The Mitotic Activity of Circulating Atypical Mononuclear Cells in Infectious Mononucleosis

By R. L. Carter

CELLS IN MITOSIS have occasionally been described in routine blood smears in infectious mononucleosis,1,4 their size and cytoplasmic features being characteristic of the large atypical mononuclear cells which occur in this condition. More recently, some information concerning the premitotic activity of these cells has come from a few autoradiographic studies5,8 in which a surprisingly high proportion have been found to take up tritium-labeled thymidine, indicating impending DNA synthesis.

Observations of this kind are particularly striking in a benign lymphoproliferative process such as infectious mononucleosis, and they prompted a more extensive study of the mitotic and premitotic activity of the circulating mononuclear cells in this condition. Three main topics have been investigated. The incidence of mitoses has been determined both in peripheral blood films and in white cell concentrates; the latter were also used to assess the uptake of \( ^{3}H \)-thymidine by means of autoradiography, and for chromosome analyses. All patients showed clinical, hematologic and serologic evidence of infectious mononucleosis. Full case histories were taken in all cases so that the experimental findings could be broadly correlated with the phase of the disease.

METHODS

Blood smears were stained by the May-Grunwald-Giemsa technic. White cell concentrates were prepared from 10–15 ml. of blood by differential sedimentation in an EDTA-dextran mixture (1 ml. of 1 per cent EDTA in 5 per cent dextran/10 ml. blood) incubated at 37 C. After 45 minutes, the leukocyte-rich supernatant plasma was separated and spun at 200 r.p.m. for 10 minutes, and films were prepared from the deposit. Conventional autoradiographic technics were employed,6,9 the concentrates being incubated with \( ^{3}H \)-thymidine (0.2 μc./ml., specific activity 2.5 c./mM) for 1 hr. without a preliminary period of in vitro culture. Kodak autoradiographic stripping plates A.R. 10 were used, and were developed after 7 days exposure. The preparations were stained with May-Grunwald-Giemsa and a differential count made on 3000 cells. Chromosome preparations were made according to the standard method of Moorehead et al.10

RESULTS

Dividing Cells in Blood Films

Two hundred and eleven peripheral blood films from 150 patients were studied. Dividing and binucleate cells were seen in five (2.4 per cent), all of them occurring during the second and third weeks of the disease. In three, the...
Table 1.—Distribution of Positive and Negative Concentrates in Relation to the Phase of the Disease

<table>
<thead>
<tr>
<th>Week</th>
<th>No. of Concentrates</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>36</td>
<td>23</td>
<td>13</td>
</tr>
</tbody>
</table>

(63.9%) (36.1%)

Table 2.—Numbers of Dividing Cells in Positive Concentrates

<table>
<thead>
<tr>
<th>No. of Dividing Cells</th>
<th>No. of Concentrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>12</td>
</tr>
<tr>
<td>6-10</td>
<td>8</td>
</tr>
<tr>
<td>11-15</td>
<td>2</td>
</tr>
<tr>
<td>&gt;15</td>
<td>1</td>
</tr>
</tbody>
</table>

Dividing Cells in White Cell Concentrates

Thirty-six concentrates were prepared from 35 patients and were examined for the presence of dividing forms, those in which dividing cells were seen being described as positive. The distribution of positive and negative concentrates in relation to the phase of the disease is shown in table 1.

The numbers of dividing cells seen were always small (table 2). Predictably, more dividing cells were seen in patients with raised leucocyte counts, but they were still quite frequently found when leucocyte counts were within normal limits (table 3). Positive concentrates contained cells at all stages of division, although cells in anaphase and telophase were infrequently seen. Representative forms are illustrated in figure 1. No dividing cells were seen in control concentrates from six healthy subjects. All of them, however, contained a few large atypical mononuclear cells, morphologically indistinguishable from those seen in infectious mononucleosis.

Incubation with Colchicine

In six cases, some of the white cell concentrates were incubated for 2 hours at 37 C. with Colcemid, using 0.1 ml. Colcemid per 1.0 ml. of concentrate. Mitoses were subsequently found in all of them, including two in which they were not seen in the nonincubated concentrates. Four concentrates prepared from normal subjects were treated in the same way, and dividing cells were subsequently demonstrated in two of them.

Autoradiography with $^3$H-Thymidine

Autoradiographs were prepared in twelve patients. The relevant findings are summarized in table 4. The uptake of tritiated thymidine was increased during the first four weeks of the disease, with peak activity in the second and third
weeks. The percentage uptake in the single autoradiograph prepared during the fifth week was within the accepted normal limits of 0.02–0.06 per cent labeling. The label was confined to the nuclei of the large atypical mononuclear cells, examples of which are illustrated in figure 2. Labeling was usually heavy, obscuring fine nuclear structure; cytoplasmic basophilia was sometimes marked. Grain counts in individual cells were not made.

Chromosome Studies (Made in Collaboration with Dr. Charles Kerr)

Chromosome preparations were made from six patients during the first three weeks of the disease. Visual karyotype analyses were performed on 30–50 cells in each case, but no qualitative or quantitative abnormalities were seen. In particular, chromosome breaks were not increased in number, being seen in <2 per cent of cells studied, a finding which is within normal limits.

DISCUSSION

During the acute phase of infectious mononucleosis, there is considerable mitotic and premitotic activity in some of the circulating atypical mononuclear

![Fig. 1.—Infectious mononucleosis. Atypical mononuclear cells in mitosis. White cell concentrates. May-Grünwald-Giesma. × 1300.](http://www.bloodjournal.org)
Table 4.—Autoradiographic Data

<table>
<thead>
<tr>
<th>Week</th>
<th>Case</th>
<th>Total W.B.C.</th>
<th>No. of Labeled Cells/3000</th>
<th>Per Cent Labeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>V. C.</td>
<td>13,600</td>
<td>96</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>S. P.</td>
<td>10,000</td>
<td>63</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>G. A.</td>
<td>21,100</td>
<td>162</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>A. K.</td>
<td>16,500</td>
<td>129</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>J. L.</td>
<td>8,800</td>
<td>90</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>R. E.</td>
<td>12,000</td>
<td>81</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>C. W.</td>
<td>10,000</td>
<td>71</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>M. N.</td>
<td>8,400</td>
<td>24</td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td>D. J.</td>
<td>10,000</td>
<td>120</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>K. S.</td>
<td>8,100</td>
<td>33</td>
<td>1.1</td>
</tr>
<tr>
<td>4</td>
<td>D. W.</td>
<td>6,600</td>
<td>27</td>
<td>0.9</td>
</tr>
<tr>
<td>5</td>
<td>J. C.</td>
<td>6,000</td>
<td>2</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Cells. Dividing forms are occasionally seen in routine blood films at this time, and can be demonstrated quite frequently in white cell concentrates, even in the absence of colchicine. Cells are most commonly seen in the early stages of mitosis, with only a few in telophase and anaphase. This may reflect a number of causes. Atypical mononuclear cells are easily damaged by exposure to anticoagulants and by centrifugation; even using short periods for separation and gentle centrifugation, damaged cells were still frequently found, some in mitosis. Secondly, it is probable that telophase and anaphase are normally of short duration. The present findings differ from those of Pearmain and Lycette, who saw no mitoses unless the concentrates were first incubated in the presence of colchicine, but only four patients were investigated and the stage of the disease was not recorded.

In the absence of preliminary morphological observations, interpretation of the autoradiographs can be difficult. Thus, in the first account of 3H-thymidine uptake by atypical mononuclear cells in infectious mononucleosis, it was suggested that the labeling might indicate impending viral, rather than cellular, synthesis of DNA. No virus particles have been identified in electron micrographs of the atypical cells, and work by Hale and Cooper, involving consecutive estimations of thymidine uptake and DNA content of individual cells, has clearly demonstrated that it is synthesis of cellular DNA which is taking place. The proportion of labeled cells found in the present study agrees with the figures from two other, smaller, series: Gavosto et al. recorded 3.8–5.2 per cent labeling in three cases, and 2.8–6.5 per cent labeling was found in five patients by Bertino et al. Bond et al. mention one remarkable case with 16.2 per cent labeling (810 cells out of 5000).

Apart from one serial study of three patients in which maximum labeling was noted "during the acute phase of the disease," little attention has been paid to the relationship between the numbers of labeled cells and the phase of the illness. In the present investigation, the largest numbers were found during the second and third weeks, at a time when the hematologic features of the disease are commonly most marked.
Fig. 2.—Infectious mononucleosis. Atypical mononuclear cells labeled with \(^{3}\text{H}\)-thymidine. \( \times 1300 \).

In all cases, labeling was confined to the nuclei of the large atypical mononuclear cells. Apart from a (variable) increase in cytoplasmic basophilia, the labeled cells showed no other morphologic features of obvious immaturity, although fine nuclear structure was often obscured in heavily labeled cells. Accurate identification of cell types in infectious mononucleosis is always difficult because the mononuclear cells are extremely pleomorphic; nevertheless, varying numbers of cells, indistinguishable from the labeled forms illustrated in figure 2, may be regularly found in ordinary blood films in this condition.

The present work deals solely with those atypical mononuclear cells which are circulating in the peripheral blood. Since many, probably the majority, lie in the tissues outside the vascular compartment, the overall mitotic and premitotic activity in the mononuclear cells is likely to be extremely high. But the consistently normal karyotypes suggest that this proliferative process, although intense, is not fundamentally deranged (c.f. the acute leukemias). It is assumed that the labeled cells divide and give rise to other mononuclear forms, since there is no current evidence for or against the view that some of the mononuclear cells seen in infectious mononucleosis may be totipotent.

Lastly, it is emphasized that similar large mononuclear cells, capable of taking up tritiated thymidine, are also described in autoradiographs made from patients with various acute infections and from normal subjects. In the latter, they are present in only small numbers so that they are difficult to find in ordinary blood films. They are, however, a constant feature of normal white cell concentrates and were present in all control concentrates in the
present study. None were seen in mitosis (in the absence of colchicine), but occasional dividing forms have been described in at least one large series of normal concentrates. The identity of these cells is unsettled. They are almost certainly not a homogeneous group, but the different claims for regarding them as young monocytes, large lymphocytes, prolymphocytes, plasmocytes and proplasmocytes (Türk cells) cannot be settled at the present time. Whatever their true nature, there is some experimental evidence that they may be totipotent and constitute "a mobile pool of primitive progenitor cells." In the light of such observations, it is clear that the whole status of the large atypical mononuclear cells seen in infectious mononucleosis needs to be reconsidered.

**Summary**

An investigation has been made into the mitotic and premitotic activity of the circulating atypical mononuclear cells which occur in infectious mononucleosis. Dividing and binucleate cells were found in 2.4 per cent of blood films and in 63.9 per cent of white cell concentrates. The number of cells in mitosis was small and they were most commonly encountered during the first three weeks of the disease; cells in prophase and metaphase predominated. The incidence of dividing cells in white cell concentrates was increased by a preliminary period of incubation with colchicine. The uptake of $^{3}H$-thymidine was shown to be increased during the first four weeks of the disease, with peak labeling in the second and third weeks; in one autoradiograph, 5.4 per cent labeling was observed. The label was confined to the nuclei of certain atypical mononuclear cells, most of which showed rather marked cytoplasmic basophilia. Karyotype analyses were consistently normal. The occurrence of similar large mononuclear cells, capable of taking up $^{3}H$-thymidine, in other pathological conditions and in normal subjects is stressed. Their origin, function and potentialities are ill-understood at the present time.

Two further autoradiographic studies dealing with the mitotic activity of atypical mononuclear cells in infectious mononucleosis have recently appeared, both of which record increased DNA synthesis. Epstein and Brecher observed peak labeling during the first two weeks of the disease; most of the labeled cells showed enhanced cytoplasmic basophilia. No variation in labeling at different phases of the disease was found by Schmid et al., who, unlike previous workers, characterize the labeled cells in some detail as plasmacytoid, monocytoid and lymphocytoid elements.

**Summario in Interlingua**

Esseva investigate le activitate mitotic e premitotic del circulante atypic cellulas mononuclear que es vidite in mononucleosis infectiose. Cellulas in division e cellulas binucleate esseva trovate in 2,4 pro cento del frottis de sanguine e in 63,9 pro cento del concentratos de leucocytos. Le numero del cellulas in stato mitotic esseva micre, e tal cellulas esseva incontrate le plus communmente durante le prime tres septimanas del morbo. Le incidentia de
mitotic activity

...cellulas in division in concentratos de leucocytos esseva augmentate par un periodo preliminari de incubation con colchicina. Le acceptation de thymidina a tritiumesseva augmentate durante le prime quatro septimanas del morbo, attingente un maximo in le secunde e le terti septimana. In un autoradiogramma, un marcation de 5,4 pro cento esseva effectuate. Le marca esseva restringite al nucleo de certe atypic cellulas mononuclear, le majoritate del quales mostrava un satis intense basophilia cytoplasmatic. Le resultatos de analyses carotypic esseva uniformemente normal. Es sublineate le occurrentia de simile grande cellulas mononuclear, capace a acceptar thymidina a tritium, in altere conditiones pathologic e mesmo in subjectos normal. Lor origine, lor function, e lor potentialitates non es ben comprendite a iste tempore.
-acknowledgments

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references


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