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An 18-mer peptide derived from the retinal S antigen induces uveitis and pinealitis in primates

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SUMMARY

S-antigen, a photoreceptor cell protein, induces a predominantly T-cell mediated autoimmune uveitis in many vertebrate animals, including primates. Because of this activity and the finding of immune responses to S antigen in patients with uveitis, this protein has been implicated in the pathogenesis of uveitis in humans. Peptide M, an 18-amino acid component of S antigen, has previously been shown to be highly uveitopathogenic in rats and guinea pigs. We report here that peptide M is immunopathogenic in some monkeys, producing inflammatory changes in eyes and pineal glands similar to those induced by native S antigen. Monkeys with disease also developed intense immune responses to peptide M, measured by the lymphocyte proliferation assay. In addition, lymphocytes from these monkeys reacted against whole S antigen. Furthermore, lymphocytes from certain monkeys immunized with whole S antigen responded well against peptide M, thus indicating that this peptide is an immunodominant epitope in these animals. Two of the four monkeys immunized with peptide M did not develop disease. Lymphocytes from these two animals did not respond in culture against the peptide. Following immunization with the whole protein, these monkeys were capable, however, of developing cellular immunity against S antigen and one of them developed disease. The possible involvement of peptide M in the pathogenesis of uveitis in humans is discussed.

Keywords S antigen primates lymphocyte proliferation uveitis pinealitis

INTRODUCTION

Intraocular inflammatory conditions which are often grouped under the term 'uveitis' are a major cause of visual impairment (National Institutes of Health, 1976; Kaplan, 1986). Although the aetiology of most of these conditions is unknown, T-cell mediated autoimmune processes are thought to play a major role in the pathogenesis of some forms of human uveitis (Faure, 1980; Kaplan, 1986; Gery, Mochizuki & Nussenblatt, 1986). This notion is supported by the finding that the retina contains several organ-specific antigens that induce inflammatory changes in susceptible animal strains which resemble those observed in certain uveitic conditions in humans (Wacker *et al.*, 1977; Faure, 1980; Meyers-Elliott *et al.*, 1983; Gery, Mochizuki & Nussenblatt, 1986; Gery *et al.*, 1986). Furthermore, some patients with uveitis show specific humoral and cell mediated immune responses toward these retinal-specific antigens (Nussenblatt *et al.*, 1980; Gregerson, Abrahams, & Thirkill, 1981; Doekes *et al.*, 1987).

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One uveitogenic retinal antigen which has been extensively investigated is S antigen, a protein of approximately 45 kD which has been found to play a pivotal role in the visual process (Pfister *et al.*, 1985; Wistow *et al.*, 1986; Shinohara *et al.*, 1987). S antigen is found in the retinas of many animal species as well as in the pineal gland, an organ that is related to vision (Kalsow & Wacker, 1973; Mirshahi *et al.*, 1984). When injected into a variety of vertebrate animals, S antigen induces a severe ocular and pineal inflammatory response—designated experimental autoimmune uveitis (EAU) and experimental autoimmune pinealitis (EAP), respectively (Wacker *et al.*, 1977; Faure, 1980; Gery *et al.*, 1986). Of particular interest has been the EAU induced by S antigen in primates (Nussenblatt *et al.*, 1981; Faure *et al.*, 1981), which resembles in many aspects the human disease entity 'birdshot retinochoroidopathy' (Nussenblatt *et al.*, 1982). The possible involvement of S antigen in the aetiology of uveitis in humans is also indicated by the finding that most of the aforementioned immune responses detected in patients with intermediate and posterior uveitis were specific toward this protein (Nussenblatt *et al.*, 1980; Gregerson, Abrahams & Thirkill, 1981; Nussenblatt *et al.*, 1982; Doekes *et al.*, 1987).

The investigation of S antigen has recently focused on the identification of epitopes responsible for its immunopathogeni-

city. We have previously identified an 18 amino acid peptide highly pathogenic for the induction of EAU in the Lewis rat and the Hartley guinea pig (Donoso *et al.*, 1987a, b; Singh *et al.*, 1988). The peptide, designated peptide M, corresponds to amino acid positions 303 to 320 (DTNLASSTIIKEGIDKTV) in bovine S antigen. More recently, the amino acid sequence of human S antigen was determined and the portion corresponding to peptide M was found to be virtually identical to that of the bovine S antigen (Yamaki, Tsuda & Shinohara, 1988).

The present study was aimed at testing the capacity of peptide M to induce cellular responses and immunopathogenic processes in primates. Some monkeys immunized with peptide M developed ocular and pineal inflammation, as well as intense lymphocyte responses toward this molecule.

MATERIALS AND METHODS

Antigens

S antigen was prepared from bovine retinas as described by Dorey, Cozette & Faure (1982). This technique involves extraction with hypotonic buffer followed by 50% ammonium sulphate precipitation, gel filtration and affinity chromatography. Minor contaminants were removed by high-performance liquid chromatography (Zigler *et al.*, 1984). Peptide M was synthesized by conventional solid-phase chemistry on a benzhydrylamine resin using an automated peptide synthesizer (Sam II, Biosearch, San Rafael, CA), as described earlier (Donoso *et al.*, 1986). Purity was monitored by reverse phase high-pressure liquid chromatography (Vydac 218 column; Vydac, Hesperia, CA). The amino acid composition of the peptide was confirmed by amino acid analysis and by a gas phase sequenator (Model 478A; Applied Biosystems, Foster City, CA).

Animals and immunization

Eight monkeys were provided by the National Institutes of Health animal facility. These comprised five *Macaca arctoides* (Nos. B0099, B8389, B8987, B9360 and B9733) and three *M. mulatta* (Nos. 62 C, AF47 and 128). The animals were treated in accordance with NIH Guidelines for Animal Use in Research. Groups of four monkeys, as indicated, were immunized with S antigen (50 μg/kg body weight), or peptide M (200 μg/kg). The antigens were emulsified in Freund's complete adjuvant (FCA), containing *Mycobacterium tuberculosis* H37Ra at 1·25 mg/ml and were injected intradermally at multiple sites in the nape of the neck. An additional adjuvant, *Bordetella pertussis* (Michigan Department of Public Health, Lansing, MI, lot 91B), was injected intravenously, $2{\cdot}5 \times 10^{10}$ bacteria/kg body weight, concurrently with the antigen emulsion. A second immunization was given 2 weeks later. Monkeys Nos. 128 and AF47 were immunized with peptide M and reimmunized 10 weeks later with S antigen, as described above. Two monkeys, one *M. arctoides* and one *M. mulatta*, were used for controls and were injected twice with FCA alone.

Treatment with cryopexy

Certain monkey eyes, as indicated, were treated with cryopexy, using a Beaver Cryo X Tractor probe (Beaver, Waltham, MA) cooled in liquid nitrogen. The probe was applied to the temporal sclera of one eye for approximately 45 sec. The affected area measured approximately 1 mm in diameter.

Disease assessment

Immunized monkeys were examined clinically for ocular inflammatory changes, by an inverted ophthalmoscope, at weekly intervals. The ocular changes were further analysed by histological examination, as described elsewhere (Nussenblatt *et al.*, 1981). Pineal inflammation was similarly determined by histological examination.

Lymphocyte proliferation assay

Peripheral blood lymphocytes collected at various intervals following immunization were separated on Isolymph gradients (Gallard-Schlesinger, Carle Place, NY) and tested for specific responses to antigens by the proliferation assay as described in detail elsewhere (Hirose *et al.*, 1987). In brief, 5×10^4 mononuclear leucocytes were cultured in quadruplicate, with or without the antigens, in round-bottom microtiter plates, in a total volume of 0·2 ml. The antigens were peptide M and S antigen, as well as purified protein derivative of tuberculin (PPD, Connaught Laboratories, Toronto, Ontario). Following incubation for 5 days, the cultures were pulsed with ^{3}H-thymidine (0·5 μCi/well) and harvested 16 h later. The ct/min values of individual cultures regularly differed from the mean values by $\leq 20\%$. The data are presented as stimulation index values (SI = mean ct/min in cultures with antigen/mean ct/min in cultures without antigen). Lymphocyte responses of immunized monkeys were determined 2 and 4 weeks after the second immunization with peptide M or S antigen. Small differences were seen between cells collected at the two intervals and the data recorded here for each monkey are those with the higher set of SI values.

RESULTS

Development of EAU and EAP in immunized monkeys

Table 1 summarizes the development of EAU in monkeys immunized with native S antigen or with peptide M. All four monkeys immunized primarily with S antigen (B8389, B8987, B9360 and 62C) developed EAU within 2–4 weeks following the second injection with the antigen. In contrast, only one monkey (B9733) developed EAU 18 days after the second immunization with peptide M. Another monkey (B0099) immunized with peptide M did not develope any detectable ocular changes for 8 weeks following immunization, but developed severe ocular inflammation in one eye 14 days after cryopexy treatment. The other two monkeys immunized with peptide M (AF47 and 128) did not demonstrate any ocular changes and also failed to develop any measurable immune response toward this peptide (see below). These two monkeys were immunized 10 weeks later with the whole S antigen and 8 weeks after that were treated with cryopexy in one eye. A mild EAU developed in both eyes of monkey AF47, and no ocular changes could be detected in monkey 128. Treatment with cryopexy did not cause EAU in the two control monkeys which were immunized with FCA alone (data not shown).

The clinical changes in monkeys immunized with peptide M included sheathing of the retinal vessels and focal greyish retinal lesions (Fig. 1). Histological examination of the retina in the enucleated eyes showed perivascular cuffing, foci of destruction of the photoreceptor cell layers and infiltrates of mononuclear cells throughout the retina (Fig. 2b). The histopathological changes in the eyes of the monkeys immunized with peptide M were indistinguishable from those observed in monkeys immu-

Table 1. Development of EAU and lymphocyte responses in monkeys immunized with S antigen or peptide M

Monkey No.	Immunizing antigen*	Cryopexy	EAU	Lymphocyte responses: antigen in culture†,‡		
				S antigen	Peptide M	PPD
B8389	S antigen	No	+	138·0	4·8	15·1
B8987	S antigen	No	+	59·1	6·1	12·6
B9360	S antigen	No	+	14·2	2·3	14·5
62C	S antigen	No	+	15·0	1·3	16·0
B9733	Peptide M	No	+	18·9	55·2	12·7
B0099	Peptide M	No	−	29·1	99·1	18·0
		Yes	+	ND	ND	ND
128	Peptide M	No	−	0·9	1·5	6·9
	S antigen	No	−	11·4	2·5	43·4
		Yes	−	ND	ND	ND
AF47	Peptide M	No	−	0·8	1·0	11·4
	S antigen	No	−	3·3	2·1	9·6
		Yes	+	ND	ND	ND

*Immunization schedules are described in Materials and Methods.

†PPD was added at 10 or 20 μg/ml; S antigen and peptide M were added at 2 μg/ml in cultures of cells from monkeys immunized with the tested antigen, or at 20 μg/ml in cultures of cells from monkeys immunized with the other antigen.

‡Stimulation index; unstimulated cultures of cells from these monkeys incorporated 340–1645 mean ct/min.

ND not done.

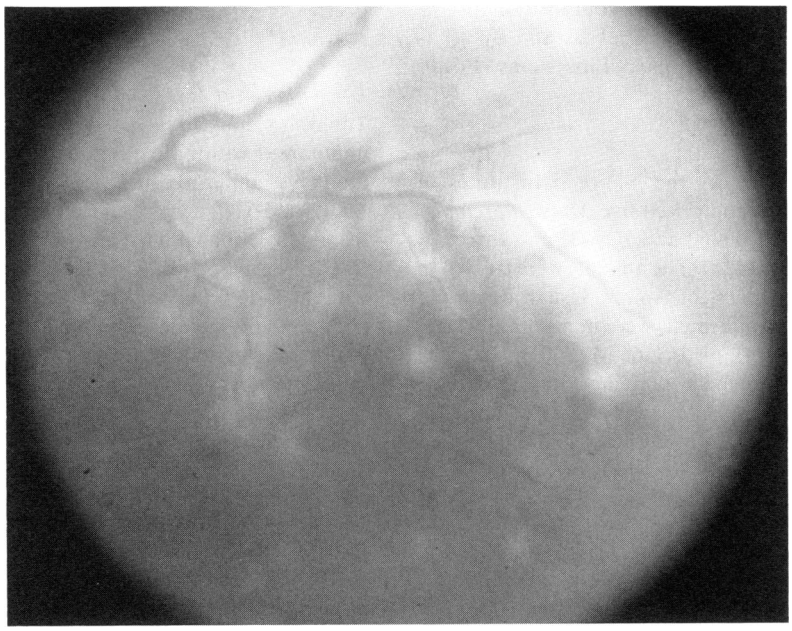

Figure 1. Clinical changes following immunization with peptide M (monkey B0099). A fundus photograph showing sheathing of the retinal vessels, grayish retinal lesions and superficial haemorrhages.

nized with native S antigen in this study and in previously reported studies (Nussenblatt *et al.*, 1981; Faure *et al.*, 1981). Both monkeys B0099 and B9733 also developed pineal inflammation, which consisted of foci of mononuclear infiltration (Fig. 3).

Immune responses in the immunized monkeys

Table 1 also records the cellular immune responses of the immunized monkeys toward S antigen, peptide M and PPD, a component of FCA. The responses were measured by the lymphocyte proliferation assay. Vigorous responses to S antigen

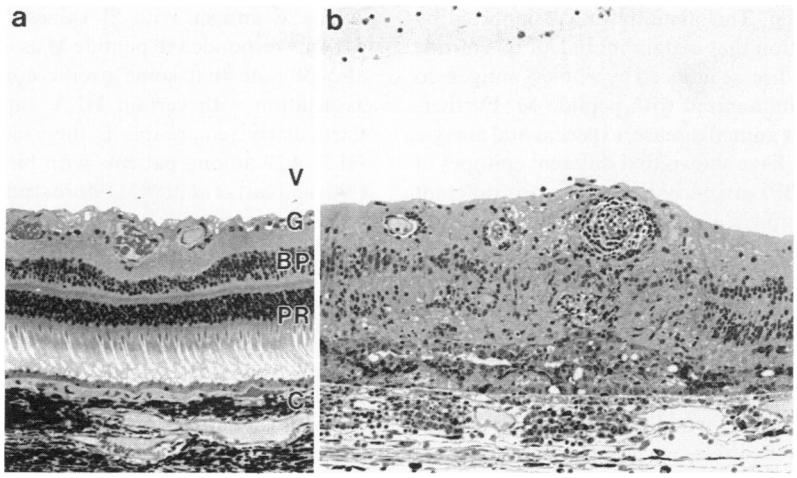

Fig. 2. Histopathological ocular changes induced by immunization with peptide M. Posterior segment of a monkey immunized with FCA alone, showing the typical stratiform morphology (a). V vitreous; G ganglion cell layer; BP biopolar cell layer; PR photoreceptor cell layer (composed of the nuclear and outer segment layers); C choroid. Posterior segment of monkey B9733 (b). Perivascular cuffing and infiltration with inflammatory cells throughout the retina. Focal destruction of the retinal cell layers. Accumulation of inflammatory cells in the vitreous and choroid (haematoxylin and eosin, magnification 120).

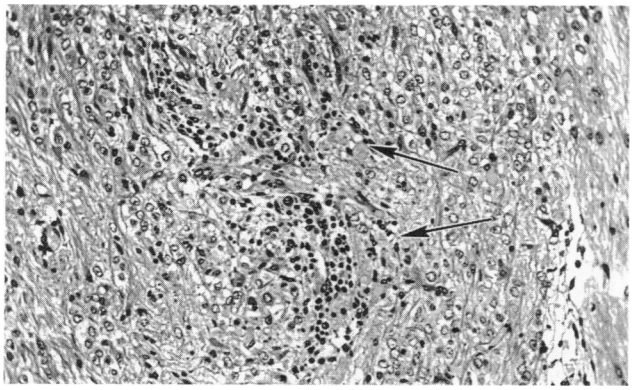

Fig. 3. Inflammatory changes in the pineal gland of monkey B9733 following immunization with peptide M. Focal infiltration with mononuclear cells (arrows) in the central area of the gland (haematoxylin and eosin, magnification 110).

were recorded in lymphocyte cultures of all four monkeys immunized with this protein, with particularly high SI levels (138·0 and 59·1) produced by cells from monkeys B8389 and B8987. Lymphocytes from these two animals reacted also with moderate SI levels (4·8 and 6·1, respectively) when incubated with peptide M.

Unlike the positive responsiveness in all monkeys immunized with S antigen, only two of the monkeys immunized with peptide M exhibited intense lymphocyte responses. Lymphocytes from these monkeys (B9733 and B0099) reacted with high levels against peptide M (SI values 55·2 and 99·1, respectively), as well as against whole S antigen (SI values 18·9 and 29·1, respectively). As mentioned above, these two monkeys are those who developed EAU and EAP following immunization with the peptide. In contrast, no responses to peptide M or S antigen were found in cultures of lymphocytes from the other two monkeys of this group (128 and AF47) following immunization with peptide M. These two monkeys responded, however, against PPD with levels similar or only slightly lower than those found with lymphocytes from the other immunized monkeys.

The lack of immune responsiveness to peptide M in monkeys 128 and AF47 was further examined by testing their responses following immunization 10 weeks later with whole S antigen. As shown in Table 1, both monkeys were capable of developing specific responses to the whole protein with moderate (128) or low (AF47) levels of lymphocyte proliferation.

Unlike the vigorous cellular responses toward peptide M in the responding monkeys, no antibodies against the peptide could be detected in their sera, using the sensitive dot-blot assay (Donoso *et al.*, 1986). In contrast, monkeys immunized with S antigen developed high levels of antibodies against the whole protein (data not shown).

DISCUSSION

Data reported here show that peptide M, that has been previously found to be highly immunopathogenic in Lewis rats and in Hartley guinea pigs (Donoso *et al.*, 1987a, b; Singh *et al.*, 1988), induces EAU and EAP in some immunized monkeys as well. It is of note that the pathological changes induced by peptide M were indistinguishable from those induced in primates by the native S antigen (Nussenblatt *et al.*, 1981, Faure *et al.*, 1981). Moreover, both the clinical and histopathological changes observed in the monkeys with EAU induced by peptide M resembled those found in eyes of patients with the uveitic condition birdshot retinochoroidopathy (Nussenblatt *et al.*, 1982).

Only two of the four monkeys immunized with peptide M developed disease. In contrast, all monkeys immunized with native S antigen in this study (Table 1) and in other studies (Nussenblatt *et al.*, 1981; Faure *et al.*, 1981) developed EAU. This finding may be explained by assuming that different determinants on S antigen serve as the immunopathogenic epitopes for different individual monkeys. Accordingly, only a portion of the monkeys are susceptible to induction of EAU and EAP by peptide M, while others are assumed to be affected by

other epitopes of S-antigen. This assumption is supported by our unpublished observation that certain inbred strains of rats that are susceptible to the disease induced by whole S antigen do not develop EAU when immunized with peptide M. Furthermore, studies with another animal disease, experimental allergic encephalomyelitis (EAE), have shown that different epitopes of myelin basic protein (MBP) are encephalitogenic for different inbred strains of mice (Pettinelli *et al.*, 1982; Zamvil *et al.*, 1986) or rats (Beraud *et al.*, 1986).

The capacity of MBP epitopes to induce EAE in mouse or rat inbred strains was found to be related to the responsiveness of lymphocytes from the immunized animals against the tested peptides (Pettinelli *et al.*, 1982; Beraud *et al.*, 1986). A similar relationship was observed in monkeys of the present study: no disease was detected in monkeys who failed to develop cellular immunity to peptide M, while these two negative monkeys were capable of developing immunity against the whole protein (Table 1), presumably against determinants other than peptide M. Furthermore, one of these monkeys (AF47) developed EAU following immunization with whole S antigen. The reason for the failure of the second monkey (128) to develop disease is not clear but could be attributed to the excessive injections with FCA which may initiate suppressive mechanisms similar to those found in animals injected with other adjuvants (Klimpel & Henney, 1978; Lichtenstein *et al.*, 1981) Repeated injections with FCA could also be one mechanism responsible for the relatively low immune responses to S antigen in monkeys 128 and AF47 (see Table 1). In addition, these two monkeys could have been 'low responders' to the S-antigen.

EAU developed in two of the immunized monkeys (B0099 andAF47) only following treatment with cryopexy (Table 1). This treatment was found in another study (deBara *et al.*, 1989) to also enhance EAU induction in rats, mainly in recipients of lymphocytes specifically sensitized to S antigen. Studies with cryopexy-treated rats have suggested that the EAU enhancement derives mainly from the breakdown of the retinal-blood barrier and infiltration of the affected tissue with lymphoid cells. These processes are assumed to enhance the immunopathogenic reaction, thus facilitating the spreading of the inflammatory reaction to areas remote from the cryopexy-affected site and even to the untreated eye, as observed in monkey AF47.

Data reported here show that the two monkeys immunized with peptide M, who reacted well to the peptide, exhibited high levels of responsiveness to whole S antigen as well (Table 1). Furthermore, lymphocytes from two monkeys immunized with native S antigen (B8389 and B8987) demonstrated significant responses to peptide M (Table 1). This finding indicates that peptide M is one of the immunodominant epitopes of S antigen in these monkeys.

The present communication is the first to report on the immunogenicity and immunopathogenicity in primates of a synthetic peptide derived from S antigen. The significance of the findings reported here is underscored by the aforementioned similarity between the clinical and pathological changes in affected monkeys and those in certain uveitic conditions in humans. Furthermore, the notion that autoimmune processes toward peptide M may play a role in the pathogenesis of uveitis in humans has been supported by data of a preliminary study in which lymphocytes from uveitis patients were tested in culture for specific responses toward S antigen and peptide M. Lymphocytes from six out of 18 tested patients responded in culture against S antigen with SI values >3.0. Four of the positive patients responded to peptide M as well (unpublished data). It is also of note that some uveitic conditions demonstrate close association with certain HLA antigens (Nussenblatt, 1980). Particularly remarkable is the exceedingly high frequency of HLA-A29 among patients with birdshot retinochoroidopathy (Nussenblatt *et al.*, 1982). More studies are needed to investigate possible associations between MHC antigens, responsiveness to peptide determinants of ocular-specific antigens and susceptibility to uveitic conditions in humans.

The information collected in this study and in future ones should be useful for a better understanding of the pathogenesis of uveitic conditions. Furthermore, knowledge of the immunodominant epitopes in S antigen and other retinal-specific antigens should be pivotal for future attempts to modulate autoimmune processes in the eye by immunological manipulations such as the induction of specific anti-idiotypic immunity by vaccination using specifically activated altered lymphocytes (Cohen, 1986; Lider *et al.*, 1987).

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