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Induction of Graft-Versus-Host Reaction in Newborn Mice by Injection of Newborn or Adult Homologous Thymus Cells.* (28640)

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Homologous adult thymus cells have been shown capable of inducing runting in newborn animals(1) and secondary disease in irradiated adult mice(2). Although these experiments indicated that thymocytes were quantitatively less effective than other lymphoid cells in producing these effects, spleen cells from animals thymectomized at birth were relatively ineffective(3).

While newborn spleen and blood cells are indicated to be incapable of producing graft-versus-host (G.V.H.) reaction(4), no studies with the newborn thymus have been reported. In the present studies with mice, no obvious quantitative differences could be found between the effectiveness of newborn and adult thymus cells in producing G.V.H. reactions.

Materials and methods. Animals: Newborn A/Hestor mice (Darwin Farms) injected within 24 hours of birth were used as recipients. Newborn (0 to 3 days old) and adult C57B1/6 mice of both sexes were used as donors.

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Cell Suspensions: The organs of the donor mice, thymus, spleen, or liver, were teased in medium 199, the cells counted, and 0.1 ml aliquots injected intraperitoneally into the experimental animals. As controls in some experiments, a portion of the thymus cell suspension was disrupted before injection either by freezing and thawing or by sonic oscillation (15 minutes, 9 kilocycles, Raytheon magneto striction oscillator). The latter treatment was estimated to disrupt at least 95% of the cells.

Spleen Index Calculation: The determination of G.V.H. reaction was by Simonsen's method of spleen assay(4). The spleen weight/body weight ratio was determined at the age of 8-10 days. Spleen indices were calculated by dividing the relative spleen weight for each experimental animal by the means of the relative spleen weights for litter-mate (non-injected) controls. Since the average spleen index of 65 control animals was 1.00 $\pm$ 0.11 S. D., animals with indices $\geqq$ 1.3 were considered to show a G.V.H. reaction.

Results. The results (Table I) show

TABLE I. Graft vs. Host Assay of Adult and Newborn C57B1 Lymphoid Cells in Newborn A Mice.

Type of cell	Newborn thymus	Adult thymus	Killed adult thymus	Newborn spleen	Newborn liver	None
Cell dose	5–15 × 10^6	5–15 × 10^6	5–15 × 10^6	2–4 × 10^6	5–9 × 10^6	
G.V.H. reaction*	19/22	28/34	6/22	0/3	3/9	1/65
Avg spleen index	2.03	2.12	1.14	1.09	1.24	1.00†

* Expressed as fraction of animals showing spleen index $\geqq$ 1.3.
† S.D. = .11.

that it is possible to cause G.V.H. reactions with C57B1 newborn thymus cells in approximately 86% of the recipient newborn A/Hestor mice. The incidence of G.V.H. reactions observed with newborn thymocytes was not significantly different from that with adult thymocytes within the same range of cells. "Killed" adult thymus cells gave a much lower incidence (27%). The finding of enlarged spleens in this case may have been due to a G.V.H. reaction caused by cells which survived the treatment with sonic oscillation, or to a reaction of the host to antigenic stimulation from the killed cells. The average spleen index was increased significantly only in the mice receiving living thymus cells. Cells from livers of newborn mice caused enlargement of the spleen in 3 out of 9 recipients, possibly due to hematopoietic cells. Newborn spleen cells were ineffective at the low dose range employed. In experiments not recorded in the Table, newborn spleen cells given together with 5 × 10^6 newborn thymus cells were found not to enhance the incidence of G.V.H. reactions obtained with the thymus cells alone.

Among the 65 non-injected control animals, only one showed a spleen index of 1.3 which is within the statistical expectation.

Discussion. The most interesting aspects of the results reported here are the apparent capacity of newborn C57B1 thymus cells to induce G.V.H. reactions in A mice, and that no striking difference between adult and newborn thymus cells was found.

Adult thymus is relatively ineffective in passively transferring immune responses although some positive results have been reported(5,6,7). After active immunization of rabbits, no antibody production can be demonstrated in the thymus(8). A low level of gamma globulin formation does occur in adult thymus tissue both in rabbits(9) and in mice (10). Newborn mouse thymus, however, does not show any γ-globulin production(10) indicating that the cells producing the G.V.H. reaction are thymocytes proper rather than cells which have migrated into the thymus.

Miller(11) was able to protect thymectomized mice with homologous or isologous whole thymus grafts but not with isologous thymus cell suspensions (5 × 10^6 cells) against the effects of thymectomy. The ineffectiveness of dissociated thymus cells in his system and the effectiveness in producing G.V.H. reactions in the present study need not contradict each other if one postulates that the presence of thymus stroma in the host is essential for effectiveness of dissociated thymus cells given. Species differences as well as differences in relative effectiveness with respect to cell dosage may also be of importance here.

Intact newborn whole thymus grafts have not been tested in the present experiments, but would seem not to produce runting in Miller's experiments(11). The slow release of thymocytes from such thymus grafts may account for this difference.

While the activity of thymus cells in producing G.V.H. reactions does not change much with age, thoracic duct and lymph node cells of adult, non-thymectomized animals are much more effective than adult thymus cells (1,2,12). Such a quantitative difference between lymphoid cells from thymus as compared to other lymphoid tissue from the adult animal might be due to a large proportion of immature cells in the thymus resulting in relatively few immunologically competent small lymphocytes(13). It is also possible that a qualitative difference exists between the two in that other lymphoid tissue but not thymus tissue receives priming stimuli with

(cross-reacting) antigens or other factors.

Summary. In the donor/recipient mouse strain combination C57B1→A, 5-15 × 10^6 neonatal thymus cells cause a G.V.H. reaction in 86% of newborn recipients. This does not differ significantly from the incidence obtained with adult thymus cells in the same dose range.

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Effect of Administration of Alkylating Agents or 6-Mercaptopurine on Serum Level of Deoxyribonuclease I in Leukemia Patients.* (28641)

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The factors influencing human deoxyribonuclease (DNase) I activity have been studied(1). A method was described for assay of this enzyme by following the rate of release of acid soluble deoxyribose-purine-containing fragments from an incubation mixture consisting of serum deoxyribonucleic acid (DNA), Mg++ and pH 7.3 buffer. Under these conditions, *in vitro* addition of nitrogen mustard (methyl-*bis*(beta-chlorethyl) amine hydrochloride) noncompetitively inhibited human serum DNase I. However, extracts prepared from human leucocytes inhibited the enzyme activity in a competitive manner(1).

Since nitrogen mustard, Myleran and 6-mercaptopurine (6-MP) are commonly used in the treatment of leukemia patients, it was of interest to determine the *in vivo* effects of these compounds on serum DNase I levels. In the present study, determinations of serum DNase I were made on normal human beings, on patients with carcinoma and on patients with leukemia. In the group of leukemia patients, DNase I levels and leucocyte counts were measured before and after therapy with nitrogen mustard, Myleran or 6-MP.

Methods. DNase I assay on serum: DNase I determinations were made on the serum samples essentially according to the procedure reported earlier(1). Two ml of serum was incubated with 2 mg deoxyribonucleic acid (DNA, sodium salt, calf thymus, Hammersten, highly polymerized; Mann Research Laboratories Inc. New York) 3 mg $MgCl_2 \cdot 6H_2O$, 0.5 ml of 0.1 M tris (hydroxymethyl) aminomethane buffer pH 7.3, and 0.1 ml toluene in a total volume of 3 ml. After 20 hours of incubation at 45°C, determinations of DNase I were made by measuring deoxyribose-purine-containing fragments in the trichloroacetic acid-soluble fraction. Eight-tenths ml of 55% trichloroacetic acid (TCA) was added to the incubation mixture and the

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