Reduced severity of Rh-haemolytic disease after anti-D immunoglobulin

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Summary

A total of 2459 Rh-negative women who received anti-D immunoglobulin after a Rh-positive pregnancy were followed up in at least one subsequent pregnancy. There was a failure of protection rate of 1.6%. Follow-up of 53 subsequent infants of mother in whom protection had failed showed that the infants were less severely affected than would have been expected. This was confirmed by a comparative statistical analysis of the present series and a series of first affected cases before anti-D immunoglobulin was available, using the antibody titre during pregnancy and the haemoglobin levels at delivery.

Introduction

It has been shown conclusively¹⁻⁵ that giving anti-D immunoglobulin to unsensitised Rh-negative women at the time of delivery of an Rh (D)-positive infant reduces the incidence of anti-D in subsequent pregnancies by a factor of 10.

Reports recording the incidence of failure have given little information on the clinical outcome of subsequent Rh (D)-positive pregnancies, but these infants seem to be less severely affected than would be expected. We describe here the clinical state of 53 infants born to Rh-negative women who had received anti-D after a previous pregnancy but whose serum was found to contain anti-D in the subsequent pregnancy.

Regional programme for administration of anti-D

If an Rh (D)-negative woman has no antibodies detected antenatally and is delivered of an Rh (D)-positive child with a negative direct antiglobulin (Coombs) test result she is offered anti-D immunoglobulin. A blood sample is taken from her immediately before the injection to test for antibodies, and the testing is performed in the laboratory of the transfusion centre. To avoid delay the injection is often given before the result of this test is known. This has resulted in 10 women receiving anti-D when anti-D was found to be present by an enzyme technique in the preinjection sample. A Kleihauer test is also performed on the preinjection sample and if the number of fetal cells is low anti-D is given. Initially the dose was 200 µg, but it has been reduced to 100 µg.⁵ If there is evidence of an abnormally high fetal-maternal bleed more than one dose is given. Anti-D is given intramuscularly and within 72 hours of delivery. In most cases an antibody test is performed six months after anti-D administration (the six-month test).

Serological tests—Serological tests for Rh (D)-antibodies are performed on all Rh (D)-negative women early in pregnancy, at the 28th week, and at 34-36 weeks, or more frequently. Standard saline, albumin, indirect antiglobulin (Coombs), and enzyme techniques are used. The enzyme technique consists of the Marsh⁶ modification of the autoanalyser method of Rosenfield and Haber⁷ and the enzyme layering method described by Boorman and Dodd.⁸

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Study of cases in which protection failed

We considered protection had failed in any woman who at anytime in a subsequent pregnancy developed anti-D that was detected by any technique.

OVERALL INCIDENCE OF FAILURES

During 1968-73 1895 women in the Yorkshire Area Health Authority area who had received the 200-µg dose of anti-D and 564 women who had received the 100-µg dose had a further pregnancy. In 32 cases treatment failed to protect from sensitisation; 28 of these women had received the 200-µg dose and 4 the 100-µg dose. It was estimated that 80% of the infants would be Rh (D)-positive, which meant a failure rate of 28 out of 1516 infants (1.8%) in the 200-µg group and 4 out of 451 cases (0.9%) in the 100-µg group. Since January 1974 a further 8 failures were found making a total of 40 first affected cases. Eleven of these women had further pregnancies, thus making a total of 53 infants and these were analysed in more detail.

DETAILED ANALYSIS OF FAILURES

The patients were divided into groups.

Group 1 consisted of 16 patients in whom the six-month test result was negative and an antibody was detected only towards the end of the subsequent Rh (D)-positive pregnancy. Antibody was first detected between the 32nd and 40th week of pregnancy. Except in two cases the antibody titre never rose above 1/10. In one case the titre rose to 1/20, but the infant was minimally affected. The highest titre reached in this group was 1/40, and the infant was moderately affected with a cord haemoglobin of 11.1 g/dl. The mean cord or capillary haemoglobin at delivery was 16.3 g/dl (range 11.1-19.8 g/dl). Four out of 16 infants required exchange transfusion, but three of these infants had a haemoglobin level above 14.6 g/dl.

Group 2 consisted of 13 patients in whom the first antibody test of the subsequent pregnancy gave a positive result and the antibody persisted throughout the pregnancy. In 10 cases the six-month follow-up test had been positive. Ten of these 13 women were delivered of an Rh (D)-positive infant. Despite the fact that antibody was detectable throughout pregnancy the infants were, at the most, only mildly affected. In only two cases did the antibody titre rise above 1/10 to a level of 1/40. The mean cord or capillary haemoglobin at delivery was 18.3 g/dl (range 16.4-21 g/dl) and nine infants had a positive direct antiglobulin test result. Exchange transfusions were performed in two of these infants, though in one case the child's haemoglobin was 16.2 g/dl.

Group 3 consisted of 10 patients who had received anti-D, although the preinjection enzyme antibody test had given a positive result. Antibodies were detected throughout the subsequent Rh (D)-positive pregnancies. Seven of these 10 women were delivered of an Rh (D)-positive infant. Apart from one case, in which the antibody titre reached 1/80, no other titre rose above 1/10, and no infant was severely affected. Six infants had a positive direct antiglobulin test result, the mean cord or capillary haemoglobin at delivery was 13.1 g/dl (range 11.1-15.2 g/dl), and exchange transfusion was performed in only two infants. The haemoglobin level at birth in these two cases was 11.1 and 11.8 g/dl.

Group 4 consisted of 11 women from the three preceding groups who had 12 further pregnancies. Of these 12 infants nine were Rh (D)-positive and in only three of these cases did the antibody titre rise above 1/10. In two cases levels reached 1/20 and in one case 1/40. The mean cord or capillary haemoglobin at delivery was 14.8 g/dl (range 10.4-17.3 g/dl) and only three infants required exchange transfusion.

Because of certain unique features one case was considered separately.

Case report—This case was the only one with a tragic outcome that we have encountered since anti-D was first introduced. Unfortunately clinical and

serological information was scanty. This woman had her first pregnancy in 1971-2, and the last antibody test performed in November 1971 gave a negative result. She delivered on 28 February 1972 and a 100- μ g dose of anti-D was given. There was no record of a preinjection antibody test being performed. During her second pregnancy she first attended the antenatal clinic at 34 weeks. An antibody test showed anti-C+D at a titre of 1/80 (40 international units). There was no fetal heart beat and a few days later she was delivered of a hydropic stillbirth. It was not possible in this case, therefore, to be sure that antibodies had not begun to develop before the anti-D immunoglobulin injection. This mother had a further Rh (D)-positive pregnancy, but although the infant was born alive its cord haemoglobin was 3.4 g/dl and it died within a few hours of severe haemolytic disease and respiratory failure.

COMPARATIVE STUDY

Analysis of the clinical outcome in these failed cases suggested that the infants were less severely affected than one would have expected. This was particularly so in cases in which an antibody was detectable throughout the whole pregnancy.

We decided, therefore, to compare the results in the 32 failures seen in 1969-73 with those in a previous series of 115 first-affected pregnancies, ones in which antibodies were detected for the first time⁹ and analysed before anti-D immunoglobulin was available. This series was from the same geographical area and laboratory, and in both series similar tests were performed antenatally and the same enzyme antibody test was performed on the maternal serum. The patient reported above (the only patient to lose her infants with Rh-haemolytic disease) has been included in the analysis though insufficient information was available for exact classification.

Two criteria were used to measure severity: the highest antibody level attained in the pregnancy, and the cord haemoglobin level.

Results

Highest antibody level in pregnancy—Results in both groups are shown in table I. Of the 115 women in the 1969 series 74 (64%) had titres of 1/20 or over, whereas only 6 (19%) of the 32 women in this series had titres over 1/20 ($\chi^2 = 20.984$, DF = 1; $P < 0.001$). Of the 18 women in this series who had antibodies throughout the pregnancy 4 (22%) had titres over 1/20 ($\chi^2 = 11.388$, DF = 1; $P < 0.001$). There were too few patients followed up through a further pregnancy (group 4) to make a satisfactory statistical comparison with the 1969 series possible.

TABLE I—Comparison between highest antibody level reached in pregnancy in first affected cases of 1969 series and present cases

Highest antibody titre in pregnancy	No (%) of cases in 1969 series	No (%) of cases in present series	
		Total cases	Cases with antibody throughout pregnancy
0-10	41 (36)	26 (81)	14 (78)
20	12 (10)	1 (3)	0
40	22 (19)	3 (9)	2 (11)
80	18 (16)	2 (6)	2 (11)
>80	22 (19)	0	0
Total	115	32	18

Cord or capillary haemoglobin level—The comparison is summarised in table II. In some cases in each group the haemoglobin was not measured. The difference was less striking than with the antibody levels, but the infants of mother treated with anti-D tended to be less severely anaemic. For statistical analysis the cases were divided into

three groups according to whether the capillary or cord haemoglobin level was over 16 g/dl, 12-16 g/dl, or less than 12 g/dl (table III).

TABLE II—Comparison between cord or capillary haemoglobin levels in first affected cases of 1969 series and present cases

Capillary or cord haemoglobin	No (%) of cases in 1969 series	No (%) of cases in present series	
		Total cases	Cases with antibody throughout pregnancy
>16 g/dl	30 (27)	14 (56)	7 (54)
12-16 g/dl	42 (38)	7 (28)	3 (23)
8-12 g/dl	24 (22)	3 (12)	2 (15)
<8 g/dl	15 (14)	1 (4)	1 (8)
Total	111	25	13

Discussion

In common with all other workers our experience has been that there is a failure rate of 1-2% after anti-D immunoglobulin prophylaxis. The failures fell into two main patterns. The first pattern appeared among women who developed an antibody late in the subsequent pregnancy (group 1). These cases were similar to cases of sensitisation occasionally seen in primigravidae and were probably due to a fetal maternal haemorrhage early in the subsequent pregnancy. The second pattern of failure occurred when the antibody was detected in the subsequent pregnancy at the first antenatal visit and persisted throughout the pregnancy. In many cases a six-month follow-up test after anti-D administration had been found positive. These failures may have been due to an insufficient dose of anti-D immunoglobulin or, more probably, because the patient was already sensitised when the anti-D was administered.

Our results support the concept of Gorman¹⁵ that Rh-immune globulin given too late to prevent immunisation "May have a moderating effect on the degree of Rh₀(D) antibody formation and the severity of disease in the subsequent pregnancy may be milder." This is particularly so with regard to the antibody levels in the pregnancies and particularly striking when the antibody is present throughout the pregnancy, even to the extent of a positive preinjection antibody test. Though the comparison is not so dramatic statistically for the cord or capillary haemoglobin levels at delivery, clinically the difference is striking. This is reflected in the stillbirth rate. Only one patient in our series lost her infant. Both Walker and Murray¹⁰ and Tovey⁹ reported an 8-10% stillbirth rate in first affected pregnancies before anti-D was available. These results contrast with the experience of Davey,¹⁶ who found nine severely affected infants, including two who died in the perinatal period, among the children of 60 Australian mothers in whom anti-D treatment had failed.

This apparent effect of anti-D on subsequent pregnancies, even when it fails to prevent sensitisation, is immunologically and clinically important. Godel *et al*¹¹ reported that even if anti-D immunoglobulin was given to women with a positive antibody test result by papain six month follow-up results were more often negative than in controls not receiving anti-D. Pollock and Ascari¹² suggested this might be a time when IgM synthesis had started before IgG production, when giving anti-D may prevent the development of the immunological memory and thus result in the failure to respond to future D stimuli.

TABLE III—Observed differences in cord and capillary haemoglobin levels between 1969 series and present series and expected differences on null hypothesis*

	16 g/dl		12-16 g/dl		12 g/dl		Total	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
1969 Series	30	35.91	42	39.99	39	35.10	111	111
Present series	14	8.09	7	9.01	4	7.90	25	25

$$\chi^2 = \sum \frac{(O-E)^2}{E} = 0.973 + 0.101 + 0.433 + 4.317 + 0.448 + 1.925 = 8.197; \text{DF} = 2; P < 0.05 \text{ (possibly significant).}$$

A further explanation, though mainly conjectural, is worthy of consideration. Before anti-D immunoglobulin was available it was not uncommon to encounter Rh-negative women who formed Rh-antibodies, but the titres remained low and they had only mildly affected infants. Possibly anti-D immunoglobulin can suppress "good" responders but not these low responders. Undoubtedly there is a point of no return, as was seen in the case outlined above: although anti-D was given the mother's second and third child both succumbed to the Rh antibodies.

The simplest explanation of why anti-D immunoglobulin protects at all is that by destroying fetal Rh (D)-positive cells it reduces the length of time the Rh antigen is exposed to potentially anti-D producing lymphocytes. This concept seems to be supported by the recent work of Woodrow *et al.*¹³ They injected Rh (D)-negative Kell-negative volunteers with D-positive Kell-positive red cells and then gave anti-Kell antibodies. They showed a reduction in the incidence of Rh antibodies. This is not, however, the only possibility.¹⁴ The suppression of anti-D may be a more complex immunological mechanism. Certainly our results suggest that the future immunological response may be altered by the administration of anti-D and also support the view that the mechanism of anti-D protection is more complex than would be expected if it were simply a matter of removal of fetal Rh-positive cells.

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Idiopathic heart block: association with vitiligo, thyroid disease, pernicious anaemia, and diabetes mellitus

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Summary

Out of 100 patients with chronic heart block 16 had one or more autoimmune disorders—namely, vitiligo (5), hypothyroidism (4), Graves's disease (1), pernicious anaemia (2), and diabetes mellitus (9). All these disorders occurred with greater frequency than normal and were more prevalent than in a group of hospital inpatients of comparable age. Autoantibodies were not increased. We suggest that among patients with idiopathic heart block there is a subgroup with multiple autoimmune disorders.

Introduction

In most cases of chronic heart block the aetiology is unknown. The commonest finding at necropsy is end-stage fibrosis of the bundle of His and its branches.^{1,2} Complete heart block has occasionally been described in thyroid disorders, including primary myxoedema³ and thyrotoxicosis.⁴ This study was instituted to assess the frequency of thyroid disease in patients with chronic heart block. In view of the autoimmune aetiology of most cases of primary hypothyroidism, together with the known associations between lymphomatous thyroiditis, pernicious anaemia, vitiligo, Addison's disease, and diabetes mellitus,⁵ the prevalence of these disorders was also determined. The serum of all patients studied was screened for organ-specific and non-organ-specific autoantibodies.

Subjects and Methods

One hundred consecutive patients with chronic heart block admitted to hospital for pacemaker implantation were studied. (Patients with acute heart block—for example, after myocardial infarction—were not included.) Altogether 49 were men and 51 women and their ages ranged from 33 to 96 years (mean 72.4 years) (table I). The duration of their disease, as judged by the time from the onset of symptoms, was 3 months to 23 years (mean 6 years). A detailed personal and family history was taken and the patients were examined for any known cause of heart block and for the presence of vitiligo, thyroid disease, diabetes, or pernicious anaemia. Biochemical screening included haemoglobin, erythrocyte sedimentation rate, urea and electrolytes, Wassermann reaction (W.R.), calcium, phosphate and alkaline phosphatase, plasma proteins, serum thyroxine and Thyopac-3 index, and, when appropriate, serum vitamin B₁₂ level. In 40 patients immunoglobulin levels were determined. Sera were collected and stored at -20°C and tested by us within three months.

Controls.—One hundred healthy people matched for age and sex with the heart-block patients were selected from a larger group tested at the Middlesex Hospital. They consisted of residents in old people's homes and, for the younger age groups, friends of rheumatoid arthritis patients who had accompanied them to hospital.

Serological Methods.—Autoantibodies were detected by a standard sandwich immunofluorescence technique using rabbit antihuman FAB conjugated with fluorescein isothiocyanate. Sera were tested at a 1/10 dilution on human thyroid and stomach and on rat liver and kidney sections.⁶ Thyroid microsomal antibodies were titrated by the Fujizoki haemagglutination test, and thyroglobulin antibody was detected by tanned red cell haemagglutination (T.R.C.).

Results

Of the 100 cases of chronic heart block 85 were clinically idiopathic and in 15 a possible cause coexisted, including ischaemic heart disease, calcific aortic valve disease, and Paget's disease. Sixteen patients had diseases associated with organ-specific autoimmunity and three had multiple disorders (table II). All had chronic heart block of unknown

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