Interference of Levamisole With Inhibition of E-Rosette Formation by Hodgkin’s Disease and Systemic Lupus Erythematosus Cytotoxic Sera

By Gennaro S. Del Giacco, Sergio Tognella, Anna L. Leone, Francesca Locci, Paolo Cornaglia, Angela Sangiuolo, and Vincenzo Grifoni

A new system has been used to test the influence of levamisole on T-cell function. Evidence has been produced that prior exposure to the drug “protects” normal human peripheral blood lymphocytes from the inhibition that cytotoxic sera from patients with Hodgkin’s disease and systemic lupus erythematosus exhibit on their E-rosette-forming capacity. Also,damage of this T-cell function already induced by Hodgkin’s sera may be partially corrected.

LEVAMISOLE, an anthelmintic drug, is known to influence the immune response, mainly in its cell-mediated arm. Improvement of clinical status and/or in vivo delayed-type reactions and in vitro E-rosette-forming ability of T lymphocytes have been observed after administration of levamisole to patients with Hodgkin’s disease (HD) and patients with other diseases involving cytoimmunodeficiency. Moreover, levamisole proved capable of directly increasing E-rosette formation when added in vitro to lymphocytes from patients with Hodgkin’s disease, or patients with solid tumors or to lymphocytes “depressed” through various mechanisms.

Impairment of the E-rosette-forming ability of lymphocytes from patients with HD has been extensively reported and variously interpreted. Studies from our laboratories have shown that the majority of HD sera exhibit complement-dependent autolymphocytotoxic and allogeneicFUNCTIONAL DEPRESSION. These and other findings suggest that a cytotoxic antibody may be responsible for T-cell functional depression.

Recently, sera from patients with systemic lupus erythematosus (SLE) containing lymphocytotoxins have been shown capable of inhibiting E-rosette formation by NPL.

This study was designed to investigate whether or not levamisole interferes, in vitro, with the activity on NPL membrane of cytotoxic sera from patients with HD or SLE.
LEVAMISOLE CYTOTOXIC SERA E-ROSETTES

MATERIALS AND METHODS

Donors. Selection of lymphocyte donors was made in order to have at disposal human peripheral blood lymphocytes (NPL) with different HLA-A and HLA-B specificities. This was assessed by microlymphocytotoxicity test23 carried out using 75 monospecific sera (NIH, Bethesda, Md.). A panel of NPL from 20 normal subjects was selected.

Complement-dependent lymphocytotoxicity of sera was evaluated against the NPL panel. Sera were selected that proved able to kill more than 15% of cells for each of the 20 NPL samples tested. As a result, serum donors were 16 patients with HD and 10 patients with SLE, all with active disease. HD patients were at different stages (II: 2; III: 10; IV: 4) and of various histologies (lymphocyte predominance: 3; nodular sclerosis: 3; mixed cellularity: 5; lymphocyte depletion: 5); 13 of 16 had not yet been treated, and 3 had been out of treatment for at least 30 days. All SLE patients were untreated. Control noncytotoxic normal serum donors were 9 healthy volunteer subjects.

Lymphocyte preparation. Mononuclear cells were separated from heparinized venous blood on Ficoll-Hypaque (Lymphoprep, Nyegaard, Oslo, Norway) gradient. Macrophages were allowed to adhere to plastic petri dishes at 37°C for 30 min. Nonadherent cells were then removed, washed thrice, and suspended in Hanks solution at 1 × 10⁶ cells/ml. Viability of lymphocytes, as assessed by trypan blue exclusion, was greater than 90%.

E-rosette-forming cells. E-rosette-forming cells were determined following the method of Papamichail et al.,24 with minor modifications.

Incubation. For incubation with sera the ratio between reactants was 300 μl of lymphocyte suspension (containing 3 × 10⁶ cells) to 150 μl of undiluted serum. Incubation took place at the previously determined optimal conditions for cytotoxic activity (HD serum 37°C for 2 hr; SLE serum 4°C for 30 min; control serum 37°C for 2 hr).

Treatment with levamisole. For treatment with levamisole (Janssen Pharmaceutica, Beerse, Belgium) the ratio was 300 μl of lymphocyte suspension (containing 3 × 10⁶ cells) to 1 ml of levamisole solution (40 μg/ml phosphate-buffered saline). Since previous experiments had demonstrated that the influence of levamisole on E-rosette formation was not substantially affected by temperature and time of contact, these factors were those already indicated as optimal for the particular serum, HD or SLE, involved in the experiment.

For the experiments in which incubation with serum and treatment with levamisole took place at the same time, 300 μl of lymphocyte suspension were added to 150 μl of undiluted serum plus 1 ml of levamisole solution. Temperature and time of contact were those optimal for serum.

Outline of experiments. Four aliquots of each lymphocyte individual sample were prepared. The first was used to determine the percentage of "native" E-rosette-forming cells (ERFC) (Table 1, experiments 1, 7, and 13). The second was treated with levamisole, washed thrice in Hanks solution, and then subdivided into two parts; one was used for control (experiments 2, 8, and 14) and the other for incubation with serum (experiments 4, 10, and 16). The third aliquot was incubated with serum, washed thrice in Hanks solution, and then subdivided into two parts, one for control (experiments 3, 9, and 15) and the other for treatment with levamisole (experiments 6, 12, and 18). The fourth aliquot was simultaneously treated with levamisole and incubated with serum (experiments 5, 11, and 17). For all samples, before ERFC determination, cells were washed thrice in Hanks solution, and viability was again assessed by trypan blue exclusion.

RESULTS

Experiments 1–6 were done using 16 NPL and 16 HD cytotoxic sera. Experiments 7–12 were done using 10 NPL and 10 SLE cytotoxic sera. Experiments 13–18 were done using 9 NPL and 9 noncytotoxic sera. Each experiment involving serum was replicate with all sera available: ERFC percentages shown in Table 1 are means ± SD of, respectively, 16 (HD serum), 10 (SLE serum), and 9 (noncytotoxic serum) individual values. The E-rosette-forming ability of NPL behaved as follows. (1) Levamisole alone had no influence (experiments 2, 8, and 14). (2) HD and SLE cytotoxic sera proved strongly inhibitory (experiments 3 and 9). (3) Previous treatment with levamisole completely protected ERFC from the inhibitory activity of HD cytotoxic sera (experiment 4). (4) Contrariwise, when
Table 1. Effect of Levamisole In Vitro on E-Rosette Formation by Normal Peripheral Lymphocytes Submitted to the Actions of Cytotoxic Sera From Patients With HD (16) or SLE (10) and of Noncytotoxic Sera From Normal Subjects (9)

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Incubation With Serum</th>
<th>Treatment With Levamisole</th>
<th>Percentage ERFC (mean ± SD)</th>
<th>Significance of Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>Untreated</td>
<td>57 ± 6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>Treated</td>
<td>56 ± 5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>HD</td>
<td>Untreated</td>
<td>50 ± 11</td>
<td>1-3 &lt;0.01</td>
</tr>
<tr>
<td>4</td>
<td>HD</td>
<td>Treated</td>
<td>51 ± 9</td>
<td>3-4 &lt;0.01</td>
</tr>
<tr>
<td>5</td>
<td>HD</td>
<td>Untreated</td>
<td>44 ± 9</td>
<td>1-5 &lt;0.01</td>
</tr>
<tr>
<td>6</td>
<td>HD</td>
<td>Treated</td>
<td>44 ± 6</td>
<td>3-6 &lt;0.01</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>Untreated</td>
<td>65 ± 5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>Treated</td>
<td>65 ± 5</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>SLE</td>
<td>Untreated</td>
<td>28 ± 7</td>
<td>7-9 &lt;0.01</td>
</tr>
<tr>
<td>10</td>
<td>SLE</td>
<td>Treated</td>
<td>44 ± 8</td>
<td>7-10 &lt;0.01</td>
</tr>
<tr>
<td>11</td>
<td>SLE</td>
<td>Untreated</td>
<td>27 ± 6</td>
<td>7-11 &lt;0.01</td>
</tr>
<tr>
<td>12</td>
<td>SLE</td>
<td>Treated</td>
<td>27 ± 7</td>
<td>7-12 &lt;0.01</td>
</tr>
<tr>
<td>13</td>
<td>None</td>
<td>Untreated</td>
<td>61 ± 9</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>None</td>
<td>Treated</td>
<td>60 ± 8</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>N</td>
<td>Untreated</td>
<td>58 ± 10</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>N</td>
<td>Treated</td>
<td>52 ± 10</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>N</td>
<td>Untreated</td>
<td>63 ± 13</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>N</td>
<td>Treated</td>
<td>59 ± 14</td>
<td></td>
</tr>
</tbody>
</table>

*Each experiment done with 16 individual sera.

*Each experiment done with 10 individual sera.

*Each experiment done with 9 individual sera. N = normal.

*Prev. = previously; Sim. = simultaneously; Subs. = subsequently.

Contact with drug was simultaneous (experiment 5) or followed (experiment 6) incubation with HD sera, their inhibitory activity was not prevented, but only slightly, although significantly, weakened. (5) Previous treatment with levamisole did not completely preserve ERFC from the inhibitory activity of SLE cytotoxic sera (experiment 10); only partial, although significant, protection was observed. (6) On the contrary, when contact with drug was simultaneous (experiment 11) or followed (experiment 12) incubation with SLE sera, their inhibitory activity was unaffected at all. (7) In control experiments using noncytotoxic sera from normal subjects (experiments 15–18) ERFC percentage remained unchanged (basal value 61 ± 9).

DISCUSSION

Levamisole is known to improve the E-rosette-forming ability of peripheral lymphocytes from subjects with diseases in which such property is impaired. Since cytotoxic sera from HD and SLE patients are able to inhibit E-rosette formation by NPL previously submitted to their action, we exploited this phenomenon to study the influence of levamisole on this T-cell function.

The results obtained confirmed that levamisole per se does not affect E-rosette-forming ability of NPL, which, on the other hand, is strongly inhibited by highly cytotoxic HD and SLE sera.
As far as the interference of levamisole with this phenomenon is concerned, we could demonstrate that previous treatment with the drug prevents such inhibitory activity completely for HD sera and partially for SLE sera. However, when contact with drug was simultaneous or followed incubation with cytotoxic sera, "protection" of ERFC from HD sera was only partial and from SLE sera was no more detectable.

Data from the literature show that levamisole corrects deficient responsiveness of T cells in terms not only of E-rosette-forming ability but also of PHA-induced phenomena. For these effects to be produced, a relatively short time of contact in vitro is required, so that a direct action on T-cell membrane must be postulated. Receptor sites might be unmasked and/or made more accessible through a molecular rearrangement of cell membrane. The promptness of such effects might be related to the changes in intracellular cyclic nucleotide levels induced by the drug.25

As far as our results are concerned, it seems reasonable first to assume that the inhibitory activity of HD and SLE cytotoxic sera involves in some way T-cell receptors for sheep red blood cells. Second, since prior contact with levamisole results in (total or partial) prevention of such inhibitory activity, some sort of competition between the drug and cytotoxic sera at the cell membrane receptor level must be admitted. However, whereas the results were clear-cut for SLE sera, in the sense that their inhibitory activity was counteracted only by previous levamisole treatment, partial correction was obtained for HD sera even when the contact with drug was postponed. These findings could indicate that inhibiting factors contained in HD and SLE cytotoxic sera differ from each other in binding strength or in stability of such binding. Reversibility of HD sera inhibitory activity, on the other hand, must be postulated in order to explain the effect of levamisole in HD both in vivo and in vitro.

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REFERENCES

9. Grifoni V: Human lymphoid cell studies and


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