Fine Structure of Leukocytes in Infectious Mononucleosis: In Vivo and In Vitro Studies

By STEVEN D. DOUGLAS, H. HUGH FUDENBERG, PHILIP R. GLADE, LAWRENCE N. CHESSIN AND HAROLD L. MOSES

THE OCCURRENCE of heterophile antibodies and morphologic abnormalities in circulating mononuclear cells which produce immunoglobulins have focused the attention of immunologists and hematologists on the clinical syndrome of infectious mononucleosis. The unique morphologic features of the peripheral leukocytes found in this syndrome have been emphasized in numerous descriptions of observations from conventional light microscopy since the early reports of Türk¹ and Downey and McKinlay.² More recently, the capacity for in vitro proliferation of peripheral blood leukocytes from patients with infectious mononucleosis has been demonstrated.³ ⁴ Although the precise identification of the cell type which proliferates in these systems is unknown, the capacity of these cells to produce immunoglobulins, interferon, complement and to phagocytose particles has been reported.³ Moreover, karyotypic markers, herpes-like particles, and aggregates of small cytoplasmic particles have been associated with these cell lines.⁵

Fine structural study permits characterization of cells with respect to cytoarchitecture and the relative extent of organelle development. Accordingly, circulating mononuclear cells from patients with infectious mononucleosis, from established cell lines derived from these patients, cell lines from Burkitt lymphoma and leukemias, were also studied in view of the known capacity of these cells for immunoglobulin synthesis and their morphologic similarity to infectious mononucleosis cells by light microscopy. These observations were correlated with our previous fine structural studies of short term lymphocyte cultures stimulated with phytomitogens.⁷ ⁸ Recent functional studies of cells of the macrophage-lymphoid-plasma cell series make precise morphologic characterization of these cell types essential.

From the Laboratory of Experimental Pathology NIAMD, Laboratory of Clinical Investigation, NIAID, National Institutes of Health, Bethesda, Maryland and Department of Medicine, University of California Medical Center, San Francisco, California.

This work was supported in part by Training Grant HE-05677 from the National Heart Institute and Contract Nonr 3656 (12) from the Office of Naval Research.

First submitted November 26, 1968; accepted for publication February 26, 1969.

STEVEN D. DOUGLAS, M.D.: USPHS Post-Doctoral Trainee in Hematology, Section of Hematology and Immunology, Department of Medicine, University of California Medical Center, San Francisco, Calif. H. HUGH FUDENBERG, M.D.: Professor of Medicine and Director of the Section of Hematology and Immunology, Department of Medicine, University of California Medical Center, San Francisco, Calif. PHILIP R. GLADE, M.D.: USPHS Post-Doctoral Trainee in Genetics, Division of Medical Genetics, Department of Medicine, Mt. Sinai School of Medicine, New York, N. Y. LAWRENCE N. CHESSIN, M.D.: Assistant Professor of Medicine, Department of Medicine, University of Rochester School of Medicine, Rochester, N. Y. HAROLD L. MOSES, M.D.: Assistant Professor of Pathology, Department of Pathology, Vanderbilt University School of Medicine, Nashville, Tenn.
LEUKOCYTES IN INFECTIOUS MONONUCLEOSIS

MATERIALS AND METHODS

Clinical Material: Peripheral Blood

Buffy coats were obtained from heparinized peripheral blood specimens obtained from 10 healthy normal volunteers and 6 patients with heterophile-positive infectious mononucleosis.

Infectious Mononucleosis Cell Lines

Sixteen continuous suspension cultures were established from 66 specimens obtained from 23 patients with heterophile-positive infectious mononucleosis. The tissue culture technics employed have been described previously. Fourteen cultures were examined by electron microscopy.

Burkitt Lymphoma and Leukemia Cell Lines

The Burkitt lymphoma P3J, Raji, AL-i and EB-2 lines were kindly provided by Drs. J. Fahey and Y. Hirshaut and were cultivated as previously described. Cell lines derived from peripheral leukocytes of patients with acute leukemia (IM-1, LK1-D and SK-L-3) were handled in a similar manner.

Electron Microscopy

Buffy coat preparations and cell culture suspensions were centrifuged at 500 r.p.m. for 20–30 minutes at 20°C and then fixed in 2 per cent glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2–4 hours. The cells were rinsed in 7.5 per cent sucrose in 0.1 M phosphate buffer, post-fixed with phosphate-buffered 1 per cent osmium tetroxide, dehydrated with graded ethanol solutions, and propylene oxide, and embedded in araldite or epon. The specimens were cut on an LKB Ultrotome, stained with uranyl acetate and lead citrate, and examined in a Philips EM 200 electron microscope.

RESULTS

Normal Peripheral Blood

In human peripheral blood, mononuclear cells were predominantly of three types: small and medium sized lymphocytes (Fig. 1), monocytes (Fig. 2) and a small number of cells which contained relatively abundant rough-surfaced endoplasmic reticulum and will be referred to as lymphoid-plasma cells (LP cells) (Figs. 3 and 4). By light microscopy LP cells are indistinguishable from small lymphocytes. The monocyte could usually be distinguished from the lymphocyte by an increased number of lysosomes and by nuclear morphology. Monocyte nuclei were often lobulated or indented and had relatively more euchromatin, whereas lymphocytes had abundant dense heterochromatin which was often present in the central portion of the nucleus.

Infectious Mononucleosis

The circulating mononuclear cells found in infectious mononucleosis have been classified according to their light microscopic appearance and previous electron microscopic studies have been limited. In the present study the cell types were distinguished by qualitative and quantitative differences in organelle development.

The three types of mononuclear cells present in normal peripheral blood also occurred in peripheral blood of infectious mononucleosis patients and there was an increase in the number of lymphoid-plasma cells (Fig. 7).
Plate I: Peripheral Blood Mononuclear Cells from Healthy Individuals.

Fig. 1.—Small lymphocyte. The heterochromatic nucleus, nucleolus and small number of electron dense lysosome-like organelles are evident. × 9,750.

Fig. 2.—Peripheral blood monocyte. The nucleus is lobulated and relatively euchromatic. The Golgi zone contains several vesicles and lysosome-like structures. × 7,800.

Fig. 3.—Peripheral blood lymphoid-plasma cell (LP cell). Numerous strands of rough surfaced endoplasmic reticulum are seen. × 7,800.

Fig. 4.—Plasmacytoid-mononuclear cell. Note well-developed endoplasmic reticulum, prominent Golgi zone and lobulated nucleus. × 7,800.

Many of the mononuclear cells appeared as typical peripheral blood monocytes (Fig. 8). An even larger number of mononuclear cells present had an increase in cytoplasm as compared to small lymphocytes and contained an increased number of lysosomes and vesicular structures. These cells frequently had clumped heterochromatin at the periphery of the nucleus, prominent nucleoli
Plate II: Peripheral Blood Mononuclear Cells from Patients with Infectious Mononucleosis.

Fig. 5.—Lymphoid cell containing many cytoplasmic vesicles, frequent ribosomes, and numerous small strands of rough surfaced endoplasmic reticulum. \( \times \) 8,100.

Fig. 6.—Lymphoid cell containing numerous ribosomal aggregates (polysomes). \( \times \) 7,150.

Fig. 7.—Lymphoid-plasma cell (LP cell) with concentric rings of rough surfaced endoplasmic reticulum. \( \times \) 7,150.

Fig. 8.—Peripheral blood monocyte. Note many lysosome-like organelles and well-developed Golgi zone. \( \times \) 6,500.

and numerous ribosomal aggregates (polysomes) in the cytoplasm (Figs. 5 and 6). Some of these cells may correspond to the Downey Type I cells as observed with Romanowsky stains.\(^2\)
Plate III: Infectious Mononucleosis Cell Lines.

Fig. 9.—Low power electron micrograph of infectious mononucleosis cell line. Most of the cells seen have varying amounts of endoplasmic reticulum and variable nuclear chromatin patterns. \( \times 2,600 \).

Fig. 10.—Lymphocytoid cell with prominent nucleolus and several electron dense lipid inclusions. \( \times 7,150 \).

Fig. 11.—This cell shows many strands of rough surfaced endoplasmic reticulum. Other features resemble small lymphocyte. \( \times 7,800 \).

Infectious Mononucleosis Cell Lines

The cell population observed in vitro was comprised of a spectrum of cell types which may be subdivided into several distinct groups. The predominant cell type was a large lymphoblastoid cell, 10-20 \( \mu \) in diameter. These cells had
Plate IV: Infectious Mononucleosis Cell Lines.

Fig. 12.—Lymphoid cell containing endoplasmic reticulum with early cisternal dilatation and dense heterochromatic nucleus. × 7,800.

Fig. 13.—Representative culture cell with euchromatic nucleus and strands of endoplasmic reticulum. × 6,100.

Fig. 14.—Blast-like cell with large nucleoli and frequent ribosomal aggregates. × 4,200.

Fig. 15.—Another blast-like cell with bizarre nuclear configuration and prominent nucleolus. × 5,850.

Euchromatic nuclei and prominent nucleoli; free (unbound) polysomes were numerous, and Golgi zones were well developed (Figs. 9, 13–15). A much smaller number of cells had many features characteristic of small lymphocytes (Fig. 10). In addition other cells, which exhibited more extensive development of rough surfaced endoplasmic reticulum often resembled the lymphoid-
Plate V: Burkitt Lymphoma Line. P.3J.

Fig. 16.—Low power micrograph showing multinucleate cells and electron dense lipid bodies. × 1,600.

Fig. 17.—Note extent of development of endoplasmic reticulum, lipid bodies and euchromatic nuclei. × 2,600.

Fig. 18.—Blast-like cell with large nucleolus, ribosomal aggregates and strands of endoplasmic reticulum. × 4,550.

plasma cells observed in the peripheral blood (Figs. 11 and 12). Infrequent cells had abundant dilated rough surfaced endoplasmic reticulum cisternae and more closely resembled plasma cells. Reticular aggregates of 22 mμ diameter particles and tubules present in association with the rough surfaced endoplasmic reticulum were frequently observed in all lines.6 Many of the cells
LEUKOCYTES IN INFECTIOUS MONONUCLEOSIS

contained frequent lysosome-like organelles, lipid bodies, and myelin figures; features which occur in many established lymphoid cell lines.8

Burkitt Lymphoma and Leukemic Cell Lines

The cell population of the Burkitt lymphoma derived cell lines was comprised of cell types similar to those observed in the cultures derived from the peripheral blood of infectious mononucleosis patients. The relative extent of organelle development in these cultured cells resembled that for the infectious mononucleosis cells and the same spectrum of cell types was present (Figs. 16–18). Occasional multinucleate cells were observed (Fig. 16). Examination of 3 cell lines (IM1, LK-1D, and SK-L-3) derived from patients with acute leukemia showed more nuclear pleomorphism, however, the same spectrum of cell types was present.

DISCUSSION

Recent investigations have demonstrated that the human circulating small lymphocyte population has a multifold potential of response to a diversity of stimuli both in vivo and in vitro. In vivo the population of circulating cells is morphologically and functionally altered during viral and bacterial infections,12 hypersensitivity phenomena22 and dysproteinemic states.19 In vitro the cells are capable of response to phytomitogens, antigens, and allogeneic lymphocytes. Following stimulation the resultant population has been shown to differ morphologically at the fine structural level in relation to the type of mitogenic stimulus.7 Moreover, some lymphoid cells have the capacity for long-term in vitro proliferation.

Morphologically, the population of small circulating lymphocytes shows a gradation in development of Golgi zones, rough surfaced endoplasmic reticulum, polysome aggregation, and lysosomes.13,14 Functional differences exist in the capacity of these cells to synthesize immunoglobulins.15 Under some circumstances the small lymphocyte is able to phagocytose organisms such as mycoplasma16 despite the fact that the number of lysosomes present in these cells are few and these organelles have been considered to be an index of heterophagic potentiality.

There are striking fine structural similarities between the population of cells which occur in short-term lymphocyte cultures from normal individuals following stimulation with phytohemagglutinin or pokeweed mitogen and the long-term established suspension cultures from patients with infectious mononucleosis, Burkitt's lymphoma, or leukemia. The similar fine structural features of cell lines derived from patients with a variety of hematologic disorders and from normal individuals maintained under conditions which differ from those employed in the present study have recently been reported.21 Some of the large blast-like cells frequently have significant development of rough surfaced endoplasmic reticulum. In this respect these cells are similar to cells observed following infection of monkey kidney culture with rubella virus17 and chick fibroblasts infected with defective Rous sarcoma virus.18

Fine structural studies of circulating peripheral blood mononuclear cells and cells cultivated in vitro derived from the circulating mononuclear cell
population reveal that a spectrum of cells is present in all cultures thus far examined. Cultures derived from peripheral blood of infectious mononucleosis patients and Burkitt lymphoma are lymphoid in morphologic features. In general these cells have fewer lysosomes than cells of the monocyte-macrophage complex. In addition, we have demonstrated that these cells have functional properties of lymphoid cells in that they lack a recognition system for IgG coated erythrocytes, a property restricted to macrophages and monocytes. A small percentage of the cells remain small lymphocytes. Others have significant development of rough surfaced endoplasmic reticulum and resemble a portion of the cells observed in cultures stimulated with pokeweed mitogen, the lymphoid-plasma cells reported in dysproteinemias, and hypergammaglobulinemic states, and a proportion of normal thoracic duct cells. A small number of normal circulating mononuclear cells and a larger number of circulating cells in patients with infectious mononucleosis are lymphoid-plasma cells (LP cells). The relationship of these cells to the plasmablast and to the mature plasma cell and their functional capabilities is not known. The morphologic response of the small lymphocyte to mitogens, possible viral infection, and to neoplasia appears to be similar. However, the relationship of the morphologic expression of host response in vivo and in vitro to the associated biochemical and immunologic events remains to be elucidated. Studies on this relationship are currently in progress.

**Summary**

The peripheral blood mononuclear cell population in patients with heterophile positive infectious mononucleosis is comprised of small and medium sized lymphocytes, monocytes, increased numbers of lymphoid-plasma (LP) cells, and large lymphocytoid cells with abundant polysomes. Cell-lines derived from these patients contain a spectrum of cells of the lymphoid plasma cell series. These cultures are morphologically indistinguishable from Burkitt lymphoma and leukemic suspension cultures. A large portion of the cells present in these continuous suspension cultures have fine structural features similar to phytomitogen stimulated lymphocytes, and to cells implicated in the immune response.

**SUMMARIO IN INTERLINGUA**

Le population del cellulas mononucleari in sanguine peripheric ab patientes con heterophilic positive mononucleosis infectiose consiste de micre e intermediemente dimensionate lymphocytos e monocytos, de augmentate numeros de cellulas lymphoido-plasmatic, e de grande cellulas lymphocytoid de, con un abundantia de polysomas. Lineas cellular derivate ab tal patientes contine un spectro de cellulas del serie a plasma lymphoide. Iste culturas es morphologicamente nondistinguiuible ab culturas suspensionate de lymphoma de Burkitt e de leucemia. Un grande portion del cellulas presente in iste continue culturas suspensionate ha caracteristicas microstructural simile a lymphocytos stimulate per un phytomitogeno e a cellulas intricate in un responsa immunologic.

**REFERENCES**


LEUKOCYTES IN INFECTIOUS MONONUCLEOSIS


Fine Structure of Leukocytes in Infectious Mononucleosis: In Vivo and In Vitro Studies

STEVEN D. DOUGLAS, H. HUGH FUDENBERG, PHILIP R. GLADE, LAWRENCE N. CHESSIN and HAROLD L. MOSES