Modification of acquired immunity in mice by imipenem/cilastatin

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The immunomodulating properties of antimicrobial drugs may have important implications for clinical practice, particularly for those patients whose immune system has been compromised. In this study, we assessed the influence of different treatments with a \(\beta\)-lactam antibiotic (imipenem/cilastatin) on several acquired immune responses of BALB/c mice; splenocyte responses to specific mitogens and to sheep red blood cells, IL-2 production and proportions of the different lympho-monocytic populations. Imipenem/cilastatin was shown to modify some lymphocyte-associated immune functions and it would be useful to investigate whether immunomodulatory effects also occur in humans.

Introduction

A biological response modifier (BRM) has been defined as an agent able to modulate effector mechanisms or mediators of host defence. Several investigations have shown antibiotics to display a BRM-like activity, thus modifying the proliferative ability of lymphocytes stimulated with antigens or mitogens, the antigen-specific primary antibody response, or the ability to produce cytokines.\textsuperscript{1,2} Because imipenem has an extremely broad antibacterial spectrum, imipenem/cilastatin is a drug of choice in the treatment of serious infections. In this study we examine the influence of a long-term chemotherapy with imipenem/cilastatin on the acquired immune response of BALB/c mice.

Materials and methods

Animals

Male BALB/c mice were used for the experiments. They were maintained under pathogen-free conditions, with free access to food and water. All investigators adhered to the national guidelines for the care of laboratory animals.

Antibiotic regimen

Imipenem/cilastatin (Merck Sharp & Dohme, S.A., Madrid, Spain) was given to mice by ip injection in 24 h intervals for 7 or 14 consecutive days. The antibiotic was diluted in sterile phosphate-buffered saline (PBS; Sigma Chemical Co., St Louis, MO, USA) and the dosages were calculated on a body weight basis, according to therapy in human medicine (4, 2, 1 and 0.5 g/70 kg/day for 7 day therapy, and 2 g/70 kg/day for 14 day therapy). Control mice received equal volumes of PBS for 7 and 14 days.

Mitogen-induced proliferation of splenocytes

Imipenem/cilastatin-treated and control mice were killed, and their spleens were aseptically removed and homogenized in sterile Hanks' balanced salt solution (HBSS; Sigma). Splenocytes were washed twice with HBSS and finally resuspended in complete medium RPMI 1640 (Sigma) supplemented with 10% heat-inactivated calf serum (Flow Laboratories, Irvine, UK), 50 \(\mu\)M 2-mercaptoethanol (Sigma), 1% penicillin G/streptomycin solution (Sigma), 1% L-glutamine (Sigma), 1% sodium pyruvate (Sigma), 5% sodium bicarbonate (Sigma) and 1% HEPES (Flow Laboratories).

Cell suspensions were adjusted to \(7 \times 10^6\) viable cells and distributed (100 \(\mu\)L/well) into 96-well flat-bottomed microtitre plates (Costar, Cambridge, MA, USA). Lipo-polysaccharide (LPS; Sigma) at a concentration of 25 mg/L, and pokeweed mitogen (PWM; Sigma) 30 mg/L were used as B-cell mitogens. Concanavalin A (Con A; Sigma) 10 mg/L and phytohaemagglutinin (PHA; Sigma) 30 mg/L were
used as T-cell mitogens. After incubation at 37°C in 5% CO₂ for 48 h, cellular proliferation was measured by colorimetric reading of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) reduction as described by Mosmann.³

**Splenic response to sheep erythrocytes**

Mice were immunized with sheep erythrocytes (SRBCs) by ip administration of 0.5 mL of 2% (v/v) SRBCs (bioMérieux, Marcy l’Etoile, France). Five days after immunization, the mice were killed and weighed, and the spleens were aseptically removed and weighed for the determination of the splenic index (the spleen weight corresponding to 25 g of body weight). The spleens were homogenized in HBSS, and the number of cells secreting anti-SRBC antibodies was determined in haemolytic plaque assays, as described by Henry.⁴

**Interleukin-2 (IL-2) assay**

Splenic cells were adjusted to 1.2 × 10⁶/mL viable cells in RPMI 1640 complete medium. Cells were stimulated by addition of Con A and incubation at 37°C in 5% CO₂ for 96 h. Supernatants were finally collected and stored at −80°C until quantitatively assayed.

Extracellular and associated IL-2 concentrations were measured by specific enzyme-linked immunosorbent assay with a murine anti-IL-2 monoclonal antibody (Mouse IL-2 ELISA Kit; Endogen, Boston, MA, USA). The optical density was measured in an ELISA Reader (Whitakker Microplate Reader 2001; Anthos Labtec Instruments, Salzburg, Austria), with a test wavelength of 450 nm and a reference wavelength of 550 nm. Values expressed are test minus reference readings.

**Analysis of lympho-monocytic populations**

Flow cytometry was used to measure the occurrence of different markers on the surface of blood cells. Fluorescein isothiocyanate (FITC)-conjugated, phycoerythrin (PE)-conjugated or quantum red (QR)-conjugated monoclonal antibodies (MoAb) to murine leucocyte markers were obtained from Sigma. Blood samples were collected from the retro-orbital plexus of mice, and incubated at room temperature with the following monoclonal antibodies: mouse CD3 antibody-FITC (clone 29B), mouse CD4 antibody-PE (clone H129.19), mouse CD8a antibody-QR (clone 53–6.7), mouse CD18 (LFA-1b) antibody-QR (a monocyte marker) (clone C71/16) and mouse CD45R antibody-PE (a B cell marker) (clone RA3-6B2). Each sample was treated with a lysing solution after incubation (Coulter Q-PREP/IMMUNOPREP Reagent system; Izasa, Barcelona, Spain) and processed for three-colour fluorescence measurements in a Coulter EPICS Elite ESP Flow Cytometer (Izasa). At least 30,000 ungated events were measured for each sample.

**Statistics**

All results are shown as means ± s.d. for five mice, tested in duplicate for each sample. Statistical analysis was performed by using two-way factorial analysis of variance. Treated and control groups were compared with the least significant difference test. A P value of <0.05 was considered significant.

**Results and discussion**

Our results show an enhanced proliferation of T and B lymphocytes in response to the specific mitogens Con A, PHA, LPS and PWM, in mice injected with 2 g/70 kg/day of impenem/cilastatin for 14 days (Table I). These results are similar to those described by other authors who have found the time of treatment with ciprofloxacin highly influential on the effects of mitogens on acquired immunity in mice.⁵ On the other hand, our results disagree with those described by Pasquier et al.,⁶ who showed no influence of impenem/cilastatin on this immune parameter in an experimental model in vitro. The differences could be attributed to metabolites of this antibiotic, which could affect biological activities that are not detectable in an in-vitro assay.

Treatment with 4 g/70 kg/day of impenem/cilastatin for 7 days induced a significant suppression in the splenic index of mice. Likewise, 4 or 0.5 g/70 kg/day of impenem/cilastatin administered to animals for 7 days, as well as prolonged therapy, caused a significant decrease in the splenic response to SRBCs (Table I). Similar antibiotic-related effects have been described by Pulverer in cefodizime-treated BALB/c mice.⁷

With regard to IL-2 production, treatment with 4 or 0.5 g/70 kg/day of impenem/cilastatin for 7 days induced a significant enhancement in the production of this cytokine by splenic lymphocytes (Table II). This effect could be mediated by a previous interaction with IL-1-producing accessory cells. Such a mechanism is corroborated by our previous results, which show similar effects of different impenem/cilastatin treatments on IL-1 production by macrophages.⁸

Total blood cell counts were similar for impenem/cilastatin-treated and control mice, but mice treated with 0.5 g/70 kg/day for 7 days showed significantly decreased B-lymphocyte and monocyte counts (Table II). This impairment in B-lymphocytic population could explain the significant decrease in the splenic response to SRBC described in this group.

We conclude from this study that impenem/cilastatin is a modifier of different lymphocyte-associated immune functions in mice; however, from a clinical point of view...
Table I. Effect of imipenem/cilastatin treatment on splenocyte response to T and B cell mitogens, and to sheep red blood cells

<table>
<thead>
<tr>
<th>Dosage of imipenem/cilastatin (g/70 kg/day)</th>
<th>Treatment (days)</th>
<th>Splenocyte proliferation (mean ± S.D., OD × 10⁻³)</th>
<th>Splenic index</th>
<th>no. plaque forming cells (× 10³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>concanavalin A</td>
<td>phytohaemagglutin</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>Control mice</td>
<td>7</td>
<td>160.7 ± 12.6</td>
<td>154.4 ± 23.0</td>
<td>136.3 ± 18.9</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>211.5 ± 25.2</td>
<td>204.6 ± 19.4</td>
<td>172.1 ± 13.6</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>144.4 ± 15.3</td>
<td>102.8 ± 27.9</td>
<td>128.2 ± 15.8</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>119.0 ± 7.7</td>
<td>155.3 ± 12.6</td>
<td>123.6 ± 15.9</td>
</tr>
<tr>
<td>0.5</td>
<td>7</td>
<td>144.5 ± 2.8</td>
<td>143.8 ± 10.8</td>
<td>147.6 ± 13.0</td>
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<tr>
<td>Control mice</td>
<td>14</td>
<td>149.6 ± 5.6</td>
<td>165.8 ± 11.7</td>
<td>149.9 ± 8.5</td>
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<tr>
<td>2</td>
<td>14</td>
<td>202.5 ± 28.8</td>
<td>217.1 ± 23.7</td>
<td>229.9 ± 27.2</td>
</tr>
</tbody>
</table>

aP < 0.05.
bP < 0.01.
cP < 0.001.

Table II. Effect of imipenem/cilastatin treatment on the different subpopulations of blood leucocytes, and on interleukin-2 production

<table>
<thead>
<tr>
<th>Dosage of imipenem/cilastatin (g/70 kg/day)</th>
<th>Treatment (days)</th>
<th>T cells CD4⁺ (%)</th>
<th>T cells CD8⁺ (%)</th>
<th>B cells (%)</th>
<th>% Monocytes per mouse</th>
<th>IL-2 OD (10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control mice</td>
<td>7</td>
<td>29.7 ± 6.3</td>
<td>12.1 ± 3.8</td>
<td>36.5 ± 10.4</td>
<td>8.7 ± 3.6</td>
<td>691.4 ± 266.1</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>31.9 ± 5.2</td>
<td>10.9 ± 1.6</td>
<td>34.6 ± 4.4</td>
<td>10.2 ± 3.1</td>
<td>2323.7 ± 620.2</td>
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<tr>
<td>2</td>
<td>7</td>
<td>34.3 ± 2.8</td>
<td>15.1 ± 2.1</td>
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<td>6.4 ± 0.4</td>
<td>576.7 ± 139.0</td>
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<td>7</td>
<td>36.4 ± 3.6</td>
<td>11.5 ± 1.7</td>
<td>33.3 ± 4.4</td>
<td>8.8 ± 2.3</td>
<td>532.7 ± 232.6</td>
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<tr>
<td>0.5</td>
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<td>39.3 ± 7.6</td>
<td>15.8 ± 3.5</td>
<td>13.6 ± 5.3</td>
<td>4.1 ± 1.2</td>
<td>2184.0 ± 626.5</td>
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<tr>
<td>Control mice</td>
<td>14</td>
<td>29.2 ± 7.5</td>
<td>11.8 ± 4.1</td>
<td>39.3 ± 14.4</td>
<td>10.4 ± 3.7</td>
<td>713.5 ± 187.0</td>
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<td>27.9 ± 5.4</td>
<td>10.6 ± 2.4</td>
<td>44.9 ± 8.7</td>
<td>10.4 ± 2.8</td>
<td>1074.0 ± 254.6</td>
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</table>

aP < 0.001.
bP < 0.01.
cP < 0.05.
it is necessary to characterize further the regulatory pathways influenced by this antibiotic, as well as to investigate whether an immunomodulatory effect of imipenem/cilastatin occurs in humans.

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References


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