CELLS INVOLVED IN THE IMMUNE RESPONSE

XI. Identification of the Antigen-Reactive Cell as the Tolerant Cell in the Immunologically Tolerant Rabbit*

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(Received for publication 6 March 1969)

Previous investigations have disclosed that normal allogeneic bone marrow cells can transfer antibody-forming capacity to irradiated rabbits (1–5). On the other hand, "primed" bone marrow (bone marrow obtained from a donor rabbit 24 hr following intravenous administration of the specific antigen) could not transfer antibody-forming capacity to the antigen with which the marrow donor had been immunized, but could transfer immunocompetence to other antigens (3, 4). It was also demonstrated that the antibody-forming cell in the marrow recipient is of host, not donor, origin (5), thus supporting the conclusion that the immunocompetent cell in the bone marrow is the antigen-reactive cell and that the bone marrow does not contain any antibody-forming cells (4–7). Furthermore, it was demonstrated that antigen-reactive cells, isolated by passage of the normal bone marrow cell suspension through an antigen-sensitized glass bead column, followed by elution of retained cells from the column (eluate), could transfer antibody-forming capacity to an irradiated rabbit, but only toward the antigen used to sensitize the glass beads (2). In contrast, the cells which passed through the antigen-sensitized glass bead column (effluent) lost the capacity to transfer immunocompetence with respect to the antigen used to sensitize the beads, but not with respect to other non-cross-reacting antigens (2).

These findings suggested a means of indentifying which of the cell types concerned with the mediation of the humoral immune response in the rabbit—the antigen-reactive cell or the antibody-forming cell—is the tolerant cell in the immunologically tolerant rabbit. As shown below, it would appear that the antigen-reactive cell is the one which is unresponsive in the latter animal.

Materials and Methods

New Zealand white rabbits were used throughout this study. The antigens used were human serum albumin (HSA) (Hyland Laboratories, Los Angeles, Calif.) and bovine gamma

* This investigation was supported by a grant from the Medical Research Council, Canada.

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globulin (BGG) (Pentex, Inc., Kankakee, Ill.). Solutions of these antigens, in various concentrations, were prepared in Medium 199 (Med-199) (Microbiological Associates, Bethesda, Md.) and sterilized by Seitz filtration.

Normal bone marrow refers to that obtained from an adult, unimmunized rabbit. Primed bone marrow refers to marrow obtained from a rabbit immunized intravenously 24 hr prior to sacrifice. Normal and primed rabbit bone marrow were obtained as previously described (2, 4). The femur and tibia were split bilaterally with a bone cutter, and the bone marrow was transferred to sterile plastic tubes (Falcon Plastics, Los Angeles, Calif.), each containing approximately 5 ml normal rabbit serum (NRS) (Microbiological Associates, Bethesda, Md.).

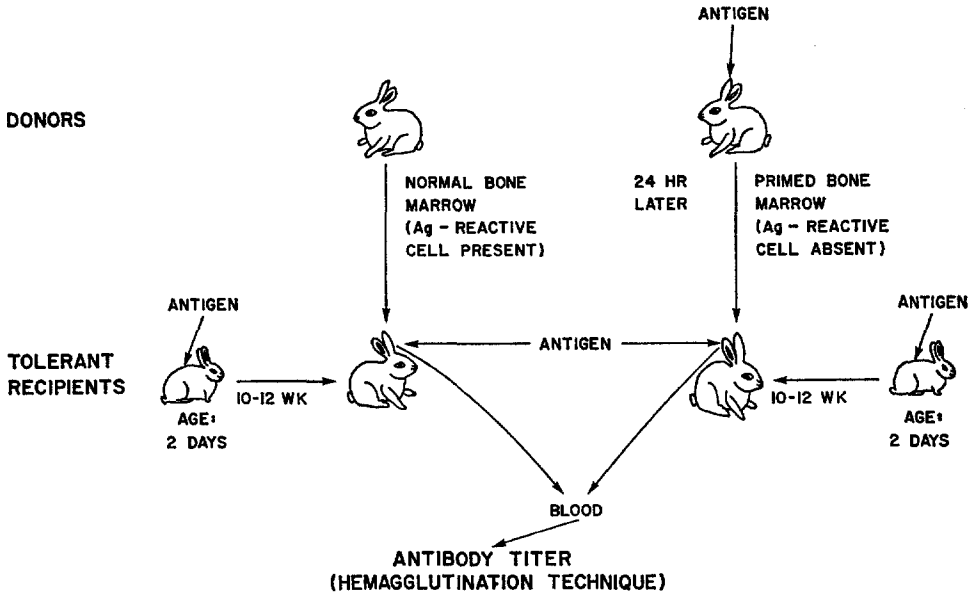

FIG. 1. Protocol of procedures followed for the demonstration of the antigen-reactive cell as the tolerant cell in the immunologically tolerant rabbit.

The tubes were vigorously shaken for several minutes and centrifuged at 600 rpm for 10 min. The fatty supernatants were decanted, and the cells were suspended in Med-199 and centrifuged once more at 600 rpm for 10 min. The cells were then resuspended in Med-199 containing 15% NRS to a concentration of 10^6 cells/ml.

The protocol followed is presented in Fig. 1. Rabbits were made tolerant to HSA or BGG by subcutaneous injection at age 2 and 5 days with a total of 200 mg of the antigen. At 10 weeks of age, each of several rabbits of each litter (2–4 rabbits) was injected with 10 mg HSA or BGG intravenously. The humoral immune response was determined during the following 4 wk, using the passive hemagglutination technique (8). The remaining rabbits of each litter (3–5 rabbits) were injected at 10 wk of age with either normal or primed allogeneic bone marrow. They were also injected with 25 mg HSA or BGG intravenously, and the humoral immune response was followed by the passive hemagglutination technique (8). In other experiments, prospective normal recipient rabbits were subjected to 800 R total body irradiation, using a ^{60}Co source, prior to being given the bone marrow cells.

RESULTS

A. Immune Response in Normal, Irradiated, and Immunologically Tolerant Rabbits to HSA and BGG, and Specificity of the Antisera.—This initial series of experiments was carried out in order to establish the nonresponsiveness of the irradiated and tolerant rabbits and the non–cross-reactivity of the two antigens selected for this investigation. Normal rabbits responded briskly to immunization with either HSA or BGG, whereas neither irradiated nor immunologically tolerant rabbits responded over a period of 40 days (Table I). Almost no cross-

TABLE I

Immune Response in Normal, Irradiated, and Tolerant Rabbits after Administration of Human Serum Albumin or Bovine Gamma Globulin

Day of bleeding after intravenous administration of HSA or BGG (25 mg)	Hemagglutination titer* of serum samples obtained after immunization of					
	Normal rabbits		Irradiated rabbits‡		Tolerant rabbits§	
	Anti-HSA	Anti-BGG	Anti-HSA	Anti-BGG	Anti-HSA	Anti-BGG
0	0	0	0	0	0	0
7	20	0	0	0	0	0
14	2,560	5,120	0	0	0	0
21	1,280	10,240	10	10	0	0
28	640	2,560	20	0	0	0
40	160	1,280	0	0	0	0

* The sera in varying dilutions were incubated with HSA-sensitized or BGG-sensitized sheep red blood cells. The titer represents the maximum dilution of the antiserum capable of effecting agglutination of the antigen-sensitized red cells. Titers less than 10 are considered negative.

‡ The rabbits were subjected to 800 R total body irradiation prior to the intravenous administration of HSA or BGG.

§ The rabbits were given 100 mg of HSA or BGG on days 2 and 5 of life. They were immunized with HSA or BGG during the 6th wk of life.

reactivity was detected between these two antigen-antibody systems by the passive hemagglutination technique (Table II), the extent of cross-reactivity being less than 0.1%.

B. Failure of Primed Bone Marrow to Transfer Antibody-Forming Capacity to Tolerant Recipients with Respect to the Priming Antigen.—Rabbits made immunologically tolerant to HSA and given HSA-primed allogeneic bone marrow were incapable of giving an immune response following immunization with HSA, but produced high-titered antisera if given normal allogeneic bone marrow cells and HSA (Table III). The converse situation was true of rabbits rendered immunologically tolerant with respect to BGG and given BGG-primed allogeneic bone marrow. Immunization of these rabbits with BGG failed to elicit an immune response, whereas recipients of normal allogeneic bone marrow cells gave good immune responses (Table IV).

TABLE II

Cross-Reactivity between Human Serum Albumin and Bovine Gamma Globulin

Antiserum tested*	Hemagglutination titers‡ of antisera after incubation with sheep red cells sensitized with	
	HSA	BGG
Rabbit anti-HSA	16,000	40
	1,280	0
Rabbit anti-BGG	20	25,600
	0	8,000

* Sera obtained from rabbits immunized with 25 mg of either HSA or BGG.

‡ Hemagglutination titers less than 10 are considered negative.

TABLE III

Immune Response of HSA-Tolerant Rabbits Given HSA-Primed or Normal Allogeneic Bone Marrow and Immunized with HSA

Day of bleeding after bone marrow transfer and intravenous administration of HSA	Antibody and antigen levels in sera of HSA-tolerant rabbits* given bone marrow from			
	Normal donors		HSA-primed donors‡	
	Anti-HSA titer§	Free HSA‖	Anti-HSA titers	Free HSA
		μg/ml		*μg/ml*
−3	0	0	0	0
5	10	ND¶	0	0.2
8	320	ND	0	0.02
14	1280	ND	0	0.01
21	1280	ND	0	0.002
28	320	ND	0	0.0003
35	80	ND	0	0
38**				
42	1280	ND	0	0.01
49	2560 (2560)‡‡	ND	40 (0)	ND
56	640	ND	20	ND

* Rabbits were given 100 mg HSA on days 2 and 5 of life. They were given either normal or primed allogeneic bone marrow at 6 wk of age.

‡ Adult rabbits received 25 mg HSA intravenously 24 hr before sacrifice.

§ The antisera were incubated with HSA-sensitized sheep red cells. Titers below 10 are considered negative.

‖ Determined by the ability of the serum to inhibit the agglutination of HSA-sensitized sheep red cells by specific antiserum.

¶ Not done.

** All rabbits received 10 mg HSA intravenously.

‡‡ Titers in parentheses after treatment of serum with 0.1 M 2-mercaptoethanol.

The failure of the tolerant recipients of primed bone marrow to mount an immune response is reflected by the presence of free antigen in the circulation, which could be detected for 3–4 wk following primary immunization (Tables III and IV).

It is interesting that the antibodies formed following secondary immunization of tolerant recipients of normal bone marrow, 38 days subsequent to primary immunization, were resistant to mercaptoethanol. On the other hand, reimmunization at day 38 of tolerant recipients of primed bone marrow, which did not produce antibodies following initial immunization, synthesized humoral antibodies which were mercaptoethanol-sensitive (Tables III and IV).

TABLE IV

Immune Response of BGG-Tolerant Rabbits Given BGG-Primed or Normal Allogeneic Bone Marrow and Immunized with BGG

Day of bleeding after bone marrow transfer and intravenous administration of BGG	Antibody and antigen levels in sera of BGG-tolerant rabbits* given bone marrow from			
	Normal donors		BGG-primed donors‡	
	Anti-BGG titer§	Free BGG‖	Anti-BGG titer	Free BGG
		μg/ml		μg/ml
−3	0	0	0	0
5	0	0.002	0	0.1
8	80	ND¶	0	0.06
14	4000	ND	0	0.007
21	1280	ND	0	0.0001
28	640	ND	0	0
35	160	ND	0	0
38**				
42	8000		10	ND
49	8000 (4000)‡‡	ND	640 (20)	ND
56	2000	ND	320	ND

* Rabbits were given 100 mg BGG on days 2 and 5 of life. They were given either normal or primed allogeneic bone marrow at 6 wk of age.

‡ Adult rabbits received 25 mg BGG intravenously 24 hr before sacrifice.

§ Titers below 10 are considered negative.

‖ Determined by the ability of the serum to inhibit the agglutination of BGG-sensitized sheep red cells by specific antiserum.

¶ Not done.

** All rabbits received 10 mg BGG intravenously.

‡‡ Titers in parentheses after treatment of sera with 0.1 M 2-mercaptoethanol.

When the tolerant recipients of primed bone marrow were tested for immunological responsiveness toward the specifie and a non–cross-reacting antigen, no immune response could be obtained with respect to the antigen used to prime the bone marrow donor, although a response to the non–cross-reacting antigen could be regularly obtained. HSA- or BGG-tolerant rabbits given normal allogeneic bone marrow responded well to immunization with HSA or BGG (Tables V and VI). However, the HSA-tolerant recipient of HSA-primed bone marrow failed to respond to stimulation with HSA but responded well to BGG (Table V), and the BGG-tolerant recipient of BGG-primed bone marrow cells failed to respond to stimulation with BGG but responded well to HSA (Table VI).

TABLE V

Immune Response of HSA-Tolerant Rabbits Given HSA-Primed or Normal Allogeneic Bone Marrow and Immunized with HSA and BGG: Specificity of the Immune Response

Day of bleeding after bone marrow transfer and intravenous administration of HSA and BGG	Hemagglutination titers of sera of HSA-tolerant rabbits * given bone marrow from			
	Normal donors		HSA-primed donors‡	
	Anti-HSA	Anti-BGG	Anti-HSA	Anti-BGG
−3	0§	0	0	0
8	160	40	0	10
12	640	2560	0	640
21	1280	1280	0	640
36	40	320	0	160
38‖				
42	2560 (1280)¶	1600 (1600)	0	1280 (1280)
50	1280	4000	40 (10)	320

* Rabbits were given 100 mg HSA on days 2 and 5 of life. They were given either norma or primed allogeneic bone marrow at 6 wk of age.

‡ Adult rabbits received 25 mg HSA intravenously 24 hr before sacrifice.

§ Titers less than 10 are considered negative.

‖ All rabbits received 10 mg HSA and 10 mg BGG intravenously.

¶ Titers in parentheses after treatment of sera with 0.1 M 2-mercaptoethanol.

TABLE VI

Immune Response of BGG-Tolerant Rabbits Given BGG-Primed or Normal Allogeneic Bone Marrow and Immunized with BGG and HSA: Specificity of the Immune Response

Day of bleeding after bone marrow transfer and intravenous administration of HSA and BGG	Hemagglutination titers of sera of BGG-tolerant rabbits* given bone marrow from			
	Normal donors		BGG-primed donors‡	
	Anti-HSA	Anti-BGG	Anti-HSA	Anti-BGG
−3	0§	0	0	0
8	80	40	20	0
12	1280	2000	640	0
21	640	2000	640	0
36	160	512	160	0
38‖				
42	8000 (8000)¶	4000 (2000)	2560 (2560)	0
50	2000	1280	1280	80 (10)

* Rabbits were given 100 mg BGG on days 2 and 5 of life. Bone marrow transfer was done in the 6th wk.

‡ Adult rabbits received 25 mg BGG intravenously 24 hr before sacrifice.

§ Titers less than 10 are considered negative.

‖ All rabbits received 10 mg HSA and 10 mg BGG intravenously.

¶ Titers in parentheses after treatment of sera with 0.1 M 2-mercaptoethanol.

It should be pointed out that only the antibodies formed in tolerant recipients given specifically primed bone marrow and antigen and reimmunized 38 days following primary immunization were mercaptoethanol-sensitive (Tables V and VI). The antibodies formed after secondary immunization in tolerant recipients of normal allogeneic bone marrow or in recipients of primed bone marrow immunized with the non–cross-reacting antigen were all mercaptoethanol-resistant (Tables V and VI).

TABLE VII

Immune Response of HSA-Tolerant Rabbits Given BGG-Primed or Normal Allogeneic Bone Marrow and Immunized with HSA and BGG

Day of bleeding after bone marrow transfer and intravenous administration of HSA and BGG	Hemagglutination titers of sera of HSA-tolerant rabbits* given bone marrow from			
	Normal donors		BGG-primed donors‡	
	Anti-HSA	Anti-BGG	Anti-HSA	Anti-BGG
−3	0§	0	0	0
8	40	320	10	10
12	1280	640	80	40
21	320	80	40	40
36	40	40	40	10
38‖				
42	1280	160	80	160
50	320	80	20	40

* Rabbits were given 100 mg HSA on days 2 and 5 of life. They were given normal or BGG-primed allogeneic bone marrow at 6 wk of age.

‡ Adult rabbits received 25 mg BGG intravenously 24 hr before sacrifice.

§ Titers less than 10 are considered negative.

‖ All rabbits were given 10 mg HSA and 10 mg BGG intravenously.

Rabbits made tolerant to HSA and given either normal or BGG-primed bone marrow cells responded with antibody formation following immunization with either HSA or BGG (Table VII). Similarly, good immune responses to both antigens were elicited in BGG-tolerant rabbits given either HSA-primed or normal bone marrow cells (Table VIII). In both cases, brisk secondary immune responses were obtained after reimmunization of the rabbits 38 days following primary immunization (Tables VII and VIII).

C. Immune Response to HSA of Irradiated Rabbits Given Bone Marrow Cells from Either HSA-Primed or HSA-Tolerant Rabbits.—Irradiated rabbits given HSA-primed or HSA-tolerant bone marrow failed to respond upon immunization with HSA, whereas irradiated recipients of normal allogeneic bone marrow cells responded well (Table IX).

TABLE VIII

Immune Response of BGG-Tolerant Rabbits Given HSA-Primed or Normal Allogeneic Bone Marrow and Immunized with BGG and HSA

Day of bleeding after bone marrow transfer and intravenous administration of HSA and BGG	Hemagglutination titers of sera of BGG-tolerant rabbits* given bone marrow from			
	Normal donors		HSA-primed donors‡	
	Anit-HSA	Anti-BGG	Anti-HSA	Anti-BGG
−3	0§	0	0	0
8	40	80	80	40
12	80	320	160	320
21	40	80	20	40
36	10	80	0	0
38‖				
42	2000	640	640	1280
50	1280	80	2560	1280

*Rabbits were given 100 mg BGG on days 2 and 5 of life. They were given normal or HSA-primed allogeneic bone marrow at 6 wk of age.

‡ Adult rabbits received 25 mg HSA intravenously 24 hr before sacrifice.

§ Titers less than 10 are considered negative.

‖ All rabbits were given 10 mg HSA and 10 mg BGG intravenously.

TABLE IX

Immune Response of Irradiated Rabbits Given Either Normal, HSA-Primed, or HSA-Tolerant Allogeneic Bone Marrow and Immunized with HSA

Day of bleeding after bone marrow transfer and intravenous administration of HSA	Hemagglutination titers* of irradiated recipients‡ given bone marrow from		
	Normal donors	HSA-primed donors§	HSA-tolerant donors‖
7	20	0	0
14	1280	0	0
21	800	0	0
28	320	0	20
42	10	0	0

* The antisera were incubated with HSA-sensitized sheep red cells. Titers less than 10 are considered negative.

‡ Recipients were subjected to 800 R total body irradiation followed by the intravenous administration of 5×10^8 bone marrow cells and 25 mg HSA.

§ Donors were given 25 mg HSA intravenously 24 hr before sacrifice.

‖ Bone marrow obtained from 6 wk old rabbits which had been injected with 100 mg HSA on days 2 and 5 of life.

DISCUSSION

The present data strongly indicate that the cell which is unresponsive in the immunologically tolerant rabbit is the antigen-reactive cell and not the antibody-forming cell. This conclusion is based on the finding that, in the tolerant

rabbit, antibody formation toward the tolerogenic antigen could be elicited if the recipients were given normal allogeneic bone marrow. This reconstitutive effect of the bone marrow, in an immunological sense, was found to be specific, since tolerant recipients of bone marrow obtained from donors primed with the tolerogenic antigen failed to form antibodies to this antigen but responded well after stimulation with a non–cross-reactive antigen. The specificity of the response in the tolerant recipient was further demonstrated by the fact that recipients made tolerant to one antigen (i.e. HSA) and given allogeneic bone marrow cells from a donor primed with a different antigen (i.e. BGG) responded with antibody formation when immunized with either of these two antigens. The interpretation of the latter findings is that the antigen-reactive cells directed to HSA, to which the tolerant recipient was made unresponsive, were present in the transferred, BGG-primed bone marrow, and therefore the tolerant recipient could successfully mount an immune response to HSA. Furthermore, the tolerant recipients of normal allogeneic bone marrow, following reimmunization 38 days after primary immunization, possessed circulating antibodies which could not be inactivated by mercaptoethanol, thus indicating that these antibodies were of a "secondary" or 7S variety, and not of a "primary" or 19S type. On the other hand, the tolerant rabbits given primed bone marrow, which did not respond following initial administration of the antigen, produced circulating antibodies after secondary immunization 38 days later which were all mercaptoethanol-sensitive; therefore these can be classified as "primary" or 19S-type antibodies. Thus one may conclude that the latter, tolerant recipients were indeed tolerant following administration of primed bone marrow and antigen, and that it was not simply a matter of antibody having been synthesized but not detected by the techniques utilized.

The present results, demonstrating that normal but not primed bone marrow could facilitate an immunological response of normal proportions in otherwise tolerant recipients, allow one to conclude that the cell which is immunologically unresponsive in the tolerant recipient is the antigen-reactive cell, which arises from cells normally residing in the bone marrow. The results also support the conclusion arrived at previously, based on investigations utilizing anti-allotype antisera to inhibit the formation of hemolytic plaques (5), that the bone marrow contains only antigen-reactive cells and is devoid of antibody-forming cells.

The data presented indicate that the immunocompetent cell (or cells) affected in the induction of the immunologically tolerant state in the neonate is probably identical with that affected in the suppression of the immune response by irradiation. In the former case the antigen-reactive cell is made tolerant and therefore immunoincompetent, and in the latter situation it is the antigen-reactive cell which is inactivated by the irradiation (5). The antigen-reactive cell must be considered to have undergone some reaction(s) in the induction of

tolerance, since the bone marrow, following induction of the tolerant state, no longer possesses cells exhibiting antigen-reactive properties directed toward the tolerogenic antigen, similar to the "primed" bone marrow following induction of an immune response. The "tolerant marrow" therefore simulates the "primed marrow" with respect to its immunoincompetence in cell transfer experiments, although the mechanisms whereby immunoincompetence is induced in the bone marrow in these two diametrically opposed immune states are probably different. The question may therefore be asked whether the antigen-reactive cell actually becomes tolerant and remains in the bone marrow in an unresponsive state, or whether it persists as an antigen-reactive cell in some other organ in the tolerant rabbit, as distinct from the antibody-forming cell (characteristic of the primary response) or the memory cell (characteristic of the secondary immune response). Since it has been demonstrated that antigen-reactive cells can interact with antigen in vitro and not be rendered tolerant or immunoincompetent (2), it is considered unlikely that interaction with antigen in vivo would have induced a tolerant state in this cell. These questions are amenable to resolution by appropriate cell transfer experiments in the laboratory.

On the basis of the data presented in this study and of findings reported previously from this laboratory (3–7), one may state unequivocally that the bone marrow in the rabbit possesses only antigen-reactive cells and no antibody-forming cells. This interpretation begs the further assertion that, in the rabbit, the antibody-forming cells originate in lymphoid organs other than the bone marrow and that their final "resting" site, following the intravenous administration of the antigen, is the spleen (5). This concept implies the existence of specific populations of bone marrow cells committed or programed to interact with a site on the antigen molecule characterized by a unique molecular composition and configuration. How can this interpretation be reconciled with a pragmatic approach based on the deduction that there cannot exist more than just a small number of bone marrow antigen-reactive cells committed to react with any particular antigen, in view of the large number of antigens known to exist (microbial, synthetic, haptenic, drugs, inanimate protein antigens, viruses, etc.)? The resolution of this dilemma rests on the probability that the above deductions are, in fact, correct but require qualification. The small number of antigen-reactive cells in the resting state directed to a particular antigen is sufficient to mediate the immune response, in view of the cells' capacity to undergo explosive proliferation following interaction with the antigen. This has been demonstrated for the thymic antigen–reactive cell in the mouse (9, 10) and the bone marrow antigen–reactive cell in the rabbit (6, 7). This proliferative activity of the antigen–reactive cells in the rabbit takes place in an organ (or organs) other than the bone marrow, since the basal activity of the "primed" rabbit bone marrow cells in vitro is not higher than normal, resting levels (7).

Where do the antibody-forming cells (AFC) originate, and where do they reside in the absence of antigenic stimulation? Attempts to resolve this problem are under way, and therefore one can only speculate. Since antibody formation can be elicited primarily in cells in the thymus (11), spleen (12–14), or draining lymph node (15–20), depending on the route of administration of the antigen (intrathymic, intravenous, and foot pad, respectively), it would appear that the potential AFC already reside in the lymphoid organs, and that the presence of antigen at these sites constitutes one of the determining factors concerned with the initiation of the local immune response. Recent findings (3–7) indicate that the committed antigen-reactive cells (ARC) vacate the bone marrow following

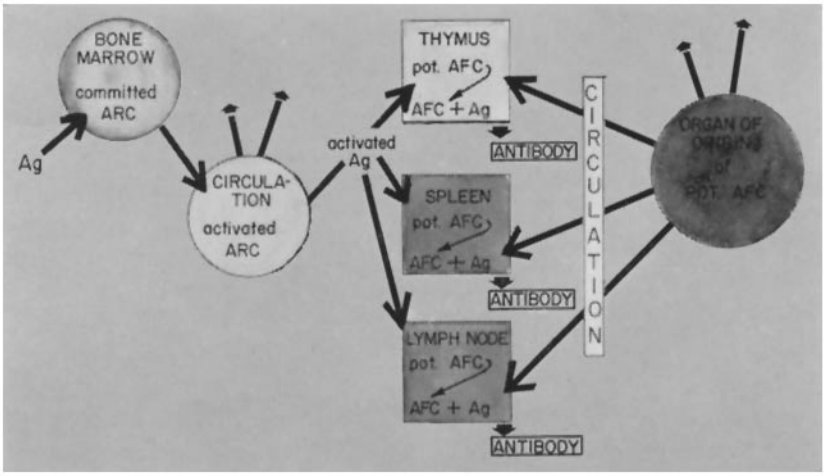

FIG. 2. Sources and destinations of antigen-reactive (ARC) and antibody-forming (AFC) cells in the rabbit following intravenous administration of antigen.

interaction with the antigen (activated antigen-reactive cells) and migrate to one or more of the lymphoid organs, where they probably transfer activated antigen to the antibody-forming cells (Fig. 2). Interaction of these cells with the activated antigen results in their transformation into memory cells, which are synonymous with Y cells (21) or antigen-recognizing, antibody-forming cells (22). The latter cells may, at this stage, be capable of forming, but not of releasing, humoral-type antibody, but they can be triggered to do so if stimulated by unprocessed or native antigen and are thereby transformed into actual antibody-forming cells or Z cells (21, 23). Depending on the type and nature of the immunization, these latter cells will masquerade as either plasma cells (15–17, 24–29) or lymphocytes (26, 30–36).

It can also be argued that antigen-reactive cells must also, to a certain extent, be dispersed and cannot, if the above discussion has any validity, be localized

solely to the bone marrow, even in the resting, unimmunized state. It would be difficult to reconcile the concept of the bone marrow as the main source of antigen-reactive cells with the fact that primary immune responses have been achieved in vitro with rabbit lymph node and spleen fragments (37–40) and mouse spleen cells (41–43). Actually, one may logically anticipate such a situation, since the bone marrow is not a static assemblage of cells, but a fluid system with the cells free to enter the bloodstream. It is therefore likely that some antigen-reactive cells will always be vacating the bone marrow, but that all the antigen-reactive cells of the specific clone, and therefore of the same antigenic specificity, can be evicted from the bone marrow following the intentional injection of a massive dose of the antigen. The scheme of cellular interactions postulated in the induction of the primary immune response in the rabbit is presented in Table X and Fig. 2.

TABLE X

Cellular Interactions Postulated in the Induction of the Primary Immune Response in the Rabbit

1. Antigen + macrophage	→	processed antigen
2. Processed antigen + antigen-reactive cell	→	activated antigen
3. Activated antigen + potential antibody-forming cell or X cell	→	memory cell or antigen-recognizing, antibody-forming cell or Y cell
4. Memory cell + native antigen	→	antibody-forming cell or Z cell
		↙
		Antibody

The concept that a multicellular system (ARC and AFC) exists to provide for the immune response is based on findings in two animal species, the rabbit and the mouse. The work in the rabbit is clear-cut. The bone marrow contains the cells which can interact with the antigen (ARC) (4, 6, 7), the manifestation of the interaction consisting of blastogenesis and mitosis (proliferation), but *not* antibody formation. Although the organ of origin of the AFC in the rabbit has not yet been established, it is definitely not the bone marrow (5). Therefore, in the rabbit, the terms ARC and AFC define exactly the functions intended: the interaction of the ARC with the antigen, leading to proliferation of the cells but not to antibody formation, and the interaction of the AFC with what is probably an activated or processed antigen moiety to form humoral-type antibodies. A similar situation apparently exists in the mouse, except that the thymus, not the bone marrow, possesses the ARC. A number of investigators have reported that an immune response can be elicited in an irradiated mouse provided that the mouse has been injected intravenously with isogenic thymus and bone marrow cells (44–47). Davies et al. (9, 10) have observed that mouse thymus cells can proliferate following stimulation with antigen but cannot

mediate an immune response in an irradiated recipient. Both Mitchell and Miller (44, 45, 47) and Taylor (48) have presented evidence strongly implicating the thymus as the source of the ARC in the mouse. Taylor (48) immunized mice with BSA, sacrificed them 24 hr later, and transferred their primed thymus cells, along with isogeneic normal bone marrow cells, to recipient irradiated mice. The latter mice were incapable of eliciting an immune response following stimulation with BSA, whereas recipients of either normal isogeneic bone marrow and thymus cells or primed isogeneic bone marrow cells and normal isogeneic thymus cells could respond (48). Results of a somewhat similar nature have been presented by Abdou and McKenna (49), who induced immunological tolerance in mice to a syngeneic spontaneous mouse tumor and then transferred thymus cells from these tolerant animals to 6 wk old mice which had been thymectomized at birth. These recipients were subsequently found to be tolerant to the tumor antigens. Both Taylor (48) and Abdou and McKenna (49) speculated that the thymus cells had been made tolerant to the specific antigens prior to their transfer to the recipient mice. However, results obtained in this laboratory indicate that a different interpretation must be considered. It has been observed that rabbit bone marrow ARC are not made tolerant following incubation, either in glass bead columns (2) or in suspension,[1] with relatively high concentrations of antigen, since these cells could then passively transfer immunocompetence to irradiated hosts with respect to the antigen(s) incubated. Thus, it is likely that the donor thymus of Taylor (48) and Abdou and McKenna (49) had been depleted of antigen-reactive cells, rather than made tolerant, after contact with antigen in vivo, in much the same manner as the rabbit bone marrow (1, 3, 6, 7) is depleted of ARC following the administration of the antigen.

Sinclair and Elliot (50) have observed that the 6 wk old mouse thymectomized at birth is capable of responding to stimulation with sheep red blood cells, but that the dose of red cells required to stimulate an immune response is approximately 100 times greater than that required to elicit a response of similar magnitude in the normal or sham-operated mouse. This observation suggests that the number of antigen-reactive cells in the mouse is reduced by a comparable figure (100-fold) in the thymectomized mouse, if one assumes a random interaction between the antigen and the antigen-reactive cell in vivo. The data support the findings of other investigators (9, 10, 47) that the mouse thymus supplies antigen-reactive cells, some of which may have seeded the other lymphoid organs prior to thymectomy but are markedly diminished in number following thymectomy. If one assumes that no emigration of antigen-reactive cells from the thymus could have taken place prior to neonatal thymectomy, it is necessary to postulate the existence of a second, if minor, source of antigen-reactive cells in the mouse. In fact, evidence has been presented implicating the spleen as a

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

source of antigen-reactive cells in the mouse (51–54). Whether the antigen-reactive cells in the spleen are wholly endogenous to that organ, or whether they may have migrated to the spleen from the same organ which seeds the thymus with antigen-reactive cells or their precursors in utero, cannot be ascertained at the present time. Thus, the thymus in the mouse serves as the counterpart of the bone marrow in the rabbit, insofar as the ARC is concerned. Recent observations by Mitchell and Miller (47) indicate that the bone marrow in the mouse is the source of the antibody-forming cell, thus apparently completing the circle in the mouse.

The spleen, which can function as a hemopoietic organ in the mouse and rat (45, 55, 56), can substitute for the combination of bone marrow and thymus cells in the transfer of immunocompetence to irradiated recipient mice (9, 46, 51–54, 57). Furthermore, reversal of the postthymectomy wasting syndrome in the mouse can be achieved by the administration of spleen cells (58–60). Such reconstituted animals also regain the ability to reject allogeneic skin grafts, indicating that the spleen in the mouse can exhibit thymic function as well. Therefore, the spleen in the mouse must contain both bone marrow–derived AFC and thymus-derived ARC, as well as other marrow- and thymus-derived cells. In fact, an interaction between two types of cells present in the mouse spleen in the induction of hemolytic foci has been alluded to by Gregory and Lajtha (61), who observed that although the number of hemolytic foci found in the spleen of an irradiated mouse given isogeneic spleen cells increased linearly with respect to the number of spleen cells injected, the number of plaque-forming cells (PFC) increased allometrically with graft size. They (61) speculated that, although the synthesis and release of antibody by the PFC can be attributed to independent activities of individual cells, the production of the PFC from precursors might be the result of an interaction between several cell types, one of which could be the antigen-reactive cell of thymic origin (9, 10, 47), which is present in the mouse spleen (58–60). Therefore, spleen cells cannot be used for the study of the sequence of cellular interactions in the immune response, since the ARC and AFC are both small lymphocytes and cannot be distinguished from each other on morphological grounds.

Although the ARC and AFC, in the rabbit, have been shown to be irradiation-sensitive and irradiation-resistant, respectively (5), observations of a similar nature have not yet been made for comparable cells in the rat or mouse. If the AFC and ARC in the latter animals should exhibit the same differential sensitivities to irradiation, a reevaluation of data obtained from investigations concerned with the transfer of immunocompetence with splenic cells to irradiated recipients would be in order to clarify which of the functional cell types, ARC or AFC, transfers the activity.

One aspect of the problem, however, remains to be clarified. What criteria should be used to distinguish the antigen-reactive cell? On the basis of the

preceding discussion, the term "antigen-reactive cell" or "antigen-sensitive cells" should be reserved only for those cells, at least in the rabbit and the mouse, which react with the antigen but do not subsequently form antibodies. However, some investigators concerned with elucidating methods for the demonstration, recognition, and localization of ARC in the mouse have greatly confused the situation, since they have utilized the term ARC to designate cells in the spleen capable of inducing "hemolytic foci" (61–64) or "bacterial immobilization in gel" (65, 66), both activities necessitating the mediation of antibodies. Armstrong and Diener (65) have stated that "the method is based on the *belief* that when these ARC are injected into a lethally irradiated host, they embed in the spleen in predictable concentrations and respond to an antigenic stimulus by proliferating and differentiating into colonies of ARC." In fact, they have stated categorically that the success of this technique is dependent upon antibody secreted by the "antigen-reactive cell." Kennedy et al. (62, 63) have said that the interpretation of their results is predicated on "two *postulated properties* of these cells: sensitivity to antigenic stimulation by sheep erythrocytes and ability to respond to this stimulation by proliferating to give rise to cells capable of hemolysin production." Playfair et al. (64) arrived at a similar conclusion. Such an interpretation is, at the very least, inconsistent with the definition of the term "antigen-reactive cell." In view of the fact that in both the rabbit (3–7) and the mouse (9, 10, 44–47) it has been demonstrated that ARC and AFC are independent cellular entities and do not differentiate one into the other, and since the mouse spleen has been shown to consist of a mixture of ARC and AFC (45, 46, 57–60), the interpretations of Armstrong and Diener (65) and of Kennedy et al. (63) rest on tenuous grounds.

In summary, the antibody-forming apparatus in the mouse has been shown to consist of two independent cellular compartments: the thymic ARC and the bone marrow AFC. In the rabbit, the bone marrow serves as the source of ARC; the organ of origin of the AFC has not as yet been defined. In both the rabbit and the mouse, the ARC appear to vacate the bone marrow and the thymus, respectively, after immunization or the induction of tolerance. The pathways taken by these cells in these two situations must be somewhat different, since the immune states which result are diametrically opposed. Future investigations will be concerned with elucidating where, as well as the mechanism whereby, the ARC transfer immunogenic information to the AFC in the immunized animal, and why they fail to do so in the tolerant animal. The defect in the tolerant rabbit lies with the ARC, since the antibody-forming cell in this animal is fully capable of synthesizing humoral-type antibodies.

SUMMARY

Rabbits were made immunologically tolerant to either human serum albumin or bovine gamma globulin by the neonatal administration of antigen. At 10 wk

of age, they were challenged with the tolerogenic antigen and found to be nonresponsive. However, these tolerant rabbits could respond with humoral antibody formation directed toward the tolerogenic antigen if they were treated with normal, allogeneic bone marrow or bone marrow obtained from a rabbit made tolerant toward a different antigen. They were incapable of responding if they were given bone marrow obtained from a rabbit previously made tolerant to the tolerogenic antigen. Irradiated rabbits were unable to respond if treated with tolerant bone marrow, but could respond well if given normal bone marrow. Since it has previously been demonstrated that the antibody-forming cell, in an irradiated recipient of allogeneic bone marrow, is of recipient and not donor origin, the data presented strongly indicate that the unresponsive cell in the immunologically tolerant rabbit is the antigen-reactive cell.

The authors wish to thank Dr. Bram Rose, Director, Division of Immunochemistry and Allergy, Royal Victoria Hospital, for his many suggestions and assistance in the preparation of this manuscript; and Miss D. Anslow and Miss M. Stevens, for the typing of the manuscript.

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