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Immunostimulatory and immunomodulatory peptides derived from the α1 domain of HLA-B27 in experimental autoimmune diseases in Lewis rats

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Abstract

Peptides derived from amino acid sequence 60–80 of HLA-B27 (B27PA, aa 60–72 and B2702PA, aa 60–80) mimic cytokeratin and are able to induce in vitro proliferation of human peripheral blood lymphocytes as well as arthritis in Lewis rats. Here we show that the pathogenic epitope recognized by autoaggressive rat T cells is located at the N-terminus of the sequence, between aa 60 and 72. A C-terminally elongated 25mer peptide (B2702.60–84) showed increased pathogenicity, indicating either a second arthritogenic epitope or an immunomodulatory region within this peptide. B2702.60–84 has been described to inhibit murine and human $CD8^+$ cytotoxic T cells (CTL) and was even successfully used for the treatment of allograft rejection. In addition to pathogenicity we have investigated the immunomodulatory effect of peptide B2702.60–84 in our rat model of experimental autoimmune uveitis (EAU), induced with retinal S-Antigen peptide PDSAg. We found that disease exacerbated following coimmunization of PDSAg with B2702.60–84. In vitro, the B27-peptide enhanced the proliferation of $CD4^+$ T cell lines specific for retinal autoantigen peptides during coincubation of B2702.60–84 with the respective antigen. Oral tolerance induction, an effective mechanism to prevent uveitis in Lewis rats, is abrogated by cofeeding peptide B2702.60–84 with the tolerogen PDSAg. In rat EAU, naturally occurring regulatory T cells and orally induced $\gamma\delta TCR^+$ suppressor cells are $CD8^+$, which might be impeded by peptide B2702.60–84. As a consequence of their abrogated suppressive capacity disease was exacerbated. We propose a similar role of HLA-B27 in man: disturbing the mechanisms down-regulating self-responses might lead to autoimmune diseases. This could explain the high association of HLA-B27 with a variety of autoimmune diseases targeting different organs or tissues.

Keywords: Autoimmune disease; HLA-B27 peptide; T lymphocytes; Uveitis; Oral tolerance; Arthritis

Introduction

HLA-B27 is the MHC molecule with the highest correlation to autoimmune diseases, such as ankylosing spondylitis (Brewerton et al., 1973) and acute autoimmune anterior uveitis (Brewerton et al., 1974). Many theories have been developed to explain the role of HLA-B27 in disease susceptibility. Up to now the role of

Abbreviations: EAU, experimental autoimmune uveitis; HSP, heat shock protein; IRBP, interphotoreceptor retinoid-binding protein; S–Ag, retinal soluble antigen

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B27 in autoimmunity has not definitely been elucidated. Recently, we could demonstrate that HLA-B27-derived peptides (B27PA, aa 60–74; B2702PA, aa 60–80) have sequence homologies with tissue-specifically expressed cytokeratin. Lymphocytes from patients with ankylosing spondylitis were shown to proliferate in response to peptide B27PA (Marker Hermann et al., 1997), and the HLA-B27- as well as cytokeratin-derived peptides were able to induce arthritis in Lewis rats. Oral application of the HLA-peptides could prevent arthritis induced with cytokeratin peptide, supporting the idea of antigenic mimicry of HLA-B27 peptide and tissue specific autoantigen (Wildner et al., 2002).

Some years ago Clayberger et al. (1993) showed that peptides corresponding to a conserved region of the α1 helix of the first domain of HLA class I molecules exhibit immunomodulatory capacity. A peptide covering amino acids 60–84 of HLA-B2702 which represents the C-terminally elongated form of the arthritogenic peptides B27PA and B2702PA was able to inhibit differentiation of T cell precursors into mature $CD8^+$ cytotoxic T cells as well as target cell lysis by mature cytotoxic T lymphocytes (Nossner et al., 1996). This effect was ascribed to the interaction of the HLA-peptide with intracellular immunophilins, similar to the action of other immunomodulatory drugs such as cylosporin A or FK506 (Friedman and Weissman, 1991; Nossner et al., 1996; Sigal and Dumont, 1992).

Here we demonstrate a new feature of HLA-B27 that differs from its role as antigen presenting element or donor of mimicry peptides. We investigated whether the immunomodulatory HLA-B27 peptide aa 60–84 (B2702.60–84) has an effect on regulatory T cells in the Lewis rat model of EAU. EAU is an intraocular inflammatory disease caused by immunization with retinal autoantigens or peptides thereof. Disease can be prevented by oral application of retinal antigens or peptides as well as peptides mimicking autoantigen (Nussenblatt et al., 1990; Thurau et al., 1991; Wildner and Thurau, 1994; Wildner et al., 1996). Regulatory or suppressor T cells induced by oral application of antigen peptides are $CD8^+$ (Lider et al., 1989; Wildner et al., 1996). We found that coimmunization of B2702.60–84 with retinal autoantigen peptide could aggravate EAU. Furthermore, coculturing T cell lines with antigen peptides and peptide B2702.60–84 resulted in enhanced antigen specific proliferation. Cofeeding of the HLA-B27 peptide with retinal autoantigen peptide PDSAg abrogated induction of oral tolerance to the retinal peptide, probably by inhibiting the tolerizing $CD8^+$ $\gamma\delta$ TCR^+ population. Immunization with peptide B2702.60–84 induced severe arthritis in Lewis rats that exceeded the disease caused by immunization with the pathogenic peptides B27PA (aa 60–74) and B2702PA (aa 60–80).

Our data suggest that a peptide processed from HLA-B27 protein including the immunomodulatory determinant of peptide B2702.60–84 might disturb regulatory $CD8^+$ T cell responses and thus promote autoimmune reactions in HLA-$B27^+$ individuals.

Materials and methods

Animals

Male and female Lewis rats were obtained from RCC (Basel, Switzerland) or bred in our own colony and used for experiments at the age of 6–8 weeks. They had unlimited access to rat chow and water. Animal experiments were approved by the Review board of the Regierung von Oberbayern. Treatment of animals conformed to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research.

Peptides

Custom peptides were purchased from Biotrend (Cologne, Germany). Sequences of HLA-B27-(B27PA, B2702PA, B2702PAS, B2702.60–84) and Cytokeratin 5-derived (Ker333, KerS) peptides are shown in Table 1. Uveitogenic peptides PDSAg (aa 342–355, FLGELTSSEVATEV) and S-Ag.286 (aa 286–297, NNRERRGIALDG; kindly provided by D. Gregerson) were from the sequence of bovine retinal soluble antigen (S-Ag). PI536 (aa 536–549, GVYLLTSHRTATAA), PI731 (aa731–744, DLYILMSHTSGSAA) and PI1137 (aa 1137–1152, KSMVILTSTVTAGTAE) were derived from human interphotoreceptor retinoid-binding

Table 1. Amino acid sequences of mimicry peptides from HLA-B27 and cytokeratin

Peptide	aa	Sequence																								
Ker333	333–353	L	D	L	D	S	I	I	A	E	V	K	A	Q	Y	E	E	I	A	N	R	S				
KerS	336–347				D	S	I	I	A	E	V	K	A	Q	Y	E										
B2702PA	60–80	W	D	R	E	T	Q	I	C	K	A	K	A	Q	T	D	R	E	N	L	R	T				
B2702PAS	63–74				E	T	Q	I	C	K	A	K	A	Q	T	D										
B27PA	60–72	W	D	R	E	T	Q	I	C	K	A	K	A	Q												
B2702.60–84	60–84	W	D	R	E	T	Q	I	C	K	A	K	A	Q	T	D	R	E	N	L	R	I	A	L	R	Y