CELLS INVOLVED IN THE IMMUNE RESPONSE

VII. The Demonstration, Using Allotypic Markers, of Antibody Formation by Irradiation-Resistant Cells of Irradiated Rabbits Injected with Normal Allogeneic Bone Marrow Cells and Sheep Erythrocytes*

By M. RICHTER,‡ M.D. and N. I. ABDOU,§ M.D.

(From The Harry Webster Thorp Laboratories, Division of Immunochemistry and Allergy, Royal Victoria Hospital, Montreal, Canada)

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In previous communications, it was demonstrated that normal rabbit bone marrow cells are capable of reacting with antigens in vitro (1–3). After administration of an antigen, the bone marrow cells lose the capacity to react to this particular antigen, whereas their reactivity to other antigens is not impaired. It was demonstrated (4, 5) that spleen cells of irradiated rabbits injected with homologous normal bone marrow cells and immunized with sheep erythrocytes produce many plaques when incubated with sheep erythrocytes and complement in the hemolysis in agar gel technique of Jerne et al. (6, 7). However, if the donor of the bone marrow cells had been injected with sheep erythrocytes 24 hr prior to sacrifice, the bone marrow recipient was unable to mount a successful immune response to the sheep red cells (4, 5). These results suggest the migration from the bone marrow of antigen reactive cells after contact with the antigen. However, the possibility that antibody-forming cells (potential or actual) may normally reside in the bone marrow and may leave the bone marrow along with the antigen-reactive cells after interaction with antigen in vivo cannot be ruled out. The study presented below was designed to determine whether the antibody-forming cells in the spleen of the bone marrow recipient are of donor or recipient origin.

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‡ Medical Research Associate, Medical Research Council, Canada.

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Methods and Materials

Only outbred, New Zealand white adult male rabbits were used in this investigation. The rabbits were identified as to their immunoglobulin allotype by double diffusion in agar. Specific anti-allotype antisera were produced in rabbits by immunization with antigen-antibody complexes. Sheep red blood cells (SRBC) were obtained as a sterile suspension in Alsever's solution. The method for preparing the bone marrow cell suspensions has been described

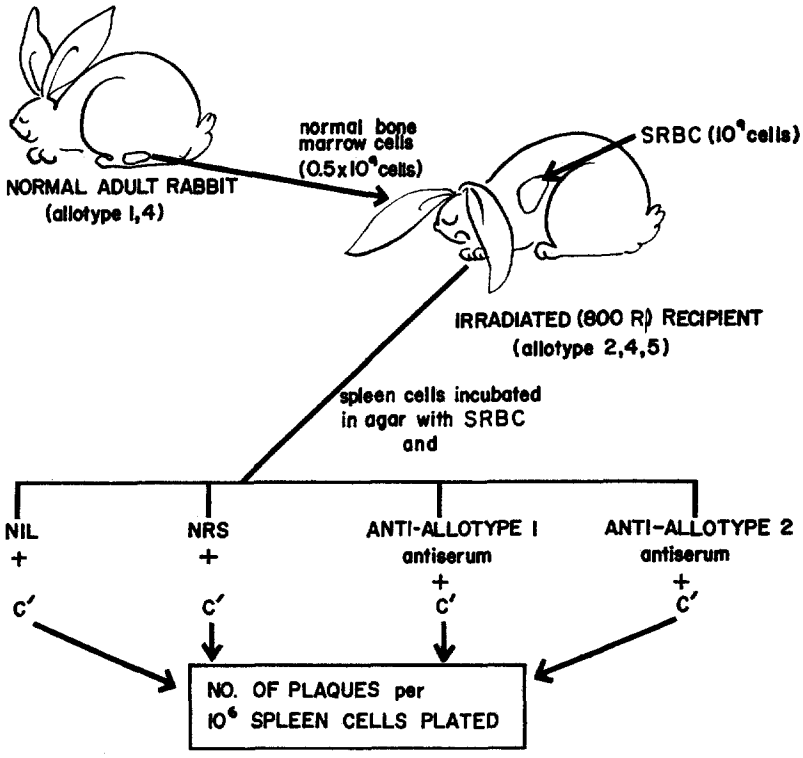

FIG. 1. Protocol for bone marrow cell transfer experiments using irradiated recipients and donor-recipient pairs of different allotypic genotype.

(3, 5). The experimental design was as follows (Fig. 1). Bone marrow cells from normal rabbits with the allotype A_1, A_4 (or A_2, A_4, A_5) were injected intravenously into normal rabbits with the allotype A_2, A_4 (or A_1, A_4) which had just been subjected to 800 R total body irradiation. The recipient rabbits were then also injected with 10^9 SRBC intravenously.

Seven days following cell transfer, the recipient rabbits were sacrificed by intravenous Nembutal, 50 mg. per kg body weight, and the spleen cells were analyzed for their capacity to produce direct plaques by the technique of Jerne et al (6, 7), with slight modifications (5). The spleen cells were incubated with anti-allotype antiserum (anti-A_1 or anti-A_2) or normal rabbit serum (NRS) and SRBC in the agar phase prior to the addition of complement. The antisera and NRS were all diluted in a 1% solution of human serum albumin (Hyland Laboratories, Los Angeles, Calif.). The technique is essentially that described by Chou et al (8). The results are expressed as the number of plaques per 10^6 spleen cells incubated.

Normal rabbit bone marrow cells were also either heat-killed by incubation in a 60°C water bath for 1 hr, or sonicated using an ultrasonic disintegrator (Fisher Ultrasonic Probe) at 16,000 cycles per second for 1 min. These preparations were injected into rabbits which had just been subjected to a dose of 800 R total body irradiation. The recipients were also given

TABLE I

The Plaque-Forming Capacity of Splenic Cells of Irradiated Rabbits Which Had Been Injected with Normal or Primed Allogeneic Bone Marrow Cells and Sheep Erythrocytes

Type of bone marrow cells transferred (0.5×10^8 mononuclear cells)	No. of plaques per 10^6 splenic lymphoid cells of irradiated recipients (day 7)*
Normal bone marrow	70 ± 10‡
Primed bone marrow	3 ± 5‡

* Recipients were subjected to 800 R total body irradiation prior to the intravenous administration of the bone marrow cells and SRBC (10^9 cells).

‡ The values represent the means and the ranges observed in approximately 50 experiments.

TABLE II

The Plaque-Forming Capacity of Splenic Cells of Irradiated ($A_{2,4,5}$) Rabbits Injected with Normal Allogeneic Bone Marrow Cells ($A_{1,4}$) and Incubated with Anti-Allotype Antiserum In Vitro

Cells incubated in vitro			No. of plaques per 10^6 splenic mononuclear cells of irradiated recipient (day 7)*
Allotype of bone marrow donor	Allotype of irradiated recipient	Anti-allotype antiserum incubated (0.2 ml)	
1, 4	2, 4, 5	Nil	52‡
1, 4	2, 4, 5	NRS	45
1, 4	2, 4, 5	Anti-1	62
1, 4	2, 4, 5	Anti-2	6
1, 4	2, 4, 5	Nil	79
1, 4	2, 4, 5	NRS	72
1, 4	2, 4, 5	Anti-1	71
1, 4	2, 4, 5	Anti-2	1

* Recipients were subjected to 800 R total body irradiation prior to the intravenous administration of the bone marrow and the SRBC (10^9 cells).

‡ Each value represents the mean of duplicate determinations; the values did not normally vary by more than ±10% from the mean.

10^9 SRBC via the intravenous route. 7 days later, the animals were sacrificed and the spleen cells analyzed for plaque-forming capacity as described above.

RESULTS

As can be seen in Table I, spleen cells of irradiated rabbits injected with SRBC and normal homologous bone marrow cells formed many plaques in

vitro, whereas spleen cells of irradiated recipients given SRBC and primed homologous bone marrow cells obtained from a donor rabbit injected with SRBC 24 hr prior to sacrifice were unable to produce plaques in vitro. The data have been presented previously (4, 5), but they are reproduced here in order to facilitate the interpretation of the subsequent data.

TABLE III

The Plaque-Forming Capacity of Splenic Cells of Irradiated Rabbits (A_1, A_4) Injected with Normal Allogeneic Bone Marrow Cells (A_1, A_2, A_4) and Incubated with Anti-Allotype Antiserum in Vitro

Cells incubated in vitro		Other reagents incubated				No. of plaques per 10^6 splenic mononuclear cells of irradiated recipient (day 7)*
Allotype of bone marrow donor	Allotype of irradiated recipient	Anti-allotype antiserum (0.2 ml)	Normal rabbit serum (NRS) 0.2 ml	Human serum albumin (HSA) 1 % 0.2 ml	Medium 199 (0.4 ml)	
A_1, A_2, A_4	A_1, A_4	Anti-1	—	HSA	—	2‡
A_1, A_2, A_4	A_1, A_4	Anti-2	—	HSA	—	79
A_1, A_2, A_4	A_1, A_4	—	NRS	HSA	—	64
A_1, A_2, A_4	A_1, A_4	—	—	—	Med 199	94

* Recipients were subjected to 800 R total body irradiation prior to the intravenous administration of the bone marrow and the SRBC (10^9 cells).

‡ Each value represents the mean of duplicate determinations; the values did not normally vary by more than ±10% from the mean.

TABLE IV

The Plaque-Forming Capacity of Splenic Cells of Irradiated Rabbits Injected with Sonicated or Heat Killed Normal Allogeneic Bone Marrow Cells

Cell preparation transferred to irradiated recipient (equivalent to 0.5×10^9 mononuclear cells)	No. of plaques per 10^6 splenic lymphoid cells of irradiated recipient (day 7)*
Whole, normal bone marrow	54‡
Sonicated, normal bone marrow	1
Heat-killed normal bone marrow	4

* Recipients were subjected to 800 R total body irradiation prior to the intravenous administration of the bone marrow and the SRBC (10^9 cells).

‡ Each value represents the mean of duplicate determinations; the values did not normally vary by more than ±10% from the mean.

As can be seen in Tables II and III, incubation of spleen cells with SRBC and anti-allotype antiserum directed to the recipient genotype completely inhibited subsequent plaque formation after the addition of complement. However, incubation of the spleen cells with anti-donor allotype antiserum or NRS had no inhibitory effect on the plaque-forming ability of the spleen cells. The entire experiment was repeated four times with essentially similar results.

As can be seen in Table IV, spleen cells of irradiated rabbits which had been injected with normal rabbit bone marrow cells gave many plaques, whereas spleen cells of irradiated rabbits which had been injected with either the heat-killed or sonicated preparations of the bone marrow did not give a greater than background number of plaques.

Insofar as the viability of these two latter cell preparations, prior to their administration into irradiated recipients, is concerned, no intact cell could be observed in the sonicate prepared from 0.5×10^9 cells. Using the dye exclusion test as an indicator of viability, less than 5% of the cells in the heat-killed preparation were viable.

DISCUSSION

Whether the antibody-forming capacity of an irradiated recipient animal which had been injected with homologous bone marrow or lymphoid cells is of donor and/or recipient origin is a question which has intrigued immunologists for several decades. However, the resolution of this problem has been difficult in view of the inability of the investigator to distinguish between the donor and recipient cells on a morphological or functional basis. The recent demonstrations by Oudin (9–12), Dray et al (13–16), Sell (17), Dubiski et al (18–20), and Chou et al. (21) that outbred rabbits can be distinguished from each other on the basis of antigenically dissimilar immunoglobulin molecules (22) suggested a specific immunologic approach to the problem. In fact, Sell and Gell (23, 24) have already verified the potential immunological nature of such a system by demonstrating the induction of blastogenesis and mitosis in lymphocyte cultures incubated with specific anti-allotype antiserum. Chou et al. (21) transferred various antigen-sensitized lymphoid cells to neonatal recipient rabbits and concluded that the antibody-forming cell in the neonatal host was of recipient origin, and not of donor origin. Antibodies in the circulation of the recipient were purified by the use of specific immunoabsorbents and were found to react only with anti-recipient allotype antiserum in vitro and not with anti-donor allotype antiserum. Nevertheless, immunoglobulins other than antibodies were found to be of donor cell origin. These investigators were unable to satisfactorily explain this dichotomy in their results. Mitchell and Miller (25, 26), using the irradiated mouse as the recipient animal and the hemolytic plaque technique as the means of assay of immunologic activity, arrived at the same conclusion with respect to the recipient origin of the antibody-forming cell. They observed that the plaques could be inhibited only by incubation of the spleen cells with anti-recipient lymphocyte antiserum and not with antiserum directed to donor lymphocytes. Results of a conflicting nature have been obtained by Harris et al. (27–29). They observed that the immune response to *Shigella* in the irradiated recipient mouse could be inhibited by prior immunization of the prospective recipient with donor white cells. They have also observed that in vitro plaque formation by spleen cells

of one strain of mice could be inhibited by incubation of these cells with antiserum directed to these cells produced in another strain of mice (30). They also transferred rabbit lymph node cells of one allotype, after in vitro incubation with *Shigella* antigen, into irradiated rabbits of a different allotype (31). The antibodies detected in the circulation of the recipients were demonstrated to be of donor, not recipient, origin. They therefore concluded that antibody formation is a property of the donor cells transferred to the irradiated recipient. Our results using the rabbits are consistent with those of Mitchell and Miller (25, 26) and Chou et al. (21) in that they unequivocally demonstrate the host origin of the antibody-forming cell. Plaque formation was inhibited when spleen cells of irradiated recipients injected with allogeneic normal bone marrow and sheep erythrocytes were incubated with antiserum directed to the recipient allotype prior to the addition of complement to the plates. No inhibition was obtained when the spleen cells were incubated with either anti-donor allotype antiserum or normal rabbit serum.

One possible explanation for the lack of correlation between our results, those of Mitchell and Miller (25, 26), and Chou et al. (21), on the one hand, and those of Harris et al. (27–31), on the other, may be related to the type of antigen used. The latter have used the *Shigella* as antigen and have assumed that the immune response induced with it is a primary one. However, in all likelihood, the donor as well as the recipient animals may have come into contact with *Shigella* antigens prior to the initiation of the experiment. Thus, the rabbit lymph node cells incubated with antigen and transferred to an irradiated recipient are, in fact, antibody-forming cells capable by themselves of initiating a secondary immune response in the irradiated recipient. On the other hand, the immune responses induced by sheep red cells and human serum proteins as antigens can be considered to be of a primary type.

It has been shown that primed bone marrow, which is defined as bone marrow obtained from rabbits 24 hr after administration of the antigen, is incapable of conferring antibody-forming capacity to irradiated recipients with respect to the antigen injected into the donor rabbit (4, 5) and the interpretation offered is that the antigen-reactive cell, normally a resident in the bone marrow, rapidly vacates the bone marrow after contact with the antigen in vivo. This hypothesis, that normal bone marrow contains the antigen-reactive cells, necessitates the additional assumption that the irradiated recipient still possesses the antibody-forming cells. It was this latter hypothesis that was tested in the current investigation.

It was demonstrated that spleen cells of irradiated recipient rabbits which were injected with normal rabbit bone marrow cells and antigen (SRBC) gave many plaques in vitro. However, if the spleen cells were incubated with antiserum directed to recipient allotype, plaque formation was inhibited whereas incubation of the spleen cells with antiserum directed to donor allotype

had no inhibitory effect on the number of plaques formed. Since identical results were achieved with both the normal and converse situations (donor A_1, A_4 into recipient A_2, A_4, A_5 and donor A_1, A_2, A_4 into recipient A_1, A_4), it may be concluded that the inhibitory effects of the anti-allotype antiserum are specific and that the antibody-forming cell in the irradiated animal is of recipient, and not donor, origin.

It is interesting to note that neither "nonspecific" inhibition nor "specific enhancement" of plaque formation by the anti-allotype antisera were observed in this investigation, although the antisera were used in varying dilutions. It has been reported that anti-allotype antiserum may, at certain concentrations, enhance the number of plaques when incubated with the cells to which it is directed (8, 52). However, incubation of the antibody-forming cells with the diluted anti-allotype antiserum generally results in an inhibition of plaque formation (8, 53, 54). It has also been reported that certain antisera possess nonspecific inhibitory activity in that they can inhibit plaque formation even if incubated with cells of a different allotype.[1] These considerations must be taken into account and adequate controls must be performed in order to ensure correct interpretation of data obtained in experiments using anti-allotype antisera as markers.

It was demonstrated that viable bone marrow cells are required in the transfer of antibody-forming capacity to irradiated recipients. Neither sonicates nor heat-killed preparation of normal rabbit bone marrow were capable of transferring antibody-forming activity. These findings, therefore, rule out any "adjuvant" effect by transferred cells, be they viable or not, and also rule out the possibility that cell extracts or cell-free preparations could transfer antibody-forming capacity or "information" to be subsequently taken up by host cells in a fashion similar to the totally in vitro system described by Fishman (36) and Adler et al. (37).

Since the antibody-forming cell was demonstrated to be of recipient origin, one must necessarily assume that it is radioresistant to 800 R and that it is the antigen-reactive cell which is radiosensitive. This interpretation is supported by the following findings: (*a*) the irradiated rabbits given 800 R irradiation, and injected with SRBC only, fail to exhibit an immune response (5); (*b*) the number of plaque-forming cells in the spleen of an irradiated recipient of normal allogeneic bone marrow cells is similar to the number observed in the spleen of a normal immunized rabbit (5); and (*c*) the bone marrow cells of irradiated rabbits lose their capacity to react with antigens in vitro and are incapable of transferring antibody-forming capacity to irradiated recipient allogeneic rabbits.[2] Experiments performed by Harris et al. (32) more than a decade ago also support our concept of the cellular events occurring during

[1] Sell, S. Unpublished observations.

[2] Richter, M. Unpublished observations.

the primary immune response. They observed that the transfer of cells of the popliteal lymph node of a rabbit injected 3 days previously with *Shigella* antigen into normal or X-irradiated recipients resulted in the formation of antibodies. However, if the recipients were irradiated within 1 hr after receiving the primed lymph node cells, the immune response was markedly suppressed in comparison with that in a nonirradiated control. These results suggest that the immunologically important cell transferred is an irradiation-sensitive antigen-reactive cell, which had migrated out of the bone marrow to the peripheral lymphoid tissues. A scheme depicting the interrelationship of these two

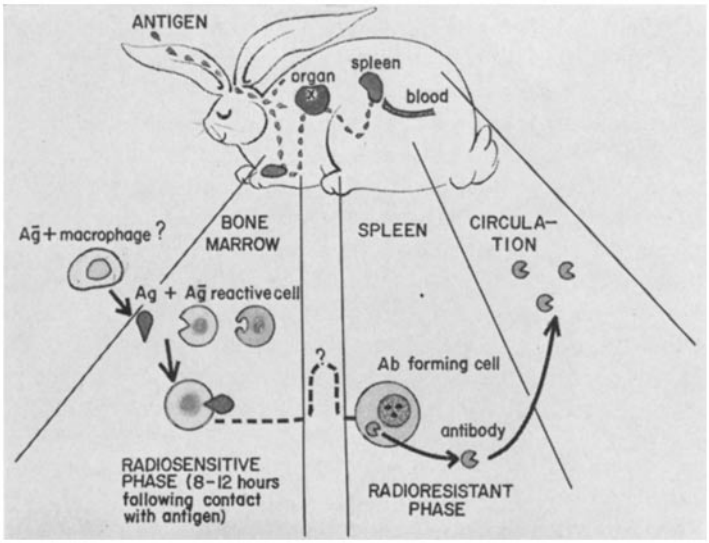

FIG. 2. The possible interrelationship of the cells mediating the primary humoral immune response (a diagramatic representation).

cell types in the induction of the primary immune response in the rabbit is presented in Fig. 2.

A question which requires consideration is whether one can define the induction of the primary response as a result of a two-cell interaction, the antigen-reactive and antibody-forming cells (25, 26, 33–35), or whether it is necessary to postulate a third cell-type, the macrophage. The evidence favoring an eminent role for the macrophage in the induction of the immune response has been well documented and has accrued from both in vitro and in vivo investigations (36–42). However, the exact stage in the immune response where the macrophage acts has hitherto not been precisely stated or defined. Evidence of a three-cell interaction has been presented by Mosier and Coppleson (43), Pribnow and Silverman (44) and Gallily and Feldman (45). Pribnow and

Silverman (44) have demonstrated that the interaction of the antigen with macrophage is the initial cellular event in the induction of the primary immune response. They observed that irradiated rabbits that received nonimmune lymph node cells and peritoneal macrophages which had been incubated with the antigen in vitro were unable to form antibody whereas normal recipients of these cells exhibited a typical primary immune response. The transfer of antigen-incubated lymph node cells, in the absence of macrophages, failed to elicit an immune response in either normal or irradiated rabbits. Furthermore, macrophages which had been irradiated after their incubation with the antigen were unable to initiate an immune response when transferred to normal recipients. The findings of Gallily and Feldman (45) also strongly imply a three-cell system. They observed that normal macrophages of mice incubated with *Shigella* antigen and transferred to mice previously exposed to 550 R were able to initiate an immune response in the recipient, whereas there was no immune response with antigen alone. However, the immune response was suppressed if the recipient mice had been subjected to 900 R irradiation. Furthermore, macrophages from irradiated donors, incubated with *Shigella,* were incapable of inducing antibody formation in irradiated recipients.

On the basis of our results, these data imply that the antigen-reactive cell in the recipient, whose functions in the immune response commence after the macrophage-antigen interaction, is irradiation-sensitive to 900 R but not to 550 R in both the mouse and the rabbit. However, it would not appear to be as irradiation-sensitive as the macrophage which is inactivated by 550 R irradiation. Therefore, the sequential transfer of immunologic information leading to the initiation of the immune response—macrophage to antigen-reactive cell to antibody-forming cell—is broken after irradiation.

In summary, we have demonstrated that the antibody-forming cells in irradiated rabbits which had received allogeneic bone marrow cells and antigen are of recipient origin and that they are relatively irradiation-resistant. These results, as well as those of other investigators, also suggest that it is the antigen-reactive cell which is irradiation-sensitive and that a third cell, the irradiation-sensitive macrophage, must be incorporated into the scheme of cell interactions leading up to antibody formation in the primary immune response (Fig. 2). Although it has been demonstrated that the bone marrow in the rabbit serves as a prime source of antigen-reactive cells (4, 5) and macrophages (46–48), and that in the rodent the cell mediating humoral and cellular immunity appears to originate in the bone marrow (25, 49–51), the organ(s) of origin of the antibody-forming cell(s) in the rabbit still remains to be determined.

SUMMARY

Bone marrow cells obtained from rabbits of one allotype were injected into irradiated rabbits of a different allotype. The recipients were also injected

with sheep red blood cells, and their spleen cells were tested for plaque-forming capacity 7 days later. Spleen cells of all recipients gave large numbers of plaques as did spleen cells incubated with antiserum directed toward donor allotype. However, incubation of the recipient spleen cells with antiserum directed toward recipient allotype completely suppressed plaque formation. These results demonstrate that antibody-formation in irradiated recipients of transferred lymphoid cells is a property of the recipient animal and that the antibody-forming cell is relatively irradiation-resistant.

It was also demonstrated that only viable normal bone marrow cells are capable of transferring antibody-forming capacity to irradiated recipient rabbits. Neither sonicates nor heat-killed preparations of normal rabbit bone marrow cells possessed this capacity.

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BIBLIOGRAPHY

1. Singhal, S. K., and M. Richter. 1968. The response of normal rabbit bone marrow cells to antigens in vitro. *Int. Arch. Allergy Appl. Immunol.* **33:**493.
2. Singhal, S. K., M. Richter, and D. G. Osmond. 1968. Cells involved in the immune response. III. Responsiveness to antigens of a lymphocyte-rich fraction of normal rabbit bone marrow. *Int. Arch. Allergy Appl. Immunol.* **34:**224.
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. Abdou, N. I., and M. Richter. 1969. Cells involved in the immune response. VI. The immune response to red blood cells in irradiated rabbits after administration of normal, primed, or immune allogeneic rabbit bone marrow cells. *J. Exp. Med.* **129:**757.
6. Jerne, N. K., A. A. Nordin, and C. Henry. 1963. The agar plaque technique for recognizing antibody-producing cells. *In* Cell-Bound Antibodies. Wistar Institute Press, Philadelphia. 109.
7. Jerne, N. K., and A. A. Nordin. 1963. Plaque formation in agar by single antibody-producing cells. *Science.* **140:**405.
8. Chou, C. T., B. Cinader, and S. Dubiski. 1967. Allotypic specificity and hemolytic capacity of antibodies produced by single cells. *Int. Arch. Allergy Appl. Immunol.* **32:**583.
9. Oudin, J. 1956. Réaction de précipitation spécifique entre des sérumes d'animaux de même aspéce. *C. R. Hebd. Séances Acad. Sci. Paris.* **242:**2489.
10. Oudin, J. 1956. L'allotypy de certains antigèns protéidiques du sérum. *C. R. Hebd. Séances Acad. Sci. Paris.* **242:**2606.

11. Oudin, J. 1960. Allotypy of rabbit serum proteins. I. Immunochemical analysis leading to the individualization of seven main allotypes. *J. Exp. Med.*, **112**:107.
12. Oudin, J. 1960. Allotypy of rabbit serum proteins. II. Relationships between various allotypes: their common antigenic specificity, their distribution in a sample population; genetic implications. *J. Exp. Med.* **112**:125.
13. Dray, S., S. Dubiski, A. Kelus, E. S. Lennox, and J. Oudin. 1962. A notation for allotypy. *Nature (London)*. **195**:785.
14. Dray, S., G. O. Young, and L. Gerald. 1963. Immunochemical identification and genetics of rabbit gamma globulin allotypes. *J. Immunol.* **91**:403.
15. Dray, S., and G. O. Young. 1958. Differences in the antigenic components of sera of individual rabbits as shown by induced isoprecipitins. *J. Immunol.* **81**:142.
16. Dray, S., and G. O. Young. 1959. Two antigenically different gamma globulins in domestic rabbits revealed by isoprecipitation. *Science.* **129**:1023.
17. Sell, S. 1966. Ig allotypes of the rabbit: Identification of a second specificity. *Science.* **153**:641.
18. Dubiski, S., Z. Dudziak, D. Skalba, and A. Dubiska. 1959. Serum groups in rabbits. *Immunology.* **2**:84.
19. Dubiski, S., J. Rapasz, and A. Dubiska. 1962. Heredity of rabbit gamma globulin isoantigens. *Acta Genet. Statist. Med.* **12**:136.
20. Dubiski, S., D. Skalba, A. Dubiska, and A. Kalus. 1959. Iso-antigens of rabbit gamma globulin. *Nature (London)*. **184**:1811.
21. Chou, C. T., S. Dubiski, and B. Cinader. 1967. Cells as antigen carriers and as immunoglobulin producers. Synthesis of antibody and allogeneic immunoglobulin after transfer of antigen-treated cells to newborn rabbits. *J. Exp. Med.* **126**:305.
22. Kelus, A. S., and P. G. H. Gell. 1967. Immunoglobulin allotypes of experimental animals. *Progr. Allergy.* **11**:141.
23. Sell, S., and P. G. H. Gell. 1965. Studies on rabbit lymphocytes in vitro. I. Stimulation of blast transformation with an antiallotype serum *J. Exp. Med.* **122**: 423.
24. Sell, S., and P. G. H. Gell. 1965. Studies on rabbit lymphocytes in vitro. IV. Blast transformation of the lymphocytes from newborn rabbits induced by antiallotype serum to a parental IgG allotype not present in the serum of the lymphocyte donor. *J. Exp. Med.* **122**:923.
25. Mitchell, J., and J. F. A. P. Miller. 1968. Immunological activity of thymus and thoracic-duct lymphocytes. *Proc. Nat. Acad. Sci. U.S.A.* **59**:296.
26. Miller, J. F. A. P., and G. F. Mitchell. 1968. Cell to cell interaction in the immune response. I. Hemolysin-forming cells in neonatally thymectomized mice reconstituted with thymus or thoracic duct lymphocytes. *J. Exp. Med.* **128**:801.
27. Harris, T. N., S. Harris, and M. Farber. 1957. Transfer of lymph node cells to recipient rabbits pre-injected with blood leucocytes of donors. *Proc. Soc. Exp. Biol. Med.* **95**:26.
28. Harris, T. N., S. Harris, and M. B. Farber. 1958. Studies on the transfer of lymph node cells. XI. Effect on the anti-Shigella agglutinin titers of recipient rabbits of the prior injection of leucocytes from the donor animals. *J. Exp. Med.* **108**:21.
29. Harris, T. N., S. Harris, and M. B. Farber. 1967. Suppression of transferred

allogeneic spleen cells in the mouse: Active induction and passive transfer by antiserum. *Immunology.* **99**:438.

30. Harris, S., T. N. Harris, and C. A. Ogburn. 1967. In vitro demonstration of suppressive antibody in mouse antisera vs. allogeneic spleen cells. *J. Immunol.* **99**:447.
31. Harris, T. N., S. Dray, B. Ellsworth, and S. Harris. 1963. Rabbit gamma-globulin allotypes as genetic markers for the source of antibody produced in recipients of *Shigella*-incubated lymph node cells. *Immunology.* **6**:169.
32. Harris, T. N., S. Harris, H. D. Beale, and J. J. Smith. 1954. Studies on the transfer of lymph node cells. IV. Effects of X-irradiation of recipient rabbits on the appearance of antibody after cell transfer. *J. Exp. Med.* **100**:289.
33. Mitchell, G. F., and J. F. A. P. Miller. 1968. Cell to cell interaction in the immune response. II. The source of hemolysin-forming cells in irradiated mice given bone marrow and thymus or thoracic duct lymphocytes. *J. Exp. Med.* **128**:821.
34. Claman, H. N., E. A. Chaperon, and R. F. Triplett. 1966. Immunocompetence of transferred thymus-marrow cell combinations. *J. Immunol.* **97**:828.
35. Davies, A. J. S., E. Leuchars, V. Wallis, R. Marchant, and E. V. Elliot. 1967. The failure of thymus-derived cells to produce antibody. *Transplantation.* **5**:222.
36. Fishman, M. 1961. Antibody formation in vitro. *J. Exp. Med.* **114**:837.
37. Adler, F. L., M. Fishman, and S. Dray. 1966. Antibody formation initiated in vitro. III. Antibody formation and allotypic specificity directed by ribonucleic acid from peritoneal cells. *J. Immunol.* **97**:554.
38. Gottlieb, A. A., V. R. Glisin, and P. Doty. 1968. Studies on macrophage RNA involved in antibody production. *Proc. Nat. Acad. Sci. U.S.A.* **57**:1849.
39. Sell, S., and R. Asofsky. 1968. Lymphocytes and immunoglobulins. *Progr. Allergy.* **12**:86.
40. Mackaness, G. B., and R. V. Blanden. 1967. Cellular immunity. *Progr. Allergy.* **11**:89.
41. Argyris, B. F. 1967. Role of macrophages in antibody production. Immune response to sheep red blood cells. *J. Immunol.* **99**:744.
42. Pearsall, N. N., and R. S. Weiser. 1968. The macrophage in allograft immunity. II. Passive transfer with immune macrophages. *RES J. Reticuloendothel. Soc.* **5**:121.
43. Mosier, D. E., and L. W. Coppleson. 1968. A three-cell interaction required for the induction of the primary immune response in vitro. *Proc. Nat. Acad. Sci. U.S.A.* **61**:542.
44. Pribnow, J. F., and M. S. Silverman. 1967. Studies on the radio-sensitive phase of the primary antibody response in rabbits. I. The role of the macrophage. *J. Immunol.* **98**:225.
45. Gallily, R., and M. Feldman. 1967. The role of macrophages in the induction of antibody in X-irradiated animals. *Immunology.* **12**:197.
46. Volkman, A. 1966. The origin and turnover of mononuclear cells in peritoneal exudates in rats. *J. Exp. Med.* **124**:241.
47. Furth, R. V., and Z. A. Cohn. 1968. The origin and kinetics of mononuclear phagocytes. *J. Exp. Med.* **128**:415.

48. Virolainen, M. 1968. Hematopoietic origin of macrophages as studied by chromosome markers in mice. *J. Exp. Med.* **127**:943.
49. McGregor, D. D. 1968. Bone marrow origin of immunologically competent lymphocytes in the rat. *J. Exp. Med.* **127**:953.
50. Lubaroff, D. M., and B. H. Waksman. 1968. Bone marrow as source of cells in reactions of cellular hypersensitivity. I. Passive transfer of tuberculin sensitivity in syngeneic systems. *J. Exp. Med.* **128**:1425.
51. Lubaroff, D. M., and B. H. Waksman. 1968. Bone marrow as source of cells in reactions of cellular hypersensitivity. II. Identification of allogeneic or hybrid cells by immuno fluorescence in passively transferred tuberculin reactions. *J. Exp. Med.* **128**:1437.
52. Weiler, E., E. W. Melletz, and E. Breuninger-Peck. 1965. Facilitation of immune hemolysis by an interaction between red cell-sensitizing antibody and gamma globulin allotype antibody. *Proc. Nat. Acad. Sci. U.S.A.* **54**:1310.
53. Robinson, W. A., J. Marbrook, and E. Diener. 1967. Primary stimulation and measurement of antibody production to sheep red blood cells in vitro. *J. Exp. Med.* **126**:347.
54. Walsh, P., P. Maurer, and M. Egan. 1967. Detection of immune response against synthetic polymers of amino acids employing the plaque-forming cell system. I. Reaction with sheep erythrocytes coated with polymer. *J. Immunol.* **98**:344.