The authors gratefully acknowledge the technical assistance of Miss Emma Radler, Anna Gruener and Dr. Edith Czaczkes, and the secretarial assistance of Mrs. Margaret R. Kauder and Miss Ann Bright.

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Received June 3, 1963. P.S.E.B.M., 1964, v115.

Effect of Neo-Natal and Adoptive Immunization on Tumor Transplant Susceptibility in Mice.* (28822)

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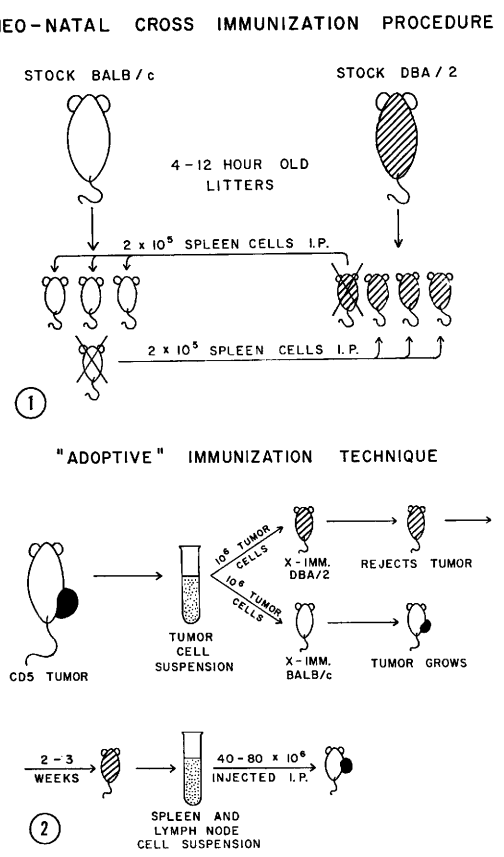
There are numerous reports in the field of tumor immunology concerning administration of immune serum from resistant animals to tumor susceptible animals ("passive" immunization), and also upon the effect of pre-immunization with heated tumor or tumor extracts ("active" immunization)(1). In general these techniques have provided protection if carried out either prior to, or soon after transplantation of tumor. They have had little success in preventing growth either of well established transplanted tumors, or of spontaneous neoplasms. A third type of immunization, called "adoptive" immunization has also been shown to be practicable (2). The technique involves transplanting functioning spleen or lymph node tissue from an immunized animal into an unimmunized animal of the same inbred strain. It has been shown that spleen and lymph nodes transferred in this way continue to produce antibodies in the new host(3). Techniques have recently been developed for rendering unrelated animals graft tolerant by neo-natal transfer of tissue(4). This makes possible the use of "adoptive" immunization between different strains of mice. Spleen and lymph nodes from a tumor resistant strain of mice might then be used to influence growth of a transplanted tumor in a tumor susceptible strain.

* This investigation was supported in part by grants from Am. Cancer Soc. and USPHS.

This paper describes neo-natal induction of graft tolerance between tumor resistant strain (DBA/2) and tumor susceptible strain (BALB/C) mice. This was followed by adoptive immunization in later life, using grafts of spleen and lymph node cells from the tumor resistant strain. Growth of previously established tumor transplants under the influence of this adoptive immunization was then studied.

Materials and methods. Mice were obtained from Jackson Memorial Laboratories, Bar Harbor, and were fed on commercial mouse pellets and tap water *ad libitum*. Two strains of mice were used, one (BALB/C) which is susceptible to the transplantable and isologous CD/5 lymphoma, and the DBA/2 mouse which is resistant to this tumor. Mice bearing CD/5 lymphoma for transplantation were kindly supplied by Dr. Morris Barrett, Nat. Cancer Inst., Bethesda, Md. Inbred mice were maintained for one generation by random mating, and for succeeding generations by brother/sister mating.

Neo-natal cross-immunization. The neo-natal cross-immunization procedure is shown schematically in Fig. 1. By using an intermittent mating schedule, numbers of BALB/C and DBA/2 litters were obtained which were born within 4-12 hours of each other. One newborn BALB/C animal was sacrificed, its spleen was removed, and a single cell suspension of spleen cells was pre-



pared in sterile isotonic saline by gently pressing the tissue through a 100-mesh stainless steel gauze. (In later experiments better cell suspensions were obtained using 40 mesh.) Each member (except one) of the newborn DBA/2 litter was then injected intraperitoneally with from 200,000 to 1,000,000 cells. Weight of the newborn spleens averaged 5 mg (range 1.5-8 mg); adult spleen, DBA/2 about 200 mg, and BALB/C about 150 mg. Suitable dilutions of cell suspensions were counted in Speirs-Levy hemocytometer chambers, and a sterile 1 ml syringe and #24 needle used for injecting.

The remaining member of the DBA litter was likewise sacrificed and its spleen cells were injected into remaining members of the BALB/C litter. This neo-natal technique should render a high percentage of BALB/C animals tolerant to grafts from their DBA partners at any time during later life without invoking either primary or secondary (graft *vs* host) homograft rejection mechanisms.

Tumor injection in neo-natally cross-immunized mice. The cross-immunized animals were allowed to mature, and at 6-8 weeks of age each animal received an injection of tumor cells. Details are shown in Fig. 2. Sterile tumor cell suspension was prepared from excised tumor by expressing fragments through 100 (or 40) mesh stainless steel gauze into a sterile bottle containing isotonic saline. Each of the cross-immunized animals then received, using a 24-gauge needle, 1,000,000 cells subcutaneously in the right groin. LD/50 for this tumor is about 50,000 cells. Tumors first became palpable in about 3-6 weeks. They were allowed to increase in size to about 500 cu mm in BALB/C animals. This size was attained usually in a further 2 weeks.

Adoptive immunization of tumor-bearing mice. When tumors in BALB/C animals had reached 500 cu mm, the tumor resistant DBA/2 partner was sacrificed. The spleen and the lymph nodes of the axillary and inguinal regions were removed and a single cell sterile suspension of the tissues was prepared and injected intraperitoneally into tumor bearing BALB/C animals. Usual dose was between 40 to 80 million mixed cells, composed of about 20% of lymph node and 80% of spleen origin. Further progress of the tumor in adoptively immunized animals was then followed.

Antibody titers. Blood was obtained from all mice at sacrifice by heart puncture. For samples taken without sacrificing the animal, an intra-orbital technique(5) was used. Titers for circulating antibody were performed on serum by an interfacial precipitation technique in Durrum tubes, using tumor homogenates clarified at 1000 $\times$ *g* as antigen. A control on quality of antigen was included by using antiserum prepared from white rats injected with tumor cell suspension. Control antiserum gave a positive titer at 1:1000.

It was originally intended to check graft efficiency of adopted spleen and lymph node cells by measuring continued production of anti-tumor antibody in tolerant hosts. However, as noted below, although DBA/2 mice

TABLE I. Precipitin Titers Against Tumor Cell Protein Following Tumor Cell Injection.

Type of mouse	No. of mice	Limiting titer against tumor cell extract* 0	1:1	1:10	1:100	1:10,000
Normal DBA/2	8	8	—	—	—	—
X-Imm DBA/2	20	13	2	3	1	1
Tumor bearing X-Imm DBA/2	3	—	—	—	1	2
Tumor bearing normal BALB/C	8	7	1	—	—	—
Tumor resistant normal BALB/C	2	2	—	—	—	—
Tumor bearing X-Imm BALB/C	6	5	1	—	—	—

* Figures in columns indicate number of mice displaying the given limiting titer. All mice were injected subcutaneously with 1 million tumor cells (CD/5 lymphoma). DBA/2 animals were bled 2-3 wk later; BALB/C at 4-8 wk. Prefix X-Imm indicates animal was desensitized by neonatal cross-immunization with spleen cells from opposite strain. Precipitin titers were determined using tumor homogenate clarified by centrifugation as antigen. Tumor used (CD/5 lymphoma) is isologous in BALB/C. For details of tumor growth in these animals, see Table II.

proved uniformly resistant to injected tumor cells, serum antibodies were detected in very few animals (Table I). Up to 4 weekly injections with 1 million tumor cells or frozen tumor homogenate failed to elicit a response in these animals. Negative results were also obtained using the sensitive gel diffusion technique of Ouchterlony(6).

Results. Tumor susceptibility in normal and neo-natally immunized mice. Normal BALB/C animals were highly susceptible to the tumor. Using 1 million cells as inoculum, 51 out of 59 animals injected developed tumors† which grew to large size and necessitated sacrifice of the animal (Table II). Tumors in these susceptible animals usually became palpable about 2 weeks following injection, then grew rapidly and were of suitable size for transplantation by about the 5th or 6th week. Normal DBA/2 animals were uniformly resistant to the tumor and none of 16 animals injected showed any signs of tumor development. Cross-immunized BALB/C animals which had been injected with DBA/2 spleen cells at birth were more resistant than normal BALB/C animals, and 26 out of 58 injected failed to develop a tumor (Table II). Injection of BALB/C spleen at birth may have lowered the resistance of DBA/2 animals somewhat, since 3 out of 35 of these animals developed tumors. These tumors after growing to about 1500 cu mm in size regressed rapidly, one completely. The other 2 subsequently began to increase in size again resulting in death of the animals.

Tumor growth following adoptive immunization. Of those cross-immunized BALB/C animals in which a tumor developed (Table II), 29 were injected with between 40 and 80 million spleen and lymph node cells from their resistant DBA/2 partners. Following this injection, 7 tumors regressed completely within a period of 1 to 2 weeks (Table III). Eight others showed partial regression followed by continued slow growth. In 14 animals adoptive immunization appeared to have no effect on tumor growth rate, although in 10 of these animals the tumor was highly necrotic upon sacrifice of the animal. In those animals showing complete regression, there was no reappearance of tumor during

† The CD/5 lymphoma arose as a spontaneous tumor in a BALB/C mouse. It is thus by definition isologous in this host. Its transplantation characteristics are such, however, that only about 90% of the transfers are successful. (Dr. Barrett, personal communication.)

TABLE II. Tumor Susceptibility of Normal and Neo-Natally Cross-Immunized Mice.

Type of mouse	No. inj	Tumors	No tumors	% immunity
Normal BALB/C	59	51*	8	13
X-Imm BALB/C	58	32†	26	45
Normal DBA/2	16	0	16	100
X-Imm DBA/2	35	3‡	32	91

* 2 of these regressed.
† 3 of these regressed.
‡ One of these later regressed completely.

Prefix X-Imm indicates animal was desensitized by neo-natal cross-immunization with spleen cells from opposite strain. At 6-8 wk of age all animals received subcutaneous injection of 1 million tumor cells (CD/5 lymphoma), in right groin. Time of first appearance of palpable tumor was about 2 wk in normal and 3-6 wk in X-Imm animals.

TABLE III. Tumor Response Following Adoptive Immunization Procedure.

Type of mouse	Total with tumor	Regressed after inj	Slowed after inj	No effect
X-Imm BALB/C	29	7	8	14*
Normal BALB/C (control)	12	0	1	11†

* Tumors were necrotic at sacrifice in 10 of these animals.

† Tumors were necrotic at sacrifice in 3 of these animals.

Animals were inj with 1 million CD/5 lymphoma cells. Tumors were allowed to increase in size to about 500 mm^3. Animals were then inj with 40-80 million cells of mixed spleen and lymph node origin from resistant DBA/2 animals. For experimental group, the corresponding DBA/2 X-Imm partner was used. For control group, normal DBA/2 animals were employed. Prefix X-Imm indicates animal was desensitized by neo-natal cross-immunization with spleen tissue from opposite strain.

the next 3 months of the experiment. A control group of 12 normal BALB/C animals bearing a tumor was injected with spleen and lymph node cells from control tumor injected DBA/2 animals. In this group there were no tumor regressions, one grew slowly following injection, and only 3 of 12 tumors were necrotic upon sacrifice of the animals (Table III).

Antibody titers in tumor injected mice. Resistance of DBA animals to tumor apparently was not associated with their ability to develop a precipitin titer against tumor protein. None of 8 normal DBA animals, and only 7 of 20 cross-immunized DBA animals injected with tumor developed a precipitin titer against tumor protein despite their uniform rejection of tumor (Table I). Conversely, the 3 cross-immunized DBA animals which did accept CD/5 tumor (Table II) displayed a high level of circulating antibodies against tumor protein, 2 of these animals showing titers in excess of 1:10,000. Of 16 tumor bearing BALB/C animals tested, 14 showed no titer at all and only 2 gave indications of a titer at the 1:1 level. Neither of 2 tumor resistant BALB/C animals tested gave a titer.

Discussion. Neo-natal injection of spleen cells from a resistant strain raised resistance to tumor in the susceptible strain from 13% to 45%. It can be concluded that this increased resistance to tumor cell injection in BALB/C mice probably represents acceptance of DBA/2 spleen cells during the neonatal procedure. Continued survival of these DBA cells then confers on the host animal some of the tumor resistant properties of the DBA/2 strain.

The failure of the subsequent adoptive immunization procedure to bring about regression in more than 25% of the tumors may be related to an insufficient dose of spleen and lymph node cells. Nossal(3) has used doses of from 180 mg to as high as 1.6 g of spleen per 20 g mouse for adoptive immunization against Salmonella. The dose of mixed spleen and lymph node used in these experiments was dictated by a 1 donor:2 hosts relationship (Table II), and corresponds to about 40 to 80 mg per mouse. Alternatively, however, since the resistance of the BALB/C mice has already been modified by the neonatal spleen injection, the partial response to subsequent adoptive immunization is not direct evidence of the success of adoptive immunization *per se.* It may represent nonspecific enhancement of the neo-natally induced resistance in these mice. Such nonspecific enhancement of tumor rejection in partially resistant mice has been noted(7).

One point of interest brought out clearly by these results is that ability to develop a serum precipitin titer against tumor protein is no criterion of ability to reject the tumor itself. It was originally intended to follow efficiency of grafting of resistant DBA spleen and lymph nodes into tumor bearing BALB/C by following the ability of these immune spleen cells to continue to produce antibody in the new host. Normal DBA/2 animals, however, uniformly failed to develop a titer against tumor protein, despite uniform failure of the tumor to grow in these animals. Similarly only 7/20 of the neonatally cross-immunized DBA/2 animals which rejected the tumor developed a titer. Highest anti-tumor titers were paradoxically displayed by 3 DBA animals which accepted tumor (Table I). That acceptance in these animals was due to differences in animals rather than tumor was shown by failure of tumor in these animals to transplant back

into normal DBA/2 animals. This is an illustration that factors associated with tissue rejection are not identical with the components of the classical immune mechanism.

Summary. 1. A strain of mice resistant to the transplanted CD/5 lymphoma (DBA/2), and a tumor susceptible strain (BALB/C), were rendered mutually graft-tolerant by neonatal cross-immunization with spleen cells. At 6-8 weeks mice of both strains were injected subcutaneously with CD/5 lymphoma, which is isologous in BALB/C mice. Growing tumors in BALB/C mice were then challenged by an "adoptive" immunization procedure using spleen and lymph nodes from resistant DBA partners. 2. Neo-natally cross-immunized BALB/C mice were more resistant (45%) to the tumor than control animals (13%). 3. Following adoptive immunization, complete tumor regression was observed in 20% and partial regression in 25% of tumor bearing animals. Corresponding figures for a control group of normal BALB/C subjected to the same adoptive immunization were: complete regression (0%), partial regression (8%). 4. Antibody titers in DBA/2 mice against tumor protein did not correlate with tumor rejection. Neo-natally cross-immunized DBA/2 mice were less resistant to tumor (91%) than normal DBA/2 (100%). Antibody titer was minimal or absent in tumor resistant DBA/2 mice, but high in those cross-immunized mice accepting the tumor.

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Received June 24, 1963. P.S.E.B.M., 1964, v115.

Circulatory Effects of a Homologous Plasma-Blood Exchange in the Dog.* (28823)

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Bliss *et al*(1) first reported that intradermal injection of homologous plasma produced well marked wheals in dogs. Infusion of homologous plasma into normovolemic dogs produced the following symptoms: a fall in blood pressure, skin edema, labored respiration, prolonged bleeding time and death of a majority of dogs within a 24-hour period. Also, the dogs did not retain the infused fluid and protein within the circulation. None of these reactions occurred when autologous blood and plasma were infused. These various responses were suggestive of a foreign protein reaction, and because they occurred with the infusion of homologous plasma as well as blood, it was suggested that an unknown "plasma factor" must be the inciting agent(1-3).

Hypervolemia, which occurred with the previous experiments, was avoided by Lawson *et al*(4) by simultaneously withdrawing a volume of blood equivalent to that infused. Under these circumstances, when autologous plasma was used for the exchange, the distribution spaces of the dye T-1824 and P^{32}-tagged erythrocytes agreed with those calculated from the dilution of the tags by the exchange. When autologous blood was transfused to increase the blood volume of normovolemic dogs, the agreement for the cell vol-

This study was supported in part by Medical Research and Development Board, Office of Surgeon General, Dept. of the Army, and in part by a grant from Nat. Heart Inst., USPHS.