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Immunology

Secretion of a chimeric T-cell receptor–immunoglobulin protein

(gene expression/gene transfer/chimeric protein)

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ABSTRACT **To produce sufficient quantities of soluble T-cell receptor protein for detailed biochemical and biophysical analyses we have explored the use of immunoglobulin–T-cell receptor gene fusions. In this report we describe a chimeric gene construct containing a T-cell receptor α-chain variable (V) domain and the constant (C) region coding sequences of an immunoglobulin γ2a molecule. Cells transfected with the chimeric gene synthesize a stable protein product that expresses immunoglobulin and T-cell receptor antigenic determinants as well as protein A binding sites. We show that the determinant recognized by the anticlonotypic antibody A2B4.2 resides on the V_α domain of the T-cell receptor. The chimeric protein associates with a normal λ light chain to form an apparently normal tetrameric (H_2L_2, where H = heavy and L = light) immunoglobulin molecule that is secreted. Also of potential significance is the fact that a T-cell receptor V_β gene in the same construct is neither assembled nor secreted with the λ light chain, and when expressed with a C_κ region it does not assemble with the chimeric $V_\alpha C_{\gamma 2a}$ protein mentioned above. This indicates that not all T-cell receptor V regions are similar enough to immunoglobulin V regions for them to be completely interchangeable.**

The T-cell receptor (TCR) appears to recognize two ligands, a protein of the major histocompatibility complex (MHC) and antigen (1–3). Thus, unlike most immunoglobulins that serve as the antigen receptors for B cells, the TCR does not recognize antigen alone but only in association with a MHC molecule. This property of the TCR is known as MHC restriction (1–4). The TCR is composed of α and β chains, which are encoded by immunoglobulin-like genes (5–8) that rearrange to form a complete variable region exon from two or three gene segments: the variable (V), diversity (D), and joining (J) regions (9–12). This assortment of gene segments provides diversity in the T-cell repertoire, which is further increased by the addition ("N-region") and deletion of variable numbers of nucleotides at the V–D and D–J or V–J joints. The V domain is attached to an immunoglobulin-like constant (C) region domain, encoded by a single exon (11–14), with other exons encoding a hinge (13, 15) and transmembrane and cytoplasmic regions (11–15). The α and β chains of the TCR are sufficient for recognition of antigen and MHC, since complementation to restore specificity occurs when α- or β-chain loss mutants are fused with each other (16) and because gene transfer of α and β chains from a T cell of known specificity into another T cell results in transfer of both specificities (17).

Most of the amino acid residues that are found to be highly conserved in all immunoglobulin V regions are also found in TCR V regions, suggesting, as do secondary structure predictions, that folding of TCR and immunoglobulin V regions is very similar (18–22). However, TCR V regions have significantly more primary sequence variability (18, 20, 22), an increased apparent rate of divergence in phylogeny (18, 22), and peaks of variability (hypervariable regions) (18, 22, 23) in addition to those noted in immunoglobulins. It has been suggested that these differences between TCR V regions and those of immunoglobulin may be due to the more complex ligand (antigen plus MHC) that must be recognized (18), but a direct test of this hypothesis, or any other model of TCR:ligand interaction, is only possible at the biochemical level. Specifically, it would be very useful to have large quantities of a soluble TCR for affinity measurements with putative ligands, crosslinking and mutagenesis studies, and also x-ray crystallography, since the cell-surface nature of the TCR and the limited quantities available have so far precluded such experiments. Our approach has been to make a chimeric protein containing TCR V domains with immunoglobulin C domains (24). Antibody molecules have been shown to be very suitable for chimeric gene studies because of their modular organization: in general, functional protein domains are encoded by separate exons. Previous work has shown that heavy (H)- and light (L)-chain V regions may be exchanged, leaving a functional antigen combining site, and that human C regions can be substituted for mouse C regions (25–28). Recently, TCR C region exons have been introduced between the V_κ and C_κ exons. Chimeric proteins were produced and used to elicit antisera that reacted with native TCR protein (29). More radical changes have also been successful, where the C region of a H chain was replaced with staphylococcal nuclease (30) and the antigen binding and enzymatic activities were retained in the chimeric protein.

The experiments described below demonstrate that a chimeric H-chain protein can be produced that has immunoglobulin and TCR antigenic determinants. This protein associates and is secreted with a normal immunoglobulin λ chain. It binds to protein A and is easily purified in milligram amounts.

MATERIALS AND METHODS

Construction of Chimeric Genes. *H-chain construction.* The upstream sequences including the IgH promotor and leader peptide (L) exon and approximately half of the L–V intron derive from a hybridoma making a dansyl-binding antibody (ref. 31; kindly provided by J. Dangl, Stanford University). The *Sal* I site was added by BAL-31 digestion (from a site in the V_H gene) into the L–V intron and blunt-end ligation into pUC19 at a site adjacent to the vector's *Sal* I site.

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Abbreviations: TCR, T-cell receptor; MHC, major histocompatibility complex; V, variable; D, diversity; J, joining; C, constant; GaMIg, goat anti-mouse IgG; H, heavy; L, light.

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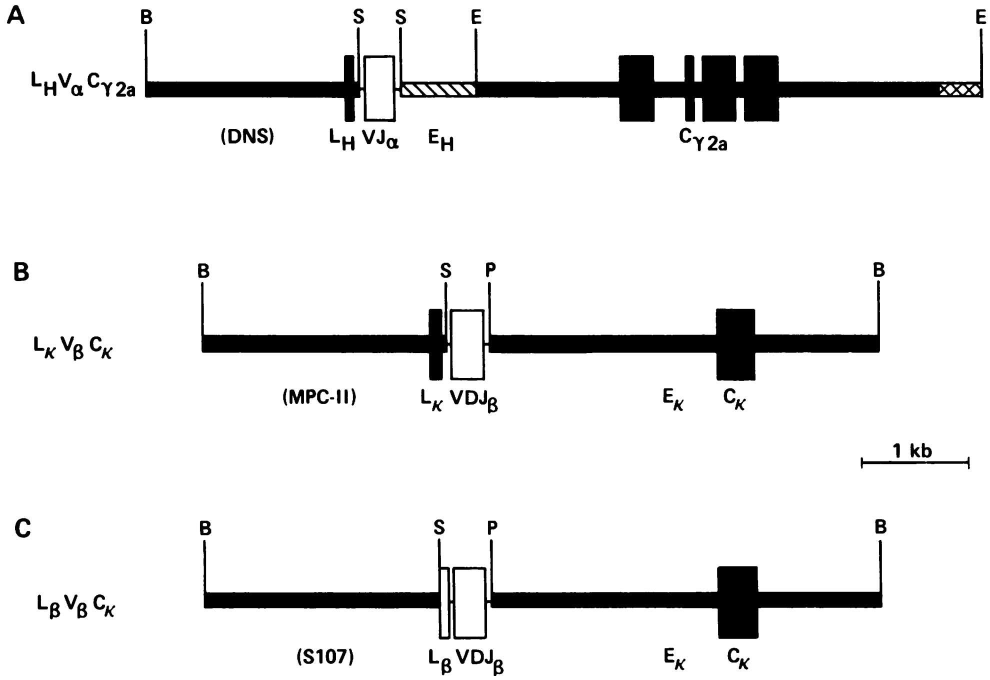

FIG. 1. Chimeric genes. Dark bars represent immunoglobulin DNA; open bars represent TCR DNA. Boxes represent protein coding regions. Restriction enzyme sites used in the construction are shown: B, *Bam*HI; S, *Sal* I; E, *Eco*RI; P, *Pst* I. (*A*) H chain. The upstream sequences, including the promotor and leader exon (L_H), come from a hybridoma producing a dansyl (DNS)-binding antibody. Diagonal shading represents the H-chain enhancer (E_H) (32) and the cross-shading represents pBR322 DNA. (*B* and *C*) L chains. In *B*, promotor and L_κ sequences come from the MPC-11 myeloma, whereas in *C*, the promotor comes from S107 but the leader comes from V_β. The TCR V regions come from the 2B4 cell line (9, 22).

The H-chain enhancer (E_H, diagonal shading; Fig. 1*A*) derives from the same IgH gene and is the ≈0.7-kilobase (kb) *Xba* I to *Eco*RI fragment (32), subcloned into pUC19 and excised as *Sal* I to *Eco*RI. The *Sal* I site therefore forms a "cassette" into which rearranged V region genes may be cloned (see below). The $C_{\gamma 2a}$ gene (a *Bgl* II fragment cloned into the *Bam*HI site of pBR322) containing all but the transmembrane and cytoplasmic domain exons was obtained from C. Hsu (Stanford University). The stippled region represents DNA derived from the region from *Bam*HI to *Eco*RI in pBR322. The TCR V regions used in this study derive from the T helper hybridoma 2B4. A rearranged "genomic" V_α clone was prepared from a cDNA clone (22) and oligonucleotide linkers (kindly synthesized by DNAX, Palo Alto, CA). The linkers provided the relevant RNA splice acceptor and donor sites, intron sequences, and *Sal* I sites, as shown in Fig. 2.

L-chain construction. A chimeric L chain was prepared in a similar manner from a *Bam*HI genomic κ clone from the cell line MPC-11 (Fig. 1*B*). The 5′ part of the gene was prepared from the *Bam*HI fragment partially digested with *Aha* III to delete the V region and about half of the L–V intron. This was subcloned into pUC19 to provide a *Sal* I site at the 3′ end. The downstream part of the gene from an *Ava* I site within $J_{\kappa 4}$ to the *Bam*HI site was subcloned into pUC19 as before and the two halves of the gene rejoined to form the L-chain cassette site. The rearranged genomic $V_{\beta 2B4}$ (9) was subcloned to flank it with *Sal* I sites and cloned into the cassette site. Because of a splicing artefact caused by the remnant of $J_{\kappa 4}$ (24), an ≈600-base-pair (bp) deletion was made from the *Pst* I site used for the $V_{\beta 2B4}$ subcloning to the *Pst* I site ≈270 bp downstream of $J_{\kappa 5}$. Thus, the 5′ *Sal* I site was lost from the finished construction used here but has been replaced in subsequent versions.

Alternate L-chain construction. A similar construct (Fig. 1*C*) containing the complete $L_\beta V_\beta$ region but using the S107 κ promotor and upstream sequences was also made. This gave identical results and the two constructions were used interchangeably.

The finished constructions, therefore, contain immunoglobulin leader and C region exons, but with TCR V regions and chimeric L–V introns (except Fig. 1*C*), of approximately the same length as in the native immunoglobulin genes. The constructions were sequenced around the cassette sites to check that they were as predicted and were inserted into the eukaryotic expression vector pSV2ΔH.gpt either individually or together in opposite transcriptional orientations. This vector allows cells to be selected for expression of the *Eco-gpt* gene by resistance to mycophenolic acid. It is a derivative of pSV2.gpt (33, 34), from which the *Hin*dIII site has been deleted.

Transfection and Analysis of Transfected Cell Lines. The Sp2/0 cell line was transfected with chimeric genes by protoplast fusion as described (34). Sp2/0 is a derivative of a hybridoma that no longer makes immunoglobulin protein (35). J558L is a mouse myeloma that produces and secretes a λ-chain protein (34). Transfected cells were selected in medium containing mycophenolic acid (GIBCO) (1 μg/ml for Sp2/0; 5 μg/ml for J558L), hypoxanthine (Sigma) (15 μg/ml), and xanthine (Sigma) (200 μg/ml). Resistant cell lines were tested for immunoglobulin synthesis by immunoprecipitation of ^{35}S metabolically labeled protein as described using the following antibodies: affinity-purified goat anti-mouse IgG (GaMIg) antiserum (Sigma), A2B4.2, a mouse monoclonal IgG_{2a} anti-TCR antibody reactive with the 2B4 TCR (36), and 14.4.4, a mouse IgG_{2a} anti-I-E^k monoclonal antibody. Immunoprecipitation was performed with fixed protein A-positive *Staphylococcus aureus* (Pansorbin, Calbiochem) coated with GaMIg or Pansorbin alone after preclearing. In some experiments (Fig. 6) immunoprecipitation was carried out using antibody-coated RIA plates (37). One-dimensional NaDodSO$_4$/PAGE was performed by the method of Laemmli (38) with Pharmacia low molecular weight standards and purified IgG_{2a} (Sigma) as markers. Unreduced samples were boiled with 150 mM iodoacetic acid and reduced samples were boiled with 25 mM dithiothreitol. After cooling, iodoacetic acid was added to reduced samples. Gels were treated for fluorography with Fluorohance (Research Products International, Mt. Prospect, IL) prior to autoradiography.

RESULTS

Expression of Chimeric Genes. Genomic clones encoding immunoglobulin H and L chains were modified to permit the expression of rearranged TCR VJ and VDJ exons in place of the existing rearranged VDJ_H and VJ_κ exons. A schematic diagram of the resulting chimeric genes is shown in Fig. 1 and the constructions are described in detail in the *Materials and Methods* section. To express these chimeric genes in B cells, we preserved intact the known immunoglobulin regulatory sequences (promotor) 5′ of the leader peptide exon (L) and 3′ of the V exon (the enhancer: E in Fig. 1). We also wished to have a convenient cassette site for the insertion of various TCR V region exons, and for this reason we have engineered unique *Sal* I sites into the vectors (due to technical reasons, the *Sal* I site of the L-chain vector is now a *Sal* I to *Pst* I site; see *Materials and Methods* and ref. 24). The TCR V regions derive from the T helper cell hybridoma 2B4 and have been described (9, 22). This T cell recognizes a cytochrome *c* peptide in the context of a MHC class II molecule (I-E^k). The V_β exon derives from a clone isolated from a genomic library of 2B4 DNA (9), whereas the V_α exon is an artificial construction made by ligating synthetic oligonucleotides that mimic the 5′ and 3′ splice sites of known TCR V_α and J_α sequences to fragments of a 2B4 VJ_α cDNA clone (22) (shown in Fig. 2). These synthetic oligomers join to sites within the coding region and end with *Sal* I "sticky ends" to allow insertion into the vector cassette site.

RNA transfer blot analysis of cells transfected only with L chain shows that chimeric L-chain mRNA is made at levels comparable to normal κ gene expression (24). In cells transfected with H- and L-chain chimeric genes, both V_α and V_β genes were expressed as mRNA of the predicted size and were appropriately spliced, as determined by cDNA sequencing (N.R.J.G., unpublished). In both cases, the level of expression of the chimeric mRNA was far greater than the expression of 2B4 TCR mRNA (ref. 24; N.R.J.G., unpublished).

Lysates from immunoglobulin-negative Sp2/0 cells transfected with chimeric H and L genes were immunoprecipitated with goat anti-mouse IgG (GaMIg) antiserum on protein A-positive *S. aureus* cells (Pansorbin) or the Pansorbin alone. The results, shown in Fig. 3, indicate that the transfected cells express both chimeric proteins—the major bands migrating close to the mouse immunoglobulin γ2a and κ markers. The chimeric L chain consistently migrates slower than the marker, with an apparent molecular mass of 29–31 kDa. The Pansorbin alone also precipitates the H chain but not the L chain. The unreduced portion of the gel shows that the H chain is precipitated by Pansorbin or GaMIg as a major species of ≈100 kDa, which indicates that the H chains exist in the cell as H_2 dimers. Thus, the H chains associate with each other but not with the L chains. These transfectants do not secrete immunoglobulin protein, either by enzyme-linked immunosorbent assay (ELISA) (the detection limit in these experiments was 3–9 ng/ml) or immunoprecipitation (data not shown). The fact that Pansorbin alone can precipitate the H chain indicates that the protein A binding region is assembled in the correct fashion.

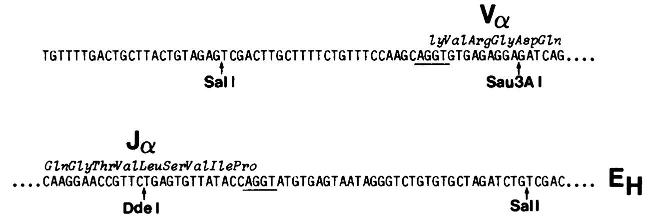

FIG. 2. Sequences of the 5′ and 3′ regions of the $V_{\alpha 2B4}$ exon derived from a cDNA clone (22) and synthetic oligomers. The IgH leader (L_H) and the 5′ part of the L–V intron derive from the H-chain gene of an anti-dansyl hybridoma (31). The 5′ oligonucleotides cover the intron from the *Sal* I site to the *Sau*3AI site in the $V_{\alpha 2B4}$ coding region. The predicted amino acid sequence is shown. The 3′ part of the VJ_α construct was made with oligonucleotides extending from the *Dde* I site in J_α to the *Sal* I site that abuts the H-chain enhancer (32) (E_H in Fig. 1) in the H-chain construct. The predicted mRNA splice donor and acceptor sequences are shown underlined.

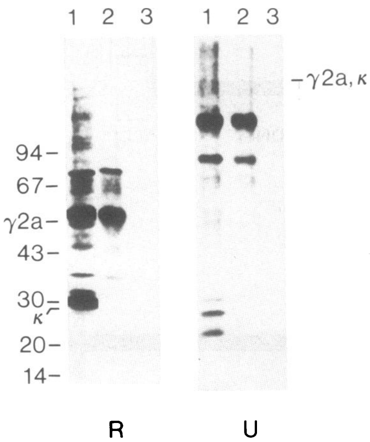

FIG. 3. Immunoprecipitation of Sp2/0 and derivatives transfected with chimeric $V_\alpha C_{\gamma 2a}$ and $V_\beta C_\kappa$ genes. A 5–15% gradient gel was run with either reduced (R) or unreduced (U) samples. Lanes 3, Sp2/0, immunoprecipitated with GaMIg. Lanes 1 and 2, transfectants precipitated with GaMIg (lane 1) or protein A alone (lane 2). Molecular masses are indicated in kDa.

Secretion of Chimeric H Chain with Native λ Chain. To distinguish between the H and L chains as the cause of the nonassociation of the chains, we transfected plasmids containing either the chimeric H-chain gene or both H- and L-chain genes into the cell line J558L, which makes and secretes a λ chain. In both cases, ELISAs on supernatants revealed secretion of immunoglobulin at levels of 0.1–1 μg/ml. Chain-specific reagents show that λ but not κ is secreted (data not shown). Fig. 4*A* shows a lysate from a nonsecreting (Sp2/0) transfectant and lysate and supernatant from a secreting (J558L) transfectant immunoprecipitated with GaMIg. The λ chain may clearly be distinguished from the $V_\beta C_\kappa$ chimera as a band of ≈26 kDa. The secreted material consists of H and λ chains only. The unreduced lanes show that, whereas there are no significant bands larger than H_2 dimers in the Sp2/0 transfectant, there are two larger bands in the J558L-derived cells, the larger of which comigrates with unreduced mouse IgG_{2a} control antibody and thus represents H_2L_2 tetramers. An intermediate band probably represents H_2L molecules. Some of this species is found in the supernatant and probably represents a degradation product rather than secreted protein. Fig. 4*B* shows that Pansorbin (i.e., protein A) precipitates the secreted protein as well as the protein from the lysate. The $V_\beta C_\kappa$ protein is only immunoprecipitated from the lysate when GaMIg is used, confirming the result in Fig. 3.

As these results suggest that the L-chain chimera is responsible for the lack of association and secretion, we tested the V_β exon in place of V_α in the H-chain vector as expressed in J558L. Of 40 transfectants, none was obtained that secreted detectable levels of immunoglobulin. Fig. 5 shows a $V_\beta C_{\gamma 2a}$ transfectant and a control $V_\alpha C_{\gamma 2a}/V_\beta C_\kappa$ transfectant (both in J558L). Although H and λ chains are made, the $V_\beta C_{\gamma 2a}$ H chains do not form H_2 dimers, and the $V_\beta C_{\gamma 2a}$ protein is not secreted. Note that little λ chain is immunoprecipitated in the $V_\beta C_{\gamma 2a}$ lane since it is not associated with H chain and the GaMIg antiserum reacts poorly with λ chain.

Expression of a TCR Clonotypic Determinant by the V_α Domain. A clonotypic monoclonal antibody made against the 2B4 hybridoma has been shown to precipitate the TCR α and β chains from that cell (36). When this antibody is used to immunoprecipitate supernatants and lysates from transfectants that express chimeric H and κ genes but secrete only H

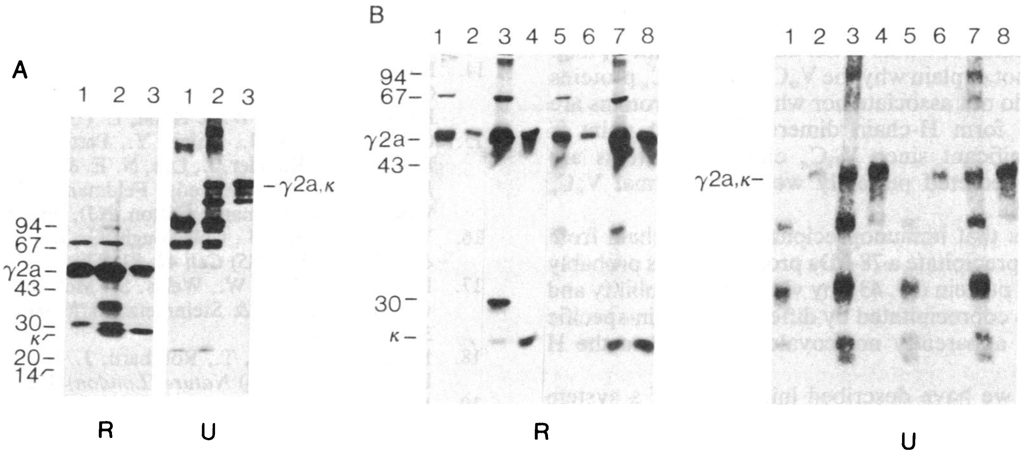

FIG. 4. (*A*) GaMIg immunoprecipitation of Sp2/0 and J558L cells transfected with chimeric H and L genes and run on a 5–15% gradient gel. Lanes 1, lysate of Sp2/0 transfectant; lanes 2, lysate; and lanes 3, supernatant from J558L transfectant. The λ chain of J558L is seen as a band running with the κ marker and the chimeric κ chain is seen as a band of ≈31 kDa. (*B*) GaMIg or Pansorbin immunoprecipitation of J558L cells transfected with chimeric H or H and L chains and run reduced on a 12.5% (R) gel or run unreduced on a 5.5% (U) gel. Lanes 1–4, GaMIg on H alone (lanes 1 and 2) or H plus L (lanes 3 and 4) transfectants. Lanes 5–8, Pansorbin precipitation of H alone (lanes 5 and 6) or H plus L (lanes 7 and 8) transfectants. Odd-numbered lanes are lysates; even-numbered lanes are supernatants. In this experiment the chimeric κ chain runs at 30 kDa and the λ chain runs with the κ marker. Molecular masses are indicated in kDa.

plus λ protein, it is able only to precipitate the H plus λ protein but not the $V_\beta C_\kappa$ protein (Fig. 6). It also does not precipitate the $V_\beta C_{\gamma 2a}$ protein but does precipitate the $V_\alpha C_{\gamma 2a}$ protein alone (data not shown). Thus, a TCR determinant is reformed in the chimeric H-chain protein, indicating that this 2B4 clonotypic determinant is an epitope of the α-chain V domain and is present whether or not this V_α is associated with another V region. This result was also obtained by infecting NIH 3T3 cells and various T-cell lines with a retrovirus containing the 2B4 α-chain cDNA. These cells then made a protein reacting with the anti-2B4 antibody (A. Korman, personal communication). We do not know whether the determinant is encoded by V, J, or junctional (N region) sequences. In related experiments using a V_β region from the C5/$V_{\beta 8}$ (18, 19) family, a chimeric $V_\beta C_\kappa$ protein is produced that reacts with an antibody known to recognize members of this V_β family on T cells (40, 41) (N.R.J.G., K.I.D., and M.M.D., unpublished), indicating that a second TCR V region determinant can also be recovered using this kind of chimeric expression system.

DISCUSSION

The data that we have here presented show that chimeric polypeptides containing TCR V regions and immunoglobulin C regions can be produced in plasma cells where they accumulate to levels comparable to native immunoglobulin molecules. These chimeric molecules seem to be in a relatively native configuration as they express immunoglobulin and TCR epitopes (as defined by GaMIg antiserum and anti-TCR V region monoclonal antibodies, respectively). The H chain is also precipitated by protein A alone (Figs. 3 and 4), indicating that two domains of the $C_{\gamma 2a}$ molecule are functioning normally, since protein A has been shown to contact residues in C_H2 and C_H3 domains when bound to immunoglobulin (42). A similar preservation of immunoglobulin and TCR antigenic determinants has been shown in the case of a TCR C_β region exon inserted into a κ-light chain (between V_κ and C_κ) and expressed in plasma cells (29). Thus, immunoglobulin–TCR chimeras are a convenient way of producing protein for immunizations designed to yield anti-TCR antibodies, helping to circumvent the difficulties inherent in the whole cell or cell-lysate immunizations currently in use.

Although the $V_\alpha C_{\gamma 2a}$ chimera does not form sulfhydryl bonds with the $V_\beta C_\kappa$ molecule, it is able to form H_2 dimers and to be assembled and secreted with a λ L chain as an apparently "normal" (H_2L_2) immunoglobulin tetramer (Fig. 4). These data indicate that although some TCR V regions are permissive for assembly with immunoglobulin C regions, others are not. This may be a V_β-specific problem since another V_β from the C5/$V_{\beta 8}$ family (18, 19) also does not associate with the $V_\alpha C_{\gamma 2a}$ protein, nor does $V_{\beta 2B4}$ associate with the λ L chain or with itself (to form H_2 dimers) when put into the H-chain expression vector in place of $V_{\alpha 2B4}$. This is interesting in view of the fact that V_β sequences have consistently shown additional hypervariable regions (18, 22, 23) and may have some structural difference that makes them non-interchangeable with immunoglobulin Vs in this type of

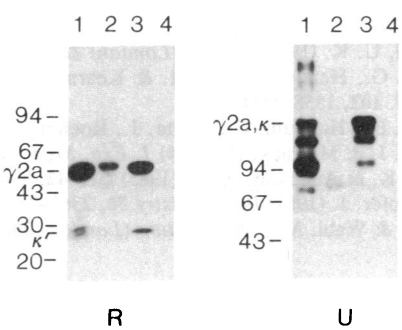

FIG. 5. GaMIg immunoprecipitation of lysate (lanes 1) and supernatant (lanes 3) from $V_\alpha C_{\gamma 2a}$ into J558L transfectants. Lanes 2 and 4, lysate and supernatant from $V_\beta C_{\gamma 2a}$ transfected into J558L. Samples were run reduced (R) or unreduced (U) on a 5–15% gradient gel. Molecular masses are indicated in kDa.

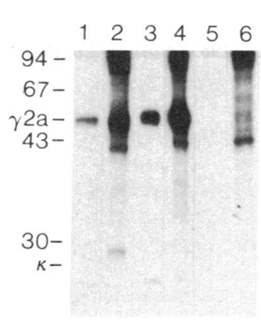

FIG. 6. $V_\alpha C_{\gamma 2a}$ into J558L transfectant was immunoprecipitated with GaMIg (lanes 1 and 2), A2B4.2 (an anti-2B4 monoclonal antibody; ref. 36) (lanes 3 and 4), or 14.4.4 (anti-I-E^k) (lanes 5 and 6) and run reduced on a 12.5% gel. Lanes 1, 3, and 5 are supernatants; lanes 2, 4, and 6 are lysates. The immunoprecipitation was performed using antibody-coated RIA plates by the method of ref. 37. Molecular masses are indicated in kDa.

expression system. Although one may argue that V_αs are more "H-chain-like" and thus better able to pair with V_λ than is V_β, this does not explain why the $V_\alpha C_{\gamma 2a}$ and $V_\beta C_\kappa$ proteins described here do not associate nor why $V_\beta C_{\gamma 2a}$ proteins are unable even to form H-chain dimers. This last point is particularly significant since $V_H C_\kappa$ chimeric proteins are assembled and secreted perfectly well with normal $V_\kappa C_\kappa$ polypeptides (26).

All treatments that immunoprecipitate the H chain from cell lysates also precipitate a 78-kDa protein. This is probably H-chain binding protein (39, 43), by virtue of its mobility and the fact that it is coprecipitated by different H-chain-specific reagents, yet is apparently not covalently linked to the H chain.

In summary, we have described initial uses of a system designed to yield secreted protein for studies on the TCR binding site. We have produced H- and L-chain polypeptides of chimeric $V_{TCR}C_{Ig}$ antibody. Although we have been unable to get complete association and secretion of the chimeric antibody, we have demonstrated the feasibility of some aspects of this system, since the chimeric H chain expresses TCR idiotypic determinants and protein A binding sites and is able to combine with immunoglobulin λ chains to form a tetrameric antibody that is secreted by the cell. Since secretion is up to a level of $\approx$2 μg/ml under optimized conditions, substantial quantities of chimeric antibody may be purified by passage of the supernatant over protein A-Sepharose columns. Other, nonsecreted, chimeric proteins can also be purified by passage over antiimmunoglobulin columns, producing useful reagents for the generation of anti-TCR antibodies.

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