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Electron Microscopic Evidence for Phagocytic Properties of Human Peripheral Mononuclear Cells Cultured with PHA

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The time course of the ultrastructural changes induced in human peripheral mononuclear cells, when cultured with PHA, has been studied. In addition to findings common to many mitotic cells, such as an increase in nuclear and nucleolar size, the presence of free polyribosomes, glycogen and lipid globules in the cytoplasma, and a high number of mitochondria, agglutinating properties due to PHA *per se* were observed in the first 12 hours.

At 72 hours certain cells developed phagocytic-like properties, i.e. they were able to incorporate both material from the extracellular compartment and syngeneic cells.

These results are discussed suggesting the possible presence of macrophages in culture or the ability of activated T cells to express it.

Peripheral blood lymphocytes undergo blast transformation and mitosis when cultured with certain lectins: Phytohemaglutinin (PHA), Concanavalin A (Con A), Pokeweed (PWM), Lipopolisaccharide (LPS) (3, 6, 13, 20). This transformation is a complex mechanism with remarkable morphological and functional changes in lymphoid cells. These reactions are generally considered as «non immunological» in contrast to the clonal response induced on sensitized lymphocytes by specific antigens; one notable difference is that the number of cells transformed by mitogen is very high (8, 9, 17).

The polyclonal activation mechanism on lymphocytes by lectins is not clearly understood. However, the significance and relevance of this phenomenon lies in 1) the morphological resemblance of mitogen transformed cells to those obtained in *in vivo* antigen responses i.e. allograft rejection and graft versus host reactions (19). 2) in the fact that the particular response to lectins may reflect the immunocompetente of lymphoid cells as demonstrated in patients with immunodeficiency diseases (7, 10) and 3) in the con-

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cordance between lymphocyte activation and malignant transformation (18).

A number of lymphocyte subpopulations have been recently described. Thus thymocytes from cortisone treated mice have been used as a «T cell» source (2). Spleen cells from thymectomised, irradiated and reconstituted mice, or bone marrow cells pretreated with anti θ and complement, are considered to be «B lymphocytes» (12, 15). In addition spleen cells from nude mice (14) or from normal mice previously treated with anti θ and complement have been used as B lymphocytes. Spleen cells from normal mice are considered to be mixtures of T and B cells. It has been found that lectins are capable of selectively inducing activation of these subpopulations: PHA and Con A trigger blast transformation of T cells, LPS of B cells and PWM of both populations (12).

T lymphocytes are transformed when cultured with PHA into large pyroninophilic cells with a prominent nucleolus-so called «activated T cells». Very little is known about these activated cells even though this activation can be quantified (12). We became interested in the early phases of this cell transformation because of the drastic morphological and functional changes that rapidly occur after mitogen treatment.

In this study we have used the electron microscope to follow morphological changes occuring in lymphocytes within the first 72 hours of PHA stimulation.

Materials and Methods

Human peripheral mononuclear cells from healthy donors were used. They were isolated from whole blood and cultured with mitogens as previously described with slight modifications (7). 3×10^6 lymphocytes/ml of Eagle's medium (Wellcome) buffered with 2.5 % of sodium bicarbonate (Wellcome) and 10 % of FCS (heated for half an hour at 56 C, Difco) were cultured in glass tubes (0.9 × 3 cm). The final concentration of PHA was 0.025 mg/ml. Cultures were harvested at 12, 24, 48 and 72 hours. At the end of each period the cell suspensions were centrifuged at 400 g for 5 minutes and the pellets were fixed (in the same tube) with glutaraldehyde buffered with phosphate saline (Ph 7.2). This material was processed with osmium dehydrated in acetone and include in Vestopal W. Sections were cut in a LKB ultramicrotome. Preparations were examined on an electromicroscope JEOL 1008 after staining with uranile acetate and lead nitrate. Control cultures without PHA were processed in the same way.

Results

After 12 hours of PHA incubation, there is an increase in cellular size, the nuclear chromatin appear more diffuse and the outline of the nucleolus and the granular material inside becomes more evident (fig. 1 a, b, c, d). We were unable to differentiate morphologically the PHA from the PWM effect on lymphocytes at this stage.

In this same period, close physical contact among cells were frequently seen. Prolongations from one cell may completely surround another (fig. 1 a). These effects might be understood as a direct action of PHA agglutinating properties on lymphocyte membranes. Also at this time large cells with high glycogen content in the cytoplasm, microfilaments and microtubules appeared (fig. 1 b, c) Some cells contained dense bodies that appeared to have been actively incorporated from the extra cellular compartment (fig. 1 c). No significant differences from the 12 hours morphological picture was observed at 24 and 48 hours.

After 72 hours of culture the transformed cells reached an even larger size, the nuclear chromatin was more diffuse and the nucleolus further extended figure 2 a). Certain changes not apparent at

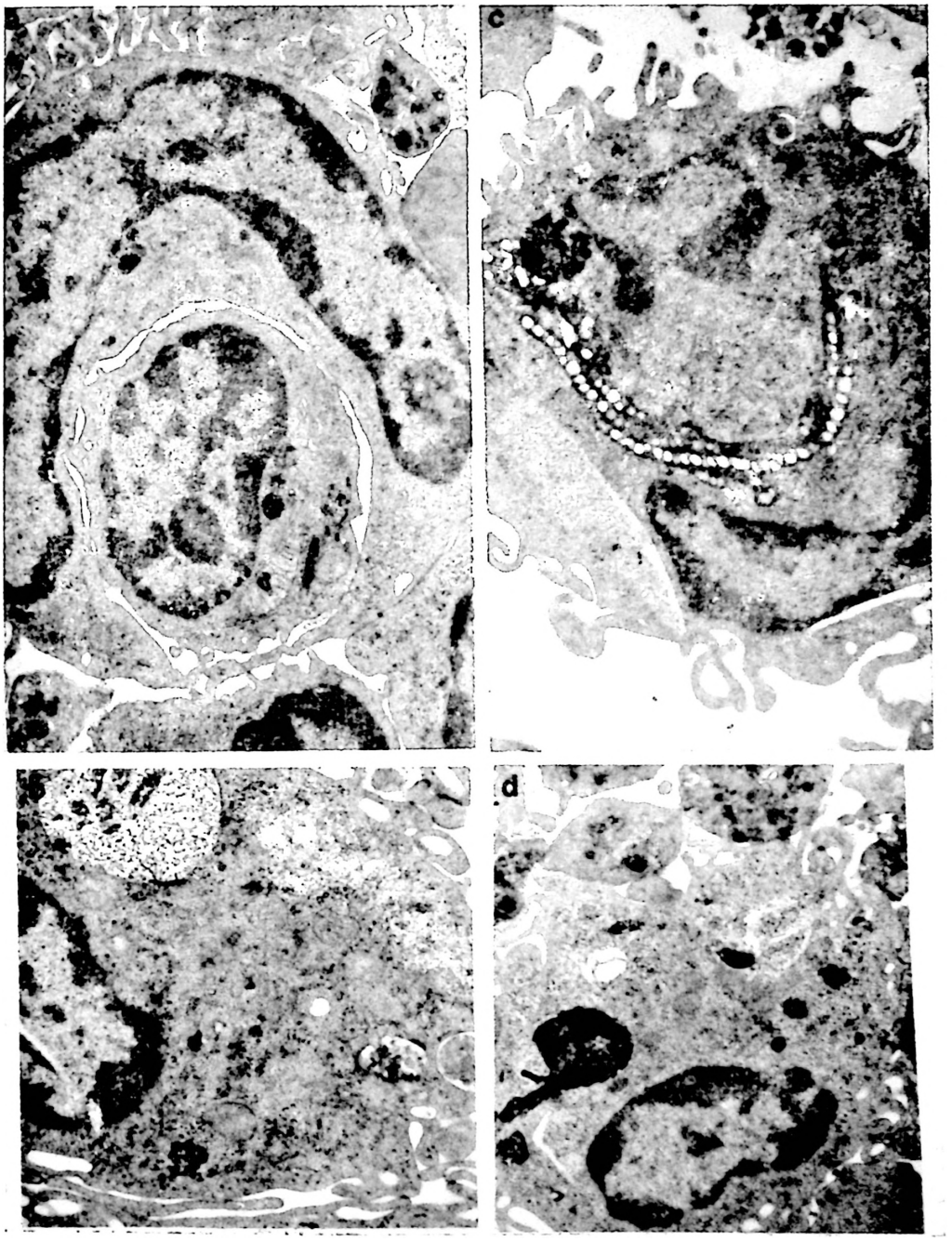

Fig. 1. *Cultures at 12 hours.*

a) One cell encompassing another.
b) Presence of glycogen granules in cytoplasm.
c) Presence of small dense bodies of varying size in cytoplasm and extra cellular compartment.
d) Cell with microfilaments.

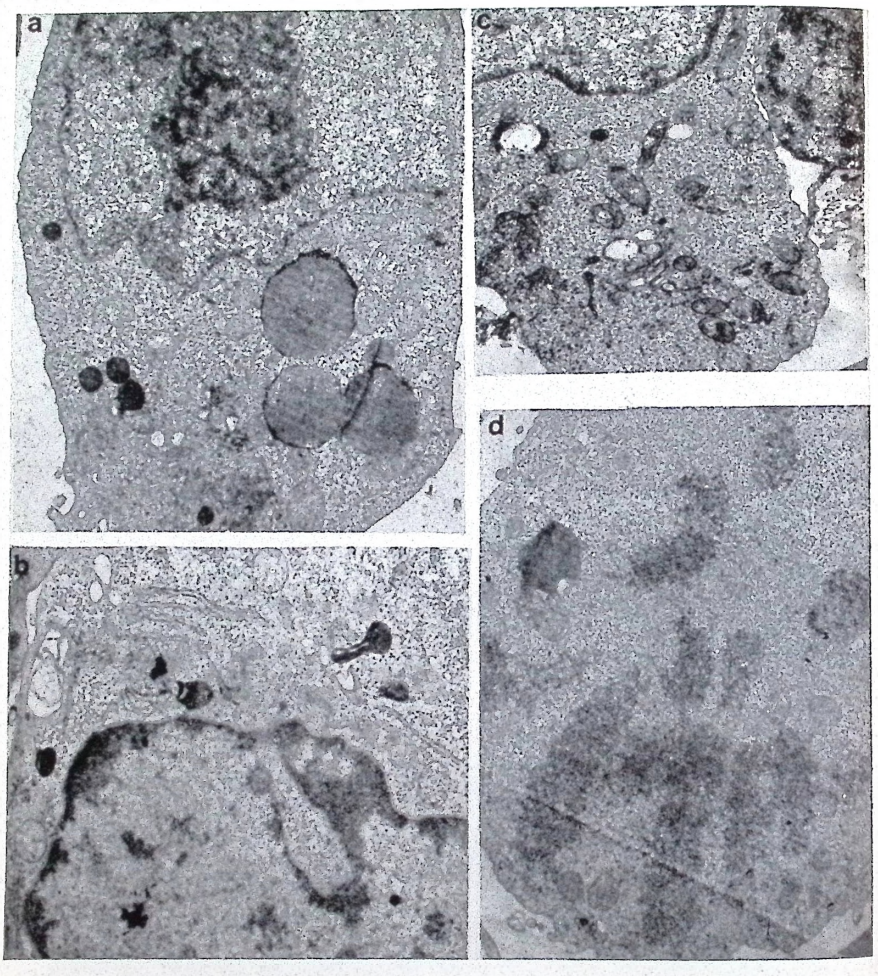

Fig. 2. *Cultures at 72 hours.*

a) Large transformed cell with diffuse nuclear chromatin and lipid globules in the cytoplasm.
b) Well developed Golgi apparatus and glycogen granules.
c) Transformed cells with high proportion of mitochondria and polyribosomes.
d) A cell in mitosis.

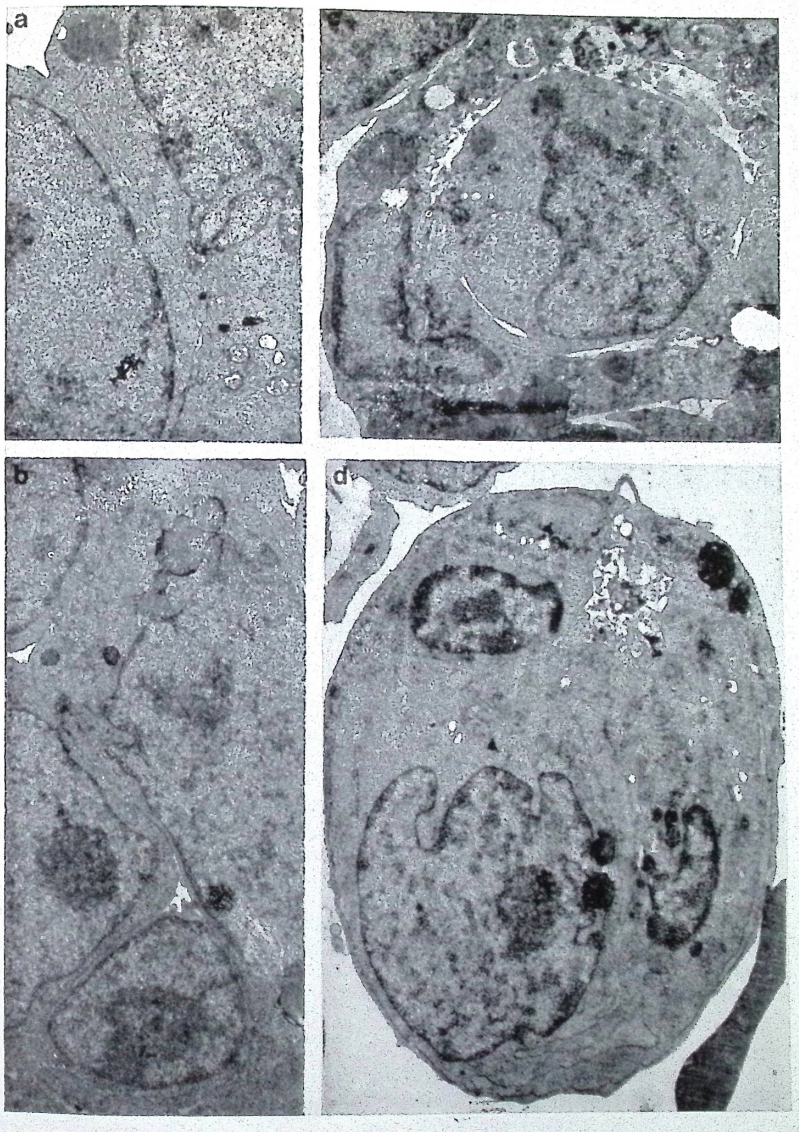

Fig. 3. *Cultures at 72 hours.*

a) y *b*) Transformed cells in close physical contact.
c) One transformed cell completely incorporated in another.
d) Presence of cell debris in phagocytic-like cells.

12 hours now appeared: a-well-developed Golgi apparatus, higher proportion of mitochondria, round liquid globules and mitotic figures (fig. 2 b, c, d). At this period it was very common to see these cells in close physical contact. Also some of these cells which appear to be undergoing degeneration were completely surrounded by others. Cell debris can be seen in the cytoplasm of the latter, phagocytic-like cells (fig. 3 a, b, c, d).

Discussion

The increase in the size of the nucleus and nucleolus and the detection of free polyribosomes, glycogen material and lipid globules in the cytoplasm is in agreement with the findings of other workers using either specific antigen or mitogen stimulation (3, 6, 13, 17) i.e. morphological features of cells undergoing mitosis. The close physical contact observed amongst the transformed cells is probably explained by the agglutinating properties of PHA, as this was absent from cultures with specific antigen.

Phagocytic-like cells were observed at 72 hours. There are two possible explanations for this phenomenon: *a*) the existence of other, non-transformed cells, in culture with phagocytic capacities, acting independently of PHA stimulation; for instance macrophages survive for long periods of time in cultures and are able to destroy other cells *in vitro* (5, 11). *b*) activated T cells may be able to develop phagocytic properties (16). This last possibility is in agreement with the finding of other authors using conventional microscopy to study the phagocytosis of iron particles (1, 4). To determine whether macrophage are present *per se* or whether the transformed cells develop macrophage-like properties, experiments are now in progress to see if cultures depleted of adherent cells retain their phagocytic properties.

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References

1. BIBERFIELD, P.: *Ultrastruc. Res.*, **37**, 41, 1971.
2. BLOMGREN, H. and ANDERSON, B.: *Cell. Immunol.*, **1**, 545, 1970.
3. COOPER, H. L.: In «Effects of drugs on the cell cycle» (Zimmerman, Padilla and Cameron, ed.). Academic Press, 1972.
4. DIAMANSTEIN, T., VOGT, W., RUHL, H. and BOCHERT, G.: *Eur. J. Immunol.*, **3**, 8, 488, 1973.
5. EVANS, R. and ALEXANDER, P.: *Nature* (London), **236**, 168, 1972.
6. FESTENSTEIN, H.: *Lancet*, i, 1982, 1968.
7. GARCÍA-PUCHE, J., MUÑOZ, E., GARRIDO, F. and OSORIO, C.: *Sangre*, **19**, 217, 1974.
8. GARRIDO, F.: Tesis doctoral, Granada (Spain), 1974.
9. GARRIDO, F., PEÑA, J., ALEMÁN, P. and OSORIO, C.: *Rev. Clin. Esp.*, **129**, 1973.
10. GARRIDO, F., PEÑA, J. and OSORIO, C.: *Sangre*, **19**, 443, 1974.
11. GARRIDO, F., SCHIRRMACHER, V. and FESTENSTEIN, H.: Immunochemistry of the cell membrane. EMBO. Ravello. Ytaly. June 1975.
12. JANOSSY, G. and GREAVES, M.: *Clin. Exp. Immunol.*, **9**, 483, 1971.
13. NOWELL, P. G.: *Cancer Res.*, **20**, 462, 1960.
14. PANTELOURIS, E. M.: *Nature*, **217**, 370, 1968.
15. RAFF, M. C.: *Transpl. Rev.*, **6**, 52, 1971.
16. ROBINSON, P. J. and ROITT, I. M.: *Nature*, **250**, 517, 1974.
17. ROITT, I. M., GREAVES, M. F., TORRIGIANI, G., BROSTOFF, J. and PLAYFAIR, J. H. L.: *Lancet*, **2**, 367, 1969.
18. SCHIRRMACHER, V. and FESTENSTEIN, H.: *J. Immunogenetics*, **2**, 1975.
19. TURK, J.: Delayed Hypersensitivity. North Holland Research Monograph, 4, 1967.
20. VAN FURTH: Mononuclear Phagocitosis. Blackwell Scientific Publications, Oxford, 1970.