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Systemic interleukin 10 administration inhibits brain tumor necrosis factor production in mice

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Abstract

Interleukin 10 is an antiinflammatory cytokine and inhibits the production of tumor necrosis factor. We have previously found that intracerebroventricular (i.c.v.) administration of recombinant human interleukin 10 inhibits brain tumor necrosis factor production induced by an i.c.v. injection of lipopolysaccharide in mice. In view of its possible pharmacological use, we have now studied whether interleukin 10 administered peripherally could inhibit brain tumor necrosis factor production. Mice were injected with recombinant human interleukin 10 (20 μg/mouse, i.v.) 10 min–24 h before lipopolysaccharide (2.5 μg, i.c.v.). Tumor necrosis factor was measured, using a bioassay, in brain homogenates 90 min after lipopolysaccharide. Recombinant human interleukin 10 administered i.v. between 10 min and 6 h before lipopolysaccharide markedly inhibited brain tumor necrosis factor production. We also measured the production of tumor necrosis factor by whole blood of these mice, and it was also markedly inhibited by recombinant human interleukin 10 treatment. In conclusion, systemic recombinant human interleukin 10 administration inhibits brain tumor necrosis factor production, suggesting its usefulness in tumor necrosis factor-mediated pathologies of the central nervous system. © 1997 Elsevier Science B.V.

Keywords: TNF (tumor necrosis factor); Interleukin 10; Brain; Lipopolysaccharide

1. Introduction

Previous reports have shown that interleukin 10 inhibits tumor necrosis factor production in vitro and in vivo and protects against lipopolysaccharide toxicity (De Waal-Malefyt et al., 1991; Gérard et al., 1993). Tumor necrosis factor is also an important pathogenetic mediator in diseases of the central nervous system. Anti-tumor necrosis factor antibodies are protective in animal models of cerebral malaria (Grau et al., 1987), experimental allergic encephalomyelitis (Ruddle et al., 1990) and bacterial meningitis (Saukkonen et al., 1990). We demonstrated earlier that interleukin 10 is an inhibitor of brain tumor necrosis factor production, as intracerebroventricular (i.c.v.) administration of interleukin 10 inhibited brain tumor necrosis factor induced by an i.c.v. injection of lipopolysaccharide (Di Santo et al., 1995). Interleukin 10 also seems to be an endogenous inhibitor of brain tumor necrosis factor production, as interleukin 10 can be produced by glial cells (Mizuno et al., 1994) and neutralization of endogenous interleukin 10 by i.c.v. injection of a monoclonal antibody upregulates lipopolysaccharide-induced brain tumor necrosis factor production (Di Santo et al., 1995). In mice with experimental autoimmune encephalomyelitis, interleukin 10 mRNA expression correlates with recovery (Kennedy et al., 1992), suggesting the possible importance of interleukin 10 in neurological diseases. Thus, interleukin 10 could be of interest in the therapy of central nervous system diseases where tumor necrosis factor production is implicated.

In this respect, it is important to know whether, to inhibit tumor necrosis factor production in the brain, interleukin 10 has to be administered i.c.v. or can be administered systemically. To clarify this point, we injected mice with recombinant human interleukin 10 i.v. Then, brain tumor necrosis factor was induced by injecting lipopolysaccharide i.c.v., as no detectable brain tumor necrosis factor can be induced if lipopolysaccharide is administered systemically at non-lethal doses (Faggioni et al., 1995b). Since administration of recombinant human interleukin 10

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to humans inhibits tumor necrosis factor production from whole blood stimulated ex vivo with lipopolysaccharide (Chernoff et al., 1995), we also obtained blood from recombinant human interleukin 10-pretreated mice and stimulated it in vitro with lipopolysaccharide to induce tumor necrosis factor production. We aimed to thus correlate the central activity of recombinant human interleukin 10 to the systemic one.

2. Materials and methods

2.1. Animals and treatments

Male CD-1 mice (25 g body weight) from Charles River Italia (Calco, Como, Italy), were used. The mice were housed five per cage and fed ad libitum. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (European Economic Community Council Directive 86/609, OJ L 358, 1, December 12, 1987; Italian Legislative Decree 116/92, Gazzetta Ufficiale della Repubblica Italiana No. 40, February 18, 1992; National Institutes of Health Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication No. 85–23, 1985).

Escherichia coli-derived recombinant murine interleukin 10 and recombinant human interleukin 10 were obtained from Schering-Plough Research Institute, Kenilworth, NJ, USA), and injected i.v. at the dose indicated. Lipopolysaccharide (phenol-extracted preparation from *Escherichia coli* O55:B5, Sigma, St. Louis, MO, USA) was injected intracerebroventricularly (i.c.v.) via a 28-gauge needle into ether-anestetized mice (Haley and McCormick, 1957; Lipton et al., 1991) at a dose of 2.5 μg/mouse in a final volume of 10 μl. Control mice received i.c.v. or i.v. saline injection.

Blood was obtained from the retro-orbital plexus under ether anesthesia and serum was prepared. Blood was collected 90 min after lipopolysaccharide injection. The brains were also removed from animals killed by exsanguination and were homogenized with a blade homogenizer (Ultra Turrax) in 4 vol (w/v) of ice-cold saline. The homogenate was then centrifuged 10 min at $9000 \times g$ in a microfuge at 4°C and the supernatant was used for tumor necrosis factor assay (Mengozzi et al., 1994). Previous work had indicated that these are the optimal times for the determination of tumor necrosis factor in lipopolysaccharide-treated mice (Sironi et al., 1992; Mengozzi et al., 1994). It should be noted that tumor necrosis factor detected in the brain under these conditions is not attributable to blood contamination. In fact, when even higher circulating tumor necrosis factor levels are induced by systemic administration of lipopolysaccharide, no tumor necrosis factor can be detected in the brain (Faggioni et al., 1995b).

2.2. Whole blood experiments

Heparinized (Liquemin, Roche, 14 U/ml) whole blood obtained from control or recombinant human interleukin 10-treated (20 μg/mouse, i.v., at the time indicated) mice was plated in 96-well tissue culture plates (100 μl/well) and incubated for 4 h at 37°C, 5% CO_2 with 1 μg/ml lipopolysaccharide (Fantuzzi et al., 1995). Plasma, obtained by centrifugation 4 h later, was used for tumor necrosis factor determination.

In the preliminary experiments designed to check for the possible species-specificity of interleukin 10, mouse whole blood was cultured with lipopolysaccharide in the presence of various doses of recombinant human interleukin 10 or recombinant murine interleukin 10. In these experiments, the effect of recombinant human and recombinant murine interleukin 10 was studied on human whole blood, obtained from healthy volunteers, with the same experimental design.

2.3. Miscellaneous determination

Tumor necrosis factor was measured by the degree of cytotoxicity on L929 cells in the presence of 1 μg/ml of actinomycin D, as previously described (Aggarwal et al., 1985), using human recombinant tumor necrosis factor as standard. The sensitivity of the assay was around 50 pg/ml.

Human recombinant interleukin 10 was measured in serum using a commercially available enzyme-linked immunosorbent assay kit (Benfer-Scheller, Milan, Italy).

3. Results

3.1. Activity of recombinant human interleukin 10 on mouse cells

In a preliminary experiment, we compared recombinant human interleukin 10 and recombinant murine interleukin 10 for their ability to inhibit tumor necrosis factor production by lipopolysaccharide-stimulated whole mouse blood. As shown in Fig. 1A both recombinant human and recombinant murine interleukin 10 inhibited whole mouse blood tumor necrosis factor production (at the lowest concentration tested, 0.1 ng/ml; inhibition was 95%, and was $>99\%$ at interleukin 10 concentrations higher than 1 ng/ml). Recombinant human interleukin 10 also inhibited tumor necrosis factor production in human whole blood while recombinant murine interleukin 10 was not active (Fig. 1B). With recombinant human interleukin 10, the inhibition of tumor necrosis factor production by human blood was not as complete as in mouse blood but was still very strong (maximal inhibition was 92% with 100 ng/ml of recombinant human interleukin 10) From these results it was concluded that the in vivo activity of recombinant human interleukin 10 could be studied in mice.

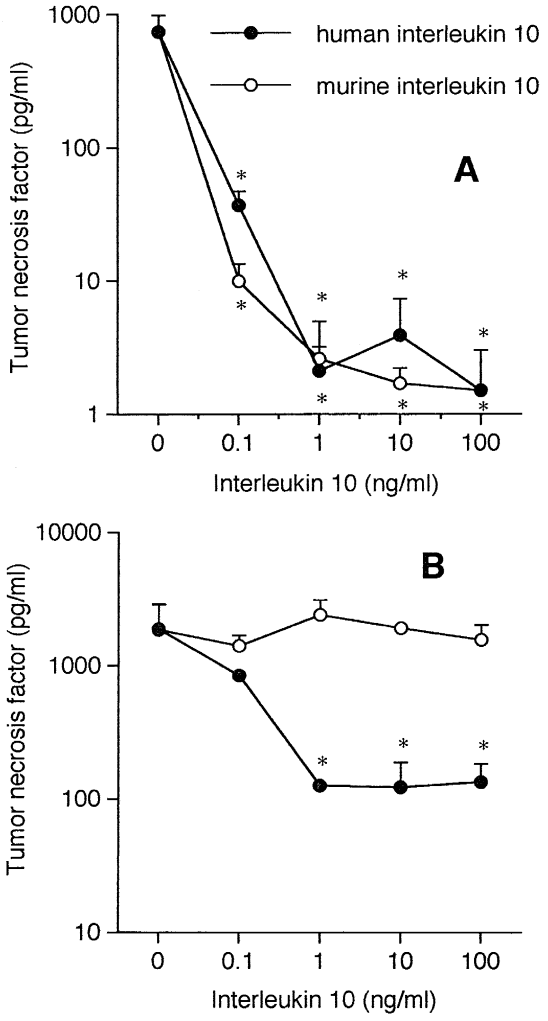

Fig. 1. Species-specificity of human and murine interleukin 10. Whole mouse blood (panel A) or whole human blood (panel B) was cultured 4 h with 1 μg/ml lipopolysaccharide and the indicated concentration of recombinant human interleukin 10 (closed circles) or recombinant murine interleukin 10 (open circles). Results are expressed as pg/ml of tumor necrosis factor produced (mean ± S.D. from duplicate cultures). $^{*}P < 0.01$ vs. control (no interleukin 10) by Student's *t*-test.

3.2. Systemic recombinant human interleukin 10 administration inhibits brain tumor necrosis factor production in vivo, and whole blood tumor necrosis factor production ex vivo

In a first set of experiments, recombinant human interleukin 10 was administered i.v. at the dose of 20 μg/mouse, at different times (10 min, 30 min, 3 h, 6 h and 24 h) before an i.c.v. injection of lipopolysaccharide (2.5 μg/mouse), and tumor necrosis factor was measured in brain homogenates 90 min after lipopolysaccharide administration. The results, shown in Fig. 2 (solid circles), indicate that i.v.-injected recombinant human interleukin 10 significantly inhibited brain tumor necrosis factor production. The effect was maximal (about 70% inhibition) when recombinant human interleukin 10 was injected between 10 min and 3 h before lipopolysaccharide.

We also evaluated the effect of recombinant human interleukin 10 administration, with the same treatment schedule, on tumor necrosis factor production by whole blood ex vivo. For this purpose recombinant human interleukin 10 was administered i.v. at the dose of 20 μg/mouse and blood was obtained at different times (10 min, 30 min, 3 h, 6 h and 24 h). Whole blood was then cultured in vitro 4 h with lipopolysaccharide (1 μg/ml). As shown in Fig. 2 (open circles) blood obtained from recombinant human interleukin 10-pretreated mice (up to 6 h of pretreatment) produced less tumor necrosis factor in vitro (> 95% inhibition), in agreement with a previous report for human volunteers (Chernoff et al., 1995).

We also performed dose–response studies where recombinant human interleukin 10 was administered i.v. at the doses indicated 30 min before lipopolysaccharide. Then in one set of experiments, lipopolysaccharide was injected i.c.v. and brain tumor necrosis factor was measured 90 min

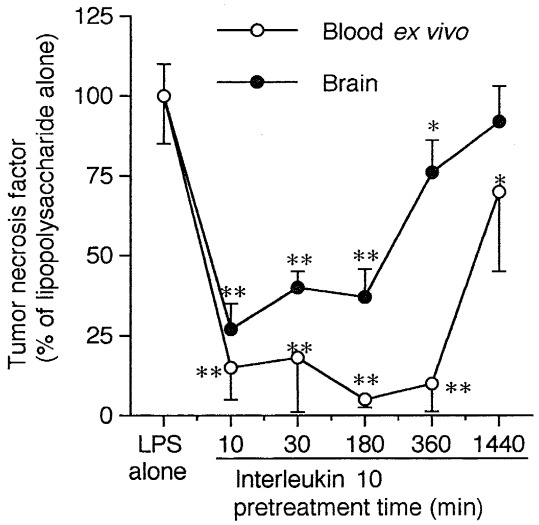

Fig. 2. Effect of systemic recombinant human interleukin 10 pretreatment on tumor necrosis factor production in vivo in the brain and ex vivo by whole blood. Solid circles: mice were pretreated with recombinant human interleukin 10 (20 μg/mouse, i.v.) at the time indicated (controls received saline alone). Then lipopolysaccharide (2.5 μg/mouse, i.c.v.) was administered, mice were killed 90 min later and tumor necrosis factor was measured in brain homogenates. Results are expressed as % of tumor necrosis factor produced in mice treated with lipopolysaccharide alone (in the experiment shown, 100% was 6.7 ng/g brain). Data are means ± S.D. (5 mice/group). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control (lipopolysaccharide alone) by Duncan's test. Open circles: mice were pretreated with recombinant human interleukin 10 (20 μg/mouse, i.v.) at the time indicated (controls received saline alone). Then blood was obtained and exposed in vitro to lipopolysaccharide (1 μg/ml). Tumor necrosis factor produced was measured 4 h later. Results are expressed as % of tumor necrosis factor produced by blood with lipopolysaccharide alone (in the experiment shown, 100% was 1.5 ng/ml). Data are means ± S.D. from triplicate cultures. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control (blood from saline-treated mice) by Duncan's test.

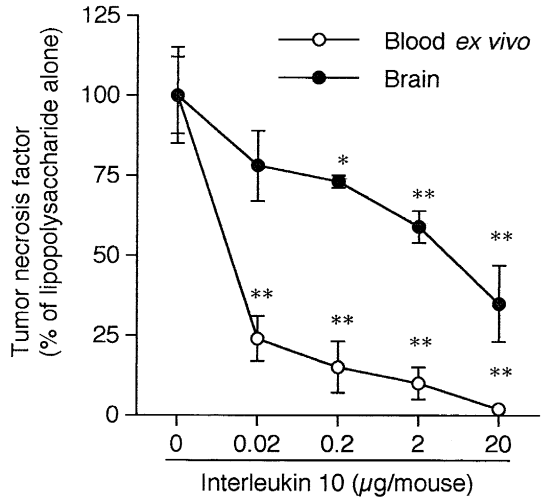

Fig. 3. Dose dependence of the effect of systemic recombinant human interleukin 10 pretreatment on tumor necrosis factor production in vivo in the brain and ex vivo by whole blood. Solid circles: mice were pretreated with various doses of recombinant human interleukin 10 (i.v., 30 min before lipopolysaccharide). Then lipopolysaccharide (2.5 μg/mouse, i.c.v.) was administered, mice were killed 90 min later and tumor necrosis factor was measured in brain homogenates. Results are expressed as % of tumor necrosis factor produced in mice treated with lipopolysaccharide alone (in the experiment shown, 100% was 5.4 ng/g brain). Data are means ± S.D. (5 mice/group). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control (lipopolysaccharide alone) by Duncan's test. Open circles: mice were pretreated with various doses of recombinant human interleukin 10 (i.v., 30 min before lipopolysaccharide). Then blood was obtained and exposed in vitro to lipopolysaccharide (1 μg/ml). Tumor necrosis factor produced was measured 4 h later. Results are expressed as % of tumor necrosis factor produced by blood with lipopolysaccharide alone (in the experiment shown, 100% was 1.5 ng/ml). Data are means ± S.D. from triplicate cultures. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control (blood from saline-treated mice) by Duncan's test.

later, while in a second set of experiments, mice were bled 30 min after interleukin 10 injection and tumor necrosis factor production from whole blood stimulated in vitro with lipopolysaccharide was measured as described above. The results are shown in Fig. 3. The inhibitory effect of recombinant human interleukin 10 on brain tumor necrosis factor levels (solid circles) was clearly dose dependent and maximal inhibition (65%) was observed at the highest dose tested (20 μg/mouse). Inhibition of tumor necrosis factor production by whole blood ex vivo was more marked and already significant at the lowest recombinant human interleukin 10 dose tested (open circles).

Finally, we investigated whether recombinant human interleukin 10 was also active when given after lipopolysaccharide. Administration of recombinant human interleukin 10 (20 μg/mouse, i.v.) 10 or 30 min after lipopolysaccharide (2.5 μg/mouse, i.c.v.) still significantly ($P < 0.01$) inhibited brain tumor necrosis factor production by 88 and 72%, respectively (tumor necrosis factor levels, 90 min after lipopolysaccharide injection, were: no interleukin 10, 4.2 ± 7.8 ng/ml; interleukin 10 + 10 min, 0.9 ± 0.1; interleukin 10 + 30 min, 1.1 ± 0.5).

3.3. Serum levels of recombinant human interleukin 10 following i.v. administration of recombinant human interleukin 10

In order to correlate the inhibition of tumor necrosis factor production ex vivo and blood interleukin 10 levels, recombinant human interleukin 10 was injected i.v. at the dose of 20 μg/mouse and its levels were measured in serum, by enzyme-linked immunosorbent assay, at the same times as used in the experiments on tumor necrosis factor production. High circulating recombinant human interleukin 10 levels were observed up to 6 h after injection (interleukin 10 levels as pg/ml, means for 3 mice per time point, were: 10 min, 500 ± 20; 30 min, 458 ± 22; 3 h, 462 ± 18; 6 h, 336 ± 18) and were undetectable (< 10 pg/ml) 24 h after injection.

4. Discussion

Our previous work had shown that interleukin 10 is an inhibitor of lipopolysaccharide-induced brain tumor necrosis factor production when administered i.c.v. (Di Santo et al., 1995). We now report that interleukin 10 is also effective to inhibit brain tumor necrosis factor production when administered systemically by the intravenous route. The effects of recombinant human interleukin 10 on brain tumor necrosis factor production had a time course similar to that found for ex vivo whole blood tumor necrosis factor production. In both cases, maximal inhibition was obtained when recombinant human interleukin 10 was given between 10 min and 6 h before lipopolysaccharide. No inhibition of brain tumor necrosis factor production was observed when lipopolysaccharide was administered 24 h after recombinant human interleukin 10.

The inhibitory effect of recombinant human interleukin 10 was dose dependent and already significant at the dose of 0.2 μg/mouse. Interestingly, recombinant human interleukin 10 was also active when injected 10 or 30 min after lipopolysaccharide.

Whole blood ex vivo tumor necrosis factor production was also inhibited in recombinant human interleukin 10-treated mice when recombinant human interleukin 10 was given up to 6 h before lipopolysaccharide. The inhibitory effect disappeared 24 h after recombinant human interleukin 10 treatment. These results correlate with the plasma kinetics of recombinant human interleukin 10 showing that high recombinant human interleukin 10 levels (0.3–0.5 ng/ml, in a range of concentrations inhibitory in vitro) could still be measured up to 6 h after i.v. injection, but returned to undetectable levels by 24 h.

It should also be noted that ex vivo tumor necrosis factor production by whole blood was more sensitive to the inhibitory effect of recombinant human interleukin 10 than was brain tumor necrosis factor production in vivo. In

fact, in whole blood ex vivo a marked inhibition (−75%) was observed even at the lowest dose of recombinant human interleukin 10, while there was complete suppression at the highest dose (20 ng/mouse).

The inhibition of brain tumor necrosis factor production by i.v.-administered recombinant human interleukin 10 is more difficult to explain. It is possible that recombinant human interleukin 10 might cross the blood–brain barrier. In fact, with respect to the central effects of peripherally injected cytokines, the question of passage across the blood–brain barrier is controversial. Opposite results have been reported for interleukin 1: there are reports that it cannot enter the brain, while others suggest that there is even an active blood-to-brain transport (discussed in Faggioni et al., 1995a). Studies with radioiodinated tumor necrosis factor indicate that this cytokine also can pass the blood–brain barrier in mice and rats (Gutierrez et al., 1993). It is also possible that peripherally administered cytokines act on the brain without passing the blood–brain barrier but by interaction with cells of regions which lack the blood–brain barrier (such as the organum vasculosum laminae terminalis) to transmit messages to the brain (Stitt and Bernheim, 1985; Blatteis, 1990; Faggioni et al., 1995a). We did not test whether interleukin 10 actually crosses the blood–brain barrier (as the enzyme-linked immunosorbent assay used for the interleukin 10 determination in plasma gave a high aspecific background in brain samples). However, it is possible that interleukin 10 crosses the blood–brain barrier, as suggested by the data reported here as well as by its documented activity following s.c. administration against experimental autoimmune encephalomyelitis (Rott et al., 1994).

In conclusion, our study indicated that inhibition of central tumor necrosis factor production can be achieved by systemic administration of recombinant human interleukin 10. It should be mentioned that this is not the first case of a cytokine which acts on the central nervous system following systemic administration. Clinical studies with interferon-β in the therapy of multiple sclerosis were first performed using an intrathecal route of administration (Jacobs et al., 1981), but it was later demonstrated that systemic (subcutaneous) administration is also effective (The IFNB Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group, 1995). Our data thus leave open the possibility of a clinical use of systemically administered interleukin 10 in tumor necrosis factor-mediated diseases of the central nervous system.

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