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AN UNUSUAL CLONOTYPIC DETERMINANT ON A CYTOTOXIC T LYMPHOCYTE LINE IS ENCODED BY AN IMMUNOGLOBULIN HEAVY CHAIN VARIABLE REGION GENE

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We have detected, in a CTL clone, an mRNA homologous to an immunoglobulin heavy chain variable region (V_H) gene segment. A full-length copy of this mRNA was cloned and sequenced, revealing that it is the transcript of an authentic, unrearranged V_H gene. The predicted protein product of this expressed V_H gene is an approximately 12-kilodalton polypeptide, with secretory signal peptide leader and no membrane-anchoring sequences. Using immunologic reagents generated against a synthetic peptide representing the carboxyl terminus of the V_H protein, we detect this protein as a clonotypic cell surface molecule. Strikingly, the anti-peptide reagents also exert effects on CTL function clonotypically. Immunoprecipitation experiments suggest that the V_H protein may be associated noncovalently with the consensus, major histocompatability complex-restricted, antigen-specific T cell receptor α- and β-chains on the cell surface of this CTL. We detect weakly cross-reactive material similar to the V_H protein in electrophoretic mobility in other CTL clones and suggest the possibility that small V_H-like molecules may constitute a novel class of receptor components with variable determinants involved in the binding of nominal antigen and contributing to overall receptor diversity.

Recent progress in elucidating the structure of antigen-specific, major histocompatability complex (MHC)-restricted receptors of thymus-derived (T) lymphocytes is founded on the assumption that T cell receptors, like antigen receptors of B cells (which are immunoglobulins [Ig]), recognize specific target molecules via unique antigen-combining sites and that these combining sites are immunologically distinguishable as idiotypic determinants. Further, because the specificity of target recognition by definition is determined by these receptors, antibodies recognizing them should exert an effect on T cell function, either by mimicking antigen and triggering T cell activation or by displacing antigen and thereby interfering with the recognition process and subsequent immune responses. A large set of monoclonal antibodies directed against clonotypic, function-associated determinants on different T cell clones recognize a class of related heterodimeric structures bearing these idiotypic determinants (1–6); these structures do resemble antibodies on the gross structural level of having constant and variable portions (7–9) and interchain and intrachain disulfide linkages (1, 2, 4, 5) as well as being genetically related by their membership in the Ig-like gene family (10–13).

The irony of this converging body of work is that it has been just such a hypothesis—that Ig-related structures compose the recognition complex of T lymphocytes—that has helped to perpetuate the enigma of the T cell receptor. Many investigators have reported that Ig heavy chain variable region (V_H)-related determinants are present on the surface of T lymphocytes (14–16). By extension from the role of Ig (and the variable regions in particular) as the antigen-binding receptors of B cells, it has been argued that T cells probably exploit the large V_H gene pool.

Just as the repertoire of antibody responses represent the diversity and abundance of independent precursors of antibody-forming (B) cells, the repertoire of T lymphocytes mediating cellular immunity appears to be very diverse and clonally distributed (17–21). The distinctive characteristic of cellular immunity is that the "antigen" recognized by T effector cells generally is some molecular species presented on the surface of a target cell in the context of a specific MHC allele (22–25). Indeed, MHC antigens play a major role in determining the pattern of responsiveness; the frequency of T cells reactive with MHC antigens (especially alloreactive) is exceedingly high. Of course, the differences in antigen recognition by B and T cells suggest that significant structural differences must distinguish their respective antigen combining receptors. Nonetheless, the reported findings of Ig-like molecules and the notion that Ig were at least partially involved in T cell antigen recognition have persisted.

Although serologic cross-reactivity does not prove genetic identity, by nucleic acid approaches investigators have searched for V_H hybridizing mRNA species in T cells. These efforts have proved largely unsuccessful. In particular, Kemp et al. (26) did not detect mRNA hybridizing with four V_H probes even under reduced stringency in eight T cell lines, and Kronenberg et al. (27), in a very careful study, could reasonably exclude the presence of V_H-related transcripts even at low copy number from three functional T cell hybridomas.

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The common failure to detect such transcripts has encouraged a timely reevaluation and discounting of the V_H model (28). Nonetheless, we have found just such a T cell-specific V_H transcript and report our efforts to understand its significance.

MATERIALS AND METHODS

Animals. Female New Zealand White rabbits (weighing 3 to 5 kg) were obtained from Pine Acres Farms, Brattleboro, VT. Female, 6-wk-old BALB/c, C_3H, DBA/2, and A/J mice were obtained from The Jackson Laboratory, Bar Harbor, ME.

Cells. Stimulator-independent, interleukin-2-dependent cytotoxic T lymphocytes (CTL)[3] lines were grown in RPMI 1640 medium supplemented with glutamine (10 mM), 2-mercaptoethanol (50 μM), HEPES buffer (10 mM), heat-inactivated fetal calf serum (10% v/v), and interleukin 2-containing EL-4 culture supernatant (3% v/v). Note that because these CTL lines are independent of stimulators, they were grown in pure cultures, so the presence of other contaminating cells (particularly Ig-producing B cells) as potential sources of V_H-like molecules can be excluded.

Interleukin 2-containing supernatants were prepared from the EL-4 thymoma cell line by culturing cells for 24 hr in serum-supplemented RPMI 1640 medium at 1×10^6 cells/ml in the presence of 10 ng/ml phorbol myristate (Sigma Chemical Co., St. Louis, MO).

Splenic blasts were prepared by culturing cell suspensions of mouse spleens in serum-supplemented medium (10^6 cells/ml) in the presence of 10 μg/ml bacterial lipopolysaccharide (Difco Laboratories, Detroit, MI) for 48 hr. Blasts were collected by centrifugation over Ficoll-Urovison (see Reference 43).

Mouse fibroblast Lta cells (also grown in serum-supplemented medium) were transfected with cloned DNA by using a calcium phosphate precipitation protocol and selected in HAT medium as described (29). The V_H cDNA of pcDFL.1 resides in an SV40-based expression vector (30). Viral sequences in the vector facilitate transcription of the cDNA insert in mammalian cells without altering the encoded translation product(s).

Nucleic acid techniques. RNA was electrophoretically fractionated in 0.8% agarose gels containing 2.2 M formaldehyde and transferred to nitrocellulose (31). Filters were preannealed for several hours in 5 × SSC (1 × SSC is 150 mM NaCl and 15 mM sodium citrate), 50% deionized formamide, 5 × Denhardt's solution (32), 20 mM sodium phosphate buffer (pH 6.5), and 250 μg/ml denatured, sheared salmon sperm DNA. Filters were then hybridized at 42°C for 16 hr with the indicated (^{32}P)-nick-translated probes in the same buffer except that the concentration of salmon sperm DNA was 100 μg/ml, Denhardt's solution was at 1 × concentration, and sodium dextran sulfate was included at 10% (w/v). Filters were rinsed several times in 2 × SSC and 0.1% sodium dodecyl sulfate (SDS), washed at relaxed stringency in the same solution at 37°C, and exposed for autoradiography. In the case described in this paper, where a positive signal was detected, the signal was present even when filters were washed at high stringency in 0.1 × SSC and 0.1% SDS at 50°C (two washes, 30 min each).

Restriction endonuclease cleavage patterns in DNA were analyzed by Southern blot analysis essentially as described (33). DNA was digested with the indicated restriction enzymes, fractionated on 1% agarose gels, and transferred to nitrocellulose. Filters were processed as above and washed at high stringency. In cases where hybridization probes contained oligo-dT runs, polyadenylic acid was included in the hybridization reaction at 50 μg/ml.

A cDNA library was prepared from DFL_{12} poly A^+ RNA by the method of Okayama and Berg (30). Plasmid DNA was prepared from this library, digested with restriction endonuclease SalI and were size fractionated by agarose gel electrophoresis. A strip of this gel was analyzed by Southern blotting as above, by using the V_H probe from the anti-(4-hydroxyl-3-nitrophenyl)acetyl (anti-NP) hybridoma P5.29.1 (34, 35). A band corresponding to the linearized full-length cDNA plasmid (2.5 kilobase pairs [kbp] cDNA plus 3.2 kbp vector = 5.7 kbp fragment) was the only hybridizing species observed; the corresponding material from the preparative gel was eluted, recircularized (see Reference 36 for methodology), and transformed into *E. coli* strain DH1 (37). Transformants were screened (37) with the same P5.29.1 V_H probe, a positive colony was purified, and plasmid DNA was prepared.

Preparation of immunologic reagents. Rabbits were immunized intradermally with 1 mg peptide-coupled bovine serum albumin (BSA) in complete Freund's adjuvant as described (38). After 1 mo, they were boosted repeatedly with the same immunogen at 15-day intervals until serum titers reached a maximum plateau (generally, four to five boosts) as measured in a solid-phase radioimmune assay (39), utilizing as antigen peptide coupled to irrelevant carrier (myoglobin) and an ^{125}I-iodinated goat anti-rabbit Ig probe. Serum was depleted of BSA reactivity on a BSA-Sepharose column and affinity purified by chromatography on a peptide-Sepharose column.

Mice were immunized with alum-precipitated, peptide-coupled BSA and boosted twice at 1-mo intervals. Spleen cells, taken 5 days after the last boost, were fused with the myeloma cell line X63.Ag8.653, and hybrids were selected in HAT medium as described (40). Supernatants were screened for the presence of peptide-binding antibodies in a solid-phase radioimmune assay (39); positive hybridomas were cloned at limiting dilution and ultimately established as malignant ascites. The IgM monoclonal anti-peptide antibody from hybrid 40.A6 was concentrated from ascitic fluid by boric acid precipitation (41). One volume of ascites was combined with 20 vol of 0.5% boric acid (w/v) and mixed by rolling for 1 hr at 4°C; the precipitated antibody was collected by centrifugation (15 min at 1500 × G, 4°C) and resuspended in and dialyzed against phosphate-buffered saline. This material was coupled to cyanogen bromide-activated Sepharose (Pharmacia; Piscataway, NJ) at a concentration of approximately 1 μg protein/μl beads.

Immunoprecipitations. Cells were metabolically labeled by culturing them at 1×10^6/ml for 16 hr in RPMI 1640 medium containing [^{35}S]methionine (100 μCi/ml; Amersham, Arlington Heights, IL) and [^{3}H]leucine (50 μCi/ml; Amersham) as the only source of those amino acids and otherwise supplemented as above except that the fetal calf serum used was first dialyzed against RPMI salts. Labeled cells were solubilized (1×10^6 cells in 200 μl) in a triple detergent extraction buffer (1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 0.1% BSA, 5 mM Na_2 EDTA, and 1 mM phenyl methyl sulfonyl fluoride in phosphate-buffered saline [PBS], and the extract was precleared with nonimmune rabbit serum (25 μg/ml) and formalin-fixed *Staphylococcus aureus* cells (50 μl of a 10% suspension in extraction buffer/reaction) for 30 min at 4°C. Precleared extracts were diluted with the extraction buffer and aliquoted into tubes (100 μl/tube) along with fixed *Staphylococcus aureus* cells (50 μl of a 10% suspension/tube). Rabbit antibodies (25 μg/ml) and soluble peptide (20 μM) were added to tubes as indicated, and the reactions were incubated at 4°C overnight. Bacteria were collected by centrifugation, washed twice in extraction buffer, then once more in extraction buffer containing 0.5 M NaCl; the bound material was eluted in SDS sample buffer. Eluted material was analyzed by SDS polyacrylamide gel electrophoresis.

Cells were surface labeled with [^{125}I]NaI in a lactoperoxidase-catalyzed reaction. Cells (5×10^6) were resuspended in 1 ml of PBS containing 2.5 μM NaI and 5 mM D-glucose (α, β equilibrium mixture). Lactoperoxidase (10 μg), 100 μCi [^{125}I]NaI, and finally 0.1 U glucose oxidase were added. The reaction proceeded for 10 min at room temperature before quenching. After washing in PBS containing 10 mM iodoacetamide, the cells were lysed in 200 μl of Nonidet P-40 (NP-40) buffer (150 mM NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM Na_2 EDTA, 0.5% NP-40, 1 mg/ml BSA, 100 mM iodoacetamide, and 1 mM phenyl methyl sulfonyl fluoride), and the extract was precleared with irrelevant antibody (in this case, mouse monoclonal anti-NP antibody, P9.37.1 (34) coupled to Sepharose (see above, "Preparation of immunologic reagents") for 30 min at 4°C. Precleared extracts were diluted with the extraction buffer and aliquoted into tubes (100 μl/tube) along with 100 μl of the indicated monoclonal antibody coupled to Sepharose (approximate antibody concentration, 250 μg/ml). After incubating at 4°C overnight, material bound to Sepharose was collected by centrifugation, washed three times in NP-40 extraction buffer, and eluted in SDS sample buffer. Eluted material was analyzed by SDS polyacrylamide gel electrophoresis.

Radiolabeled and immunoprecipitated material was analyzed by one-dimensional SDS polyacrylamide gel electrophoresis (42). Reduced samples were prepared by heating at 100°C for 5 min in sample buffer (42) containing 5% 2-mercaptoethanol; unreduced samples were prepared in buffer without 2-mercaptoethanol. Gels were dried and autoradiographed for visualization of samples; those containing ^{3}H-labeled material were first fluorographed.

CTL functional assays. CTL-mediated cytotoxicity was assayed essentially as described by Pohlit et al. (43). Splenic blast target cells were labeled with [^{51}Cr]sodium chromate and derivatized with activated succinimide esters of the indicated haptens as described (43) except that fluorescein was added to targets in the isothiocyanate form, and this coupling was performed at 37°C at a pH of 8.9 in an otherwise identical buffer. Effector CTL and targets were washed and resuspended in RPMI medium containing heat-inactivated fetal calf serum (10% v/v). Effectors were distributed to wells in 100 μl at

[3] Abbreviations used in this paper: CTL, cytotoxic T lymphocyte; kb, kilobase; kbp, kilobase pair; kD, kilodaltons (m.w. × 10^{-3}); NP-40, Nonidet P-40; NP, (4-hydroxy-3-nitrophenyl)acetyl; NIP, (5-iodo-4-hydroxy-3-nitrophenyl)acetyl; SP, 3-(*p*-sulfophenyldiazo)-4-hydroxyphenyl acetyl; TK, thymidine kinase.

2×10^5/well and dilutions. Where indicated, additions were made in 50 μl to each well. Antibodies were incubated with effectors for 30 min at 37°C before the addition of targets; concanavalin A (Con A) was added immediately before targets. Targets (2×10^4) then were added to each well so that the final volume in each well was 200 μl, and the effector to target cell ratios were as indicated. Plates were incubated at 37°C for 5 hr, and ^{51}Cr released into supernatants was then determined. The percent of ^{51}Cr specifically released was calculated as described (43).

Interferon-γ (IFN-γ) was assayed as an I-A-inducing activity in a modification of procedures described (44, 45). Briefly, cells of a clone of the macrophage line P388.D1, which was selected for highly inducible I-A expression (D. Gold, personal communication), were incubated for 72 hr with various dilutions of the culture supernatants to be tested (2×10^5 cells/well with 200 μl of the dilution in complete medium). Each dilution was tested in triplicate wells. Cells were then fixed with glutaraldehyde (46) and processed in a cellular radioimmune assay by using the monoclonal anti-I-A^d antibody MKD-6 (44) and ^{125}I-labeled monoclonal rat anti-mouse κ-chain antibody, 187.1 (47). Results were calculated in units of IFN-γ/ml, with the number of units being defined as equal to the inverse of the lowest dilution giving an I-A-specific signal at least fourfold above background.

RESULTS

A V_H mRNA. We, like other investigators (26, 27), screened mRNA from a collection of clonal lines of functional T lymphocytes with a variety of V_H probes. In most cases, no V_H-hybridizing mRNA was observed. However, in two cases, CTL clones having specificity for fluoresceinated targets bearing the "d" allele of the mouse major histocompatability H-2D molecule were found to synthesize a unique mRNA species hybridizing with a V_H probe representative of the family of anti-NP (34, 35) antibodies (Fig. 1). Because these two CTL were derived from the same mixed lymphocyte culture and share fine specificities (20), we believe them to be related, and we report our work on only one of them, DFL_{12}. It should be noted from Figure 1 that the DFL_{12} V_H-hybridizing mRNA is of low abundance and larger in size than authentic Ig γ_1 heavy chain mRNA (2.5 vs 2.1 kilobases [kb]).

To learn more about this mRNA, we isolated a cDNA clone of the message. A cDNA library of DFL_{12} was prepared in the Okayama-Berg expression vector pcD (30) and screened. A clone designated pcDFL.1 was isolated and sequenced (Fig. 2).

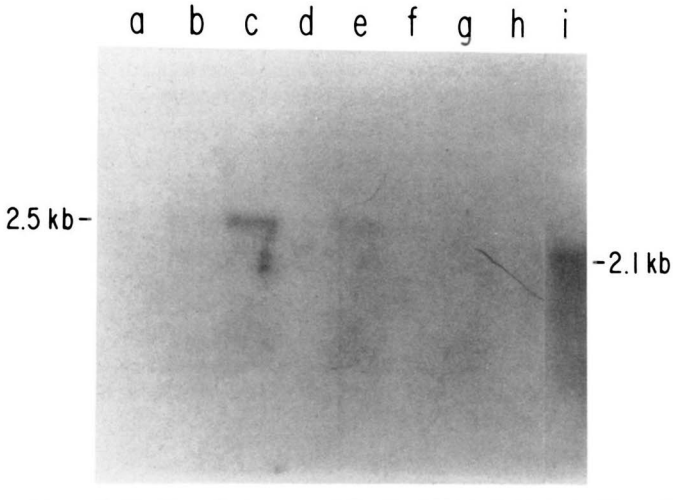

Figure 1. Northern blot screen of T cell mRNA hybridizing with an Ig V_H probe. RNA (poly A^+, 10 μg per lane) was electrophoresed through a 1% agarose formaldehyde gel and transferred to nitrocellulose. T cell sources of RNA on this filter (20): lanes a) DFL_{16}, b) DFL_{13}, c) DFL_{12}, d) DFL_4, e) DFL_2, f) BNP_1, g) CSP_2, and h) B6.1. Hybridization was with a V_H-specific probe derived from the anti-NP hybridoma P5.29.1 (34). Ig γ_1 heavy chain-enriched RNA from the same anti-NP hybridoma (1.0 ng) was included (lane i).

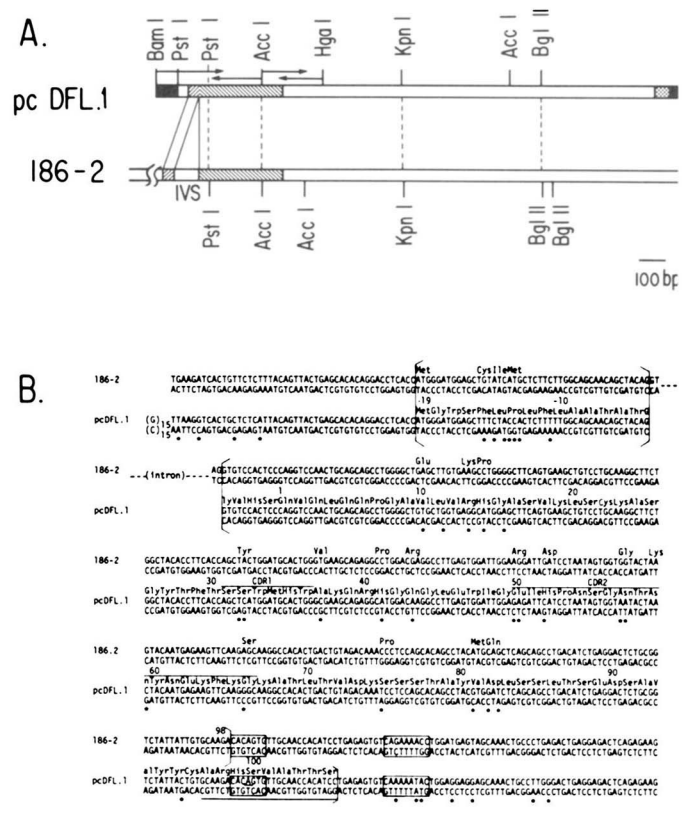

Figure 2. Structure and sequence of the V_H cDNA clone from DFL_{12}. The cDNA clone, pcDFL.1, is compared with the unrearranged anti-NP V_H genomic segment from clone 186-2 (48). In the map at top (*A*), restriction endonuclease sites at the ends of the cDNA insert (BamHI and the left-most [5′] PstI site) are derived from the cloning vector; black boxes represent vector sequences. The cDNA is derived from a polyadenylated mRNA and was synthesized with an oligo-dT-primed vector (30); it therefore ends with a oligo-dA/oligo-dT run (*stippled*). The coding region of the cDNA (*hatched*) was sequenced (see Reference 69) by the strategy indicated with arrows. A sequence comparison of the coding region of pcDFL.1 and clone 186-2 is presented below (*B*). Nucleotide differences are indicated with asterisks below the line; resulting amino acid differences are shown above. The oligo-dG/oligo-dC track at the beginning of the cDNA arose in the process of cDNA synthesis (30). The V_H reading frame is shown in brackets; it is interrupted in 186-2 by an intron (labeled IVS in *A*) that has been accurately removed by splicing in the mRNA represented by pcDFL.1. Amino acid numbering is in agreement with typical Ig protein sequences (see Reference 70). Residues −19 through −1 constitute the signal peptide, which is cleaved in transport of the polypeptide through the membrane of the endoplasmic reticulum; residues 31 through 36 and 50 through 66 constitute, respectively, the first and second antigen interactive "complementarity determining regions" (CDR1 and CDR2 as labeled). Ig gene rearrangement occurs normally upstream of the heptamer and nonamer sequences (*boxed*) that form a putative rearrangement site; thus in a complete Ig heavy chain gene, coding by a V_H gene segment would end by residue 98 as indicated. However, the coding capacity of pcDFL.1 continues beyond this point and ends after another seven amino acid residues. A nine-amino acid synthetic peptide was prepared from this predicted carboxyl terminal sequence (*underlined*) and was used in generating antibodies unique to the unrearranged V_H gene product.

It was immediately apparent that pcDFL.1 represents the transcript of an authentic V_H gene. Indeed, the V_H sequence of pcDFL.1 is highly homologous to the V_H gene identified as the germ line predecessor of the NP^b family in C57BL/6 mice, designated 186-2 (48). The cDNA sequence reveals normal features of Ig V_H transcription (49, 50): initiation occurs appropriately upstream of the signal peptide encoding leader sequence, which is spliced to the body of the variable region. However, transcription in this case continues through the heptamer and nonamer sequences (boxed in Fig. 2*B*) that form one of the putative recognition sites where Ig gene rearrangement occurs in

the creation of a complete heavy chain Ig gene. The transcript ends with a polyadenylate tail about 1.5 kb further downstream. Thus this cDNA is the full-length representation of the transcript of an unrearranged Ig V_H gene. Nucleotide differences between clones pcDFL.1 and 186-2 (29 nucleotide substitutions in the coding region leading to 18 amino acid differences) may represent mouse strain differences between DBA/2, from which the DFL_{12} cell line was derived, and C57BL/6, from which the 186-2 clone was isolated. The overall similarities in both coding and flanking regions (Fig. 2) suggest that pcDFL.1 represents a transcript of a DBA/2 gene related or equivalent to the 186-2 gene.

The notion that this mRNA comes from an unrearranged V_H gene was further examined by comparing the restriction endonuclease cleavage patterns of DNA from the DFL_{12} line with germ-line DNA from the parental DBA/2 mouse strain. DFL_{12} cell DNA and DBA/2 liver DNA were digested with several restriction enzymes and analyzed by Southern hybridization (Fig. 3). Although hybridization with the complete pcDFL.1 insert (lanes a and b) identifies a large number of homologous fragments, typically indicative of the large size of the NP-V_H gene family, a unique probe derived from the 3′ end of pcDFL.1 (BglII to BamHI, see Fig. 2*A*) hybridizes to very few bands and reveals no differences between DFL_{12} and DBA/2 (Fig. 3, lanes c through h). Examination of the blots suggests that no gross rearrangements are discernible within at least 20 kbp of this V_H gene.

The open reading frame apparent in the sequence of pcDFL.1 (Fig. 2*B*) encodes what begins as a normal Ig V_H protein (48). A 19-amino acid leader signal peptide is fused to the body of the V_H gene. This V_H sequence extends 98 amino acids to the normal site of Ig rearrangement (the heptamer and nonamer sequences, see above) and then continues! A stop codon is found eight codons further downstream, midway between the heptamer and nonamer blocks in the 23-bp spacer region. In sum, a 124-amino acid polypeptide is encoded by this mRNA. This predicted protein, of approximately 15.4 kilodaltons (kD) could be expected to be secreted by virtue of its amino-terminal signal sequence, yielding a processed protein of about 13.3kD from which the signal peptide had been removed. There are no obvious hydrophobic membrane anchoring stretches in the molecule.

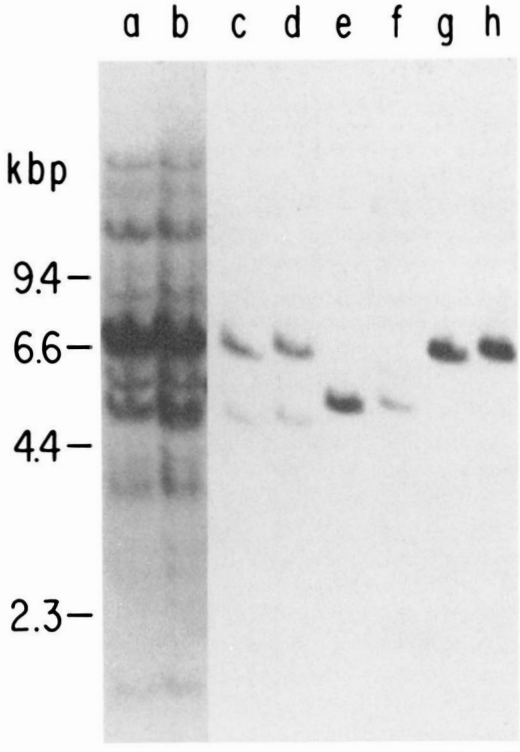

Figure 3. Southern blot analysis of the V_H gene. DNA from DFL_{12} cells (lanes a, c, e, and g) and from parental DBA/2 mouse kidney (lanes b, d, f, and h) were digested with restriction endonucleases EcoRI (lanes a through d), HindIII (lanes e and f), and BamHI (lanes g and h) and transferred to nitrocellulose filters after electrophoresis. One filter was hybridized with a probe prepared from the complete BamHI insert (see Fig. 2) of pcDFL.1 (lanes a and b); the other filter was probed with only a 3′ fragment (BglII to BamHI, see Fig. 2) of pcDFL.1 (lanes c through h).

Identification of the V_H protein. In an effort to determine whether the predicted V_H protein actually is translated from the mRNA described above, and further, to explore possible functional implications of that protein, we generated a specific immunologic reagent. A synthetic nine-amino acid peptide corresponding to the carboxyl terminus of the predicted V_H protein (underlined in Fig. 2*B*) was used to immunize rabbits after coupling to BSA as carrier. Anti-peptide-specific antibodies were prepared from immune rabbit serum by affinity chromatography (see *Materials and Methods*); this preparation (600 μg/ml protein) was relatively unreactive with carrier and highly reactive with peptide, binding significantly even at 32 ng/ml (Fig. 4*A*).

DFL_{12} cells were metabolically labeled for 16 hr with [^{35}S]methionine and [^{3}H]leucine and extracted in detergent buffer (see *Materials and Methods*). Immune complexes with anti-peptide antibodies were precipitated with fixed *Staphylococcus aureus* cells and visualized after SDS polyacrylamide gel electrophoresis. Figure 5, lane a, shows the metabolically labeled material precipitated from DFL_{12} cells and run under reducing conditions; lane d represents a comparable precipitation from an unrelated CTL line, CSP_2 (derived from a C_3H mouse and having specificity for H-2K^k targets derivatized with the hapten 3-(*p*-sulfophenyldiazo)-4-hydroxyphenyl acetyl [SP], Reference 43), which does not express a V_H message (see Fig. 1). Although a band corresponding in mobility to the predicted 13kD polypeptide is not observed, a pair of bands of lower apparent m.w., centered about 12kD are readily seen in DFL_{12} and not CSP_2. However, the large number of other bands precipitated from both cells compromises any conclusion from this comparison. Higher salt washes did not reduce appreciably the "background" precipitation seen. We therefore resorted to comparing precipitations made with and without an excess of unlabeled free peptide as competitor. In this analysis, only competable bands are considered to be specifically immunoprecipitated. Excess free peptide was added to an identical sample of the DFL_{12} extract, and the mix was immunoprecipitated as in lane a. Figure 5, lane b, reveals that most material was not competable and therefore not specifically precipitated. In contrast, the approximately 12kD doublet of DFL_{12} was competed dramatically by free peptide. These two bands presumably correspond to processed protein products of the unrearranged V_H mRNA (see below). Some other, higher m.w. bands also are lost from lane b. Again, the high level of background makes it difficult to characterize these here; the possibility that there may be other molecules associated and co-precipitated with the 12kD V_H protein will be

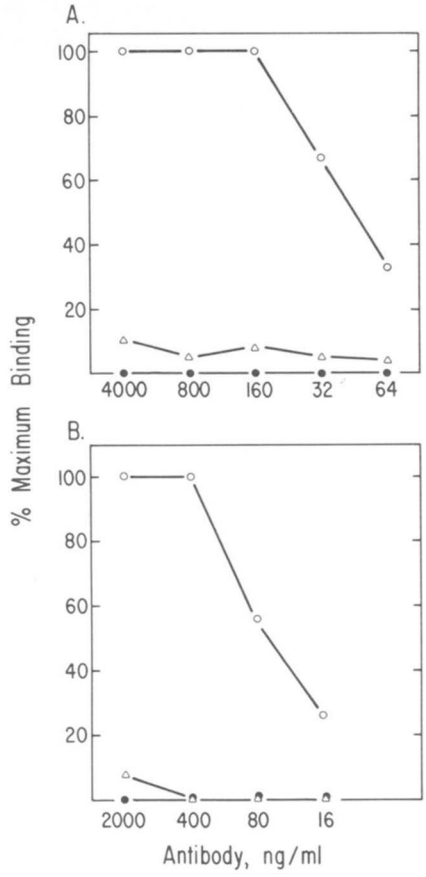

Figure 4. Reactivity of anti-peptide reagents. Specific binding to various antigens was measured in a solid-phase radioimmune assay. *A*, Binding of affinity-purified rabbit anti-V_H-peptide antibodies was monitored by using an [^{125}I]iodinated goat anti-rabbit Ig probe. Antibody dilutions were prepared in PBS containing normal goat serum at 10 mg/ml final protein concentration as described in *Materials and Methods*. *B*, A similar analysis of the binding of the 40.A6 monoclonal mouse anti-peptide antibody, probed with an [^{125}I]iodinated monoclonal rat anti-mouse κ-chain antibody, 187.1 (Reference 47). Antigens: V_H-peptide coupled to irrelevant carrier, myoglobin (*open circles*), uncoupled myoglobin (*closed circles*), and uncoupled (immunizing) carrier, BSA (*triangles*).

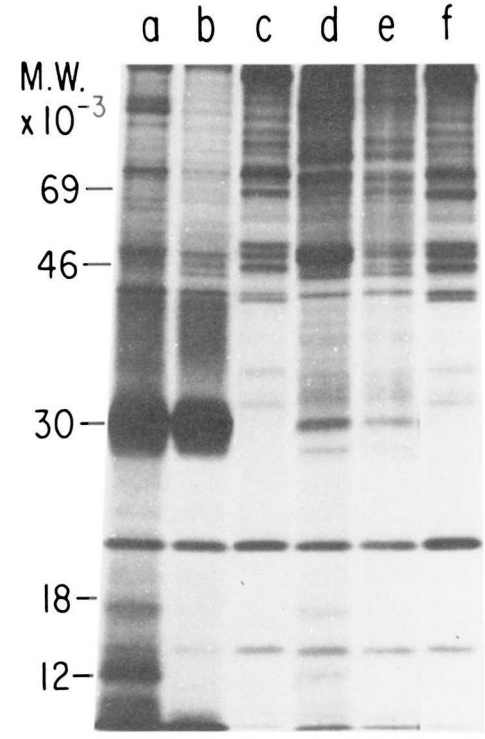

Figure 5. Identification of V_H proteins with anti-V_H-peptide antibodies. DFL_{12} (lanes a through c) and CSP_2 (lanes d through f) cells were analyzed for the expression of the predicted V_H proteins by immunoprecipitation from metabolically labeled cell extracts. Cells were labeled for 16 hr with [^{35}S]methionine and [^{3}H]leucine, extracted in detergent buffer and immunoprecipitated (see *Materials and Methods*) with affinity-purified anti-peptide antibodies and fixed *Staphylococcus aureus* cells (lanes a and d). Identical reactions were performed in the presence of excess (20 μM) unlabeled peptide as competitor (lanes b and e). Material precipitated with nonimmune rabbit serum is displayed in lanes c and f. Precipitated proteins were visualized by SDS polyacrylamide gel (15%) electrophoresis under reducing conditions, followed by fluorography and autoradiography.

discussed below.

Careful inspection reveals that a faint doublet similar to the V_H doublet of DFL_{12} is specifically precipitated from CSP_2 cells (Fig. 5, lanes d and e). Although CSP_2 cells do not synthesize a V_H mRNA like that found in DFL_{12} cells (Fig. 1), this doublet may represent material distantly related immunologically. Indeed, similar low m.w. doublet have been observed with these antibodies in several CTL lines examined (all lacking detectable V_H homologous transcripts; data not shown), suggesting that such proteins might be a common feature of these cells (discussed below).

Another explanation of these findings is that the two 12kD bands in DFL_{12} are not encoded by the NP-V_H mRNA and are precipitated adventitiously by the anti-peptide antibodies. To test this, the V_H cDNA clone was transfected into mouse fibroblast Lta cells along with the herpes simplex virus thymidine kinase (*tk*) gene. pcDFL.1 contains the V_H cDNA insert in an SV40-derived expression vector (30). Viral sequences in the vector facilitate transcription of the cDNA insert in mammalian cells without altering the encoded translation product.

Thymidine kinase positive (TK^+) colonies were screened for the presence of pcDFL.1 DNA and mRNA (data not shown). Positive colonies were grown for protein analysis. Precipitation experiments were performed as with the CTL lines above, and the results are shown in Figure 6. Untransfected L cells (not shown) and L cells transfected with the *tk* gene alone (lanes a and b) did not synthesize a precipitable 12kD doublet, whereas a transfectant that received both the *tk* gene and pcDFL.1 produced a doublet that could be specifically precipitated with the anti-peptide antibodies and appeared identical in mobility to the authentic DFL_{12} doublet (lanes d and e). Thus, the V_H mRNA of DFL_{12}, represented by the cDNA clone pcDFL.1, does encode the 12kD doublet, and these two polypeptides are specifically detected by the anti-peptide antibodies.

Functional correlation of the V_H protein. Interest in Ig V_H products as components of the T cell recognition complex has been based largely on observations that sera reactive with V_H determinants (idiotypic or framework) interfere with T cell function (14–16). In an effort to determine whether the NP-V_H proteins of DFL_{12} might play some functional role in this CTL, experiments were performed to test whether our V_H reactive anti-peptide antibodies interfered with DFL_{12} function.

DFL_{12} is a clonal CTL line derived from a fluorescein-specific syngeneic mixed lymphocyte culture (20). DFL_{12} displays high cytotoxic activity on fluoresceinated H-2D^d targets but shows little killing of H2 inappropriate (e.g., H2^k) targets or unfluoresceinated targets (Fig. 7*A*). When DFL_{12} cells were pretreated with the anti-peptide anti-

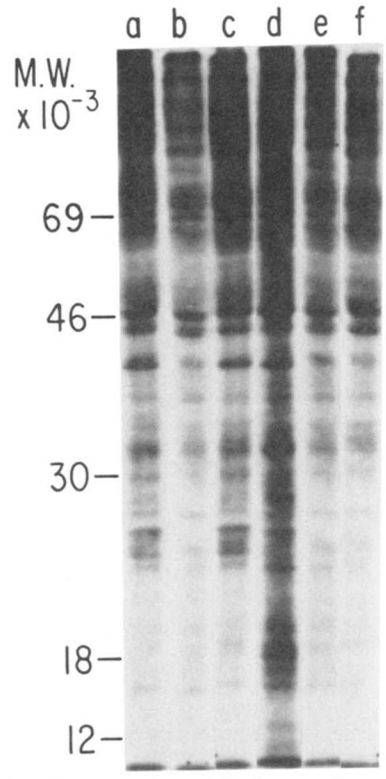

Figure 6. Identification of V_H proteins by transfection. Mouse Lta cells were cotransfected with the DFL_{12} cDNA clone, pcDFL.1, and the herpes simplex virus *tk* gene. TK$^+$ colonies were selected in HAT medium and screened for the presence of pcDFL.1. A positive colony was analyzed for expression of the V_H proteins by immunoprecipitation (lanes d through f). As a control, an L cell clone transfected with the HSV *tk* gene alone was analyzed (lanes a through c). Cells were labeled for 16 hr with [^{35}S] methionine and [^{3}H]leucine and extracted and immunoprecipitated as in Figure 5 with affinity-purified anti-peptide antibodies (lanes a and d). Identical reactions were performed in the presence of excess (20 μM) unlabeled peptide as competitor (lanes b and e). Precipitations with nonimmune rabbit serum are shown in lanes c and f. Precipitated proteins were visualized after SDS polyacrylamide gel electrophoresis under reducing conditions.

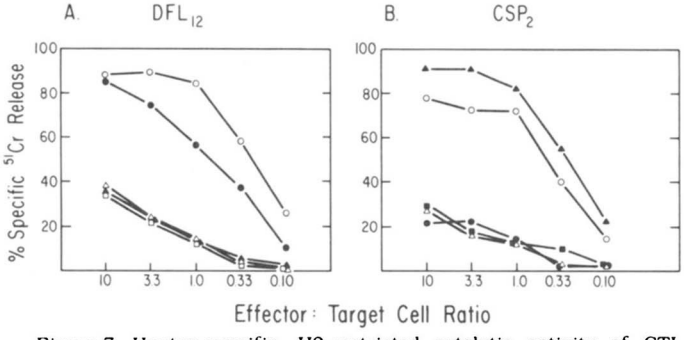

Figure 7. Hapten-specific, H2-restricted cytolytic activity of CTL clones. Splenic LPS blasts were labeled with [^{51}Cr]sodium chromate and derivatized with haptens as described in *Materials and Methods*. Target cells were distributed to 96-well plates at 2×10^4/well. Effector CTL cells (DFL_{12} in *A* and CSP_2 cells in *B*) were added at the indicated ratios and allowed to lyse targets for 5 hr. Plates were centrifuged briefly, and ^{51}Cr released into the supernatant was measured. Spontaneous ^{51}Cr release was generally less than 10% of input counts. Targets: Blasts from A/J mice (H-2K^{k}D^d) derivatized with fluorescein isothiocyanate (*open circles*), NP-*O*-succinimide (*closed circles*), SP-*O*-succinimide (*closed triangles*), and not derivatized (*open triangles*); C_3H (H2^k) blasts derivatized with fluorescein isothiocyanate (*open squares*) and DBA/2 (H2^d) blasts derivatized with SP-*O*-succinimide (*open squares*).

bodies for 30 min before the addition of labeled targets, their cytotoxic activity was inhibited dramatically (Fig. 8*A*, Expts. a and d). Control rabbit serum had no effect on target lysis by DFL_{12} (Fig. 8*A*, Expt. h). That this antibody-blocking activity is specifically due to anti-V_H-

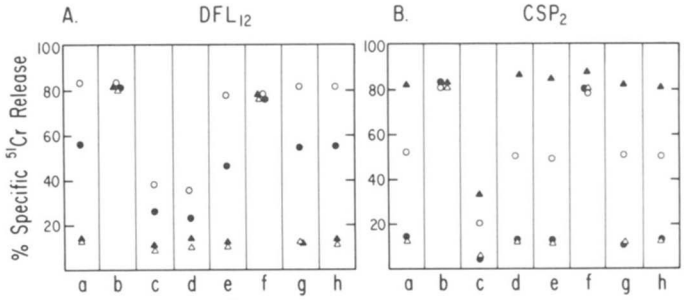

Figure 8. Lectin and antibody effects on CTL activity. DFL_{12} (*A*) and CSP_2 (*B*) cells were treated as described below and incubated with ^{51}Cr-labeled and hapten-derivatized A/J blast targets as in Figure 7, at an effector to target cell ratio of 1. Experiments: a) control with no additions (compare with Fig. 7); b) ConA (20 μg/ml final concentration) was added at the same time as targets (t = 0); c) 3155 monoclonal mouse antibody against Lyt-2 (Reference 52) was added to CTL cells 30 min before the addition of targets (t = −30) at a final concentration of 25 μg/ml; d) rabbit anti-peptide antibodies were added at t = −30 at a final concentration of 150 μg/ml; e) rabbit anti-peptide antibodies were added as in experiment d with excess free peptide (100 μM final concentration); f) rabbit anti-peptide antibodies were added as in experiment d, and ConA was added as in experiment b; g) 40.A6 monoclonal mouse anti-peptide antibody (150 μg/ml final concentration) was added at t = −30; h) nonimmune rabbit serum (150 μg/ml final concentration) was added at t = −30.

peptide reactivity is demonstrated by the abolition of blocking by co-addition of excess peptide competitor with the antibodies (compare Fig. 8*A*, Expts. d and e). Thus, just as in the precipitation experiments above, the ability of peptide to fully compete the activity of the antibodies implicates the V_H-encoded 12kD polypeptides in a functional role.

The addition of the lectin Con A to DFL_{12} cells pretreated with anti-peptide antibodies also restored their ability to lyse targets (Fig. 8*A*, Expt. f). Because Con A permits stable CTL-target conjugates to form nonspecifically (51, and Fig. 8*A* and *B*, Expts. b), this result suggests that the anti-peptide antibodies interfere with killing by inhibiting the normal binding to DFL_{12} of appropriate targets and subsequent conjugate formation. By inference, the V_H determinant bound by the anti-peptide antibodies might play a role in specific target recognition by DFL_{12}.

For comparison, the unrelated CTL, CSP_2, was analyzed similarly. CSP_2 shows specificity for SP-derivatized H-2K^k targets and is relatively unreactive on underivatized or H2-inappropriate targets (20; and see Fig. 7*B*). Whereas the anti-peptide antibodies inhibited DFL_{12} lysis of appropriate targets by greater than 50%, the same antibody preparation strikingly had no effect on CSP_2 killing (Fig. 8*B*, Expts. a and d). Both CTL lines were equally susceptible to blocking by a monoclonal antibody reactive with the cell surface molecule Lyt-2 (Fig. 8*A* and *B*, Expts. c) (52).

As an internal control in these experiments, the same target cells were used throughout. Because DFL_{12} is restricted to the H-2D^d molecule and CSP_2 to K^k, targets were prepared from splenic blasts of A/J mice (haplotype K^{k}D^d). Note that CSP_2 cells react with these cells when fluoresceinated about half as well as they lyse SP-derivatized targets (Fig. 7*B*). This anti-fluorescein reactivity also was unaffected by the anti-peptide antibodies (Fig. 8*B*, Expt. d); thus the susceptibility to lysis of fluoresceinated targets per se is not affected by the anti-peptide antibodies.

These results, demonstrating that the anti-V_H-peptide antibodies block specific killing by DFL_{12} clonotypically,

are especially noteworthy in that the antibodies were prepared on the basis of reactivity with the V_H carboxyl terminal synthetic peptide and were not selected for blocking activity. This also provides a possible explanation for the relatively high concentrations of antibodies needed to effect killing inhibition (Fig. 9*A*): although the antibodies react well with peptide (Fig. 4), they may bind only weakly to the native protein on viable cells.

Localization of the V_H protein. Presumably, the anti-V_H-peptide antibodies exert their inhibition of DFL_{12} killing by binding to V_H protein molecules that are exposed on the cell surface. It could be expected that the V_H protein would be transported to the cell's exterior (because the V_H mRNA encodes a signal peptide; see above and Fig. 2); however, because it has no intrinsic membrane-anchoring segment, the V_H protein can only be present on the cell surface if it is held there by other integral membrane molecules. To explore these predictions, we wished to test whether V_H molecules were accessible to labeling in a lactoperoxidase-catalyzed cell surface iodination reaction and also to identify molecules that coprecipitate with the V_H protein and that might serve as putative anchoring structures.

The rabbit anti-peptide antibodies, although quite specific in clonotypic blocking activity, were limited in their usefulness in immunoprecipitation analysis, due to the problem of high nonspecific background precipitation (see Fig. 5). To overcome this limitation, a new anti-V_H-peptide reagent was prepared. A B cell hybridoma, designated 40.A6, producing a monoclonal anti-V_H-peptide antibody (see Fig. 4*B*) was derived from a mouse immunized with the same carboxyl terminal nine amino acid synthetic peptide coupled to BSA (see *Materials and Methods*). To facilitate immunoprecipitation experiments, the 40.A6 antibody was covalently linked to Sepharose beads.

Cells were surface labeled with [^{125}I]NaI in a lactoperoxidase-catalyzed reaction, and a soluble extract was prepared with NP-40 lysis buffer. The 40.A6 monoclonal anti-peptide antibody precipitated a single band of approximately 12kD from DFL_{12} cells (Fig. 10, lane a), whereas no such material was precipitated from CSP_2 cells (Fig. 10, lane c). Comparison of this 12kD singlet to the doublet precipitated from metabolically labeled cells (see Fig. 5, lane a, and Fig. 6, lane d) identifies it as the lower m.w. species of the two. This is consistent with the interpretation that at least the lower of the two bands

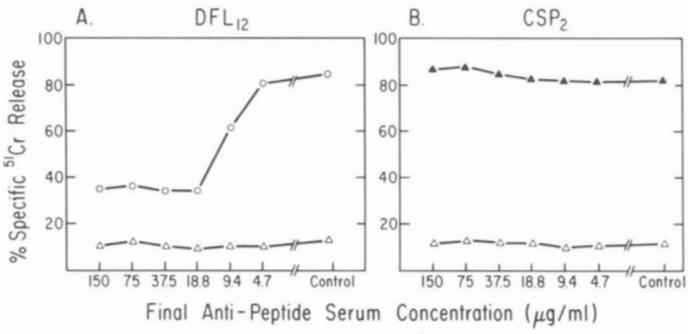

Figure 9. Titration of rabbit anti-peptide antibodies. The activity of rabbit anti-peptide antibodies were titrated in CTL-mediated target lysis reactions as in Figure 8, experiments d. Affinity-purified rabbit anti-peptide antibodies and dilutions in control nonimmune rabbit serum (as in Fig. 8, Expts. h) were prepared to final rabbit serum concentration of 150 μg/ml.

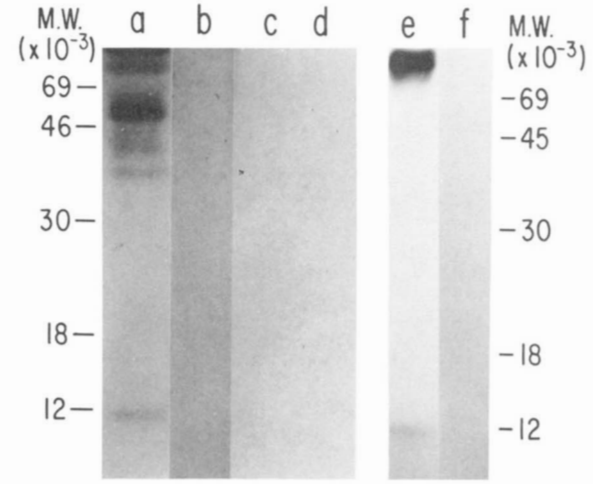

Figure 10. Analysis of cell surface V_H protein. DFL_{12} (lanes a, b, e, and f) and CSP_2 (lanes c and d) cells were subjected to lactoperoxidase-catalyzed cell surface radioiodination. ^{125}I-labeled material was extracted in an NP-40 lysis buffer under nonreducing conditions and subjected to immunoprecipitation (see *Materials and Methods*) with Sepharose beads to which had been coupled the 40.A6 monoclonal mouse anti-NP-V_H-peptide antibody (lanes a, c, and e) or "irrelevant" P9.37.1 (34) anti-NP monoclonal antibody (lanes b, d, and f). Precipitated material was split and resuspended either in reducing (lanes a through d) or nonreducing (lanes e and f) buffer before SDS polyacrylamide gel (18%) electrophoresis.

encoded by the V_H message represents the processed protein from which the signal peptide has been cleaved in transport across the membrane and to the cell surface. Further evidence for this view comes from experiments in which culture supernatants of metabolically labeled DFL_{12} cells are precipitated with the anti-peptide antibody. Again, only the lower of the two V_H bands can be detected (data not shown).

It should be noted that the precipitations with 40.A6 are much cleaner than with the rabbit antibodies; only a few other bands (discussed below) are brought down with the V_H material. However, because antibody bound beads were used and the effective antibody concentration was much higher than before (approximately 250 μg/ml vs 25 μg/ml with the rabbit antibodies), the specificity test of free peptide competition was no longer feasible. Therefore, as a test of specificity, we rely on comparison both between different CTL cell lines (compare Fig. 10, lanes a and c) and between 40.A6 antibody and an "irrelevant" monoclonal—in this case an authentic anti-NP antibody (compare Fig. 10, lanes a and b, and lanes c and d). By these criteria the 12kD protein is again identified by precipitation with 40.A6 antibody as the translation product of the V_H mRNA in DFL_{12} cells. Although the presence of similar cross-reactive molecules in other CTL lines (for example, CSP_2; see Fig. 5, lane d) was suggested with the anti-peptide antibodies, the monoclonal anti-peptide antibody 40.A6 does not react with this material (Fig. 10, lane c).

The observation that the V_H molecules are accessible to lactoperoxidase-catalyzed iodination and the implication that they are involved in target cell recognition by DFL_{12} suggest that they indeed are present on the cell surface. What holds these molecules to the membrane? A diffuse band of approximately 45kD is reproducibly and specifically coprecipitated with the 12kD V_H protein by 40.A6 antibody (Fig. 10, lane a). Of course, the most interesting T cell surface molecules of this size are the α and β chains of the heterodimeric T cell receptor. The

TABLE I

IFN-γ Induction in CTL clones[a]

Cells Treated with	DFL_{12}	CSP_2
	IFN-γ Secreted (U/ml)	
Control	<1	<1
Con A	9	12
40.A6 Sepharose Beads	3	<1
P9.37.1 Sepharose Beads	<1	<1

[a] CTL were cultured in normal medium (control) and with Con A (4 μg/ml) or Sepharose beads to which had been coupled the 40.A6 monoclonal mouse anti-NP-V_H-peptide antibody or P9.37.1 (Reference 34) monoclonal mouse anti-NP antibody (50 μg/ml final antibody concentration). Cultures were initiated at 5×10^5 cells/ml, and supernatants were harvested after 24 hr. Secreted IFN-γ was quantitated in an indirect radioimmunoassay as described in *Materials and Methods*.

two chains of the receptor form a disulfide-linked integral membrane structure of about 90kD on the surface of functional T cells; if the 45kD material in lane a of Figure 10 represents α- and β-chains, identical precipitates prepared in nonreducing conditions should migrate as a 90kD band. Lane e presents the results of such an experiment. Although the 12kD V_H molecule remains as in lane a, the 45kD band is not present and a new band of 90kD has appeared. We conclude that the 12kD V_H protein may by held noncovalently to the cell surface through an association with heterodimeric T cell receptor α- and/or β-chains.

More functional correlations. Just as we employed the 40.A6 monoclonal antibody to clarify and extend the immunoprecipitation results obtained with the rabbit anti-peptide antibodies, we hoped to confirm the functional correlations made. Unfortunately, 40.A6 antibody has no effect on target cytolysis by DFL_{12} (or any other) cells even at very high concentrations (Fig. 8*A* and *B*, Expts. g).

Another physiologic CTL function that can be readily monitored is the production of IFN-γ. Triggering of CTL through receptor aggregation (mediated by targets, antibodies directed at the receptor complex, or lectins) induces IFN-γ secretion (44). As measured in an indirect assay that exploits the ability of IFN-γ to induce MHC Class II molecules on the surface of antigen-presenting cells (see *Materials and Methods*), DFL_{12} cells and CSP_2 cells could be induced by Con A to secrete 9 and 12 U of IFN-γ, respectively, whereas no detectable IFN-γ (<1 U) was made without stimulation (Table I). DFL_{12} but not CSP_2 cells could also be induced to secrete low levels (3 U) of IFN-γ by polyvalent 40.A6 anti-peptide antibody beads (Table I). The 40.A6 clone was selected solely on the basis of its binding to the synthetic peptide, and again, it is notable that this monoclonal anti-V_H-peptide reagent exerts a clonotypic effect on DFL_{12} function.

DISCUSSION

A substantial body of work accumulating over the last several years argues that Ig V_H transcripts are not common in functional T cells. Among the more than 30 T cell clones that have been examined (26, 27, and this report), only the one V_H mRNA that we describe here has been observed.

The DFL_{12} transcript is quite unusual. It is the product of an unrearranged V_H gene. Although unrearranged heavy chain constant region genes are normally transcribed (53, 54), properly initiated transcription of variable region genes has been thought to occur only after rearrangement to diversity, joining, and constant gene segments (55, 56). Recently, Yancopoulos and Alt (57) found that unrearranged V_H gene segments are transcribed early in B cell ontogeny, especially preceding V_H rearrangement. The NP-V_H mRNA we describe is transcribed by a mature T cell. It is present at approximately 0.005% of total DFL_{12} cell mRNA (fivefold to tenfold lower than the level of T cell receptor α- and β-chain mRNA (12) and early B cell V_H mRNA (57)). We do not know how this one variable gene has been activated uniquely and stably.

We have presented an analysis of the translation product of this unrearranged V_H gene, relying on immunologic reagents generated against a corresponding synthetic peptide. We have demonstrated that the V_H transcript encodes a protein of approximately 12kD that is expressed as a clonotypic cell surface molecule on DFL_{12} cells.

Rabbit anti-peptide antibodies that recognize the V_H-encoded protein also specifically inhibit the cytotoxic activity of DFL_{12} cells. Effects of antibodies on T cell activity (in the absence of complement) can indicate roles for the recognized epitopes in immune function (58). Clonotypic effects, in particular, have identified antibody-analogous receptor idiotopes (1–5). In most cases (1, 3–5; but see 6), these clonotypic antibodies inhibit normal T cell responses to appropriate antigens, presumably by interfering conformationally or sterically with receptor binding and conjugate formation (51). Our results suggest that the V_H molecule also belongs in the class of function-associated, clonotypic (i.e., receptor-like) molecules. Another interpretation of the blocking data is that the binding of antibodies to V_H molecules exerts a more global effect on the CTL cell surface—for example, by altering membrane fluidity and normal signal transduction. This sort of effect has been suggested to occur as a result of lectin interactions with B cell surfaces (59). It also is possible that the blocking activity of the antibodies resides in a CTL-reactive, contaminating component, distinct from the anti-V_H-peptide reactivity. The fact that free V_H peptide competitively inhibits this blocking activity just as it competes with the V_H protein in precipitation experiments argues against this possibility. The lack of blocking activity for a monoclonal mouse anti-V_H-peptide antibody could support the argument that it is not the anti-peptide activity of the antibodies that is responsible for blocking; alternatively, it may reflect a greater selectivity for the non-native determinants of the V_H molecule by the monoclonal reagent. We take the weak but clonotypic IFN-γ inducing activity of this monoclonal antibody on the V_H-expressing CTL as further evidence of a function-associated role for the V_H protein.

On the basis of precipitation experiments with labeled cell surface material, we do think that the V_H protein may be directly involved through its association with what is, in the minimal consensus view, the MHC-restricted, antigen-specific receptor of T lymphocytes: a heterodimeric, disulfide-linked complex of two 45kD polypeptides (1, 2, 4, 5). Material resembling receptor α- and β-chains by the criteria of susceptibility to lactoperoxidase-catalyzed iodination, electrophoretic mobility, and the presence of interchain disulfide linkages coprecipitates with the cell surface form of the V_H protein.

This offers a unifying means of interpreting the func-

tional data. Blocking by the anti-peptide antibodies occurs at an early step in the interaction of the CTL with its target, one which can be circumvented by the lectin Con A, i.e., the recognition phase. Similarly, the induction of IFN-γ by monoclonal anti-peptide antibody results from antibody triggering of the receptor complex. Even if the V_H molecule is not directly involved in actual target binding, its proximity to that binding site allows antibodies recognizing it to sterically or conformationally affect receptor function.

Moreover, this view provides an explanation for the means by which the V_H molecule, lacking any membrane-anchoring sequence, is held noncovalently to the cell surface. Ig domains are known to aggregate; even in the absence of intermolecular disulfide bridges, authentic Ig molecules maintain their structural and functional integrity (60). Perhaps the V_H protein—itself little more than an Ig domain—associates with one of the Ig-like domains (10–13) of the α- or β-chains of the receptor.

If the V_H protein is yet another component of the receptor complex (51, 61, 62), what function does it serve? Beyond the peculiarity of a T cell with any V_H mRNA, we were struck by the fact that the T cell we have studied, DFL_{12}, is a CTL prepared specifically against fluoresceinated targets; it transcribes a V_H belonging to the family that encodes NP-reactive antibodies. In B cells, the variable regions of antibodies reactive with NP and fluorescein are distinctly unrelated (D. Kranz; T. Imanishi-Kari; personal communications). Surprisingly, DFL_{12} shows significant cytotoxic activity for NP-derivatized targets (see Fig. 7), about 60% of the fluorescein-specific activity for which it was selected. DFL_{12} does not share one other feature of the NP^b antibody response. The family of NP^b antibodies is characteristically heteroclitic for the related hapten, (5-iodo-4-hydroxy-3-nitrophenyl)acetyl (NIP), binding more strongly to NIP than to NP (35). DFL_{12}, on the other hand, lyses NIP-derivatized targets just as well as it does NP-modified ones (data not shown).

An antibody's combining site for antigen is created by the variable regions of both an Ig heavy chain and an Ig light chain together; a V_H alone does not form this antigen-binding pocket (60). Note that Kraig et al. (63) found that T and B cells that share specificity for the same antigen (and in some cases are reported to share idiotypic determinants) do not express related V_H genes, a result that follows from the more general lack of any detectable V_H transcription in T cells. DFL_{12} provides an exception to both generalizations.

Perhaps the small V_H protein contributes to a hapten-specific binding moiety when complexed appropriately with other Ig-like domains in the context of the MHC-restricted receptor. The model that T cells recognize, through a single receptor, the combination of nominal antigen and MHC determinants on antigen-presenting and target cells is supported by a wealth of experimental evidence (6, 64–66). However, this notion does not exclude the interaction of distinct MHC or antigenic epitopes with the receptor, nor the presence within a single receptor complex of components concerned principally with one or the other of those epitopes. We suggest the possibility that the V_H protein we have described is one such component of the receptor complex of DFL_{12} cells. Its role is in the creation of a binding site for the haptenic part (for example, a phenolic group of fluorescein or a related substituted phenyl group from NP or NIP) of the complex antigen of the target cell—not alone, but in structural complementation with other components of the DFL_{12} receptor.

We have seen, in other CTL, weakly cross-reactive proteins of mobility similar to the DFL_{12} V_H polypeptides in studies performed with our rabbit anti-V_H-peptide antibodies. Might this indicate that small V_H-like molecules are a common feature of T cells? Gately and Martz (67) observed T cell-specific proteins with electrophoretic mobilities similar to the V_H-like molecules we report here. Note that the V_H cross-reactive material is not precipitated from other T cells with the monoclonal mouse anti-peptide antibody; presumably the monoclonal reagent does not react with some more common determinant represented in the peptide and detected by the rabbit antibodies. These small proteins must be only distantly related; certainly, we know that the genes encoding them are not homologous to Ig V_H genes on the level of nucleic acid hybridization!

We hypothesize that these small proteins represent a class of elements that noncovalently associate through Ig-like domains with other elements of T cell receptors. Structurally, this is reminiscent of the association of invariant β_2-microglobulin (68) with polymorphic Class I MHC gene products. Functionally, however, we imagine these small molecules as playing a quite different role: they are adding another level of variability to receptor composition, thereby contributing to the overall diversity of T cell immune receptors and responses.

Our interpretation of the role of the V_H protein in DFL_{12} cells—that it is functionally significant and, more sweepingly, possibly representative of a novel class of receptor elements—remains to be proved. The association between the V_H protein and receptor α- and β-chains will be confirmed only by comparing immunoprecipitations performed with α- and/or β-specific reagents (not yet available for DFL_{12}) and anti-V_H antibodies. Most important, the functional significance of the inferred role of the V_H protein in the receptor complex awaits a genetic analysis.

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