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Blood Group Antigens I, i, H and HI on Monkey, Rabbit and Guinea Pig Erythrocytes[1] (37856)

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(Introduced by J. F. Mohn)

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The human blood group antigens I and i were found by Wiener *et al.* (1) to be present on the erythrocytes of a wide variety of animal species in addition to man. Among the animals studied, rabbits were shown to be I-positive and monkeys to be i-positive. The Ii status of guinea pigs was not reported. Marty *et al.* (2) examined the red cells of one guinea pig and reported the apparent absence of the I and i antigens in this species because the erythrocytes failed to absorb anti-I and anti-i antibodies from human antisera specific for these antigens. This report describes the results of the examination of the red cells of rabbits, monkeys, and guinea pigs for the presence of I, i, HI, and H blood group antigens.

Materials and Methods. Antisera. All of the antisera used in this study were of human origin. The anti-I (Hal), anti-i (Obr) and anti-i (Hog) antisera were obtained from group A donors, the anti-HI (Hil) serum from a group B donor, and the anti-H (Pec) serum from a group O_h (Bombay) individual.

Test Cells. Whole blood from human donors was collected by venipuncture and placed into ACD anticoagulant solution, PHS formula A. Whole blood from rabbits, monkeys, and guinea pigs was obtained by cardiac puncture and mixed with ACD solution, PHS formula B. All blood samples were maintained aseptically and were used up to 14 days following collection or were stored as frozen red cells in the vapor phase of liquid nitrogen at −150° according to the method of Mohn, Bowman and Cunningham (3).

Preparation of absorbed antisera. For the experiments described in Table I, one aliquot of each of the anti-I, anti-H, and anti-HI antisera were absorbed 6–9 times at 4° for 30 min with 0.1 vol of washed, human adult red cells; the anti-i antiserum was absorbed with human newborn umbilical cord cells. A second set of aliquots of each of the four unabsorbed antisera was absorbed at least two times with monkey erythrocytes, a third set with rabbit cells, and a fourth set with guinea pig red cells.

Preparation of red cell eluates. Eluates containing the anti-I, anti-H, and anti-HI antibodies were prepared by adsorption onto and elution from human adult group OI erythrocytes. Human group Oi red cells were used to prepare the anti-i eluate. Equal volumes of washed, packed cells and antiserum were mixed at 4° for 30 min. After centrifugation at 4°, the supernatant serum (absorbed) was removed. The packed cells were washed 3× at 4° in PBS (phosphate-buffered saline solution, pH 7.4) and then resuspended in 1 vol of PBS. Elution was carried out for 15 min in a shaking water bath at 42°. The eluate was recovered after

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TABLE I. Reduction of the Agglutination Scores of Anti-I, Anti-i, Anti-H, and Anti-HI Sera after Absorption with Human and Animal Erythrocytes When Examined with Human, Monkey, Rabbit, and Guinea Pig Test Cells at 4°.

Antiserum absorbed with red cells of:	Human test cells				Animal test cells		
	OI	Oi[a]	Bi	Ai	Monkey	Rabbit	G. Pig
	Part I. Anti-I (Hal) serum (gr. A_1)						
Human (BI)	55	32	34		2	23	33
Monkey	3	0			22	0	2
Rabbit	25	13			1	37	34
Guinea pig	23	11			2	37	37
	Part II. Anti-HI (Hil) serum (gr. B)						
Human (A_1BI)	45	13		21	0	19	26
Monkey	2	0			3	0	2
Rabbit	13	8			1	29	19
Guinea pig	14	5			2	18	29
	Part III. Anti-H (Pec) serum (gr. O_h)						
Human (A_1BI)	21	16	21	43	2	2	1
Monkey	2	1			30	2	2
Rabbit	13	11			2	21	1
Guinea pig	13	12			1	0	14
	Part IV. Anti-i (Obr) serum (gr. A)						
Human (Bi cord)	9	32	45		21	2	13
Monkey	1	20			19	6	2
Rabbit	1	1			1	10	3
Guinea pig	1	1			3	2	20

[a] Cord cells.

centrifugation in a heated centrifuge at 42°.

Hemagglutination Reactions. Serial two-fold dilutions of the absorbed sera and red cell eluates were prepared in PBS solution in 0.1 ml volume in 10 × 75 mm tubes to which an equal volume of a 2% (v/v) suspension of erythrocytes in PBS solution was added. Following incubation at 4° for 30 min, the tubes were centrifuged and returned to the water bath at 4° for an additional 5 min. The agglutination reactions were then read, graded and scored as previously described (4).

Results. When conducting direct agglutination experiments using human antisera and animal erythrocytes, it is not always possible to recognize with confidence the specific reactions under study due to the presence of heteroagglutinins in human sera for animal red cells. It is especially difficult when the titer of the specific antibody under study is equal to or less than the titer of the heteroagglutinins. Such was the case in our direct agglutination experiments, especially with the anti-H serum (Pec) and the anti-i serum (Obr). Absorption experiments were therefore carried out in which aliquots of each of the anti-I, anti-i, and anti-H, and anti-HI sera were absorbed separately with human, monkey, rabbit or guinea pig red cells and the absorbed sera examined in direct agglutination experiments with human adult, human cord, monkey, rabbit and guinea pig test cells. The results of these experiments are presented in Table I as the *reduction* in agglutination scores after absorption of the sera. The larger the score, the greater the amount of antibody removed by the absorbing cells.

In Part I, absorption of the human anti-I serum (Hal) with human adult group BI erythrocytes resulted in a marked reduction in the agglutination scores with the human group OI and Oi test cells and with the rabbit and guinea pig cells but not with

those of the monkey. Absorption of an aliquot of the serum with rabbit red cells and another aliquot with guinea pig cells yielded similar score reductions. Absorption of an aliquot with monkey erythrocytes removed only the heteroagglutinins specific for monkey cells; there was no reduction in anti-I activity. These results indicate the presence of the I antigen on the rabbit and guinea pig red cells and its absence from the monkey cells. Activity was removed for the human group OI and to a lesser extent for the human Oi cells as was expected.

In Part II, the absorption pattern of the anti-HI serum (Hil) was similar to that seen with the anti-I serum (Hal) indicating that the red cells of rabbits and guinea pigs possessed both the H and I antigens and that the monkey cells were devoid of these antigens.

It was the case with the *Hil* and *Hal* sera that the anti-I and anti-HI antibodies, respectively, were in higher titer than the heteroagglutinins. Consequently, the results with *both* the animal and the human cells provided information about the presence or absence of the respective antigens.

Since anti-H antibodies in the serum (Pec) obtained from a group O_h Bombay individual were in lower titer than the heteroagglutinins, only the results with the human test cells (Part III) were useful in recognizing the presence or absence of H substance on the absorbing cells. The results indicate that the H antigen was present on the rabbit, guinea pig, and the human group ABI cells but was absent from the red cells of the monkey.

In Part IV, absorption of the anti-i serum (Obr) with the human newborn cord cells (group Bi) not only removed anti-i activity for the monkey and human cells as expected but also reduced the agglutination with the guinea pig test cells suggesting the presence of i antigen on the guinea pig cells; no significant reduction was seen with rabbit test cells. Absorption of the serum with monkey cells removed only the activity for monkey cells and the human group Oi cells; any removal of anti-i activity for guinea pig test cells was masked by the heteroagglutinins for these cells not absorbed by the monkey cells. The absorptions with the human group Bi (cord) and rabbit cells indicate that the rabbit cells did not have the i antigen. Absorption of the sera with the guinea pig cells failed to remove anti-i activity for the human group Oi test cells. This result is in contrast with that obtained after absorption with human group Bi cells leaving the i-status of guinea pig erythrocytes in doubt.

In an effort to confirm the I-status and to resolve the i-status of guinea pig erythrocytes, an absorption–elution experiment was performed. Anti-I serum (Hal) was absorbed with human adult group OI red cells and anti-i serum (Hog) diluted 1:10 with PBS was absorbed with human cord group Oi cells at 4°. After washing the absorbing cells in PBS at 4°, heat-eluates were prepared in PBS at 42°. Serial two-fold dilutions of each of the two eluates were then examined in a direct agglutination experiment at 4° with guinea pig, rabbit, monkey, human adult group OI and human cord group Oi test cells suspended in PBS. The results of this experiment are presented in Table II as the direct agglutination scores.

TABLE II. Agglutination Scores of Red Cell Eluates of Anti-I Serum (Hal) and Anti-i Serum (Hog) with Human, Monkey, Rabbit, and Guinea Pig Erythrocytes at 4°.

	Test cells				
Eluates	Human adult group OI	Human cord group Oi	Monkey	Rabbit	Guinea pig
Anti-I (Hal)[a]	46	27	7	54	53
Anti-i (Hog)[b]	0	58	61	7	34

[a] Eluted from human adult gr. OI red cells after absorption of undiluted anti-I serum (Hal).

[b] Eluted from human cord gr. Oi red cells after absorption of anti-i serum (Hog) diluted 1:10.

The anti-I eluate agglutinated the guinea pig test cells as strongly as the human adult group OI cells and those of rabbits. The human cord group Oi cells were agglutinated also but less strongly since these cells are not entirely free of the I antigen. The monkey test cells were very weakly agglutinated reflecting a small amount of anti-i in the anti-I serum (Hal).

The anti-i eluate unequivocally agglutinated the guinea pig test cells although less strongly than the monkey and human cord group Oi test cells. Human adult group OI test cells were not agglutinated. The rabbit test cells, which were more strongly I-positive than the human adult cells, were only weakly agglutinated reflecting the presence of anti-I in low titer in the anti-i serum (Hog). From the results of this experiment, it would appear that the guinea pig red cells possessed both I and i blood group antigens.

An additional absorption–elution experiment was performed in which 1 vol of the potent anti-i serum (Hog) diluted 1:256 in PBS was absorbed with 1 vol of packed, washed human cord group Oi red cells and this absorbed serum was saved as was a portion of a heat-eluate prepared of the human cord absorbing cells at 42°. One volume of the eluate was further absorbed with 1 vol of guinea pig cells at 4° and this absorbed-eluate was also saved. A heat-eluate was then prepared from the guinea pig cells used in this absorption. The unabsorbed serum, serum absorbed with cord cells, eluate of the cord cells, eluate absorbed with guinea pig cells and the eluate of the guinea pig cells were examined at the same time by direct agglutination at 4° in a titration experiment with guinea pig, monkey, rabbit, human adult group OI, human adult group O_hI (Bombay), and human cord group Oi test cells. The results of this experiment are presented in Table III.

The unabsorbed anti-i serum gave high agglutination scores with the guinea pig, monkey and human cord group Oi test cells. Low scores were given with the rabbit, human adult group O_hI and group OI test cells reflecting the presence of anti-I in low titer at this dilution (1:256) of the serum Hog. Absorption of the serum with human cord group Oi cells reduced the scores with the guinea pig, monkey, and human cord group Oi test cells and removed all anti-I activity due to the fact that cord cells contained some I antigen. The heat-eluate prepared from the cord cells used for absorption contained high activity for the i-positive cells and for the guinea pig cells and also gave low scores with the I-positive cells. Absorption of this eluate with guinea pig cells removed considerable anti-i activity and all of the anti-I activity as well. The heat-eluate prepared from the guinea pig cells used for absorption contained a good yield of anti-i antibodies. Anti-I antibodies were also demonstrated due to the presence of the I antigen on the guinea pig cells. The results of this experiment indicate unequivocally that the guinea pig erythrocytes contained the i-antigen.

TABLE III. Direct Agglutination Scores of Unabsorbed Anti-i Serum (Hog), Serum Absorbed with Human Oi Cells, Eluate of Human Oi Cells, Eluate Absorbed with Guinea Pig Cells, and Eluate of Guinea Pig Cells with Human, Rabbit, Monkey, and Guinea Pig Erythrocytes at 4°.

	Anti-i serum (Hog) 1:256				
Test cells	Unabsorbed serum	Serum absorbed with human (cord) cells	Eluate of human (cord) cells	Eluate absorbed with G. pig cells	Eluate of G. pig cells
Guinea pig	56	37	44	20	40
Monkey (i)	68	42	60	21	46
Human (Oi cord)	48	28	35	0	30
Rabbit (I)	16	0	14	0	12
Human (O_hI)	7	0	7	0	7
Human (OI)	1	0	0	0	1

Discussion. Since human adult I-positive red cells have some i-antigen and human cord i-positive erythrocytes possess a small amount of I-substance, it was expected that absorption of the anti-I and anti-i sera with either human adult or human cord cells would remove antibody activity for both test cells. This is readily seen in Parts I and II of Table I.

In Part II or Table I, absorption of the anti-i serum (Obr) with human cord i-positive erythrocytes removed antibody activity for both the cord and guinea pig test cells indicating that guinea pig red cells are i-positive. However, absorption of the serum with guinea pig cells did not remove activity for the human cord i-positive test cells. This contradiction was difficult to explain except on grounds that two absorptions with the guinea pig cells may have been insufficient to remove enough antibody to produce detectable reductions in agglutination scores. In Tables II and III, absorption of the potent anti-i serum (Hog) with human cord cells again removed antibody activity for guinea pig test cells. It was shown in Table III that guinea pig erythrocytes did remove anti-i activity from the eluate of the cord cells used to absorb the anti-i serum (Hog) and that the eluate of the guinea pig absorbing cells contained anti-i antibody activity for human cord i-positive test cells. These observations indicate, that guinea pig red cells possess the i-antigen.

Summary. Anti-I, anti-i, anti-H and anti-HI sera of human origin were used to examine the erythrocytes of rabbits, monkeys, and guinea pigs for the presence of I, i, H, and HI blood substances. In direct agglutination experiments with the four antisera after they had been absorbed with rabbit, monkey, guinea pig, human group OI or human group Oi (cord) red cells, it was evident that the erythrocytes of rabbits possessed I, H, and HI but no i substances. Monkey red cells had i but no I, H, and HI blood group activity. Guinea pigs were shown to have I, H, and HI, antigens but the i-status of their erythrocytes remained equivocal. Further absorption elution experiments revealed unequivocally that guinea pig red cells possessed the i blood group antigen.

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