row(9-11). Lorenz and Congdon(12) and van Bekkum and Vos(11) succeeded in prolonging the survival time of a few lethally irradiated mice treated with GPBM. This would indicate at least temporary transplantation of donor cells, if it can be assumed that transplantation of donor cells is necessary for even an extension of survival time of lethally irradiated mice.

We found that (a), during maximum depression period after varying X-ray doses, mice responded beter to sheep than to rat antigens(5) and (b), recovery rate was faster for sheep antigens than for rat antigens(1,3). Published results and findings here reported substantiate our hypothesis: antibody-producing cells of normal adult mice can differentiate between and respond to closely and distantly related antigens with equal effect. The degree of X-ray injury to the recognition and response mechanisms decreases with the foreignness of the antigen.

*Summary.* Bone marrow from species closely and distantly related to the mouse was injected after various X-ray doses to the total body. Rat, hamster, guinea pig, and rabbit bone marrow persisted in normal, nonirradiated mice for not more than 1 day. The minimum X-ray dose that would permit persistence of rat, hamster, guinea pig, and rabbit bone marrow in mice for more than 3 days was 400, 600, 1150, and 1500 r, respectively. The minimum X-ray dose that permitted (a) temporary transplantation of rat and hamster bone marrow in mice was 500 and 600 r, respectively, and (b) prolonged transplantation of rat and hamster bone marrow in mice was 800 and 950 r, respectively. The significance of these findings is discussed.

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## Effects of Immunized Parental Strain Bone Marrow on Lethally Irradiated $F_1$ Hybrid Mice. (24647)

G. E. COSGROVE, JR., A. C. UPTON, E. E. SCHWARTZ,* C. C. CONGDON AND T. MAKINODAN

*Biology Division, Oak Ridge National Lab.,† Oak Ridge, Tenn.*

Nonirradiated bone marrow cells inoculated into irradiated recipients transplant and prevent death from hemopoietic failure. However, a syndrome called "foreign bone marrow reaction" occurs in many animals receiving antigenically foreign marrow cells after whole-body irradiation(1). Mice so afflicted seem to recover from radiation-induced injury but soon lapse into progressively wasting state, and die 30-90 days after treatment. Since implantation of adult parental spleen cells in irradiated $F_1$ mice hastens death if donor is pre-sensitized to recipient(2), this experiment was undertaken to determine effects of pre-sensitized parental bone marrow on survival of irradiated $F_1$ hybrids.

* Present address: Albert Einstein Medical Center, Philadelphia, Pa.

† Operated by Union Carbide Corp. for U. S. Atomic Energy Comm.

TABLE I. Effects of Pre-sensitization of Bone Marrow Donors on Survival of Lethally Irradiated Recipients: C3H and 101 Strain Combinations.

| Recipient | Donor | Immunologic status of donor: Antigenic material | Wk inj. before sacrifice | Cumulative mortality at day* 30 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|---|
| (101 × C3H)F$_1$ | (101 × C3H)F$_1$ | | | 2/35 | 2/35 | 2/35 | 2/35 |
| (C3H × 101)F$_1$ | (C3H × 101)F$_1$ | | | 0/20 | 1/20 | 1/20 | |
| (101 × C3H)F$_1$ | 101 | | | 4/55 | 6/55 | 7/55 | |
| *Idem* | C3H | | | 0/30 | 0/30 | 0/30 | 2/30 |
| (C3H × 101)F$_1$ | 101 | | | 5/61 | 10/61 | 11/61 | 12/61 |
| *Idem* | " | (C3H × 101)F$_1$ tissues | 1 | 1/39 | 6/39 | 6/39 | 6/39 |
| (101 × C3H)F$_1$ | " | C3H spleen | 1 | 1/12 | 1/12 | 1/12 | 1/12 |
| (C3H × 101)F$_1$ | " | (C3H × 101)F$_1$ tissues | 3 | 2/20 | 14/20 | 16/20 | 16/20 |
| (101 × C3H)F$_1$ | " | C3H spleen | 4 or more; each wk inj. | 18/28 | 23/28 | 25/28 | 28/28 |
| *Idem* | C3H | 101 spleen | *Idem* | " | " | " | |
| " | 101 | Rat " | " | 0/12 | 0/12 | 0/12 | 0/12 |
| " | (101 × C3H)F$_1$ | 101 " | " | 0/7 | 0/7 | 0/7 | 0/7 |
| " | *Idem* | C3H " | " | 0/9 | 0/9 | 0/9 | 0/9 |
| 101 | " | | | 14/34 | 17/34 | 18/34 | 19/34 |
| " | C3H | | | 12/40 | 18/40 | 24/40 | 24/40 |
| C3H | 101 | | | 5/10 | 7/10 | 10/10 | 10/10 |
| " | (101 × C3H)F$_1$ | | | 7/31 | 8/31 | 8/31 | 8/31 |

* No. dead/No. in group.

TABLE II. Effects of Pre-sensitization of Bone Marrow Donors on Survival of Lethally Irradiated Recipients: C57BL and 101 Strain Combinations.

| Recipient | Donor | Immunologic status of donor: Antigenic material | Wk inj. before sacrifice | Cumulative mortality at day 30 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|---|
| (C57BL × 101)F$_1$ | (C57BL × 101)F$_1$ | | | 14/66 | 14/66 | 14/66 | 14/66 |
| " | 101 | | | 16/60 | 16/60 | 18/60 | 21/60 |
| " | C57BL | | | 31/130 | 68/130 | 106/130 | 115/130 |
| " | 101 | C57BL spleen | 1 | 11/25 | 15/25 | 17/25 | 18/25 |
| " | C57BL | 101 " | 1 | 5/20 | 11/20 | 14/20 | 15/20 |
| " | 101 | C57BL " | 4 | 7/10 | 9/10 | 10/10 | 10/10 |
| " | C57BL | 101 " | 4 | 3/14 | 11/14 | 13/14 | 14/14 |
| 101 | (C57BL × 101)F$_1$ | | | 29/36 | 35/36 | 35/36 | 35/36 |
| C57BL | " | | | 33/34 | 33/34 | 33/34 | |
| 101 | C57BL | | | 34/40 | 37/40 | 38/40 | |
| C57BL | 101 | | | 40/40 | 40/40 | 40/40 | 40/40 |

* No. dead/No. in group.

*Methods.* Adult (C3H × 101)F$_1$, (101 × C3H)F$_1$, (C57BL × 101)F$_1$, 101, C3H, and C57BL mice were given single LD$_{100/30}$ X-ray doses of 800-900 r to the whole body. Mice were placed within separate compartments of rotating lucite drum during exposure. Physical factors were 250 kvp, 30 ma, 3 mm of Al added filtration, 0.44 mm of Cu hvl, 82-94 cm target-to-mouse distance, 90-98 r/minute. Different groups of mice received 1 femur-equivalent (about 12 million nucleated cells) of nonimmunized or pre-immunized parental strain bone marrow cells within 1 to 5 hours after irradiation. Other groups received F$_1$ homologous or isologous bone marrow cells (Tables I, II). Marrow cell suspensions were prepared in sterile Tyrode's solution as outlined by Congdon and Urso(3), or in sterile saline. Parental strain bone marrow donors were adults 12 weeks to 1 year old. Mice were pre-immunized by intraperitoneal inoculation of suspensions of 1) pooled (C3H × 101)F$_1$ thymus, liver, and spleen cells at least 3 weeks before sacrifice, 2) pooled (C3H × 101)F$_1$ liver, spleen, and kidney cells 1 week before sacrifice, and 3) homologous parental spleen cells 1 week before sacrifice or at weekly intervals 4 or more

weeks before sacrifice (Tables I, II). For immunization controls, 101 strain mice were given weekly injections of spleen cells from adult rats for at least 4 weeks. In each instance, at least 10 million nucleated cells were inoculated/injection.

*Results.* Mortality 30-120 days after inoculation was low in (101 × C3H)$F_1$ and (C3H × 101)$F_1$ animals treated with isologous marrow, non-immunized parental marrow, and marrow from parental donors immunized 1 week or less before sacrifice (Table I). Conversely, survival among mice treated with marrow from donors immunized 3 or more weeks before sacrifice was poor beyond 30 days. Sensitization of donor against rat tissue did not influence mortality. Use of (101 × C3H)$F_1$ marrow to protect 101 or C3H parental strain recipients caused little delayed mortality. However, delayed mortality was high when 101 marrow was used to protect mice of C3H strain, and vice versa. In (C57BL × 101)$F_1$ mice, non-sensitized C57BL parental strain marrow gave poor survival after first 30 days (Table II). Pre-immunization of donors of either 101 or C57BL parental strains increased delayed mortality in (C57BL × 101)$F_1$ recipients. In parental strain mice protected with (C57BL × 101)$F_1$ marrow, 30-day mortality was high, as it was when marrow of one parental strain was used to protect the other.

Histological changes in $F_1$ hybrids sacrificed or dying after administration of pre-immunized parental bone marrow consisted principally of granulomatous proliferation of reticuloendothelial elements of the white pulp of spleen, cortex of lymph nodes, and Peyer's patches, a reaction similar to that caused by injection of parental or homologous spleen cells(2) but proceeding more slowly.

*Discussion.* Although administration of homologous or heterologous bone marrow is usually associated with delayed mortality in lethally irradiated recipients(1), the outcome is variable when parental strain marrow cells are transplanted into $F_1$ hybrids, mortality varying from strain to strain(4-7). One basis for this variability is the varying extent of histocompatibility differences between donor and recipient at the *H-2* locus(7,8). For example, in the present experiment, marrow cells from C3H or 101 donors, which are identical at the *H-2* locus ($H\text{-}2^k$) (R. D. Owen, personal communication), failed to consistently provoke delayed mortality in (101 × C3H), or reciprocal, $F_1$ recipients unless pre-sensitized to them. In contrast, marrow cells from C57BL donors, which differ at the *H-2* locus from 101 mice ($H\text{-}2^b$ and $H\text{-}2^k$, respectively), caused much delayed mortality in (C57BL × 101)$F_1$ recipients. The greater percentage of delayed mortality in (C57BL × 101)$F_1$ recipients injected with C57BL cells, as opposed to 101 cells, cannot be accounted for. It may conceivably denote either greater antigenicity of the $F_1$ to the C57BL parent than to the 101 parent by virtue of undefined differences at the *H-2* locus or other loci, or greater ability of C57BL than 101 marrow cells to initiate antibody production. Similarly, failure of nonsensitized 101 or C3H marrow cells to cause delayed death in (101 × C3H), or reciprocal, $F_1$ recipients cannot be explained in the face of high delayed mortality, such parental strain cells caused when transplanted into the homologous parent, unless it is assumed that the $F_1$ was less antigenic to parent than was the homologous parent. The latter may have been the case if antigenicity was determined by gene dose, as suggested by Uphoff(9), since the homozygous parent contained twice the gene dose of the heterozygous $F_1$ for any given antigen. Presumably, undefined histocompatibility differences between C3H and 101 mice at the *H-2* locus or other loci elicited the lethal reaction observed in (101 × C3H), or reciprocal, $F_1$ recipients when reactivity of parental strain donors was enhanced by pre-sensitization. Pre-sensitization to nonspecific (rat) antigens was without effect, as anticipated.

Enhancement of foreign bone marrow reaction by pre-sensitization of donor against the recipient is notable in that it denotes heightening of antibody-forming potentiality of injected bone marrow cells. The effect is the reverse of that obtained by transplantation of fetal hemopoietic tissue(10). It is also noteworthy that pre-sensitization did not increase the marrow's lethality to the level of

spleen or other lymphoid tissues(2,11-14) or exert any detectable effect unless carried out well in advance of transplantation.

If the foreign bone marrow reaction is similar to foreign spleen reaction in pathogenesis, as suggested by histopathologic resemblances, it could be initiated by lymphoid cells in bone marrow. In this case, differences between effectiveness of spleen and marrow would represent merely numerical differences in numbers of reactive, antibody-forming cells present in each tissue. On the other hand, the characteristically different time sequences of the 2 reactions suggest the differences between effects of marrow and spleen result from qualitative dissimilarities, yet to be elucidated. Since antibody-forming cells, not normally detectable in bone marrow, may be found there in highly immunized animals(10), the effects of pre-sensitization may conceivably represent release of such cells from spleen and lymph nodes and their migration to other sites, including the marrow.

*Summary.* Pre-sensitization of parental strain donor mice against $F_1$ hybrid recipients accentuated the foreign bone marrow reaction caused by injection of parental marrow cells into lethally irradiated $F_1$ recipients. This effect occurred with use of parental strains (101 and C3H) that were histocompatible at the *H-2* locus, as well as with those (C57BL and 101) that were histoincompatible at this locus. These results are interpreted to indicate the importance of relative antibody-forming potency of implanted cells, as well as the extent of histocompatibility differences in pathogenesis of the homograft reaction.

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## Adenylic Acid Deaminase Activity in Spectrum of Rat Tumors and in Normal Liver.* (24648)

SHUNG-KAI CHAN, THOMAS A. MCCOY AND DONALD E. KIZER
*Biomedical Division, Samuel Roberts Noble Fn., Ardmore, Okla.*

Deamination of adenosine-5′-monophosphate (AMP) to inosine-5′-monophosphate (IMP) is catalyzed by the enzyme 5′-adenylic acid deaminase. This enzyme was demonstrated in skeletal muscle and in water-soluble fraction of smooth muscle, liver and kidney. Its isolation in crystalline form from rabbit skeletal muscle was recently reported. The above work was summarized by Lee(1). Greenstein *et al.*(2) observed deamination of yeast adenylic acid (presumably adenosine-3′-monophosphate) by several normal tissues of rat and mouse and by a mouse hepatoma. Recently Fodor *et al.*(3) showed that homogenates of both growing and regressing Flexner-Jobling carcinomas deaminated adenosine-5′-monophosphate, while their activity towards the 3′-isomer was much lower. Our purpose was to determine the 5′-adenylic acid deaminase activity in a number of rat transplantable tumors, and to ascertain whether activity in hepatomas resulting from dye carcinogenesis

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