BONE MARROW FUNCTION

I. Peripheral T Cells Are Responsible for the Increased Auto-antiidiotype Response of Older Mice

By YOUNG TAI KIM, EDMOND A. GOIDL,* CHRISTIANE SAMARUT,
MARC E. WEKSLER, G. JEANETTE THORBECKE, AND
GREGORY W. SISKIND

From the Division of Allergy and Immunology and the Division of Geriatrics and Gerontology, Department of Medicine, Cornell University Medical College, New York 10021; the Department of Pathology, New York University School of Medicine, New York 10016; and the *Department of Microbiology, University of Maryland School of Medicine, Baltimore, Maryland 21202

Recently (1) we have shown that, with age, there are changes in B cell clonal expression as manifested by differences in the idiotypes (Id) produced and an increase in the magnitude of the auto-anti-Id response. The increased auto-anti-Id response can be transferred to young recipients with splenic lymphoid cells from aged animals (2). However, lethally irradiated mice reconstituted with bone marrow (BM) from aged donors behave like young mice in that they manifest only modest auto-anti-Id responses (2), suggesting that the BM of old and young animals is similar. Furthermore, it has been shown that splenic T cells obtained from old mice modify the response of BM-reconstituted irradiated mice to behave in a manner typical of old animals with respect to auto-anti-Id production (2). On the basis of these observations we have proposed the hypothesis that the changes in Id expression and auto-anti-Id production in old animals are due to shifts in clonal distribution among the long-lived peripheral T cells, as a consequence of life-long interactions with internal and environmental antigens. This hypothesis predicts that if the peripheral lymphoid system of an old animal is acutely depleted of cells while the BM is left intact (e.g., irradiation with BM shielding) and the animals are allowed to repopulate their peripheral lymphoid system from their own marrow, they should behave like young mice with respect to auto-anti-Id production and it should be possible to influence this pattern of recovery transfer of peripheral T cells from donors of different ages. These predictions are borne out by the present results.

Materials and Methods

Animals, Irradiation, and Cell Transfer. C57BL/6J male mice were used. Old animals were 18 mo old, or older, while young mice were 8 wk old. Mice were anesthetized with tribromo ethanol, and placed in a lead shield that protected the bone marrow of their rear legs, head, and part of their spinal column, while permitting irradiation of their

spleen, thymus, and main axillary and inguinal lymph nodes (G. Fernandez, personal communication). They were exposed to 800 rad gamma irradiation from a Gammator M (Radiation Machinery Corp., Parsippany, NJ) and were then housed under laminar flow conditions. This dose of radiation has been found to be 100% lethal for nonshielded C57BL/6J mice.

Preparation of Antigens and Hapten. Aminoethylated polyacrylamide beads (PAA) were prepared according to the methods of Inman and Dintzis (3) and were trinitrophenylated (TNP-PAA) as previously described (4). Trinitrophenylated Ficoll (TNP-F) was prepared by coupling TNP-lysine (Sigma Chemical Co., St. Louis, MO) to Ficoll 400 (Pharmacia Fine Chemicals, Piscataway, NJ) (5).

Immunization. Mice were immunized by the intravenous injection of 10 μg TNP-F and killed by cervical dislocation; then splenic anti-TNP plaque-forming cells (PFC) were assayed.

Cell Culture. Primary anti-TNP PFC responses were elicited in spleen cell culture using the relatively T-independent antigen, TNP-PAA, as described (4).

PFC Assay and the Determination of Cells Whose Secretion Is Inhibited by Auto-anti-Id. Anti-TNP PFC in individual mouse spleens or individual culture dishes were assayed by a slide modification of the Jerne plaque technique using 2,4,6-trinitrophenylated sheep red blood cells (TNP-SRBC) as described (1, 2). Also, anti-TNP PFC assays were performed with concentrations of TNP-EACA (epsilon aminocaproic acid) in the agar ranging from 1×10^{-9} to 1×10^{-5} in half-log units. In some cases, in the presence of low concentrations of TNP-EACA (1×10^{-9} to 10^{-7} M), a significant augmentation of PFC occurs above the number detected in the absence of hapten. We have demonstrated that such hapten-augmentable PFC are B cells whose secretion of antibody has been inhibited by the binding of auto-anti-Id to cell surface antibody molecules (2).

Determination of Auto-anti-Id by Enzyme-linked Immunosorbent Assay (ELISA). 0.2 ml of serial dilutions of serum in 0.1 M carbonate buffer, pH 9.4, was added to polyvinyl microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) and the plates were held at room temperature for 2 h. The plates were washed with 0.1% Tween 20 in phosphate-buffered saline (PBS) three times and held at room temperature for 2 h with 2% bovine serum albumin in 0.1 M carbonate buffer, pH 9.4. They were then washed with 0.1% Tween in PBS, held at room temperature for 6 h with alkaline phosphatase (calf intestine type VII; Sigma Chemical Co.) conjugated to affinity-purified anti-TNP antibody, washed with 0.1% Tween in PBS, and incubated with paranitrophenyl phosphate in 1 M Tris-HCl buffer, pH 8.1. Absorbance at 405 nm was determined with a multi-reader (model EL307; Bio-Tek Instruments Inc., Burlington, VT). Background binding was determined with age-matched normal sera. Conjugation of alkaline phosphatase to the affinity-purified anti-TNP antibody was carried out with 0.2% glutaraldehyde (6).

Results

Recovery of the Immune System of Mice Irradiated While Their BM Is Protected by Lead Shielding. 8-wk-old C57BL/6J male mice were subjected to 800 rad gamma radiation with their BM shielded. Mice were killed at various intervals 1–7 wk after irradiation and their spleen cells were tested for the ability to mount an in vitro primary anti-TNP PFC response to TNP-PAA. The number of spleen cells recovered was very low 1 and 2 wk after irradiation, and progressively increased to achieve approximately normal levels at 6 wk after irradiation (Fig. 1). Spleen cells obtained 1 and 2 wk after irradiation and cultured with TNP-PAA produced few if any anti-TNP PFC. Thereafter, the anti-TNP PFC response increased progressively and, at 6 wk after irradiation, was essentially equivalent to that of unirradiated mice (Fig. 2). Based on these data, a recovery period of 6–7 wk was allowed between irradiation and challenge in all subsequent experiments.

Effect of Irradiation of the Peripheral Lymphoid System on Auto-anti-Id Production by Old Mice. Mice of different ages were irradiated with their BM shielded and
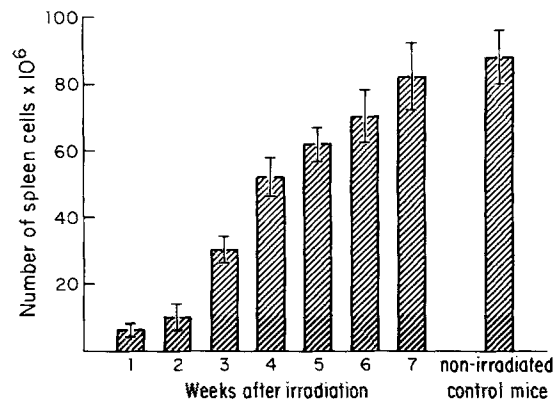
FIGURE 1.   Recovery of splenic cells after irradiation (800 rad) of C57BL/6 mice with their BM shielded. Mice were sacrificed at weekly intervals, and their spleens were removed and teased into single-cell suspensions. The number of spleen cells recovered is plotted against time after irradiation. Each data point represents an average of results on five individual mice.
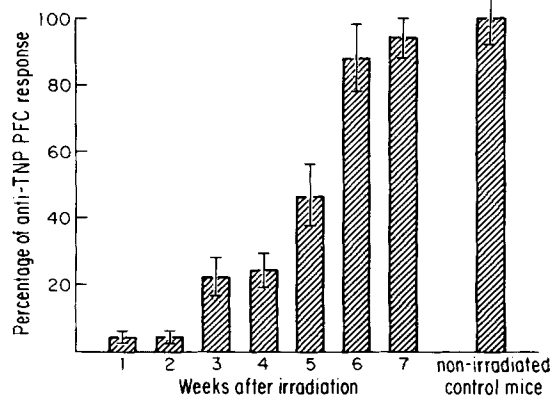

FIGURE 2.   Recovery of the capacity of spleen cells (from C57BL/6 mice irradiated (800 rad) with their BM shielded) to mount an in vitro anti-TNP PFC response to TNP-PAA. Culture conditions are described in Materials and Methods. The data represent the percentage of the response of simultaneously cultured cells from nonirradiated normal donors (averages of five individual mice). The direct anti-TNP PFC response of cells from control (nonirradiated) donors is also the average of five mice.

allowed to recover for 6 wk before intravenous immunization with 10 μg TNP-F. 6 d after immunization the mice were killed and their splenic hapten-augmentable PFC responses were assayed. Nonirradiated normal mice were similarly immunized and assayed.

As shown in Table I, 70% of the normal old mice had >20% hapten-augmentable PFC soon after immunization, compared with only 30% of normal young mice. After recovery from irradiation, only 23% of old mice had >20% hapten-augmentable PFC. In addition, the mean percentage of hapten-augmentable cells for this group was significantly lower than for the nonirradiated old mice.

Sera from similarly treated mice, obtained 6 d after TNP-F injection, were tested for auto-anti-Id by ELISA. The results in Table II confirm the finding

TABLE I

Hapten-augmentable PFC Response of Mice Immunized With TNP-F After Recovery from Irradiation With Their BM Shielded

Mice			Anti-TNP PFC per spleen	Incidence of mice having >20% hapten-augmentable PFC	Average percent (±SE) of hapten-augmentable PFC
Group	Age	Treatment			
1	8–10 wk	None	10,277 ± 1,607	6/21 (29%) 9/28 (32%) 7/22 (32%)	18.8 ± 2.2
2	8–10 wk	Irradiated	7,190 ± 923	10/21 (48%)	24.9 ± 4.5
3	18–24 mo	None	5,765 ± 1,158	17/26 (65%) 14/17 (82%)	44.9 ± 4.6
4	18–24 mo	Irradiated	5,875 ± 1,807	6/26 (23%) 5/22 (23%)	21.3 ± 3.5

C57BL/6 mice of the age indicated were untreated or were exposed to 800 rad gamma irradiation with their BM shielded. 6 wk later the mice were immunized with 10 μg TNP-F, injected intravenously. The mice were sacrificed 6 d later and their splenic anti-TNP PFC response and hapten-augmentable PFC response were assayed. Data are presented as mean percent ± SE. The difference between groups 3 and 4 in the average percent hapten-augmentable PFC is statistically significant ($P < 0.05$; Student's t test).

TABLE II

Concentration of Auto-Anti-Id in Serum of Mice Immunized with TNP-F After Recovery from Irradiation With Their BM Shielded

Mice			Binding of anti-TNP antibody (A_{405nm})
Group	Age	Treatment	
1	18–24 mo	None	0.398 ± 0.069
2	18–24 mo	Irradiated	0.294 ± 0.042
3	8–10 wk	None	0.275 ± 0.044
4	8–10 wk	Irradiated	0.279 ± 0.037

C57BL/6 mice of the age indicated were untreated or were exposed to 800 rad gamma irradiation with their BM shielded. 6 wk later the mice were immunized with 10 μg TNP-F intravenously. 6 d after immunization the mice were bled and their serum assayed for auto-anti-Id by ELISA. Data are presented as mean absorbance (405 nm) ± SD for groups of six mice.

TABLE III

Influence of Splenic T Cells from Donors of Various Ages on the Auto-anti-Id Response of Mice that Have Recovered from Irradiation With Their BM Shielded

Group	Age of irradiated mice	Age of T cell donor	Anti-TNP PFC per spleen	Incidence of mice having >20% hapten-augmentable PFC	Average percent (±SE) hapten-augmentable PFC
1	8 wk	None	14,447 ± 3,800	3/12 (25%)	17.0 ± 4.5
2	8 wk	8 wk	10,133 ± 2,340	5/18 (28%)	15.6 ± 2.8
3	8 wk	24 mo	15,144 ± 3,400	12/16 (75%)	30.9 ± 4.5
4	18 mo	None	10,470 ± 3,100	3/11 (27%)	15.5 ± 2.9
5	18 mo	8 wk	11,043 ± 2,800	2/12 (17%)	9.8 ± 4.2
6	18 mo	18 mo	8,067 ± 2,200	10/14 (71%)	35.5 ± 5.7

Mice of the indicated age were irradiated (800 rad) with their BM shielded. 7 d later they were injected intravenously with 2 × 10⁷ splenic T cells (prepared by passage of spleen cells over a nylon wool column) from donors of the indicated age. 7 wk after irradiation mice were immunized with 10 μg TNP-F, intravenously; 6 d later they were sacrificed and their spleens assayed for anti-TNP PFC and hapten-augmentable PFC. Data are presented as mean percent ± SE. The differences between groups 2 and 3 and between groups 5 and 6 in average percent hapten-augmentable PFC are statistically significant ($P < 0.05$; Student's t test).

that old mice produce more auto-anti-Id than young mice. Old mice that had recovered from irradiation with BM shielding produced low levels of auto-anti-Id, comparable to those produced by young mice. Thus, by both assays for auto-

anti-Id production (hapten-augmentable PFC and ELISA), old mice that had recovered from irradiation behaved like young mice.

Influence of Peripheral T Cells on the Auto-anti-Id Response of Mice that Have Recovered from Irradiation With BM Shielding. Old or young mice were irradiated while their bone marrow was shielded. 1 wk later the animals received splenic T cells from either 8-wk-old or 18–24-mo-old donors. 6 wk after T cell transfer, the mice were immunized with 10 μg TNP-F. The PFC responses (Table III) show that, in both young and old irradiated mice, the age of the T cell donor determines whether the magnitude of the hapten-augmentable PFC response is typical of that of young animals or of old animals.

Discussion

We previously concluded, on the basis of cell transfer studies (2), that the BM of old and young mice function similarly with respect to the anti-Id repertoire they generate. Although some disagreement exists (7), this interpretation is consistent with the results of others who have suggested that BM of old and young mice is similar with respect to stem cell activity (8, 9). In addition, Zharhary and Klinman (10) have shown that the degree of diversity of the B cell repertoire specific for PR8 influenza virus is comparable in old and young mice. Using cell transfer methods, we have obtained evidence that the age-related increase in auto-anti-Id is determined by characteristics of the peripheral (splenic) T cell population (2); we hypothesized that the altered distribution of Id is the consequence of Id–anti-Id interactions between the newly arising lymphoid cells and the long-lived peripheral T cell population.

To further test this hypothesis we have used mice that were irradiated with their BM shielded and then allowed to recover by spontaneous repopulation of their peripheral lymphoid system from their own BM. The dose of irradiation was essentially 100% lethal to nonshielded mice. The lead shield blocked only 40% of the radiation from the Cs source used. Nevertheless, almost all shielded mice remained alive and regained immune function. The results show that such autologously reconstituted old mice behave like young animals in their response to TNP-F, in that they generate low auto-anti-Id responses as measured by two different assay techniques (ELISA and hapten-augmentable PFC). If irradiated mice (old or young) repopulate their peripheral lymphoid system in the presence of splenic T cells transferred to them from old donors, the anti-TNP responses are like those of old mice; if they repopulate their peripheral lymphoid system in the presence of splenic T cells from young donors, their responses are like those of young mice. The fact that the immunologic behavior of recipients of equal numbers of peripheral T cells is determined by the age of the T cell donor suggests that a T cell deficiency is not responsible for these findings.

The results reported are consistent with the hypothesis that the BM B cells of old and young mice are similar in the spectrum of Id they can produce. Some of the differences between old and young mice seem to be the consequence of Id–anti-Id interactions between cells, arising from the BM and long-lived peripheral T cells.

Summary

After immunization with trinitrophenyl (TNP)-Ficoll, mice produced both anti-TNP antibodies and auto-anti-idiotype (auto-anti-Id) antibodies specific for

the anti-TNP antibody. Older animals produced more auto-anti-Id than did young animals. When mice were exposed to a normally lethal dose of irradiation while their bone marrow (BM) was partially shielded, they survived and slowly (6 wk) regained immune function, as indicated by the number of nucleated cells in their spleen and the in vitro primary plaque-forming cell (PFC) response of their spleen cells to TNP-treated aminoethylated polyacrylamide beads. Recovery is presumably the result of repopulation of the peripheral lymphoid system by cells originating in the BM. By enzyme-linked immunosorbent assay (ELISA), and by hapten-augmentable PFC assay, we show that, after recovery from irradiation with their BM shielded, old animals produce low auto-anti-Id responses, like those of young animals. The transfer of splenic T cells into mice irradiated with their BM shielded provided evidence that the magnitude of the auto-anti-Id response is controlled by the peripheral T cells. Thus, mice that received splenic T cells from aged donors produced high levels of auto-anti-Id while those that received splenic T cells from young donors produce low levels of auto-anti-Id.

References

1. Siskind, G. W., A. F. Schrater, G. J. Thorbecke, M. E. Weksler, and E. A. Goidl. 1982. The role of auto-anti-idiotype antibody in the regulation of the immune response. *Cell. Immunol.* 66:34.
2. Goidl, E. A., J. W. Choy, J. J. Gibbons, M. E. Weksler, G. J. Thorbecke, and G. W. Siskind. 1983. Production of auto-anti-idiotypic antibody during the normal immune response. VII. Analysis of the cellular basis for the increased auto-anti-idiotype antibody production by aged mice. *J. Exp. Med.* 157:1635.
3. Inman, J. K., and H. M. Dintzis. 1969. The derivatization of cross-linked polyacrylamide beads. Controlled induction of functional groups for the preparation of special purpose biochemical absorbents. *Biochemistry.* 8:4074.
4. Kim, Y. T., M. E. Weksler, and G. W. Siskind. 1981. Antigen-induced *in vitro* inhibition of immune responsiveness. *Cell. Immunol.* 14:44.
5. Mitchell, G. F., J. H. Humphrey, and A. B. Williamson. 1972. Inhibition of secondary anti-hapten responses with the hapten conjugated to type 3 pneumococcal polysaccharide. *Eur. J. Immunol.* 2:460.
6. Avrameas, S. 1969. Coupling of enzymes to proteins with glutaraldehyde. *Immunochemistry.* 6:43.
7. Farrar, J. J., B. E. Loughman, and A. A. Nordin. 1974. Lymphopoietic potential of bone marrow cells from aged mice: comparison of the cellular constituents of bone marrow from young and aged mice. *J. Immunol.* 112:1244.
8. Ogden, D. A., and H. S. Micklem. 1976. The fate of serially transplanted bone marrow cell populations from young and old donors. *Transplantation (Baltimore).* 22:287.
9. Harrison, D. E., C. M. Astle, and J. W. Doubleday. 1977. Stem cell lines from old immunodeficient donors give normal responses in young recipients. *J. Immunol.* 118:1223.
10. Zharhary, D., and N. R. Klinman. 1984. B cell repertoire diversity to PR8 influenza virus does not decrease with age. *J. Immunol.* 133:2285.