Erythrocytes in Human Transplantation: Effects of Pretreatment with ABO Group-Specific Antigens

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ABSTRACT Erythrocyte group antigens A and B can act as potent and group-specific transplantation antigens in man. ABO group-incompatible recipients pretreated with such antigens have rejected skin allografts obtained from donors incompatible for the same antigens in an accelerated (4–5 days) or white graft manner. Skin grafts applied to the same recipients from ABO-compatible donors were accorded first-set survival times. Intact erythrocyte suspensions and antigens isolated from hog (A substance) and horse (B substance) stomachs, were equally capable of inducing this type of allograft sensitivity. The latter observation broadens the spectrum of heterologous antigens capable of inducing allograft sensitivity in the mammalian host and provides a readily available, heat-stable, and water-soluble source of antigens for further studies of allograft rejection mechanisms in man.

INTRODUCTION

Erythrocyte groups were first implicated in human transplantation in 1919 by Shawan’s report that the use of blood grouping principles in skin grafting could result in prolonged allograft survival (1). The exact role of such antigens in conditioning allograft responses has, however, remained obscure for some years (2). The studies of Brown and McDowell (3) and of Woodruff and Allan (4) appeared to indicate that blood groups were of no particular significance in conditioning the rejection of skin allografts in man. In addition, Medawar (5, 6) provided cogent evidence that erythrocytes were not active as individual- or strain-specific transplantation antigens in experimental animals. In a different experimental setting, however, Barrett and Hansen (7) showed that erythrocyte stromata could sensitize mice to tumor transplants. More recently, Griffiths and Crikelair (8) and Kuhns, Rapaport, Lawrence, and Converse (9) described anti-A and/or anti-B antibody responses in human recipients of transplantation antigens (skin graft, leukocyte extracts) obtained from ABO-incompatible donors, once again implicating erythrocyte antigens in experimental allograft responses.

The possibility that erythrocyte antigens might also be of importance in organ transplantation was initially noted by Simonsen and Sorensen and Simonsen, Buemann, Gammeltoft, Jensen, and Jorgensen (10–12), who suggested as early as 1949 that the sharing of antigens by kidneys and erythrocytes indicated that gross biological incompatibilities between donor and recipient might be eliminated by avoidance of incompatibilities in the ABO group antigens. It is of interest in this regard that original reports by Hume, Merrill, Miller, and Thorn (13) of renal transplantation in man included one blood group O recipient of a
transplant obtained from a group B donor. This transplant ceased to function on the 7th post-operative day, and, although other variables were also implicated, the authors concluded that renal transplantation would be unwise in the face of major blood group incompatibilities. This observation has influenced Hume et al.'s selection of donors and recipients for renal transplantation since that time (14–17). Woodruff (18), Hamburger, Vaysse, Crosnier, Aubert, and Dormont (19), Goodwin, Mims, and Kaufman (20), and Murray and Harrison (21) have also expressed support for the concept that renal allografts should not be performed across major ABO blood group incompatibility barriers. It was not, however, until the carefully documented clinical studies of Starzl et al. (22–25) that the influence of ABO group incompatibility upon the fate of human renal allografts became fully established. Kidneys transplanted across major ABO group barriers were shown to risk a particularly rapid and violent type of rejection, whose tempo and intensity were related to a significant extent to pretransplantation anti-A or anti-B isoantibody titers in the recipients (26). A recent report of the Kidney Transplant Registry fully confirms this concept (27), and the observations of Jacobson and Najarian (28) that pretreatment of dogs with serologically incompatible erythrocytes may induce in the recipients a decrease in survival times of kidney transplants obtained from donors of the same erythrocyte group suggests that some form of isosensitization may have been implicated in the mediation of such responses.

It is the purpose of this study to assess the role of erythrocyte group antigens in human transplantation under experimental conditions designed to delineate the circumstances under which such antigens might be capable of inducing rapid allograft rejection. In preliminary experiments, blood group O recipients immunized with AB erythrocytes were noted to reject skin allografts obtained from other donors belonging to blood group AB in an accelerated manner, whereas grafts obtained from group O donors were rejected in first-set fashion (29). The present report describes responses to 94 ABO blood group-compatible and incompatible skin allografts in 19 recipients pretreated with ABO group-compatible or incompatible erythrocytes, or with water-soluble A, B, and O (H) antigens. The results indicate that pretreatment with A or B erythrocyte group antigens in the form of erythrocytes, or as water-soluble substances, induces in blood group O recipients a state of hypersensitivity to skin allografts obtained from donors of the same incompatible erythrocyte group (A or B). This sensitivity is expressed in the recipients by the white graft or accelerated rejection of the ABO-incompatible transplants. In contrast, skin grafts obtained from blood group O donors and applied to the recipients at the same time are accorded normal first-set survivals. The serum antibody responses observed in the recipients as a result of pretreatment and of subsequent challenge with skin allografts are described, with particular reference to their possible relationship to the types of graft responses observed.

METHODS

Selection of donors and recipients. Skin and erythrocyte donors and recipients were selected from a stable population of healthy volunteers known not to transmit homologous serum hepatitis on the basis of their previous records of blood and/or skin donations. These individuals were members of the panel of blood donor volunteers of the Institut de Recherches sur les Maladies du Sang, Laboratoire D’Immu-Hématologie, Hôpital Saint-Louis, Paris, France. Complete erythrocyte, leukocyte, platelet, and serum group determinations were available for this entire panel.

Basic plan of experiment. (1) Six group O recipients were injected with A\textsubscript{B}, A\textsubscript{O}, or B erythrocytes. 2 wk later they were tested with skin grafts obtained from group O, A\textsubscript{B}, A\textsubscript{A}, A\textsubscript{O}, and B donors.

(2) Four group A\textsubscript{1} recipients were injected with A\textsubscript{1} erythrocytes and tested with group O and group A\textsubscript{1} grafts 2 wk later.

(3) Five group O recipients were injected intradermally with soluble A or B substances; they were tested 2 wk later with grafts from group O donors, and with grafts obtained from group A\textsubscript{1} or B donors, respectively. In addition, one group A\textsubscript{1} recipient was pretreated with soluble B substance, and was then tested with grafts obtained from group B and group O donors. The final recipient in this series was a group A\textsubscript{1} subject who was pretreated in similar fashion with O (H) substance, and was tested with grafts obtained from donors of blood groups A\textsubscript{B}, B, and O.

Earlier studies of allograft rejection responses in man have indicated that the usual first-set skin allograft survival time is 10–12 days. In this study, as in previous reports, the accelerated (4–5 days) or white graft rejection of skin allografts have been interpreted as a manifestation of hypersensitivity of the host to the individual-specific and/or group-specific antigens present in the

Erythrocytes in Transplantation 2207
donors of such grafts. The white graft reaction has also
been considered as an expression of a higher state of
sensitivity than that expressed by graft rejection at 4-5
days (30-38).

Materials used for pretreatment of recipients. Ali-
quot of blood were obtained from each donor. They
were defibrinated in order to eliminate the blood plate-
lets, and they were then freed of leukocytes by seven or
eight successive sedimentations in Dextran (6% solution,
mol wt 200,000). No leukocytes were detectable in
the majority of the final erythrocyte suspensions used;
occasional lymphocytes were noted in some instances but
they never exceeded 3000-4000 cells in each preparation.
Soluble A substance was obtained from commercial
sources. This material, extracted from hog gastric mu-
cosa, was analyzed by Dr. Elvin A. Kabat of Columbia
University. It contained 58 gamma/ml of nitrogen; 295
gamma/ml of N-acetyl glucosamine; 136 gamma/ml of
galactose, and 94.5 gamma/ml of fucose. Soluble O (H)
substance extracted from hog gastric mucosa in similar
fashion, and containing the same nitrogen concentration
was also obtained from Dr. Elvin A. Kabat. This ma-
terial differs from soluble A substance only by the ab-
sence of the amino-sugar determinants present in A sub-
stance (39). Soluble B substance isolated from horse
stomach lining was obtained from commercial sources
(Knickerbocker Laboratories). Its composition has previ-
ously been described in detail by Baer, Kabat, and
Knaub (40).

The final erythrocyte preparations were suspended in
pyrogen-free isotonic saline solution, and were injected
intradermally in divided doses of 0.1-0.2 ml, into the
deltoid region of the shoulders of the recipients. The
water-soluble A, B, and O (H) substances were injected in
similar volumes in the same regions. In those in-
stances where intravenous injections of erythrocyte sus-
pensions were employed, the latter were also resuspended
in isotonic saline solution before injection.

Schedule of sensitization of the recipients. (1) Four
group O recipients were injected with A,B erythrocytes,
given in 4 consecutive wk injections of 6.4-9 x 10^6 cells.
The cells were injected intravenously in two recipients and
intradermally in the other two individuals. 1 wk after
the last injection, each subject received three skin allografts
from A,B and A,B donors, and three grafts obtained
from group O donors. Two other group O indi-
viduals received intravenous injections of similar amounts
of group A or of group B erythrocytes, respectively.
1 wk after the last injection, they were also tested with
grafts obtained from three group O donors, and with grafts
obtained from three group A or group B donors,
respectively.

(2) Four group A individuals were pretreated in
similar fashion with group A erythrocytes, injected in-
travenously in two recipients, and intradermally in the
other two instances. The erythrocyte doses used were
similar to those used above. 1 wk after the last injection,
all recipients were tested with grafts obtained from the
donor of the group A erythrocytes, and with grafts ob-
tained from three other group A, individuals. Because of
donor-recipient incompatibilities encountered in other
erthrocyte and serum group antigens, this experiment
also permitted an assessment of the role of the antigens
C, Le^a, M, N, Fy^a, P, S, and Gm^a in influencing allo-
graft responses under similar conditions.

(3) Seven individuals received intradermal injections
of soluble blood group substances A, B, and O (H).
The first four subjects received intradermal injections
of soluble A substance. Two individuals (HOG and
BAR) received 3 mg of A substance weekly for 4 con-
ssecutive wk, and were tested with skin grafts obtained
from three group O donors and from three group A
donors 1 wk later (i.e., 28 days after the first injection
of A substance). A third subject (VAI) received the
first two injections of A substance, but developed a mas-
sume inflammatory reaction at the injection site, which
resulted in a reduction of his last sensitizing dose to 0.3
mg of A substance. The fourth subject (BAR) only re-
ceived the first two doses of A substance (i.e., 6 mg)
for similar reasons. Subjects VAI and BAR were both
tested with grafts obtained from three group O and
three group A donors at 28 days after the 1st sensitizing
injection.

Comparable amounts of O (H) substance were in-
jected into a group A,B recipient, who was tested with
three grafts obtained from A,B donors, two grafts ob-
tained from O donors, and one graft obtained from a B
donor, 28 days after the first injection of O (H) sub-
stance. Two other recipients were injected in similar
fashion with soluble B substance, including one individual
of blood group O (TIC), and one group A recipient
(FIL). Both subjects were tested with grafts obtained
from three group O donors, and with grafts obtained
from three group B donors, 28 days after the 1st in-
jection of B substance.

Methods of grafting and of graft observation

The methods of grafting and of graft observation have
been described in detail in previous publications (30, 32,
34, 41). All grafts were full-thickness skin specimens
measuring 11 mm in diameter, placed on the anterior
surface of the forearms of the recipients. The transplants
were observed daily; gross and stereomicroscopic criteria
were employed for the diagnosis of graft rejection.
Such criteria included cessation of blood flow and throm-
busis in the superficial graft vessels, graft cyanosis, and
edema, and the development of erythema and induration
around the grafts (41). In the case of the white graft
reaction, the absence of vascularization, dead white color
of the grafts, and their evolution into a tan-colored
eschar, provided the landmarks for recognition of this
type of response (32).

Serologic Studies. Serum samples were obtained from
the recipients before pretreatment, and at weekly intervals
thereafter, until the 4th wk after graft rejection. The sera
were preserved at -22°C, and were inactivated at
56°C for 30 min before use. Each aliquot was tested for
its anti-A and anti-B hemagglutinin titers by standard
hemagglutination tests, utilizing 1% suspensions of hu-
man blood group A and B erythrocytes. The hemagglutinin titers are presented in the tables and text as reciprocals of the highest serum dilution at which definite agglutination occurred. Parallel hemolysin titer determinations were also performed by standard techniques.

Selected serum samples were diluted to 1:4 and were incubated for 1 hr at 37°C with equal volumes of 0.2 M 2-mercaptoethanol; they were then used immediately in hemagglutination tests (42). The immunoglobulin properties of high-titered antisera were also studied by sucrose density gradient ultracentrifugation (43). A volume of 0.5 ml of selected serum samples, at a dilution corresponding to 32–64 agglutinating U, was layered over a sucrose density gradient (10–40%). Separation was performed in a swinging bucket rotor (Spinco ultracentrifuge) at 95,000 g for 16 hr. Successive fractions were obtained by the drop collection method, and were examined by hemagglutination tests against group A and B erythrocytes after dialysis against saline.

RESULTS

(1) Pretreatment of group O recipients with ABO-incompatible erythrocytes. As noted in Table I, 18 skin allografts obtained from blood group O donors were accorded first-set survival times (6–13 days) in recipients pretreated with A₂B, A₁, or B erythrocytes. Skin grafts from group AB (A₁B or A₂B) donors were rejected in the same subjects as first-set grafts (6–8 days) in five instances, and in an accelerated manner (4–5 days) in three cases. Four other group AB grafts were accorded white graft rejection. The white graft responses occurred in recipients pretreated intravenously with A₂B erythrocytes.

In similar fashion, recipients CHAL and PHIL, who had received A₁ and B erythrocytes, respectively, rejected skin grafts obtained from other A₁ and B donors in accelerated fashion (4–5 days).

(2) Pretreatment of group A₁ recipients with A₁ erythrocytes. The results of pretreatment of group A₁ recipients with A₁ erythrocytes are illustrated in Table II. This technique failed to sensitize the recipients to skin allografts obtained from the erythrocyte donors or from other group A₁ individuals. All grafts were rejected as first-set grafts (8.5–15 days). These results provide an additional control for possible effects of minute concentrations of leukocyte contaminants in the erythrocyte preparations used in this study. The first-set rejection of grafts obtained from the specific erythrocyte donors indicates that such leukocyte contaminants did not have a significant influ-

<table>
<thead>
<tr>
<th>RECIPIENT</th>
<th>METHOD OF PREIMMUNIZATION EMPLOYED</th>
<th>SURVIVAL OF SKIN ALLOGRAFTS (DAYS)</th>
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<tr>
<td></td>
<td>ROUTE (cells)</td>
<td>AB GROUP</td>
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<tr>
<td>POU</td>
<td>3 x 10¹⁰</td>
<td>i.d.*</td>
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<td></td>
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</tr>
<tr>
<td></td>
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<tr>
<td>CHAS</td>
<td>3 x 10¹⁰</td>
<td>i.d.</td>
</tr>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAU</td>
<td>3 x 10¹⁰</td>
<td>i.v.†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHAC</td>
<td>3 x 10¹⁰</td>
<td>i.v.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHAL</td>
<td>3.6 x 10¹⁰</td>
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<td></td>
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<tr>
<td>PHI</td>
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* intradermal.
† intravenous.
§ white graft reaction.

Erythrocytes in Transplantation 2209
### TABLE II

**ERYTHROCYTE ANTIGENS IN HUMAN TRANSPLANTATION—EFFECTS OF PRETREATMENT OF A1 RECIPIENTS WITH A1 ERYTHROCYTES**

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donors</th>
<th>Route of Immunization</th>
<th>Erythrocyte Dose (cells)</th>
<th>Erythrocyte or Serum Group Incompatibilities Between Donors and Recipients</th>
<th>Survival Time of A1 Skin Allografts (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHA</td>
<td>FAU*</td>
<td>INTRAVENOUS</td>
<td>$4 \times 10^{10}$</td>
<td>$\text{Le}^b$ $\text{c}$ $\text{Le}^b$ $\text{E}$ $\text{c}$ $\text{Le}^a$ $\text{c}$</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>DRE</td>
<td></td>
<td></td>
<td></td>
<td>9</td>
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<tr>
<td></td>
<td>LHE</td>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>GOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOU</td>
<td>DRE*</td>
<td>INTRAVENOUS</td>
<td>$4 \times 10^{10}$</td>
<td>$\text{Le}^b$ $\text{c}$ $\text{Le}^b$ $\text{N}$ $\text{Le}^b$ $\text{D}$ $\text{E}$ $\text{c}$ $\text{Gm}^a$</td>
<td>10</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.5</td>
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<tr>
<td></td>
<td>FAU</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>GOH</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>MOR</td>
<td>LHE*</td>
<td>INTRADERMAL</td>
<td>$4 \times 10^{10}$</td>
<td>$\text{F}<em>{y}^{\text{PM}}$ $\text{F}</em>{y}^{\text{PM}}$ $\text{F}_{y}^{\text{PM}}$ $\text{Le}^b$</td>
<td>11</td>
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<td></td>
<td></td>
<td>15</td>
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<tr>
<td></td>
<td>DRE</td>
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<td></td>
<td></td>
<td>10</td>
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<tr>
<td></td>
<td>GOH</td>
<td></td>
<td>$\text{Le}^b$ $\text{F}_{y}^{\text{PM}}$ $\text{Le}^a$</td>
<td>$\text{P}$ $\text{S}$ $\text{Gm}^b$ $\text{P}$ $\text{S}$ $\text{Gm}^b$ $\text{P}$ $\text{S}$ $\text{Gm}^b$</td>
<td>12</td>
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<tr>
<td>GAU</td>
<td>GOH*</td>
<td>INTRADERMAL</td>
<td>$4 \times 10^{10}$</td>
<td>$\text{Le}^b$ $\text{c}$ $\text{Le}^b$ $\text{P}$ $\text{S}$ $\text{Gm}^b$ $\text{P}$ $\text{S}$ $\text{Gm}^b$</td>
<td>9</td>
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<tr>
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<td>FAU</td>
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<td>11</td>
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<tr>
<td></td>
<td>DRE</td>
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</tbody>
</table>

* Donor of erythrocyte suspension used in pretreatment of the recipient.

### TABLE III

**ERYTHROCYTE ANTIGENS IN HUMAN TRANSPLANTATION—EFFECTS OF PRETREATMENT OF RECIPIENTS WITH SOLUBLE BLOOD GROUP SUBSTANCES**

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Blood Group</th>
<th>Method of Preimmunization Used</th>
<th>Soluble Blood Group Substance Employed</th>
<th>Route of Administration</th>
<th>Dose (mg)</th>
<th>Survival of Skin Allografts (Days) Obtained from Donors of Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOG</td>
<td>0</td>
<td>A Substance</td>
<td>i.d. *</td>
<td>12</td>
<td></td>
<td>$\text{A}^1$ $\text{B}$ $\text{A}^1$ $\text{B}$</td>
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<tr>
<td>JAN</td>
<td>0</td>
<td>A Substance</td>
<td>i.d.</td>
<td>12</td>
<td></td>
<td>$\text{A}^1$ $\text{B}$ $\text{A}^1$ $\text{B}$</td>
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<tr>
<td>VAI</td>
<td>0</td>
<td>A Substance</td>
<td>i.d.</td>
<td>12</td>
<td></td>
<td>$\text{A}^1$ $\text{B}$ $\text{A}^1$ $\text{B}$</td>
</tr>
<tr>
<td>BAR</td>
<td>0</td>
<td>A Substance</td>
<td>i.d.</td>
<td>6</td>
<td></td>
<td>$\text{A}^1$ $\text{B}$ $\text{A}^1$ $\text{B}$</td>
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<tr>
<td>TIC</td>
<td>0</td>
<td>B Substance</td>
<td>i.d.</td>
<td>9</td>
<td></td>
<td>$\text{A}^1$ $\text{B}$ $\text{A}^1$ $\text{B}$</td>
</tr>
<tr>
<td>FIL</td>
<td>A1</td>
<td>B Substance</td>
<td>i.d.</td>
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<tr>
<td>COL</td>
<td>A1B</td>
<td>H Substance</td>
<td>i.d.</td>
<td>6</td>
<td></td>
<td>$\text{A}^1$ $\text{B}$ $\text{A}^1$ $\text{B}$</td>
</tr>
</tbody>
</table>

* i.d. = Intradermal.
ence upon the results observed. Review of other erythrocyte and serum group incompatibilities present between the A, erythrocyte donors and the recipients studied here also indicates that group antigens C, Le^b, M, N, Fy^a, P, and S were inactive as transplantation antigens under these experimental conditions.

(3) Pretreatment of group O, A, and A,B recipients with soluble blood group substances. 42 grafts obtained from group O, A, B, and A,B donors were applied to seven group O, A, and A,B recipients after pretreatment with watersoluble A, B, or O (H) blood group substances, respectively. As noted in Table III, group O recipients of A substance rejected nine grafts obtained from group A donors as white grafts; three other A,B grafts were rejected in an accelerated manner (4 days). The 12 group O grafts placed on the same recipients were accorded first-set survival times (7–22 days).

When two other recipients (group O subject TIC and group A, subject FIL) were tested with grafts obtained from group B and group O donors after pretreatment with soluble B substance, the group B grafts were rejected as white grafts in five instances and in an accelerated manner (4–5 days) in one case. The six group O grafts applied to the recipients were accorded first-set survival times (8–12 days).

The final recipient was a group A,B individual (COL) pretreated with O (H) substance. This subject rejected three grafts from A,B donors, one graft from a group B donor, and two grafts from group O donors as first-set grafts (7–11.5 days).

(4) Isoagglutinin titers in recipients of blood group antigens and skin allografts. Fig. 1 illustrates isoagglutinin titers observed in group O recipients pretreated with A, and B erythrocytes and tested with group O, A, or B skin allografts. None of the recipients developed any significant levels of anti-A or anti-B isoantibody. Pretreatment of group O recipients with soluble A substance, however, resulted in significant elevations in anti-A antibody titers (44). As noted in Fig. 2, two subjects received 4 consecutive wk 3 mg doses of A substance. The first recipient (JAN) had a preimmunization anti-A titer of 8; this rose to 128 1 wk later, and was 64 at the time of the third injection of A substance. It remained at that level for the remainder of the study. JAN accorded accelerated rejection responses to three grafts obtained from A, donors. The second subject (HOG) had an anti-A titer of 64 before immunization; this rose to 512 2 wk after the first injection of A substance, and remained at that level while three group A skin grafts were rejected as white grafts.

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Figure 1 Erythrocyte antigens in human transplantation. Serum antibody responses in recipients of ABO-incompatible erythrocytes.

Erythrocytes in Transplantation 2211
local reactions induced by injection of this with smaller doses of VAI received only 0.3 mg of A substance on the 21st day; the isoagglutinin titer was 512 at that time, and remained at this level at the time of white graft rejection of three group A1 grafts. The second recipient (BAR) had a base line anti-A antibody titer of 28; this titer rose to 8192 within 1 wk. At this time, the second injection of A substance also evoked an intense inflammatory reaction, and pretreatment was discontinued. This subject’s anti-A titer remained 8192 during the next 2 wk, and reached 32,778 at the time of white graft rejection of three skin transplants obtained from group A1 donors. Moderate increases in anti-B antibody titers occurred in parallel with the anti-A responses described.

The isoagglutinin responses observed in recipients of B substance are illustrated in Fig. 3. Subject TIC (blood group O) whose base line anti-B antibody titer was 32, received 9 mg of B substance. The titer rose to 512 2 wk after the first injection of B substance, and was 1024 at the time of skin grafting. Three grafts obtained from B donors were rejected as white grafts by this recipient. At that time, his anti-B titer was 512. TIC's anti-A antibody titer rose to 128 after the second injection of B substance, and was 64 at the time of skin grafting and of graft rejection. The second recipient, FIL (blood group A1) had an anti-B antibody titer of 2 before sensitization; it was 512 1 wk later, and rose to 1024 at the time of the last injection of B substance. This subject accorded

Figure 2 Erythrocyte antigens in human transplantation. Serum antibody responses in group "O" recipients of soluble "A" substance.

Two other group O recipients were pretreated with smaller doses of A substance because of the local reactions induced by injection of this material. The presensitization anti-A titer of the first subject (VAI) was 32; it rose to 1024 2 wk later. VAI received only 0.3 mg of A substance on the 21st day; the isoagglutinin titer was 512 at that time, and remained at this level at the time of white graft rejection of three group A1 grafts. The second recipient (BAR) had a base line anti-A antibody titer of 28; this titer rose to 8192 within 1 wk. At this time, the second injection of A substance also evoked an intense inflammatory reaction, and pretreatment was discontinued. This subject’s anti-A titer remained 8192 during the next 2 wk, and reached 32,778 at the time of white graft rejection of three skin transplants obtained from group A1 donors. Moderate increases in anti-B antibody titers occurred in parallel with the anti-A responses described.

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that observed after (31, 32, 36, 37) transplantation antigens. Serum antibody responses in recipients of soluble "B" substance.

white graft responses to two group B grafts, and an accelerated rejection response (4–5 days) to a 3rd group B graft. His anti-B antibody titer was 128 at that time. The anti-A and anti-B hemolysin titers paralleled these results.

Addition of 2-mercaptoethanol to selected serum samples did not significantly affect the anti-A or anti-B hemagglutinating activity of high-titer antiserum. Sucrose density gradient ultracentrifugation of such sera indicated that their anti-A or anti-B hemagglutinating activity was localized to the IgG fraction. These results demonstrate that the serum antibody responses induced in recipients of soluble blood group substances were primarily of an IgG nature (42, 43).

DISCUSSION

The ability of blood group O recipients to reject in an accelerated manner skin grafts obtained from group AB, A, or B donors after pretreatment with erythrocytes of the corresponding group indicates that such erythrocytes can act as potent and group-specific transplantation antigens in man. Under the experimental conditions described, such erythrocytes have induced in the recipients a state of hypersensitivity to skin allografts similar to that observed after pretreatment of the host with donor-specific (31, 32, 36, 37) or group-specific (33, 34, 45) transplantation antigens. The results suggest an experimental explanation for the decreased survival of kidney allografts transplanted across major ABO-incompatibility barriers (21–27), and are in agreement with the report of Jacobson and Najarian (28) that treatment of dogs with group-incompatible erythrocytes may decrease the survival time of renal allografts in the recipients. The failure of group A recipients to accord accelerated rejection responses to skin grafts obtained from group A donors after pretreatment with group A erythrocytes lends further support to the concept that the type of allograft sensitivity induced by incompatible human erythrocytes may be of a group-specific, rather than an individual-specific character. This possibility is strengthened by the first-set survival time accorded by these recipients to skin grafts obtained from the specific erythrocyte donors.

The accelerated and/or white graft rejection of group A and B skin grafts in group O recipients pretreated with the corresponding soluble blood group substance illustrates the potential effectiveness of soluble antigens as transplantation antigens in man. The isolation and solubilization of biologically active extracts of mammalian transplantation antigens has become recognized as one of the most difficult problems in transplantation biology. The present study reports the existence of a type of water-soluble, heat-stable antigens capable of
inducing strong allograft sensitivity in man. The implication of amino-sugars (glucosamine, galactosamine) as determinants of the A or B blood group specificity of such antigens (39) also suggests a potential approach to further studies of the biochemical specificity of the human transplantation antigens. The possible involvement of such determinants in conditioning responses of human recipients to allografts is strengthened by the observation that pretreatment of a group A,B recipient with O (H) substance failed to affect his responses to skin grafts obtained from group O, A,B, or B donors.

The ability of soluble antigens isolated from hog (A substance) and horse (B substance) stomachs to induce allograft sensitivity in man broadens the range of heterologous antigens implicated in the induction of mammalian transplantation responses. In this regard, the results may be pertinent to Brent, Medawar, and Ruszkiewicz's description of serologic cross-reactions between soluble A substance, pneumococcal polysaccharide, and the H-2 antigens of the mouse (46), and to the observation that group A streptococci and staphylococci can induce strong allograft sensitivity in rodents (47-51). The results are also in harmony with the recent detection of heterophile hemagglutinins directed against sheep, guinea pig, and rat erythrocytes in recipients of human transplantation antigens (52).

The mechanisms responsible for the accelerated and/or white graft rejection of grafts obtained from group A, B, or AB donors in group O recipients of the corresponding incompatible erythrocyte group antigens are not clear at this time. Humoral agents would appear to be implicated by the apparent relationship between serum isoagglutinin levels and the incidence of white graft responses in recipients of soluble blood group substances. This interpretation is consonant with Wilson and Kirkpatrick's observation of a similar relationship between preformed anti-A and/or anti-B antibody titers in the host and the rapid rejection of ABO-incompatible renal allografts (26). It would also provide a possible explanation for the occasional white graft responses described in group O recipients of skin allografts obtained from group A donors in the absence of any obvious pretreatment (53). Indeed, the presence of blood group A substance in commercial peptones (39) has introduced this antigen as a contaminant in bacterial vaccines, toxoids, and other materials administered parenterally to human recipients. Such treatment may result in the development of significant antibody responses in individuals lacking this antigen (54), and may be related to the recipient's subsequent response to tissue transplants obtained from group A donors.

Involvement of cellular effector mechanisms in the allograft responses described in this report is suggested, however, by the absence of detectable serum isoantibody levels in some pretreated recipients who rejected blood group-incompatible grafts in an accelerated and/or white graft fashion. The possible role of cellular factors in this regard is also strengthened by the demonstration that leukocyte extracts obtained from specifically sensitized donors are capable of mediating individual-specific skin allograft rejection (accelerated rejection) in man (55).

It is possible, however, that the mechanisms of graft rejection operative under the present experimental conditions are not related to any of the types of allograft responses described previously. A definitive conclusion on these possibilities awaits the biological testing of the cellular and/or humoral factors associated with this type of response. The relative capacity of such factors to transfer ABO erythrocyte group-specific skin allograft sensitivity to normal recipients is currently under investigation.

**ADDENDUM**

Results described in this report were recently confirmed by Visetti, Scudeller, Leigheb, and Cepellini. 1967. Importanza dei sottogruppi: A1-A2 e della reazione: crociante A-B per la sopravvivenza di allograft in vitro. *Minerva Dermatol.* 42: 563. These authors have also shown that A1 is a stronger transplantation antigen than A2, and that subjects sensitized with either soluble substance A or B could become cross-sensitized to skin allografts obtained from donors belonging to the other blood group. The latter observation is in keeping with the close structural similarities between A and B substances demonstrated by Kabat (39).

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2214  *Rapaport, Dausset, Legrand, Barge, Lawrence, and Converse*
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