Effects of L-156,602, a C5a Receptor Antagonist, on Mouse Experimental Models of Inflammation

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Received July 10, 1992

L-156,602, a C5a receptor antagonist, was found as an immunosuppressant with preferential effects on delayed-type hypersensitivity (DTH) in our screening program and it was shown that L-156,602 suppressed the efferent phase of DTH. Here, we tested its effects on experimental models of inflammation induced in mice. L-156,602 did not suppress serotonin- and carrageenan- induced inflammation while it completely suppressed concanavalin A-induced inflammation 4 hr after elicitation. The inflammation appeared 24 hr after the elicitation with concanavalin A and it was significantly suppressed by L-156,602. Muramyl dipeptide (MDP)-induced acute joint inflammation was also significantly suppressed by L-156,602. These results demonstrated the unique immunomodulating properties of L-156,602 in mouse experimental models of inflammation.

We have established a novel screening method for substances that modulate antibody production and delayed-type hypersensitivity (DTH) in vivo and showed that functions of B cells, type 1 helper T cells (Th1), type 2 helper T cells (Th2), and inflammatory cells could be separately evaluated in vivo in that system.1) L-156,602 which was first reported as a C5a receptor antagonist2) was found as a DTH-specific immunosuppressant in our screening system.3) We showed that the agent specifically suppressed the DTH response induced by trinitrobenzene sulfonic acid (TNBS) while antibody productions against sheep red blood cells (SRBC) and Brucella abortus were slightly enhanced.3) When DTH and antibody production were induced by i.v. immunization of mice with trinitrophenol (TNP)-conjugated SRBC, L-156,602 significantly suppressed DTH response while it greatly augmented antibody production.3) L-156,602 suppressed the efferent phase of DTH that was induced by direct injection of Th1 and its relevant antigen into hind footpads.3) These results suggested that L-156,602 had unique immunomodulating effects on inflammatory reactions as well as the humoral immune system. To characterize the anti-inflammatory properties of L-156,602, we investigated the effects of L-156,602 on mouse experimental models of inflammation.

Materials and Methods

Animals. Specific pathogen free CDF1, BALB/c Nu/+ , BALB/c Nu/Nu, (female, 6–8 week old) mice were obtained from Charles River Japan Co., Ltd. (Tokyo). C.B-17 scid/scid mice (female, 6–8 week old) were purchased from Clea Co., Ltd. (Tokyo).

Reagents. Serotonin and carrageenan were products of Research Biochemical Inc. (Nakik, MA) and Wako Pure Chemical Indust. Ltd. (Tokyo), respectively. Concanavalin A (con A) and muramyl dipeptides (MDP) were obtained from Sigma Chemical Co. (St. Louis, MO).

Induction of inflammation. Forty microliters of serotonin (25 µg/ml), carrageenan (2 mg/ml), or con A (2.5 or 5 mg/ml) was injected into the left hind-footpad. Increase in footpad thickness was evaluated with a dial caliper (Ozaki Mfg. Co., Ltd., Tokyo). Adjuvant arthritis was induced by the method of Koga et al.4) MDP (400 µg in saline) was i.v. injected into the tail veins of BALB/c mice. Twenty-four hours later, mice were examined and graded for severity of arthritis by the method of Wood et al.5) Lesions of the extremities distal to the bend of the knee were graded on a scale of 0–4 based on the number of joints involved and the degree of erythema and swelling. The maximum score was thus 16.

Results

Since the compound was shown to significantly suppress the efferent phase of DTH that was induced by direct injection of Th1 and its relevant antigen into hind footpads,3) we further investigated anti-inflammatory properties of L-156,602 using mouse experimental models. The responses against serotonin were evaluated 2 hr after

Fig. 1. Course Analysis of Serotonin-, Carrageenan-, and Con A- induced Inflammation.

Forty microliters of serotonin (25 µg/ml), carrageenan (20 mg/ml), or con A (5 mg/ml) was injected into subcutaneous tissue of hind footpads of CDF1 mice. At the indicated time later, increases in footpad thickness were measured. Means of 3 mice are shown. Vertical bars, SD.
Effects of L-156,602 on Inflammations

Table I. Characterization of Concanavalin A-induced Inflammation

<table>
<thead>
<tr>
<th></th>
<th>Increase in footpad thickness* (10⁻² cm)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>4 h</td>
</tr>
<tr>
<td>Exp. 1</td>
<td></td>
</tr>
<tr>
<td>BALB/c nu/+</td>
<td>12.5 ± 1.8</td>
</tr>
<tr>
<td>BALB/c nu/nu</td>
<td>11.3 ± 1.4</td>
</tr>
<tr>
<td>Exp. 2</td>
<td></td>
</tr>
<tr>
<td>C.B-17 +/+</td>
<td>10.2 ± 0.3</td>
</tr>
<tr>
<td>C.B-17 scid/scid</td>
<td>8.0 ± 0.5</td>
</tr>
</tbody>
</table>

Con A (Exp. 1; 200 μg, Exp. 2; 100 μg) was injected into a hind footpad. Increase in footpad thickness was measured 4 and 24 hours later.

* Mean ± SD, n = 3

Table II. Effects of L-156,602 on MDP-induced Inflammation

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Score*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Saline)</td>
<td>5.5 ± 1.3</td>
</tr>
<tr>
<td>0.25</td>
<td>4.0 ± 1.8</td>
</tr>
<tr>
<td>0.5</td>
<td>0.8 ± 1.0b</td>
</tr>
</tbody>
</table>

BALB/c mice were i.v. injected with 400 μg of MDP. Twenty-four hours after the elicitation, mice were examined and graded for severity as described in Materials and Methods. L-156,602 was injected i.p. as described in the legend of Fig. 2.

* Mean ± SD, n = 5.

b Statistically significant compared with saline treated group (p < 0.01; double-tailed Student's t-test).

Fig. 2. Effects of L-156,602 on Serotonin-induced Inflammation.

Serotonin (1 μg/40 μl) was injected into subcutaneous tissue of hind footpads of CDF1 mice. Two hours later, increases in footpad thickness were measured. L-156,602 was injected i.p. on days -2, -1, and 0 (day 0 corresponds to the elicitation day). Means of 3 mice are shown. Vertical bars = SD.

Fig. 3. Effects of L-156,602 on Carrageenan-induced Inflammation.

Forty microliters of carrageenan (20 mg/ml) was injected into subcutaneous tissue of hind footpads of CDF1 mice. Four and twenty-four hours later, increases in footpad thickness were evaluated. Schedule of treatment with L-156,602 was the same as in Fig. 2.

Elicitation because the inflammation reached its maximum after 2 h and no swelling could be observed after 4 h (Fig. 1). In contrast, carrageenan-induced inflammation was long-lasting. The maximum swelling was observed 4—8 h later and was still significant 48 h later. The inflammation was evaluated 4 h and 24 h after elicitation (Fig. 1). L-156,602 was hardly effective on both serotonin- and carrageenan-induced inflammations (Figs. 2 and 3).

Lewis et al. reported that subplantar administration of Con A into the rat hind paw induced swelling which was still present 48 h later.6 Con A also induced large swellings when it was injected into subcutaneous tissue of hind footpads of CDF1 mice. At the doses over 100 μg/footpad, swelling reached a maximum 24 h later and lasted for less than 72 h (Fig. 1). When 25 μg of Con A was used, maximum swelling was observed 4 h later (data not shown). Since Con A was known to be a mitogen for T cells, we first saw if activation of T cells was involved in Con A-induced inflammation using nude mice, which lack mature T cells because of the absence of a thymus, and scid mice, which lack T and B cells because of a deficiency in the recombinaise responsible for DNA-rearrangement of T and B cell receptors7] (Table I). In both cases, Con A induced significant footpad swelling as compared with the reference mice. This result implies that Con A-induced swelling is independent of T cell activation. L-156,602 completely suppressed the Con A-induced swelling 4 h later at the dose of 0.5 mg/kg (Fig. 4). The suppressive effect on the swelling after 24 h was less than that after 4 h although it was still statistically significant. MDP-induced adjuvant arthritis was also

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suppressed by L-156,602 (Table II). Significant effects of L-156,602 were observed at the dose of 0.5 mg/kg although a slight reduction of body weight was observed in this experimental group.

Discussion

L-156,602 has been reported as a C5a receptor antagonist and a cyclic hexadepsipeptide. This group is known to include antibiotics against Gram-positive bacteria and anti-tumor agents. We screened selective immunomodulating substances among microbial metabolites and found that Streptomyces sp. A1502 (FERM P-12448) produced a DTH-specific suppressing compound which was identical with L-156,602. We showed that the antibiotic inhibited the effenter phase of DTH, suggesting that C5a is involved in the reaction.

Serotonin is a vasoactive amine and is thought to interact with local vascular serotonin receptor, which enhances vascular permeability and footpad swelling. L-156,602 was ineffective in this swelling response (Fig. 2). An accumulation of macrophages was reported to be important for carrageenan-induced inflammation. Vinegar et al. distinguished two phases in the swelling of this inflammation. The first phase (30–90 min) is maintained by the release of histamine and serotonin and the second phase (2–6 h) by prostaglandins. L-156,602 did not significantly suppress carrageenan-induced inflammation 4 and 24 h later, suggesting that C5a is not involved in the accumulation of macrophages and release of prostaglandins although effects of the agent on the first phase of the inflammation were not ascertained (Fig. 3). On the contrary, con A-induced oedema was completely suppressed 4 h later by the agent. Lewis et al. showed that serotonin but not histamine plays a major role in the early phase (0.5–1.5 h) of con A-induced inflammation. Since C5a induces degranulation from mast cells, causing the release of serotonin, L-156,602 might suppress con A-induced oedema 4 h later through C5a antagonism. Nagaoka et al. demonstrated that con A induced marked accumulation of leukocytes, especially macrophages, in the peritoneal cavity between 16 and 48 h after the administration which could not be suppressed by anticomplementary agents (FUT-175 and K-76 COONa), bromophenacetyl bromide, nordihydroguaiaretic acid, and indomethacin. It is, therefore, likely that L-156,602 suppressed con A-induced swelling only partially 24 h later because of the infiltration of macrophages, which was consistent with ineffectiveness of L-156,602 in carrageenan-induced inflammation.

MDP has been reported to be the minimal structure necessary for the immunoadjuvant activity of cell wall peptidoglycans. Unlike joint inflammation induced by bacterial cell walls, acute joint inflammation induced by MDP is not followed by chronic, recurrent, and erosive arthritis. Kawasaki et al. and Ramanathan et al. reported that MDP did not activate a complement cascade although some derivatives of MDP were effective in its activation. Since L-156,602 significantly suppressed the arthritis induced by MDP, further studies are necessary to find whether the suppression of MDP-induced inflammation by L-156,602 is dependent on antagonism of the C5a receptor.

This study demonstrated that L-156,602 had specific effects on inflammations. To clarify the mode of anti-inflammatory action of L-156,602 as well as involvement of C5a in L-156,602-sensitive inflammations, it might be necessary to use anti-C5a antibody or C5a together with L-156,602.

Acknowledgment. Authors thank Dr. Toshitaka Koga for his advice on adjuvant arthritis.

References