SPONTANEOUS FORMATION OF ROSETTES BY AUTOLOGOUS HUMAN MONOCYTE-MACROPHAGES AND LYMPHOCYTES IN CELL CULTURES

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During study on cell cultures from total human blood leukocytes, we found a rosette-shaped cell association formed by monocytes and lymphocytes. Considering the immunologic roles of these two cell types, we decided to study such cell association, the results being reported here together with data on the frequency of their ocurrence in cell cultures of human leukocytes of healthy subjects, at different culture times.

Total leukocytes were obtained from blood of healthy human donors (9 women, 8 men; mean age 34, range 20-62). Venous blood was drained into syringes previously treated with phenol-free heparin. The plasma containing the white cells were seeded in TC 199 medium plus penicilin-streptomycin.

Samples of cultures were prepared at 48 and 96 hours by slight shaking of the respective flask. The cells were centrifugated at 200 g for 10 minutes and the supernatant was discarded. The cell pellet was gently resuspended in drops of TC 199 medium. Drops of this cytopreparation were put on glass slides for 5 min in a humidified chamber, and then dessicated by centrifugation on a disk perpendicular to the axis of the centrifuge, as previously described [1]. The cytopreparations were stained with May Grünwald-Giemsa, and observed at a light microscope, at 400 x. In considering that the monocytes were found singly and centrally located in the rosettes we have described, the quantification of the rosettes was made as the percentage of monocytes forming rosettes, by studying 50 of such cells in each case. A rosette was considered when three or more lymphocytes surrounded a monocyte.

In all cases we observed the presence of rosettes formed by monocyte/lymphocytes. Their mean number was 5.2 (SD 3), at 48 hs, and 8% (SD 4.2) at 96 hs with respect to the total of monocyte-macrophages [2, 3] counted in the cytopreparations. Figures 1 and 2 show aspects of these rosettes. The central cell was always a monocyte surrounded by small lymphocytes. The nucleus of the monocyte was typical in some of these rosettes (Fig. 1) and in others it presented features that suggest cell conversion to macrophages (Fig. 2).

The results show the appearance of rosette formation by cultured monocyte-macrophages and lymphocytes obtained from circulating blood. To our knowledge, this is the first report on the occurrence of this phenomenon with such cells from peripheral blood. We think that the preparation of the cocultured cells for their observation in the way we described has permitted the phenomenon to be found.

These spontaneously-formed rosettes appear as a *selective* cell-cell association. In fact, although the total leukocytes from each donor were seeded in the flask culture, the rosettes were produced only among monocyte-ma-

crophages and lymphocytes. It is obviously a phenomenon produced at the cells' membrane level and, perphaps, related to the antigen presentation. We think of interest to elucidate this aspect in further work.

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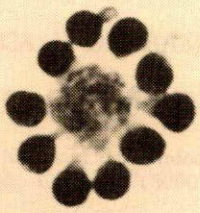

Fig 1

FIGURE 1: Rosette formed by a monocyte surrounded by small lymphocytes. Human total leukocytes culture. 48 hours. Stain: May grünwald-Giemsa. 400 x.

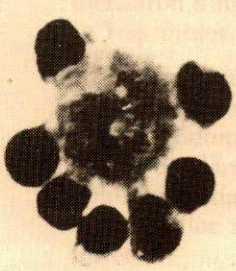

Fig 2

FIGURE 2: Rosette formed by a monocyte-macrophage surrounded by small lymphocytes. Note the nuclear shape of the macrophage. Human total leukocytes culture. 96 hours. Stain: May Grünwald-Giemsa. 800 x.