## Effect of *in Vitro* Irradiation of Cells in Graft-Versus-Host Reactions[1] (35771)

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A severe and frequently fatal graft-versus-host (GVH) reaction may result from the administration of allogeneic immunologically competent lymphoid cells to a host deficient in cell-mediated immunity (1, 2). High speed centrifugation, freeze thawing, and radiation have been used to free the blood or blood products of immunocompetent cells prior to their administration to patients. The capacity of lymphocytes to respond to phytohemagglutinin (PHA) and incorporate radiolabeled thymidine following irradiation has been used to measure the amount of immunocompetence remaining (3, 4). From certain of these studies (3), it has been maintained that even large doses of irradiation do not inhibit immunocompetent cells as revealed by the capacity of those cells to respond by *in vitro* transformation to PHA. This evidence has been taken as a warning that irradiation *in vitro* may not be a reliable method of eliminating the hazard of GVH reactions. Coifman *et al.* (5) have recently shown that this *in vitro* analysis of lymphocyte function following irradiation may not be a valid assessment of immunocompetence. The following experiments were designed to reassess the effects of irradiation on a well-defined biological function of immunocompetent cells using an *in vivo* GVH assay.

This study demonstrated that *in vitro* irradiation, under optimum conditions of oxygenation and cell numbers, can effectively inhibit a sufficient proportion of immunocompetent cells to keep the hazard of GVH reactions to a minimum. It is clear, however, from the results reported herein, that, when assayed by this function like so many others, irradiation in moderate doses may leave a residue of cells capable of initiating a GVH reaction.

*Methods.* Inbred mice of C3Hf/Bi (these mice will be referred to as C3H in the text) and A/jax and their $F_1$ hybrids of these strains were used. The inbred strains originated from the colonies of the late Drs. J. J. Bittner and C. Martinez and have been maintained in our laboratories by rigorous inbreeding. A detailed description of the strains have been reported recently (6) and designed as U. of M. colony sublines (Umc).

The graft-versus-host assay as described by Simonsen (7) was performed in young C3H $\times$ A $F_1$ mice injected intraperitoneally with spleen cells of parental A strain. Litters of 8–10-day-old C3H $\times$ A $F_1$ hybrids were divided into three groups of two or three mice each. These groups were: (a) an experimental group given irradiated spleen cells of parental strain A; (b) negative controls given irradiated syngenic spleen cells; and (c) positive controls given nonirradiated parental A strain spleen cells. Six spleens were pooled; and the spleen cell suspensions were prepared in cold minimal essential medium (Grand Island Biological Co., Grand Island, New York, Cat. No. 138). Viability of the suspended cells, determined by trypan blue exclusion, ranged from 75 to 95%. Cells were counted in a hemocytometer and the concentrations were adjusted to the desired number of cells in approximately 0.2 ml of medium.

[1] Aided by grants from the U.S. Public Health Service (AI-08677, AI-00798 and NS-02042), National Foundation-March of Dimes, and The American Heart Association.

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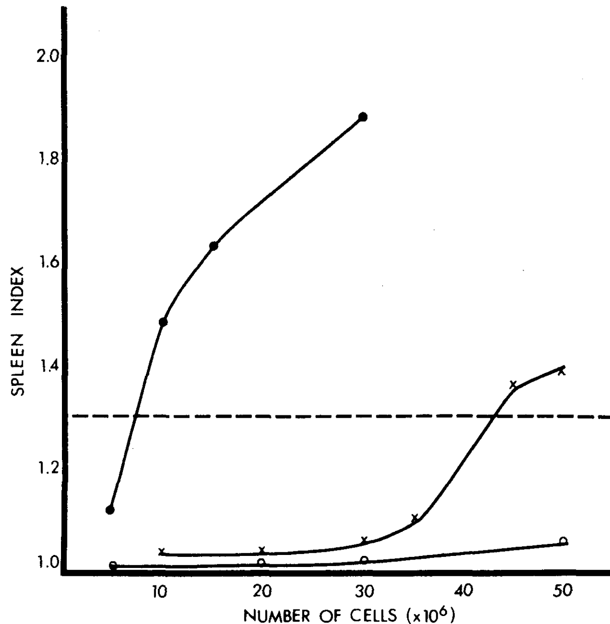


FIG. 1. Spleen indices of C3H × A $F_1$ hybrid mice following injection of parental A spleen cells: (●) normal A cells; (×) A cells irradiated with 1500 R; (○) A cells irradiated with 2000 R.

Eight days after cell injection, the animals were killed, their body and spleen weights were determined, and a relative spleen weight (mg/10 g of body wt) was calculated. The spleen index was determined by dividing the mean relative spleen weight of the experimental groups by the mean relative spleen weight of the negative control. Spleen indices of greater than 1.30 are considered indicative of significant GVH reactivity.

Six to eight litters were tested at each of the cell doses. All radiation was administered by a GE Maximar, 220 kVp, without iometer, delivering an air dose of 462 R/min through a 1 mm Al filter. The spleen cells in Falcon test tubes (16 × 150 mm) (Falcon Plastics, division of Bio Quest, Oxnard) were irradiated following adequate oxygenation ($pO_2 > 70$ mm Hg.)

*Results*. Figure 1 shows the results obtained when different numbers of parental strain A spleen cells were administered intraperitoneally to $F_1$ hybrids. Nonirradiated spleen cells produced spleen indices of 1.3 or greater when $10 \times 10^6$ spleen cells were injected. The spleen indices increased proportionately when greater numbers of cells were given. When the spleen cells were irradiated with 1500 R *in vitro* prior to injection, significant spleen indices were not observed with $35 \times 10^6$ cells/mouse, although the spleen indices with $35 \times 10^6$ cells were higher than with $20 \times 10^6$ cells. When $45 \times 10^6$ spleen cells treated with 1500 R were injected, however, significant spleen indices of greater than 1.30 were obtained. Following irradiation with 2000 R, no significant splenomegaly was observed, even with cell doses of $50 \times 10^6$/mouse. While not significantly different, the spleen indices were slightly greater for cell doses of $50 \times 10^6$ cells than for $20 \times 10^6$ treated with 2000 R.

*Discussion*. Our results indicate that, for the graft-versus-host reaction as measured by the Simonsen assay, the response of $F_1$ hybrid mice to nonirradiated parental spleen cells varies with the number of cells injected. *In vitro* irradiation of the donor parental spleen cells prior to injection can suppress the capacity of these immunocompetent cells to produce a GVH reaction as measured by the Simonsen assay. When as many as $35 \times 10^6$ spleen cells which had been irradiated with 1500 R were administered, the spleen

indices of the recipient mice were less than 1.30. The spleen indices, while not significantly different from each other, appeared to increase as the number of injected irradiated cells increased. This observation may indicate that this dose of irradiation does not inhibit all the immunocompetent cells, but that among the cells injected, the number which tolerated the irradiation is insufficient to launch a GVH reaction. However, when a large number of cells is administered, the number of functionally surviving cells is sufficient to produce a detectable reaction.

A radiation dose of 2000 R prevented significant GVH reactivity when the cell inocula contained as many as $50 \times 10^6$. It may, of course, be extrapolated that even 2000 R would be inadequate to prevent GVH reactions if greater numbers of cells could have been administered.

The sensitivity of cells to irradiation differs with each target cell type. Cells within the lymphoid series also vary in their susceptibility to irradiation (8–11). Adequate oxygenation of the target tissue has been shown to be important for the maximum effect of irradiation (12).

The survival curves of irradiated mammalian cells are exponential. This indicates that a given dose of radiation will inhibit a given number of cells and if larger numbers of cells are irradiated greater numbers of cells will survive. The surviving cells have been sublethally injured (13). Simularities in the repair processes of lethal and sublethal radiation injuries suggest that these two types of cell damage are qualitatively similar but quantitatively different (14). Puck and coworkers (15, 16) observed that many tissues, while no longer able to replicate following high doses of radiation, could still grow and be metabolically active.

Our study demonstrates that *in vitro* irradiation under optimum conditions of oxygenation and cell numbers can effectively inhibit the capacity of lymphoid cells to initiate GVH reactions. Such treatment should, under most circumstances, eliminate the clinical hazard of GVH reactions. The influence studied, like so many others attributable to irradiation, is not absolute; and residual cells, capable of initiating GVH reactions, could be a clinical hazard under appropriate circumstances.

These observations are in agreement with those of Blackett (17) who showed that *in vivo* irradiation of parental rat lymphoid cells inhibited the capacity of these spleen cells to elicit a GVH reaction in $F_1$ hybrid recipients. Extracorporeal irradiation of blood in passage from parental to $F_1$ hybrid rats has suggested that two populations of lymphoid cells exist differing in radiosensitivity, which are capable of inducing GVH reactions (18). These studies confirm the previous *in vitro* analysis of radiation injury to cells but have the advantage of being able to study a well-defined *in vivo* biological function of immunocompetent cells.

With the increasing use of immunosuppressive agents in clinical medicine (organ transplantation, cancer therapy, autoimmunity, and bone marrow transplantation) and the deficiencies of cell-mediated immunity produced by these agents, the need for administering blood or blood products free of immunocompetent cells to these patients is crucial. The serious hazard of administering immunocompetent cells to patients with defective cell-mediated immunity is clear (1).

It is generally accepted that 3000 R delivered to a well-oxygenated unit of human blood, known to contain approximately $1–5 \times 10^9$ lymphocytes, can be safely administered to patients with deficiencies of both cellular and humoral immunity (5). This amount of irradiation will effectively eliminate a sufficient number of immunocompetent cells contained in that volume of blood to prevent a sufficient immunological assault upon the susceptible host. If multiple transfusions are given however, the accumulative effect of those sublethally injured immunocompetent cells remaining in each unit of irradiated blood may be sufficient to initiate a GVH reaction. When immunocompetence is to be eliminated, while leaving granulocytes and platelets functionally intact, even higher doses than the usually recommended 1–3000 R may be necessary to reduce the hazard of GVH to acceptable levels. Whenever possible, as for example when only red blood cells are

needed, methods designed to eliminate all immunocompetent cells such as freeze-thawing and centrifugation techniques should be used.


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Received Feb. 8, 1971. P.S.E.B.M., 1971, Vol. 137.