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Rapid communication

2-Arachidonylglycerol, an endogenous cannabinoid, inhibits tumor necrosis factor-α production in murine macrophages, and in mice

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Abstract

2-Arachidonylglycerol (2-AG) inhibits the production in vitro of tumor necrosis factor-α (TNF-α) by mouse macrophages, as well as in mice. It has no effect on the production of nitric oxide (NO). The effect on TNF-α is enhanced when 2-AG is administered together with 2-linoleylglycerol (2-Lino-G) and 2-palmitylglycerol (2-PalmG), an 'entourage effect' previously noted in several behavioral and binding assays. 2-AG also suppresses the formation of radical oxygen intermediates.

Keywords: Endocannabinoid; Radical oxygen intermediate; Cytokine

Two types of endogenous cannabinoids have been identified, the most thoroughly investigated compounds being arachidonylethanolamide (anandamide) and 2-arachidonylglycerol (2-AG). Although these endocannabinoids, anandamide in particular, have been the object of investigations in various systems, their physiological roles are not clear (Mechoulam et al., 1998). In view of the anti-inflammatory action of plant and synthetic cannabinoids, and of the presence of endocannabinoids and of cannabinoid receptors in organs associated with immune regulation, a plausible role attributed to the endocannabinoid system is an anti-inflammatory one. Indeed, anandamide has been shown to exhibit anti-inflammatory effects (Molina-Holgado et al., 1997) and 2-AG suppresses interleukin-2 (Ouyang et al., 1998). For a review of the effects of cannabinoids on the immune system, see Klein et al. (1998).

Since tumor necrosis factor-α (TNF-α) is an important inflammatory mediator (Newton and Decicco, 1999), inhibition of its production by a body constituent may reflect a role for the endogenous ligand in anti-inflammatory processes. We now report that 2-AG inhibits in vitro TNF-α production in murine peritoneal macrophages, as well as in mice. Macrophages were chosen for their active participation in innate and immune functions. They phagocytize and destroy many infectious agents, and act as effector cells in both humoral and cell mediated immune responses. However, macrophages can also cause a wide array of inflammatory diseases.

As cannabinoids have been shown to have anti-oxidative properties (Mechoulam et al., 1998), we also looked into the action of 2-AG on the formation of radical oxygen intermediates. These are highly toxic species, formed in many biological reactions. We found that 2-AG suppresses the formation of these radicals.

Mouse (thioglycollate-elicited) peritoneal macrophages (harvested from C57BL/6 mice) were incubated in vitro with various concentrations of 2-AG (0.05–50 μg/ml) together with either lipopolysaccharide (LPS), which triggers TNF-α production, or with LPS and interferon-gamma, which together elicit the production of nitric oxide (NO). 2-AG was dissolved in ethanol and then diluted (1:500) in Dulbecco's modified Eagle's medium containing 5% fetal calf serum. In the concentrations studied, 2-AG was not toxic to macrophages, assessed either by trypan blue or erythrosin B dye exclusion. TNF-α production was determined by bioassay using BALB/c CL.7 cells as targets (Gallily et al., 1997). NO was determined on the basis of the accumulated nitrite in the supernatants of the treated macrophages using Griess reagent (Gallily et

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al., 1997). Radical oxygen intermediates in macrophages were determined by a luminol-enhanced chemiluminescence response assay, employing biolumat LB9500T, Berthold, Wildbad, Germany (Avron and Gallily, 1995).

For the determination of the effect of 2-AG on TNF-α levels in serum of LPS-treated mice, female C57BL/6 mice 9–11 weeks old, weighing 20–23 g were injected i.p. with either 5 mg/kg LPS alone or with LPS (5 mg/kg) and 2-AG (10 mg/kg) or with LPS (5 mg/kg) and 2-AG (1 mg/kg), 2-linoleylglycerol (2-Lino-G, 10 mg/kg) and 2-palmitylglycerol (2-PalmG, 5 mg/kg). TNF-α concentration in serum peaked after 90 min when the mice were bled and serum TNF-α activity (titer) was bioassayed, as described above.

As seen in Fig. 1A, a dose response TNF-α inhibition by 2-AG was noted in vitro. At concentrations of 50 and 10 μg/ml inhibition of 97% and 90%, respectively was observed after 24 h of incubation. Even at concentrations of 0.1 μg/ml 2-AG, 50% inhibition of TNF-α production was still noted. 2-AG (25 μg/ml) also suppressed the production of zymosan-induced radical oxygen intermediates in macrophages by more than 85% after 40 min (chemiluminescence peak of control macrophages: 10,012 cpm; 2-AG treated ones: 1390 cpm). However, addition of 2-AG (50–0.05 μg/ml) to macrophage cultures activated by both LPS and interferon-gamma, did not suppress NO generation after 24 h incubation (assay with 25–50 nM $NO_2^-/NO_3^-/10^6$ cells).

Inhibition of TNF-α production was also seen in mice (Fig. 1B). When 2-AG (10 mg/kg) was administered together with LPS, 73% inhibition of TNF-α was observed. Co-administration of LPS with 2-AG (1 mg/kg), 2-Lino-G (10 mg/kg), and 2-PalmG (5 mg/kg) inhibited TNF-α production almost totally.

We have previously shown that 2-Lino-G and 2-PalmG, which accompany 2-AG in the body, although inactive in both in vivo and in vitro assays (including binding to the cannabinoid receptors), enhance 2-AG activity (Ben-Shabat et al., 1998). This enhancement, which we have named 'entourage effect' is also seen here. 2-AG (1 mg/kg) in the presence of the 'entourage' compounds, was more active than a higher dose of 2-AG (10 mg/kg).

Anandamide has also been shown to inhibit TNF-α production (in astrocytes), however, its effect is relatively modest (Molina-Holgado et al., 1997). Contrary to the results reported now with 2-AG on macrophages, anandamide also blocked NO release in astrocytes.

In summary, the present report is the first one that records the effect of the endocannabinoid 2-AG on TNF-α, on NO and on radical oxygen intermediates production. 2-AG inhibits TNF-α formation and radical oxygen intermediates in vitro without affecting NO generation. 2-AG also suppresses TNF-α production in vivo.

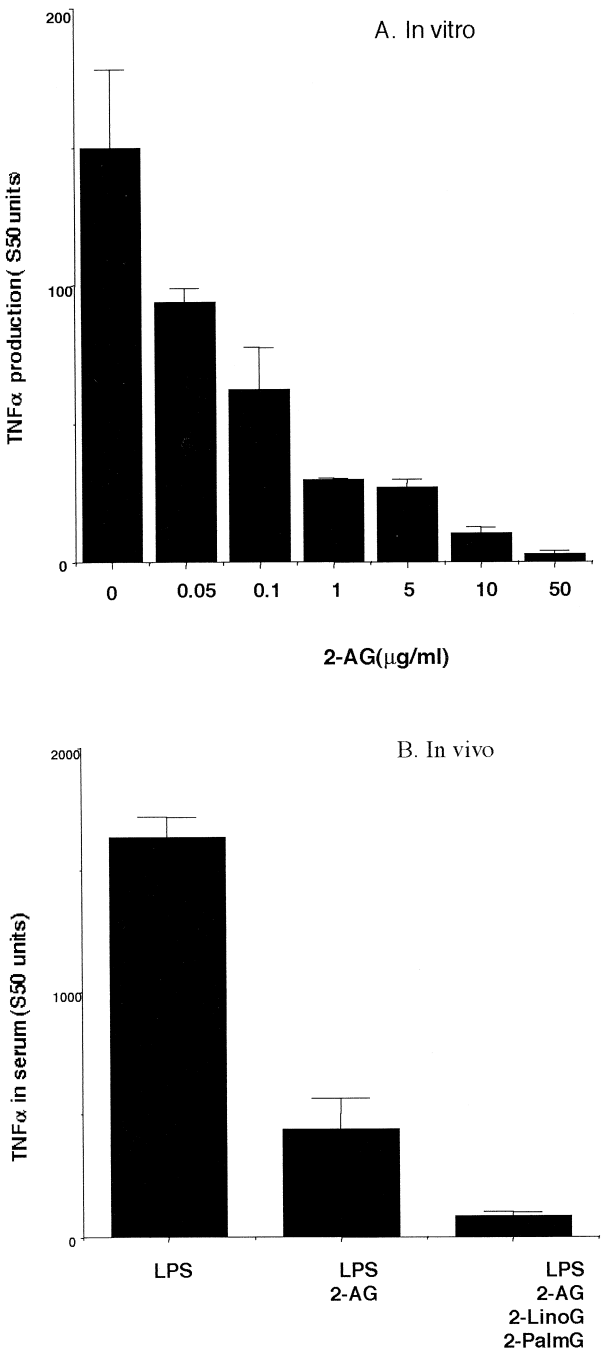

Fig. 1. (A) TNF-α titer in the macrophage supernatants was determined by bioassay using BALB/c CL.7 cells as targets (Gallily et al., 1997). TNF-α titer was expressed in S50 units, defined as the reciprocal of the test supernatant dilution required to destroy 50% of the target CL.7 cells. No effect of 2-AG on clone CL.7 target cells was seen. The above data are from a representative experiment (done in tetraplicates) of four experiments performed. The differences in TNF-α titer were all significant ($p < 0.05$) determined by Mann–Whitney *U*-test. (B) Female C57BL/6 mice were injected (i.p.) with either LPS (5 mg/kg) alone, or with 2-AG (10 mg/kg) and LPS (5 mg/kg), or with a mixture of 2-AG (1 mg/kg), 2-PalmG (5 mg/kg), 2-Lino-G (10 mg/kg) and LPS (5 mg/kg). The method for measurements in serum is presented in the text. Data in both (A) and (B) are given in mean S50 ± SEM.

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