MINIREVIEW

Synthetic Autoantigens of Immunoglobulins and T-Cell Receptors: Their Recognition in Aging, Infection, and Autoimmunity (43801)

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Abstract. **Immunoglobulins and their close relatives, the antigen-specific T-cell receptors, are recognition proteins that express structures which readily serve as self-immunogens. Healthy humans can produce antibodies against variable region-defined recognition structures termed idiotypes, as well as against constant region structures, and the levels of these can increase markedly in autoimmune disease; e.g., rheumatoid factors are autoantibodies directed against a conformational determinant of the γ heavy chain. More recent analyses employing synthetic peptide technologies and construction of recombinant T-cell receptors document that autoantibodies directed against both variable and constant region markers of the α/β T-cell receptor occur in healthy individuals. Alterations in levels of antibody, usage of IgM or IgG isotypes, and specificity for particular peptide-defined regions vary with natural physiological processes (aging, pregnancy), with artificial allografting, with retroviral infection, and with the inception and progression of autoimmune disease (e.g., rheumatoid arthritis, systemic lupus erythematosus). Two of the major autoimmunogeneic regions of the Tcr α/β are "constitutive" markers inasmuch as all individuals tested produce antibodies against these regions. The most frequently observed autoantibodies are against Tcr Vβ CDR1 and Fr3 markers. It is hypothesized that these are normally involved in immunoregulation. Autoantibodies usually are not detected against CDR2 region determinants, or the "private idiotypes" defined by the CDR3 region, or the highly conserved FR4 segment specified by the joining gene segment. However, autoantibodies against the CDR2 of the Tcrα chain occur in some SLE patients, and healthy pregnant women produce antibodies against the common peptide determinant expressed by the joining gene and the beginning of the Cα or Cβ domain. Although the precise role of the naturally occurring autoantibodies in immunoregulation remains to be determined, modification of the course of autoimmune diseases in experimental rodent models (experimental allergic encephalomyelitis) has**

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been successfully carried out by immunization with synthetic peptides corresponding to the CDR2 and Fr3/CDR3 segments, and immunization of humans with synthetic Vβ CDR2 segments may prove helpful in multiple sclerosis. Moreover, infusion of intravenous immunoglobulins has been successful in the treatment of many autoimmune diseases, including examples where levels of T cells bearing particular Vβ gene subsets were elevated. The recent knowledge gained from T-cell receptor structural analysis and antigenic modeling holds promise for determining the roles of particular variable domain structures in antigen recognition MHC-restriction and immunoregulation, and in the development of synthetic and recombinant reagents for modulation of autoimmune and infectious diseases. [P.S.E.B.M. 1994, Vol 207]

Immunoglobulins and their close relatives the α/β T-cell receptors are examples of syngeneic proteins that express structures which readily serve as self-immunogens. These are the combining site-defined markers or idiotypes that are recognized by T cells (1–3) and induce the formation of anti-idiotypic antibodies (4–6) that serve as part of a regulatory network (7, 8). Studies with antibodies have been carried out with a great degree of precision because of the availability of large quantities of monoclonal protein and the application of hybridoma technology coupled with accurate 3-dimensional characterization by x-ray crystallography (9, 10). The situation with respect to T-cell receptors is less clear because they have not yet been produced in sufficient quantity even though gene sequence is available (11, 12) to allow antigenic or 3-dimensional characterization of the intact proteins. In our initial studies, we approached the problem of antigenic and structural characterization of T-cell receptors by focusing upon the sequence of the Tcr β chain which is homologous to Ig λ light chain (11–13). Our objectives were (i) to use computer analyses of sequences and predicted structures to identify a "universal" peptide antigen shared between the variable domains of Tcrs and light chains (14–17); and (ii) to use comprehensive peptide synthesis (18) to duplicate the complete covalent structure of a human Tcr β chain (19, 20). These peptides served as the basis for mapping epitopes shared between Tcrs and Ig-L chains (20, 21). In order to calibrate the procedures, we began by duplicating the sequence of the human monoclonal λ light chain Mcg by a nested set of overlapping synthetic 16-mer peptides and mapping epitopes recognized by xenoantisera (21) and natural human autoantibodies (22). The availability of milligram quantities of intact λ chain of known structure allowed us to test for the binding of antipeptide antibodies to the native structure. We used competitive inhibitions to ascertain the degree to which conformations of peptides in solution mimicked the antigenic structures of the same sequences in the intact structure. Parallel approaches to investigate the reactivities of anti-Tcr peptides with intact molecules were carried out using Western blot analyses (23), immunocytofluorescence with monoclonal human T cells, and the construction of a recombinant single chain Vα/Vβ structure.

The tie-in of the synthetic antigen approaches to autoimmunity and immunoregulation arose in our hands from control experiments in which sera of unimmunized humans (19, 22), mice, and rabbits were tested for IgG binding to the overlapping sets of peptides duplicating Tcrβ (19) and Igλ chains (22). The normal IgG pools of these species bound to a limited number of peptides, and these corresponded to the same sequence regions in the β and λ chains. Natural autoantibodies occur in diverse vertebrate species including sharks (24), bony fishes (25), mice (26), and humans (27). These autoantibodies react with a large set of self-antigens such as DNA, thyroglobulin, cytoskeletal proteins, the senescent cell antigen (28), and the constant regions of IgG heavy chain (29). Thus, it was worthwhile to characterize the autoantibodies to Tcrs in healthy individuals and in those with autoimmune disease to determine the parameters of expression of these antibodies and to elucidate their possible functions in immunoregulation. The importance of such autoantibodies in clinical medicine is suggested by the successful use of intravenous immunoglobulins as therapy for Kawasaki's disease (30), multiple sclerosis (31), acute idiopathic thrombocytopenic purpura (32, 33), and experimental rodent autoimmune uveoretinitis (34). It can be hypothesized that the diseases are ameliorated by the action of autoantibodies specific for individual sets of Tcr domains that modify the expression or function of T cells bearing these particular Vβ regions (35).

We will use the data available on the structure of immunoglobulins and on the construction of synthetic and recombinant idiotypes (3, 4, 36–40) in conjunction with recent modeling of Tcrs (17, 20, 41–44) to provide a framework for interpreting the antigenic and functional structures of Ig/Tcr V and C domains. The levels and isotype expression (IgM or IgG) of autoantibodies to Tcr peptide-defined regions are dependent upon the following conditions: aging, autoimmune disease, allograft transplantation, pregnancy, and retroviral in-

fection. We will review the present state of knowledge regarding autoantibodies to synthetic Tcr/Ig autoantigens in each situation. However, we emphasize that this set of phenomena is a recently discovered one. At best, we can only suggest guidelines to the development of novel approaches to determine the roles of such antibodies in the diverse problems of immunoregulation occurring under particular conditions.

Molecular Modeling and Epitope Mapping of Tcr α/β

Based upon the homology between T-cell receptors and immunoglobulin light chains, we (17, 20) and other investigators (41–44) have predicted the 3-dimensional structures of the T-cell receptors. In general, these models closely resemble those of immunoglobulin domains including V/V interactions to form the combining site, but the accuracy of these predictions must be verified by crystallography on individual molecules. Our approach was based on the substantial homology between the human Tcr β chain and λ myeloma light chain Mcg. The 3-dimensional model of the β chain serves as an aid in interpreting our data and also provides insight to possible functions of antigenic portions of the molecule. Overall, it is relatively straightforward to align sequences corresponding to predicted β bands, but the uncertainties arise in modeling the loop structures. Our modeling was based on a completely characterized Mcg λ light chain with the alignment given in Figure 1. Others have carried out superpositions using a large set of light chains (43). Major features of the model were similar from either derivation.

The Mcg starting model, illustrated in Figure 2 (top panel), shows more than 35% sequence identity to the V domain, J segment, and the C domain of the Tcr β chain. A model of the Tcr β chain constructed on the Mcg template is presented in the lower panel of Figure 2. Deletions and insertions are indicated by shaded sections in the β chain model, and these can be cor-

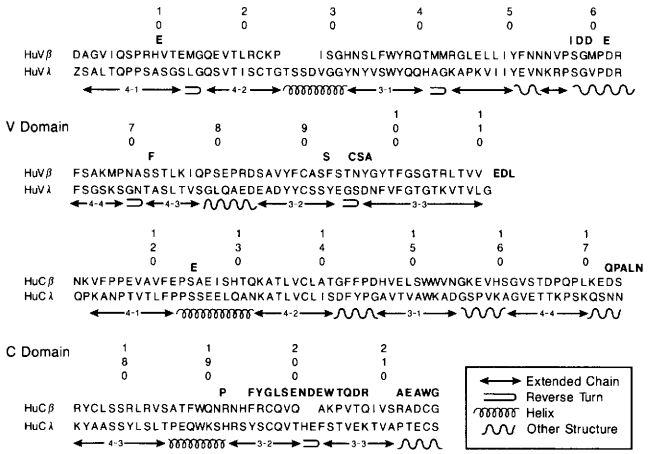

Figure 1. Alignment of the amino acid sequences of a human T cell β chain (YT35) and a human λ chain (Mcg) to emphasize possible similarities in their 3-dimensional structures. The known structural features of the Mcg light chain are schematically listed below the appropriate one-letter codes for the amino acid residues. Shortened structures are indicated by gaps in the sequence and insertions are designated by placement of the code letters above the corresponding segment. This figure was adapted from Edmundson *et al.* (16).

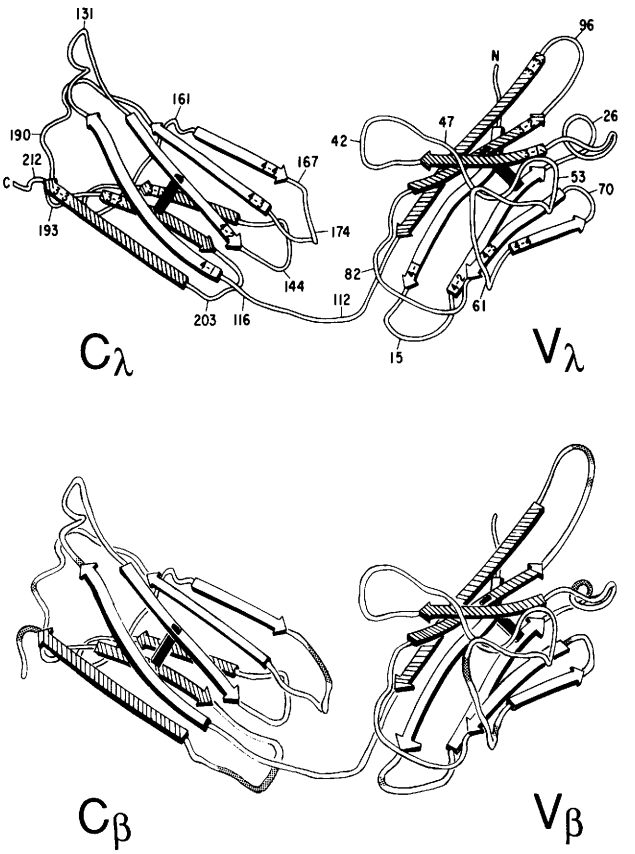

Figure 2. Comparison of the structure of the Mcg λ chain (upper panel) with a model of the T-cell receptor β chain (lower panel). Sequences of the two chains are aligned and correlated with the 3-dimensional structures as shown in Figure 1. Deletions and insertions are indicated by dotted sections in the β chain model. Note that only a few alterations have to be made in the structure of the V domain of the λ chain to accommodate the sequence of the β chain. These changes include a shortening of the first hypervariable loop (CDR1) of the β chain (segment containing residue 26 in Mcg), a lengthening of the third hypervariable loop (CDR3, labeled by residue 96) and an extension of the framework region (Fr3) connecting the second hypervariable loop (CDR2, marked by residue 53) with the pleated sheet strand designated 4–4. In the model of the Cβ domain, we have chosen to maintain the defining features of the immunoglobulin fold, namely the 3-stranded (striated) and 4-stranded (white) β-pleated sheets illustrated in the upper panel. With this assumption, two sets of additional residues in the β chain loop out from the globular domain structure into the space between the V and C domains. The "switch region" connecting the V and C domains and the C-terminal segment (far left) are elongated. The drawing of the Mcg light chain was made by Kathryn R. Ely and taken from Edmundson *et al.* (16).

related with the alignment in Figure 1. Only minor alterations were required in the structure to accommodate the sequence of the β chain. These adjustments include a shortening of the CDR1 of the β chain (the segment containing Residue 26 in Mcg), a lengthening of the CDR3 (indicated by Residue 96), and an extension of Fr3 that connects the CDR2 (marked by Residue 53) with the pleated sheet strand designated 4–4. The model of the Cβ domain maintains the defining features of the immunoglobulin fold; namely, the 3-stranded (striated) and 4-stranded (white) β-pleated sheets illustrated in the Tcr β structure. In this model, two sets of additional residues in the β chain loop out of the globular domain structure in the space between the V and C domains. The "switch" region connecting the V and C domains and C-terminal segment are elongated. Furthermore, the loop in the Cβ separating β strands 4.3 and 4.4 is longer than that in the Cλ (containing residues 167 and 174) and similar increased length is observed for the loop connecting β bands 3–2 and 3–3 (containing residue 203 in Cλ).

To map the antigenic determinants on the proposed Ig-like domains of the Tcr β chain, we applied the comprehensive synthetic peptide approach for the determination of continuous antigenic sites of proteins (18) using 16-mer peptides that overlapped one another by five residues. We used the λ light chain of Mcg as a model to validate the approach for immunoglobulins. Major λ-specific determinants recognized by polyclonal rabbit antisera (21) corresponded to the N-terminal portion of the V region and C-terminal segment of the Cλ domain. These determinants were essentially the same in the peptide and the protein as they are linear and devoid of major conformational folding. Other strongly antigenic segments such as Vλ Fr3 (residue 78–93) and a portion of the C domain (residues 177–192) showed strong conformational dependence. The "switch peptide" (residues 100–115) was likewise bound by xenoantisera directed against λ chains, and this region showed considerable cross-reactivity with Tcr Jβ segments. The Tcr β peptide segment that showed the highest degree of cross-reactivity using rabbit antisera to human κ and λ chains was the "switch peptide," corresponding to the λ segment labeled with Residues 112 and 116 in Figure 2. Other reactivities were directed against the CDR1 and Fr3 segments of the Vβ domain and against C-domain segments corresponding in position to Cλ 174–203. These reactivities represented true cross-reactions because similar binding properties were exhibited by affinity-purified antibodies to these peptides (20). Antibodies against human γ heavy chain did not react with any Tcr β peptides. The Fr4 sequence specified by Jβ segment shows strong homology to κ and λ light chains and Tcr α and β chains. These segments are responsible for some of the broad cross-reactions demonstrated between the immunoglobulin and Tcr protein (16, 17, 23). The positively charged residues (lysine or arginine) in the sequence FGXGT (K or R) LV have been found to be essential for the cross-reaction between light chains and Tcrs (16). Models illustrating the prominence of this residue in the context of the 3-dimensional structure of Fr4 is presented in Figure 3.

We carried out control studies to determine baseline binding of human immunoglobulins to Tcr β (19), α (46), and λ light chains (22), with the novel finding that natural antibodies to limited numbers of peptide-defined determinants are present for all three molecules. The distribution of antipeptide binding by normal IgG subsets is illustrated in Figure 4, which shows the binding in enzyme-linked immunosorbent assay (ELISA) of two polyclonal intravenous immunoglobulin preparations (IVIG) to the set of peptides duplicating the Tcr β chain. Both IVIG preparations have strong activity against Peptide 3, 8, and 17, but one preparation also shows activity towards Peptide 11 and 21. This binding is carried out by specific antibodies, as evidenced by our ability to affinity purify the antibodies with individual specificities and also to specifically block the binding using free peptides (19). The affinity-purified IgG antibodies comprised a small proportion of the IVIG pool (<0.1% of the total IgG) and were found to be polyclonal by isoelectricfocusing analysis and by the presence of κ and λ light chains. Natural IgG antibodies of mouse and rabbit also bind to the same peptides. This finding is not surprising because there is strong homology between some mouse Vβ genes and their human counterparts; for example, the human Vβ8 and Vβ6 genes form a family with murine Vβ 11 genes. Furthermore, antibodies di-

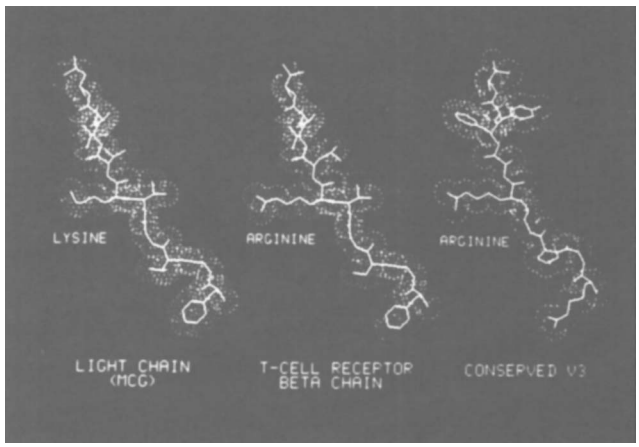

Figure 3. Comparison of antigenically cross-reactive peptides representing the variable domain FR4 of human λ light chain Mcg, the T-cell receptor β chain, and the conserved sequence within the V3 loop of HIV-1 gp120. The structure of the λ light chain segment was determined by x-ray crystallography (27–29) and the T-cell receptor segment modeled on it (12, 23) with the HIV-1 V3 loop analyzed on the basis of homology. The peptides are presented using the van der Waals surface dot representations.

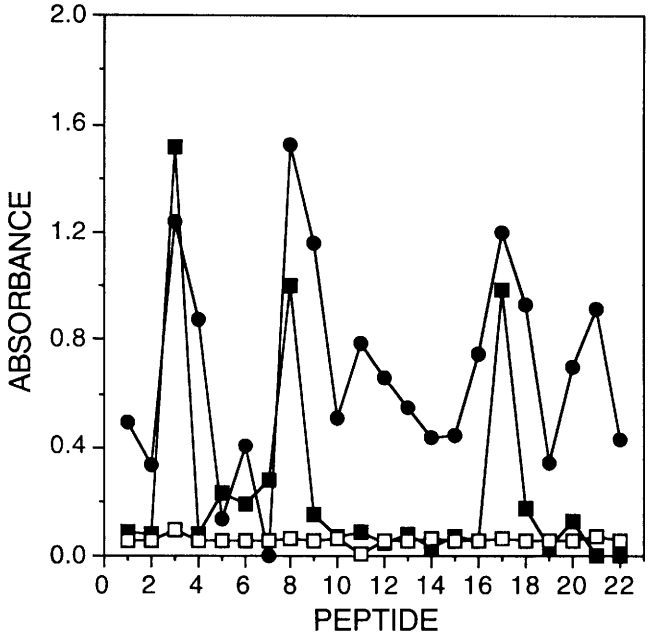

Figure 4. Binding in ELISA of normal human polyclonal IgG immunoglobulins to synthetic overlapping peptides modeling the immunoglobulin VJC domain in the human Tcrβ chain. (■), Gammagard IgG preparation, Baxter Healthcare; (●), Sandoglobulin preparation, Sandoz Pharmaceuticals; (□), negative control.

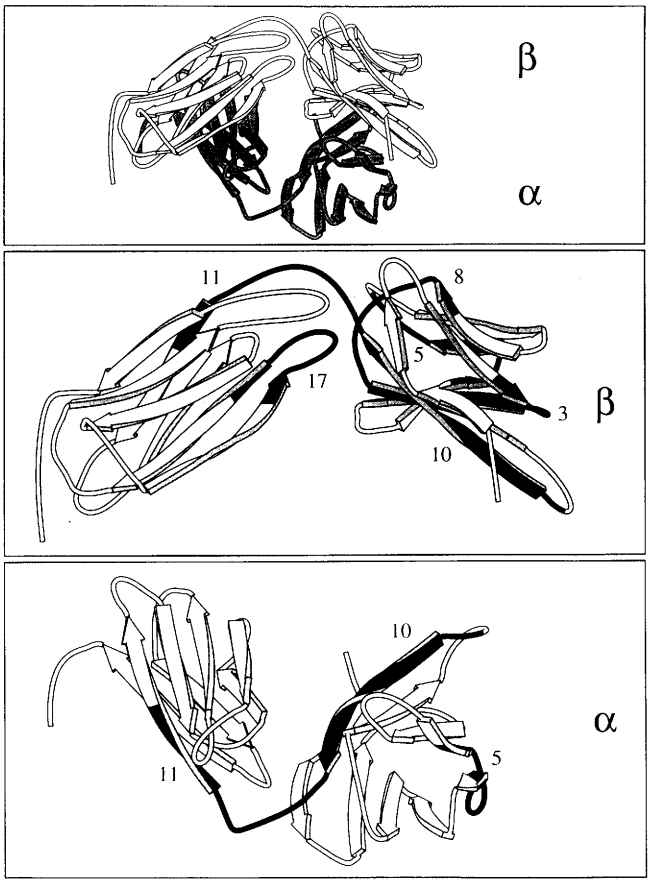

Figure 5. Three-dimensional model of the immunoglobulin domains of the α/β Tcr. The constant region is on the left and the variable region on the right. The chains are designated α and β, and the individual peptides indicated are those in Table I. Upper figure is the intact α/β structure, middle figure is the β chain and lower is the α. The model was constructed on the basis of homology with light chains as above, but the program RIBBON was used in this depiction (19, 20).

rected against synthetic peptides corresponding to human Jβ and Cβ sequences bind readily to their murine counterparts (23). Figure 5 is a model of the Ig-domains of Tcr α/β illustrating the locations of particular peptides relevant to immunomodulation, natural immunity, and specific immunization in certain disease or physiological states.

We have analyzed intravenous immunoglobulin preparations from healthy and HIV-infected individuals, and have assayed sera from more than 150 individuals representing healthy people in the age range 20–90, rheumatoid arthritis patients, pregnant women, and individuals infected with coccidiomycosis for autoreactivity with synthetic Tcr antigens. Salient autoantigenic peptide segments which proved to be of general reactivity or showed selectivity for particular health-related states are listed in Table I. Constitutive levels of autoantibodies to Tcr β Peptides 3, 8, and 17 occur in the majority of individuals. Likewise, many individuals show low levels of autoantibodies to λ light chain Peptides 3, 8, and 17. As represented in the predicted structure in Figure 5, β3 contains the entire CDR1 sequence and a portion of the Fr2 segment. β8 is a portion of the Fr3 sequence and β17 is a large loop in the Cβ domain that projects forward towards the Vβ. The asparagine serves as a potential glycosylation site. Figure 2 shows that the λ peptides also correspond to CDR1, Fr3, and a small loop in the Cλ domain. The CDR1 peptides can be considered public idiotopes in the sense that the members of particular Vβ subgroups would all contain this sequence, irrespective of the CDR3 private idiotope presented. The Fr3 determinants are of interest because this region is not part of the combining site for antigen but would be specific for the products of particular Vβ genes and would be exposed on the outer surface of the molecule. This region has been implicated in the recognition of superantigens by Vβ segments (47, 48), and it corresponds to segments involved in forming V_Ha allotypes in rabbits (49). The β5 and α5 segments are of interest because these correspond to the CDR2 regions of the Tcrs. We have not found human natural antibodies directed against the Vβ CDR2, but this region is clearly highly antigenic and exposed in Tcrs occurring on the surfaces of T cells. Antibodies have been produced in rabbits against this peptide. These stain T cells by immunocytofluorescence and react in Western blots with solubilized Tcr β chains and a recombinant single chain Vα/Vβ construct we have produced. By analogy with properties of anti-idiotypic sera against light chains of rheumatoid factors (38), we would con-

Table I. Salient Autoantigenic Peptide Segments

	Synthetic sequence	Description
β3	C K P I S G H N S L F W Y R Q T	CDR1/Fr2; public Vβ "idiotope"; major autoantigenic site
β8	K I Q P S E P R D S A V Y F C A	Fr3; major autoantigenic site; marker for Vβ gene families
β17	Q P L K E Q P A L N D S R Y C L	Cβ large loop in man; N-glycosylation site; major autoantigen
β5	L L I Y F N N N V P I D D S G M	CDR2; low levels of autoantibodies in normals; rabbit antibodies bind strongly to T cells and SCVαVβ; public Vβ idiotope
α5	L L L K Y T S A A T L V K G I N	CDR2; Public Vα idiotope; major autoantigen in SLE; minimal autoantibody in normals
Jβ	A N Y G Y T F G S G T R L T V V	Cross-reactive set containing the Fr4 segment specified by the joining gene segment. Mimicked by the constant segment within the HIV-1 V3 loop
Jα	S A S K I I F G S G T R L S I R	
Mcg10	V F G T G T K V T V L G Q P K A	
α11	R L S I R P N I Q N P D P A V Y	"Switch peptide" continuous with Fr4
β11	T R L T V V E D L N K V F P P E	Usually not autoantigenic; autoantibodies found in pregnancy

sider this region to constitute a public idiotope restricted to particular Vβ gene subgroups. Only low levels of autoantibodies, if any, to the corresponding segment of the α chain (α5) occur in most human sera. However, sera from SLE patients from North Carolina have elevated levels of IgG antibodies to this peptide by comparison with normal healthy women who are pregnant (51).

The set of peptides (α10, 11; β10, 11; Mcg10) containing the Fr4 segment that is encoded by the joining region gene of Tcrs and light chains is of interest because the FGXGT+L motif is highly conserved in Tcrs and light chains of species widely spread in vertebrate evolution (13, 15, 17). Usually, there is little detectable natural reactivity directed against this region of the molecule. In fact, we have been unable to immunize rats and mice with this peptide (16). Rabbits respond quite well because they express light chains with a negatively charged glutamic acid (E) at Position 12, as opposed to positively charged arginine (R) or lysine (K) residues. Rabbit antibodies directed against the synthetic Jβ peptide cross react in ELISA with the Jα and Jλ peptides illustrated here.

We have recently found that IgG preparations from plasma of humans infected with HIV-1 contain antibodies directed against the Jβ segment (52). This is because there is a conserved stretch of the form GPG*RAF (Y or V) in the major neutralizing determinant V3 loop of the gp120 glycoprotein of HIV-1 (53). As illustrated in Figure 3, the conserved V3 peptide is sufficiently similar to the Tcr peptide to cause the generation of antibodies by antigenic mimicry (52). In addition to the J peptides, pregnant women have autoantibodies directed against the α11 and β11 peptides that overlap the Jβ and V3 segment but continue through the "switch region" into the constant domain.

Structural and Functional Interpretation of Autoantigenic Sites on Tcrs

Mice and other species contain natural antibodies directed against V_H peptides of the phosphorylcholine binding myeloma protein TEPC 15 (54). Antibodies to human Tcr occur in the natural alloimmunization process during pregnancy (55, 56) and in renal transplantation (57). The results of epitope mapping using comprehensive peptide synthesis facilitate the localization of these determinants. Antibodies against Tcr Vβ8.2 appear in B10.pL mice in the recovery phase of exper-

imental allergic encephalomyelitis induced by immunization with myelin basic protein (58, 59). It is important to determine the function of these naturally occurring antibodies. Such autoantibodies can bind directly to intact molecules. For example, in the case of the natural and induced antibodies to the λ light chain, antibodies produced against intact immunoglobulins contain subsets reactive with individual peptides (21, 22). Moreover, affinity-purified natural human antibodies to the peptide segments, notably Mcg 8 (Fr3), bind to the intact light chain in ELISA and Western blot analysis.

Reaction with the intact molecule is an initial stage in determining whether the natural antibodies might serve an immunoregulatory function. We have approached this problem using either affinity-purified natural human or induced rabbit antipeptide antibodies. Their capacity to react with intact T cells has been determined by fluorescence or with a single chain recombinant Vα/Vβ Fv structure in ELISA and Western blot analysis. As illustrated in Table II, both the rabbit and human affinity-purified antibodies directed against variable region determinants bind to the recombinant Vα/Vβ Fv. As a control, antibodies directed against the Cβ peptide β17 do not react with the variable region domains. Polyclonal IgG from HIV-infected individuals binds to the recombinant Vα/Vβ and the level of binding is increased by affinity purification with a synthetic peptide corresponding to the conserved portion of the V3 loop (52).

Table II. Binding of Affinity-Purified Autoantibodies and Induced Rabbit Antibodies to Recombinant Single Chain (RSC) Vα/Vβ Fv and to Intact T Cells

(A) Binding to RSC Vα/Vβ in ELISA Antibody	Sum titer (cumulative ELISA absorbancy control subtracted)
Rabbit anti-β3	5.8
Rabbit anti-β5	10.8
Rabbit anti-β8	10.7
Rabbit anti-β17	0.0
Human anti-β3	0.9
Human anti-β8	1.1
Human anti-β17	0.3
HIVIG (unfractionated)	0.9
H-anti-HIV-gp120-V3	3.4

(B) Immunocytofluorescent binding of affinity-purified rabbit anti-Tcr β peptide antibodies and controls to human T cells (% of positive cells)

	JURKAT	Peripheral T cells (CD3+)
Conjugate control	2.4	0.1
Anti-ovalbumin	7.3	11.1
Anti-CD3	85.1	(100)
Anti-β1	8.5	13.2
Anti-β5	74.8	35.1
Anti-β8	34.1	12.6
Anti-β11	20.8	19.3
Anti-β17	5.4	ND
Anti-β20	4.8	12.3

Because of background problems, it is difficult to carry out clear cut immunocytofluorescence studies on human cells using human IgG immunoglobulins (60). However, use of the rabbit antisera illustrates that some peptide determinants are exposed whereas others are unavailable. Antibodies to β1, β5, and β8 bind to Tcr associated with the cell surface, with the anti-β5 binding most strongly. We interpret the negative results with the β17 to result from occlusion of the determinant either because it is a glycosylation site or because of steric hindrance from proteins comprising the CD3 complex. Further evidence for steric effects rises from the observation that antibodies against β20, a C-terminal peptide, likewise do not bind to Tcr α/β on the intact cell. Rabbits have high levels of natural IgG antibodies to the β3 peptide, and purposeful immunization with this antigen decreases the levels of antibody relative to the natural condition (62). However, based upon screening of more than 30 human IgG myeloma proteins, we found that the λ/γ1 protein PRE (62) binds strongly to the β3 peptide in ELISA and Western blot, whereas it is negative for the other peptides. This monoclonal immunoglobulin also stains JURKAT T cells by immunocytofluorescence. The staining of a CD3 negative variant PRT3 is much less than that of the intact α/β-bearing cell, substantiating the conclusion that the binding is directed towards Tcr. We have obtained parallel results using sera from lupus (SLE) patients that were shown to bind in immunocytofluorescence to JURKAT but not to the CD3 deficient variant. These antibodies reacted with T-cell receptor α and β peptides and with the recombinant Vα/Vβ construct.

Knowledge of the 3-dimensional structure of immunoglobulins and of the production and analysis of synthetic idiotypic determinants (3, 4, 63) serves as the basis in interpreting our present results with T-cell receptors and autoantibodies directed against particular peptide-defined epitopes. Antibodies directed against CDR1 determinants usually occur in both the IgM and IgG pools of unimmunized individuals as well as in sera of individuals with autoimmune diseases. The CDR1 region is clearly exposed in the V_L/V_H structure of immunoglobulins and contributes to forming the combining site. It is conceivable that this determinant may play an important role in antigen recognition or in immunoregulation involving all the members of a particular Vβ gene set because these sequences are shared by all members of the individual Vβ gene sets. The α/β Tcrs do not show affinity maturation with time

after immunization because there is not the accumulation of somatic mutations necessary for this to occur as it does in Ig heavy and light chain V regions (65). Nevertheless, the Vβ-germline-gene defined CDR1 may play a crucial role in the recognition of sites on MHC involved in presentation, or it may serve as a site for autoregulation either by the autoantibodies or autoreactive T cells. Helper T cells can be specifically generated against immunoglobulin idiotypes (1–3). CDR1 of the light chain is a large, highly accessible loop which protrudes into solvent for all immunoglobulin structures, and is therefore well suited for regulatory role via interactions with macromolecules.

Parallel arguments can be made for the Fr3 segment, which likewise is characteristic of a particular Vβ or Vα germline gene. While located on the exterior of the molecule, however, Fr3 is not associated with the complementarity determining regions. Thus, it might be more appropriate to consider the Fr3 determinants as analogs of the V_Ha allotypes of the rabbit (49), which are noncombining site determinants formed from residues lying in Fr1 and Fr3. This portion of the T-cell receptor β chain has been proposed to serve in the recognition of superantigens (48), a role consistent with the structural and serological properties presented here. Moreover, synthetic peptides corresponding to the Fr1 and Fr3 segments of appropriate murine T cells block the presentation of the Mls superantigen by MHC-restricted spleen cells, but do not block binding of a peptide bearing the Mls epitope (66).

It is of interest that significant quantities of autoantibodies to the private idiotype defined largely by CDR3 were not found in any of the individuals or Igs preparations studied. Individual markers in this region would be considerably less frequent because this region is formed from recombination of V, D (in heavy chains and Tcrβ), and J gene segments (11). Despite the lack of natural autoantibodies to CDR2 and CDR3 determinants, these structures, particularly CDR2 (β5 peptide) are highly immunogenic in rabbits. The antibodies bind strongly to an intact recombinant Vα/Vβ construct and to the Tcr α/β expressed on the surface of T cells. The one situation where we observed elevated levels of IgG autoantibodies to the CDR2 of Vα (α5 peptide) was in women with SLE.

The peptide specified by the joining gene segment contains an N-terminal portion that is part of CDR3 and a C-terminal segment specifying Fr4. Davie and his colleagues (4) have produced xenoantisera directed against synthetic peptides corresponding to J_H observing that this peptide contains both idiotypic and conserved determinants. We have found that the natural levels of antibodies to the Jβ peptide are extremely low and, furthermore, that it is extremely difficult to immunize mice and rats with the highly conserved peptide. However, we found that pools of IgG prepared from individuals infected with HIV contained appreciable levels of antibodies to the marker (52). Since the determinant would be expected to be exposed in the intact conformation and it contains charged residues strongly implicated in its antigenicity (16), the failure to respond most probably results from a self-tolerance mechanism. Breaking of T-cell tolerance has been demonstrated in mice infected by nematodes (62), so it is conceivable that other infections could have similar effects. The production of antibodies to the Jβ segment in HIV-infected individuals probably results from antigen mimicry. We suggest that the individuals respond to the conserved portion of the major neutralizing V3 loop, which, as shown in Figure 3, strongly mimics the autologous Tcr (52). Consequently, the induced antibodies cross-react with intact recombinant Vα/Vβ and with the Jβ peptide.

The Fr4 segment leads into the "switch peptide" that bridges the V and C domains. Antibodies against this segment do not occur in quantity in most individuals, again because of a probable self-tolerance mechanism. However, this determinant is readily recognized by xenoantisera (14), and the levels of IgG autoantibodies against this region rise substantially in the natural alloimmunization process of pregnancy (51). The last constitutive autoantigenic determinant is a portion of the Cβ which loops out from between the 4–4 and 4–3 β bands. This loop projects forward towards the variable domain and shows variation in length when human and mouse Cβ domains are compared. There is also considerable length variation in the corresponding segments of Tcr α chain and immunoglobulin light chains. The human Cβ segment contains a glycosylation site which is not present in the light chains. However, the processed peptide is normally immunogenic in many cases because of the frequency of autoantibodies directed against this region.

Comparisons of Human Autoreactive Peptide Segments

The preceding data for both Tcrβ and Ig λ light V domains indicate that CDR1 and Fr3 segments are usually autoantigenic in unimmunized individuals, but that natural autoantibody levels to CDR2, CDR3, and Fr4 markers are low to nondetectable. With the recent interest in developing synthetic peptides to ameliorate autoimmune disease involving restricted sets of T cells such as experimental autoimmune encephalomyelitis (EAE) in rodents (58, 59, 67–69), it is worthwhile to compare the selection of peptides in the EAE model with the peptides and the Tcr Vα/Vβ model developed here. The essential features of both the EAE and Mls superantigen models are (i) the capacity of T cells to respond is MHC-restricted and (ii) restricted subsets of Vβ genes predominate in the responses; e.g., Vβ8.2

and 13 in murine EAE (67) and Vβ6 in the Mls response (66). Because the goal is to obtain immunogenic Tcr peptides that selectively ameliorate the particular disease, investigators have compared Tcr sequences of reactive and unreactive T cells and chosen regions designed to confer maximal selectivity without affecting other responses. The EAE studies thus focused upon regions that correspond to CDRs 2 and 3 in the model proposed here.

Offner *et al.* (68) were able to modulate the course of the disease by immunizing with a synthetic peptide containing a portion of Fr2 and all of CDR2 from the susceptible Vβ8 sequence (residues 39–59). The homologous peptide from the nonreactive Vβ14 sequence was used as a negative control. Furthermore, immunization with synthetic peptides corresponding to the C-terminal portion of Vβ Fr2 and the CDR2 segment specifically prolonged allograft survival in mice (94). The feasibility of immunization with CDR2-containing Tcrβ-derived peptides for the treatment of specific human autoimmune diseases was documented in multiple sclerosis where some patients generate T-cell clones specific for myelin basic protein that preferentially use Tcr Vβ genes from the Vβ5.2 and Vβ6.1 families (95, 96). Injection of low doses of peptides (100–300 μg) in physiological solution did not induce broad spectrum immunosuppression but resulted in the production of peptide-specific T-cell immunity and antibodies. These results parallel studies using the CDR2 segment of Igs to generate anti-Ids (38, 63).

Synthetic peptides corresponding to CDR3 segments were successfully used by Howell *et al.* (68) and by Kumar and Sercarz (58) to modulate the disease. One of the peptides (residues 76–101) used in the latter study contains the murine Vβ8.2 homolog of the Fr3 (β8) peptide described here, as well as the CDR3. A 17-mer peptide, LILELATPSQTSVYFCA, representing the Fr3 segment (compare with Fr3 segments in Fig. 6) was as effective in binding to the immunoregulatory T cells in EAE as was the full-length Fr3/CDR3 peptide (V. Kumar and E. E. Sercarz, personal communication). In addition to the efficacy of Vβ CDR3 peptides in protecting against the development of EAE, the synthetic Jα segment of the major rat α chain was also effective (69).

The studies of Pullen *et al.* (48) using site-directed mutagenesis to identify residues involved in MHC-restricted Vβ-mediated recognition of the Mls superantigen were supported by MacNeil *et al.* (66), who used synthetic Fr1 and Fr3 peptides to block the activation. Although the low levels of natural autoantibodies to Tcr-CDR2 and CDR3 indicate that these determinants are usually not immunogenic, purposeful immunization of mice with autologous peptides can generate both T-cell responses and antibody production (70). Immunizations with T-cell peptides frequently use peptide dissolved in physiological saline, thereby showing that the peptides alone act in an immunoregulatory manner and break tolerance. In the light of the studies with synthetic Tcr peptides in immunodulation, it is interesting that human IgG autoantibody levels are raised against a CDR2 marker (α5) in SLE and the Fr4/C-domain (α11; β11) switch peptides in pregnancy. These changes in immunoregulation, thus, represent an actual autoimmunization process that may parallel reactions occurring in animals experimentally immunized with the corresponding autologous peptides.

Fine Specificity of Human Autoantibodies for Individual Vβ Gene Products

Human autoantibodies to peptide-defined Tcr segments have properties expected of induced antibodies inasmuch as they can occur in the IgG isotype and show great specificity for particular peptides, as well as the capacity to react with the intact molecules. It is worthwhile to ask whether the specificity of these antibodies is comparable to that achieved in purposeful xenoimmunization and whether the human antibodies can distinguish among products of distinct Vβ genes. Figure 6 is a schematic diagram illustrating the arrangement of human Vβ segments, the selection of one of these by a rearrangement with a D and J segment, and the representation of the major autoantigenic Vβ peptides in their location within the Vβ structure. A small set of sequences representing distinct Vβ genes is illustrated to show that these sequences remain constant within the progeny of a particular Vβ family but display considerable variation among different families. On the basis of this variation, the Fr3 region is considered as a fourth hypervariable region but is not a CDR (44, 71, 72). The products of the individual Vβ segments usually are present in the range of 2%–20%

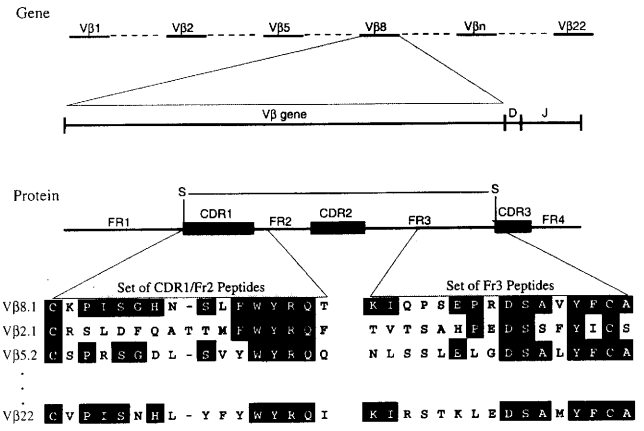

Figure 6. Diagram illustrating the location of the Tcr Vβ autoantigenic regions. The human Tcr Vβs are comprised of at least 22 different families. Although some residues are conserved, there is considerable sequence variation between the different families in these regions.

in the pool of α/β Tcr-expressing T lymphocytes (73, 74), so antibodies raised against particular peptides would be expected to react with a reasonable selection of expressed Vβ sequences.

Both IgM and IgG autoantibodies binding these peptides occur, with the level of IgM autoantibodies being higher in healthy young individuals than that of IgG (61, 75). In order to investigate the fine structure for individual Vβ sequences of human autoantibodies and to gain information regarding V_H and V_L usage, we screened 70 Epstein-Barr transformed B cells for their capacity to produce IgM antibodies reactive with Tcr peptides. One such antibody that showed exquisite specificity for the Vβ8.1 (β3) peptide was discovered (76). As shown in Figure 7A, the IgM κ immunoglobulin produced by the B-cell line IARC 307 bound only to this peptide when tested against a set of CDR1 homologs representing different human and murine Vβ sequences. The inhibition curve shown in Figure 7B, gives 50% inhibition using approximately 20 nanomoles of soluble peptide. This antibody was not polyreactive inasmuch as it did not bind to a series of other test antigens including thyroglobulin, ovalbumin, fetuin, double and single-stranded DNA, and peptides corresponding to Vα and Vλ. mRNA was isolated from the B-cell line and the cDNA was analyzed using polymerase chain reaction with a set of primers for distinct V_H and V_L sequences. Amplified products were cloned and sequenced. The translated protein sequences of the isolated V_H and V_L are given in Table III which also includes the sequence of a murine IgM monoclonal antibody to β3. This antibody was detected in hybridomas formed by fusing spleens of moth-eaten mice, a genetic variant characterized by massive production of autoantibodies. The particular hybridoma producing the anti-Vβ CDR1 (β8) was kindly provided by Dr. C. A. Bona of Mount Sinai School of Medicine (clone UN37–5). The comparison of the V_H sequences with databases revealed that the human heavy chain belongs to the V_HIII family and shows greater than 99% identity to the germline sequence DP54 (45). The J_H segment used by the protein corresponds to J6C. The sequences of CDRs 2 and 3 are distinct from those described for rheumatoid factors, autoantibodies to DNA, and characterized polyreactive antibodies. The V_H and V_L usage in autoantibodies to Vβ CDR1 distinguishes them from rheumatoid factors, a result consistent with the difference in specificity of the two sets of antibodies (50). The Vκ expressed is a member of the VκIII family and is more similar to the VκIIIa sequences than it is to members of the VκIIIb family, which is often associated with rheumatoid factors (38). The natural murine antibody to the β3 peptide expresses a V_HIII gene that is representative of the J606 family (77). It may prove to be of interest that three murine monoclonal IgM antibodies reactive with thymus cells used the J606 family (78).

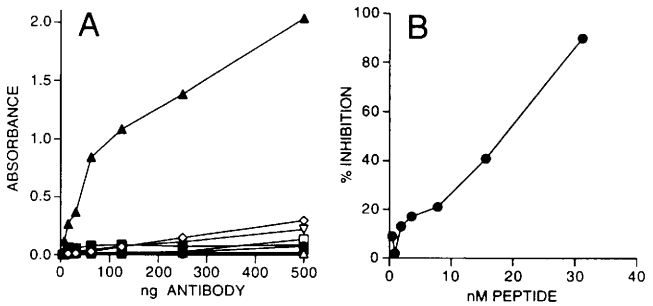

Figure 7. (A) Binding of IARC307 autoantibody in ELISA to synthetic peptide CDR1 homologs from several Tcr Vβ families of man and mouse and to peptides of the same region of Tcr Vα (PY14) and Igλ (Mcg). (▲), Vβ8.1H; (△), Vβ2.1H; (◆), Vβ5.2H; (○), Vβ6.3H; (□), Vβ1M; (■), Vβ8.1M; (▽), Vβ8.2; (◇), Igλ; (●), Vα. The H suffix indicates a human sequence and M a mouse sequence. (B) Inhibition of IARC307 autoantibodies binding to the Vβ8.1 CDR1 region peptide with free peptide.

Analysis of human IgG autoantibodies to homologs corresponding to the β8 (Fr3) peptide illustrate that these antibodies possess a specificity comparable to that in the induced secondary response of rabbits. Figure 8 illustrates binding of affinity-purified rabbit (A) and human (B) antibodies against the β8 (Vβ8 sequence) to the peptide used for affinity purification and to a set of five homologous peptides representing distinct Vβ genes. Both antibodies discriminate among the set with best reactivity against the antigen used for immunization and selection. Both sera give the next best reactivity against the Vβ6.3 homolog which shows the highest degree of identity (63% identity in the peptide and 56% in overall Vβ structure). In general, the order of specific binding reactions parallel one another with a single exception: the affinity-purified human autoantibody shows a stronger reactivity against the Vβ2.1 homolog than does the rabbit antibody.

Analysis of expression of individual Vβ genes using PCR technology indicates that individuals tend to express most, if not all, of the genes and that there is individual variation that has not been reliably correlated with autoimmune disease (79, 80). We would expect that usage of isotypes and anti-Vβ specificities of autoantibodies in individuals would reflect their genetic background (particularly with respect to HLA), their disease history with respect to autoimmunity and infection, and altered physiological conditions including aging and pregnancy. In practice, the set of six Fr3 homologs used here can be used to provide an overall profile of individual reactivities. This is illustrated in Figure 9 which compares anti-Fr3 profiles of a set of individuals before (Left) and after (Right) heart transplantation. There is individual variation as represented by the error bars, but these data illustrate the increase in the level of IgG autoantibodies that occurs following

Table III. Comparative Alignments of CDR2 and CDR3 from Human and Murine Monoclonal Autoantibody V_HIII with Corresponding Segments of Human Autoantibodies

(A) CDR2 {V_HIII}	
Hu Anti-β3	N I K Q D G S E K — — Y Y V D S V K G
Mu Anti-β3	E I R N K A N N H A T Y Y A E S V K G
Anti-DNA[a] (SLE, pathogenic)	F I S E S G S N T — — Y Y V D S V K G
Anti-IgG (RF)	V I S Y D G S N K — — Y Y A D S V K G
Polyreactive	G T G G R G G S T — — N Y A D S V K G
RF (Po/Id+)	W K Y E N G N D K — — H Y A D S V N G
Anti-DNA (Hybridoma)	A I S G S G G S T — — Y Y A D S V K G
(B) CDR3	
Hu Anti-β3	C A R — G G — — — — S G W W V Y Y Y Y M D V W G
Mu Anti-β3	C T R — S G — — — — — — — — — S Y W Y F D V W G
Anti-γG (RF; Po/Idt)	C A R D A G P Y V — S P T F — — — — — F A H W G
Polyreactive	C A K — G G V E L A S T K P S S Y W Y F D L W G
Anti-DNA (#1)	C A R — G R M W E R — — W F G E S P P F D Y W G
Anti-DNA (#2)	C A K Q V L Y Y G — S G S Y H W — — — F D P W G
Anti-RH	C A R E V T M V R G V R R — — Y Y G — M D V W G

[a] Sequences are taken from Kabat (64) except for the pathogenic anti-DNA autoantibody which is from Chastagner (93).

allograft transplantation. This finding is consistent with a study reporting that autoantibodies to T-cell receptors arise as a consequence of renal transplantation in humans (57).

Features of Autoantibody Production to Defined Tcr α/β Peptide Regions

Table IV lists major observations made regarding the appearance and properties of IgM and IgG autoantibodies to peptide-defined regions of Tcr α/β chains. Variation in the levels of autoantibodies occurs in healthy individuals as well as in autoimmune disease and infection. A study of age dependence of serum IgM and IgG autoantibodies from healthy individuals in the age range 20–88 indicated that IgM autoantibodies tend either to stay at the same level throughout life or to decrease with increasing age (61, 75). By contrast, IgG autoantibodies tend to increase with age, particularly those autoantibodies reacting with some of the peptides corresponding to CDR1 sequences from different Vβ subgroup families (61). This finding is consistent with studies showing that the capacity to mount primary responses to external antigenic challenge decreases with age while the capacity to mount secondary responses can increase (81).

Figure 10 gives a comparison of human IgM and IgG autoantibody levels to Tcr β peptides and control proteins in healthy individuals, patients suffering from osteoarthritis (OA), and patients suffering from the autoimmune diseases rheumatoid arthritis (RA) and SLE. The common dietary antigen ovalbumin is included in the IgG comparisons because 70%–80% of normal individuals express high titer IgG antibodies to this glycoprotein (61, 75). Antiovalbumin antibody can thus serve as a monitor of the functioning of the secondary immune response. RA patients express relatively large quantities of rheumatoid factors which are usually of the IgM class and bind to Fc (Cγ2/Cγ3) conformational determinants of human Igγ chains (29). The specificity of the autoantibodies to T cells and the gene usage described to date establish that these antibodies are not rheumatoid factors. The RA patients illustrated here showed an overall increase in IgM autoantibodies to the CDR1 determinant and to the Cβ region loop by comparison with serum reactions of normal humans and OA patients. Seven SLE sera represented here were previously shown to react with hu-

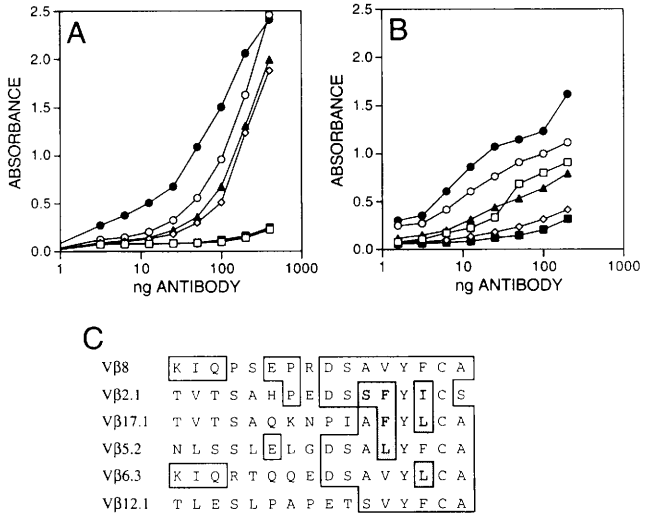

Figure 8. Quantitative ELISA of affinity-purified rabbit antibody to Tcr Fr3 peptide β8 (A) and affinity-purified natural human IgG antibody to β8 (B) against a set of homologous Tcr peptides corresponding to the Fr3 segment. (●), Vβ8; (□), Vβ2.1; (◇), Vβ5.2; (○), Vβ6.3; (△), Vβ12.1; (■), Vβ17.1. (C) Alignment of the Tcr Fr3 peptide homologs.

man T cells by cytotoxicity or immunocytofluorescence. A statistical comparison of individual data for normals versus the corresponding RA reactivities to the particular peptide group using the Wilcoxon 2-sample test indicated significant binding to the β3 peptide ($P = 0.016$) and to the β17 (Cβ loop) peptide ($P = 0.004$). The natural IgG response to ovalbumin in RA patients was significantly lower than that of the other groups, a finding consistent with the general anergy often observed in these patients. Individual RA patients can express extremely high titer (>10,000) IgM autoantibodies against Tcr peptides. Overall, the highest IgM titers we observed to the β3 peptide were found in these patients. Although the IgG titers are lower, some patients have strong IgG reactivities with titers >1,000 against Tcr peptides. In addition to the SLE patients, we included eight unselected SLE patients in the study. The SLE patients differ from those suffering from RA in having normal IgG responses to ovalbumin, and in showing elevated IgG autoantibodies to a range of Vβ and Cβ peptide defined determinants. The IgM reaction in this SLE subset is higher to the Vβ CDR1 peptide than in unselected SLEs that are comparable to normals. Figure 11 (left side) contains the antipeptide autoantibody profiles of an SLE patient whose serum IgM stains the T cell JURKAT in immunocytofluorescence and is negative on a variant lacking CD3. The serum was tested for its IgM and IgG autoantibodies against Tcr α and Tcr β and λ overlapping peptides. On the right side of Figure 11 are the profiles of a RA patient. The sera were tested at dilution of 1/200. The SLE serum shows strikingly different profiles against all of the overlapping peptide sets. In the first place, the IgG reactivity is stronger in all cases and for Tcr α (A) and β (B) chain peptides shows a much wider distribution of activities. In addition, the SLE patient shows strong IgG reactivities against Mcg light chain Peptides 3, 4, and 8.

Both IgM and IgG autoantibody levels against Tcr

Table IV. Properties of Autoantibodies to Defined TCR α/β Peptide Regions

1. Occur naturally in both IgM and IgG immunoglobulins of man, mouse, and rabbit. The fraction of IgG autoantibody is less than 0.1%.
2. IgM levels tend to be higher in young people (less than 40 years of age) and tend to decline with age. IgG levels can increase with age.
3. The levels of anti-Tcr IgM autoantibodies are elevated in rheumatoid arthritis relative to normal individuals.
4. The levels of IgG autoantibodies to Tcr determinants are elevated in systemic lupus erythematosus, particularly in those individuals whose sera bind to human T cells in immunocytofluorescence.
5. Monoclonal human IgM and IgG and murine IgM autoantibodies to the Vβ CDR1 region have been characterized. These are extremely specific for particular peptides, can bind intact molecules, and have V_HIII and VκIIIa sequences distinct from those of known autoantibodies (e.g., polyspecific Abs rheumatoid factors, anti-DNA).
6. Allograft transplantation in man correlates with an increase in IgG autoantibody to the Vβ Fr3 region.
7. The level of IgG autoantibodies directed against peptides corresponding to the "switch" region linking the V and C domains of Tcr α/β chains increases in pregnancy.
8. Infection of C57Bl/6 mice with the radiation-induced defective LP-BM5 strain of the murine leukemia retrovirus induces polyclonal B-cell proliferation which includes production of IgG autoantibodies to a large set of Vβ CDR1 peptide homologs. Reactivity against murine Vβ8.1 CDR1 continues to increase selectively following the decline of other autoantibodies.

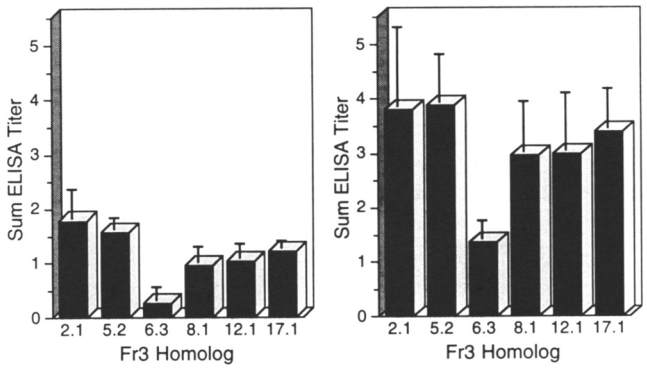

Figure 9. Autoantibody activity in four heart transplant patients to various Tcr-Vβ family CDR1-region peptide homologs. Results are expressed as the Sum ELISA Titer (area of the ELISA curve) with the standard error. (Left), Pretransplant; (Right), Post-transplant.

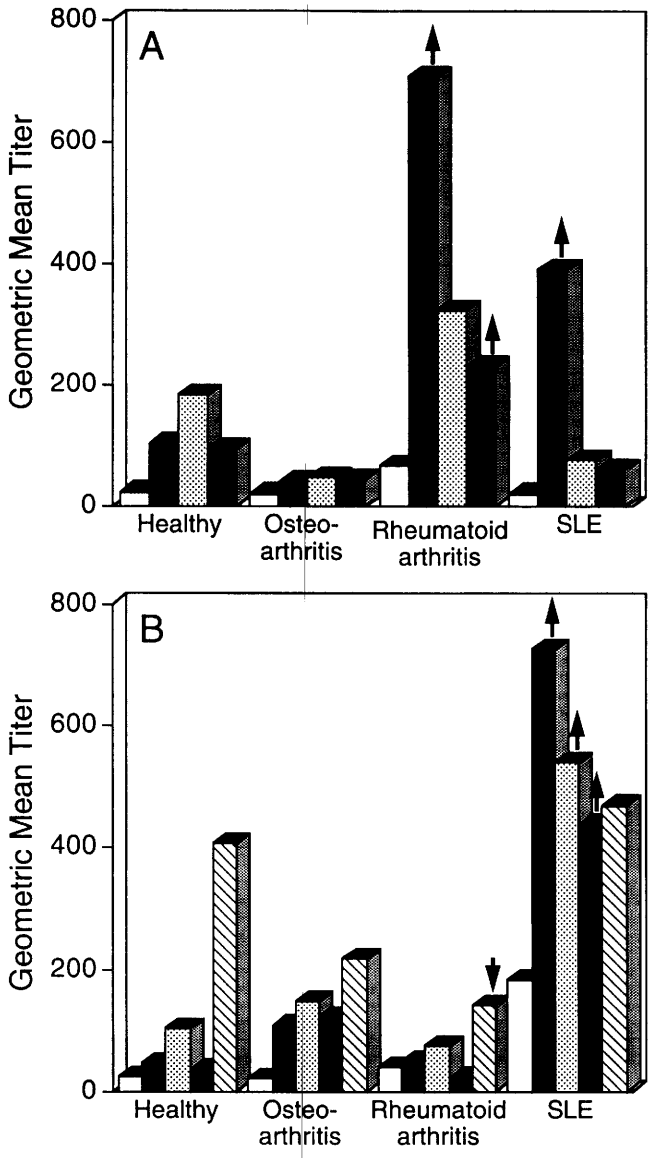

Figure 10. Comparison of human IgM and IgG autoantibody levels to Tcr β peptides and control proteins. Healthy individuals (32 total) and patients suffering from osteoarthritis (10 total), rheumatoid arthritis (14 total), or systemic lupus erythematosus (7 total) were tested. Activity in ELISA was measured against CDR1 peptide (solid), Fr3 peptide (light shade), constant region peptide (dark shade) ovalbumin (stripe), and uncoated negative control (open). The results are expressed as geometric mean titers. A down arrow indicates a statistically significant lower value than for normals. An up arrow is a significantly higher value.

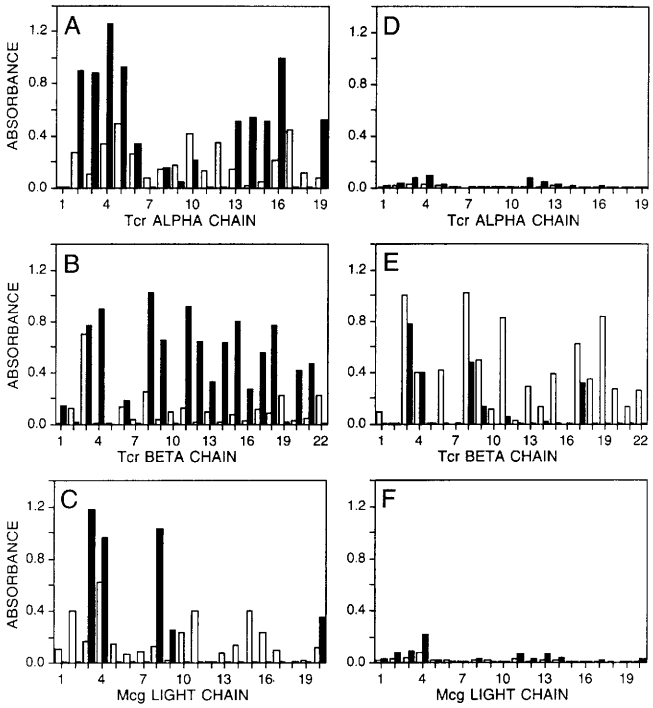

Figure 11. Comparison of IgM (open bar) and IgG (solid bar) binding activity of serum from an SLE patient (A, B, C) and an RA patient (D, E, F) to overlapping synthetic peptides duplicating the Ig domains of Tcr α (A, D), Tcr β (B, E), and the λ light chain Mcg (C, F). The RA patient was one of two showing the highest binding to peptides of 14 patients studied. The SLE patient was one of a group whose serum reacted with human T cells in immunocytofluorescence.

α and λ light chain peptides are extremely low in the RA patient. The reaction against the β peptides is relatively typical for RA sera inasmuch as the IgM activity is higher than the IgG and major reactivity is found against peptides β3, β8, and β17. Thus, the RA serum suggests a prolonged IgM response against a limited set of peptides with a deficient secondary IgG reaction, whereas the SLE profiles indicate a strong secondary response against a variety of peptides. In addition to the IgG autoantibody activity with its requisite dependence on T cells, differences in antigen processing are suggested because of the presence of antibodies directed against most segments of the Tcr molecule.

The selection of human autoantibodies for certain regions of Tcr α/β chains as a function of disease or physiological condition is illustrated in Figure 12. These data were obtained for three groups: healthy and pregnant women and a group with SLE in the University of North Carolina. To determine selectivity, identical assays were carried out using homologous sets of peptides from the α chain, the β chain, and λ light chain. All three groups have IgG autoantibodies directed against the CDR2 segment of the Vα (peptide α5), but the level of reactivity in the SLEs is significantly higher than that of the normal or pregnant women. Furthermore, the same sera show negligible reactivity to the homologous CDR2 peptides from the Tcr β chain and the Mcg light chain. The same three sets of sera are tested on the right hand side against "switch" peptides of Tcr α(α11), Tcr β(β11) and light chain (Mcg11). No reactivity is shown against the light chain peptide but pregnant women show elevated levels of activity against both the Tcr α and Tcr β peptides. This result is consistent with the natural alloimmunization process that occurs in pregnancy, but its relevance to the selective maintenance of an immunosuppressed state remains to be established.

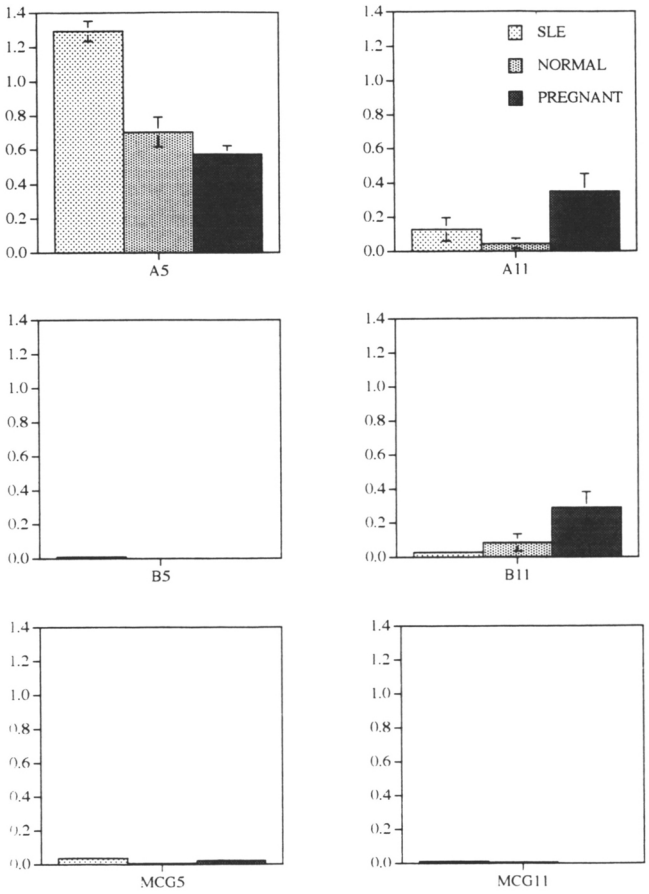

Figure 12. Quantitative comparison of binding of sera from 15 normal women, 15 pregnant women, and 30 women suffering from systemic lupus erythematosus for their capacity to bind CDR2 region peptides (α5, β5, and Mcg5) and switch region peptides (α11, β11, and Mcg11). Data are expressed as mean ± SE of sera assayed at a dilution of 1:200.[97]

Autoantibodies to T-Cell Receptors in Retroviral Infection

If the antibodies directed against Tcrs serve an immunoregulatory function and their increased production results from a disruption of the regulatory network, infectious agents that disrupt the immune system should have an effect on the levels and specificities of the antibodies. Humans infected with HIV suffer massive dysregulation of their immune systems in which there is an initial hyperactivity of lymphocytes and an increased production of immunoglobulins, followed by a loss of functional T cells and the generation of immune deficiency (82). Comparison of autoantibodies present in polyclonal IgG preparations from uninfected humans and those infected with HIV disclosed that the HIV-infected individuals produced substantially increased levels of autoantibodies to the Tcr CDR1, Fr3, and C region peptides (52). This result is consistent with a dysregulation of the immune system resulting in increased polyclonal stimulation of B cells. In addition, autoantibodies against the Jβ segment were found, and the homology between Fr4 and the conserved stretch of the V3 loop of HIV-1 of gp120 suggests that these antibodies arose as a result of immunization with the V3 determinants. Thus, antigenic mimicry between the conserved portion of the V3 loop and the Jβ Fr4 sequence results in the production of antibodies cross-reactive with the self-determinant (52). Infection of C57BL/6 mice with a mixture containing a defective variant of murine leukemia virus (LP-BM5) and a helper virus induces a parallel series of events, including lymphoid hyperplasia and hypergammaglobulinemia followed by the appearance of acquired immune deficiency (AIDS) (83, 84).

The murine model has many similarities to human AIDS even though the initial target of the retrovirus is B cells rather than CD4+ T cells. Infected mice show pronounced increases in the levels of IgG antibodies to T-cell receptor peptides, as illustrated in Figure 13. The binding of IgG immunoglobulins of uninfected mice is compared with those of animals 6 weeks after infection. There are striking increases in the levels of autoantibodies to peptides β3, β8, and β17. In the time course of infection, the antibodies appear after 2 weeks of infection and reach peak levels at approximately 6 weeks. The peak levels are maintained until the 12th week, after which there is a general decline. The hypergammaglobulinemia has essentially the same kinetics (85, 86). However, the appearance of the antibodies against T-cell receptor peptide defined determinants is not strictly a reflection of the hypergammaglobulinemia, as illustrated in Table V. This table lists the percentage of sera within each group showing substantial reactivity (titers >100) against a set of synthetic peptides duplicating the CDR1 region of human and murine T-cell receptor β chains. There is strong reactivity against all the CDR1 segments at 12 weeks, a result which is consistent with a general polyclonal activation. However, by 16 weeks there is a selectivity in the reaction that suggests an active process of autoimmunization. This is particularly apparent in the retained reactivity to the murine Vβ 8.1 and 8.2 sequences. Moreover, the levels of IgG antibodies to other antigens including the CDR1 of the λ light chain and to distinct antigens including ovalbumin do not show this amplified behavior following retroviral infection. It remains to be determined whether production of these autoantibodies plays a role in the pathogenesis of immune deficiency following retroviral infection, and whether this process can be modified by treatment either with peptides or with specific autoantibodies. The association of autoantibodies directed against Tcrs with viral infection is of interest because autoimmune reactions are found in viral in-

[3]Wang E, Marchalonis JJ, Winfield JB, unpublished data.

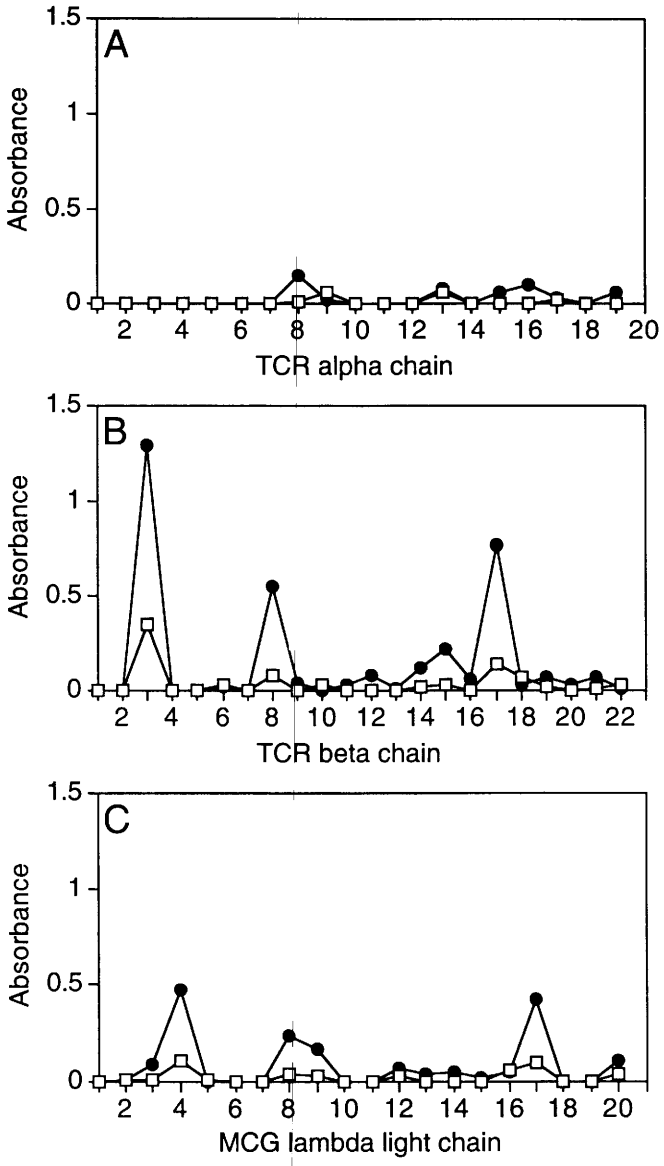

Figure 13. Activity of pooled sera from LP-BM5 virus-infected C57BL/6 mice (MAIDS) to sets of overlapping peptides modeling (A) Tcr α chain, (B) Tcr β chain, and (C) Mcg light chain. ELISA assays were done in quadruplicate with sera diluted 1:200. (●), Infected mice; (□), age-matched uninfected mice.

fections (87, 88) and viruses have been proposed as causative agents in autoimmune diseases like multiple sclerosis (89).

Concluding Remarks

We have used a novel synthetic peptide approach to map autoreactive determinants of human T-cell receptors. We determined that autoantibodies specific for peptide-defined regions bind to intact T-cell receptor V domains using a single chain recombinant Vα/Vβ FV construct. We propose that the regions of Tcrs that carry out recognition and regulatory roles are the same as those of immunoglobulins that govern these processes; notably, we speculate that epitopes defined by the CDR1 and Fr3 segments act as regulatory markers for products of individual Vβ genes. A primary function of natural antibodies is probably regulatory inasmuch as 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 or endogenous antigens. Regulatory IgG autoantibodies function in the removal of aged blood cells expressing the senescent cell antigen (28), and these autoantibodies recognize peptide determinants expressed by modified self-antigens (90). The presence of autoantibodies to peptide defined determinants of human T-cell receptors in commercial preparations of intravenous immunoglobulins may contribute to the success of such preparations in the treatment of autoimmune diseases (32, 33, 91). Further clinical importance of the findings presented here are suggested by the efficacy of some IVIG preparations in the treatment of Kawasaki's disease, an acute multiple system vasculitis of unknown ideology that is a major cause of acquired heart disease in children. These patients show significantly elevated levels of circulating Vβ2- and Vβ8.1-positive T cells compared with other groups (32, 33, 91). These levels decrease, as does the severity of the symptoms following treatment with intravenous Igs. Kawasaki's disease might result from a process resembling activation by superantigens which results in an increased percentage of T cells bearing particular Vβ gene products. Thus, treatment either with autoantigenic peptides or antibodies directed against these peptides may modulate the course of the disease. The existence and the specificity profiles of the autoantibodies we have described lead to a number of challenging questions. Can the antibodies themselves act as superantigens in selectively stimulating subsets of α/Tcr-bearing cells? Is the effect of such antibodies always suppressive? The V-region segments found to be autoantigenic are exposed in the predicted 3-dimensional folding and have been implicated in antigen recognition (CDR1), in the binding of MHC markers (CDR1), or in the recognition of superantigens (Fr3). Extensive studies are in progress using rodent EAE, a proposed animal model for multiple sclerosis, testing either immunization with antibodies or T-cell receptor peptides (58, 59, 66–69) to modulate or eliminate the disease symptoms. The T-cell receptor peptides usually used in these studies correspond to CDR2 and CDR3 segments of antibody or Tcrs and were chosen to duplicate selective idiotypes. The autoreactive peptides considered here may represent advantages for the study of immunoregulation. For example, more than 30% of Vβ sequences listed in Kabat *et al.* (64) show at least 50% identity to the autoreactive CDR1 sequence studied in detail here in human and murine systems. This finding may suggest that the epitope disclosed could serve as a public

Table V. Percentage of Significantly Reactive Sera (8–10 Animals per Group)

CDR1 Vβ Peptide	16 Days preinfection	12 Weeks postinfection	16 Weeks postinfection
Hu Vβ8 (pepβ3)	0%	63%	13%
Hu Vβ2.1	0%	63%	25%
Hu Vβ5.2	11%	50%	75%
Hu Vβ6.3	11%	50%	38%
Mu Vβ	11%	75%	35%
Mu Vβ8.1	11%	88%	100%
Mu Vβ8.2	11%	100%	63%

regulatory idiotope. The Fr3 region is not directly involved in the binding of nominal antigen, but it may be implicated in activation either by superantigen or interaction with MHC products. There is sufficient homology among the protein and gene sequences of Tcrs of man, mouse, and rabbit to allow the design of immunochemical and molecular studies to establish the regulatory role of Tcr autoantigens.

Most individuals, both healthy and those suffering from autoimmune disease, express constitutive antibodies against the putative immunomodulatory epitopes. In addition, autoantibodies were found that have a restricted distribution suggesting an actual process of immunization. The most notable among these are the autoantibodies against switch peptides found in the sera of pregnant women and antibodies against the Jβ segment in HIV-infected individuals that most probably reflect antigen mimicry. Different reactions against distinct portions of the same molecule indicate that the differences may occur in processing of antigen as a function of age or in the other physiological states considered. B cells can present immunoglobulin peptides to T cells via MHC interactions (92), and T cells can recognize immunoglobulin idiotopes (1–3), a necessary stage in the induction of IgG autoantibodies to B cells. The observed reactivity to λ light chain peptides is consistent with this. The prevalence of IgG autoantibodies to immunoregulatory determinants and to other segments of T-cell receptors indicates that helper T cells must also possess the capacity to react against characteristic determinants of other T-cell receptors. It is of interest to determine whether T cells can present peptides to other T cells in an MHC-requiring fashion, or whether this stimulation requires accessory cells such as macrophages. An understanding of the functions and immunoregulatory interactions involving autoantibodies to T-cell receptors has great promise for the elucidation of mechanisms of immune regulation and for modifying the disease processes in autoimmunity and retroviral infection.

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