Impaired production of mononuclear cell procoagulant activity in chronic lymphocytic leukaemia

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SUMMARY Chronic lymphocytic leukaemia (CLL) is associated with a low incidence of thrombotic complications, or disseminated intravascular coagulation (DIC), or both, despite the frequent occurrence of severe infections. We have investigated the capacity of blood mononuclear cells to produce procoagulant activity when stimulated with bacterial endotoxin in 16 patients with untreated chronic lymphocytic leukaemia (CLL). Procoagulant activity generated by patients' cells after prolonged incubation with endotoxin was significantly lower than that produced by cells from a matched control group (p < 0.001). The defect could not be attributed to an inhibitory effect of leukaemic lymphocytes. It is suggested that in CLL the monocyte has an intrinsic functional abnormality of the procoagulant response to endotoxin and possibly to other stimuli. These findings help explain why CLL patients do not develop thrombotic complications despite the high incidence of severe infectious diseases.

Many haematological malignancies and solid tumours are associated with vascular thrombosis and/or disseminated intravascular coagulation (DIC).1 2 The frequency of these haemostatic complications is exceedingly high when patients with malignancy are exposed to stimuli affecting the haemostatic system such as surgery or infection.1 3 In contrast, chronic lymphocytic leukaemia (CLL) is associated with a low incidence of thrombotic manifestations and/or DIC.4 5 This is notable considering the frequent occurrence of severe infectious diseases in CLL patients.4 The reason for this discrepancy is unknown.

Normal human peripheral blood mononuclear cells generate a potent procoagulant activity, identified as tissue factor when exposed to bacterial endotoxin and other stimulants (immune complexes, complement proteolytic products, mitogens, etc). They are therefore capable of triggering blood coagulation through the extrinsic pathway.6 7 It is now generally accepted that the monocyte is the cellular source of procoagulant activity.5 8 9 Although it is not yet known whether blood mononuclear cell procoagulant activity is of any relevance for physiological haemostasis, several lines of evidence indicate that it may play a major role in the activation of intra- and/or extravascular coagulation occurring in malignancy, immunological diseases and severe human infections.6 10 11 In this context the observation of Komp and Donaldson12 that patients with Gram-negative sepsis complicating acute leukaemia and with severe leucopenia presented no signs of DIC is of interest. They postulated that leucocytes are necessary for DIC to occur as the result of endotoxaemia. This is supported by evidence that rabbits made leucopenic with cytotoxic drugs are protected against endotoxin-induced DIC and that the infusion of leucocytes though not platelets restores the endotoxin-mediated coagulative changes.13

We suggest that in patients with CLL, mononuclear blood cells may be defective in their ability to produce procoagulant activity in response to endotoxin and that this explains the low incidence of thrombosis and/or DIC in CLL.

Material and methods

PATIENTS

Sixteen patients with B cell type CLL, seven women and nine men, aged 42–72 yr (mean: 63), all newly

Accepted for publication 14 July 1982
ISOLOGATION OF MONONUCLEAR CELLS

Blood anticoagulated with trisodium citrate (0.015 M final concentration) was centrifuged for 15 min at 1300 rpm and platelet-rich plasma (PRP) was removed. Blood mononuclear cells were isolated from the remaining blood diluted with citrated phosphate-buffered saline (9 vol PBS + 1 vol 3.8% (wt/vol) trisodium citrate) by the Ficoll-Hypaque (Lymphoprep, Nyegaard, Oslo, Norway) gradient technique.14 Cell preparations were washed four times with citrated PBS containing 0.5% (wt/vol) bovine albumin and 0.1% (wt/vol) glucose and suspended in Hanks' balanced salt solution (Difco Laboratories, Detroit, Michigan). Final cell suspensions from patients and controls contained more than 97% blood mononuclear cells. The ratio of platelets to white cells in each preparation was always less than 0.5:1 as determined by light microscopy. Monocytes were identified by cytochemical reactivity for alphanaphthyl acetate esterase.15 T lymphocytes by spontaneous rosette formation with sheep red blood cells (E rosettes)16 and B lymphocytes by the detection of surface immunoglobulins using the direct membrane immunofluorescence method.17 The patients' blood mononuclear cell preparations comprised 2-8% monocytes (mean: 3.7%), 3-22% T lymphocytes (mean: 10.5%) and 47-88% B lymphocytes (mean: 66.5%). In controls the percentages of blood mononuclear cell subpopulations were: monocytes: 11-24% (mean: 19%); T lymphocytes: 61-75% (mean: 64%); B lymphocytes: 15-27% (mean: 21%). In some instances patients' or control blood mononuclear cells were depleted of monocytes by allowing them to ingest carbonyl iron (Graf srl, Milan, Italy), followed by recentrifugation on lymphoprep. These preparations contained less than 1% monocytes. Cell viability assessed by the trypan blue test was more than 95%. Blood mononuclear cells of each patient were isolated simultaneously with those of a sex- and age-matched control.

INCUBATION OF BLOOD MONONUCLEAR CELLS AND EVALUATION OF PROCOAGULANT ACTIVITY

The capacity of blood mononuclear cells to produce procoagulant activity in response to endotoxin was studied as follows. Each patient's and control suspension was adjusted to 0.5 × 10⁶ monocytes/ml and, after addition of 20% BaSO₄-adsorbed human serum, was mixed with endotoxin (10 μg/ml of Serratia marcescens LPS, W. Difco Laboratories) and incubated at 37°C. At predetermined intervals (0 and 4 h) the coagulant activity generated in the incubation mixture was evaluated by a one-stage recalcification time using the following test system: 0.1 ml mixture, 0.1 ml normal plasma (pool from at least three normal subjects) and 0.1 ml 0.025 M CaCl₂. The assay was performed with intact and disrupted (repeated freezing and thawing) cells. Results were expressed in arbitrary units by comparison of the clotting times of the intact or disrupted cells with a standard curve of clotting times produced by dilutions of rabbit brain thromboplastin suspension. One hundred units of thromboplastin cause normal plasma to clot in 30 s. All tests were performed in duplicate in prewarmed plastic tubes. BaSO₄-adsorbed human serum contained less than 0.01 U/ml of factors IX, VII and X.

Results

The Figure (upper panel) shows the procoagulant activity generated by patients' and control blood mononuclear cells after 4 h incubation with endotoxin, as measured by clotting times in the recalcification assay. Procoagulant activity was significantly reduced (longer clotting times) in the patients' group using both intact and disrupted cells (mean ± SE: 65.6 ± 2.7 vs 49.7 ± 1.8; p < 0.001 and 42.5 ± 1.8 vs 30.3 ± 1.1; p < 0.001 respectively, by Student's t test). No activity (clotting times longer than 300 s) was observed in control and patients' blood mononuclear cells immediately after isolation. When expressed in arbitrary thromboplastin units, the amount of procoagulant activity generated by patients' blood mononuclear cells was about one-fourth that produced by control cells (Figure, lower panel).

No correlation was found between the number of total lymphocytes or T lymphocytes and procoagulant activity levels in patients' preparations (r = 0.3 p > 0.5 and r = 0.08, p > 0.5) respectively. Moreover the number of T lymphocytes per monocyte was not statistically different in control and patients' blood mononuclear cell preparations (mean ± SE: 3.58 ± 0.2 vs 2.97 ± 0.37, p > 0.1). In some experiments normal mononuclear leukocytes were enriched with lymphocytes from the same donor to produce a variety of lymphocyte/monocyte ratios with the same number of monocytes/ml. The ratios were chosen to approximate those seen in patients. The Table

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diagnosed, were studied before treatment. White blood cell count ranged between 8.2 × 10⁹ and 300 × 10⁹/l (mean: 51.8 × 10⁹/l). No severe bacterial infection was present at the time of investigation. Results of screening studies of the plasma coagulation system including activated partial thromboplastin time, one-stage prothrombin time and fibrinogen levels were within the normal range in all patients.

A control group consisted of 16 apparently healthy subjects, seven women and nine men, aged 40-67 yr (mean 56).
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Discussion

Endotoxin-stimulated blood mononuclear cells have significantly reduced procoagulant activity in a group of patients with CLL studied before treatment. The defect was seen consistently not only with intact cells but also upon cell disruption, a procedure which makes available the total cellular procoagulant activity content. This suggests a decreased procoagulant activity.

It must be emphasised that some patients' blood mononuclear cell preparations contained a large proportion of lymphocytes which could have influenced the generation of monocyte procoagulant activity. This is unlikely, however, since enrichment of normal blood mononuclear cells with lymphocytes from the same donor in quantities sufficient to approach the lymphocyte/monocyte ratios seen in patients did not interfere with monocyte procoagulant activity.

There are several possible explanations for the hyporesponsiveness of blood mononuclear cells from CLL patients described here. First, leukaemic B lymphocytes may inhibit the monocyte procoagulant response. This is unlikely since the generation of procoagulant activity by blood mononuclear cells from control subjects was not affected by leukaemic lymphocytes.

T lymphocytes appear to participate to some extent in the induction of monocyte procoagulant activity by various stimuli. In our study the number of T lymphocytes did not differ significantly in patients' and control incubation mixtures. Although several studies have suggested that T cells from patients with CLL are functionally normal, a possible hitherto unrecognised T cell defect cannot be excluded.

Finally in CLL the monocyte may have an intrinsic defect of the procoagulant response to endotoxin and other stimuli. Multiple monocyte enzymatic deficiencies and abnormal monocyte chemotactic response have been recently reported in CLL patients.

Our findings may have clinical implications. First they suggest that blood mononuclear cell procoagulant response to various stimuli is a useful measure to assess the monocyte functional integrity in CLL. On the other hand the reduced capacity to produce procoagulant activity may explain why patients with CLL are not prone to thrombotic complications and/or DIC despite the high incidence of severe infectious diseases.

This work was partially supported by a contract from the Italian National Research Council ("Clinical Pharmacology and Rare Disease") and by a grant of the Association of Cancer Research.

Procoagulant activity generated by patients' and control mononuclear cells after four hours of incubation with endotoxin. Upper panel: clotting time in the one-stage recalcification assay. Lower panel: cellular PCA expressed in arbitrary thromboplastin units (see Material and methods).

Procoagulant activity generation by control MNC enriched with control or patients' lymphocytes

<table>
<thead>
<tr>
<th>MNC enriched with:</th>
<th>PCA (one stage clotting time) s ± SEM</th>
<th>(n = 3) lymphocyte/monocyte ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Control lymphocytes</td>
<td>52.1 ± 2.2</td>
<td>49.8 ± 1.1</td>
</tr>
<tr>
<td>Patients' lymphocytes</td>
<td>51.6 ± 2.8</td>
<td>53.0 ± 1.9</td>
</tr>
</tbody>
</table>

shows that, whatever the lymphocyte/monocyte ratio, the generation of procoagulant activity by normal blood mononuclear cells upon stimulation with endotoxin was unaffected. In another series of experiments variable amounts of patients' lymphocytes were added to a fixed number of control blood mononuclear cells. No inhibition of endotoxin-induced procoagulant activity was observed (Table).
Judith Bagott, Gigliola Brambilla, Vanna Pistotti and Vincenzo de Ceglie helped prepare the manuscript.

References


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doi: 10.1136/jcp.36.1.37