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Prevention of maternal RHO (D) sensitization

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Prevention of Maternal Rh₀ (D) Sensitization

by

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The College of Medicine in the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Medicine

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Omaha, Nebraska

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Prevention of Maternal Rh\textsubscript{0} (D) Sensitization

History

Erythroblastosis fetalis is an ancient disease; the hydropic form was probably known to Hippocrates. However, it was not until 1932 that three forms—hydrops, icterus gravis, and anemia of the newborn—were shown to be the same disease. 26 Landsteiner and Wiener 47 discovered the RH antigen in 1940. The antigen was first found on the red cell of the rhesus monkey, and hence its name, Rh factor. In 1941, Levine 50 recognized the relationship between erythroblastosis fetalis and atypical agglutinins in the mothers' serum which were identical to the anti-Rh agglutinin of Landsteiner. Several of Levine's patients had transfusion reactions after delivery when they received blood from their husbands. He suggested that the infant inherited something from the father to which the mother had antibody. The antibody then crossed the placenta where it caused the damage typical of erythroblastosis fetalis. This theory is the basis of Rh isoimmunization.

Levine 48 later added fetal-maternal ABO blood group incompatibility to the causes of erythroblastosis fetalis and confirmed Landsteiner's finding that Rh factor is inherited as a mendelian dominant gene.

The neonatal mortality of the disease was greatly reduced in 1947 when Diamond 23 proposed exchange transfusions. Liley 51 introduced the daring concept of intra-uterine transfusions as the next step in therapy in 1964.

Two groups of investigators, working independently and using different approaches, found a method for preventing erythroblastosis
fetalis early in the 1960's. Finn and Clark in Liverpool, England were impressed with Levine's finding that ABO incompatibility between the mother and the fetus seemed to prevent isoimmunization of the mother to the Rh factor. This was believed to be because the ABO agglutinins cleared the fetal red cells from the mother's blood before Rh isoimmunization could take place. They reasoned, "It seemed that circulating Rh positive fetal cells might be similarly eliminated by the administration of anti-D (anti-Rh\textsubscript{0}), thus preventing sensitization of the mother." They were able to prove this by injecting Rh positive blood and serum from Rh sensitized women into Rh negative male volunteers.

At the same time, Freda, Gorman, and Pollack in the United States were basing their work on the classical findings of Theobald Smith. While working with Diphtheria toxin-antitoxin mixtures in 1909, Smith showed that "... an excess of antitoxin reduces the possibility of producing an active immunity, and may extinguish it altogether." Freda's group was able to prevent Rh-negative isoimmunization in male volunteers with anti-Rh gamma-globin. These results suggested an effective method of preventing hemolytic disease of the newborn caused by Rh\textsubscript{0} isoimmunization.

Research in the past decade has followed three principal directions. Kleihauer reported a simple and sensitive method for identifying fetal red cells in adult blood in 1957. Many workers have used this technique to determine the frequency, timing and volume of transplacental fetal-maternal hemorrhage. Other investigators have clarified
the maternal immune response and attempted to find how it is suppressed by passive gamma-globin. The third approach has been clinical trials with passively administered gamma-globin in Rh<sup>−</sup> negative mothers who have delivered Rh<sup>+</sup> positive infants. Several large series have reported this treatment to be nearly one-hundred percent effective in preventing Rh isoimmunization.

Diamond recently commented on the unique history of hemolytic disease of the newborn due to the Rh factor: "Rarely has it been our good fortune to have a disease recognized, its cause clearly determined, its treatment successfully developed to a great extent, and then its prevention found, all in one generation."

**Incidence**

The Rh<sup>−</sup> antigen (D) occurs in about 85% of the Caucasian population. It is present in only 70% of the Basques, 75% of the Negros, and is rarely absent in Chinese, Japanese, and American Indians. About 12% of families are at risk because of Rh incompatibility, but sensitization takes place in only about 12% of these or 1.4% overall. There are about 400,000 risk pregnancies per year in the U.S. and 20,000 to 40,000 new sensitizations if no preventive treatment is used. These estimates agree with Smith’s series of 12,297 pregnancies in which 173 women or 1.4% became immunized to Rh<sup>+</sup>.

Hemolytic disease of the newborn occurs in about 1.8% of all deliveries. This is caused by ABO incompatibility in 1.2%, Rh<sup>−</sup> incompatibility in 0.6%, and rarely by other blood factors.
mortality in Rh affected infants has been reported as 36% when the antibody appeared before the fifth month and 15% when it first appeared after the sixth month. The stillbirth rate in Rh hemolytic disease has remained about 15% resulting in about 5,000 stillbirths each year in the United States. 26,30

Transplacental hemorrhage

The first event in Rh hemolytic disease is the leakage of fetal red cells into the maternal circulation. This leakage is thought to take place in the placenta, through breaks in fetal capillaries in the chorionic villi. The fetal cells then enter the intervillous space and the mother's general circulation. Research on transplacental hemorrhage (TPH) has contributed heavily to the development of a method of preventing maternal isoimmunization to the Rh\textsubscript{o} antigen.

Most workers use the Kleihauer \textsuperscript{44} technique to detect TPH. This is an acid elution method that destroys adult red cells. The preserved fetal red cells then show up against a background of adult ghost cells. Fetal hemorrhages as small as 0.1 ml. can be identified in the maternal blood by this technique. \textsuperscript{88} This method is also used to quantify TPH; five cells per 50 low power microscope fields is equal to 0.25 milliliters of fetal blood. \textsuperscript{38} Although there is good agreement with results from immunofluorescent methods, \textsuperscript{19} the Kleihauer technique has several possible errors that makes comparison of data from different laboratories difficult. Fetal cells contain hemoglobin F almost exclusively, but adult cells may also contain small amounts of hemoglobin F. Which makes them show up with the Kleihauer technique.
Woodrow found that hemoglobin F not due to fetal cells occurs in 6.3% of blood samples from pregnant women, in 2% of those from non-pregnant women, and 1.9% of those from a general hospital population. One-fourth of all slides have some irregularly stained cells and "definite foetal cells" are occasionally seen in non-pregnant women. Woodrow suggests that three cells per 50 low power fields or 0.15 ml. be the minimum criterium for TPH.

Other factors should be considered when studying TPH data. Fetal cells have a normal survival time of about 80 days in the maternal circulation. Thus, there is an additive effect from frequent small hemorrhages as the pregnancy approaches term. Hindemann showed that fetal red cells are absorbed from the adult peritoneal cavity and sometimes do not appear in the circulation for as long as 13 days after they are introduced. He also found that late invasion of the maternal blood by fetal red cells occurs in 3% of vaginal deliveries. This possible transabdominal route could explain maternal sensitization when no fetal cells were found immediately post-partum.

The frequency of TPH cannot be stated simply. Many factors seem to influence it, including how often fetal cells are searched for in the maternal blood and how generous are the worker's criteria for TPH. (See table 1). Cohen believes that small transplacental hemorrhages are physiologic. Clayton found that fetal cells are present at some time during pregnancy in 72% of pregnant patients when their blood is examined at each pre-natal visit. Other factors that alter the frequency of TPH will be discussed below.
Table 1

Transplacental Hemorrhage

<table>
<thead>
<tr>
<th>Author</th>
<th>Clinical Material</th>
<th>Fetal cells found</th>
<th>Amount considered positive</th>
<th>Large Hemorrhage</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finn</td>
<td>250 random deliveries</td>
<td>11.7%</td>
<td>any</td>
<td>greater than 5 ml. in 1.5%</td>
<td></td>
</tr>
<tr>
<td>Zipursky</td>
<td>normal obstetric cases</td>
<td>15.8%</td>
<td>any</td>
<td>greater than 0.5ml. in 0.5%</td>
<td>Kleihauer technique</td>
</tr>
<tr>
<td>Brown</td>
<td>165 normal deliveries</td>
<td>50%</td>
<td>any</td>
<td>greater than 0.5ml. in 6.8%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>132 with ABO compatible infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>33 with ABO incompatible infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohen</td>
<td>622 deliveries of ABO compatible infants</td>
<td>50%</td>
<td>any</td>
<td>0.5ml.-40ml. in 10%</td>
<td>Kleihauer and immuno-fluorescent techniques</td>
</tr>
<tr>
<td>Woodrow</td>
<td>200 random pregnancies</td>
<td>12%</td>
<td>30%</td>
<td>any</td>
<td>greater than 3.0ml. in 3%</td>
</tr>
<tr>
<td>McLarey</td>
<td>233 random pregnancies</td>
<td>54.3%</td>
<td>any</td>
<td>greater than 0.5ml. in 10.3%</td>
<td>Kleihauer technique</td>
</tr>
<tr>
<td>Woodrow</td>
<td>700 random pregnancies</td>
<td>3.5%</td>
<td>19.8%</td>
<td>70.15 ml.</td>
<td>Kleihauer technique</td>
</tr>
<tr>
<td>Author</td>
<td>Clinical Material</td>
<td>Amount considered positive</td>
<td>Large Hemorrhage</td>
<td>Comments</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------------</td>
<td>----------------------------</td>
<td>------------------</td>
<td>-----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Clayton 17</td>
<td>94 random and 100 Rh negative pregnant women</td>
<td>72%</td>
<td>any</td>
<td>greater than 0.1ml. in 10.8% Kleihauer technique tested each prenatal visit before onset of labor</td>
<td></td>
</tr>
<tr>
<td>Schneider 71</td>
<td>850 pregnancies</td>
<td>15.6%</td>
<td>greater than 0.05 ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zipursky 87</td>
<td>948 deliveries of ABO compatible infants</td>
<td>6.2%</td>
<td>greater than 0.1ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>242 deliveries of ABO incompatible infants</td>
<td>0.8%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohen 20</td>
<td>Rh positive mothers with ABO incompatible infant</td>
<td>52%</td>
<td>20%</td>
<td>any</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rh negative mothers with ABO incompatible infants</td>
<td>32%</td>
<td>5%</td>
<td>any</td>
<td></td>
</tr>
<tr>
<td></td>
<td>All ABO compatible mothers</td>
<td>50%</td>
<td></td>
<td>any</td>
<td></td>
</tr>
<tr>
<td>Clarke 13</td>
<td>1000 Rh negative women</td>
<td>18.6%</td>
<td>greater than 0.25ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>458 with Rh positive ABO compatible infants</td>
<td>20.2%</td>
<td>greater than 0.25ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>348 with Rh negative ABO compatible infants</td>
<td>4.2%</td>
<td>greater than 0.25ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>119 with Rh positive ABO incompatible infants</td>
<td>4.0%</td>
<td>greater than 0.25ml.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Table 1 (con't)*
The time of the sensitizing dose of fetal cells is an important and sometimes controversial point. Fetal red cells are detected in the maternal blood as early as the 14th week of gestation and in 12% of pregnancies by the 20th week. 17

McLarey 51 found fetal cells in 54% of his patients between 21 and 42 weeks of gestation. He found no increase in frequency during the third trimester but showed that the volume of TPH increases as term nears. Woodrow 84 using stricter criteria, found evidence for TPH only during the third trimester. Woodrow 84 and Schneider 71 studying women at term, demonstrated TPH in only 12% and 15.6% respectively.

Several studies have shown the incidence of fetal cells in maternal blood postpartum to be about 50%. 9,19,20 Woodrow 84 found TPH greater than 0.15 ml. in 19.8% of patients after delivery. In a similar study he reported fetal cells post-partum in 23% of patients who had no evidence of TPH before delivery. 83 Clark 12 made retrograde injections of tagged red cells through the umbilical vein at delivery. He found significant TPH in about one-third of his patients.

The volume of fetal-maternal hemorrhage varies from a trace to a reported 250 ml. 53 Most evidence suggests that the larger hemorrhages usually occur at term and during delivery. McLarey 54 found that TPH greater than 0.5 ml. occurred in 10% of pregnancies at some time before delivery. Clayton 17 found the same incidence for TPH greater than 0.1 ml. during pregnancy. Hemorrhages larger than 0.25 ml. have been reported in 9% and 16% of post-partum patients. 13,83 Recently, massive TPH has been described as the cause of newborn anemia and maternal trans-
fusion reactions. 5,19,53,63 These very large hemorrhages occur in about 1% of pregnancies. 19,29

Fetal maternal ABO incompatibility has a significant effect on TPH. Both the incidence and volume of TPH are decreased in ABO incompatible pregnancies as compared to compatible pregnancies. 9,13,29,55,87,88 This difference may be due to rapid clearance of incompatible cells from the maternal circulation; however, Cohen 20 showed normal survival of ABO incompatible fetal cells in cases of massive TPH.

Cesarean section, large placentae, manual removal of the placenta, and cervical laceration at delivery all are associated with increased risk of TPH. 9,55,58,88 Transabdominal amniocentesis does not seem to increase TPH if done with ease. 10,85 Zipursky 88 reported an increased incidence in primiparas; however, other workers found no significant relationship between TPH and maternal age, parity, previous abortion, infarcted placentae, duration of labor, or Apgar of the infant. 9,54 Finally, fetal-maternal Rh incompatibility does not seem to protect against TPH as effectively as ABO incompatibility. 13,54,87

These data suggest that transplacental hemorrhage, while common during pregnancy, is rare before the third trimester. Fetal cells are present post-partum in about 50% of women and are present in volumes greater than 0.25 ml. in about 10.81

Immunization

The Rh antigen is much more complicated than first thought. There are three pairs of allelomorphic genes and corresponding antigens in
the Rh system. These genes are inherited as mendelian dominants and recessives which give rise to eight phenotypes. 26 (see Table 2). In addition, the Rh\textsubscript{0} (D) antigen is made up of several components, some of which may be absent in certain individuals. These people, designated D\textsuperscript{u}, are Rh positive but make antibody to the complete D antigen. 6 Grobbelaar 37 believes that, "Many, possibly all, Rh\textsubscript{0} antisera are polyvalent and contain a spectrum of antibodies of different specificities, anti-Rh\textsubscript{0}, anti-Rh\textsuperscript{A}, etc." The following discussion will be limited to the Rh\textsubscript{0} antigen which is the most antigenic and the only one which commonly causes hemolytic disease of the newborn. 26,76

Smith 73 screened 12,297 random pregnancies and found that 1.4% were immunized to Rh\textsubscript{0}. When only Rh negative women are studied, the rate of immunization increases with the volume of fetal cells present after delivery. (See Table 3). Several workers agree that volumes of TPH smaller than 0.1 ml. cause sensitization of from 2.6 to 4.6% of Rh negative women. 71,83,86 Zipursky 86 found that 15.6% of Rh negative mothers were immunized by TPH greater than 0.1 ml. Finn 28 and Woodrow 83 reported a 20% incidence with TPH greater than 0.25 ml. The risk of immunization may reach a limit of 50% as the volume of TPH rises above 5 to 10 ml. 82

Although the risk is less, more women may be immunized when no fetal cells or only small volumes are demonstrated after delivery. 83,86 Delayed transabdominal passage of fetal cells into the maternal circulation has been suggested as the cause of immunization when no TPH is found after delivery. 41
Table 2
Rh<sub>o</sub> Isoimmunization

<table>
<thead>
<tr>
<th>Author</th>
<th>Material</th>
<th>ABO of infant</th>
<th>Number</th>
<th>Volume TPH</th>
<th>Immunized</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schneider 71</td>
<td>Rh negative women</td>
<td>Any</td>
<td>39</td>
<td>greater than 0.05 ml.</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Finn 28</td>
<td>Rh negative</td>
<td>Any</td>
<td>130</td>
<td>greater than 0.25 ml.</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>Woodrow 83</td>
<td>Rh negative primiparas</td>
<td>Compatible</td>
<td>126</td>
<td>none</td>
<td>4</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>less than 0.25 ml.</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.25 ml to 3.0 ml.</td>
<td>1</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>greater than 3.0 ml.</td>
<td>2</td>
<td>67</td>
</tr>
<tr>
<td>Pollack 66</td>
<td>Rh negative women</td>
<td>Compatible</td>
<td>726</td>
<td>not tested</td>
<td>51</td>
<td>7</td>
</tr>
<tr>
<td>Sullivan 78</td>
<td>Rh negative</td>
<td>Compatible</td>
<td>84</td>
<td>none</td>
<td>2</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>54</td>
<td>any</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zipursky 86</td>
<td>Rh negative</td>
<td>Compatible</td>
<td>472</td>
<td>Less than 0.1 ml.</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>440</td>
<td>greater than 0.1 ml.</td>
<td>5</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>32</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The time at which maternal sensitization occurs is critical and somewhat controversial. Most studies show that maternal antibodies to the Rh factor develop within 6 months when none are found at delivery. Woodrow feels that most significant TPH occurs at or just before labor and that immunization begins at that time. Since small TPH occurs frequently during pregnancy and more women are sensitized by small volumes of fetal cells, Zipursky believes that most immunization occurs before labor. Chown found anti-Rh antibodies in 2% of Rh negative primigravidas at the time of delivery. Using sensitive tests, Krieger found that twice as many Rh negative mothers were immunized during pregnancy as during labor and delivery. Many of these would not be detected by direct Coombs tests on cord blood or by routine post-partum serologic tests. Bergstrom found Rh antigen on the red cells of a 38 day old fetus and suggests immunization by a previous early abortion as the cause of antibodies in primagravidas. The higher risk of immunization during a second pregnancy suggests that some women with antibodies are not identified.

As would be expected from its effect on TPH, ABO incompatibility protects against Rh isoimmunization. The incidence of ABO incompatible matings in the general population is 35%, while in those matings which result in Rh hemolytic disease of the newborn the incidence is only 13 to 25%. It has been shown in Rh negative male volunteers that ABO incompatible Rh positive blood is less capable of causing Rh isoimmunization. Stern showed that this protective effect is not due to maternal anti-A
or anti-B globulin blocking the Rh antigen sites on the fetal cells. He felt that the theory of clonal competition for antibody best explained this protection: "The basic concept of this theory presupposes that presence of clones of antibody-forming cells, each of which produces a specific globulin which is identical with an antibody for a specific antigen. Exposure to the antigen results in proliferation of the corresponding clone which then synthesizes large amounts of this globulin and thus brings about appearance of circulating antibody. The theory explains differences between responses to primary and secondary exposure to the antigen." If, for instance, the Rh negative Type O mother has a large clone producing anti-A globulin when exposed to fetal cells with both A and Rh_0 antigens, the anti-A clone will take up most of the fetal cells. This would allow little chance for the potential anti-Rh_0 close to be stimulated. 77

Hemolyzed cells are less antigenic than intact cells. 65,71 Pollack 65 believes that complement fixing antibody of the ABO system hemolyzes fetal cells and they are cleared by the liver and kidney before Rh immunization can occur. Mollison 57 suggests that the ABO incompatible fetal cells are taken up by the macrophages in the liver which is an unfavorable site for antibody formation.

Whatever the mechanism, ABO incompatible fetal cells are not found as often in the maternal circulation and are not as capable of Rh_0 isoimmunization as are compatible cells. However, this protection is not complete.

Other factors seem to influence the incidence of Rh_0 immunization.
Taylor found that the Rh type of the grandmother is significant in Rh hemolytic disease. An Rh negative woman with an Rh positive mother was more likely to have an affected infant by the third pregnancy than a woman with an Rh negative mother. It is possible that these women were sensitized in-utero by their mothers.

As expected, there is an increased risk of immunization with complicated deliveries, probably a function of increased TPH.

There is also a variable individual response to Rh subo antigen in all studies. Not all women are immunized even with very large TPH. Although the rate of immunization of volunteers increases with repeated exposure and large amounts of antigen, some Rh negative women do not produce antibodies even after many Rh positive pregnancies. Taylor found that 88% of the women who produced antibodies, did so by three Rh positive, ABO compatible pregnancies. Woodrow believes that pregnant women in general may be less susceptible to antigen stimulation.

The maternal immune system usually responds to the Rh subo antigen on the surface of fetal red cells with antibody production. Both macrophages and lymphoid tissue are involved in this response. The mother makes two types of antibody to the Rh subo antigen. The first to appear is a macroglobulin, IgM, which has a molecular weight of 1,000,000, a sedimentation constant of 195, and is most active in a saline medium. This antibody is present in the serum for about one week then begins to decrease as the second type of antibody increases. This antibody, IgG, has a molecular weight of 160,000, a sedimentation constant of 75, and is most active in an albumin medium. It has been suggested that IgG
is responsible for the end of IgM production. The immune response will be discussed in greater detail below.

The placenta is selective in allowing antibody to cross into the fetal circulation. The IgM macroglobins cannot cross while the IgG antibodies are actively transported. The transfer occurs directly across the chorionic villi into the fetal circulation rather than indirectly via the amnionic fluid. All fetal antibody is acquired from the mother. The fetal gamma globin level remains low until 4 months of pregnancy then rises to the maternal level at 9 months and may slightly exceed it at term. After birth, the infant begins to produce his own antibody as the maternal antibody disappears. The child's antibody level does not reach adult levels again until about two years of age. (See fig. 1). In the fetal circulation the anti-Rh\(_{o}\) antibody destroys fetal red cells and produces the changes characteristic of erythroblastosis fetalis.

The mother is only rarely affected by Rh\(_{o}\) isoimmunization. An erythroblastotic maternal syndrome consisting of rapid fluid retention without hypertension or proteinuria has been described in mothers with severely affected fetuses. All symptoms and signs disappear after fetal death or delivery but increase in severity with the next pregnancy. Rarely, large TPH causes transfusion reactions in Rh immunized mother.
Prevention of Immunization

Since Levine described the cause of erythroblastosis fetalis, interest has turned toward finding a way to prevent the disease. Several workers have looked for ways to stop the leakage of fetal cells across the placenta. Doolittle reduced the immunization rate of Rh negative mothers with a delivery routine that stressed drainage and spontaneous delivery of the placenta. Messer used an adren-ergic drug that increases capillary resistance to injury and
lessens but does not eliminate TPH.

The second approach is to limit the maternal immune response. Hackel 39 found that components of nucleic acids lower the titer of anti-Rh serum in vitro. Sugars and amino sugars produce a similar effect. 8,62 The amino sugars are part of the polysaccharide blood group antigens and probably combine with antibody to lower titers. 62

The most successful method of preventing Rh hemolytic disease is to suppress maternal antibody production with passive antibody administration. Finn 29 and Freda 33 are credited with the discovery of this method. In the first experiments Rh negative male volunteers received both Rh positive blood and anti-Rh antibody. The antibody protected them from isoimmunization to the Rh₀ antigen and produced no side effects. 15,16,29,31,33,87 Clinical trials in postpartum Rh negative women were then begun and a great deal of data is now available. (See Table 3). These studies show that anti-Rh₀ gamma-globulin is almost 100% effective in preventing the appearance of anti-Rh₀ antibodies in the maternal serum after delivery of a Rh positive infant. 28, 40,66,67,71 In addition, some of these patients have been followed through a second Rh positive pregnancy without evidence of immunization. 28,40,66, 67,71 In addition, some few patients have been treated after more than one Rh positive pregnancy with equally good results after as many as four treatments. 40

A great deal more is known about the mechanism of immune response and how it is prevented than was known at the time this treatment was
discovered. Finn assumed that the passive antibody would eliminate the fetal cells before the mother was immunized. Studies with Cr \(^5\) tagged Rh negative red cells show that passive anti-Rh antibody does increase the clearance of these cells. The speed of clearance is dose related; the rate of red cell destruction is proportional to the square root of the amount of antibody on the cells. (See figure 2) Delayed clearance of fetal cells is associated with maternal immunization in cases of massive TPH protected with small doses of passive antibody. Clarke suggested that 95% of the incompatible cells have to be removed within 24 hours to prevent immunization.

Most authors now believe that clearance of the fetal cells is not as important as antigen competition in the prevention of maternal immunization. They base this belief upon what is presently known about normal antibody production.

There are two principal theories of the mechanism of antibody production at the present time. The clonal selection theory was discussed above in connection with the protective effects of ABO incompatibility. This theory does not require the continued presence of antigen for antibody production and has been largely supplanted by the instructional theory. According to this theory, antigen is taken up by macrophages which have the capacity to induce antibody formation in the lymphoid tissue. The macrophages produce messenger RNA in response to antigen. The messenger RNA carries information for IgM production to the lymphoid tissue. The macrophages are also
thought to release RNA-antigen complexes which seek out the already committed lymphoid cells. The antigen in the complex selects the proper cells and gains entrance for the RNA. These cells then produce IgG. This theory requires continued antigen stimulation for antibody production and still provides an explanation for the enhanced response to a second dose of antigen. The early appearance of IgM in the immune response tends to support this theory.

Figure 2

Relationship of Red Cell Clearance to Dose of Passive Antibody

0.3 ml. of Rh-positive blood amounts are figures against curves
Gamma-globulin 10 minutes later in micrograms
Siskind recently modified this theory to include the affinity of circulating antibody as a control mechanism. There is a marked difference in the affinity of antibody for antigen. This affinity increases as the immune response continues. Siskind suggested that antibody forming cells have antibody-like molecules on their surface which captures antigen. They are then stimulated to produce more antibody. Cells with the highest affinity molecules would have the best chance to capture antigen and would produce antibody with high affinity. This would explain how circulating antibody prevents hyperimmunization by feedback to the antibody producing cells.

These theories help explain some observations about the suppression of the immune response with passive antibody. The passive anti-Rho antibody is thought to combine with the Rho antigen and prevent it from stimulating lymphoid cells to produce antibody. The site of this interaction is not known for certain but is probably extra-circulatory. It is known that Rh antibodies cause red cells to be sequestered and broken down in the spleen. Pollack believes that passive antibody prevents macrophages from processing antigen and producing messenger RNA. The antibody may also compete with lymphoid cells for RNA-antigen complexes necessary for continued IgG production.

Passively administered IgG antibody is more effective in suppressing active immunization than IgM antibody. Smaller doses of high affinity antibody prevent immunization as well as larger doses
of low affinity antibody. \textsuperscript{56,72} Since IgG is produced late in the immune response it would be expected to have a high affinity for antigen and could successfully compete with the lymphoid cells.

One disturbing result that was found in some trials was significant enhancement of immunization when passive antibody was given. \textsuperscript{15,18,22,65,72} This usually occurred when the doses of gamma globin were relatively small. Pollack \textsuperscript{65} was able to demonstrate this effect in Rh negative men. (See Fig. 3) Siskind \textsuperscript{72} produced similar results with very small doses of high affinity antibody. He believes that small doses of antibody cause suppression of only the low affinity lymphoid cells, allowing the high affinity cells to produce relatively more antibody than usual.

\textbf{Figure 3}

Enhancement of the Immune Response

\begin{center}
\begin{tikzpicture}
\begin{axis}[
    width=\textwidth,\n    height=0.5\textwidth,\n    ybar,\n    bar width=10pt,\n    ymin=0,\n    ymax=15,\n    ylabel=Number of patients,\n    xlabel=Dose of gamma-globulin in micrograms,\n    symbolic x coords={0, 1, 10, 20, 40},\n    xtick=data,\n    ytick={0, 5, 10, 15},\n    legend style={at={(0.5, 0.85)},\n                  anchor=north,\n                  legend columns=-1,\n                  draw=white!15!black,\n                  fill=white,\n                  font=\footnotesize,\n                  nodes={text=black,\n                         font=\scriptsize}},\n]
\addplot[\textcolor{red}{pattern=north east lines},\n         pattern color=red] coordinates {(0, 5) (1, 10) (10, 20) (20, 20) (40, 5)};
\addplot[\textcolor{blue}{pattern=horizontal lines},\n         pattern color=blue] coordinates {(0, 5) (1, 10) (10, 20) (20, 20) (40, 5)};
\addplot[\textcolor{green}{pattern=dots},\n         pattern color=green] coordinates {(0, 5) (1, 10) (10, 20) (20, 20) (40, 5)};
\addplot[\textcolor{orange}{pattern=north west lines},\n         pattern color=orange] coordinates {(0, 5) (1, 10) (10, 20) (20, 20) (40, 5)};
\addplot[\textcolor{purple}{pattern=crosshatch},\n         pattern color=purple] coordinates {(0, 5) (1, 10) (10, 20) (20, 20) (40, 5)};
\end{axis}
\end{tikzpicture}
\end{center}

\textit{tested} \hspace{1cm} \textit{immunized} \hspace{1cm} (Each received 5 ml. of Rh+ blood)
The amount of passive antibody necessary to suppress the maternal immune response appears to depend upon the dose of antigen present. The passive antibody is measured in micrograms of anti-Rho gamma-globin. Equally good results are obtained with doses of 200 to 300 micrograms as with 1000 micrograms. Most of the treatment failures that have been reported are cases of very large fetal-maternal hemorrhage. Hughes-Jones suggests that the absolute number of unbound antigen sites is the limiting factor in immune suppression. There are about 12,000 Rho antigen sites on each fetal cell: With TPH of 400 milliliters, 10,000 micrograms of gamma-globin would be necessary to bind one-half of the antigen sites and suppress immunization. Seventy-five micrograms will occupy 10% of the sites on one milliliter of fetal cells and prevent immunization. The affinity of the antibody for antigen as well as the dosage would affect the number of bound sites.

There is some disagreement about when the passive antibody should be administered. Suppression is more difficult late in the immune response but has been accomplished in animals as long as 40 days after immunization. Siskind produced late suppression with large doses of high affinity antibody. Some authors believe that immunization usually occurs during pregnancy rather than after delivery. Zipursky found that anti-Rho gamma-globin given during pregnancy is effective in preventing immunization and does no harm to the fetus. The half-life of human gamma-globin is about 30 days and would provide prolonged protection if given during pregnancy.
In most large series the antibody is given within 72 hours of delivery with excellent results. 28,66

Another important question is which patients should be protected? Certainly, all Rh negative mothers with Rh positive ABO compatible infants should receive gamma-globulin if they are not already immunized. The presence of fetal cells in the maternal circulation after delivery should not be a requirement for protection. 66,78 Although ABO incompatibility is protective it is not completely effective. 49,76 These patients with ABO incompatible infants probably should also receive anti-Rh₀ antibody. 66 In addition, treatment has been recommended after early abortions and any manipulation likely to cause transplacental hemorrhage. 3,64

There is very little risk to this treatment. The danger of serum hepatitis has been eliminated by use of gamma-globulin preparations. Clarke 14 found that there is about a 6% incidence of maternal sensitization to gamma-globulin. This also occurs with blood transfusions and rarely causes symptoms. 14 A few local skin reactions at the site of injection and fevers lasting 24 hours have also been reported. 13 There seems to be no damage to the immune system in general and there is no increase in infections in those treated. 14

Passive Rh antibody treatment may select against Rh-positive fetuses in subsequent pregnancies. Finn 28 found that 50% of the treated Rh-negative women had Rh-positive infants with the next pregnancy. This is a significant difference from the expected 80% incidence.
It is not yet clear whether this represents true selection or, if so, what are its implications.

Discussion

There can be little doubt that anti-Rho gamma-globulin is a safe and effective method of suppressing maternal Rho immunization. The treatment is still expensive; the price of one standard dose of anti-Rho gamma-globulin is $50.00 at the University of Nebraska Hospital. However, the cost has been lowered and is expected to decrease further.

The development of this treatment has contributed a great deal to the basic understanding of the immune system. The human Rho factor is an important research tool because the antigen is normally absent in a significant number of people, is naturally introduced into pregnant women, and is easily identified with the Kleihauer technique. It is understandable that this is the first disease of an immune nature for which a specific method of prevention has been developed.

Several questions about this method still need to be resolved. There is some evidence that maternal isoimmunization usually begins during pregnancy. However, passive antibody given after delivery still effectively suppresses maternal antibody production. Animal studies have shown that the immune response can be suppressed as long as 40 days after antigen is introduced. It is possible, then, that some of the treated mothers who have no antibody six months after delivery actually were immunized during pregnancy and then suppressed by passive gamma-globulin at delivery. There is a marked difference in the sensitivity of the serologic tests used in the clinical series and small
amounts of maternal anti-Rh₀ antibody may be overlooked. If this is true, these women would be capable of an amnestic immune response during a subsequent Rh₀ positive pregnancy. This problem will be resolved when more women have been followed through several Rh₀ positive pregnancies. It may be necessary to give gamma-globulin during pregnancy as Zipursky suggests.

Another interesting question is raised by this line of thought. Theoretically, the maternal immune response could be suppressed by very large doses of IgM passive antibody. This antibody does not cross the placenta and could do no damage to the fetus. Perhaps erythroblastosis fetalis could be prevented even when the mother is already immunized by giving IgM early in pregnancy.

There are other possibilities. If antibody could be altered so that it would still compete with the lymphoid cells for antigen but could not produce the changes seen in auto-immune diseases, passive antibody could be used to treat these disorders. Perhaps small doses of unaltered, very high affinity antibody could accomplish this.

Whatever the future applications of these principles, it seems likely that hemolytic disease of the newborn caused by the Rh₀ antigen will soon go the way of smallpox, diphtheria, and polio.

**Summary**

In the past 30 years Rh₀ hemolytic disease of the newborn has been recognized, its cause found, and its prevention developed. This disease occurs in about 0.6% of all deliveries and has a stillbirth rate of about 15%, resulting in 5,000 stillbirths each year in the
United States.

The leakage of fetal red cells across the placenta into the maternal circulation is the first event in Rhₐₐ hemolytic disease. Many factors influence this transplacental hemorrhage; but in general, TPH is very common during pregnancy, occurs during delivery in about one-half of all patients, and is greater than 0.25 ml. in about 10%.

The mother then produces antibodies to the Rhₐₐ antigen on the fetal red cells. The rate at which maternal isoimmunization occurs depends on the dose of fetal cells, the ABO types of the mother and fetus, and the variable individual response of the mother. It is not clear whether immunization usually occurs during pregnancy or after delivery.

In affected pregnancies the anti-Rhₐₐ IgG antibodies cross the placenta and destroy fetal red cells. There have been attempts to interrupt this process at several points. The most successful method is suppression of the maternal immune response with passively administered anti-Rhₐₐ gamma-globulin. According to the instructional theory of antibody production, the passive antibody competes with the lymphoid cells and macrophages for antigen. As expected, high affinity antibody is more effective than low-affinity antibody and the antigen-antibody ratio is critical in immune suppression. The phenomenon of enhancement of the immune response with small doses of passive gamma-globulin can also be explained with the present theories.

Passive anti-Rhₐₐ gamma-globulin is a safe and very effective method of preventing Rh-hemolytic disease of the newborn. There is still some
question about the optimal time of administration and whether the treatment is still effective after several Rh-positive pregnancies. Continued clinical trials will soon answer both questions. The principles discovered during the development of this treatment may be applicable to other immune diseases.
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