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Immunologically Competent Cells in Adult Mouse Liver: Studies with Parent-to-Hybrid Radiation Chimeras. (26113)

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Evidence has been accumulated that tissues other than spleen, lymph nodes, and bone marrow contain immunologically competent cells. Peripheral white blood cell fractions (1,2), thymus(3,4), and liver, kidney, and heart muscle(4) contain cells able to confer adoptive immunity to transplantation antigens and to cause rejection of heterologous or homologous bone marrow following implantation in X-irradiated mice.

To obtain further evidence of the immunologic potentialities of adult liver tissue, we grafted liver cells into sublethally irradiated F_1 hybrids, using inbred parental strain mice as donors. In this genetic situation, it is expected that only immunologically active cells of the grafted liver can react positively against the F_1 hybrid antigens. Therefore, a fatal reaction should occur in the F_1 recipients of certain strain combinations, similar to the wasting disease described after parental spleen(5,6), thymus(7), or leukocyte(8) implantation.

Methods. Sixteen- to 20-week-old male and female (C57BL $\times$ 101)F_1, (C3H $\times$ 101)F_1, and (101 $\times$ C3H)F_1 recipient mice were exposed to a single dose of total-body radiation of 600 or 900 r. Mice were irradiated in a revolving partitioned lucite cage under the following conditions: 300 kvp, 20 ma, 4.7 mm of Be inherent and 3 mm of Al added filtration, 100 cm target-to-mouse distance, hvl 0.45 mm of Cu, 76 r/min.

Within 1 hour after irradiation, each 600-r irradiated mouse received intraperitoneally a suspension of parental strain liver cells, equivalent to 0.25 of a whole liver in 0.5 to 1 ml of Tyrode's solution. Liver cell suspensions were prepared by chopping the organ with scissors and by repeated aspiration of small fragments through a syringe. The preparation was finally passed through a wire mesh screen, and penicillin was added.† Each 900-r irradiated mouse received intravenously within 1 hour, *via* the tail vein, 1 femur-equivalent (about 10×10^6 nucleated cells) of isologous bone marrow (IBM). Subsequently these mice were given an intraperitoneal inoculation of parental liver cells equivalent to 0.1 of a whole liver. Marrow

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† The final suspension of liver cells contained in all instances a mixture of parenchymal, littoral, and biliary cells; these are referred to throughout the text as simply "liver cells."

TABLE I. Survival of 600-r Irradiated F_1 Hybrid Mice Injected with Parental Strain Liver Cells.

Recipients	Donors	90-day survival of recipients: No. treated	No. surviving	% surviving	Time of death (days after irrad.)
(C57BL × 101)F_1	Radiation control	30	30	100	
	(C57BL × 101)F_1	25	24	96	18
	C57BL	40	0	0	13–25
	101	40	0	0	12–17
(C57BL × 101)F_1 + IBM	C57BL	30	20	66	25–53
	101	40	32	80	19–56
(C3H × 101)F_1	Radiation control	20	19	95	17
	(C3H × 101)F_1	20	17	85	12–13
	C3H	20	15	75	14–57
	C3H presensitized to 101	20	0	0	18–51
	101	20	7	35	33–87
	101 presensitized to C3H	20	0	0	15–30
(101 × C3H)F_1	Radiation control	20	18	90	68
	C3H	20	10	50	52–90
	C3H presensitized to 101	20	0	0	28–77
	101	20	11	55	25–40
	101 presensitized to C3H	20	0	0	15–33

cells were suspended in Tyrode's solution according to the method described by Congdon and Urso(9). The tissues were chilled throughout the procedure. Some groups of (C3H × 101)F_1 and of (101 × C3H)F_1 mice received preimmunized parental liver cells; donor mice were preimmunized to the reciprocal parent of the F_1 combination by intraperitoneal implantation of 10×10^6 spleen cells 4 weeks before killing. Donor mice were killed by bleeding at the age of 3 to 5 months. In certain instances referred to, liver donors were anesthetized with Nembutal before killing and perfused with 20 ml of Tyrode's solution injected *via* the left cardiac ventricle. When liver donors were irradiated, the exposure conditions were identical to those described for the recipients. The term "frozen" liver indicates that the liver cell suspension was slowly frozen and kept overnight at −10°C before thawing and injection. No attempt was made to avoid the Eichwald-Silmser effect(10,11) by appropriate matching of donors and recipients according to sex and strain, but no evidence for sex-determined histoincompatibility was noted in the experiments with liver transplantation. For this reason, the sex of the donors and recipients is not indicated in the results presented.

Results. Effects on survival. All the 600-r irradiated (C57BL × 101)F_1 mice receiving parental strain liver cells died 12 to 25 days after treatment (Table I). In the second week after irradiation, the animals developed a condition resembling the foreign spleen reaction(5) showing weight loss, ruffling of hair, and atrophy of skin and lymphatic tissue. Both parental strains were effective in inducing the disease in 100% of the animals, although survival time after implantation of 101 liver cells was somewhat shorter. Of the irradiated F_1 control mice, all survived the observation period of 90 days with or without receiving isologous liver cells. Parental liver cells did not affect survival of nonirradiated F_1 hybrids. Mortality after inoculation of C3H liver cells into 600-r irradiated (101 × C3H)F_1 and (C3H × 101)F_1 mice was contrastingly low. The same F_1 hybrids showed a higher mortality, however, 80 to 90 days after inoculation of 101 liver cells. Liver cells from presensitized parental mice caused greater and earlier mortality among both experimental groups. Injection of lethally irradiated (900 r) mice with IBM did not alter the killing effect of parental liver in either strain combination (C57BL × 101)F_1 or (101 × C3H)F_1 (Fig. 1). At this higher radiation dose level, 0.1 equivalent of a mouse liver was enough to cause the disease in (C57BL × 101)F_1 mice. In the other hy-

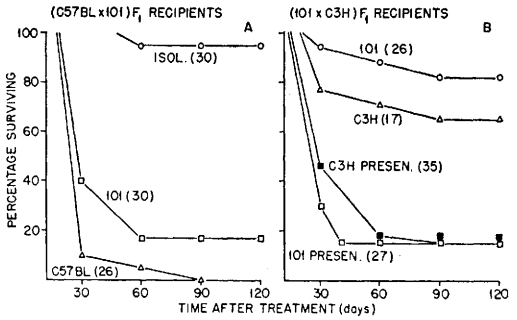

FIG. 1. Survival of 900-r irradiated F_1 hybrid mice inj. with isologous bone marrow (10×10^6 nucleated cells) and parental-strain liver cells (0.1 liver equivalent). Numbers in parentheses indicate No. of mice.

brid, unsensitized 101 and C3H liver cells gave rise to a cumulative mortality of about 25%. After presensitization of the donors, mortality rate increased markedly in the F_1 recipients, as it occurs in the corresponding sublethally irradiated groups. Isologous liver cells did not modify the acute radiation death pattern in lethally irradiated (C57 BL $\times$ 101)F_1 recipients. Inoculation of IBM into 600-r irradiated (C57BL $\times$ 101)F_1 mice prevented the killing effect of subsequently inoculated C57BL or 101 liver cells, at least during the first 90 days, in 74% of the treated animals. Mortality of the (C57BL $\times$ 101)F_1 recipients implanted with perfused C57BL liver cells occurred later than in those with liver cells from unperfused livers, but 100% mortality was nevertheless reached (Table II). Among the recipients implanted with 101 perfused liver cells, 15% are surviving after 90 days. When the parental liver was irradiated *in vivo* with 950 r of X rays within one hour before killing of the donor, or the cell suspension was frozen and thawed before injection, no mortality was observed in the irradiated F_1 hybrid recipients (Table II).

Pathological effects. Histological changes in the 600-r irradiated (C57BL $\times$ 101)F_1 hybrids injected with C57BL or 101 liver cells were found mainly in the hemopoietic and lymphoid tisues. Although hemopoietic elements in the white and red pulp of the spleen, in lymph nodes, and in bone marrow were actively recovering from radiation-induced atrophy by the second week, lymphoid follicles remained moderately atrophic. Six to 10 days after treatment, the lymphoid cells in the germinal centers were replaced with proliferating reticuloendothelial cells. This reaction was milder, however, than that after injection of parental spleen cells(5), even when the stimulus was exaggerated by injecting the equivalent of 2 livers per recipient. At time of death, the bone marrow was cellular; therefore, the immediate cause of death was not lack of bone marrow regeneration. In the 900-r irradiated F_1 mice infused with IBM and with parental liver cells, the red pulp of the spleen and the bone marrow were cellular at time of death. The lymphoid system was atrophic in all animals and follicles of the spleen and lymph nodes showed marked granulomatous proliferation of reticuloendothelial elements, similar to that described.

Discussion. Implantation of adult par-

TABLE II. Survival of 600-r Irradiated (C57BL $\times$ 101)F_1 Mice Injected with Parental Strain Liver Cells.

Donors	Treatment of donor	Treatment of liver	No. mice inj.	No. survivors 30 days	No. survivors 90 days
C57BL	None	None	40	0	0
101	None	None	40	0	0
(C57BL $\times$ 101)F_1	None	None	25	24	24
C57BL	"	Perfused with Tyrode's solution	20	7	0
101	"	Perfused with Tyrode's solution	20	7	3
C57BL	"	Frozen and kept overnight at −10°C	10	10	10
101	"	Frozen and kept overnight at −10°C	10	10	10
C57BL	950 r total-body 1 hr before killing	None	25	25	25
101	950 r total-body 1 hr before killing	None	25	25	25
(C57BL $\times$ 101)F_1	950 r total-body 1 hr before killing	None	10	10	9

ental-strain liver cells into (C57BL × 101)F_1 mice was followed by a fatal disease when the recipients were irradiated with a sublethal dose of X rays (600 r) or with a lethal dose (900 r) followed by subsequent inoculation of isologous bone marrow. Inasmuch as this effect was not caused by sublethal irradiation alone, by lethal irradiation and IBM infusion, or by irradiation and treatment with isologous F_1 liver cells, we infer that the observed reaction resulted from immunogenetic differences between F_1 hybrids and parental tissues. The similarity of these findings with those of the foreign spleen reaction(5,6) and the ability of transplanted liver cells to confer adoptive immunity(4) suggest that immunologically competent cells present in the grafted liver can give rise to an *in vivo* antigen-antibody reaction with consequent wasting disease.

The pathologic findings indicate that mice receiving parental liver cells do not die of bone marrow aplasia. Changes observed in spleen and lymph nodes, characterized by replacement of lymphoid elements with reticuloendothelial cells in the germinal centers, are similar to those observed during the foreign spleen reaction(5,6,12) and are of the same type, even if less pronounced, as the changes described by Congdon *et al.*(13) in lethally irradiated mice given homologous spleen cells intravenously.

Administration of adult parental liver cells to (C3H × 101)F_1, or reciprocal F_1, mice leads to different results probably because there is less pronounced immunogenetic difference between the latter two parents at the *H-2* and/or other loci(14), both parental strains being *H-2K* (R. D. Owen, personal communication). In this situation, parental liver cells failed consistently to provoke early and high mortality in irradiated F_1 recipients unless presensitized to the reciprocal parent of the hybrid. The mortality occurring after implantation of unsensitized 101 and C3H liver cells in 600-r irradiated F_1 mice is probably due to minor antigenic differences, reported also by Cosgrove *et al.*(15) in bone marrow transplantation experiments with the same mouse strains. A smaller amount of parental liver cells (0.1 liver equivalent) caused a mortality of about 25% in 900-r irradiated (101 × C3H)F_1 mice protected against radiation death with IBM. Also in the 900 r irradiated (C57BL × 101)F_1 hybrid 0.1 liver equivalent was enough to induce the wasting disease, whereas in 600-r irradiated F_1 recipients 0.25 equivalent was required. Radiation dose and liver cell dose are probably interrelated factors in determining the foreign liver reaction. The enhancement of the foreign liver reaction by presensitization of donors to the reciprocal parent denotes further the immunological nature of the reaction and suggests, according to Makinodan *et al.*(16) and to Cosgrove *et al.*(15), that the differences in immunologic effectiveness between various tissues reflect differences in number of existing antibody-forming cells.

By use of skin-grafting technics, commercially obtained strain 101 mice used in this laboratory have been shown to be incompletely homogeneous (Congdon, personal communication). But in these experiments, in which recipients were sublethally irradiated, if the 101 liver antigens were not identical with the 101 antigens of the (C3H × 101)F_1 or (C57BL × 101)F_1 constitution, the F_1 presumably should have rejected the liver, and deaths would not be expected. Data obtained with liver cells from perfused animals, after removal of circulating peripheral blood cells, showed that immunologically competent cells were still present in the liver in sufficient numbers to cause the graft-versus-host reaction. But their number was reduced, as one can argue from the delay in appearance of the killing effect and from the reduction in mortality It is possible that perfusion procedure not only removes the residual blood cells from the liver vessels but also some of the littoral reticuloendothelial cells. This does not exclude, of course, the possibility that the immunologically active cells might have been diffusely infiltrating lymphoid cells.

After having been frozen and thawed or after having been heavily irradiated *in vivo*, parental liver cells lost their ability to react immunologically in irradiated F_1 recipients. This is presumptive evidence that a viable cell

was involved in the described foreign liver disease.

Protection of lethally irradiated (C57BL × 101)F_1 mice with IBM did not prevent the occurrence of the wasting disease after parental liver cell implantation. This finding could be easily explained by the assumed incapacity of the F_1 antibody-forming cells to react immunologically against parental antigens. In addition to this, it is well known that the antibody-forming cell content of normal mouse bone marrow tissue is very low (16). On the other hand, in sublethally irradiated F_1 hybrids, IBM infusion prevented the effect of parental liver cells and allowed 74% survival of the treated recipients; 100% survival was also noted when parental liver cells were introduced in nonirradiated F_1 hosts.

The need of irradiation for occurrence of a foreign spleen or liver reaction in a parent-to-hybrid combination has been related to lack of proliferation stimulus(5) and lack of proper space(6) for the grafted immunologically active cells in a nonirradiated adult recipient. Furthermore, it has been postulated that antibody-forming cells of certain genetic constitutions might be more or less sensitive to an excess of antigen(12) and sometimes be destroyed. The latter situation should not, however, be modified by recipients' whole-body irradiation. On the other hand, there is some evidence that the F_1 hybrid may react immunologically against parental-strain tissues. For example, Popp and Sapp(17) have observed spontaneous reversions in lethally irradiated (C57BL × 101)F_1 mice carrying an active transplant of C57BL bone marrow, and Cudkowicz *et al.*(18) have observed a high incidence of death (15 to 60 days after treatment) in (C57BL × 101)F_1 hybrids given a lethal whole-body radiation dose, parental bone marrow (C57BL or 101), and isologous liver cells. Also, the reduced incidence of foreign liver disease in 600-r irradiated (C57BL × 101)F_1 mice receiving isologous bone marrow could be explained in terms of a positive reaction of the F_1 against the parental tissues. That this reaction cannot be attributed to sex-determined histoincompatibility(10,11) between hybrid antibody-forming cells and parental liver antigen is evident from its occurrence even when F_1 recipient, F_1 bone marrow donor, and parental liver donor were females. The F_1-against-parent reaction could therefore occur in nonirradiated or in sublethally irradiated-isologous bone marrow restored animals of this strain combination but not after heavy irradiation of the F_1 recipient's immune mechanism.

Summary. 1. Implantation into irradiated (C57BL × 101)F_1 hybrids of viable liver cells from adult donor mice of the parental strains caused a wasting disease followed by death in over 90% of the recipients. 2. This "foreign liver disease" occurred after a sublethal dose of radiation was given to the recipients or after a lethal dose followed by infusion of isologous bone marrow. It did not occur in nonirradiated recipients but occurred in only 26% of sublethally irradiated F_1 hybrids infused with isologous bone marrow. 3. In a strain combination in which the F_1 hybrid was homozygous at the *H-2* locus, and the parents should therefore have been antigenetically relatively compatible, the "foreign liver disease" occurred late after treatment and was enhanced by presensitization of the donor to the reciprocal parent of the hybrid. It was shown that radiation dose and liver cell dose were inter-related factors in determining the development of the disease. 4. Deaths are ascribed to an *in vivo* immunologic reaction after antibody production by the grafted parental cells against F_1 antigens. Nevertheless, there is evidence of a possible immune response by the F_1 hybrid against the parent.

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Growth of Laboratory and Naturally Occurring Strains of Eaton Agent in Monkey Kidney Tissue Culture. (26114)

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There has been renewed interest in the agent recovered in the embryonated egg from patients with atypical pneumonia by Eaton *et al.*,(1). Until recently considerable controversy has surrounded the role of this agent in respiratory illness. Studies carried out in the past few years, however, have provided evidence that Eaton agent is associated with human respiratory disease. Thus, 80 to 95% of patients with cold agglutinin positive atypical pneumonia were found to develop fluorescent stainable antibody for the agent (2,3). In addition, a controlled epidemiologic study in military recruits indicated that Eaton infection was associated with febrile upper respiratory illness as well as atypical pneumonia(4). Approximately 10% of children hospitalized with lower respiratory tract illness in Washington, D.C. developed a serologic response to this agent(5).

‡ The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

Early studies were hindered by the difficulty of working with an agent whose only index of activity was the production of lung lesions in a portion of, but not all, inoculated hamsters or cotton rats(1,6,7). Application of the fluorescent antibody technic resulted in a more quantitative assay system and permitted demonstration of specific antigen in the bronchial epithelium of the experimentally infected chick embryo(8). Employing this technic it was possible to recover a limited number of strains from patients with pneumonia by serial amniotic egg passage(3, 8,9). This procedure, however, is difficult, involved, and clearly limits the quantity of epidemiologic information which can be obtained. Simpler laboratory technics are needed to facilitate the recovery and identification of the agent before extensive field studies necessary to an understanding of the natural history of infection are possible.

This report will describe preliminary studies which indicate that Eaton agent propagates in several different types of tissue