Permissive recognition of immunodominant determinants of the retinal S-antigen in different rat strains, primates and humans

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Abstract

The majority of antigenic peptides exhibit restriction in their interaction with the MHC molecules on antigen-presenting cells of different haplotypes. Certain peptides, however, are ‘permissive’: they bind strongly to different MHC molecules and are selected as the immunodominant epitopes by animals using these MHC gene products. Here we show for the first time that several peptides from four regions of the sequence of human S-antigen (H-SAg), a retinal-specific protein, demonstrate high levels of permissiveness. Each of these peptides was found to be immunodominant in at least some of four inbred rat strains and five cynomolgus monkeys, immunized with whole H-SAg. Moreover, some of these peptides were recognized by lymphocytes from normal controls and four patients with uveitis who responded against the H-SAg molecule. On the other hand, the permissive peptides stimulated marginal or no response in cultures of Lewis rats injected with adjuvant alone, or rat and human cell lines specific to other antigens, thus demonstrating that these peptides do not carry any non-specific mitogenic activity. One peptide, 29, which was found immunodominant in the monkeys, the uveitis patients and Lewis rats, is highly immunopathogenic in this rat strain. No good correlation between immunodominance and immunopathogenicity was found with other H-SAg peptides. The finding of cross-species permissiveness among peptides of H-SAg and similar observations with myelin proteins suggest that permissiveness could be quite prevalent among peptides of immunopathogenic antigens.

Introduction

The immune response against a whole protein is targeted toward a small number of peptide determinants, the immunodominant epitopes, while the majority of the protein’s sequences are ‘cryptic’ and do not elicit any detectable response (1,2). A pivotal property of immunodominant peptides is their strong affinity to the MHC molecules on antigen-presenting cells (APC) of the responding animal (1,3–5). Due to the polymorphism of MHC molecules, the majority of immunogenic peptides bind strongly to only a single or a small number of MHC molecules (6–9). Certain peptides, however, were found to exhibit ‘permissiveness’ or ‘promiscuity’ in their capacity to bind to a large number of different MHC molecules, of one or more species, and to be recognized by T cells restricted by these MHC molecules (4,10–14). The majority of permissive peptides have been found in the sequence of microbial proteins such as viral (10), mycobacterial (11,12) or plasmodial (13,14) components, or tetanus toxin (4,10). In addition, several peptides of the myelin basic protein (MBP) (5) and proteolipid protein (PLP) (15) were found to be permissive in their binding to different MHC molecules. Moreover, a number of these peptides were found to be encephalitogenic in experimental animals (15–17) and to be recognized by lymphocytes from patients with multiple sclerosis (5,18–20).

The retina contains at least four tissue-specific proteins that are immunopathogenic and induce intraocular inflammation.
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Fig. 1. Sequence of H-SAg and its 40 overlapping peptides.

Table 1. HLA haplotypes of the patients and normal controls

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<tr>
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<td>Patient 3</td>
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* Determined by the Histocompatibility Laboratory, University of Michigan Hospitals.

(uveitis) when injected into experimental animals (21–23). One of these proteins, designated ‘S-antigen’ (SAg) or ‘arrestin’, is a 48 kDa glycoprotein that plays a pivotal role in the vision process (24,25). SAg is highly uveitogenic in several species (21,22,24,26,27) and is thought to be a target for immunopathogenic processes that mediate uveitic conditions in humans (21,22,28). In accord with this notion, a large portion of patients with uveitis were found to respond to SAg by the lymphocyte proliferation assay (21,22,28–30). In addition, similar to observations with the response to MBP (18,31), cellular responses to SAg were also detected in normal controls (32,33).

The goal of the present study was to identify peptide determinants of human SAg (H-SAg) that are immunodominant in animals immunized with the whole molecule, as well as those that are recognized by human donors who respond against this protein. Forty overlapping peptides that span the entire sequence of H-SAg were tested and several peptides exhibited permissiveness by being immunodominant in different rat strains, several individual monkeys, as well as some human donors.

Methods

Antigens
Recombinant human SAg (rH-SAg) was prepared as described elsewhere (34), purified protein derivative (PPD)
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containing Mycobacterium tuberculosis H37 Ra, at 2.5 mg/ml (Difco, Detroit, MI). Control rats were injected with the adjuvant emulsified with saline. The monkeys were immunized with the antigen, emulsified with Hunter’s adjuvant (CytRx, Norcross, GA), at 40 µg/kg body weight, injected into the nape of the neck. All animal procedures conformed to Institutional guidelines and the ARVO Resolution on Use of Animals in Research.

Human subjects

After informed consent was obtained, blood samples from eight male donors with positive lymphocyte response against whole rH-SAg were obtained. They included four normals (62, 22, 32 and 23 years old respectively) and four patients with the following uveitic conditions: 1, 4 years old, with chronic non-granulomatous anterior uveitis; 2, 46 years old, with Behcet’s disease; 3, 45 years old with idiopathic intermediate uveitis; 4, 31 years old with idiopathic intermediate uveitis. The HLA haplotypes of all donors are detailed in Table 1.

HLA typing

HLA typing of most donors was carried out by the NIH Typing Laboratory; normal donor 2 was typed by the Histocompatibility Laboratory, University of Michigan Hospitals, Ann Arbor, MI. The donor haplotypes were determined by serology or by DNA analysis, as indicated, using standard methods.

Primary lymphocyte cultures

Lymphocytes were obtained from the draining lymph nodes of the rats or from the peripheral blood of the monkeys and node cells from rats of different inbred strains. The rats were human donors. Rat lymph nodes and monkey blood samples immunized with rH-SAg and the lymphocyte assays were carried out as detailed in Methods. The peptides were tested at 100 µg/ml. The recorded data are means of SI values of two rats of each strain; the single cell suspensions were prepared by Miles-Yeda (Rehovot, Israel). Forty overlapping 20-mer peptides, except for the last fragment measuring 15 amino acids (Fig. 1), spanning the entire H-SAg sequence (24), and peptide 1181–1191 of bovine interphotoreceptor retinoid-binding protein (IRBP) (35) were provided by Applied Biosystems (Foster City, CA, USA). The peptides were synthesized by solid-phase chemistry using t-butyloxy carbonyl derivatives of the amino acids and purified by HPLC to at least 95% purity.

Animals and immunization

Male rats of four inbred strains, Lewis, ACI, Buffalo and Brown Norway (BN), 8–10 weeks old, were supplied by Harlan Sprague Dawley (Indianapolis, IN). Five female cynomolgus monkeys, weighing 2.5–3 kg, were provided by the NIH Animal Facility. The rats were injected into one hind footpad with 50 µg rH-SAg, emulsified with complete Freund’s adjuvant containing Mycobacterium tuberculosis H37 Ra, at 2.5 mg/ml (Difco, Detroit, MI). Control rats were injected with the adjuvant emulsified with saline. The monkeys were immunized with the antigen, emulsified with Hunter’s adjuvant (CytRx, Norcross, GA), at 40 µg/kg body weight, injected into the nape of the neck. All animal procedures conformed to Institutional guidelines and the ARVO Resolution on Use of Animals in Research.

Results

Proliferative responses of lymph node cells from rats immunized with rH-SAg

Groups of rats of four inbred strains were immunized with rH-SAg and examined for the development of experimental
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Fig. 3. Proliferative responses to the 40 peptides of H-SAg by PBL of cynomolgus monkeys immunized with rH-SAg. The peptides were tested at 100 µg/ml (A) or 10 µg/ml (B). See Methods for more detail. The mean c.p.m. ± SEM in the unstimulated control cultures of the individual monkeys were as follows: 3809, 292 ± 22; 3813, 370 ± 74; 3822, 511 ± 81; 3826 and 297 ± 31. The responses (SI values) of the four monkeys to rH-SAg, at 10 and 2 µg/ml, were as follows: 3809, 189.6 and 167.1; 3813, 208.6 and 186.3; 3822, 20.9 and 21.5; 3826, 162.0 and 120.6.

Autoimmune uveoretinitis and cellular immunity against rH-SAg and the 40 overlapping peptides of its sequence. Severe ocular inflammation developed in the Lewis rats, less intense disease was seen in eyes of ACI and Buffalo rats, whereas no ocular changes were detected in the BN rats. The rat strains differed also in the intensity of their cellular response to the 40 peptides (Fig. 2), with the Lewis and ACI rats showing higher levels of proliferative responses than the BN and Buffalo animals. Although the patterns of response to individual peptides also varied among the different strains, it is remarkable that immunodominant peptides were selected by all four strains almost exclusively from three regions of the molecule, i.e. sequences 61–90 (peptides 7 and 8), 171–200 (peptides 18 and 19) and 281–310 (peptides 29 and 30). Moreover, several peptides were immunodominant in two or three rat strains, and peptide 8 was immunodominant in all four rat strains.

Proliferative responses of peripheral blood lymphocytes (PBL) from immunized monkeys

All five monkeys immunized with rH-SAg developed severe intraocular inflammation. Proliferative responses of lymphocytes from four of these animals against the 40 peptides are summarized in Fig. 3. All four monkeys responded vigorously to the whole SAg preparation and to a relatively large number of peptides. Although there are differences among individual animals in their response to the 40 peptides, it is noteworthy that certain peptides were immunodominant in all four monkeys. These commonly dominant peptides include sequences designated 1, 3, 5, 8, 13, 18, 19, 29 and 30. In addition, several other peptides (15, 21, 22, 32 and 33) strongly stimulated lymphocytes from some but not all monkeys. The peptides were examined at two concentrations, 10 and 100 µg/ml, and considerable variability was noted among the peptides in their stimulatory capacity at the two concentrations. Peptides 1, 8, 29 and 30 were similarly active at the two concentrations, whereas the responses to the other peptides were usually much lower at 10 than at 100 µg/ml. The effect of peptide concentration was further examined by measuring the proliferative responses of lymphocytes from the fifth immunized monkey (3850) against nine stimulatory peptides at four concentrations, 0.1, 1, 10 and 100 µg/ml (Fig. 4). As seen in Fig. 4, only two peptides, 29 and 30, were stimulatory at all four concentrations; peptides 1, 3 and 8 again showed similar activity at 10 and 100 µg/ml, but had
Fig. 4. Proliferative responses of blood lymphocytes of monkey 3850 to different concentrations of nine immunodominant H-SAg peptides. The monkey was immunized and its lymphocytes were tested as detailed in Methods. The mean c.p.m. ± SEM in the unstimulated control cultures was 898 ± 66.

no significant effect at the lower concentrations, while peptides 5, 13, 18 and 19 were strongly stimulatory only at 100 µg/ml.

Proliferative responses of human PBL
PBL from four healthy donors and four patients with uveitis, that responded against rH-SAg, were also tested for their proliferative response against the 40 overlapping peptides of this protein. The patterns of response of the four healthy donors (Fig. 5) were quite variable, with only one peptide, 18, being stimulatory to lymphocytes of all four individuals. Other peptides, that stimulated lymphocytes of two or three donors, included 3, 8, 13 and 30. Much higher uniformity was observed in the pattern of response of the uveitis patients (Fig. 6), with all four individuals responding well against peptides 1, 8, 18 and 29. Moreover, patients 1, 2 and 3 responded with a remarkably uniform pattern, that closely resembled that seen in the monkeys’ responses (Fig. 3). Despite the similarity in their pattern of peptide recognition, the four patients were found to differ in their MHC haplotypes (Table 1).

Permissive peptides of H-SAg do not carry non-specific mitogenic properties
To test whether the highly permissive peptides stimulate lymphocytes by a non-specific mitogenic activity, the 40 H-SAg peptides were added (at 100 µg/ml) to cultures of lymph node cells of control Lewis rats injected with the adjuvant emulsified with saline. As seen in Fig. 7(A), no proliferation was stimulated by most peptides and only a slight response was detected in cultures incubated with peptide 8. In contrast, lymph node cells from rats immunized with rH-SAg responded with high SI levels against peptides 29, 30, 32 and 33, at both 100 and 10 µg/ml (Fig. 7B and C). The lack of non-specific mitogenicity by the 40 peptides was further demonstrated by the finding that the peptides had no effect in cultures of cell lines of human lymphocytes specific to PPD, or rat lymphocytes specific for H-SAg peptide 35, or peptide 1181–1191 of bovine IRBP (‘R15’) (Fig. 8). On the other hand, the cell line lymphocytes responded vigorously to their specific antigens (including H-SAg peptide 35), as well as to the mitogens Con A (rat cells) or PHA (human cells) (Fig. 8).

Discussion
Data recorded here show that certain regions of the H-SAg sequence harbor peptide determinants that were selected for immunodominance by the immune systems of animals with different MHC make-ups. Three of these regions, at sequences 61–90, 171–200 and 281–310, encompass peptides that were immunodominant in at least some of the four rat strains (Fig. 2), the five monkeys (Figs 3 and 4) and the eight human donors (Figs 5 and 6). In addition, sequence 1–20 was found to be recognized by all H-SAg immunized monkeys and the majority of human donors. The highest level of permissiveness was exhibited by peptide 8, that was dominant in all four rat strains, all monkeys and seven of the eight human donors. Also of note are peptide 18, that was recognized by all human donors and the five monkeys, and peptides 29 and 30, that stimulated vigorous responses by lymphocytes of Lewis rats, all monkeys and three of the uveitis patients.

The peptides used in the present study consisted of 20 amino acids each and it is possible that different portions of some of these peptides were selected as the dominant epitopes by immune systems of different animals. Additional studies, with truncated peptides, should be carried out, therefore, in order to analyze the fine specificity of the
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Fig. 6. Proliferative responses to the 40 H-SAg peptides by PBL of four patients with uveitic conditions. The peptides were tested at 100 µg/ml and the assay as well as the patients' conditions are described in Methods. The mean c.p.m. ± SEM in the unstimulated control cultures of the individual patients were: 1, 735 ± 43; 2, 478 ± 70; 3, 489 ± 29; 4, 860 ± 27. The responses (SI values) of the patients to rH-SAg, at 10 and 2 µg/ml, were as follows: 1, 8.1 and 9.9; 2, 15.5 and 24.3; 3, 5.5 and 10.3; 4, not determined.

Remarkable differences were seen among the levels of response stimulated by the immunodominant peptides (Figs 2–6). In addition, the peptides differed markedly in their minimal stimulatory concentration in culture. This parameter was investigated in the present study with monkey lymphocytes. Several peptides were found to stimulate lymphocyte responses only at 100 µg/ml but not at 10 µg/ml (Fig. 3). Moreover, only peptides 29 and 30 stimulated significant responses when tested at the low concentrations of 1.0 and 0.1 µg/ml (Fig. 4). The relationship between immunodominance of peptides and their minimal stimulatory concentration in culture was depicted in our previous study with rats immunized with peptides derived from another retinal protein, IRBP. The minimal stimulatory concentration of an immunodominant peptide was lower by at least four or five orders of magnitude than those of cryptic peptides of the same protein (39). Our observations are in accord with the finding that a direct correlation exists between immunodominance of peptides and their affinity toward MHC molecules (1, 3–5); it is assumed that high affinity enables peptides to effectively bind to the APC and provide stimulation even at low concentrations. Additional studies are being set up to measure the affinity of the H-SAg peptides toward MHC molecules and compare this feature with their immunodominance.
The present study made it possible to compare the immunodominance and permissiveness of H-SAg peptides with their capacity to induce experimental autoimmune uveoretinitis; the same 40 peptides tested here were examined in another study for their uveitogenicity in Lewis rats (40). The data are summarized in Table 2 and show a partial correlation between the two immunological activities. One peptide, 29, that is dominant in the Lewis rat (Fig. 2), was also highly uveitogenic in this strain (40). Peptide 29 was also immunodominant in the tested monkeys (Figs 3 and 4) and the four uveitis patients (Fig. 6). A high level of uveitogenicity in Lewis rats was also exhibited by peptide 19 (40), that was cryptic in Lewis rats, but was immunodominant in ACI rats (Fig. 2) and in the tested monkeys (Fig. 3). On the other hand, no uveitogenicity was demonstrated in Lewis rats by peptide 8, that was moderately immunodominant in these rats (Fig. 2) and showed the highest level of permissiveness, and by peptides 1 and 18, that were dominant in monkeys (Fig. 3) and most human donors (Figs 5 and 6). Also of note is the finding that peptide 35, that encompasses the epitope with the highest uveitogenicity in Lewis rats (41), stimulated just a minimal response in lymphocyte cultures of these animals (Fig. 2) and showed a low level of immunodominance in only one human donor (Normal 1, Fig. 5).

The relationship between immunopathogenicity of peptides and their immunodominance (as determined by lymphocyte
proliferation) was examined in Lewis rats with several other antigenic systems. A good correlation was observed between these two properties with the major encephalitogenic peptide of MBP (sequence 68–84) (17,42,43) and the highly uveitogenic peptide of bovine IRBP (sequence 1177–1191) (35). On the other hand, Gregerson et al emphasized the dissociation between uveitogenicity of several peptides of bovine SAg and their capacity to stimulate lymphocyte proliferation (44). The lack of complete correlation between immunopathogenicity of peptides and their capacity to stimulate vigorous lymphocyte proliferation in culture is in accord with the observations that the process by which lymphocytes acquire immunopathogenicity in culture is not necessarily accompanied by measurable proliferation (45,46).

There is no known approach to directly examine whether peptides which are immunodominant in patients with autoimmune diseases are the target for the pathogenic process. The present study examined the responses of a small number of humans, four normal donors and four uveitis patients. The normal controls showed a low level of uniformity, except for their common response to peptide 18 (Fig. 5). On the other hand, the pattern of response of the patients to the 40 peptides exhibited a remarkable level of uniformity and it resembled that of the immunized monkeys (Figs 3 and 6). Additional cases should obviously be tested, to further investigate the reproducibility of this observation.

In summary, data recorded here show for the first time that SAg, an ocular-specific protein, encompasses several peptide determinants that exhibit high levels of permissiveness by being selected as immunodominant peptides by the immune systems of different rat strains, cytomolgus monkeys and humans. Our observations with SAg peptides are in line with findings of permissiveness among peptides of MBP (5,18,19) and PLP (15) and suggest that permissiveness is quite prevalent among peptides of immunopathogenic proteins. More studies, with additional antigens, are needed to further examine this notion.

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Abbreviations

APC antigen-presenting cell
Con A concanavalin A
H-SAg human S-antigen
IRBP interphotoreceptor retinoid-binding protein
MBP myelin basic protein
PBL peripheral blood lymphocyte
PHA phytohemagglutinin
PLP proteolipid protein
PPD purified protein derivative
rH-SAg recombinant human S-antigen
SAg S-antigen
SI stimulation index

References

specific antigens and immunopathogenic processes they provoke. Progr. Retinal Res. 5:75.


