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Effect of Cortisone in Delineating Thymus Cell Subsets in Advanced Age[1] (38851)

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The possible etiologic/pathogenetic role of immune dysfunction in the process of aging has received increasing attention in recent years. Whereas normal immune functions decrease markedly with age, there is an increase in autoimmune manifestations (1, 2). It has been proposed that the thymus plays an important role in determining these alterations (3). The following study in a long-lived mouse strain was done to determine the age-related changes in the mixed lymphocyte reactivity of thymus cells from normal and cortisone-treated mice.

Material and Methods. Animals. Two- and 21-month-old male C57BL/6J mice were obtained from Jackson Memorial Laboratories, and housed six to a cage. They were not regrouped after death of any members of a cage. The mice were fed a diet of standard Purina Laboratory Chow. After being sacrificed the animals were examined and those with gross pathologic alterations were excluded from this study.

Cortisone treatment. The mice were injected intraperitoneally with 125 mg of cortisone acetate per kilogram of body weight (cortisone acetate, 25 mg/ml, The Upjohn Company, Kalamazoo, MI).

Mixed lymphocyte reaction. Two days after the cortisone treatment the reactivity of thymus cells was assessed using the mixed lymphocyte reaction (MLR) as described elsewhere (4). Special care was taken to avoid contaminating the thymus cell preparation with parathymic lymph nodes. Mitomycin C-treated CBA spleen cells were used as stimulator cells. Controls consisted of mitomycin C-treated syngeneic cells whose ^{3}H-thymidine counts were subtracted from those of the experimental cell cultures. The MLR of thymus cells from young and old cortisone-treated mice and noninjected animals were compared. In each experiment the thymuses from four to six mice were pooled.

Results. After cortisone treatment there was a remarkable decrease in the number of cells obtained from the thymus. The mean number of cells obtained per thymus was 30.1×10^6 in young nontreated mice and 2.5×10^6 in young cortisone-treated mice, 9.8×10^6 in old nontreated mice, and 0.9×10^6 in old cortisone-treated mice. The viabilities of the cell suspensions were between 75% and 93% in the nontreated groups and between 89 and 97% in the cortisone-treated groups. No difference was noted in the viabilities between cells derived from young and old mice. Table I shows the mixed lymphocyte reactivity of thymus cells in various groups studied. Each value represents the mean of four separate experiments. There was no significant difference in the mixed lymphocyte reactivity or in unstimulated baseline reactivity between the young and old mice when nontreated animals were compared ($P = 0.56$ and 0.17, respectively). After cortisone treatment, the remaining thymus cells from young donors showed a 12-fold increase in reactivity over that of the untreated young mice ($P = 0.001$). However, no increase of reactivity was observed when cells from cortisone-treated old mice were compared to those of untreated old mice ($P = 0.4$). In addition to the effect of cortisone treatment on total reactivity in the young mice, a 9-fold increase in the background counts was found ($P = 0.01$), whereas in the old mice the increase in both the total counts and background counts was about 2-fold.

Discussion. An essential role in initiating and supporting immune competence has been ascribed to the thymus. It is well known that neonatal thymectomy is followed by severe immunological defects (5–7). Studies on adult thymectomy alone or combined with whole-body irradiation or antilympho-

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TABLE I. EFFECT OF CORTISONE TREATMENT OF THE MIXED LYMPHOCYTE REACTIVITY OF THYMUS CELLS OF 2- AND 27-MO-OLD C57BL/6J MICE.

Age group (in months)	Untreated mice		Cortisone-treated mice	
	Stimulated (allogeneic cells)	Unstimulated (syngeneic cells)	Stimulated (allogeneic cells)	Unstimulated (syngeneic cells)
2	1142[a] ± 519	126 ± 45	13,107 ± 1260	1117 ± 174
27	1569 ± 458	433 ± 170	2682 ± 1145	726 ± 317

[a] The values represent cpm of four separate experiments (four to six) mice per experiment) ± 1 standard error of the mean.

cyte serum treatment demonstrated that the thymus has a continuing influence on immunological responsiveness throughout adult life (8–10). Yet neonatal or adult thymocytes show only minimal immune competence, with the exception of a steroid-resistant cell subpopulation that has immune competence comparable to peripheral lymphoid cells and comprises only about 5%–10% of the thymus lymphoid cells (11). The sequence of maturation of thymic lymphocytes seems to involve differentiation from steroid-sensitive, theta-rich, H-2 poor, cortical cells without immune competence to steroid-resistant, theta-poor, H-2 rich medullary cells reactive to phytohemagglutinin, pokeweed mitogen, and concanavalin-A, as well as reactive to allogeneic cells in the mixed lymphocyte culture, graft-versus-host reaction, and cell-mediated lysis assays (12, 13). Some controversy exists as to whether this mature population of the thymus leaves the organ to become peripheral T-cells or whether these cells are a persistent population in the thymus and should not necessarily be the same as peripheral T-cells despite certain behavioral similarities (14). Certain studies also suggest that the thymus contains two cortisone-resistant subpopulations: a non-reactive precursor that matures in the periphery as shown by transfer experiments and a reactive T-cell which is already mature in the thymus (15).

After cortisone treatment in young mice the thymus cell population, composed by definition of the cortisone-resistant cells, showed a 12-fold increase in mixed lymphocyte reactivity compared to age-matched controls, and also a significant increase in background reactivity of these cells was seen. However, no significant increase in reactivity of thymus cells was seen in the old mice. Thus, in addition to the observed age-related decrease in the total number of cortisone-resistant thymus cells, their mixed lymphocyte reactivity was much lower than expected, and not significantly different from the non-cortisone-treated controls. The disproportionate decrease in mixed lymphocyte reactivity of the cortisone-resistant cells from the old mice suggests that a significant proportion might be analogous to the immature subpopulation of cortisone-resistant cells described by Mosier and Cantor (15).

The reason for the observed age-related increase in background counts is not entirely clear. This phenomenon has also been seen regularly in our other aging studies and probably reflects clonal diversification of lymphoid cells with age. Similarly, the observed increase in background counts in the young cortisone-treated mice compared to the untreated controls raises the possibility that cortisone influences cell reactivity through additional mechanisms beyond its influence on the relative cell-maturation proportions in the thymus.

The age-related decline in the thymus of both the number and mixed lymphocyte reactivity of cortisone-resistant cells accompanies the general decline in immunocompetence of peripheral T-cells, as demonstrated in studies on the mixed lymphocyte reaction (16), cell-mediated cytotoxicity reaction (17), resistance to transplantable allogeneic and xenogeneic tumor grafts, and response to mitogens (18). Similarly, these alterations accompany the development of autoimmune phenomena with age. The thymus clearly has a significant influence in autoimmune disorders as shown by the early appearance of these disorders after neonatal thymectomy (19, 20), the high levels of anti-DNA antibodies in nude mice (21), and the reversibility of these abnormalities by thymus grafts (21, 22).

These associations suggest that the mature cortisone-resistant thymus cells might have a significant role in maintaining self-tolerance. Whether or not the cells that prevent

autoimmune phenomena are the same as the suppressor T-cells found to regulate the immune response to some foreign antigens remains to be determined. It has been shown that the suppressor T-cells described in the immune response to type III pneumococcal polysaccharide (SSS-III) reside in the thymus and that there is a decline in their function with age in NZB mice (24). A similar decline of suppressor cells in the thymus in truly old mice from long-lived strains has not been reported.

Our present finding of a marked decline with age in the number of mature cortisone-resistant cells in the thymus suggests that these cells could be related to or actually be suppressor cells.

Summary. The mixed lymphocyte reactivity of thymus cells from normal and cortisone-treated, young and old C57BL/6J mice was evaluated. The results indicated that while in the young mice cortisone treatment caused a 12-fold increase in the reactivity of thymus cells, it had no effect on the response of thymus cells from old mice. The significance of the disappearance of cortisone-resistant mature thymus cells in old mice is discussed regarding the general decline in immunocompetence and the increase of autoimmune manifestations in old age.

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