Tac Antigen Forms Disulfide-Linked Homodimers†

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ABSTRACT: Interleukin 2 (IL-2) is responsible for stimulating T-cell proliferation via interaction with specific, high-affinity membrane receptors. Although an IL-2-binding protein has been identified by virtue of its reactivity with a monoclonal antibody that competes with IL-2 for binding (anti-Tac), the complete and precise structure of functional IL-2 receptors is still unknown. To define further the composition of IL-2 receptors, both IL-2 itself and anti-Tac were used as ligands to adsorb membrane proteins for analysis by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (NaDodSO_4/PAGE). A variety of experimental approaches yield results indicating that the Tac antigen, which migrates as a single protein on NaDod-SO_4/PAGE under reducing conditions, is also expressed as disulfide-linked homodimers and oligomers. Examined under nonreducing conditions, both activated normal human T cells and cell lines from patients with adult T cell leukemia (HUT-102; MT-1) express Tac antigen homodimers (M_r 105 000) in addition to monomers (M_r 54 000). Formation of the disulfide bond is not a consequence of the experimental procedures used to isolate the proteins for analysis, inasmuch as identical results are obtained when the receptor proteins are iodinated and extracted in the presence of *N*-ethylmaleimide or prepared for electrophoresis in the absence of heat denaturation. Accordingly, these findings point to Tac antigen associating with itself preferentially. The physiologic significance of homodimer and oligomer formation, especially as it relates to the formation of high-affinity IL-2 receptors, is presently unknown.

Until now the structure of the interleukin 2 (IL-2) receptor (Robb et al., 1981) has eluded complete description despite considerable effort utilizing both biochemical and gentic approaches. Initial attempts to analyze IL-2 receptor structure exploited monoclonal antibodies (Uchiyama et al., 1981a,b) that prevented binding of radiolabeled IL-2 to both activated T cells and IL-2 receptor$^+$ cell lines derived from patients with adult T cell leukemia (ATL) (Leonard et al., 1982). The earliest experiments showed that these antibodies, designated anti-Tac, precipitate a single broad band (M_r 47 000–53 000) from radioiodinated cell membranes as analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (NaDod-SO_4/PAGE) (Leonard et al., 1982). The very first reports identified only this single Tac antigen, whether or not reducing agents were utilized (Leonard et al., 1982, 1983). In addition, subsequent pulse–chase experiments with biosynthetic radiolabeling approaches using amino acid and carbohydrate residues confirmed these initial findings and indicated that a single protein of about M_r 33 500 is glycosylated prior to its insertion into the membrane as the mature monomeric Tac antigen (Leonard et al., 1983). However, Wano et al. (1984) noted subsequently that a larger moiety (M_r 115 000) was also immunoprecipitated with anti-Tac, provided cells were biosynthetically radiolabeled for periods longer than 16 h.

To generate more definitive data as to the nature of the Tac antigen, anti-Tac was used to purify sufficient membrane protein for amino acid sequence determination, followed by the identification of cDNA clones using synthetic oligonucleotides as probes (Leonard et al., 1984; Nikaido et al., 1984). The nucleotide sequence of Tac cDNA predicts a single core protein of M_r 28 500 with a typical hydrophobic transmembrane sequence just prior to a short hydrophilic cytoplasmic carboxyl-terminal domain of only 13 residues. Transfection of this cDNA into COS cells results in the transient expression of a surface protein capable of binding both IL-2 and anti-Tac (Leonard et al., 1984; Nikaido et al., 1984). Although these data yielded information on the primary structure of Tac antigen, further experiments suggested that additional components were necessary to impart the attributes of authentic IL-2 receptors to this protein. Thus, cDNA transfection into non T cells only results in the expression of low-affinity, nonfunctional IL-2 binding sites (Greene et al., 1985; Sabe et al., 1986), whereas transfection of the Tac cDNA into cells of the T-cell lineage reportedly makes for both high- and low-affinity IL-2 receptors (Hatakeyama et al., 1985; Kondo et al., 1986a). Moreover, only transfectants expressing a high-affinity site are capable of signal transduction in response to IL-2. Consequently, one proposal is that another gene product, expressed specifically by T cells, might somehow associate with the Tac antigen to convert these low-affinity IL-2 binding sites into high-affinity, fully functional IL-2 receptors (Kondo et al., 1986b). In this regard, even before the isolation of the Tac cDNA, an additional protein about twice the size of the Tac antigen had already been detected (M_r 113 000, Leonard et al., 1983; M_r 115 000, Wano et al., 1984). However, this larger protein was observed only intermittently, and even when present, it was invariably radiolabeled less intensely than the Tac antigen, thereby making it of dubious significance other than being a nonspecific contaminant of the immunoprecipitates.

To reexamine the nature of the molecules expressing the Tac epitope, especially to determine whether molecular sizes distinct from Tac antigen could be discerned, anti-Tac and IL-2 itself were used as affinity adsorbents to select proteins for analysis. Our results described here reveal that the Tac

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protein also exists as disulfide-linked homodimers and oligomers.

Materials and Methods

Cell Cultures. The ATL cell lines HUT-102B2 (Poiesz et al., 1980) and MT-1 (Miyoshi et al., 1981) were maintained in RPMI 1640 medium (Grand Island Biological Co.) supplemented with 10% heat-inactivated fetal calf serum (Sterile Systems). Activated human T cells were prepared by stimulating peripheral blood mononuclear cells with anti-T3 monoclonal antibody (Ortho Pharmaceutical Corp.) for 3 days and then culturing them for an additional 10 days with 125 pM IL-2 (Cantrell & Smith, 1983). To ensure IL-2 receptor reexpression in the absence of IL-2 production by these synchronized (G_0/G_1) cell populations, phorbol 12,13-dibutyrate (PdBu, 50 ng/mL, Consolidated Midland) was used as the immunostimulatory reagent (Cantrell & Smith, 1984; Stern & Smith, 1986). After incubation for 6 h in the presence of PdBu, the cells were washed and cultured for 12 h to allow for maximal IL-2 receptor expression as described (Cantrell & Smith, 1984; Stern & Smith, 1986).

Monoclonal Antibodies. The monoclonal antibody 1HT4 (mouse immunoglobulin (Ig) G_{2a}), which recognizes the same epitope of the human IL-2 receptor as does anti-Tac (Teshigawara et al., 1987), was provided by Dr. Ellis L. Reinherz (Dana Farber Cancer Institute). 1HT4 Affi-Gel 10 (Bio-Rad) was prepared according to the manufacturer's instructions.

IL-2. Recombinant IL-2 derived from *Escherichia coli* and purified to homogeneity (Kato et al., 1985) was supplied by Takeda Chemical Industries, Ltd. For affinity column chromatography, it was conjugated to Affi-Gel 10 (1 mg/mL of gel).

Iodination of Cell Surface Proteins and Precipitation with Anti-Tac. Surface iodination was carried out by the lactoperoxidase method as described (Reinherz et al., 1983) with (10–20) × 10^6 cells/mCi of $Na^{125}I$ (Amersham Corp.). The labeled proteins were extracted with a lysis buffer (RIPA) containing 1% Triton X-100. Proteins were precipitated with anti-Tac and *Staphylococcus aureus* Cowan I cells after prior clearing with control IgG and staphylococcal cells (Ritz et al., 1985). In some experiments *N*-ethylmaleimide (NEM, 5 mM) was used during the various extraction procedures to prevent artifactual oxidative disulfide bond formation. NEM was used prior to iodination, and during iodination, lysis, immunoprecipitation, and electrophoresis.

Gel Electrophoresis. $NaDodSO_4$/PAGE was performed as described by Laemmli (1970). Two-dimensional $NaDodSO_4$/PAGE (the first dimension under nonreducing conditions and the second dimension under reducing conditions) was performed as described (Ritz et al., 1985) with the exception that the strip excised from the nonreducing gel was heated to 100 °C for 2 min in a microwave oven in the presence of the reducing agents (100 mM dithiothreitol (DTT) and 10% 2-mercaptoethanol). ^{14}C-Methylated protein standards were purchased from Amersham.

Peptide Mapping. The cleavage products of IL-2 receptor proteins after limited proteolysis were analyzed according to the method of Cleveland et al. (1977). Proteases used were papain, α-chymotrypsin, and *S. aureus* V8 protease (Sigma). Briefly, dimer and monomer receptor proteins were extracted from the gel with 0.125 M tris(hydroxymethyl)aminomethane hydrochloride (pH 6.8)–0.5% $NaDodSO_4$–10% glycerol–0.0001% bromophenol blue. The extract was boiled for 5 min and then incubated at 37 °C for 1 h in the presence of the proteases (4, 20, and 100 μg/mL). The reactions were terminated by boiling for 2 min after addition of 2-mercaptoethanol (10%) and $NaDodSO_4$ (2%). $NaDodSO_4$/PAGE of the cleavage products was performed by using a 15% acrylamide gel.

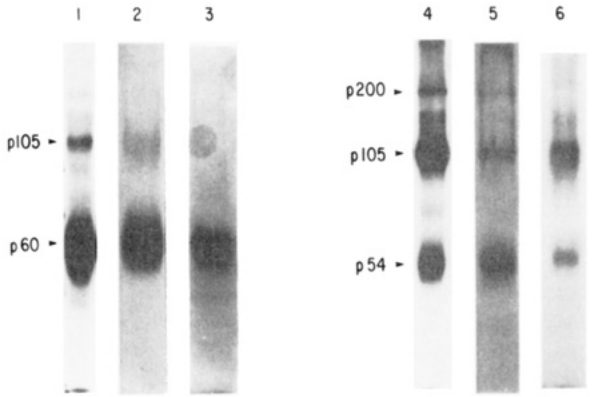

FIGURE 1: $NaDodSO_4$/PAGE analysis of Tac antigen immunoprecipitates. Lanes 1–3, reducing conditions; lanes 4–6, nonreducing conditions. Lanes 1 and 4, HUT-102B2; lanes 2 and 5, MT-1; lanes 3 and 6, stimulated T cells cultured in the presence of IL-2. Acrylamide concentration was 7.5%. Autoradiography was performed at −70 °C for 2 days for HUT-102B2 and MT-1 and for 2 weeks for stimulated T cells with Kodak X-Omat R X-ray film and a Du Pont intensifying screen.

Results

Gel Electrophoresis of Anti-Tac- and IL-2-Reactive Proteins under Nonreducing vs. Reducing Conditions. Three different cells were chosen for initial analysis. The HUT-102 cell line, derived from an ATL patient, expresses large numbers of anti-Tac-reactive molecules [(0.5–1.0) × 10^6] of which only 1% are high-affinity IL-2 receptors. For comparison, another ATL cell line was selected (MT-1) that also expresses large numbers of anti-Tac-reactive molecules [(2–3) × 10^5 molecules/cell] but no detectable high-affinity IL-2 receptors (Fujii et al., 1986). In contrast to these ATL cell lines, activated normal human T cell blasts express 50–100-fold fewer anti-Tac-reactive molecules [(2–10) × 10^3 molecules/cell], of which generally 10% are high-affinity IL-2 receptors (Smith & Cantrell, 1985). Representative $NaDodSO_4$/PAGE analyses of anti-Tac immunoprecipitates of radioiodinated surface components are shown in Figure 1. Under standard reducing conditions (20 mM DTT), the predominant species immunoprecipitated consistently from all three cell types was a broad band migrating between M_r 55 000 and 65 000 (lanes 1–3). In addition, a less prominent species of M_r 105 000 (p105) was also visualized readily in the precipitates from HUT-102 (lane 1) and MT-1 (lane 2), but was generally undetectable in the normal T cell immunoprecipitates (lane 3).

Distinctly different profiles were observed when the same immunoprecipitates were analyzed under nonreducing conditions (lanes 4–6). Two major bands (M_r 54 000 and 105 000, designated p54 and p105, respectively) were consistently present in the immunoprecipitates of all three cells. In addition to these two species, at times we observed a minor M_r 200 000 band, as well as larger aggregates that hardly penetrated the separating gel. It is noteworthy that these three cell types all expressed both p54 and p105, as the ratios of high-affinity to low-affinity IL-2 binding sites differ considerably among them. Consequently, the $NaDodSO_4$/PAGE patterns of these anti-Tac immunoprecipitates do not make for an easily discernible correlation of receptor affinity with molecular size. For example, the immunoprecipitate from HUT-102 cells (lane 4) contains equivalent amounts of p54 vs. p105, yet the great majority (98–99%) of binding sites expressed by these cells are of the low-affinity class.

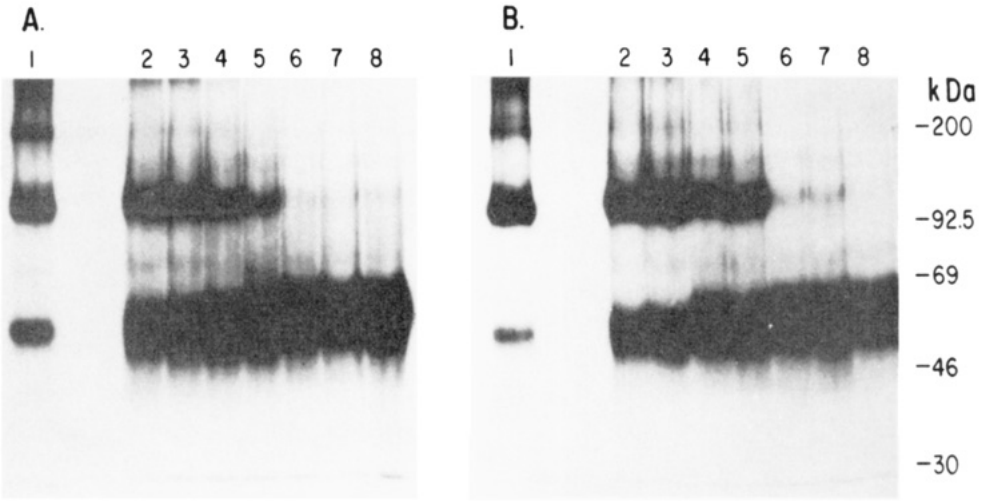

FIGURE 2: $NaDodSO_4$/PAGE analysis of affinity-purified IL-2 receptors under various DTT concentrations. Anti-Tac (A) and IL-2 (B) column-purified HUT-102B2 IL-2 receptor proteins were subjected to reduction under various DTT concentrations. $NaDodSO_4$/PAGE was performed on a 7.5% acrylamide gel. Lane 1, DTT was omitted; lanes 2–8, DTT was added at 0.1, 0.3, 1, 3, 10, 30, and 100 mM.

From these results, it appeared that p105 was reduced to the broad band migrating at M_r 55000–65000. To investigate this possibility and to discern whether the profiles of Tac antigen as analyzed by $NaDodSO_4$/PAGE were similar to those of IL-2 binding proteins, radiolabeled membranes from HUT-102 cells were solubilized and affinity-purified by using both solid-phase anti-Tac and solid-phase IL-2. As shown in Figure 2, the $NaDodSO_4$/PAGE patterns for anti-Tac-purified proteins were identical with those for IL-2 column-purified proteins. Under nonreducing conditions, both preparations contained p54 and p105, although p105 was slightly more predominant in the IL-2 column-purified preparation compared with the proteins adsorbed by anti-Tac (Figure 2, lanes 1). In seeking optimal reducing conditions, DTT concentrations were varied from 0.1 to 100 mM (lanes 2–8). It is evident from this analysis that p105 disappeared as the DTT concentration was increased: simultaneously, the band at p54 became progressively broader, likely a result of cleavage of intrachain disulfide bonds. It is noteworthy that DTT concentrations in excess of 30 mM are necessary to completely reduce p105. Moreover, as shown in Figure 3, identical results were obtained with two-dimensional $NaDodSO_4$/PAGE (the first dimension under nonreducing and the second dimension under reducing conditions) with anti-Tac-purified HUT-102 radiolabeled membranes; after reduction with 100 mM DTT plus 10% 2-mercaptoethanol, p105 and all larger anti-Tac-purified radiolabeled proteins migrated at essentially identical molecular sizes, ranging from M_r 55000 to M_r 65000. It is noteworthy that complete reduction of the proteins separated in the strip of gel used for the first dimension was observed only after the gel slice was heated to 100 °C for 2 min in a microwave oven.

Prevention of Oxidation Artifacts. Although these results made the conclusion inescapable that the p54 Tac protein complexes with another protein to form larger disulfide-linked species, it was far from clear whether p105 exists as such in the native state: alternatively, oxidative disulfide bonds might form during iodination, or after lysis of the cells during the procedures necessary prior to $NaDodSO_4$/PAGE. To explore this possibility, all procedures were performed in the presence of 5 mM NEM, which irreversibly alkylates all free sulfhydryl groups, thereby preventing oxidation. (A covalent bond is formed readily between any free sulfhydryl groups and NEM. Once formed, this bond cannot be oxidized, even by an iodine radical, which would be present during the iodination process

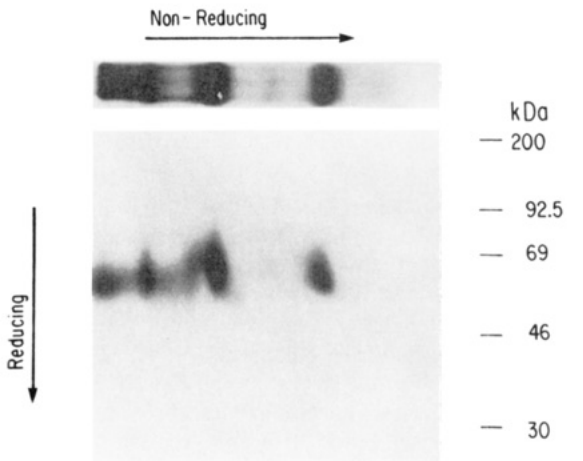

FIGURE 3: Two-dimensional $NaDodSO_4$/PAGE analysis of affinity-purified HUT-102B2 receptor proteins. Anti-Tac column-purified receptor proteins were electrophoresed under nonreducing conditions for the first dimension. The second dimension was electrophoresed under reducing conditions (100 mM DTT, 10% 2-mercaptoethanol, heated at 100 °C for 2 min in a microwave oven).

itself.) As displayed in Figure 4A, p54 and p105 were readily detectable regardless of whether NEM was present or not during all steps subsequent to iodination (lane 1 vs. lane 2). Also, it is important to note that NEM used during the iodination reaction (lane 3) resulted in a decreased but equal ratio of p54 and p105 compared with that of lanes 1 and 2. Therefore, the effects of NEM on the enzymes used to catalyze the iodination reaction actually suppressed the amount of ^{125}I incorporated, but did not change the relative amounts of p54 vs. p105.

Oxidative disulfide formation resulting in artifacts has been particularly troublesome during denaturing steps just prior to $NaDodSO_4$/PAGE, especially when immunoprecipitated proteins are unfolded by heating to 100 °C to allow maximum binding of $NaDodSO_4$. Accordingly, anti-Tac HUT-102 immunoprecipitates were examined under nonreducing conditions without heating. As is evident in Figure 4B, the $NaDodSO_4$/PAGE patterns are indistinguishable whether or not the samples were boiled. Consequently, the p105 protein is formed as a result of disulfide linkage of p54 to another protein of approximately the same molecular size (M_r 50000–55000), and this complex is expressed in the native membrane state.

Peptide Mapping. To ascertain whether p105 is a homo-

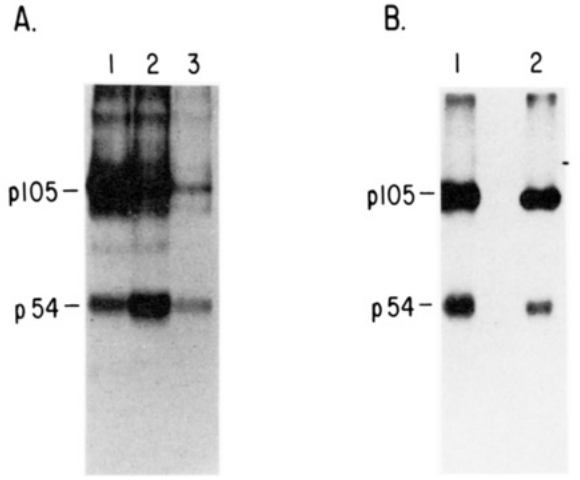

FIGURE 4: Effect of NEM (A) and heat treatment (B) on receptor proteins prior to electrophoresis. (A) HUT-102 surface proteins were iodinated, extracted with 1% Triton X-100, and precipitated with anti-Tac. (Lane 1) Control, no NEM present; (lane 2) NEM (5 mM) present during all procedures subsequent to iodination; (lane 3) NEM (5 mM) present during iodination and all subsequent procedures. $NaDodSO_4$/PAGE was carried out on a 10% acrylamide gel. (B) Iodinated proteins from HUT-102 were extracted and precipitated as described in part A. The precipitated receptor proteins were boiled for 5 min (lane 1) or kept at room temperature (lane 2) prior to $NaDodSO_4$/PAGE. Electrophoresis was carried out on a 7.5% acrylamide gel.

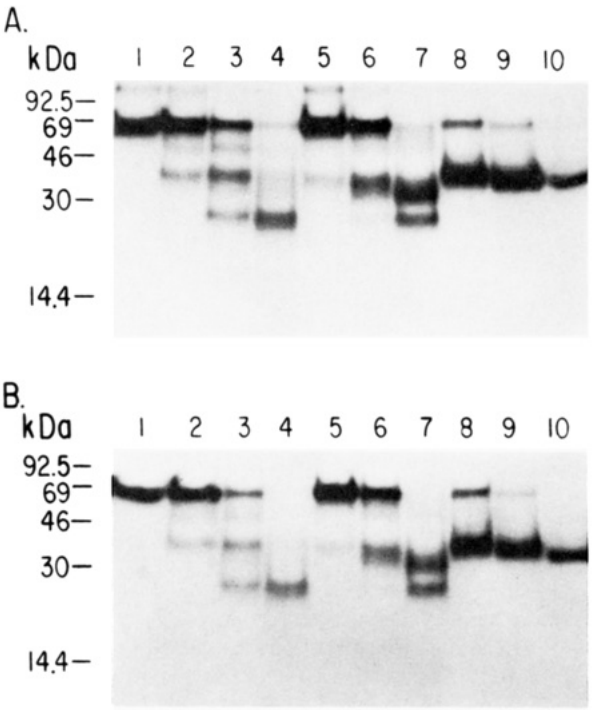

FIGURE 5: Peptide maps of dimer (A) and monomer (B) IL-2 receptors. Dimer and monomer receptors of HUT-102B2 were extracted from the corresponding nonreducing gel by sample buffer. The extract was boiled for 5 min and incubated at 37 °C for 1 h with the indicated concentrations of proteases. (Lane 1) Without digestion; (lanes 2–4) digestion with 4, 20, and 100 μg/mL papain; (lanes 5–7) digestion with 4, 20, and 100 μg/mL α-chymotrypsin; (lanes 8–10) digestion with 4, 20, and 100 μg/mL *S. aureus* V8 protease. $NaDodSO_4$/PAGE was performed on a 15% acrylamide gel.

dimer of p54 or a heterodimer of two different proteins of similar size, both p54 and p105 were analyzed by $NaDodSO_4$/PAGE after limited proteolytic digestion with three different proteases. HUT-102 cells were surface radioiodinated and immunoprecipitated with anti-Tac, and p54 and p105 were excised and extracted from a nonreducing gel. The resulting proteins were subjected to proteolytic digestion with three different concentrations each of papain, α-chymotrypsin, and *S. aureus* V8 protease and then analyzed via $NaDodSO_4$/PAGE. The results, shown in Figure 5, revealed identical peptide profiles for p105 (A) vs. p54 (B), except that an additional faint band of partially reduced p105 was visible in several lanes of the analysis of p105. Consequently, these results are consistent with p105 as a disulfide-linked homodimer of p54.

Discussion

The p105 disulfide-linked Tac homodimer provides an accounting for the protein observed intermittently (Leonard et al., 1983; Wano et al., 1984) when anti-Tac immunoprecipitates were examined under standard reducing conditions; their p113–115 likely consisted of only partially reduced homodimers. Actually, it is puzzling how the profile of p54 and p105, so visible upon $NaDodSO_4$/PAGE analysis under nonreducing conditions, went undetected for so long. However, nonreducing gel patterns are curiously absent from most reports on the nature of the IL-2 receptor protein, probably because Leonard et al. (1982) first stated that there was no difference between the patterns seen under reducing vs. nonreducing conditions. In effect, subsequent investigators must have simply neglected to investigate gels under both reducing and nonreducing conditions. Moreover, in subsequent investigations these same investigators employed biosynthetic radiolabeling techniques to examine the size of proteins precipitable with anti-Tac. Leonard et al. (1983) labeled cells overnight with tritiated glucosamine prior to electrophoresis under nonreducing conditions and only observed a single radiolabeled protein (M_r 45000). However, Wano et al. (1984) remarked that a larger moiety (M_r 115000) could be observed, but only when the biosynthetic labeling procedure using [^{35}S]methionine was allowed to proceed for longer than 16 h. Recent analysis of the murine IL-2 receptor by Malek and Korty (1986) is pertinent in this regard, as two major proteins (M_r 58000 and 110000) under both nonreducing and reducing conditions were found. These investigators speculated that the p110 is a homodimer of p58, but they were perplexed by their inability to reduce the p110 to p58 and concluded that peptide maps of the two moieties were indicated. From our experience, it is likely that the p58 and p110 murine proteins of Malek and Korty are analogous to the human p54 and p105 described here; their conditions would have been insufficient to completely reduce the disulfide bonds of the p110 homodimer. Likewise, their conditions for reduction using two-dimensional $NaDodSO_4$/PAGE (i.e., 100 mM DTT, without heating) were probably inadequate. We found that both 100 mM DTT and 10% 2-mercaptoethanol were necessary, as was heating in a microwave oven, to achieve total reduction, especially if the p105 homodimer is embedded in the gel matrix used for the first dimension.

In the analysis of the p105 Tac homodimer, we pondered considerably whether the pattern observed could be the result of oxidative disulfide formation, which might well have occurred upon cell lysis or during the steps necessary for iodination, immunoprecipitation, and preparation for $NaDodSO_4$/PAGE. Within the limits of available experimentation, it seems most improbable that the p105 homodimer is an artifact. First, it is the predominant species reactive with either anti-Tac or IL-2 from every cell tested, regardless of the receptor density; one might expect cells with a high density of Tac antigen to form more disulfide-linked homodimers after lysis than those with a relative paucity of receptors. Second, the use of NEM throughout all procedures, including iodi-

nation, had no effect on the ratio of p54 vs. p105. Finally, the $NaDodSO_4$/PAGE profiles are identical regardless of whether the immunoprecipitates are denatured by heating prior to electrophoresis. Especially pertinent in this regard is the report by Malek and Korty (1986), who used Western blot for analysis to obviate oxidative iodination of the surface proteins: murine T cells (concanavalin A stimulated splenocytes and CTLL cells) express two distinct proteins (p58 and p110) reactive with the 7D4 monoclonal antibody. Given these considerations, alternative methods of determining the size of IL-2 binding proteins, especially as they exist in intact membranes, appear warranted.

Throughout the course of these experiments we also considered the possibility that p105 is made up of a heterodimer of two distinct chains rather than a homodimer of p54. The reduction of p105 to such a broad band spanning M_r 55 000–65 000 certainly provided an ample range to accommodate two different proteins having similar sizes. However, the increase in molecular size observed after reduction would also be explained readily by the reduction of internal disulfide bonds of p54. Similarly, the broadness of the $NaDodSO_4$/PAGE pattern after reduction is explicable by variable glycosylation of p54. Indeed, removal of the carbohydrate residues using hydrogen fluoride failed to generate more than one smaller core protein (T. Ciardelli and K. Kato, unpublished observation). The identical peptide maps found for p54 and p105 essentially eliminated any further consideration of a heterodimer: only another protein that binds IL-2 but cannot be surface radioiodinated could account for the data. Therefore, the only interpretation that remains feasible after consideration of all of the information consists of a homodimer as the correct configuration for p105.

Although it is of interest that the Tac protein is found expressed as monomers and dimers and perhaps as larger oligomers as well, this by itself does not account for the phenomenon of high-affinity IL-2 receptors vs. low-affinity IL-2 binding sites: MT-1 cells, which have only low-affinity binding sites detectable, express both p54 and p105 in essentially the same pattern as HUT-102 cells (1% high-affinity sites) and normal T cells (10% high-affinity sites). Accordingly, alternative explanations must now be sought that do account for the obvious 1000-fold difference in affinity (K_d = 10 pM vs. 10 nM) displayed by these two classes of receptors. In particular, we must now reconsider the heretofore remote possibility that the true, high-affinity IL-2 receptor may be comprised of additional IL-2 binding proteins encoded by an entirely separate gene, quite distinct from the Tac gene. In this regard, we have recently uncovered a novel IL-2 receptor expressed by a cell line established from a patient with T-cell acute lymphoblastic leukemia (Teshigawara et al., 1987). This finding is especially relevant as these cells do not express cell surface Tac antigen nor do they manifest detectable Tac mRNA. Moreover, this unique IL-2 binding site appears to be of intermediate size (M_r 75 000) compared with p54 and p105. Similar findings have also recently been reported by Sharon et al. (1986) and Tsudo et al. (1986). Therefore, further experiments are essential to elucidate fully the structure and functional relationships between these apparently separate and distinct forms of the IL-2 receptor. However, it is likely that this newly discovered IL-2 binding protein and the p54 Tac protein are expressed as non-disulfide-linked α,β heterodimers and that, by associating with one another, they make for high-affinity IL-2 receptors. This situation is reminiscent of the T8 glycoprotein, which becomes linked to T6 molecules on thymocytes but forms homodimers and oligomers on the surface of mature T cells that lack T6 (Snow & Terhorst, 1983). Thus, by analogy to the molecules comprising the immunoglobulin gene family, many of which form natural associations, IL-2-binding proteins could well be members of a lymphokine receptor family, in which related but distinct molecules favor interaction with one another. It remains to be determined whether the IL-2 receptor p75 α chain preferentially associates with p54 (Tac) monomers or dimers, but it is conceivable that multimeric complexes of α chains and β chains are important for IL-2-mediated signal transduction.

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A Calorimetric Study of the Thermotropic Behavior of Pure Sphingomyelin Diastereomers†

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ABSTRACT: The phase-transition properties of sphingomyelins were investigated in detail with totally synthetic, chemically and stereochemically pure (2*S*,3*R*)-(*N*-stearoylsphingosyl)-1-phosphocholine (D-*erythro*-C_{18}-SPM) (**1**) and the corresponding 2*S*,3*S* isomer (L-*threo*-C_{18}-SPM) (**2**). Heating scans of an unsonicated dispersion of **1** right after hydration showed a main transition (I) at 44.7 °C (ΔH = 6.8 kcal/mol). Upon incubation at 20–25 °C a second transition (II) appeared at 36.0 °C (ΔH = 5.7 kcal/mol). The two gel phases were designated as G_α and G_β phases, respectively. The G_β phase was also metastable and relaxed to a third gel phase (G_γ) upon incubation below 10 °C. Conversion of the G_γ phase to the liquid-crystalline phase occurred via two new endotherms at 33.4 °C (2.6 kcal/mol) (III) and 43.6 °C (8.0 kcal/mol) (IV) as well as a main transition at 44.7 °C (9.5 kcal/mol). Possible interpretations have been proposed to account for the observed phase transitions. The L-threo isomer **2** showed similar thermotropic behavior to dipalmitoylphosphatidylcholine (DPPC): a "main transition" at 44.2 °C (6.0 kcal/mol), a "pretransition" at 43.1 °C (1.8 kcal/mol), and upon incubation at 7 °C for 2 weeks, a very broad "subtransition" at ca. 35 °C. The results are substantially different from previous studies of sphingomyelins using mixtures of stereoisomers. Mixing of **1** with **2**, **1** with DPPC, and **2** with DPPC removed the metastability of the gel phase and resulted in a single transition.

Thermal phase transitions of sphingomyelins (SPM)[1] have been a subject of numerous investigations over the last decade, as summarized in two recent reviews (Barenholz & Thompson, 1980; Barenholz & Gatt, 1982). Three different types of sphingomyelins have been used for these studies: (a) natural sphingomyelins isolated from various sources (Calhoun & Shipley, 1979a; Shipley et al., 1974; Barenholz et al., 1976), (b) semisynthetic SPM obtained from natural compounds through the deacylation–reacylation sequence (Calhoun & Shipley, 1979b; Cohen et al., 1984; Maulik et al., 1986), and (c) totally synthetic SPM (Barenholz et al., 1976; Maulik et al., 1986; Estep et al., 1979, 1980, 1981).

The natural sphingomyelins have D-erythro 2*S*,3*R* configuration at the sphingosine moiety, but the fatty acyl residue is usually a mixture of several species depending on the sources of isolation, and the long-chain base may also be heterogeneous (sphingosine is the major component, but dihydrosphingosine, C_{20}-sphingosine, etc., may be present as minor components) (Barenholz & Gatt, 1982). The semisynthetic SPM should be stereochemically pure. However, we have found that the commercially available semisynthetic SPM is indeed a mixture of D-erythro 2*S*,3*R* and L-threo 2*S*,3*S* isomers (Bruzik, 1987).[2] The totally synthetic SPM was obtained as a mixture of en-

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[1] Abbreviations: C_{18}-SPM, (*N*-stearoylsphingosyl)-1-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; HPLC, high-pressure liquid chromatography; Mops, 3-(*N*-morpholino)propanesulfonic acid; SPM, sphingomyelin; $\Delta T_{1/2}$, half-width of the transition; T_m, main transition temperature.

[2] In accordance with the 1976 recommendations by IUPAC–IUB Commission on Biochemical Nomenclature (1978) on the nomenclature of lipids, the sphingomyelin derived from (2*S*,3*R*)-sphingosine is designated as D-*erythro*-sphingomyelin or simply as sphingomyelin. The 2*S*,3*S* diastereomer of sphingomyelin is defined as L-*threo*-sphingomyelin.

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