ELECTRON MICROSCOPY OF HUMAN LYMPHOCYTES STIMULATED BY PHYTOHAEMAGGLUTININ

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Mononuclear cells from the human peripheral blood undergo an intense proliferative reaction when they are cultured in the presence of the mitogen phytohaemagglutinin (10). This proliferation is accompanied by a marked change in the morphology of the cells. Observation by light microscopy shows that they increase in size, reaching diameters of up to 30 μ. The transformed cells are highly mobile when discrete, but tend to be quiescent when in contact with one another. Their nuclei are enlarged and contain one or more nucleoli; vesicles of varying size and density are scattered throughout their cytoplasm (5). This preliminary report describes the main morphological features of the new organization of human lymphocytes stimulated in this way, as revealed by electron microscopy.

MATERIALS AND METHODS

Cells were obtained either from the peripheral blood or from the thoracic duct lymph of patients undergoing thoracotomy. The cells were cultured in 20 ml screw-cap bottles using the method of Moorehead *et al.* (9). After 3 days' culture the supernatant was removed and cold 1 per cent osmium tetroxide (veronal-acetate buffer: pH 7.4) was poured gently onto the undisturbed cells. Two minutes later the cell layer was broken up and fixation was then continued for 1 to 1½ hours. Following dehydration in ethanol the cells were stained for 1 to 12 hours in methyl/butyl methacrylate (1:4) containing 0.5 per cent uranyl nitrate. They were then embedded in the same mixture, polymerization occurring at 58°C in 24 to 48 hours. Sections were cut with glass knives on a Cambridge (A. F. Huxley pattern) ultramicrotome and mounted on carbon-coated grids. They were examined in the Elmiskop I operating at a beam voltage of either 40 or 60 kv and equipped with either a 50 or 20 μ objective aperture.

RESULTS

The ultrastructure of freshly isolated lymphocytes from the peripheral blood was in all respects similar to that described by several authors and reviewed by Bessis and Thiéry (2). Freshly isolated lymphocytes from thoracic duct lymph are derived from a medium which, though isosmotic with blood, has a lower protein content (6). The appearance of these cells in the electron microscope was not affected by this difference in environment. The small lymphocytes had very sparse, finely granular cytoplasm containing few organelles. The latter consisted of a few large oval mitochondria, some vesicles of micropinocytotic origin, infrequent isolated ergastoplasmic lamellae and very occasionally, a "compound vacuole" (8). A few ribosomes were distributed throughout the cytoplasm. The Golgi apparatus, when present, was very poorly developed. This paucity of cytoplasmic organelles was only slightly less marked in large lymphocytes. The nuclei were commonly deeply notched, often in more than one plane. The ultrastructure of lymphocytes from control cultures did not differ from that of the freshly isolated cells (Fig. 1).

The electronmicrographs in Figs. 2 to 5 show the appearance of cells from 3-day cultures containing phytohaemagglutinin. There was a profound increase in the complexity of the cytoplasm of such cells. Mitochondria were usually more abundant, sometimes markedly so, as in Fig. 5, and were occasionally of elongated or bizarre form. The Golgi apparatus was invariably highly developed and one or both centrioles were often seen (Fig. 4). The most marked cytoplasmic change was the greatly increased ribosome content. The lamellae of the ergastoplasm were usually

more abundant than in the normal but most of the ribosomes were dispersed, singly or in "rosettes," throughout the cytoplasm. Micropinocytosis was clearly increased in these cells and resulted in the presence in their cytoplasm of large numbers of vesicles. Some of these were empty, some were of the "compound vacuole" type, others contained granules as yet unidentified. There was a decrease in the density of chromatin in the nuclei of the transformed cells; evaginations of the outer nuclear membrane, similar to those in Fig. 3, were sometimes seen. The nucleolus was commonly "bobbined" or diffuse in appearance.

An occasional feature of the cytoplasm of transformed lymphocytes was the presence of crystal-like structures (Fig. 5) having a periodicity of about 280 A. These structures, which so far have been seen only in cells derived from blood, do not appear to be surrounded by any membrane.

DISCUSSION

There is now considerable evidence to suggest that the small lymphocytes, diploid resting cells, are the principal cells to be stimulated when blood is cultured with phytohaemagglutinin (4). It is possible however that the large lymphocytes present, which are normally dividing cells, may contribute to this proliferative reaction. Thoracic duct lymph contains a more uniform population of mononuclear cells than peripheral blood, and the action of phytohaemagglutinin on these cells was to produce a type of transformation similar to that seen in blood cultures. The proportion of large lymphocytes in the initial cultures was small (1.5 to 2.5 per cent); yet 15 to 20 per cent of the cells were transformed after 3 days. This further suggests that small lymphocytes can be transformed by phytohaemagglutinin. The precise origin of the individual transformed cells examined was unknown but their appearance in the electron microscope was markedly different from that of either small or large lymphocytes. Nor did they closely resemble the earlier cells of the lymphocyte series as seen in the nodes or marrow, although they had many of the characteristics of immature cells. They were in many ways similar to the cells arising in the spleen in homograft reactions (3).

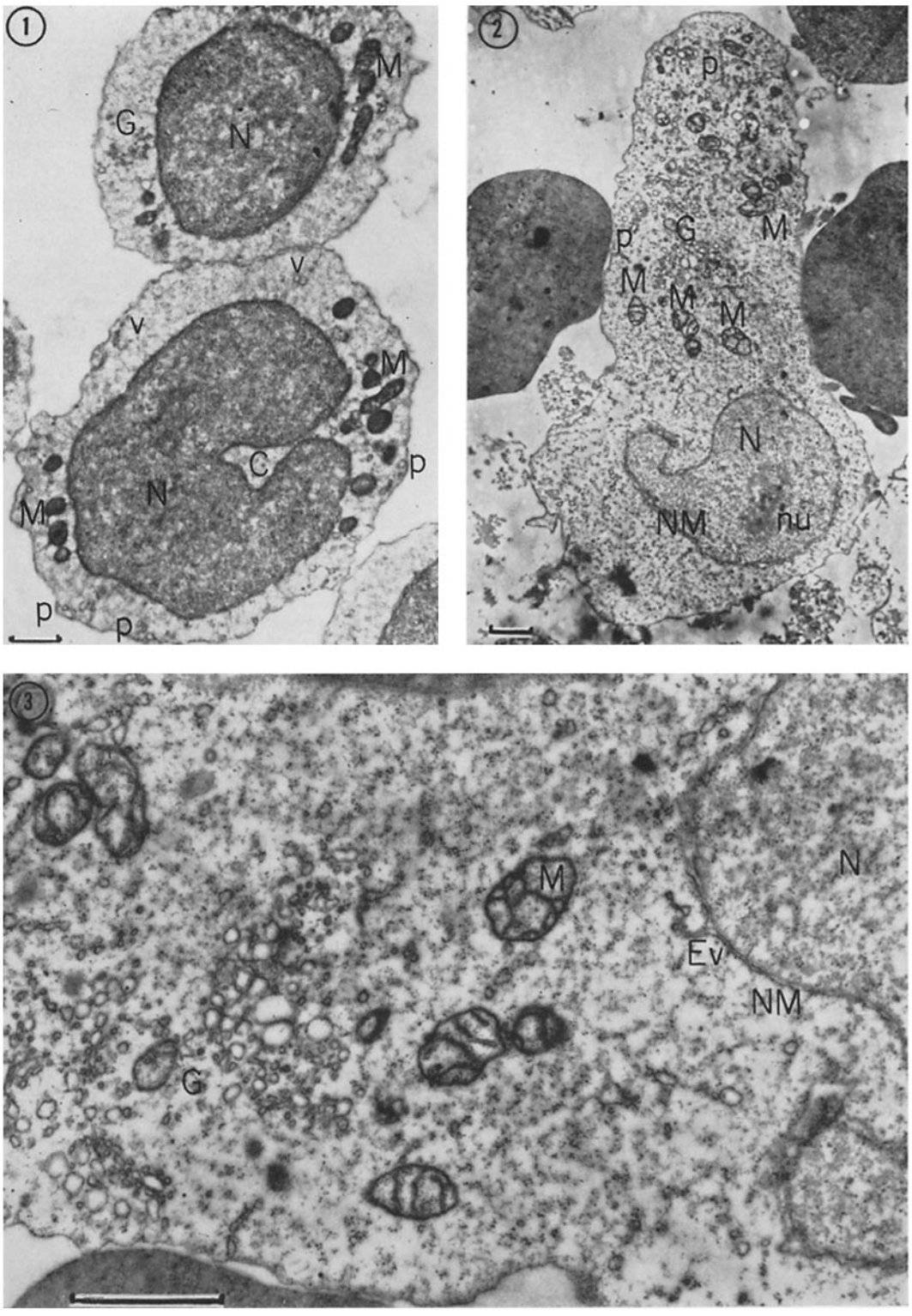
The greatly increased cytoplasmic content of ribosomes in the transformed cells is indicative of active protein synthesis, this may be associated with the growth of the cells and the synthesis of new organelles. The highly developed Golgi apparatus and the evidence of increased micropinocytosis are a reflection of the altered organization in the cytoplasm associated with cell division.

Protein crystals and "crystal-like structures" have been reported in the cytoplasm of leukaemic lymphoid cells (1, 7). Such structures, however, have not been observed in normal lymphocytes. The crystal-like structures we have seen in phyto-

FIGURE 1 Normal small lymphocytes from human thoracic duct lymph. This field includes two cells, and parts of two others from a 24-hour "control" culture, *i.e.* containing no phytohaemagglutinin. Their ultrastructure does not appear to differ from that of freshly isolated cells. One of the nuclei (*N*) shows a typical deep cleft (*C*). The mitochondria (*M*) are fairly large and few in number. Micropinocytosis is taking place (*p*) and the vesicles resulting from it can be seen (*v*). The poorly developed Golgi region (*G*) is present in the upper cell. × 6,500.

FIGURE 2 A transformed lymphocyte from a 3-day culture containing phytohaemagglutinin. The magnification is the same as in Fig. 1, for comparison. Two red cells, a red cell ghost, and some cell debris are also present. The elongated shape of this cell is indicative of its highly motile state at the moment of fixation. The decrease in density of chromatin in the nucleus (*N*) and the "bobbined" nucleolus (*nu*) are apparent. The nuclear double membrane (*NM*) is typically irregular. The cytoplasm contains numerous free ribosomes, somewhat bizarre mitochondria (*M*), an extensively developed Golgi apparatus (*G*), and numerous vesicles. There is evidence of very active micropinocytosis (*p*). × 6,500.

FIGURE 3 Part of the transformed lymphocyte of Fig. 2, at higher magnification. The Golgi apparatus (*G*) and part of the nucleus (*N*) are included. An evagination (*Ev*) of the abnormal nuclear membrane (*NM*) can be seen. Branching of the cristae occurs in the mitochondrion at *M*. × 22,000.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haemagglutinin-stimulated lymphocytes are of unidentified composition. Quaglino *et al.* (12) have reported the presence of "blocks of PAS-positive material" in the cytoplasm of such cells. It seems possible that these are identifiable with the "crystals" seen in the present investigation. If so, then they are possibly composed of glycoprotein.

These observations indicate the latent potential of the lymphocyte to change its structure and metabolism. This effect is so profound that the transformed cell acquires many of the ultrastructural characteristics of a cancer cell (11).

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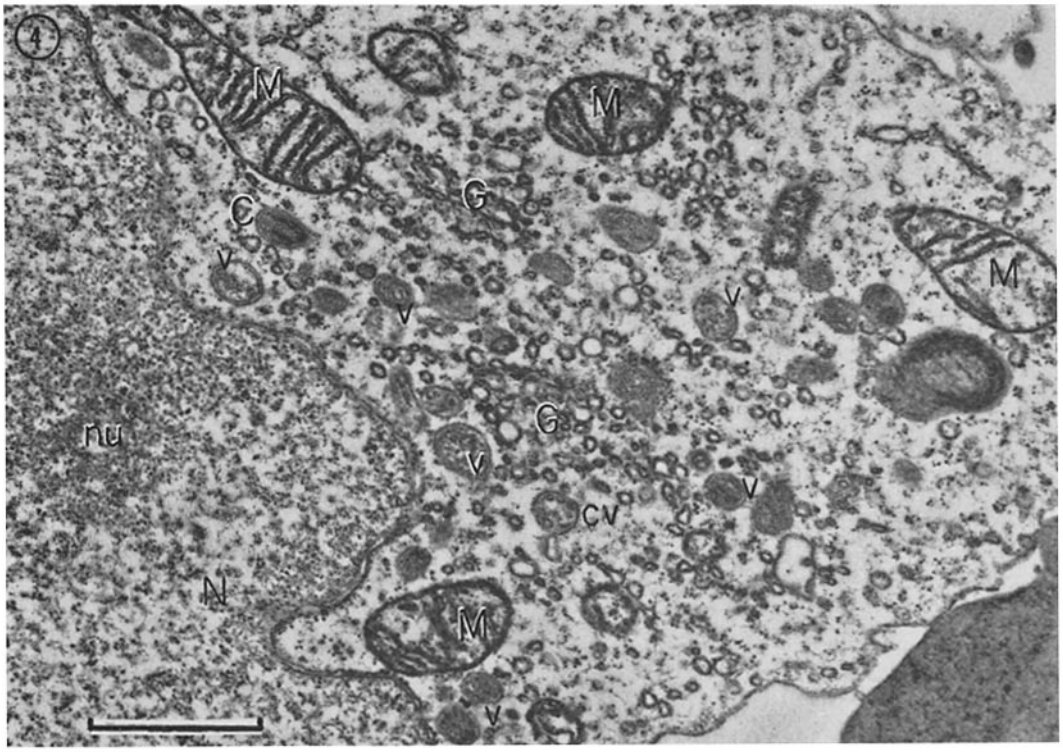
FIGURE 4 Part of a transformed lymphocyte from a 3-day culture containing phytohaemagglutinin. A red cell is also included (bottom right). Part of the nucleus (*N*) containing chromatin of low density and diffuse nucleolar material (*nu*) is shown. The cytoplasm is rich in ribosomes and in organelles. These include mitochondria (*M*), vesicles and tubules of the Golgi apparatus (*G*), a centriole (*C*), compound vacuoles (*CV*), micropinocytotic vesicles and occasional ergastoplasmic lamellae. Numerous vacuoles with double membranes are also present (*V*) which contain granules of unidentified composition. × 22,000.

FIGURE 5 A transformed lymphocyte from a 3-day culture containing phytohaemagglutinin. This is a cross-section of a cytoplasmic process (similar to that in the cell shown in Fig. 2) flanked by two red cells. Apart from the organelles described above, this electron micrograph also shows one of the crystal-like structures (*Cr*) described in the text. Numerous mitochondria are also present; in some of these the section is cut parallel to the cristae, which appear as flat plates (*cr*). × 17,500.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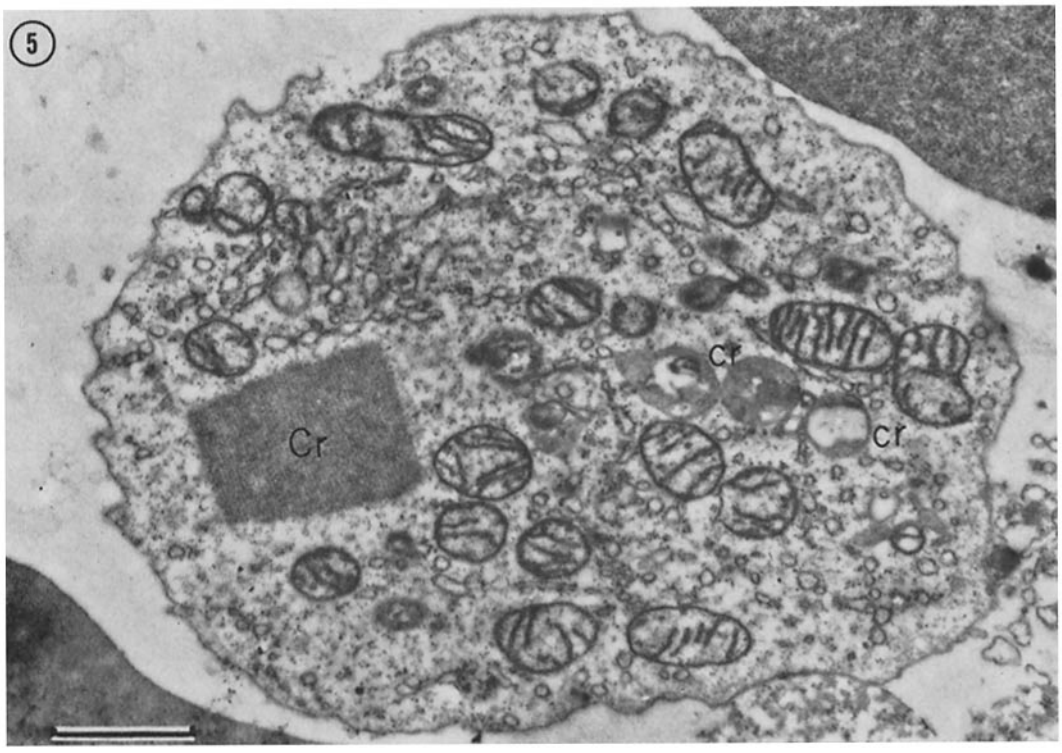
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