The "Infectious Mononucleosis Cell"
A Cytochemical Study

By Peter Galbraith, W. J. Mitus, Mohan Gollerkeri and William Dameshek

The nature of the abnormal cells appearing in the peripheral blood in infectious mononucleosis has been the subject of controversy. It is now generally accepted that they belong to the lymphocytic series although an occasional worker holds out for their monocytic origin. Recently, Paegle, using the electron microscope, found that most of the cells in the blood of patients with infectious mononucleosis contained organelles similar to those of the cells of the lymphocytic series and did not have an ergastoplasm. He was unable to distinguish between the three cell types described by Downey.

It is the purpose of this study to characterize the infectious mononucleosis (IM) cells cytochemically in an attempt to throw further light on their origin, nature and function. The study was restricted largely to the atypical lymphocytes as they appeared in the peripheral circulation, although imprint preparations of biopsied lymph nodes were investigated in two cases.

Materials and Methods

Blood was obtained from seven patients with documented infectious mononucleosis. All patients gave a positive heterophil antibody test. Blood smears: studies were performed using phase microscopy; supravital staining; periodic acid Schiff reaction, with and without diastase digestion; methyl-green pyronine; sudan black; dithizone; reactive sulphydryl groups (in smears fixed in 10 per cent neutral formalin); lactic acid dehydrogenase; D. P. N. diaphorase (in smears fixed in formal-calcium); alkaline phosphatase; acid phosphatase; non-specific esterase; phosphorylase; acidine orange fluorescence; and Nadi oxidase. In four cases, 5 ml. of blood was incubated with 1 μc. of H3-thymidine and H3-cytidine, separately, and after 20 minutes, slides were made from theuffy coat. These were subjected to autoradiography using the method described by Jofest.

Fluorescent antibody studies were done on the blood smears of six of the seven patients using the method described by Coons, in which fluoresceine labeled antihuman gamma globulin was used.

In two patients, aspiration and biopsy of a lymph node was performed. The imprints were examined with the use of the following stains: Wright-Giemsa; periodic acid Schiff reaction, with and without diastase digestion; methyl-green pyronine; Sudan black; acid phosphatase; alkaline phosphatase; phosphorylase; acidine orange fluorescence; and Nadi oxidase. Routine histologic studies were also performed. In one case, lymph node imprints and histologic sections were examined using the Coons' fluorescent technic.

Results

The results are presented in table 1.

In fresh preparations at room temperature, the IM cells studied under...
THE "INFECTIOUS MONONUCLEOSIS CELL"

Table 1.—Cytochemistry of the Infectious Mononucleosis Cells

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</thead>
<tbody>
<tr>
<td>P.A.S.</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>P.A.S. diastase</td>
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<td>-</td>
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<td>-</td>
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<tr>
<td>Methyl green pyronine</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Acridine orange</td>
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<tr>
<td>I.D.H., D.P.N.H.- diaphorase</td>
<td>++</td>
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<td>+++</td>
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<td>+++</td>
<td>++</td>
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<tr>
<td>Sudan black</td>
<td>±</td>
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<td>±</td>
<td>±</td>
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<td>±</td>
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<tr>
<td>Phosphorylase</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<td>+</td>
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<td>Acid phosphatase</td>
<td>+++</td>
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<tr>
<td>Non-specific esterase</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Reactive-SH groups</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>Alkaline phosphatase</td>
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<tr>
<td>Peroxidase</td>
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<tr>
<td>&quot;Nadi&quot; oxidase</td>
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<tr>
<td>Dithizone</td>
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<tr>
<td>Fluorescent antibody</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Uptake of H³-thymidine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H³-cytidine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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Phase microscope were observed to be stationary, but to effect minor changes in the shape of their nuclei and cytoplasm, occasionally projecting languid pseudopodia (figs. 1 and 2). Six to eight elongated and fat mitochondria were usually found. The outstanding cytochemical findings were the presence of numerous glycogen granules in the cytoplasm (fig. 3), weak pyroninophilia (fig. 4), weak acridine orange fluorescence at the periphery of the cell (fig. 7), and a moderate to strong reaction for acid phosphatase (fig. 5). The cytochemical studies, which were helpful in the differentiation between IM cells, lymphocytes, and monocytes are summarized in table 2. We were unable to distinguish cytochemically the three abnormal cell types described by Downey. Autoradiographic studies demonstrated that H³-thymidine was incorporated in vitro by the atypical cells, which though few in number, were heavily labeled (fig. 6).* H³-cytidine, on the other hand, labeled the cells less heavily, although many of the normal appearing lymphocytes shared cytidine incorporation. No labeling of the IM cells with the fluorescent antihemoglobin took place in the peripheral blood.

Histologic examination of the lymph nodes showed marked follicular and reticulum cell hyperplasia. In some areas, the architecture of the lymph node was obliterated and was strongly suggestive of neoplastic proliferation.

Lymph node aspirations and imprints in two cases showed the following cells types: (1) small and large lymphocytes; (2) primitive cells, 20-30 μ in diameter with large nuclei containing fine chromatin network and occasional nucleoli (figs. 8 and 9). The cytoplasm was moderately abundant and stained pale blue with Wright-Giemsa. These cells constituted approximately 15-20 per cent of all the lymph node cells; (3) large cells, 30-50 μ in diameter, moderately large nuclei with fine chromatin and distinct nucleoli. Cytoplasm was abundant and stained intensely blue with Wright-Giemsa. These

*With Wright-Giemsa counterstain, in spite of some distortion, it was possible to discriminate between "atypical lymphocytes" and normal appearing lymphocytes.
cells, which had the appearance of "immunoblasts," as described by Dameshek,\textsuperscript{37} constituted 5 per cent of the cells counted; (4) rare plasma cells and reticulum cells were seen. Histochemical and immunofluorescent studies of the two main cell types are summarized in table 3 (figs. 10 and 11). Mitotic figures were common.

**Discussion**

The majority of the cytochemical tests indicated that the basic cellular constituents and the enzymatic functions common to most living cells, and in particular the lymphocytes and monocytes, were present in the IM cells. The high glycogen content of these cells, as manifested by the presence of diastase digestible granules, has been noted by us before\textsuperscript{5,26} and was confirmed in the present study. Together with the observed increased in phosphorylase, this may be taken to indicate an altered glycogen metabolism. The presence of glycogen in a granular form is similar to that found in the neoplastic cells which occur in chronic lymphocytic leukemia or lymphosarcoma. Monocytes differ in that glycogen staining is diffusely positive throughout the cytoplasm.

The strongly positive reaction for acid phosphatase was a surprising finding. Lymphocytes, both normal and neoplastic, are usually negative or at most contain a few granules, although we have observed occasional "lymphosarcoma cells" which gave a strongly positive reaction. Monocytes and reticulum cells are moderately positive. It is possible, but not proved, that the increase of this enzyme is linked with the degree of motility of the cells observed in fresh preparation at room temperature over the period of 1–2 hours. IM cells showed some motility under phase microscopy, and this was distinctly greater than that of normal lymphocytes. In this connection, it is interesting that the motile and phagocytic cells in the peripheral blood (neutrophilic polymorphonuclears and monocytes) are acid phosphatase positive.
Fig. 3.—IM cell in the peripheral blood. PAS stain; numerous cytoplasmic granules are present. X 1100

Fig. 4.—IM cell and neutrophil in the peripheral blood, methyl green pyronine. Cytoplasmic pyroninophilia of the IM cell is weak to moderate. X 1100

Fig. 5.—IM cell in the peripheral blood, acid phosphatase stain. X 1100

Fig. 6.—IM cell in the peripheral blood showing numerous granules overlying the nucleus and thus indicating H\(^3\) thymidine incorporation. X 1100

Fig. 7.—IM cell in the peripheral blood showing slight fluorescence limited to the edge of the cytoplasm. Acridine orange stain. X 1100
Fig. 8.—Lymph node aspirate from a case of infectious mononucleosis. The cells appear primitive, but the cytoplasmic basophilia is not marked. Wright-Giemsa stain.

Fig. 9.—Lymph node imprint from a case of infectious mononucleosis. Three of the large, primitive cells have light-staining cytoplasm while one stains darker. Wright-Giemsa stain.

Fig. 10.—Lymph node imprint from a case of infectious mononucleosis. Most of the large, primitive cells are weakly pyroninophilic, but two cells at the periphery of the group are markedly pyroninophilic.

Fig. 11.—Lymph node imprint from a case of infectious mononucleosis. Acid phosphatase stain.

Studies of the comparative cytochemistry of the IM cell (table 2) showed similarities to cells of both the lymphocytic and monocytic series. In 1937, Gall suggested that lymphocytes might transform into monocytes and Rebbuck's more recent studies seem to confirm this view. Downey and Stasney described transitions from reticuloendothelial cells to lymphocytes and Moeschlin considered IM cells as lymphatic monoblasts and monocytes. The cytochemical changes indicating similarities to monocytes further suggested this possible relationship. It is also of interest that in the bone marrow and in the lymph nodes of infectious mononucleosis, both lymphoid and reticulum cell hyperplasia are frequently encountered.

Our autoradiographic studies, as shown by the in vitro incorporation of H\(^3\)-thymidine, indicated an active proliferation of the IM cells. The elongated and swollen mitochondria probably indicated increased functional activity rather than retrograde degenerative changes, although the latter possibility could not be excluded.

No indications of the production of antibody by the IM cells of the blood
Table 2.—Comparative Cytochemistry of Infectious Mononucleosis Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Lymphocytes</th>
<th>I.M. Cells</th>
<th>Monocytes</th>
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<tbody>
<tr>
<td>P.A.S.</td>
<td>+++</td>
<td>+++-</td>
<td>+</td>
</tr>
<tr>
<td>granules</td>
<td>granules</td>
<td>diffuse</td>
<td></td>
</tr>
<tr>
<td>P.A.S. diastase</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methyl green pyronine</td>
<td>pyroninophilia</td>
<td>±</td>
<td>pyroninophilia</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>negative</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>(rare granules)</td>
<td>granules</td>
<td>granules</td>
<td></td>
</tr>
<tr>
<td>Sudan black</td>
<td>-</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Non-specific esterase</td>
<td>negative</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>(rare granules)</td>
<td>(fine granules)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive-SH groups</td>
<td>++</td>
<td>+++</td>
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were found. Weak basophilia with Wright-Giemsa stain, faint pyroninophilia and negative cytoplasmic fluorescence with acridine orange, all indicated low levels of RNA, a substance essential for protein synthesis and present in large quantities in protein producing cells (e.g., plasma cells and pancreatic exocrine cells). IM cells showed only slight incorporation of H3-cytidine in their cytoplasms. Fluorescent antibody studies were negative, and under the electron microscope these cells lacked a lamellar ergastoplasm. All these findings fail to support the concept that the IM cell of the blood produces a humoral antibody. On the other hand, relatively few large cells (hemocyto- blasts, immunoblasts) found in sectioned material and in the imprints of lymph nodes showed distinct pyroninophilia, a positive acridine orange fluorescence and positivity with Coon’s fluorescent technic (table 3).

IM cells appear to differ from the lymphoid cells found in various other immunologic phenomena. For example, the “immunologically competent cells” described by Czitober et al. which appeared in the peripheral blood of rabbits following injection of typhoid vaccine were strongly basophilic, strongly pyroninophilic and demonstrated strong acridine orange fluorescence. These cells did not stain for acid phosphatase and contained few glycogen granules. In addition, they fluoresced brightly when examined with the fluorescent antibody technic of Coons. Earlier, Braunsteiner et al. had shown that these cells possessed an ergastoplasm. André et al. have made extensive studies of the cells involved in the homograft reaction. These cells had cytochemical features similar to the immunocompetent cells described by Czitober et al., but were morphologically more primitive. Recent electron microscopic studies have shown that these cells are very rich in free ribosomes, but contain no rough endoplasmic reticulum (ergastoplasm).

It can be said that the IM cell of the blood differs distinctly from the immunocytes and from the large cells (immunoblasts) involved in the homograft reaction. They appear to have no role in the production of humoral antibody, and it is not possible as yet to ascribe to them a definite function. They have many similarities to malignant lymphoid cells; thus, they might represent a neoplastic type of proliferative response to a viral agent.

Our preliminary studies of lymph nodes showed the presence of two dis-
tinct types of abnormal cells (table 3). The more common was a primitive cell with faintly basophilic cytoplasm. These cells seemed very similar, both morphologically and histochemically, to the IM cells of the peripheral blood; in addition, they showed no immunologic activity. On the other hand, the larger, strongly basophilic cells gave a strong pyronin and acridine orange reaction as well as positive immunofluorescence. These cells could possibly represent an immunologic response to the presumed virus and might indeed, be responsible for the production of abnormal proteins, including the heterophil antibody found in the disease.

**Summary**

The abnormal cells in the peripheral blood of patients with infectious mononucleosis were studied by available histochemical technics and with immunofluorescent and autoradiographic technics. The findings indicated a close relationship of these cells to lymphocytes, although the strong acid phosphatase reaction observed in the infectious mononucleosis (IM) cells was not usually seen in normal lymphocytes, but rather in monocytes and reticulum cells. There was no evidence of antibody production by the IM cells of the peripheral blood.

Studies of the lymph nodes showed two abnormal cell types: one, similar to the IM cells in the peripheral blood but more immature, and the second, a primitive cell with various indications of immunologic competence.

**Summario in Interlingua**

Le cellulas anormal in le sanguine peripheric de patientes con mononucleosis infectiose esseva studiate per medio del existente technicas histochemic e con le adjuta de technicas immunofluorescente e autoradiographic. Le constatationes indicava un intime relation de iste cellulas con le lymphocytes, ben que le forte reaction de phosphatase acide observate in le cellulas de mononucleosis infectiose non esseva observate usualmente in lymphocytes normal sed plus tosto in monocytes e cellulas de reticulo. Esseva trovate nulle evidentia pro le production de anticorpore per cellulas de mononucleosis infectiose in le sanguine peripheric.
THE "INFECTIOUS MONONUCLEOSIS CELL"

Studios de nodo lymphatic monstrava duo typos de cellulas anormal. Le un esseva simile al cellulas de mononucleosis infectiose in le sanguine peripheric sed plus immatur. Le secunde esseva un typo de cellula primitive con varie indicationes de competentia immunologic.

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