

LYMPHOID PRECURSORS: THYMUS INDEPENDENT ANTIBODY PRODUCTION^{1,2}

MARVIN L. TYAN, LEONARD A. HERZENBERG AND PRISCILLA R. GIBBS

From the Naval Radiological Defense Laboratory, San Francisco, California 94135,³ and the Department of Genetics, Stanford University School of Medicine, Stanford, California 94305⁴

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Thymectomized and nonoperated adult mice were lethally irradiated and given liver cells from embryos of various ages. The resultant chimeras were hyperimmunized with sheep erythrocytes, and cell suspensions from their spleens were used in a hemolytic plaque assay employing specific anti-allotype antisera as developing agents in order to distinguish between cells producing host and donor-type γG_{2a} (7S) antibodies. It was found that lymphoid stem cells taken from embryos as early as the 10th day of gestation (i.e., before the appearance of the thymic rudiment) were able to produce γG_{2a} anti-SRBC antibodies in thymectomized hosts. The number of donor-type "developed" plaques found in the spleens of thymectomized hosts was approximately one-tenth that found in their nonoperated controls.

Sensitization of the hosts to SRBC before irradiation and the transfer of embryonic liver cells did not increase the number of donor-type PFC.

Adult thymectomized mice exposed to a potentially lethal dose of wholebody irradiation and protected with hemopoietic and lymphoid stem cells from bone marrow or fetal liver subsequently show a marked impairment of their ability to reject foreign skin grafts and have a severely diminished capacity to produce antibody to some but not all antigens (1-4). These studies have shown that the primary response to sheep erythrocytes (SRBC), while considerably diminished by thymectomy-irradiation, is not totally abrogated in the majority of mice. It is not known, however, whether this response is host in origin or due to antibody production by the transferred lymphoid cells and thus a manifestation of an innate function of these stem cells.

It has been shown recently that lymphoid precursors obtained from the livers of embryos as early as the 9th to 12th days of gestation (i.e.,

prior to the appearance of the thymic rudiment) give rise to cells which are able to produce γG_{2a} immunoglobulins in thymectomized-irradiated hosts as well as they do in nonoperated controls (5, 6). Despite apparently adequate levels of donor immunoglobulins, these thymectomized chimeras were unable to produce detectable antibody to the synthetic polypeptide (T,G)-A--L (a polylysine backbone with side chains of poly-DL-alanine and terminating in short random sequences of tyrosine and glutamic acid), suggesting that the formation of specific antibody to at least certain antigens is more thymus dependent than is immunoglobulin production *per se*. However, it was subsequently noted that the majority of these thymectomized chimeras produced relatively good antibody responses to sheep erythrocytes if repeatedly challenged. The purpose of the present experiments was to determine the extent of donor stem cell participation in the response to SRBC antigens. This was done with the use of specific anti-allotype sera to "develop" and identify host and donor γG_{2a} plaque-forming cells in the spleens of these thymectomized chimeras.

MATERIALS AND METHODS

Hosts. Thymectomized and nonoperated (C57L × A)F₁ (LAF₁) mice were used throughout as hosts. Completeness of thymectomy was con-

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³ M. L. Tyan. Present address: Stanford Research Institute, Menlo Park, Calif. 94025.

⁴ L. A. Herzenberg and P. R. Gibbs.

firmed macroscopically when the mice were killed for the plaque-forming cell (PFC) assay. The mice underwent thymectomy 2 or more weeks prior to irradiation. Sham thymectomies were not done, as previous work (1-3) has shown that this operative procedure has no effect on immunologic responsiveness.

Irradiation. The LAF₁ mice received 870 rad whole-body x-radiation (250 kvp, 15 ma; HVL 1.5 mm Cu).

Liver cell donors. C57Bl/6 × C57Bl/6 embryos were used as donors of liver cells. The livers were dissected free of maternal blood or tissue contamination, minced and suspended in cold TC 199 (Difco Laboratories, Detroit, Mich.) and injected i.p. into the host shortly after irradiation. Each recipient received from 25 to 75 × 10⁶ viable nucleated cells. The estimate of the age of gestation was based on the following criteria: 9 to 10 days, a well developed yolk sac, a conspicuous heart and early pigmentation of the liver; 11 to 12 days, a well pigmented liver and the absence of any evidence of a thymic rudiment; 13 to 20 days, the presence of the thymus and the relative size and development of the fetus. This host-donor combination was selected because of the readily distinguishable γ G_{2a} allotypes (7).

Immunizations. Forty-five days after irradiation the chimeras were given 0.2 ml of a 20% suspension of SRBC i.p.; this was repeated weekly for from 4 to 8 weeks and the mice were bled and killed 10 days after the last injection (between 90 and 120 days after irradiation). In some experiments the host mice were given SRBC i.p. twice in the 3 weeks before irradiation. In addition, certain of the chimeras were immunized 45 and 100 days after irradiation with the synthetic polypeptide (T, G)-A--L (6).

Serum titrations. The mice were bled from the retro-orbital plexus on the day of death. The sera were titrated for SRBC agglutinins in 1.5% PVP, and the titers are expressed as the reciprocal of the log₂. The sera were tested for antibodies to (T, G)-A--L as previously described (6).

Assay method for PFC. The details of this method have been described previously (8-10). Briefly, cell suspensions were made from the spleens of the chimeras and samples were plated on three sets of slides for determining the number of direct or "undeveloped" γ M plaque-forming cells, and, using specific anti-allotype sera, the number of "developed" or γ G_{2a} (7S) host or donor PFC. The majority of assays were done in tripli-

cate and the results of the individual assays were always in close agreement. All assays were done using as controls spleen cells from sensitized non-irradiated mice producing γ G_{2a} antibody of known host or donor allotype. The number of "developed" plaques was calculated by subtracting the average number of "direct" plaques produced by a given spleen from the average number of PFC found on the slides developed for host or donor γ G_{2a} allotype. In most instances when donor 7S PFC were found the number of "developed" donor plaques was at least twice the number of "direct" γ M plaques. Normal mice in our colony (NRDL) have a background of 10 to 100 γ M PFC/spleen (11).

Assay of serum immunoglobulins. The sera were assayed for donor-type γ G_{2a} globulins by a semi-quantitative double diffusion-in-gel method (12). In a few cases the sera were quantitatively tested for donor γ G_{2a} globulins by an inhibition of precipitation assay using standard serum pools of donor allotype as a reference (13).

In summary, thymectomized and nonoperated mice were lethally irradiated and protected with cells from the livers of 9- to 20-day-old embryos. Subsequently, these mice were immunized with SRBC and a synthetic polypeptide (T, G)-A--L; their sera were tested for antibodies to these antigens. Cell suspensions from their spleens were used on a hemolytic plaque assay employing specific anti-allotype sera as developing agents in order to distinguish between cells producing host and donor-type γ G_{2a} (7S) anti-SRBC antibodies. In addition, the amounts of donor-type γ G_{2a} globulins in the sera of the thymectomized and nonoperated mice were compared by means of semi-quantitative and quantitative methods.

RESULTS

Serum titrations. The mean anti-SRBC hemagglutinin titers produced by the thymectomized chimeras were about four doubling dilutions lower than those of the nonoperated controls (Table I). The mice used in the plaque assay had anti-SRBC titers somewhat higher than the mean (6.0 ± 2.0 vs 4.2 ± 2.9), since it was soon found that mice with serum titers below 1:4 produced no 7S and few γ M plaques. The mean titers varied little with respect to the age of the fetal liver donors and more than half of the mice responded with titers of 1:4 or greater. On the other hand, none of the thymectomized mice

TABLE I

Response to SRBC and origin of γG_{2a} plaque-forming cells (PFC) in thymectomized (T_x) and nonoperated, lethally irradiated LAF₁ mice given liver cells from C57Bl/6 embryos

Age of Fetal Liver Donor	Host T_x	Response to SRBC			Origin and Type of Plaque-Forming Cells					
		No. mice	Log ₂ titer mean \pm S.D.	No. of Mice	γG_{2a} PFC					No. of donor γG_{2a} PFC/spleen ^a
					Donor only	Host and donor	Host only	"Direct" PFC only	No. PFC	
<i>days</i>										
10-12	Yes	22	3.4 \pm 3.4	10	2	4	1	1	2	1,280 (200-3500)
	No	2	8.0	1		1				18,000
13-16	Yes	19	4.3 \pm 2.7	10	4	2		2	2	2,070 (250-9,000)
	No	3	7.3 \pm 1.1	2	1	1				19,000 (18-20,000)
17-20	Yes	95	4.8 \pm 2.8	42	10	12		17	3	3,470 (400-15,000)
	No	23	7.4 \pm 0.9	3	1	2				22,000 (20-25,000)

^a Spleens of thymectomized hosts containing donor γG_{2a} plaques. Mean number of donor PFC in all such spleens, 2815. Mean including spleens not containing donor PFC, 1500.

TABLE II

Response to the synthetic polypeptide (T,G)-A-L by thymectomized (T_x) and nonoperated, lethally irradiated LAF₁ mice given liver cells from C57Bl/6 embryos^a

Age of Fetal Liver Donor	Host T_x	Mean Response to (T,G)-A-L	
		No. mice	% ppt. ^b \pm S.D.
<i>days</i>			
10-12	Yes	6	3 \pm 4.2
	No	2	60 \pm 8.0
13-16	Yes	5	1 \pm 2.0
	No	3	46 \pm 5.6
17-20	Yes	24	1 \pm 0.5
	No	20	52 \pm 12.5

^a Certain of the data have been reported previously (6).

^b Percentage of antigen precipitated by sera diluted 1:250.

tested responded to two immunizations with (T,G)-A-L (Table II).

Immunoglobulin assays. Ninety-three of the 94 chimeras tested had donor-type γG_{2a} globulin in their sera. As shown in Table III, quantitative and semi-quantitative assays for donor-type γG_{2a} globulins in the sera of these chimeras revealed that, if anything, the levels of donor γG_{2a} tended to be higher in the thymectomized hosts than in the nonoperated controls.

PFC assays. Six of 10 thymectomized mice which received liver cells from embryos 12 days of age or younger had significant numbers of donor-type γG_{2a} PFC in their spleens. Similarly six of 10 mice which received liver cells from 13- to

TABLE III

Production of γG_{2a} globulins by C57Bl/6 fetal liver cells in lethally irradiated thymectomized and nonoperated LAF₁ mice

Host Thymectomy	Quantitative Assay		Semi-Quantitative Assay	
	No. of mice	Donor γG_{2a} ^a	No. of mice	Donor (0-4 ⁺)
<i>mean (range)</i>				
Yes	13	18 (8-30)	54	2.6
No	9	12 (8-20)	18	2.2

^a Percentage of specific γ -globulin allotype present in the sera as compared to that present in the standard reference sera.

16-day embryos and 22 of 42 mice given cells from 17- to 20-day fetuses were found to have donor "developed" PFC in their spleens. The mean number of donor 7S PFC found in the spleens of thymectomized chimeras was approximately 10-fold lower than in the nonoperated controls. One of 62 thymectomized mice produced only host-type "developed" PFC.

As the hosts used in these experiments are known to have a normal background response to SRBC, the above results could not rule out the participation of surviving sensitized host macrophages (14) or other antigen processing or "memory" cells in the initiation of antibody production by the donor cells. Therefore, thymectomized LAF₁ mice were injected with SRBC twice in the 3 weeks before irradiation and cell transfer. The chimeras were challenged with SRBC as noted previously. While this procedure resulted in an earlier appearance of anti-SRBC agglutinins and

TABLE IV

Response to SRBC by presensitized, thymectomized lethally irradiated LAF₁ mice given liver cells from C57Bl/6 embryos

Host Sensitized	SRBC Titer		Origin and Type of PFC (No./Total; Mean and Range)					
	No. of mice	Mean ^a	Host γ G _{2a}		Donor γ G _{2a}		"Direct" PFC	
No	62	6.0 \pm 2.0	32/62	1,057 (0-11,000)	34/62	1,500 (0-15,000)	56/62	2,434 (0-23,000)
Yes	37	7.6 \pm 0.6	16/20	1,050 (0-3,500)	8/20	461 ^b (0-2,000)	19/20	2,170 (0-7,500)

^a Reciprocal log₂ and S.D.

^b Mean number of donor PFC in all 20 spleens. Mean number in spleens containing donor PFC, 1,150 (350 to 2,000).

higher titers (7.6 \pm 0.6 vs 4.2 \pm 2.9), the number of mice having donor-type 7S PFC and the mean number of donor plaques was, if anything, decreased (Table IV). There was a slight increase in the number of mice having host-type "developed" plaques. The fetal liver cells used in these experiments were from 10 to 20 day gestations. The one recipient of 10-day liver cells had 2000 donor-type PFC in its spleen.

DISCUSSION

These results demonstrate that in the absence of the thymus, lymphoid precursors obtained from embryonic liver are able to produce γ G_{2a} antibody to sheep erythrocytes. When transferred to thymectomized-irradiated hosts, lymphoid precursors obtained from embryos before, as well as after, the appearance of the thymus produce γ G_{2a} immunoglobulins at least as well as they do in nonoperated mice. Thus, immunoglobulin production *per se* appears to be largely, if not entirely, thymus-independent (5). Similarly, it was found that lymphoid stem cells taken from embryos of the same age (10 to 12 days, as well as later) were able to produce anti-SRBC but not anti-(T,G)-A-L antibodies in thymectomized hosts. However, the number of donor-type plaques found in the spleens of thymectomized hosts was only one-tenth that found in the non-operated controls.

These data, then, suggest that lymphoid precursors have an innate ability to respond to at least certain antigens, and that with respect to the response to SRBC the "maturing" influence of the thymus on the responding stem cell population, in this and perhaps other experimental models (15, 16), is primarily quantitative in nature. That is, the thymus promotes an increase

in the number of effector cells committed to this response but does not appear to be involved in antigen recognition, processing or in the initiation of antibody production. This may or may not be true with regard to other antigens.

The finding that specific responsiveness on the part of the thymectomized chimeras was observed with an antigen to which the mice had a normal background before treatment suggested that host macrophages (14) or other antigen-processing cells may have been involved in the response of the donor cells to SRBC. However, presensitization of the hosts did not result in an increased number of donor-type plaque-forming cells. Thus, it would appear that there was little, if any, interaction between surviving host "memory" or antigen processing cells and the donor effector cells.

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