Fetal Liver and Adult Thymus Cells: Absence of Synergism in Graft-versus-Host Reactions[1] (34392)

MARVIN L. TYAN[2]
(Introduced by Leonard J. Cole)
Naval Radiological Defense Laboratory, San Francisco, California 94135

It has been shown that an increased antibody response to sheep red cells results when dissociated thymus or thoracic duct cells are allowed to interact with lymphoid stem cells of bone marrow origin in lethally irradiated adult or thymectomized newborn mice (1–4). Further, it has been demonstrated that this augmented response is due primarily to antibody production by the lymphoid precursors contained in the marrow (3, 4). On the other hand, using a graft-versus-host model, it has been shown that dissociated thymus cells from fetal or newborn mice are incapable of promoting the maturation of the lymphoid precursors contained in fetal liver (5). Further, there have been reports recently in which varied experimental models have failed to reveal a synergism between thymus cells from adult mice and bone marrow in cell-bound immune reactions (6–8). The purpose of the present experiments was to determine whether a synergistic interaction in graft-versus-host reactions would result from the admixture of lymphoid stem cells from mouse fetal liver and cells from adult thymus.

Materials and Methods. The presence of mature lymphoid cells or their precursors in adult thymus or fetal liver was demonstrated by means of a modified parental-F_1, graft-versus-host, method (9, 10). In contrast to tissues which contain mature lymphoid cells, fetal liver from a homozygous donor does not produce deaths when injected into a sublethally irradiated F_1 hybrid, one parental strain of which is identical to that of the donor. However, if after 60 days the spleen and lymph nodes of this primary host are injected into a second F_1 hybrid (one parental strain identical to that of the liver cell donor but the second parental strain differing from the second parent of the primary host), a significant number of deaths will occur within 60 days. Thymectomy of the first host prevents this effect, as the thymus is essential for the maturation of these lymphoid precursors in the first host (11). It was felt that if a synergistic interaction did occur between cells of thymic origin and lymphoid stem cells in cell-bound immune reactions, this interaction would manifest itself in our experimental model by a mortality rate among the primary recipients of both types of cells greater than that produced by thymus cells alone. Alternatively, the interaction might result in maturation of stem cells tolerant of the primary host (5); this would result in no change in the mortality rate of the primary hosts, but rather in an increase in the number of deaths among secondary recipients given lymphoid tissues from thymectomized primary hosts.

In all experiments involving parental fetal liver cells each primary host was given 50×10^6 viable nucleated cells from 17- to 20-day-old A/HeJ embryos intraperitoneally. The recipients of dissociated thymus cells were given 20 or 50×10^6 nucleated cells from 10- to 14-week-old A/HeJ donors intraperitoneally. The thymuses were dissected carefully in an effort to eliminate contamination with mediastinal lymph nodes or peripheral blood. In some experiments the primary recipients underwent thymectomy 1 week prior to irradiation and cell transfer.

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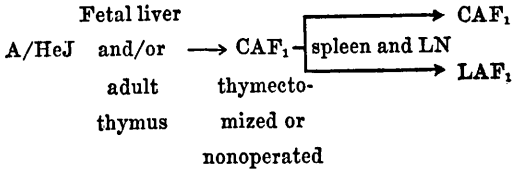
[2] Present address: Stanford Research Institute, Menlo Park, Calif. 94025.

TABLE I. Sixty-Day Mortality among Sublethally Irradiated Primary and Secondary F_1 Hybrid Recipients of Parental (A/HeJ) Fetal Liver and/or Adult Thymus Cells.

Fetal liver (50×10^6)	Thymus cells ($\times 10^6$)	Thymectomy primary host	60-day Mortality (no./total)		
			Primary host	Secondary host	
			LAF_1	CAF_1	LAF_1
+	—	—	0/20	29/30	0/10
+	—	+	0/19	0/28	
—	20	—	12/20	2/8	0/6
—	20	+	2/20	10/28	0/8
—	50	—	5/10	1/5	0/5
—	50	+	3/10	1/7	0/7
+	20	—	0/10	7/10	0/10
+	20	+	0/20	7/28	0/10
+	50	—	0/20	16/20	0/20
+	50	+	0/20	6/20	0/20
—	—	—	0/17	0/34	0/10
—	—	+	0/24	1/40	0/10

All primary and secondary hosts received 500 rads of whole-body X-radiation prior to injection (250 kVp, 15 mA; HVL 1.5 mmCu; 28 rads/min). Noninjected, irradiated mice served as primary controls. Sixty days after the injection of fetal liver and/or adult thymus cells the survivors were killed. The spleen and lymph nodes of each were gently disrupted separately; the resultant cell suspensions were injected into two sublethally irradiated secondary recipients (in many instances one of the secondary hosts was syngeneic to the primary host). All primary hosts were 12-week-old male (BALB/c × A)F_1 (CAF_1) mice, and secondary hosts were either 12–14-week-old (C57L × A)F_1 (LAF_1) or CAF_1 mice. The mice were housed randomly, 6–10 to a cage. The experimental design may be summarized as follows:



Results. (Table I). There were no deaths among the primary recipients of fetal liver cells or among the secondary hosts given lymphoid cells from the thymectomized primary recipients of liver cells. However, there was a high mortality rate when the secondary hosts received spleen and lymph node cells from nonoperated primary recipients. No deaths occurred among the secondary hosts when they were syngeneic with the primary recipients of liver and/or adult thymus cells, demonstrating that lymphoid precursors develop specific tolerance for the transplantation isoantigens of the primary host (5).

Deaths occurred among all groups of primary hosts given adult thymus cells. There was a significant decrease in the mortality rate ($p<0.001$) among the thymectomized primary hosts given 20×10^6 cells when comparison is made with their nonoperated controls; however, there was no significant difference in the mortality rates in the groups given 50×10^6 cells. When compared to the control groups, there was a low but highly significant mortality rate ($p<0.001$) among the secondary recipients of thymus cells from thymectomized and nonoperated hosts. When fetal liver cells and adult thymus cells were given concurrently, no deaths occurred among the primary hosts. This was a highly significant decrease in the mortality rate noted when thymus cells alone were given ($p<0.001$). Further, there was no indication that the

addition of liver cells to the thymus cells increased the mortality rate among the secondary hosts given lymphoid tissues from thymectomized primary recipients.

Discussion. Clearly the present data do not demonstrate a synergism in graft-versus-host reactions between thymus cells from adult mice and the lymphoid precursors contained in fetal liver. Rather, as has been reported previously (8), the addition of lymphoid stem cells seemed to inhibit the capacity of thymus cells to produce a graft-versus-host reaction. It is not clear from these experiments whether this latter effect is due to a specific interaction of the cells in a process independent of or competitive with cell-bound immune responses (*e.g.*, antibody production) or whether it is due to a nonspecific effect such as competition for space in the host lymphoid tissues. Further, it would seem clear that dissociated thymus cells from adult mice, as well as those from fetal and newborn mice, are incapable of inducing a measurable degree of maturation of the lymphoid precursors in fetal liver; the intact structure of the thymus seems to be necessary (5).

The occurrence of a small but significant number of deaths among the secondary recipients of thymus cells from thymectomized as well as from nonoperated primary hosts suggests that in addition to stem cells and completely mature lymphoid cells (those which can produce deaths in the primary hosts) the thymus contains cells which are functionally intermediate. That is, the stem cells derived from fetal liver or bone marrow may undergo at least two stages of functional maturation, one of which is certainly under the influence of the thymus. In the first stage, which most likely takes place in the thymus, the stem cell gains the *potential* to participate in cell-bound immune reactions without losing its ability to develop specific tolerance. In the next stage the cell which has been seeded to the peripheral lymphoid tissues becomes tolerant of antigens in its environment prior to gaining full immunological competence as a result of thymic or nonthymic factors. Thereafter, this effector cell is unable to develop specific tolerance and if involved in a cell-bound immune response is consumed by a process described as "allergic death" (12). Obviously this hypothesis requires further experimental exploration.

Summary. Parental fetal liver cells were unable to produce a measurable graft-versus-host reaction in sublethally irradiated F_1 hybrids. No synergism between fetal liver cells and thymus was observed in graft-versus-host reactions.

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