Impairment of neutrophil chemotaxis by serum from patients with chronic lymphoproliferative disease

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SUMMARY The sera of 74 individuals with chronic lymphoproliferative disease were screened for the presence of inhibitory activity against neutrophil chemotaxis. This was present in more than half the patients with IgA myeloma and Hodgkin's disease but was less common in chronic lymphocytic leukaemia, lymphocytic lymphoma and non-IgA paraproteinaemia. Heating the sera prior to testing frequently enhanced inhibitory activity particularly in myeloma and lymphoma.

It is well known that people with chronic lymphoproliferative disease (LPD) may have significant impairment of host defence mechanisms including that of impaired acute inflammatory response. Subjects with multiple myelomatosis (MM), chronic lymphocytic leukaemia (CLL), lymphocytic lymphoma (LL), and Hodgkin's disease (HD), have been reported to have increased susceptibility to infections. Granulocyte dysfunction may contribute to this problem especially if induced by extracellular factors associated with the underlying disorders.

The following mechanisms may be considered:

Neutropenia as a result of "marrow failure" may occur consequent upon replacement of the marrow by tumour, suppression by tumour products, or damage by the myelotoxic effects of chemotherapy.

Immunoglobulin (Ig) deficiency is particularly seen in MM and CLL. It is one of the diagnostic criteria for the former, and is present in at least half of the patients with the latter. Whatever the cause, the resultant lack of Ig, especially of IgG, leads to loss in efficiency of the classical complement activatory mechanisms and to poor opsonisation of infecting microorganisms. Complement activation is required for the optimal functioning of neutrophils via chemotaxis and phagocytosis.

Drug treatment Although the deleterious effects of drugs upon granulocyte function have seldom been considered in the selection of drugs used, they probably contribute significantly to neutrophil "dysfunction". Vinblastine is known to damage the cytoskeletal system of the leucocytes, corticosteroids in large sustained doses act on several aspects of granulocyte function and some antibiotics given routinely for infections in neutropenic patients are also reported to cause impairment of neutrophil function.

Inhibitory substances associated with a number of LPD, chiefly myeloma and Hodgkin's disease, have been reported to reduce cell responsiveness or to inactivate chemotaxins themselves. (Other tumours have been also reported to be associated with suppressor substances against chemotaxis).

We have undertaken a search for such inhibitors using a suitable method for screening as we have recently described. Some characteristics of these substances have also been noted.

Material and methods

Seventy-four patients with lymphoproliferative disease attending the Southampton group of hospitals and 13 normals were tested for the presence in their serum of inhibitory material which would impair the migration of normal neutrophils towards a chemotactic mixture (normal pooled serum). As far as possible an aliquot of serum taken before treatment was tested, but sometimes it was necessary to test patients' sera during the intervals between therapy.

TEST SERA AND NORMAL SERA Aliquots from glass-clotted whole blood were kept at -20°C until tested.
POOLED AB SERUM (PABS)
This was prepared from pools of three or more donors of AB blood group, and was used as the standard chemotaxin. The pools were all pretested to ensure that they produced a "leading front"21 (ie the distance moved by the fastest 2 cells) of at least 100 μm when used at a 10% dilution in Hanks' basal salt solution with 0-2% human serum albumin added (HBSS-HSA).

INDICATOR CELLS
These were obtained from a small panel of volunteer staff donors who were of blood group O. Twenty millilitres of blood was added to 100 I.U. heparin and then dextran-sedimented. The leucocytes were washed twice and resuspended in HBSS–HSA to give a concentration of about 4 × 10⁹ leucocytes/l.

The details of the apparatus and methods have been described elsewhere.22 Our development of the Addison and Babbage raft modification23 of Boyden's chamber24 permitted us to screen up to 15 sera simultaneously ensuring maximal similarity of experimental conditions.

We had previously described an assessment of chemotaxis based on the percentage cumulative sum of migrating cells at five different levels into a 3 μm membrane (see Fig. 1). However, this series of patients included some with extremely strong inhibition of chemotaxis and a curve instead of a straight line was obtained when cell number was plotted against distance moved. By plotting the natural logarithmic value of x (ln x), a straight line was obtained, and the gradient was easier to visualise and measure. We therefore used the formula y = m (ln x) + c, rather than y = mx + c where y = % cumulative sum of migrating cells; x = depth (μm) into membrane where cells were counted; c = constant; m = the gradient of the line that was best fit for the set of points available. Fig. 2 shows the correlation of the m values from both these assessments.

SCREENING ROUTINE

(i) Negative control
The cells were allowed to migrate towards HBSS–HSA—that is, there was no gradient and no chemotaxin. (We had already established earlier that the minimum acceptable degree of spontaneous migration was 15 μm and the maximum was 40 μm, derived from over 50 separate screening tests.

(ii) Positive controls
We used 20% and 10% dilutions of PABS (or other) in HBSS–HSA in the lower "raft" compartments. The degree of chemotaxis evoked by PABS was recorded as "m" (see above) and any changes observed in the mixtures were adjusted to a percentage change compared with 10% PABS. The 20% PABS was a second control for the experiments where test serum was incubated with PABS (see (iv) below).

Sample assays

(iii) 10% Test Serum in HBSS–HSA
This was used in the lower "raft" compartment. This tested the intrinsic chemotactic activity of the serum.

(iv) 100 μl neat test serum
This was preincubated with 100 μl of neat PABS at

![Fig. 1 A comparison of the linear plot and the semilogarithmic plot (see text).](http://jcp.bmj.com/)

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![Graph showing correlation of migration gradient by two methods of calculation.](image)

Fig. 2  Correlation of the migration gradient by two methods of calculation (the figures are in absolute values).

![Graph illustrating the migration gradient of normal neutrophils towards PABS.](image)

Table 1  Migration gradients of normal neutrophils towards PABS

<table>
<thead>
<tr>
<th>Mean ± SD</th>
<th>Range (Mean ± 2 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% in HBSS-HSA (× 42)</td>
<td>−45.0 ± 7.1 -30.8 to −59.2</td>
</tr>
<tr>
<td>20% in HBSS-HSA (× 41)</td>
<td>−43.1 ± 6.4 -30.3 to −55.9</td>
</tr>
</tbody>
</table>

![Graph illustrating the migration gradient of normal neutrophils towards normal control sera.](image)

Table 2  Migration gradients of normal neutrophils towards normal control sera

<table>
<thead>
<tr>
<th>Mean ± SD</th>
<th>Range (Mean ± 2SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% in HBSS-HSA (× 20)</td>
<td>−52.3 ± 7.1 -38.1 to −66.5</td>
</tr>
<tr>
<td>20% in HBSS-HSA (× 16)</td>
<td>−44.7 ± 5.1 -34.5 to −54.9</td>
</tr>
</tbody>
</table>

![Graph illustrating the percentage of sera showing inhibition.](image)

Table 3  Percentage of sera showing inhibition (that is, chemotaxis reduced by > 15% of the values obtained using PABS alone)

<table>
<thead>
<tr>
<th>Type (and number) of subjects screened</th>
<th>Normals (13)</th>
<th>CLL (18)</th>
<th>MM (15)</th>
<th>LL (31)</th>
<th>HD (11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>28% (5)</td>
<td>47% (7)</td>
<td>29% (9)</td>
<td>64% (7)</td>
<td></td>
</tr>
</tbody>
</table>

room temperature for half an hour. The mixture was then made up to 1 ml with HBSS-HSA. This represented a final concentration of 10% PABS and 10% test serum. The rest of the chemotaxis plate was similarly made up with other sera to be screened in the manner of steps (iii) and (iv). It was usual to screen 10 sera but up to 15 was possible; each serum was tested in duplicate.

If a serum was shown on screening to have inhibitory activity, the test was repeated to see if the activity was affected by heating (56°C for 30 min) or by overnight dialysis (at 4°C with phosphate-buffered saline at pH 7.2).

**Results**

Figure 1 illustrates how the migration curve of the semilogarithmic plot was altered from the curve to a straight line using the natural logarithm of x. Figure 2 shows that the gradients derived from each plot correlated well with each other and conclusions were interchangeable providing comparisons were made in kind—that is, the gradients from the linear plot should not be compared with those from the logarithmic plot.

Table 1 gives the computed values of m obtained
as described for 11 pools of AB serum estimated on a total of 42 occasions at 10% and 41 occasions at 20% dilution in HBSS–HSA.

Table 2 gives the similarly calculated values of m for normal control sera. This was used as a basis for comparing test sera (10%) under study. We chose a 15% (one SD) or more reduction as suggestive of the need for further study of the serum. The results were obtained from experiments separately performed in order to obtain the range for normal people.

Table 3 gives the results observed in the normal population (“well” subjects) and the four disease groups. The m values of the preincubated mixtures of PABS and test sera were compared with the m value of PABS 10% for that day. The rationale used was that: if the test sera was “inert” as far as chemotaxis was concerned then the chemotactic effect of the mixture would remain the same as that of the 10% PABS positive control and if there was an enhancement (which might be expected, due to increased serum content) it would not be significant unless greatly different from the 20% PABS positive control for the day; however, if an inhibitor activity was present the stimulant effect of PABS would be reduced. Again a greater than 15% inhibition was considered an indication to retest the serum.

Figure 3 gives the histogram results of the m values (m values are negative but in the interest of clarity the sign has been omitted throughout as it does not alter the outcome). A proportion of sera from all four disease conditions are associated with a percentage of m values showing greatly reduced intrinsic chemotactic activity of the sera.

Table 4 shows the analysis of paraproteinaemic sera by their Ig groups. IgM is not associated with inhibitory activity in any of our cases.

Finally, Table 5 shows the results of the partial characterisation studies performed on some of the test sera. (Not all the positive sera could be retested because some stored aliquots were insufficient for these procedures.)

**Table 4**  Incidence of inhibition of chemotaxis in paraproteinaemia

<table>
<thead>
<tr>
<th>IgA myeloma (9)</th>
<th>Non-myeloma with IgA excess (2)</th>
<th>IgG myeloma (6)</th>
<th>Lymphoma with IgM excess (4)</th>
<th>IgA* (2)</th>
<th>IgG* (2)</th>
<th>α cells</th>
<th>γ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>56% (5)</td>
<td>100% (2)</td>
<td>33% (2)</td>
<td>0</td>
<td>&quot;100%&quot;</td>
<td>0</td>
<td>&quot;100%&quot;</td>
<td></td>
</tr>
</tbody>
</table>

IgA* = purified IgA paraprotein.
IgG* = purified IgG paraprotein.
α cells = sonicated cytoplasm of IgA producing cells.
γ cells = sonicated cytoplasm of IgG producing cells.
"100%" = complete inhibition of PABS.

**Table 5**  Effect of storage, dialysis and heating on serum inhibitors

<table>
<thead>
<tr>
<th>Groups retested</th>
<th>Storage</th>
<th>Dialysis</th>
<th>Heating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>NC (3)</td>
<td>NC (3)</td>
<td>NC (3)</td>
</tr>
<tr>
<td>Diseased, but “negatives” on screening</td>
<td>NC (3)</td>
<td>†33% (3)</td>
<td>†66% (3)</td>
</tr>
<tr>
<td>MM (IgA) inhibitors</td>
<td>†100% (4)</td>
<td>Variable</td>
<td>†75% (4)</td>
</tr>
<tr>
<td>MM (IgG) inhibitors</td>
<td>Variable</td>
<td>Variable</td>
<td>†100% (3)</td>
</tr>
<tr>
<td>LL inhibitors</td>
<td>†100% (3)</td>
<td>†100% (2)</td>
<td>Variable</td>
</tr>
<tr>
<td>CLL inhibitors</td>
<td>†100% (3)</td>
<td>†100% (2)</td>
<td>Variable</td>
</tr>
<tr>
<td>HD inhibitors</td>
<td>†80% (5)</td>
<td>NC (3)</td>
<td>Variable</td>
</tr>
</tbody>
</table>

NC = no change.
% ( ) = percentage of number retested.
† = loss or reduction of inhibitory activity.
= emergence or enhancement of inhibitory activity.

**Discussion**

Significant inhibitory activity was not observed in normal sera; none was induced by storage, dialysis, nor by heating of aliquots used for preincubated mixtures. Sera from 7/15 myeloma patients were inhibitory, this effect increasing after storage, or after heating. Heated, stored sera were the most inhibitory.

In other groups of patients, storage seemed to cause a loss of inhibitory activity. This, however, could be restored by heating, particularly in those sera from the lymphoma group. Thus it would seem that in these patients there may be two components—one labile and observed in the fresher samples only, and the other heat-enhanceable and survives storage.
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We suggest that in the myeloma group, storage and heating enhanced inhibitory activity, or alternatively caused a diminution in native “antagonists” to the inhibitory activity. This latter view is in accordance with that held by others and van Epps and Williams have demonstrated that although normal sera rarely develop inhibitory activity even after heating, sera from patients with initially demonstrable chemotactic inhibitory activity showed a considerable increase in activity after heating, and additional individuals with chemotactic inhibitory activity were detected by this means.

Conclusions

Many patients with a variety of lymphoproliferative disorders show significant serum inhibitors to chemotaxis and these are usually enhanced by heating sera in vitro. At least two types of inhibitory activity may be present in the non-myeloma lymphoproliferative disorders studied in vitro. Because of the different properties of such inhibitors their screening should include heating of the test serum which might unmask some inhibitors.

An awareness of neutrophil dysfunction in such patients and their consequent vulnerability to infection on this ground as well as the others mentioned is important.

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References


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