Atypical Immunologic Tolerance in a Human Blood Group Chimera*

By PHILLIP STURGEON, DOROTHY T. McQuiston, ROBERT SPARKES, JOEL SOLOMON AND EUGENE V. BARNETT

A PERMANENT DOUBLE POPULATION of red blood cells may result from interfetal transplantation of hemopoietic tissue between nonidentical human1 or bovine2 twins. The condition was originally referred to as "chimerism." If the term, in its application to humans, is restricted to the equivalent of the bovine twins, possibly 14 additional cases have been reported.3-9 In all the relevant cases, the host's natural iso-hemagglutinins against the transplanted erythrocytes were absent. Aberrations in early embryonic or in prezygotic mechanisms also may result in genetically distinct blood cell populations.10-13 The resultant individuals are referred to as "mosaics" and show, in addition, generalized tissue chimerism.

In this paper "blood chimera" or "chimera" refers to an individual having a dual blood cell population which results from vascular anastomoses between dizygotic fetuses.14 The term "tissue chimera" refers to a more generalized chimerism which may include double populations of blood cells.

In one of the four reported tissue chimeras the blood cell differences did not involve the ABO system;10 in the other three, type O was associated with type A or B cells. In two of the latter, as in the blood group chimeras, incompatible isoagglutinins were not detected at any time.11,12 In the third the serum contained a weak anti-A on one occasion which did not react with the subject's cells and two years later was not demonstrable.

The person considered here appears to a blood group chimera, but unlike other cases, he is not a known twin and incompatible iso-hemagglutinins are present. The purpose of our studies was to determine which of the natural mechanisms resulting in dual cell populations is involved and to evaluate the...
nature of the immunologic adjustments which permit tolerance of a presumed foreign graft in the presence of circulating incompatible antibody.

MEDICAL BACKGROUND

The propositus, Robert Murray, is a Caucasian man, born September 10, 1913, who donated blood four times between November 1963 and February 1966; his cells and serum reacted as type O.* However, on the last two donations his cells, on crossmatch with the serum of a type O recipient, gave faint agglutination. Tests of his cells with anti-A in these laboratories showed weak agglutination in a field of unagglutinated cells. However, his own serum agglutinated strongly both A1, A2 and B cells. The possibility that this was one of the weaker variants of type A was soon dispelled when one of us (D. McQ.) observed a "mixed field" type of agglutination in tests of his cells with anti-M and with anti-S.

Robert is of northern European ancestry and has led a vigorous, healthy, normal life with no unusual illness; he has never received a blood transfusion. He is one of four sibs among whom there is a pair of fraternal twins. Although both of Robert's parents are deceased, older sibs and relatives who can recount the occasion of his birth have no knowledge of a co-twin. He is married, has three children and two grandchildren, all normal and healthy.

Physical examination reveals a robust, lean, healthy, light-complexioned man. There is no body asymmetry, heterochromia, gynecomastia or hypospadias; no abnormalities are noted in any of the organ systems.

METHODS

Robert's "native blood" was divided into its two cell components by differential agglutination using either anti-A, Dolichos anti-A1, or anti-M and the "agglutinates" were separated from the "free" or "inagglutinable" cells and dispersed with blood group substance.

Typing of Robert's native cells and particularly the dispersed agglutinated cells was complicated by spontaneous agglutination, when undiluted typing sera were employed. Weak false positive reactions also occurred upon addition of antiglobulin reagents but, for the most part, these were easily distinguished from the stronger specific reactions.

The routine serologic methods were those which have been standard in these laboratories over the past five years. All tests were done at least in duplicate by two technicians working independently and different reagents were used whenever possible.

Special quantitative studies were done of erythrocyte A and H antigens, of serum immunoglobulins using Hyland immuno-plates, of βC globulin, and of immunoglobulins in eluates prepared from red cells. Total hemolytic complement was determined and the agglutinated cells were tested with anti-γ-A, -G, -M and βC in microtiter plates with rabbit anti-human serum. The monospecificity of the immunoglobulin-specific anti-sera was confirmed by immunoelectrophoresis and by Ouchterlony double diffusion against whole human serum.

Survival of the native, agglutinated and free populations of red cells was followed by the 51Cr method. Reticulocyte counts on the separated samples were done by the brilliant cresyl blue method.

*The authors are indebted to Mrs. Teresa Atchison, Chief Technologist, and Dr. Herbert Lorberbaum, Medical Director of the Four Counties Red Cross Blood Center, San Jose, California for bringing this case to their attention and particularly for assistance in obtaining many of the blood samples required for this investigation.

†Hyland Laboratories, Inc., Los Angeles, California 90039

†We are indebted to Dr. William G. Figueroa for these tests.
ATYPICAL IMMUNOLOGIC TOLERANCE

Buccal sex chromatin analysis was made on slides prepared from both left and right sides and were read individually. Drumstick analysis was performed on Wright's stained blood smears; the number of cells with drumsticks per 500 neutrophils was determined. Chromosome analysis was performed on blood, bone marrow and skin, using modifications of standard technics. All samples were obtained the same day. Dermatoglyphic analysis was done on bilateral rolled fingerprints and palmprints. Evidence for asymmetry was evaluated by simple pattern comparisons and by digital ridge counts.

Serum group specific component (Gc) phenotypes were determined by Dr. Bern.

RESULTS

The phenotypes of the proband's (Robert Murray) native, free and agglutinated cells, and those from the other members of the family are given in Table 1. The nature of the s, Fyb, and Lu* reactions of Robert's agglutinated cells is doubtful because of their tendency to agglutinate spontaneously. The family tree with the most probable genotypes is given in Figure 1.

Four commercial anti-A sera gave weak to moderate positive reactions with Robert's native cells by a centrifugation technic; with two of the reagents free cells were clearly evident. Tests by a slide technic gave equivocal to weak reactions with many free cells; similar results occurred with anti-A1 of both plant and human origin. The reactions were not enhanced with seven different anti-A,B sera and the gross proportion of free cells remained the same. Mixed reactions were also noted with anti-M and -S and in one of two tests with anti-Lu*. All reactions with four anti-B sera were negative.

The specificity of the native cells' reactions with anti-A was confirmed by absorption and heat elution. The titer of the eluate with A1 cells was 1/8 and with A2 cells was 1/4. The strength of the A antigen was compared with normal type A1 and A2 cells in titrations of anti-A and anti-A,B; the end points with the latter cells varied from 1/256 to 1/512, whereas with the native cells they were 1/8 to 1/16. With an anti-A1 reagent (Dolichos) the titers with normal A1 and with the native cells were 1/8 and 1/4 respectively.

The possibility that the free cells might be a weak A variant was also studied. Both enzyme treated and untreated cells were tested with 13 different anti-A and several anti-A,B reagents; they did not agglutinate. Furthermore, absorption and elution studies with these cells and with those of Lyle (Robert's type O brother) eliminated the possibility of their being the weakest of A variants, A*.

Quantitative hemagglutination studies of the native blood in April, 1966 with several human anti-A and anti-A,B and with anti-A1 (Dolichos) gave values of 42.6 ± 8 per cent A cells. A second study in June, 1966 showed 35.02 ± 2.0 per cent A cells. On both occasions, the same percentage of cells was agglutinated by anti-A, anti-A,B and anti-A1; there is, therefore, no indication of an array of heterogeneous A phenotypes.

Quantitative anti-A assays were performed with the native blood, A1 and A3 cells in parallel with agglutinated cells using Dolichos lectin (Fig. 2). The differences in their slopes show that the native cells are qualitatively distinct from A3 cells but are similar, if not identical, to the A1 antigen of standard A1 cells; quantitatively they are much reduced in potency.

In three separate experiments using different commercial anti-A sera, the log2 potencies of A1 and the agglutinated cells differed by six to eight doses.
Table 1.—Blood Types of the Robert Murray Kinship

<table>
<thead>
<tr>
<th></th>
<th>Murray, Lyle</th>
<th>Tucker, Virginia</th>
<th>Murray, Jean</th>
<th>Murray, Robert</th>
<th>Murray, Wife of Robert</th>
<th>Murray, Kathleen</th>
<th>Moll, JoAnn</th>
<th>Murray, Richard</th>
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<td>A1B</td>
<td>B</td>
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<td>O</td>
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<td>Lu(a−)</td>
<td>Lu(a+)†</td>
<td>Lu(a−)</td>
<td>Lu(a+)†</td>
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<td>H</td>
<td>AH</td>
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</table>

* Many free cells.
† Suggestion of free cells.
at the 50 per cent level of agglutination. One experiment is shown in Figure 2 where the difference is 8.4 log₂ doses; the experiment illustrated by Figure 3A yielded a log₂ difference of 6.8 doses. In each case, the slopes of the regression lines do not differ significantly. In contrast anti-A₁ assays with the agglutinated cells and with the A₁ cells of Robert's wife and of daughters Kathleen and JoAnn revealed differences of 0.6-1.2 log₂ doses (Fig. 3B). Again, slopes are not significantly different.

With anti-A,B the slopes show a statistically significant difference and the prozone with the agglutinated cells is especially pronounced (Fig. 3C). The log₂ difference is 3.3 but the nonparallelism of the A₁ and agglutinated cells' assay curves makes this calculation less useful for comparative purposes.

Assays with Ulex europaeus anti-H showed that the agglutinated cells had more H reactivity than the type O free cells (Fig. 3D) and the slopes are virtually identical. The only A variants giving such results with Ulex are Aₓ and Aᵧ. The log₂ potency difference between the agglutinated cells and reference A₁ cells, assayed with Ulex, was 4.8 tubes; again, this calculation
Fig. 2.—Quantitative anti-A assays performed with Robert's cells compared to those with two A variants.

Fig. 3.—Quantitative anti-A and anti-H assays. Curves labeled “Robert” are from assays performed with agglutinated cells.
has limited utility because of the difference in slopes of the two curves. This wide difference is somewhat unusual. In assays of 50 random A1 cells, we found that H reactivity ranged from 5.9 log₂ doses greater than that of our reference A1 cells to 2.2 log₂ doses less, the limit of our Ulex reagent; the median was + 0.7 log₂ doses. One experiment showing assays of the two highest scoring A1 cells is shown on Figure 3E. H. L. is an A'O male; A. L. is his 13 year old group O daughter. The genotype of R. J. is unknown. The reference A1 cell (J.M.S.) is from a known A'O. Note that even the strongly reactive H. L. and R. J. cells give assay curves with slopes only slightly greater than those of the reference A1 cell and far lower than that obtained with the O cells of A.L. In assays with anti-A1 however, R. J., H. L. and the reference A1 cell gave identical slopes and log₂ potencies that covered a range of less than 0.5 dose.

Tests of Robert's serum at room temperature with six examples of type A1, four of A2 and two of type B cells gave positive reactions; whereas there was none with his own nor with ten examples of type O cells. The saline isoagglutinin titers against A1, A2, and B cells were 1/16, 1/8, and 1/8 respectively at 16 C.; at 37 C. the endpoints occurred at one dilution less, and with enzyme (Ficin) treated cells the endpoints occurred at one dilution greater. Absorption and elution studies showed that his serum sensitized the weak A variants A3, A5, and A9. The anti-A could be removed completely from his serum by two absorptions with equal volumes of either A1 or A2 cells at 4 C. Possibly there was some loss in anti-B titer with this treatment; the converse obtained following absorption with type B cells. "Cross reactivity" was also demonstrated when the appropriate eluates were tested with ficinized A1, A2, and B cells.

The blood group activity of Robert's serum was neutralized by commercial blood group substance and by saliva from secretors of A but not by saliva of type A nonsecretors nor from secretors of only H. A careful search of the serum for other isoantibody activity by a variety of methods at 16 and 37 C. was negative; specifically, anti-M and anti-S were not demonstrable, and autoagglutination did not occur at either temperature.

From examination of the family tree given in Figure 1 it is apparent that the A antigen in Robert's blood must be determined by the same gene as that responsible for the A determinants of Virginia's type AB blood. Robert's serum was absorbed repeatedly with type B cells at 4 C. until it would no longer agglutinate the absorbing cells nor those of Jean (whose B antigen must be determined by the same gene as that which determines Virginia's). Virginia's cells were then tested with the absorbed serum; strong agglutination, comparable to that noted with other type A1 cells, occurred.

Mercaptoethanol treatment of the serum essentially eliminated the 16 C. saline anti-A and -B activity in Robert's serum. The indirect antiglobulin activity, however, was unchanged and, perhaps, even enhanced by this treatment while the saline agglutinating activity against ficinized cells was substantially reduced but not eliminated. These findings are consistent with the presence of both IgM (and/or IgA) and IgG classes of isoagglutinins. Type A2 cells were sensitized in his serum at 4 C. for 12 hours and eluted into 6 per cent.
bovine albumin by heating to 56 C. for seven minutes. Eluates were also prepared by mixing the sensitized cells with a solution of commercial A and B substance. Quantitation of immunoglobulins, Table 2, revealed only \( \gamma G \) globulins; the quantities of \( \gamma A \) and \( \gamma M \) did not differ significantly from the type O control cells.

Reagent anti-A was absorbed four times in sequence at 37 C. for 15 minutes with thoroughly washed packed native cells in the ratio of five volumes of serum to four of cells. Parallel absorptions of reagent anti-A were also done with normal \( A_1 \) and \( A_2 \) cells in the ratio of 5/1.6. Samples from each absorption were set aside for saline titrations at 37 C. with type \( A_1 \) and \( A_2 \) cells, different from those used in the absorptions, and with Robert's native cells and with Virginia's cells. The results summarized in Table 3A show after one or more in vitro absorptions of a reagent anti-A that an antiserum is prepared having the characteristics of Robert's in vivo serum. A similar study using reagent anti-A,B serum gave comparable results. Other absorptions performed at room temperature (25 C.) in parallel with equal volumes of \( A_1 \) and Robert's native cells and with O cells revealed some interesting differences between the anti-A specificities of group O and group B sera. As shown in Table 3B, six 25 C. absorptions with Robert's cells were required to exhaust the activity of the group B serum against normal \( A_1 \) cells but were not sufficient to do so with the group O serum.

Robert's undiluted saliva was added in equal volumes to each tube in a serial dilution of a reagent anti-A which was then tested with type \( A_1 \), \( A_2 \) and native cells. There was no reduction in titer. Similar results were obtained with anti-B. Robert's saliva diluted 1/2 inhibited completely an anti-H reagent and at dilutions of 1/4 and 1/1000 it inhibited anti-Le\(^a\) and -Le\(^b\) respectively. His undiluted serum (after absorption of the A isoagglutinins) showed no inhibition of reagent anti-A. Robert's salivary phenotype is therefore that of a secretor of H, Le\(^a\), and Le\(^b\) corresponding to the O, Le(a-b+) type of his unagglutinated cells.

Repeated direct tests of the native cells with an antiglobulin reagent of unknown immunoglobulin specificity gave weak reactions. With a specific anti-\( \gamma M \) reagent there were many moderate to large sized agglutinates in a background of free cells. The agglutinated cells (following disassociation) also gave strong reactions without free cells; his free cells did not react.

<table>
<thead>
<tr>
<th>Cell Types Used in Absorption of Robert's Serum</th>
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<tbody>
<tr>
<td>( \gamma G ) 12.18 ( \mu /ml. )</td>
<td>11.76 ( \mu /ml. )</td>
<td>2.2 ( \mu /ml. )</td>
</tr>
<tr>
<td>( \gamma A ) 3.3 ( \mu /ml. )</td>
<td>3.3 ( \mu /ml. )</td>
<td>5.2 ( \mu /ml. )</td>
</tr>
<tr>
<td>( \gamma M ) 3.9 ( \mu /ml. )</td>
<td>3.9 ( \mu /ml. )</td>
<td>2.9 ( \mu /ml. )</td>
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</table>

- Heat eluted into 6 per cent bovine albumin.
- Eluted into AB substance.
- Heat eluted into AB substance in 6 per cent bovine albumin.
Table 3A.—Influence on Titer of a Reagent Anti-A Absorbed at 37 C. with Robert's and Other Type A Cells

<table>
<thead>
<tr>
<th>Cells Used in Titrations</th>
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<th>A2</th>
<th>A3</th>
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<tr>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Absorbed with:</td>
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</tr>
<tr>
<td>A1</td>
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<td>1/16</td>
<td>-</td>
</tr>
<tr>
<td>Virginia</td>
<td>-</td>
<td>1/16</td>
<td>+</td>
</tr>
<tr>
<td>A2</td>
<td>0</td>
<td>1/32</td>
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<tr>
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<td>1/128</td>
<td>-</td>
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<tr>
<td>After First Absorption</td>
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<td></td>
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<tr>
<td>Absorbed with:</td>
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<tr>
<td>A1</td>
<td>0</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Virginia</td>
<td>-</td>
<td>1/8</td>
<td>+</td>
</tr>
<tr>
<td>A2</td>
<td>0</td>
<td>1/32</td>
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<td>1/128</td>
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<td>After Second Absorption</td>
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<td>A1</td>
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<td>+</td>
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<tr>
<td>A2</td>
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<td>Robert</td>
<td>0</td>
<td>1/128</td>
<td>1/128</td>
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* Pluses indicate the strength of reaction with undiluted serum where ½ and ¼ dilutions were not tested and the ¼ dilution gave a negative reaction.

Table 3B.—Influence on Titration Score of Group B and Group O Sera Absorbed at 25 C with A1, Robert's and O Cells.

<table>
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<th>Absorbed Sera Tested with A1 Cells</th>
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<th>Group O Serum</th>
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<td>Robert</td>
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<tr>
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<td>1st absorption</td>
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<td>2nd absorption</td>
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<td>0</td>
<td>3</td>
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<td>6th absorption</td>
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The tendency toward mixed field clumping of Robert's native cells when exposed to serum from type AB and A donors was studied. Out of 19 inactivated sera, 16 produced a trace to a slight reaction; his own serum did not. With the serum giving the strongest reaction, all other members of the kinship were tested; none reacted. The agglutinated fraction of his cells gave reactions of moderate to strong intensity involving all cells; the reaction was detectable through 1/4 dilutions of the serum in saline and was not significantly altered.
by enzyme treatment of the cells nor by testing at 16 rather than 37 C. The
free cells did not react with any of the sera. This reaction did not occur in
saline, 6 per cent human albumin nor in 2 per cent Cohn fractions III, 1 per
cent IV-I, 0.5 per cent IV-I and 5 per cent V.
Heat eluates of Robert's native and agglutinated cells gave negative re-
action in saline at 16 C. with A1 or A2 cells and also when tested by the
ficinized or indirect γM antiglobulin technic. Mixed agglutination of native
cells with A1 cells did not occur. A 1/3200 dilution of commercial A substance
was found just capable of completely inhibiting the agglutination of A1 cells
by a 1/16 dilution of anti-A. The dilute A substance was exposed to an equal
volume of washed native cells and the cells removed by centrifugation. No
loss of inhibiting activity of the treated substance could be demonstrated.
Appropriate controls using artificially sensitized cells were positive.
Studies by the antiglobulin titration technic of the materials absorbed to the
native cells (Table 4) revealed the presence of γG, γA and γM im-
munoglobulins and β1C globulin. The reagents in lower titer gave false
positive reactions with normal O cells. Nevertheless, the titer achieved with
the native cells was significantly greater than that achieved with normal A
cells. The β1C globulin level of Robert's serum was 155 mg. per cent and the
total hemolytic complement level was 97 units. Quantitation of immuno-
globulins in the whole serum revealed a concentration of γG of 960 mg. per
cent, γA of 206 mg. per cent, and γM of 34 mg. per cent. The lower limit
of normal for γM is 50 mg. per cent.
51Cr auto survival using native blood was done and the rate of decay was
followed in the native blood as well as in both the agglutinated and the free
cells. The specific activities of the native blood and of the fractions were
calculated relative to their hemoglobin content. No significant difference was
found in the survival times (see Fig. 4). Reticulocyte counts of the two
fractions were within normal limits and not significantly different.
Buccal sex chromatin analysis showed per 100 cells from each side: right,
2 per cent positive; left, 1 per cent positive. A drumstick count on neutrophils
in the peripheral blood showed none in 500 cells.
The results of chromosome analyses are summarized in Table 5. The karyo-
types from both the blood and bone marrow show a consistent normal XY
male pattern. The chromosome counts in the fibroblast culture established
from a skin biopsy from the left side (biopsy from right side failed to grow)

<table>
<thead>
<tr>
<th>Test</th>
<th>Red Cells</th>
<th>Anti-w.s.*</th>
<th>Anti-γG</th>
<th>Anti-γA</th>
<th>Anti-γM</th>
<th>Anti-B1C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robert</td>
<td>0</td>
<td>4†</td>
<td>4</td>
<td>8</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* w.s.—whole serum
† Reciprocal of highest dilution of antiglobulin reagent resulting in agglutination of test red blood cells.
showed considerable aneuploidy with changes from the diploid number 46 all being in the hypodiploid direction. However, all cells have five G group chromosomes indicating the presence of a Y chromosome. Further, the chromosome loss in the hypodiploid cells was random. The results in the fibroblast culture all suggest that the aneuploid cells are due to technical artifact and that the skin chromosome complement is also nonmosaic 46 XY.

The dermatoglyphic analysis shows the axial palmar tri-radii are in the t position bilaterally. Digital patterns show five ulnar loops on the right side with a total ridge count of 68; on the left side one radial and four ulnar loops are present with a total ridge count of 54. The right-left digital ridge count difference is thus 14. Comparison of the findings in our subject with a large group of persons studied by Holt does not indicate an unusual degree of asymmetry.

Studies of the group specific component (Ge) factors demonstrated that

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**Table 5.—Results of Chromosome Analyses**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>42</th>
<th>43</th>
<th>44</th>
<th>45</th>
<th>46</th>
<th>Karyotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Marrow</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All normal XY</td>
</tr>
<tr>
<td>Blood</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All normal XY</td>
</tr>
<tr>
<td>Skin (left)</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>36</td>
<td>Six full karyotypes of 46 chromosome cells are normal XY. All cells have 5 G-group chromosomes</td>
</tr>
</tbody>
</table>
Robert is type 2–1 and his siblings are either 2–1 or 2–2; his wife is 2–2 and their three children are all 2–1.

**Discussion**

From the data presented and according to the classification employed in this paper the subject appears to be a “blood chimera” as the result of an intrauterine transfusion through chorionic vascular anastomoses with an undetected twin. His two erythrocyte populations differ by three blood factors in two blood group systems or gene loci and probably by a fourth factor in a third system. A more generalized chimerism is not evident from gross physical examination, from dermatoglyphic studies or from the cytogenetic analyses including studies for X chromosome mosaicism.

A number of possible mechanisms could be responsible for these findings. Pattern gene loci exist for certain characteristics, such as hair color in the Waardenburg syndrome; to date this situation has not been described for the blood types. Variation in gene expression occurs for X-linked conditions in the female due to the single active X phenomenon or due to a variegated position effect when genes are transferred between euchromatic and heterochromatic regions of chromosomes. Neither of these seems likely because the chromosome analyses showed only a single X chromosome and there were no recognizable chromosome rearrangements. Theoretically cytoplasmic genes could account for the observations but there is no indication that the genes for red cell types are of this nature. Because three genetic loci are probably involved, both somatic crossing-over and somatic mutations seem unlikely. There is also no evidence that somatic segregation and reduction have occurred to account for the observations. Because no cells with an XX sex chromosome constitution were found, it is unlikely that a maternal-fetal cell exchange has occurred. Also he never received a blood transfusion or other transplantation.

While double fertilization, i.e., fertilization by two sperm, could account for the findings there is no evidence that tissues other than the red blood cells are involved in the observed chimerism. The temporary acquisition of new erythrocyte antigens secondary to disease is an equally unlikely explanation inasmuch as the subject is well, the condition has been constant over the past three years, and because differences have been found in multiple genes.

The explanation of the subject’s dual cell population simply on the basis of blood chimerism is wanting, however, because of the absence of a co-twin. However recently an instance of possible blood chimerism was reported in an individual who had no twin. Nevertheless in our subject’s family there is, by history, twinning in other generations, and his sibs include a pair of fraternal

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*It is possible, though unlikely, that two sperm bearing Y chromosomes and differing in two or possibly three blood group genes but bearing more or less identical genes for skin pigmentation, eye color, body build and dermatoglyphic symmetry could be involved. Also, since the subject is a secretor of only H substance, the sperm bearing the A₁ gene would have to bear se and that bearing the O gene, Se and both ova would have to be se; there is, however, no evidence of a se gene in this kinship. Finally, were these conditions met, one would expect to find substantial amounts of Le⁺ salivary substance unless the A₁ tissue cells were le/le; there is no evidence of the latter gene in this kinship.
twins. Furthermore, although the subject was the product of what is recalled as a normal pregnancy, he was of low birth weight but thrived under relatively primitive conditions. *

Conditions for the establishment of blood chimerism can exist in early embryonic life. In the bovine embryo the primordia of both the germ and the hemopoietic cells have been observed in the circulation of 25 to 31 day old fetuses. While normal pregnancy, he was of low birth weight but thrived under rela-

Conditions for the establishment of blood chimerism can exist in early embryonic life. In the bovine embryo the primordia of both the germ and the hemopoietic cells have been observed in the circulation of 25 to 31 day old fetuses. During a comparable age in human fetuses primordial germ cells complete their migration from the yolk sac to the gonadal ridge. Since this is the stage when the exchange of embryonic cells most likely takes place leading to bovine chimerism it may be that human chimerism is also established at this time of development. The conclusion that our subject's phenotype prior to these events was type O is based on finding he is a salivary secretor of H but not of A substance.

Besides the absence of a co-twin, the subject presented here is unique among human blood chimeras because his type A transplant has not suppressed formation of the corresponding isoagglutinin. Nevertheless his anti-A does readily agglutinate a sib's blood whose A antigen must be the product of the same gene as that which determines the A antigen of the transplanted cells. However, except for his serum's failure to agglutinate his transplanted A cells, it cannot be distinguished from ordinary anti-A or A,B.

In contrast to the almost normal characteristics of the subject's anti-A, the A cells of the transplant show substantial modification. Fifty per cent agglutination of his A cells required approximately 256 times the concentration of antibody necessary for 50 per cent agglutination of control A cells. Qualita-

The agglutination of the cells in inactivated serum and in the direct anti-

*We are grateful for the assistance given to us by the Registrar of Cripple Creek, Colorado, Ethel M. Pedrie, who sought out the birth certificate and physician's records for information pertaining to the medical circumstances of Robert's birth.
and attenuated A antigen may not be related, it appears quite possible that they are.

A hypothetical mechanism responsible for the two phenomena is one of specific first and second stage antigen-antibody complexing of bivalent antibody to a single particle (the red cell) bearing multiple antigenic sites. These antibodies would then become effectively monovalent and agglutination would not occur. The absence of mixed agglutination with normal A1 cells, and of reactivity with soluble A antigen might also be explained. Such an interaction of antibody with the red cell is consistent with recent theories on the gross structure of antibody molecules, with studies on the nature of binding of immune rabbit 7S antibody to human A1 cells, with theories on the mechanism of interaction between virus and antibody and with electron micrographs which are interpreted as showing bivalent attachment of single antibody molecules to wart and influenza virus particles. However, in our subject, the evidence suggests that the red cell bound material is predominantly IgM in nature and, if such antibody is polyvalent, with estimates varying from six to ten binding sites, it is more difficult to visualize how a single cell bearing multiple antigenic sites might tie up all of the specific groups of a molecule having a rigid symmetrical structure. However, if the valences have an effective polar disposition at the extremities of "long, very flexible legs" such would still be possible. Total binding of all valences of 19S Forssman antibody by sheep erythrocyte membrane is strongly suggested in electron micrographs. Such a configuration could leave exposed the reaction site for antiglobulin sera. At the same time it could be more stable, more resistant to elution and to interaction with other antigen than configurations involving bridging between two relatively massive cells. Thus the absence of shortened 51Cr red cell survival could also be explained and a plausible explanation can be set forth for the availability of binding sites on the transplanted cells that react with standard anti-A but not with the host's isoagglutinins.

This explanation assumes that there is variation in binding affinities of A antigens and of anti-A. The anti-A of the host, absorbed by stable interaction with the transplant, would be comprised of antibody with weak affinity and would not complex with the remaining antigens because of their weak antibody-binding affinities. Whereas a standard reagent, not depleted of its stronger binding anti-A molecules, could interact with the transplanted cells' uncomplexed weak A antigens and conversely, the host's anti-A could react with normal A1 cells whose stronger antibody-binding sites were not blocked. The absorption experiments are entirely consistent with this hypothesis as are all of the quantitative hemagglutination studies with one exception; that is, the H slope is more like that of type O cells.

On the basis of the antigen-antibody complexing postulated above, the unique serologic characteristics of the A1 transplant in, and its "tolerance" by the host is explained. In the classic sense, however, such a serologic adaption may not fit the definition of tolerance. If "... the tolerant state is due to a central failure of the immune response rather than to an interference with its inception or fulfillment (author's italics)..." then our subject's physiologically
ATYPICAL IMMUNOLOGIC TOLERANCE

nonreactive state is not one of tolerance. The argument might be advanced, however, that his own lymphoid tissue is producing anti-A non-reactive with antigenic determinants of the transplant’s antigen but reactive with all other A antigen determinants. However, against this is the fact that our subject’s serum agglutinates the A cells from his sister whose A antigen is the product of the same gene as that which determines his A antigen. The immunologic studies indicate strongly that tolerance herein is the result of modification of the transplanted antigen rather than suppression of the host’s central immunologic response. It would be more appropriate to conclude, therefore, that his state is not one of “tolerance” but rather one where the physiologic consequences of immune activity have been thwarted.

This immunologic situation bears a closer resemblance to the abrogation of the rabbit’s response to skin homograft resulting from the prior intravenous injection of living epidermal or blood cells or with the use of both killed cells or with passive transfer of serum. There are similarities to the phenomenon of “split” tolerance. The situation also may be akin to the phenomenon of “immunologic enhancement” of tumor grafts with prior exposure of the host to cells from the donor.

Although there is much evidence that tolerance in this case is directly associated with modification of the transplanted antigen by antibody, the “O like” slope of the anti-H assay curve is consistent with a more direct biochemical modification. If the host is capable of biochemical modification and augmentation of the transplant’s H antigen, it is reasonable to expect this would be associated with a reciprocal attenuation of the A antigen. Attenuation of the transplant’s A antigen and augmentation of H could be mediated by anti-A interfering with the enzymatic addition of N-acetylgalactosamine to H-active chains at the membrane surface. The induction of tolerance by this mechanism also would not be that of central inhibition, as postulated to be operating in blood group chimeras, but would be more one of “afferent inhibition.”

Although our subject’s state of immunologic unresponsiveness is apparently unique among blood group chimeras, there are many examples of the coexistence of human red cell antigens and specific antibodies and their complexing in the absence of physiologic consequences. Instances of autoantibody specific for one or more of the host’s Rh-Hr antigens are known. In some cases active hemolysis has been absent in spite of evidence of antibody binding at physiologic temperatures. A positive direct Coombs’ test in a healthy individual without evidence of hemolytic anemia is not an uncommon observation in the blood grouping laboratory. Moreover, a case of fulminating autoimmune hemolytic anemia that reached a state of compensation without clinical or laboratory evidence of hemolysis, despite the persistence of a strongly positive direct antiglobulin test has been observed.

The apparent contradiction of antibody in the presence of its antigen is also seen in cases of B-like antigen acquired by A subjects. This has been observed in people 57 to 85 years old, most of whom had gastrointestinal cancer.
a bacterial enzyme is thought to confer B specificity on some nonspecific substance present in all red cells.

The transitory presence during pregnancy of specific antibody was also observed in the first reported case of human chimerism.\textsuperscript{65} Should similar observations be made in other cases, studies for changes in strength of the transplanted antigen and the adsorption of $\gamma$-globulin coincident with the appearance of antibody would be particularly illuminating.

**Summary and Conclusions**

A blood specimen from a normal male donor was found to have both type A\textsubscript{1}, MNS and O, Ns cells. Gross physical examination, chromosome analysis, bilateral dermatoglyphic comparisons, blood grouping and secretor studies lead to the conclusion that interfetal transplantation of erythropoietic tissue is the most likely basis for the state of blood chimerism in this individual. Unlike other well-documented examples of human blood group chimerism there is no co-twin. However, a strong history of twinning in the family, a pair of fraternal twins among the sibs and a neonatal history of low birth weight but full maturity indicates that the individual may have been a twin. It is postulated that a co-twin died and was absorbed in early fetal life.

Another unusual feature of the case is the persistence of anti-A isoagglutinins in the type O host despite the A\textsubscript{1} transplant. The isoagglutinin reacts at body temperatures with the product in another sib of the A gene responsible for the transplanted A antigen. There is $\gamma$-globulin, predominantly $\gamma$M, on the transplanted cell surfaces, but there is no evidence of accelerated blood production or destruction. Quantitative studies of the transplanted A antigen reveals it to be greatly reduced in potency but qualitatively of type A\textsubscript{1}; its H is possibly enhanced and qualitatively like that of type O.

Because of the contradictory immunologic features in this case, consideration is given to unusual mechanisms of tolerance which might permit a transplant to survive in the presence of specific antibody. The favored hypothesis is that multivalent binding of iso-antibody molecules to multiple antigenic sites on single red cells is involved. This type of complexing could account for the reduction in antigen strength yet normal red cell survival. The data presented are also consistent with the possibility of biochemical modification of the transplant cell's antigens. This is indicated not only by attenuation of its A antigen but also by suggestive augmentation of its H antigen. Considerations are given to how such modification could be mediated serologically by host antibody.

To date, central inhibition of erythrocyte isoagglutinin production has been the implied mechanism responsible for transplant compatibility in cases of both bovine and human blood group chimerism. Nevertheless, in this case, whatever the mechanism might be, central inhibition of antibody to at least one of the transplant's antigens is not operating. Therefore, human blood group chimerism may be achieved by means other than that of classic immunologic tolerance; otherwise A B O antigens would have to be considered as other than transplant antigens.
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SUMMARIO IN INTERLINGUA

Esseva trovate que un specimen de sanguine ab un normal donator mascule contineva cellulas typo $A_1$, MNS e cellulas typo $O$, Ns. Le macro-examination physic, analyse chromosomatic, bilateral comparationes dermatoglyphic, grupamento de sanguine, e studios secretori supporta le conclusion que transplantation interfetal de tissu erythropoietic es le base le plus probable pro le stato de chimerismo sanguinee in iste individuo. Per contrasto con altere bendocumentate exemplos de chimerismo de grupo de sanguine human, il existe in iste caso nulle fratemo bigeminal. Tamen, un forte tendentia de gemination in le historia del familia, un par de geminos fraterne inter le consanguineos immediate del subjecto, e antecedentes neonatal de un basso peso natal (associate con maturitate complete) indica que iste probando eseva possiblemente un gemino. Es postulate que le secunde gemino moriva e eseva absorbate in un phase precoce del vita fetal.

Un secunde aspecto inusual de iste caso es le persistentia de isoagglutininas anti-A in le hospite de typo $O$ in respecto de un transplantation de $A_1$. Le isoagglutinina reage a temperatura corporee con le producto in un altere cofraterno del gen A responsable pro le transplantate antigeno A. Il existe globulina $\gamma$, predominantemente $\gamma M$, al superficies del transplantate cellulas, sed il ha nulle evidentia de un accelerate production o destruction de sanguine. Studios quantitative del transplantate antigeno A revela que illo es grandement e reducute in potencia sed remane qualitativemente del typo $A_1$. Su H es possiblemente promovite e qualitativamente simile a illo de typo $O$.

A causa del caracteristicas immunologic contradictori in iste caso, attention es prestate a inusual mechanismos de tolerantia que permitte possiblemente le supervivencia de un transplanta in le presentia de anticorpore specific. Le hypothese preferite es que multivalent ligage de molecules iso-anticorporee a multiple sitos antigenic al superficie de erythrocytos individual es lo que occurre. Iste tipo de complexation explica possiblemente le reduction in le potencia antigenic in association con normal supervivencia erythrocytic. Le datos presentate es etiam compatibile con le possibilitate de un modification biochimic del antigenos del cellulas transplantate. Isto es suggestionate non solmente per le attenuation de su antigeno. A sed etiam per le evidentia pro un augmento de su antigeno H. Es prendite in consideration le question de como un tal modification pote esser mediate serologicamente per anticorpore del hospite.

Usque a iste tempore, inhibition central del production de isoagglutinina erythrocytic ha essite acceptate como le mechanismo responsabile pro le compatibilitate de transplantas in casos de bovin e human chimerismo de grupo sanguinee. Nonobstante, in le presente case—sia que sia le mechanismo—inhibition central de anticorpore anti al minus un del antigenos del transplanta non es presente. Per consequente, chimerismo de grupo de sanguine human pote esser effectuat per medios altere que illo del classic tolerantia immunologic. Alteremente antigenos A B O deberia esser considerate como altere que antigenos de transplanta.

ADDENDUM

Subsequent to the acceptance of this paper for publication, Robert's blood was re-examined to determine the current state of the serological characteristics in this case. Tests of his native blood with anti-A revealed a dual cell population; A antigen was demonstrated by absorption and elution of the anti-A. The specificity of the eluate was confirmed by its reaction with type A cells and not with type O or B. It was further substantiated by its neutralization by saliva from a secretor of A but not by those containing all other blood group substances. The serum isoagglutinins remained unchanged. However, at the suggestion of Dr. Ruth Sanger, absorptions and elution studies to confirm the M and S types were undertaken. These were negative for M or S. The latter findings make genetic mechanisms such as somatic mutation more difficult to exclude in this case; nevertheless, its essential characteristic—the tolerance of type A cells by a group O individual without suppression of anti-A—is atypical.

ACKNOWLEDGMENT

The authors are deeply indebted to Mr. Robert Murray who willingly traveled the
length of California on many occasions to provide us with the fresh blood specimens, and who in spite of good health, submitted to the many uncomfortable diagnostic procedures required to make this a complete study. Without his extraordinary and enthusiastic cooperation and his keen interest, the extent of this study would have been greatly curtailed.

REFERENCES


ATYPICAL IMMUNOLOGIC TOLERANCE


Atypical Immunologic Tolerance in a Human Blood Group Chimera

PHILLIP STURGEON, DOROTHY T. MCQUISTON, ROBERT SPARKES, JOEL SOLOMON and EUGENE V. BARNETT

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