PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 925–928 (1975)

Alteration of Bone Marrow–Thymus Cell Synergism in Hereditary Asplenic and Adult Splenectomized Mice (38661)

LILIANA B. WARGON[1], B. B. LOZZIO, AND C. J. WUST

The University of Tennessee Memorial Research Center and Hospital and Department of Microbiology Knoxville, Tennessee 37920

Development of the immune response to an antigen such as sheep erythrocytes (Srbc) is thought to require the interaction of at least three distinct cell types: (a) An adherent cell, probably a macrophage; (b) the thymus-dependent (T), and (c) the bursal derived, or bone marrow equivalent of mammals (B) lymphocytes (1, 2). These lymphocytes in peripheral lymphoid tissue possess properties which distinguish them from similar cells in the tissue of origin. Thus, thymus cells during differentiation and/or migration to lymphoid tissue lose the TL antigen and some of their theta antigen while obtaining more H-2 determinants (3). In the thymus, the cell is referred to as antigen reactive while the postmitotic daughter cells which settle in the spleen are referred to as specific inducer cells that are capable of triggering B cells (4). B lymphocytes have a greater sensitivity to inhibition by anti-immunoglobulin, or anti-mu chain sera in spleen than they do in bone marrow (5).

The new environment in which T and B lymphocytes settle may be essential for development of full immunocompetence by affecting differentiation or by certain cellular interactions. It has been postulated, for example, that the spleen exerts a pronounced effect upon T and B cells which allows them to take part in the response resulting in IgM synthesis (6).

Recently, we have reported that congenital asplenic mice have a marked deficiency of immunoglobulin production in the primary and secondary immune responses to sheep erythrocytes (7). Also, we have shown that a neonatal spleen cell transplant markely enhanced interferon production in congenitally asplenic mice to the extent that they were able to produce amounts of interferon approximately the same as normal littermates with spleen (8). The growth of the spleen graft was associated with hyperplasia of the majority of bone marrow cells including lymphoid cell (9). These results prompted us to explore the T and B cell synergistic ability leading to antibody formation of adult congenitally asplenic mice prior to and after transplantation of spleen cells at birth. Adult splenectomized littermates were also studied.

Material and Methods. Animals. Mice that are genetically asplenic in the heterozygous condition (Dh/+, dominant hemimelia) and normal littermates with spleens (+/+, homozygous), hereinafter described as "asplenic" and "normal", respectively, were used in this study. A partially inbred colony of asplenic mice was developed by brother–sister matings and have been maintained here (UTMRC) for the past three years. Splenectomy and sham-splenectomy was performed in 1-mo-old mice of both sexes as indicated previously (7). Mice were used 60–80 days after surgery because these mice remained anemic for at least 45 days after splenectomy (10).

Neonatal spleen cell transplants (SCT). Spleen cells were obtained from 1-mo-old normal donors (+/+) and 20 $\times$ 10^6 cells, in 0.1 ml of saline, were injected subcutaneously into both asplenic and normal littermates within 20 hr after birth. The number of spleen cells to be injected was determined on the basis of the number of nucleated cells in the spleen of a newborn mouse, which ranged from 15 to 23 $\times$ 10^6 cells. These mice were then held for 2 mo before being used in the plaque forming cells experiments. An autopsy was made in all transplanted mice to determine the macroscopic appearance of the graft. Tissue sections were also examined by standard techniques. The macroscopic and

[1] Predoctoral Research Trainee from the Facultiad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina.

microscopic appearance of spleen grafts has been described (8, 9).

Irradiation and reconstitution experiments. Normal recipients, 2 mo of age, were given 850R with an X-ray source as described elsewhere (11). Sixteen hr later viable bone marrow (B) and/or thymus (T) cells (20×10^6 cells each) in 0.2 ml of Eagle's Minimal Essential Medium with Hank's balanced salt solution containing 40 units of heparin were injected intravenously (iv). Cell viability was determined using trypan blue as indicated previously (7). On the fourth day after irradiation, 0.2 ml of a 10% suspension of washed Srbc was given iv. Mice were killed on the 14th day, spleen cell suspensions were made and the number of plaque-forming cells (PFC) was determined.

Plaque-forming cells assay. Direct (19S) PFC were detected according to the method of Jerne, Nordin and Henry (12) as reported previously (7). Indirect (7S) antibody forming cells were developed using goat-anti-serum to mouse 7S gamma globulin (Hyland, Inc., Costa Mesa, CA) diluted 100-fold, a concentration found to give optimal development of indirect PFC (7) following the method of Dresser and Wortis (13).

Results. The cooperation of B and T cells in forming PFCs in the spleen of irradiated recipients is presented in Table I. Several conclusions can be drawn from these data: (a) There was a basal level of 300–400 PFC/spleen when B cells alone and T cells from asplenic mice were used in reconstitution; (b) when B cells and T cells were taken from normal (+/+) or sham-splenectomized mice, reconstitution results in a significant ($P < 0.02$) higher number of PFC with 62% being IgM producers; (c) when B and T cells were taken from splenectomized mice the number of PFC was similar to that found in normal (+/+) mice but only 29% were IgM producers; (d) in general, reconstitution of irradiated recipients with B and T cells from asplenic or splenectomized normal mice results in a lower number of 19S PFC.

Eighty percent of the mice given a neonatal SCT developed visible masses of splenic tissue, rich in lymphocytes and hematopoietic elements, at the site of the injection and surrounding area. The transplanted cells proliferate and differentiate well as indicated by the formation of nodular (2–8 mm in diameter) masses of spleen cells within the subcutaneous tissue. These masses were very rich in lymphocytes and have all the hematopoietic elements peculiar to the normal mouse spleen. In Table II are presented the data obtained when normal and asplenic donors, which had been given a subcutaneous SCT, were used as a source of B and T cells. The results indicate that a SCT did not restore either the ability of B cells from asplenic mice to produce IgM in normal numbers or to cooperate with T cells in irradiated recipients.

Discussion. Our data indicate that the ab-

TABLE I. BONE MARROW–THYMUS CELL COOPERATION OF CONGENITALLY ASPLENIC AND SPLENECTOMIZED MICE.

Genotype of syngeneic cells transplanted		No. of irradiated recipients	Number of PfC/spleen[a]		% 19S	Total no. of PFC/spleen
Bone marrow	Thymus		19S	7S		
+/+	+/+	19	536 ± 112	326 ± 73	62	862
Dh/+	Dh/+[a]	9	171 ± 80	150 ± 80	53	321
S	S[a]	10	286 ± 160	685 ± 110	29	971
SS	SS[a]	5	610 ± 208	500 ± 180	60	1010
+/+	Dh/+	9	290 ± 144	114 ± 74	72	404
Dh/+	+/+	9	100 ± 100	200 ± 115	33	300
+/+	none	9	400 ± 120	0	100	400
Dh/+	none	10	240 ± 65	80 ± 53	75	320
S	none	8	250 ± 120	180 ± 49	58	430
SS	none	5	316 ± 164	0	100	316

[a] Dh/+, S, and SS denote congenitally asplenic, splenectomized, and sham-splenectomized mice, respectively. The number of PFC given is the mean ± one SD observed.

TABLE II. EFFECT OF A SPLEEN CELL TRANSPLANT AT BIRTH ON THE BONE MARROW-THYMUS CELL COOPERATION OF CONGENITALLY ASPLENIC MICE.

Genotype of syngeneic cells transplanted		Number of irradiated recipients	Number of PFC/spleen[a]			% 19S
Bone marrow	Thymus		19S	7S	Total	
+/+	+/+	17	565 ± 180	420 ± 128	985	59
Dh/+	Dh/+	14	170 ± 95	168 ± 66	338	50
+/+	Dh/+	8	300 ± 300	100 ± 50	400	75
Dh/+	+/+	8	200 ± 150	133 ± 84	333	60

[a] Values are mean ± one SD.

sence of spleen in donor mice results in a lowered capacity of B and/or T cells to reconstitute in appropriate irradiated recipients a normal degree of immunocompetence. Both B and T cells from asplenic mice appear to be defective in their cooperative response to Srbc. This lowered capacity is directly related to a diminished number of IgM producers and lower levels of serum IgM antibody (7, 14). It appears that a regulatory role of spleen is essential to develop cooperative functioning of either B or T cells during embryogenesis and perinatal life (15). Such regulation may be the response of cells to the microenvironment of the spleen.

The fact that we were unable to get restoration of T-B cooperation by transplanting spleen cells at birth stands in agreement with the results of Battisto *et al.* (6) who achieved only minimal, at best, restoration of the response by giving an entire spleen, on two occasions, intraperitoneally to young adult mice with spleen agenesis.

Another important finding of the present study concerns the marked shift from IgM to IgG producers shown by B and T cells from adult splenectomized animals transplanted in irradiated recipients. The proportional number of PFC of IgG type produced by T and B cells from asplenic mice, was similar to that of normal littermates. Thus, the shift from IgM to IgG PFC could only be seen when T and B cells from mice splenectomized were used, but not with those cells from mice with spleen agenesis. In addition, B cells from the asplenic and splenectomized mice produced a significant number of PFC of IgG type, whereas B from normal and sham-splenectomized mice produced only IgM.

Since B cells alone do not synthesize IgG in the absence of T cells (15) or T cell-replacing factor (16) we must assume that numerous T cells were present among marrow cells from asplenic and splenectomized animals or that T replacing factor was synthesized in the recipients of B cells from mice without spleen but not in those getting B cells from mice with spleen.

The possibility that the spleen influences the immune competence, early in fetal life, is based on several observations: (a) Spleen cells may have antigens recognized by neonatal thymus cells but to which tolerance develops in the adult (17, 18); (b) the spleen secretes a factor which participates in the regulation of the in vitro primary immune response (19); (c) normal T and B cells do not collaborate when they are derived from an hereditarily asplenic mouse (6, present study) or from neonatally splenectomized mice (20–21); and (d) B-cell differentiates further in the splenic environment (22).

Summary. The cooperation between bone marrow (B) and thymus (T) cells was markedly impaired in mice with congenital asplenia. The deficiency of IgM producers could not be corrected by neonatal transplantation of spleen cells. The use of T and B cells from splenectomized donors results in a marked shift from 19S to 7S antibody forming cells.

1. Chan, E. L., Mishell, R. I., and Mitchell, G. F., Science **170**, 1215 (1970).
2. Raff, M. D., Nature (London) **242**, 19 (1973).
3. Boyse, E. A., and Old, J. J., Ann. Rev. Genet. **3**, 269 (1969).
4. Ito, T., Kino, T., and Cudkowicz, G. J., Immunology **110**, 596 (1973).

5. Mond, J. J., and Thorbecke, G. J., J. Immunol. **110,** 605 (1973).
6. Battisto, J. R., Borek, F., and Bucsi, R. A., Cell Immunol. **2,** 627 (1971).
7. Lozzio, B. B., and Wargon, L. B., Immunology **27,** 167 (1974).
8. Lair, S. V., Brown, A., and Lozzio, B. B., Proc. Soc. Exp. Biol. Med. **146,** 475 (1974).
9. Lozzio, B. B., and Machado, E. A., Exp. Hematol. **3,** (in press) (1975).
10. Lozzio, B. B., Amer. J. Physiol. **222,** 290 (1972).
11. Lopez, A., and Lozzio, B. B., J. Reticuloendothelial Soc. **12,** 324 (1972).
12. Jerne, N. K., Nordin, A. A., and Henry, O. *in* "Cell Bound Antibodies" (B. Amos, H. Kiprowski, eds.) p. 109 Wistar Gust Press Philadelphia (1963).
13. Dresser, D. W., and Wortis, H. H., Nature (London) **208,** 859 (1965).
14. Battisto, J. R., Cantor, L., Borek, F., Goldstein, A., and Cabrerra, E., Nature (London) **222,** 119 (1969).
15. Chiller, J. M., Weigle, W. O., Contemp. Topics Immunobiol. **1,** 119 (1972).
16. Schimpl, A., and Wecker, E., J. Exp. Med. **137,** 547 (1972).
17. Carter, J., and Wegman, T. G., Cell. Immunol. **7,** 402 (1973).
18. Howe, M. L., Goldstein, A. L., and Battisto, J. R., Proc. Nat. Acad. Sci. U.S.A. **67,** 613 (1970).
19. Watson, J., and Thoman, M., Proc. Nat. Acad. Sci., U.S.A. **69,** 594 (1972).
20. Bucsi, R. A., Borek, F., and Battisto, J. R., J. Exp. Med. **136,** 761 (1972).
21. Shillcock, J. A., Pappas, F., and Battisto, J. R., Fed. Proc. **32,** 966 (Abs.) (1973).

Received September 6, 1974. P.S.E.B.M., 1975, Vol. 148.