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Immunology

Sequences of the cell-attachment sites of reovirus type 3 and its anti-idiotypic/antireceptor antibody: Modeling of their three-dimensional structures

(hemagglutinin/epitope computer modeling/encephalitis/sequence similarity/peptides)

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ABSTRACT **Previous studies have identified an area of amino acid sequence similarity shared by the reovirus type 3 cell-attachment protein σ 1 and an anti-idiotypic/antireceptor monoclonal antibody (mAb) 87.92.6 that mimics reovirus type 3 by attaching to the same cell-surface receptor. We found that synthetic peptides corresponding to this area of primary sequence similarity bind a neutralizing mAb 9BG5 against which the mAb 87.92.6 is directed. The synthetic peptides compete with mAb 87.92.6 and reovirus type 3 for binding by mAb 9BG5 and displace mAb 87.92.6 and reovirus type 3 from binding to the cell-surface reovirus type 3 receptor. Such observations show that the shared primary structure between reovirus type 3 σ 1 polypeptide and antireceptor mAb 87.92.6 defines the oligopeptide neutralizing/cell-attachment epitope of reovirus type 3. Computer modeling of this epitope, by use of sequence similarities of known immunoglobulin hypervariable loop conformations, permits an examination of the rudimentary three-dimensional structure of this epitope.**

We investigated reovirus type 3 interactions with cellular receptors using antireceptor antibodies developed by an anti-idiotypic approach. A neutralizing antireovirus type 3 monoclonal antibody (mAb) termed 9BG5 was used to immunize mice, and an anti-idiotypic mAb denoted 87.92.6 was developed, which binds mAb 9BG5 and mimics the intact virus by binding to cell-surface receptors specific for reovirus type 3 (1–3). mAb 87.92.6 competes with reovirus type 3 for binding to specific cellular receptors, thereby mimicking the binding domain of the viral cell-attachment protein σ 1 (the viral hemagglutinin). This domain is implicated in the neutralizing-antibody response to reovirus type 3 (4, 5). Immunization of BALB/c mice with mAb 87.92.6 elicits neutralizing antibodies to reovirus type 3 (6). These data indicate that mAb 87.92.6 biologically resembles the epitope on σ 1 that interacts with the cellular receptor for reovirus type 3.

The nucleic acid sequences of the heavy- and light-chain variable regions of mAb 87.92.6 are known (7), and these sequences are similar to that of the reovirus type 3 σ 1 protein. A 16-amino acid sequence in the reovirus type 3 σ 1 protein, encompassing amino acids 317–332, resembles a combined sequence encompassing the second complementarity-determining regions (CDR IIs) of the mAb 87.92.6 heavy- and light-chain variable regions (V_H and V_L, respectively). Specifically, amino acids 43–51 of the V_H resemble amino acids 317–324 of σ 1, and amino acids 46–55 of V_L resemble amino acids 323–332 of σ 1 (7). Synthetic peptides corresponding to amino acids 317–332 of the σ 1 protein, 43–56 of the V_H sequence, and 39–55 of the V_L sequence were prepared to probe the significance of this shared homology.

MATERIALS AND METHODS

Peptide Synthesis and Conjugation. Peptides were synthesized using a model 430A Applied Biosystems peptide synthesizer (Applied Biosystems, Foster City, CA). Deprotection and release of the peptide from the solid-phase support matrix were accomplished by treating the protected peptide on the resin with anhydrous HF containing 10% (vol/vol) anisole or 10% (vol/vol) thioanisole for 1–2 hr at 0°C. The peptides were extracted with either ethyl acetate or diethyl ether and then dissolved in 10% (vol/vol) aqueous acetic acid and filtered to remove the resin. After lyophilization, the composition and purity of the peptides were determined with both amino acid analysis and reverse-phase high-performance liquid chromatography.

V_H and V_L peptides, both bearing an amino-terminal cysteine, were conjugated by mixing equimolar amounts in 0.1 M ammonium bicarbonate, pH 8.0, and stirring vigorously overnight at room temperature exposed to air. The conjugates were then lyophilized.

V_H or V_L was coupled to bovine serum albumin (BSA) by incubating BSA at 6 mg/ml with peptide at 6 mg/ml in 0.1 M sodium bicarbonate/0.1% glutaraldehyde and stirring overnight. The conjugates were dialyzed against distilled water and lyophilized.

RIA Procedure. The wells of 96-well V-bottom polystyrene plates (Dynatech Laboratories, Alexandria, VA) were coated with 2.5 μg of peptide by evaporation. Peptide-coated wells were washed three times with phosphate-buffered saline (PBS), blocked with 2% BSA/PBS/0.1% NaN_3 for 1 hr at 37°C, and washed; then iodinated antibody [purified over a staphylococcal protein A column and radioiodinated by the chloramine-T method (8)] was diluted to the number of cpm indicated in 1% BSA/PBS/0.1% NaN_3. After incubation at 4°C overnight the wells were washed, cut out, and the radioactivity counted. The cpm of mAb 9BG5 bound to blank wells was subtracted from cpm of 9BG5 bound to peptide-coated wells. Nonspecific binding to peptides was corrected for by subtracting from this value a similar value determined for an iodinated isotype-matched control mAb A11.

Polystyrene wells were coated with purified mAb 87.92.6 or control IgM,K antibody HO22.1 by incubation of purified antibody (purified on a goat antimouse IgM column), diluted

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Abbreviations: BSA, bovine serum albumin; PBS, phosphate-buffered saline; CDR II, second complementarity-determining regions; V_H and V_L, heavy- and light-chain variable regions, respectively; mAb, monoclonal antibody; Reo peptide, reovirus type 3 hemagglutinin peptide.

in 0.1 M $NaHCO_3$, pH 9.5, to 1 μg/ml with 50 μl per well, and incubated overnight at 4°C. The wells were washed, blocked as above, and washed again, and a mixture of radioiodinated mAb 9BG5 and peptides (at the concentrations noted) were added for 1 hr at 37°C. The wells were washed and counted as above. Specific cpm bound was always determined by subtracting cpm bound to blank wells coated with BSA from cpm bound for mAb 87.92.6-coated wells.

Binding of Reovirus Type 3 to Antibody and Cells. Staphylococcal protein A (Sigma) was diluted to 5 μg/ml in 0.1 M $NaHCO_3$, pH 9.6, and 50 μl per well was dispensed into 96-well polystyrene plates and incubated overnight at 4°C; the wells were decanted, washed three times in PBS, and blocked with 2% BSA/PBS/0.1% NaN_3 for 1 hr at 37°C. The wells were decanted, washed three times in PBS, and mAb 9BG5 or A11 diluted to 10 μg/ml in 1% BSA/PBS/0.1% NaN_3 was added (50 μl/well) for 1–3 hr at 37°C. The wells were decanted and washed three times in PBS. Competitors were added at the concentrations noted (100 μl per well), diluted in 0.5% BSA/5 mM phosphate buffer/0.45% NaCl, and preincubated for 45–60 min at 23°C. After preincubation with inhibitors, radioiodinated reovirus type 3 particles diluted in 1% BSA/PBS/0.1% NaN_3 were added (5–10 × 10^5 cpm per well), and incubation was continued for 45 min. Wells were decanted, washed eight to ten times with PBS, and the cpm bound was determined.

Murine L cells (4, 5) were suspended at 10^6 cells per ml in 1% BSA/PBS/0.1% NaN_3, and 50 μl (5 × 10^4 cells) was added to each well of a 96-well microtiter plate and preincubated with inhibitors at the concentrations noted for 45–60 min at 23°C. Equivalent input cpm of radioiodinated reovirus type 3 type 1 or variant K particles was added in 50 μl (700,000–1,250,000 cpm per well) and incubated for 45 min. The cells were washed three times in 1% BSA/PBS/0.1% NaN_3, and specific cpm bound was determined, as noted in Fig. 2*B*.

Flow Cytometry. R1.1 cells (10^7 cells/ml) (3) were incubated in 1% BSA with or without V_L–BSA, V_H–BSA, or peptides as indicated for 45 min. mAbs 87.92.6 or HO-13-4 were added at the concentrations noted for an additional 30 min, and fluorescence was monitored as described (1).

RESULTS AND DISCUSSION

Interaction of Neutralizing mAb 9BG5 with Peptides. The antireceptor mAb 87.92.6 binds to both the reovirus type 3 receptor and the neutralizing mAb 9BG5 (2). We hypothesized that peptides derived from similar areas of mAb 87.92.6 and the reovirus type 3 σ 1 protein (7) might have similar properties. The peptides synthesized to test this hypothesis are shown in Fig. 1; the reovirus hemagglutinin peptide (Reo peptide) corresponds to amino acids 317–332 in the type 3 viral hemagglutinin. Computer modeling predicts this area to be predominantly a β-sheet configuration and to include a β-turn. The V_L peptide represents amino acids 39–55 of the light-chain variable region of mAb 87.92.6 and includes the CDR II. Modeling predicts this area also to predominantly be a β-sheet and to include a β-turn. The V_H peptide contains amino acids 43–56 of the heavy-chain variable region of mAb 87.92.6, including a portion of the CDR II of the heavy chain.

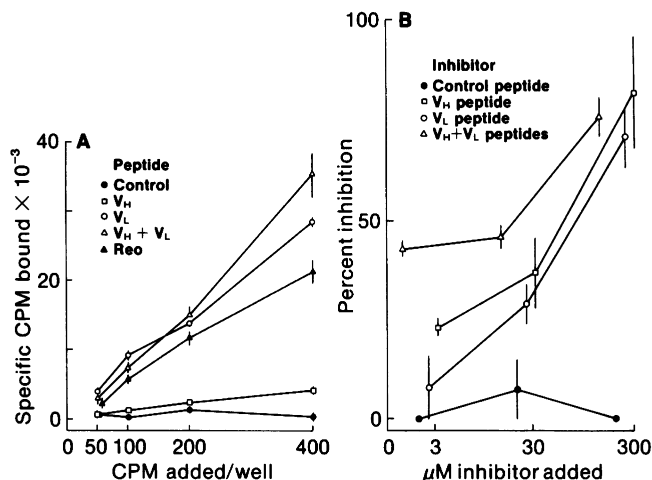

FIG. 2. Neutralizing antireovirus type 3 mAb 9BG5 interacts with modeled peptides. (*A*) Specific binding of ^{125}I-labeled 9BG5 to peptides was determined by RIA. Specific cpm (CPM) of 9BG5 bound to peptide-coated wells is shown versus the number of cpm added to each well. Mean ± SD for triplicate wells is shown. (*B*) Competition of binding of mAb 9BG5 to mAb 87.92.6-coated wells by peptides was done by a similar protocol. Binding of ^{125}I-labeled 9BG5 to wells coated with irrelevant mouse IgM,K antibody HO22.1 resembled binding to blank wells. Percent inhibition was determined by subtracting specific cpm bound with inhibitor from specific cpm bound without inhibitor, dividing this by cpm bound without inhibitor, and multiplying the result by 100. The mean ± SEM of values from two experiments is shown. Control peptide A was used in these studies.

The control peptides, whose linear sequences are unrelated to V_H, V_L, and the Reo peptide, are also shown.

We reasoned that these peptides might be recognized by the anti-reovirus type 3 neutralizing mAb 9BG5. The results of a RIA determining the specific binding of purified radioiodinated mAb 9BG5 to the wells of microtiter plates coated with the peptides are shown in Fig. 2*A*. Virtually no binding to control peptide A or to the V_H peptide was seen in this assay. Significant immunoglobulin binding to the V_L peptide, the carboxyl-terminal sequence of which strongly resembles the Reo peptide carboxyl terminus, was noted. Strong binding to the Reo peptide by mAb 9BG5 was also evident. The V_H and V_L peptides linked together by using an amino-terminal cysteine residue (V_H + V_L peptide) showed the highest level of mAb 9BG5 binding. Binding of the mAb 9BG5 neutralizing antibody to the V_L peptide and Reo peptide indicates that the area of sequence similarity between V_L and Reo peptides (amino acids 323–332 of the σ 1 protein) may be involved in the neutralizing epitope.

We also tested the ability of these peptides to inhibit the binding of mAb 9BG5 to the anti-idiotype mAb 87.92.6. As shown in Fig. 2*B*, both V_L and V_H peptides could significantly inhibit binding of ^{125}I-labeled mAb 9BG5 to microtiter wells coated with mAb 87.92.6. These findings indicate that mAb 9BG5 can interact with either the V_H or the V_L peptide in the liquid phase. The Reo peptide was too poorly soluble for use in liquid-phase assays. When the V_H + V_L peptide was used, inhibition of binding was more marked on a molar

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VH   (Cys) Gln Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly
            ●   o   o       ●   ●   ●       o
Reo        Gln Ser Met --- Trp Ile Gly Ile Val Ser Tyr Ser Gly Ser Gly Leu Asn
                                        o   o       ●   ●   ●   ●       ●   o
VL   (Cys) Lys Pro Gly Lys Thr Asn Lys Leu Leu Ile Tyr Ser Gly Ser Thr Leu Gln
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FIG. 1. Synthetic peptides containing the similar sequence of mAb 87.92.6 and reovirus type 3 hemagglutinin (Reo). The peptides were synthesized by solid-phase methods. Sequences are aligned with maximum homology. ●, Identical amino acids; o, amino acids of the same class. Control peptides used included A, Lys-Ser-Gly-Asn-Ala-Ser-Thr-Pro-Gln-Gln-Leu-Gln-Asn-Thr-Leu-Asp-Ile-Arg-Gln-Arg, and B, Cys-Asn-Gly-Ser-His-Val-Pro-Asp-His-Asp-Val-Thr-Glu-Glu-Arg-Asp-Glu.

basis; this indicates that the complex $V_H + V_L$ peptide more completely mimics the site of interaction of mAb 87.92.6 with mAb 9BG5.

Inhibition of mAb 9BG5 Binding to Reovirus by Modeled Peptides. By using a competitive binding assay, we examined whether these peptides specifically block the interaction between mAb 9BG5 and reovirus type 3. Reovirus type 3, but not reovirus type 1, competed with ^{125}I-labeled reovirus type 3 for binding (data not shown), indicating the specificity of the assay. Although the control peptide did not significantly affect reovirus type 3 binding to mAb 9BG5, V_L peptide markedly inhibited binding (Fig. 3). V_H peptide alone did not inhibit ^{125}I-labeled reovirus type 3 binding to mAb 9BG5. $V_H + V_L$ peptide was not significantly more inhibitory than V_L peptide alone in this assay (data not shown). These results indicate that amino acids 323–332 of the reovirus type 3 hemagglutinin specify the site of interaction of mAb 9BG5 with reovirus type 3.

Modeled Peptides Inhibit mAb 87.92.6 Binding to the Reovirus Type 3 Receptor. The neutralizing epitope on reovirus type 3, recognized by mAb 9BG5, is involved in binding to the reovirus type 3 receptor (1, 2, 9). As mAb 87.92.6 binds both to mAb 9BG5 and the reovirus type 3 receptor, the V_L peptide might also interact with the viral receptor. To test this hypothesis, we asked whether the V_H and V_L peptides, coupled to a protein carrier, could specifically block mAb 87.92.6 binding to the reovirus type 3 receptor on murine thymoma (R1.1) cells. As shown in Fig. 4*A*, preincubation of R1.1 cells with V_L peptide, coupled to BSA (V_L–BSA), blocked the binding of mAb 87.92.6, showing interaction of V_L–BSA with the reovirus type 3 receptor. This blocking effect was specific, as preincubation of R1.1 cells with V_L–BSA had no effect on the binding of mAb HO-13-4, an isotype-matched control mAb that binds to the Thy-1.2 molecule present on the R1.1 cell surface (Fig. 4*B*). Thy-1.2 and the reovirus type 3 receptor are expressed at equivalent densities on R1.1 cells (2). As another control, we showed that V_H–BSA has no inhibitory effect on mAb 87.92.6 binding when used at the same concentration as V_L–BSA (Table 1). These data indicate a direct interaction of the V_L peptide with the reovirus type 3 receptor and imply that amino acid residues 46–55 of the mAb 87.92.6 light-chain variable region, and 323–332 of the type 3 σ1 protein, directly interact with the reovirus type 3 receptor.

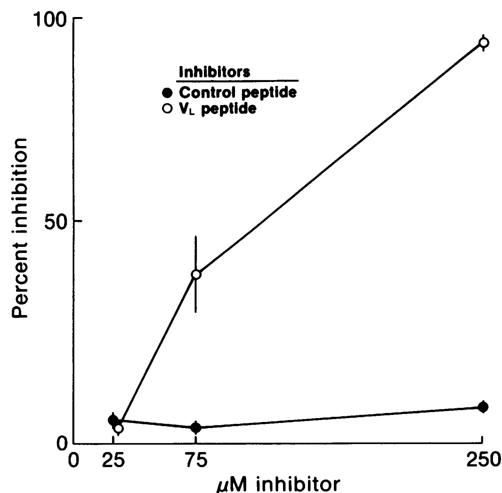

FIG. 3. V_L peptide inhibits binding of reovirus type 3 particles to mAb 9BG5. In this experiment 6700 cpm were bound to mAb 9BG5-coated wells, and 500 cpm were bound to control (mAb A11)-coated wells without inhibitors; control peptide B was used. The mean ± SD of binding inhibition (determined as noted in Fig. 2) of replicate wells is shown.

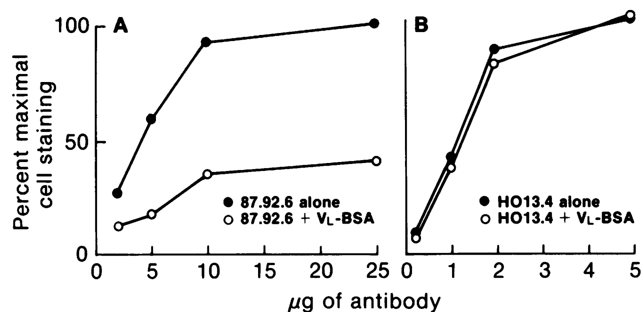

FIG. 4. (*A*) V_L peptide inhibits binding of antireovirus receptor mAb 87.92.6 to R1.1 cells. Binding of V_L–BSA to the reovirus type 3 receptor was determined by its ability to compete for binding with anti-type 3-receptor mAb 87.92.6. (*B*) V_L peptide does not inhibit binding of isotype-matched control mAb HO13.4. V_L–BSA was added at 200 μg/ml. The maximal percent positive mAb values were as follows: mAb 87.92.6, 15.3%; mAb HO13.4, 97%.

In contrast, uncoupled V_L peptide was unable to successfully compete with mAb 87.92.6 for receptor binding (Table 1). When V_H and V_L peptides were coupled together, significant inhibition of mAb 87.92.6 binding was seen. This fact implies that the same structural or conformational factors important in mAb 87.92.6 binding by mAb 9BG5 are also important in the interaction of mAb 87.92.6 with the reovirus type 3 receptor on R1.1 cells.

Although the complex of $V_H + V_L$ peptide appears to more completely mimic the site of interaction in the anti-idiotype (mAb 87.92.6)—idiotype (mAb 9BG5) interaction (Fig. 2) and the interaction between mAb 87.92.6 and the receptor, in keeping with the general idea of such interactions (10, 11), the effective addition of the V_H sequence may stabilize the conformational properties of the putative V_L portion of the complex. The structural similarities between the putative V_L loop in mAb 87.92.6 and the Reo peptide help elucidate residues essential for antibody and receptor recognition and binding as well as residues that may stabilize secondary structure essential to presenting the antigenic site.

V_L Peptide Inhibits Reovirus Type 3 Binding to L Cells. Murine L cells have specific receptors for reovirus type 3. Prior studies indicate specific saturable binding of radiolabeled reovirus type 3 to L cells that can be inhibited by unlabeled reovirus type 3 (12). We used this assay to determine whether V_L peptide could inhibit radioiodinated reovirus type 3 from binding to L cells. As shown in Fig. 5*A*, V_L peptide markedly inhibited reovirus type 3 binding to L cells, whereas a control peptide had no effect; this inhibition was consistently found in multiple assays. V_H peptide and several other control peptides had no effect on reovirus type

Table 1. Inhibition of antibody binding

Inhibitor	Positive, % mAb HO13.4	Positive, % mAb 87.92.6
Experiment 1		
None	99	60
V_H–BSA	99	67
V_L–BSA	99	20
Experiment 2		
None	99	85
V_L alone	92	80
$V_H + V_L$	88	33

Percent positive for mAb on flow cytometry is shown in each instance uncorrected. In experiment 1, antibody was added at 10 μg per sample with inhibitors added at 1 mg/ml, whereas in experiment 2, mAb HO13.4 was added at 10 μg per sample, mAb 87.92.6 was added at 25 μg per sample; inhibitors were present at 500 μg/ml.

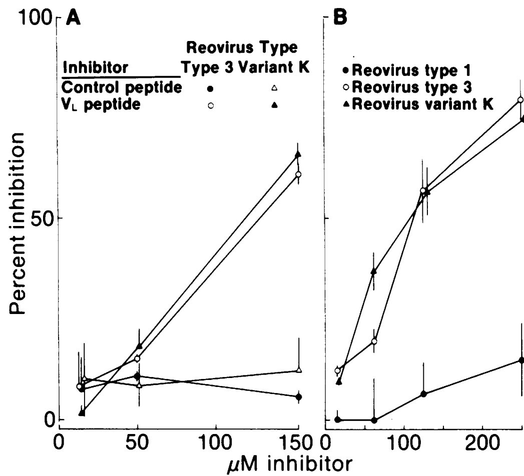

FIG. 5. (*A*) V_L peptide inhibits binding of reovirus type 3 and variant K to L cells. Mean ± SD percent inhibition of binding from replicate wells is shown versus final competitor concentration. Control peptide B was used. (*B*) Radioiodinated reovirus binding to L cells.

3 binding to L cells. V_H peptide coupled to V_L peptide could also inhibit reovirus type 3 binding, but with no greater efficacy than free V_L peptide (data not shown). V_L peptide also inhibited binding of reovirus type 3 to R1.1 cells and murine B104 neuroblastoma cells (data not shown; ref. 3). This binding inhibition is a property of the hemagglutinin, as radioiodinated reovirus type 1 binding to L cells was not significantly inhibited by V_L peptide (Fig. 5*B*), whereas binding of reassortant virus 1-HA.3 (bearing the reovirus type 3 hemagglutinin gene with all other genome segments derived from reovirus type 1; refs. 4 and 5) was inhibited (13). Thus V_L peptide interacts specifically with the reovirus type 3 receptor, and amino acid residues 323–332 of the hemagglutinin are implicated in the interaction between reovirus type 3 and its receptor on L cells. The inability to augment inhibition by the coupling of V_H peptide to V_L peptide shows that, although the V_H peptide epitope is important in mAb 87.92.6 binding to mAb 9BG5 and the reovirus type 3 receptor, reovirus type 3 does not use the V_H peptide portion of the hemagglutinin sequence to directly interact with the receptor or mAb 9BG5.

Elucidation of the Mechanism by Which Reovirus Type 3 Variant K Escapes Neutralization by mAb 9BG5. In prior studies, antigenic variants of reovirus type 3 were developed by growing reovirus type 3 in the presence of 9BG5 neutralizing mAb (14); these variants had diminished virulence compared to reovirus type 3. Several of these variants were subjected to nucleotide sequencing (15), revealing an alteration in either amino acid 419 or amino acid 340 of the reovirus type 3 hemagglutinin. One of these variants, designated variant K, had a Glu → Lys alteration at residue 419, markedly diminished neurovirulence, and altered tissue tropism (15)—as well as being resistant to neutralization by mAb 9BG5. We analyzed variant K binding to L cells using radioiodinated virus as described. We found specific binding of variant K to receptors on L cells, which was inhibited by unlabeled variant K and unlabeled reovirus type 3 but not by reovirus type 1, whereas radioiodinated reovirus type 3 binding to L cells was inhibited by unlabeled variant K (data not shown). These data indicate that reovirus type 3 and variant K use the same receptor on L cells.

We next investigated binding of variant K by mAb 9BG5. In solid-phase RIA with polystyrene wells coated with viral particles, we found high levels of mAb 9BG5 binding to reovirus type 3 and negligible binding to variant K (data not shown). In the assay described in Fig. 2, we found high levels of binding of radioiodinated reovirus type 3 to mAb 9BG5, diminished but significant binding of variant K, and no significant binding of reovirus type 1 (10,000 cpm of type 3; 1500 cpm of variant K; and <100 cpm of type 1 virus bound to mAb 9BG5 with equivalent input counts of virus). These data indicate a marked difference in the ability of mAb 9BG5 to interact with the hemagglutinin on variant K, compared to the reovirus type 3 hemagglutinin.

Although variant K has diminished affinity for binding to mAb 9BG5, it still uses the same epitope as the parent type 3 virus for binding to murine L cells (Fig. 5*B*). V_L peptide inhibits reovirus type 3 and variant K binding to L cells with equivalent efficacy. In contrast, no significant inhibition of reovirus type 1 binding to L cells is seen. V_L peptide inhibition of variant K binding is specific, as several control peptides and V_H peptide were unable to inhibit variant K binding (Fig. 5, and data not shown).

We conclude that variant K uses the same residues on the viral hemagglutinin as the parent virus to attach to L cells but escapes neutralization by mAb 9BG5 by limiting the ability of mAb 9BG5 to interact with this site on the hemagglutinin.

Predicted Structure of the Cell-Attachment Site of Reovirus Type 3 and Its Internal Image. These data support a structural similarity between the reovirus type 3 cell-attachment epitope and its internal image on the mAb 87.92.6 V_L CDR II. We reasoned that the structure of the mAb 87.92.6 V_L CDR II and the reovirus type 3 cell-attachment epitope would be similar to other immunoglobulin hypervariable loops with similar amino acid sequences. After searching the protein data base, we selected two immunoglobulin CDR II sequences, both with crystallographically defined three-dimensional structures, that had sequence similarity to the reovirus type 3 hemagglutinin sequence and the mAb 87.92.6 V_L CDR II sequence (Fig. 1).

NEWM is a human IgG1 antibody, and the sequence of its heavy-chain CDR II, compared to the reovirus type 3 hemagglutinin sequence, shares 6 identities and 4 conservative substitutions. Similarly, the sequence of the V_L CDR II of REI (a human light-chain dimer) and of the mAb 87.92.6 V_L CDR II share 11 identities and 1 conservative substitution.

Computer modeling of these structures was accomplished by making appropriate amino acid side-chain substitutions and performing energy minimization calculations on the resultant structures. The reovirus hemagglutinin epitope was modeled after both the REI and NEWM CDR II structures—with the best energy minimization obtained for the NEWM structure. Fig. 6*A* shows the predicted structure of the mAb 87.92.6 V_L CDR II in the area of highest sequence similarity with the reovirus type 3 hemagglutinin, whereas the corresponding region of the reovirus type 3 hemagglutinin is shown in Fig. 6*B*. The region of the β-turn in these structures has the greatest degree of sequence similarity, and for both this region is predicted to form into a turn structure by the Chou and Fasman algorithms (16). The degree of sequence similarity between these regions and their predicted locations lead us to postulate that this β-turn area is involved in the interaction with mAb 9BG5 and the reovirus type 3 receptor. Note that, although the backbone configurations of these turns differ, the positions of the amino acid side chains are nearly identical (Fig. 6*C*).

IMPLICATIONS

By demonstrating sequence similarity between the σ 1 cell-attachment protein of reovirus type 3 and the antireceptor mAb 87.92.6, we could localize the cell-attachment site of reovirus type 3 with some precision. If attachment of an organism to specific cellular receptors is important in the pathogenesis of infection by that agent, the approach outlined here should result in the general ability to determine the

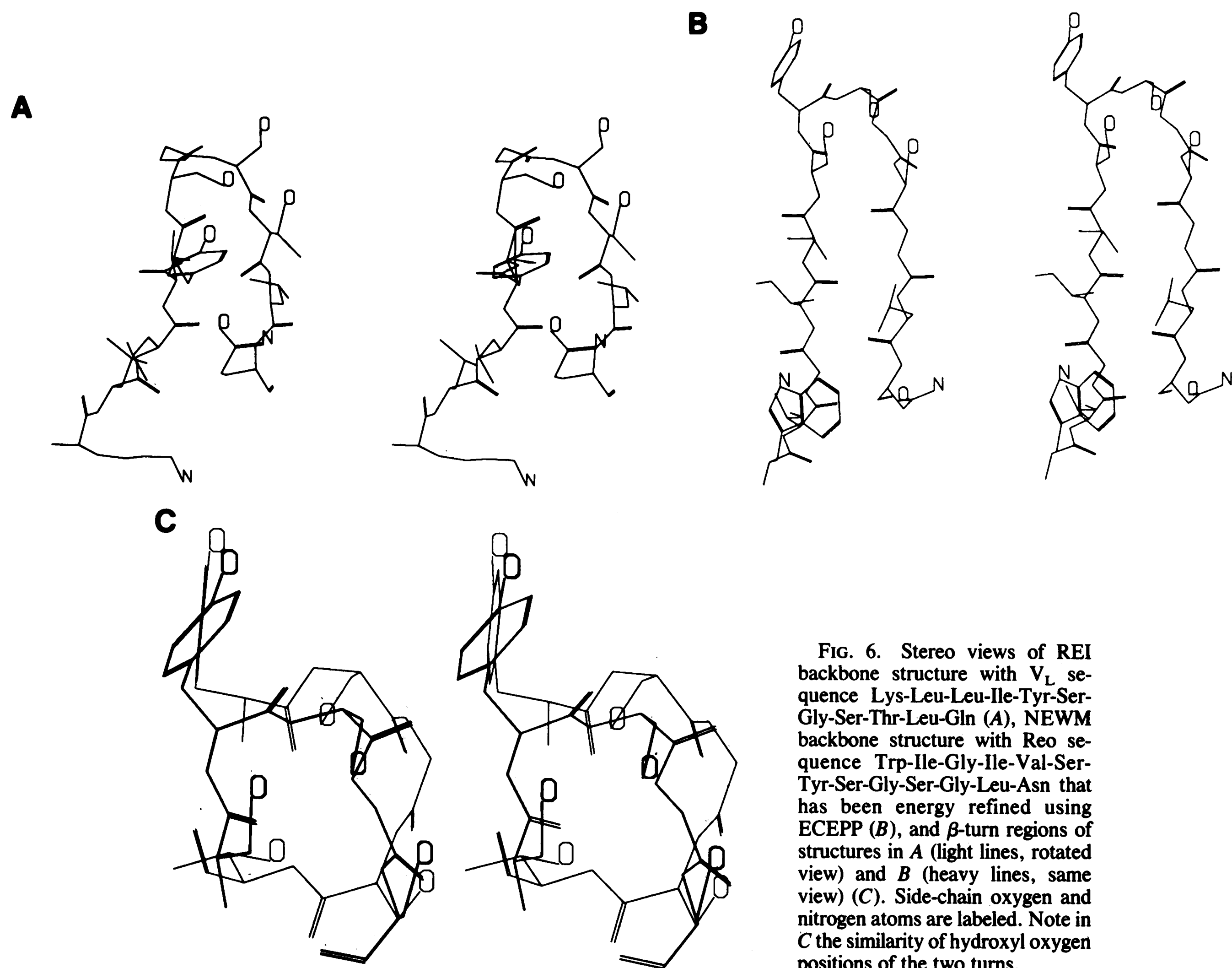

FIG. 6. Stereo views of REI backbone structure with V_L sequence Lys-Leu-Leu-Ile-Tyr-Ser-Gly-Ser-Thr-Leu-Gln (*A*), NEWM backbone structure with Reo sequence Trp-Ile-Gly-Ile-Val-Ser-Tyr-Ser-Gly-Ser-Gly-Leu-Asn that has been energy refined using ECEPP (*B*), and β-turn regions of structures in *A* (light lines, rotated view) and *B* (heavy lines, same view) (*C*). Side-chain oxygen and nitrogen atoms are labeled. Note in *C* the similarity of hydroxyl oxygen positions of the two turns.

oligopeptide epitopes involved in pathogen–receptor interactions. This approach should also apply more widely to other receptor–ligand interactions and, for polypeptide ligands, may permit the identification of the precise epitopes bound. Potentially this strategy could lead to the design of biologically active substances that would interact with specific receptors in predictable ways.

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