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T CELL RECONSTITUTION BY THYMIC HORMONES IN AGING

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As the crucial role of the thymus in T cell differentiation was recognized (Miller, 1962; Miller & Osoba, 1963), immunologists have been engaged in a search for an endocrine function of the thymus. This interest was based on the demonstration that thymus grafts enclosed in a cell-impermeable Millipore chamber (Osoba & Miller, 1963), pregnancy (Osoba, 1973), or cell-free thymic extracts (Bach & Carnaud, 1976) may restore immune competence in neonatally thymectomized mice. Restoration of immune competence is exhibited in graft vs. host reactivity, skin allograft rejection and cell-dependent antibody responses (Stutman et al., 1970). Thymus grafts in Millipore chambers also lead to restoration of the circulating thymic hormone levels (Dardenne et al., 1974). Similar results were also obtained with chambers containing an epithelial thymoma, thus demonstrating the epithelial origin of thymic hormones (Stutman et al., 1969).

The thymus appears to secrete several hormone-like products that influence the T cell maturation pathways. Although the thymic hormones isolated in different laboratories are all peptidic, they differ in chemical structure, mechanism of action and cell subsets they specifically activate. Some of these peptides act earlier in central lymphoid tissues leading the progeny of stem cells towards lymphoid maturation, others regulate the proliferation of the mature T cells thus contributing to the maintenance of helper, suppressor and cytotoxic T cell compartments in the periphery (Doria & Frasca, 1987).

During senescence the immune system exhibits a decline of immune responsiveness to exogenous antigens and an increased incidence of autoimmune phenomena. Most of the decline in immune responsiveness to exogenous antigens arises from alterations in regulatory T cell populations rather than in other cell compartments of the immune system (Doria et al., 1987).

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Profound defects have been detected in the helper T cell (Th) population of old mice. Antigen-specific Th cells have been classified in two types, based on different properties such as the surface phenotype and the mode of action. One cell type (Th1) recognizes epitopes of the antigen molecule in association with MHC-encoded class II antigens (Ia) and interacts with B cells through an antigen bridge of physically linked carrier and hapten determinants (cognate B cell activation). The other cell type (Th2) is not MHC-restricted and does not require physical linkage of carrier and hapten determinants for effective T-B cell cooperation (polyclonal B cell activation). Both mechanisms may operate synergistically (Tada et al., 1978).

Th cell activity declines with advancing age: it is already reduced to 64% in mice at 6 months of age but is not completely lost (30% residual activity) at 24 months of age. The age-related decline in Th cell activity can be attributed to reduction either in the pool size of Th cell subpopulations or in their ability to interact synergistically in the generation of helper activity. The decrease in Th cell activity has been attributed to thymus involution (Hirokawa & Makinodan, 1975) and subsequent reduced concentration of thymic factors involved in T cell maturation (Bach et al., 1973). The precursor frequency of Th cells is, indeed, decreased in old mice although the progeny of each precursor cell maintains full capacity to produce lymphokines and to proliferate (Miller, 1984).

Th cells produce interleukin 2 (IL-2), a growth factor for T and B lymphocytes, in response to mitogens, antigens and other lymphokines. Aging negatively affects IL-2 production which has been found to decline 170-fold in mice from 3 to 22 months of age. Since the decline in Th cell activity during this life period is only 4-fold, it appears that the dependence of helper activity from IL-2 is not absolute as other factors, probably less affected by aging, compensate the decreased IL-2 production and contribute to the maintenance of a reduced Th cell activity in old mice.

Several studies were carried out in our laboratory to investigate immunological effects of synthetic thymosin α_1 , a 28 amino acid residues peptide with thymic hormone-like activity. Thymosin α_1 was the first to be purified, sequenced (Goldstein et al., 1977), and synthesized (Wang et al., 1979), by solution or solid phase methods, among the several peptides present in the calf thymus extract Fraction 5. In 1980, Wetzel et al. have reported the isolation and complete chemical characterization of N^{α} – desacetylthymosin α_1 using recombinant DNA procedures (Wetzel et al., 1980). Several tests performed both in vivo and in vitro have show that thymosin α_1 is 10-1000 times more active than Fraction 5 in promoting T cell differentiation.

Our results showed that injection of immunodeficient old mice with thymosin α_1 recovers to a significant extent Th cell activity, IL-2 production and IL-2 receptor expression by spleen cells (Frasca et al., 1986). These biological activities are restricted to the first 14 amino acids of the thymosin α_1 molecule. Table I shows the results of four independent experiments in which spleen cells from aging (3-18 month old) mice, primed with Horse Red Blood Cells (HRBC) 4 days before culture, were assayed for Th cell activity, IL-2 production and IL-2 receptor expression. Mice were left uninjected or injected with 10 ug thymosin α_1, 5 ug N_{14} (N-terminal amino acid residues 1-14) or 5 ug C_{14} (C-terminal amino acid residues 15-28) synthetic fragment of the thymosin α_1 molecule, 3 days before HRBC priming. Results indicate that helper T cell activity, IL-2 production and IL-2 receptor expression are impaired by aging but are restored to a large extent by a single injection of 10 ug thymosin α_1. Injection of an equimolar amount of the N_{14} fragment is at least as effective as the entire molecule in restoring these T cell activities, whereas injection of the C_{14} fragment has no demonstrable effect. It is noteworthy that thymosin α_1 and its N_{14} fragment are effective when injected in 6, 12 and 18 month old mice, but not in 3 month old mice. Our data support the possibility that the thymosin α_1-induced enhancement of Th cell activity in old mice results from an increase in the T cell precursor frequency.

TABLE I

Enhanced helper T cell activity, IL-2 production and IL-2 receptor expression by spleen cells from aging mice, uninjected or injected with thymosin peptides

Exp. No.	Age (months)	Treatment	Helper T cell activity	IL-2 production	IL-2 receptor expression
1	3	none	100	100	100
	3	α_1 (10 μg)	107	78	93
	3	N_{14} (5 μg)	106	82	77
	3	C_{14} (5 μg)	116	84	95
2	3	none	100	100	100
	6	none	57	47	25
	6	α_1 (10 μg)	74	87	99
	6	N_{14} (5 μg)	104	85	66
	6	C_{14} (5 μg)	59	23	18
3	3	none	100	100	100
	12	none	53	52	0
	12	α_1 (10 μg)	81	71	89
	12	N_{14} (5 μg)	114	78	134
	12	C_{14} (5 μg)	43	28	9
4	3	none	100	100	100
	18	none	43	0	23
	18	α_1 (10 μg)	74	47	82
	18	N_{14} (5 μg)	86	99	81
	18	C_{14} (5 μg)	46	0	0

Results are expressed as percent of 3 month old untreated controls.

TABLE II

Precursor frequency of proliferating T cells from mice of different ages uninjected or injected with thymosin peptides

Exp. No.	Age (months)	Treatment	Precursors per 10^4 cells
1	3	none	27 (1.35)
	3	α_1 (10 μg)	41 (1.28)
	19	none	9 (1.65)
	19	α_1 (10 μg)	23 (1.38)
2	3	none	79 (1.21)
	3	N_{14} (5 μg)	91 (1.20)
	19	none	24 (1.37)
	19	N_{14} (5 μg)	95 (1.19)
3	3	none	42 (1.28)
	3	C_{14} (5 μg)	51 (1.25)
	20	none	11 (1.57)
	20	C_{14} (5 μg)	13 (1.51)

Numbers in parentheses are error factors by which precursor frequencies should be multiplied or divided to obtain the variations due to one standard error.

Results from limiting dilution analysis of T cell precursors (Table II) indicate that injection of thymosin α_1 or its N_{14} fragment 3 days before the assay increases the frequency of mitogen responsive T lymphocytes in old, but not in young mice, whereas injection of the C_{14} fragment is devoid of any effect in both young and old mice. Thus, thymosin α_1 amplifies the pool of T cell precursors in immunodeficient old mice.

Our studies on the T cell precursor frequency were performed on spleen cells which comprise an extrathymic T cell pool of mature T cells and immature T cells undergoing the final maturation steps (Weissman et al., 1975; Stutman, 1978; Scollay et al., 1984). The effects observed after the short time interval between the injection of thymosin peptides and the assay are likely due to induced proliferation of mature T cells rather than to enhanced T cell maturation (Frasca et al., 1987). This rapid amplification of the mature T cell compartment is a biological effect of practical value for the therapy of age-related T cell immunodeficiency.

These results have been extended in humans, using the synthetic pentapeptide TP-5, constituted by amino acid residues 32-36 of the thymic hormone thymopoietin (Goldstein et al., 1979). Injection of TP-5 in elderly subjects has been shown to be effective in enhancing proliferative responses to mitogens and IL-2 production by peripheral blood mononuclear cells (Meroni et al., 1987; Table III).

The results so far obtained in both mice and humans suggest possible ways for therapeutic intervention in aging. Thus, as shown herein, helper T cell reconstitution by thymic hormones may lead to an increase of immune reactivity to exogenous antigens.Furthermore, a similar therapeutic approach, under appropriate conditions that preferentially enhance suppressor T cell activity, may prevent or mitigate the increased expression of autoimmune reactivity. In conclusion, the use of thymic hormones is very promising in the treatment of age-related immune disfunctions but requires accurate protocols to reach antithetic objectives, such as the amelioration of immune responsiveness to pathogens and the alleviation of autoimmune reactions.

TABLE III

Proliferative response to mitogens (PHA, Con A, PWM) and IL-2 production by PBM from six elderly subjects before (T_0) thymopentin treatment and 4 weeks (T_1), 12 weeks (T_2), and 24 weeks (T_3) after the end of treatment.

Time of treatment	PHA	Con A	PWM	IL-2
T_0	27 ± 6	12 ± 5	9 ± 2	2 ± 1
T_1	97 ± 14	36 ± 4	15 ± 3	20 ± 4
T_2	115 ± 8	49 ± 10	24 ± 4	26 ± 9
T_3	48 ± 11	27 ± 6	N.D.	9 ± 4

Elderly subjects (71-92 years old) were injected subcutaneously with 50 mg thymopentin 3 times/week over a period of 4 weeks. The proliferative response is expressed as mean c.p.m.x10^3 ± SE. The IL-2 production is expressed as mean units/ml ± SE.

REFERENCES

BACH, J.F. & CARNAUD, C., 1976. Thymic Factors. *Prog. Allergy, 21* :418-484.

BACH, J.F.; DARDENNE, M. & SALOMON, J.B., 1973. Studies on thymus products. IV. Absence of serum "Thymic activity" in adult NZB and (NZB x NZW) F1 mice. *Clin. Exp. Immunol., 14* :247-258.

DARDENNE, M.; PAPIERNIK, M.; BACH, J.F. & STUTMAN, O., 1974. Studies on thymus products. III. Epithelial origin of the serum thymic factor. *Immunology, 27* :299-304.

DORIA, G.; ADORINI, L. & FRASCA, D., 1987. Immunoregulation of the antibody responses in aging mice, p. 143. In: *Aging and the Immune Response: Cellular and Humoral Aspects*, E. Goidl, ed., Marcel Dekker Inc., New York.

DORIA, G. & FRASCA, D., 1987. Thymic hormones as immunoregulatory molecules: an overview p. 109. In: *Immunoregulation and Thymic Hormones*, F. Dammaco, ed., Masson; Italia.

FRASCA, D.; ADORINI, L. & DORIA G., 1987. Enhanced frequency of mitogen-responsive T cell precursors in old mice injected with thymosin α_1. *Eur. J. Immunol., 17* :727-730.

FRASCA, D.; ADORINI, L.; MANCINI, C. & DORIA, G., 1986. Reconstitution of T cell function in aging mice by thymosin α_1. *Immunopharmacology, 11* :155-163.

GOLDSTEIN, A.L.; LOW, T.L.K.; Mc ADOO, M.; Mc CLURE, J.; THURMAN, G.B.; ROSSIO, J.; LAI, C.Y.; CHANG, D.; WANG, S.S.; HARVEY, C.; RAMEL, A.H. & MEIENHOFER, J., 1977. Thymosin α_1: isolation and sequence analysis of an immunologically active thymic polypeptide. *Proc. Natl. Acad. Sci. USA, 74* :725-729.

GOLDSTEIN, G.; SCHEID, M.P.; SCHLESINGER, D.H. & VAN WAUWE, J., 1979. A synthetic pentapeptide with biological activity characteristic of the thymic hormone thymopoietin. *Science, 204* :1309-1310.

HIROKAWA, K. & MAKINODAN, T., 1975. Thymic involution: effect on T cell differentiation. *J. Immunol., 114* :1659-1662.

MERONI, P.L.; BARCELLINI, W.; FRASCA, D.; SGUOTTI, C.; BORGHI, M.O.; DE BARTOLO, G.; DORIA, G. & ZANUSSI, C., 1987. In vivo immunopotentiating activity of thymopentin in aging humans: increase of IL-2 production. *Clin. Immunol. Immunopathol., 41* :151-159.

MILLER, J.F.A.P., 1962. Immunological significance of the thymus in the adult mouse. *Nature, 195* :1318-1320.

MILLER, J.F.A.P. & OSOBA, D., 1963. Role of the thymus in the origin of immunocompetence, Vol. 16, p. 12. In: *Nature and Origin of Immunologically Competent Cells*, Wolsterholme & Knight, eds., Churchill, London, CIBA Found. Study Group.

MILLER, R.A., 1984. Age-associated decline in precursor frequency for different T cell-mediated reactions, with preservation of helper or cytotoxic effect for precursor cell. *J. Immunol., 132* :63-68.

OSOBA, D., 1973. Effect of pregnancy on the restoration of immunological responses in neonatally thymectomized mice. Vol. 2, p. 293. In: *Contemporary Topics in Immunobiology*, Carter, R.L. & Davies, A.J.S., eds., Plenum Publishing, New York.

OSOBA, D. & MILLER, J.F.A.P., 1963. Evidence for a humoral thymus factor responsible for the maturation of immunological faculty. *Nature, 199* :653-655.

SCOLLAY, R.; BARTLETT, P. & SHORTMAN, K., 1984. T cell development in the adult murine thymus: changes in the expression of the surface antigens Ly 2, L3T4 and B2A2 during development from early precursor cells to emigrants. *Immunol. Rev., 82* :79-104.

STUTMAN, O., 1978. Intrathymic and extrathymic T cell maturation. *Immunol. Rev., 42* :138-184.

STUTMAN, O.; YUNIS, E.J. & GOOD, R.A., 1969. Carcinogen-induced tumors of the thymus. III. Restoration of neonatally thymectomized mice with thymomas in cell impermeable chambers. *J. Natl. Cancer Inst., 43* :499-502.

STUTMAN, O.; YUNIS, E.J. & GOOD, R.A., 1970. Studies on thymus function. I. Cooperation effects of thymic function and lymphohemopoietic cells in restoration of neonatally thymectomized mice. *J. Exp. Med., 132* :583-595.

TADA, T.; TAKEMORI, T.; OKUMURA, K.; NORAKA, M. & TOKUHISA, T., 1978. Two distinct types of helper T cells involved in the secondary antibody response: Independent and synergistic effects of Ia^- and Ia^+ helper T cells. *J. Exp. Med., 147* :446-458.

WANG, S.S.; DOUVAN KULESHA, I. & WINTER, D.P., 1979. Synthesis of thymosin α_1. *J. Am. Chem. Soc., 101* :253-254.

WEISSMAN, I.L.; MASUDA, T.; OLIVE, C. & FRIEDBERG, S.H., 1975. Differentiation and migration of T lymphocytes. *Is. J. Med. Sci., 11* :1267-1269.

WETZEL, R.; HEYNEKER, H.L.; GOEDDEL, D.V.; JHURANI, P.; CREA, R.; LOW, T.L.K.; Mc CLURE, J.E.; THURMAN, G.B. & GOLDSTEIN, A.L., 1980. Production of biologically active N α-desacetylthymosin α_1 in *E. coli* through expression of a chemically synthesized gene. *Biochemistry, 19* :6096-6104.