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The biological effect of three thymosin fraction 5 polypeptides in the murine mixed lymphocyte reaction

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Summary. The biological effects of three thymosin fraction 5 polypeptides, designated as β_{10}, β_4, and α_1 were tested in the MLR of mouse splenocytes, lymph node cells and thymocytes against syngeneic or allogeneic stimulators. It was found that all three polypeptides, after *in vivo* and *in vitro* treatment of the responder cell population, could enhance the allogeneic MLR. These polypeptides were also able to induce significant syngeneic MLR in systems where responder cells were used against irradiated syngeneic splenocytes. In addition, while β_4 was shown to have a weak stimulatory effect on allogeneic MLR utilizing thymocytes as the responder cell type, α_1 could strongly induce such responses when syngeneic splenocytes were included into the culture system.

Preincubation of purified mature T cells or thymocytes with α_1 has shown these cells to be the target of this polypeptide action. Thus, it appears that thymosin fraction 5 polypeptides not only initiate differentiation processes of immature T cells, but also exert their effects on mature T lymphocytes.

Abbreviations; α_1, thymosin fraction 5 polypeptide α_1; APC, antigen-presenting cells; β_4, thymosin fraction 5 polypeptide β_4; β_{10}, thymosin fraction 5 polypeptide β_{10}; FCS, fetal calf serum; LN, lymph node cells; MLR, mixed lymphocyte reaction; PBS, phosphate-buffered saline; SC, spleen cells; S-MLR, syngeneic mixed lymphocyte reaction.

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INTRODUCTION

Previous studies based on the restoration of immunological competence in thymectomized animals utilized thymic grafts or cell-free extracts, suggested the existence of thymus extracts, referred to as thymosin, capable of inducing differentiation of immature cells of bone marrow origin into mature lymphocytes with functional activities (Bach *et al.*, 1971; Goldstein, Slater & White, 1966). Fractionation of thymus extracts resulted in factors with diverse biological properties, such as *in vivo* lymphopoietic activity (Klein, Goldstein & White, 1965), *in vitro* induction of helper and suppressor cells in the sheep red blood cell system (Ahmed *et al.*, 1979), increased sensitivity of bone marrow rosette-forming cells to a certain drug (Goldstein *et al.*, 1972) induction of Lyt surface markers on bone marrow cells from nu/nu mice (Ahmed *et al.*, 1979), induction of *in vitro* antibody responses in athymic mice (Armerding & Katz, 1975) and influence on *in vitro* proliferative responses of human and mouse lymphocytes (Ahmed *et al.*, 1979; Kaufman, 1980).

A partially purified thymosin preparation termed 'thymosin fraction 5' has been extensively studied for biological activity, as well as in clinical trials (Goldstein, Low & Thurman, 1980). Thymosin fraction 5 is a potent immunopotentiating preparation and can act in lieu of the thymus gland to reconstitute some immune functions in thymus-deprived or immuno-

deprived individuals. Analytical polyacrylamide gel electrophoresis and isoelectric focussing have demonstrated that fraction 5 consists of 10–15 major components, and 20 or more minor components with molecular weights between 1000 and 15,000 (Bach, Dardenne & Pleau, 1977).

Although it appears to be clear that thymus factors act on thymus-derived lymphocytes inducing differentiation and activation processes, the mechanism of thymosin action on the immune system has not been elucidated. Thus, a major effort in our studies is to understand the cellular events by which the thymus gland may exert control over T-cell development and activity.

The first attempt in this direction was undertaken to assess modulation of the *in vitro* proliferative responses of mouse lymphocytes, taken from various lymphoid organs, against allogeneic or syngeneic targets in the mixed lymphocyte reaction. In this respect, three different polypeptides were tested, designated as β_4 (Low, Hu & Goldstein, 1981), β_{10} (Erickson Vitanen *et al.*, 1984), and α_1 (Low *et al.*, 1979). These peptides were originally isolated from thymosin fraction 5, but were also found (β_4, β_{10}) in a number of other cell types (Hannapel *et al.*, 1982; Erickson-Vitanen *et al.*, 1984). The data presented in this work show that not only thymocytes, but also mature thymus-derived lymphocytes are influenced in their biological activity by these polypeptides. These studies establish new possibilities to test the activity of thymosin fraction 5 polypeptides. In addition, they impart more understanding of the target cell for thymic factors and the possible mechanism of their action.

MATERIALS AND METHODS

Mice

Males of strains DBA/1, AKR and BALB/c were obtained from our animal colony at the Hellenic Anticancer Institute.

Injection protocol

Mice were injected once intraperitoneally with 65 ng of the thymosin fraction 5 polypeptides in 1 ml of PBS.

Thymosin fraction 5 polypeptides

All three polypeptides were kindly provided by Dr B. L. Horecker at the Roche Institute of Molecular Biology, Nutley, NJ 07110. For a description of procedures used for the isolation of each one of these polypeptides, see Low *et al.*, 1981 (for β_4), Erickson-Vitanen *et al.*, 1984 (for β_{10}) and Low *et al.*, 1979 (for α_1).

Cell preparation

Single cell suspensions were prepared from the spleen, inguinal and para-aortic lymph nodes and the thymus 3 days after the injection of the polypeptides. T cells were obtained from spleen cells which were passed through a nylon-wool column (Baxevanis *et al.*, 1982), and then were incubated for 1 hr at room temperature on plastic dishes coated with goat anti-mouse immunoglobulin (Sigma) (Mage, McHugh & Rothstein, 1977). Non-adherent cells were harvested, tested for the expression of Thy 1.2 antigen by indirect immunofluorescence (98% were Thy 1.2 positive), and used as a source of T cells. Irradiated (3300 rads) spleen cells served as stimulators and, when indicated, as the source of antigen-presenting cells (APC).

Lymphocyte cultures

The culture medium was RPMI 1640 supplemented with 10% FCS, antibiotics, 2 mM L-glutamine, and 5×10^{-5} M 2-mercaptoethanol. Treatment of cells with the peptide α_1 was carried out by 16-hr incubation of 4×10^6 cells per ml in 50-ml flasks in a total volume of 5 ml culture medium. The final concentration of the polypeptide was 12·5 ng/ml. The treated cells were washed three times at 1000 r.p.m. for 10 min before being added to the cultures.

In the mixed lymphocyte reaction, cell cultures were set up in a total volume of 0·2 ml in microculture plates (Sterilin), consisting of 3×10^5 responders and 6×10^5 irradiated (3300 rads) splenocytes which served as stimulators and, where indicated, 1×10^5 irradiated (3300 rads) splenocytes syngeneic to the responders served as the source of APC. In the same volume, the polypeptides were also included at a final concentration of 75 ng/ml for β_{10} and β_4 and 25 ng/ml for α_1. The cultures were kept for 4 days at 37° in a humidified atmosphere of 5% CO_2. One μCi of [^{3}H]methyl thymidine was added in 50 μl of culture medium for the last 16–24 hr of culture.

RESULTS

Effect of thymosin fraction 5 polypeptides on allogeneic MLR

Spleen cells from DBA/1 mice are able to develop

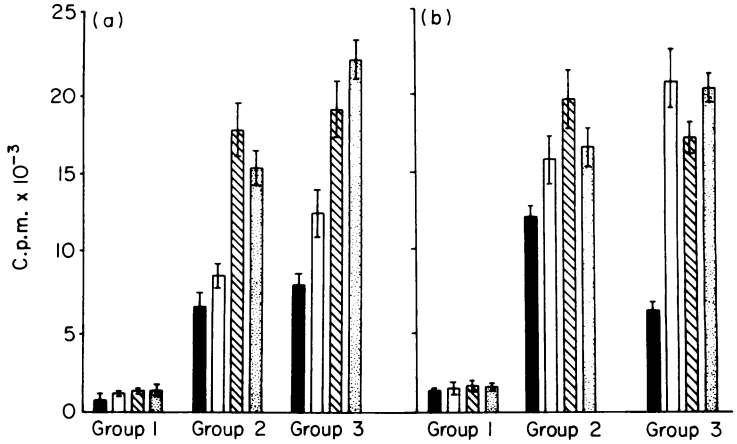

Figure 1. (a) DBA/1 males were injected once intraperitoneally with 65 ng of β_{10}, β_4 and α_1 in 1 ml PBS or with PBS alone. Three days later, SC from each group were collected, adjusted to 3×10^6/ml and tested for their ability to react against irradiated (3300 rads) BALB/c or AKR SC (6×10^6/ml). All groups were set up in triplicate: (■), PBS; (□), β_{10}; (□), β_4; (▤), α_1. Group 1, DBA/1 SC; Group 2, DBA/1 sc anti-BALB/c SC; Group 3, DBA/1 SC anti-AKR. (b) Fresh DBA/1 SC (3×10^5/well) were cultured as responders against AKR or BALB/c irradiated (3300 rads) stimulators (6×10^5/well) in a total volume of 200 μl for 4 days in microplates. The polypeptides β_{10}, β_4 (15 ng/well) and α_1 (5 ng/well) were included in the same volume (200 μl/well) for the entire culture period. (■), RPMI; (□), β_{10}; (□), β_4; (▤), α_1. Group 1, DBA/1 SC; Group 2, DBA/1 SC anti-BALB/c SC; Group 3, DBA/1 SC anti-AKR SC. Counts per minute (c.p.m.) refer to the mean of three independently performed experiments ± the standard deviation (SD).

significant primary MLR to allogeneic irradiated stimulators, such as splenocytes from AKR or BALB/c mice. However, this response could be enhanced by three different thymosin fraction 5 polypeptides. This is shown in two different sets of experiments with either the polypeptides injected *in vivo* or added into the cell cultures *in vitro* (Fig. 1a and 1b). DBA/1 mice were split in four groups, each consisting of four mice injected with the polypeptides β_4, β_{10} and α_1 or PBS as a control. Pooled spleen cells from each group were tested *in vitro* for their ability to mediate MLR against irradiated splenic stimulators from the AKR and BALB/c strains. Fig. 1a shows that splenic DBA/1 responses against the allogeneic BALB/c or AKR stimulators significantly enhanced in the presence of β_4 and α_1, whereas the polypeptide β_{10} showed a weaker enhancing effect. In another experiment, we tested the effect of the three polypeptides on the same allogeneic combination when added into the cell cultures *in vitro*. As shown in Fig. 1b, no significant difference could be noticed in terms of the enhancing effect for all three polypeptides. Finally, when DBA/1 inguinal and para-aortic lymph node cells were used as responders against the allogeneic AKR stimulators, the α_1 but not the β_4 (B_{10} was not tested) polypeptide enhanced the response (both tested *in vitro*) (Fig. 2).

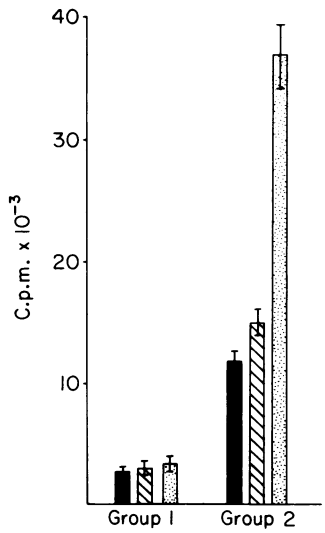

Figure 2. Fresh DBA/1 LN were cultured as responders (3×10^5 well) against AKR irradiated (3300 rads) SC (6×10^5/well): (■), RPMI; (□), β_4; (▤), α_1. Group 1, DBA/1 LN; Group 2, DBA/1 LN anti-AKR SC. C.p.m. and SD as for Fig. 1.

Effect of peptides on syngeneic MLR

The experiments just described indicate that three distinct thymosin fraction 5 polypeptides are capable

Table 1. Response of DBA/1 spleen cells (SC) or lymph node cells (LN) against irradiated syngeneic stimulators after *in vitro* and *in vivo* treatment with thymosin fraction 5 polypeptides

Agent	DBA/1 SC & DBA/1 SC (c.p.m.)*	DBA/1 LN & DBA/1 SC	DBA/1 SC (c.p.m.)	DBA/1 LN (c.p.m.)
(a)				
PBS	1015±205	NT‡	801±45	NT
β_{10}	*5947±1280*†	NT	1108±186	NT
β_4	*20,111±2890*	NT	1635±546	NT
α_1	*9462±2022*	NT	1597±250	NT
(b)				
RPMI	2498±649	1012±159	1155±98	1527±512
β_{10}	*8124±1059*	NT	1900±133	NT
β_4	*12,004±2690*	*7164±236*	1297±403	1507±222
α_1	*7282±1418*	*3936±231*	1440±253	1542±331

DBA/1 males were injected intraperitoneally with 65 ng of β_{10}, β_4 and α_1, or PBS as a control. After 3 days, SC from each group (3×10^5/well) were separately tested as responders against (a) irradiated (3300 rads) DBA/1 SC (6×10^5/well), or (b) fresh DBA/1 SC or LN cells were separately tested as responders against DBA/1 SC (3300 rads) in the presence of β_{10}, β_4 (15 ng/well of each) and α_1 (5 ng/well). C.p.m. refer to the mean of 3–4 independently performed experiments ± the standard deviation (SD).

* Mean c.p.m. ± SD of four replicate experiments.

† Values in italics are significantly higher than corresponding control values ($P < 0{\cdot}05$).

‡ NT, not tested.

of increasing the responding status of DBA/1 splenocytes against allogeneic stimulators. We next asked the question whether substances exerted comparable effects on responses against self class II products (Ia), referred to as syngeneic MLR (Glimcher *et al.*, 1981; Yamashita, Ono & Nakamura, 1982). Testing DBA/1 spleen cells treated *in vivo* (Table 1a) or *in vitro* (Table 1b) with β_4, β_{10} and α_1 for responses against syngeneic irradiated splenocytes, we found that all three polypeptides could induce S-MLR, with the strongest effect seen in the presence of β_4. Syngeneic MLR could also be induced when DBA/1 lymph node cells were used as responders. The stimulation obtained here was, however, lower than that obtained with splenocytes as responders (Table 1b).

Effect of peptides on MLR using thymocytes as responders

The results presented thus far demonstrate a substantial effect of the thymosin fraction 5 polypeptides in the development of stronger reactivities of splenocytes and lymph node cells against allogeneic and syngeneic stimulators. The next step was to investigate the effect of the isolated polypeptides on lymphocytes at less mature stages of functional differentiation. Accordingly, we analysed the biological activity of α_1 and β_4 on the allogeneic and syngeneic MLR of DBA/1 thymocytes against irradiated AKR or DBA/1 splenocytes. The data in Fig. 3 show that, in the presence of the polypeptide β_4, significant, although low, responses against syngeneic stimulators could be performed (Groups 3 and 5). In contrast, no effect could be demonstrated when the polypeptide α_1 was added into the cultures (Groups 3 and 5). Testing the allogeneic response, we found that β_4 could activate the DBA/1 thymocytes against the AKR stimulators, whereas α_1 showed no significant effect (Group 6). Surprisingly, when 1×10^5 DBA/1 irradiated splenocytes were added into the cultures (actually a range of $0{\cdot}5 \times 10^5$–$2{\cdot}5 \times 10^5$ splenocytes added per well showed equal effects (data not shown)) then, in the presence of α_1, significant high responses could be performed, whereas no substantial differences could be seen in the presence β_4 (Group 4).

Effect of the polypeptide α_1 on purified T-cell populations and thymocytes

The mixed lymphocyte reaction is known to be a T-cell mediated response (Conen & Howe, 1973; Rich & Rich, 1974). In order to confirm the role of the T cell as the target cell of the action of the polypeptides, we designed experiments in which highly purified DBA/1 cells were incubated for a period of 16 hr with the polypeptide α_1 at a final concentration of 12·5 ng/ml. T

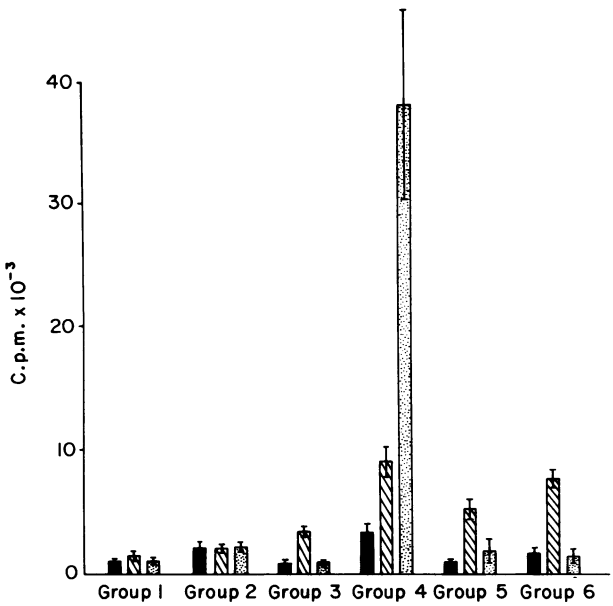

Figure 3. As for Fig. 1. Here, DBA/1 thymocytes (3×10^5/well) were used as responders against irradiated AKR or DBA/1 SC (6×10^5/well). Where indicated, 1×10^5 irradiated syngeneic DBA/1 SC were added into the culture. (■), RPMI; (□), β_4; (▦), α_1. Group 1, DBA/1 Thymocytes (Thy); Group 2, DBA/1 Thy+DBA/1 SC (c.p.m.) SC*; Group 3, DBA/1 Thy+ DBA/1 SC* anti-DBA/1 SC; Group 4, DBA/1 Thy+ DBA/1 SC* anti-AKR SC; Group 5, DBA/1 Thy anti-DBA/1 SC; Group 6, DBA/1 Thy anti-AKR SC. The cell number in every group was kept constant by the addition of irradiated (3300 rads) responders (e.g. in the first group, 7×10^5 irradiated thymocytes were added/well).

* 1×10^5 of DBA/1 SC were added per well.

cells were then extensively washed to remove unbound polypeptides and tested for allogeneic responses against the irradiated AKR stimulators in the presence or absence of syngeneic irradiated splenocytes. As a control, DBA/1 cells were incubated with culture medium alone. In order to see whether the α_1 polypeptide was acting on the responder T-cell population via the syngeneic APC (included in the splenic population) or even via the allogeneic stimulators, the DBA/1 and/or the AKR irradiated splenocytes were tested (similarly to the T cell with α_1 (or RPMI as a control) and then were used in the MLR assay.

As can be seen from Table 2, significant anti-AKR responses were obtained only in the presence of DBA/1 irradiated splenocytes (Groups 1–4). As demonstrated in Groups 15–18, the treatment of responder T cells with α_1 resulted in a three- to four-fold enhancement of the allogeneic MLR. The treatment with α_1 of the irradiated DBA/1 or AKR splenic populations appeared to have no effect on the outcome of the MLR (Groups 5–9). Irradiated splenocytes (treated or non-treated) did not show any reactivity when cultured together or alone (Groups 19–26). Finally, treated T cells did not proliferate in the absence of either the syngeneic macrophages or the stimulators, or both (Groups 10–14). Similar data were obtained when thymocytes were used as the responder population (Table 3). These data clearly demonstrate that mature thymus-derived lymphocytes (T cells) and precursor T cells (thymocytes) are the direct targets of at least one thymosin fraction 5 polypeptide (α_1).

DISCUSSION

In the experiments reported here, we have investigated the implication of three polypeptides, α_1, β_4 and β_{10}, of thymosin fraction 5 in the regulation of the immune response. For this, we chose a functional assay detecting proliferative responses of responder cells against irradiated stimulator cells (referred to as MLR) (Rich & Rich, 1976) where T cells (as responders) are activated by the recognition of foreign

Table 2. Effect of α_1 on DBA/1 T cells

Groups	Cells added into the cultures* DBA/1 T cells	DBA/1 Spleen cells	AKR Spleen cells	C.p.m.†
1	+	−	−	824±68
2	+	+	−	1105±147
3	+	−	+	2618±518
4	+	+	+	16,900±5715
5	+	+‡	−	2116±215
6	+	−	+‡	2074±725
7	+	+‡	+‡	15,446±4149
8	+	+‡	+	18,553±7341
9	+	+	+‡	18,505±4316
10	+‡	−	−	1581±532
11	+‡	+	−	1748±430
12	+‡	+‡	−	1858±136
13	+‡	−	+	2483±482
14	+‡	−	+‡	1848±548
15	+‡	+	+	*64,589±8171*§
16	+‡	+‡	+	*67,544±5764*
17	+‡	+	+‡	*65,282±7496*
18	+‡	+‡	+‡	*69,443±8856*
19	−	+	+	1468±305
20	−	+‡	+‡	1259±319
21	−	+	−	1155±350
22	−	+‡	−	1553±423
23	−	−	+‡	1562±291
24	−	−	+	1035±18
25	−	+‡	+	1096±228
26	−	+	+‡	1533±609

DBA/1 T cells (3×10^5/well) were cultured as responders against irradiated (3300 rads) AKR SC (6×10^5/well) in the presence of irradiated (3300 rads) DBA/1 SC (1×10^5/well) for 4 days in microculture plates in a total volume of 200 μl. As controls, DBA/1 T cells were cultured alone, or with AKR SC (3300 rads), or with DBA/1 SC (3300 rads). As further controls, irradiated AKR or DBA/1 SC were cultured alone or in mixtures.

* The number of cells in Groups 1–18 was kept constant by the addition of irradiated (3300 rads) responder cells (e.g. in Group 1, 7×10^5 irradiated DBA/1 T cells were added per well).

† Mean c.p.m. ± SD of four replicate experiments.

‡ The corresponding cell population was treated with α_1 (see Materials and methods).

§ Values in italics are significantly higher than corresponding control values ($P < 0.05$).

Table 3. Effect of α_1 on DBA/1 thymocytes

Group	Cells added into the cultures* DBA/1 Thymocytes	DBA/1 Spleen cells	AKR Spleen cells	C.p.m.†
1	+	−	−	494±177
2	+	+	−	834±219
3	+	−	+	797+199
4	+	+	+	677±394
5	+	+‡	−	754±182
6	+	−	+‡	659±109
7	+	+‡	+‡	778±225
8	+	+‡	+	696±149
9	+	+	+‡	966±71
10	+‡	−	−	687±289
11	+‡	+	−	1163±567
12	+‡	−	+	1195±250
13	+‡	+	+	9569±1819§
14	+‡	+‡	+	*11,542±2986*
15	+‡	+	+‡	*9549±2505*
16	+‡	+‡	+‡	*12,281±2934*

Details and footnotes as for Table 2, except that DBA/1 thymocytes were used as responders.

class II products (Ia) on the allogeneic stimulators (B cells or macrophages).

In the course of our studies using two different protocols, we have been able to demonstrate both *in vivo* and *in vitro* enhancing effects by all three polypeptides in allogeneic responses (Fig. 1a, b and Fig. 2.). Irrespective of the method used, the biological effect obtained was almost identical, except in the case of β_{10}. This discrepancy, namely that β_{10} showed high biological activity *in vitro* but only a weak one when administered *in vivo*, could be explained by a possible neutralization of the activity of this polypeptide *in vivo* due to plasma factors. Alternatively, it could be that concentration effects were responsible. Our recent data support the latter possibility, since increased doses of β_{10} injected *in vivo* have shown significant enhancing effects on the allogeneic MLR (C. N. Baxevanis *et al.*, unpublished data). In addition, the finding that β_4, although enhancing the allogeneic responses of DBA/1 splenocytes, does not have any effect on allogeneic responses of DBA/1 lymph node cells, may suggest a differentiation on the level of T-cell clones sensitive to various thymosin polypeptides.

Our results, so far, are in agreement with previous reports in the mouse system (Armerding & Katz, 1975; Kaufman, 1980), where it was reported that thymic

factors could increase the reactivity of murine or human lymphocytes against histoincompatibile stimulators.

Interestingly enough, these polypeptides were able to induce syngeneic MLR *in vitro* (Table 1, Fig. 3) in cultures where the concentration of self-Ia was not, by itself, high enough to generate such anti-self responses (Glimcher *et al.*, 1981; Yamashita *et al.*, 1982). This finding is of importance since (i) it bypasses an artificial system (high enrichment for Ia^+ cells) of inducing self-MLR *in vitro*, and (ii) various treatments of T-cell subpopulations with the relevant polypeptides will induce an activation pathway towards self-Ia products where the various T-cell types and T-T interactions involved in it will be easier to analyse.

Another interesting finding in the present work was the ability of α_1 to induce strong allogeneic MLR in thymocyte cultures only when syngeneic irradiated spleen cells were co-cultured (Fig. 3). Although it is early to draw any conclusions from these data, it can be suggested that α_1, in the presence of syngeneic regulatory cells, may have a specific effect on the differentiation of thymocytes into functional mature T cells. This finding also suggests the requirement of macrophages to present alloantigens to syngeneic responder T cells (see also Table 2).

The studies with thymocytes have also shown that in the presence of β_4, a two-fold stimulation could be obtained, irrespective of whether syngeneic irradiated splenocytes were present in the cultures. We believe that this is the first report directly proving that two different thymosing fraction 5 polypeptides exert different biological effects. In addition, the stimulation obtained with α_1 plus syngeneic splenocytes was significantly higher than that obtained with β_4 in the presence or absence of syngeneic splenocytes. This could indicate either the presence of two distinct subsets among the thymocyte population, each one reactive to one of the polypeptides but never to both, or that both polypeptides act on the same population with the α_1 acting on a more mature cell which arises after the action of β_4 on the precursor cell. Work is in progress to elucidate these possibilities.

In conclusion, it is clear that factors such as the thymosin fraction 5 polypeptides β_{10}, β_4 and α_1 can alter significantly the cellular interaction controlling the immune system, thus resulting in functionally important changes. The mode of action of these polypeptides, as well as the cell–cell interactions involved, are now under investigation.

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REFERENCES

AHMED A., WONG D.M., THURMAN G.B., LOW T.L.K. & GOLDSTEIN A.L. (1970) T-lymphocyte maturation: cell surface markers and immune function induced by T-lymphocyte cell-free products and thymosin polypeptides. *Ann. N.Y. Acad. Sci.* **332,** 81.

ARMERDING D. & KATZ D.H. (1975) Activation of T and B lymphocytes *in vitro*. IV. Regulatory influence on specific T cell functions by a thymus extract factor. *J. Immunol.* **114,** 1248.

BACH J.F., DARDENNE M., GOLDSTEIN A.L., GULA A. & WHITE A. (1971) Appearance of T-cell markers in bone-marrow resette-forming cells after incubation with thymosin, a thymic hormone. *Proc. natn. Acad. Sci. U.S.A.* **68,** 2734.

BACH J.F., DARDENNE M. & PLEAU J.M. (1977) Biochemical characterization of a serum thymic factor. *Nature (Lond.)*, **266,** 55.

BAXEVANIS C.N., ISHII N., NAGY Z.A. & KLEIN J. (1982) H-2 controlled suppression of T-cell response to LDH-B. Characterization of the LDH-B suppressor pathway. *J. exp. Med.* **156,** 822.

CONEN L. & HOWE M.L. (1973) Synergism between subpopulations of thymus-derived cells mediating the proliferative and effector phases of the mixed lymphocyte reaction. *Proc. natn. Acad. Sci. U.S.A.* **70,** 2707.

ERICKSON-VITANEN S.E., RUGGIERI S., NATALINI P. & HORECKER B.L. (1984) *Thymosin β_{10}, A New Analogue of Thymosin β_4 in Mammalian Tissues*, Vol. 9. Academic Press, New York (in press).

GLIMCHER L.H., LONGO D.L., GREEN I. & SCHWARZ R.H. (1981) Murine syngeneic mixed lymphocyte response. I. Target antigens are self Ia molecules. *J. exp. Med.* **154,** 1652.

GOLDSTEIN A.L., GULA A., ZAKO M.M., HARDY M. & WHITE A. (1972) Purification and biological activity of thymosin, a hormone of the thymus gland. *Proc. natn. Acad. Sci. U.S.A.* **69,** 1800.

GOLDSTEIN A.L., LOW T.L.K. & THURMAN G.B. (1980) Thymosin: basic properties and clinical application in the treatment of immunodeficiency diseases and cancer. In: *Polypeptide Hormones* (eds R. F. Beers Jr. and E. G. Bassett), P. 449. Raven Press, New York.

GOLDSTEIN A.L., SLATER F.D. & WHITE A. (1966) Preparation assay and partial purification of a thymic lymphocytopoietic factor (thymosin). *Proc. natn. Acad. Sci. U.S.A.* **56,** 1010.

HANNAPEL E., XU G.J., MORGAN J., HEMPSTEAD J. & HORECKER B.L. (1982) Thymosin β_4: a ubiquitous peptide in rat and mouse tissues. *Proc. natn. Acad. U.S.A.* **79,** 2172.

KAUFMAN D.B. (1980) Maturational effects of thymic hormones on human helper and suppressor T cells: effects of FTS and thymosin. *Clin. exp. Immunol.* **39,** 722.

KLEIN J.J., GOLDSTEIN A.C. & WHITE A. (1965) Enhancement of *in vivo* incorporation of labeled precursors into DNA and total production of mouse lymphocytes after administration of thymic extracts. *Proc. natn. Acad. Sci. U.S.A.* **53,** 812.

LOW T.L.K., HU S.K. & GOLDSTEIN A.L. (1981) Complete animo acid sequence of bovine thymosin β_4: a thymic hormone that induces terminal deoxynucleotidyl transferase activity in thymocyte populations. *Proc. natn. Acad. Sci. U.S.A.* **78,** 1162.

LOW T.L.K., THURMAN G.B., MCADOO M., MCCLURE J., ROSSIE J.L., NAYLOR P.H. & GOLDSTEIN A.L. (1979) The chemistry and biology of thymosin. Isolation, purification and biological activities of thymosin α_1 and polypeptide β_1 from calf thymus. *J. biol, Chem.* **254,** 981.

MAG M.G., MCHUGH L.L. & ROTHSTEIN T.L. (1977) Mouse lymphocytes with and without surface immunoglobulin: preparative scale separation in polysterene tissue culture dishes coated with specifically purified anti-immunoglobulins. *J. Immunol. Meth.* **15,** 47.

RICH S.S. & RICH R.R. (1974) Regulatory mechanisms in cell mediated immune responses. I. Regulation of mixed lymphocyte reactions by alloantigen-activated thymus-derived lymphocytes. *J. exp. Med.* **150,** 1588.

RICH S.S. & RICH R.R. (1976) Regulatory mechanisms in cell-mediated immune responses. III. I-region control of suppressor cell interactions with mixed lymphocyte reactions. *J. exp. Med.* **143,** 672.

YAMASHITA U., ONO G. & NAKAMURA H. (1982) The syngeneic mixed lymphocyte reaction in mice. II. The I region control of suppressor T cell activity induced in the syngeneic mixed lymphocyte reaction. *J. Immunol.* **128,** 1010.