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Immunology

Definition of the HLA-A29 peptide ligand motif allows prediction of potential T-cell epitopes from the retinal soluble antigen, a candidate autoantigen in birdshot retinopathy

(major histocompatibility complex class I proteins/peptide binding motif/autoimmune uveoretinitis)

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ABSTRACT **The peptide-binding motif of HLA-A29, the predisposing allele for birdshot retinopathy, was determined after acid-elution of endogenous peptides from purified HLA-A29 molecules. Individual and pooled HPLC fractions were sequenced by Edman degradation. Major anchor residues could be defined as glutamate at the second position of the peptide and as tyrosine at the carboxyl terminus. *In vitro* binding of polyglycine synthetic peptides to purified HLA-A29 molecules also revealed the need for an auxiliary anchor residue at the third position, preferably phenylalanine. By using this motif, we synthesized six peptides from the retinal soluble antigen, a candidate autoantigen in autoimmune uveoretinitis. Their *in vitro* binding was tested on HLA-A29 and also on HLA-B44 and HLA-B61, two alleles sharing close peptide-binding motifs. Two peptides derived from the carboxyl-terminal sequence of the human retinal soluble antigen bound efficiently to HLA-A29. This study could contribute to the prediction of T-cell epitopes from retinal autoantigens implicated in birdshot retinopathy.**

Birdshot retinochoroidopathy (BSR) is a rare autoimmune chronic inflammatory uveitis characterized by its association with the HLA class I allele *HLA-A29*, among the strongest between HLA class I and disease (1, 2). HLA-A29 is present in >90% of the cases, while it is detected in <5% in the healthy Caucasian population. Additionally, HLA-A29 could be a predisposing genetic factor to other idiopathic forms of retinal vasculitis (3), which are an important cause of loss of vision in the U.S.A. and in Western Europe. Uveitis may also be a manifestation of other autoimmune diseases, such as anterior uveitis, which is associated with HLA-B27 and spondylarthropathies (4) or Behçet's disease, which is associated with HLA-B51 (5).

Two subtypes of HLA-A29 have been described, A29.1 and A29.2 (6), having a single amino acid (aa) difference at aa position 102 outside the peptide-binding groove (7). Both subtypes may be equally associated with BSR (8), although the association between HLA-A29.1 and BSR is still a matter of controversy (7). Experimental models of autoimmune uveoretinitis (EAU) mimicking many aspects of the human disease can be induced in rodents (for review, see ref. 9) upon immunization with retinal evolutionary conserved autoantigens: retinal soluble antigen (S-Ag) and interphotoreceptor–retinoid-binding protein. S-Ag can also induce EAU in primates (10) and elicits proliferative cellular responses in some BSR affected individuals (11–13). It is a 48-kDa dominant protein in the interphotoreceptor cells of the retina that plays a role in the phototransduction cascade (14). Peptides encompassing the 343–362 sequence of S-Ag are the most pathogenic in the Lewis rat EAU model (15–17). Cell-transfer experiments in EAU (18), as well as the beneficial effects of immunosuppressive agents such as cyclosporine in EAU (19) and human BSR (20) clearly demonstrate the central role of T-cell-mediated autoimmunity in this disease.

T cells recognize a structural complex composed of antigenic peptides bound to the groove of the restricting HLA molecule. Crystallographic studies of peptide-bound HLA class I molecules (for review, see ref. 21), synthetic peptide *in vitro* binding experiments, and peptide elution followed by HPLC fractionation and sequencing have succeeded in defining the rules of peptide binding for several HLA class I alleles (for review, see ref. 22). HLA class I-bound peptides have a size constraint commonly of 8–10 aa. The nature of the preferred anchor residues that are usually found at the second position (P2) and at the carboxyl terminus (C terminus) of the peptide is dictated by the composition of the so-called B and F pockets in the antigenic binding site of the HLA class I molecule. However, little is known about peptide-binding requirements for HLA-A29, although this information is an essential prerequisite for the definition of putative immunogenic T-cell epitopes. For that reason, we have characterized natural peptides eluted from HLA-A29.2. This motif was validated by *in vitro* binding studies with polyglycine synthetic peptides and allowed to select six peptides from the retinal S-Ag sequence which were tested similarly.

MATERIAL AND METHODS

Cell Lines. The homozygous Epstein–Barr virus-transformed human B-cell line Sweig [HLA-A29 (A*2902), B61 (B*4002), and Cw2 (Cw*02022)] was cultured in roller bottles in RPMI 1640 medium/10% fetal calf serum/2 mM glutamine/penicillin G at 50 units/ml and streptomycin at 50 μg/ml. Cells were pelleted and stored at −80°C. Approximately 3×10^9 cells were used in each separate HLA purification and peptide fractionation experiment. As a source of HLA-B44 molecules for binding experiments, the Epstein–Barr virus-transformed human B-cell line PF97387 (HLA-A29, B44) was used. Murine L fibroblasts J26 (23) already transfected with human β_2-microglobulin (β2m) were used as

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Abbreviations: BSR, birdshot retinochoroidopathy; EAU, experimental autoimmune uveitis; S-Ag, retinal soluble antigen; β2m, β_2-microglobulin; mAb, monoclonal antibody; TFA, trifluoroacetic acid.

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transfection recipients. HLA-A*2902 cDNA was obtained from a patient suffering from BSR (7). The *Hin*dIII–*Sal* I cDNA insert was cloned into pMAMneoBlue^R expression vector (Clontech, Palo Alto, CA), and transfection was carried out with Transfectam (IBF, Villeneuve la Garenne, France). High-expressing transfectants were selected by cytofluorometry, cloned by limiting dilution, and cultured in geneticin (250 μg/ml) containing Dulbecco's modified Eagle's complete medium (GIBCO/BRL).

HLA Purification. Pellets were lysed in 50 mM Tris·HCl, pH 7.4/150 mM NaCl/5 mM EDTA/1% Nonidet P-40 with protease inhibitors: 1 mM phenylmethanesulfonyl fluoride, 10^{-4} M iodoacetamide, 1% (vol/vol) aprotinin, trypsin inhibitor at 10 μg/ml, 10^{-5} M pepstatin, 10^{-5} M leupeptin (all from Sigma). After centrifugation at 100,000 × *g* for 60 min, supernatants were passed through sequential affinity columns as in ref. 24. Specific columns were cyanogen bromide-activated Sepharose 4B columns (Pharmacia, Saint Quentin en Yvelines, France) coupled to purified anti-HLA monoclonal antibodies (mAbs) (10–20 mg per column). SFR8-B6, a Bw6-specific mAb (25), was used for HLA-B61 purification. HLA-B and C molecules were further depleted by passage on one to three B1.23.2 mAb columns. Remaining HLA-A29 material was captured by W6/32 mAb (anti-HLA-A, B, C) columns. Column elutions were done under basic conditions (50 mM diethylamine, pH 11.5). The eluted fractions were immediately neutralized with 1 M Tris·HCl, pH 7.4 and concentrated by ultrafiltration on a Centriprep-30 cartridge (Amicon, Beverly, MA). The quantity and the purity of HLA molecules were estimated by the bicinchoninic acid protein assay reagent (Pierce Chemical Co., Rockford, IL) and by SDS/PAGE analysis. Columns coupled to normal mouse immunoglobulins were treated identically as the specific mAb-coupled columns, thus providing an internal control for each purification.

Peptide Elution and HPLC Fractionation. Peptides were acid-eluted by treatment with 0.1% (vol/vol) trifluoroacetic acid (TFA) at room temperature and collected by centrifugation through a 5-kDa cut-off device (Amicon). The extracts were further concentrated to 100 μl using SpeedVac (Savant Instruments, Inc., Farmingdale, NY). Reversed-phase HPLC was done on a C_{18} column (ODS C_{18}, 4.6 × 150 mm, Beckman Instruments, Gagny, France) using Beckman system Gold instrumentation (solvent module 126-UV detector 166). The gradient consisted of 0.05% TFA in H_2O/0.05% TFA in acetonitrile 91:9 for 10 min followed by a linear increase to 35% acetonitrile/0.05% TFA over 60 min. Absorbance was monitored at 220 nm; the flow rate was 500 μl/mn. Fractions of 500 μl were collected on a Gilson 203B collector.

Automated Edman Degradation Sequencing. HPLC profiles of peptides eluted from purified HLA molecules and mock-treated material were compared. Dominant single peaks were sequenced individually, whereas remaining fractions eluting between 15 min and 60 min were pooled, lyophilized, and redissolved in a small volume of 0.1% TFA in water. Microsequencing by N-terminal Edman degradation was done by using an Applied Biosystems, Inc. (Foster City, CA), 473-A or 494 protein sequencer. Interpretation of pool sequencing data was performed following the convention of Falk *et al.* (26). Data base searches were performed in the National Biomedical Research Foundation (NBRF-PIR) data base (release 44, March 1995).

Synthetic Peptides and *in Vitro* Binding Assay. Peptides prepared by solid-phase synthesis were purchased from Neosystem (Strasbourg, France). *In vitro* binding assays were performed essentially as described in Gnatic *et al.* (27). Briefly, purified HLA molecules were denatured in 12.5 mM NaOH, pH 11.7/1.5 M urea for 1 hr at 4°C. The heavy chains and β2m were separated from endogenous peptides on a Sephadex G25 column (PD10, Pharmacia). Exogenous β2m (2 μg/ml) and 6 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) (Sigma) were added just before exogenous peptides at 0.1 μM or 10 μM. After a 24-hr incubation at 4°C, reassembled HLA molecules (0.5 μg in 100 μl) were incubated overnight at 4°C in 96-well microtiter plates (Nunc Maxisorp, Denmark) coated with mAb B9.12.1 for HLA-A29 or mAb B1.23.2 for HLA-B61 and HLA-B44 (1 μg of purified immunoglobulin). Correctly folded HLA complexes were revealed with anti-β2m M28 mAb coupled to alkaline phosphatase and measured at 360/460 nm in a Microfluor reader (CytoFluor 2300, Millipore, Saint Quentin en Yvelines, France).

RESULTS

HLA-A29 Peptide Consensus Sequence. HLA-A29 natural endogenous ligands were acid-eluted from W6/32 immunoaffinity-purified A29 molecules obtained from the B-cell line Sweig. Dominant single peaks and the remaining specific pooled fractions were subjected to Edman's degradation sequencing since it has been shown that both pool and single-peptide sequences should be done in parallel to avoid possible biases if only dominant individual sequences were analyzed (28). Fractions present in the W6/32 HPLC profile and not in the B1.23.2 one were preferentially selected. Individual sequences were obtained from HPLC fractions of three separate experiments and are shown in Table 1. A primary dominant sequence was obtained in all the fractions, although in most instances other aa were present in lower yield. Ten sequences are 9 aa long, five are 10 aa, and one is 8 aa in length. Background due to free aa at the first NH_2 terminal residue precluded in most cases a precise assignment at this position. All sequences had glutamate as the second peptide residue (P2), which appears as the dominant anchor of this motif. A second anchor residue was tyrosine (6/16 peptides) or a small hydrophobic residue (leucine, alanine, or valine) at the C terminus (6/16 peptides). Phenylalanine or a hydrophobic residue (isoleucine, valine) was frequently found at the third position of the peptide (P3). Sequences from fractions 2-44 and 3-47 matched intracellularly expressed proteins, respectively, hsp90 α chain and ubiquitin. The latter peptide has also been found as a natural ligand for HLA-B40 (29).

Pool sequencing was performed for HLA-A29 from three different experiments. As a control, pool sequencing was also performed on HLA-B61-eluted peptides from Sweig, confirming the previously reported glutamate as an anchor at position

Table 1. HLA-A29 individual peptide sequence

Fraction	Sequence	Yield
1-35	XEVDVEY-Y	4,5
1-44	XEFDTFESY	6,8
1-46	XEIELILEY-	9,1
2-28	XE-PMAEA-	3
2-34	XEDDQQQALV	3,2
2-44*	XEKYIDQEEL	8
2-47	XEFFPEYYYY	3,3
2-48	XEFQEHYEY	10
2-55	XEFQVYL-Q	6
2-58	XEIQINVQ	3,5
3-26	XEVNNVALL	
3-30	PEMSV--LL	
3-46	XEIGAGATGA	22
3-47†	XESTLHLVL	17
3-53	XEFPLVV-L	6
3-56	XEFTL-LAY	

Initial yield was calculated from the repetitive yield of the phenylthiohydantoin-derivatized amino acid and is expressed in pmol. X denotes the first residue of the peptide, preferentially S, T, G, A, K, or V; -, not defined.

*Homologous to human hsp90 α chain-(481–490).

†Homologous to human ubiquitin-(64–72).

2 (data not shown). At the C terminus we found leucine, the HLA-B40 C-terminal reported anchor (29). The results for HLA-A29 are summarized on Table 2. A 9-aa-length motif with anchor residues at P2 (glutamate) and C terminus (predominantly tyrosine and, at a lesser extent, leucine) and an auxilliary anchor at P3 (phenylalanine, isoleucine, valine, alanine, or leucine) are the hallmarks of HLA-A29 natural ligands. Additionally, a slight increase of signal for methionine at P2 was observed in the three pool sequencing experiments but was not found in the individual sequences. A significant signal for proline at position 4 was detected in the pool and in two individual sequences. A slight increase of signal at position 5 for isoleucine and valine and for tyrosine at position 7 was also found. No specific residue was overrepresented at position 6 or 8.

The HLA-B allele of the Sweig cell line is *HLA-B61*, for which the peptide motif also shows a glutamate as P2 anchor. Although HLA-A29 molecules were depleted of HLA-B61 through passages over B1.23.2 columns, any possible bias in our data due to a contamination of HLA-A29 by HLA-B61 was definitively excluded by conducting additional experiments with HLA-A29 transfectants. Murine J26 L cells were transfected with the A*2902 cDNA, and highly expressing clones were selected. HLA-A29 molecules were purified as previously described. The amount of material obtained did not allow us to perform individual sequences but instead pool sequencing. Due to a high background of free amino acids at the first cycle, all signals were decreased in the second cycle with the exception of glutamate. At the third cycle, phenylalanine and isoleucine signals increased. No other significantly interpretable signal was observed until P9, where tyrosine was the only signal to increase. These data, together with those obtained using the B-cell line Sweig, unambiguously confirmed glutamate at P2 and tyrosine at P9 as peptide anchors for HLA-A29.2 natural ligands.

***In Vitro* Polyglycine Peptide-Binding Assay.** To experimentally assess the relevance of the motif found for peptide binding to HLA-A29, we used an *in vitro* assembly assay, taking advantage of the recovery of a stable conformation of denatured heavy chains in the presence of exogenous human β2m and allele-specific peptides (27). HLA-B61 and HLA-B44 molecules that have similarities with HLA-A29 with regard to their peptide motif were tested in parallel. Reproducible results were obtained with different preparations of purified material. A complete list of the peptides used in this study is presented in Table 3 and the results are shown in Fig. 1. These data are typical of those obtained in five separate experiments. Peptides K-9-Y(F) and K-9-Y(I) corresponding to sequences of dominant HPLC peaks of peptides eluted from HLA-A29 (fractions 1-46 and 2-48) and peptides with a combination of glutamate at P2, phenylalanine or valine at P3 and tyrosine or leucine at the C terminus were synthesized. Glycine backbone residues were chosen because they allow good peptide flexibility and do not have a side chain that could impair peptide binding. The peptides corresponding to HLA-A29 natural ligands bound specifically to HLA-A29 and not to HLA-B61 or to HLA-B44 (Fig. 1). The NP383-391 HLA-B27-associated peptide (30) was found repeatedly negative with these alleles (data not shown). With polyglycine peptides, distinctive patterns of reactivity were observed with HLA-A29, -B61, and -B44, although some peptides could bind to several alleles, as expected from their motifs. Optimal binding requirements for HLA-A29 could be deduced (Fig. 1*A*). Peptide G-9-Y(G) did not bind efficiently, showing that in the polyglycine context the two main anchors, glutamate at P2 and tyrosine at P9 alone are not sufficient and that an auxiliary anchor at P3 could be necessary. The 9-mer peptide G-9-G(F) bound weakly, showing the need for a carboxyl-terminal anchor, in addition to preferred residues at P2 and P3 to optimize binding. Such optimal binding was obtained with peptide G-9-Y(F), in agreement with the proposed motif: glutamate at P2, phenylalanine at P3, and tyrosine at P9. Peptide G-9-Y(V) bound less efficiently, indicating that in this context phenylalanine at P3 was preferred. The 9-mer G-9-Y(F) and the 10-mer G-10-Y(F) peptides bound equally well to HLA-A29. A similar analysis for HLA-B61 (Fig. 1*B*) showed that the optimal binding occured with the 9-mer G-9-L(F). The pattern of peptide binding was also different on HLA-B44 (Fig. 1*C*). Reactivity of polyglycine peptides showed a requirement for a carboxyl-terminal residue other than glycine and a good efficiency of peptides with tyrosine or leucine at this position. A prominent role of the P3 residue was not evidenced because G-9-Y(G) with glycine was as reactive as other peptides with phenylalanine or valine at P3. Therefore, peptide binding to HLA-B44 seems less dependent upon the P3 residue than HLA-A29 peptide binding.

Table 2. HLA-A29 motif defined by pool sequencing

	Position								
Residues	1	2	3	4	5	6	7	8	9
Anchor or auxiliary anchor		**E**	F						**Y**
Other preferred		M	V I A K L	P	V		Y		L

Boldface letters indicate proposed anchor residues. Criteria used to evaluate the pool sequencing are those of Falk *et al.* (26).

Table 3. Synthetic peptides used

Name	Sequence	Comments
K-9-Y(F)	KEFQEHYEY	HPLC fraction 2–48
K-9-Y(I)	KEIELILEY	HPLC fraction 1–46
NP383-391	SRYWAIRTR	HLA-B27-associated
V-9-L(Q)	VEQVANVVL	S-Ag-(245–253)
V-10-Y(Q)	VEQVANVVLY	S-Ag-(245–254)
K-9-L(G)	KEGIDRTVL	S-Ag-(317–325)
G-9-A(L)	GELTSSEVA	S-Ag-(344–352)
S-9-F(V)	SEVATEVPF	S-Ag-(349–357)
F-9-L(E)	FEEFARHNL	S-Ag-(379–387)
G-9-Y(G)	GEGGGGGGY	
G-9-Y(F)	GEFGGGGGY	
G-9-L(F)	GEFGGGGGL	
G-9-G(F)	GEFGGGGGG	
G-10-Y(F)	GEFGGGGGGY	
G-9-Y(V)	GEVGGGGGY	

The length of the peptide is indicated between its first and last position; the aa in parentheses is the aa residue at P3.

HLA Binding of S-Ag Peptides. Screening of the sequence of human S-Ag (31), did not reveal peptides with the canonical HLA-A29 peptide consensus motif. For instance, no 9-mer peptide was found with glutamate at P2 and tyrosine at P9. Because some natural eluted peptides from HLA-A29 also carried leucine or alanine at P9 (see Table 1), we synthesized six peptides with these characteristics, including one 10-mer peptide with glutamate at P2 and tyrosine at its last position and one 9-mer with phenylalanine at P9 (Table 3). We excluded peptides with a carboxyl-terminal residue absent or rarely found in natural peptides. The six selected peptides were tested on HLA-A29, -B61, and -B44, and results from one typical experiment out of three are shown (Fig. 2). Two peptides (V-9-L and V-10-Y) did not bind significantly to any one of these alleles (data not shown). Among the other four peptides, all had different reactivities on the three alleles. S-9-F(V) bound preferentially to HLA-A29 but also to HLA-B44 and to HLA-B61. G-9-A(L) bound equally well to both

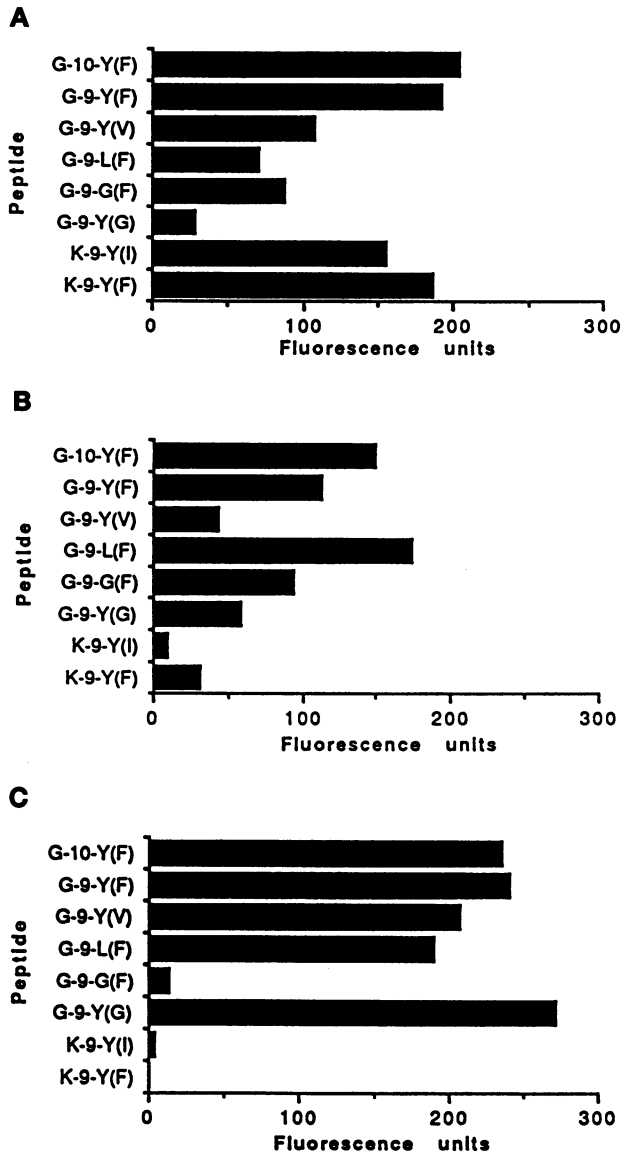

FIG. 1. Association of polyglycine peptides (for sequences, see Table 3) at 10 μM with HLA-A29 (*A*), HLA-B61 (*B*), and HLA-B44 (*C*) purified molecules. Results are expressed as fluorescence units at 360/460 nm after substraction of the background level obtained from wells incubated without peptide.

HLA-A29 and -B61 but not to -B44. K-9-L(G) and F-9-L(E) bound preferentially to HLA-B61 and more weakly to HLA-A29 and to HLA-B44.

DISCUSSION

The definition of allele-specific peptide binding motifs is an important step to gain insight into the molecular mechanisms of HLA and disease association (32, 33). We describe in this paper the peptide motif of HLA-A29, the allele linked to BSR, a severe form of autoimmune uveitis. The length (9 aa) and the position of main anchors at the second and at the carboxyl terminus of the peptide are common features among HLA class I-associated peptides. How can these anchors accomodate HLA subpockets—namely, the P2 (glutamate), P3 (phenylalanine or hydrophobic) and carboxyl-terminal (tyrosine or leucine) anchors respectively with pockets B, D, and F in accordance with the x-ray structure of peptide bound to HLA class I (21)? As an increasing number of peptide consensus motifs have been defined for HLA class I alleles (22), comparisons of polymorphic residues in these pockets allow some conclusions to be drawn. B pocket residues of different HLA alleles are compared in Table 4. Among the Aw19 group of serological crossreactivity, Gln-63 and Leu-62 are specific to HLA-A29 (34) and account for -A29 epitopes recognized by monospecific alloantisera or mAb (34, 35). Peptide motifs have been defined for HLA-A31 and -A33 (22). For these alleles residue at P2 is not considered as a dominant anchor but is preferentially occupied by an hydrophobic or aromatic residue.

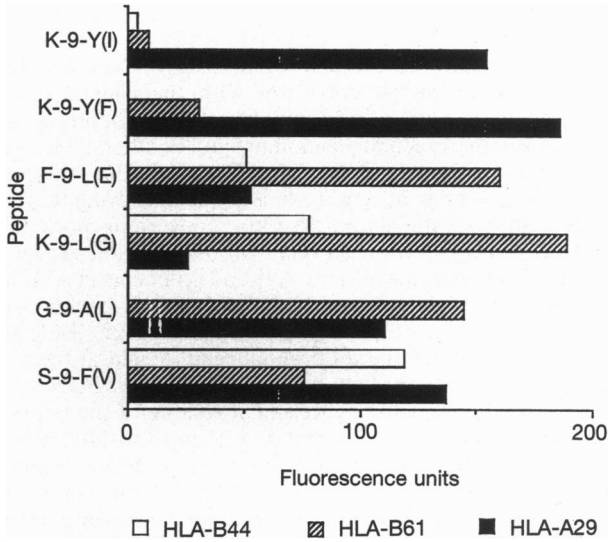

FIG. 2. Induction of HLA-A29, -B61, and -B44 assembly in the presence of retinal S-Ag peptides. Sequences of the peptides are shown in Table 3. Results are expressed as in Fig. 1.

Table 4. Anchor residues and HLA pockets in different alleles

Alleles	Peptide residues	HLA pockets						
		B-pocket residues						
	P2	9	24	45	63	66	67	70
HLA-A29	E	T	A	M	Q	N	V	Q
HLA-A31	L/V/Y/F	T	A	M	E	N	V	Q
HLA-A33	A/I/L/F/Y/V	T	A	M	E	N	V	Q
HLA-A3	L/V/M	F	A	M	E	N	V	Q
HLA-A11	V/I/F/Y	Y	A	M	E	N	V	Q
HLA-B44	E	Y	T	K	E	I	S	N
HLA-B60,-B61	E	H	T	K	E	I	S	N
		D-pocket residues						
	P3	70	73	97	99	114	156	
HLA-A29	F/V/I/A/K/L	Q	T	M	Y	R	L	
HLA-A31	F/L/Y/W	H	I	M	Y	Q	L	
HLA-A33	L/K	H	I	M	Y	Q	L	
HLA-A3	F/Y	Q	T	I	Y	R	L	
HLA-A11	M/L/F/Y/I/A	Q	T	I	Y	R	Q	
HLA-B44	M/I/L/D	N	T	R	Y	D	D	
HLA-B60	A/V/I/L/M/F	N	T	S	Y	N	L	
HLA-B61	F/I/L/V/Y/W	N	T	S	Y	N	L	
		F-pocket residues						
	C-terminus	77	80	95	116			
HLA-A29	Y	N	T	I	D			
HLA-A31,-A33	R	D	T	I	D			
HLA-A3,-A11	K/Y/F	D	T	I	D			
HLA-B44	Y/F	N	I	I	D			
HLA-B60	L	S	N	L	Y			
HLA-B61	V	S	N	L	Y			

Motifs and sequences from alleles *HLA-A29* (A*2902), *-A31* (A*3101), *-A33* (A*3302), *-A3* (A*0301), *-A11* (A*1101), *-B44* (B*4402 and B*4403), *-B60* (B*40012), and *-B61* (B*4006) are presented (22).

HLA-A31 and -A33 sequences in pocket B are identical and have only one difference with HLA-A29 at position 63, glutamine in HLA-A29 instead of glutamate in -A31 and -A33. This change in charge correlates with the change in the preferred P2 residue, Gln-63 possibly allowing peptides with glutamate at their second position to bind. In other alleles with a glutamate P2 anchor, HLA-B44, -B60, and -B61, the positively charged lysine at residue 45 is probably playing this role by its contacts with glutamate (36). Concerning pocket D, HLA-A29 and -A3 have an identical composition except at residue 97 (methionine in HLA-A29 and isoleucine in A3), and both prefer phenylalanine at P3. Notably, the D-pocket composition is markedly different between HLA-A29, -B61, and -B44, accounting for the differential requirement at the peptide third position for binding to these molecules. Pocket F is in contact with the carboxyl-terminal residue of the peptide. Sequence comparisons show that Asp-77 and Asp-116 are both needed for the binding of a positively charged residue, arginine or lysine (22). Substitution of Asp-77 by Asn-77 correlates with the binding of tyrosine instead of arginine as seen by comparing HLA-A29 to -A31 and -A33 molecules. The same association of Asn-77 and Asp-116 is shared with HLA-B44, which also binds peptides with an aromatic carboxyl-terminal residue. Altogether, our results agree with the current knowledge of the structure of HLA class I-bound peptides.

Because a good correlation has been shown between class I peptide binding and immunogenicity of potential T-cell epitopes (37), we first attempted to validate the motif defined in an *in vitro* binding assay. Two peptides synthesized according to HLA-A29-eluted peptides, K-9-Y(F) and K-9-Y(I), bound this allele specifically. Next we have used polyglycine synthetic peptides to define preferred residues at P3 or at the carboxyl terminus for an optimal HLA-A29 binding compared to HLA-B44 and B61 alleles. This assay clearly shows the requirement for another residue than glycine at P3, aromatic or hydrophobic according to the data of peptide elution.

Although S-Ag immune response is not unique to patients with BSR and could also occur in patients with other forms of uveitis, S-Ag is considered a model autoantigen in this disease (1, 9, 11–13) and is highly uveitogenic in animal models (9). For that reason, we synthesized S-Ag peptides in accordance with the HLA-A29 motif, which were tested in the *in vitro* refolding assay. Two out of six peptides, G-9-A(L) and S-9-F(V), bound HLA-A29 efficiently. Both are located in the carboxyl-terminal sequence of S-Ag, between aa 344 and 357. Interestingly, this part of the molecule was reported to contain the major pathogenic site of the human or bovine molecules in the Lewis rat EAU model (15–17) and also induced proliferative responses in about one-third of the patients (16). This part of the molecule could also play a crucial role in HLA-B27-associated uveitis due to cross-reactive proliferative responses with an HLA-B27-derived peptide (38). Until now, HLA-A29-restricted cytotoxic responses directed against S-Ag have not been reported in BSR. This is a difficult task—particularly in this disease in which samples from affected tissues can be hardly obtained. This study could provide necessary tools for an accurate prediction of HLA-A29-associated peptides and for subsequent functional analysis in affected patients or in HLA-A29 transgenic animals.

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