Rh Immunization Following Incompatible Blood Transfusion and a Possible Long-term Complication of Anti-D Immunoglobulin Therapy

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Anti-D immunoglobulin is now widely used to prevent Rh-negative mothers from being sensitized by Rh-positive fetal erythrocytes. The administration of anti-D following the transfusion of large amounts of Rh-positive erythrocytes into Rh-negative individuals has been less uniformly successful in preventing primary immunization (Bowman and Chown, 1968), and occasional mild (Woodrow *et al*, 1968) or severe (de Veber, 1968) reactions to plasma containing anti-D have been noted. The present case report demonstrates that while a large dose of anti-D immunoglobulin may be given with safety, a patient who is already weakly sensitized to the D antigen will subsequently show a rapid secondary immune response. Furthermore, a complication of unknown importance is the development of a positive direct anti-human globulin reaction on the patient's red cells; this reaction appearing 6 months after the immunoglobulin treatment.

Case History

A 33-year-old multiparous housewife, blood group O Rh negative was admitted to the Casualty Department in a state of collapse. A diagnosis of haemorrhage from an ectopic pregnancy was made. At laparotomy a ruptured tubal pregnancy of approximately 6 weeks duration was found; the abdominal cavity containing about 2 litres of blood. During the operation the patient received 2 units of group O Rh-positive blood (approximately 400 ml of red cells) followed by 5 units of O Rh-negative blood. Her postoperative course was satisfactory and without gynaecological complication. An attempt to destroy the incompatible red cells by injection of anti-D immunoglobulin was considered advisable, as initial testing of the pretransfusion serum failed to reveal any irregular antibodies and the direct antiglobulin test was negative. When later repeat tests of the pre-transfusion serum revealed that the patient was in fact weakly sensitized to the D antigen, immunoglobulin therapy was stopped.

The patient was seen at regular intervals for the subsequent 12 months. Eight months after the first ectopic pregnancy, intraperitoneal haemorrhage from a second ectopic pregnancy occurred and this again required emergency laparotomy.

Anti-D Therapy

Forty hours after the mismatched transfusion, the patient received 860 micrograms of anti-D immunoglobulin as a single intramuscular injection. During the next 5 days, pulse, temperature, and blood pressure were recorded every 4 hours. A fluid balance chart was maintained and the oral fluid intake kept above 3 l/24 hours. The subsequent dosage of anti-D is illustrated in the Figure. The total dose over 96 hours was 6860 micrograms, and this was given as intramuscular injections of 860, 1200, 1400, 1400, and 2000 micrograms at 24-hour intervals. The clinical state was monitored closely after each injection. There was a slight increase in pulse rate and temperature 2-3 hours after each injection, but no other untoward effects were noted.

Before, during, and after the blood transfusion and subsequent to the anti-D therapy the following parameters were measured; haemoglobin, PCV, serum bilirubin, plasma haemoglobin, haptoglobin, α hydroxybutyrate dehydrogenase (α HBDH), the clearance of the D positive cells from the circulation, and the titre of anti-D.

Materials and Methods

Haemoglobin, PCV, serum bilirubin, plasma haemoglobin, and haptoglobin were all measured by standard methods (Dacie and Lewis, 1968); α HBDH estimation was performed by the method described by Wilkinson (1962).*

Anti-D immunoglobulin was obtained from the Lister Institute.†

Screening tests for irregular antibodies were performed by manual techniques using saline at 12° C and

* We are grateful to Dr A. C. Pollard, Fulham Hospital, London W6 for performing the α HBDH estimations.

† Prepared on behalf of the Department of Health and Social Security by the Blood Products Laboratory, Lister Institute, Elstree, Hertfordshire.

TABLE
RESULTS OF PARAMETERS STUDIED

	Day								
	1	2	3	4	5	6	7	8	14
Haemoglobin (g/100 ml)	11.5	11.1	11.1	11.7	11.9	11.9	10.7	10.7	11.7
Bilirubin (mg/100 ml)	—	—	0.6	0.5	1.5	1.2	0.4	0.3	—
Plasma haemoglobin (mg/100 ml)	—	6.6	4.5	1.5	8.0	13.9	6.3	1.0	1.0
α HBDH (units/100 ml)	—	160	—	200	240	520	520	380	240
Haptoglobin (mg/100 ml)	—	20	55	90	55	Nil	Nil	Nil	35
Anti-D titre (μg/ml)	0.005	0.005	0.005	0.005	0.15	12.1	25	95	162
Percentage survival of D positive cells	—	100	100	100	60	40	10	3	—

37° C, albumin addition, anti-human-globulin and enzyme techniques (bromelin, papain, and ficin). A comprehensive panel of frozen genotyped red cells was used throughout the investigation.

Quantitation of anti-D was carried out using the Technicon Autoanalyzer by the method described by Judd and Jenkins (1970).

Post-transfusion survival of Rh-positive cells was followed by using the automated Ashby differential agglutination techniques (Szymanski *et al*, 1967).

Results

As mentioned above, initial testing of the pre-transfusion serum by albumin and indirect antiglobulin techniques failed to detect anti-D, and anti-D immunoglobulin therapy was started in order to prevent primary immunization to the D antigen.

Subsequent investigations of this pretransfusion sample of blood showed the patient to be group O cde/cde, direct antiglobulin test negative. The serum contained weak anti-D agglutinins demonstrated by the indirect antiglobulin test, papain and ficin premodified cell techniques, and by the one-stage bromelin technique at pH 7.3.* Cells from the two Rh-positive donors used in the transfusion gave positive reactions against the patient's serum on repeat cross-matching by indirect antiglobulin and enzyme (bromelin) techniques.

In view of these findings the anti-D immunoglobulin therapy was stopped as there was no reason to suppose that a secondary immune response to the D antigen could be modified in any way.

The Figure illustrates the rapid rise of anti-D concentration following the transfusion from a level of less than 0.005 μg/ml to 162 μg/ml at 14 days. The anti-D titre 72 hours after the start of immunoglobulin therapy was 12.5 μg/ml, a value much higher than the passive level possible from the injected anti-D alone, which indicates that most of this anti-D had come from the patient's immune

response. At this time 40% of the D positive cells were still in the circulation.

The other results are summarized in the Table. It will be seen that nearly all 400 ml of the foreign Rh-positive cells were cleared from the circulation within 96 hours of starting anti-D immunoglobulin therapy. The biochemical changes resulting from this catabolism of approximately 120 g of haemoglobin over a known period of time are shown. The changes in αHBDH can be ascribed to the haemolysis since there was no evidence of liver or muscle disease (Plummer *et al*, 1963).

Samples of blood from the patient were sub-

FIG. Changes in relation to anti-D therapy.

* The presence of weak anti-D antibody was confirmed by Dr Goldsmith, Blood Group Reference Laboratory, Gatliff Road, Off Ebury Bridge Road, London SW1.

mitted to full serological investigation at intervals during the subsequent 12 months. Initial post-transfusion samples showed a negative direct antiglobulin test, mixed field agglutination with anti-D sera and free anti-D in the patient's serum.

The picture changed as the transfused Rh-positive cells disappeared and as the secondary response caused a rapid rise in anti-D titre. A weakly positive direct antiglobulin test was noted on the 3rd, 4th, and 5th days after transfusion. This test became negative by the 6th day after transfusion. Details are given in the Table.

The re-appearance of a direct antiglobulin test 6 months after treatment was surprising. Serological investigation of a sample taken at this time showed that the patient's cells were sensitized with IgG auto-antibody. An eluate prepared from the cells showed a warm type nonspecific antibody reacting equally strongly with all of a comprehensive panel of genotyped cells including D—/D—. The serum contained anti-D to a titre of 20.4 µg/ml, together with a trace of both anti-C and a non-specific warm type antibody. The latter was detected by enzyme techniques only.

Three months later, ie, 9 months following treatment, the anti-D titre had fallen to 8.8 µg/ml and the direct antiglobulin test was slightly weaker. It was not possible to elute antibody off the red cells, but there was still a trace of nonspecific warm type antibody in the serum. The investigation of this sample, however, was complicated by the fact that the patient had received a further 6 pints of Rh-negative blood 5 weeks earlier to treat haemorrhage from a second ectopic pregnancy. A final sample taken one year after treatment showed a negative direct antiglobulin test. The serum now contained anti-D to a titre of 3.5 µg/ml with a trace of anti-C. No other irregular antibody was present.

Discussion

This report provides further evidence that in cases of Rh incompatible transfusion, the use of anti-D immunoglobulin intramuscularly in large doses is a relatively safe procedure and unlikely to be accompanied by any immediately harmful effects of red cell breakdown. Adverse reactions to anti-D have occurred when whole plasma containing anti-D was injected but not when purified preparations of anti-D were given (de Veber, 1968).

Haemolysis was predominantly extravascular in type although there was a slight transient elevation of plasma haemoglobin. The D positive cells were cleared from the circulation between 24 and 120 hours from the start of anti-D therapy, but the

patient's own immune response probably assisted in their removal. Bilirubin reached peak concentration at 48 hours and plasma haemoglobin at 72 hours. α HBDH reached its peak a little later at between 72 and 96 hours. Haptoglobins were depleted by 72 hours. The well defined transient elevation of α HBDH activity confirms that the measurement of the two anionic lactate dehydrogenase isozymes (LDH₁ and LDH₂) is a useful indication of haemolysis, at least when this is mainly extravascular in type. The non nucleated red cell contains mainly two lactate dehydrogenase isozymes LDH₁ and LDH₂ (*Nutrition Reviews*, 1966), and it has been shown that in the absence of liver or muscle disease, α HBDH activity is mainly dependent on the concentration of these two anionic lactate dehydrogenase isozymes (Plummer *et al*, 1963).

The injection of 6860 µg of anti-D immunoglobulin, if rapidly absorbed would produce a peak plasma level of about 3 µg/ml of anti-D in this patient. The very rapid rise of anti-D to a level approaching 100 µg/ml within 7 days of the Rh positive transfusion is typical of a secondary response in a previously immunized recipient. The case offers further evidence that anti-D immunoglobulin is unable to suppress a secondary response even when the initial anti-D is present in trace quantity.

The most interesting feature of this case has been the development of a positive direct antiglobulin test and the identification of a warm type nonspecific auto-antibody on the cells, and free in the serum 6 months after administration of the massive dose of immunoglobulin. Full clinical and haematological investigation has failed to reveal any other cause for this auto-antibody. The patient was not taking any drugs and had not had any recent infections. There was no evidence of excessive haemolysis. The direct antiglobulin test remained positive for at least 3 months but was negative by one year after treatment.

The appearance of auto-antibodies during an isoimmune red cell response is discussed by Mollison (1967). The rare cases of post-transfusion thrombocytopenia may be due to a similar mechanism. Morrison and Mollison (1968) suggested that the combination of platelet with anti-platelet antibody in some way acts as a complex foreign to the immune system. The resultant antibody then cross reacts with the patients platelets to produce thrombocytopenia. P.L. Mollison (personal communication) offers a similar theory to explain our findings. The auto-antibody was provoked in response to a massive dose of foreign antigen, ie, the combination

of Rh-positive cells and anti-D immunoglobulin. The resultant antibody then cross reacted with the patients own red cells. Bowman *et al* (1961) described a similar case, and while not strictly comparable, it should be noted that Cook (1971) discovered two Rh-negative male volunteers who produced positive direct antiglobulin tests within 6 to 8 weeks of primary immunization with small intravenous doses of Rh-positive red cells. The positive antiglobulin tests appeared simultaneously with the iso anti-D and persisted for about five months. It is worth noting the possibility of auto-antibody formation in the event of this being detected in follow-up samples from women who have been treated with anti-D immunoglobulin.

Summary

Rhesus-positive red cells (400 ml) were transfused into an Rh-negative patient who was at that time weakly sensitized to the D antigen. The foreign cells were eliminated from the circulation over a period of 5 days by large doses of anti-D immunoglobulin assisted by a rapid immune response. Biochemical changes subsequent to this haemolysis were studied and no adverse clinical effects were observed.

Six months later the patient developed a positive direct antiglobulin reaction which persisted for 3 months. The significance of the auto-antibody production and its relation to the previous immunoglobulin therapy is discussed.

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