Mouse red cell rosette formation and the colchicine sensitivity test: relative usefulness in the differential diagnosis of chronic lymphocytic leukaemia and B lymphocytic lymphoma

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SUMMARY  Mouse erythrocyte (M) rosette formation and colchicine sensitivity were compared for their ability to differentiate chronic lymphocytic leukaemia (CLL) from B cell non-Hodgkin's lymphoma (NHL) with overspill.

Twenty-two cases of CLL and eight of NHL were studied along with 31 normal adults. Results from the patients in both tests differed significantly from the controls but colchicine sensitivity failed to differentiate them further. M rosettes, on the other hand, while increased in some patients with NHL were, without overlap, much more numerous in those with CLL, and clearly distinguished the two conditions.

A significant autolymphocytotoxic effect of plasma from both study groups was also noted which was not found in the controls.

Where chronic lymphocytic leukaemia (CLL) presents with atypical morphology or a low white count and more malignant B cell non-Hodgkin's lymphomas (NHL) display blood and bone marrow dissemination, distinguishing these conditions morphologically and immunologically can be difficult. Since the prognosis and treatment are not the same a simple discriminatory test is potentially useful.

Selective ultrasensitivity of CLL lymphocytes to killing by low concentrations of colchicine was shown in 1972 by Thomson et al, and this characteristic is thought to be a sensitive method not only of detecting such abnormal cells but also of assessing the size of the population. Alternatively, more than 95% of cases of CLL have been reported to have a large proportion of peripheral lymphocytes with a specific ability to form rosettes with mouse erythrocytes; this proportion varying between 40% and 90%.

To assess the ability of these two tests to distinguish CLL lymphocytes from those of other B lymphoproliferative disorders, a study was undertaken comparing the two techniques in a selected group of patients where conventional criteria allowed a confident diagnosis of CLL or NHL.

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Material and methods

PATIENTS STUDIED

Adult patients encountered over two years were examined. Diagnosis of CLL was made on the basis of clinical findings and an increase in non-T lymphocytes staining either weakly or not at all for surface immunoglobulin. B cell NHL was diagnosed on the basis, in addition to the clinical picture, of increased abnormal lymphocytes in the blood which stained strongly for surface immunoglobulin. In all cases where the lymphocytes were tested with specific light chain typing sera their monoclonal nature was established.

Eight other patients with non-T lymphoproliferative disease encountered during the study period were excluded from analysis as their findings prevented confident classification.

Normal controls were adult members of hospital staff or outpatients with non-haematological, non-infective complaints.

TECHNIQUES

Lymphocytes were separated from 5 ml heparinised blood by centrifugation on a Ficoll-Triosil mixture, washed twice and resuspended in medium 199 at a concentration of $0.25 \times 10^6/0.4$ ml. T lymphocytes
were identified by sheep-E-rosette formation.
Surface immunoglobulin was detected by direct
immunofluorescence of $1 \times 10^6$ lymphocytes which
had been incubated at 37°C in medium 199 for at
least 30 min and washed to remove extrinsic bound
immunoglobulin.

**MOUSE (M) ROSETTE FORMATION**
Mouse red cells were obtained from CBA or C57B1
mice, which have both been shown to give the same
results. They were washed three times and made up
to 1% vol/vol in medium 199/20% fetal calf serum,
and were shown to give the same results after storing
for up to 2 weeks at 4°C. Lymphocytes were tested in
duplicate by mixing $0.25 \times 10^6$ with $0.25$ ml
mouse red cells, supplementing with 20% fetal
calf serum, centrifuging gently (200 g) for 5 min
at room temperature and then incubating at room
temperature for 1 h. After gentle resuspension
and staining with 0.3% methylene blue the cells were
examined in a haemocytometer and the percentage
of 200 lymphocytes with more than two mouse red
cells adhering was counted.

**COLCHICINE SENSITIVITY TEST**
Lymphocytes were tested in duplicate for sensitivity
to colchicine at three molar concentrations ($10^{-7}$M,
$10^{-6}$M and $10^{-5}$M) compared with a background
without colchicine. Lymphocytes ($0.25 \times 10^6$) in
medium 199 supplemented to 30% with autologous
plasma or fetal calf serum were incubated with and
without colchicine at 37°C for 24 h. The proportion
of pyknotic cells was determined on a cytocentrifuge
preparation made from each culture, wet-fixed in
Susa’s fixative for 10 min, washed in running tap
water for 15 min, stained for 2 min in Mayer’s
haemalum and washed for a further 15 min. The
slides were dried quickly with a fan and examined
“blind,” with the label obscured, under a light
microscope. Five hundred cells were examined per
slide by the same observer, and each was counted as
pyknotic or not. Pyknotic cells were shrunken,
rounded and contained densely staining nuclear
material. The mean percentage of pyknotic cells in
cultures without colchicine was subtracted from the
mean percentage pyknotic at each dose.

**STATISTICAL METHODS**
Student’s $t$ test was used to determine the significance
of the difference between mean values from patients
and controls. The paired $t$ test was used to compare
cell death in autologous plasma and fetal calf
serum. Correlations were done using Pearson’s
product moment correlation coefficient.

**RESULTS**
Twenty-two patients with CLL, eight with NHL and
31 controls were studied. The lymphocyte counts
ranged from $285 \times 10^9/l$ to $60 \times 10^9/l$ in the CLL
patients and from $74 \times 10^9/l$ to $60 \times 10^9/l$ in those
with NHL. The mean, range and standard error of
the proportion of mouse rosetting and colchicine
sensitive lymphocytes in the groups studied are shown
in the Table.

It was found at an early stage that the percentage
of cells dying in CLL or NHL plasma without the
addition of colchicine was often very high (37-95%),
much higher than the control backgrounds. Incubating
cells from nine of these patients (six CLL,
three NHL) in both autologous plasma and fetal
calf serum led to the discovery that in the latter, in
all cases, the backgrounds were much lower (11-54%)
mostly within the normal range (5-24%). The
difference between the results in the two types of
supplement was highly significant ($p < 0.001$)
indicating that patient’s plasma had a lymphotoxic
effect. Following this discovery all patient colchicine
sensitivity cultures were incubated with fetal calf
serum rather than autologous plasma as supplement.
This cytotoxic effect was not seen in the control
subjects.

Chronic lymphocytic leukaemia lymphocytes were
ultrasensitive to colchicine at all three doses com-
pared to the controls ($p < 0.001$), as were those from
the NHL patients ($p < 0.001$). The two groups of
patients did not differ significantly from each other,
however ($p > 0.1$).

Mouse E rosetting and colchicine sensitivity test results

<table>
<thead>
<tr>
<th></th>
<th>% mouse E rosettes</th>
<th>% pyknotic cells with colchicine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^{-7}$M</td>
<td>$10^{-6}$M</td>
</tr>
<tr>
<td><strong>CLL</strong></td>
<td>Mean</td>
<td>56.1</td>
</tr>
<tr>
<td>Patients SEM</td>
<td>3-3</td>
<td>7.2</td>
</tr>
<tr>
<td>Range</td>
<td>29-80</td>
<td>5-77</td>
</tr>
<tr>
<td><strong>NHL</strong></td>
<td>Mean</td>
<td>5-5</td>
</tr>
<tr>
<td>Patients SEM</td>
<td>1-9</td>
<td>6-3</td>
</tr>
<tr>
<td>Range</td>
<td>0-15</td>
<td>18-52</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>Mean</td>
<td>1-6</td>
</tr>
<tr>
<td>SEM</td>
<td>0-3</td>
<td>1-2</td>
</tr>
<tr>
<td>Range</td>
<td>0-8-5</td>
<td>0-12</td>
</tr>
</tbody>
</table>
not, nor did they overlap with the NHL values (Figure).

Finally the numbers of colchicine-sensitive and M rosetting cells in each patient were compared to each other and to the absolute lymphocyte counts (as logarithms). Although there was a tendency for the proportion of abnormal cells measured by either technique to correlate with the number of circulating lymphocytes (apart from M rosettes in NHL) this did not achieve significance \( p < 0.1, > 0.05 \). Similarly, there was no correlation between abnormal cell numbers estimated by the two techniques.

Discussion

It is clear from this study that M rosette formation can be used with reasonable confidence to distinguish CLL lymphocytes from the neoplastic cells of B cell NHL. The colchicine sensitivity test, on the other hand, cannot.

As the main object was to define a useful discriminatory test in an occasionally confusing clinical situation it would be unwise to draw any further conclusion from these findings on the specificity of either technique, or what particular cell (if any) each identifies.

![Diagram](https://example.com/diagram)

**Mouse red cell rosette numbers in patients with CLL and NHL and normal controls.**

Colchicine sensitive cells in lymphomas other than CLL have been noted before \(^5\) and are thought to represent a fairly wide variety of immature, or abnormal, B cells.\(^5\) Normal B cells, however, are not apparently colchicine-sensitive, either those circulating\(^5\) or those in lymph nodes.\(^6\) Our own results would confirm that peripheral mixed normal lymphocytes are also resistant, and so the test may be helpful in distinguishing neoplastic from reactive lymphoproliferation.

M rosette formation, in contrast, appears to be a fairly specific feature of the CLL lymphocyte and has rarely been observed in other conditions. Exceptions include plasmacytoma, Waldenström's macroglobulinaemia,\(^7\) and a proportion of patients with hairy cell leukaemia.\(^8\) As none of these disorders is likely to be confused with CLL if appropriate protein studies and cytochemistry are performed, they do not diminish the usefulness of the test. Ability to form M rosettes probably marks a fairly specific stage of B cell differentiation to which the CLL lymphocyte corresponds.

The lack of correlation between colchicine-sensitive cells and M rosettes formed by our patients and their lymphocyte counts is not surprising as a similar lack of correlation has been previously noted\(^9\) and presumably simply reflects a variation in the number of normal lymphocytes present.

The incidental discovery of a cytotoxic effect of patients' plasma on their own cells is of more interest. Other workers have noted that CLL lymphocytes survive less well in cultures without colchicine than normal lymphocytes,\(^5\)\(^9\) and a non-antibody lymphotoxic factor in serum from a patient with CLL has been demonstrated using a cytotoxicity test.\(^10\) CLL serum has also been found to inhibit lymphocyte protein and nucleic acid synthesis induced by PHA.\(^11\)\(^12\) but the nature and specificity of such inhibitory and cytotoxic activity, however, remains obscure at present.

We acknowledge the help of Dr R Walker, who provided the mouse red blood cells, and that of the physicians at the Royal Hallamshire Hospital whose patients we studied.

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Requests for reprints to: Dr JS Lilleyman, Department of Haematology, The Children's Hospital, Sheffield S10 2TH, England.
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