Immunosuppressive Action In Vitro by a Bovine Spleen Extract

V. H. Morales and E. García-Giralt

Institut de Cancérologie et d’Immuno génétique, Hôpital Paul Brousse, 94-Villejuif, France

An extract from bovine spleen which has been shown to have immunosuppressive properties, inhibited the stimulation of DNA synthesis in mouse lymphocytes by phytohaemagglutinin and by allogeneic cells. It was also found that the lymphocytes from animals treated with the extract did not respond in vitro to an antigenic stimulation.

We have previously reported that a protein obtained from bovine spleen (BSE) by alcoholic fractionation\(^1\) had immunosuppressive properties as measured in vivo by the graft versus host reaction and in vitro by the haemolsyin plaque forming test\(^2,3\). This protein was also found to be tissue specific but not species specific concerning the inhibition of DNA synthesis in vivo\(^4\).

Stimulation of lymphocytes in vitro either by phytohaemagglutinin (PHA) or by histocompatibility antigens in mixed lymphocyte culture (MLC) is considered representative of the delayed hypersensitivity reaction in vivo\(^4\). The present study demonstrates the inhibition of these two reactions by BSE.

Material and Methods

**BSE preparation**

Bovine spleen obtained from 1 — 2 years old animals was dissected and the capsule and fibrous tracts removed. The cells from the remaining tissue were washed 3 times with saline solution resuspended in 100 ml of distilled water and left for one hour. The suspension was then homogenized during 1 min with an Ultra-Turrax homogenizer, left 30 min and then centrifuged at 17,000 rpm for 45 minutes. The pellet was discarded and the supernatant dialysed against deionized water for 24 hours. The precipitation produced by dialysis was eliminated by centrifugation. Ethanol (96%) was then added drop by drop to the supernatant until a 75% ethanol concentration was reached. The suspension was centrifuged at 10,000 rpm for 20 min, the precipitate discarded and the supernatant was evaporated under vacuum. This fraction with a 2 — 10 mg concentration of protein per ml was used as BSE. All the extraction procedure was performed at 4 °C.

Transformation by PHA

Spleen cells from 8 weeks old (C57Bl/6 X DBA/2) F1 mice were passed through a nylon mesh column and counted in a hemacytometer. The lymphocytes were suspended in 2 ml of medium at a concentration of 5 x 10\(^6\)/ml in 16 x 125 mm Falcon plastic screw-top tubes and incubated at 37 °C in a humidified 5% CO\(_2\) atmosphere for 72 hours. The nutrient medium was RPMI supplemented with 5% heat inactivated AB human serum. PHA P (Difco) was added at the beginning of incubation to a final concentration of 10 μg/ml. Tritiated thymidine (H3-TdR) (1 μC/ml) was added for 24 hours on the 2nd day of culture. The cultures were centrifuged separately, washed 3 times with 2% perchloric acid (PCA) at 4 °C, resuspended in 3 ml of 15% PCA and incubated at 65 °C for 4 hours. The material obtained was then centrifuged and 0.1 ml of the supernatant was added to 10 ml of scintillation liquid. Radioactivity was measured in a Packard liquid scintillation spectrometer\(^5\).

**Mixed lymphocyte culture**

Spleen cells from C57Bl/6 mice were mixed with spleen cells from DBA/2 mice irradiated with 6000 r from a cobalt source (CO\(_60\)), in the same culture conditions as described above. The concentration of cells was 2.5 x 10\(^6\)/ml of the former and 5 x 10\(^6\)/ml of the latter. Five days later, 1 μC/ml of H3-TdR was added and the DNA extracted after 12 hours of labeling. Radioactivity was measured as described above\(^6,7\).

References

Results

Table I shows the results obtained with PHA stimulation of lymphocytes in the presence of 50 μg/ml of BSE added when the culture was started. The inhibition of lymphocyte transformation was decreased by 40% in the cultures with BSE ($P < 0.01$). The presence of BSE in the cultures which did not receive PHA did not influence H3-TdR uptake. This is in agreement with our previous results, where no changes could be seen on cells left for 3 days in the presence of high doses (200 μg/ml) of BSE.

<table>
<thead>
<tr>
<th>Lymphocytes cultivated with</th>
<th>PHA Absent</th>
<th>PHA Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>10616 (± 1632)</td>
<td>43186 (± 988)</td>
</tr>
<tr>
<td>BSE</td>
<td>11434 (± 834)</td>
<td>28534 (± 8243)</td>
</tr>
</tbody>
</table>

Table I: Mean * C.P.M./culture found in lymphocyte cultures stimulated with PHA in the absence or in the presence of BSE. Values within parentheses represent the 95% confidence limits. * Mean of 5 cultures.

Table II shows the results obtained in an experiment performed with lymphocytes from mice treated with 8 mg per mouse of BSE during 3 consecutive days before sacrifice. H3-TdR incorporation in unstimulated lymphocytes from controls or treated mice was nearly the same. Stimulation by PHA is decreased by 41% in lymphocytes from treated mice when compared with the controls ($P < 0.001$).

<table>
<thead>
<tr>
<th>Lymphocytes cultivated with</th>
<th>PHA Absent</th>
<th>PHA Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>8100 (± 1424)</td>
<td>48040 (± 5268)</td>
</tr>
<tr>
<td>BSE</td>
<td>9211 (± 488)</td>
<td>28699 (± 4820)</td>
</tr>
</tbody>
</table>

Table II: Mean * C.P.M./culture found in lymphocyte cultures stimulated in vitro with PHA obtained from control mice and from mice treated with BSE. Values within parentheses represent the 95% confidence limits. * Mean of 5 cultures.

Table III shows that in the MLC, the responding cells from mice treated with BSE incorporated 50% less H3-TdR than the lymphocytes from non treated mice. A short decrease of H3-TdR uptake in the non stimulated lymphocytes from treated C57Bl/6 mice is also illustrated in Table III.

<table>
<thead>
<tr>
<th>Lymphocytes from mice treated with</th>
<th>Irradiated C57Bl/6 lymphocytes + non irradiated C57Bl/6 lymphocytes</th>
<th>Irradiated DBA/2 lymphocytes + non irradiated C57Bl/6 lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSE</td>
<td>3806 (± 590)</td>
<td>7347 (± 828)</td>
</tr>
<tr>
<td></td>
<td>2365 (± 216)</td>
<td>3208 (± 256)</td>
</tr>
</tbody>
</table>

Table III: Mean * C.P.M./culture found in MCC of lymphocytes where the responding cells were obtained from mice treated with BSE. Values within parentheses represent the 95% confidence limits. * Mean of 5 cultures.

Discussion

BSE is a protein obtained from bovine spleen by alcoholic fractionation. It has a significant inhibitory activity on DNA synthesis in established cell lines obtained from the peripheral blood of normal donors of patients with acute lymphoblastic leukemia¹, RNA and protein syntheses however, are not inhibited during the first hours of culture¹. We have also previously reported that BSE inhibits DNA synthesis in mouse lymphoid tissue, decreases the number of plaque forming cells in the spleens of mice immunized by sheep red blood cells when added in vivo and inhibits the graft versus host reaction and the human lymphocyte transformation in vitro by PHA². The molecular weight of this protein is 45 000³.

The data obtained show that BSE inhibits the stimulation of lymphocytes in vitro by PHA and that lymphocytes from mice treated with BSE incorporate less H3-TdR when cultivated in the presence of PHA or of irradiated allogeneic cells. This effect is different from what has been reported for antilymphocytic serum which induces lymphoblastic transformation but blocks the lymphocytic response to a non specific (PHA) or specific (antigen) stimulation and inhibits the response to an allogeneic stimulation (MLC)⁹.

The mechanism of action of BSE is unknown, however the data obtained so far indicates that BSE inhibits DNA synthesis in vitro and in vivo and that the in vivo inhibition is tissue specific⁴. Thus, probably the immunosuppressive action is, at least, partly due to the inhibition of DNA synthesis. Furthermore, since cells taken from BSE treated mice do not respond to the antigenic stimulation in vitro, it seems the action is on the immune competent cells rather than on the antigen.