3.1.12 PW

THE VITRO EFFECTS OF THYMIC EPITHELIAL CULTURE SUPERNATANTS (TES) AND T CELL GROWTH FACTORS (TCGF) ON CTL FUNCTIONS OF THYMOCYTES. Ada M. Kruisbeek. NIH, Bethesda, Md. 20205 (on leave of absence from REPGO-TNO-Institutes, Rijwykte, The Netherlands).

We have previously shown that both PHA, Con A and MLR reactivity can be induced in PNA^+-thymocytes by TES. We now report that: (1) H-2 restricted CTL against TNP-self and CTL against alloantigens can also be induced by TES, and (2) that TCGF, the putative T helper cell product, allows these functions to appear in PNA^+ thymocytes. In contrast to TCGF which acts only when added after the stimulating signal, TES acts when preincubated with cells for 24 hr at 37°. This confirms that TCGF acts on activated T cells (as reported by others), while TES changes the PNA^+ subpopulation in such a way that CTL effectors can be generated. Data on the effects of TES on Thy 1 and Lyt phenotype distribution are presently collected. We hypothesize that: a) PNA^+ thymocytes include a population of competent CTL precursors; b) TCGF bypasses the need for helper T cells in this otherwise unresponsive cell population; c) TES acts via induction of helper T cells which in turn help the already present CTL precursors to develop into effector CTL.

3.1.13 PW

T CELL DIFFERENTIATION PATHWAYS IN THE RABBIT THYMUS. Leene,W.,Roholl,P.,Hoeben,K. Lab.of Histology and Cell Biology,Univ.of Amsterdam,P.O.Box 60.000,1005 GA Amsterdam

To study differentiation pathways in the rabbit thymus, lymphocytes isolated from the organ were separated into three peak fractions of low,medium and high density and characterized morphometrically on the EM level with a semi-automatic quantitative analyser(MOP-AMO1).It appeared that the three density classes can be distinguished according to the amount of euchomatin per nuclear volume (approx.36% of this volume being occupied by

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The specific role of the thymus in the ontogeny of mammalian 'T-cells' was investigated in the marsupial quokka. Quokka pouch young of neonatally thymectomized mothers were totally thymectomized prior to the first appearance of any lymphoid cells anywhere in the body. The detailed ontogeny of cells having the classical characteristics of mammalian 'T-cells' was compared in thymectomized and control animals.

Pre-lymphocytopoiesis thymectomy resulted in a delay in the appearance of 'T-cells', however approximately 4 months after thymectomy 'T-cells' appeared and eventually developed to near normal levels in blood and various lymphoid organs.

The appearance of classical 'T-cells' in pre-lymphocytopoiesis thymectomized quokkas unequivocally demonstrates that the thymus is not uniquely responsible for 'T-cell' ontogeny in the quokka. The effects of neonatal or in utero thymectomy in many other mammalian species suggests that this extra-thymic pathway to the development of 'T-cells' is a phonomenon common to mammalian immunology in general.

3.1.15

CELL POPULATIONS IN MOUSE THYMUS IDENTIFIED BY ACID ALPHA-NAPHTHYL-ACETATE ESTERASE (ANAE)
Manconi, P.E., Ennas, M.G., Paghi, L., Zaccheo, D. Insitute of Hygiene, University of Cagliari and Institute of Anatomy, University of Genoa, Italy.

ANAE histochemistry is considered as an useful marker for mouse and human lymphocyte subpopulations. B and n lymphocytes appear devoid of ANAE activity. About 80 % T blood cells bear ANAE activity in form of a single cytoplasmic spot, 20 % in form of multiple granules. This last type of reaction identifies T cells and mitogen-activated cells. Conflictuary results have been obtained concerning ANAE activity of thymus cells. In our hands about 90 % thymocytes are ANAE positive, the great majority bearing multiple cytoplasmic granules. The prevalence of ANAE positive cells is related to animal age. Epithelial cells show diffused ANAE reaction, and analogous pattern is found in the pole of lymphocytes in contact with them. This finding suggest a possible role of ANAE in thymocyte differentiation.

3.1.16 PW

LIMITING DILUTION ANALYSIS OF CYTOLYTIC T LYMPHOCYTE PRECURSOR CELLS (CTL-P) IN NUDE MICE.

J.L. Maryanski and J.-C. Cerottini, Ludwig Institute for Cancer Research, Lausanne, Switzerland.

A sensitive system of limiting dilution mixed leukocyte microculture (micro MLC) was used to detect and to quantitate CTL-P in the spleens of C57BL/6 nu/nu (nude) mice. Although the frequency of CTL-P was 40-100 times lower in nude mice than in normal mice, approximately one in 20,000-40,000 nude spleen cells could be identified as a CTL-P (directed against DBA/2 alloantigens). The cytolytic progeny of nude spleen cells cultured in micro MLC were sensitive to treatment with monoclonal anti-Thy 1.2 and complement. Priming nude spleen cells in conventional MLC expanded the pool of CTL-P 60-fold, such that by day 7 of culture the frequency of CTL-P was one in 80 cells. This microculture system may be useful for analyzing the specificity repertoire and function of pre-thymic T cells.

3.1.17

TRANSIENT HOST POPULATION IN THYMUS REPOPULATION OF RADIATION BONE MARROW CHIMERAS. B. J. Mathieson, S. O. Marrow, U. Hammerling*, and A. Singer. NIH, Bethesda, MD. 20205 and *Memorial Sloan Kettering Institute, New York, N.Y.

The Ly phenotype of thymocytes from radiation bone marrow chimeras shortly after bone marrow transfer was analyzed using flow microfluorometry. Cells appearing in the thymuses of these mice between 12 and 26 days post irradiation (950R) and transfer were typed for Lyt 1, Lyt 2, and Ly 9 antigens. The chimeras used were: B6-Ly-1^a→ B_6, B_6-Ly-2^a→ B_6, B_6-Ly-1^a→ (B10xB10.A)F_1, and C_3H.SW → B10. While at 9 days post transfer thymuses of the chimeras contained fewer than 10^5 cells, by 12 days a normal complement of thymocytes could be obtained. However, at 12 days, greater than 98% of the thymus cells were of host origin, as determined by Ly phenotype. Thymus cells 15 days post transfer were approximately 50% donor and 50% host by Ly phenotype. By 19 days, the cells were greater than 98% donor phenotype. This time course was identical for all chimeric combinations. These results demonstrate the existence of a radiation resistant host cell component, capable of thymus repopulation, which may be important in further understanding the role of the thymus in T cell differentiation.