

Leukocyte Antigen-Antibody Reaction and Lysis of Paroxysmal Nocturnal Hemoglobinuria Erythrocytes

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The interaction between leukocyte antigens and specific antibodies can cause lysis of the red cells of paroxysmal nocturnal hemoglobinuria (PNH) *in vitro*. It is suggested that the activation of complement ensuing from the antigen-antibody interaction is responsible for PNH cell lysis. The clinical implications of this finding are briefly outlined.

THE TRANSFUSION OF COMPATIBLE WHOLE BLOOD to patients suffering from paroxysmal nocturnal hemoglobinuria (PNH) is known to sometimes cause a hemolytic reaction. It has been suggested that leukocyte isoantibodies present in the patient's serum through interaction with the donor's leukocyte antigens are responsible for lysis of PNH red cells.¹ As yet, it has not been clarified why this interaction should cause an exacerbation of hemolysis of PNH cells. In PNH, the basic abnormality is an intrinsic defect of the red cell membrane which renders the erythrocyte abnormally sensitive to the lytic action of complement (C).^{2,3} Yachnin and Ruthenberg⁴ suggested that PNH erythrocyte lysis might depend upon the evolution in the fluid phase (i.e., in plasma or serum) of hemolytically active C components which are capable of injuring the cell membrane despite the lack of an antibody coat. Available data suggest that any mechanism which leads to activation of C in the fluid phase, as for example an antigen-antibody reaction, could result in increased hemolysis of PNH red cells. In the work reported here, evidence is presented that interaction between leukocyte antigen and isoantibody can cause PNH cell lysis *in vitro*.

MATERIALS AND METHODS

Blood was collected into acid-citrate-dextrose solution from two PNH patients and red cells were washed three times with saline before use. Six ABO-compatible fresh sera containing leukocyte cytotoxic isoantibodies were obtained from polytransfused patients by drawing the blood without anticoagulant and allowing it to clot for 2 hours at room temperature. The same procedure was employed to collect the sera from normal subjects. Leukocyte cytotoxic isoantibodies were detected by the dye-exclusion method described by Engelfriet and Britten.⁵ The titer of isoimmune sera was 1:1; the percentage of stained cells varied from 20 to 90 per cent, according to the sera and leukocytes used. Sera did not contain red cell isoantibodies: negative reactions were obtained in the saline, bromelin and

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Table 1.—Effect of Leukocyte Antigen-Antibody Reaction on PNH Red Cells

Addition to Immune Serum (0.5 ml.)	Serum	Serum	Serum
	Z.L. (20)•	B.G. (30)•	D.R. (90)•
1 × 10 ⁸ reactive leukocytes (0.05 ml.)	18	5	23
1 × 10 ⁸ nonreactive leukocytes (0.05 ml.)	—	—	—
Inert serum (0.05 ml.)	—	—	—
0.2 N HCl (0.05 ml.)	30	9	41

The hemolytic test system is described in the text. Numbers indicate per cent hemolysis. Dashes indicate that no hemolysis present.

•Numbers in parentheses indicate the percentage of trypan blue-stained leukocytes given by the serum when tested with reactive leukocytes in the dye-exclusion method.

antiglobulin tests using suitable test cells (Selectogen, Ortho). Leukocytes to be used in lysis tests were obtained by dextran sedimentation of defibrinated blood from normal subjects and patients suffering from chronic lymphatic leukemia. Leukocyte suspensions were centrifuged for 10 minutes at 1300 × g. The supernatant serum was kept for control (inert serum, see below) and the cell buttons, containing leukocytes contaminated with red cells, were washed once with saline. Leukocytes used in the test had been previously shown to react with sera containing isoantibodies.

One volume (0.05 ml.) of packed PNH red cells and one volume (0.05 ml.) of cell button (containing either 1 × 10⁸ or 0.5 × 10⁸ or 0.25 × 10⁸ leukocytes) were added in this order to 10 volumes (0.5 ml.) of isoantibody-containing sera. Mixtures were incubated for 45 minutes at 37°C. Tubes were then centrifuged and the intensity of hemolysis was determined spectrophotometrically by the cyanmethemoglobin method; the intensity was expressed as a percentage of complete (100 per cent) lysis. Control tubes containing isoimmune sera + PNH red cells + inert serum, isoimmune sera + PNH red cells + leukocytes not reacting with isoantibodies, and normal (nonimmune) sera + PNH red cells + leukocytes were run in parallel. Another control was set up where, in addition to PNH cells and reactive leukocytes, isoimmune sera which had been previously inactivated by heating at 56°C for 30 minutes were used. This procedure is known to destroy C but not the cytotoxic antibody.⁶ The acid-hemolysis test was carried out according to Dacie and Lewis,⁷ using both normal and isoimmune sera.

RESULTS

Results of a typical experiment are shown in Table 1. Similar data were obtained using red cells from the other PNH patient and two other isoimmune sera.

Hemolysis was observed in the tubes where leukocytes reacting with fresh isoimmune sera were placed. Lysis appeared to be C-dependent since no hemolysis occurred when the isoimmune sera had been previously inactivated. The intensity of lysis was less than that obtained in the acid-hemolysis test and varied according to the serum used: it was not proportional to the percentage of trypan blue-stained cells given by the serum in the dye-exclusion method. Surprisingly, one of the isoimmune sera used, although giving a positive acid-hemolysis test, failed to work in the experiment. It was from one of the two PNH patients under study. At the moment we are unable to give an explanation for this negative result.

The intensity of lysis varied according to the number of leukocytes used, being evident with 1 × 10⁸ cells and nil below 0.5 × 10⁸ cells. Almost pure lymphocytes were as effective in the experiment as a mixture of granulocytes and lymphocytes.

DISCUSSION

The above results suggest that the interaction between leukocyte antigen and antibody can cause PNH cell lysis *in vitro*. It appears likely that the activation of C ensuing from the antigen-antibody interaction is responsible for PNH cell lysis. Apparently, PNH cells behave as an indicator of this interaction. If this is so, PNH red cells could be used to detect C activation *in vitro* carried out by some antigen-antibody reactions such as those involving leukocytes. Preliminary results indicate that this possibility exists and that both PNH and PNH-like cells can be employed. The latter are normal red cells treated with the sulfhydryl compound AET⁸ and have been shown to be a good substitute for PNH red cells in some *in vitro* hemolytic systems.^{8,9}

From a clinical point of view, the above findings support the opinion that the hemolytic transfusion reactions which occur in some PNH patients may be caused by the interaction between transfused leukocytes and specific antibodies present in the patients' serum. The use of white cell-poor suspensions of red cells seems to be advisable for transfusion in these patients.

Note added in proof: Since this paper was submitted for publication, Lachmann and Thompson (J. Exp. Med. 131:643, 1970) reported that PNH red cells are extremely sensitive to "reactive hemolysis," i.e. a C-mediated hemolysis initiated at the C5 stage by a stable complex (C56) generated by C activation at a distance. This observation could explain our findings.

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