Proc. Natl. Acad. Sci. USA
Vol. 78, No. 10, pp. 6411–6415, October 1981
Immunology

Characterization of T-cell surface proteins bound by heterologous antisera to antigen-specific T-cell products

(T-lymphocyte/antigen receptor/membrane)

ROBERT E. CONE*†, ROBERT W. ROSENSTEIN*‡, JAMES H. MURRAY*, G. MICHAEL IVERSON*‡, WLODZIMIERZ PTAK§, AND RICHARD K. GERSHON*‡

Departments of *Pathology and †Surgery, Yale University School of Medicine; ‡Howard Hughes Medical Institute, Laboratory of Cellular Immunology, Yale University, New Haven, Connecticut 06510; and §Copernicus University, Kracow, Poland

Contributed by Richard K. Gershon, June 22, 1981

ABSTRACT Heterologous antisera specific for murine T-cell antigen-recognition molecules were prepared by immunization of rabbits with dinitrophenyl-specific murine T-cell suppressor factors that had been purified by hapten-affinity chromatography. The antisera (*i*) bind to antigen-specific T-cell products that differ in their antigen-recognizing specificity; (*ii*) absorb the specific suppressor activity in preparations containing suppressor factors; (*iii*) stain all $Lyt2^+$ T cells brightly in indirect immunofluorescence examination, stain some $Lyt1^+$ cells (with low intensity), and do not stain B cells; (*iv*) precipitate cell membrane proteins from T cells that bear striking structural resemblance to the antigen-specific molecules used for immunization. These results suggest that, like B cells, there is a commonality between antigen-specific effector molecules released by T cells and their membrane-associated receptors.

The specificity of the immune system stems from the presence of a multiplicity of lymphocytes having membrane-associated receptors that distinguish various antigens. As a result of the initial interaction between membrane receptors and antigen, the induced cells proliferate or differentiate or both and produce soluble effector molecules that have the same specificity as their membrane receptors. B-lymphocyte-derived effector molecules (immunoglobulins) resemble their membrane-bound counterparts in that they bear the same variable region, and in some cases constant region, antigenic determinants. Consequently, antisera to immunoglobulin isotypic and allotypic determinants have facilitated the detection and isolation of membrane immunoglobulins and provided the evidence that the membranous form of these immunoglobulins serves as B-cell antigen-recognition structures (for review, see refs. 1 and 2).

Like B cells, T lymphocytes possess cell surface receptors for antigen that appear to bear immunoglobulin variable region heavy chain gene products (2, 4) and produce biologically active molecules that also bear these products (5). However, the structural properties of T-cell antigen-recognition molecules are not well defined; one reason for this is that these molecules do not bear epitopes commonly associated with immunoglobulin constant regions (3, 4, 6, 7). Thus, a general lack of reagents that detect putative constant region markers on T-cell antigen-recognition structures has hampered the isolation of these molecules in large amounts for structural analysis. We have described the isolation and partial characterization of antigen-specific suppressor molecules (TSF) released *in vitro* by T cells obtained from mice rendered tolerant to the haptens trinitrophenyl and dinitrophenyl (TNP and DNP, respectively) (8). TSF, as isolated by hapten-affinity chromatography, is a 68,000-dalton polypeptide that bears neither detectable immunoglobulin constant region determinants nor antigenic determinants encoded by genes in the major histocompatibility complex.

In this report, we describe the production of heterologous antisera to TSF and the use of these reagents to detect and isolate T-cell surface molecules that share antigenic determinants with TSF. The results indicate that these antisera detect antigenic determinants shared by different soluble antigen-specific T-cell products and cell surface proteins borne by many (but not all) T lymphocytes. Moreover, the cell surface proteins bound by anti-TSF sera exhibit marked structural similarity to TSF.

MATERIALS AND METHODS

Animals. CBA/J, BALB/c, or C57BL/6 mice were obtained from The Jackson Laboratory, and maintained in the Department of Comparative Medicine, Yale University. New Zealand White rabbits were used for immunization.

Production of Rabbit Anti-TSF (RaTSF) Sera. TSF was prepared as described (8). The purified TSF used for immunization was eluted from an antigen affinity column and was a single 68,000-dalton polypeptide chain as judged by gel electrophoresis of ^{125}I-labeled TSF or staining with Coomassie blue (8).

Rabbits received an intramuscular injection of 100 μg of TSF emulsified in complete Fruend's adjuvant followed 2 weeks later by 100 μg subcutaneously in incomplete Freund's adjuvant. Subsequent boosts at 1- to 2-month intervals were by subcutaneous injection of 50–100 μg in phosphate-buffered saline (P_i/NaCl), pH 7.2, or, after a 4-month interval, by intravenous injection of 100 μg of TSF mixed with 1 mg of complexes of polyadenylate and polyuridylate (poly A:U, Boehringer).

Immunoassays. *Solid phase on microtiter plates.* TSF at 1 μg/ml (determined by Lowry protein analysis) was coated onto flexible plastic microtiter wells as described (8).

One hundred microliters of serial dilutions (1:2) of antisera to be tested for binding to TSF was incubated in TSF-coated microtiter wells for 18 hr at 4°C. The wells were washed and bound antibody was detected by the addition of ^{125}I-labeled goat anti-rabbit immune globulin as described (8).

Solid phase on Sepharose beads. Anti-TSF IgG was prepared by ammonium sulfate precipitation, and the IgG fraction was coupled to cyanogen bromide-activated Sepharose 4B (9) at a coupling ratio of 1 mg/ml of beads. ^{125}I-Labeled TSF was added to 200 μl of 50 mM Tris·HCl/10 mM EDTA/0.15 M NaCl, pH 8.0/0.05% Triton X-100. Anti-TSF-Sepharose beads were then

Abbreviations: DNP, dinitrophenyl; P_i/NaCl, phosphate-buffered saline; RaTSF, rabbit antisuppressor factor serum; SARIg, sheep anti-rabbit IgG serum; TNP, trinitrophenyl; TSF, antigen-specific T-cell-derived suppressor factor.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

added to ^{125}I-labeled TSF, and the mixture was incubated 1 hr at 4°C with occasional shaking. The beads were then washed with 0.05% Triton X-100/Tris and bound radioactivity was determined.

Indirect immunoprecipitation. ^{125}I-labeled TSF in 0.05% Triton X-100-Tris was mixed with 2–4 μl of anti-TSF and incubated for 1 hr at 4°C. After this incubation, a 10-fold volume of sheep anti-rabbit immunoglobulin serum (SARIg) was added, and precipitation was allowed to proceed overnight. Alternatively, a 10-fold volume of fixed 10% (vol/vol) *Staphylococcus aureus* Cowan strain I (Calbiochem, Pansorbin) was added, and the mixture was held 15 min at 4°C before washing.

Immunofluorescence Studies. Splenic T and B lymphocytes and Lyt subsets were prepared according to the method of Wysocki and Sato (10). For separation of T and B cells, 50 μg of affinity-purified goat anti-mouse immunoglobulin and 50 μg of normal goat immunoglobulin in 10 ml of 0.05 M Tris buffer, pH 9.5, were added to 100 × 15 mm Fisher polystyrene dishes. After 40 min at room temperature, soluble material was decanted off and the precipitates were washed four times with P_i/NaCl/1% calf serum. Spleen cells (2×10^7) suspended in 3 ml of P_i/NaCl were poured onto each dish, and the plates were incubated on a level surface at 4°C for 70 min. Nonadherent (T-cell-enriched) cells were harvested by decanting. The dishes were washed with P_i/NaCl/calf serum, and the adherent cells were obtained by incubating the plates with 5 ml of P_i/NaCl/calf serum at 37°C for 30 min. The entire surface of the plate was then flushed with a Pasteur pipette. More than 45% of the cells applied to the plate were recovered. The nonadherent population was 65% Thy-1 positive and <1% surface immunoglobulin positive. The adherent population was >90% surface immunoglobulin positive and <1.5% Thy-1 positive.

Lyt1$^+$,2$^-$ and Lyt2$^+$ splenic T cells were prepared in similar fashion by absorption of plate-purified splenic T cells to dishes coated with monoclonal rat anti-mouse Lyt2 antibody (a gift from J. Ledbetter, Stanford University). Lyt1$^+$,2$^-$ T cells were recovered as the nonadherent fraction of cells and Lyt2$^+$ cells were recovered from the adherent fraction.

Cell suspensions were stained for Lyt antigens by incubation at 1×10^6/ml with biotin-conjugated anti-Lyt antibody for 15 min. The cells were then washed through a layer of calf serum, suspended in a 2 μg/ml solution of fluorescein-labeled avidin and incubated at room temperature for 15 min. The cells were then centrifuged through a layer of calf serum and >100 cells were examined in wet mount on a fluorescence microscope. Cell staining was done in 0.02% NaN_3.

For staining with rabbit antisera, 50 μl of a 1:10 dilution of antiserum were added to the cells and the mixture was held on ice for 45 min. The cells were then washed, suspended in fluorescein-labeled SARIg and incubated at room temperature for 15 min. The cells were then washed and examined as described above.

Isolation of Cell Surface Proteins. CBA/J thymocytes or spleen cells prepared as described (11) and splenic T cells prepared by negative selection on goat anti-mouse immunoglobulin plates were radiolabeled by lactoperoxidase-catalyzed cell surface radioiodination (11). Lactoperoxidase (EC 1.11.1.7) was obtained from Sigma. In general, $2–5 \times 10^7$ cells were labeled with 2 mCi of ^{125}I or ^{131}I (1 Ci = 3.7×10^{10} becquerels; New England Nuclear). ^{125}I-Labeled surface proteins were obtained by lysis of labeled cells with 0.05% Triton X-100 in Tris/EDTA buffer as described (11).

Aliquots of dialyzed centrifuged lysates were mixed with RaTSF or normal rabbit serum for 1 to 2 hr at 4°C and immune complexes were isolated by precipitation with SARIg or absorption to *Staphylococcus* as described above. In some experiments, cell lysates were mixed with RaTSF–Sepharose or normal rabbit gamma globulin-Sepharose for 2 hr at 4°C and then washed.

NaDodSO$_4$/Polyacrylamide Gel Electrophoresis. Immunoprecipitated ^{125}I-labeled surface proteins were dissolved or eluted from Staphylococcus or RaTSF-Sepharose beads by boiling in electrophoresis sample buffer/2% 2-mercaptoethanol and resolved by NaDodSO$_4$/polyacrylamide gel electrophoresis as described (11, 12). ^{131}I-Labeled reduced and alkylated MOPC 104E (μ,λ) and ovalbumin were used as molecular weight markers.

RESULTS

Assay of Anti-TSF by Solid Phase Radioimmunoassay. Anti-TSF activity in the sera was routinely detected by either indirect immunoprecipitation of ^{125}I-labeled TSF or by binding of anti-TSF to TSF-coated microtiter wells. In general, 10–20 μl of anti-TSF could bind maximal amounts (40–70%) of ^{125}I-labeled TSF when assayed by indirect immunoprecipitation. For rapid assay, TSF-coated plates were more efficient. Binding of anti-TSF was detected by addition of ^{125}I-labeled affinity-purified sheep anti-rabbit immunoglobulin. As shown in Fig. 1, anti-TSF (R–11) exhibited specific binding to a dilution of 1:80 when tested against DNP-specific TSF. Similar results were obtained when RaTSF was tested against TNP- or oxazalone-specific TSF. Antisera specific for IgM, IgG, or bound bovine serum albumin did not bind significantly to TSF and conversely anti-TSF showed no binding activity to microtiter plates coated with bovine serum albumin, mouse serum albumin, or normal mouse IgG. When competition radioimmunoassays were performed using anti-TSF conjugated to Sepharose beads (Fig. 2), it was found that 5 μg of TSF completely inhibited binding of anti-TSF-Sepharose beads to ^{125}I-labeled TSF. Approximately 20% inhibition was obtained with 200 μl of normal mouse serum as compared with 7% inhibition with an equivalent amount of normal rabbit serum. Based on the titration curve, CBA/J normal mouse serum contained 0.5–2 μg of inhibitor per ml of non-immune serum. Absorption of culture supernates containing DNP-, TNP-, or oxazalone-specific TSF (8) to anti-TSF-Sepharose removed suppressor activity from the supernatants that could be recovered by elution of the beads with 0.2 M Na_2CO_3.

Binding of Anti-TSF to Lymphocytes. The results presented above indicated that anti-TSF was highly specific for TSF and did not bind to common serum proteins, including immuno-

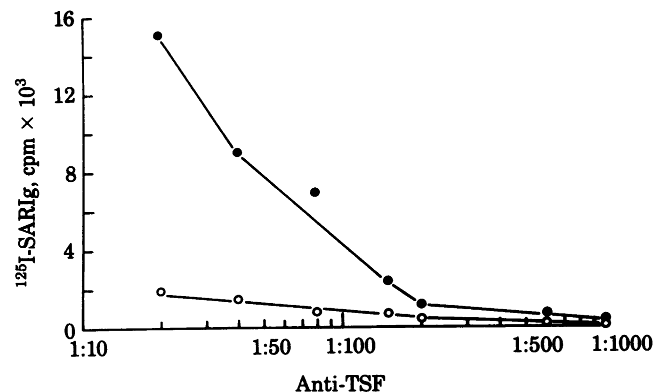

FIG. 1. Assay of anti-TSF on TSF-coated microtiter plates. Dilutions of RaTSF or normal rabbit serum were added to TSF-coated wells of microtiter plates. After incubation and washing, ^{125}I-labeled affinity-purified SARIg was added, and the plates were incubated further and washed. The wells were then punched out and assayed for radioactivity. ●, RaTSF; ○, normal rabbit serum.

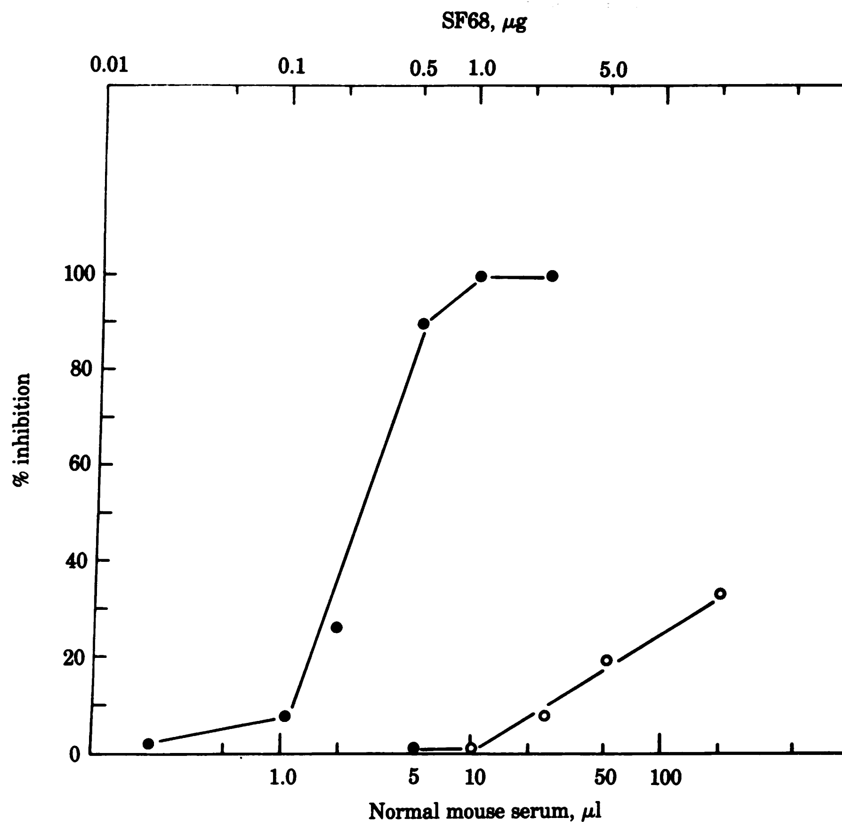

FIG. 2. Competition radioimmune assay with TSF. Twenty-five microliters of μl RaTSF-Sepharose beads were incubated with TSF or normal mouse serum and ^{125}I-labeled TSF was then added. After further incubation, the beads were washed and radioactivity was determined. Binding in the absence of TSF or normal mouse serum was 40%. •, RaTSF-Sepharose, TSF, and ^{125}I-labeled TSF; ○, TSF-Sepharose, normal mouse serum, and ^{125}I-labeled TSF.

globulins. Anti-TSF could detect, however, lymphocyte cell surface antigens as shown in Table 1. By indirect immunofluorescence examination, anti-TSF stained ≈80% of CBA/J thymocytes, although the staining was dull. Approximately 40–50% of spleen cells were stained by this reagent with bright speckled fluorescence. The selective binding of anti-TSF to cells was further demonstrated by the observation that 60–80% of thy-1$^+$ plate-purified splenic T cells were brightly stained while splenic B cells were not reactive with this serum. Moreover, nearly all Lyt2$^+$ cells were stained while only 50% of Lyt1$^+$,2$^-$ cells stained (and these with low intensity). Thus, anti-TSF appears to be specific for T cells and shows some selectivity for Lyt2$^+$ T cells.

To further test the cell specificity of anti-TSF and the relationship of cell surface determinants recognized by anti-TSF to those carried by TSF, absorption studies with cells were performed. Absorption of 10 μl of anti-TSF with 2×10^8 CBA/J or C57B1/6 thymocytes or spleen cells resulted in a 10-fold reduction in titer for binding to TSF in plate binding assays. To further quantitate the ability of cells to absorb anti-TSF, a dilution of anti-TSF was absorbed with $2–600 \times 10^5$ plate-purified splenic T or B cells. Nearly maximal absorption of activity was obtained with as few as 1×10^6 T cells whereas 6×10^7 B cells did not affect the ability of the antiserum to stain the cells.

Table 1. Indirect immunofluorescent staining of lymphocytes with RaTSF

	Cells stained, %					
Antiserum	Thymus	Spleen	Splenic B*	Splenic T†	Lyt1$^+$,2$^-$‡	Lyt1$^-$,2$^+$§
RaTSF	80 (weak)	36	1.7	55	52 (weak)	98
Anti-Thy-1	NT	48	2.8	66	NT	NT
Anti-κ	0	39	66	0	NT	NT

NT, not tried.
* Prepared by absorption and elution from goat anti-mouse immunoglobulin plates.
† Nonadherent cells from goat anti-mouse immunoglobulin plates.
‡ Nonadherent splenic T cells from anti-Lyt-2 plates.
§ Splenic T cells bound and eluted from anti-Lyt-2 plates.

Immunoprecipitation of Cell Surface Proteins with Anti-TSF. To identify cell surface proteins bound by anti-TSF, thymocytes, spleen cells, and plate-purified splenic T cells were radiolabeled by lactoperoxidase-catalyzed cell surface iodination and detergent-soluble labeled proteins were precipitated with anti-TSF serum or anti-TSF IgG-Sepharose beads. Approximately 1% or 2% of the radiolabeled proteins were bound by 20 μl of anti-TSF and 0.3–0.5% were bound by normal rabbit serum or rabbit IgG-Sepharose beads. Absorption of the antiserum with 2×10^8 spleen cells or thymocytes reduced the amount of cell surface proteins bound to that obtained when 1 μl or 6 μl, respectively, of unabsorbed antiserum was used. Moreover, preincubation of anti-TSF Sepharose beads with 5 μg of TSF reduced by 93% the amount of ^{125}I-labeled thymocyte or spleen cell membrane proteins bound by anti-TSF-Sepharose, whereas 25 μl of normal mouse serum inhibited binding by only 10–15%.

Membrane proteins bound by anti-TSF were resolved (after reduction and alkylation) by $NaDodSO_4$/polyacrylamide gel electrophoresis. As shown in Fig. 3, the profile of cell membrane proteins bound by anti-TSF varied with the manner in which the cell membrane proteins were isolated. Thymocyte or splenic T-cell membrane proteins isolated by indirect immunoprecipitation with anti-TSF and SARIg serum were resolved into three peaks with apparent M_r of 68,000, 45,000, and 25,000 comprising 16%, 53%, and 31% of the resolvable radioactivity. The electrophoresis patterns obtained from thymocyte, splenic T-cell, or (not shown) spleen cell membrane proteins were qualitatively similar. If cell surface proteins were isolated by precipitation with anti-TSF serum and staphylococcus, the

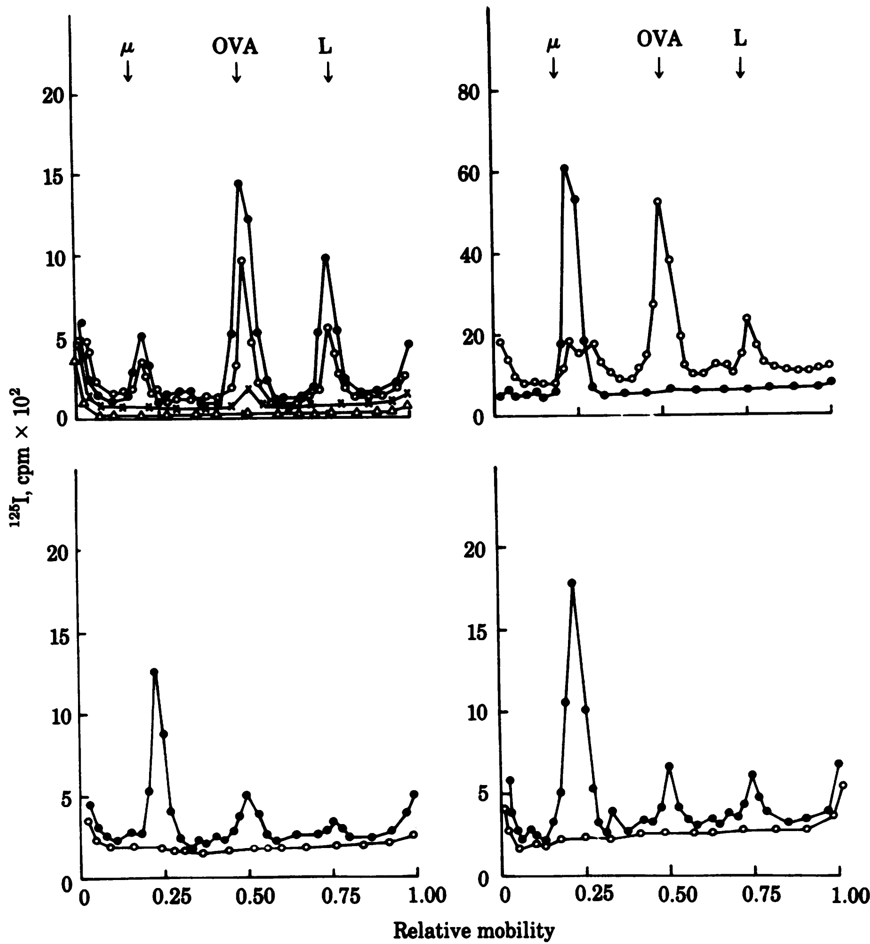

FIG. 3. NaDodSO$_4$/polyacrylamide gel electrophoresis analysis of ^{125}I-labeled cell surface proteins bound by RaTSF. Plate-purified CBA/J splenic T cells or thymocytes (2 × 10^7) were surface labeled with ^{125}I by lactoperoxidase-catalyzed radioiodination. The labeled cells were lysed with 0.05% Triton X-100 and detergent-soluble proteins were reduced, alkylated, and isolated. (*A*) Isolation by RaTSF serum and SARIg serum; •, splenic T cells and RaTSF, ○, thymocytes and RaTSF; x, splenic T cells and normal rabbit serum; △, thymocytes and normal rabbit serum. (*B*) ^{125}I-Labeled TSF (•) and ^{125}I-labeled TSF precipitated by RaTSF and SARIg (○). (*C*) Splenic T-cell membrane proteins bound by RaTSF and staphylococcus (•) or normal rabbit serum and staphylococcus (○). (*D*) Splenic T-cell membrane proteins bound by RaTSF-Sepharose (•) or normal rabbit Ig-Sepharose (○).

major peak of radioactivity (75% of the total) was associated with the 68,000-dalton polypeptide and the remainder with polypeptides of 45,000 and 25,000 daltons. Membrane proteins bound by anti-TSF-Sepharose beads were similar in their electrophoresis profiles to those bound by anti-TSF-staphylococcus. Immunoprecipitation of cell membrane proteins first with anti-TSF serum and staphylococcus or anti-TSF-Sepharose removed all proteins bound by anti-TSF serum and SARIg serum and, conversely, immunoprecipitation with anti-TSF serum and SARIg serum removed all proteins bound by anti-TSF and staphylococcus or anti-TSF-Sepharose. Thus, although the profile of cell membrane proteins varied with the different isolation systems, all three methods appear to bind the same polypeptides.

These results raised the possibility that the method of precipitation might alter the configuration of the proteins isolated. To test this hypothesis, we precipitated ^{125}I-labeled TSF according to the three methods. Immunoprecipitation of TSF by anti-TSF and staphylococcus or anti-TSF-Sepharose did not significantly alter the 68,000-dalton TSF polypeptide, although occasionally small peaks of 45,000 and 25,000 daltons were detected. In contrast, immunoprecipitation of ^{125}I-labeled TSF with anti-TSF serum and SARIg serum resulted in a loss of the 68,000-dalton polypeptide and the appearance of polypeptides with apparent M_r of 45,000 and 25,000. These results support our contention that the indirect immunoprecipitation system using anti-TSF and sheep anti-rabbit sera may result in proteolysis of cell membrane proteins bound by anti-TSF.

DISCUSSION

Our results demonstrate a commonality of epitopes between antigen-specific polypeptides released by T lymphocytes and cell surface proteins present on a majority of T lymphocytes that can be recognized by heterologous antisera raised against one group of soluble antigen-specific (anti-DNP) T-cell products. As these antisera bind soluble antigen-specific T-cell polypeptides specific for different antigens (8), the results suggest that some T cells, like B cells, release antigen binding proteins that share epitopes with each other and with cell surface molecules likely to be membrane antigen-recognition structures. In addition, competition radioimmune assays demonstrated that molecules sharing antigenic determinants with T-cell antigen-specific polypeptides occur in normal serum at low levels (1 or 2 μg/ml). It thus appears likely that some immunologically active T-cell products are present in the circulation and that these levels may increase on immunization, as is the case for B-cell-derived immunoglobulins.

The observation that anti-TSF binds neither to common serum proteins nor to B lymphocytes but does bind a majority of splenic T cells provides further evidence for the T-cell origin of TSF (8). However, even the staining of T cells by anti-TSF is selective. The preferential staining of Lyt2$^+$ vs. Lyt1$^+$,2$^-$ cells by anti-TSF could be due to differences in display, site density, or turnover (13) of Lyt1$^+$,2$^-$ receptors. Moreover, Lyt1$^+$,2$^-$ and Lyt2$^+$ cells may bear isotypically distinct receptors. Thus, anti-TSF may be specific for Lyt2$^+$ "isotypes" and crossreactive with Lyt1$^+$,2$^-$ isotypes.

T-cell membrane proteins bound by anti-TSF bear structural similarities to soluble TSF used for immunization. Both TSF and cell surface proteins bound by anti-TSF are 68,000-dalton polypeptides that can be converted to 45,000- and 25,000-dalton fragments during immunoprecipitation. Moreover, preliminary studies show that membrane proteins bound by anti-TSF can

occur, like TSF (8), as non-covalently linked dimers and as monomers.

The striking structural similarity between TSF and T-cell membrane proteins that share epitopes with TSF contrasts with the differences between B-cell membrane IgM and its soluble counterpart (pentameric IgM). Whether TSF is actually derived as a shed T-cell membrane product or is a distinct soluble analogue of T-cell membrane antigen-recognition structures remains to be investigated.

Taken together, our results on the molecular characteristics of T-cell membrane proteins bound by anti-TSF are consistent with those obtained by others (3, 14, 15) working with heterologous or anti-idiotypic antibodies that bind antigen-specific T cell-derived proteins. Moreover, it has been found that heterologous antisera prepared in rabbits (H. Binz and H. Frischknect, personal communication) against a rat T-cell-derived allospecific proteins and in sheep (J. Mattingly and C. Janeway, Jr., personal communication) against a murine T-cell-derived sheep erythrocyte-specific suppressor factor crossreact with murine DNP-specific TSF and T-cell membrane proteins bound by RaTSF. Conversely, RaTSF crossreacts with rat allospecific T-cell proteins (16). These results further support our contention that RaTSF has specificity for epitopes shared by many T-cell antigen-recognition structures and that these structures may show a high degree of evolutionary conservation. Although T-cell membrane proteins bound by RaTSF do not bear immunoglobulin isotypic determinants, they exhibit similar structural properties to T-cell surface proteins bound by rabbit antisera to immunoglobulin variable region framework determinants (17) and preliminary evidence suggests that these can be bound by antivariable region reagents. Thus, it is likely that T-cell surface receptors for antigen, like their B-cell counterparts, bear variable region and constant region domains that are shared with soluble circulating structures that perform immunobiological functions.

We thank Ms. Pallas Lo and Robyn Gershon for outstanding technical assistance and Ms. M. Ainley for preparation of the manuscript. This work was supported in part by Grants CA 14216 and CA 23887 from the National Cancer Institute, AI 16942 from the National Institutes of Health, and PCM 79-21779 from the National Science Foundation. J.H.M. was a postdoctoral fellow supported by National Institutes of Health Training Grant AI 07019.

1. Warner, N. L. (1974) *Adv. Immunol.* **19,** 67–216.
2. Eichmann, K. (1978) *Adv. Immunol.* **26,** 211–254.
3. Binz, H. & Wigzell, H. (1976) *Scand. J. Immunol.* **5,** 559–571.
4. Binz, H. & Wigzell, H. (1977) *Contemp. Top. Immunobiol.* **7,** 133–177.
5. Tada, T. & Okumura, K. (1979) *Adv. Immunol.* **28,** 1–80.
6. Cone, R. E. (1977) *Prog. Immunol.* **3,** 47–56.
7. Cone, R. E. & Rosenstein, R. (1980) *Mol. Immunol.* **17,** 913–924.
8. Rosenstein, R. W., Murray, J. H., Cone, R. E., Iverson, G. M., Ptak, W. & Gershon, R. K. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5821–5825.
9. Axen, R., Porath, J. & Ernbach, S. (1967) *Nature (London)* **214,** 1302–1304.
10. Wysocki, L. J. & Sato, V. C. (1978) *Proc. Natl. Acad. Sci. USA* **73,** 2476–2480.
11. Cone, R. E. & Brown, W. (1976) *Immunochemistry* **13,** 571–579.
12. Emerson, S. G., Reilly, P. & Cone, R. E. (1979) *J. Immunogenet.* **6,** 87–97.
13. O'Connor, S., Eardley, D., Shen, F-W, Gershon, R. K. & Cone, R. E. (1980) *Mol. Immunol.* **17,** 913–924.
14. Rubin, B., Hentel-Wulff, B. & Kimura, A. K. (1979) *J. Supramol. Struct., Suppl.* **3,** p. 256 (abstr.).
15. Kontiainen, S. & Feldmann, M. (1979) *Thymus* **1,** 59–79.
16. Cone, R. E., Murray, J., Rosenstein, R. W., Ptak, W., Iverson, G. M. & Gershon, R. K. (1981) in *Immunoglobulin Idiotypes and their Expression,* ICN-UCLA Symposium (Academic, New York), in press.
17. Cone, R. E. & Rosenstein, R. W. (1980) *Mol. Immunol.* **17,** 913–924.