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Immunology

Human autoantibodies reactive with synthetic autoantigens from T-cell receptor β chain

(autoimmunity/IgG/peptides/molecular modeling)

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ABSTRACT **We used mapping with synthetic overlapping peptides in combination with molecular modeling to analyze the IgG antibodies that humans naturally produce against human T-cell receptor β chains and to localize the recognized peptide autoantigens in the three-dimensional structure of the molecule. Healthy individuals produce low levels of antibodies against T-cell receptor peptides, and these can be increased in autoimmune diseases. We characterized the reactivities in detail because IgG molecules reactive with self peptides occur in preparations of intravenous immunoglobulin and can be isolated by immunoaffinity chromatography. Natural IgG antibodies were directed against three major peptides. One corresponds to the first complementarity-determining region of the variable region. A second corresponds to the third framework of the variable region. The third is located in the constant region and is predicted to be a loop that extends out of the β-barrel structure. This peptide is one that would give a characteristic structural distinction between the β-chain constant region and the constant regions of immunoglobulin light chains to which β chains are homologous. The capacity to bind these peptides is found in small fractions of normal polyclonal IgG, which contains both κ chains and λ chains. The activity is antibody-like in being confined to the Fab fragment and in its capacity to discriminate among homologous synthetic peptides corresponding to distinct β-chain variable-region genes. We propose that a recognition and regulatory process naturally occurs that parallels the immune network for the regulation of the production of antibodies.**

Humans produce antibodies to a variety of self antigens without noticeable ill effects (1–4). The production of antibodies themselves is regulated in part by autoimmune responses to defined portions of the antibodies, most notably combining site-related markers or idiotypes (5, 6) and constant-region markers detected by IgM antibodies termed rheumatoid factors (7). Because of the crucial importance of immunoglobulin-like T-cell receptors (TCRs) in the initiation of specific immunity, we chose to focus on natural human antibodies to synthetic peptides based on human TCR β-chain gene sequence. We assessed by enzyme-linked immunosorbent assay (ELISA) the capacity of sera to react with synthetic TCR peptide autoantigens. These sera were obtained from ostensibly healthy individuals 20–90 years of age and from patients with rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE). Hexadecapeptides representing the TCR YT35 β chain, which contains $V_\beta 8$, $D_\beta 1.1$, $J_\beta 1.2$, and $C_\beta 1$ segments (8), were overlapped by 5 residues to model the antigenic structures (9, 10) of the TCR β fragment homologous to immunoglobulin λ light chain (11).

We found that reaction of human IgG with particular synthetic TCR autoantigens occurs in low levels in healthy individuals and can rise in autoimmune diseases such as RA. We present immunochemical data characterizing the nature and locations of the TCR β peptides recognized by the natural human antibodies. Our data show that human IgG autoantibodies recognize TCR β segments corresponding to idiotype, restricted variable-region framework, and constant-region markers.

MATERIALS AND METHODS

Human Sera and IgG. Sera of healthy individuals of both sexes in the age range 20–60 years and of patients with RA or SLE were obtained through the Division of Rheumatology, Department of Internal Medicine, College of Medicine, Arizona Health Sciences Center, Tucson. Sera of healthy individuals in the age range 70–90 years were obtained through the courtesy of John T. Boyer (Division of Geriatric Medicine, Department of Internal Medicine, College of Medicine, Arizona Health Sciences Center, Tucson). Peptide binding studies were performed on sera of 29 healthy adults under 60 years of age, 21 healthy elderly individuals 70–90 years of age, 14 RA patients, and 8 SLE patients. The results of this study will be reported in detail elsewhere; data given here are representative of the human groups. Commercial human IgG (immune globulin intravenous [human], Baxter Healthcare, Santa Ana, CA, or Sandoglobulin, Sandoz Pharmaceutical) was prepared as a starting solution of 50 mg/ml in water. The Sandoglobulin was a gift of Sandoz Pharmaceutical.

Peptides and Antibodies. The gene sequence of human TCR β YT35 (12) was used to construct a series of 22 synthetic hexadecapeptides that overlapped by 5 residues. These were synthesized by the University of Arizona Biotechnology Center, using an Applied Biosystems peptide synthesizer. Purity was determined by amino acid composition and sequence analysis. When necessary, peptides were purified by reverse-phase HPLC. Rabbit antibody to TCR peptide β8 was raised as described (13).

Competitive ELISA. Beginning with 500 μg of free peptide, we made 2-fold serial dilutions of peptide and incubated each dilution with a constant amount of antibody for 30 min. These were incubated on peptide-coated plates for 1 hr, and the standard ELISA procedure was then followed. These procedures are described in detail elsewhere (10, 14).

Affinity Purification of Human IgG. Peptide (5 mg) in 0.1 M sodium carbonate (pH 8.0) was incubated with 1 g of activated CH Sepharose 4B (Pharmacia) at room temperature for 2 hr. The Sepharose was then washed with Tris-buffered saline (TBS: 150 mM NaCl/25 mM Tris, pH 8.0), treated with

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Abbreviations: TCR, T-cell receptor; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

1 M ethanolamine (pH 8) for 1 hr to block unreacted sites, washed with TBS, and poured into a column (6 cm × 0.8 cm). The coupling procedure was essentially as described by the manufacturer.

Human IgG (50 mg/ml) was diluted to 25 mg/ml with TBS, and 4 ml of this dilution was applied to the column at a rate of 5 ml/hr. After washing, the retained antibodies were eluted with 1 M propionic acid in 1-ml fractions and immediately brought to neutral pH with 3 M Tris (pH 9). The eluates were then characterized by ELISA. Detailed descriptions of these procedures are given elsewhere (14).

RESULTS AND DISCUSSION

Reaction of Human Immunoglobulins with Synthetic Overlapping TCR β Peptides. Fig. 1 compares the profiles of peptide reactivities among representative sera from a healthy 50-year-old woman (*A*), a healthy 74-year-old woman (*B*), a patient suffering from SLE (*C*), a patient with RA (*D*), and two preparations of commercial IgG used for prophylactic purposes (*E* and *F*). The commercial IgG preparations lacked readily detectable IgM (3), and the observed patterns reflect only IgG binding activities.

The normal serum in Fig. 1*A* shows weak reactivities against peptides β3 and β8 that are apparent at the 1:200 dilution but are lost at the higher dilutions. The serum of the elderly individual (Fig. 1*B*) reacts with peptide β8 but shows only marginal activity against other peptides, including β3 and β4, β13, and β17. The serum of the SLE patient (Fig. 1*C*) shows definite activity against peptides β3 and β8 and also reacts with peptides 17 and 20. The strongest serum reaction is shown by the RA patient (Fig. 1*D*), whose serum gives definite activities against peptides β3, β8 (and β9), and β17 that are detectable at dilutions of 1:200 and 1:1000. The commercial IgG preparations (Fig. 1 *E* and *F*) show definite reactivities against peptides β3, β8, and β17. The strength of reactivity and the range of activities of the Sandoz IgG preparation (Fig. 1*E*) is greater than that shown for the Baxter

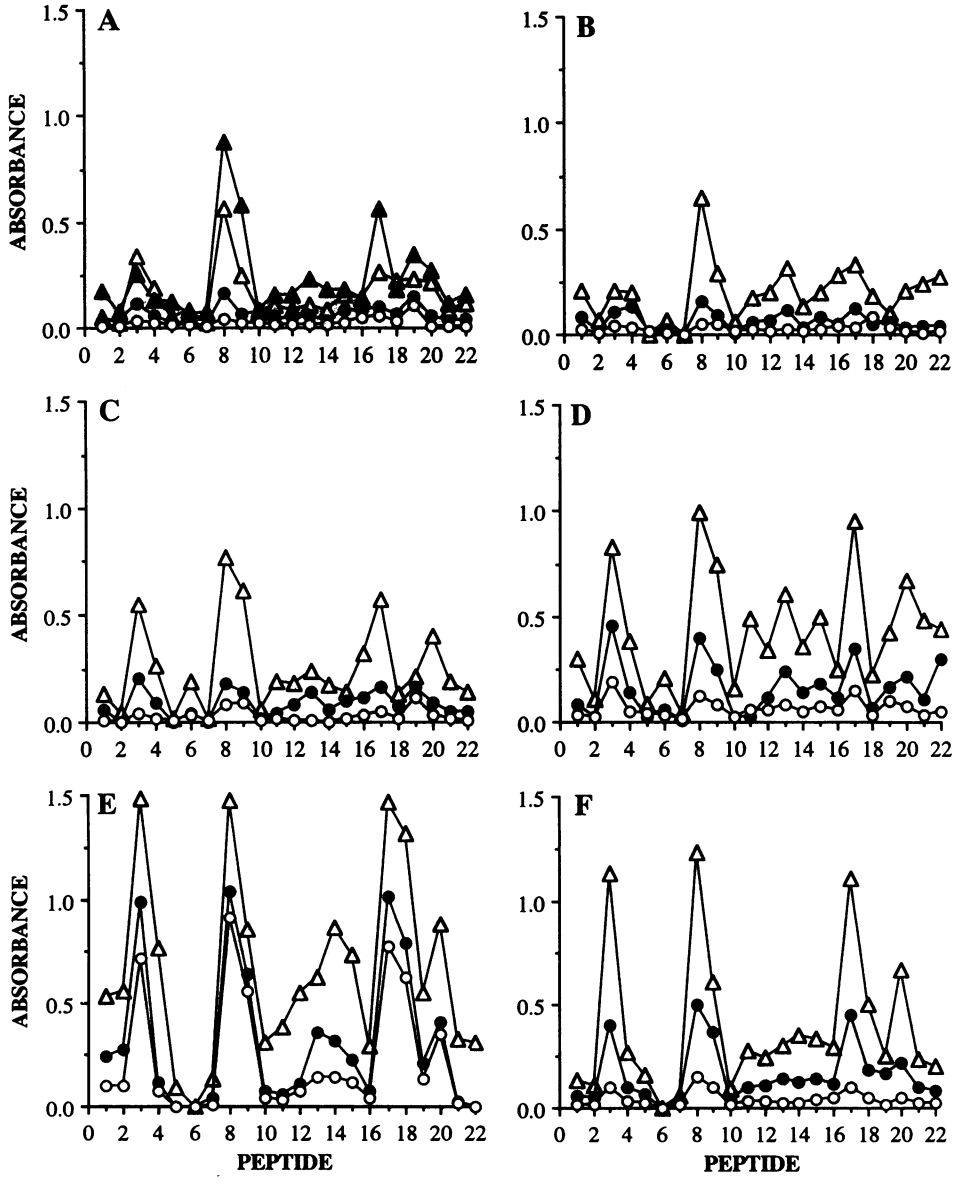

FIG. 1. Analysis by ELISA titration of the reactions of human immunoglobulins with individual ordered overlapping peptides modeling human TCR β sequence. Samples were serum of a normal 50-year-old woman (*A*), serum of a normal 74-year-old woman (*B*), serum of a patient suffering from SLE (*C*), serum of a patient with RA (*D*), commercial polyclonal IgG (Sandoglobulin) (*E*), and commercial polyclonal IgG (Baxter) (*F*). The sera or globulin preparations were tested at dilutions of 1:200 (△), 1:1000 (●), and 1:5000 (○). In *A* a polyclonal IgG isolated from a pool of 14 normal sera was tested at a dilution of 1:200 (▲). In *A–D*, rabbit antisera directed against both human IgM and IgG were used to obtain a total profile of the natural antibody activity.

preparation (Fig. 1*F*), although both bind the same three major peptides. The sequences of these autoantigenic peptides are as follows: β3, CKPISGHNSLFWYRQT; β8, KIQPSEPRDSAVYFCA; and β17, QPLKEQPALNDSRYCL.

We prepared IgG from pooled sera of 14 normal individuals by affinity chromatography on protein A/G (Pharmacia) and tested this preparation for its capacity to bind the set of overlapping TCR peptides (Fig. 1*A*). The profile was similar to the normal serum pattern inasmuch as the binding to peptide β8 was strongest. Binding to peptides β3 and β17 also occurred.

IgG Autoantibody Activity Against TCR Peptides. The preceding data show that human serum IgM and IgG can bind directly in solid-phase assays to synthetic peptides predicted from TCR sequence. We carried out a number of studies to determine whether this binding was antibody activity rather than protein/peptide interaction that was not dependent upon the presence of an antibody combining site. In one test, we prepared Fab fragments from human IgG and found that these were responsible for the binding profiles shown in Fig. 1. Two other criteria were also invoked. Solid-phase immunoadsorbents consisting of β3, β8, or β17 were used to determine whether the antibody activities could be isolated by immunoaffinity chromatography and in which total fraction of the IgG. The second criterion was based on whether the binding could be inhibited by the peptides used in the isolation.

As shown in Fig. 2, small distinct subsets comprising <0.1% of the starting polyclonal IgG bound to these peptides. These antibodies could be eluted with 1 M propionic acid. The specific subsets were enriched ≈1000-fold relative to the starting material. Although specific for individual peptides, they were polydisperse in that they contained both κ and λ chains. Strong inhibition of the binding of the affinity-purified anti-β3 to β3 could be obtained by using the homologous peptide. Peptide β8 showed moderate inhibition, but peptide β17 did not inhibit the binding of the affinity-purified antibody. This latter finding suggests that the binding affinity was either too high or too low to be characterized under these conditions.

Fine Specificity of Natural and Induced Antibody for the β Peptide Sequences. The TCR β chain modeled in these studies was based on $V_{\beta}8$ gene sequence (8). One of the major peptides recognized by human antibodies is peptide β8, which corresponds to the third framework of the variable

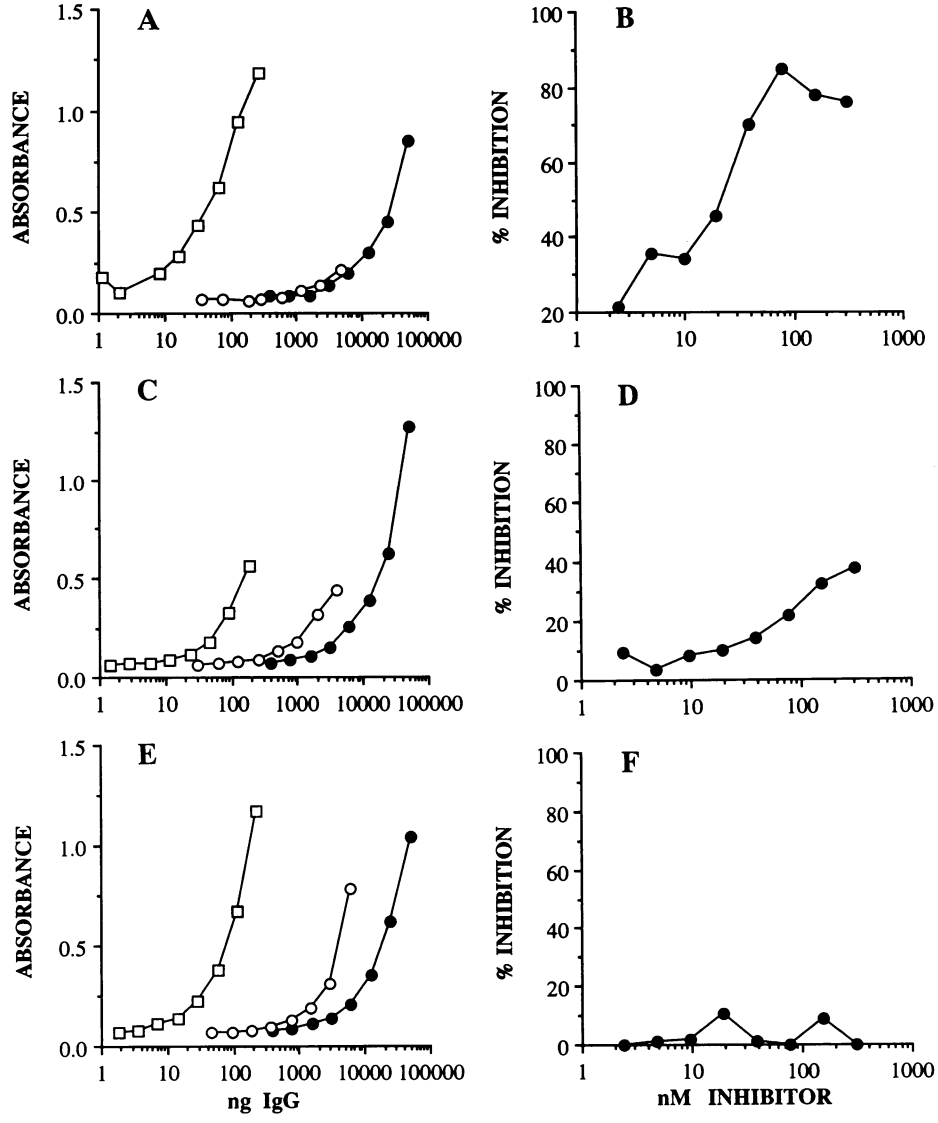

FIG. 2. (*Left*) Quantitative analysis by direct ELISA binding of commercial polyclonal IgG (Baxter) (●), antibody affinity-purified on peptide columns (□), and the "unbound," or "flow-through," fraction (○) against the peptide β3 (*A*), β8 (*C*), or β17 (*E*). (*Right*) Inhibition of the binding of affinity-purified anti-peptide antibody by the corresponding soluble peptide: β3 (*B*), β8 (*D*), or β17 (*F*).

region (13). This region contains amino acid sequences that vary among V_β genes and contribute to defining the particular V_β subset. We synthesized hexadecapeptides from five β8 homologs and tested the capacity of affinity-purified natural human antibodies and induced rabbit antibodies directed against the β8 peptide to discriminate among members of the set. The set contained the peptides shown in Fig. 3 *Right*.

In ELISA titrations the affinity-purified human IgG antibody to β8 shows (Fig. 3*B*) a hierarchy of reactivity with binding to β8 > β6.3 > β2.1 = β12.1 > β5.2 = β17.1. The affinity-purified subpopulation of a rabbit antiserum raised against peptide β8 (Fig. 3*A*) reacts strongly with β8 and also strongly, but to lesser degree, with peptides β6.3, β5.2, and β12.1. It does not react significantly with the peptides β2.1 and β17.1. Thus, both natural human IgG antibodies and induced rabbit IgG antibodies show a hierarchy of reactivities allowing discrimination among the V_β subsets. It is interesting that the V_β8 and V_β6.3 sequences show strong crossreactivity, because these two are related to one another (63% identity in this peptide and 56% overall) and also to the murine V_β11 family by analysis with the computer programs of Feng and Doolittle (15). The immune rabbit and natural human antibodies both react poorly, if at all, with peptide β17.1, which shows only 25% identity to the V_β8.1 sequence in peptide β8.

CONCLUSIONS AND SPECULATIONS

Normal individuals of diverse species continually produce antibodies to a variety of self components (1–4, 7, 16, 17). Our data show that low levels of IgM and IgG antibodies against synthetic TCR β peptide autoantigens occur in the blood of healthy humans and are increased in some autoimmune diseases. Kaveri *et al.* (18) have reported that people and mice express naturally occurring antibodies that bind to a peptide located in the complementarity-determining region 2/framework 3 sequence of the heavy-chain variable region of the murine myeloma protein S107/T15. We find that naturally occurring IgM antibodies are more frequent than IgG (unpublished work). The presence of the latter indicates a requirement for T-cell help and suggests that TCRs naturally recognize other TCR V_α/V_β structures expressing distinct idiotypes or subgroups. The distribution of the prominent TCR peptides involved in binding shows that natural IgG antibodies can recognize structures equivalent to idiotypes, framework residues, and the constant region. The existence of T cells specifically reactive with the idiotypic determinants on other T cells has been described (19).

Fig. 4 presents a three-dimensional model of the TCR β chain that was constructed on the basis of sequence similarities with human λ-type light chains and maps the localization of the peptides that react with human autoantibodies. The major peptides correspond in position to the first complementarity-determining region of the variable region (β3), to a loop in the third framework of the variable region (β8), and to a large loop in the constant region (β17) that extends out of the β-barrel structure of the constant region. Based upon their predicted positions, these peptides would be accessible both to antibodies and to TCRs. The localization of an antigenic determinant to the third framework region is interesting because rabbit heavy-chain variable-region *a* allotypes are conformational determinants consisting of residues contributed from the first and third frameworks, both of which are exposed on the outer face of the folded structure (23). With respect to TCRs, it is noteworthy that the third framework region of the molecule, rather than the combining site for antigen, is implicated in the binding of "superantigens" (24). Peptide β3, which corresponds to complementarity-determining region 1, could be considered to represent an idiotypic site on the human TCR. Our results show that naturally occurring antibodies, and presumably TCRs, can be directed against antigenic regions of self TCRs just as antibodies and TCRs can be directed against characteristic regions of self immunoglobulins. Antibodies and T cells directed against idiotypic determinants are generated in immune responses, and these play a part in subsequent regulation of the particular response (5, 6). IgM antibodies directed against constant regions of γ heavy chain are produced in secondary immune responses (7).

We propose that parallel recognition and regulatory processes are directed toward the corresponding regions of TCRs as are directed against immunoglobulins. We also suggest that a primary function of natural antibodies is regulatory in the sense that they are directed against self antigen-specific recognition molecules. Moreover, they may play a part in the modulation of specific immune responses following the introduction of exogenous antigens. Regulatory IgG autoantibodies have been shown to function in the removal of aged red blood cells expressing senescent cell antigen (25), and it has been shown that these autoantibodies recognize peptide determinants expressed by modified self antigens (26). The presence of autoantibodies to defined human TCR peptides in commercial preparations of intravenous immunoglobulins may lead to an understanding of the success of such preparations in the treatment of autoimmune diseases (3).

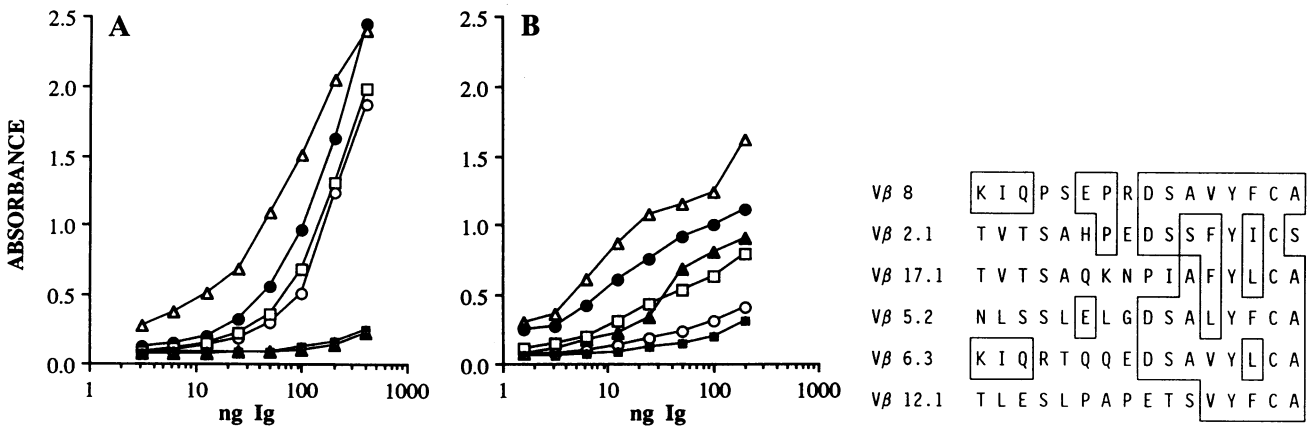

FIG. 3. Quantitative ELISA of binding of affinity-purified rabbit antibody to peptide β8 (*A*) and affinity-purified natural human IgG antibody to β8 (*B*) to a set of homologous TCR peptides corresponding to the β8 segment. The designation of V_β subgroups (genes) is that of Toyonaga and Mak (8). △, V_β8; ▲, V_β2.1; ○, V_β5.2; ●, V_β6.3; □, V_β12.1; ⊞, V_β17.1. The actual peptide sequences are given at right.

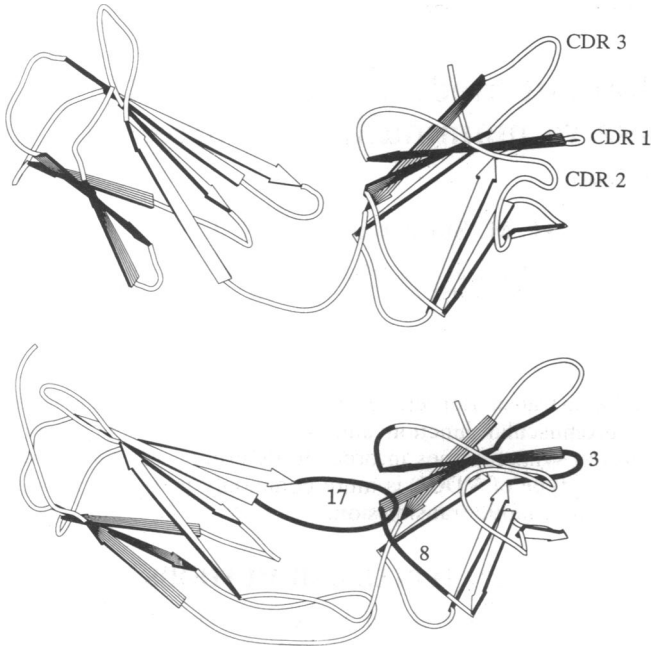

FIG. 4. Locations of TCR β-chain peptides that react with human autoantibodies. A model of part of the β chain was constructed on the basis of sequence similarities with human λ-type light chains (13). The three-dimensional structure of the Mcg λ chain (20) (*Upper*) was used as the initial template for the β-chain model (*Lower*). These models were generated with the aid of the program RIBBON (21). In the Mcg model the light-chain variable (V_L) domain is on the right and the constant (C_L) domain is on the left. Hypervariable loops contributing to the antigen binding site are labeled CDR 1, 2, and 3 (complementarity-determining regions; ref. 22). Arrows represent segments of antiparallel β-pleated sheets. The three-stranded sheets are shaded and the four-stranded layers are in white. Corresponding segments in the β chain are assumed to have the same general secondary structures as in the λ chain. Alignment of the sequences of the V domains of the two proteins posed no major problems with respect to the three-dimensional features, and only small differences were noted. The presence of three insertions made the alignments of the C domains less straightforward. We chose to maintain the three- and four-stranded layers as the basic structural motif and loop out the additional residues as shown in the model of the β chain (see loop marked 17, the loop below it, and the carboxyl-terminal segment). The hexadecapeptides 3, 8, and 17 represent segments shown in black and numbered in the β-chain model. Peptide 3 includes CDR 1, peptide 8 consists of residues from framework region 3, and peptide 17 contains one of the inserts in the C domain. Note that all three peptides correspond to loops that should be accessible to antibodies, as predicted from the reactivities of the peptide mimics alone (see Fig. 1).

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