ABO incompatibility: Immunocytochemical findings in hemolytic transfusion reactions

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ABO incompatibility plays a major role in fatal transfusion reactions. However, a conclusive serological post mortem diagnosis may be a problem since the corpora delicti, namely the incompatible red cells (IRC), are quickly eliminated from the circulation by immunohemolysis and phagocytosis. On the other hand, IRC can be detected in the body tissues of the recipient using morphological methods. ISHIYAMA et al. (1977) and KEIL et al. (1983) reported encouraging results with the mixed cell agglutination reaction (MCAR). A better sensitivity and a higher resolution can be attained with more modern immunoenzymatic methods (PEDAL et al. 1986).

MATERIALS AND METHODS

Out of 23 autopsy cases after confirmed ABO-incompatible blood transfusions or erythrocyte transfusions, routinely formalin-fixed and paraffin-embedded samples of various tissues were available. The essential serological and clinical data were taken from documents of the Institutes of Forensic Medicine.

The transfusion volumes varied between 10 ml and 9.450 ml. Twenty recipients were blood group O; they had received A blood by mistake 15 times and B blood by mistake five times. Of three recipients with blood group B, two had received blood of group A and one had received blood of group A_1B. The survival times were between five hours and 17 days. The cause of death was hemolytic shock in all cases.

After deparaffination, and inhibition of endogenous peroxidase with 1 % H_2O_2-methanol and trypsin pretreatment, the antigens A and B were visualized with the standard PAP method in 4 µm sections. The incubation steps were:

1. monoclonal anti-A/anti-B Seraclone^R, Biotest, D-6050 Offenbach, cat. no. 801315/801340 (1:10)
2. rabbit antiserum to mouse IgM, Bionetics, distribution by Fresenius, D-6370 Oberursel/Ts., cat. no. 8403-09 (1:500)
3. swine antiserum to rabbit Ig (bridge antibody), Dakopatts, D-2000 Hamburg, cat. no. Z 196 (1:200)
4. PAP (rabbit), Dakopatts, D-2000 Hamburg, cat. no. Z 113 (1:100)

AEC was used as chromogen; counterstaining with hematoxylin.

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RESULTS

IRC in large blood vessels

IRC, clearly identified by their staining behaviour, were present in large vessels as isolated cells or as circulating red cell agglutinations. These findings were constant in cases of massive transfusions and short survival times. However, therapeutic exchange transfusions as well as longer survival times resulted in complete disappearance of IRC from large vessels.

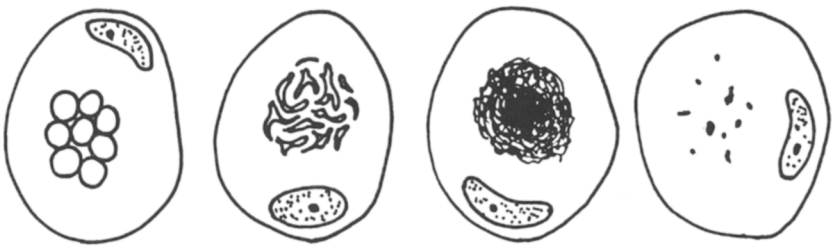
IRC in small blood vessels

With their occurrence in large vessels, IRC were simultaneously found in the blood capillaries. In some instances, lots of IRC were present in the terminal vessels whereas they had disappeared from the large vessels. The sinusoidal capillaries of the liver, spleen, the adrenal cortex and the bone marrow as well as the terminal vascular bed in the lungs and the kidneys proved to be sites of predilection for the occurrence of IRC, whereas the capillaries of the myocardium and the central nervous system showed strikingly few IRC.

IRC in mononuclear macrophages

Even after the shortest survival time of five to six hours, massive phagocytosis and intracellular degradation of IRC was to be observed in the stellate cells of the liver and in mononuclear macrophages of the spleen and the adrenal cortex. Four patterns which are to be interpreted as phases of a degradation process can be distinguished:

I	closely packed intact IRC within macrophages;
II	collapse of the IRC membranes;
III	condensed, amorphous, globular masses;
IV	granular IRC membrane residues.



I II III IV

Schematic: Representation of phagocytosis and intracellular degradation of incompatible erythrocytes by macrophages

The correlation between survival times and morphology

The morphological equivalents of shock were to be found in all cases. Within increasing survival time, the picture transformed from acute shock to the more complex pattern of protracted shock with its typical complications.These findings, however, ar not the subject of this study. The following will deal with the relation between the visualizability of IRC and survival time.

After survival times under 24 hours (n = 11), IRC were constantly present in the terminal vascular bed and were mostly also present in larger vessels. In all cases which could be evaluated, an intensive phagocytosis was already found in the liver, spleen and the adrenals. After very low transfusion volumes, IRC remnants occurred almost exclusively in macrophages.

After survival times of two to five days (n = 8), residues of IRC could be demonstrated exclusively in macrophages, but only after transfusion volumes of at least 450 ml. In four of these cases in which IRC were practically no longer demonstrable in normal tissues, they could be visualized without problems in necrotic tissue areas or in older hemorrhages. It is evident that the IRC had escaped phagocytosis in these unperfused tissues.

After survival times of eight to 17 days (n = 4), the results of attempts at immunocytochemical visualization of IRC were always negative.

survival time (days)	incompatible red cells in large vessels	small vessels	macro-phages	hemmorrhages, necroses
0 - 1	+/-	+	+	n.p.
2 - 5	-	-	+/-	+
8 - 17	-	-	-	n.p.

Table: Demonstration of incompatible red cells (IRC), located intravascularly and in macrophages, depends on the survival period. Note the persistence of IRC in necroses and hemorrhages.

n.p. = not present in our material

CONCLUSIONS

The immunocytochemical findings make evident the two mechanisms by which IRC are eliminated from the circulating blood of the recipient. On the one hand, a redistribution of IRC takes place from the larger vessels into the capillary system even at short survival times. Since this process is evidently favored by the reduced perfusion in shock, the IRC are especially numerous in the capillaries of the "organs of shock", the lungs and the kidneys. On the other hand, in agreement with experimental findings (JANDL et al. 1957), massive phagocytosis of IRC, especially in the liver and the spleen, is already observed even after a very short time.

Serological detection of incompatible red cells is restricted to their presence in the circulation. Immunocytochemistry, however, additionally detects incompatible red cells or erythrocyte membrane remnants in the peripheral vascular region, in macrophages, and in necroses or hemorrhages. In this way, positive detection of an incompatible transfusion succeeds even after minimal transfusion doses or after a long survival time. The method is indispensable if blood samples are not available and/or if the question of a transfusion incident is to be investigated retrospectively on formalin-fixed autopsy material.

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