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Immunology

Antigenic mimicry of natural L-peptides with retro-inverso-peptidomimetics

(reversal of peptide bond/liposomes/monoclonal antibodies to short peptides/kinetic analysis of peptide–antibody interactions)

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ABSTRACT **Three analogues of the model peptide of sequence IRGERA corresponding to the COOH-terminal residues 130–135 of histone H3 were synthesized, and their antigenicity, immunogenicity, and resistance to trypsin were compared to those of the natural L-peptide. The three analogues correspond to the D-enantiomer, containing only D-residues, and two retro-peptides containing NH-CO bonds instead of natural peptide bonds. The chirality of each residue was maintained in the retro-peptide and inverted in the retro-inverso-peptide. Antibodies to the four peptide analogues were produced by injecting BALB/c mice with peptides covalently coupled to small unilamellar liposomes containing monophosphoryl lipid A. Each of the four peptide analogues induced IgG antibodies of various subclasses. The IgG3 antibodies reacted similarly with the four analogues, whereas antibodies of the IgG1, IgG2a, and IgG2b isotypes showed strong conformational preferences for certain peptides. The retro-inverso-peptide IRGERA mimicked the structure and antigenic activity of the natural L-peptide but not of the D- and retro-peptides, whereas the retro-peptide IRGERA mimicked the D-peptide but not the L- and retro-inverso-peptides. The equilibrium affinity constants (K_a) of three monoclonal antibodies generated against the L- and D-peptides with respect to the four peptide analogues were measured in a biosensor system. Large differences in K_a values were observed when each monoclonal antibody was tested with respect to the four peptides. The use of retro-inverso-peptides to replace natural L-peptides is likely to find many applications in immunodiagnosis and as potential synthetic vaccines.**

The development of neuropeptides, peptide hormones, peptide antibiotics, or peptide-based synthetic vaccines is strongly impaired by the high susceptibility of peptides to proteolysis, which limits, *inter alia*, parental and oral administration. For many years intense work has been focused on the synthesis of peptide analogues in the search for mimics with enhanced activity and biological half-lives. Examples of modifications introduced in peptides are the replacement of L-amino acid residues by D-amino acids or by unnatural residues (e.g., sarcosine and β-alanine) and the modification of peptide bonds (1–3). These changes provide pseudopeptides or peptidomimetics with a higher metabolic stability, since most natural proteases cannot cleave D-amino acid residues and nonpeptide bonds. An important problem encountered with such peptide analogues is the conservation of their biological activity. Recently, the D-form of human immunodeficiency virus type 1 protease has been synthesized (4). As could be expected, the enantiomeric protein displayed reciprocal chiral specificity as the enzyme was unable to cleave the normal L-substrate but did hydrolyze its D-enantiomer. In contrast, Wen and Laursen (5) showed that both the L- and D-form of an α-helical antifreeze polypeptide bound equally well to the same achiral ice substrate, whereas Wade *et al.* (6) found that the L- and D-enantiomers of several channel-forming antibiotic peptides were equally active.

Modified peptides could also be useful as potential synthetic vaccines if they could induce antibodies that recognized the natural (unmodified) antigenic structure of the pathogen and neutralized its infectivity. In comparison with the considerable amount of work describing the production and cross-reacting properties of antibodies to natural synthetic peptides, relatively little is known regarding the immune response against peptide analogues, especially against D-peptides and peptides containing reversed bonds. Several authors have claimed that D-peptides would probably possess little or no immunogenicity, since they could not be processed and presented to the major histocompatibility complex molecules for recognition by helper T cells or cytotoxic lymphocytes (4, 7). Dintzis *et al.* (8) reported the lack of immune response to an all-D-amino acid protein, rubredoxin, whereas the L-protein enantiomer induced a strong IgG antibody response. These results confirmed older observations showing the poor immunogenicity of D-amino acid polymers.

In contrast with these results, we have recently described the generation in mice of antibodies against an all-D-hexapeptide of sequence IRGERA corresponding to the COOH-terminal residues 130–135 of histone H3 (9). Antibodies of the IgG3 isotype induced against either the D- or L-peptide were able to bind both the homologous peptide and its mirror enantiomer. Anti-L-peptide antibodies of the IgG1, IgG2a, and IgG2b isotypes did not cross-react with the D-peptide.

In the present study, we have prepared two other IRGERA analogues, a retro-inverso analogue and a retro analogue, which contain reversed peptide bonds (i.e., -NH-CO- instead of -CO-NH-). The chirality of each residue was maintained in the retro-peptide and inverted in the retro-inverso-peptide. As the reversal of peptide bonds in a retro-inverso-peptide leads to a L-peptide-related analogue, we postulated that this analogue should be recognized by anti-L-peptide antibodies of the IgG1, IgG2a, and IgG2b isotypes. Since IgG3 antibodies induced against the L- or the D-peptide cross-reacted equally well with the L- and D-peptides, we anticipated that this class of antibodies would react with both retro-inverso- and retro-peptides. We have analyzed the immunogenicity and antigenicity of retro analogues as well as their resistance to trypsin in comparison with the L- and D-peptides. The four peptide analogues were tested in ELISA with polyclonal and

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Abbreviations: mAb, monoclonal antibody; BSA, bovine serum albumin; FAB, fast atom bombardment.

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monoclonal anti-IRGERA peptide antibodies. Equilibrium affinity constants of monoclonal antibodies (mAbs) obtained from mice immunized against the L- and D-peptides toward the four peptide analogues were measured.

MATERIALS AND METHODS

Histone H3 and Peptides. Chicken erythrocyte histone H3 was isolated and purified as described (10). Three analogues of the model peptide of sequence IRGERA corresponding to the COOH-terminal residues 130–135 of histone H3 were produced. Preparation and purification of L- and all-D IRGERA peptides have been described (9). The two new analogues, the retro-inverso- and retro-peptides, were synthesized as the L- and D-peptides by the solid-phase methodology in Boc chemistry on a *p*-methylbenzhydrylamine resin (Applied Biosystems) (11). Protected amino acids were from Neosystem (Strasbourg, France). The (*R,S*)-2-methylmalonic acid monobenzyl ester obtained by alcoholysis of 2,2,5,-trimethyl-1,3-dioxane-4,6-dione (12) was incorporated into the peptide chain as a racemate. HF cleavage, purification, HPLC, fast atom bombardment (FAB)-MS analysis, and circular dichroism measurements were performed as described (9, 11, 13).

Peptide Carrier Conjugation. To allow the coating of peptides in a direct solid-phase ELISA test, IRGERA analogues were conjugated to bovine serum albumin (BSA) as described (9). For immunization of mice, peptides were covalently coupled to preformed small unilamellar vesicles containing monophosphoryl lipid A (9, 14).

Antisera and mAbs. Antisera were obtained by immunizing BALB/c mice with liposome-associated peptides as described (9). mAbs to the L- and D-peptides were prepared by standard fusion protocols (15). Detailed descriptions of the generation and characterization of the mAbs will be reported elsewhere. The reactivity of three mAbs, 4x11 (derived from a mouse immunized against the L-peptide) and 11x2 and 11x7 (derived from a mouse immunized against the D-peptide) are described in this paper.

ELISA and Kinetic Analysis of mAb Binding. The ELISA procedure (direct binding and competition experiments) was as described (9). For real-time binding experiments, a BIAcore biosensor system (Pharmacia Biosensor) was used (16–19). To immobilize peptides to the sensor chip, the standard procedure described (20) for cysteine-containing peptides was used.

Direct binding experiments and competition assays were performed as described (17). mAbs 4x11, 11x2, and 11x7 (200 nM) were incubated with the competitor peptides used at a molar excess of 1.75–800 over antibody. Antibody kinetic constants were measured as described (17).

Resistance to Trypsin. Resistance of the four peptide analogues to trypsin was tested using the proteolytic enzyme covalently immobilized on 3.2-mm-diameter nylon spheres (21). The specific activity of the enzymatic spheres was equivalent to 18 nmol of *p*-toluenesulfonyl-L-arginine methyl ester hydrolyzed per min per nylon sphere. Protease digestion was initiated by immersing 15 enzymatic spheres into 1 ml of a peptide solution (150 μg/ml) maintained at 25°C under constant agitation. The digestion was performed in Hepes-buffered saline at pH 7.4 during 5–240 min. The reaction was stopped by removing the enzymatic spheres. Peptide cleavage was evaluated immunochemically by measuring the capacity of the remaining peptide to compete with the binding of mAb 11x2 to the immobilized L-peptide.

RESULTS

Synthetic Peptides. Four peptides were used in this study (Fig. 1). The parent peptide IRGERA corresponds to the COOH-terminal end of histone H3, which has been extensively studied in this laboratory (14, 22). A cysteine and two additional glycine residues were added at the NH_2-terminal end to allow selective conjugation of the peptides to liposomes or to BSA and to enhance accessibility of peptides bound to carriers.

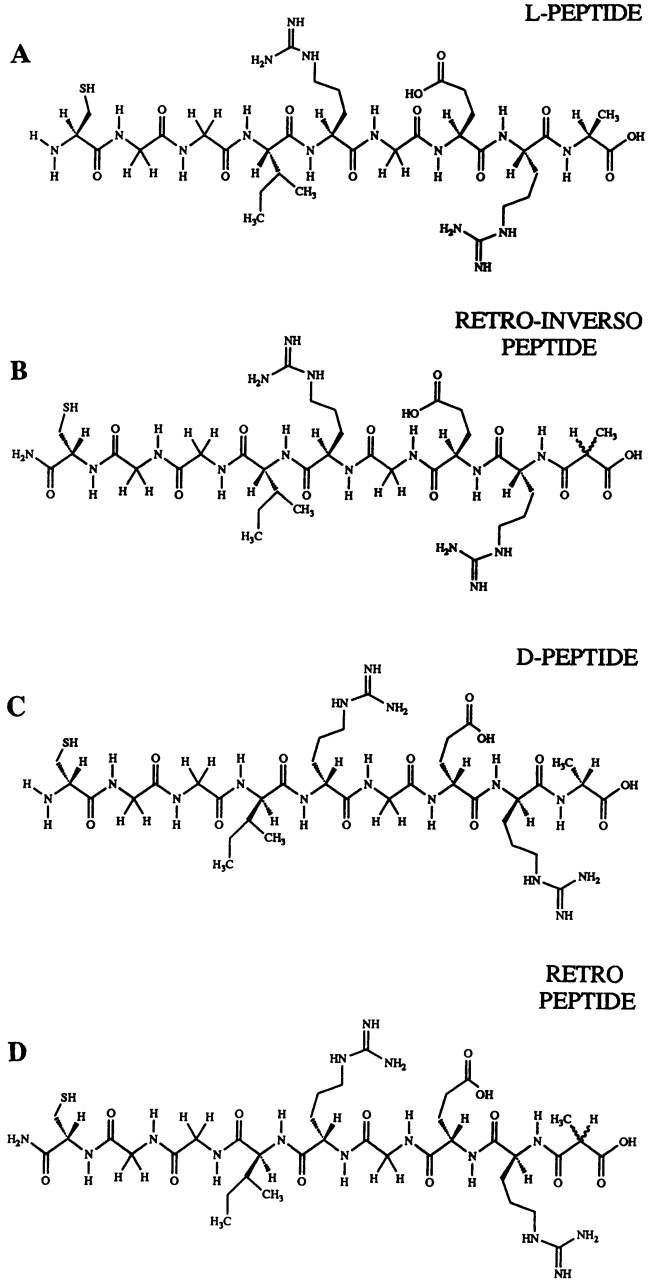

FIG. 1. Schematic representation of the natural (L) IRGERA peptide and peptide analogues.

Fig. 1 shows the structural relationships between the L-peptide and the three structurally related analogues used in this study—namely, the D-enantiomer (9) and the end group-modified retro and the retro-inverso analogues. The retro-inverso analogue is obtained by replacing the normal L-amino acid residues by the corresponding D-amino acids and by reversing the direction of the peptide backbone. This results in maintenance of the side chain topochemistry—i.e., the original spatial orientation of all side chains is retained (23). In the case of the retro analogue, the backbone is reversed but the chirality of amino acids in the sequence is retained, resulting in a noncomplementary side chain topochemistry

between this analogue and the parent L-peptide. This retro analogue is thus topochemically related to the D-enantiomer. However, in such linear peptides, the two pairs of topochemically related peptides do not share end group and charge complementarity. To solve this end-group problem, a *gem*-diaminoalkyl residue can be introduced at the amino terminus and a 2-substituted malonic acid can be introduced at the carboxyl terminus (23). However, monoacyl *gem*-diaminoalkyls are hydrolyzed, and one should expect the half-life of peptides incorporating such residues to be 10–50 hr at 25°C (24). Because of this and because the NH_2-terminal cysteine is not part of the epitope, we chose to use the cysteine residue in its L-form and a carboxaminated termination. The (*R,S*)-2-methylmalonic acid monobenzyl ester [FAB-MS (*m/e*): 208 (M^+)] was incorporated into the peptide as a racemate, thereby generating a pair of diastereoisomers. The two diastereoisomers of both retro and retro-inverso analogues were identified by analytical HPLC, but the separation was not good enough to allow the diastereoisomers to be purified. The mixtures of diastereoisomers were considered to be pure on the basis of analytical HPLC and mass spectrometry [FAB-MS (*m/e*): 946.3 (MH^+) for both retro- and retro-inverso-peptides; expected values 946.45].

The negative ellipticity found at 198 nm in the CD spectra of the L-peptide and retro-peptides indicates an unordered form. As mentioned previously for the parent L-peptide and its D-enantiomer (9), the CD spectra of retro and retro-inverso analogues are mirror images (data not shown).

Polyclonal Antibodies to IRGERA Analogues. Groups of two BALB/c mice were injected with the four IRGERA analogues conjugated to liposomes. The reaction of antibodies to the four peptides with the peptide analogues and with H3 was measured in a direct ELISA format. A strong antibody response was found against the four peptides (data not shown). In the case of L-peptide, antibodies belonged to the IgG1, IgG2a, IgG2b, and IgG3 subclasses. The IgG3 antibody response appeared slightly later than the IgG1, IgG2a, and IgG2b antibody response. In the case of retro-inverso-, D-, and retro-peptides, the IgG3 antibody response was predominant. It should be noted that although the IgG3 antibody response to retro-inverso- and retro-peptides was particularly strong, the duration of the antibody response was similar to that induced against L- and D-peptides.

The antibodies of IgG1, IgG2a, and IgG2b isotypes raised against the L- and retro-inverso-peptides recognized both the L- and retro-inverso-peptides as well as histone H3 but not the D- and retro-peptides. Conversely, antibodies of IgG1, IgG2a, and IgG2b isotypes raised against the D- and retro-peptides recognized both D- and retro-peptides but not the L- and retro-inverso-peptides. Antibodies of IgG3 isotype induced against the four peptides cross-reacted equally well with all four peptide analogues and with H3. Immunoreactivity of peptide conjugates was confirmed in a competitive binding assay with free peptides in solution (Table 1).

mAbs to L- and D-IRGERA Analogues. From a panel of mAbs to various IRGERA analogues, we selected three antibodies on the basis of their reactivity in ELISA with the four peptide analogues. mAb 4x11 (IgG1) was generated from the spleen cells of a mouse immunized with the L-peptide; it reacted in ELISA with the L- and retro-inverso-peptides but not with the D-peptide and only slightly with the retro-peptide. mAbs 11x2 and 11x7 (both IgG3) were generated from the spleen cells of a mouse immunized with the D-peptide; they reacted in ELISA with the four IRGERA analogues as well as with the parent histone H3.

The binding of these mAbs to the four peptide analogues was measured in the BIAcore using peptides covalently linked to the dextran matrix through their free SH group. Kinetic rate constants and equilibrium affinity constants of the three mAbs for the four peptide analogues are shown in

Table 1. Recognition in competitive ELISA of IRGERA and IRGERA analogues by mouse antibodies induced against homologous and analogue peptides

Antigen used as inhibitor	MEI_{50}*			
	Anti-L	Anti-RI	Anti-D	Anti-R
	IgG1, IgG2a, and IgG2b response			
Peptide L	10	80	—	—
Peptide RI	10	10	—	—
Peptide D	—	—	10	20
Peptide R	—	—	5	10
H3	5	18	—	—
	IgG3 response			
Peptide L	10	50	5	70
Peptide RI	50	10	10	70
Peptide D	10	70	5	30
Peptide R	5	70	50	10
H3	5	60	5	90

Microtiter plates were coated with 2 μM peptide conjugated to BSA by means of *N*-succinimidyl 3-(2-pyridyldithio)propionate (carrier to peptide molar ratio = 1:10) and allowed to react with mouse antisera raised against the homologous peptide (serum dilution = 1:500) and preincubated with the various peptides and with H3 used as inhibitor. A control peptide corresponding to the sequence 149–158 of tobacco mosaic virus protein was used as internal control and had no effect on the antibody binding. Anti-mouse IgG peroxidase conjugates were both diluted 1:5000. Molar excesses of inhibitor peptide are expressed over peptide coated into the wells of microtiter plates and were calculated as described (9). L, natural L-peptide; RI, retro-inverso-peptide; D, D-peptide; R, retro-peptide; —, no detectable cross-reactivity (up to a competitor peptide concentration of 125 μg/ml).

*Molar excess of inhibitor required to inhibit 50% of the binding between anti-peptide antibodies and homologous antigens.

Table 2. The equilibrium affinity constants (K_a) of mAbs 4x11, 11x2, and 11x7 for their homologous peptides were 3×10^6 M^{-1}, 1.3×10^{10} M^{-1}, and 1.2×10^7 M^{-1}, respectively (Table 2). It is noteworthy that both mAbs 4x11 and 11x7 showed at least a 50-fold lower K_a value for the homologous peptide than for an heterologous peptide—namely, the retro-inverso-peptide in the case of mAb 4x11 and the retro-peptide in the case of mAb 11x7 (Table 2). mAb 11x2 reacted with all four peptides. However, compared with the affinity for the homologous peptide, K_a values were 30-fold lower for the heterologous retro-peptide and 10^3- to 10^4-fold lower for the L- and retro-inverso-peptides (Table 2).

Table 2. Kinetic rate constants and equilibrium affinity constants of mAbs 4x11, 11x2, and 11x7 for the four IRGERA analogues

mAb	Peptide used as antigen	$k_a \times 10^{-3}$, $M^{-1}s^{-1}$	$k_d \times 10^5$, s^{-1}	$K_a \times 10^{-6}$, M^{-1}
4x11	L	3 ± 0.2	100 ± 0.3	3
	RI	18 ± 0.5	8 ± 0.2	225
	D	NB	—	—
	R	NB	—	—
11x2	L	2 ± 0.3	100 ± 0.2	2
	RI	13 ± 0.6	160 ± 0.3	8
	D	130 ± 0.3	1 ± 0.4	13,000
	R	5 ± 0.5	1 ± 0.3	500
11x7	L	NB	—	—
	RI	NB	—	—
	D	3 ± 0.4	25 ± 0.5	12
	R	6 ± 0.6	1 ± 0.4	600

NB, no binding. Association (k_a) and dissociation (k_d) rate constants are the mean values obtained in two to four independent experiments. L, natural L-peptide; RI, retro-inverso-peptide; D, D-peptide; R, retro-peptide. mAb 4x11 is an anti-L-peptide antibody (IgG1). mAbs 11x2 and 11x7 are both anti-D-peptide antibodies (IgG3).

Table 3. Recognition in competitive BIAcore assays of IRGERA and IRGERA analogues by mAbs 4x11, 11x2, and 11x7

Peptide used as inhibitor	MEI_{50}*			
	L-peptide		D-peptide	
	4x11	11x2	11x2	11x7
L	1	1	10	—
RI	1	3	15	—
D	—	1	10	10
R	—	0.5	25	10

L, natural L-peptide; RI, retro-inverso-peptide; D, D-peptide; R, retro-peptide. —, No detectable cross-reactivity (up to a competitor peptide concentration of 250 μg/ml).
*Molar excess of inhibitor peptide (over 200 nM mAb) required to inhibit 50% of the binding between mAb and the given peptide.

To confirm the difference in immunoreactivity of different peptide analogues, inhibition experiments with the three mAbs were conducted in the BIAcore system. The three mAbs preincubated with various concentrations of the four peptide analogues were allowed to react with the homologous peptides immobilized on the sensor surface. As shown in Table 3, the binding of mAb 11x2 to L- and D-peptides was inhibited by the four peptide analogues (50% inhibition of antibody binding observed with a 0.5- to 25-fold molar excess of different peptides over antibody). The binding of mAb 4x11 to L-peptide was inhibited only by the free L- and retro-inverso-peptides, whereas the binding of mAb 11x7 to the D-peptide was inhibited only by the free D- and retro-peptides. These results are thus in complete agreement with those obtained in the direct ELISA format described above.

When the reactivity of mAbs with the four peptide analogues was compared in ELISA and in the BIAcore system, in some cases a poor correlation was found (Table 4). For instance, similar OD values were obtained in ELISA when mAb 11x2 was allowed to react with the L- and D-peptides, whereas K_a values as measured in the biosensor system differed by four orders of magnitude.

Resistance of Peptide Analogues to Trypsin. One of the potential advantages of using peptide analogues containing D-amino acid residues or reversed peptide bonds rests in their much higher resistance to proteases, which could increase their immunogenicity compared to natural L-peptides (6, 8). We have tested the resistance of peptide analogues to trypsin whose specificity is based upon positively charged lysine and arginine side chains. We used the enzyme covalently immobilized on nylon spheres and measured the residual capacity of proteolyzed peptides to compete with the L-peptide antigen for the binding of mAb 11x2 in the BIAcore. mAb 11x2 was used in this experiment because it recognized all four analogues in solution (Table 3). The advantage of using the enzyme immobilized on nylon spheres lies in its enhanced stability, the absence of contamination of the digests, and an easy control of proteolysis (21). As shown in Fig. 2, the L-peptide was rapidly digested under these conditions. Half of its antigenic activity was lost after 7 min and no activity remained after 10 min. The retro-peptide was slightly less sensitive (half-life, 9 min; complete loss of activity, 40 min) whereas the D- and retro-inverso-peptides were much more resistant to trypsin (half-lives of 73 and 67 min, respectively; complete loss of activity, not yet observed after 240 min).

Table 4. Reactivity in ELISA of mAbs 4x11, 11x2, and 11x7 with the four IRGERA analogues and equilibrium affinity constants of the three mAbs for the four analogues

mAb	Test	Reactivity with peptide			
		L	RI	D	R
4x11	ELISA (OD)	1.29	1.12	0.19	0.40
	BIAcore (K_a, M^{-1})	3×10^6	2×10^8	NB	NB
11x2	ELISA (OD)	2.75	1.78	2.92	1.92
	BIAcore (K_a, M^{-1})	2×10^6	8×10^6	1×10^{10}	5×10^8
11x7	ELISA (OD)	0.47	0.89	1.45	0.78
	BIAcore (K_a, M^{-1})	NB	NB	1×10^7	6×10^8

NB, no binding. For ELISA, microtiter plates were coated with 2 μM peptide conjugated to BSA and allowed to react with mAb (4 μg/ml). Anti-mouse IgG-peroxidase conjugates were diluted 1:5000. L, natural L-peptide; RI, retro-inverso-peptide; D, D-peptide; R, retro-peptide.

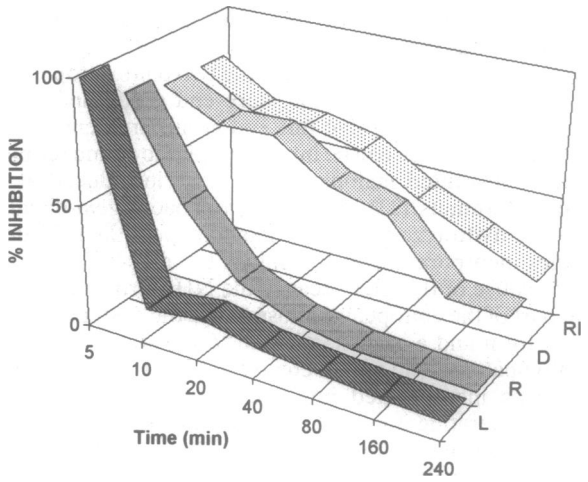

FIG. 2. Resistance of the four peptide analogues to trypsin covalently immobilized on nylon spheres. The digestion mixture incubated during different times at 25°C was allowed to incubate first with mAb 11x2 (4 μg/ml) and was then injected over the L-peptide immobilized on the activated dextran matrix. Results are expressed as the percentage of inhibition of the binding of mAb 11x2 to immobilized L-peptide by the competitor peptides subjected to trypsin. L, L-peptide; R, retro-peptide; D, D-peptide; RI, retro-inverso-peptide.

DISCUSSION

Retro-inverso analogues of natural peptides represent one of the most widely used peptidomimetic approaches to the design of bioactive molecules. This modification, which affects the backbone of peptides but not the orientation of side chains, has been introduced in many biologically active peptide analogues (1). However, there is little information on the antigenicity and immunogenicity of such compounds. The present work was carried out with a model hexapeptide of sequence IRGERA, which corresponds to the COOH-terminus of histone H3. In its free form, this peptide is non-immunogenic (14).

The major findings of this study can be summarized as follows. As far as immunogenicity is concerned, IgG antibodies could be raised readily against the three types of peptide analogues tested, the all-D-peptide and the retro- and retro-inverso-peptides, when they were associated with small unilamellar vesicles containing the nontoxic adjuvant monophosphoryl lipid A. As observed previously (9), a strong IgG3 antibody response was obtained in all cases. It is noteworthy that when the L- and analogue peptides were injected in mice as ovalbumin–peptide conjugates in the presence of Freund's adjuvant, a relatively high IgG3 antibody response was also obtained, although the overall immune response was lower. The IgG3 antibodies obtained after immunization with ovalbumin–peptide conjugates showed the same binding characteristics as those of the IgG3 antibodies obtained after immunization with peptide analogues coupled to liposomes (data not shown).

As shown previously (9), antibodies of IgG3 isotype induced against the IRGERA L- and D-peptides cross-reacted equally well with both L- and D-peptides, and in addition they also recognized the retro- and retro-inverso-peptides. This lack of discrimination potential of the IgG3 antibodies is puzzling and, as discussed previously (9), may be related to the self-association and concomitant increased binding shown by these antibodies. The presence of the linking residues CGG is not responsible for the IgG3 binding, since the same reactivity was observed with the IRGERA hexapeptide tested as inhibitor. In contrast, antibodies of IgG1, IgG2a, and IgG2b isotypes were able to differentiate the four peptides. The retro-inverso-peptide IRGERA was found to mimic the natural L-peptide but not the D- and retro-peptides; conversely, the retro-peptide mimicked the D-peptide but not the L- and retro-inverso-peptides. Most interestingly, antibodies of the IgG1, IgG2a, and IgG2b isotypes induced against the retro-inverso-peptide IRGERA recognized the parent protein histone H3 in solution, whereas antibodies of these isotypes induced against the D- and retro-peptide IRGERA did not.

mAbs produced from mice immunized against the L- and D-peptides were tested with the four peptide analogues in ELISA, and their kinetic rate constants and equilibrium affinity constants for the four analogues were measured using the BIAcore biosensor system. The three mAbs (one anti-L-peptide mAb of IgG1 isotype and two anti-D-peptide mAbs of IgG3 isotype) showed a similar pattern of reactivity in ELISA as found with polyclonal antibodies. The lack of correlation between some OD values in ELISA and the K_a values (Table 4) may be due to the low diffusion rate in microtiter wells leading to fast antibody reassociation and very low apparent rates of dissociation (25, 26). The different microenvironments of the peptides in the dextran layer of the sensor chip and as BSA conjugates in the ELISA plate may also be responsible for the observed differences. In the BIAcore system, mAb 11x7 (anti-D-peptide IgG3) bound both D- and retro-peptides but not the L- and retro-inverso-peptides, thus showing a pattern of reactivity similar to that of IgG1, IgG2a, and IgG2b polyclonal antibodies raised against the D-peptide.

Interestingly, mAb 11x2 (anti-D-peptide IgG3) bound D-peptide with a K_a around 1.3×10^{10} M^{-1} and retro-peptide with a K_a of only 5×10^8 M^{-1}. It bound the L- and retro-inverso peptides with K_a values of 1.9 and 7.9×10^6 M^{-1}, respectively. It is likely that the cross-reactivity of mAb 11x2 was detectable in this test because its affinity for the homologous D-peptide was particularly high.

It is noteworthy that both mAb 4x11 (anti-L-peptide IgG1) and mAb 11x7 (anti-D-peptide IgG3) bound heterologous peptides with a higher K_a (Table 2). Such examples of heterospecificity have been described with different mAbs (27–30).

The use of retro-inverso analogues to replace natural L-peptides is likely to find many applications in immunological research including diagnostics and therapy. The retro-inverso-peptide IRGERA, which was used as a model, was much more stable than the L-parent peptide to trypsin digestion. It mimicked the L-peptide and induced a strong antibody response toward the L-peptide and the parent protein H3. This type of peptide analogue may thus be useful for developing potential synthetic vaccines. If the mode of binding of such peptide analogues on the membrane of B and T lymphocytes is similar to that of natural peptides, retro-inverso-peptides may also represent interesting tools to study the interaction of peptides with HLA molecules or T-cell receptors. This approach may be useful in the development of new immunointervention strategies (31, 32).

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