ANTIGEN-SPECIFIC T LYMPHOCYTE CLONES

III. Papain Splits Purified T Suppressor Molecules into Two Functional Domains

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T cell clones can synthesize homogeneous proteins that mediate T cell recognition and function (1-6). We have defined a clone of Ly-2+ T-suppressor (Ts) cells that expresses cell-bound receptors for antigen and secretes a 70,000-mol wt glycoprotein that specifically binds to antigen and mediates antigen-specific suppressor activity. It is likely that the secreted protein represents a modified form of the cell-bound receptor, as is the case for B cells (5, 6).

The structural basis of antigen-specific suppression by this purified glycoprotein has not been established. Digestion of immunoglobulins (Ig) with proteolytic enzymes has provided important insights into the structural basis of antibody activity (7-10). We have used this approach to study the functional organization of the 70,000-mol wt suppressor protein. Papain splits the 70,000-mol wt molecule into two peptides of 45,000 and 24,000 mol wt. We have isolated these fragments from the purified 70,000-mol wt parent molecule and defined their functional, biochemical, and antigenic properties.

Materials and Methods

Biosynthetic Labeling and Purification of Specific Ts Molecules. Biosynthetic labeling of the 70,000 mol wt protein and purification of this protein to homogeneity from supernatant material has been described in detail (5, 6).

Enzymatic Digestion and Isolation of the Enzymatic Fragments. Proteolytic digestion of the purified 70,000-mol wt protein was carried out as follows: 10,000 cpm of the purified 70,000-mol wt protein was incubated with papain (10 μg/ml) in 1 ml phosphate-buffered saline (PBS) containing 2.5% glycerol for 1-2 h at 37°C. One aliquot of the reaction mixture was removed at different intervals for analysis in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). The enzymatic fragments formed after digestion were separated by Sephacryl S-200 column chromatography (80 × 1 cm) in the same buffer.

Binding of (35S)-labeled Proteins to Erythrocytes or Erythrocyte Glycopeptides. Sheep erythrocytes (SRBC) or burro erythrocytes (BRBC) (10^7 cells/ml) were incubated with 1-3 ml of different internally labeled protein preparations (10^5 cpm) for 2 or 24 h at 4°C. The erythrocytes (RBC) were pelleted by centrifugation (1,000 g), washed three times with PBS, and dissolved in Laemmli sample buffer (11) for subsequent analysis in SDS-PAGE or scintillation counting. Ethanol-extracted glycopeptides from SRBC were conjugated to Sepharose 4B with cyanogen bromide.
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bromide (2 mg protein/ml beads), as described previously (5). Cell supernatant material was mixed with the immunoadsorbent beads (0.1 ml beads/ml supernatant) in 5-ml syringe columns before incubation at 4°C, with occasional shaking. The columns were then extensively washed with PBS, followed by elution with Sorensen's glycine-HCl (pH 2.4) buffer and immediate neutralization with excess Tris-HCl (2 M, pH 7.2).

Biological Assay for Suppressive Activity. Inhibition of anti-RBC helper function was tested as described previously (5, 6). Briefly, 10^6 Ly-1 helper cells from SRBC- or BRBC-immune donors were incubated with different proteins for 6-72 h. Cells were then washed with PBS and tested for specific RBC helper activity after addition to cultures containing 10^6 B cells and the homologous erythrocyte. In all cases, anti-RBC plaque-forming cells (PFC) and total (“reversed”) PFC were determined from triplicate cultures, as previously described (5, 12).

Antiserum. Antiserum with specificity for framework regions of V_H chains of Ig were prepared in rabbits after immunization with the myeloma protein MOPC-315, as described previously (13). The MOPC-315 affinity-purified Ig fraction of this antiserum reacted with myeloma proteins MOPC-315, MOPC-460, and, to a lesser extent, T15 and S107. This was because of binding to the heavy (H) chain fragments as judged by direct binding to purified MOPC-315 H-chains and lack of reactivity to constant (C) and to light (L) chains. It should be noted, however, that the reagent does not precisely correspond to that reported by Ben-Neriah et al. (13) since only a restricted number of myeloma proteins were bound by this antibody, in contrast to the considerably wider reactivity of the reagent described by Ben-Neriah et al. (15).

PAGE. Electrophoresis was carried out in 0.7-mM-thick slab gels using a modification of the method of Laemmli, as described previously (11). Fluorographic treatment has routinely been used to enhance sensitivity and to reduce exposure time for the radioautographs (14).

Extraction of Proteins from Polyacrylamide Gels. After resolution of proteins in SDS-PAGE, these proteins were extracted from the gels in the presence of SDS as described (14). The gel was cut in horizontal bands corresponding to the positions of test and control proteins; each fraction was ground up and placed in a small tube. Proteins present in each slice were extracted after incubation with 2 ml 2% SDS for 2 h at room temperature, followed by incubation at 4°C overnight, separated from SDS and gel debris by passage through Sephadex G-25 columns, and concentrated by vacuum dialysis.

Solid-Phase Radioimmunoassay. 0.1 ml of protein solutions was incubated at room temperature for 1 h in polyvinyl microtiter plates. The plates were washed 10 times with PBS; 0.2 ml of 2% gammaglobulin-free horse serum in PBS was then added to quench and decrease nonspecific binding. 0.1 ml of antiserum was applied and incubated for 1 h at room temperature. After washing the plates 10 times with PBS, 40,000 cpm of ^125I-goat anti-rabbit F(ab')_2 was added for an overnight incubation at 4°C. The plates were washed 10 times with tap water before determination of bound radioactivity.

Results

Evidence for Antigen-induced Cleavage of the 70,000-mol wt Protein. The Ts clone CI.Ly23/4 secretes a 70,000-mol wt glycoprotein that binds specifically to glycophorin from SRBC and specifically suppresses the primary antibody response to SRBC (5, 6). Metabolically labeled proteins (50,000 cpm) from the supernatant of this clone were incubated for 2 h with SRBC or BRBC. After the RBC were washed three times, 1 aliquot of the RBC pellet was dissolved in Laemmli buffer. 5,200 cpm of protein bound to SRBC and 810 cpm to BRBC, and analysis in SDS-PAGE showed that 70-80% of the radioactivity bound to SRBC had an apparent mol wt of 70,000 (Fig. 1A) and no discrete protein bound to BRBC (5; and data not shown). To determine whether a more prolonged interaction with antisera might result in breakdown of the 70,000-mol wt binding protein, the second aliquot of the washed RBC pellet was incubated an additional 22 h at 4°C. Analysis of the pelleted proteins in SDS-PAGE showed a loss of the 70,000-mol wt band and a concomitant appearance of a sharp peak at 45,000 mol wt and a broader one at ~24,000-mol wt (Fig. 1B). The sum of
Fig. 1. After a 2-h incubation of internally labeled peptides from the Ts clone with SRBC, the erythrocytes were pelleted, washed three times, and an aliquot was analyzed in SDS-PAGE. (See Materials and Methods for precise conditions of incubation.) This short incubation was intended to detect peptides that bind to erythrocytes using conditions that minimize degradation. Virtually all of the internally labeled peptides bound to SRBC resolved at 70,000 mol wt (A). To determine whether additional interaction between SRBC-binding peptides and SRBC might result in degradation, a second aliquot of the washed erythrocyte pellet was incubated an additional 22 h. Analysis of SRBC-bound material after this period shows that degradation of the 70,000-mol wt material has occurred, resulting in the appearance of two smaller peptides of 45,000 and 24,000 mol wt. This result does not indicate retention of antigen-binding activity by either of these 70,000-mol wt breakdown products. To resolve this, we incubated Cly23/4 supernatant 24 h alone or with SRBC. After a 24-h incubation in the absence of RBC, the supernatant was then incubated for 2 h with SRBC. The SRBC were washed three times, and the pellet was dissolved in buffer. Virtually all of the bound counts per minute (>85%) had apparent 70,000 mol wt (C). This experiment showed that 24-h incubation of the 70,000-mol wt protein in the absence of antigen did not result in binding by smaller molecular weight internally labeled peptides. We therefore attempted to induce degradation during this period by incubating the internally labeled supernatant material with antigen (SRBC) for 24 h. At the end of this period, the RBC were pelleted, washed three times, and the pellet was dissolved in Laemmli buffer. Analysis of these internally labeled proteins bound to SRBC showed a 50% reduction in 70,000-mol wt peptides and the appearance of a 24,000- but not a 45,000-mol wt fraction binding to SRBC (D). K, 1,000 mol wt.

The counts per minute in these two peaks estimated by densitometry was approximately equal to the counts per minute present in the 70,000 mol wt protein bound to RBC before the additional 22-h incubation (Fig. 1A), suggesting a relationship between the 70,000-mol wt protein and the two smaller peptide fractions.

Although incubation of SRBC-bound 70,000-mol wt material resulted in appearance of two smaller peptides that might represent breakdown products, these data did not indicate whether these smaller peptides retained antigen-binding activity. To address this question, internally labeled cell supernatant material was incubated for 22 h at 4°C in the absence of antigen. The supernatant was then incubated for 2 h
with SRBC. After washing the SRBC three times, the pellet was dissolved in Laemmli buffer. Virtually all of the bound counts per minute (>85%) had apparent mol wt of 70,000 (Fig. 1 C). This experiment showed that 24-h incubation of the 70,000-mol wt protein in the absence of antigen did not result in binding by smaller molecular weight internally labeled peptides. We therefore attempted to induce degradation during this period by incubating the internally labeled supernatant material with antigen (SRBC) for 24 h. At the end of this period, the RBC were pelleted, washed three times, and the pellet was dissolved in Laemmli buffer. Analysis of these internally labeled proteins bound to SRBC showed a 50% reduction in 70,000-mol wt peptides and the appearance of a 24,000- but not a 45,000-mol wt fraction binding to SRBC (Fig. 1 D). The above findings indicate that antigen induces cleavage of the 70,000-mol wt peptide into two major subunits and suggest that the 24,000- but not the 45,000-mol wt subunit binds to antigen.

It is unlikely that proteases from SRBC or contaminating leukocytes (which we are unable to detect in our SRBC suspensions) cause this degradation because virtually identical results are obtained after elution of internally labeled peptides from columns coated with SRBC glycopeptides (obtained after elution from SDS-containing polyacrylamide gels [see Materials and Methods]). Immediate elution (Fig. 2) reveals that almost all of the bound peptides have a 70,000 mol wt according to Sephacryl chromatography; elution after 4 h of incubation on the columns (at 4°C) (Fig. 2 B) reveals that a substantial fraction of the bound material resolves at ~45,000 and 23,000 mol wt. This confirms the results of Fig. 1 A and B using antigen that is highly unlikely to contain active proteases.

Suppressive Activity of Cl.Ly23/4 Peptides after Sephacryl Fractionation. We have previously reported that freezing and thawing of CI.Ly23/4 supernatant in the absence of protease inhibitors results in a small amount of biologic activity in fractions corresponding to approximate mol wt of 40,000 to 50,000 and 20,000 to 25,000 after fractionation on Sephacryl S-200 columns (6). 1 aliquot of CI.Ly23/4 supernatant containing internally labeled peptides was frozen and thawed before application to S-200 columns. The majority of internally labeled proteins (75–85%) had a mol wt of

![Fig. 2. Sephacryl S-200 column chromatography of SRBC glycopeptide-binding proteins from Cl.Ly23/4 supernates. Internally labeled Cl.Ly23/4 supernatant material was eluted immediately (C) or after 6 h (O) from columns by low pH buffer and fractionated by Sephacryl S-200 chromatography on 100- × 2-cm columns equilibrated with PBS containing 2% glycerol, 1 mM, phenylmethylsulfonylfluoride, and 100 mM KCl. K, 1,000 mol wt.](image-url)
20,000–100,000. Approximately 15 fractions were tested for antigen-binding activity and suppressive activity. Virtually all specific antigen-binding activity was present in fractions corresponding to ~70,000 and 25,000 mol wt (Fig. 3A). Specific suppressive activity (as judged by the ability of the supernatant to inhibit T-helper (Th) activity of Ly-1 cells after a 6-h incubation) was confined to the 70,000-mol wt fractions which suppressed anti-SRBC Th cells but not anti-horse RBC (HRBC) Th activity. However, after prolonged incubation (72 h), fractions from 70,000–90,000 mol wt as well as fractions of ~45,000 mol wt inhibited anti-SRBC Th activity response (Fig. 3). No suppressive activity was observed in the 25,000-mol wt fractions.

The above information, taken together, indicates that 45,000-mol wt polypeptides can inhibit Th activity nonspecifically and may represent breakdown product(s) of the 70,000-mol wt antigen-specific suppressive molecule. Although this breakdown can be accelerated in the presence of antigen (Fig. 1), it might be expected that long-term incubation of Ly-1 T cells and 70,000-mol wt protein would result in breakdown of the 70,000-mol wt material and the appearance of nonspecific activity in the 70,000-mol wt fraction. This was tested. Ly-1 cells were preincubated with different fractions of internally labeled peptides and $10^8$ SRBC or HRBC for 72 h (Fig. 3). In contrast to a 6-h incubation (where the 70,000-mol wt fraction suppressed specifically), incubation of 70,000-mol wt peptides for 72 h with Ly-1 cells immune to SRBC or HRBC resulted in suppression of Th activity to either erythrocyte.

Suppressive Activity of Antigen-binding Proteins Extracted from SDS Gels. After affinity chromatography using columns coated with SRBC glycopeptides, internally labeled peptides corresponding to 70,000, 45,000, and 24,000 mol wt are detected in SDS-PAGE (see above; and M. Fresno, unpublished results) and by Sephacryl S-200 chromatography (5). Occasionally, a 33,000-mol wt band is also detected. The former peptides were extracted from polyacrylamide gel slices, along with a control slice lacking detectable internally labeled proteins in the region corresponding to 90,000 mol wt. After elution of SDS, the 70,000-mol wt protein retained specific suppressive activity after extraction from the gel (Table I). Peptides of 45,000 mol wt also mediated suppression; however, this activity was not specific for SRBC. Fractions having apparent 24,000 mol wt and a control gel slice of 90,000 mol wt were not suppressive. This directly confirms the biologic activity of these polypeptides after chromatographic separation. Moreover, because identical results were obtained using fractions obtained after Sephacryl chromatography (Fig. 3), potential sensitivity of these different peptides to SDS cannot account for the above results.

Proteolytic Degradation of the Purified 70,000-mol wt Protein. Unequivocal definition of the 45,000-mol wt and 24,000-mol wt peptides was obtained by digestion of biosynthetically labeled 70,000-mol wt protein after purification of the protein to >95% homogeneity, as described previously (6) (Fig. 4). Incubation of the protein with several different proteases, including pepsin and trypsin, resulted in many small heterogeneous peptides. Digestion with papain reproducibly yielded two distinct peptides: incubation with 1 or 10 µg/ml of papain for 5–120 min at 37°C yielded internally labeled protein that resolved in SDS-PAGE into two fractions of 45,000 and 24,000 mol wt (Fig. 4). These two smaller peptides were present in the same ratio after digestion for 5–60 min in two different experiments using both 10 and 20 µg/ml of papain. Estimation of the radioactivity contained in each band after papain incubation for 60 min, followed by electrophoresis in polyacrylamide gels, indicated
Fig. 3. Sephacryl S-200 chromatography of Cl.Ly23/4. 4-h [35S]methionine-pulsed supernates from Cl.Ly23/4 cultures in serum-free media were fractionated in Sephacryl S-200 columns (Materials and Methods). The fractions were tested for: (A) binding to 10⁷ SRBC (○) or 10⁷ BRBC (□); (B) effects on Th activity of SRBC-immune Ly-1 cells. 10⁸ Ly-1 cells were preincubated with different fractions (final dilution: 1:1,000) and 10⁶ SRBC for 6 h (○) or 72 h (■). Ly-1 cells were washed twice with PBS-2% FCS before mixing with 10⁷ nonimmune B cells and 10⁶ SRBC. Anti-SRBC PFC were enumerated 4 d later. PFC responses induced by Ly-1 cells that had been preincubated for 24 h or 72 h with Sephacryl buffer were 1,120 ± 360 and 980 ± 60, respectively; (C) effect on Th activity of Ly-1 cells after preincubation for 72 h of supernatant fractions with either SRBC (△) or HRBC (▲). Ly-1 cells were then tested for anti-SRBC or anti-HRBC Th activity. OVA, ovalbumin; STI, soybean trypsin inhibitor; LYS, lysozyme; K, 1,000 mol wt.

Biological Activities of the Enzymatic Fragments. The enzymatic fragments obtained after papain digestion were separated using Sephacryl S-200 chromatography. Two major peaks of counts per minute in the region of the 45,000- and 24,000-mol wt markers were detected, confirming the results of the SDS-PAGE analysis. In addition, a small amount of counts per minute activity was detectable in the 70,000-mol wt region, probably representing undigested protein (Fig. 5A). Column fractions corresponding to these three peaks, together with fractions having apparent mol wt of recovery of ~75% of the counts per minute contained in the undigested 70,000-mol wt parent molecule.
# Table I

Suppressive Effects of Sheep Glycophorin-binding Proteins

| Preincubation for 24 h | PFC/culture | Percentage of control PFC response after preincubation with mol wt |
|-----------------------|-------------|---------------------------------------------------------------|
|                       |             | 90,000 | 70,000 | 45,000 | 24,000 |
| 1. Ly-1 cells + SRBC  | Anti-SRBC   | 88     | 8      | 36     | 90     |
|                       | Total       | 118    | 108    | 54     | 97     |
| 2. Ly-1 cells         | Anti-SRBC   | 88     | 88     | 33     | 108    |
|                       | Total       | 88     | 92     | 63     | 91     |
| 3. B cells            | Anti-SRBC   | 109    | 109    | 117    | 109    |
|                       | Total       | 117    | 119    | 78     | 108    |
| 4. B cells + SRBC     | Anti-SRBC   | 103    | 98     | 98     | 118    |
|                       | Total       | 123    | 123    | 79     | 99     |
| 5. Ly-1 + B cells + SRBC | Anti-SRBC | 90     | 10     | 52     | 81     |
|                       | Total       | 108    | 132    | 62     | 99     |

33,000 and 15,000 ("control" fractions), were tested for biologic activity. More than 95% of the protein present in the 70,000-mol wt fraction bound to SRBC and <3% bound to BRBC. 70% of the counts per minute in the 24,000-mol wt fraction also bound to SRBC, and 4% bound to BRBC, confirming the results of antigen-incubation experiments (Fig. 1 D). Although the 15,000-mol wt fraction also contained significant amounts of specific binding activity, the small number of counts per minute in this fraction and its poor resolution on Sephacryl S-200 suggested that this probably represented contamination by material trailing the 24,000-mol wt protein (Fig. 5 A). Neither the 45,000- nor the 33,000-mol wt fractions displayed binding activity. Tests for suppressive activity showed that only the 70,000- and 45,000-mol wt fractions suppressed (Fig. 5 C). Suppression by the 70,000-mol wt protein was greater than suppression exerted by the 45,000-mol wt material, although the former contained three- to fivefold less internally labeled protein than the latter. In addition, suppression by the 70,000- but not the 45,000-mol wt peptide was specific since the former inhibited Th activity to SRBC but not BRBC, whereas the latter inhibited the response to both erythrocytes.

It is unlikely that papain contributed to the biologic activities of the digested proteins because (a) >95% of the counts per minute of trace-labeled papain eluted from Sephacryl S-200 columns ~30,000 mol wt (fractions 75, 76) and (b) because the biologic activities of the 70,000-, 45,000-, and 24,000-mol wt internally labeled peptides obtained after Sephacryl chromatography of frozen-thawed CLy23/4 supernatant in the absence of papain (Fig. 5) are virtually identical to the properties of the peptides obtained after papain digestion of the purified 70,000-mol wt molecule.
We also tested the polypeptides for suppressive activity at three different concentrations (Table II). At all concentrations, the 70,000-mol wt protein was suppressive. The 45,000-mol wt peptide also exerted suppression, albeit less efficiently. The 24,000-mol wt peptide did not exert significant suppressive activity even at the highest concentration tested.

Parallel analysis of the purified 70,000-mol wt protein after incubation for 1 h (or 24 h) with buffer (in the absence of papain) showed that the protein was stable at 37°C and localized as a single sharp peak in Sephacryl S-200 at apparent mol wt of 70,000 (Fig. 5 B). More than 95% of the protein in this fraction bound specifically to SRBC and the protein inhibited anti-SRBC but not anti-BRBC helper function (Fig. 5 B and D).

To define antigenic determinants present on peptides secreted by Cl.Ly23/4, cell fractions of internally labeled protein separated by Sephacryl S-200 columns were
incubated with antisera that recognize determinants or V_H chains of some myeloma proteins (see Materials and Methods) for 1 h before development with 125I-goat anti-rabbit F(ab\') This antiserum strongly reacted with the purified 70,000-mol wt protein and the 23,000-mol wt enzymatic fragment either by radioimmunoassay (Fig. 5 E
and F) or immunoprecipitation (data not shown) but did not bind to the 45,000-mol wt fragment (Fig. 5 E). This is a provocative finding. Although genetic interpretations of this type of cross-reaction should be extremely guarded (16), it does provide a useful serologic means of distinguishing between the two subunits of the 70,000-mol wt peptide.

Discussion

With a method that allows production of large numbers of continuously propagatable cloned cells, we have defined both Th and Ts cell clones (3). CI.Ly23/4 is an SRBC-specific T suppressor clone that expressed surface receptors for the glycoporphin expressed by SRBC (5). Analysis of this T cell clone has suggested that these cells share several major characteristics with antibody-forming B cells. Ts cells and B cells display similar numbers of surface receptors that bind to antigen in the absence of major histocompatibility complex products. Both respond to signals from inducer T cells by secretion of antigen-binding proteins.

The Ly-2+ T cell clone secretes 70,000-mol wt proteins that bind to antigen and mediate suppression. Picogram amounts of purified antigen-binding proteins specifically inhibit production of antibody in response to the antigen (6). In addition to the 70,000-mol wt protein, 45,000- and 24,000-mol wt proteins have been routinely observed under conditions where proteolysis was not carefully controlled (Fig. 3 and reference 5). Fractionation of biosynthetically labeled supernatant proteins by size in Sephacyr S-200 demonstrated small amounts of 24,000-mol wt peptides that bound specifically to SRBC but did not suppress, as well as 45,000-mol wt peptides without binding activity but capable of suppressing PFC responses to all RBC tested (albeit less efficiently than the 70,000-mol wt fraction [Table II]). These studies also show that interaction of the 70,000-mol wt protein with antigen markedly increases its susceptibility to degradation, resulting in breakdown into these two major peptides: 45,000 and 24,000 mol wt according to mobility in SDS-PAGE (Fig. 1).

Although SDS-PAGE denatures proteins, some biologically active proteins retain substantial activity after recovery from SDS-containing gels (14). We show that the 70,000-mol wt protein retains specific suppressive activity after extraction from these gels, directly confirming results of separation of antigen-specific suppressive peptides according to size. In addition, the 45,000-mol wt peptide obtained from the gel suppressed nonspecifically and to a lesser extent than the intact 70,000 mol wt molecule; the 24,000-mol wt band did not suppress but specifically bound to antigen (Table I).

Degradation of Ig with proteolytic enzymes has provided important insights into the structural basis of this molecule's biological activity and specificity for antigen (7–10). We used this approach to study the functional organization of the 70,000-mol wt suppressor protein. We found that although the 70,000-mol wt protein is sensitive to digestion by several different proteases, including pepsin and trypsin, papain yielded the most reproducible cleavage: this enzyme split and purified the 70,000-mol wt antigen-binding molecule into two peptide subunits of 45,000 and 24,000 mol wt (Fig. 2). These subunits were relatively resistant to further degradation and represented 70–85% of the digested product after 5–120 min of digestion. Because this restricted cleavage of the 70,000-mol wt protein was a reproducible characteristic of the protein, we defined the biologic activity of the two cleavage products. The 45,000-mol wt
subunit retained suppressive activity, although activity was 5–50-fold less potent than the intact 70,000-mol wt peptide; the 24,000-mol wt peptide bound specifically to SRBC but did not suppress (Fig. 5, Table II). Thus, the two breakdown products obtained after papain digestion displayed the same size and biologic activities as the 45,000- and 24,000-mol wt peptides obtained after incubation of 70,000-mol wt material with antigen. The two peptides were also distinguished serologically. An antiserum made against myeloma proteins MOPC-315 that probably recognizes some framework regions in \( V_H \) sequences (see Materials and Methods) reacted with the 70,000-mol wt parent molecule and the 24,000-mol wt peptide. A rabbit serum prepared against trinitrophenyl-specific T cell suppressor factor, which reacts with determinants on several T-suppressor factors specific for different antigens (15), reacted with the 70,000-mol wt molecule and the 45,000- but not the 24,000-mol wt subunit. The biologic and serologic properties of the 70,000-mol wt molecule and the 45,000- and 24,000-mol wt peptides are summarized in Table III.

The combined activities of the separate 45,000- and 24,000-mol wt peptides after papain digestion of the 70,000 mol wt protein accounted for the biologic activity of the parent molecule (summarized in Table III), and the peptides probably represent two distinct domains of the Ts protein. However, it is formally possible that the 24,000-mol wt peptide is a breakdown product of the 45,000-mol wt peptide. This is unlikely, since the ratio between the 45,000- and 23,000-mol wt peptides remains approximately equal and constant throughout the entire period of digestion (5–60 min). Moreover, if the peptides resulted from sequential cleavage, the “first” enzymatic cut of the 70,000-mol wt protein must result in a 45,000-mol wt product that has lost both antigen-binding sites and \( V_H \) determinants, whereas a “second” cleavage of the 45,000-mol wt protein yields a 23,000-mol wt peptide which reexpresses both sites. Nonetheless, definitive evidence that the 45,000- and 23,000-mol wt peptides represent independent regions of the 70,000-mol wt parent molecule requires amino acid sequencing or peptide mapping of the three molecules.

Papain digestion of Ig chains produces two fragments, Fc and F(ab). The F(ab) monomer (heavy \([H] \) chain) has an average 22,000 mol wt, carries the antigen-binding site, and contains sequences encoded by \( V_H \) genes. The Fc fragment has a mean mol wt of 50,000, carries the biologic activity of different classes of Ig, and is encoded by \( C \) genes. Each \( C \) gene product displays characteristic “isotypic” determinants and can be serologically defined by antibodies. Ts molecules purified from cloned T cells also appear to consist of two functionally distinct domains formed after cleavage by papain: a variable (V) region (23,000 mol wt) that binds specifically to antigen but lacks suppressive activity and a second constant (C) region (45,000 mol wt) that does

|                | mol wt | Isoelectric point | Antigen binding | Suppression | Serologic reaction |
|----------------|--------|------------------|-----------------|-------------|------------------|
|                |        |                  |                 | Antigen-specific | Nonspecific     | Anti-Ts factor | Anti-"V_H" |
| Purified Ts    | 70K    | 5.0              | ++              | ++          | +                | +              |
| Peptide A      | 45K    | 5.6              | -               | -           | +                | +              |
| Peptide B      | 24K    | ?                | +               | -           | -                | -              |
not bind antigen but suppresses antibody responses to a variety of antigens. The 45,000-mol wt portion of this molecule appears to share serologic determinants with partially purified Ts proteins that are likely to be specific for other antigens (15). Since these determinants are not detected on proteins synthesized by cloned inducer T cells, they may represent isotypic determinants on T cell molecules that suppress immune responses.

We have shown previously that picogram amounts of the purified monoclonal 70,000-mol wt protein suppresses the entire antibody response to a complex cellular antigen (5, 6). The structural properties of the molecule described in this report are consistent with the following mechanism of action by single T cell molecules: binding of the 70,000-mol wt protein to Th cells that display the foreign antigenic determinant may be followed by increased sensitivity of the Ts molecule to surface proteases on target Th cells and release of the 45,000-mol wt subunit. This subunit suppresses both antigen-specific target Th cells as well as other Ly-1 Th cells which have bound to closely associated determinants displayed by the foreign erythrocyte. The net effect of this reaction is suppression of an immune response to a complex foreign protein by Ts molecules specific for one or several sites on the foreign protein. An important feature of this mechanism is that it ensures efficient regulation of immunity to a large array of foreign molecules by a relatively small number of Ts clones. We are directly testing this hypothesis by studying the interaction between the purified Ts protein and an SRBC-specific Th clone.

Summary

Purified molecules (70,000 mol wt) from a T-suppressor (Ts) clone bind to sheep erythrocyte glycophorin and specifically suppress the response to this antigen. Papain splits purified 70,000-mol wt Ts molecules into two peptides: mol wt 45,000 and 24,000. The 45,000-mol wt peptide nonspecifically suppresses antibody responses to several antigens and lacks antigen-binding activity. The 24,000-mol wt peptide does not suppress but retains antigen-binding activity. The results indicate that papain splits the Ts molecule into a "constant" region responsible for function and a "variable" region responsible for antigen-binding. Since binding of the 70,000-mol wt molecule to antigen also results in release of the 45,000 mol wt subunit, this cleavage may allow Ts molecules specific for one determinant to suppress immunity to complex foreign proteins.

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