CELLS INVOLVED IN THE IMMUNE RESPONSE

VIII. The Relationship between the Loss and Reappearance of Antigen-Reactive Cells and Immune Responsiveness After Irradiation of Normal Adult Rabbits*

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The successful induction of the primary humoral immune response in the rabbit has been shown to require the mediation of two cell types, the antigen-reactive cell and the antibody-forming cell (1, 2). It has been established that the antigen-reactive cell in the rabbit originates in the bone marrow (1, 3, 4); however, the organ of origin of the antibody-forming cell has not been adequately investigated. A third cell type, the macrophage, must also be considered to play a role in the induction of the primary immune response (5–8), although definitive evidence as to the exact nature of its role still remains to be realized. Cell transfer experiments have demonstrated that the inhibition of the immune response in the rabbit by irradiation is due to the destruction of the antigen-reactive cell since the antibody-forming cell is irradiation resistant (2). Furthermore, it has also been established that in irradiated rabbits given allogeneic normal bone marrow cells and antigen, the antibody-forming cells originate from recipient or host cells and not from donor cells (2). It was therefore concluded that normal rabbit bone marrow contains antigen-reactive cells and no antibody-forming cells. The present investigation was undertaken to establish whether a direct relationship exists between the presence of the antigen-reactive cell and the recovery of immune responsiveness in irradiated rabbits.

Methods and Materials

The protocols for the experimental procedures carried out in this investigation are diagrammatically presented in Fig. 1.

Adult New Zealand white rabbits were used throughout this study. The antigen used was sheep red blood cells (SRBC) obtained as a sterile cell suspension in Alsever's solution. The immunizing dose of SRBC was 10^9 cells, always given via the intravenous route.

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Using a Cobalt-60 source, the rabbits were subjected to doses of irradiation ranging from 800 to 1400 R whole body irradiation. The skin-source distance was 200 cm, the field size was 50 x 50 cm, the colorimeter size was 20 x 20 cm, and the output was 6.97 R/min. The rabbits were then injected with the antigen at varying intervals of time thereafter. The rabbits were sacrificed 7 days after immunization and their spleen cells were analyzed for antibody-forming capacity by the hemolysis-in-gel (plaque) technique of Jerne et al. (9, 10).

Other rabbits were irradiated and immediately given bone marrow cells (0.5×10^9 cells) obtained from normal or irradiated (800 R) donors. The method of obtaining a bone marrow cell suspension has been previously described (1, 3). These rabbits were also given SRBC intravenously at predetermined times thereafter. The immune response was determined by

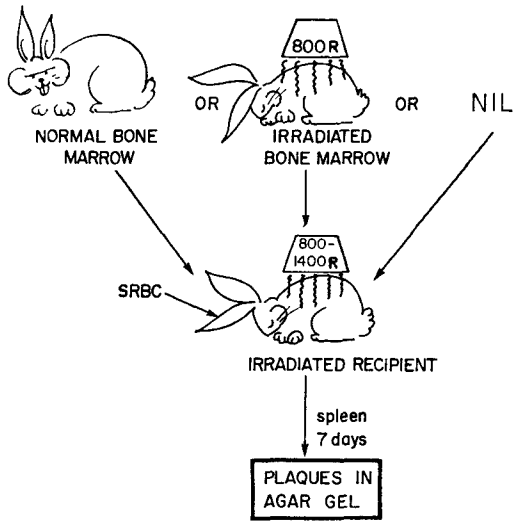

Fig. 1. Protocol for the demonstration of radiosensitivity of the antigen-reactive cel (ARC) in rabbit bone marrow.

the direct agglutination technique and the hemolysis-in-gel (plaque) technique of Jerne et al (9, 10).

Primed bone marrow cells refer to cells obtained from a rabbit immunized with the antigen 24 hr prior to sacrifice.

RESULTS

As can be seen in Table I, spleen cells of irradiated (800 R) rabbits immunized with SRBC and either not given any cells or injected with bone marrow cells of primed or irradiated (800 R) donor rabbits could not form hemolytic plaques when incubated with SRBC in agar gel. On the other hand, spleen cells obtained from an immunized unirradiated rabbit or an irradiated (800 R) rabbit given normal allogeneic bone marrow cells and immunized with SRBC formed abundant plaques upon incubation with SRBC.

The effect of varying the dose of irradiation on the capacity of recipient rabbits of normal allogeneic bone marrow cells to elicit an immune response following stimulation with SRBC is presented in Table II. A dose of 800 R

TABLE I

The Effect of Irradiation and the Administration of Primed or Irradiated Allogeneic Bone Marrow on the Plaque-Forming Capacity of Spleen Cells of Irradiated Recipient Rabbits

Bone marrow donor treated as follows	Recipient rabbit treated as follows	SRBC (10^9 cells) injected in recipient as follows	No. of plaques per 10^6 splenic lymphoid cells incubated with SRBC*
—	Untreated‡	Yes	72
	Irradiated (800R)‡	Yes	3
Normal untreated	Irradiated (800R)	Yes	70
Irradiated (800R)§	Irradiated (800R)	Yes	2
Primed‖	Irradiated (800R)	Yes	3
—	Untreated‡	No	4

* Rabbits were sacrificed 7 days following the injection of the SRBC. Each value represents the mean of duplicate determinations.

‡ Rabbits were not injected with any donor cells.

§ Donor rabbits were subjected to 800R total body irradiation several hours prior to sacrifice.

‖ Primed bone marrow was obtained from donor rabbits 24 hr following the injection of the SRBC.

TABLE II

The Plaque-Forming Capacity of Spleen Cells of Rabbits Subjected to Varying Doses of Irradiation and Given Normal Allogeneic Bone Marrow Cells

Dose of irradiation administered	No. of plaques per 10^6 splenic lymphoid cells 7 days following the administration of bone marrow and SRBC*
Nil	70
800	68
1000	2
1200	<1
1400	<1

* Normal allogeneic bone marrow (5×10^8 cells per recipient) and SRBC (10^9 cells) were given intravenously immediately following irradiation. Each value represents the mean of duplicate determinations.

total body irradiation was the maximum the recipient could tolerate and still be capable of forming antibodies, whereas exposure of the recipient to 1000 R or more resulted in complete inhibition of the immune response.

Rabbits exposed to 800 R and immunized with SRBC at varying times thereafter were incapable of eliciting a humoral immune response during the

first 3 wk following irradiation. Recovery of immune responsiveness occurred about 3–4 wk following irradiation (Table III). By 5 wk, the capacity of the rabbit to respond greatly exceeded that of a normal unirradiated rabbit (Tables

TABLE III

The Plaque-Forming Capacity of Spleen Cells of Rabbits Injected with Sheep Red Blood Cells (SRBC) at Varying Intervals of Time Following Irradiation (800R)

SRBC (10^9 cells) injected at following days subsequent to irradiation	No. of plaques per 10^6 splenic lymphoid cells*
1	1
7	1
14	24
21	25
28	103
63	80
98	95
105	71

* The rabbits were subjected to 800R total body irradiation prior to the injection of the SRBC. They were sacrificed 7 days later and tested for plaque-forming capacity to SRBC. Each value represents the mean of duplicate determinations.

TABLE IV

The Plaque-Forming Capacity of Irradiated (800R) Rabbits Reconstituted with Allogeneic Normal Bone Marrow and Given Sheep Red Cells (SRBC) at Varying Intervals of Time

SRBC (10^9 cells) given at following days subsequent to irradiation and bone marrow transfer	No. of plaques per 10^6 splenic lymphoid cells of irradiated recipients of normal allogeneic bone marrow and SRBC*
1	64
7	49
14	37
21	130
28	300
68	60
98	84

* Rabbits were subjected to 800R total body irradiation and injected with normal allogeneic bone marrow cells (5×10^8 cells) and SRBC (10^9 cells). They were sacrificed 7 days later. Each value represents the mean of duplicate determinations.

III and IV). However, if the irradiated rabbits were given normal allogeneic bone marrow cells immediately following irradiation, the response to SRBC was maximum at 1 wk and diminished over the following 2 wk (Table IV). The capacity of the spleen cells of the recipients to form plaques rose sharply by 4 wk, and then returned to normal levels.

The bone marrow cells transferred immediately following irradiation from

irradiated (800 R) donors to irradiated (800 R) recipients were incapable of conferring antibody-forming capacity to these animals. The capacity of the bone marrow to transfer immunocompetence was regained somewhat if the marrow was transferred 2 or 4 wk following irradiation of the bone marrow donor. The bone marrow completely recovered the capacity to transfer antibody-forming capacity if transferred 6 wk following irradiation (Table V).

TABLE V

The Plaque-Forming Capacity of Spleen Cells of Irradiated (800R) Rabbits Given Allogeneic Bone Marrow Cells Obtained from Irradiated (800R) Donor Rabbits

Irradiated donor sacrificed and bone marrow transferred (5×10^8 cells) at following days subsequent to irradiation	No. of plaques per 10^6 recipient* splenic lymphoid cells incubated with SRBC
1	4
14	18
28	21
42	43

* Irradiated rabbits were sacrificed 7 days following the administration of the allogeneic irradiated bone marrow cells and the SRBC (10^9 cells) and the spleen cells were analyzed for plaque-forming capacity. Each value represents the mean of duplicate determinations.

TABLE VI

The Antibody-Forming Capacity of Irradiated Rabbits Immunized with Sheep Red Blood Cells (SRBC)

Day of injection of SRBC relative to day of irradiation (Day 0)*	Antibody titers‡ at the following days after injection of the antigen		
	7	10	14
1	0	0	0
7	0	0	0
14	0	0	0
28	0	320	640
42	1280	2560	2560

* The rabbits were subjected to 800R total body irradiation and injected with 10^9 SRBC intravenously.

‡ Expressed as the inverse of the dilution of the serum capable of agglutinating sheep red blood cells. Titers less than 10 are considered to be negative.

As can be seen in Table VI, rabbits subjected to 800 R total body irradiation were incapable of responding with antibody formation until approximately 4 wk had elapsed from the day of irradiation. By 6 wk their capacity to synthesize humoral antibody had attained normal levels.

DISCUSSION

Results of previous investigations have disclosed that only the bone marrow cells in the normal rabbit can be stimulated by antigens in vitro to undergo

blastogenesis and mitosis. Cells of the other lymphoid organs of the normal rabbit do not possess this capacity to respond in vitro (3). Using a number of antigens (sheep red cells, horse red cells, human serum albumin, bovine gamma globulin), we have also observed that the normal rabbit bone marrow consistently contains antigen-reactive cells (ARC) directed toward each of these antigens. On the other hand, cells capable of transferring immunocompetence to sheep erythrocytes have at times been detected in the sacculus rotundus and the circulating blood.[1] The spleen, popliteal lymph node, appendix, and thymus appear not to possess ARC, since they are generally incapable of transferring a significant degree of immunocompetence to 800 R irradiated rabbits.[1] It was therefore tentatively concluded that the bone marrow in the rabbit constitutes the main, if not the only, source of the ARC (1, 2).

The present investigation was carried out in an attempt to answer the following questions: (*a*) Is there a specific relationship between the degree of immunocompetence following total body irradiation and the presence of viable antigen-reactive cells (ARC)? (*b*) Do the ARC recover following immunosuppressive irradiation? (*c*) If they do recover, does the rate of reappearance of the ARC following irradiation correspond to the extent of recovery of immunocompetence? (*d*) Do the ARC reappear in the bone marrow, their original organ of habitation?

The initial experiments were carried out in order to establish the dose of irradiation which would be optimal for the series of experiments contemplated. Unirradiated or irradiated adult rabbits were injected with antigen (SRBC) along with bone marrow cells obtained from normal, irradiated (800 R), or primed donors. When their spleen cells were analyzed for plaque-forming capacity 7 days later, only the spleen cells of the untreated immunized rabbits and the immunized irradiated rabbits given normal bone marrow cells responded. Therefore, irradiation of the donor with 800 R was sufficient to deplete its bone marrow of ARC (Table I). Furthermore, it was also demonstrated that the administration of 800 R total body irradiation to the recipient rabbit was the maximum which the animal could tolerate and still possess antibody-forming cells (AFC) in its spleen 7 days later. Subjecting recipients of normal bone marrow cells (ARC) to 1000 R or more prior to the cell transfer resulted in complete destruction of the antibody-forming apparatus in the recipient. The cells mediating this latter function have been demonstrated to be of host origin and are not contained in the bone marrow cells transferred (2). The dose of irradiation administered (800 R) in the subsequent experiments was, therefore, one which effectively destroys the ARC but does not harm the antibody-forming cells (AFC) to any significant degree. Therefore, the failure of such irradiated rabbits to respond with humoral antibody formation following anti-

[1] N. I. Abdou and M. Richter. Unpublished results.

genic stimulation can be attributed to the lack of immunologically competent ARC. In cell transfer experiments, no ARC could be detected in the bone marrow of an irradiated (800 R) rabbit immediately following irradiation, whereas slight recovery of the ARC was evident by 2 wk. The immune responsiveness of the irradiated (800R) rabbit was reestablished to normal levels by 4 wk after irradiation (800 R) (Table VI) at a time when the ARC were shown to have reappeared in these animals (Table III and V). These findings were confirmed under somewhat different conditions (Table IV) in that irradiated rabbits were given normal allogeneic bone marrow cells and antigen. In this case, the plaque-forming capacity of the recipients' spleen cells diminished during the first 3 wk after the transfer of the bone marrow cells. This demonstrated the recovery of the antigen-recognizing apparatus in the irradiated rabbit followed by rejection of the transplanted allogeneic bone marrow cells. Complete recovery of the irradiated rabbits was not attained until 4 wk post-irradiation when the number of plaque-forming spleen cells detected was far greater than the number observed in the unirradiated immunized rabbit. Thus, it appears that the loss of immune responsiveness of the rabbits in the immediate postirradiation period is a direct consequence of the lytic action of irradiation (800 R) on the radiosensitive ARC and that recovery of the immune responsiveness is directly related to the reemergence of the ARC population of cells.

Our present knowledge concerning the radiosensitivity of the various types of cells involved in the different phases of the immune response is still incomplete (11, 12). The immunosuppressive action of irradiation could variously be attributed to its effect on the macrophage, and/or the antigen-reactive cell, and/or the actual antigen-processing step, and/or the antibody-forming cell. Harris and Noonan (13) have shown that rat peritoneal macrophages are less sensitive to 750 R total body irradiation than the circulating leukocytes. Mouse macrophages, or at least those macrophages which exhibit immunologic functions, were found to be radiosensitive to 550–600 R (6, 14). Rabbit immunologically active macrophages were completely inactivated following exposure in vivo to 750 R (15). Hege and Cole (16) could not suppress the background antibody plaque-forming cells by 500 R total body irradiation, a dose found to suppress cellular proliferation and the production of plaque-forming cells (17). Cells producing 19S antibodies were shown to be more sensitive to irradiation than those producing 7S antibodies (18). It has also been shown that the induction phase (latent period) of the immune response is radiosensitive, whereas the antibody-forming phase of the immune response is much more radioresistant (19). Our observations are in agreement with these findings; we have demonstrated that the ARC is radiosensitive to 800 R, whereas the AFC is much more radioresistant (2). Davies et al. (20) found that the response of the ARC to stimulation by antigen is characterized by brisk cell division and

proliferation. These cells and their progeny display increased nucleic acid metabolism and take deep basophilic stains (21). It is this phase of cell division which is very radiosensitive (22). On the other hand, the antibody-producing cells were found to be relatively radioresistant (19, 23).

The exaggerated immune response observed in the irradiated rabbits 4–5 wk after irradiation would imply an abrupt recovery of the ARC population by transformation from irradiation-resistant precursor stem cells followed by synchronous division and proliferation of these cells to greater than normal levels. Feedback-inhibition, which undoubtedly operates at the cellular level to control the proliferation of the different cell lines, would then forestall any further proliferation of that particular cell line with a return to normal levels by the 8th wk following irradiation. This delayed quasi-stimulatory effect of irradiation on the immune response in the rabbit has previously been observed by Dixon and McConahey (24) in an entirely in vivo system. They demonstrated that the immune response to a number of protein antigens could be enhanced by subjecting the immunized rabbits to 500 R total body irradiation 2 hr–2 days following the injection of the antigen. The enhanced immune response was attributed to depletion of the cells of the lymphoid tissues by the irradiation followed by a disproportionate proliferation of the antigen-stimulated immunocompetent cells which were resistant to the lytic effects of the irradiation. A similar enhancing effect of irradiation on the immune response has also been reported by Gengozian and Makinodan (25) and Morgan et al. (26) who irradiated mice 4–6 days following injection of the antigen, and by Vlahovic and Stankovic (27) who irradiated guinea pigs after injection of horse serum proteins. Taliaferro and Taliaferro (11, 19) noted that the immunodepressing effects of sublethal doses of irradiation were transient, with recovery commencing within 1 wk following a low dose of irradiation and within several weeks following exposure to larger doses of irradiation. Recovery of immune competence was found to be uniformly followed by an overcompensatory proliferation of the lymphoid tissue and an enhanced immune response. However, Silverman and Chin (28) and Fitch et al. (29) were unable to substantiate the immunoenhancing effect of irradiation on the primary immune response in the mouse.

The data presented in the present study confirms the greater radiosensitivity of the ARC as compared to the AFC in the normal rabbit (2). This strongly implies that the suppressive effects of irradiation on the primary immune response in the rabbit can be directly attributed to inactivation or death of the unstimulated antigen-reactive cell population. The present data implicate only two cell types—the antigen-reactive cell and the antibody-forming cell—in the induction of antibody synthesis. The exact role of the macrophage, if any, in the facilitation of the immune response in the rabbit is still a controversial subject (14, 30).

SUMMARY

By appropriate irradiation and cell transfer experiments, a direct correlation was observed between the presence of viable and immunologically active antigen-reactive cells and the capacity of the rabbits to respond following immunization. Rabbits given 800 R total body irradiation were unable to elicit a humoral immune response nor did they possess significant numbers of antigen-reactive cells. The ability to respond with humoral antibody formation did not reappear until antigen-reactive cells could be detected. These results strongly indicate that the presence of competent antigen-reactive cells are necessary for the successful induction of the humoral immune response in the rabbit.

BIBLIOGRAPHY

1. Abdou, N. I., and M. Richter. 1969. Cells involved in the immune response. VI. The immune response to sheep red blood cells in irradiated rabbits following administration of normal, primed or immune allogeneic rabbit bone marrow cells. *J. Exp. Med.* **129:**757.
2. Richter, M., and N. I. Abdou. 1969. Cells involved in the immune response. VII. Antibody formation by radioresistant cells of irradiated rabbits injected with normal allogeneic bone marrow cells and sheep erythrocytes. *J. Exp. Med.* **129:** 1261.
3. Singhal, S. K., and M. Richter. 1968. Cells involved in the immune response. IV. The response of normal and immune rabbit bone marrow and lymphoid tissue lymphocytes to antigens in vitro. *J. Exp. Med.* **128:**1099.
4. Abdou, N. I., and M. Richter. 1969. Cells involved in the immune response V. The migration of antigen-reactive immunocompetent cells out of the bone marrow following antigen administration. *Int. Arch. Allergy Appl. Immunol.* **35:**330.
5. Gershon, H., and M. Feldman. 1968. Studies on the immune reconstitution of sublethally irradiated mice by peritoneal macrophages. *Immunology.* **15:**827.
6. Gallily, R., and M. Feldman. 1967. The role of macrophages in the induction of antibody in X-irradiated animals. *Immunology.* **12:**197.
7. Unanue, E. R., and B. A. Askonas. 1968. The immune response of mice to antigen in macrophages. *Immunology.* **15:**287.
8. Argyris, B. F. 1968. Role of macrophages in immunological maturation. *J. Exp. Med.* **128:**459.
9. Jerne, N. K., A. A. Nordin, and C. Henry. 1963. The agar plaque technique for recognizing antibody-producing cells. *In* Cell Bound Antibodies. B. Amos and H. Koprowski, editors. Wistar Institute Press, Philadelphia. 109.
10. Jerne, N. K., and A. A. Nordin. 1963. Plaque formation in agar by single antibody-producing cells. *Science (Washington).* **140:**405.
11. Taliaferro, W. H., L. G. Taliaferro, and B. N. Jaroslow. 1964. Radiation and Immune Mechanisms. Academic Press, New York.
12. The Effects of Ionizing Radiations on Immune Processes. C. A. Leone, editor. Gordon and Breach, Science Publishers, Inc., New York. 1962.
13. Harris, J. W., and T. R. Noonan. 1967. Early changes in peritoneal leucocytes of

whole-body X-irradiated rats. *Int. J. Radiat. Biol. Related Stud. Phys. Chem. Med.* **13**:183.

14. Mitchison, N. A. 1969. The immunogenic capacity of antigen taken up by peritoneal exudate cells. *Immunology.* **16**:1.
15. Pribnow, J. F., and M. S. Silverman. 1967. Studies on the radiosensitive phase of the primary antibody response in rabbits. I. The role of the macrophage. *J. Immunol.* **98**:225.
16. Hege, J. S., and L. J. Cole. 1967. Antibody plaque-forming cells in unsensitized mice. Specificity and response to neonatal thymectomy, X-irradiation and PHA. *J. Immunol.* **99**:61.
17. Kennedy, J. C., J. E. Till, L. Siminovitch, and E. A. McCulloch. 1965. Radiosensitivity of the immune response to sheep red cells in the mouse, as measured by the hemolytic plaque method. *J. Immunol.* **94**:715.
18. Svehag, S., and B. Mandel. 1964. The formation and properties of poliovirus-neutralizing antibody. II. 19S and 7S antibody formation: Differences in antigen dose requirement for sustained synthesis, anamnesis, and sensitivity to X-irradiation. *J. Exp. Med.* **119**:21.
19. Taliaferro, W. H., and L. G. Taliaferro. 1954. Further studies on the radiosensitive stages in hemolysin formation. *J. Infect. Dis.* **95**:117.
20. Davies, A. J. S., E. Leuchars, V. Wallis, and P. C. Koller. 1966. The mitotic response of thymus-derived cells to antigenic stimulus. *Transplantation.* **4**:438.
21. Coons, A. H. 1959. Some reactions of lymphoid tissue to stimulation by antigens. *Harvey Lect.*, **53**:113.
22. Stender, H. S., D. Strauch, and H. Winter. 1962. Effects of non-specific agents on the antibody response in irradiated animals. *In* The Effects of Ionizing Radiations on Immune Processes. C. A. Leone, editor. Gordon and Breach, Science Publishers, Inc., New York. 315.
23. Nossal, G. J. V. 1959. Plasma cell proliferation following whole body irradiation. *Aust. J. Exp. Biol. Med. Sci.* **37**:499.
24. Dixon, F. J., and P. J. McConahey. 1963. Enhancement of antibody formation by whole body X-irradiation. *J. Exp. Med.* **117**:833.
25. Gengozian, N., and T. Makinodan. 1958. Relation of primary antigen injection to time of irradiation on antibody production in mice. *J. Immunol.* **80**:189.
26. Morgan, P., N. P. Sherwood, A. A. Werder, and K. Youngstrom. 1960. Studies on anaphylactic shock in the mouse. III. Effects of single whole-body X-irradiation of 500 Roentgens. *J. Immunol.* **84**:324.
27. Vlahovic, S., and V. Stankovic. 1961. Studies on whole-body X-irradiation effects on anaphylactic hypersensitivity in guinea pigs. *Acta Allergol.* **16**:329.
28. Silverman, M. S., and P. H. Chin. 1955. The effect of whole body X-irradiation of mice on immunity to tetanus toxoid. *J. Immunol.* **75**:321.
29. Fitch, F. W., R. W. Wissler, M. Lavia, and P. Barker. 1956. The timing of antigen injection relative to whole body X-irradiation and the development of circulating antibody and the splenic histologic reaction in the rat. *J. Immunol.* **76**: 151.
30. Loewi, G., A. Temple, A. P. P. Nind, and M. Axelrad. 1969. A study of the effects of anti-macrophage sera. *Immunology.* **16**:99.