Survival of Group A Erythrocytes Following Bone Marrow Transplantation for Reconstitution of Lymphopenic Hypogammaglobulinemia

By M. M. Azar, R. A. Gatti, E. J. Yunis, J. Swanson, and R. A. Good

The survival of chromium-labeled group A erythrocytes was measured in a patient who had previously received two bone marrow transplants for reconstitution of lymphopenic hypogammaglobulinemia. The patient was of blood group A before transplantation; the donor of blood group O. The patient's erythrocytes are now virtually 100% group O. Anti-B titers are present; anti-A antibodies are not demonstrable. The cells producing these isohemagglutinins are of donor origin; the donor has anti-A titers of 1:64 in saline and 1:256 by antihuman globulin test as well as anti-B titers. No evidence of immunological destruction of group A erythrocytes was found in this patient suggesting that the immune system of the donor may have become tolerant of the group A substance of the recipient.

In the chimeric state produced by marrow transplantation, it is not known whether there is mutual immunologic tolerance or a low-grade immunity compatible with normal production and survival of two separate populations of cells. Recent evidence from long-lasting radiation chimeras in dogs suggests that immunity against host-type fibroblasts is present in the lymphocyte system of donor origin and that blocking antibodies produced by the recipient may prevent any destructive action on the recipient host. This competing immunologic relationship is thought to be the basis for persistence of the marrow and absence of a demonstrable continuing graft-vs.-host disease. An opportunity to look at this question in another way was provided when we were able to establish a chimeric state in a child with severe dual system immunodeficiency. The initial marrow transplant reconstituted this child with respect to both immunological systems. It also produced a typical graft-vs.-host reaction that seemed gradually to have subsided. But...
cause the recipient child was of blood group A and the donor blood group O, the immunological system of the donor reacted against the A antigens of the host and produced first, a direct Coombs'-positive hemolytic anemia and then later an aregenerative pancytopenia. Treatment of this complication by a second marrow transplant established a long-lasting chimeric state that did not lead to a graft-vs.-host reaction and in which virtually all of the circulating red cells were those of donor origin.3 It was the purpose of the present investigation to determine, 2 yr after the first transplant, whether the new immunological system of the recipient continued to be active against the host's red blood cells or whether a state of tolerance had developed. If the former situation existed, accelerated production and destruction of the group A cells would be expected; if the latter, the A cells would be expected to have normal survival time.

**CASE HISTORY**

A 5-mo-old male child (D.C.) with severe combined immunodeficiency disease (lymphopenic hypogammaglobulinemia)4 was given bone marrow and peripheral blood leukocytes from his 8-yr-old sister who was histocompatible when matched by the mixed leukocyte culture technique. The recipient was of blood group A De MM kk Fy(a+) and the marrow donor group O DeE MM kk Fy(a+). Results of titration studies on donor's serum were as follows: anti-A, saline, 1:64, immune 1:256; anti-B, saline 1:32, immune 1:256. Within the first month after the first bone marrow transplant, the population of A cells decreased from 100% to 90%. A positive direct Coombs' test developed at the time of onset of fever, vomiting, bloody diarrhea, irritability and a coarse, erythematous, maculopapular rash over his face, arms, and back.2 These findings are characteristic of the graft-vs.-host reaction (GVH) in man as described by Miller.5 Hepatosplenomegaly was found and the hemoglobin level, at that time, was 6 gm 100 ml. These relationships are shown in Fig. 1. No specific antibody was eluted from the coated erythrocytes at this time; however, 120 days after the onset of the GVH reaction only 0–5% of group A cells could be detected.

Approximately 3 mo after the first marrow engraftment, a second transplant was done in an attempt to correct an aregenerative pancytopenia.3 Within 2 wk, platelet, hemoglobin,
... Fig. 2.—Graph showing survival of chromium-labeled group A erythrocytes in group O patient D.C., chimera 95% O, 5% A, counts corrected for ⁵¹Cr decay.

and leukocyte levels began to increase; 1 mo later, all hematological parameters were within normal ranges and the normal values have persisted to the present.

Blood group substances A, H, Lewis A and B were repeatedly detected in saliva during the 2-yr period. In addition, the blood group A substance present in the patient's serum was tested by the hemagglutination inhibition technique against three randomly selected O individuals. Thus, DC serum was estimated to completely inhibit anti-A antibody in a titer of 1:8. Anti-B isoagglutinins were typically absent in the child's serum initially but appeared soon after the first bone marrow transplant and were present for more than 2 yr following the transplant. Anti-A activity was absent throughout the complete follow-up. An attempt was made to stimulate the patient's lymphocytes in vitro with group A specific substance, but no response was elicited in either patient or controls with good anti-A antibody titers. This finding may indicate that A substance like other polysaccharide antigens does not elicit cell-mediated immunity.

**In Vivo Studies**

Survival of blood group A cells was studied to determine the presence of acquired anti-A activity. Twenty-two months after the first bone marrow transplant, 25 ml ⁵¹Cr-labeled red blood cells from a compatible unrelated donor of group A CDe/ce were administered. A young male who had given blood five times in the previous 12 mo was chosen as the donor because previous recipients of his blood showed no complications. The red cell survival was followed for 60 days by doing daily 5-min counts on a single limb and precordium during the first week and 3 times weekly on the precordium thereafter. Probes aimed at liver and spleen were carried out to rule out any source of error or to detect any early sequestration of injected erythrocytes. The transfused cells showed a survival curve perhaps longer than normal; the ⁵¹Cr half life (Fig. 2) was about 43 days. The direct Coombs' test remained negative throughout and Cr-labeled cells were found in the peripheral blood 60 days after injection. Repeatedly over the 2-yr period, a search was made for anti-A antibodies using standing techniques in saline and at no time were such antibodies demonstrable. Blood group A substance was found in at least normal concentrations in the child's saliva and serum indicating that the recipient of the marrow in this instance was a secretor of the A substance. The child's complement levels were normal for his age.

**Discussion**

A graft-vs.-host disease appeared in this patient with severe dual system immunodeficiency 1 wk following the initial bone marrow transplantation. By
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the 10th day after the transplantation, the direct antiglobulin test became positive, reaching maximal reactivity by day 14 when the hemoglobin value was at its lowest. Four months later, after a steady decrease of the A cell population the number of host red cells became almost negligible. Since a close relationship between GVH, direct Coombs' reactivity and the pancytopenia was observed, the question of whether low-grade immunity or tolerance to the donor cells exists in this chimeric state in man seems pertinent. In the former case, one would expect to find anti-A activity in the serum as evidence of much activity directed toward circulating erythrocytes. Failure to determine specificity of the red-cell-bound antibodies that produced the initially positive direct globulin test was disappointing.

The elution studies which were attempted at the critical interval of transient Coombs' positivity, however, were not exhaustive and it is possible that such antibodies were present and were just not eluted. It seems more likely that the antibody on the red cells at this time was not attributable to anti-A determinants, but perhaps to other antigens on the erythrocytes as a consequence of the graft-vs.-host reaction. In our more lateral studies, however, where adequate efforts at elution were carried out failure to demonstrate any anti-A antibodies that are elutable from the red cells could be an indication of a postulated tolerance against A cells or simply a measure of the very few A cells present in the circulation. It has been extensively postulated that in experimental chimeric animals tolerance exists or that tolerance and immunity coexist. Recently this position has been questioned and evidence has been forthcoming that apparent tolerance may be a function of a very special form of immune reaction.7 In the situation under study here, it seems likely that with the existence in serum of antibody capable of combining with a red blood cell antigen, the Coombs' test might be positive, and the survival of the newly injected group A cells shortened.8 This would be expected whether or not the antibody was inhibitory to reactions of lymphocytes against erythrocytes or other host cells.7 Thus, in a chimeric patient the absence of detectable Coombs' positivity and normal hemoglobin levels and red cell counts together with normal survival time for 51Cr-tagged cells could be taken as evidence for the existence of an unresponsiveness of the immunologically competent cells, originally of donor origin, that had established the functional immunological system of the chimeric child. The remote possibility that the tagged A cells administered for this study might lead to termination of the putative tolerant state required consideration. Our findings of the gradual decrease in numbers of administered A cells with normal half-life values and lack of immune elimination argue against this possibility. We would expect that free antibody of any type against A cells should result in a more rapid elimination of the group A erythrocytes injected. Again such immune elimination was not observed, and if any variation was present, the cells survived longer than would be expected. The slightly prolonged survival of transfused erythrocytes may be explained by the nature of the donor cells. The latter were derived from a young male who had repeatedly donated blood in the previous year. He could have had a slightly younger population of red blood cells on the average than
that of the usual donor. If this were the case, it might account for their slightly prolonged survival.

We conclude from these studies that most likely the donor’s lymphoid cells in this long-lasting chimera did not continue to produce antibodies against the host’s blood group A antigens. This finding seems in keeping with earlier experimental observations in mice that apparently showed that donor cells could develop tolerance during graft-vs.-host reactions. The fact that the donor lymphocytes continued to produce anti-B antibodies indicates that such responses are basically intact, but responses to the patient’s own antigens are in some way specifically suppressed. The mechanism of this suppression remains enigmatic at present writing; however, it could reflect a clonal elimination of immunologically competent cells or inactivation of competent cells as a consequence of the immune response itself. The possibility cannot be absolutely excluded that large amounts of blood group A substance, more readily available to anti-A antibody formed by donor’s cells than are blood group antigens on the surface of the erythrocytes, could act to interfere with demonstration of anti-A titers and even with immune elimination of injected A cells. Such a phenomenon would resemble previously accepted explanations of immunological paralysis produced by injections of large doses of pneumococcal polysaccharides in mice. Recent experiments suggest, however, that in mice the mechanism of tolerance induction with polysaccharide antigens is similar to that involved in tolerance to protein antigens in mice. The normal levels of serum complement and high levels of A substance, although compatible with production of small amounts of antibody, argue against massive complement utilization and absorption by antigen-antibody complexes with the secreted A antigen. Our findings, reflecting immunological unresponsiveness to red cell antigens, do not rule out the coexistence of cellular immunity and blocking antibody to tissue antigens as has been found in other chimeras.

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