No irregular erythrocyte antibodies observed after bone allografts in 144 patients

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Red cell blood group antigens present in bone allografts may cause the formation of irregular erythrocyte antibodies (IEA). In the literature, 4 such cases have been described; all were Rh(D) negative females who had received Rh(D) positive bone and had made anti-Rh(D).

To investigate the immunization by red cells after bone allografting, a retrospective and a prospective study was carried out. Altogether 144 patients were tested for the presence of IEA, before as well as after obtaining frozen allogeneic bone in orthopedic or maxillo-facial surgery. In 9 patients, the tests disclosed IEA preoperatively. No new IEA were detected postoperatively in the 144 patients, including 30 Rh(D) negative patients who received Rh(D) positive bone.

We conclude that formation of IEA is a rare phenomenon. Nevertheless, it is recommended to give only Rh(D) negative bone to Rh(D) negative girls and women of childbearing age.

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The most immunogenic antigens in bone allografts are cell surface-related histocompatibility antigens (Muscolo et al. 1976, Friedlaender 1983, Prolo and Rodrigo 1985, Horowitz and Friedlaender 1987). Residual erythrocytes can also induce an immunologic response against blood group antigens (Curtiss and Herndon 1955), resulting in the formation of irregular erythrocyte antibodies (IEA).

Curtiss and Herndon (1955) were the first to report on the immunogenicity of blood group antigens in allogeneic bone. Weinberg et al. (1959) demonstrated that bone, free of marrow, contains blood group A and B antigens. Besides these reports, 4 cases have been described of red cell antibody production, probably related to previous bone allografting (Hill et al. 1974, Johnson et al. 1985, Jensen 1987, Larsen and Eiskjær 1987). All cases were Rh(D) negative women, with Rh(D) antibodies after receiving bone from Rh(D) positive subjects.

In view of these data, the increasing number of allogeneic bone transplants in our hospital gave rise to concern about red cell sensitization in our patients after bone allografting. A combined retrospective and prospective study was carried out to investigate this problem.

Patients and methods

120 patients were investigated retrospectively and 24 prospectively. The sera from all patients were preoperatively tested for the presence of IEA. The patients in the retrospective group had orthopedic (n 91) or maxillo-facial surgery (n 29), between 1983 and 1986 (Tables 1 and 2). From this group, blood samples were collected 1–3 years after the operation, since antigen release (total resorption of the graft), and therefore antibody production may take long a time.

The 24 patients in the prospective group received allogeneic bone in orthopedic surgery between 1986 and 1987 (Table 1). Blood samples were collected after 2 weeks, 3, 6, 9 and 12 months. We could not obtain all 5 blood samples from each patient. However, the blood from all patients was tested after 12 months. None of the patients was treated with immunosuppressive drugs.

Bone

After harvesting the bone, swab cultures were taken and the bone was preserved at –80 °C for at least 6 weeks. No cryoprotectives or other chemicals were used. Bones with positive bacteriological cultures were discarded. At the time of this study the bone was obtained from our casual bone supply which did not meet the requirements of the present day especially regarding the transfer of viral infections. Nowadays

Table 1. Indication for orthopedic bone allografting

	Retrospective	Prospective
Total hip revision	68	16
Osteotomy	5	2
Spondylosis (5–16 levels)	4	4
Arthrodesis	4	
Knee arthroplasty	2	
Bone cyst	2	
Fracture	2	1
Pseudarthrosis	1	
Chondrosarcoma	1	
Osteomyelitis	2	
Shoulder endoprosthesis		1
Total	91	24

Table 2. Indication for maxillo-facial bone allografting

	Retrospective
Le Fort osteotomy	14
Paranasal bone onlays	5
Chin plastic	3
Höfer osteotomy	3
Kuffner osteotomy	2
Reconstruction processus alveolaris	1
Fracture treatment	1
Total	29

donors are screened for the presence of HIV, lues and hepatitis B and C viruses with due observance of an incubation time of 3 months.

After having been thawed, the bone was implanted as cancellous bone chips. Most of the bone was derived from femoral heads; only 6 patients received fibula, rib, iliac or ankle bone. The fibula and iliac bones were used as whole transplants. 106 patients received bone from one donor, 32 patients from 2 donors and 6 patients from 3 donors.

Blood group serology

All sera were screened for IEA, according to standard methods (American Association of Blood Banks 1990), with a 2-cell screening set (Selectogen®, Ortho-Diagnostic Systems, Raritan, NJ, U.S.A.), with the following blood group antigens: CcDEe, Kk, Fy^aFy^b, Jk^aJk^b, Xg^a, Le^aLe^b, MNSs, P_1. The following different techniques were used: saline room temperature and saline 37 °C; 45 minutes incubation at 37 °C with papain pretreated red cells; indirect antiglobulin test after incubation with 30% albumin for 45 minutes at 37 °C.

Results

Preoperatively, 9 patients had IEA (2 anti-Fya; 2 anti-Kell; 2 anti-D and anti-C; 1 anti-C; 2 non-specific antibodies). Postoperatively, they had no further new antibodies. The other 135 patients did not have IEA before the operation and remained negative after bone allografting. These 135 patients included 37 patients who were Rh(D) negative, 30 of whom received Rh(D) positive bone.

Discussion

Over the last decades, knowledge about the immune response after bone allografting has increased. HLA-antigens, as well as blood group antigens, have been claimed to have been immunogenic. Curtiss and Herndon (1955) appear to be the first to examine the immunogenicity of blood group antigens in allogeneic bone. They interchanged fresh knees of blood group A negative dogs with those of A positive dogs. In 2 out of 5 A negative animals, antibodies against A positive red blood cells were detected. Weinberg et al. (1959) demonstrated that human bone free of marrow contains blood group A and B antigens. Except for these reports, 4 cases of red cell antibody induction related to bone allografting have been described. In all cases Rh(D) negative females received bone from Rh(D) positive donors. Hill et al. (1974) reported a case of hemolytic disease in a newborn, due to antibodies in the blood of a primigravid mother. Thirteen years earlier, at the age of 14, she received a bone allograft; she had never received a blood transfusion. Johnson et al. (1985) detected anti-C and anti-G (related to the Rh(D) system) antibodies in a 23-year-old female, with a negative history of blood transfusion or pregnancy. 3 years earlier she had received frozen bone allografts from 2 donors. The authors emphasized the relationship between the amount of grafted bone and immunization, as did Friedlaender (1984), who stated that the immune response after frozen bone allografting is dose-related. Johnson suggested the use of prophylactic anti-Rh(D) immune globulin or Rh(D) bone matching for Rh(D) negative women of childbearing age in order to prevent immunization. These measures, however, do not prevent the formation of other antibodies like anti-Kell, anti-Kidd, etc. Larsen and Eiskjaer (1987) detected anti-C, anti-D and anti-E

antibodies in a Rh(D) negative 13-year-old girl, 1 year after she had received frozen cancellous bone chips from 3-4 Rh(D) positive donors. Jensen (1987) recently reported a case of Rh(D) immunization after bone allografting. 3 months after the implantation of frozen cancellous bone from 3 donors, a 13-year-old girl appeared to have formed anti-D, -C and -E.

In our opinion, only the cases of Larsen and Eiskjaer (1987) and Jensen (1987) give solid evidence of red cell antibody formation due to bone allografting. The other cases refer to women of childbearing age. Despite their negative history regarding previous pregnancy or abortion, this can never be ruled out completely. Furthermore, it is known that Rh(D) antibodies can be formed during the first pregnancy (Eklund and Nevanlinna 1986).

We wondered how many of our patients were sensitized to red cell antigens after bone allografting. With pre- and postoperative blood investigations, no newly formed IEA were detected. Because of the antigenicity of Rh(D) antigens, we analyzed the number of patients at risk, i.e., all 30 Rh(D) negative patients who were negative for Rh(D) antibodies preoperatively and received Rh(D) positive bone. None of them developed anti-D after the operation.

The number of patients is too small to draw any conclusions about the risk of being sensitized after bone allografting, but we can conclude that induction of IEA after bone allografting is a rare phenomenon.

As immunogenicity is dose-related, we have to recognize that the amount of transplanted bone in our series was small, except for 38 patients who received bone from several donors. The question remains whether these quantities are sufficient to stimulate antibody formation. We know that occasionally very small amounts of red cells can be immunogenic. Mollison et al. (1987) reported amounts of 0.5 mL and Jakobowicz et al. (1972) even 0.012–0.032 mL fresh red cells to be sufficient to induce IEA formation. We do not know if this is true also of frozen red cells present in the bone. Therefore further investigation, by means of registration of bone donor blood groups and testing of large groups of patients for IEA, is desirable.

We agree with Johnson et al. (1985) to give Rh(D) negative girls and women of childbearing age only Rh(D) negative bone, since this is a very simple measure. For other red cell antigens, matching is associated with more problems. In blood transfusion practice the typing of blood for other antigens than ABO and Rh(D) is not routine. Besides, the theoretical incidence of IEA, other than anti-Rh(D) is approximately 1% per unit transfused (Giblett 1977), and it is probably lower in bone allografting.

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