Cytokine-mediated inflammatory hyperalgesia limited by interleukin-10

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- 1 The effect of interleukin-10 (IL-10) upon the hyperalgesic activities in rats of bradykinin, tumor necrosis factor α (TNF α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), interleukin-8 (IL-8), prostaglandin E₂ (PGE₂) and carrageenin were investigated in a model of mechanical hyperalgesia.
- 2 Hyperalgesic responses to bradykinin (1 μ g) were inhibited in a dose-dependent manner by prior treatment with IL-10 (1–100 ng).
- 3 Hyperalgesic responses to TNF α (2.5 pg), IL-1 β (0.5 pg) and IL-6 (1.0 ng) but not to IL-8 (0.1 ng) and PGE₂ (50 ng and 100 ng) were inhibited by prior treatment with IL-10 (10 ng).
- 4 Hyperalgesic responses to carrageenin (100 μ g) were inhibited by IL-10 (10 ng) when this cytokine was injected before but not after the carrageenin.
- 5 A monoclonal antibody to mouse IL-10 potentiated the hyperalgesic responses to carrageenin (10 μ g) and TNF α (0.025 pg) but not that to IL-8 (0.01 ng).
- 6 In *in vitro* experiments in human peripheral blood mononuclear cells (MNCs), IL-10 (0.25–4.0 ng ml⁻¹) inhibited in a dose-dependent manner PGE₂ production by MNCs stimulated with IL-1 β (1–64 ng ml⁻¹) or endotoxin (lipopolysaccharide, LPS, 1 iu = 143 pg ml⁻¹) but evoked only small increases in IL-1ra production.
- 7 These data suggest that IL-10 limits the inflammatory hyperalgesia evoked by carrageenin and bradykinin by two mechanisms: inhibition of cytokine production and inhibition of IL-1 β evoked PGE₂ production. Our data suggest that the latter effect is not mediated via IL-10 induced IL-1ra and may result from suppression by IL-10 of prostaglandin H synthase-2 (COX-2).

Keywords: Inflammatory hyperalgesia; tumor necrosis factor α ; bradykinin; interleukin-1; interleukin-6; interleukin-8; interleukin-10; interleukin-1 receptor antagonist; ELISA; prostaglandin H synthase-2; cyclo-oxygenase-2

Introduction

In a rat paw pressure test, carrageenin-evoked hyperalgesia has been shown to result from the combined effect of the release of cyclo-oxygenase products and sympathomimetic amines (Nakamura & Ferreira, 1987). In this model, carrageenin caused hyperalgesia by releasing bradykinin, which initiated a cascade of cytokine release (Ferreira *et al.*, 1993). The proposed sequence of events was that bradykinin stimulated the release of tumour necrosis factor α (TNF α), which: (i) induced interleukin-1 β (IL-1 β) and interleukin-6 (IL-6), which stimulated the production of cyclo-oxygenase products and (ii) induced interleukin-8 (IL-8) which stimulated production of sympathomimetics (Cunha *et al.*, 1992; Ferreira *et al.*, 1993).

In recent years a number of cytokines have been described which inhibit the production of TNF α , IL-1 β , IL-6 and IL-8 (cytokines that are generally regarded as pro-inflammatory). One such 'antagonist cytokine' is IL-10. IL-10, which is produced by murine Th2 lymphocytes and monocytes, inhibits production of cytokines by murine Th1 lymphocytes (Fiorentino *et al.*, 1989) and is believed to play a role in inhibiting delayed type hypersensitivity responses (Zlotnik & Moore, 1991; Howard & O'Garra, 1992) and to suppress macrophage functions such as class II expression (De Waal Malefyt *et al.*, 1991a), adhesion (Fiorentino *et al.*, 1991) and the synthesis of cytokines including IL-1, IL-6, IL-8, G-CSF, GM-CSF and TNF α (De Waal Malefyt *et al.*, 1991b; Fiorentino *et al.*, 1991; Bogdan *et al.*, 1991; Oswald *et al.*, 1992).

Given the capacity of IL-10 to inhibit the production of the pro-inflammatory cytokines TNF α , IL-1 β , IL-6 and IL-8, we have investigated the possibility that IL-10 limits inflammatory hyperalgesia by diminishing the cytokine-mediated hyperalgesic responses to carrageenin and bradykinin.

Methods

Nociceptive test

A constant pressure of 20 mmHg was applied to the hind paws of rats and discontinued when they presented a typical freezing reaction (reaction time). This reaction was characterized by a reduction of escape movements: animals usually made several attempts to escape from the position imposed by the experimental situation. These were followed by alterations in respiratory frequency with the onset of a typical shivering reaction. The intensity of hyperalgesia was quantified as the variation in reaction time (Δ reaction time) obtained by subtracting values measured 3 h after administration of hyperalgesic substances from (control) reaction times measured before injection at zero time (Ferreira *et al.*, 1978).

Experimental protocol

In vivo measurements Hyperalgesia was measured 3 h after injection of bradykinin (1–100 μ g), TNF α (0.025 and 2.5 pg), IL-1 β (0.5 pg), IL-6 (1.0 ng), IL-8 (0.01 or 0.1 ng), PGE₂ (50 and 100 ng) and carrageenin (10 and 100 μ g), each injected in

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100 μ l, into the hind paws (intraplantar, i.pl.) of rats. IL-10 (1–100 ng in 100 μ l, i.pl.) or saline control (100 μ l) was injected into the (same) hind paws, 30 min before or 2 h after the hyperalgesic substances. A monoclonal antibody to mouse IL-10 (150 μ g in 50 μ l, i.pl.) or a control antibody (150 μ g in 50 μ l, i.pl.) was injected into the (same) hind paws, 30 min before hyperalgesic substances.

In vitro assays PGE₂ and IL-1 receptor antagonist (IL-1ra) in media conditioned by human peripheral blood mononuclear cells (MNCs, 5×10^6 cells ml⁻¹) incubated for 16 h with IL-1 β , IL-10 and endotoxin (lipopolysaccharide, LPS) were quantified by enzyme immunoassay (Amersham plc, Amersham, UK) and ELISA, respectively. The cells were isolated from the buffy coats of heparinised human blood by density gradient centrifugation (Poole *et al.*, 1989). The protocol for ELISA of IL-1ra was based upon a protocol for ELISA of IL-6 described previously (Taktak *et al.*, 1991). Briefly, microtiter plates (Immunoplate Maxisorp, NUNC) were coated with immunoaffinity purified sheep anti-IL-1ra polyclonal antibodies (100 μ l of a 2 μ g ml⁻¹ solution in PBS buffer) at 4°C for 16–24 h. After washing with assay buffer (0.01 M phosphate, 0.05 M NaCl, 0.1% Tween 20, pH 7.2), 100 μ l of 1% ovalbumin (Sigma, Poole, Dorset) in PBS coating buffer were added to each well except reagent blank wells and the wells covered and incubated at 37°C for 1 h. After washing with assay buffer, 100 μ l of standard (recombinant IL-1ra, NIBSC preparation 92/644, 7.8–10,000 pg ml⁻¹) or sample were added to each well and incubated at room temperature for 4 h. After washing with assay buffer, 100 μ l/well of biotinylated immunoaffinity purified sheep anti-IL-1ra polyclonal antibodies (diluted 1/1000) were added to each well and the wells covered and incubated at room temperature for 1 h. After washing with assay buffer, colour was developed using avidin-peroxidase (Dako Ltd, High Wycombe, UK) and 1, 2-phenylenediamine dihydrochloride (orthophenylene diamine, OPD, Sigma, Poole, Dorset). The enzyme reaction was stopped by adding 150 μ l 1 M H₂SO₄ to each well and absorbance at 490 nm was measured.

Results are presented as means with s.e.mean of groups of 5 animals for the *in vivo* measurements and of triplicate wells (using pooled cells from two donors) for the *in vitro* assays. Three independent *in vitro* assays were performed, each using blood from different donors of the same blood group. Differences between responses were evaluated by ANOVA, followed by Bonferroni *t* test. Formal statistical tests are not reported for differences where means differed by more than three times the larger s.e.mean.

Materials

Drugs Recombinant human IL-1 β , IL-6, IL-8 (72 amino acids), IL-10, TNF α , IL-1ra and *E. coli* 0113:H10:K- endotoxin (lipopolysaccharide, LPS) were NIBSC preparations coded 86/680, 88/514, 89/520, 92/516, 87/650, 92/644 and 84/650. The specific activities of these materials are IL-1 β : 100,000 international units (iu)/1 μ g/ampoule, IL-6: 100,000 iu/1 μ g/ampoule, IL-8: 1,000 units/1 μ g/ampoule, IL-10: 5,000 units/1 μ g/ampoule, TNF α : 40,000 iu/1 μ g/ampoule, IL-1ra: 10 milli-units/10 μ g/ampoule, LPS: 14,000 iu/2 μ g/ampoule. PGE₂ was a gift from the Upjohn Co (U.S.A.). Carrageenin was a gift from the FMC Corporation (Philadelphia, U.S.A.). Bradykinin was purchased from Sigma (St. Louis, U.S.A.). The monoclonal IgM antibody to mouse IL-10, SX-1, was provided by Professor F. Liew (University of Glasgow, UK). The control monoclonal antibody was a purified unrelated IgM raised against ovalbumin in our laboratory.

Animals Male Wistar rats, 130–180 g, housed in temperature controlled-rooms (22–25°C) with water and food *ad libitum* until use.

Results

Inhibition by IL-10 of the hyperalgesic responses to bradykinin TNF α , IL-1 β and IL-6

Injection of bradykinin (1 μ g, i.pl.) into one hindpaw of rats evoked hyperalgesia, measured 3 h after injection (Figure 1a). IL-10 (1–100 ng/paw), injected 30 min before bradykinin (1 μ g/paw), dose-dependently inhibited the hyperalgesic response measured 3 h after injection of bradykinin (Figure 1a). Although no effect was seen with IL-10 at 1 ng/paw, IL-10 at

Figure 1 Inhibition by interleukin-10 (IL-10) of the hyperalgesic responses to bradykinin, tumour necrosis factor α (TNF α), IL-1 β and IL-6. Responses were measured 3 h after injection (in 100 μ l, i.pl.) of bradykinin, TNF α , IL-1 β and IL-6. IL-10 (1–100 ng in 100 μ l, i.pl., open columns) or saline (Sal, 100 μ l, i.pl., solid columns) was given 30 min before hyperalgesic substances. Panel (a) shows the intensity of hyperalgesia in injected paws in response to bradykinin (1.0 μ g, solid column) and its inhibition by IL-10 (1–100 ng in 100 μ l, i.pl., open columns). Panel (b) shows the effect of saline (Sal) and IL-10 (10 ng in 100 μ l, i.pl.) on responses to TNF α (TNF, 2.5 μ g), IL-1 β (IL-1, 0.5 μ g) and IL-6 (1.0 ng). Mean $\pm$ s.e.mean in groups of 5 rats are shown; *P < 0.05; **P < 0.01, ***P < 0.001.

Figure 2 Effect of interleukin-10 (IL-10) on the hyperalgesic responses to IL-8 and prostaglandin E₂ (PGE₂). Responses were measured 3 h after injection (in 100 μ l, i.pl.) of IL-8 and PGE₂. IL-10 (10 ng in 100 μ l, i.pl., open columns) or saline (100 μ l, i.pl., solid columns) was given 30 min before hyperalgesic substances. Panel (a) shows the effect of saline (Sal) and IL-10 (10 ng in 100 μ l, i.pl.) on the response to IL-8 (0.1 ng). Panel (b) shows the effect of saline (Sal) and IL-10 (10 ng in 100 μ l, i.pl.) on the response to PGE₂ (50 and 100 ng). Mean $\pm$ s.e.mean in groups of 5 rats are shown.

10 and 100 ng/paw inhibited responses to bradykinin (1 μg /paw) by $53 \pm 7\%$ ($P < 0.001$) and $92 \pm 4\%$ ($P < 0.001$), respectively. IL-10, 1–100 ng/paw, injected 30 min before saline had little effect upon Δ reaction times, which were 2.7 ± 0.7 , 0.3 ± 0.7 and 0.8 ± 1.0 s for 1, 10 and 100 ng/paw IL-10. IL-10 (10 ng/paw), injected 30 min before the cytokine, also inhibited hyperalgesic responses to TNF α (2.5 pg/paw, $-71 \pm 8\%$, $P < 0.01$), IL-1 β (0.5 pg/paw, $-75 \pm 10\%$, $P < 0.001$) and IL-6 (1.0 ng/paw, $-43 \pm 12\%$, $P < 0.05$, Figure 1b). The Δ reaction time for IL-10, 10 ng/paw, injected 30 min before saline was 1.1 ± 1.6 s.

Figure 3 Inhibition by interleukin-10 (IL-10) of the hyperalgesic response to carrageenin. Responses were measured 3 h after injection (in 100 μL , i.p.) of carrageenin. IL-10 (10 ng in 100 μL , i.p., open columns) or saline (100 μL , i.p., solid columns) was given 30 min before or 2 h after carrageenin. Panel (a) shows the effects of saline (Sal) and IL-10 (10 ng in 100 μL , i.p.) given 30 min before carrageenin on the response to carrageenin (Cg, 100 μg). Panel (b) shows the effects of saline (Sal) and IL-10 (10 ng in 100 μL , i.p.) given 2 h after carrageenin on the response to carrageenin (Cg, 100 μg). The Δ reaction times for IL-10, 10 ng/paw, injected 30 min before and 2 h after saline were 1.1 ± 1.6 s and 1.0 ± 3.3 s, respectively, data not shown. Mean $\pm$ s.e.mean in group of 5 rats are shown; * $P < 0.001$.

Figure 4 Effect of a monoclonal antibody to interleukin-10 (IL-10) on the hyperalgesic responses to carrageenin, tumour necrosis factor α (TNF α) and IL-8. Responses were measured 3 h after injection (in 100 μL , i.p.) of hyperalgesic substances. The monoclonal antibody to IL-10 (IL-10Ab, 150 μg in 50 μL , i.p., open columns) or a control antibody (CAB, 150 μg in 50 μL , i.p., hatched columns) or saline (50 μL , i.p., solid columns) was given 30 min before carrageenin (10 μg), TNF α (0.025 pg) and IL-8 (0.1 ng). The Δ reaction times for control antibody and monoclonal antibody to IL-10 were 2.6 ± 0.9 s and 1.7 ± 1.6 s, respectively, data not shown. Mean $\pm$ s.e.mean in groups of 5 rats are shown; * $P < 0.05$.

Failure of IL-10 to inhibit the hyperalgesic responses to IL-8 and PGE₂

IL-10 (10 ng/paw), injected 30 min before the hyperalgesic stimulus, failed to inhibit responses measured 3 h after injection of IL-8 (0.1 ng/paw, Figure 2a) and PGE₂ (50 and 100 ng/paw, Figure 2b).

Inhibition by IL-10 of the hyperalgesic response to carrageenin

Injection of carrageenin into one hindpaw of rats (100 μg /paw) evoked hyperalgesia, measured 3 h after injection (Figure 3a). IL-10 (10 ng/paw) injected 30 min before carrageenin inhibited carrageenin-evoked hyperalgesia by $81 \pm 8\%$ ($P < 0.001$) whereas IL-10 (10 ng/paw) injected 2 h after the carrageenin had no significant effect upon the response (Figure 3b). The Δ reaction times for IL-10, 10 ng/paw, injected 30 min before and 2 h after saline were 1.1 ± 1.6 s and 1.0 ± 3.3 s, respectively.

Potential by a monoclonal antibody to IL-10 of the hyperalgesic response to carrageenin and TNF α but not IL-8

A monoclonal antibody to IL-10 (150 μg /paw) but not a control antibody (150 μg /paw), injected 30 min before the

Figure 5 Inhibition by interleukin-10 (IL-10) of IL-1 β -evoked and lipopolysaccharide (LPS)-evoked immunoreactive prostaglandin E₂ (PGE₂) production by human peripheral blood mononuclear cells (MNCs, 5×10^6 cells ml^{-1}) incubated for 16 h with IL-10 (0.25–4.0 ng ml^{-1}), IL-1 β (1–64 ng ml^{-1}) and LPS (1 μg ml^{-1}). IL-10 (vertically hatched columns), IL-10+IL-1 β (1 ng mg^{-1} , solid columns), IL-10+IL-1 β (4 ng mg^{-1} , horizontally hatched columns), IL-10+IL-1 β (16 ng mg^{-1} , cross hatched columns), IL-10+IL-1 β (64 ng mg^{-1} , open columns), and IL-10+LPS (1 μg ml^{-1} , diagonally hatched columns). S.e.mean of triplicate wells using pooled cells from two donors are shown. (Three independent assays were performed.)

stimulus, potentiated hyperalgesic responses measured 3 h after injection of carrageenin ($10 \mu\text{g/paw}$, $+59 \pm 23\%$, $P < 0.05$) and $\text{TNF}\alpha$ (0.025 pg/paw , $+69 \pm 11\%$, $P < 0.05$) but not responses measured 3 h after IL-8 (0.1 ng/paw , $+11 \pm 12\%$, $P < 0.05$, Figure 4). The Δ reaction times for control antibody and monoclonal antibody to IL-10 were $2.6 \pm 0.9 \text{ s}$ and $1.7 \pm 1.6 \text{ s}$, respectively.

Inhibition by IL-10 of IL-1 β -evoked PGE₂ production by human peripheral blood mononuclear cells (MNCs)

IL-1 β ($1\text{--}64 \text{ ng ml}^{-1}$) evoked dose-dependent increases in the production of immunoreactive PGE₂ by MNCs; LPS ($1 \text{ iu} = 143 \text{ pg ml}^{-1}$) evoked an increase in the production of immunoreactive PGE₂ similar to that seen with IL-1 β (64 ng ml^{-1} , Figure 5). IL-10 ($0.25\text{--}4.0 \text{ ng ml}^{-1}$) inhibited in a dose-dependent manner responses to IL-1 β and LPS, with the highest dose of IL-10 (4.0 ng ml^{-1}) abolishing responses to IL-1 β and reducing the response to LPS by over 90% (Figure 5).

Induction of IL-1ra by IL-10, IL-1 β and LPS in MNCs

IL-10 ($0.25\text{--}4.0 \text{ ng ml}^{-1}$) evoked small (dose-dependent) increases in the production of immunoreactive IL-1ra by MNCs (Figure 6). IL-1 β ($1\text{--}64 \text{ ng ml}^{-1}$) and LPS (1 iu ml^{-1}) evoked larger increases in secretion of immunoreactive IL-1ra by MNCs; the effects of IL-10 and IL-1 β and of IL-10 and LPS were not additive (Figure 6).

Figure 6 Induction of immunoreactive interleukin-1 receptor antagonist (IL-1ra) by IL-10, IL-1 β and lipopolysaccharide (LPS) in MNCs. Immunoreactive IL-1ra production by human peripheral blood mononuclear cells (MNCs, $5 \times 10^6 \text{ cells ml}^{-1}$) incubated for 16 h with IL-10 ($0.25\text{--}4.0 \text{ ng ml}^{-1}$), IL-1 β ($1\text{--}64 \text{ ng ml}^{-1}$) and LPS (1 iu ml^{-1}). IL-10 (vertically hatched columns), IL-10 + IL-1 β (1 ng ml^{-1} , solid columns), IL-10 + IL-1 β (4 ng ml^{-1} , horizontally hatched columns), IL-10 + IL-1 β (64 ng ml^{-1} , cross hatched columns), IL-10 + IL-1 β (64 ng ml^{-1} , open columns), and IL-10 + LPS (1 iu ml^{-1} , diagonally hatched columns). Vertical bars are s.e.mean of triplicate wells using pooled cells from two donors are shown. (Three independent assays were performed.)

Discussion

IL-10 inhibits the production of the pro-inflammatory cytokines $\text{TNF}\alpha$, IL-1 β , IL-6 and IL-8 and upregulates the expression of the IL-1 receptor antagonist, IL-1ra, (Howard & O'Garra, 1992; Jenkins *et al.*, 1994). Consequently IL-10 has therapeutic potential in acute and chronic inflammatory diseases. To investigate the possibility that IL-10 could inhibit inflammatory hyperalgesia, bradykinin was chosen as the first inflammatory mediator against which to test IL-10 since bradykinin initiates the release of a cascade of pro-inflammatory cytokines following administration of carrageenin in a model of inflammatory hyperalgesia (Ferreira *et al.*, 1993).

The capacity of a single injection of IL-10 to abolish hyperalgesia evoked by a potent pro-inflammatory stimulus such as carrageenin confirms the anti-inflammatory activity reported for IL-10 in other systems (Fiorentino *et al.*, 1989, 1991; Bogdan *et al.*, 1991; Zlotnik & Moore, 1991; De Waal Malefyt *et al.*, 1991a,b; Howard & O'Garra, 1992; Oswald *et al.*, 1992; Jenkins *et al.*, 1994). That IL-10 inhibited hyperalgesic responses to $\text{TNF}\alpha$ and IL-6 but not to IL-8 and PGE₂ was expected since the hyperalgesic activities of $\text{TNF}\alpha$ and IL-6 depend upon induction of cytokines (IL-1 β and IL-8, and IL-1 β , respectively). In contrast, IL-8 induces hyperalgesic sympathomimetic amines (Cunha *et al.*, 1991) and PGE₂ sensitizes nociceptors (Ferreira, 1972), actions which are not sensitive to inhibition by IL-10. Consequently, the data obtained are consistent with the cascade of mediators proposed for this model, in which the sequence of events appears to be as follows. Carrageenin stimulates release of bradykinin, which stimulates the release of $\text{TNF}\alpha$. This (i) induces IL-6 and IL-1 β , which stimulate the production of cyclo-oxygenase products and (ii) induces IL-8 which stimulates local production of sympathetic amines (Cunha *et al.*, 1991, 1992; Ferreira *et al.*, 1993).

The finding that IL-10 inhibited hyperalgesic responses to carrageenin when the IL-10 was injected before but not after the carrageenin are consistent with the reported inhibitory action of IL-10 on the production of $\text{TNF}\alpha$, IL-1 β , IL-6 and IL-8 (de Waal Malefyt *et al.*, 1991b; Fiorentino *et al.*, 1991; Bogdan *et al.*, 1991; Oswald *et al.*, 1992), the pro-inflammatory cytokines that transduce the hyperalgesic activity of carrageenin (Ferreira, *et al.*, 1988; Cunha *et al.*, 1991; 1992). That endogenous IL-10 has a significant role in limiting cytokine-mediated inflammatory hyperalgesia is indicated by the finding that, in rats, a monoclonal antibody to mouse IL-10 potentiated hyperalgesic responses to carrageenin and $\text{TNF}\alpha$. The lack of effect of the antibody neutralizing endogenous IL-10 on the response to IL-8 was expected since, as stated above, IL-8 releases hyperalgesic sympathomimetics (Cunha *et al.*, 1991) and therefore does not rely upon IL-10-sensitive cytokine production to evoke hyperalgesia.

A finding that required further study was the capacity of IL-10 to inhibit IL-1 β -evoked hyperalgesia. IL-1 β stimulates the production of cyclo-oxygenase products, such as PGE₂ (Seibert *et al.*, 1990) which sensitize nociceptors (Ferreira, 1972), i.e., IL-1 β evokes hyperalgesia by a mechanism that does not require IL-10-sensitive production of pro-inflammatory cytokines. Therefore IL-10 must have inhibited IL-1 β -evoked hyperalgesia by other means. Since IL-10 can induce the IL-1 receptor antagonist, IL-1ra (Howard *et al.*, 1992; Jenkins *et al.*, 1994), experiments were performed to investigate the possibility that induction of IL-1ra by IL-10 contributed to the inhibition by IL-10 of IL-1 β evoked hyperalgesia. These experiments were performed *in vitro* using human peripheral blood mononuclear cells (MNCs), cells that are a major source of IL-10 (Howard & O'Garra, 1992) and which are sensitive to suppression by IL-10 of inflammatory cytokine production (Ralph *et al.*, 1992). IL-10 abolished IL-1 β -induced PGE₂ production by MNCs while having little effect upon IL-1 β -induced production of IL-1ra, suggesting that IL-10 induction of IL-1ra contributed little to the inhibition by IL-10 of IL-1 β -induced PGE₂ production.

In addition to its potent inhibitory effect on IL-1 β -induced PGE₂ production, IL-10 inhibited (by more than 90%) LPS-evoked PGE₂ production. Similarly, IL-10 inhibited, by 80–95%, concanavalin A (ConA)-induced monocyte prostaglandin H synthase-2 (PGHS-2/COX-2) and completely inhibited detectable PGHS-2 protein (Mertz *et al.*, 1994). Taken together, these data support the notion that suppression by IL-10 of PGHS-2, rather than some activity of IL-10 against IL-1 β , accounted for the inhibition by IL-10 of PGE₂ production evoked by IL-1 β and LPS, although it should be noted that in the *in vitro* studies of Mertz *et al.* (1994) optimal expression of COX-2 mRNA and protein occurred at 4 h and 18 h, respectively after stimulation with ConA, whereas the *in vivo* hyperalgesic response to IL-1 β was maximal at 1 h (Ferreira *et al.*, 1988). A proportion of this difference could be accounted for by the time required for ConA to induce endogenous mediators such as IL-1 β . It is conceivable that inhibition by IL-10 of an effect of IL-1 β on phospholipase A₂ (PLA₂) may

have contributed to the diminished PGE₂ production since IL-1 upregulates cytosolic (>>secretory) PLA₂ gene expression in human synovial cells (Angel *et al.*, 1994).

In summary, the data reported above suggest that IL-10 limits the inflammatory hyperalgesia evoked by carrageenin and bradykinin by two mechanisms: inhibition of cytokine production and inhibition of IL-1 β evoked PGE₂ production. The latter effect does not appear to be mediated via IL-10-induced IL-1ra and may result from suppression by IL-10 of prostaglandin H synthase-2 (COX-2). The capacity of IL-10 to inhibit concomitantly both IL-1 β production and IL-1 β -stimulated PGE₂ production helps to ensure that the cascade of mediators that cause inflammatory hyperalgesia is closely regulated.

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