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Autoimmune uveitis and antigenic mimicry of environmental antigens

Gerhild Wildner*, Maria Diedrichs-Möhring

Section of Immunobiology, Dept. of Ophthalmology, Ludwig-Maximilians-University, Mathildenstr. 8, 80336 Munich, Germany

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Abstract

Autoimmunity directed against antigens of immune privileged sites, which are hidden from the immune system by blood-organ-barriers, is difficult to explain: it would require already activated cells to enter the tissue where the respective autoantigens are sequestered. Autoimmune uveitis, a sight-threatening inflammatory disease of the eye, is such an example. To induce disease autoreactive T cells must have been activated outside the eye to pass the blood-retina-barrier and then crossreact with retinal autoantigen. We have described two environmental peptides mimicking a highly pathogenic epitope from retinal S-antigen. One mimicry antigen is from rotavirus, a common pathogen causing gastroenteritis, the other from bovine milk αs2casein, a frequent nutritional protein ought to induce oral tolerance. Lewis rats develop uveitis after immunization with both mimicry peptides and casein protein. However, these mimicry antigens failed to induce oral tolerance for protection from uveitis, suspecting that they rather induce immunity than tolerance. Humoral and cellular immune responses to these antigens are enhanced and more frequent in patients with uveitis compared to healthy individuals. Our findings suggest that multiple environmental antigens mimic autoantigens and might cause autoimmune diseases by eliciting defensive immune responses, however, they are not necessarily useful for therapeutic tolerance induction.

Keywords: Autoimmune uveitis; Peptides; Molecular mimicry; Rotavirus; Bovine milk casein

1. Autoimmune diseases despite immune privilege

Autoimmune uveitis is an inflammatory disease affecting the inner eye, with a prevalence of approximately 2‰ in developed countries. $CD4^+$ Th1 cells recognizing retinal autoantigens recruit other leukocytes to the eyes, where they cause an inflammatory reaction that destroys photoreceptors and neuronal cells and leads to decreased vision or even to blindness.

In the Lewis rat model of experimental autoimmune uveitis (EAU) disease can be induced by immunization with retina specific autoantigens such as S-Ag (S-antigen) or IRBP (interphotoreceptor retinoid-binding protein), moreover, peptides derived from these proteins are pathogenic as well [1]. However, most ocular antigens are sequestered from the immune system behind the blood-retina barrier, which can only be passed by already activated T lympho-

Abbreviations: EAU, experimental autoimmune uveitis; TCR, T cell receptor; S-Ag, retinal S-antigen.

* Corresponding author. Tel.: +49-89-51603888; fax: +49-89-51603045.

E-mail address: Gerhild.Wildner@med.uni-muenchen.de (G. Wildner).

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cytes. The immune privilege of the eye usually prevents destructive intraocular immune reactions, but for initiation of inflammation the invading lymphocytes have to be preactivated outside the eye and probably stably polarized Th1-type memory cells [2].

We therefore propose that the immune response leading to uveitis must be initiated outside the eye by antigens mimicking ocular proteins. These antigens can be other self-proteins, such as previously described peptides derived from HLA-molecules [3,4], or provided by environmental proteins encountered during infection (pathogens) [5,6] or nutrition (food antigens) [6,7]. The proteins include peptides that mimic retinal S-Ag and are uveitogenic if rats were immunized subcutaneously.

The potential role of infections to initiate autoimmune diseases was supported by Lukashev et al., who reported uveitis in children following an outbreak of echovirus infections in Siberia [8]. These authors, however, did not show antigenic similarities between echovirus and retinal proteins. In addition to uveitis, antigenic mimicry of pathogens and/or food antigens was also described for other autoimmune diseases, such as myocarditis [9], diabetes [10] and encephalomyelitis [11–14].

2. Uveitogenicity and immunogenicity of environmental mimicry antigens

Gastrointestinal infections normally elicit defensive immune responses, whereas food antigens are not attacked due to oral tolerance induction. Oral tolerance is an important mechanism to avoid adverse immune reactions to soluble nutritional antigens [15,16], which are often resorbed without being denatured or degraded [17]. Failure of oral tolerance usually leads to food allergies by initiating a predominant Th2 response, whereas application of nutritional antigens during gastrointestinal infections can lead to a Th1 response [18]. While food allergies often develop in atopic individuals with a genetic predisposition to a deviant Th2 immune response, gastrointestinal induction of autoimmune responses, which often belong to the Th1-type, should also be regarded as a deviant immune response based on a respective susceptible genetic background.

Proteins from bovine milk are frequent food allergens [19], but are also often suspected to be antigenic triggers for autoimmunity. Humans are the only species consuming milk from other species, especially from cattle, even as adults. Interestingly, the incidence of allergies to bovine milk and of autoimmune diseases is increased in industrialized countries of the western hemisphere, where milk proteins are a dominant component especially of processed food. Milk is the first diet of a neonate with an immature and thus still impaired immune system, therefore milk contains substances to directly defend pathogens [20] and stimulate the immune system [21]. Calves fail to develop oral tolerance [22], which implies a different immunological handling of cow's milk proteins in the gut compared to the human situation.

For autoimmune uveitis, we have described antigenic mimicry between a peptide from retinal S-Ag (PDSAg) and a peptide ('Cas') from αs2casein, a major component of bovine milk, and, moreover, a peptide from surface protein vp4 of rotavirus ('Rota'), a common gastrointestinal pathogen. Retinal S-antigen is immunogenic in man and highly uveitogenic in rats [6] (Fig. 1a). Peptides Rota and Cas and even the complete αs2casein protein were uveitogenic in rats by subcutaneous immunization with complete Freund's adjuvant (Fig. 1a). With respect to the fact that s.c. application is a very unusual way to get in contact with gastrointestinal viruses or milk proteins, we tried to imitate a more 'natural' way of antigen contact by cofeeding the peptides, S-antigen and casein protein with native cholera toxin, which triggers gastrointestinal Th1 responses in rats and thus breaks oral tolerance. Surprisingly, only casein protein, but neither S-antigen nor one of the peptides induced disease by the oral way, although none of the epitopes that are pathogenic by subcutaneous application will be digested by intestinal proteases. This led to the speculation that immunostimulatory peptides described for the sequence of αs2casein might have an additional pathogenic effect [21].

Although the sequences of the peptides from retinal S-Ag, rotavirus and casein display high similarities and suggest trimolecular mimicry, we did not observe crossreactivities of rat T cell lines with all three peptides. Rota- as well as Cas- specific T cells (generated in vivo by immunization followed by in vitro-propagation with the same peptide) are highly crossreactive with PDSAg, but only Cas-specific T cell lines weakly crossreact also with peptide Rota. T cell lines with

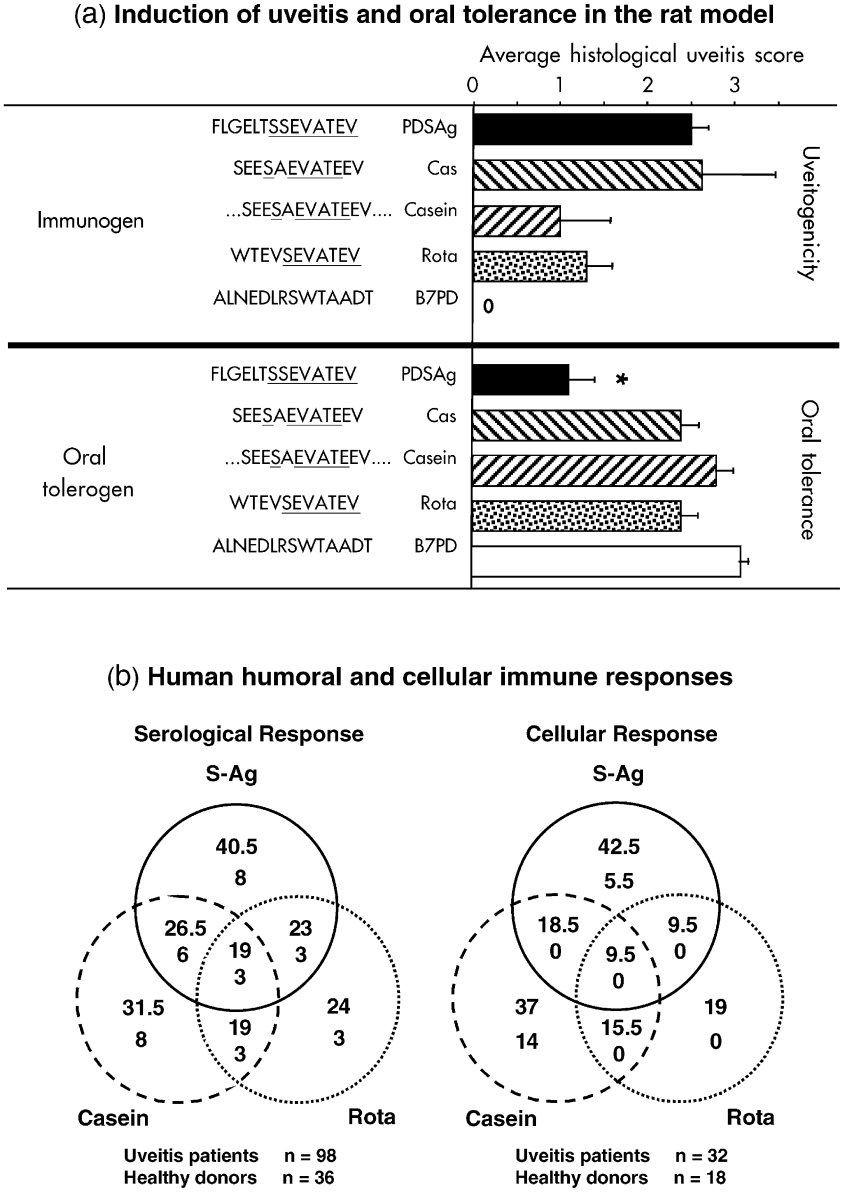

Fig. 1. Immune responses to mimicry antigens: (a) Lewis rats were immunized or orally tolerized with peptides and casein protein as indicated. Similar amino acids in the peptide sequences are underlined, shown as one-letter-code. B7PD is a control peptide from HLA-B7, which is neither pathogenic nor tolerogenic [3]. The incidence of uveitis is 90% of rats for PDSAg, 24% for Cas, 50% for casein and 30% for Rota. 'Average histological uveitis score' describes the stages from minimal cellular infiltrates in the retina (score 0.5) to total destruction of the retinal architecture. 'Uveitogenicity' represents the average uveitis score from positive rat eyes only. To test oral tolerance induction, rats were fed with peptides Rota, Cas or casein protein prior to subcutaneous immunization with PDSAg. Asterisk indicates significant amelioration of uveitis. (b) Sera from uveitis patients (anterior and posterior uveitis) and healthy donors were tested for reactivity to S-Ag/PDSAg, Rota and Cas/casein in ELISA, peripheral blood lymphocytes in proliferation assays with the same antigens. Frequencies of reactivity (%) are shown (uveitis patients: upper numbers; healthy donors: lower numbers). Measurements were regarded as positive if they exceeded the mean value of healthy donors to the respective antigen+2 standard deviations. Overlapping fields represent those samples, which react to two (or all three) antigens as indicated.

specificity for PDSAg do not corecognize Rota, Cas or casein protein but are highly specific for PDSAg only, which points to a better presentation of PDSAg by MHC-class II and/or a higher T cell receptor avidity. Anderson et al. [23] have made similar observations with a more artificial model of PLP-specific murine T cell clones and altered peptide ligands. In their hands, some T cell clones are more crossreactive than others, although they are all activated with the same peptide. Our findings indicate that in EAU, a T cell response to environmental antigens crossreacts with autoantigen, but not that a primary autoimmune response accidently also recognizes foreign antigens. Furthermore, rat T cell lines specific for the whole casein protein are uveitogenic after adoptive transfer and proliferate in response to peptide Cas, but not to the S-Ag peptide PDSAg. An explanation could be the recognition of two different epitopes on peptide Cas, whereas the predominant response is not crossreactive with PDSAg. T cells recognizing both, peptides Cas and PDSAg, may be a minor population within the casein-specific T cell line and not detectable by proliferation assays, which is typical for an 'unfocused' cross-reactivity [24].

Humoral and cellular immune responses from uveitis patients in comparison to healthy donors revealed increased reactivity to S-Ag/-peptide, casein/-peptide and rotavirus peptide (Fig. 1b). 19% of the tested serum samples reacted to two or even all three antigens, and 9.5 to 18.5% of peripheral blood lymphocyte samples proliferated in response to more than one antigen. As in the rat model, the lack of general crossreactivity between all three antigens observed in patients is inconsistent with the concept of trimolecular mimicry.

3. Failure of oral tolerance induction with casein- and rotavirus antigens to prevent EAU

Whereas rotavirus infections are expected to elicit a defensive immune response, milk proteins should generally induce oral tolerance, suggesting that milk consumption under 'normal' conditions might be able to prevent uveitis due to crossreactivity. However, in the rat model we failed to elicit oral tolerance to S-Ag peptide induced uveitis by feeding Rota peptide, Cas peptide or casein protein, although these antigens were mimics of retinal autoantigen with respect to pathogenicity (Fig. 1a). If this reflects the human situation, bovine milk casein would not be able to induce oral tolerance protecting from uveitis, but rather induce autoimmunity. With respect to the fact that bovine milk proteins are the most frequent food allergens, especially in children, one could speculate about the immunomodulatory capacities of milk proteins and their effect on the human GALT. The immunological effects of the peptides from rotavirus and bovine casein are in striking contrast to the previously desribed HLA-B peptide B27PD [3], although they are all mimicking the same S-Ag epitope represented by peptide PDSAg. While rotavirus and casein peptides are uveitogenic but not orally tolerogenic, the HLA peptide B27PD is poorly uveitogenic but highly tolerogenic after oral application. This indicates that not all pathogenic antigens are useful tolerogens and that the immune mechanisms ruling oral tolerance do not always use the same antigens than the effector responses, as we have previously shown [25] with altered peptide ligands from PDSAg.

Take-home messages

- Surface protein vp4 from rotavirus, a gastrointestinal pathogen, and bovine milk αs2casein share amino acid sequences with retinal S-antigen, an autoantigen, which is immunogenic in uveitis patients and pathogenic in the Lewis rat model of experimental uveitis;
- Oral immunization with αs2casein causes uveitis in rats;
- Mimicry peptides from rotavirus and milk casein do not induce oral tolerance to prevent uveitis in rats;
- Uveitis patients show more frequently and enhanced humoral and cellular responses to the mimicry peptides Rota and Cas, αs2casein and retinal S-Ag/S-Ag-peptide PDSAg compared to healthy individuals; and
- Breaking oral tolerance to food antigens or defensive immune responses against pathogenic epitopes, both mimicking autoantigens, are potential initiating events for autoimmune diseases like uveitis.

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The World of Autoimmunity; Literature Synopsis

Methylprednisolone in the treatment of Guillain-Barre syndrome

van Koningsveld et al. (Lancet 2004;363:192) examined whether methylprednisolone, when added to standard treatment with intravenous immunoglobulin (IVIg) improves outcome of Guillain-Barre syndrome patients compared with IVIg alone. They analyzed the outcome of 225 patients. In the group treated with Methylprednisolone and IVIg, the disability scores increased by one grade or more in 68% of patients compared with only 56% in the IVIg alone group, yielding odd ratio of 1.68 (0.97–2.88). However, after adjustment for age and degree of disability at entry, the odd ratios turned into significant. The authors concluded that even though no significant difference was found between the treatment groups, the potential importance of the combination between both drugs warrants further investigation.