# Further Evidence for Human A-Like Antigens on Marmoset Red Cells.* (31683)

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The presence of human-like ABO blood group antigens and antibodies in various subhuman primates has been known for a number of years. Relatively little attention has been given, however, to the study of these blood groups in marmosets and other lower primates. Wiener *et al*(1) and Moor-Jankowski *et al*(2) have tested the cells and sera of a total of 31 marmosets of several species and have classified them as belonging to Group A on the basis of salivary tests, which indicate the secretion of A and H-like, but not B substance. They have also suggested that marmosets do not necessarily have an A-like red cell antigen since human anti-A absorbed with chimpanzee O cells reacts much less strongly with marmoset cells than with human A cells. They indicated, however, that marmosets, like other New World monkeys do have a B-like red cell antigen, although this differs somewhat in specificity from the human B antigen.

Gengozian(3), reporting studies in *Tamarinus nigricollis,* has also demonstrated B-like red cell antigens and A-like salivary substances. In addition, he has given evidence suggesting the presence of an A-like antigen on marmoset red cells. He demonstrated this by the agglutination of human A cells by eluates of anti-A made from marmoset cells, even though the marmoset cells themselves were not agglutinated by either the anti-A or the eluate.

Until the present study, no attempts to study marmoset red cell antigens with the use of heteroimmune reagents have been reported. The present work has been directed toward this aim in an effort to further characterize the ABO-like blood groups of marmosets with *Oedipomidas oedipus* as the experimental animal. Also, observations on the reactions of cells and sera of *O. oedipus* are reported.

*Materials and methods.* Marmosets from a breeding colony of about 125 animals were bled of up to 5 cc by femoral venipuncture. Cells obtained from blood collected into ACD were washed 3 times with isotonic saline before use. Human test cells, antisera, and anti-A and -H lectins were obtained from Hyland Laboratories (Los Angeles, Calif.). Heteroagglutinins were produced in rabbits immunized by a 2-week course of twice-weekly intravenous injections of 0.4 cc of 50% washed marmoset cells suspended in saline. Isoimmunizations of marmosets were attempted by a similar series of injections intraperitoneally. Sera from rabbits and marmosets were collected 10 to 14 days after the last injection.

Agglutination tests were made in saline and read after centrifugation for 1 minute at 1,000 rpm. In all direct tests, marmoset sera were initially diluted 1:2 with saline to eliminate nonspecific agglutination of human cells. Titrations were performed by conventional methods. Absorptions and elutions were done with the technique of Landsteiner and Miller (4).

*Results. Reactions of human and marmoset red cells with immune heteroagglutinins.* Reactions of rabbit anti-marmoset red cell reagents are shown in Table I. Preimmunization rabbit sera showed no reactivity against human or marmoset red cells. The unabsorbed immune reagents reacted strongly with all cells. Absorption of the antisera with either human B or O cells removed only nonspecific agglutinins for human cells and left agglutinins specific for human A and marmoset cells in 6 of the 7 sera. When the reagents were absorbed with human A cells, there was no noticeable effect on their reactions with marmoset cells. Thus the antisera contained agglutinins specific for marmoset cells in addition to anti-A-like antibodies. To further examine the specificities of the

* This investigation was supported in part by USPHS Grants 5T1 HE 5133-10 and HE 06578.

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TABLE I. Reactions of Human and Marmoset Red Cells with Rabbit Anti-Marmoset Sera Before and After Absorption with Human Red Cells.

| | Rabbit antisera† | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cells* | R380 | R337 | R275 | R334 | R308 | R324 | R434 | Absorbed with |
| A | 3+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | Unabsorbed |
| B | 3+ | 4+ | 3+ | 3+ | 3+ | 4+ | 4+ | |
| O | 3+ | 4+ | 2+ | 3+ | 2+ | 4+ | 4+ | |
| M | 4+ | 3+ | 4+ | 4+ | 4+ | 4+ | 4+ | |
| A | — | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | O cells |
| B | ± | 1+ | 1+ | 1+ | — | — | 1+ | |
| O | — | — | ± | — | — | — | — | |
| M | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | |
| A | — | — | — | — | — | — | — | A cells |
| B | — | ± | — | — | — | ± | ± | |
| O | — | — | — | — | — | — | — | |
| M | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | |
| A | — | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | B cells |
| B | — | — | — | — | — | — | — | |
| O | — | — | — | — | — | — | — | |
| M | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | 4+ | |

* A, B, and O = human $Rh_o$ cells; M = marmoset cells.
† Reactions graded from — (no agglutination) to 4+ (a single clump of agglutinated cells).

antibodies against marmoset cells, one serum was chosen for more detailed analysis. Results of absorptions and elutions performed with this reagent are shown in Table II.

Titers of the antiserum against human A cells and marmoset cells were not significantly affected by absorption with either B or O cells. Absorption with human A cells had little effect on the titer against marmoset cells, but did remove nonspecific agglutinins against human cells as shown by the reduction in titers of the reagent for B cells and the removal of activity against O cells. This non-specific activity against B and O cells was also removed when either B or O cells were used for absorption. Absorption with marmoset cells reduced the activity of the reagent against A cells, as well as removed the non-specific anti-B and -O antibodies.

TABLE II. Reactions of Rabbit-Anti-Marmoset Serum No. R434 with Human and Marmoset Red Cells.

| | Reaction with cells tested: † | | | |
|---|---|---|---|---|
| | A | B | O | M |
| Antiserum | $(Log_2)^{-1}$ titer | | | |
| Unabsorbed | 8 | 7 | 5 | 12 |
| A-absorbed | —* | 3 | — | 10 |
| B-absorbed | 7 | — | — | 11 |
| O-absorbed | 8 | 2 | — | 11 |
| Marmoset-absorbed | 4 | — | — | 3 |
| Eluate from: | | | | |
| A cells | 2 | 2 | — | 5 |
| B cells | 5 | — | — | 7 |
| O cells | — | — | — | 2 |
| Marmoset cells | 4 | — | — | 7 |

* $(Log_2)^{-1}$ titer $<2$.
† A, B, and O = human $Rh_o$ cells; M = marmoset cells.

Eluates prepared from the absorbing cells gave somewhat surprising reactions (Table II). Although the eluate from A cells reacted only weakly with A cells, it strongly agglutinated marmoset cells. In addition, this eluate contained a weak specific agglutinin for B cells. The eluate from B cells reacted strongly with marmoset cells, and also with A cells, but not with B cells. The eluate from O cells reacted only weakly with marmoset cells. The eluate from marmoset cells reacted strongly with marmoset cells and also with A cells. This eluate was the only eluate that contained the agglutinins corresponding to those removed in the absorption treatment, and likewise demonstrated clearly a common specificity between human A and marmoset cells.

*Reactions of normal marmoset sera with human A, B, and O cells.* The reactions of direct tests of 25 normal marmoset sera with human red cells are shown in Table III. All cells tested were $Rh_o$. Of the sera tested, 16 contained a natural B agglutinin. One sample contained both A and B agglutinins

TABLE III. Reactions of Normal Marmoset Sera with Human Red Cells.

| No. of sera tested | Human cells A | B | O |
|---|---|---|---|
| 1 | + | + | — |
| 1 | — | + | + |
| 14 | — | + | — |
| 9 | — | — | — |
| Total 25 | 1 | 16 | 1 |

and another agglutinated only B and O cells. Nine of the sera failed to react with human red cells. Although serial dilutions of the sera were not tested, agglutination of A and O cells did not occur at serum dilutions of 1:10 but strong specific reactions with B cells were seen at this dilution.

These results are similar to those of Gengozian(3) in which 18 of 30 *T. nigricollis* were found to have serum containing B agglutinins. Wiener *et al*(1) found no B agglutinins in tests from the sera of 2 *O. oedipus*.

*Reactions of human anti-A and anti-B reagents with marmoset red cells.* The results of titrations of human anti-A and anti-B reagents against marmoset red cells are shown in Table IV. Although reactions are observed with both anti-A and anti-B, the anti-B titers are considerably higher than those with anti-A against cells of all animals except No. 308. Cells from the remaining animals reacted with anti-B titers equal to or only slightly less than human B cells.

Although the differences in titers between anti-A and anti-B for marmoset cells suggest the presence of a B-like antigen in 5 of the 6 animals tested, the possibility of nonspecific reactions or differences in the strength of the human antisera could not be eliminated. Therefore, eluates of anti-A and anti-B were prepared from their respective human red cell antigens and tested against human and marmoset cells. The results are shown in Table IV. The anti-B eluate reacted strongly with cells from all marmosets except No. 308. No reactions were observed with the anti-A eluate. Absorptions of human anti-A and anti-B reagents with marmoset red cells failed to remove the reactivity of these reagents for human cells. Tests with anti-A and anti-H lectins were also negative. These reactions, in suggesting the presence of a B-like, but not an A-like antigen of the cells of *O. oedipus*, agree with reactions observed in *T. nigricollis* (3) and other species(1,2).

*Tests for immune isoantibodies in* O. oedipus. No isoantibodies were detected in sera from 6 marmosets immunized with homologous red cells. Although the immunization schedule was not vigorous and may not have stimulated detectable antibody production, another possible explanation for failure to produce isoagglutinins may be in the fact that marmosets frequently exhibit chimerism (5). This has been shown to affect the myelopoietic and lymphopoietic systems(6,7) and presumably also affects the red cells. If so, the difficulty in producing isoantibodies is greatly increased.

*Discussion.* Tests on the heteroagglutinins produced against marmoset cells clearly suggested the presence of an A-like antigen on the marmoset red cell. This was shown by the reduction in titer of the antiserum against human A cells after absorption with marmoset cells, and the subsequent recovery of agglutinins for human A, but not B or O cells in the eluate from marmoset cells (Table II). That the specificities of the human and marmoset A antigens are different is apparent since marmoset cells were not agglutinated specifically by human anti-A reagents or eluates. Also, the absorption of the rabbit reagent with human A cells reduced the reactivity of the reagent against marmoset cells

TABLE IV. Reactions of Marmoset Red Cells with Human Anti-A and Anti-B Antisera and Eluates.

| Cells | Reaction with: Antiserum Anti-A | Antiserum Anti-B | Eluate Anti-A | Eluate Anti-B |
|---|---|---|---|---|
| | $(Log_2)^{-1}$ titer | | Pos (+) or neg (—) for activity | |
| M308* | 3 | 5 | — | — |
| M380 | 4 | 7 | — | + |
| M337 | 3 | 8 | — | + |
| M334 | 2 | 8 | — | + |
| M324 | 3 | 7 | — | + |
| M275 | 2 | 7 | — | + |
| Human A | 8 | 0 | + | — |
| " B | 0 | 8 | — | + |
| " O | 0 | 0 | — | — |

* Numbers represent individual marmosets tested.

only slightly, and the absorption of human anti-A reagents with marmoset cells likewise failed to diminish the reactivity of the anti-A for human A cells. The difference in specificity of these antigens has been further pointed out in the finding of Gengozian(3) that eluates from marmoset cells reacted with human anti-A, but not agglutinated, will agglutinate human A cells. These differences suggest that although the marmoset and human A antigens may differ considerably, they undoubtedly share certain factors.

In addition to anti-A activity, the rabbit antiserum contained a weak specific agglutinin for human B cells. This agglutinin was present in the serum after absorption with either A or O cells. In view of the evidence suggesting a B-like antigen on the marmoset red cell, it is surprising that greater anti-B activity was not found in the rabbit antiserum. Since the relative antigenicity of the ABO blood group antigens in rabbits seems to be A>C>B(8), probably the rabbits simply failed to respond to the B-like antigen of the marmoset red cells.

The reactions of the eluates from the A and B cells are also of interest. The recovery of agglutinins for both A and B cells in the eluate from A cells is suggestive of the presence of a "cross-reacting" type of antibody. This cross-reactivity is also suggested by the findng that the eluate from B cells caused agglutination of only human A and marmoset cells. The evaluation of the significance of these findings is difficult since only one serum was studied in detail. It is tempting to speculate that perhaps the marmoset cell may contain an antigen intermediate in specificity between the human A and B factors, or possibly a factor resembling the C factor discussed by Wiener and Ward (9) and that this factor is involved in the reaction of the "cross-reacting" antibody observed. Marmoset cells apparently possess both A- and B-like factors; how these may be related to each other and to their human counterparts is not yet clear.

*Summary.* Absorption and elution tests using immune heteroagglutinins produced in rabbits immunized with red cells of the marmoset *Oedipomidas oedipus* have further indicated the presence of an A-like antigen on the marmoset cells. In addition, the rabbit antiserum was found to have properties suggesting the presence of a "cross-reacting" type of antibody against human A and B cells. Although marmoset cells appear to have both A- and B-like red cell antigens, considerable differences in the reactions of these antigens compared to those of human A and B cells were seen.

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Received September 23, 1966. P.S.E.B.M., 1967, v124.