DNA and RNA Synthesis of Circulating Atypical Lymphocytes in Infectious Mononucleosis

By Lois B. Epstein and George Brecher

It is now generally agreed that the atypical mononuclear cells associated with infectious mononucleosis are members of the lymphocyte family, but little is known about physiologic differences that may exist between the abnormal cells and normal lymphocytes. One approach to an understanding of such differences has been the study of nucleic acid synthesis. Using radioautographic technics, Gavosto et al. found that a higher than normal proportion of leukocytes synthesized DNA in the peripheral blood of patients with infectious mononucleosis. The present study was undertaken to evaluate this observation further and to determine the relationship of RNA and DNA synthesis to the peripheral white blood cell count and the proportion of atypical lymphocytes during the course of the disease.

Materials and Methods

Blood samples were obtained from 12 male and 1 female patients ranging in age from 14-26 years. All patients had documented infectious mononucleosis with malaise, fever, sore throat, and lymphadenopathy, heterophile agglutination titers greater than 1/224, and atypical lymphocytes in the peripheral blood. The onset of the disease was dated from the appearance of malaise and at least one other symptom (fever, sore throat, headache). An average of three blood samples was obtained from each patient during the course of the disease, with the earliest sample being obtained on the fourth day of the disease. In addition, 6 blood samples from 6 normal individuals were collected.

In aliquots of 35 samples from the 13 patients with infectious mononucleosis and the 6 samples from controls, DNA synthesis was measured in vitro by incubating blood in a heparinized dextran-saline solution containing H3-thymidine (final concentration 1.8 μc./mL), as described by Bond et al. Similar incubation mixtures with H3-cytidine were used to study RNA synthesis in aliquots of 18 of the 35 samples detailed above. After a 50-minute incubation period, the leukocyte-rich supernatant was withdrawn and centrifuged at 1,000 rpm for 10 minutes at room temperature. Smears for radioautography were prepared from the cell button and treated as previously described. All radioautographs were exposed for 5 days. After developing and staining, 1,000 mononuclear cells per slide were examined and grain counts determined. Only cells with 11 or more grains were considered to be positively labeled.

Results

I. Incorporation of H3-Thymidine

The results of 35 experiments in which the per cent of mononuclear cells in DNA synthesis was measured by the in vitro incorporation of H3-thymidine

From the National Institute of Arthritis & Metabolic Diseases and The Clinical Center, National Institutes of Health, Bethesda, Md.

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*Specific activity, 1.9C/mM, Schwarz Bioresearch, Inc.

†Specific activity, 1.5C/mM, Schwarz Bioresearch, Inc.
is shown in figure 1, middle. The per cent of mononuclear cells labeled was greatest during the first 2 weeks of illness and gradually declined to control values (0.0 per cent–0.2 per cent) during the following 3 weeks. The highest value obtained was 9.4 per cent on the eighth day of illness. All of these data were obtained using a total incubation period of 1 hour and an H³-thymidine concentration of 1.8 µc./ml. in the final incubation mixture. Ten additional experiments were performed using 2- and 3-hour incubation periods, and four additional experiments were performed using up to four times the concentration of H³-thymidine. In all cases the results were not significantly different from those obtained with the original conditions. Also the average number of grains per labeled cell did not vary significantly with the progression of the disease.

II. Incorporation of H³-Cytidine

The results of 18 experiments in which the per cent of mononuclear cells synthesizing RNA was measured by the in vitro incorporation of H³-cytidine are shown in figure 1, top. As was observed with H³-thymidine, the proportion of cells labeled with H³-cytidine was greatest during the first 2 weeks of illness, but it returned to control values after 4 weeks of illness.

III. Relationship of H³-Thymidine and H³-Cytidine Incorporation to White Blood Cell Count and Differential

In figure 1, bottom, the per cent of mononuclear cells that were atypical lymphocytes is plotted as a function of the days after onset of illness. It is quite apparent that whereas the per cents of mononuclear cells in RNA and DNA synthesis returned to normal values in 4 and 5 weeks respectively, the per cent of mononuclear cells that were atypical lymphocytes approached the normal value (0.5 per cent) much more slowly.

The values for the white blood cell counts, the absolute number of lymphocytes and monocytes during the course of the disease are shown in figure 2. Since the patients' own WBC counts prior to illness are not known, it is impossible to determine when the total lymphocytes returned to normal. The values did appear to stabilize within 3 weeks, even though atypical lymphocytes persisted much longer.

IV. Description of Cells that Incorporate H³-Thymidine and H³-Cytidine

Of the 35,000 mononuclear cells examined after the 1-hour incubations with H³-thymidine, 712 cells were labeled with 11 or more grains. Of these, 75 per cent were large cells (9–16 µ in diameter) with indented nuclei and with varying amounts of cytoplasm that was usually foamy and moderately to intensely basophilic. Another 20 per cent were similar except for the presence of nonindented nuclei. The remaining labeled cells were either smaller forms of the cells just described or large cells with indented nuclei and very pale cytoplasm.

Of the 18,000 mononuclear cells examined after the one hour H³-cytidine incubations, 520 cells were labeled with 11 or more grains. Of these, 95 per
Fig. 1.—(Top) The percentage of mononuclear cells in RNA synthesis as measured by H3-cytidine uptake. (Middle) The percentage of mononuclear cells in DNA synthesis as measured by H3-thymidine uptake. (Bottom) The percentage of mononuclear cells that were atypical lymphocytes.

cent were the large cells with indented nuclei and varying amounts of cytoplasm that were usually foamy and moderately to intensely basophilic. Only 4 per cent were the large cells with nonindented nuclei. Mitotic figures were not searched for in this group of slides.

DISCUSSION

Identification of atypical lymphocytes in smears of blood samples incubated in dextran-saline mixtures for measurement of DNA and RNA synthesis is not possible with certainty. Even after the 50-minute incubation used in the present experiments, the morphology of both normal and atypical lymphocytes undergoes mild changes and in radioautographs the grains overlying the
cells further interfere with identification. It is presumably for this reason, that even the gross classification of labeled cells showed such discrepancies as 75 per cent of large cells with indented nuclei in the thymidine experiments, and 95 per cent in the cytidine series. Notwithstanding the uncertain direct identification, there is good statistical evidence that most of the labeled cells were atypical lymphocytes. In the direct blood smears of patients with infectious mononucleosis 9-43 per cent of all mononuclear cells were atypical lymphocytes, whereas 2-10 per cent of all mononuclear cells were in DNA synthesis in the radioautographs. In normal controls, only 0.2 per cent of all mononuclear cells were in DNA synthesis in autoradiographs. Atypical lymphocytes morphologically similar to those seen in infectious mononucleosis comprised only 0.5 per cent of mononuclear cells in normal man.9 These figures strongly support the tentative conclusion of the morphologic examination that virtually all DNA synthesizing cells in patients with infectious mononucleosis are atypical lymphocytes.

The results confirm earlier observations of a high percentage of cells in DNA synthesis in the peripheral blood of patients with infectious mononucleosis.8 Not unexpectedly, RNA synthesis was also readily demonstrable in atypical lymphocytes. The percentage of cells in RNA and DNA synthesis were similar, and presumably the same cells were involved.

It may be assumed that the mononuclear cells in DNA synthesis are pre-
paring to undergo mitosis. However, only 0.01 per cent of the cells were actually in mitosis in radioautographs. It is unlikely that this represents an artifact due to the 50-minute incubation in dextran-saline. In none of the direct smears in which at least 200 cells were counted were mitoses noted. The paucity of mitoses in the peripheral blood in infectious mononucleosis is further attested by the general experience in our own and other laboratories.

Several explanations for this apparent discrepancy are possible: (1) the period of DNA synthesis may be very long relative to the period of actual mitosis; (2) the cells may synthesize DNA without ever dividing; (3) the DNA synthesis observed may be that of an intracellular virus rather than of nuclear DNA; (4) the cells may divide only extravascularly possibly because plasma contains some mitosis inhibiting factor or they may circulate only briefly, so that the chance of a mitosis occurring in the peripheral blood becomes negligible.

The following observations make the first possibility unlikely. The grain counts observed in the cells of the patients with infectious mononucleosis are similar to those in transformed "blast-like" cells grown in culture with phytohemagglutinin when comparable H\(^3\)-thymidine concentrations are used. This would suggest that the rate of DNA synthesis and the time required for it are the same in both types of cells. The DNA synthesis time was estimated to be between 12 and 30 hours in the transformed cells. If a mitotic time of 30 minutes is assumed, one mitosis should occur among every 24 to 60 cells in DNA synthesis. This figure is approached in cultures, but not in infectious mononucleosis blood.

The second possibility is unlikely for two reasons. If cells were to synthesize DNA without going on to subsequent division one would expect to find a large proportion of the cells in the premitotic resting phase (G\(_2\)) or existing as polyploid forms. Hale and Cooper have shown that neither situation occurs.

In regard to the third possibility, virus-like particles have never been demonstrated in the nuclei of peripheral blood cells of patients with infectious mononucleosis. However, Reinauer has observed particles having the morphology of viruses in the cytoplasm of a few such cells and it is not possible to rule out the occurrence of viral DNA synthesis.

At the present time the fourth possibility, that the cells in DNA synthesis divide extravascularly, appears the most likely.

Nearly all of the labeled cells appear to be atypical lymphocytes. However, atypical lymphocytes persist long after DNA and RNA synthesis have returned to control values. It therefore is likely that active proliferation of atypical lymphocytes is restricted to a relatively short period early in the disease, at which time a representative sample of such cells, many in active DNA synthesis, appears in the peripheral blood. Apparently, enough of these cells are produced early in the disease so that they continue to be released into the periphery, although they are no longer proliferating.

The present experiments attest to the biologic difference of atypical lympho-
cytes from the normally circulating small lymphocytes. This is further supported by the observations of MacKinney,\textsuperscript{14} that the atypical lymphocytes are incapable of transformation and division under conditions of culture which have been shown previously to induce transformation of small lymphocytes into dividing "blast-like" cells.\textsuperscript{15} MacKinney’s observations also appear incompatible with the suggestion made recently,\textsuperscript{16} that the atypical lymphocyte and the "blast-like" transformed cells are, in fact, the same cell.

**SUMMARY**

The percentages of mononuclear cells synthesizing DNA and RNA in serial studies of blood from 13 patients with infectious mononucleosis were determined. Early in the disease a high percentage of atypical lymphocytes were in DNA synthesis but this percentage decreased rapidly as the disease progressed. Late in the disease many atypical lymphocytes were still present but few, if any, were synthesizing DNA. Similar results were found for RNA synthesis.

Presumably active proliferation of atypical cells in the tissues is restricted to an early period of the disease, whereas release of such atypical cells may continue for a considerable period.

**SUMMARIO IN INTERLINGUA**

Esseva determinate le procentage del cellulas mononucleari synthetisante acido deoxyribonucleic e acido ribonucleic in studios serial de sanguine ab 13 patientes con mononucleosis infectiose. In stadios precoce del morbo, un alte procentage de atypic lymphocytos esseva occupate in le synthese de acido deoxyribonucleic, sed iste procentage declinava rapidemente durante que le morbo progredeva. Tarde in le curso del morbo multe lymphocytos esseva ancora presente, sed pauches (o nulles) continuava synthetisar acido deoxyribonucleic. Simile resultatos esseva trovate quanto al synthese de acido ribonucleic.

Il pare que le active proliferation de atypic cellulas in le tissus es restringite a un precoce periodo in le curso del morbo, ben que le liberation de tal cellulas atypic pote continuar durante un considerabile periodo.

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Lois B. Epstein, M.D., Research Medical Officer, Laboratory of Experimental Pathology, National Institutes of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

George Brecher, M.D., Chief, Hematology Service, Clinical Pathology Department, Clinical Center National Institutes of Health, Bethesda, Md.
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LOIS B. EPSTEIN and GEORGE BRECHER