Observations in clinical and experimental ocular autoimmunity

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Chapter 5

Immunogenicity and Pathogenicity of Human S-Ag Determinants in Rat Strains

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Sumeet Mainigi

Submitted for publication
Abstract

Aim: The present study was initiated to determine rat strain susceptibility to developing uveitis following immunization with immunopathogenic determinants of human S-Antigen (S-Ag), as identified in the LEW rat.

Method: LEW, F344, COP, WKY, WF and BN rats were immunized with peptides 180-200, 280-300, 340-360, 350-370 of the human S-Ag sequence. Immunoproliferative responses were determined at day 14, antibody titers and pathologic score was established at day 30. The influence of pertussis toxin on disease severity was also assessed.

Results: Strains sharing the same RT-1 (class II antigen) with LEW were most sensitive to disease induction, though COP strain did not develop disease following immunization with any determinant. In this strain, antibody titers were elevated to all determinants at day 30. The WF strain also developed disease with all determinants except 280-300, though its RT-1 antigen differs from LEW. Lymphocyte proliferation indices at several determinant concentrations revealed similar profiles in strains developing disease.

Conclusion: Susceptibility to uveitis following immunization with determinants of human S-Ag is not limited to a single RT-1 complex. Additional factors play a role in disease induction, possibly relating to the induction of a Th1 versus a Th2 profile.

Introduction

Experimental autoimmune uveitis (EAU) is a T-cell mediated autoimmune disease that can be induced in rodents and non-human primates by immunization with antigens such as soluble antigen (S-Ag), a retinal arrestin. EAU closely resembles the pathologic changes observed in certain human forms of uveitis, and serves as a model for these sight-threatening diseases. As with all T-cell mediated processes, EAU is induced after the antigen is processed by antigen presenting cells (APC) and presented via the major histocompatibility complex (MHC) to the T cell receptor (TCR).

Uveitis susceptibility varies considerably between different strains of rats and mice. While Lewis rat are highly susceptible to EAU, Fisher rats, which share the same class II antigen, are much more resistant and require the addition of pertussis adjuvant for disease induction. In addition, whereas most antigens are specifically class II restricted, there exists a small set of antigenic determinants which are capable of eliciting an immune and/or pathogenic response from more than one class II antigen among the various strains of a given species. A subgroup of these antigens can even elicit a response in several animal species.

The present study aimed at determining the immunogenic and pathogenic potential of certain determinants of human S-Ag in a variety of rat strains. The chosen sequences were all highly pathogenic in the Lewis rat, with one corresponding to the immunodominant sequence and capable of inducing EAU at a very low immunizing dose. Certain chosen determinants were capable of only recognizing self in lymphocyte proliferation assays, while others were also capable of recognizing whole S-Ag. The chosen MHC profiles varied from complete homology with Lewis.
Table 1: Peptide sequence, immunologic and pathologic characteristics in the Lewis (LEW) rat:

<table>
<thead>
<tr>
<th>Determinant Location (hSAg)</th>
<th>Sequence</th>
<th>Immunogenicity</th>
<th>Pathogenicity</th>
<th>Bovine S-Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>180-200</td>
<td>VQHAPLEMGPQPRAEATWQF</td>
<td>+</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>280-300</td>
<td>TLTLLPLLANRERRGIALD</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>340-360 *</td>
<td>GFLGELTSSEVATEVPFRLM</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>350-370</td>
<td>VATEVPFRLMHPQPEDPAKE</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

Ability of lymphocytes from rats immunized with the determinant to recognize S-Ag in culture.

* Determinant able to induce disease at a dose < 10 μgm. The immunodominant epitope for human S-Ag is nested in this sequence.

class II antigen, to partial or total absence of homology. Thus, a response map to human S-Ag determinants in a variety of rat strains could be established.

Methodology:

**Peptide synthesis and sequence selection:**
Oligomeric peptides of human S-Ag measuring 20 amino acids in length were synthesized by Applied Biosystems (Foster City, CA, USA) using t-butyloxycarbonyl derivatives of amino acids on an automated solid-phase peptide synthesizer and were purified by HPLC to at least 95% purity. The amino acid composition of each peptide was verified using amino acid analysis and automated gas-phase sequencing. The sequence of each studied peptide as well as its immunogenicity and pathogenicity in the Lewis rat is summarized in table 1.

**Immunization protocol and follow-up:**
Male rats between 8 – 10 weeks of age were purchased from Harlan Sprague Dawley Microbiological Associates (Walkerville, MD). The following rat strains were studied: Lew, F344, COP, WKY, WF, BN. Animals were immunized with a single subcutaneous injection, in the hind footpad of complete Freund’s adjuvant containing a final concentration of 100(g/rat of peptide determinant and 2.5 mg/mL of Mycobacterium tuberculosis strain 37RA (Difco, MI). Half of the animals used for the assessment of the pathological response were given an intravenous injection

Table 2: Pathologic score at day 30 following immunization with or without Pertussis

<table>
<thead>
<tr>
<th>Rat Strain*</th>
<th>Bovine S-Ag</th>
<th>180-200</th>
<th>280-300</th>
<th>340-360</th>
<th>350-370</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lew†</td>
<td>4.0</td>
<td>4.0</td>
<td>3.0</td>
<td>4.0</td>
<td>3.0</td>
</tr>
<tr>
<td>F344</td>
<td>3.0</td>
<td>2.0</td>
<td>1.0</td>
<td>0</td>
<td>3.0</td>
</tr>
<tr>
<td>COP</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WKY†</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WF</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BN†</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* In superscript is given the RT-1 (MHC class II) for each rat strain

* Numbers correspond to the determinant location within the human S-Ag sequence.

+ corresponds to the animals with addition of pertussis toxin, and – to animals which did not receive pertussis. Each data point is the average of 4 animals.
of Pertussis toxin. Animals used in the lymphocyte proliferation assays were killed on day 12 to 14, while the animals used for evaluation of pathogenicity were killed on day 30. For each assay, 4 animals were used. Pathology was graded based on the degree of cellular infiltration and the degree of retinal damage as stated elsewhere. The study was approved by the Animal Care Committee of the National Eye Institute and adhered to the policies established by the Public Health Service on the Humane Care and Use of Laboratory Animals.

**Lymphocyte proliferation assay:**
Mononuclear cells taken from the draining inguinal nodes of immunized animals were tested on day 12 to 14. Cells were cultured at concentration of $1.5 \times 10^6$ cells/mL in 96 well flat-bottomed microtiter plates (Costar, Cambridge MA) containing 0.2 mL of RPMI supplemented with glutamine and 1% heat inactivated normal rat serum, and 2 mercapto-ethanol. All cultures were performed in quadruplicate against the peptide used for immunization, serially diluted from 100 $\mu$g/mL down to 0.001$\mu$g/mL. Concanavullin A was used as a positive control of proliferation. Cultures were incubated for 4 days at 37°C in 5% CO₂. For the last 12 hours, prior to harvesting at 5 days, each well was pulsed with $^3$H-thymidine (New England Nuclear, Boston, MA; 2 Ci/mmol, 0.5 $\mu$Ci per 10 $\mu$L/well). Results are expressed as a stimulation index (SI = mean c.p.m. in stimulated cultures/mean c.p.m. in unstimulated control cultures).

**ELISA assays:**
Serum collected on animals killed on day 30 was used to determine the IgG antibody present against the immunizing peptide. The peptide was diluted to 0.5 $\mu$g/mL in PBS (pH 7.36) and plated overnight at 4°C on 96-well flat-bottomed Immulon IV plates (Dynatech). Plates were washed x3 with PBS and incubated with 2% bovine serum albumin (BSA) in PBS for 4 hours at room temperature followed by a repeat wash. Fifty microliters of sera diluted to 1:40 in 1% BSA-PBS-Tween 20 (0.05%) (PBS-T) was added to triplicate wells and incubated for 2.5 hours at room temperature. Plates were washed three times in PBS-T, left in PBS-T for 10 minutes followed by an additional 3 washes. Fifty microliters of the alkaline phosphatase-conjugated secondary antibody goat anti-rat IgG (kpl, Gaithersberg, MD) (1:1000) in PBS-T was added, and the plates incubated for 1 hour at room temperature. Plates were washed three times in PBS-T, once in de-ionized distilled water, and once in p-nitrophenyl phosphate disodium buffer (NPP; Sigma), before adding the chromogenic agent. After further incubation, the optical density (OD) was read at 490 nm with an ELISA plate reader equipped with the appropriate filters (Molecular Devices, Menlo Park, CA).

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>Bovine S-Ag</th>
<th>180-200</th>
<th>280-300</th>
<th>340-360</th>
<th>350-370</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lew⁺</td>
<td>3.7</td>
<td>3.7</td>
<td>17.0</td>
<td>22.5</td>
<td>16.5</td>
</tr>
<tr>
<td>F344⁻⁺</td>
<td>2.0</td>
<td>2.0</td>
<td>8.0</td>
<td>3.6</td>
<td>8.0</td>
</tr>
<tr>
<td>COP⁻⁺</td>
<td>1.8</td>
<td>1.8</td>
<td>2.9</td>
<td>14.5</td>
<td>1.2</td>
</tr>
<tr>
<td>WKY⁺</td>
<td>2.2</td>
<td>2.2</td>
<td>31.0</td>
<td>25.7</td>
<td>18.0</td>
</tr>
<tr>
<td>WF⁺</td>
<td>1.7</td>
<td>1.7</td>
<td>1.9</td>
<td>10.7</td>
<td>5.7</td>
</tr>
<tr>
<td>BN⁻</td>
<td>2.3</td>
<td>2.3</td>
<td>1.6</td>
<td>1.6</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* In superscript is given the RT-1 (MHC class II) for each rat strain
* Numbers correspond to the determinant location within the human S-Ag sequence.
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elicited no response in all remaining species, while determinant 180-200, in the presence of pertussis did cause a very mild degree of inflammation in F344, WKY and WF.

Lymphocyte proliferative responses were studied in two ways. At a fixed antigen dose of 10 μg/mL, proliferation responses showed a dissociation between proliferation and pathogenicity (table 3). COP which appeared resistant to disease induction nonetheless showed a strong proliferative response to determinant 340-360. The strongest proliferative responses were observed in WKY, and were comparable or above those observed with LEW. This was also evident when studying the proliferative response over a range of antigen concentrations (Figure 1-4). Sharing the same RT-1 lead to a proliferative response at a lower antigen concentration than when the RT-1 locus was different, with the exception of determinant 180-200, which did not appear to elicit much of a proliferative response in any strain except at relatively high concentrations. For immunoproliferative determinants, a difference of 10 to 1000 fold in the minimum peptide concentration was noted between strains sharing the "I" locus and those which did not. WKY gave an equal or higher proliferative response than LEW, though this did not translate in a pathogenic response.

B cell stimulation was also studied in the form of serum antibody levels to the immunizing peptide. There was little correlation between the presence of an antibody titer and the development of a cellular immune response or the presence of ocular disease. Note that while BN rats showed little proliferative ability to all tested peptides, it did show high antibody titers to determinant 340-360. Similarly, COP which did not proliferate well to antigenic stimuli in vitro, gave a high antibody response to all determinants, either in absolute terms or relative to other tested strains.

Discussion

The immune response in mice has been carefully dissected in a number of autoimmune models. It appears to begin as a balanced cytokine response which then becomes polarized toward a type 1 T helper cell response (Th1) in disease-susceptible animals, and a type 2 response (Th2) in disease-resistant genotypes. The final immune profile can also be influenced by the presence or absence of adjuvants (incomplete versus complete Freund’s adjuvant), or the addition of pertussis toxin. In this study, an attempt was made to determine rat strain susceptibility to uveitis when immunized with immunopathogenic determinants of S-Ag. The influence of pertussis toxin was also assessed with regards to its ability to favor an immunopathogenic response.

Strains which contained the same class II MHC as the lewis rat were more likely to develop disease, but this susceptibility was not always present. In the case of COP no disease induction was seen. In this particular strain, a high antibody titer was observed to all immunized determinants, possibly suggesting that a strong Th2 response had favored B cell proliferation. Addition of pertussis toxin did help in eliciting a pathologic response to determinant 180-200 and heightened the pathogenic response in all pathogenic determinants in the WKY rat strain.

Lymphocyte proliferative response profiles in susceptible strains were similar irrespective of the RT-1 locus. A recent theory suggests that the interaction between T-cell receptor, peptide and MHC can determine Th1/Th2 dominance. According to this theory, Th phenotype is determined by the ability of a given MHC to bind antigen. If present in sufficient quantity, a Th1 response re-
sults. Plotting lymphocyte proliferation against a wide range of antigen concentrations, allows one to determine the proliferative potential of a given antigen, which in a crude way reflects the binding affinity to MHC and TCR. An analysis of the cytokines secreted in the presence of the determinants at each concentration would more clearly define the exact Th profile but was beyond the scope of this study. Additional factors also play an important role. In the case of F344, lymphocyte proliferation was low to certain determinants, despite clearly inducing a Th1 profile. Expression of co-stimulatory factors, fluctuations in the cytokine microenvironment, as well as neuro-endocrine factors can all influence the level of pathologic response.

In summary, we have shown that certain determinants of human S-Ag are capable of causing an immunopathologic response in a number of rat strains not all of which share the same class II MHC antigen as the LEW rat. In addition, with the exception of COP, antibody titers were variable and did not appear to correlate with disease susceptibility. Finally, susceptible rat strains appeared to generate similar lymphoproliferative response curves. Further studies on cytokines released in lymphocyte proliferation assays from these various strains would help to further delineate the role of Th1 and Th2 cells in both susceptible and resistant strains.

References

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