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E-Rosettes in Human Lymphocytes. Enhancement and Depression after Incubation of Lymphocytes with Papain and Trypsin

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A study of E-rosette formation after incubation of lymphocytes with trypsin and papain is shown. The rosette formation ability of lymphocytes pretreated with papain or trypsin is compared with controls.

Incubation with trypsin markedly impars rosette formation but a complete recovery is reached when lymphocytes are cultured during 6-8 hours.

Incubation with papain has an opposite effect; E-rosette formation is notably increased. It is suggested that B-lymphocytes or a subpopulation of T-lymphocytes, which are normally non rosette forming, may account for this phenomenon.

A high percentage of peripheral human lymphocytes have the capacity to bind sheep red blood cells (SRBC) in a non-immune way, forming direct rosettes (E-rosettes) (2, 3, 4).

Several recent papers confirm that the T-lymphocyte population is directly responsible for the formation of this spontaneous rosettes (2, 8, 11). The utilization of this property affords the possibility to study the proportion of T-lymphocytes in the peripheral blood of normal individuals as well as in several pathological circumstances.

However, very little is known about the structure and behaviour of the lymphocyte membrane receptors with capacity to bind SRBC.

In the present work, we have studied the rosette formation ability of lymphocytes obtained from fifty normal individuals and also, the effect of previous incubations of lymphocytes with trypsin and papain on this phenomenon.

Materials and Methods

Twenty ml of perypheral blood were obtained from healthy donors. Lymphocytes were isolated according to techniques described elsewhere (5). After washing twice in Hanks solution (pH 7.2, Difco),

the concentration was adjusted to 3×10^6 cells/ml.

Trypsin (Wellcome, 5 %) was used at 0.25 % in PBS (Phosphate buffered saline solution) and Papain (Merck, 1/350) was prepared according to the method described by PORTER (9), dissolving 15 mg in 100 ml of 0.1 M phosphate buffer, pH 7, 0.1 M cysteine and 0.02 M EDTA.

Whenever the enzyme effect was essayed, 1 ml of a solution of trypsin or papain, as described in the preceding paragraph, was taken for each 3×10^6 cells. Lymphocytes were incubated 30 minutes at room temperature, keeping a control without enzymes at the same time and the same temperature. As papain controls, we also tested lymphocytes with phosphate buffer, cysteine, EDTA and all together

Lymphocytes were washed twice in Hanks solution after enzyme treatment and concentration was now adjusted to 3×10^6 cells/0.5 ml. The next step was performed mixing 3×10^6 lymphocytes/0.5 ml with 0.5 ml of a suspension of SRBC (0.05 % in Hanks solution), during 5 minutes at 37° C. Centrifugation at 1,200 rpm was followed by incubation at 4° C during 1 hour 30 minutes. The supernatan was removed and the top layer of the pellet was gently resuspended by shaking. One drop of the cell suspension was mounted on a glass slide, and covered by a coverslip. 300 lymphocytes were counted and all lymphocytes binding more than three SRBC were considered positive.

To study the recovery in rosette formation after trypsin treatment, incubation at 37° C was performed during 2, 4, 6, 8 and 18 hours, suspending 3×10^6 cells in 1 ml of tissue culture medium (M.E.M. Eagle's, Wellcome) buffered with sodium bicarbonate (7).

Results

The rosette formation in normal individuals and with the previously described method, varies between 50 to 65 %.

Trypsin incubation reduced this number to figures of 2 to 3 %. Further incubation at 37° C of lymphocytes pretreated with trypsin, is followed by a progressive recovery in the rosette forming capacity. Figure 1 shows this recovery in relation

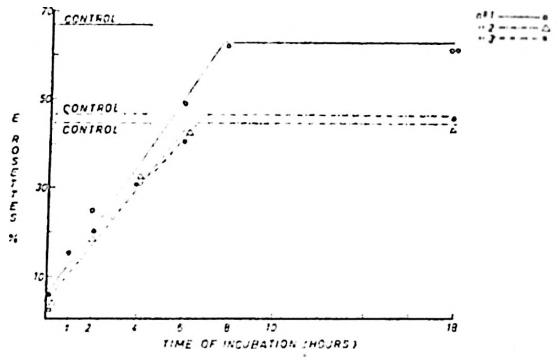

Fig. 1. *Recovering of non Immune rosette forming capacity after treatment of lymphocytes with trypsin.*
The base capacity is totally recovered after 6-8 hours of incubation.

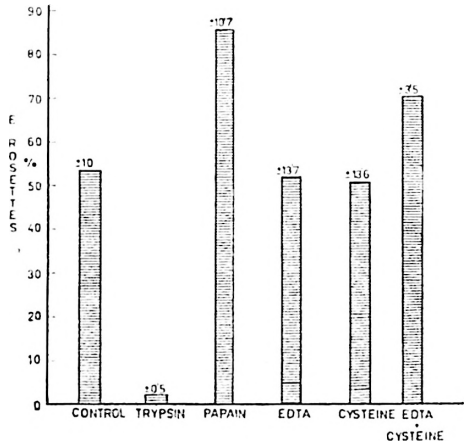

Fig. 2. *Percentage of human lymphocytes forming E-rosette in base conditions and after treatment of lymphocytes with trypsin and papain.*
As specific papain control, results obtained with EDTA and cysteine are also shown.

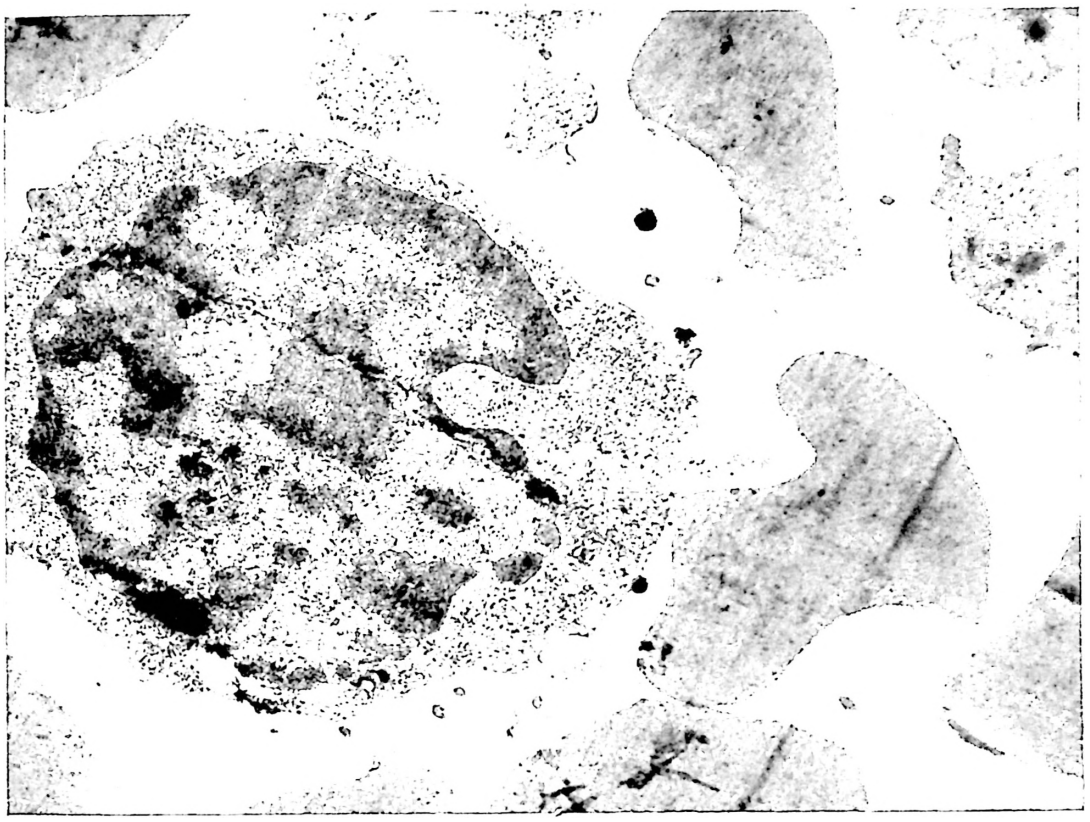

Fig. 3. *T-cell rosettes micrograph.*
Lymphocyte from human peripheral blood after incubation with sheep erythrocytes. One contact site between SRBC and lymphocyte membranes is evident (× 24,000).

to the incubation time. At 6-8 hours the base rosette forming capacity has been totally reached; and, when incubation time is prolonged until 18 hours, the number of formed rosettes does not increase anymore. The rosette regeneration rate was uniform for the different cases and unrelated to the base level.

Papain incubation has shown a different effect. After incubation with papain the rosette formation increased 20 to 25 % (fig. 2). Phosphate buffer, cysteine and EDTA separately have no effect on rosette formation, while phosphate buffer with EDTA and with cysteine at the same concentrations used to prepare the papain active solution also increased the rosette base percentage. Nevertheless, this increase only reached 10 %.

Discussion

The abrupt decrease observed in rosette formation after incubation with trypsin, seems to indicate that T-lymphocyte membrane receptors for SRBC (figs. 3 and 4) are sensitive structures to this proteolytic enzyme. In any case, the used trypsin doses did not impair cell viability, as it was proved in phase contrast microscopy.

The regeneration of these lymphocyte membrane receptors has a progressive

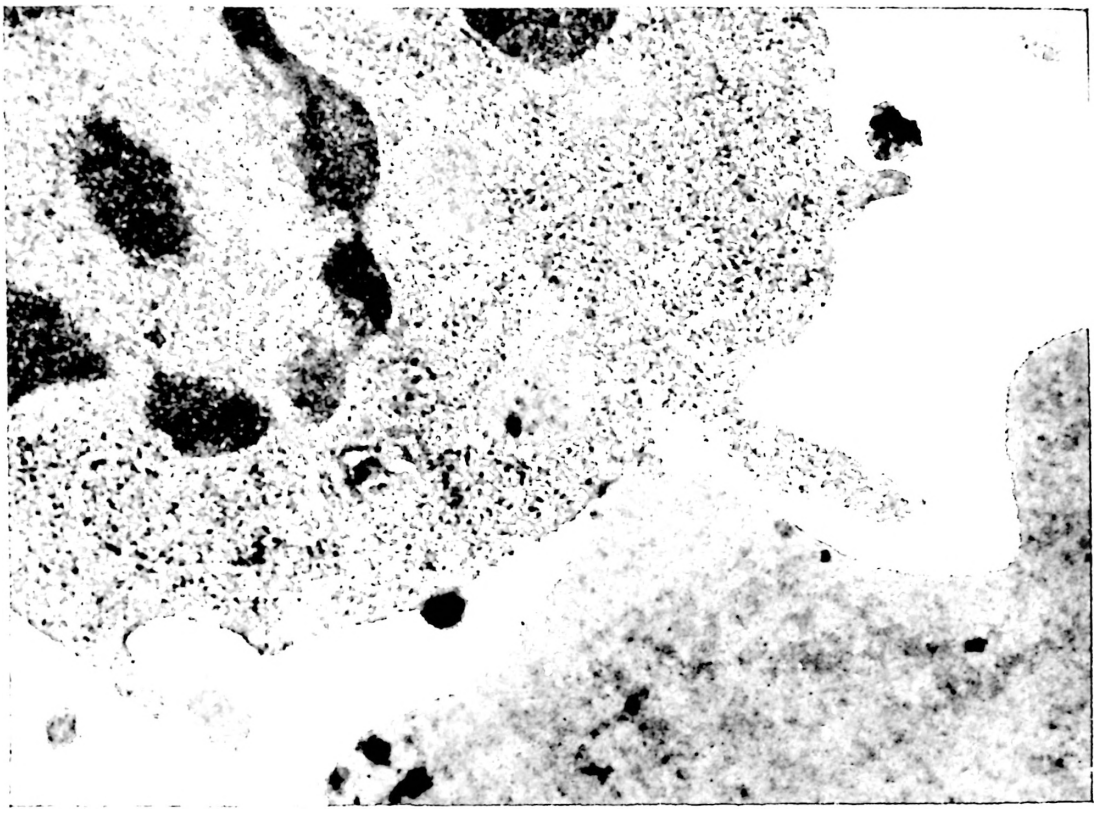

Fig. 4. *High power micrograph of erithrocyte receptor lymphocyte.* This figure shows intimate membrane-membrane interaction between reactive lymphocyte and sheep red blood cell (× 60,000).

and constant rythm until reaching the rosette forming base level, when lymphocytes are incubated at 37° C in tissue culture medium. The required time to recover the total rosette forming capacity is markedly lower to that found by JONDAL *et al.* (8). Perhaps these discrepances are due to the tissue culture medium properties.

Since prolongation of culture does not increase the rosette forming base level, it appears to be that the same cell population, before and after trypsin treatment, is able to bind SRBC. Therefore, other cells (B-lymphocytes) would not modify their non rosette forming property, when cultured.

The treatment of lymphocytes with papain has surprisingly shown completely opposite results to those got with trypsin. Similar results have been reported by BENWICH *et al.* (1), working with neuraminidase.

Since papain incubation, with the described method, did not impair cell viability, we think that the increased number of formed rosettes is absolute and could be due to primarily non rosette forming cells which have acquired the E-rosette forming property (6).

As it is known, the base rosette forming cell population are T-lymphocytes (11). Furthermore, anti-human Ig sera do not inhibit the rosette formation (4). Hence,

the involved receptors could not be immunoglobulins. These data suggest two possibilites: 1st. Papain action could be the destruction of cell surface immunoglobulins, enhancing a more closed contact between B-lymphocyte receptors and SRBC. This hypothesis implies that B-lymphocytes have also E-rosette forming receptors which are normally masked by surface immunoglobulins. 2nd. Papain action could modify T-cell membrane, enhancing rosette formation in a subpopulation of T-lymphocytes, which are normally non rosette forming.

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