Brief Note

Lymphocyte RNA Synthesis in Infectious Mononucleosis:
The Response to Phytohemagglutinin in Vitro

By Arnold D. Rubin

In normal individuals, most circulating lymphocytes are small and show no mitotic activity. When cultivated in vitro with phytohemagglutinin (PHA), these cells enlarge and divide. In contrast, cellular enlargement, DNA synthesis, and at times mitosis have been observed to occur spontaneously in a high proportion of circulating lymphocytes of patients with infectious mononucleosis (IM). When these cells are incubated in vitro for 48 to 72 hours, they take on the morphologic appearance of normal lymphocytes and respond normally to PHA.

We have shown previously that PHA produces a marked stimulation in the synthesis of nonribosomal RNA in cultures of normal lymphocytes. This effect is detectable within 1 hour after exposure to PHA. The purpose of the present study is to compare the effects of PHA on RNA metabolism in suspensions of normal and IM lymphocytes immediately after isolation from the circulation and before the occurrence of any morphologic changes.

Methods

Suspensions of lymphocytes (93 to 98 per cent pure) were isolated from the blood of normal volunteers and from six patients during the acute phase of IM according to a previously published technic. Differential counts were performed on the blood of all six IM patients; 60 to 80 per cent of the lymphocytes were morphologically atypical. This percentage was unchanged by the procedure which separated lymphocytes from other circulating leukocytes. Incubations were carried out in Eagle's minimal essential medium with 15 per cent calf serum and additional glutamine (2 mM). Parallel suspensions were treated with PHA or incubated in the absence of mitogen. After a one hour incubation, tritiated uridine (10 μg./ml., 7.3 C./mmol.) was introduced, and 30 minutes later the cells were harvested. Undegraded RNA was isolated from the lymphocytes by extraction with 60 C. phenol-sodium dodecyl sulfate and sedimented across a 5 to 20 per cent sucrose gradient. Thirty serial fractions were collected through a puncture in the bottom of the centrifuge tube. Ultraviolet absorbency at 260 μm. (OD260) and radioactivity (CPM) were measured for each fraction.

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First submitted March 9, 1966; accepted for publication May 20, 1966.

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The cooperation of the Tufts University Health Service and of Dr. Clark W. Heath is gratefully acknowledged.

BLOOD, VOL. 28, NO. 4 (OCTOBER), 1966
RESULTS

The left half of Figure 1 shows a typical result obtained when suspensions of normal lymphocytes were incubated for 1 hour, with and without PHA. The sedimentation pattern of bulk cellular RNA is represented by the ultraviolet absorbency. Peaks of absorbency at 28S, 18S, and 4S served as markers for comparative sedimentation rates. In unstimulated suspensions of normal lymphocytes, labeled RNA (representing in each case 80 to 90 per cent of incorporated radioactivity) sedimented mostly with the heavier fractions (20 to 50S). Normal lymphocytes incubated with PHA for 1 hour yielded higher levels of labeled RNA, most of which was included in a broad band sedimenting from 6 to 30S. The right half of Figure 1 shows a typical result obtained when blood lymphocytes from a patient with IM were treated in the same manner. Identical results were obtained with lymphocytes from all six patients with IM. Labeled RNA from unstimulated suspensions of IM cells sedimented mostly in peaks at 45S and 4S. The levels of labeled RNA were 10 times those of normal lymphocytes. PHA treatment of IM cells for 1 hour resulted in diminution in the previously elevated levels of labeled RNA. The species of RNA most inhibited were contained within the 45S and 4S peaks. In contrast with normal lymphocytes, PHA treatment evoked no increase in the rate of synthesis of 6 to 30S RNA. DNA content of these suspensions, as measured by perchloric acid extraction, was unchanged 1 hour after PHA treatment. Therefore, loss of cells could not account for the inhibition of RNA synthesis.

DISCUSSION

Treatment of normal lymphocytes with PHA results in an abrupt shift from a nongrowing state to one of cell enlargement and mitosis. This shift, which is accompanied by an early increase in RNA synthesis, is associated with an increase in newly synthesized RNA sedimenting in the range of 6 to 30S. As previous studies have established that this type of RNA is nonribosomal, it is probable that this material represents varieties of messenger RNA concerned with regulating the shift from the resting state to active growth.

IM lymphocytes, which appear to be rapidly proliferating in the absence of any definite stimulus, were found to synthesize RNA at 10 times the rate of normal unstimulated lymphocytes. This high rate of RNA synthesis might be anticipated in a population of cells rapidly proliferating with increased metabolic requirements. Furthermore, as RNA metabolism in such a population of cells already would have been set in a pattern to support active growth, PHA treatment could not induce the shift from a resting state. The lack of increased production of 6 to 30S RNA following treatment of IM cells with PHA might be considered a reflection of the spontaneous mitotic activity of freshly isolated IM lymphocytes. However, mitotic activity in itself does not prevent the PHA-induced stimulation of lymphocyte RNA synthesis. Rubin and Cooper have shown that PHA-activated normal lymphocytes, even during the peak of the growth response, can still be induced to synthesize 6 to 30S RNA at increased rates if additional PHA is added to the culture medium. Consequently, there
must exist a basic difference in RNA metabolism between normal lymphocytes and freshly isolated IM cells.

It is not clear why PHA treatment results in an inhibition of RNA synthesis in IM lymphocytes. This phenomenon appears to be a characteristic response of IM lymphocytes and may represent a peculiar effect of PHA on the RNA metabolism of these cells.

When IM lymphocytes are incubated in vitro for 48 to 72 hours, they assume the characteristics of normal lymphocytes, and PHA stimulated the production of 6 to 30S RNA in much the same fashion as observed in cultures of normal lymphocytes. The freshly isolated IM lymphocytes, under a continued stimulus to proliferate, may be "locked on" a particular metabolic pattern so that RNA synthesis is no longer regulated in the usual way. Such a stimulus would appear to be of limited duration, since the IM patient fully recovers from the hematologic manifestations of his disease and the IM lymphocytes revert to normal in culture. The circumstances under which PHA influences lymphocyte RNA synthesis is under investigation as a possible approach to the regulation of cell differentiation.

SUMMARY

Circulating lymphocytes were isolated from normal individuals and from patients with IM. IM lymphocytes synthesized RNA at 10 times the rate of

Fig. 1.—Density gradient sedimentation of RNA from lymphocytes incubated for 1 hour: 30 minutes exposure to uridine H, 10 μc./ml. Dotted line, ultraviolet absorbency (OD 260): closed circles with broken lines, radioactivity, counts/min., unstimulated cells; open circles with unbroken lines, radioactivity, PHA stimulated cells.
normal lymphocytes. A 1-hour exposure to PHA did not result in the stimulation of 4 to 30S RNA synthesis which occurs in a similarly treated normal lymphocyte. RNA synthesis in IM lymphocytes actually appeared to be depressed by this short exposure to PHA.

**SUMMARIO in Interlingua**

Lymphocytos circulante esseva isolate ab individuos normal e ab patientes con mononucleosis infectiose. Le lymphocytos in mononucleosis infectiose synthetisava acido ribonucleic 10 vices plus rapidemente que lymphocytos normal. Le exposition durante 1 hora a phytohemagglutinina non resultava in le stimulation del synthese de acido ribonucleic ab 4 ad 30S le qual occurre in similemente tractate lymphocytos normal. De facto, le synthese de acido ribonucleic per lymphocytos in mononucleosis infectiose pareva esser deprimite per iste breve exposition a phytohemagglutinina.

**ACKNOWLEDGMENTS**

The author wishes to express his appreciation for the advice and encouragement of Drs. William Dameshek and Robert S. Schwartz of the New England Medical Center Hospitals.

**REFERENCES**

5. Rubin, A. D.: Observations to be published.
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