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AGE-RELATED FUNCTIONAL CHANGES IN THE THYMIC STROMAL TISSUE[1]

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Dr. Hoecker's illustrious career began with his development of one of the first mouse H-2 serology laboratories in the world, shortly after his return to Chile from a sabbatical visit to Dr. Peter Gorer's laboratory in Great Britain. Thus, together with Drs. Peter Gorer, George Snell, Bernard Amos and others, he began his pioneering studies defining the biology of the H-2 system. Their efforts led to our current understanding of the Major Histocompatibility Complex (MHC) and, in turn, the genetic basis of how the immune system distinguishes self from nonself. Today, a major emphasis of MHC research is focused on the thymus, for the thymic stromal tissue is intimately involved in the generation of MHC restricted T cells. It would be fitting, therefore, to honor Dr. Hoecker by summarizing our efforts in characterizing the changes which occur in the thymic stromal tissue with age.

Our discussion on age-related changes in the thymic stromal tissue will be prefaced by a brief comment on how aging alters T cell-dependent

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for their ability to mount a humoral or cell-mediated immune response in an immunologically neutral environment of x-irradiated old and young syngeneic recipients, respectively; i.e., (Y --> 0) and (0 --> Y) (Albright and Makinodan, 1966; Goodman and Makinodan, 1975; Price and Makinodan, 1972). The results showed that both intrinsic and extrinsic changes affect the immune response, but much of the age-related alteration can be attributed to changes in the donor cells, i.e., (Y --> Y) > (Y --> 0) > (0 --> Y) > (0 --> 0).

As to the influence of the cellular milieu, it could be due to an accumulation of a deleterious substance of molecular or viral nature, or it could be due to a loss of an essential factor. In this regard, Antonaci *et al.* (1983) recently detected a dissociable inhibitory factor on the surface of circulating lymphocites of old but not young humans.

Cellular changes. Changes in immune response with age can result from (a) a change in the number of immune cells; (b) a shift in the enhancing and suppressive activities of regulator cells, or (c) qualitative changes in immune cells. Al three types of change have been observed (Goidl, 1987).

A. *Quantitative Changes*

In humans it was found via the use of monoclonal antibody reagents that the total number of circulating lymphocytes decreases with age by about 15%, due primarily to a decrease in the number of T cells (Ligthart *et al.*, 1985; Nagel *et al.*, 1983; O'Leary *et al.*, 1983). In mice the pattern and magnitude of change in the number of T cells was found to vary with the tissue and organ source; in general, the number does not change appreciably with age (Kay *et al.*, 1979). Thus, cell loss does not appear to contribute significantly to the changes in T cell-dependent immune functions with age.

B. *Imbalance in Regulatory Cells*

Although often contradictory, the results of studies on regulatory cells suggest that a shift in the regulatory activities of T cells and monocytes/macrophages is occurring with age. However, the shift could reflect qualitative changes as well, at least in the case of certain T cell populations. This suspicion stems from the demonstration that the proportion of T cells with enhancing and suppressor markers does not change appreciably with age as discussed earlier and that T cells undergo qualitative changes with age as will be discussed.

hypoxanthine-aminopterin-thymidine medium suggests that these metabolic changes are reflective of changes at the genomic level. The studies of individual aging mice by Inamizu *et al.* (1986) further show that an inverse correlation exists between the frequency of 6-thioguanine resistant T cells and the ability of T cells to produce IL-2 or proliferate in response to mitogenic stimulation. This would mean that old mice with a high frequency of HPRT-deficient T cells tend to have a reduced T-cell function and those with a low frequency of HPRT-deficient T cells tend to have a heightened T-cell function. Of course, this does not necessarily mean that specific mutation of the HPRT gene is responsible for the decline in T cell activities with age. However, it validates the use of 6-thioguanine resistant T cells as cellular probes to assess the role gene alterations play in T cell aging.

III. Age-related changes in the thymus

The thymus is involved in four important functions which may be affected by aging: a) the production of chemotactic factors which attract prethymic stem cells to the gland (Pyke and Bach, 1979), (b) the induction of self tolerance and major histocompatibility complex restriction (Zinkernagel and Doherty, 1979), (c) the production of hormones that act on intrathymic and postthymic immature T cells and that influence the peripheralization of T cells (Stutman, 1978), and (d) the production of hormones that modulate a neuroendocrine circuit (Hall and Goldstein, 1981). To date, four thymic peptides with hormonal activities have been chemically defined: thymic humoral factor (Trainin *et al.*, 1975), thympoietin (Schlesinger and Goldstein, 1975), thymosin α_1 (Goldstein *et al.*, 1977), and thymulin (Dardene *et al.*, 1977). These hormones are found in the thymic epithelial cell (Hirokawa *et al.*, 1982a; Haynes *et al.*, 1983) and therefore are presumed to be synthesized by them. However, very little is known of what role other stromal cells (fibroblasts, macrophages, and reticular cells) play in the differentiation and regulation of the parenchymal T cells.

The thymus starts to involute at around sexual maturity in humans (Boyd, 1932) and mice (Santisteban, 1960), which coincides with the cessation of prethymic stem cell migration into the thymus, as judged by heterochronic parabiosis studies (Kay, 1984), and with the onset of age-related decline in T cell-dependent antibody response (Makinodan and Peterson, 1962). This suggests that thymic involution contributes to the decline in T - cell-dependent immunity with age. Evidence in support of this view is substantial. One example is that adult thymectomy of autoimmune-susceptible and autoimmune-resistant mice accelerates the de-

thymus can be looked upon as the pacemaker of aging for the T cell arm of the immune system.

In order to understand more specifically how the thymus is aging physiologically, a series of studies were initiated to characterize the influence of age on the stromal cells. Thymic adherent cells obtained from mice ranging in age from 1 day to 20 months were cultured *in vitro* for one month with weekly collection of the supernatant. The supernatants were then assessed for their ability to augment or inhibit the antigen/mitogen responses of indicator thymocytes obtained from 2-or 4-week-old mice and of indicator prethymic splenic cells obtained from 3-month-old syngeneic nude mice (Sato *et al.*, 1984). The results revealed that intrathymic but not prethymic T indicator cells are responsive to the modulating influence of the supernatant. It is likely therefore that the prethymic stem cells require a direct contact with the adherent cells in order to undergo differentiation. The results further showed that the ability of thymic adherent cells to synthesize an augmenting factor declines drastically between 2.5 and 5 months of age, which is consistent with the findings derived from histologic and thymic graft studies (Hirokawa *et al.*, 1982a; Hirokawa and Makinodan, 1975; Hirokawa *et al.*, 1982b). Perhaps the most interesting findings in terms of homeostasis are those indicating that the thymic adherent cells can also synthesize an inhibitory factor. Thus, thymic adherent cells of newborn mice were shown to synthesize both the augmenting factor and the inhibitory factor, while those of young adult mice synthesize primarily the augmenting factor and those of 20-month-old mice synthesize primarily the inhibitory factor.

Our current effort has been centered on the inhibitory factor because it is produced primarily by the involuted thymus of aging mice (Kinohara *et al.*, 1985). Our initial study showed that the inhibitory factor suppresses the proliferation of crude IL-2 stimulated, peanut agglutinin-agglutinable (PNA^+) immature thymocytes and PNA^- mature thymocytes, but not of specific T blast cells, nor of cells of the cytolytic T lymphocyte line (CTLL-2). These results suggest that the inhibitory activity of the factor could be T cell differentiation stage-specific. Our gel filtration analysis, using ultrogel AcA54, then showed that the inhibitory activity of the supernatant of thymic adherent cells of young mice was found mainly in the 68,000 fraction, whereas that of involuting thymus of aging mice was polydispersed. The latter supernatant was subjected to DEAE ion exchange chromatography, and the fraction with the highest inhibitory activity was then subjected to gel filtration, using Sephacryl S-300. The results showed that the activity was confined to the 68,000 fraction, which also contains serum albumin. However, albumin itself had

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