

A NEW HLA-LINKED T CELL MEMBRANE MOLECULE,  
RELATED TO THE  $\beta$  CHAIN OF THE CLONOTYPIC  
RECEPTOR, IS ASSOCIATED WITH T3<sup>†</sup>

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The MHC-restricted T cell recognition phenomenon remains of central interest in cellular immunology. Recent functional, biochemical, and molecular studies (reviewed in 1–4) provide abundant information concerning distinctive properties of the clonotypic T cell recognition structures expressed in stage III thymocytes and virtually all major subclasses of mature peripheral T cells. At the protein level, the T cell antigen receptor was identified as a heterodimeric glycoprotein comprised of disulfide-linked  $\alpha$  and  $\beta$  chains. In humans, the  $\alpha$  chain has a molecular mass in the range of 46–49 kD, and is acidic. The  $\beta$  chain appeared to be a smaller glycoprotein with a molecular mass in the range of 38–43 kD, and has a more variable pI (slightly acidic to basic) (5–7). Furthermore, cDNAs corresponding to the characterized human and mouse  $\alpha$  and  $\beta$  chains were cloned and sequenced (8–13). The genes encoding  $\alpha$  and  $\beta$  chains of the T cell receptor appeared to have striking similarities to immunoglobulin genes in both sequence and organization (8, 10–19), and are rearranged during T cell differentiation (9, 11, 12, 14, 15, 17, 20, 21). Although the T cell receptor genes are closely related to those encoding the immunoglobulin molecules, a clear difference between the genes encoding the  $\alpha/\beta$  T cell receptor and the immunoglobulin molecules was observed (reviewed in 4). In addition, a third rearranged gene ( $\gamma$ ) with properties similar to the  $\alpha$  and  $\beta$  genes has been recently characterized (22–24). The cell surface protein produced by this gene remains unidentified. The T cell antigen receptor is associated on the membrane of human T cells with the T3 molecular complex (25, 26). The T3 molecular complex was identified as a structure consisting of two glycosylated proteins of ~28 and 22 kD, and a nonglycosylated hydrophobic protein of 20 kD (27, 28). Although evidence that favored the possibility of dual recognition of antigen and the MHC restriction element by the single  $\alpha/\beta$  T cell antigen receptor has

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This study was supported by National Institutes of Health (Bethesda, MD) grants AI-21579 (to Y. Bushkin), CA-40192 (to D. Posnett), and AI-18321 (to C. Y. Wang), and by American Cancer Society grant IM-420 (to B. Pernis).

<sup>†</sup> This paper is dedicated to Dr. Henry G. Kunkel, of the Rockefeller University, who died on December 14, 1983, and was instrumental in the initial phase of this research.

been recently reported (3, 29–31), the mechanisms of MHC-restricted antigen recognition by T cells have not been clarified.

Considerable work in this laboratory has focused on the biochemical characterization of the  $\alpha/\beta$  T cell receptor molecule expressed on the cells of patient (SU) with Sezary syndrome (7, 32–34). SU leukemia cells have been previously characterized as morphologically typical Sezary leukemia cells. Unlike other T cell clones (35) and leukemias (7), they are unresponsive to activation by Sephadex-bound anticolonotypic antibody (S511) recognizing the  $\alpha/\beta$  T cell receptor expressed on these cells (7). Normal T cells bearing the S511 molecule have been identified as a minor subset (~2%) of resting peripheral blood T cells (32, 33). Previous studies (7) have shown that the S511 heterodimeric molecule is slightly different from other reported clonotypic structures expressed on various leukemia clones, having molecular masses of 38 and 43 kD (two chains) and slightly acidic pIs for each chain. Our attempts to further characterize this T cell receptor molecule on SU cells have led to a surprising observation. The A1.4 mAb, recognizing an antigenic determinant on the  $\beta_2$  microglobulin ( $\beta_2m$ )<sup>1</sup>-free HLA heavy chain, coprecipitated from SU leukemia cell lysates a molecule of 38 kD. By peptide mapping experiments, this 38 kD molecule appeared to be similar but not identical to the  $\beta$  chain of the T cell receptor expressed on the same SU leukemia cells (34). We therefore believe that these cells express two related but distinct molecules: (a) the classical  $\alpha/\beta$  T cell antigen receptor, and (b) a  $\beta_2m$ -free HLA heavy chain-like molecule (henceforth referred to as  $\beta_2m$ -free HLA) noncovalently associated with a 38 kD  $\beta$  chain-like molecule (the 38/43 kD heterodimer). We wondered whether this latter structure might be associated with T3, in a way similar to that of the  $\alpha/\beta$  T cell receptor, and that such an association might give a clue to its functional significance.

This paper describes in detail the biochemical features of this  $\beta$  chain-like 38 kD molecule and its association with the  $\beta_2m$ -free HLA heavy chain on the surface of SU leukemia cells. We also present the evidence for an association of both the  $\alpha/\beta$  T cell receptor and the 38 kD chain with the T3 complex, as shown by comodulation experiments. The possible significance of this newly described structure is discussed.

## Materials and Methods

**Cells.** Cells derived from the peripheral blood of healthy donors and patients with Sezary syndrome or T cell chronic lymphocytic leukemia were obtained by Ficoll-Hypaque density centrifugation.

**Monoclonal Antibodies.** mAb S511 (IgG2b) reacting with the idiotype-like molecule on leukemia cells of patient SU has been described previously (32). The A1.4 antibody (IgG1) recognizing an SDS-stable framework determinant expressed on the heavy chain of HLA-A,B,C molecules was developed in our laboratory using Con A-activated T cells as the immunogen. This mAb has been used extensively in previous studies of human class I molecules (36, 37). Other anti-HLA antibodies used in the present study were W6/32 (38), and an mAb (anti-HLA) developed by D. Capra and associates (University of Texas, Dallas). Anti-human  $\beta_2m$  mAb, designated as BBM.1, has been described previously (39). The Leu-4 antibody was kindly provided by R. L. Evans (Memorial Sloan-Kettering Cancer Center).

<sup>1</sup> Abbreviations used in this paper: 2D, two-dimensional;  $\beta_2m$ ,  $\beta_2$ -microglobulin; endo-F, endoglycosidase F; TL, thymus leukemia.

*Antigenic Modulation In Vitro.*  $3 \times 10^7$  leukemia cells were suspended in regular medium at a concentration of  $10^7$  cells/ml. Freshly isolated PBMC from healthy donors were added to the cell suspension in 50-ml Falcon tubes at a final concentration of 20%. For complete Leu-4/T3 antigen modulation, 150  $\mu$ l of Leu-4 antibody supernatant was added to 1 ml of suspended cells, followed by incubation for 6 h at 37°C and 5% CO<sub>2</sub>. Partial modulation of Leu-4/T3 antigen was achieved by adding 100  $\mu$ l of Leu-4 antibody three times at 2-h intervals. After 6 h of incubation, all cultures were transferred to a small Falcon culture flask for an additional 1-h incubation in the same medium. Nonadherent cells were then collected, washed three times with PBS, and used for radioiodination or immunofluorescence staining.

*Cytofluorographic Analysis.* This has been described elsewhere (40).  $10^4$  modulated cells per sample were incubated with a saturating concentration of Leu-4 mAb followed by staining with fluorescein-conjugated goat anti-mouse IgG. Fluorescence distributions were determined with a fluorescence-activated cell sorter (FACS IV; Becton Dickinson Immunocytometry Systems, Mountain View, CA).

*Radioiodination and Immunoprecipitation.*  $3.0$ – $7.5 \times 10^7$  cells were radioiodinated using the lactoperoxidase method with 5–15 mCi of Na<sup>125</sup>I (Amersham Corp., Arlington Heights, IL) and lysed in 2 ml of buffer composed of 0.5% NP-40 plus 1 mM PMSF (Sigma Chemical Co., St. Louis, MO) in PBS (pH 8.3) without Ca<sup>2+</sup> and Mg<sup>2+</sup>. Before immunoprecipitation, bovine hemoglobin (Sigma Chemical Co.) was added to the cell lysates at a final concentration of 1% to reduce nonspecific binding of labeled material to antibody-coated beads. Immunoprecipitation was carried out by incubation of the labeled cell lysates with antibody-coated goat anti-mouse IgG agarose beads (Sigma Chemical Co.). The material was eluted from the beads with Laemmli sample buffer (41) for reducing (with 2% 2-ME) and nonreducing SDS-PAGE, or with IEF sample buffer (36) for two-dimensional (2D) gel electrophoresis.

In sequential immunoprecipitation experiments, a 150- $\mu$ l aliquot of labeled cell lysate was first incubated with an excess amount of W6/32 antibody-coated goat anti-mouse IgG agarose beads. This step was performed twice, and the absorbed lysate was subjected to the subsequent clearing step with A1.4 antibody (three times) followed by precipitation with S511 antibody and SDS-PAGE analysis.

*Treatment with Endoglycosidase F (Endo-F).* This method was performed as described elsewhere (37).

*Immunoblotting.* The method of Johnson et al. (42) was used with minor modifications. Briefly,  $1.5 \times 10^7$  cells were lysed in 200  $\mu$ l of Laemmli sample buffer, and insoluble nuclear material was removed by passage of the lysate through a piece of gauze before application on an SDS-PAGE slab. Proteins separated on SDS-PAGE were transferred to a nitrocellulose sheet at 55 V for 2.5 h.

*SDS-PAGE and 2D Gel Electrophoresis.* SDS-PAGE was performed according to Laemmli (41) on vertical slab gels of 11% acrylamide. The 2D analysis was performed as described elsewhere (36). IEF slab gels for first-dimensional resolution of 2D analysis were constructed with pH 3.5–10 ampholines (LKB Instruments, Gaithersburg, MD). The slabs were then cut into strips for the second-dimension resolution by SDS-PAGE.

*Chymotryptic Peptide Mapping.* This was done according to Elder et al. (43). Radioiodinated and sequentially immunoprecipitated molecules (Fig. 5) were isolated from slices of Laemmli slab gels and subjected to chymotrypsin digestion (50  $\mu$ g/ml of enzyme in 1% ammonium bicarbonate, pH 8.3) for 18 h at 37°C.

## Results

*The 38 kD Molecule Is Immunoprecipitated with A1.4 Antibody from Radioiodinated Sezary Leukemia Cell Lysates.* As illustrated in Fig. 1, the S511 mAb precipitated from radioiodinated cell lysates of the Sezary leukemia patient SU the  $\alpha/\beta$  T cell receptor molecule consisting of two disulfide-linked polypeptide chains with molecular masses of 43 and 38 kD, respectively (lane 2). The A1.4 mAb, recognizing an SDS-stable framework determinant on the heavy chain of HLA,

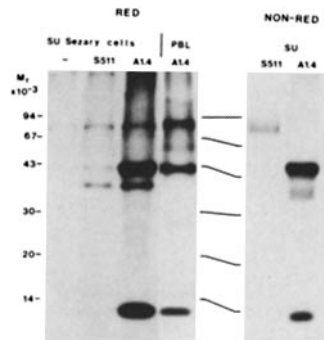


FIGURE 1. SDS-PAGE of the  $\alpha/\beta$  T cell receptor and HLA molecules precipitated from radioiodinated Sezary leukemia and normal PBL lysates. Reducing conditions, lanes 1-4: beads alone, cell lysate of patient SU reacted with S511 antibody, A1.4 antibody, and PBL lysate reacted with A1.4 antibody. Nonreducing conditions, as in lanes 2 and 3.

precipitated from the same cell lysate a 38 kD molecule similar in mobility to the  $\beta$  chain, in addition to the 43 kD heavy HLA chain and its accompanying  $\beta_2m$  (Fig. 1, lane 3). In contrast, no such 38 kD band was observed in anti-A1.4 precipitate from radioiodinated peripheral blood lymphocyte lysate (Fig. 1, lane 4). SDS-PAGE under nonreducing conditions revealed that this 38 kD molecule is not disulfide-linked to any other chain, since no higher molecular mass component similar to the 80 kD T cell receptor was observed in anti-A1.4 precipitates (Fig. 1, lanes 5 and 6). The lack of interchain disulfide bonding with the 38 kD polypeptide precipitated by the A1.4 antibody suggested that this chain was not identical to the  $\beta$  chain of the T cell receptor molecule expressed on the same cells. Therefore, we decided to determine the molecular mass of both polypeptides more accurately. The 38 kD bands precipitated by A1.4 and S511 antibodies were both excised from the gel (Fig. 1), eluted with Laemmli sample buffer and resubmitted to SDS-PAGE. This procedure revealed minor but reproducible differences in the mobility of both 38 kD chains on SDS-PAGE, the A1.4 band migrating slightly faster than the S511  $\beta$  chain band (data not shown).

14 other T cell leukemias, including 5 patients with Sezary syndrome and 2 with T cell chronic lymphocytic leukemia were further screened for the presence of a similar 38 kD band in anti-A1.4 precipitates. A second patient (PR) with Sezary syndrome was found to express a similar 38 kD molecule. As seen in Fig. 2, the A1.4 antibody precipitated from the radioiodinated cell lysate of this patient a 38 kD chain in addition to the HLA heavy chain and  $\beta_2m$  (lane 2). As in the case of patient SU (not shown), only A1.4 antibody was able to precipitate this 38 kD molecule but not other anti-HLA and anti- $\beta_2m$  antibodies (Fig. 2).

*Similarity in IEF Patterns Between the 38 kD chain and the  $\beta$  Chain of the T Cell Receptor from the Same Leukemia Cells.* We further characterized S511 and A1.4 immunoprecipitates from SU leukemia cell lysates by 2-D (IEF-SDS-PAGE) gel analysis. The 38 kD molecule precipitated with A1.4 antibody has not only the same pI as the  $\beta$  chain of the T cell receptor precipitated with S511 antibody from the same cell lysate, but also has a very similar IEF pattern (Fig. 3). To compare the glycosylation of these two 38 kD chains, endo-F digestion was

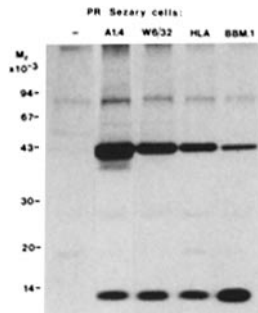


FIGURE 2. SDS-PAGE of radiiodinated cell lysate of patient PR with Sezary syndrome reacted with various anti-HLA and anti- $\beta_2m$  antibodies. Lanes 1–5: beads alone, A1.4, W6/32, anti-HLA developed in Dr. D. Capra's laboratory, and BBM.1.

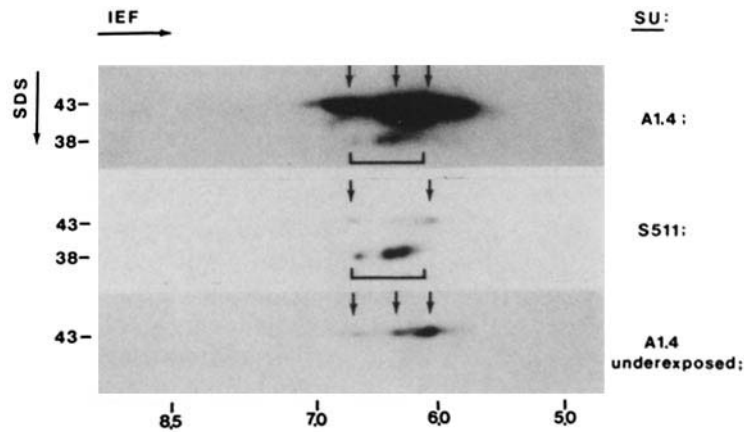


FIGURE 3. 2D gel patterns of the  $\alpha/\beta$  T cell receptor precipitated by S511 antibody, and the 38 kD and HLA heavy chain molecules precipitated by A1.4 antibody from the radiiodinated cell lysate of patient SU. The 43 kD chain of each immunoprecipitate is designated by arrows, whereas the 38 kD chain is designated by brackets. Only segments of the autoradiographs are shown.

performed. Treatment of both immunoprecipitates with endo-F, which cleaves high-mannose and complex *N*-linked carbohydrates (44) resulted, in both cases, in the replacement of 38 kD chains by bands migrating at  $\sim 34$  kD (Fig. 4). However, the band corresponding to the deglycosylated 38 kD chain coprecipitated by A1.4 ran as a more diffuse and slightly faster band than the deglycosylated  $\beta$  chain precipitated by S511. Thus, SDS-PAGE, 2D gel electrophoresis, and endo-F digestion were unable to clearly distinguish the two 38 kD chains, although minor differences were suggested by these experiments.

The 43 kD  $\alpha$  chain of the T cell receptor and the HLA heavy chain resolved in the same pI range (Fig. 3). It appeared, however, that the 43 kD chains from both precipitates displayed somewhat different IEF patterns, more complex for the 43 kD chain precipitated by A1.4 antibody than for that precipitated by S511 antibody.

*The 38 kD Chain and the  $\alpha/\beta$  T Cell Receptor Are Different Molecules as Shown by Sequential Immunoprecipitation.* Similarities, in both molecular mass and IEF

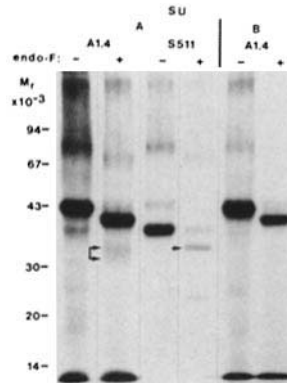


FIGURE 4. SDS-PAGE of A1.4 and S511 immunoprecipitates digested with endo-F. A, SU leukemia cell lysate reacted with A1.4 and S511 antibodies followed by digestion of precipitates with endo-F as described (37). The 34 kD bands corresponding to deglycosylated 38 kD chains are designated by an arrow and a double-arrow bracket. Studies with  $\alpha$  and  $\beta$  chains of the T cell receptor isolated from SDS-PAGE slabs (Y. Bushkin, unpublished data) indicated that the band migrating at  $\sim 24$  kD (lane 4) is nonspecific. B, the same experimental conditions as in A, except that SU leukemia cells were modulated by excess of Leu-4 antibody before iodination and immunoprecipitation with A1.4 antibody. This modulation results in disappearance of the 38 kD chain precipitated by A1.4 as described in the text and Fig. 9.

patterns of the 38 and 43 kD chains precipitated by A1.4 antibody, to the  $\alpha$  and  $\beta$  chains of the T cell receptor precipitated by the S511 antibody from SU leukemia cell lysates prompted us to examine the structural relationship between these molecules. In particular, we asked whether these two 38/43 kD molecules expressed on the same cells were indeed different molecules. Labeled SU cell lysate was precleared with W6/32 antibody, which recognizes the HLA heavy chain in association with  $\beta_2m$  (45), and then subjected to sequential immunoprecipitations with A1.4 and S511 antibodies. As seen in Fig. 5, reaction of W6/32-precleared SU cell lysate with A1.4 antibody resulted in precipitation of both 43 and 38 kD chains, but not  $\beta_2m$  (lane 3). Since preclearing with W6/32 antibody completely depleted the cell lysate of  $\beta_2m$ -associated HLA heavy chains precipitated by W6/32 (lane 2), the 43 kD band seen in the A1.4 precipitate (lane 3) may represent  $\beta_2m$ -free HLA. The intensity of the 43 kD band precipitated by A1.4 was strongly reduced by preclearing with W6/32 as expected, since A1.4 also precipitates  $\beta_2m$ -associated HLA heavy chains (Figs. 1 and 2). We next reacted sequentially the W6/32- and A1.4-precleared SU cell lysate with A1.4 (Fig. 5, lane 4) or S511 antibody (lane 5). The intensity of labeled  $\alpha$  and  $\beta$  chains of the T cell receptor precipitated by S511 antibody from this precleared SU cell lysate (Fig. 5, lane 5) appeared to be identical to that precipitated from untreated lysate (lane 6). Likewise, preclearing with S511 antibody did not decrease the intensity of the two bands precipitated by A1.4 antibody (data not shown). These results provided clear evidence that the 43 and 38 kD chains precipitated by A1.4 antibody from SU cell lysates, and the  $\alpha/\beta$  T cell receptor expressed on the same cells are different molecules.

Coprecipitation of both the 43 and 38 kD chains by A1.4 is probably due to a noncovalent association, since the A1.4 antibody recognizes only the 43 kD chain, as determined by immunoblotting experiments (Fig. 6). Here it was shown

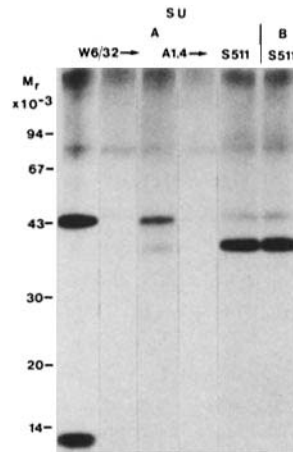


FIGURE 5. SDS-PAGE of sequential immunoprecipitation of radioiodinated SU leukemia cell lysate with W6/32, A1.4, and S511 antibodies. *A*, sequential precipitations. *B*, parallel precipitation. For complete absorption, each preclearing step with W6/32 or A1.4 antibody was performed three times. Only first (lanes 1 and 3) and last (lanes 2 and 4) absorptions are shown. Each immunoprecipitate was done with an equal amount of the original SU cell lysate.

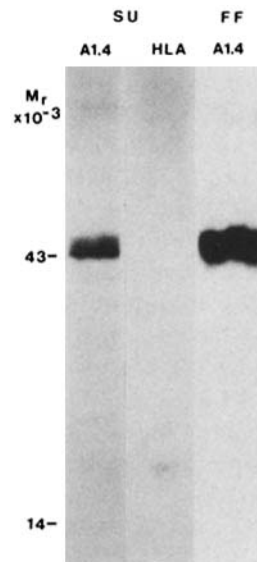


FIGURE 6. Immunoblotting: A1.4 antibody reactivity with SDS-denatured HLA heavy chains from different T cell leukemias. SU and FF leukemia (5) cell lysates were prepared and separated on SDS-PAGE as described in Materials and Methods. Nitrocellulose blots were reacted with A1.4 (lanes 1 and 3) or with an anti-HLA antibody recognizing an SDS-sensitive antigenic determinant (lane 2), followed by reaction with radioiodinated goat anti-mouse IgG.

that A1.4 binds to the SDS-denatured HLA heavy chain. A similar result was observed with lysates from two different T cell leukemias: SU cells (Fig. 6, lane 1), which express the 38 kD molecule coprecipitated by A1.4, and FF cells (lane 3), which do not express this molecule. Thus, the antigenic determinant recognized by A1.4 is located on a nonpolymorphic regions of the typical  $\beta_2m$ -

associated HLA heavy chain and the  $\beta_2m$ -free HLA. It is not present on the 38 kD molecule coprecipitated by A1.4 from SU and PR cell lysates (Figs. 1 and 2). These results favor a noncovalent association of the  $\beta_2m$ -free HLA and the 38 kD molecule, rather than sharing of an antigenic determinant by both chains.

*Structural Similarity Between the  $\alpha/\beta$  T Cell Receptor and the HLA/38 kD Heterodimer Expressed on the Same Cells, as Shown by Chymotryptic Peptide Mapping.* Although sequential immunoprecipitation experiments suggested that the  $\alpha/\beta$  T cell receptor and the HLA/38 kD heterodimer expressed on the same SU leukemia cells are distinct molecules, chymotryptic peptide mapping experiments revealed some similarities. The 38 and 43 kD chains immunoprecipitated from precleared lysates and analyzed by SDS-PAGE in the sequential precipitation experiment (Fig. 5, lanes 1, 3, and 5) were excised from the gel and subjected to peptide mapping. As shown in Fig. 7, the 38 kD chain precipitated by A1.4 and the 38 kD  $\beta$  chain of the T cell receptor precipitated by S511 shared 50% of tyrosine-containing peptides, although some of the peptides on these maps were unique for each molecule. Likewise, the comparison of peptide maps of the  $\beta_2m$ -free 43 kD chain precipitated by A1.4 and the 43 kD  $\alpha$  chain of the T cell receptor, precipitated by S511, revealed similarity but not identity between these molecules (Fig. 8, B and C).

Of special interest was the comparison between peptide maps of the HLA heavy chain precipitated by W6/32 and the  $\beta_2m$ -free 43 kD chain precipitated by A1.4 from the cell lysate depleted of  $\beta_2m$ -associated HLA (Fig. 8, A and B). The salient finding was that the peptide map of the 43 kD chain precipitated by A1.4 clearly contained four peptides not present in the peptide map of the HLA heavy chain precipitated by W6/32. These additional peptides were found in spite of preclearing the lysate with W6/32. At present, it is not clear whether this difference is due to the fact that the  $\beta_2m$ -free HLA heavy chain may be more accessible to the label than its  $\beta_2m$ -associated counterpart, or to the fact that the  $\beta_2m$ -free 43 kD chain actually represents an HLA-like chain, distinct from HLA heavy chain at the amino acid level. Generation of mAbs specific for the 38 kD and  $\beta_2m$ -free 43 kD chains precipitated by A1.4 antibody may help to answer these questions.

*Both the  $\alpha/\beta$  T Cell Receptor and the 38 kD Chain Are Associated with the T3 Molecular Complex on the Surface of SU Leukemia Cells.* Structural similarity between the 38 kD molecule and the  $\beta$  chain of T cell antigen receptor from the same SU cells have prompted us to investigate whether this 38 kD molecule could be comodulated with the T3 molecular complex similarly to comodulation of T3 with the  $\alpha/\beta$  T cell receptor (7, 32, 46). As seen in Fig. 9A, the Leu-4 mAb precipitated from the unmodulated radioiodinated SU leukemia cell lysate the T3 molecular complex, consisting of three bands of 28, 24, and 22 kD, respectively (lane 1). The S511 mAb precipitated from the same lysate the 38/43 kD  $\alpha/\beta$  T cell receptor molecule (Fig. 9, lane 2). The A1.4 antibody immunoprecipitated HLA heavy chain and  $\beta_2m$  (as did W6/32 [Fig. 9, lane 3]), and also coprecipitated the 38 kD chain (lane 4). As shown in Fig. 9B, partial modulation of the T3 molecular complex from the surface of SU cells with Leu-4 antibody, followed by radioiodination, immunoprecipitation, and SDS-PAGE resulted in the loss of both the 38 kD molecule coprecipitated by A1.4 antibody



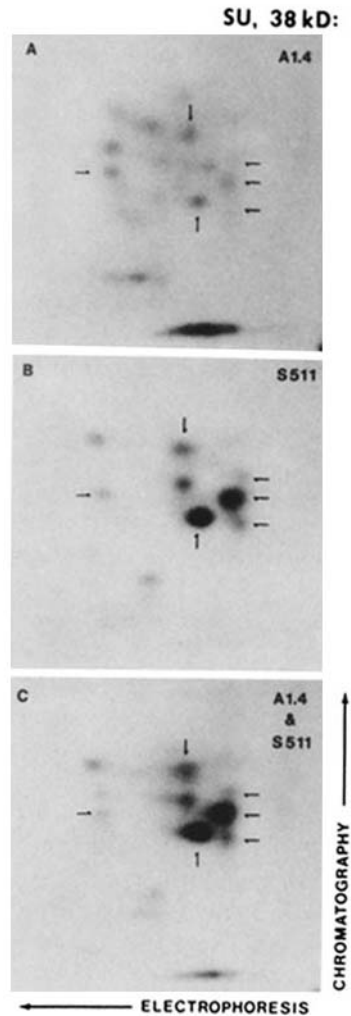


FIGURE 7. Comparison among  $^{125}\text{I}$ -chymotryptic digests of the 38 kD chain and the  $\beta$  chain of the T cell receptor precipitated by A1.4 and S511 antibodies. Molecules were isolated from the W6/32-precleared lysate and separated on a slab gel as shown in Fig. 5. Chymotryptic digests were analyzed on thin-layer cellulose plates by electrophoresis in the first dimension and chromatography in the second dimension. *A*, peptide map of the 38 kD chain coprecipitated by A1.4 antibody (from Fig. 5, lane 3); *B*, peptide map of the  $\beta$  chain of the  $\alpha/\beta$  T cell receptor precipitated by S511 antibody (from Fig. 5, lane 5); *C*, mixture of *A* and *B*. Tyrosine-containing peptides common for both chains are designated by arrows.

and the 22–24 kD light chains of T3 (lanes 2 and 4). However, the 28 kD heavy chain of T3, as well as the  $\alpha$  and  $\beta$  chains of the T cell antigen receptor were not modulated from the cell surface, and remained accessible to radioiodination (Fig. 9B, lanes 3 and 4) under these conditions. A recent report (26) shows that the  $\beta$  chain of the human T cell antigen receptor is predominantly associated with the 28 kD heavy chain of T3 molecular complex on the cell surface. Accordingly, complete modulation of T3 from the surface of SU cells resulted

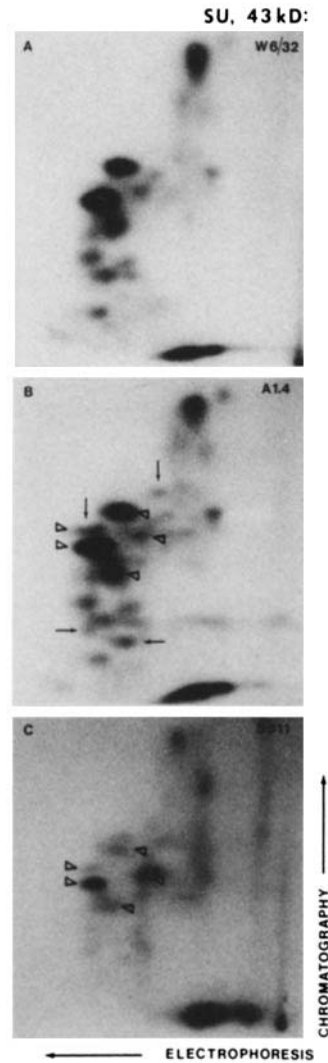


FIGURE 8. Comparison among  $^{125}\text{I}$ -chymotryptic digests of the 43 kD chains precipitated by W6/32, A1.4, and S511 antibodies. The 43 kD molecules were isolated from the slab gel (Fig. 5), digested with chymotrypsin, and analyzed as described in Fig. 7. A-C, peptide maps of: HLA heavy chain precipitated by W6/32 antibody (from Fig. 5, lane 1),  $\beta_2\text{m}$ -free HLA heavy chain precipitated by A1.4 antibody (from Fig. 5, lane 3), and  $\alpha$  chain of the  $\alpha/\beta$  T cell receptor precipitated by S511 antibody (from Fig. 5, lane 5). Tyrosine-labeled peptides present in the  $\beta_2\text{m}$ -free 43 kD chain precipitated by A1.4 antibody and absent in the HLA heavy chain precipitated by W6/32 antibody are designated by long arrows. Peptides designated by stemless arrows are common to the  $\beta_2\text{m}$ -free 43 kD chain precipitated by A1.4 antibody and the  $\alpha$  chain of the  $\alpha/\beta$  T cell receptor.

in comodulation of the  $\alpha/\beta$  T cell receptor with the remaining 28 kD heavy chain of T3 (Fig. 9C, lanes 1 and 2).

The cytofluorographic analysis carried out parallel to radioiodination and SDS-PAGE experiments was in accordance with the biochemical data in the sense

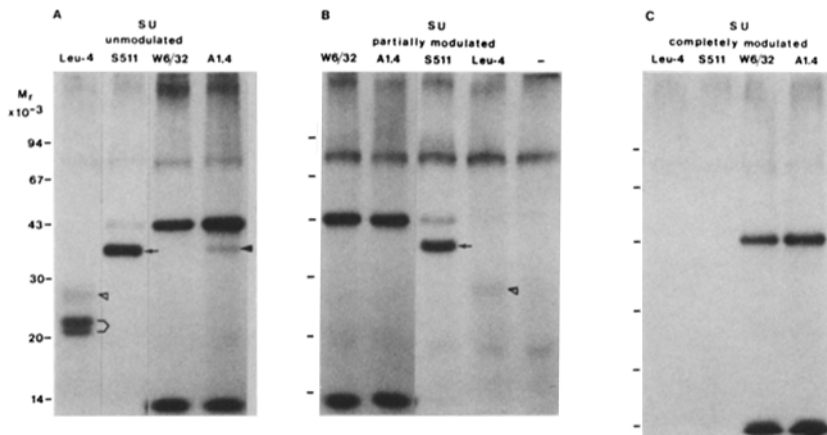


FIGURE 9. Comodulation of the  $\alpha/\beta$  T cell receptor and the 38 kD molecule with the T3 molecular complex on the surface of SU leukemia cells. *A* shows immunoprecipitates obtained from unmodulated radioiodinated SU leukemia cell lysate. Lanes 1–4: labeled cell lysates reacted with Leu-4, S511, W6/32, and A1.4 antibodies, respectively. Arrow designations: triangle bracket for the 22–24 kD chains of T3 molecular complex, open stemless arrow for the 28 kD chain of T3, blackened arrow for the 38 kD ( $\beta$ ) chain of T cell receptor, and blackened stemless arrow for the 38 kD chain coprecipitated by A1.4 antibody. *B* shows immunoprecipitates from partially modulated SU cells. Lanes 1–5: W6/32, A1.4, S511, Leu-4, and beads alone. *C* shows immunoprecipitates from completely modulated SU cells. Lanes 1–4: Leu-4, S511, W6/32, and A1.4.

that leukemia cells completely modulated with Leu-4 antibody were totally negative for Leu-4 and S511 immunofluorescence staining (not shown).

### Discussion

The results presented in this paper clearly indicate that at least some T cell clones, represented by human T cell leukemias such as SU, synthesize a 38 kD glycoprotein that is similar but not identical to the  $\beta$  chain of the classical  $\alpha/\beta$  38/43 kD T cell antigen receptor. The 38 kD molecule coexists with the  $\alpha/\beta$  T cell receptor on the same cell, since generation of two different clonotypic antibodies to SU cells (32) has confirmed the clonal nature of this leukemia. Similarity between the  $\beta$  chain of the T cell receptor and the 38 kD molecule was shown by endo-F digestion, 2D (IEF–SDS–PAGE) electrophoresis, and most significantly, chymotryptic peptide mapping using precleared lysates (Figs. 3, 4, and 7). The latter method revealed that ~50% of peptides are shared between the two chains. Nevertheless, these two glycoproteins are distinct molecules, as was clearly evidenced by sequential immunoprecipitation experiments and the chymotryptic peptide maps (Figs. 5 and 7). Several observations suggest that the 38 kD, HLA-associated chain coprecipitated by A1.4 antibody does not have an identical amino acid sequence to the  $\beta$  chain of the  $\alpha/\beta$  T cell receptor: (a) absence of the interchain disulfide bonds characteristic for the  $\beta$  chain of the T cell antigen receptor; (b) differences in peptide composition between the two chains; (c) sequential immunoprecipitation experiments showed a clear difference between these two chains. However, comodulation experiments (discussed below) have revealed that both the 38 kD HLA-associated chain and the  $\beta$  chain of the

T cell receptor are associated with the T3 complex on the cell surface of SU leukemia cells. Therefore, some biochemical and structural similarities between these two distinct 38 kD chains allow us to hypothesize that both chains are related.

Of special interest is the observed coprecipitation of the 38 kD molecule with a  $\beta_2m$ -free HLA. Our interpretation that the 43 kD chain of the 38/43 kD heterodimer precipitated by A1.4 antibody from SU leukemia cell lysates is a  $\beta_2m$ -free HLA heavy chain is based on the following observations: (a) this antibody recognizes an SDS-stable framework determinant on the HLA heavy chain, as evidenced by immunoblotting experiments (Fig. 6); (b) chymotryptic peptide maps of the 43 kD chain precipitated by the A1.4 antibody from SU cell lysates, and maps of the 43 kD chain precipitated by the A1.4 antibody from SU lysates depleted of the W6/32-reactive 43 kD chain are nearly identical (Fig. 8). On these peptide maps, however, four weakly labeled peptides (out of 19) are unique for the 43 kD chain precipitated by A1.4 antibody. The appearance of these four peptides might be due to higher accessibility of the  $\beta_2m$ -free HLA heavy chain to the radioactive label, or alternatively, might indicate the presence of a distinct HLA heavy chain-like molecule such as a human analog of thymus leukemia (TL), for example. However, murine TL heavy chains are characteristically associated with  $\beta_2m$  (47). In addition, comparison of chymotryptic and tryptic peptide maps of murine TL and H-2 molecules has revealed little similarity in the composition of the peptides (37, 48). Thus the data presented herein support the argument that the A1.4-reactive  $\beta_2m$ -free 43 kD chain is not a human TL equivalent. Immunoblotting experiments revealed that the A1.4 antibody recognizes a determinant of the SDS-denatured 43 kD chain and not the associated 38 kD chain. This does not favor the interpretation that A1.4 recognizes identical epitopes on HLA heavy chains and on an unrelated 38 kD cell membrane molecule. An association of the 38 kD chain with the  $\beta_2m$ -free HLA heavy chain could be due to noncovalent hydrogen bonds similar to the association of  $\beta_2m$  and HLA heavy chain (reviewed in 49).

Coprecipitation of a 38 kD chain by A1.4 is not a unique feature of SU leukemia, since the same anti-HLA antibody precipitated a similar 38 kD chain from another human T cell leukemia (PR). The 38 kD chain precipitated from PR leukemia cell lysates has both pI and molecular mass slightly different from that of the 38 kD chain expressed on SU leukemia cells (Y. Bushkin, unpublished data). Coprecipitation by A1.4 antibody was observed in 2 of 14 T cell leukemias tested. It is important to determine what subset of normal T cells expresses this new 38/43 kD heterodimer. While no such heterodimer is precipitated from resting peripheral blood T cells, a very small amount of the 38 kD chain is coprecipitated by A1.4 antibody from the cell lysates of alloactivated polyclonal T cells (Y. Bushkin, N. Mohaghehpour, and E. G. Engleman, unpublished data). A 38 kD polypeptide may be synthesized by a minor subpopulation of these alloactivated T cells. Thus the presence of the 38/43 kD heterodimer on the cell surface may depend on the state of activation of the T cell.

The data presented in this paper show that the 38 kD molecule coprecipitated by A1.4 is associated with the T3 complex. Like the  $\alpha/\beta$  T cell receptor, it is comodulated with the T3 complex, as shown in experiments that involved the

complete disappearance of the T3 complex from the membrane of SU cells exposed to the Leu-4 antibody. In addition, examination of partially modulated cells shows that the T3 complex may be dissociated, with disappearance of the two smaller chains (22 and 24 kD) and persistence of the larger (28 kD) component. We are presently investigating the precise conditions that determine this partial modulation of the T3 complex and the role of accessory cells (40). The dissociation of the 28 kD chain from the 22 and 24 kD components of the T3 complex has been observed before (50) in normal human peripheral blood T cells exposed to Leu-4 antibodies. When the cells exposed to Leu-4 lost the smaller chains of T3 but retained the 28 kD chain, they also lost the 38 kD molecule but retained their clonotypic T cell receptor molecule. This observation is consistent with the fact that the  $\alpha/\beta$  T cell receptor is associated primarily with the 28 kD chain of T3 (26), and supports the finding that the 38 kD molecule and the clonotypic T cell antigen receptor are separate entities. These findings suggest that, at least in SU cells, the T3 complex may serve as an aggregation center of different molecules, since different components of the T3 complex appear to react with the  $\alpha/\beta$  T cell receptor and the 38/43 kD heterodimer.

In conclusion, the 38 kD chain expressed on the surface of SU leukemia cells is noncovalently associated with  $\beta_2m$ -free HLA as detected by mAb A1.4. This 38 kD chain has significant structural homology to the  $\beta$  chain of the clonotypic T cell receptor expressed on the same cells. Nevertheless, the 38 kD chain is a distinct molecule. Its association with the T3 molecular complex suggests a functional role in T cell activation. One possible interpretation is that this 38 kD chain represents the still unidentified protein product of the  $\gamma$ -chain gene, or of yet another member of the T cell receptor gene family. Currently we are investigating the possibility that these 38 kD, HLA-associated chains from SU and PR leukemia cells contain both constant and variable peptides similar to the  $\alpha$  and  $\beta$  chains of the classical T cell antigen receptor (51, 52).

### Summary

The 38 kD molecule is noncovalently associated with  $\beta_2$  microglobulin ( $\beta_2m$ )-free HLA heavy chain-like molecule, and thus forms a second heterodimer distinct from the clonotypic  $\alpha/\beta$  T cell receptor expressed by the same clone of leukemia cells. This second heterodimer (38 kD/HLA) is variably expressed and appears to be associated with the T3 molecule. We suggest, therefore, that it has a functional role in T cell activation.

*Received for publication 30 December 1985*

### References

1. Acuto, O., M. Fabbi, A. Bensussan, C. Milanese, T. J. Campen, H. D. Royer, and E. L. Reinherz. 1985. The human T-cell receptor. *J. Clin. Immunol.* 5:141.
2. Pernis, B., and R. Axel. 1985. A one and a half receptor model for MHC-restricted antigen recognition by T lymphocytes. *Cell.* 41:13.
3. Yagüe, J., J. White, C. Coleclough, J. Kappler, E. Palmer, and P. Marrack. 1985. The T cell receptor: the  $\alpha$  and  $\beta$  chains define idiotypic, and antigen and MHC specificity. *Cell.* 42:81.

4. Robertson, M. 1985. The present state of recognition. *Nature (Lond.)*. 317:768.
5. Acuto, O., S. C. Meuer, J. C. Hodgdon, S. F. Schlossman, and E. L. Reinherz. 1983. Peptide variability exists within the  $\alpha$  and  $\beta$  subunits of the T cell receptor for antigen. *J. Exp. Med.* 158:1368.
6. Kappler, J., R. Kubo, K. Haskins, C. Hannum, P. Marrack, M. Pigeon, B. McIntyre, J. Allison, and I. Trowbridge. 1983. The major histocompatibility complex-restricted antigen receptor on T cells in mouse and man. V. Identification of constant and variable peptides. *Cell*. 35:295.
7. Posnett, D., R. D. Bigler, Y. Bushkin, D. E. Fisher, C. Y. Wang, L. F. Mayer, N. Chiorazzi, and H. G. Kunkel. 1984. T cell antiidiotypic antibodies reveal differences between two human leukemias. *J. Exp. Med.* 160:494.
8. Yanagi, Y., Y. Yoshikai, K. Leggett, S. P. Clark, I. Aleksander, and T. W. Mak. 1984. A human T cell-specific cDNA encodes a protein having extensive homology to immunoglobulin chains. *Nature (Lond.)*. 308:145.
9. Hedrick, S. M., D. I. Cohen, E. A. Nielsen, and M. M. Davis. 1984. Isolation of cDNA clones encoding T cell-specific membrane-associated protein. 1984. *Nature (Lond.)*. 308:149.
10. Hedrick, S. M., E. A. Nielsen, J. Kavaler, D. I. Cohen, and M. M. Davis. 1984. Sequence relationships between putative T-cell receptor polypeptides and immunoglobulins. *Nature (Lond.)*. 308:153.
11. Chien, Y. H., D. M. Becker, T. Lindsten, M. Okamura, D. I. Cohen, and M. M. Davis. 1984. A third type of murine T-cell receptor gene. *Nature (Lond.)*. 312:31.
12. Saito, H., D. M. Kranz, Y. Takagaki, A. C. Hayday, H. N. Eisen, and S. Tonegawa. 1984. A third rearranged and expressed gene in a clone of cytotoxic T lymphocytes. *Nature (Lond.)*. 312:36.
13. Sim, G. K., J. Yagüe, J. Nelson, P. Marrack, E. Palmer, A. Augustin, and J. Kappler. 1984. Primary structure of human T-cell receptor  $\alpha$ -chain. *Nature (Lond.)*. 312:771.
14. Siu, G., S. P. Clark, Y. Yoshikai, M. Malissen, Y. Yanagi, E. Strauss, T. W. Mak, and L. Hood. 1984. The human T cell antigen receptor is encoded by variable, diversity and joining gene segments that rearrange to generate a complete V gene. *Cell*. 37:393.
15. Malissen, M., K. Minard, S. Mjolsness, M. Kronenberg, J. Goverman, T. Hunkapiller, M. B. Prystowsky, Y. Yoshikai, F. Fitch, T. W. Mak, and L. Hood. 1984. Mouse T cell antigen receptor: structure and organization of constant and joining gene segments encoding the  $\beta$  polypeptide. *Cell*. 37:1101.
16. Gascoigne, N. R. J., Y. H. Chien, D. M. Becker, J. Kavaler, and M. M. Davis. 1984. Genomic organization and sequence of T-cell receptor  $\beta$ -chain constant- and joining-region genes. *Nature (Lond.)*. 310:387.
17. Siu, G., M. Kronenberg, E. Strauss, R. Haars, T. W. Mak, and L. Hood. 1984. The structure, rearrangement and expression of the D $\beta$  gene segments of the murine T-cell antigen receptor. *Nature (Lond.)*. 311:344.
18. Clark, S. P., Y. Yoshikai, S. Taylor, G. Siu, L. Hood, and T. W. Mak. 1984. Identification of diversity segment of human T-cell receptor  $\beta$ -chain, and comparison with the analogous murine element. *Nature (Lond.)*. 311:388.
19. Patten, P., T. Yokota, J. Rothbard, Y. H. Chien, K. I. Arcai, and M. M. Davis. 1984. Structure, expression and divergence of T-cell receptor  $\beta$ -chain variable regions. *Nature (Lond.)*. 312:40.
20. Toyonaga, B., Y. Uanagi, N. Suci-Foca, M. Minden, and T. W. Mak. 1984. Rearrangements of T-cell receptor gene YT35 in human DNA from thymic leukemia T-cell lines and functional T-cell clones. *Nature (Lond.)*. 311:383.
21. Minden, M., B. Toyonaga, K. Ha, Y. Yanagi, B. Chin, E. Gelfand, and T. W. Mak.

1985. Somatic rearrangement of T-cell antigen receptor gene in human T-cell malignancies. *Proc. Natl. Acad. Sci. USA.* 82:1224.
22. Saito, H., D. Kranz, Y. Takagaki, A. C. Hayday, H. N. Eisen, and S. Tonegawa. 1984. Complete primary structure of a heterodimeric T cell receptor deduced from cDNA sequences. *Nature (Lond.)* 309:757.
23. Hayday, A. C., H. Saito, S. D. Gillies, D. Kranz, G. Tanigawa, H. N. Eisen, and S. Tonegawa. 1985. Structure, organization, and somatic rearrangement of T-cell gamma genes. *Cell.* 40:259.
24. Kranz, D. M., H. Saito, M. Heller, Y. Takagaki, W. Haas, H. N. Eisen, and S. Tonegawa. 1985. Limited diversity of the rearranged T-cell  $\gamma$  gene. *Nature (Lond.)* 313:752.
25. Meuer, S. C., O. Acuto, R. E. Hussey, J. C. Hodgdon, K. A. Fitzgerald, S. F. Schlossman, and E. L. Reinherz. 1983. Evidence for the T3-associated 90K heterodimer as the T-cell antigen receptor. *Nature (Lond.)* 303:808.
26. Brenner, M. B., I. S. Trowbridge, and J. L. Strominger. 1985. Cross-linking of human T cell receptor proteins: Association between the T cell idiotype  $\beta$  subunit and the T3 glycoprotein heavy subunit. *Cell.* 40:183.
27. Borst, J., S. Alexander, J. Elder, and C. Terhorst. 1983. The T3 complex on human T lymphocytes involves four structurally distinct glycoproteins. *J. Biol. Chem.* 258:5135.
28. Kanellopoulos, J. M., N. M. Wigglesworth, M. Owen, and M. J. Crumpton. 1983. Biosynthesis and molecular nature of the T3 antigen of human T lymphocytes. *EMBO (Eur. Mol. Biol. Organ.) J.* 2:1807.
29. Hunig, T., and M. Bevan. 1982. Antigen recognition of cloned cytotoxic T lymphocytes follows rules predicted by the altered self hypothesis. *J. Exp. Med.* 155:111.
30. Hansburg, D., E. Heber-Katz, T. Fairwell, and E. Appella. 1983. Major histocompatibility complex-controlled, antigen-presenting cell-expressed specificity of T cell antigen recognition. *J. Exp. Med.* 158:25.
31. Marrack, P., R. Shimonkevitz, C. Hannum, K. Haskins, and J. Kappler. 1983. The major histocompatibility restricted antigen receptor on T cells. IV. An anti-idiotypic antibody predicts both antigen and I-specificity. *J. Exp. Med.* 158:1635.
32. Bigler, R. D., D. E. Fisher, C. Y. Wang, E. A. Rinnooy Kan, and H. G. Kunkel. 1983. Idiotypic-like molecules on cells of a human T cell leukemia. *J. Exp. Med.* 158:1000.
33. Bigler, R. D., D. N. Posnett, and N. Chiorazzi. 1985. Stimulation of a subset of normal resting T lymphocytes by a monoclonal antibody to a crossreactive determinant of the human T cell antigen receptor. *J. Exp. Med.* 161:1450.
34. Bushkin, Y., H. G. Kunkel, B. Pernis, and C. Y. Wang. 1985. Evidence for a second receptor-like molecule in a human T-cell leukemia. In: *Cell Biology of the Major Histocompatibility Complex*. B. Pernis and H. Vogel, editors. Academic Press, New York. 173-183.
35. Meuer, S. C., J. C. Hodgdon, R. E. Hussey, J. P. Protentis, S. F. Schlossman, and E. L. Reinherz. 1983. Antigen-like effects of monoclonal antibodies directed at receptors on human T cell clones. *J. Exp. Med.* 158:988.
36. Bushkin, Y., M. J. Chorney, E. Diamante, S. M. Fu, and C. Y. Wang. 1984. Biochemical characterization of the human T6 antigen: A comparison between T6 and murine TL. *Mol. Immunol.* 21:821.
37. Bushkin, Y., M. J. Chorney, E. Diamante, C. Lane, S. M. Fu, and C. Y. Wang. 1985. Biochemical characterization of a p43,12 complex: comparison to human and murine class I molecules. *Mol. Immunol.* 22:695.
38. Barnstable, C. J., W. F. Bodmer, G. Brown, G. Galfre, C. Milstein, A. F. Williams, and A. Ziegler. 1978. Production of monoclonal antibodies to group A erythrocytes,

- HLA and other human cell surface antigens—new tools for genetic analysis. *Cell*. 14:9.
39. Brodsky, F., W. F. Bodmer, and P. Parham. 1979. Characterization of a monoclonal anti- $\beta$ 2-microglobulin antibody and its use in the genetic and biochemical analysis of major histocompatibility antigens. *Eur. J. Immunol.* 9:536.
  40. Rinnooy Kan, E. A., E. Platzer, K. Welte, and C. Y. Wang. 1984. Modulation induction of the T3 antigen by OKT3 antibody is monocyte dependent. *J. Immunol.* 133:2979.
  41. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* 227:680.
  42. Johnson, D. A., J. W. Gautsch, J. R. Sportsman, and J. H. Elder. 1984. Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. *Gene Anal. Technol.* 1:3.
  43. Elder, J. H., F. Jensen, M. Bryant, and R. Lerner. 1977. Polymorphism of the major envelope glycoprotein (gp 70) of murine C-type viruses: virion associated and differentiation antigens encoded by a multi-gene family. *Nature (Lond.)* 267:23.
  44. Elder, J. H., and S. Alexander. 1982. Endo- $\beta$ -N-acetylglucosaminidase F: endoglycosidase from *Flavobacterium meningosepticum* that cleaves both high-mannose and complex glycoproteins. *Proc. Natl. Acad. Sci. USA.* 79:4540.
  45. Krangel, M. S., H. T. Orr, and J. L. Strominger. 1979. Assembly and maturation of HLA-A and HLA-B antigens in vivo. *Cell.* 18:979.
  46. Meuer, S. C., K. A. Fitzgerald, R. E. Hussey, J. C. Hodgdon, S. F. Schlossman, and E. L. Reinherz. 1983. Clonotypic structures involved in antigen-specific human T cell function. Relationship to the T3 molecular complex. *J. Exp. Med.* 157:705.
  47. Vitetta, E. S., J. W. Uhr, and E. A. Boyse. 1975. Association of a  $\beta$ 2-microglobulin-like subunit with H-2 and TL alloantigens on murine thymocytes. *J. Immunol.* 114:252.
  48. Yokoyama, K., E. Stoekert, L. J. Old, and S. G. Nathenson. 1981. Structural comparisons of TL antigens derived from normal and leukemia cells of TL<sup>+</sup> and TL<sup>-</sup> strains and relationship to genetically linked H-2 major histocompatibility complex products. *Proc. Natl. Acad. Sci. USA.* 78:7078.
  49. Ploegh, H. L., H. T. Orr, and J. L. Strominger. 1981. Major histocompatibility antigens: the human (HLA-A, -B, -C) and murine (H-2K, H-2D) class I molecules. *Cell.* 24:287.
  50. Rinnooy Kan, E. A., C. Y. Wang, L. C. Wang, and R. L. Evans. 1983. Noncovalently bonded subunits of 22 and 28 kD are rapidly internalized by T cells reacted with anti-Leu-4 antibody. *J. Immunol.* 131:536.
  51. Reinherz, E. L., S. C. Meuer, K. A. Fitzgerald, R. E. Hussey, J. C. Hodgdon, O. Acuto, and S. F. Schlossman. 1983. Comparison of T3-associated 49- and 43-kilodalton cell surface molecules on individual human T-cell clones: evidence for peptide variability in T-cell receptor structures. *Proc. Natl. Acad. Sci. USA.* 80:4104.
  52. Kappler, J., R. Kubo, K. Haskins, J. White, and P. Marrack. 1983. The mouse T cell receptor: comparison of MHC-restricted receptors on two T cell hybridomas. *Cell.* 34:727.