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# Segmental Homology between T-Cell Receptors and Immunoglobulin Variable Regions: Evidence that Antisera to Synthetic $J_H1$ Peptide React with Murine and Human T-Cell Products[1] (42034)

ANNE MACKEL-VANDERSTEENHOVEN,* GERARDO R. VASTA,* MYRON J. WAXDAL,† AND JOHN J. MARCHALONIS*

*Department of Biochemistry, Medical University of South Carolina, Charleston, South Carolina 29425; and the †Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205*

*Abstract.* To determine precisely the nature of serological determinants shared between T-cell surface molecules and immunoglobulin variable regions, the capacity of antisera directed against a synthetic peptide corresponding to the entire $J_H1$ region of classical immunoglobulin plus five residues of the D region were tested for their capacity to bind to T-cell membranes and isolated T-cell products. The anti-$J_H1$ antisera reacted with normal and monoclonal *in vitro* grown T-cell lines as judged by microhemagglutination and binding in enzyme-linked immunosorbent assays. Immunologically cross-reactive membrane components disclosed by immunoblot transfer analysis ("Western blots") consisted of major components in the molecular weight range 30–35,000 and minor components in the range 65–70,000. The major product of the human T-cell leukemia line MOLT-3 had an approximate mass of 34,000 Da, a value consistent with the predicted size of the molecule specified by the recently described putative T-cell receptor gene YT35. The 65 to 70,000-Da components are most probably tightly associated dimers of the 30 to 35,000-Da forms. It was possible to align the $J_H$ sequences of molecules reactive with the anti-$J_H1$ antisera and other characterized $V_H$ sequences of molecules known to be cross-reactive with T-cell products. This facilitated a comparison disclosing clear segmental homology between the protein sequence derived from the YT35 gene and immunoglobulin $V_H$ framework regions sharing approximately 50% of sequence identity. The identification of $V_H$-related T-cell products (termed $V_T$-bearing molecules) with products of putative T-cell receptor genes gained further support by N-terminal sequence of the 68,000-Da product of the 70-N2 T-cell line which showed homology to the predicted N-terminal region of the YT35 product. These serological and protein chemical data, coupled with the comparison to gene sequence, show that T-cell components that bear serological determinants cross-reactive with $V_H$ show segmental homology with products of putative T-cell receptor genes and immunoglobulin $V_H$. 

Although it has been known for many years that T cells can synthesize products serologically related to immunoglobulins (1–5), the determinants recognized were not classical immunoglobulin constant region markers and the precise relationship between the cross-reactive T-cell products and immunoglobulins was uncertain. Recent results have indicated that human (6–8) and murine (9, 10) T cells synthesize surface markers related to heavy chain variable regions and that these products are involved in the specific binding of antigen (2–5, 8–12) and in antigen-specific T-cell function (8, 9, 13), making them leading candidates for the role of antigen-specific T-cell receptors. In this paper, we use antibodies directed against a synthetic peptide consisting of 16 residues corresponding to a portion of the D (CDR3)[2] region of

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[2] Abbreviations used: CDR, complementarity determining region; ELISA, enzyme-linked immunosorbent assay; HBSS, Hanks' balanced salt solution; BSA, bovine serum albumin; $V_TM$, T-cell molecule related to immunoglobulin $V_H$ regions; KLH, keyhole limpet hemocyanin; NRS, normal rabbit serum; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis.



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$V_H$ and the entire $J_H1$ (21) sequence to detect and partially characterize components of human and murine T cells expressing molecules bearing this determinant. Our results are consistent with the conclusion that T cells produce molecules specified by the recently described putative T-cell receptor genes (14–16), notably the MOLT-3 line, and that these products show homology to immunoglobulin $V_H$ in the J region and an overall segmental homology in framework regions including the N-terminus.

**Materials and Methods.** *Cells.* Normal thymus lymphocytes were prepared from 4- to 8-week-old male and female ICR mice as described previously (17). The *in vitro* grown tumor lines WEHI-7.1, MOLT-3, and 70-N2 were grown as previously described (18). WEHI-7.1 is a BALB/c line of suppressor phenotype (C. Spellman, personal communication); MOLT-3 a human T cell of amplifier phenotype (19); 70-N2 a marmoset T cell of amplifier phenotype (20).

*Antisera.* Rabbit antisera to murine and marmoset (70-N2) $V_T$Ms were prepared as described previously (17, 18). Defined rabbit antisera against a 16 residue $J_H$ peptide (WYFDVWGAGTTVTVSS) with a sequence corresponding to the complete $J_H1$ region (WGAGTTVTVSS) and the C-terminal 5-residues of the D-region of MOPC-104E (kindly provided by Dr. J. Davie and Dr. M. Seiden, Washington University, St. Louis [Ref. (21)] was prepared as follows: New Zealand white rabbits were injected intradermally with 0.5 mg of antigen emulsified in complete Freund's adjuvant (1:1). An intradermal booster injection was given 2 weeks later and consisted of 0.5 mg antigen emulsified in incomplete Freund's adjuvant (1:1). Blood was collected from the ear at 2-week intervals.

Rabbit antisera to keyhole limpet hemocyanin (KLH) was prepared as described above. A rabbit antibody produced against a synthetic peptide corresponding to a stretch of influenza virus hemagglutinin was a gift of Dr. Davie and Dr. Seiden (21).

*Cell extraction and preparation of T-cell antigens.* Formic acid extraction of washed and packed cell preparations was performed as described by Mackel *et al.* (17). Purified murine IgT was prepared as described previously (17).

*Mouse myelomas.* MOPC-104E ($\lambda$, $\mu$), MOPC-315 ($\lambda$, $\alpha$), and TEPC-15 ($\kappa$, $\alpha$) were purchased from Litton Bionetics, Inc. (Charleston, S.C.). The human myeloma protein McE (22) was the gift of Dr. G. W. Litman (Sloan-Kettering Institute N.Y.)

*Cell microagglutination assays.* were performed in 96-well Terasaki plates (Robbins Scientific, Mountain View, Calif.). Test cells were washed three times in Hanks' balanced salt solution containing 10% bovine serum albumin (HBSS–10% BSA) and resuspended in HBSS–10% BSA at concentrations of $3 \times 10^6$ cells/ml. Twofold serial dilutions of rabbit antisera or preimmune rabbit sera (control) in HBSS–10% BSA were placed in the levels (5 $\mu$l) and equal volumes of the cell suspensions were added. The plates were gently Vortex-mixed for 10 sec and incubated at room temperature ($25 \pm 3°C$) for 1 hr. Agglutination was read under microscope and graded from 0 to 4 for degree of agglutination. Autoagglutination controls were the substitution of rabbit sera by HBSS–10% BSA.

*Enzyme-linked immunosorbent assay (ELISA).* The ELISA was performed as described by Mackel *et al.* (17).

*Enzyme-linked immunoelectrotransfer blot.* The immunoblot was performed using the method described by Tsang *et al.* (23). Briefly, following electrophoresis, SDS–polyacrylamide gels were electrophoretically transferred onto nitrocellulose paper using a Trans-Blot cell (Bio-Rad Lab). Once transfer was complete, the paper was rinsed with phosphate-buffered saline, pH 7.4, containing 1% gelatin (gelatin solution) three times for 15-min each. The nitrocellulose was then treated with the primary rabbit antibody (1:100) in 1% gelatin solution for 2 hr at room temperature. Following three, 15-min washes with the 1% gelatin solution, the nitrocellulose was treated with the secondary antibody, a peroxidase-conjugated goat anti-rabbit IgG (Cappel Lab., Cochranville, Pa.) at a 1:1000 dilution in 1% gelatin solution for 2 hr at room temperature. The nitrocellulose paper was then washed three times with PBS, pH 7.4. The paper was then exposed for 10–20 min to the substrate solution 4-chloro-1-napthol containing 0.03% $H_2O_2$. Once the reaction has developed sufficiently, the nitrocellulose paper was washed

in distilled water overnight and allowed to air-dry in the dark.

*Microsequence analysis.* This was performed on 200 μg of $V_T$-related product of the 70-N2 cell isolated by immune affinity chromatography on antibody of goat antiserum to human $F(ab')_1$ as described elsewhere (24, 25). The $V_TM$ molecule had an apparent mass of 68 kDa. Microsequence analysis was performed as described by Waxdal *et al.* (26) using [$^{35}$S]phenylisothiocyanate (Amersham Searle; sp act 250–300 Ci/mole) to detect N-terminal residues.

**Results.** The enzyme-linked immunosorbent assay was used to detect binding of rabbit antisera to the synthetic $J_H1$ peptide to test immunoglobulins, plasma membranes of T cells, and to isolated T-cell products. Figure 1 illustrates a callibration experiment in which specific binding (absorbency with the anti-$J_H1$ peptide minus absorbency with the anti-KLH) is titrated versus the murine myeloma MOPC-104E and TEPC-15 which possess a 16-mer sequence identical to that of the immunizing antigen and MOPC-315 which has a murine $J_H2$ sequence. Conformation obviously plays a role in determining the degree of reactivity because the binding to TEPC-15 is better than that to MOPC-104E even though both have the same J region sequence. In addition, the capacity of the antiserum to pick up other $J_H$ sequences as illustrated by the reaction with MOPC-315. Seiden *et al.* (21) also detected these types of reactions in testing anti-$J_H$ in binding

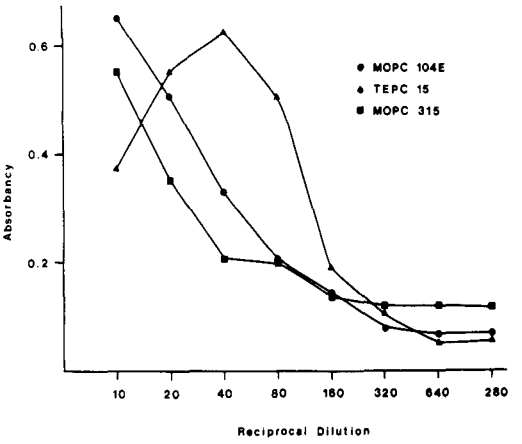



FIG. 1. Reactivity measured by ELISA of rabbit antisera against the synthetic $J_H$ peptide with the defined murine myeloma proteins MOPC-104E, TEPC-15, and MOPC-315. Rabbit antiserum to keyhole limpet hemocyanin (KLH) was used as a control, and the control valves were subtracted point by point from the anti-$J_H$ valves in constructing this figure. The wells were coated with 0.5 μg of antigen. The absorbency was measured at 414 nm.

and in inhibition assays. We also found that the antiserum reacts with immunoglobulins of other species, for example McE which has a human $J_H2$ sequence (22) reacted to a degree approximately 25% of that shown by the MOPC-104E molecule.

The capacity of the rabbit anti-$J_H$ sera to react with normal murine thymus, a murine monoclonal T-cell leukemia, and monoclonal primate *in vitro* T-cell lines (MOLT-3 and

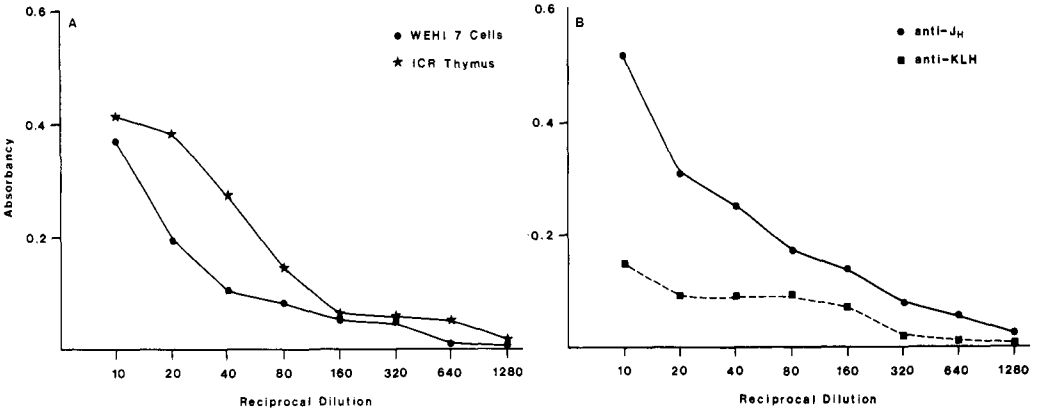



FIG. 2. Reactivity measured by ELISA of rabbit anti-$J_H$ serum with formic acid extracts of (A) WEHI-7 cells, ICR thymocytes, and (B) MOLT-3 cells. The KLH control was subtracted from the anti-$J_H$ values in 2A as indicated in Fig. 1. The wells were coated with 5 μg antigen.

70-N2) was tested by ELISA and by microhemagglutination assay. As shown in Fig. 2, the anti-$J_H$ serum bound to the plasma membrane of MOLT-3 and to the plasma membranes of murine thymus and WEHI-7 cells. Figure 2B shows both the anti-$J_H$ and anti-KLH binding, whereas only the specific binding is illustrated in Fig. 2A. Microhemagglutination titrations testing anti-$J_H$, anti-KLH, and normal rabbit serum for their capacity to agglutinate intact whole human MOLT-3 T cells and marmoset 70-N2 cells are shown in Fig. 3. Both monoclonal, *in vitro* T-cell lines are agglutinated by the anti-$J_H$, with the reaction against the 70-N2 cells being stronger. In previous studies using antisera against human $V_H$ determinants, the 70-N2 line possessed the greatest amount of $V_H$-

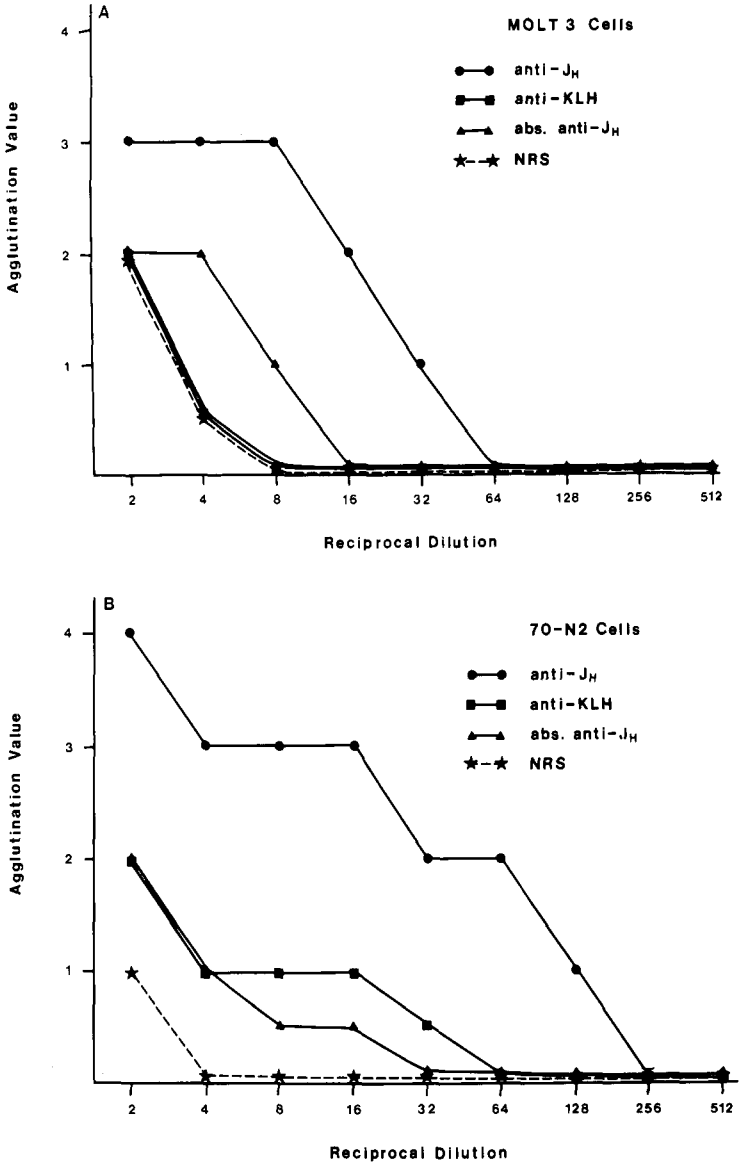



FIG. 3. Reactivity of anti-$J_H$ serum, anti-KLH, anti-$J_H$ serum absorbed with solid phase MOPC-104E, and normal rabbit serum, NRS as assayed by microhemagglutination. (A) agglutination titration of MOLT-3 cells. (B) agglutination titration of 70-N2 cells.

cross-reactive material as judged by flow cytometry (6). The binding of anti-$J_H$ to both lines was substantially eliminated by absorption of the antiserum with insolubilized MOPC-104E-Sepharose, indicating the specificity of the binding reaction.

The anti-$J_H$ serum bound to immune affinity-purified $V_T$-bearing molecules (17) of murine thymus as illustrated in Fig. 4. The immune affinity-isolated product (inset A) was prepared using a solid phase immunoadsorbent consisting of the IgG fraction of rabbit anti-mouse $V_TM$ (17) coupled to Sepharose. The product originally was a single component of apparent mass 68 kDa, but degraded spontaneously upon standing (27) into the components (44–46; 22–30 kDa) shown here. The anti-$J_H$ serum (21) bound slightly better to the purified $V_TM$ than did the anti-$V_TM$ (17) used in the original isolation. Antibody to viral hemagglutin-peptide/KLH (21) resembled normal rabbit serum in lack of binding.

The capacity of the anti-$J_H$ serum to react with specific membrane components was illustrated by immunoblot transfer analysis using solubilized plasma membranes of thymus, the WEHI-7 line, the MOLT-3 T cell line, and the 70-N2 line. As shown in Fig. 5, the Western blots show that essentially two size range of products are identified by the anti-$J_H$ serum. These are indicated by the arrowheads. In MOLT-3 (lane B, Fig. 5A), two specifically precipitated components are observed; one has an apparent mass of 34 kDa and the other approximately 66 kDa. The specifically bound material from the WEHI-7 membrane had apparent masses of approximately 35 and 65 kDa with a component at 14 kDa also observed (lane C, Fig. 5A). Normal murine thymus (lane D, Fig. 5A) has detectable material in the range of 14, 30–35, and 65 kDa. Although there are quantitative differences between the higher-molecular-weight bands and corresponding bands visualized by anti-KLH, we did not consider these components to be specific. Specific components occur at approximately 30 kDa (major) and 70 and 68-kDa in the 70-N2 membrane preparation (lane A, Fig. 5B).

**Discussion.** We have found that rabbit antibodies raised against a synthetic $J_H1$ peptide (21) bind to the surface of normal and monoclonal T lymphocytes and react with products in the size range 30–35 and 65–70 kDa in apparent mass. This observation further defines the nature of cross-reactions between T-cell products and immunoglobulin heavy chain variable regions which have been observed for both idiotypic (8, 9, 11, 28) and nonidiotypic markers (6, 10, 18, 29). Because one of the cell lines which we studied, MOLT-3, possesses an immunoglobulin-like T-cell specific gene (14), we thought it worthwhile to compare the sequences of the YT35 T-cell receptor gene with sequences of the $V_H$

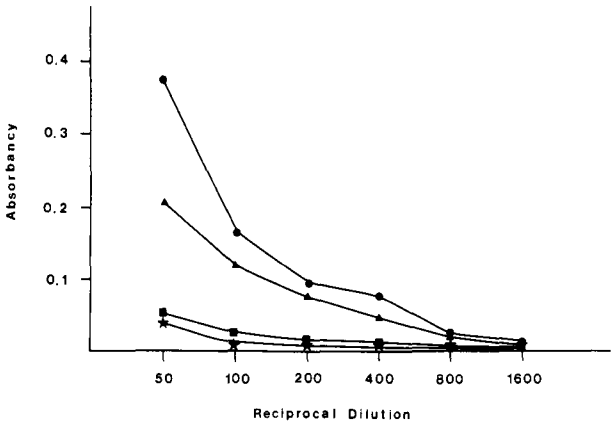



FIG. 4. Reactivity in the ELISA of rabbit anti-$J_H$ peptide (●), anti-influenza virus hemagglutinin (HA) (■), anti-mouse IgT (▲), and normal rabbit serum (NRS) (★) with affinity-purified IgT ($V_TM$) isolated from murine thymocytes. Wells were coated with 0.25 μg of antigen. (A) product of murine thymocytes isolated by immune affinity chromatography on rabbit anti-mouse IgT ($V_TM$) Sepharose and run on 10% SDS–PAGE. (B) molecular weight markers on 10% SDS–PAGE.

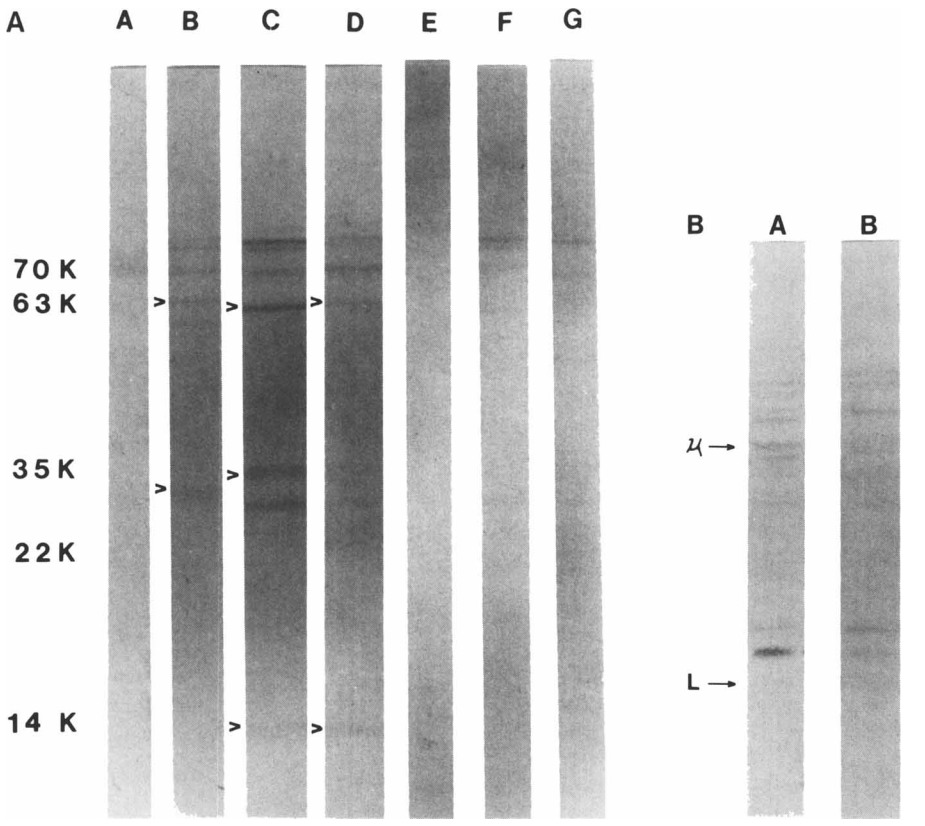
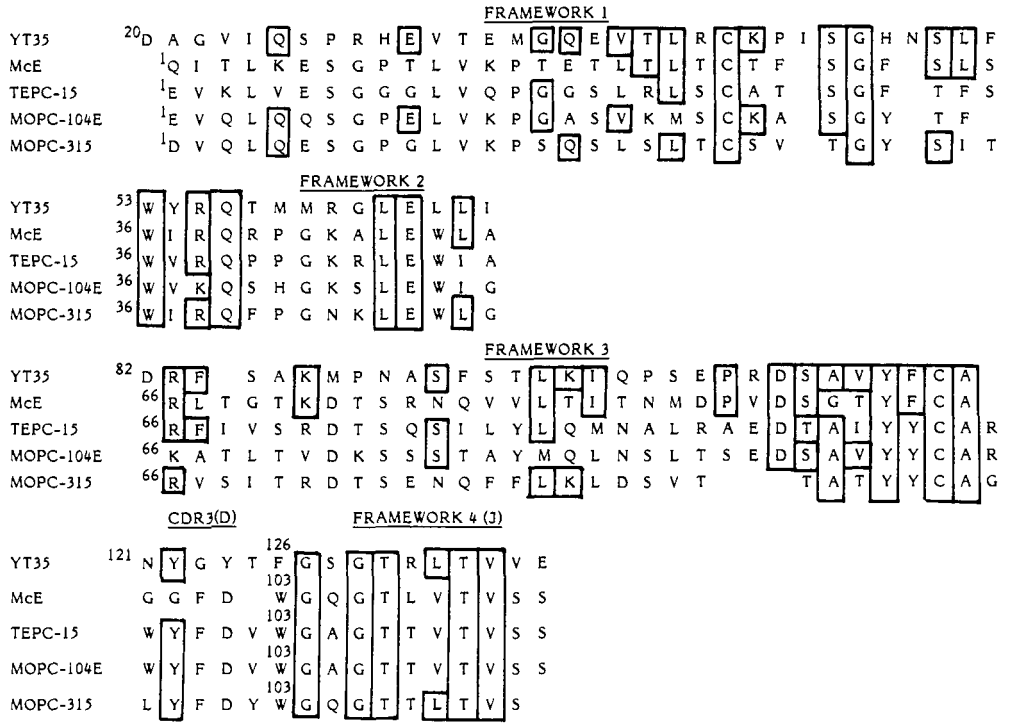



FIG. 5. Immunoblot analyses of 10% SDS–polyacrylamide gels electrophoretically transferred to nitrocellulose paper. (A) lane A, MOPC-104E, lane B, formic acid extract of MOLT-3 cells, lane C, formic acid extract of WEHI-7 cells, lane D, formic acid extract of ICR thymocytes, lane E, formic acid extract of MOLT-3 cells, lane F, formic acid extract of WEHI-7 cells, lane G, formic acid extract of ICR thymocytes. Gel slices A–D were stained with the rabbit anti-$J_H$ peptide antisera. Gel slices E–G were stained with the rabbit anti-KLH antisera. Arrowheads denote unique bands present compared to the appropriate control. (B) Resolution of formic acid solubilized membrane of the 70-N2 T-cell line. Lane A, anti-$J_H$; lane B, anti-KLH. Positions at which immunoglobulin light chains (L) and $\mu$ chains ($\mu$) migrate are indicated.

framework regions of monoclonal immunoglobulins bearing the 16-mer sequence of the synthetic peptide, TEPC-15 and MOPC-104E, with MOPC-315 which has a $J_H2$ sequence but reacts strongly with the anti-$J_H$ sera, and with the sequence of a human myeloma protein McE which is the prototype molecule for the Hv(1) allotypic marker (30). In addition to the anti-$J_H1$ data reported here, rabbit antisera against the human Hv(1) allotype bind significantly to the MOLT-3 plasma membrane as determined by ELISA assay. At a 1/80 dilution the binding of anti Hv(1) to the MOLT-3 membrane was 1.1 $A_{414}$nm units whereas the binding of normal rabbit serum gave <0.1 $A_{414}$nm units. Table I shows an alignment of the framework sequences of the derived YT35 sequence and its comparison with those of the monoclonal immunoglobulins. This was done by first aligning visually the J region sequences and also the stretch of D region sequence in our test peptide. We then found it easy to align regions corresponding to frameworks 1, 2, and 3. Substantial identity was found in comparing the frequency of matches of the $V_T$ of YT35 with the corresponding $V_H$ segments as follows: FR1, 41%; FR2, 43%; FR3, 50%; and $J_H$, 55% identity. This alignment is consistent with the serological data and buttresses previous studies by our group showing V region cross-reactions between

TABLE I. COMPARISONS OF FRAMEWORK REGIONS OF MOLT-3 PUTATIVE T-CELL RECEPTOR GENE WITH SEQUENCES OF CROSS-REACTIVE IMMUNOGLOBULIN $V_H$




defined immunoglobulins and T-cell products (18, 27, 31). The degree of identity within all of these stretches is greater than that which we have previously shown to be sufficient to allow idiotope cross-reactions between immunoglobulins and non-immunoglobulin molecules of corresponding specificities (32, 33). The overall segmental homology is strengthened by our finding that antibodies to $V_H$a allotypes bind to purified $V_T$Ms (27) because these antisera detect markers dependent upon residues occurring in the first and third $V_H$ frameworks (34).

We believe that the subunit mass of the products we have detected from the various T-cell lines is in the range of 30–35 kDa and that the 65- to 70-kDa molecule represents a dimer of this basic unit. This conclusion is consistent with work of other investigators studying antigen-specific T-cell factors (5, 11, 35–37) and also with our studies that the 68-kDa molecule can be made to dissociate into components in the range 25–30 kDa by reduction and alkylation in the presence of 6 *M* guanidine–HCl (27). This subunit size is consistent with that expected of the product of the YT35 gene (14). Direct support for the conclusion that our $V_T$-related products are specified by genes comparable to those of putative T-cell receptors is obtained by comparing the first 10 N-terminal amino acid sequence residues we have obtained for the immune affinity-purified 70-N2 product $V_T$M-1) with those derived from the YT35 gene sequence as follows:

YT35: *D A G V I* Q S P R *H*

$V_T$M-1: *D* D ? *V I* A W V S *H*.

We provide the first direct evidence that T cells produce a molecule which has a stretch of sequence cross-reactive with defined $J_H$ determinant(s) and an N-terminus similar to that of the predicted product of a putative T-cell receptor gene. Other serological cross-reactions buttress the conclusion that the molecule possesses segmental homology to immunoglobulin $V_H$ regions (5, 18, 25, 27, 31). Antisera to the $J_H$ peptide show cross-reactions with other $J_H$ regions, e.g., $J_H$2

sequence (both human and murine) and react with immunoglobulins of other species including sharks. We found these sera to react with immunoglobulins in a manner paralleling that described by Seiden *et al.* (21). It is tempting to speculate whether or not the 30- to 35-kDa $V_T$-bearing products described here can be identified as the $\alpha$ or $\beta$ chains of the MHC-restricted heterodimer which has been reputed to be a T-cell receptor (38–40). We feel that this identification is not warranted at the present time for two reasons: first, $V_H$-related T-cell products bind antigen directly (2, 8, 9, 35–37), whereas the $\alpha/\beta$ heterodimer does not (39). Furthermore the molecular weights of the subunits described here are lower than those which would be expected for the $\alpha$, $M_r$ 45,000, or the $\beta$ $M_r$ 40,000, subunits of the MHC-restricted heterodimer. We believe it most likely that the type of products described here and in other studies of $V_H(V_T)$-related T-cell products (43), will turn out to be the antigen-binding subunits of complexes involving additional regulatory subunits necessary for help or suppression. Although the N-terminal sequence of at least one of our products is similar to that of "$\beta$-gene" products (41), $V_T$-related molecules bear immunoglobulin ($V_H$-cross-reactive) serological determinants and bind antigen (3, 42), whereas the MHC-restricted T-cell receptor neither binds antigen (39) nor expresses immunoglobulin cross-reactive determinants (44). It is possible, however, that serological cross-reactions between "$\beta$-gene" products and $V_H$-segments will be detected since this result is consistent with the appreciable sequence homology expected (45).

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