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PLATELET BINDING IN MONONUCLEAR CELL PREPARATIONS FROM PERIPHERAL BLOOD

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Summary.—We have investigated the relative binding affinity of white blood cells for platelets, during the centrifugation and washing procedures used for separating mononuclear cells from peripheral blood. In wet cell preparations the majority of monocytes (>90%) showed platelet binding, compared to 14% of lymphocytes and 38% of polymorphs. Monocytes also had greater numbers of attached platelets per cell and demonstrated considerable variation in their affinity for platelets. The phenomenon was affected by the calcium concentration of the medium. Our observations are of value for identifying monocytes in wet cell preparations. The technique may also be useful for studying platelet adhesion in vascular disease.

IN THE ISOLATION of mononuclear cells from anticoagulated blood the preparations are always contaminated with numerous platelets. During the repeated washing and centrifugation required to remove platelets, they bind and rosette with large mononuclear cells. We have investigated this phenomenon to determine its value in distinguishing monocytes from lymphocytes. The morphological distinction may be difficult, particularly in the case of large lymphocytes, as both cell types overlap in size (Arenson, Epstein and Seeger, 1980). At present non-specific esterase staining (Ornstein, Ansley and Saunders, 1976) is probably the best method for identifying monocytes. We have also assessed platelet-monocyte binding as a means of obtaining a more pure lymphocyte preparation. We consider that this *in vitro* phenomenon can be used to further our understanding of platlet adhesions in life, particularly in relation to endothelium in vascular disease.

MATERIALS AND METHODS

Cell separation.—Cells were isolated from heparin (20 u/ml), preservative-free or EDTA (1 mg/ml) anti-coagulated blood from 15 healthy volunteers using sterile techniques. Blood was mixed with Dextraven 110 (Fisons) in a 10:1 ratio and left to settle for 30–60 min, to remove red cells. Following centrifugation (400 *g*, 24 min) on Ficoll-Hypaque (Sg, 1·078, 20°) mononuclear cells and platelets were obtained as a dense band at the plasma interface and polymorphonuclear leucocytes (polymorphs) as a pellet on the bottom of the tube. Platelets were isolated separately by centrifuging blood or buffy coat on Ficoll–Hypaque (Sg. 1·6063, 20°) at 250 *g* for 20 min.

Platelet binding to white blood cells.—Platelet binding was studied in washed mononuclear cell preparations in buffy coat preparations and in mixed polymorph-platelet suspensions. Buffy coat cells and mononuclear cell preparations were twice washed in Eagle's medium (MEM) and centrifuged for 10 min at 400 *g*, before observing platelet binding. In experiments to assess the effects of Ca^{2+} on platelet binding, the cells and platelets were centrifuged in PBS containing EDTA (1 mg/ml). Polymorphs were added to platelets which had been separated from the same volume of blood and polymorph-platelet suspensions were subjected to the same washing and centrifuging procedures as the mononuclear cell and buffy coat preparations.

Platelet binding was observed by phase contrast microscopy in wet cell preparations. Mononuclear cells were graded by size into small (small lymphocytes) and large cells (large lymphocytes and monocytes). Smears were made from cells resuspended in serum, fixed in methanol or 15% formalin-dextrose and stained with Giemsa and/or non-specific esterase (Ornstein *et al.*, 1976).

Lymphocyte purification by removal of platelet-

bound monocytes.—Unwashed platelets, separated from 4 ml of blood, were diluted to 15 ml with PBS for counting. One ml of the platelet suspension (25×10^6 cells) was placed in sterile flat-bottomed tubes (14 mm diam.) which were centrifuged (650 *g*) for 5 min. Following centrifugation, gluteraldehyde (5 μl of a 25% solution in saline) was added to each tube so that the platelets were firmly attached to the bottom of the tube. After 5 min incubation (20°) the tubes were gently washed 4 times with PBS to remove the gluteraldehyde. Autologous mononuclear cells were added (1 ml containing 1×10^6 cells) and the tubes were again centrifuged at 180 *g* for 5 min. The cells were then resuspended and recovered after 5 min incubation (20°) using a Pasteur pipette.

RESULTS

In Ficoll–Hypaque preparations, an average of 16·1% (±5·2) of white cells (range 9–34%) were large mononuclears (monocytes and large lymphocytes). Most (92·7%) showed platelet binding (Table I). In comparison, only 14·0% of small lymphocytes and 37·9% of polymorphs bind platelets in both buffy coat preparations and polymorph-platelet mixtures. Platelet aggregation in these preparations was minimal. However, increasing the time, force of centrifugation and the number of washings, increased the clumping of platelet aggregated cells.

We confirmed that platelet binding was predominantly a characteristic of monocytes in wet cell preparations, by establishing that most of the large platelet-binding cells were NSE positive.

In the cell population demonstrating platelet binding, the affinity of platelets for monocytes was much higher than for lymphocytes or polymorphs (Figure). In Giemsa stained smears we found that approximately 30% of lymphocytes and polymorphs bound platelets and in 50% of these cells only one platelet was attached. The binding of 3 or more platelets was a feature of 65% of monocytes compared to about 5% of lymphocytes and polymorphs (Table II).

TABLE I.—*Platelet binding to white blood cells in unstained wet preparations*

	No. of cells counted	Percentage of cells binding platelets
Large cells (monocytes and large lymphocytes)	94 (±21·6) n=6	92·7 (±3·7)
Small lymphocytes	383 (±186) n=6	14·0 (±8·3)
Polymorphonuclear leucocytes	231 (±55·6) n=7	37·9 (±20·0)

TABLE II.—*Platelet binding to white blood cells in stained smear preparations*

	No. of cells counted	Percentage of cells binding platelets No. of platelets/cell				
		1	2	3	>3	Total
Monocytes	200 (±100) n=6	9·6 (±6·3)	15·7 (±10·3)	14·9 (±3·5)	49·6 (±18·7)	89·8 (±6·1)
Lymphocytes	340 (±174) n=5	18·7 (±6·0)	7·3 (±1·5)	1·7 (±1·0)	2·5 (±2·8)	30·2 (±9·9)
Polymorphs	186 (±35) n=7	16·3 (±7·8)	9·9 (±5·2)	3·9 (±2·0)	1·3 (±2·0)	31·4 (±12·3)

TABLE III.—*Platelet binding to large mononuclear cells in unstained wet preparations separated from EDTA or heparin anticoagulated blood using Ficoll–Hypaque*

	No. of cells counted (monocytes and large lymphocytes) n=4	Percentage of cells binding platelets No. of platelets/cells				
		1	2	3	>3	3+>3
EDTA (1 mg/ml)	94·5 (±42·3)	27·3 (±5·9)	13·7 (±7·0)	10·6 (±7·8)	8·3 (±5·4)	18·9
Heparin (20 u/ml)	82·8 (±32·0)					92·0 (±6·3)

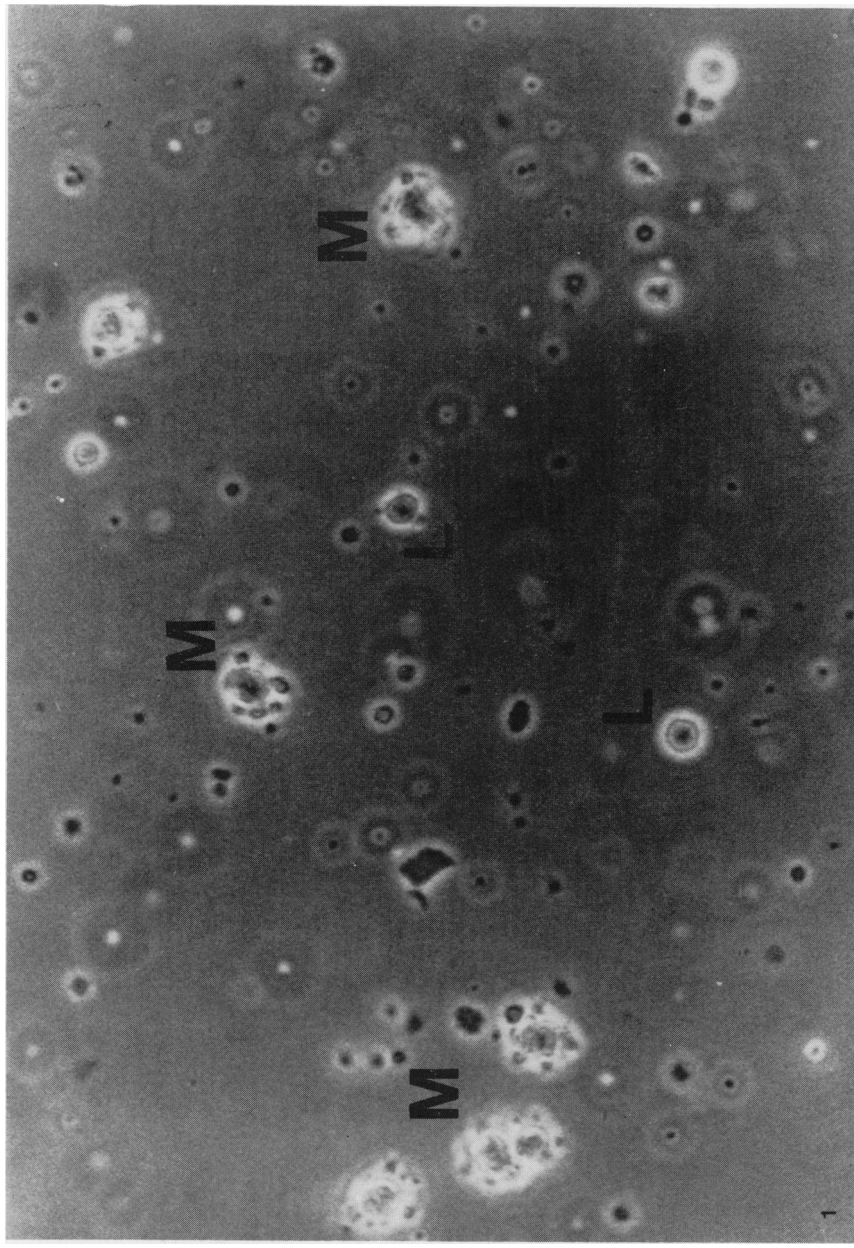

FIGURE.—Monocytes (M) showing many attached platelets compared to lymphocytes (L) with few or none. Wet preparation, phase, contrast. ×400.

The importance of Ca^{2+} in facilitating platelet binding was shown by comparing the binding of cells derived from blood containing heparin or EDTA as anti-coagulant (Table III). In EDTA treated samples, the number of monocytes binding platelets fell to 60% and the number of cells with 3 or more attached platelets was reduced from 92–18·9%.

The centrifugation of mononuclear cells on to gluteraldehyde-fixed platelets in MEM selectively removed monocytes (M) and increased the purity of lymphocytes (L) from an initial L:M ratio of 4·8 (± 1·08) to 17·2 (± 6·0), compared to 11·2 (± 2·9) in plain tubes (n = 4). In MEM supplemented with 5% human serum (n = 3), the respective L:M ratios were 8·5 (± 1·9), (± 16·7) and 24·4 (± 6·1).

DISCUSSION

We have demonstrated that in the preparation of mononuclear cells from blood, platelets bind preferentially and in greater numbers to monocytes, than to lymphocytes or polymorphs. Over 90% of large mononuclear cells in Ficoll–Hypaque preparations showed platelet binding, and of these 90% were identified as monocytes by non-specific esterase staining.

We observed that monocytes demonstrated different affinities for platelets, which may substantiate reports that they exist in different stages of maturity (Horwitz *et al.*, 1977). The functionally more mature monocytes appear to bind larger numbers of platelets (Arenson *et al.*, 1980).

The development of platelet adhesion is associated with a change in shape and deformation of the membrane (Vermylen, 1978). Although the nature of the cell receptor site involved in adhesion is ill defined, it is possibly associated with fibrinogen/fibrin transition products (Tangen *et al.*, 1971, Sherman and Lee, 1977). Adhesion *in vitro* is probably due to the alteration of membrane properties, caused by centrifugation. We have shown that the phenomenon is dependent on the presence of Ca^{2+}. Platelet binding may also be influenced by plasma proteins for Djaldetti and Fishman (1978) found that serum from a patient with hypogammaglobulinaemia had an abnormally high capacity to facilitate platelet-monocyte binding. Platelet satellitism around neutrophils (Larson and Pierre, 1977) has usually been observed in smears prepared from EDTA anti-coagulated blood.

The phenomenon of platelet binding affects a number of techniques. It decreases cell yield during cell separation procedures, and causes inaccuracies in automated cell counting (Larson and Pierre, 1977). The results of lymphocyte rosetting techniques may also be affected.

We attempted to make use of the monocyte-platelet binding phenomenon, to increase the purity of lymphocyte suspensions. Although the centrifugation of mononuclear cells on to gluteraldehyde-fixed platelets selectively removed monocytes and increased the percentage of lymphocytes in these preparations, the degree of purity achieved did not warrant further development of this technique. Probably the selective aggregation of monocytes by thrombin in the presence of platelets (Arenson *et al.*, 1980) is a more effective technique for purifying lymphocytes. Our study suggests that this occurs through the aggregation of monocyte-bound platelets.

Whether platelet binding directly, or indirectly, through the release of pharmacological agents, activates monocytes and alters their functional properties *in vitro* is unknown.

In conclusion, the characteristic binding of platelets to monocytes *in vitro* facilitates their identification and may correlate with the functional maturity of monocytes. As platelet adhesion is difficult to study *in vitro*, in the absence of platelet aggregation, monocyte-platelet binding may prove useful for studying the mechanism of platelet adhesion in vascular disease.

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