PAPERS AND ORIGINALS

Mechanism of Rh Prophylaxis: An Experimental Study on Specificity of Immunosuppression

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British Medical Journal, 1975, 2, 57-59

Summary

The mechanism by which Rh immunization is prevented by IgG anti-D was investigated by studying the specificity of immunosuppression. 62 D-negative Kell(K)-negative male volunteers were given two successive stimuli of 1 ml D-positive K-positive red cells. Thirty-one of the volunteers were also given 13-14 µg of IgG anti-K immediately after each stimulus, the others acting as controls. Anti-D developed in 11 of the 31 controls and in one of the 31 volunteers who had received anti-K. This marked suppression of the anti-D response by IgG anti-K was accompanied by the rapid clearance of the injected red cells to the spleen. This shows that the predominant mechanism that must be operating when IgG anti-D prevents Rh immunization is not antigen specific but is one that must involve the whole red cell, probably through destruction within splenic macrophages.

Introduction

Experimental studies in human volunteers,^{1 2} controlled clinical

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trials,^{3 4} and extensive clinical experience in many countries have established the efficacy of the passive administration of IgG Anti-D in the prevention of Rh immunization. The exact mechanism by which immunosuppression is obtained is still a matter for debate, though it is associated with a rapid clearance of the circulating Rh-positive red cells, mainly to the spleen.¹ In considering the possible mechanisms involved it would be of great value to know whether the administered anti-D prevents the formation of antibodies to the D determinant only or whether the antibody response to other blood group determinants on the red cells is also prevented.

To obtain evidence on the mechanism of antibody suppression from a study of Rh-negative mothers treated with IgG anti-D would require careful antibody screening of many treated mothers, together with an accurate knowledge of the expected incidence of antibodies of other specificities in untreated mothers after pregnancy. Sufficient information on this point is not available and we therefore studied the specificity of immunosuppression in male volunteers.

Experimental Methods

We decided to base the study on the Rh and Kell systems, and initially considered the possibility of administering anti-D together with D-positive K-positive red cells to D-negative K-negative volunteers and so determine whether or not anti-D would prevent an anti-K as well as an anti-D response. Because, however, the K blood group determinant is less immunogenic than D we expected that this approach would require the injection of considerable volumes of blood to many recipients to obtain an adequate incidence of anti-K in controls not given anti-D. We therefore decided to see whether administering IgG anti-K would prevent the anti-D response when D-positive K-positive red cells were given to D-negative K-negative recipients.

Recipients.—Male blood donors typed as group Arr and K negative were interviewed in groups. The purpose, nature, and potential risks of the experiment were explained to them in detail. Those who volunteered to take part were introduced into the study in small groups at intervals.

IgG Anti-K.—Material was obtained by plasmapheresis on three separate occasions from a healthy D-positive woman who had lost two infants from haemolytic disease due to anti-K. Plasmapheresis had previously been performed therapeutically during pregnancy.⁵

The Cohn F II fraction was prepared by the blood products laboratory of the Lister Institute, Elstree, and was made available in 2-ml ampoules for intramuscular injection. It contained no anti-D and the concentration of anti-K was estimated to be 3.3 mg/l with an equilibrium constant of about 2.5×10^{10} l/mol.

Donor Erythrocytes.—The same donor was used throughout. His red cells were group A K-positive, the Rh phenotype was CDce, and the probable genotype was CDe/cde. His blood had been used for transfusion many times without evidence of any untoward effects on the recipients. The amount of red cells used each time was 1 ml, and they were labelled with ^{51}Cr before injection into the recipients for the purpose of clearance studies.

Clearance Studies.—A pilot investigation on three D-negative K-negative volunteers established that the intramuscular injection of either 2 ml or 4 ml of the anti-K resulted in the virtually complete clearance of 1 ml of the donor's D-positive K-positive red cells from the circulation within 24 hours. We decided to use 4 ml of the IgG anti-K preparation as the standard dose throughout the study.

EXPERIMENTAL PROCEDURES

We expected that only some recipients would develop antibody after one stimulus and that to limit the number of volunteers a succession of stimuli with red cells would be necessary. Each volunteer in the experimental group was to receive anti-K with each successive injection of red cells while the controls would receive only red cells each time. Volunteers were introduced into the study in groups of about six and were divided randomly into two groups—experimental and control. Where the numbers were odd the next set of volunteers were assigned so as to keep the total numbers in the two groups as near equal as possible. After a baseline sample of blood was obtained for antibody tests each volunteer was given 1 ml of D-positive K-positive ^{51}Cr -labelled red cells intravenously. Thirty minutes later those in the experimental group were given 4 ml IgG anti-K by intramuscular injection. Clearance of the red cells was studied by taking ^{51}Cr counts at 30 minutes and 24 hours after the injection of the red cells.

As the experiment proceeded we decided that the results of antibody tests would be assessed after each volunteer had received two stimuli with Rh-positive blood and that when the point was reached where 10 people had developed anti-D no further volunteers would be introduced into the experiment. We tried to maintain the interval between the two stimuli fairly constant but for practical reasons this was not possible and the interval between them varied between 15 and 70 weeks. Nevertheless, excepting those people who developed anti-D after the first stimulus, the average interval between stimuli was 35.6 weeks in the experimental subjects and 36.1 weeks in the controls.

Out of 32 volunteers in the experimental group and out of 33 controls, 31 in each group completed the study. Tests for antibodies were carried out on samples of serum taken just before the second stimulus and at least 10 weeks after it. Standard tests in saline and albumin together with antiglobulin and enzyme methods were applied.

Results

CLEARANCE STUDIES

In most volunteers given anti-K virtually all the injected Rh-positive K-positive red cells were cleared within 24 hours. Occasionally about 5% activity remained at 24 hours after injection of the red cells.

One control subject showed a reduced survival of the red cells of the first stimulus and was found to have an antibody which was active at temperatures below 37°C . The specificity of the antibody was uncertain but there was no evidence that it included anti-D. It subsequently disappeared and clearance of the second injection of red cells was normal and no immune antibody was found. In two other controls reduced survival was found with the second stimulus though antibodies were not detectable at that time. One of the two developed anti-D during the subsequent weeks but the other had no detectable antibody.

Four subjects, two controls, and two who had received anti-K, were studied by surface counting over heart, liver, and spleen. In the two subjects given anti-K a considerable excess of activity was recorded over the spleen 21 hours after the injection of the labelled red cells. This suggested that sequestration of the cleared red cells was predominantly in the spleen.

TESTS FOR ANTIBODY

The numbers of subjects who developed anti-D after each stimulus are shown in the table. The difference between the two groups was significant ($P=0.0013$) and by the end of the experiment 11 of the 31 controls had developed anti-D compared to only one of the 31 men given anti-K. The 24-hour clearance of the injected red cells in this one volunteer who developed anti-D in spite of having received anti-K was 97%. Two control subjects produced, in addition to anti-D, anti-K in one instance and anti-Bu^a in the other. No person who did not develop anti-D developed any other antibody. One of the controls who developed anti-D and the anti-K treated volunteer who developed anti-D had had a previous blood transfusion. The details were carefully checked and it was clearly established that Rh-negative blood had been used in each case.

Results of Tests for Antibodies after Each Stimulus with D-positive K-positive Red Cells

	1st Stimulus		2nd Stimulus		Total with Anti-D
	No. Tested	No. with Anti-D	No. Tested	No. with Anti-D	
Controls	31	7*	24	4	11
Group given anti-K	31	1	30	0	1

*Anti-K appeared in one man and anti-Bu^a in another in addition to anti-D.

Discussion

Our results show that when D-positive K-positive red cells are injected into D-negative K-negative recipients together with an amount of IgG anti-K sufficient to produce rapid clearance of the cells to the spleen the active anti-D response is greatly suppressed. The main implication is that the antibody response is prevented by a mechanism which does not depend on the specificity of the passive antibody but that immunosuppression must involve the whole red cell. Two mechanisms for antibody-mediated immunosuppression which would be expected to show specificity for the corresponding antigen have been put forward (this is assuming that steric hindrance does not occur, see below). The first is that high-affinity IgG antibody molecules given passively compete with the antibody receptor sites of B-lymphocytes for antigenic determinants, thus blocking the interaction between the determinants and the potential antibody-producing cell.⁶ The second mechanism is that the combination of antigen and antibody suppresses the B lymphocyte itself and renders it unresponsive to the antigen.^{7,8} As in both these mechanisms the prevention of the immune response involves the antigen-specific B lymphocytes it would be expected that were either or both mechanisms predominant in our experiment the anti-D response would not have been prevented by the passive anti-K.

In this context it is relevant that when D-positive K-positive red cells are treated *in vitro* with excess IgG anti-K it is still possible to agglutinate the cells normally with anti-D. This suggests that the D and K antigenic sites on the red cell are not spatially so related to each other that when IgG anti-K is attached to K sites the D sites are rendered inaccessible to anti-D molecules. Such steric hindrance cannot therefore be adduced as a likely mechanism by which the D antigen loses its immunogenicity.

A process affecting the whole red cell presumably involves its uptake by a macrophage followed by destruction of the blood group determinants or their diversion from immunogenic sites. When Rh-positive red cells are incompatible on the ABO system with the serum of Rh-negative recipients they are largely removed as microagglutinates or haemolysates to the liver, where rapid phagocytosis occurs and immunocompetent tissues are absent. The anti-D response is very much suppressed. In the case of IgG anti-D and anti-K, however, clearance is to the spleen and it is likely that the coated red cells become attached to macrophages at Fc-receptor sites and rapid endo-

cytosis follows. This is presumably followed in turn by rapid degradation of cell membranes and associated antigens.

Clearly rapid clearance and phagocytosis of Rh-positive red cells by anti-D, anti-K, anti-A, or anti-B all lead to definite suppression of the anti-D response. There is also evidence that this suppression is not always complete. Clinically a Rh-negative mother may develop anti-D after a first ABO-incompatible Rh-positive pregnancy. In a study in male volunteers five out of 32 Rh-negative subjects produced anti-D after a series of injections of ABO-incompatible Rh-positive red cells.⁹ Nevertheless, when a similar series of injections of ABO-compatible Rh-positive cells coated in vitro with anti-D were given none of the recipients developed anti-D. This suggests that anti-D is more suppressive than anti-A or anti-B and raises the possibility that though a predominant mechanism of immunosuppression is shared by these antibodies anti-D may have a further effect—such as the ability to block any D antigen that escapes the destructive process within macrophages.

PRACTICAL IMPLICATIONS

Most cases of haemolytic disease of the newborn are due to anti-D, and the current practice of typing mothers and their infants for the D antigen and administering anti-D where there is incompatibility has resulted in an appreciable decrease in the incidence of the disease (B. M. Hibbard, personal communication) and in deaths due to it.¹⁰ The formation of any other antibodies in these mothers will probably also be largely prevented by the administration of anti-D. Nevertheless, very occasionally a blood group antigen other than D, present in the fetal cells, may escape the destructive process and survive to immunize the mother. There is a hint that this may be so.¹¹

In relation to organ transplantation our findings may have relevance to attempts to modify the primary immune response in the recipient to foreign antigens introduced with the graft—for example, on passenger leucocytes. Antibodies directed at any determinant on the donor cells should suppress the immune response to other determinants present.

We are greatly indebted to the blood donors who volunteered for this experiment. The IgG anti-K was prepared by Dr. W. d'A. Maycock and Mr. L. Vallet at the Lister Institute of Preventive Medicine, and we thank them for their help. We thank Dr. N. C. Hughes-Jones for estimating the concentration of anti-K.

This work was supported by the Research Committee of the Mersey Regional Area Health Authority.

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Comparison of Intravenous AH 5158 (Ibidomide) and Propranolol in Asthma

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British Medical Journal, 1975 2, 59-61

Summary

The cardiac and bronchial effects of AH 5158 and propranolol were compared in a double-blind, placebo-controlled intravenous study on 10 asthmatics. AH 5158, like propranolol, is a non-cardioselective β -adrenoceptor-blocking drug, but unlike propranolol it also has an α -adrenoceptor-blocking action. Both drugs produced equivalent cardiac (β_1) blockade, but propranolol produced bronchoconstriction, whereas AH 5158 did not. Hence the α -blocking action of AH 5158 seems to prevent the bronchoconstrictor effects of propranolol in these patients.

Introduction

β -Adrenoceptor antagonists are often used for angina, hyper-

tension, and cardiac arrhythmias. They are not, however, specifically cardioselective, and one of the first introduced, propranolol, may lead to or aggravate bronchoconstriction in asthma.¹ This risk is less but not absent with cardioselective drugs such as practolol and acebutolol.²⁻⁴ There is evidence that α -adrenoceptors are present in the bronchial muscle and that stimulation of these—for example, by exercise, methoxamine, or histamine—leads to bronchoconstriction.⁵⁻⁷ This can be prevented in asthmatics by pretreatment with α -receptor-blocking drugs such as thymoxamine, phentolamine, phenoxylbenzamine, and indoramin.⁷⁻⁹

AH 5158 (ibidomide) (5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl) amino] ethyl] salicylamide) is a recently developed drug which, like propranolol, blocks both β_1 - and β_2 - receptors but also has some α -receptor-blocking activity.^{10 11} Possibly, therefore, this α -blocking action may prevent bronchoconstriction when the drug is given to asthmatics. We have investigated this by comparing the effects of AH 5158 and propranolol in a placebo-controlled trial on asthmatic patients.

Subjects and Methods

Ten asthmatic outpatients were studied (six men and four women). All gave their consent and understood that their asthma might worsen temporarily. Their mean age was 31.8 (range 18-48) years and their mean weight 65.8 (range 54-79) kg. Eight had extrinsic

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