Microangiopathic haemolytic anaemia: The phenomenon of red cell adherence

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SYNOPSIS Examination by scanning electron microscopy of erythrocytes from three cases of microangiopathic haemolytic anaemia demonstrated the presence of adherent cells. Although in some cases this appeared to be due to a thin strand, presumably fibrin, between the erythrocytes, in most instances there was intimate contact and this was invariably associated with damage to the red cell surface membrane.

It is considered that the adherence is predominantly due to altered physico-chemical properties of the surface membrane of the red blood cell and that further morphological abnormalities may result from the intravascular stresses to which the paired cells are subjected.

Microangiopathic haemolytic anaemia is a haemolytic disorder secondary to disease of small blood vessels and is characterized by the presence of fragmented and distorted erythrocytes in the peripheral blood (Brain, Dacie, and Hourihane, 1962). Of particular interest is its association with disseminated intravascular coagulation (Bull and Brain, 1968), a pathological process which may be triggered off by a number of aetiological mechanisms such as release of tissue thromboplastin, anoxia, anoxaemia, and endothelial damage (McKay, 1968). The cause of the distortion and fragmentation of red blood cells which are such constant features of microangiopathic haemolytic anaemia is considered to be mechanical trauma occurring within small blood vessels (Brain *et al.*, 1962; Bull and Brain, 1968; Bull, Rubenberg, Dacie, and Brain, 1968).

In the present study scanning electron microscopy was used to examine blood from three cases of microangiopathic haemolytic anaemia. Adherent erythrocytes were readily demonstrated in each case and evidence is presented to show how this phenomenon may result in further destruction of the red blood cell.

Materials and Methods

Specimens of venous blood were taken directly into 0.5% phosphate-buffered glutaraldehyde (pH 7.4) at room temperature. After 15 to 30 minutes' fixation they were centrifuged at 500 revs/min for five minutes, and then washed six times in double-distilled water. One drop was placed on a coverslip and allowed to dry in a dust-free atmosphere. After coating the dried specimens with a thin film of gold they were examined with the Cambridge Stereoscan electron microscope.

SUBJECTS STUDIED

Ten specimens from eight normal subjects and five specimens from three patients with microangiopathic haemolytic anaemia were studied: brief details of the latter are as follows.

Case 1

Acute onset of unknown aetiology associated with acute renal failure and peripheral vein thrombosis.

Case 2

Insidious onset in a case of longstanding rheumatoid arthritis.

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Case 3

Chronic renal failure and hypertension.

In all three patients the criteria for diagnosis of microangiopathic haemolytic anaemia were: (a) the presence of burr cells and schistocytes in the peripheral blood; (b) a reticulocyte count of 5% or more; and (c) a haemoglobin less than 9.0 g/100 ml.

Results

NORMAL CONTROLS

The erythrocytes were regular, biconcave discs 7 to 8 μ in diameter. Anisocytosis was minimal and poikilocytosis absent. The surface of the mature erythrocyte was smooth and featureless. A small proportion of the red blood cells possessed a solitary papillomatous-like projection directed into the lumen of the cell. The erythrocytes were evenly distributed on the preparation and showed no tendency to clump (Fig. 1).

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As expected, many bizarre forms were seen. In contrast to the even distribution of erythrocytes from normal subjects, red blood cells from cases of microangiopathic haemolytic anaemia showed a tendency to irregular clumping (Fig. 2).

In addition to the gross morphological abnormalities many of the red blood cells had an abnormal surface membrane. Particularly striking in case 2 were large numbers of smaller than normal spheroidal cells in which the membrane

was irregularly heaped up, giving an appearance not unlike a ball of wool (Fig. 3). Although these cells resembled lymphocytes they were, in fact, smaller and, moreover, they constituted about 6% of the total erythrocyte population which corresponded to the proportion of spherocytes in this patient's peripheral blood. Since the diameter and general morphology were also comparable it is considered, therefore, that they are the spherocytes of microangiopathic anaemia.

In addition to a number of tears and membrane defects, observed in specimens from all three cases, the surface of some erythrocytes was characterized by coarse corrugations whilst in others this was thrown into very many small, irregular, bleb-like eruptions (Fig. 4).

Adherent cells were noted in all specimens from patients with microangiopathic haemolytic anaemia. They were not seen in any of the preparations from normal control subjects. Various modes of adherence were observed and tearing of the surface membrane at points of contact was readily demonstrated. In most instances the affected cells were intimately opposed to each other (Fig. 5), but some cells were joined by spinous processes which occasionally resulted in a torn membrane (Fig. 6). This type was particularly prominent in case 1. Furthermore, a few cells were joined by thin intercellular strands which did not appear to be derived from either surface membrane. This was observed chiefly in case 3.

Further examination of the five specimens demonstrated that projections from the surface of erythrocytes may be formed by the separation of adherent cells.

Fig. 1 Scanning electron microscopy, showing even distribution of erythrocytes from a normal control subject ($\times 1,300$).

Fig. 2 Scanning electron microscopy, showing clumping of erythrocytes in microangiopathic haemolytic anaemia ($\times 1,200$).

Fig. 3 Scanning electron microscopy from case 2, showing typical 'wool-ball' appearance of spherocytes ($\times 5,400$).

Fig. 4 Scanning electron microscopy from case 1, showing adherent erythrocytes and abnormal red cell surface membrane ($\times 6,700$).

Fig. 5 Scanning electron microscopy from case 2, showing adherent erythrocytes with cell membrane damage ($\times 7,700$).

Fig. 6 Scanning electron microscopy from case 3, showing tearing of red cell membrane by spinous process of an adherent cell ($\times 15,200$).

Discussion

Many different terms have been applied to the abnormal distorted erythrocytes found in certain haemolytic anaemias. Thus 'burr cells', 'spicule cells', 'helmet cells', and 'triangular cells' are frequently described. A feature which is common to all of these is the presence of spiny projections extending from some point on the periphery. Ehrlich (1891), who first described these abnormal erythrocytes, considered them to be the result of circulating chemical substances secondary to anaemia, but within recent years attention has

been focused on mechanical factors. Following a study on patients with microangiopathic haemolytic anaemia, Brain *et al* (1962) considered that the common link between the haemolytic anaemia and the underlying disease was the presence of pathological changes in small blood vessels. They suggested that an important factor in the pathogenesis of the haemolysis is direct contact between red cells and the diseased vessel wall. More recently, Bull and Brain (1968) have demonstrated that red cells can fragment when a rapidly moving erythrocyte encounters a thin fibrin strand.

It appears to be fairly well accepted that contact between erythrocytes and diseased blood vessels is of prime importance in the production of the abnormal erythrocytes of microangiopathic haemolytic anaemia. However, it has not been shown previously that red cell destruction may continue after the initial damage has taken place, although Venkatachalam, Jones, and Nelson (1968) observed frequent close juxtaposition of erythrocytes to endothelial cells in experimentally produced microangiopathic haemolytic anaemia in rats. These authors suggested that red cells may become adherent to endothelium and then fragment as a result of shearing stresses. In another report of experimentally produced microangiopathic haemolytic anaemia in rabbits, Rubenberg, Regoezi, Bull, Dacie, and Brain (1968) observed that erythrocytes in wet preparations tended to adhere to each other and also to glass surfaces. They concluded that this effect was mediated through fibrin which had become attached to one of the red cell surfaces.

Salisbury and Clarke (1967) have demonstrated the potential of the scanning electron microscope in the study of erythrocytes. This instrument, which has greater depth of focus and higher resolution than the light microscope, has proved of value in demonstrating pathological changes, particularly of the red cell surface membrane.

In this study examination by scanning electron microscopy revealed erythrocytes connected in a variety of ways. In one subject only some cells were joined by a thin strand passing between them. Although contamination of the preparation cannot definitely be excluded, these strands were not observed in any of the control specimens; neither have they been seen in other conditions. It is believed that they are most probably fibrin. The majority of adherent cells, however, were in intimate contact and this was invariably associated with some alteration of the surface membrane. The third type of adherence was by means of spinous processes which appeared to be formed by the separation of cells which were only connected by a small area of surface membrane. On occasion this resulted in a torn surface membrane. In neither of the last two modes of adherence was there any evidence of interposed fibrin.

The negative electrical charge at the surface of normal erythrocytes ensures their functional and structural independence within the circulation. Alteration in the physico-chemical properties of the surface membrane of the red cell may result in the loss of the surface charge and the associated natural repulsion between cells. It is perhaps significant that both fibrinogen and globin, a histone, can increase the adhesiveness of erythrocytes (Ponder, 1966). The importance of fibrin in the pathogenesis of this condition has already been mentioned, but the presence of free haemo-

globin, released from erythrocytes in the affected vessels, may also contribute to the observed phenomenon.

The resolution of the scanning electron microscope is inadequate for the demonstration of antibodies which may be present on the surface membrane. The direct antihuman globulin tests, however, were repeatedly negative in the three patients and red cell antibodies are not considered a feature of this condition. A small proportion of cells were connected by fibrin strands, presumably acquired during passage through the affected vessels. Apart from these, the most likely explanation of the phenomenon of adherence between erythrocytes is alteration of the physico-chemical properties of the red cell surface membrane with a consequent increase in physical affinity between cells.

Although it is accepted that many of the abnormal cells observed in microangiopathic haemolytic anaemia reflect mechanical trauma occasioned within the damaged vasculature, it is apparent that further red cell damage may occur when adherent erythrocytes are subjected to the stresses of the vascular flow, and that, in some cases, these forces are sufficient to break the contact at its weakest point with the formation of the spicules which are a feature of this condition.

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