

## Autoantibodies in Acquired Hemolytic Anemia With Special Reference to the LW System

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Most autoantibodies in patients with warm antibody autoimmune hemolytic anemia (AIHA) have specificity within the Rh system. Using rare cells such as -D- and Rh<sub>null</sub> cells, Weiner and Vos (1963) described specificity against normal cells (nl), partially deleted cells (pdl), and deleted cells (dl). Recently, autoantibodies which failed to react with Rh<sub>null</sub> cells that were of anti-U specificity have been described. It was suggested that the "Rh related" autoantibodies that cannot be identified as specific Rh antibodies may be anti-U. In the present study we examined eluates from the red cells of eight patients with AIHA using a panel of extremely rare cells and cross-absorption and elution techniques. We demonstrated autoantibody specificities not definable without the rare cells and, further, defined heterogeneity of the LW antigen. Autoantibodies with U

specificity occurred in three eluates only. It was always present with an antibody of another specificity. Six of the eluates contained anti-LW, two anti-nl, five anti-pdl, three anti-dl, and one anti-e. Absorption and elution studies using the rare Rh-positive LW-negative (Mrs. Bigelow) showed that anti-pdl may in fact represent anti-LW + LW<sub>1</sub> and that Mrs. Bigelow may represent a weak variant of LW. Injection of her red cells into guinea pigs produced an anti-LW that reacted similarly to the antibody produced by injecting Rh-positive LW-positive cells. An analogy to the ABO is suggested that normal Rh-positive LW-positive cells represent LW<sub>1</sub>, Rh-negative LW-positive cells represent LW<sub>2</sub>, Mrs. Bigelow represents LW<sub>3</sub> and Rh<sub>null</sub> cells represent the only true LW-negative (lw).

**P**ATIENTS WITH autoimmune hemolytic anemia (AIHA) of the warm antibody type may form mixtures of specific autoantibodies which react with all, or almost all, human red cells.<sup>1</sup> The specificity of the antibodies in these mixtures can only be resolved by selective absorption and elution and often depends on the availability of red cells having many different genotypes. Using these cells it is possible to demonstrate that autoantibodies almost always possess specificities against "Rh-associated antigens."<sup>1,2</sup> Red cell eluates which show the presence of antibodies against well-defined Rh antigens or anti-U frequently also possess antibodies against "Rh-associated antigens." Reports

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that autoantibodies are directed against other blood group antigens such as Kidd,<sup>3</sup> Kell,<sup>4,5</sup> and Xg<sup>a6</sup> are rare.

This study describes the antibody specificities of selected eluates from red cells of patients with AIHA. The use of a large panel of red cells including several examples of very rare phenotypes revealed autoantibody specificities that could not have been detected without the use of such cells. Further, the recognition of these autoantibody specificities extends previous observations about anti-LW antibodies in AIHA,<sup>7</sup> establishes the existence of anti-LW variants, and suggests further complexities of the LW system.

### MATERIALS AND METHODS

The AIHA patients reported in this study were selected for more detailed investigation because their red cell eluates had on previous occasions shown the presence of multiple antibodies.

The selected panel of red cells used (see Table 2, below) had been stored in a buffered glycerol citrate solution at  $-20^{\circ}\text{C}$ . Frozen cells were recovered by Weiner's method<sup>8</sup> using Visking dialysis tubing. In no instance did the frozen and thawed red cells react differently from fresh cells before storage.

Antiglobulin tests were performed using standard technique and with an antiglobulin serum that had previously been evaluated in detail.<sup>9</sup>

Red cell eluates were absorbed with the appropriate type of washed packed red cells using equal volumes of eluate and cells. Following incubation at  $37^{\circ}\text{C}$  for 1 hr the red cells were washed four times in saline. Antibody elution was then performed on a 50% saline suspension of red cells. The ether elution procedure of Vos and Kelsall<sup>10</sup> was used, modified only in that ether was added directly to the 50% suspension of packed washed red cells. The sequence of absorption and reelution used for the differentiation of the type of specific antibody to be recovered is detailed in the Results section.

Guinea pigs were given biweekly subcutaneous injections of a 5% suspension of washed cells in an equal volume of Freund's complete adjuvant. This produced intense antibody responses against a variety of cells within a period of 8-10 wk. In our experience, the method of Levine and Celano<sup>11</sup> in which guinea pigs were given six intraperitoneal injections of a 25% suspension of cells at intervals of 3-4 days resulted in considerably lower responses. In our absorption studies the highest dilution of guinea pig serum giving the strongest reaction with the immunizing red cells was accepted as the optimal starting dilution. Using this procedure we avoided repeated absorption of the antibody with valuable red cells. The range of antibody titer values was between 1:512 and 1:4096.

#### Definitions of Antibody Specificity (See Table 1)

Specificities of the antibodies in this study were defined as reported previously.<sup>1,2</sup>

*Antibodies for well-defined red cell antigens.* These antibodies showed distinct specificity against any of the well-defined blood group antigens such as C, c, E, e, Kell, and Fy<sup>a</sup>. Since red cell eluates often reacted with all normal cells (those without Rh gene deletions), absorption studies were necessary to demonstrate such specificity.

**Table 1. Autoantibodies in Acquired Hemolytic Anemia and Their Serological Reactivity for Selected Red Cells<sup>1</sup>**

	Anti-nl	Anti-pdl	Anti-dl	Suggested Antigenic Make-up of Red Cells
"Normal cells" CDe/cde, cDE, cde, cde/cde, etc.	+	+	+	nl, pdl, dl
"Partially deleted" Cells -D-/-D- or cD-/cD-	-	+	+	pdl, dl
"Fully Deleted" Cells ---/--- (Rh <sub>null</sub> )	-	-	+	dl

**Table 2. Antiglobulin Reactions of Selected AIHA Red Cell Eluates**

Red Cell Type	Red Cell Eluates From Acquired Hemolytic Anemia Patients							
	BN	BL	SP	CT	FR	MT	VP	BS
cDE/cDE, LW+, U+	512*	16	256	4	32	128	64	256
cde/cde, LW+, U+	256	16	256	4	32	128	64	256
CDe/CDe, LW+, U-	512	16	256	4	32	128	64	256
cDE/cde, LW+, U-	256	16	256	4	32	128	64	256
CDe/cde, LW-, U+	256	16	256	4	8	128	64	256
-D-/-D-, LW+, U+	256	16	256	4	32	128	64	32
---/---, LW-, U+	8	4	256	4	0	0	64	0
---/---, LW-, U-	0	4	256	0	0	0	64	0

\*Titers are expressed as reciprocals of highest dilution giving definite positive results.

*Antibodies reactive for "normal" red cells (anti-nl).* Anti-nl antibodies were defined as those that react with all normal human red cells possessing the common Rh phenotypes CDe/cDE, CDe/CDe, cde/cde, and the like, but fail to react with partially deleted red cells of the phenotype -D-/-D- as well as the fully deleted Rh<sub>null</sub> red cells ---/---.

*Antibodies reactive for partially deleted red cells (anti-pdl).* Anti-pdl antibodies were defined as those that react with red cells of the phenotype -D-/-D- but fail to react with Rh<sub>null</sub> bloods. Anti-pdl also reacts with normal red cells since such erythrocytes also contain the pdl antigen.

*Antibodies reactive for Rh<sub>null</sub> red cells (anti-dl).* Anti-dl antibodies were defined as those that react with Rh<sub>null</sub> red cells. Such antibodies also react with all normal and with partially deleted red cells.

**RESULTS**

Table 2 records the indirect antiglobulin titer values of eight selected AIHA eluates tested against a panel of red cells capable of differentiating a variety of specific antibodies. Six of these 8 eluates revealed significant differences in reactivity when using cells of varying phenotypes, thus suggesting that mixtures of specific autoantibodies may be involved.

Table 3 summarizes the results of antibody determinations performed on the

**Table 3. Specificities of Autoantibodies Recognized by Selective Absorption and Elution Studies Using Different Panels of Red Cells**

Patients	Antibodies in Eluates From Patients' Red Cells Recognized by:	
	Panel 1	Panel 2
	CDe/CDe, LW+, U+	CDe/CDe, LW+, U- -D-/-D-, LW+, U+
	cDE/cDE, LW+, U+	cDE/cDE, LW+, U+ ---/---, LW-, U+
	cde/cde, LW+, U+	cde/cde, LW+, U+ ---/---, LW-, U-
	-D-/-D-, LW+, U+	cDE/cde, LW+, U-
	---/---, LW-, U-	CDe/cde, LW-, U+
BN	anti-pdl	anti-pdl, anti-U
BL	anti-pdl, anti-dl	anti-pdl, anti-dl, anti-LW
SP	anti-pdl, anti-dl	anti-pdl, anti-dl, anti-LW
CT	anti-pdl	anti-LW, anti-U
FR	anti-pdl	anti-pdl, anti-LW
MT	anti-nl, anti-pdl	anti-nl, anti-pdl, anti-LW
VP	anti-dl	anti-dl, anti-U
BS	anti-nl, anti-e, anti-pdl	anti-nl, anti-e, anti-LW
Observed	13 specific autoantibodies	20 specific autoantibodies

**Table 4. Absorption and Elution Experiments with Red Cell Eluate FR**

Step No.	Procedure	Test Cells					
		1 cDE/cDE LW+, U+	2 CDe/CDe LW+, U-	3 CDe/cde LW-, U+	4 -D- LW+, U+	5 Rh <sub>null</sub> LW+, U+	6 Rh <sub>null</sub> LW-, U-
1	Original eluate	32*	32	8	32	0	0
2	Original eluate after one absorption with test cell No. 4	0	0	0	0	0	0
3	Original eluate after one absorption with test cell No. 3	4	4	0	4	0	0
4	Re-elution of antibody from test cell No. 4 (step 2)	16	16	4	16	0	0

\*Titers are expressed as reciprocals of highest dilution giving definite positive results in the indirect antiglobulin test.

same eluates using two different sets of panel cells. The findings show that cell panel 1 seemed to be limited in its scope to detecting anti-U and anti-LW because it lacked the required cell types to differentiate anti-U from anti-nl and anti-pdl and also anti-LW from anti-pdl. Restricted availability of rare cell types, particularly the CDe/cde, LW-negative, U-positive cells of Mrs. Bigelow<sup>12</sup> and the Japanese Rh<sub>null</sub>, LW-negative, U-positive<sup>13</sup> blood reduces the opportunity of isolating the full spectrum of autoantibodies.

Table 4 shows the results of specificity testing for red cell eluate FR. This autoantibody had previously been classified as anti-pdl<sup>2</sup> (see also Table 3), using a more limited panel of test cells. The demonstration of anti-LW specificity and the close association of this antibody with anti-pdl became apparent when the rare CDe/cde, LW-negative (Mrs. Bigelow) red cells were included in the present investigations. As indicated in *Step 1* (Table 4): The antibody in eluate FR reacted more strongly with LW-positive cells than with the LW-negative cells of Mrs. Bigelow. The eluate did not react with the two Rh<sub>null</sub> LW-negative red cells indicating the presence of anti-pdl, or anti-pdl + nl and possibly anti-LW as well. *Step 2*: Absorption of the original eluate with test cell number 4, (-D-, LW+, U+) which has pdl, LW and U antigens but does not have nl, completely removed all antibody activity, indicating probable pdl or pdl + LW specificity. *Step 3*: The original eluate after one absorption with test cell number 3 (CDe/cde, LW-, U+) retained antibody activity only for LW-positive test cells. *Step 4*: Reelution from test cell number 4 (-D-, LW+) following step 2, established the presence of an antibody that sensitized LW-positive red cells more strongly than the LW-negative red cells of Mrs. Bigelow (test cell 3). This eluate was subsequently tested against over 200 random samples of blood to determine if other examples of weak positive reactions similar to the weak reactions observed with Mrs. Bigelow's red cells could be demonstrated, but none was found.

**Table 5. Absorption Experiments With Red Cell Eluate PM**

Before Absorption		Reactions With Test Cells				
		CDe/cDE LW+	CDe/cde LW+	cde/cde LW+	CDe/cde LW-	Rh <sub>null</sub> LW-
		++++	++++	++++	+++	0
Absorbing Cells	Rh <sub>null</sub> ·LW-	+++	+++	+++	++	0
	CDe/cde, LW-	+++	++	+	0	0
	cde/cde, LW+	+	0	0	0	0
	CDe/cDE, LW+	0	0	0	0	0
	Baboon	0	0	0	0	0
	Monkey	0	0	0	0	0
	Sheep	++++	++++	++++	++++	0
	Rabbit	++++	++++	++++	++++	0
Horse	++++	++++	++++	++++	0	

Table 5 illustrates the results obtained when a further eluate (P.M.), which reacted with all cells tested, except Rh<sub>null</sub>, was absorbed with various cells including animal cells. One absorption of the eluate with Mrs. Bigelow's CDe/cde LW-negative cells removed activity for her cells completely and reduced the reaction against cde/cde LW-positive and CDe/cde LW-positive cells. Absorption with cde/cde LW-positive cells removed the activity against Mrs. Bigelow's cells and CDe/cde LW-positive cells and reduced the activity against CDe/cDE LW-positive cells. Absorption with CDe/cDE LW-positive cells removed all antibody activity. Baboon and monkey cells also removed all antibody activity whereas absorption with sheep, rabbit, or horse cells had no effect. These results extend the observations made in Table 4.

One may interpret such data as follows: If Mrs. Bigelow's cells represent a weak variant of LW, the antibody recovered from the -D-/-D-, LW-positive sensitized red cells (Table 4, step 4) may be described as an anti-LW rather than as anti-pdl. The implication of this observation is that Rh<sub>null</sub> red cells would remain as the true LW-negative bloods as originally described by Levine et al.<sup>11</sup> The ability of anti-pdl to react more strongly with LW-positive test cells (Table 4, step 4) and very weakly with the LW-negative test cells of Mrs. Bigelow, may be interpreted to indicate that anti-pdl is anti-LW + anti-LW<sub>1</sub>, which may be compared with a group B serum with anti-A + anti-A<sub>1</sub>. If such an analogy exists then it could be anticipated that absorption of anti-LW + LW<sub>1</sub> (anti-pdl) by a subgroup of LW (e.g., Mrs. Bigelow) should leave unabsorbed anti-LW<sub>1</sub> activity and that the eluate from the absorbing red cells should contain anti-LW and react with LW-positive cells. This hypothesis was confirmed following absorption of the original eluate with Mrs. Bigelow's red cells (Table 4, step 3). The results showed that these red cells not only removed the LW activity from anti-LW + LW<sub>1</sub> (anti-pdl), but also considerably reduced the strength of anti-LW<sub>1</sub>, a feature which parallels the preparation of anti-A serum which has been absorbed with group A<sub>2</sub> cells.

The results shown in Table 5 confirm and extend these findings as monkey and baboon cells are known to be rich in LW antigen. The results further suggest that homozygous D-positive cells have more LW antigen than heterozygous D-positive cells, which have more LW antigen than D-negative cells.

**Table 6. Absorption Experiments Using Guinea Pig Antisera to Rh-Positive, LW-Negative (Mrs. Bigelow) Cells**

		Reactions with Test Cells				
		CDe/cDE Lw+	CDe/cde Lw+	cde/cde Lw+	CDe/cde Lw-	Rh <sub>null</sub> Lw-
Before Absorption		++++	++++	++++	++++	++++
Absorbing Cells	Rh <sub>null</sub> . Lw-	++++	++++	+++	++	0
	CDe/cde. Lw-	++++	+++	+	0	0
	cde/cde. Lw+	++	(+)	0	0	0
	CDe/cDE. Lw+	0	0	0	0	0
	Baboon	0	0	0	0	0
	Monkey	0	0	0	0	0
	Sheep	++++	++++	++++	++++	++++
	Rabbit	++++	++++	++++	++++	++++
Horse	++++	++++	++++	++++	++++	

To confirm that Mrs. Bigelow does possess a weak LW antigen her cells were injected into guinea pigs. The results are shown in Table 6. The antibody formed by injection of Mrs. Bigelow's cells was exactly the same as that seen following injection of LW-positive cells. In all the immunization experiments, whether Rh-positive, LW-negative or Rh-positive, LW-positive cells were used the guinea pigs always produced two types of antibody: one that reacted with all human cells and could be removed with one absorption with Rh<sub>null</sub> cells; and secondly an antibody that reacted exactly the same as the eluate shown in Table 5. The reactions of the latter antibody were only apparent following absorption of the guinea pig serum with Rh<sub>null</sub> cells. The guinea pig antibody for Rh-positive, LW-positive red cells could always be completely absorbed with a single absorption with Rh-positive, LW-positive cells and red cells from baboons and monkeys, suggesting that these red cells possess an abundance of LW sites.

Autoantibodies reactive for Rh<sub>null</sub> cells were observed in nearly 40% of 51 red cell eluates from AIHA red cells investigated.<sup>2</sup> They can be found either singly or in combination with other Rh-related autoantibodies. The activity of autoantibodies for Rh<sub>null</sub> red cells can be removed by absorption using any human or nonhuman primate red cells but not by sheep, rabbit, or horse red cells. This is illustrated by the results obtained with eluate K.P. shown in Table 7. These findings suggest that the specificity of the antibody may be directed against the "nucleus of the Rh substance" as postulated by Wiener, Gordon, and Gallop.<sup>14</sup> The ability of Rh<sub>null</sub> cells to separate this autoantibody from other varieties of Rh-related antibodies influenced Wiener and Vos<sup>1</sup> to classify this autoantibody as anti-dl (i.e., "deletion").

Guinea pigs injected with the original Rh<sub>null</sub> red cells of Vos et al.<sup>15</sup> produced antibodies which were identical to those recovered in the eluates from the red cells of some patients with AIHA (see Table 7). In no instance did we observe the production of other known varieties of Rh-related antibodies, e.g., anti-LW in guinea pigs injected with Rh<sub>null</sub> red cells.

Table 8 shows the results obtained with a third variety of Rh-related autoantibody often recovered from the red cells of AIHA patients. These antibodies

**Table 7. Absorption Experiments With Red Cell Eluate KP**

		Reactions With Test Cells				
		CDe/cDE Lw+	CDe/cde Lw+	cde/cde Lw+	CDe/cde Lw-	Rh <sub>null</sub> Lw-
Before Absorption		++++	++++	++++	++++	++++
Absorbing Cells	Rh <sub>null</sub> . Lw-	0	0	0	0	0
	CDe/cde. Lw-	0	0	0	0	0
	cde/cde. Lw+	0	0	0	0	0
	CDe/cDE. Lw+	0	0	0	0	0
	Baboon	0	0	0	0	0
	Monkey	0	0	0	0	0
	Sheep	++++	++++	++++	++++	++++
	Rabbit	++++	++++	++++	++++	++++
Horse	++++	++++	++++	++++	++++	

fail to agglutinate partially deleted -D-/-D-, LW-positive red cells and Rh<sub>null</sub> cells but do react with all other red cells (i.e., anti-*nl*). Their specific activity seems to be directed towards a region of the Rh antigen representing the CcEe sequence. The antibody can be completely absorbed by Rh-positive, LW-negative and Rh-positive, LW-positive cells but not with red cells from baboons and monkeys. In our immunization studies we were never successful in producing this type of antibody in guinea pigs. Experiments are now in progress to establish whether antibodies to the CcEe region may be more readily produced in nonhuman primates, which like the donor of the partially deleted -D-/-D-red cells are known to lack these particular determinants.

**DISCUSSION**

Nugent, Colledge, and Marsh,<sup>16</sup> and Marsh, Reid, and Scott<sup>17</sup> recently reported anti-U as a specific autoantibody in AIHA. They also postulated that the "Rh-related" autoantibodies which often fail to be identified as specific Rh antibodies may in fact be anti-U. In this study we were able to examine the reactions of red cell eluates from eight AIHA patients against a selected combination of extremely rare types of red cells. Absorption and elution experiments showed autoantibodies with U specificity to be found in some, but not all, preparations of red cell eluates.

In eight selected AIHA eluates we observed two examples of anti-*nl*, five anti-*pdl*, three anti-*dl*, six anti-LW, one anti-*e*, and three anti-U autoantibodies.

**Table 8. Absorption Experiments Using a Selected Red Cell Eluate**

		Reactions With Test Cells					
		CDe/cDE Lw+	CDe/cde Lw+	cde/cde Lw+	CDe/cde Lw-	-D-/-D- Lw+	Rh <sub>null</sub> Lw-
Before Absorption		++++	++++	++++	++++	0	0
Absorbing Cells	CDe/cde. Lw-	0	0	0	0	0	0
	cde/cde. Lw+	0	0	0	0	0	0
	CDe/cDE. Lw+	0	0	0	0	0	0
	-D-/-D-. Lw+	++++	++++	++++	++++	0	0
	Baboon	++++	++++	++++	++++	0	0
	Monkey	++++	++++	++++	++++	0	0

This represents an 85% incidence of antibodies with specificities related to the Rh system and a 15% incidence of anti-U. This would tend to support the suggestion that some association exists between Rh and U antigens.<sup>18</sup>

The pdl autoantibody described by Weiner and Vos<sup>1</sup> is often found in AIHA eluates and is known to react with all human red cells except Rh<sub>null</sub>. This antibody previously could not be classified as anti-LW because it sensitized Mrs. Bigelow's "LW-negative" red cells. Of particular interest in the present study was the observation that Mrs. Bigelow's LW-negative red cells could separate anti-pdl into anti-pdl plus anti-LW (Table 4). It appears that Mrs. Bigelow, who so far has been accepted as LW-negative, has a weak form of the LW antigen present. The absorption studies were confirmed by injecting Mrs. Bigelow's cells into guinea pigs and demonstrating the presence of anti-LW after absorption with Rh<sub>null</sub> cells. It is tempting to suggest an analogy to the ABO system and postulate that Rh-positive cells represent LW<sub>1</sub>, Rh-negative cells represent LW<sub>2</sub> (as suggested by Levine,<sup>11</sup> and Mrs. Bigelow represents LW<sub>3</sub>. Rh<sub>null</sub> appears to be the true LW-negative (? lw). It will be interesting to see if studies of Rh-negative, LW-negative samples will show them to have a weaker form of LW than Mrs. Bigelow, who is Rh-positive. An apparent criticism of this analogy is that if Mrs. Bigelow represents LW<sub>3</sub>, comparable to A<sub>3</sub> in the ABO system, then the anti-LW in her serum should react with only LW<sub>1</sub> (i.e., Rh-positive) red cells, comparable to the anti-A<sub>1</sub> found in the sera of some A<sub>3</sub> individuals. However, the anti-LW reacts strongly with Rh-positive (LW<sub>1</sub>) cells and weakly with LW<sub>2</sub> (Rh-negative) cells. It is possible that the results are simply reflecting a quantitative difference rather than a qualitative difference. Even though anti-A<sub>1</sub> usually does not agglutinate A<sub>2</sub> cells, some workers have reported that anti-A<sub>1</sub> found in A<sub>x</sub> individuals reacts with both A<sub>1</sub> and A<sub>2</sub> cells.<sup>19</sup> We know there is a quantitative effect, as anti-A<sub>1</sub> can be removed by several absorptions with A<sub>2</sub> cells. This demonstrates that anti-A<sub>1</sub> does indeed react with A<sub>2</sub> cells. By analogy anti-LW<sub>1</sub> reacts with LW<sub>1</sub> strongly and LW<sub>2</sub> weakly. The only difference between the LW<sub>1</sub> reactions and the reactions of anti-A<sub>1</sub> with A<sub>1</sub> and A<sub>2</sub> cells is that LW reactions are demonstrated by strong agglutination versus weak agglutination whereas the A<sub>1</sub>, A<sub>2</sub> reactions are demonstrated by agglutination versus absorption. The over-all results of our studies suggest that LW represents a spectrum of variations from LW<sub>1</sub>, LW<sub>2</sub>, LW<sub>3</sub>, and maybe LW<sub>4</sub>. Rh<sub>null</sub> cells may be the only true LW-negative cells, and there appears to be a variation in LW even between homozygous D-positive and heterozygous D-positive individuals which adds to the evidence that D and LW are related in some way.

#### REFERENCES

1. Weiner W, Vos, GH: Serology of acquired hemolytic anemias. *Blood* 22:606, 1963
2. Vos GH, Petz LD, Fudenberg HH: Specificity of acquired haemolytic anaemia autoantibodies and their serological characteristics. *Br J Haematol* 19:57, 1970
3. Van Loghem JJ vd Hart M: Varieties of specific autoantibodies in acquired haemolytic anaemia. *Vox Sang* 4:2, 1954
4. Dausset J, Colombani J: The serology and the prognosis of 128 cases of autoimmune hemolytic anemia. *Blood* 14:1280, 1959
5. Fluckiger P, Ricci C, Usteri C: Zur Frage der Blutgruppenspezifität von Autoantikörpern. *Acta Haematol* 13:53, 1955
6. Yokoyama M, Eith DT, Bowman M: The first example of auto-anti-Xg<sup>a</sup>. *Vox Sang* 12:138, 1967



7. Celano MJ, Levine P: Anti-LW specificity in autoimmune acquired hemolytic anemia. *Transfusion* 7:265, 1967
8. Weiner W: Reconstitution of frozen red cells. *Lancet* i:1264, 1961
9. Garratty G, Petz LD: An evaluation of commercial antiglobulin sera with particular reference to their anti-complement properties. *Transfusion* 11:79, 1971
10. Vos GH, Kelsall GA: A new elution technique for the preparation of specific immune anti-Rh serum. *Br J Haematol* 2:342, 1956
11. Levine P, Celano MJ: Agglutinating specificity of LW factor in guinea pig and rabbit anti-Rh serums. *Science* 156:1744, 1967
12. De Veber LL, Clark GW, Hunking M, Stroup M: Maternal anti-LW. *Transfusion* 11:33, 1971
13. Ishimori T, Hasekura H: A Japanese with no detectable Rh blood group antigens due to silent Rh alleles or deleted chromosomes. *Transfusion* 7:84, 1967
14. Wiener AS, Gordon EB, Gallop C: Studies on autoantibodies in human sera. *J Immunol* 71:58, 1953
15. Vos GH, Vos D, Kirk RL, Sanger R: A sample of blood with no detectable Rh antigens. *Lancet* i: 14, 1961
16. Nugent ME, Coledge KI, Marsh WL: Autoimmune hemolytic anemia caused by anti-U. *Vox Sang* 20:519, 1971
17. Marsh WL, Reid ME, Scott EP: Autoantibodies of U blood group specificity in autoimmune haemolytic anaemia. *Br J Haematol* 22:625, 1972
18. Schmidt PJ, Lostumbo MM, English LT, Hunter OB: Aberrant U blood group accompanying Rh<sub>null</sub>. *Transfusion* 7:33, 1967
19. Issitt PD: *Applied Blood Group Serology* (ed. 1). Oxnard, Spectra Biologicals, 1970.