Topological Mimicry of Cross-reacting Enantiomeric Peptide Antigens*

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Rabbit polyclonal antibodies against multimeric peptide antigens were found to cross-react to a significant extent with topologically related variants of the parent antigen, where the chirality of each amino acid residue (inverso derivatives), or the peptide sequence orientation (retro derivatives), was inverted or where both modifications were simultaneously introduced (retro-inverso derivatives). All peptide variants displayed similar recognition properties for antibodies and similar dose-dependent inhibitory effects on the interaction between immobilized parent antigen and corresponding antibodies. Importance of peptide side chain topology on antigenicity was evaluated analyzing the recognition properties of two sequence-simplified parent peptide variants, one lacking of the side chains in the sequence odd position and the other in even position. These two variants, prepared introducing glycine residues alternatively in the parent peptide sequence, were found to cross-react to a significant extent with the original antibody raised against the parent peptide. Analysis of molecular models of peptide enantiomeric variants in the elongated all-trans configuration suggested that the topological equivalence of alternating side chains could lead to the formation of similar recognition surfaces, thus mimicking the parent peptide antigenic structure.

Peptides biological activity is most often dependent upon well defined primary and secondary structure elements, even if in many cases peptide topology can be altered, by amino acid chirality inversion (inverso modification) (1–3) or by peptide reversal of amino acid sequence (retro modification) (4), without altering the peptide functional activity (1–4). Retro-inverso modification of biologically active peptides is the most common peptidomimetic approach used to enhance peptide stability against the action of proteolytic enzymes and thus prolong their half-life in vivo (5, 6). In addition, this type of modification, leaving unaltered the topology of the peptide side chain and interconverting carbonyl and amide groups from their position, constitutes a useful approach to elucidate the role of peptide backbone polarity in molecular recognition events (7). End group modified retro-inverso peptides are isomers of linear peptides in which the direction of the sequence is reversed, the chirality of each amino acid residue is inverted, and the end groups are modified to obtain close complementarity and full topological relationship to the parent peptide. Retro-inverso peptide have been found to retain recognition properties (8) or biological activity as well as the parent peptide in many cases (9–11), once their end groups were suitably modified.

While the effect of peptide chirality and backbone modification has been extensively investigated in terms of biological reactivity, a limited amount of work has been carried out in order to assess the importance of peptide topochemistry on antibody recognition. Earlier studies suggested the lack of cross-antigenicity between enantiomeric peptides (12–14), as well as between enantiomeric proteins, such as rubredoxin and its all-o analogue (15). More recently the stereochemical requirements for peptide antigenicity have been investigated with renewed interest, since preliminary studies in a limited number of cases suggested the existence of antibody cross-recognition with topologically related variants of the parent peptide. In the first study, cross-recognition between the COOH-terminal fragment 130–135 of histone H3 (IRGERA) and the corresponding all-o analogue was experimentally observed with antibodies of the IgG3 isotype raised in mice against the parent peptide (16). In addition, it was shown that antibodies raised against the all-o peptide variant were able to recognize the all-L parent peptide equally well. In both cases, the corresponding antibodies recognized also the parent protein histone H3. On the other hand, anti-parent peptide antibodies of the IgG1, IgG2a, and IgG2b isotypes failed to bind the all-o peptide. The analysis of peptide topology importance on antigenicity has been extended in a further study, evaluating the effect of backbone polarity reversal (retro derivative), or amino acid chirality and backbone inversion (retro-inverso derivative), on antibody recognition (17). Antibodies to the peptide analogues were produced in BALB/c mice and showed a strong correlation between cross-recognition properties and peptide topology, since mouse antibodies of the IgG1, IgG2a, and IgG2b isotypes against the parent peptide recognized only the peptide retro-inverso derivative, while antibodies of the same isotype against the inverso (all-o) peptide recognized only the corresponding retro-peptide and vice versa. These observations were supported by other studies on the cross-antigenicity between a cyclic peptide analogous to the third complementarity-determining region (CDR3) in immunoglobin and the corresponding retro-inverso isomer (18). Retro-inverso peptides are strongly topologically correlated with the parent peptide, since the resulting side chain disposition is similar to the parent peptide, but carbonyl and amide groups are interconverted from their positions (6, 7). Similarly, inverso peptide are strongly correlated with the retro derivative of the parent peptide for the same reason, while no apparent structural relationship can be envisioned between retro and retro-inverso peptides, as well as between the inverso and the all-L parent peptide, since in both cases the peptides display an enantiomeric relationship. The stringent side chains topological equivalence between linear all-L peptides and the corresponding retro-inverso derivatives, or between retro and inverso peptides, could provide a sufficient explanation for the observed

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cross-antigenicity, while cross-recognition between parent antigen and its retro or inverse variants apparently lack of a rational mechanistic explanation.

In this study we have investigated the cross-recognition properties of polyvalent antibodies raised in rabbits against a 15-residue peptide (P15) able to bind interleukin 2 and at the same time to inhibit its interaction with the p55 interleukin 2 receptor subunit (19). The peptide antigen was produced in a tetrameric form (MAP-P15) starting from a tetradentate lysine core for direct immunization (20), and cross-antigenicity with a series of topochemically related peptides, such as inverse, retro, and retro-inverse variants was evaluated in direct binding and competition experiments by ELISA. To further examine the role of side chain topology of enantiomeric antigens on cross-antigenicity, two other variants of the parent peptide were prepared, where the parent peptide amino acid sequence was changed in order to display only side chains in alternating position (1, 3, 5, 7, ... or 2, 4, 6, 8, ...) of the parent peptide original sequence, by replacing corresponding residues with glycine residues. Recognition properties of sequence-simplified variants were then evaluated by direct and competitive ELISA experiments.

**MATERIALS AND METHODS**

**Peptide Synthesis**—Peptides were produced by solid phase peptide synthesis following the Fmoc methodology on a fully automated model 431A Applied Biosystems peptide synthesizer, software version 1.2. Multimeric peptides used for immunization were produced starting from a tetradentate (MAP-P15) lysine core as reported previously (21). After resin cleavage, low molecular weight contaminants were removed by dialysis against 0.1 mM acetic acid. Linear peptides were purified by RP-HPLC on a RP-8 Lichrosphere 10-μm 10-mm inner diameter column. Peptide identity was confirmed by amino acid analysis and determination of molecular weight by TOF-MALDI mass spectrometry.

Preparation of Anti-MAP Antiserum—Rabbit anti-MAP antiserum was prepared using the peptides without carrier conjugation. Six New Zealand rabbits were immunized with peptides (50–200 μg) dissolved in 0.15 M sodium chloride, 0.05 M sodium phosphate buffer, pH 7.3, and emulsified with complete Freund’s adjuvant containing hIL-17Rv (2 mg/ml) by injections in the rear foot pads. Two weeks later the same immunogen was administered subcutaneously. After two subsequent boosters, blood samples were collected from each animal for monitoring antibody titers. One week after the last boost, animals were bled and sera stored at −80 °C until use. Antibodies were then purified by affinity chromatography on MAP-P15 affinity column as described previously (21) (final concentration, 20 μg/ml).

Antibody Affinity Purification on Peptide Columns—Affinity column with immobilized peptides (+)P15 and (-)P15 were prepared by incubating 10 mg of peptide dissolved in 10 ml of 0.1 M sodium bicarbonate buffer, pH 8.5, with 1.0 g of Eupergit C30 N (epoxy-activated methacrylate gel, Sigma) as described previously (21). Samples of anti-MAP-P15 antibodies (200 μg), previously purified on a MAP-P15 affinity column, were loaded on the (+)P15 or (-)P15 affinity columns equilibrated at a flow rate of 1 ml/min with 25 mM Tris, pH 7.2. After elution of unbound material, the eluent was changed to 0.1 M acetic acid to eluted bound antibody. Extent of purification was followed by ELISA, evaluating fractions reactivity on plates coated with the parent antigen MAP-P15.

Conjugation to Carrier Protein—Conjugation of linear peptides to BSA was carried out by mixing 5 mg of peptide dissolved in 1 ml of 50 mM PBS, pH 7.0, with 15 mg of bovine serum albumin dissolved in 1 ml of the same buffer and incubating the mixture with 2 ml of 0.2% glutaraldehyde solution in the same buffer. After 1 h of incubation at room temperature, 1 ml of 1 M glycine solution was added and 1 h later the conjugates were extensively dialyzed against 50 mM PBS, pH 7.0. Peptide-BSA conjugates were characterized by TOF-MALDI mass spectrometry, indicating that in all cases an average of eight peptide chains were coupled to BSA.

ELISA—Polystyrene microtiter plates were incubated overnight at 4 °C or for 2 h at room temperature with 50 μg/ml MAP solution or 100 μg/ml peptide-conjugated solution (100 μl/well) in 0.1 M sodium carbonate buffer, pH 8.5. After five washings of the microtiter plates with PBS, the wells were saturated with 200 μl of PBS containing 3% BSA, for 1 h at room temperature, to block the uncoated plastic surface. Plates were then washed again with PBS and filled with samples containing anti-MAP antibodies at varying concentrations, previously diluted with PBS containing 0.5% BSA (PBS-B).

For competition experiments, various concentrations of inhibitor (MAP, conjugated peptide, or linear peptide) were incubated for 3 h at 37 °C with anti-MAP antibodies. The mixture was then added to MAP- or BSA-conjugated peptide-coated wells.

After 1 h of incubation the plates were washed five times with PBS. For anti-MAP antibody detection, wells were filled with 100 μl of a horseradish peroxidase labeled goat anti-rabbit (IgG) immunoglobulin solution diluted 1:1000 with PBS-B. The plates were then left to stand for 1 h at room temperature in a humid covered box, washed with PBS 5 times, an then filled with chromogenic substrate solution consisting of 1 mg/ml o-phenylenediamine in 0.1 M sodium citrate buffer, pH 5.0, containing 5 mg hydrogen peroxide. The absorbance at 450 nm was determined with a model 2250 EIA Reader (Bio-Rad).

**RESULTS**

**Peptide Antigens**—The peptide used for immunization was produced by solid phase peptide synthesis starting from a tetradentate lysine core following the Fmoc methodology. Amino acid analysis and determination of molecular weight by laser desorption mass spectrometry confirmed the intended chemical nature. Following the same synthetic methodology, the corresponding linear peptide (VRGLWLAPADLDAR) was assembled and purified by semipreparative RP-HPLC. The inverse derivative was prepared using amino acid in the d configuration (VRLGWLLAPADLDAR), the retro inverting the sequence COOH to NH2 terminus (RADLDAPALLWGLRV), while the retro-inverse assembling the retro sequence with amino acids in the d configuration (RADDPALLWLVR). No end group modification was carried out, since preliminary experiments with peptide smaller fragments suggested that the main epitope was distant from the peptide antigen amino or carboxyl terminus. As a control, a peptide containing all the amino acids of the parent linear antigen was prepared scambling the amino acid sequence. Peptides were all purified by RP-HPLC and fully characterized by amino acid analysis and determination of molecular weight by laser desorption mass spectrometry (TOF-MALDI). Sequences of the peptides used in this study are shown in Fig. 1.

**Antigenicity of Retro, Retro-Inverse, and Inverso Variants**—Rabbit immunization with MAP-P15 induced a strong immune response even after the 1st boost, as detected by ELISA on microtiter plates coated with the multimeric antigen, while preimmunum serum was unreactive. Antibody binding was dose-dependent and specific, since soluble MAP-P15 was able to inhibit the interaction between antibodies and immobilized MAP-P15 in a dose-dependent manner (not shown). Unrelated tetrameric peptides or BSA had no effect on the interaction, as well as scrambled linear P15 coupled to BSA. Crude serum was first purified by affinity chromatography on MAP-P15 antigen columns as described previously (21). Affinity-purified antibody was then tested for its ability to recognize the linear parent peptide, the retro, retro-inverse, and inverse derivatives, conjugated to BSA to facilitate coating on microtiter plates for ELISA determination. All the four topologically related variants, adsorbed on microtiter plates at roughly the same concentration, reacted in a similar way with affinity-purified polyclonal antibodies, in a dose-dependent fashion (not

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1 The abbreviations used are MAP, multimeric antigenic peptide; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; Fmoc, 9-fluorenylmethoxycarbonyl; RP-HPLC, reversed-phase high performance liquid chromatography; PBS, phosphate-buffered saline; TOF-MALDI, time-of-flight matrix-assisted laser desorption ionization.
Binding to BSA was negligible, as well as binding to scrambled linear antigen conjugated to BSA, thus providing evidence of the sequence specificity of the interaction. The polylysine core used for MAP-P15 synthesis apparently was not immunogenic, since antibodies did not cross-react with two other unrelated tetrameric peptides with the same lysine core (not shown). Binding was inhibited in the presence of soluble BSA-conjugated antigens, in a dose-dependent manner (not shown), while BSA or scrambled parent peptide conjugated to BSA had no effect on the interaction, thus providing further evidence of interaction specificity. Cross-recognition between the peptide variants was further confirmed by competition experiment on microtiter plates coated with the parent antigen MAP-P15. As shown in Fig. 2, all the peptide variants conjugated to BSA were efficiently able to displace the interaction between anti-MAP-P15 antibodies and MAP-P15 immobilized on microtiter plates in a dose-dependent manner, and interaction was reduced at 50% of its original value by BSA-peptide conjugates at roughly the same concentration (Table I). Scrambled linear antigen, on the other hand, had no effect on the interaction, thus providing additional support to the sequence dependence of these interactions. Similar dose-response inhibition profiles were observed with free peptides, without conjugation to BSA (not shown).

Antibody Cross-recognition with Sequence-simplified Peptide Variants—While the observed antibody cross-recognition with the retro-inverso derivative of the parent antigen could be ascribed to the close side chains topological equivalence of the two isomers, no structural similarity between retro and inverso derivatives could be invoked to explain cross-reactivity. In order to study further this intriguing behavior, a molecular model of all the peptide variants was built in the extended all-trans configuration. The parent peptide and its retro-inverso analogue display a superimposable side chain topology, with main differences, as expected, only at the level of peptide backbone, where carbonyl groups are replaced in the retro inverso isomer by amide groups and vice versa. Assuming that the peptide backbone does not take part in stabilizing contacts with the antibody, the molecular surface formed by the side chains residues in the two variants is very similar. The same is true considering the other two antigen variants, the retro and the inverso isomers, which display, as seen before, a superimposable side chains disposition. On the other hand, enantiomeric antigens, such as parent peptide P15 and its inverso derivative, or the retro-inverso and retro variants, by definition, are char-

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**FIG. 1.** Sequences of the linear peptide P15 and its tetrameric form MAP-P15 used for immunization and the corresponding retro, retro-inverso, inverso variants, and sequence-simplified variants (+G)P15 and (-G)P15. Lower-case notation is used for amino acids in the D configuration.

**FIG. 2.** Inhibition of the binding between anti-MAP-P15 antibody and immobilized MAP-P15 by BSA-conjugated linear peptide P15 ( ), retro P15 ( ), inverso P15 ( ), retro-inverso P15 ( ), and scrambled P15 ( ). Data are representative of five different experiments.

**TABLE I**

| Competitor [µg/ml] | Inhibitor* | IC50<sup>a</sup> [µg/ml] |
|-------------------|------------|--------------------------|
|                   | P15        | 7                        |
|                   | Retro P15  | 4                        |
|                   | Inverso P15| 50                       |
|                   | Retro-inverso P15 | 20                   |
|                   | Scrambled P15 | NI<sup>b</sup>         |
|                   | (+G)P15    | 23                       |
|                   | (-G)P15    | 20                       |

* All peptides conjugated to BSA via glutaraldehyde-mediated coupling.

<sup>a</sup> Concentration of peptide BSA conjugates required to reduced to 50% the binding of anti-MAP-P15 antibodies to microtiter plates immobilized MAP-P15.

<sup>b</sup> No inhibition up to a concentration of 1000 µg/ml.
characterized by a nonsuperimposable side chains arrangement. A more detailed analysis of enantiomeric antigens suggested the possibility of aligning only the side chains of alternating residues, such as residues in position 1, 3, 5, 7, ... or alternatively in position 2, 4, 6, 8, ... (Fig. 3). In this configuration, the carbonyl and amide groups in the backbone are not superimposable, but located in close proximity. This structural analysis suggests that enantiomeric antigens could display similar recognition surfaces formed by alternating residues, thus providing a possible explanation for the observed cross-antigenicity. In order to test this hypothesis, parent peptide variants were prepared where alternating residues were replaced by glycine residues. Two sequence-simplified variants were synthesized, one with glycine residue substitutions in the parent peptide even sequence position (VGLGWGLPGDG-DGR) ( (+G)P15 variant) and the other with glycine replacement in the odd position (GRGGGLGAGLGAG) ( (–G)P15 variant). Molecular models of these two latter variants are shown in Fig. 4. Sequence-simplified variants were then conjugated to BSA and then tested for their ability to recognize the antibody.
FIG. 5. Cross-recognition properties of sequence-simplified peptide variants. A, binding of affinity-purified anti-MAP-P15 antibody to immobilized P15 ( ), (+)P15 (●), (−)P15 (▲), and to scrambled P15 (△) conjugated to BSA, adsorbed to microtiter plates at concentration 5 μg/well. B, inhibition of the binding between anti-MAP-P15 antibody and immobilized MAP-P15 by P15 ( ), (+)P15 (●), (−)P15 (▲), and scrambled P15 (△) conjugated to BSA. Data are representative of five different experiments.

TABLE II

| Immobilized peptide | Antibody recovery<sup>a</sup> | Immunoreactivity recovery<sup>b</sup> |
|---------------------|-----------------------------|------------------------------------|
| P15                 | 95                          | 94                                 |
| (+)P15              | 92                          | 91                                 |
| (−)P15              | 89                          | 90                                 |

<sup>a</sup> Determined by SDS-polyacrylamide gel electrophoresis analysis of fractions corresponding to the column bound and unbound material in comparison with the amount of antibody initially loaded on the column.

<sup>b</sup> Determined by ELISA on microtiter plates coated with MAP-P15.

DISCUSSION

Experimental evidences of antigenic cross-recognition between linear antigens and the corresponding retro-inverso isomers have been reported only recently in at least three different cases. In the first study, monospecific rabbit antibodies raised against the parent multimeric antigen. As detected by direct binding ELISA experiments, affinity-purified anti-MAP-P15 antibody recognized both sequence-simplified variants in a dose-related manner (Fig. 5A). Antibody binding to parent antigen MAP-P15 was inhibited by soluble (+)P15 and (−)P15 variants in a dose-dependent fashion, and peptide conjugates concentration yielding 50% binding inhibition was very close and comparable with that of BSA-P15 conjugates (Fig. 5B and Table I). Once again, scrambled P15 conjugated to BSA had no effect on these interactions.

Antibody Affinity Purification on Sequence-simplified Parent Peptide Variants Columns—Extent of cross-recognition between sequence-simplified variants and parent antigen was further examined by affinity chromatography on columns prepared by immobilizing (+)P15 and (−)P15 on Eupergit C30N, a preactivated affinity medium. Monospecific antibodies previously purified on the MAP-P15 affinity column were then eluted on the two columns, and as a positive control, also on an affinity column prepared with the linear parent peptide antigen P15, equilibrated with a neutral buffer. After elution of columns unbound material, the buffer was changed to acidic conditions to elute bound antibody. Fractions corresponding to the unbound and bound material were collected for SDS-polyacrylamide gel electrophoresis analysis and ELISA determination of antibody reactivity using microtiter plates coated with the parent multimeric antigen MAP-P15. As shown in Table II, in all cases immunoreactivity was found in the columns bound fractions, while negligible activity was found in the columns breakthrough, and SDS-polyacrylamide gel electrophoresis analysis indicated that almost all the antibody loaded on the columns was recovered in the bound fractions. Elution of crude serum samples on the same columns confirmed the presence of immunoreactivity only in the columns adsorbed fractions. These results suggest that the entire anti-parent peptide antibody population recognizes the two sequence-simplified variants, equally well as the parent antigen.

Experimental evidences of antigenic cross-recognition between linear antigens and the corresponding retro-inverso isomers have been reported only recently in at least three different cases. In the first study, monospecific rabbit antibodies were used as conformational probes to demonstrate surface similarities between a cyclic peptide analogous to the third complementarity-determining region (CDR3) in immunoglobulin and the corresponding retro-inverso isomer (18). In the second study, poly- or monoclonal antibodies raised against the COOH-terminal hexapeptide of histone H3 by injecting BALB/c mice with peptides covalently coupled to small unilamellar liposomes containing monophosphoryl lipid A, exhibited a clear cross-reactivity with the retro-inverso derivative (17, 22). Similarly, antibodies raised against the retro derivative cross-reacted with the inverse derivative, but not with the parent linear antigen or the retro-inverso form, at least for antibodies of the IgG1, IgG2a, and IgG2b isotypes. Only antibodies of the IgG3 isotype displayed a generalized cross-reactivity, as in our case, with the parent peptide and the retro, retro-inverso, and inverse derivatives. In the third very recent study, similar cross-reactivity was found between the major antigenic site of foot-and-mouth disease virus and its end group-modified retro-inverso isomer (23). Cumulatively these results ruled out the importance of the peptide backbone as the common structural recognition element providing cross-reactivity between linear antigens and the corresponding topologically related variants, since retro-inverso peptides, while structurally similar to the linear parent peptide in the side chain disposition, have totally inverted carbonyl and amide groups in the backbone. The hypothesis of the limited role of peptide backbone on antibody recognition was further supported from data deriving from a different study, where it has been shown that pseudopeptide analogues of the COOH-terminal hexapeptide of histone H3 obtained by systematically replacing, in each analogue, one
peptide bond at a time by a reduced peptide bond Y (CH$_2$–NH), maintained recognition properties for poly- or mononal antibodies raised against the original parent peptide (24). In our study, antigenic cross-recognition was found not only between the parent peptide and its retro-inverso derivative, or between the inverso and the retro derivatives, but with the complete set of topologically related peptides, to the same extent and with similar characteristics. These observations are in agreement with data on the cross-recognition properties of IgG3 mouse monovalent antibodies against the COOH-terminal histone H3 hexapeptide with the all-o derivative (16, 17). While structural similarities between retro-inverso and parent peptide, or between inverso and retro derivatives of the parent peptide, are clearly evident from a simple visual inspection of the peptide molecular models, and could constitute the simplest reason to explain cross-reactivity, no structural relatedness can be apparently detected between the parent peptide and its inverso or retro derivative. Inverso peptides are mirror images of the linear parent peptide, and consequently also the side chains are oriented in a nonsuperimposable way. The same is true for the retro and retro-inverso derivatives. But if enantiomeric peptides are aligned in the trans configuration, side chains in alternating residues (1, 3, 5, . . . or alternatively 2, 4, 6, . . .) can be superimposed, thus leading to topologically equivalent molecular surfaces formed by alternating residues. The epitope recognized by the antibody in this case is formed not by a linear array of residues, but by alternating residues. This suggests that a peptide antigen can display two recognition surfaces, one formed by residues in odd position, the other by residues in even position, and that probably both surfaces can take part in antibody recognition events, even independently. The cross-recognition dependence on the alternating array of side chains has been confirmed by experiments with sequence-simplified variants of the parent peptide, which represent a molecular dissection of the parent peptide side chains topology. Peptide variants, where only parent peptide side chains in alternating position were left, still retained a certain degree of cross-antigenicity. These findings have considerable significance for a more detailed understanding of antigen-antibody interaction and could constitute an interesting route to design sequence-simplified peptides able to cross-react with antibodies of predetermined specificity. In addition, the observed cross-reactivity between peptides displaying complementary side chains could suggest the possibility of incorporating a 2-fold antibody specificity on peptide antigens, by generating chimeric peptides characterized by side chains in alternating position belonging respectively to two different antigens. The structural model proposed in our study to explain cross-recognition between enantiomeric antigens is based on the assumption that peptide binding occurs in an extended and flexible conformation. A growing number of linear peptide antigens are seen to adopt an extended conformation upon binding to the corresponding antibodies (25–27). Similarly, extended conformations dominate T-cell epitope-major histocompatibility complex of class I or II complexes (28–30).

Our study also points out that, at least in the case systems analyzed, topologically related variants of the parent antigen do not need end group modification to display cross-antigenicity.

The generality of the phenomenon of antigenic cross-reactivity has also been confirmed with two other polyclonal antibodies raised against a multimeric 13 residue peptide (GRFKYL-HFRRHL) and a 14-residue peptide (RKFLAGLRARRLKF). In both cases the same type of antigenic cross-recognition was observed with the complete series of topochemically related peptides.2

Inverso- and retro-inverso peptides are particularly stable to proteolytic treatments since their inverted chiral configuration is not easily recognized by enzymes, making these derivatives useful for their prolonged half-life in vitro and in vivo. The stability and cross-antigenicity of retro-inverso and inverso peptides could provide a novel and interesting route for the treatment of various diseases associated to the immune system (31).

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