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STUDIES ON THE TOLERANCE OF THE IMMUNE SYSTEM OF MOUSE CHIMERAS

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Adult mice made tolerant of skin homografts by neonatal injection of homologous hemopoietic cells, and adult mice lethally x-irradiated and subsequently injected with homologous hemopoietic cells are known to be cell chimeras. Indeed, injection of homologous hemopoietic cells into normal neonatal or irradiated adult mice is followed by take and proliferation of the transferred cells. Direct evidence for the existence of cell chimerism in tolerant mice was presented by Trentin and Sessions,[1] who were able to identify donor cells by use of a chromosomal marker. In radiation chimeras, the presence of donor cells was clearly shown several times and by various techniques.[2]

It is generally accepted that the hemopoietic tissues commonly used to induce tolerance or to restore lethally irradiated mice, i.e., spleen or bone marrow, contain potential or actual antibody-forming cells. The observation of runt disease in tolerant mice of some donor-recipient strain combinations (interpreted as being due to a graft-anti-host immune reaction) indicates that immunologically competent donor cells can colonize the injected newborns and confer a chimeric feature to the developing immune system. Likewise, repopulation of the irradiated host by competent donor cells is admitted to occur in radiation chimeras. The appearance of secondary disease in these animals indeed is referred to a graft-anti-host immune reaction. If the host immune system recovers from radiation damage, as might be the case in mice exposed to a 30-day LD_{100} of X-rays, some time after irradiation and hemopoietic treatment the immune system of the animal should be a mosaic of host and donor cells. Therefore, on the basis of the foregoing facts and arguments, immunologically competent cells of host and donor types may be expected to coexist in both tolerant mice and radiation chimeras.

Frequency and severity of runt and secondary diseases depend upon the strain combinations used. Longevity of the tolerant mice and radiation chimeras that escaped or survived runt or secondary disease implies that some time after their transplantation the immunologically competent donor cells either have disappeared or ceased to react against the host antigens, or never started this reaction.

Persistence of skin grafts and hemopoietic cells of donor type in tolerant mice several months after the neonatal injection indicates that the host immune system remains unable to react against antigens of the donor type. Presence of donor hemopoietic cells in long-term radiation chimeras suggests that the host immune system, if ever recovered from radiation damage, is unreactive against the hemopoietic graft.

The present study is an experimental analysis of the immune system of tolerant mice and radiation chimeras several months after the injection of the homologous hemopoietic cells. The aim was the identification of host and donor antibody-forming cells and evaluation of their functional state.

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Methods and Results

Agglutinin response to heterologous antigens was induced in tolerant mice and radiation chimeras. Their spleen cells were then analyzed in a discriminant test to find out whether host or donor cells were responsible for the agglutinins produced to the heterologous antigen. Technical details have been published elsewhere.[3,4]

Tolerant mice. C3H newborn mice of both sexes were injected intravenously with 1×10^7 nucleated spleen cells from CBA adult female mice. Two months after the neonatal injection, 22 C3H mice were grafted with CBA skin from adult donors of the same sex as the recipients. The skin grafts showed no macroscopic signs of rejection during the observation time of three months. In contrast, CBA skins grafted onto 12 age-control C3H mice, noninjected at birth, showed complete necrosis in 11.7 ± 0.4 days.

That the tolerant mice were chimeras could be inferred from the detection of donor type antigens in their spleens. This was performed by injecting spleen cells from tolerant mice, at the time of their sacrifice for the identification test (see below), into normal C3H mice, which were then tested for transplantation immunity against CBA antigens by a method previously described.[4]

When five months old, 11 tolerant mice, 10 normal C3H, and 10 normal CBA mice were injected intraperitoneally with 1 ml. of one per cent rat RBC. Twelve days later, blood was collected individually for serum titration of antirat RBC agglutinins. The mean $\log_2$ titer of tolerant mice was found normal, that is, not significantly different ($P = 0.20$) from that of normal C3H and CBA mice. This result indicates that the unresponsiveness of C3H mice to CBA skin grafts was specific, for the tolerant mice were able to respond with normal vigor to an antigen unrelated to C3H and CBA strains.

None of the tolerant mice showed any sign of runt disease at any time before and after the injection of rat RBC.

Identification of the immune system of tolerant mice. Normal C3H and CBA adult mice were immunized by intraperitoneal injection of 1×10^7 nucleated spleen cells from adult CBA or C3H mice, respectively, and 10 days later were given a total-body X-ray dose of 700 r. Within two hours after the irradiation, the immunized mice of each type were divided in four groups of 7 to 15 animals. A control group received intravenously 1 ml. of Tyrode's solution. The others received intravenously one of the following inocula in 1 ml. volume of Tyrode's solution: 24×10^6 nucleated spleen cells from C3H, CBA, or tolerant C3H mice. All donor mice had been injected intraperitoneally with 1 ml. of one per cent rat RBC 12 days earlier, and each cell suspension was prepared from a pool of five to six spleens. Immediately after the intravenous injection, all groups were given intraperitoneally 1 ml. of one per cent rat RBC. Six days later, the recipients were decapitated, and blood was collected individually for serum titration of antirat RBC agglutinins. The results are presented in TABLE 1.

The Table shows that the antirat RBC agglutinins detected in the irradiated recipients were produced only by the secondary response of the transferred spleen cells. Spleen cells from C3H or CBA donors did not produce agglutinins when transferred into irradiated recipients specifically immunized to the spleen cell donor. Spleen cells from tolerant C3H mice produced agglutinins when transferred into irradiated C3H mice immunized to CBA tissues, but did not yield agglutinins when transferred into CBA mice immunized to C3H tissues. This finding demonstrates that the secondary response of spleen cells from the tolerant mice was due only to the C3H cells, that is, to the host cells.

TABLE 1

PRESENCE (+) OR ABSENCE (0) OF ANTIRAT RBC AGGLUTININS IN THE SERUM OF RECIPIENT MICE

Irradiated recipients given rat RBC	Spleen cell donors sensitized to rat RBC			
	None	C3H	CBA	Tolerant C3H
C3H anti CBA	0	+	0	+
CBA anti C3H	0	0	+	0

Radiation chimeras. Twelve-week-old male and female (101 × C3H) F_1 mice were given a total-body X-ray dose of 900 r (30-day LD_{100}). Within four hours after irradiation, they were injected intravenously with 2×10^7 nucleated bone marrow cells from (C57Bl/6 × DBA/2) F_1 donors of the same age and sex as the recipients.

Five months after the bone marrow injection, mice that survived secondary disease were found to be cell chimeras, for their peripheral blood was shown to have donor type cells by the agglutination technique of Gorer and Mikulska.[5] Twenty of these long-term survivors, 15 normal (101 × C3H) F_1, and 15 normal (C57Bl/6 × DBA/2) F_1 mice of the same age as the chimera hosts were injected intraperitoneally with 1 ml. of one per cent rat RBC. Eighteen days later, blood was collected for serum titration of antisheep RBC agglutinins. The mean $\log_2$ titer of the radiation chimeras was found subnormal, that is, significantly lower ($P = 0.05$) than that of the normal mice.

Identification of the immune system of radiation chimeras. Normal (101 × C3H) F_1 and (C57Bl/6 × DBA/2) F_1 adult mice were immunized by intraperitoneal injection of 1×10^7 nucleated spleen cells from adult (C57Bl/6 × DBA/2) F_1 or (101 × C3H) F_1 mice, respectively, and 18 days later were given a total-body X-ray dose of 900 r. Within two hours after the irradiation, the immunized mice of each type were divided in four groups of 10 animals. A control group received intravenously 1 ml. of Tyrode's solution. The others received intravenously one of the following inocula in 1 ml. volume of Tyrode's solution: 12×10^6 nucleated spleen cells from (101 × C3H) F_1, (C57Bl/6 × DBA/2) F_1 mice, or radiation chimeras. All donor mice had been injected intraperitoneally with 1 ml. of one per cent sheep RBC 18 days earlier and each cell suspension was prepared from a pool of four to five spleens. Immediately after the intravenous injection, all groups were given intraperitoneally 1 ml. of one per cent sheep RBC. Six days later, the recipients were sacrificed, and blood was collected individually for serum titration of antisheep RBC agglutinins. The results are given in TABLE 2.

TABLE 2

PRESENCE (+) OR ABSENCE (0) OF ANTISHEEP RBC AGGLUTININS IN THE SERUM OF RECIPIENT MICE

Irradiated recipients given sheep RBC	Spleen cell donors sensitized to sheep RBC			
	None	(101 × C3H) F_1	(C57Bl/6 × DBA/2) F_1	(101 × C3H) F_1 chimeras
(101 × C3H) F_1 anti-(C57Bl/6 × DBA/2) F_1	0	+	0	0
(C57Bl/6 × DBA/2) F_1 anti-(101 × C3H) F_1	0	0	+	+

This Table shows that the antisheep RBC agglutinins found in the irradiated recipients were produced only by the secondary response of the injected spleen cells. Spleen cells from (101 × C3H) F_1 or (C57Bl/6 × DBA/2) F_1 donors did not produce agglutinins when transferred into irradiated recipients specifically immunized to the spleen cell donor. Spleen cells from radiation chimeras produced agglutinins when transferred into irradiated (C57Bl/6 × DBA/2) F_1 mice immunized to (101 × C3H) F_1 tissues, but did not form agglutinins when transferred into (101 × C3H) F_1 mice immunized to (C57Bl/6 × DBA/2) F_1 tissues. This result demonstrates that the secondary response of spleen cells from the radiation chimeras was due only to the (C57Bl/6 × DBA/2) F_1 cells, that is, to the donor cells.

Discussion

In the experiments presented, tolerant mice and radiation chimeras were shown by indirect and direct techniques, respectively, to be mosaics of host and donor cells. Agglutinin response to heterologous antigens was induced in both types of chimeras, and resulted normal in tolerant mice and subnormal in radiation chimeras. Their spleen cells were found able to give a secondary agglutinin response when transferred into proper recipients and challenged again with the test antigen. Only cells of host or donor type where found responsible for the agglutinin production by spleen cells transferred from tolerant mice or radiation chimeras, respectively. Since the method of identification used was based on a secondary agglutinin response, it follows that also the primary response to the heterologous antigen should have been produced by host cells in the tolerant mice and by donor cells in the radiation chimeras. The nondetectability of immunologic activity to the heterologous antigen by donor cells in the tolerant mice and by host cells in the radiation chimeras implies that the undetected cells either were absent at the time of the antigen challenge or incapable of responding to it. Both possibilities have been discussed previously.[3,4] These negative results raise the question of the sensitivity of the method used. Preliminary controls showed that in order to detect a secondary agglutinin response against rat RBC in the serum of irradiated CBA mice, preimmunized to C3H tissues and given the test antigen, at least 5×10^5 spleen cells needed to be transferred from CBA mice sensitized against rat RBC. Similarly, a threshold of 7×10^5 spleen cells was found when irradiated (101 × C3H) F_1 mice preimmunized to (C57Bl/6 × DBA/2) F_1 tissues were used as recipients and sensitized (101 × C3H) F_1 mice, as donors. However, it should be pointed out that the sensitivity of the method for detecting the immunologic activity of cells grown in chimeric tissues may be different, and. therefore, cannot be estimated from these types of controls. While the finding of only donor cells being responsible for antibody production in homologous radiation chimeras is in agreement with previous results,[2] the lack of donor type immune activity in tolerant mice might seem at variance with what was found by Michie *et al.*[6] These authors analyzed the chimerism of the immune system of tolerant mice, making use of Simonsen's G.V.H. assay,[7] whereby immunologic competence of cells is evaluated by measuring their capability to induce spleen enlargement when injected into susceptible recipients. They reported that cells from mice supposed to be "specifically" tolerant could induce spleen enlargement when transplanted into recipient mice carrying antigenic components unrelated to the host and donor strains used in the induction of tolerance. In some experiments, the transplanted cells responsible for the spleen enlargement were found to be of host type, while in other experiments, of both host and donor types. These results would suggest that host and donor type cells, potentially capable

of a primary immune response, were both present in some of the tolerant mice. However, since their mice had not been tested for the specificity of tolerance, it is difficult to predict which type of immune system would actually have responded in the tolerant animals, had they been challenged with antigens unrelated to the host and donor strains.

The present findings allow an attempt to evaluate the functional state of the immune system of tolerant mice and radiation chimeras. The host immune system of tolerant mice, being the only one responsible for the primary agglutinin production elicited in these animals by rat RBC, can be considered in a tolerant state, for it was capable, while tolerating a homologous skin graft, to respond with *normal* vigor to antigens unrelated to the host and donor strains. In the light of this observation, the definition of tolerance (specific unresponsiveness), so far referred to the whole animal,[8] can also be applied to the host immune system.

Longevity of the radiation chimeras suggests that at the time of the experiment, the donor immune system was not reacting against the host antigens. However, this unresponsiveness was not specific, for the primary response to sheep RBC produced in the chimera by the donor immune system was *subnormal.* Hence, unlike tolerant mice, the radiation chimeras analyzed in the present work had an immune system that cannot be considered in a tolerant state. This conclusion is in contrast with the claim that the donor immune system of long-term radiation chimeras is "specifically" unresponsive to the host antigens and, therefore, in a tolerant state.[9-11] The specificity of such unresponsiveness was demonstrated by the normal response against antigens unrelated to the host and donor strains, which was displayed by donor type lymphoid cells when transferred from radiation chimeras to suitable test recipient mice. However, the rate of differentiation and proliferation of the immunologically competent cells, which affects the intensity of an immune reaction, may have changed upon their transfer from the chimeric tissues to the test recipients. Therefore, a normal degree of response by retransplanted donor type cells does not prove that also in the chimera the donor type immune system was capable of reacting with normal vigor to the test antigen. On the contrary, direct estimate of antibody production in the chimeras, followed by identification of the immune system responsible for it, provided sufficient information to rule out the possibility that the radiation chimeras analyzed in the present study had a tolerant immune system. This conclusion, however, need not conflict with the possibility that individual donor cells were tolerant. A smaller number of antibody-forming cells in the chimeras, compared to that in normal mice, may indeed account for the subnormal efficiency of the chimera immune system to react against the heterologous antigen.

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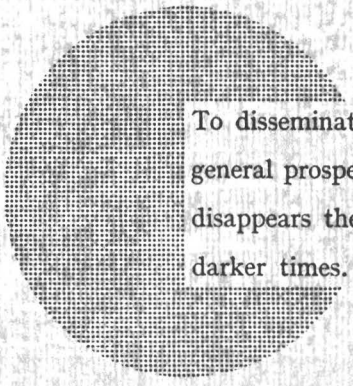

To disseminate knowledge is to disseminate prosperity — I mean general prosperity and not individual riches — and with prosperity disappears the greater part of the evil which is our heritage from darker times.

Alfred Nobel