Biochemical Analysis of Human T Lymphocyte Differentiation Antigens T4 and T5

Abstract. *Two major functionally distinct T cell subsets in man have been defined with heteroantiserums and monoclonal antibodies directed against stable cell surface antigens that appear during thymic ontogeny. A monoclonal antibody to T4 antigen (anti-T4) is reactive with the peripheral inducer T cell population while a monoclonal antibody to T5 antigen (anti-T5) is reactive with the cytotoxic and suppressor population. Immunoprecipitation and electrophoresis on sodium dodecyl sulfate polyacrylamide gel were used to show that on human thymocytes or peripheral T cells the T4 antigen is a single 62,000-dalton glycoprotein while the T5 antigen is a complex of two glycoproteins, one being 30,000 daltons and the other 32,000 daltons. Similar glycoproteins have been isolated with antibodies to murine Lyt 1 and Lyt 2,3 antigens. Both the antigens defining the phenotypes of inducer and suppressor populations in man and mouse are structurally homologous.*

Profound changes in cell surface antigens mark the stages of T cell ontogeny (*1*). In man, the earliest cells within the thymus bear antigens shared by some bone marrow cells but lack mature T cell antigens. With maturation, thymocytes acquire a TL-like antigen and concurrently express antigens defined by two monoclonal antibodies (anti-T4 and anti-T5). The $T4^+$, $T5^+$, HTL^+ thymocytes account for approximately 70 percent of the total thymic population. With further maturation, thymocytes lose HTL antigens, acquire two mature T cell antigens (T1 and T3 antigens), and segregate into $T4^+$ and $T5^+$ subsets. Immunologic competence is partially acquired at this stage but is not fully developed until thymic lymphocytes are exported (*1*).

On peripheral T cells, however, the T4 and T5 antigens are represented on reciprocal subsets of mature T cells. Thus, the T4 antigen is found on approximately 55 to 65 percent of peripheral T cells (*2*) while the T5 antigen is present on 20 to 30 percent of peripheral T cells (*3*). These two subsets correspond to the previously defined $T_{H_2}^-$ and $T_{H_2}^+$ subsets and represent the circulating inducer and cytotoxic suppressor populations in man (*4*). Thus, the $T5^+$ subset in man appears to be analogous to the murine Lyt $2^+,3^+$ subset which mediates both cytotoxic and suppressor functions, whereas the $T4^+$ T cell subset is analogous to the murine Lyt 1^+ subset (*5*) which provides inducer (helper) functions. We now report the biochemical characterization of the T4 and T5 antigens.

Both T4 and T5 undergo immunoprecipitation from a Nonidet P-40 lysate of human thymocytes labeled with tritiated sodium borohydride after a mild oxidation with a low concentration of sodium periodate (*6*). With this technique, sialic acids are specifically oxidized; after reduction with tritiated borohydride, either radioactive 5-acetamido-3,5-dideoxy-L-arabino-2-heptulosonic acid or 5-acetamido-3,5-dideoxy-L-arabino-2-octulosonic acid is formed (*6*). The glycoprotein, detected by anti-T4, has a molecular size of 62,000 daltons (Fig. 1). The T5 appears on the polyacrylamide gel as a doublet of two glycoproteins of 30,000 and 32,000 daltons (Fig. 1). As a control monoclonal antibody to $\beta 2$ microglobulin was used (A88). Since only the larger subunit of the HLA-A and HLA-B antigens is a glycoprotein [the smaller one being $\beta 2$-microglobulin (12,000 daltons)] (*7*), only one band at 45,000 daltons can be detected (Fig. 1). When isolated from thymocytes, this band may also contain human Qa-like or TL-like antigens (*8*). The T4 can also be precipitated from a lysate prepared from human thymocytes metabolically labeled with L-[^{35}S]methionine. Its molecular size as determined by SDS-polyacrylamide gel electrophoresis is 62,000 daltons.

The target antigens detected by anti-T4 and anti-T5 can be precipitated from a solubilized (in NP-40) membrane preparation of human peripheral blood T lymphocytes. T cells purified on a nylon wool column were activated with phytohemagglutinin and cultured in the presence of L-[^{35}S]methionine. Electrophoresis on SDS-polyacrylamide gel shows that T4 has the same molecular size under reducing and nonreducing conditions (Fig. 2). In contrast, the T5 antigen is a 76,000-dalton protein under nonreducing conditions (Fig. 3). Under reducing conditions the target antigen for T5 is on one of two subunits (30,000 to 32,000 daltons) on human T lymphocytes (Fig. 3). Similar results were obtained

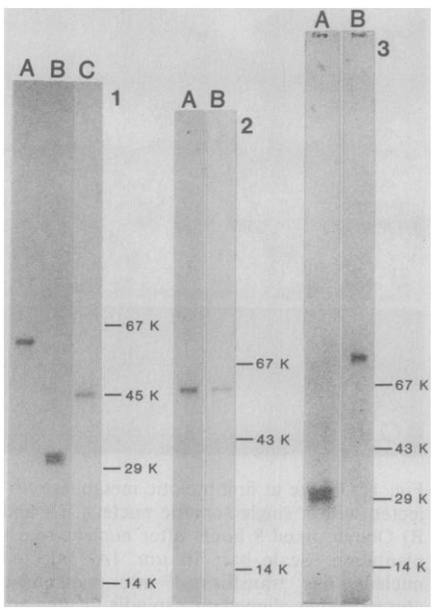

Fig. 1 (left). Electrophoretic analysis of immunoprecipitates made with monoclonal antibodies to T4, to T5, and $\beta 2$ microglobulin of lysate from ^{3}H-labeled human thymocytes. Normal thymus gland was obtained from patients aged 2 months to 14 years who had portions of their thymuses removed during corrective cardiac surgery. The thymic fragments were finely minced and pressed through a stainless steel mesh. The cells were next purified on a Ficoll-Hypaque density gradient (*12*), then labeled with periodate and tritiated sodium borohydride (*6*) and lysed in 1 percent NP-40. After a preliminary incubation of 1 hour with normal mouse immunoglobulin G (IgG) and rabbit antiserum to mouse IgG, portions of the lysates (100 μl) were incubated with 20 μg of monoclonal antibodies and an optimal amount of rabbit antiserum to mouse IgG for 16 hours at 4°C. After several washings with buffers containing 0.2 percent NP-40 and 0.5 percent deoxycholate, the precipitates were solubilized in the sample buffer containing 2-mercaptoethanol and subjected to electrophoresis for 15 hours. For the 5 to 15 percent gradient polyacrylamide gel electrophoresis a modification of the Laemmli system was used (*13*). The internal molecular size markers were phosphorylase B (94,000 daltons), bovine serum albumin (67,000 daltons), ovalbumin (43,000 daltons), aldolase (29,000 daltons), and cytochrome c (14,000 daltons). The gels were subjected to fluorography according to (*14*). (A) Monoclonal antibody to T4; (B) monoclonal antibody to T5; (C) monoclonal antibody to $\beta 2$ microglobulin. Fig. 2 (center). SDS-polyacrylamide gel electrophoresis of immunoprecipitates made with monoclonal antibody to T4 (anti-T4) of lysates from ^{35}S-labeled human T lymphocytes. Nylon wool purified human T lymphocytes were incubated with 0.25 μg of PHA per milliliter of culture medium (RPMI 1640). At day 3, cells were labeled with L-[^{35}S]methionine (*15*). Immunoprecipitates were made and analyzed as described (legend to Fig. 1). (A) Antibody to T4, reducing conditions. (B) Antibody to T4, nonreducing conditions (without 2-mercaptoethanol). Fig. 3 (right). SDS-polyacrylamide gel electrophoresis of immunoprecipitates made with anti-T5 of lysates from ^{35}S-labeled human T lymphocytes. Phytohemagglutinin-activated human T lymphocytes were labeled with [^{35}S]-methionine (*15*). Immunoprecipitates were made and analyzed as described (Fig. 1). (A) Antibody to T5, reducing conditions. (B) Anti-T5, nonreducing conditions (without 2-mercaptoethanol).

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with ^{125}I-labeled T lymphocytes (data not shown).

Comparison of the molecular size determined for T4 62,000-dalton with that of the Lyt 1.1 antigens from murine thymocytes shows a striking similarity. Alloantiserum to Lyt 1.1 precipitated a 67,000-dalton glycoprotein and an 87,000-dalton glycoprotein that could be labeled with tritiated sodium borohydride and galactose oxidase but not with the ^{125}I-labeled lactoperoxidase (*9*). A monoclonal xenoantiserum [termed 53-7.3 (*10*)] detected a 70,000-dalton glycoprotein on ^{125}I-labeled C57B1/6 thymocytes (*10*).

Murine Lyt 2 and Lyt 3 antigens were found on cell surface glycoproteins from thymocytes labeled with ^{125}I-labeled lactoperoxidase (*10, 11*). Immune precipitations carried out with alloantiserums to Lyt 2 and Lyt 3 detected a 35,000-dalton protein for each marker on C57B1/6 thymocytes (*11*). However, in contrast, a monoclonal rat antibody (53-6.7) precipitated a complex of two subunits of approximately 30,000 and 35,000 daltons that was resolved under reducing conditions (*10*). Under nonreducing conditions, a 65,000-dalton glycoprotein was found. These molecular sizes are similar to those determined for T5 (30,000 to 32,000 daltons).

Our results plus the previous functional data indicate that the glycoproteins recognized by the monoclonal antiserums to T4 and T5, respectively, are the human homologs of the Lyt 1 and Lyt 2,3 antigens. Isolation and further characterization of these cell surface markers will be important in determining the precise role of T4 and T5 in the functions of the cells that express them. Information obtained in such studies would also aid in our understanding of the differentiative pathways of human T lymphocytes.

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References and Notes

1. E. Reinherz and S. Schlossman, *Cell*, in press.
2. E. L. Reinherz, P. C. Kung, G. Goldstein, S. F. Schlossman, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4061 (1979).
3. _____, *J. Immunol.*, in press.
4. R. L. Evans, H. Lazarus, A. Penta, S. F. Schlossman, *J. Immunol.* **120**, 1423 (1978); E. L. Reinherz and S. F. Schlossman, *ibid.* **122**, 1335 (1979).
5. H. Cantor and E. A. Boyse, *Cold Spring Harbor Symp. Quant. Biol.* **51**, 23 (1977).
6. C. G. Gahmberg and L. C. Anderson, *Ann. N.Y. Acad. Sci.* **312**, 240 (1978).
7. P. Cresswell, T. Springer, J. L. Strominger, M. J. Turner, H. M. Grey, R. T. Kubo, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2123 (1974).
8. J. Michaelson, L. Flaherty, E. Vitetta, M. D. Poulik, *J. Exp. Med.* **145**, 1066 (1977).
9. P. J. Durda, C. Shapiro, P. D. Gottlieb, *J. Immunol.* **120**, 53 (1978).
10. J. A. Ledbetter and L. A. Herzenberg, *Immunol. Rev.* **47**, 65 (1979).
11. P. J. Durda and P. D. Gottlieb, *J. Exp. Med.* **144**, 476 (1976).
12. A. Boyum, *Scand. J. Clin. Lab. Invest. Suppl.* **21**, 97 (1968).
13. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
14. W. M. Bonner and R. A. Laskey, *Eur. J. Biochem.* **46**, 83 (1974).
15. C. Terhorst, R. Robb, C. Jones, J. L. Strominger, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4002 (1977).
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Excitatory and Inhibitory Effects of Serotonin on Sensorimotor Reactivity Measured with Acoustic Startle

Abstract. *Serotonin infused into the lateral ventricle in rats produced a dose-dependent depression of the acoustic startle reflex. When infused onto the spinal cord, serotonin produced a dose-dependent increase in startle. Thus the same neurotransmitter can modulate the same behavior in opposite ways, depending on which part of the central nervous system is involved.*

Serotonin is often considered to be an important behavioral inhibitor (*1*). However, there are data that are difficult to reconcile with this conclusion (*2*). The acoustic startle response, a simple reflex behavior, is modified by changes in serotonin levels. Small amounts of serotonin or serotonin agonists, infused directly into the hippocampus (*3*) or the ventricles (*4*), depress startle (*5*), suggesting that serotonin inhibits this behavior. However, markedly increasing the levels of serotonin in the brain and spinal cord (*6, 7*) or administering drugs that mimic serotonin, heightens startle (*8*), suggesting that serotonin is excitatory.

Excitatory or inhibitory effects of neurochemicals on behavior may depend on the location and nature of the receptors involved. We now report that serotonin depresses acoustic startle when infused into the forebrain (intraventricularly), but heightens this response when infused onto the spinal cord (intrathecally).

Male albino rats (300 to 400 g) were anesthetized with chloral hydrate and implanted with cannulas into the lateral ventricles or with catheters into the spinal cord (*9*). Twenty-four hours later, an injection of the monoamine oxidase inhibitor pargyline (25 mg/kg) was given (*10*). One hour later, the rats were placed in a startle apparatus and subjected to noise bursts every 20 seconds for 15 minutes (*11*). The animals were then infused with various doses of serotonin dissolved in saline (*p*H 7.4). The animals with cannulated lateral ventricles received saline or 0.78, 3.12, 12.5, 50, or 200 μg of serotonin. The spinal animals received saline or 12.5, 25, 50, 100, or 200 μg of serotonin. (Doses are based on the salt weight of serotonin creatinine sulfate.) There were three rats in each of the experimental and control groups. A total of 10 μl of fluid was administered to each animal at the rate of 4 μl/min (*12*). The rats were returned immediately to the test chamber and given noise bursts every 20 seconds for 20 minutes. Next, the rats with cannulated ventricles were infused with 10 μl of 0.5 percent Fast Green FCF dye. Fifteen minutes later they were perfused, and their brains were examined to ensure adequate infusion of the dye. Animals in which one or both ventricles were incompletely infused (8 percent) or whose catheters showed signs of being clogged (17 percent) were not included in the data.

Figure 1 shows that serotonin caused a rapid decrease in startle amplitude when infused into the lateral ventricle and a rapid increase when infused onto the spinal cord. As shown in Fig. 2, both of these effects were directly related to the amount of serotonin infused. An overall analysis of variance with doses common to both placements (saline and 12.5, 50, and 200 μg of serotonin) revealed a significant dose × placement interaction, F (3, 12) = 7.14, $P < .01$. Subsequent analyses indicated a dose-related depression of startle when serotonin was infused into the lateral ventricle, linear F (1, 12) = 20.04, $P < .001$, and a dose-related excitation of startle when serotonin was infused onto the spinal cord, linear F (1, 12) = 17.35, $P < .001$ (*13*).

Infusion of serotonin into the two areas also had opposite effects on other types of motor activity. Doses ($\geq$ 3.12 μg) that depressed startle when given intraventricularly produced catalepsy (*14*). In contrast, doses that increased startle when given intrathecally produced tremor of the hind quarters, indicative of a localized serotonin "syndrome" (*9, 15*).

Recent single-unit recording studies indicate that there are different types of

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