Stimulation of Interferon Production in Mice and in Mouse Spleen Leukocytes by Analogues of BL-20803

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Various structural analogues of the interferon inducer BL-20803 exhibited close agreement between ability to stimulate interferon production in the intact mouse and in cultures of spleen adherent leukocytes.

In several reports we have suggested that the interferon-eliciting activity of the low-molecular-weight compound BL-20803 is directed at certain leukocytes, of which the adherent (A) spleen cell is the paradigm (2, 9). Among the lines of evidence offered, we have shown that spleen A cells, when recovered from mice treated with the compound and then cultured in vitro, actively produced interferon. Furthermore, direct exposure of spleen A cells to BL-20803 elicited about 2.5 to 8 times as much interferon as did treatment of spleen nonadherent cells. Finally, pretreatment of mice with rabbit antiserum to mouse spleen A cells inhibited their subsequent interferon response to BL-20803 by at least 65%.

The synthesis in our laboratories of a large number of analogues of BL-20803 (compound 5 in Table 1), which have varying abilities to elicit interferon in mice (1), has provided us an additional opportunity to test the involvement of the spleen A cell in the animals’ interferon responses by comparing interferon production in the whole animal and in spleen A cell cultures stimulated by the various analogues. In an exact correlation, there would be the same rank order of activity between in vivo and in vitro treatment. The data in Table 1 compare the ability of compounds to stimulate interferon in the mouse in terms of minimal effective dose (MED) with interferon stimulation of isolated A cells at two concentrations of compounds, 3.1 and 0.78 μg/mg. In general, those compounds most active in vivo were the most potent interferon inducers in vitro. None of the compounds that failed to stimulate A cells were active in the intact animal. On the other hand, a few of the analogues, e.g., compounds 15, 16, 17, 19, and 24, appeared to elicit at least a small amount of interferon from A cells but had no discernible stimulating effect in intact mice. In the particular case of compound 17, the in vitro activity was as pronounced as the activities of those analogues that were unequivocally stimulatory in the intact mouse. It seemed likely that these results reflected interference by somatic processes with the compound’s interaction with the target cells in adequate concentration or in an active form. As mouse blood is known to be rich in esterases, it seemed possible that in the body the methyl ester at the 7 position of compound 17 was converted to the corresponding carboxylic acid, thus forming the known inactive compound 18 (see Table 1).

We tested this possibility in the following way. A stock solution of compound 17 was prepared in phosphate-buffered saline at pH 7.2 containing 1.8 mg of compound per ml. A 0.5-ml portion of the solution was distributed to separate tubes, each containing an equal volume of (i) unheated mouse serum; (ii) mouse serum that had been heated to 56°C for 30 min, or (iii) phosphate-buffered saline. All samples were incubated at 36°C for 30 min. Portions of 0.1 or 0.2 ml were diluted in RPMI medium containing 10% fetal bovine serum, and various dilutions were added to A cell cultures to test their ability to stimulate interferon. These dilutions were selected such that at least one dilution would contain a concentration of compound 17 within its nontoxic, active range of approximately 0.4 to 6 μg/ml, as has already been reported for BL-20803 (2). At equal dilutions of 1/270, there appeared to be more active compound in the heated mouse serum than in the unheated serum, as indicated in Table 2 by the threefold greater amount of interferon stimulated by the heated serum sample. More precise quantitation was not possible because this biological test has two limitations: (i) at high or borderline toxic levels, the compound either abolished or depressed interferon yields; and (ii) only that amount of compound in excess of the binding capacity of the serum contributed to biological activity (0.5 ml of serum could bind at least 100 μg of compound 17).
### Table 1. Interferon-stimulating ability of BL-20803 (compound 5) and its analogues

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>R</th>
<th>R¹</th>
<th>X</th>
<th>Interferon stimulation</th>
<th>A cells (U/10⁶ cells)</th>
<th>Mouse MED (mg/kg)</th>
<th>M (µg/mg)</th>
<th>L (µg/mg)</th>
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<tr>
<td>1</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>5,7-di-CH₃O</td>
<td>25 to 50</td>
<td>20 67</td>
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<td>2</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>6,7-di-CH₃</td>
<td>50</td>
<td>29 14</td>
<td></td>
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<tr>
<td>3</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>6,7-di-CH₃O</td>
<td>50</td>
<td>27 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>7-CH₃O</td>
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<td>57</td>
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<tr>
<td>5</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>H</td>
<td>200</td>
<td>42 5</td>
<td></td>
<td></td>
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<tr>
<td>6</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>H</td>
<td>200</td>
<td>41 10</td>
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<tr>
<td>7</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>H</td>
<td>200</td>
<td>20 10</td>
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<td>8</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
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<td>7-CH₃OH</td>
<td>200</td>
<td>7 3.4</td>
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<td>9</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>6-C₆H₅</td>
<td>200</td>
<td>7 &lt;1.5</td>
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<td>10</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>6-C₂H₅</td>
<td>400</td>
<td>10 2</td>
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<tr>
<td>11</td>
<td>HNCH₂CHOHCH₂N(C₆H₅)₂</td>
<td>CH₃</td>
<td>7-Cl</td>
<td>400</td>
<td>16 7</td>
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<td>12</td>
<td>S-(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>H</td>
<td>&gt;50⁶</td>
<td>&lt;1 &lt;1</td>
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<tr>
<td>13</td>
<td>HNCH₂CHOHCH₂N(C₆H₅)₂</td>
<td>CH₃</td>
<td>H</td>
<td>&gt;100⁶</td>
<td>&lt;2 &lt;2</td>
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<td>14</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>5-Cl,8-CH₃O</td>
<td>&gt;100⁶</td>
<td>1.8 1</td>
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<td>15</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>7-CF₃</td>
<td>&gt;100⁶</td>
<td>3 2.7</td>
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<tr>
<td>16</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>7-OH</td>
<td>&gt;300</td>
<td>7 3.6</td>
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<td>17</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>7-CO₂CH₃</td>
<td>&gt;300</td>
<td>40 14</td>
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<td>18</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>7-COOH</td>
<td>&gt;300</td>
<td>&lt;3 &lt;3</td>
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<tr>
<td>19</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>Cl</td>
<td>&gt;400</td>
<td>15 &lt;3</td>
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<tr>
<td>20</td>
<td>NH₂</td>
<td>CH₃</td>
<td>H</td>
<td></td>
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<td>21</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>6-CH₃C-NH</td>
<td>&gt;400</td>
<td>&lt;3 &lt;3</td>
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<tr>
<td>22</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>5-NO₂</td>
<td>&gt;400</td>
<td>&lt;3 &lt;3</td>
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<td>23</td>
<td>H</td>
<td>CH₃</td>
<td>7-CH₃CH₃NH</td>
<td>&gt;400</td>
<td>2 2</td>
<td></td>
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<tr>
<td>24</td>
<td>HN(CH₂)₃N(CH₃)₂</td>
<td>CH₃</td>
<td>5-NH₂</td>
<td>&gt;400</td>
<td>3.4 2</td>
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<tr>
<td>25</td>
<td>H</td>
<td>CH₃</td>
<td>5-NH₂CH₃N(CH₃)₂</td>
<td>&gt;400</td>
<td>1.6 1</td>
<td></td>
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</tr>
</tbody>
</table>

*a* Toxic at 100 mg/kg.

*b* Toxic at 300 mg/kg.

*c* Toxic at 200 mg/kg.

The remaining 0.8 to 0.9 ml of unheated and heated mouse serum samples of compound 17 were lyophilized, and the residues were each extracted with chloroform containing 10% (vol/vol) triethylamine. As shown by vapor-phase chromatographic analysis, the unheated and heated serum samples contained 68 and 273 µg of unaltered ester, respectively. The residue from the unheated serum sample remaining after extraction was suspended in chloroform containing N,N-dimethylformamide, and this supernatant was shown to contain less than 1 µg of compound 17. The suspension was then acidified with 1 N hydrochloric acid and treated with diazomethane to convert any carboxylic acid present to the corresponding methyl ester. The mixture was evaporated, and the residue was extracted as above with chloroform-triethylamine. By vapor-phase chromatographic analysis, this residue now contained 215 µg of compound 17. Thus, active esterases in the unheated serum converted a significant proportion of the available compound 17 to the inactive 7-carboxyl derivative, compound 18. The 68 µg of unaltered ester detected in the unheated serum sample probably represented the
Table 2. Effect of mouse serum on the ability of compound 17 to stimulate interferon production by mouse spleen A cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Interferon production (U/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unheated mouse serum</td>
<td>1/30</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>1/90</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>1/270</td>
<td>20.8</td>
</tr>
<tr>
<td>Heated mouse serum</td>
<td>1/90</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>1/270</td>
<td>61.9</td>
</tr>
<tr>
<td></td>
<td>1/810</td>
<td>10.7</td>
</tr>
<tr>
<td>Phosphate-buffered saline</td>
<td>1/270</td>
<td>12.3a</td>
</tr>
<tr>
<td></td>
<td>1/810</td>
<td>12.3</td>
</tr>
<tr>
<td></td>
<td>1/2,430</td>
<td>2.9</td>
</tr>
</tbody>
</table>

* This sample was partially toxic to the spleen A cells.

amount by which the hydrolytic capacity of the esterases was exceeded. In another study, we found that 300 μg of compound 17 was completely inactivated by the enzymatic activity contained in 0.5 ml of unheated mouse serum.

The results support our general conclusion from prior studies that the spleen A cell, possibly representative of a certain class of fixed macrophages, is readily stimulated by BL-20803 to produce interferon. This observation is now extended to other active, structurally related compounds. The A cell response appears to be a more sensitive indication of a compound’s interferon-inducing potential than the intact mouse, obviously due to the optimum conditions in the in vitro culture system for unimpeded contact between compound and target cell. The structural specificity of the BL-20803 series of compounds is also emphasized by the loss of activity resulting from the relatively small change in the substituent grouping when the 7-methyl ester is converted to the 7-carboxyl derivative.

LITERATURE CITED

