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Immunology

Changes in surface antigens of immature thymocytes under the influence of T-cell growth factor and thymic factors

(lymphocyte differentiation/interleukin II/thymic hormones)

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ABSTRACT **Peanut agglutinin-positive thymocytes, peanut agglutinin-negative thymocytes, cortisone-resistant thymocytes, and unfractionated thymocytes were prepared from congeneic C57BL/6 Tla mice. By using surface iodination and immunoprecipitation of solubilized antigen with specific antisera (e.g., anti-H-2D, anti-TL, anti-$Qa^2/_3$, and anti-gp70), the released specific antigens were electrophoresed on polyacrylamide gels, and their radioactivity was measured. The relative percentages of surface antigens H-2D, TL, $Qa^2/_3$, and gp70 were 3.2%, 47.5%, 2.5%, and 46.8%, respectively, for peanut agglutinin-positive thymocytes; 31.8%, 4.4%, 32.7%, and 31.1%, respectively, for cortisone-resistant thymocytes; 13.2%, 28.7%, 12.3%, and 45.8%, respectively, for peanut agglutinin-negative thymocytes; and 7.7%, 27.1%, 4.3%, and 60.9%, respectively, for unfractionated thymocytes. After incubation with thymosin fraction V or T-cell growth factor (interleukin II) for 20 hr, the changes in surface antigens of peanut agglutinin-positive thymocytes closely correlated with their normal maturation (i.e., H-2D increases and TL decreases). Thymic factors (e.g., thymosin α_1, thymopoietin pentapeptide, facteur thymic serique) had only small or no effects on surface antigens of peanut agglutinin-positive thymocytes. The results suggest that peptides yet to be identified in thymosin fraction V may play an important role in intrathymic evolution and that T-cell growth factor is possibly a peripheral signal derived from activated T cells that modulates T-cell receptors and may be a critical regulator of intrathymic cellular development.**

The thymus plays an important role in the differentiation and functional maturation of thymus-derived (T) lymphocytes. The general pathway appears to be that precursors in bone marrow and spleen, termed prothymocytes, migrate to the thymus gland, where they are processed and then seeded into the peripheral lymphoid compartment. One of the characteristics of the progression of lymphocytes along their differentiation pathway is change in their cell surface receptors. Immature thymocytes have a high amount of Thy-1 and TL antigens and a low amount of H-2 on their cell surface (1). Whereas on mature thymocytes and peripheral T cells TL is absent and Thy-1 antigen is reduced in quantity, the amounts of H-2 antigen are increased (1, 2). $Qa^2/_3$ antigens have low expression in mature lymphocytes (2, 3). There is also some evidence to show that the expression of the major envelope protein (gp70) of murine leukemia virus (MuLV) type C is related to the maturation of T lymphocytes (4).

Recently, several factors have been extracted from the thymus, some of which possess the ability to promote or regulate the differentiation and maturation of T lymphocytes. Use of the Komuro and Boyse assay has indicated that prothymocytes under the inductive influence of thymic hormones like thymosin fraction V, thymosin α_1, facteur thymique serique (FTS), and thymopoietin$_{32-36}$ (TP_5) *in vitro* express the surface marker Thy-1 (refs. 5 and 6; M. Scheid, personal communication).

The details of intrathymic evolution are still not completely clear. The general assumption has been that some immature cortical thymocytes move into the thymic medulla under the influence of the thymic microenvironment and possibly its hormonal secretions. There they undergo maturational changes, and finally some such cells are thought to travel to the periphery (7). An alternative view, which to us is more persuasive, is that the cortical thymocytes leave the thymus through the juxtamedullary sinuses and contribute to the peripheral T-cell population and subsequently experience further differentiation steps (8). In any case, an important question concerns how the most immature thymocytes mature in the thymus and what factors are responsible for or contribute to their maturation.

Based on the above considerations, we established a model for studying the maturation of thymocytes and the effects of some thymic factors and a factor derived from mature T cells—i.e., T-cell growth factor (TCGF), also called interleukin 2 (IL-2). Our model includes thymocytes in different stages of maturation—e.g., unfractionated thymocytes, most of which are immature thymocytes; cortisone-resistant (C^R) thymocytes, a population of mature thymocytes; thymocytes agglutinated by peanut agglutinin (PNA^+), a population of cortical origin that contains immunologically incompetent, immature thymocytes; and thymocytes not agglutinated by PNA (PNA^-), a population of more mature cortical and medullary thymocytes. We used immunochemical isolation and detection methods to compare the differences of certain surface antigens on these thymocyte populations. The results show that intrathymic lymphocytes reflect different stages of maturation with different surface antigen patterns. Furthermore, our data indicate that thymosin fraction V and TCGF may be among the regulators of the expression of thymocyte surface antigens.

MATERIAL AND METHODS

Animals. Female (8- to 12-wk-old) C57BL/6 Tla (B6TL+) mice congeneic with C57BL/6, having received only the *Qa-Tla* region from the A strain, were used for all experiments and

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Abbreviations: PNA, peanut agglutinin; PNA^+, peanut agglutinin positive; PNA^-, peanut agglutinin negative; C^R, cortisone resistant; TCGF, T-cell growth factor (also called interleukin II); FTS, facteur thymic serique; TP_5, thymopoietin$_{32-36}$; MuLV, murine leukemia virus; kDa, kilodaltons.

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were provided from the breeding colony of the Sloan–Kettering Institute.

Antisera. Anti-H-2D^b {(B6-H-2^k × HTI)F$_1$ anti-EL4}, anti-TL {(B6 × A.Tla^b)F$_1$ anti-ASL-1}, and anti-Qa^2/3 (B6.K anti-B6) were provided by F. W. Shen of Sloan–Kettering Institute. Goat anti-Rauscher-MuLV-gp70 serum was provided by R. Lerner and J. Elder of Scripps Clinic and Research Foundation (La Jolla, CA).

Preparation of Mouse Thymocytes. Normal thymuses and atrophied thymuses from mice 72 hr after treatment with hydrocortisone (hydrocortisone acetate, Merck, Sharp & Dohme; 2.5 mg per mouse by intraperitoneal injection) were removed aseptically and minced in minimal essential medium (GIBCO). Thymocytes were passed through a 200-mesh stainless steel sieve. The cells were pelleted by low-speed centrifugation (1,000 rpm) at 4°C for 5 min, washed with minimal essential medium, and counted. The final cell pellet was resuspended in RPMI 1640 medium supplemented with 5% heat-inactivated fetal calf serum, 25 mM Hepes (Sigma), 50 μM mercaptoethanol (Eastman), 2 mM glutamine (GIBCO), penicillin (100 units/ml), and streptomycin (100 μg/ml) (abbreviated complete media). The total cells from a normal thymus were designated unfractionated thymocytes; those from cortisone-treated mice were designated C^R thymocytes.

Normal total thymocytes were separated into PNA$^+$ and PNA$^-$ thymocytes essentially as described by Reisner *et al.* (9). Briefly, thymocytes suspended in minimal essential medium at a concentration of 4–8 × 10^8 cells per ml were mixed with an equal volume of peanut agglutinin (1 mg/ml; Miles-Yeda, Rehovot, Israel), allowed to agglutinate for 2–5 min at room temperature, and then layered over a 10-ml cushion of 2.5% bovine serum albumin (Sigma) in saline solution. The aggregated cells sedimented rapidly and were collected after 2–5 min at room temperature. The nonagglutinated cells were aspirated from the top of the cushion 5 min later. The cells were freed from PNA by washing twice with 0.2 M galactose (Sigma) in phosphate-buffered saline containing 1% bovine serum albumin, followed by two washes with minimal essential medium containing 1% bovine serum albumin. Cells from both the bottom and the top were subjected to the PNA agglutination procedure a second time. Double PNA$^+$ and PNA$^-$ thymocytes were used in our experiment.

Incubation of PNA$^+$ Thymocytes with TCGF and Thymic Factors. PNA$^+$ thymocytes were suspended in complete media at concentrations of 2.5–5 × 10^6 cells per ml. Ten ml of this cell suspension was incubated individually with the following factors: purified TCGF obtained from S. Gillis (designated G-TCGF) at a concentration of 1:10 (this preparation had been determined to be free of other lymphokine activities) (10) and rat TCGF (CR-TCGF, Collaborative Research, Waltham, MA) at a concentration of 1:10. The following thymic hormone preparations were used: thymosin fraction V and thymosin α_1 (a gift from A. Goldstein and T. Low, George Washington University School of Medicine, Washington, DC) at concentrations of 100 μg/ml and 1 μg/ml, respectively; FTS (a gift from J. F. Bach, Hopital Necker, Paris, France) at a concentration of 1 ng/ml; and TP5 (a gift from G. Goldstein, Ortho Pharmaceuticals, Raritan, NJ) at a concentration of 0.1 μg/ml. Cell culture with complete medium containing 5% heat-inactivated fetal calf serum but without any factors was a control.

The cell suspensions were incubated in a humidified incubator with 5% CO_2/95% air at 37°C for 20 hr as described by Touraine *et al.* (11). After incubation, the cell viabilities were checked (above 80%), and the cells were then washed twice with cold phosphate-buffered saline and suspended in 1 ml of cold phosphate-buffered saline for iodination.

Iodination of Cell Surface Antigens and Preparations of Cell Lysates. Cell surfaces were iodinated with ^{125}I and lysed with Nonidet P-40 (Shell Oil, Rockville, MA) essentially as described by Tung *et al.* (12). Cells suspended at concentrations of 2.5–5 × 10^7 cells per ml in phosphate-buffered saline were iodinated by incubation with 200 μg of lactoperoxidase (Sigma) and 2 mCi of Na^{125}I (Amersham; 1 Ci = 37 GBq) at room temperature. The reaction was initiated by addition of 25 μl of 0.03% H_2O_2, with a second addition 5 min later. Five min after the second addition, the reaction was terminated by washing the cells twice with 50 ml of cold phosphate-buffered saline. Radioiodinated cells were then lysed by addition of 1 ml of 0.5% Nonidet P-40/0.5% sodium deoxycholate/20 mM Tris, pH 7.6/50 mM NaCl/2% Trasylol/10 mM phenylmethylsulfonyl fluoride (Sigma). After centrifugation the cleared supernatant was removed for immune precipitation.

Immune Precipitation of Specific Surface Antigens. The cell lysates were first cleared of material that precipitated nonspecifically; 20 μl of normal B$_6$TL+ mouse serum was added to the cell lysates, which were then mixed and kept on ice for 45 min. Then 40 mg of washed *Staphylococcus aureus* cells (Pansorbin; Calbiochem) was added. After 15 min at room temperature, the lysates were clarified by centrifugation at 3,000 rpm for 15 min. Aliquots of the cleared lysate were added to protein A-Sepharose CL 4B (Pharmacia, Uppsala, Sweden) to which the corresponding specific antiserum (see below) had been adsorbed. They were then shaken for 60 min at room temperature and subsequently washed twice with solutions containing 1 M NaCl, 20 mM Tris (pH 7.4), and 0.5% Nonidet P-40.

Specific antiserum was adsorbed to protein A-Sepharose CL 4B as follows: 20 μl of corresponding antiserum was added to 100 μl of 10% protein A-Sepharose CL 4B, shaken at room temperature for 60 min, and then washed twice with phosphate-buffered saline containing Nonidet P-40. Protein A-Sepharose coated with normal mouse serum was used as a blank.

The antibody and antigen were released from protein A-Sepharose by heating for 2–5 min in a boiling-water bath in sample buffer containing 2% NaDodSO$_4$, 5% mercaptoethanol, 100 mM Tris (pH 6.8), and 10% glycerol.

NaDodSO$_4$ Slab Gel Electrophoresis and Autoradiography. Slab gel electrophoresis was performed essentially as described by Laemmli (13). The separating and stacking gels were 10% and 5% polyacrylamide, respectively; phosphorylase *b* [94 kilodaltons (kDa)], albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa) were used as markers (Pharmacia).

After electrophoresis, the gels were stained with Coomassie brilliant blue, destained with 8% acetic acid/5% methanol/1% glycerol, and then dried under vacuum. The dried gels were exposed to x-ray film (Kodak XR-5, Eastman) with Lightning Plus intensifying screen (DuPont) at −70°C.

For measuring the radioactivity of the iodinated antigens, we used the developed film as a guide to cut the corresponding bands of gels, which were assayed in a gamma scintillation spectrometer; cpm of the gel band with antiserum minus cpm of the same-size gel to which normal mouse serum had been added was considered as the radioactivity of the iodinated antigens.

Because in each experiment the radioactivity of the ^{125}I varied, data were calculated as the relative amount of antigen expressed as the percentage of the total radioactivity of the antigens.

RESULTS

Comparison of Surface Antigens in Mouse Thymocytes. Most thymocytes are immature (>80%) and are very sensitive to cor-

tisone treatment. For example, the cell number per thymus from the mice was $1.06 \pm 0.26 \times 10^8$ (mean value from 86 mice) before cortisone treatment and 2.3×10^6 per thymus (mean value from 28 mice) after this treatment. The surface antigens of unfractionated and C^R thymocytes are shown in Fig. 1. As the autoradiography in Fig. 1 Upper shows, the major polypeptide of H-2 antigen migrated with a molecular mass of 45 kDa. TL antigen, the prominent bands of $Qa^2/_3$, and gp70 were located at 45, 40, and 70-kDa positions, respectively. Quantitatively, H-2 and $Qa^2/_3$ of the C^R thymocytes were present in much higher proportion than were those of unfractionated thymocytes, but TL and gp70 were present in lower proportion. Qualitatively, the mobilities of H-2, TL, and $Qa^2/_3$ on $NaDodSO_4$/polyacrylamide gel electrophoresis were similar in both C^R and unfractionated thymocytes, but the mobility of gp70 appeared to be different in these two kinds of thymocytes. Indeed the mobility of the gp70 that was from C^R thymocytes was somewhat faster than that from unfractionated thymocytes. The quantitative results (Fig. 1 *Lower*) indicate that the relative amounts of these four antigens differ greatly in C^R and unfractionated thymocytes. Although most of the unfractionated thymocytes are immature, they still contain a minor population of mature thymocytes. To separate the immature population from the mature population from the same thymocyte suspension, thymocytes were separated into PNA^+ and PNA^- cells with two cycles of treatment with peanut lectin. The percentage of total recovery and viability of PNA^+ cells after the first cycle of agglutination were $42 \pm 10\%$ and $90 \pm 3\%$, respectively, and were $10 \pm 5\%$ and $88 \pm 10\%$, respectively, for PNA^- thymocytes. After a second cycle of agglutination, the cell number had been reduced to two-thirds of those recovered from the first cycle of agglutination, but the viability remained >90%.

The surface antigens of the two populations of thymocytes were detected by the procedures described (Fig. 2). As expected, H-2 and $Qa^2/_3$ were extremely low in double-rosetted PNA^+ thymocytes as compared with PNA^-, C^R, and unfractionated thymocytes. TL and gp70 were high in PNA^+ thymocytes. TL and gp70 in PNA^- thymocytes were lower than in PNA^+ thymocytes but higher than in C^R thymocytes. The gp70 of PNA^- thymocytes shows two bands on gel electrophoresis, one of which is faster than that from PNA^+ thymocytes.

To further analyze whether there were qualitative differences in the surface antigens, especially gp70, among different populations of thymocytes, the corresponding gel bands were cut and partially digested with *S. aureus* V8 protease (14). Similar peptide mapping of H-2, TL, and $Qa^2/_3$ was confirmed for both unfractionated and PNA^- thymocytes; however, a striking difference was observed in the peptide mapping of the various thymocyte populations. The slower migrating (upper) part of the band shared by both PNA^+ and PNA^- thymocytes showed similar peptide maps. The faster migrating part (lower part of the band) of gp70 of PNA^- cells, not seen in PNA^+ cells, differed greatly from that of PNA^+ cells. The peptide mapping of

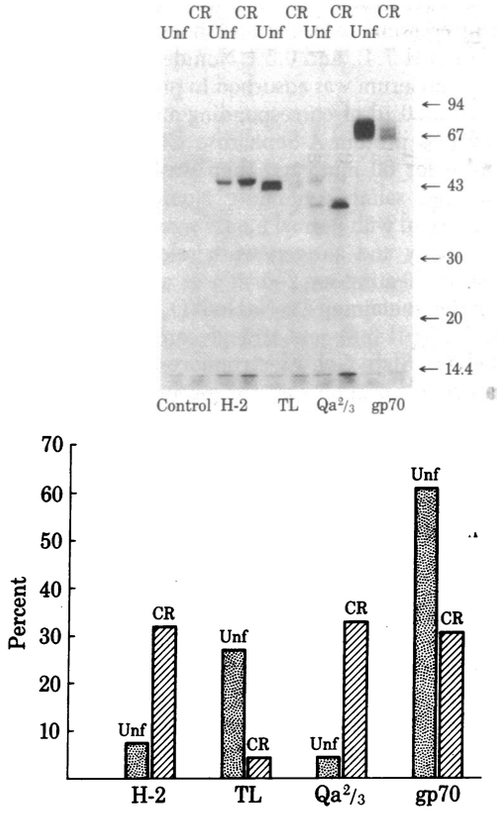

FIG. 1. Surface antigens of unfractionated and C^R thymocytes. Unfractionated and C^R thymocytes were obtained from 8- to 12-wk-old female C57BL/6 Tla^a mice. The same number of cells were surface-iodinated with ^{125}I and immunoprecipitated with normal mouse serum-coated or with anti-H-2, anti-TL, anti-$Qa^2/_3$, or anti-gp70 antiserum-coated protein A-sepharose CL 4 B. Released antigens were run on $NaDodSO_4$/polyacrylamide gels. The dried gels were autoradiographed as described. A representative of two virtually identical experiments is shown. (*Upper*) Autoradiograph (48-hr exposure) of the surface antigens. (*Lower*) Relative percentages of the antigens. Sizes are shown in kDa.

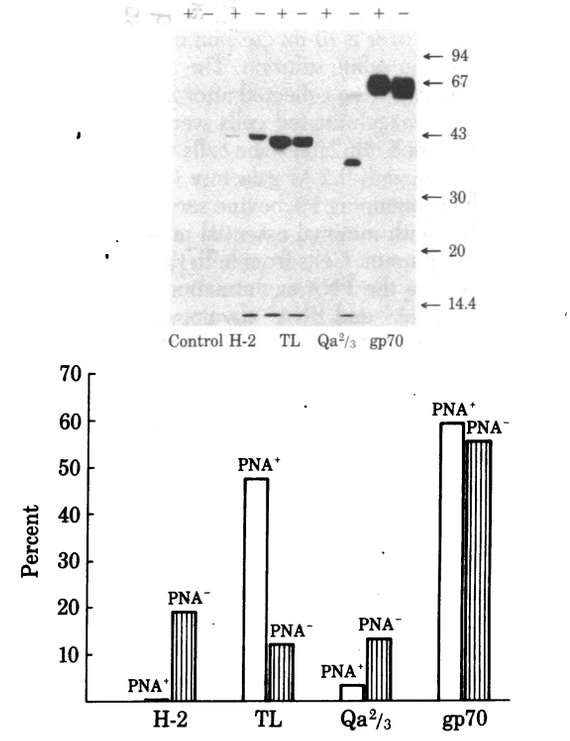

FIG. 2. Surface antigens in PNA^+ and PNA^- thymocytes. Normal thymocytes were treated with PNA to separate them into PNA^+ and PNA^- thymocytes. The same number of PNA^+ and PNA^- thymocytes was surface-iodinated, and the receptors were isolated as described. A representative experiment with double rosetted cells is shown. A total of four experiments were performed, two with single rosetting and two with double rosetting. The results of each type of experiment were virtually identical and consistent with the result presented. (*Upper*) Autoradiograph (48-hr exposure) of surface antigens. Lanes: +, PNA^+; −, PNA^-. (*Lower*) Relative percentage of the antigens in PNA^+ and PNA^- thymocytes. Sizes are shown in kDa.

gp70 in C^R thymocytes and peripheral lymphocytes was just like the pattern of the lower part of the band in PNA^- cells. The peptide mapping of gp70 in unfractionated thymocytes exhibited the characteristics of both the PNA^+ and PNA^- types.

Effects of TCGF and Thymic Factors on the Surface Antigens in PNA^+ Thymocytes. The results indicate that PNA^+ thymocytes possess surface antigens characteristic of the immature thymocytes as shown in other studies; therefore, this population of cells was selected as an *in vitro* system for studying the maturation or differentiation, or both, of normal thymocytes.

Freshly prepared PNA^+ thymocytes were incubated with TCGF, thymosin V, thymosin α_1, TP_5, and FTS for 20 hr as described. At the end of the incubation, after checking cell viabilities (>80%), the surface antigens were detected as described (Table 1). The data was corrected to be based on the same number of cells, i.e., 5×10^7 cells per sample.

After incubation of PNA^+ thymocytes with TCGF, the proportion of H-2 increased significantly and TL decreased significantly (Table 1). Two preparations of TCGF were tested in our experiments. The purified G-TCGF seemed to be more active in changing the apparent percentage of these two antigens. The less-pure commercially obtained TCGF also influenced expression of these two markers; however, it also decreased expression of gp70 on PNA^+ thymocytes. No difference was observed in peptide maps of gp70 in control thymocytes and thymocytes after incubation with either TCGF (data not shown). After incubation with TCGF, $Qa^2/_3$ increased slightly, but the change was not significant. H-2 and TL changed significantly after PNA^+ thymocytes were incubated with thymosin V (100 μg/ml)—i.e., H-2 increased and TL decreased. The effect of the other thymic factors, thymosin α_1, TP5, and FTS, on the surface antigens of PNA^+ thymocytes were analyzed, but only smaller or no changes were observed with the concentrations of these factors used. The only significant finding was an increase in H-2 with FTS. The results suggest that TCGF and components of thymosin fraction V are factors that can modulate T-cell surface antigens and may be considered from these data to be regulators of intrathymic cellular development.

Table 1. Radioactivity of surface antigens of PNA^+ thymocytes after incubation with TCGF and thymic factors

Incubation factor*	Radioactivity, cpm ± SD			
	H-2	TL	$Qa^2/_3$	gp70
Control (10)	346 ± 100	3,009 ± 961	305 ± 96	5,090 ± 1,291
G-TCGF (4)	800 ± 143 $P < 0.001$	1,049 ± 436 $P < 0.01$	232 ± 88 NS	4,257 ± 1,005 NS
CR-TCGF	681 ± 330 $P < 0.01$	1,436 ± 1,342 $P < 0.01$	290 ± 137 NS	1,899 ± 1,509 $P < 0.001$
Thymosin V (4)	523 ± 173 $P < 0.05$	1,499 ± 193 $P < 0.05$	144 ± 83 NS	4,903 ± 1,596 NS
Thymosin α_1 (2)	353 ± 103 NS	1,726 ± 200 NS	190 ± 130 NS	3,588 ± 611 NS
FTS (2)	574 ± 100 $P < 0.05$	2,990 ± 368 NS	157 ± 109 NS	6,467 ± 1,897 NS
TP_5 (3)	340 ± 5 NS	2,726 ± 1,210 NS	112 ± 2 NS	5,416 ± 2,256 NS

After autoradiography, with the x-ray film as a guide, the corresponding antigen bands on dried gels were cut and radioactivity was assayed in a γ-scintillation spectrometer. All the data were corrected as 5×10^7 cells per sample and are expressed as cpm ± standard deviation. *P*, Student *t* test as compared with control; NS, not significant.

*Figure inside the parenthesis is the number of experiments.

DISCUSSION

The thymus consists of mixed populations of thymocytes in different stages of maturation. Cortical thymocytes, a subpopulation of immature thymocytes, differ from medullary thymocytes, a subpopulation of mature thymocytes, both in the antigens expressed on their surface and in immunological functions. Cortical thymocytes can be agglutinated by PNA, but medullary thymocytes cannot. Cortical thymocytes are sensitive to cortisone, whereas medullary thymocytes are insensitive to cortisone. Using these characteristics, we analyzed the surface antigen pattern on thymocytes in different stages of maturation.

Murine H-2D antigen is a gene product of the major histocompatibility complex located on the 17th chromosome (15). This antigen is present only in low concentration on immature thymocytes and increases in concentration on mature thymocytes (1). TL antigens are specified by the *Tla* locus, which is situated near the *H-2D* locus on the 17th chromosome (16). TL antigens are expressed only on lymphocytes at an early stage of their differentiation and on some leukemic cells (4). $Qa^2/_3$ antigens, whose genes are also located on the 17th chromosome between *H-2D* and *Tla*, are present only on mature thymocytes (17). Another surface antigen, gp70, the major envelope glycoprotein of MuLV is known to be expressed on lymphocytes of certain mouse strains (e.g., C57BL/6-Tla^a) which rarely, if ever, express complete virus (18). The expression of gp70 is also related to the state of differentiation of the cells (4).

These results reveal that there are extremely low levels of H-2 and $Qa^2/_3$ but high levels of TL and gp70 on PNA^+ thymocytes and that there are high levels of H-2 and $Qa^2/_3$ and low levels of TL and gp70 on C^R thymocytes. The surface antigen pattern of PNA^- thymocytes, except for a high gp70, is similar to that of C^R thymocytes. The C^R-thymocyte population represents approximately 3% of the total, whereas the PNA^--thymocyte population after double rosetting represents about 7%. The difference between C^R and PNA^- thymocytes may be due to the presence of some immature thymocytes, which have large amounts of gp70 among the cells of the PNA^--thymocyte population. The surface antigen pattern of unfractionated thymocytes has characteristics of a mixture of PNA^+ and C^R thymocytes.

It is now well established that a functional thymus is an essential requirement for the maturation, proliferation, development, and expression of immunological competence of the T lymphocyte. Although it is still not clear how the thymus exerts control over T-cell development, it appears that a hormonal mechanism may be one component in the system for controlling T-cell differentiation. For the purpose of testing the responsiveness of immature thymocytes to what might be considered intrathymic influences, the changes of surface antigens on the PNA^+ thymocytes under the influence of *in vitro* incubation with some putative thymic hormones or TCGFs were studied.

The thymic factors tested were thymosin fraction V, a relatively crude preparation comprising approximately 35 polypeptides with molecular weights ranging from 1,000 to 15,000 (19); thymosin α_1, a fully defined polypeptide of known sequence consisting of 28 amino acid residues with a molecular weight of 3,108 (20); TP_5, a synthetic pentapeptide corresponding to residues 32–36 of thymopoietin (21); and FTS, a synthetic nonapeptide (22). Each of those factors has been shown to induce T cells to express θ antigen in the Komuro and Boyse assay (refs. 5 and 6; M. Scheid, personal communication). The effects of a T-cell product, TCGF, of both rat (as CR-TCGF) and mouse origin (G-TCGF) to produce changes of surface receptors in PNA^+ thymocytes were also examined. These two

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preparations of TCGF were both able to increase basal thymidine incorporation and to potentiate concanavalin A-induced thymidine incorporation in unfractionated and PNA^- thymocytes.

Some cell surface antigens in PNA^+ thymocytes were changed significantly after a 20-hr incubation with thymosin fraction V because H-2D increased and TL decreased. Only a small change or no change was observed with the other thymic factors tested. The changes indicate that thymosin fraction V contains a component(s) that is (are) not thymosin α_1, TP5, or FTS and that promotes the maturation of cortical thymocytes *in vitro*. The changes are correlated with normal maturation (1). The results indicate that the cortical, immature thymocytes under the influence of thymosin fraction V undergo maturational changes, a finding compatible with the view that hormonal mechanisms play a role in intrathymic development.

No change occurred with $Qa^2/_3$ after incubation with either thymic hormones or TCGF. Even during the normal T-cell maturation, the change of $Qa^2/_3$ was not great in comparison with changes in H-2 expression. $Qa^2/_3$ is present in much lower apparent concentration than H-2, thus $Qa^2/_3$ antigens may not change to a measurable extent under the experimental conditions used. Abbott *et al.* (3) have reported that tumor necrosis factor modulates $Qa^2/_3$ in addition to TL, Thy 1, and H-2 on PNA^+ thymocytes. In PNA^+ thymocytes after incubation with CR-TCGF, the gp70 content also decreased significantly, but this change did not occur after incubation with purified TCGF or thymosin V. This difference is presumably due to the presence of other contaminating components in the less-purified CR-TCGF preparation. What this factor is remains to be determined.

The functional significance of the observed changes in cell surface receptors after induction with thymosin fraction V and TCGF is not clear. The TCGF preparations used in this study increased basal thymidine incorporation and potentiated concanavalin A-induced thymidine incorporation in unfractionated thymocytes (data not shown), as has been demonstrated by others (10). In PNA^+ thymocytes, TCGF increased to a lesser degree both basal and mitogen-induced thymidine incorporation. It is not clear whether this smaller response results from a contamination of cells already responsive to mitogens or whether a small fraction of cells were induced to mature to the point of being mitogen-responsive.

Given the well-established role of TCGF as a comitogen, providing a necessary second signal after initial activation by mitogen (10), and the general consensus that immature thymocytes are not mitogen responsive (9), we would prefer to interpret the influence of both thymosin fraction V and TCGF within the context of differentiation-promoting influences. This interpretation is consistent with what is known about thymic hormones, but represents an alternative concept in the biological action of TCGF. Under the action of TCGF (a factor not known to be present in thymosin fraction V), H-2d and TL increased consistently and significantly on PNA^+ thymocytes. TCGF is released from activated mature T cells and may function as a peripheral signal. Acting on cells in the thymus, it would appear to promote the expression of surface markers characteristic of mature thymocytes and, thus, to induce the maturation of PNA^+ thymocytes. The notion that there exists a signal from the periphery to regulate replenishment of T cells is an appealing one. It further suggests that TCGF may possibly be a factor active in modulating T-cell receptors and, thus, a factor that acts as a critical regulator of intrathymic development. It remains to be determined whether immature thymocytes bear receptors for TCGF and thymic hormones and what mechanisms are involved in their influences at both the cytoplasmic and nuclear level, and what functional changes are consequent to their influences.

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