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Leukemia-like Pattern of the DNA, RNA, and Protein Content of Individual Mononuclear Cells in the Peripheral Blood of Patients with Infectious Mononucleosis[1,2]

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SUMMARY

The deoxyribonucleotide, total nucleotide, and protein content of single mononuclear cells derived directly from the peripheral blood buffy coats of patients with infectious mononucleosis were determined cytochemically by means of ultramicrospectrophotometry and ultramicrointerferometry. A small fraction of cells in the populations derived from each patient was found to be in the S- or G-2-phase of DNA synthesis. The intercellular variation in the total nucleotide and the protein content per cell in these cell populations was greater than that in comparable populations of normal lymphocytes.

There were indications for the presence of more than one kind of population of mononuclear cells in the peripheral blood of patients with infectious mononucleosis. The evidence suggests that there might be one nondividing, non-RNA, and nonprotein-accumulating cell population; one dividing, and one nondividing, but RNA- and protein-accumulating population in each of the specimens so examined. The population characterized by large total nucleotide and mass values appeared to be identical with the population of so-called atypical mononuclear cells, and may well be genetically distinct from the other mononuclear cells in such populations.

The cytochemical patterns observed in these populations derived directly from the peripheral blood buffy coats of patients with infectious mononucleosis were clearly similar to those previously described in populations of leukemic blast cells similarly derived directly from patients with acute leukemia. These cytochemical observations may well indicate similarities in the growth kinetics of mononuclear blood cell proliferation in infectious mononucleosis and acute leukemia.

INTRODUCTION

Previous studies, utilizing the biophysical instrumentation developed for the cytochemical analysis of cell populations (13–20, 36, 37), described the increased intercellular variability in the amounts of nucleic acids and proteins in individual lymphoblastic cells derived directly from the peripheral blood of patients with acute leukemia as compared to the intercellular variability of these cytochemical parameters among individual cells in populations of normal blood lymphocytes (26, 27). In these studies, there was evidence for the presence of a population of non-DNA synthesizing, nondividing, but RNA- and protein-accumulating cells in the populations of lymphoblastic cells derived from patients with acute leukemia, and the existence of genetically different subpopulations in such populations of lymphoblastic cells could not be excluded.

In order to determine whether the patterns of increased intercellular variability observed in populations of lymphoblastic and myeloblastic cells derived from acute leukemia were characteristic of populations of neoplastic cells alone, or, as well, might be found in populations of cells derived from benign lymphoproliferative disorders, similar cytochemical analyses of mononuclear cells derived directly from the peripheral blood buffy coat of patients with infectious mononucleosis were undertaken.

MATERIALS AND METHODS

Eight patients with infectious mononucleosis were studied (Table 1), each of which presented a typical clinical and hematologic picture. Six of these patients had a positive Paul-Bunnel-Davidsohn test after absorption of their serum with guinea pig renal tissue. The other two patients (R. P. and J. L.) were included in the series although their Paul-Bunnel-Davidsohn tests were not specific, as adjudged by absorption with

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Table 1

Patient[a]	Age	Total WBC × 10^{-3}	Total mononuclear cells (% of total WBC)	Atypical mononuclear cells (% of total WBC)	Paul-Bunnel test	RNA:DNA ratio		
						Uncorrected	Corrected[b] (Method *a*)	Corrected[b] (Method *b*)
A.A.	26	12.1	79	12	1:320	0.40	0.26	<0.10
P.B.	15	8.7	60	35	1:320	<0.10	<0.10	<0.10
R.P.	15	19.2	71	28	1:80[c]	0.58	0.45	0.22
A.L.	14	10.9	52	18	1:2560	0.44	0.42	0.39
J.L.	15	11.0	69	31	1:20[c]	0.46	0.35	0.29
B.R.	16	20.5	83	42	1:160	0.30	0.26	0.19
Br.R.	17	7.3	75	13	1:80	0.45	0.38	0.44
B.T.	24	10.0	82	16	1:80	0.52	0.61	0.66
Normal blood lymphocytes						<0.10	<0.10	<0.10

RNA:DNA ratio in mononuclear blood cells from patients with infectious mononucleosis.

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[b]According to methods *a* and *b* as described by Killander (34).

[c]Nonspecific, *cf.* text.

guinea pig renal tissue. All patients had received penicillin prior to admission to the hospital.

Isolation of Cells and Preparations of Specimens. White blood cells were isolated from venous blood according to a minor modification of a previously described method (25, 27). Approximately 10 ml of venous blood was drawn by cubital puncture into an EDTA Vacutainer (B-D, Columbus, Nebraska) containing 12 mg ethylenediaminetetraacetate (EDTA), the tubes immediately placed in crushed ice, and the entire isolation procedure (24) thereafter done at 4°C. The blood was mixed with a solution of 6% bovine fibrinogen (Sigma Chemical Company, St. Louis, Missouri) in saline and placed at 60° to the horizontal for 30 min to permit the separation of red blood cells. The WBC-rich supernatant was removed and centrifuged at 300 × *g* for 7 min, the supernatant discarded, and the resulting pellet washed once with 5 ml of 5% dextrose in distilled water containing 1 mg/ml of EDTA. The pellet was well suspended in 2 ml of saline, and 6 ml of distilled water were then added during vigorous shaking for 30 seconds to lyse the remaining red blood cells. Isotonicity was restored by the addition of 2 ml of 3.6% saline; the suspension was recentrifuged, the supernatant discarded, and the pellet resuspended in a few drops of saline. Each suspension was then spread carefully on a series of quartz slides and immediately fixed by freeze-substitution in liquid propane held at –196°C in a liquid nitrogen bath; this was immediately followed by immersion in absolute ethanol held at –70°C for 48 hr in an alcohol-CO_2 bath which was then allowed to equilibrate to room temperature (+20°C). All preparations were stored in fresh absolute ethanol until examined, at which time they were passed through graded (absolute) alcohols to distilled water. One set of slides was then passed to glycerin and mounted in redistilled glycerin ($n = 1.455$) under quartz cover slips which were sealed with paraffin. Duplicate slides were stained by the Feulgen reaction and mounted in DePeX (Gurr, London).

Cytochemical Analyses. Cytophotometric measurements were done in the high-resolution, rapid-scanning, ultramicrospectrophotometer and the high-resolution, rapid-scanning, ultramicrointerferometer described elsewhere (18, 20, 36, 37) on 50–100 randomly selected cells in each population. In each instance, the individual cells so examined were located on appropriate "maps," in order to permit determination of mass and total nucleotide content on the same individual cells. The preparations were then stained with May-Grünewald-Giemsa, and the "mapped" cells were classified as mononuclear or polymorphonuclear.

The DNA content of individual Feulgen-stained cells was determined at 546 mμ. The mean Feulgen-DNA content of polymorphonuclear cells in each preparation was taken to represent 1 relative unit (RU), and all DNA determinations on individual mononuclear cells in that preparation were referred to this mean Feulgen-DNA value for polymorphonuclear cells. Cells with Feulgen-DNA values of *circa* 1 RU were considered to be 2c-cells, c being the haploid DNA content, and cells with Feulgen-DNA values between 2c and 4c were considered to be intermediate cells.

The total nucleotide content of individual unstained cells was determined in ultraviolet light at 265 mμ (E_{tot} 265). The nonspecific extinction at 315 mμ (E_{tot} 315) was in the range of 10–20% of the E_{tot} 265 per cell. Determination of mass and E_{tot} 265 on the same individual mononuclear cells resulted in correlation coefficients of 0.61–0.86 (mean 0.74), in cell populations derived from the different patients (Chart 1), indicating a significant degree of consistency in the nucleic acid content per unit dry mass of the individual cells in the same population. Thus, the E_{tot} 265 value represented a good measure of the relative total nucleotide content of these individual cells, as has been observed in similar studies with other kinds of cells (13–17, 19, 26–28, 33, 34, 39).

The mean relative RNA content of individual cells was computed from determination of the non-DNA total extinction at 265 mμ as described previously (33, 34). The mean DNA content of normal lymphocytes which contain negligible amounts of RNA (*circa* 10% of the total nucleotide content) has been shown to be 14–15 relative UV units (26–28, 33, 34), and the amount of DNA contained in blood polymorphonuclear cells has been found to be essentially similar (27). The mean E_{tot} 265 value attributable to DNA, therefore, could be estimated

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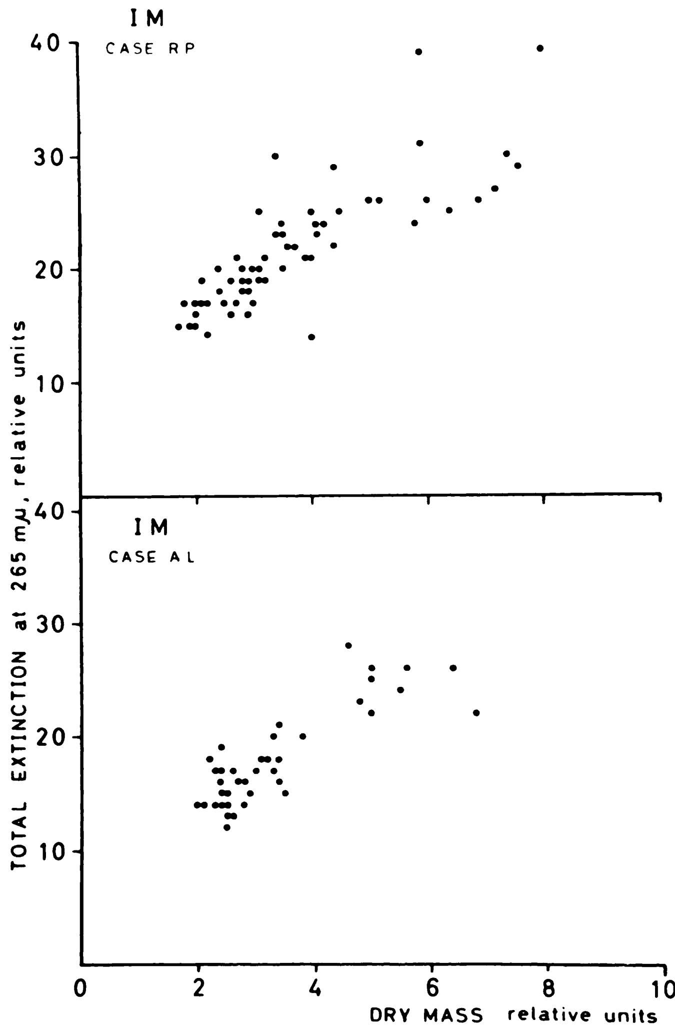

Chart 1. Total extinction at 265 mμ (E_{tot} 265) of individual mononuclear cells plotted against the mass values of the same cells in cell populations from two different patients with infectious mononucleosis (IM) (R. P., [corr. coeff. 0.82] and A. L., [corr. coeff. 0.77]).

from determination of the Feulgen-DNA content and the total E_{tot} 265 of the same individual cells, using normal lymphocytes or polymorphonuclear cells as reference controls for the Feulgen reaction. The E_{tot} 265 value attributable to DNA was then subtracted from the total E_{tot} 265 to obtain the non-DNA total extinction at 265 mμ as an estimate of the RNA content of the cell. The uncorrected RNA:DNA ratio was calculated as the ratio of mean non-DNA total extinction at 265 mμ to mean DNA total extinction at 265 mμ (Table 1). A somewhat more accurate estimate of the RNA:DNA ratio could be obtained by correction of the E_{tot} 265 values for nonspecific extinction at 315 mμ (13, 14, 34, 39) and for the absorption due to proteins (33, 34). Such corrections (Table 1) have been made according to previously described methods (34), and the true RNA:DNA ratio probably lies between these two corrected values.

The mass (dry mass) of individual cells as determined in the ultramicrointerferometer was presumed to represent an approximate measure of the total protein content of the cell since it is well established that proteins constitute 80–90% of the total cell mass (14, 34).

RESULTS

DNA Content (Table 2; Charts 2, 3). In all of the cell populations derived from the peripheral blood buffy coats of each individual patient, more than 90% of the individual mononuclear cells had a DNA content similar to that characteristic of polymorphonuclear (2c) cells present in the same cell population. However, in each of these populations of mononuclear cells, there was a minor proportion of cells which contained intermediate or 4c amounts of DNA, indicating that these cells were in the S- or G-2-phase of DNA synthesis. Cells with a DNA content greater than that designated as 4c were not observed in these populations.

Total Nucleotides and RNA. The degree of intercellular variability in the total nucleotide content of the individual mononuclear cells in these populations was significantly greater than that observed among normal lymphocytic cells (Table 2; Charts 2, 3). In each of the cell populations derived from the peripheral blood buffy coats of each individual patient, a portion (usually the predominant portion) of the individual mononuclear cells had E_{tot} 265 values similar to those characteristic of normal blood lymphocytes. However, a smaller portion of these mononuclear cells (usually about the same percentage of the population as that classified as atypical mononuclear cells in the differential count) had significantly greater E_{tot} 265 values. The mean E_{tot} 265 of individual cells in this portion of the population usually was about twice that of the individual cells in the remainder of the mononuclear cells in the population. Using the same method for the calculation of the RNA:DNA ratio as has been used in previous studies with other kinds of cells (27, 34), the mean RNA:DNA

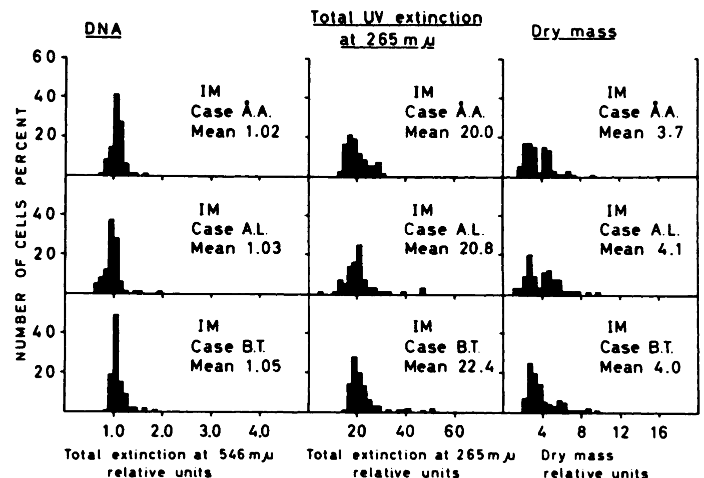

Chart 2. Frequency distribution of DNA, E_{tot} 265, and mass per cell in populations of mononuclear cells derived from individual patients with infectious mononucleosis (IM).

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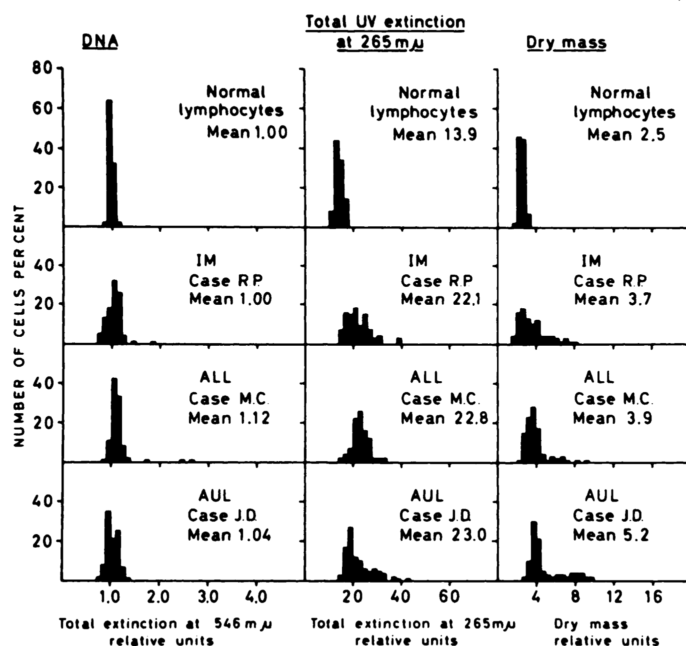

Chart 3. Comparison of frequency distributions of DNA, E_{tot} 265, and mass per cell in normal lymphocytes and mononuclear cell populations derived from individual patients with infectious mononucleosis (IM) and acute leukemia: ALL, acute lymphoblastic leukemia; AUL, acute unclassified leukemia (from data considered elsewhere [27]).

ratio varied from <0.10 to 0.66 in the cell populations derived from individual patients (Table 1).

Mass. The degree of intercellular variability and the mass values of individual mononuclear cells in the populations derived from these individual patients also was significantly greater than that characteristic of normal blood lymphocytic cells (Table 2). The frequency distribution pattern of mass per individual mononuclear cell was similar to the frequency distribution pattern of the E_{tot} 265 values for the same individual cells in the same populations. Again, in some cases there appeared to be two kinds of mononuclear cells; the mean mass value of the cells in the portion of the population characterized by the higher mass values was usually about twice the mean mass value of those in that portion characterized by the lower mass values (Charts 2, 3). Again, too, the percentage of cells in the portion of the population characterized by high individual mass values usually was of the same order of magnitude as that percentage of the population classified as atypical mononuclear cells in the differential count.

DISCUSSION

Thus, in the present studies, there are indications for the presence of cytochemically different populations of mononuclear cells in the peripheral blood of patients with infectious mononucleosis. The frequency distribution patterns of the DNA content per individual mononuclear cell clearly indicates the presence of DNA-synthesizing (intermediate, or 4c) cells in the peripheral blood of patients with infectious mononucleosis, as has also been described elsewhere (22, 31). Such an observation is in agreement with the reported presence of mitotic cells in the peripheral blood of such patients (11, 12, 43) and the ability of buffy coat cells derived from the peripheral blood of patients with infectious mononucleosis to incorporate tritium-labeled thymidine when cultured *in vitro* (8–10, 24, 29–31, 38, 42). However, the number of such DNA-synthesizing cells was low (less than 10%); this was also true for the number of DNA-synthesizing cells observed in the peripheral blood buffy coats of patients with acute leukemia as described elsewhere (27). These observations might be interpreted to indicate that the G-1 phase (in relation to the S- and G-2-phases) is prolonged in either instance or that, in such peripheral bloods, there are one dividing and one or more nondividing subpopulations of mononuclear cells. The morphologic evidence for the maturity of most mononuclear cells in infectious mononucleosis favors the latter or, perhaps, a combination of both of these interpretations. It is of particular interest that cytochemical (27) as well as autoradiographic

Table 2

Patient	Feulgen-DNA relative units (mean)	% of mononuclear cells in S- or G-2-phase	Total extinction at 265 mμ: relative units		Dry mass: relative units	
			Mean	Coeff. of variation	Mean	Coeff. of variation
A.A.	1.02	2	20.0	22.6	3.7	40.4
P.B.	1.09	3	13.4	22.3	3.5	42.4
R.P.	1.00	2	22.1	23.5	3.7	41.9
A.L.	1.03	3	20.8	36.5	4.0	43.5
J.L.	1.00	0	20.5	21.3	4.3	41.5
B.R.	1.07	8	19.5	22.1	3.6	31.8
Br.R	1.00	0	20.3	11.9	4.8	30.1
B.T.	1.05	7	22.4	31.1	4.0	41.0
Normal blood lymphocytes						
	1.00	0	13.9	10.9	2.5	13.0

Feulgen unit DNA, total ultraviolet extinction at 265 mμ (E_{tot} 265), and mass of mononuclear cells from peripheral blood of patients with infectious mononucleosis.

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(35) studies of human leukemic blast cells derived from acute leukemia similarly indicate the presence of one (probably small) dividing and one (morphologically more mature) nondividing subpopulation of leukemic cells in the peripheral blood.

The degree of intercellular variability in the total nucleotide content and the mass per individual mononuclear cell in infectious mononucleosis was significantly greater than that characteristic of normal blood lymphocytes and thus was similar to that observed in populations of leukemic blast cells derived from patients with acute leukemia (27). As in the case of cell populations deriving from acute leukemia, it is unlikely that, in the cell populations deriving from infectious mononucleosis, this degree of intercellular variability was due to a distribution of cells in various phases of mitotic cycle. Far too few cells were found to be engaged in active DNA synthesis to account for the relatively high frequency of cells characterized by large total nucleotide and mass values, and further, cell populations which contained a large number of cells characterized by large mass values did not contain a greater number of cells in active DNA synthesis than did those cell populations which contained but a small number of individual cells characterized by large mass values.

It seems more likely, therefore, that there is more than one population of mononuclear cells in the peripheral blood of patients with infectious mononucleosis, a hypothesis similar to that suggested recently as the result of similar cytochemical studies on populations of human leukemic blast cells derived from patients with acute leukemia (27).

These observations, together with other evidence (29, 31, 42), suggest that there might be one nondividing, non-RNA, and nonprotein-accumulating cell population (2c cells, with low total nucleotide and mass values); one dividing (2c–4c cells, with low or high total nucleotide and mass values) and one nondividing, but RNA- and protein-accumulating cell population (2c cells, with low to high, or only high total nucleotide and mass values). The subpopulation characterized by large total nucleotide and mass values, obviously identical with the population of so-called atypical mononuclear (Downey or McKinlay) cells (23), may well be genetically different from the other mononuclear cells in the peripheral blood of patients with infectious mononucleosis.

Morphologic distinction between the mononuclear cells in infectious mononucleosis and leukemia cells is often difficult, and certain other similarities between infectious mononucleosis and leukemia are well known (21, 22). The data presented herein clearly indicate that there are also a number of cytochemical similarities between the mononuclear cells in the peripheral blood of patients with infectious mononucleosis or acute leukemia, such cytochemical similarities perhaps reflecting similarities in the kinetics of *in vivo* proliferation of mononuclear cells in either of the two diseases. As in the case of leukemia, continuous cultures of lymphocytic cells have been derived from the peripheral blood buffy coats of patients with infectious mononucleosis (40), and it is of interest that the incidence of "lymphoblastoid transformation" occurs with greater frequency in cultures derived from children with infectious mononucleosis than in comparable cultures derived from children with acute leukemia or other disease entities (4–7). Although the possible role, with respect to this difference in the incidence of "transformation", of prior chemotherapy in those patients with leukemia from whom these specimens were derived cannot be evaluated at the present time, extensive cytologic studies failed to reveal detectable differences among these "transformed" cells, irrespective of the clinical diagnosis of the patient from whom the cultures were derived (41). It is of further interest that a number of continuous cultures of monocytic cells derived from the bone marrows of patients with infectious mononucleosis have been heterotransplanted to rats, wherein the resulting tumors exhibit the histologic characteristics of reticulum cell sarcomas[4], and the development of lymphomas in newborn Syrian hamsters following implantation of human lymphoblasts derived from patients with acute leukemia has been described (1–3). The possible significance of such similarities has been emphasized by the recent report suggesting the Epstein-Barr virus (EBV) as a possible "common denominator" establishing a tenuous relationship between infectious mononucleosis and Burkitt's lymphoma (32). Waterhouse and Lapidus (44) recently reported a case of infectious mononucleosis associated with a mass in the anterior mediastinum, the precise nature of which was unknown. Although this may be the first recorded instance of such an occurrence, it was suggested that infectious mononucleosis be considered in the differential diagnosis of masses in the anterior mediastinum.

Such similarities are indeed of interest, in view of the manifest biologic differences between these kinds of mononuclear cells as implied by differences in the usual clinical courses of infectious mononucleosis and leukemia in man. It is intriguing to postulate, for example, that the initial biologic insult precipitating disease in either instance might be similar, as suggested by Benyesh-Melnick *et al.* (6) and Pope (40), or, perhaps, even identical, the biologic factors determining ultimate direction or extent of progression being attributes of the individual host and its response to the initial biologic insult.

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