Lymphokine-induced macrophage aggregation: involvement of cyclic-GMP and microtubules

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Abstract

Human lymphokine (LK) is known to induce guinea-pig macrophage aggregation. This effect can be quantitatively measured with a Born modified platelet aggregometer. This method has been well correlated with the state of delayed hypersensitivity. Previous findings about the mechanism of action of the aggregated indicated that this aggregation is not due to an increase in the cellular level of c-AMP. It is doubtful whether c-AMP is a messenger of the LK action. Using a radio-immunonassay, small significant increases were found in c-GMP levels of LK-aggregated macrophages. In addition, exogenous dibutyryl c-GMP and carbamylcholine induced a macrophage aggregation, as did the divalent cation ionophore A-23187. These data, together with the fact that LK-induced macrophage aggregation is inhibited by colchicine and at 0°C, suggest that microtubule polymerization is involved in this process.

The biological activities of lymphokines (LK) are increasingly numerous. The formation of cellular clumps is one of these manifestations. Aggregation of macrophage or neutrophil polymorphonuclear leukocyte (PMN) has been associated with lymphokine starting from the classical paper of DAVID et al. in 1964 [6]. Because of the absence of a reliable method for measuring this effect, very few authors gave attention to it. We have recently developed a method for the quantitation of macrophage aggregating factor (MAF) by measuring the light absorbance of cell suspensions using a modified Born platelet aggregometer [15]. In comparison with the most common semi-quantitative method for assessing LK activity, i.e. the inhibition of spontaneous migration of indicator cells induced by migration inhibitory factor (MIF), the nephelometric method offers practical advantages. It is easy to set up, quantitative, reproducible and more sensitive. Furthermore, it allows the study of early events of LK activity: aggregation is maximal at 30 min after addition of preformed LK. The prevalent opinion is that macrophage aggregation is merely a particular way in which MIF affects macrophage.

Another field where cellular clumps are involved has recently received attention. Plugging of small blood vessels by leukocyte aggregates is supposed to be a mechanism of tissue injury in many non-immune inflammatory diseases. This plug has been implicated in the increased vascular permeability classically associated with acute inflammation. Chemotactic factors (formyl-methionyl-leucyl-phenylalanine, C5aFr), induce human neutrophil aggregation in vitro. When infused into rabbits, these same chemotactic factors induce neutropenia and monocytopenia. It is thought that similar events can occur in humans during states such as pulmonary dysfunction in haemodialysed patients, sudden blindness with retinal infarction after trauma or acute pancreatitis (Purtscher's syndrome), myocardial infarction, adult respiratory distress syndrome, endotoxaemia, sepsis and serum sickness [4, 10]. Because of this, and because aggregation is such a non-specific parameter, we have carried out studies on the mechanism of LK-induced macrophage aggregation.

We have previously shown that macrophage aggregation is not a passive agglutination but is dependent on the metabolic integrity of the macrophage. This has been determined by the effect of the glycolytic inhibitors 2-deoxyglucose and iodoacetamide which inhibited LK-induced macrophage aggregation [11]. This phenomena seems dependent upon the presence of calcium and magnesium ions. We have found that calcium ionophore A23187 (a lipophilic bivalent cation capable of loosely complexing with Ca2+ and transporting it into the cell) caused a large aggregation at 10−6 mol/l, similar to that induced by LK, and this was reversed in the presence of EDTA [11]. It has previously been suggested that LK induces an influx of divalent cations as a primary event [7]. The intracellular level of Ca2+ has also been shown to regulate human PMN cell aggregation induced by chemotactic factors and it has been suggested that Mg2+ may foster the formation of cell–cell adhesions [11]. Ca2+ can be considered as a coupler of cyclic nucleotide formation or microtubule assembly and Pick et al. [11] noted that the observed polymerization of macrophage tubulin may be mediated by either high or low intracellular Ca2+ levels together with changes in the cyclic-GMP/cyclic-AMP ratio.

Experiments in which exogenous c-AMP and agents by leukocyte aggregates is supposed to be a mechanism of tissue injury in many non-immune inflammatory diseases. This plug has been implicated in the increased vascular permeability classically associated with acute inflammation. Chemotactic factors (formyl-methionyl-leucyl-phenylalanine, C5aFr), induce human neutrophil aggregation in vitro. When infused into rabbits, these same chemotactic factors induce neutropenia and monocytopenia. It is thought that similar events can occur in humans during states such as pulmonary dysfunction in haemodialysed patients, sudden blindness with retinal infarction after trauma or acute pancreatitis (Purtscher's syndrome), myocardial infarction, adult respiratory distress syndrome, endotoxaemia, sepsis and serum sickness [4, 10]. Because of this, and because aggregation is such a non-specific parameter, we have carried out studies on the mechanism of LK-induced macrophage aggregation.

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raising its intracellular level were added to macrophages together with LK indicate a possible antagonistic effect of c-AMP towards LK-induced macrophage aggregation. Thus db-c-AMP plus theophylline and L-isoproterenol but not D-isoproterenol (the biologically inactive isomer) significantly depressed LK-induced macrophage aggregation [16]. Furthermore, LK had no effect on macrophage c-AMP levels after 30 min (Table 1). Many workers have previously studied the possible involvement of c-AMP in the mechanism of action of MIF: c-AMP decreased MIF activity and levels after 30 min (Table 1). Many workers have previously studied the possible involvement of c-AMP in the mechanism of action of MIF: c-AMP decreased MIF activity and levels after 30 min (Table 1). In view of these results, it is thought unlikely that c-AMP acts as a second messenger. On the other hand, it is of interest that we have noted a significant increase in c-GMP levels 30 min after addition of LK (Table 1). In this context, raised c-GMP levels have been obtained in human PMN 3 min after the addition of LIF [2]. It is known that surface phenomena mediated by alterations in microtubular function are associated with increased c-GMP levels [12]. Moreover, while db-c-GMP had no effect on LK-induced macrophage aggregation, it was possible to produce aggregation with db-c-GMP at $10^{-2}$ and $10^{-6}M$.

The fact that ionophore A 23187, and LK-induced macrophage aggregation are inhibited by colchicine (a microtubular disruptive agent) implies that microtubules may be directly involved in this process. In this respect, it has been already suggested that tubulin polymerization is an essential step in the mechanism of migration inhibition and that this may be mediated via Ca$^{2+}$ and c-GMP [13]. Furthermore, spontaneous aggregation of human PMN is also inhibited by colchicine at $10^{-6}-10^{-4}M$ [8]. But it is interesting to note that this drug had no effect on aggregation of human PMN in response to chemotactic factor [9] and only moderate attenuation on aggregation of PMN cell induced by C5a at $10^{-2}-10^{-4}M$ [5].

Aggregation is a non-specific parameter but its mechanism seems to differ from cell to cell between inducing agents. Our results indicate that microtubules may well be involved in the mechanism of action of MAF possibly by the well-known effects of microtubules on cell surface topography [17].

Chemotactic factor-induced PMN aggregation is temperature dependent [9] as is LK-induced macrophage aggregation, the maximum aggregating activity being obtained at $37^\circ C$. In this context, it is of interest to note that one of the most effective means of causing tubulin depolymerization is cooling the cells to $4^\circ C$. We have observed a spontaneous macrophage aggregation which is optimal at $37^\circ C$ and totally absent at $4^\circ C$. This natural tendency for macrophages to aggregate in vitro is potentiated by MAF. We have found a significant decrease in c-GMP levels of temperature-aggregated macrophages (Table 2). These levels (3.10 pmol/mg protein) are less than those obtained with lymphokine-induced macrophage aggregation (4.8 pmol/mg protein). Thus, it could be that lymphokine acts as a constraint on c-GMP consumption.

The results presented here suggest that LK foster microtubule generation and tubulin polymerization by a mechanism dependent upon an elevation in the cellular level of c-GMP.

Lymphokine was prepared by stimulating human lymphocytes with concanavalin A as described [16]. Aggregation was measured as described [15]. Cyclic nucleotide measurements were done by radioimmunoassay with the cell suspensions which gave the maximum aggregation for a given LK concentration. Results are means ± S.E.M. of five separate preparations.

### Table 1

<table>
<thead>
<tr>
<th>Concentration of LK stock solution</th>
<th>Aggregation (cm) test-control</th>
<th>c-AMP</th>
<th>T</th>
<th>c-GMP</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>8.1 ± 1.9</td>
<td>15.40 ± 0.76</td>
<td>17.50 ± 2.32</td>
<td>3.50 ± 0.50</td>
<td>4.80 ± 0.90a</td>
</tr>
<tr>
<td>14</td>
<td>8.3 ± 0.8</td>
<td>16.67 ± 1.20</td>
<td>18.70 ± 1.64</td>
<td>3.15 ± 0.16</td>
<td>3.75 ± 0.21b</td>
</tr>
<tr>
<td>8</td>
<td>6.2 ± 1.2</td>
<td>18.25 ± 0.92</td>
<td>18.00 ± 1.04</td>
<td>3.19 ± 0.24</td>
<td>3.19 ± 0.24</td>
</tr>
<tr>
<td>2</td>
<td>5.6 ± 1.4</td>
<td>17.20 ± 1.47</td>
<td>18.60 ± 1.17</td>
<td>3.35 ± 0.22</td>
<td>3.66 ± 0.37</td>
</tr>
<tr>
<td>0.8</td>
<td>2.7 ± 0.6</td>
<td>17.84 ± 1.57</td>
<td>16.50 ± 1.19</td>
<td>3.25 ± 0.35</td>
<td>3.32 ± 0.35</td>
</tr>
</tbody>
</table>

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* Significant increase ($p < 0.05$). b Significant increase ($p < 0.01$) by paired Student's $t$-test.

### Table 2

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Aggregation</th>
<th>c-GMP levels (pmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>-</td>
<td>6.85 ± 1.35</td>
</tr>
<tr>
<td>37</td>
<td>+</td>
<td>3.10 ± 0.40</td>
</tr>
</tbody>
</table>

Results are means ± S.E.M. of six separate preparations.

* $p < 0.05$.

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### References

