BINDING OF PATHOGENIC LIGANDS BY HUMAN PLACENTAL ERYTHROCYTES

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Fc-receptors for IgG are located on the surface of the membranes of many peripheral blood cells including T and B lymphocytes, monocytes, macrophages, and polymorphonuclear leukocytes [2, 13]. More recently Fc-receptors have also been found on erythrocyte membranes [15]. A detailed study of the role of Fc-receptors has shown that the main function of these formations is to remove from the circulation only aggregated IgG or immune complexes [2]. Their formation is linked with interaction between antibody and antigen, which under normal conditions are eliminated. If for some reason or other this does not happen, these pathological formations can lead to the development of disease: renal damage, or autoimmune diseases [6]. During binding of immune complexes with Fc-receptors, the cells release neutral and acid hydrolases, metabolites of amino acids such as prostaglandins, and leukotrienes [12]. If macrophages are activated, active forms of oxygen are produced [5]. Meanwhile, as a result of attachment of immune complexes or aggregated immunoglobulins to erythrocytes no activating processes of any kind are observed. It has been shown that the main role in ridding the serum of immune complexes is played by macrophages. These experimental data also have been confirmed by clinical observations [11]. However, despite the large number of investigations which had indicated the essential role of blood cells in these processes in pregnant women, the question of the role of erythrocytes in adsorption of pathogenic ligands has not yet been fully explained. This aspect is particularly important from the clinical point of view, for an increase in the content of immune complexes has been found in the serum of pregnant women even in the course of uncomplicated pregnancy [1].

The aim of this investigation was to evaluate the possible role of human placental erythrocytes in the adsorption of pathogenic ligands.

EXPERIMENTAL METHOD

Pieces of placenta were taken from 20 women immediately after labor. The pieces were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 4 h. The material was then dehydrated in alcohols and acid and embedded in paraffin wax. Placental sections were stained with hematoxylin and eosin. For immunohistochemistry we used rabbit antibodies against human IgG ("Boehringer," Germany). Colloidal gold particles were prepared by the method in [4]. The optimal amount of antirabbit antibodies (determined by Zsigmondy's test) was mixed with gold sol, the pH of which had previously been adjusted to 8.0 with 0.2 M K_2CO_3 solution, after which the complex was centrifuged at 20,000*g* for 30 min and the residue resuspended in 1 ml phosphate buffer, containing polyethylene-glycol (PEG), with mol. wt. of 20,000, in a concentration of 0.2 mg/ml. After dewaxing the sections were

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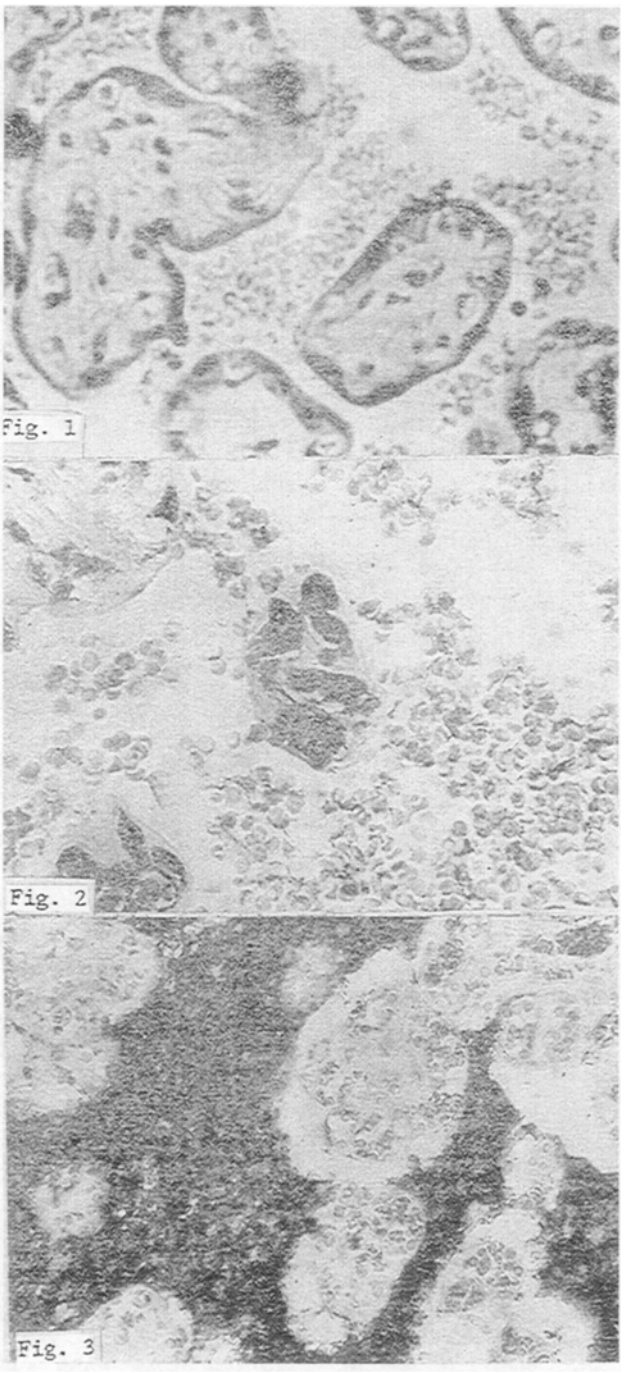

Fig. 1. Structure of placenta after normal childbirth. Hematoxylin and eosin.

Fig. 2. Staining of fetal blood flow for IgG under normal conditions.

Fig. 3. Distribution of colloidal gold label in placenta. Homogeneous staining of maternal erythrocytes.

taken through decreasing concentrations of ethanol to phosphate buffer. The sections were then incubated for 30 min in 1% bovine serum albumin solution. They were then incubated at 37°C in a humid chamber with primary antibodies against human IgG. After careful washing for 3 min the samples were incubated with antirabbit antibodies, conjugated with colloidal gold particles for 2 h, washed with phosphate buffer, and mounted in balsam without counterstaining. As the control, sections not incubated with specific antibodies and also sections treated with secondary antibodies only were used.

EXPERIMENTAL RESULTS

The structure of the placenta immediately after labor, when stained with hematoxylin and eosin, was usual in all cases. The main mass of the villi differed in diameter and the capillary and vascular network was well represented. The trophoblast was virtually homogeneous, consisting essentially of a syncytium with occasional cells of the cytotrophoblast lining it. The intervillous space varied in width and, as a rule, contained many maternal erythrocytes (Fig. 1).

On immunohistochemical investigation of placental sections the IgG circulating in the fetal blood stream was stained (Fig. 2). Meanwhile, in two cases (compensated heart disease and nephropathy) the colloidal gold label was found in the maternal blood stream (Fig. 3). The degree of staining was unequal, for some erythrocytes stained very darkly, others, especially between large villi, stained less intensively.

In view of the presence of Fc-receptors for IgG on cells of the trophoblast, it is considered that the placenta is a sorbent of antibodies against various fetal antigens [8]. Some workers directly associate a fall in the titer of antibodies with their adsorption in the placenta, on the grounds that adsorption of immunoglobulins is an essential factor for the normal course of pregnancy [14]. When these data are evaluated, it has to be noted that only cell membranes of the placenta were studied in the investigations cited. The role of erythrocytes in the removal of aggregated immunoglobulins and immune complexes from the circulation still awaits study.

Pregnancy, even with a normal course, is accompanied by a raised level of circulating immune complexes, especially at the end of pregnancy. In the case of certain complications (toxicosis, nephropathy) the content of immune complexes in the serum rises sharply [14]. An important role in the onset of any pathology undoubtedly involves the ability of different cells to eliminate immune complexes. Until now this role has been ascribed to nucleated peripheral blood cells and to placental membranes. As regards the ability of erythrocytes, which possess a sufficiently high level of Fc-receptors, this problem has not yet been investigated.

It can be concluded from the results obtained in this study that erythrocytes of pregnant women, in the case of pathology, adsorb immune complexes or aggregated IgG on their membranes, for freely circulating IgG are not bound with the Fc-receptors of erythrocytes [2]. It is difficult to overestimate the role of erythrocytes in these processes, for their number in the serum in hundreds of times greater than the number of cells in the peripheral blood. It will be quite obvious that the functional role of these cells may be very important from this point of view.

For the first time we have thus shown by an immunohistochemical method that under pathological conditions human placental erythrocytes are actively involved in the adsorption of pathogenic complexes.

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