkephalins were obtained commercially (Sigma Chemical Co., St. Louis, MO) and were used as received after verification of purity using reversed phase HPLC. Twelve male, albino, New Zealand rabbits (ABC Rabbitry, Pomona, CA), weighing 2–3 kg, were used for each enkephalin. Tears, aqueous humor, and anterior segment tissues were collected from these rabbits as previously described. Tissue homogenates, 20% w/v, were prepared in isonotic KCl using a Potter-Elvehjem tissue homogenizer followed by centrifugation at 3,020× g in a Sorvall RC-5B refrigerated superspeed centrifuge (DuPont Instru-