Depression of prothymosin-α production in murine thymus correlates with staphylococcal enterotoxin-B-induced immunosuppression

Teresa L.K. Low, Tair-Long Pan and Yee-Shin Lin[1]

Departments of Biochemistry and [1]Microbiology, National Cheng Kung University Medical College, Tainan, Taiwan 70101, Republic of China

Received 22 May 1990; revised version received 4 July 1990

Prothymosin-α (ProTα) and thymosin-β_4 (Tβ_4) were isolated from murine thymus and characterized by microsequence analysis. Murine Tβ_4 has an identical sequence to bovine Tβ_4, whereas murine ProTα is highly homologous to rat ProTα. Murine ProTα differs from rat ProTα at two positions, Glu^{100} and Asp^{108} of the rat sequence are substituted by aspartic and glutamic acid, respectively, in murine ProTα. The amount of ProTα in murine thymus was found to be reduced after in vivo treatment with staphylococcal enterotoxin-B (SEB), a superantigen which stimulates T cells bearing specific V_β receptors. Results from the anti-SRBC (sheep erythrocyte) plaque-forming cell assay showed that the antibody response of the spleen cells from these animals was also suppressed. On the other hand, the amount of Tβ_4 was not changed significantly. Our studies suggest that the suppression of SEB on antibody response correlates with the depression of ProTα production in the thymus.

Prothymosin-α; Thymus; Staphylococcal enterotoxin-B; Immunosuppression

1. INTRODUCTION

Thymic hormones play an important role in promoting the maturation and differentiation of lymphoid precursor cells into immunocompetent T cells [1]. Two of the thymic hormones, prothymosin-α (ProTα) and thymosin-β_4 (Tβ_4), have been isolated from various animal and tissue sources [2-5]. ProTα contains thymosin-α_1 [6] sequence at its amino terminus. Biologically, ProTα enhances resistance to opportunistic infections [7] and restores mixed lymphocyte responses [8]. Tβ_4 induces terminal deoxynucleotidyl transferase (TdT) in vivo and in vitro [9] and stimulates release of leuteinizing hormone [10].

Staphylococcal enterotoxin-B (SEB), a superantigen which stimulates T cells bearing specific V_β receptors, has been shown to induce suppressor cell populations which inhibit both humoral and cell-mediated immune responses [11-15]. However, the mechanism of such immunosuppression is not clear. In order to evaluate the role of thymic hormones in SEB-induced immunosuppression, we have determined the concentration of ProTα and Tβ_4 in murine thymus after in vivo treatment of SEB.

Correspondence address: T.L.K. Low, Department of Biochemistry, National Cheng Kung University Medical College, Tainan, Taiwan 70101, Republic of China

Abbreviations: ProTα, prothymosin-α; Tβ_4, thymosin-β_4; SEB, staphylococcal enterotoxin-B; TdT, terminal deoxynucleotidyl transferase; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; SRBC, sheep erythrocytes; PFC, plaque-forming cell; SEA, staphylococcal enterotoxin-A

To identify murine ProTα and Tβ_4, they were first isolated from normal murine thymus and characterized by microsequence analysis. Thymic extracts from SEB-treated groups were prepared and fractionated by reversed phase high-performance liquid chromatography (HPLC). Peaks corresponding to murine ProTα and Tβ_4 were collected and quantitated by amino acid analyses. In addition, the plaque-forming cell responses of the spleen cells were studied. Our results suggest that immunosuppression induced by SEB injection correlates with the thymic hormone modulation.

2. MATERIALS AND METHODS

2.1. *Materials*

Trifluoroacetic acid (TFA) and fluorescamine (Fluram) were obtained from Pierce. Trypsin-TPCK was purchased from Cooper Biomedical. Acetonitrile was a product of Merck (Frankfurt, FRG). All other chemicals were of reagent grade and were used without further purification.

2.2. *Isolation and characterization of murine ProTα and Tβ4*

Murine thymic tissues were excised from young BALB/c mice (4–6 weeks of age). Tissue extract was prepared as described previously [2]. Fresh tissues were cut into pieces and boiled for 5 min in water. The chilled suspension was homogenized with polytron (Brinkman) and centrifuged at 16 000 × *g* for 30 min. The supernatant was filtered, titrated to pH 3 and again centrifuged. The lyophilized crude extract (30 mg by Lowry method [16]) was chromatographed on Sephadex G-100 (1.6 × 100 cm) in 0.01 M ammonium bicarbonate, pH 8.0. Peptides in each protein pool were purified by HPLC (Waters Ass.) using a reverse phase column (μBondapak C_{18} column, 30 × 0.39 cm). Solvent A contained 0.05% TFA and solvent B contained acetonitrile with 0.05% TFA. A linear gradient of 10–25% B in 60 min was used. Amino acid analysis was carried out using a Beckman 6300 analyzer. Digestion of purified peptides with trypsin was performed in 1% ammonium bicarbonate at pH 8.0 for 2.5 h at 37°C.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

Cyanogen bromide cleavage was performed in 70% formic acid. The tryptic digests or cyanogen bromide fragments were fractionated by HPLC. Peptides which were poorly resolved by HPLC were purified by high voltage paper electrophoresis at pH 1.9 (Savant, Farmingdale, NY). Sequence analysis was carried out using a gas-phase sequencer (Applied Biosystems, Model 477A) with an on-line PTH analyzer (Model 120A).

2.3. *In vivo treatment with SEB or saline*

BALB/c mice, 6–8 weeks of age, were obtained from National Cheng Kung University Animal Center. Mice (4 per group) were injected intravenously with 0.2 ml SEB (Sigma, St. Louis, MO, 100 μg/ml in saline) per animal. Each mouse in the control groups received 0.2 ml saline. They were sacrificed 4 h, 1 day, 2 days, 3 days, 4 days or 7 days post SEB injection. In addition, 5 days prior to sacrificing, each animal was also primed with 0.5 ml of 0.1% sheep erythrocytes (SRBC) intraperitoneally.

2.4. *Isolation and quantitation of ProTα and Tα4 in SEB- or saline-treated mice*

Thymus glands were removed immediately from the sacrificed mice. Tissues from each experimental group were pooled and weighed. They were extracted as described above. The crude extracts were fractionated by HPLC. Peaks which corresponded to murine ProTα and Tβ4 were collected, lyophilized and analyzed. The amount contained in each peak was estimated by amino acid analysis.

2.5. *Plaque-forming cell assay*

The anti-SRBC plaque-forming cell (PFC) response was determined by the Cunningham slide modification of the Jerne hemolytic plaque assay [17]. Single cell suspensions were prepared from immunized mouse spleens. Triplicate determinations of the PFC response were performed for each group. Only the direct (IgM) PFC responses were measured. Results are expressed as PFC/10^8 cells.

3. RESULTS AND DISCUSSION

Murine ProTα and Tβ4 were isolated from pooled thymic tissues by heating, homogenization, centrifugation, gel-filtration and HPLC on reverse phase C_{18} column. The yield of purified Tβ4 was 50 μg/g wet thymic tissue and 95 μg/g for purified ProTα. The primary structures of murine Tβ4 and ProTα were determined by sequence analyses of purified tryptic peptides and cyanogen bromide fragments. As shown in Fig. 1, the amino acid sequence of murine Tβ4 is identical to bovine or porcine Tβ4 [2,5]. The primary structure of murine ProTα was determined except for a segment consisting of residues 53–89. It contains the thymosin-α1 at its amino terminus. Murine ProTα differs from rat ProTα [18] at two positions, Glu^{100} and Asp^{108} of the rat sequence are substituted by aspartic acid and glutamic acid, respectively, in murine ProTα.

We next investigated the effects of SEB treatment on the thymic hormone production in the thymus. Thymic extracts were prepared from each experimental group and fractionated by HPLC. Characterization of peaks 1 and 2 in Fig. 2 as Tβ4 and ProTα, respectively, has been accomplished by: (i) amino acid compositional analyses; and (ii) comparison of retention time in HPLC elution profiles with those of purified murine Tβ4 and ProTα. Figs. 2A–D show the HPLC elution profiles of crude thymic extracts prepared from mice which were sacrificed 4 h, 2 days, 3 days or 7 days post SEB injection. Figs. 2E–H are the profiles from saline-treated groups. Table I lists the quantitative results calculated from amino acid analyses. The concentration of ProTα in the thymus was reduced two days post SEB injection. The lowest value was reached at 3 days. The changes were not as obvious for Tβ4 with only slight increases at 1 day to 3 days post SEB injection. Finally, we studied the effect of in vivo SEB treatment on the immune response in the spleen using an anti-SRBC plaque-forming cell (PFC) assay. Our results, as shown in Table II, showed that the anti-SRBC PFC response was suppressed when SEB was given to mice for 2 days, but not for 1 day or 4 h. The suppressive activity induced by SEB sustained up to 7 days. Taken together, our data suggest that there is a good correlation between the depression of ProTα production in the thymus and the suppression of antibody response in the spleen which was mediated by SEB treatment.

It has been demonstrated that suppressor cell populations, capable of suppressing both humoral and cell-mediated immune responses, are activated by SEB [11–15]. Furthermore, the SEB-induced mitogenesis requires the participation of both Ia and the T-cell antigenic receptor complex [19–21]. The activation of suppressor cells with SEB appears to occur through the induction of the T-cell subpopulation expressing the $Ly\text{-}1^+,2^-$, $Ly\text{-}1^-,2^+$ or $Ly\text{-}1^+,2^+$ cell surface phenotypes. However, the mechanism of such immunosuppression is not clear. Most recently, Marrack et al. [22] provided evidence that the toxicity of SEB in mice is mediated by T cells. They also suggested that the SEB-activated T cells secrete different lymphokines which in turn exert toxic effects. Our studies suggest that such immunosuppression or toxic effects might involve the modulation of ProTα and other thymic hormones in the thymus.

By Northern blot analysis, ProTα mRNA was found to be most abundant in the thymus [23]. However, the ProTα gene is also expressed in many other tissues including ovary, kidney, brain, heart, intestine, lung and spleen, suggesting a general role for this protein. In thymus, ProTα gene expression changes during the maturation of T-lymphocytes, increasing from pre-T cells to immature thymocytes and decreasing in mature thymocytes, spleen Ig^- lymphocytes and circulating lymphocytes. Thus, the change in ProTα concentration in the thymus upon SEB stimulation might represent a process of T-cell activation and differentiation which resulted a redistribution of T-cell populations. Furthermore, it has been reported that several-fold increase of ProTα mRNA was induced upon in vitro treatment of human peripheral blood lymphocytes with staphylococcal enterotoxin A (SEA) [24]. The differential effect exerted by these enterotoxins on the thymus and peripheral blood lymphocytes suggests the existence of different mechanisms due to variations in cell popula-

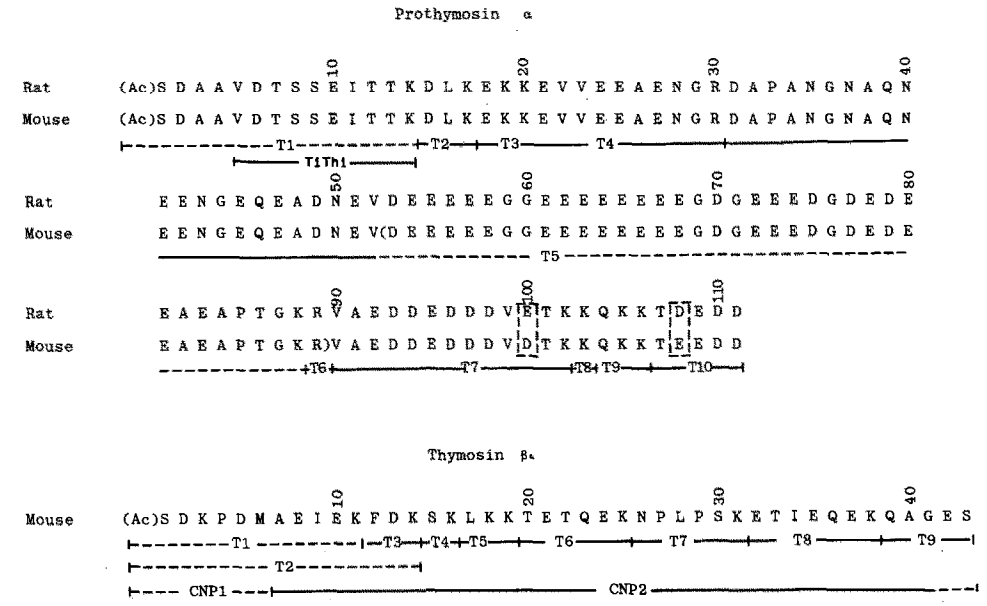

Fig. 1. The amino acid sequence of murine prothymosin-α (ProTα) and thymosin-β_4 (Tβ_4). Peptides generated by treatment with trypsin, thermolysin and cyanogen bromide are designated T, Th and CN, respectively. Sequences established by automated sequence analysis are shown by solid lines. Unsequenced portions are shown by dashed lines. The N-terminal ends of both ProTα and Tβ_4 were blocked and were not identified. The tryptic peptides of murine ProTα are placed by homology with the sequence of rat ProTα [18]. Residues differing between the two ProTα molecules are boxed.

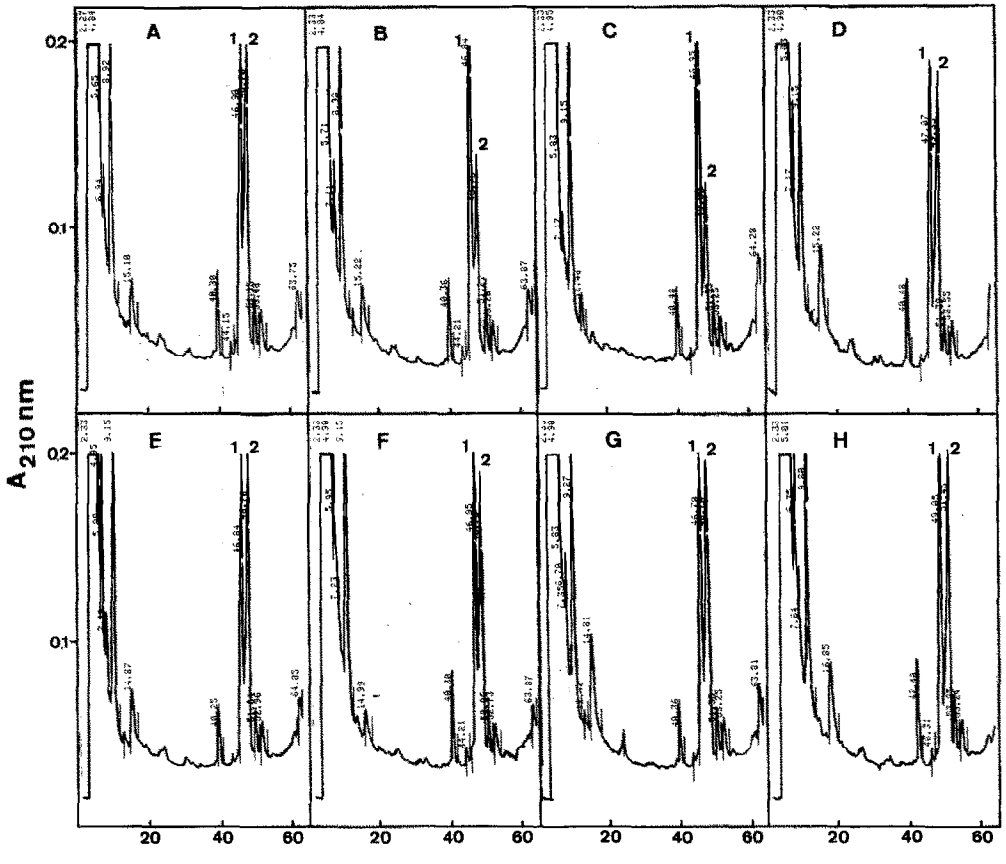

Fig. 2. Effect of in vivo SEB treatment on ProTα production in murine thymus. (A) Mice were sacrificed 4 h post-SEB injection. Crude thymic extract was prepared and fractionated by HPLC. Peaks 1 and 2 were identified as Tβ_4 and ProTα, respectively. The peaks were collected and quantitated by amino acid analysis. (B–D) HPLC profiles of thymic extracts from mice which were sacrificed 2 days, 3 days and 7 days post SEB injection. (E–H) HPLC profiles of thymic extracts from saline-treated groups. Mice were sacrificed 4 h, 2 days, 3 days and 7 days post saline treatment.

Table I

Concentration of ProTα in murine thymus after in vivo treatment with SEB

Days after treatment[a]	ProTα[b]			Tβ4[b]	
	SEB	Saline	% Decrease	SEB	Saline
Exp. 1					
1/6	140	137	−2	62	54
1	118	123	4	62	51
2	68	126	46	60	57
3	56	137	59	63	56
4	105	113	7	55	55
7	113	118	4	46	48
Exp. 2					
1/6	130	126	−3	42	43
1	106	113	6	58	41
2	52	120	57	50	45
3	46	127	64	57	44
4	85	99	14	41	45
7	95	109	13	36	34

[a]Amounts expressed as μg/g thymus tissue, based on amino acid analysis result of HPLC separated peptides. Mice without saline treatment contained 130 μg of ProTα and 55 μg of Tβ4 per gram of thymus tissue. Results from two experiments are shown. [b]BALB/c mice were treated with 20 μg SEB in 0.2 ml or with 0.2 ml saline i.v. on various days

Table II

Effect of in vivo SEB treatment on anti-SRBC plaque-forming cell response

Days after treatment[a]	PFC/10^8 cells (± SD)[b]		
	SEB	Saline	% Decrease
1/6	33 060 ± 1850	40 530 ± 1730	18
1	22 300 ± 730	23 060 ± 2180	3
2	370 ± 170	30 450 ± 2320	99
3	3 910 ± 1160	44 560 ± 3680	91
4	2 250 ± 350	28 830 ± 2500	92
7	1 860 ± 1790	50 440 ± 2500	92

[a]BALB/c mice were treated with 20 μg SEB in 0.2 ml or with 0.2 ml saline i.v. on various days

[b]Mice were immunized with 0.5 ml of 0.1% SRBC i.p. and the anti-SRBC plaque-forming cell (PFC) responses were determined after 5 days. The PFC response for positive control (without saline or SEB treatment) was 35 900 ± 1350/10^8 splenocytes, and the negative control was 50 ± 50/10^8 splenocytes. Average from a group of 5 mice in a representative experiment is shown (± SD)

tions. It was found that the depression of ProTα appeared on days 2 and 3 after SEB treatment and returned to normal on day 4, whereas the anti-SRBC plaque-forming cell response was sustained for a longer period of time. The possible explanations for these observations include: (i) these two events may occur via different mechanisms; (ii) the anti-SRBC response may be the secondary effect which lasts longer due to the induction of suppressor cells or other specific suppressions. Further studies are needed to reveal the significance of the correlation between ProTα and the immune response mediated by SEB.

Acknowledgements: This research is supported in part by Grants NSC 78-0412-B006-15, NSC 78-0412-B006-33 and NSC 79-0412-B006-11 from the National Science Council, Republic of China. The skillful technical assistance of Shu-Chen Hu is gratefully acknowledged.

REFERENCES

[1] Low, T.L.K. and Goldstein, A.L. (1984) in: Immune Modulation Agents and Their Mechanisms (Fenichel, R.L. and Chirigos, M.A. eds) pp. 135–162, Marcel Dekker, New York.
[2] Low, T.L.K. (1988) Asia Pacific Commun. Biochem. 2, 93–102.
[3] Pan, L.X., Haritos, A.A., Wideman, J., Komiyama, T., Chang, M., Stein, S., Salvin, S.B. and Horecker, B.L. (1986) Arch. Biochem. Biophys. 250, 197–201.
[4] Haritos, A.A., Blacher, R., Stein, S., Caldarella, J. and Horecker, B.L. (1985) Proc. Natl. Acad. Sci. USA 82, 343–346.
[5] Low, T.L.K. and Goldstein, A.L. (1982) J. Biol. Chem. 257, 1000–1006.
[6] Low, T.L.K. and Goldstein, A.L. (1979) J. Biol. Chem. 254, 987–995.
[7] Haritos, A.A., Goodall, G.J. and Horecker, B.L. (1984) Proc. Natl. Acad. Sci. USA 81, 1008–1011.
[8] Baxevanis, C.M., Reclos, G.J., Panneerselvam, C. and Papamichail, M. (1988) Immunopharmacology 15, 73–84.
[9] Hu, S.K., Low, T.L.K. and Goldstein, A.L. (1981) Mol. Cell. Biochem. 41, 49–58.
[10] Rebar, R.W., Miyake, A., Low, T.L.K. and Goldstein, A.L. (1981) Science 214, 669–671.
[11] Donnelly, R.P. and Rogers, T.J. (1982) Cell. Immunol. 72, 166–177.
[12] Lin, Y.S., Patel, M.R., Linna, T.J. and Rogers, T.J. (1986) Cell. Immunol. 103, 147–159.
[13] Lin, Y.S. and Rogers, T.J. (1986) Cell. Immunol. 102, 299–306.
[14] Holly, M., Lin, Y.S. and Rogers, T.J. (1988) Immunology 64, 643–648.
[15] Taub, D.D., Lin, Y.S., Hu, S.C. and Rogers, T.J. (1989) J. Immunol. 143, 813–820.
[16] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.

[17] Cunmingham, A.J. and Szenberg, A. (1968) Immunology 14, 599–600.
[18] Frangou-Lazaridis, M., Clinton, M., Goodall, G.J. and Horecker, B.L. (1988) Arch. Biochem. Biophys. 263, 305–310.
[19] Fleischer, B. and Schrezenmeier, H. (1988) J. Exp. Med. 167, 1697–1707.
[20] Fraser, J.D. (1989) Nature 339, 221–223.
[21] White, J., Herman, A., Pullen, A.M., Kubo, R., Kappler, J.W. and Marrack, P. (1989) Cell 56, 27–35.
[22] Marrack, P., Blackman, M., Kushnir, E. and Kappler, J. (1990) J. Exp. Med. 171, 455–464.
[23] Gomez-Marquez, J., Segade, F., Dosil, M., Pichel, J.G., Bustelo, X.R. and Freire, M. (1989) J. Biol. Chem. 264, 8451–8454.
[24] Eschenfeldt, W.H. and Berger, S.L. (1986) Proc. Natl. Acad. Sci. USA 83, 9403–9407.