A Comparison of the Immunologic Function of Thymus Cells at Varying Stages of Maturation[1] (34545)

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The role of the thymus in immunologic maturation of the newborn and adult irradiated mouse is firmly established (1). However, from morphologic and functional studies, it would appear that the activity of the thymus is not constant throughout life (2).

In an attempt to study systematically the relationship between the age of the thymus and its immunologic function, an assay was developed (3) based on the work of Miller *et al.* (4) in the adult mouse. In this system, injection of calibrated numbers of isolated thymus cells from 2-week-old syngeneic donors significantly restored immunologic reactivity to thymectomized radiation chimeras. This procedure overcame some of the difficulties of technique and interpretation encountered when comparing grafts of intact thymic tissue and made it possible to quantitate roughly the immunologic effect of a constant number of thymus cells from donors of varying ages.

In this paper, the differences in the restorative capacity of thymus cells from fetal, neonatal, 4-week-old, and 1-year-old donors are described.

Materials and Methods. Mice of the $C_3Hf/HeHa$ strain from Health Research Inc., West Seneca, N. Y. were used throughout. Female mice, aged 7 weeks, were thymectomized or sham thymectomized as described by Miller (5). One week later, they received 850 R of X-irradiation followed by a therapeutic injection of approximately 5×10^6 bone marrow cells from 8-week-old syngeneic female donors, as previously described (3). Control groups of animals consisted of sham thymectomized and thymectomized radiation chimeras. All experimental groups were made up of thymectomized radiation chimeras which, less than 24 hr after irradiation, were injected intravenously with thymus cells. Thymus cell donors were either late third trimester fetal mice, neonatal mice within 24 hours of birth, 4-week-old or 1-year-old syngeneic mice. In each study group the cells were pooled from a number of donors of the same age and 5×10^6 cells were injected intravenously into each recipient mouse. In a later experiment, most of the above groups were repeated and one additional group received 20×10^6 fetal thymus cells.

The standard dose of 5×10^6 thymus cells was selected because a previous study showed that this number restored immunologic competence to a significantly higher level than in the thymectomized controls but still clearly lower than the sham thymectomized chimeras (3). Thus, this number of cells should permit detection of differences in the restorative capacities of thymus cells from donors of varying ages.

Hemagglutinin titer to sheep red cell antigens was studied as an estimate of immunologic competence. Thirty days after irradiation all recipient mice were injected intraperitoneally with 0.2 ml of a 20% suspension of washed sheep red blood cells (SRBC) in saline. The mice were bled from the retro-orbital sinus of the eye before the injection of SRBC and 7, 14, and 21 days later. Serum antibody titers were expressed as logarithms (to the base 2) of the reciprocal of the greatest dilution showing macroscopically visible agglutination. Animals with naturally occurring sheep cell agglutinins were eliminated from the study. In one experiment, the

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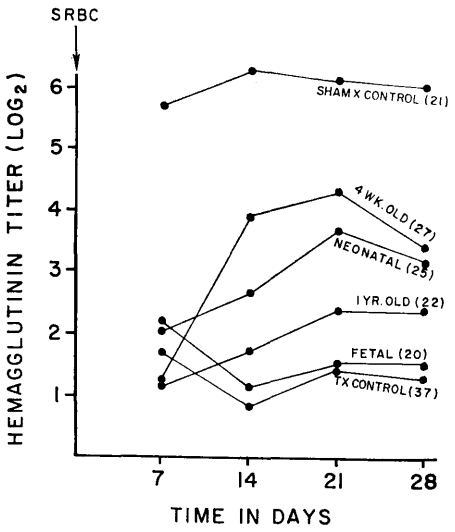

FIG. 1. Serum hemagglutinin titers after sheep red blood cell injection in adult thymectomized radiation chimeras injected with thymus cells from donors of varying ages. The number of mice per group is given in parentheses.

21-day blood sample was also used to measure the mononuclear white cell levels.

Additional studies, using the Cunningham and Svenberg (6) modification of the hemolytic plaque technique of Jerne *et al.* (7), were carried out to detect direct (19*S*) and indirect (7*S*) plaque-forming cells (PFC). The antimouse gamma globulin rabbit serum used in this assay was purchased from a commercial source. The immunization procedure was the same as that for the detection of hemagglutinins.

Results. Figure 1 depicts the effect of injecting 5×10^6 thymus cells of different ages. The mean antibody titers 21 days after antigen injection of all thymectomized groups injected with thymus cells remained significantly lower than that of the sham thymectomized control radiation chimeras ($p < 0.001$). The thymectomized groups injected with thymus cells from neonatal or 4-week-old donors achieved higher mean antibody titers than the thymectomized control group ($p < 0.001$); however, the group injected with thymus cells from 1-year-old donors was only marginally improved over the thymectomized controls ($p < 0.025$) and the group given 5×10^6 fetal thymus cells failed to show significant restoration ($p > 0.5$).

On statistical analysis of the 21-day mean antibody titers of the groups injected with 5×10^6 thymus cells, the restorative capacity of thymus cells from fetal donors was found to be similar to that of cells from 1-year-old donors ($p < 0.200$) but significantly lower than that of cells from neonatal or 4-week-old donors ($p < 0.001$). These latter two groups did not differ significantly from each other ($p < 0.050$) and achieved higher titers than the group injected with cells from one-year-old donors ($p < 0.005$).

Table I gives the results of a second experiment which showed that the injection of 20×10^6 thymus cells from fetal donors afforded a mean titer significantly higher than the injection of 5×10^6 such cells ($p < 0.001$); this higher number of fetal cells gave immunologic restoration equal to that observed in the groups injected with 5×10^6 thymus cells from neonatal or 4-week-old donors ($p < 0.5$).

The peripheral mononuclear white cells levels of some of the mice referred to in Table I are shown in Table II. The level of the thymectomized controls was about half that of the sham thymectomized group. All

TABLE I. Hemagglutinin Titers 21 Days After SRBC Injection in Adult Thymectomized Radiation Chimeras Injected with Fetal Thymus Cells.

Groups	Hemagglutinin titer ($\log_2$) ± SD	No. of mice	*p* values cf. Tx control
Tx controls[a]	1.05 ± 1.34	20	—
Sham Tx controls	6.63 ± 0.72	16	<0.001
Tx + 5×10^6 fetal thymus cells	2.08 ± 1.71	13	>0.5
Tx + 20×10^6 fetal thymus cells	4.45 ± 1.13	11	<0.001
Tx + 5×10^6 neonatal thymus cells	4.13 ± 0.92	15	<0.001
Tx + 5×10^6 4-week-old thymus cells	4.76 ± 1.15	17	<0.001

[a] Tx = thymectomized.

[2] Cappel Laboratories, Inc., Dowington, Pa.

TABLE II. Number of Mononuclear Cells/mm³ of Peripheral Blood of Reconstituted Thymectomized Radiation Chimeras.

Groups	Mean no. of mononuclear cells/mm³ of peripheral blood ± SE	No. of mice	p values (cf. Tx controls)
Tx controls	2771 ± 331	10	—
Sham Tx controls	5259 ± 647	10	<0.001
Tx + 5 × 10⁶ fetal thymus cells	3034 ± 167	10	>0.5
Tx + 20 × 10⁶ fetal thymus cells	2279 ± 193	10	>0.5
Tx + 5 × 10⁶ neonatal thymus cells	2908 ± 370	10	>0.5
Tx + 5 × 10⁶ 4-week-old thymus cells	3992 ± 628	10	>0.2

the reconstituted thymectomized groups showed no improvement over the thymectomized controls except for the group injected with thymus cells from 4-week-old donors but this was not significant ($p < 0.2$).

Control and most reconstituted groups of mice were assayed individually for the number of direct PFC in their spleens 4 days after immunization with SRBC. No significant increase in the number of plaques was produced by the antimouse gamma globulin at this time. As shown in Table III, there was some rise above that of the thymectomized controls in the number of direct PFC/10^6 spleen cells of the reconstituted mice. However, the increase was not significant and did not approach the level of the sham thymectomized controls. There was little difference between the reconstituted groups.

Both direct and indirect plaques were studied 7 days after immunization and the results are shown in Table IV. The spleens were again assayed individually, part of each cell suspension being used for the detection of direct PFC and part for the indirect. The results of the indirect PFC are not corrected for the presence of direct PFC within the total obtained. This total may not include all direct PFC since they may have been partially inhibited by the antimouse gamma globulin antiserum (8). However, the relative differences between the indirect and direct PFC responses can be compared since the direct PFC response was assayed at the same time.

The values for direct PFC 7 days after immunization were lower than the 4-day values, except for the thymectomized group injected with thymus cells from 4-week-old donors where the difference was slight. Day 7 is, therefore, presumably past the time of the peak 19S response. The statistical differences between the groups were similar to those at day 4.

Of the indirect PFC responses, those of the thymectomized group reconstituted with thymus cells from 4-week-old donors were significantly higher than the thymectomized controls ($p < 0.005$) but this was not found with the group injected with cells from 1-year-old donors ($p < 0.4$). The difference between these two reconstituted groups was not significant ($p < 0.2$) and both were still far below the sham thymectomized controls.

Discussion. Recent studies (9, 10) suggest the existence of synergism between thymus cells and antibody-forming precursor cells of the bone marrow. The present experiments based on the production of serum hemagglutinins to sheep RBC show that thymus cells from donors of differing ages possess varying abilities to confer immunologic maturation presumably upon bone marrow-derived lymphoid cells. Thus, five million thymus cells

TABLE III. Mean Numbers of Direct PFC/10^6 Spleen Cells of Reconstituted Thymectomized Radiation Chimeras 4 Days After Antigen Injection.

Groups	No. of direct PFC/10^6 spleen cells ± SE	No. of mice	p values (cf. Tx controls)
Tx controls	15 ± 2	10	—
Sham Tx controls	91 ± 15	10	<0.001
Tx + neonatal thymus cells	27 ± 5	7	<0.050
Tx + 4-week-old thymus cells	22 ± 5	10	<0.4
Tx + 1-year-old thymus cells	49 ± 17	10	<0.1

TABLE IV. Mean Numbers of Direct and Indirect PFC/10^6 Spleen Cells of Reconstituted Thymectomized Radiation Chimeras 7 Days After Antigen Injection.

Groups	No. of direct PFC/10^6 spleen cells ± SE	No. of indirect PFC/10^6 spleen cells ± SE	No. of mice	p values (cf. Tx controls) Direct PFC	p values (cf. Tx controls) Indirect PFC
Tx controls	7 ± 2	11 ± 4	7	—	—
Sham Tx controls	47 ±13	747 ±244	3	<0.005	<0.001
Tx + 4-week-old thymus cells	25 ± 7	123 ± 31	6	<0.025	<0.005
Tx + 1-year-old thymus cells	15 ± 3	55 ± 30	7	<0.1	<0.4

from late-term fetal donors afforded virtually no restoration of immune reactivity of thymectomized radiation chimeras, whereas thymus cells from newborn or 3-week-old donors manifested significant restoration. The deficiency observed in the thymus cells of late-term fetal donors was not absolute but quantitative, since an inoculum of 20 million such cells showed a significant restorative capacity equal to that of five million thymus cells from neonatal or 4-week-old syngeneic donors. The results observed in the postnatal groups suggest that thymus cells taken from donors within 24-hr of birth possess restorative capability equal to that observed in thymus cells from 4-week-old mice, but this capacity diminished by 1 year of age.

These findings are consistent with earlier reports on the pattern of thymic development in the perinatal period. In a study of mouse thymus differentiation, Ball (11) observed, during the late gestation period, a rapid increase in the absolute number of cells and a decrease in cell size which reflected "the rise of a new cell type, the small lymphocyte." Both he, and Axelrad and Van der Gaag (12) showed, in the postnatal thymus, a continuing increase in the proportion of small lymphocytes until the maximum size of the thymus is reached. The greater proportion of large undifferentiated cells in the fetal thymus may well be incapable of the restorative function described here, and this could explain the difference in action between the fetal and neonatal thymus cell inocula. Admittedly, the identity of the active component in the thymus cell suspensions is unknown. There is additional evidence which suggests changes in thymic cellular activity throughout the perinatal period. Clark observed alterations with age in the type of inclusions in epithelial cells of the thymic medulla which are indicative of secretory activity (13). This activity appeared to "develop late in fetal life, accelerate after birth and moderate about 2 weeks after birth." It may be a reflection of this activity that is observed in the work reported here and may explain the similarity in action between neonatal and 3-week-old thymus cells.

In the graft-*versus*-host assay of Simonsen (14), thymus cells from neonatal and adult mice were equally reactive (15, 16). The present observations on thymus cells from neonatal and 4-week-old donors indicate the same is true of their ability to induce the production of circulating antibody.

Using the hemolytic plaque-forming technique, thymectomized radiation chimeras reconstituted with thymus cells were deficient in production of direct PFC (19*S*) both 4 and 7 days after antigen injection. These were times at which sham thymectomized chimeras were capable of good responses. However, 7 days after immunization, the thymectomized group injected with thymus cells from 4-week-old donors, produced significantly higher numbers of indirect PFC (7*S*) than the thymectomized controls. Similar restoration was not found in the group which received thymus cells from 1-year-old donors. If mouse agglutinins to sheep red cells are 7*S* globulins (17), it would appear that this component of the immunologic system is the one more readily restored to thymectomized mice by the injection of certain thymus cells.

Of the thymus cells tested in this work, those from 4-week-old donors appear to be the most effective.

It was suggested by Eidinger and Pross (18) Shearer *et al.* (19) and others that 19*S* and 7*S* globulins are produced by different populations of progenitor cells. It could, therefore, be argued that the thymus cell inocula studied here are largely deficient in activity responsible for 19*S* globulin production, whereas, the function related to 7*S* antibody production appears to be low in fetal thymus, to rise after birth with a peak possibly at 4 weeks and to be low again in the old mouse.

In the reconstituted groups given thymus cells from neonatal or 4-week-old donors, the serum antibody titers were in closer approximation to the levels measured in the sham thymectomized group than was observed when these groups were analyzed for the number of PFC. This discrepancy may reflect homeostatic regulation of the antibody forming cells of the sham thymectomized group since their high number of plaque-forming cells was not reflected in excessive levels of circulating antibody.

Summary. Injections of mouse thymus cells from fetal, neonatal, 4-week- and 1-year-old syngeneic donors were compared for their ability to potentiate the production of serum antibody to sheep red blood cells in thymectomized, irradiated recipients previously injected with syngeneic bone marrow. Five million fetal thymus cells afforded no restoration of the immunologic response and one-year-old thymus cells gave marginal improvement. The apparent deficiency of the fetal thymus cell inoculum was quantitative since 20 million of these cells proved to be as active as five million neonatal thymus cells. Neonatal and 4-week-old thymus cells induced significant and virtually equal immunologic reactivity in terms of serum antibody titers. Immunologic restoration estimated by the number of spleen direct PFC was insignificant in all the groups given thymus cells. There was no increase in the number of indirect PFC observed in the experimental groups except for the one treated with 4-week-old thymus cells and here the restoration was significant but incomplete. These findings are discussed with reference to the cellular changes in the thymus in the perinatal period.

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