British Journal of Pharmacology (1997) 121, 417–424

Cytokines, nerve growth factor and inflammatory hyperalgesia: the contribution of tumour necrosis factor α

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1 Peripheral inflammation is characterized by heightened pain sensitivity. This hyperalgesia is the consequence of the release of inflammatory mediators, cytokines and growth factors. A key participant is the induction of the neurotrophin nerve growth factor (NGF) by interleukin-1β (IL-1β).

2 Tumour necrosis factor α (TNFα) has been shown both to produce hyperalgesia and to upregulate IL-1β. We have now examined whether the induction of TNFα in inflammatory lesions contributes to inflammatory sensory hypersensitivity by inducing IL-1β and NGF.

3 The intraplantar injection of complete Freund's adjuvant (CFA) in adult rats produced a localized inflammation of the hindpaw with a rapid (3 h) reduction in withdrawal time in the hot plate test and in the mechanical threshold for eliciting the flexion withdrawal reflex.

4 The CFA-induced inflammation resulted in significant elevation in the levels of TNFα, IL-1β and NGF in the inflamed paw. In the case of TNFα, an elevation was detected at 3 h, rose substantially at 6 h, peaked at 24 h and remained elevated at 5 days, with similar but smaller changes in the contralateral non-inflamed hindpaw. No increase in serum TNFα was detected at 24 h post CFA injection.

5 Intraplantar recombinant murine TNFα injections produce a short-lived (3–6 h) dose-dependent (50–500 ng) increase in thermal and mechanical sensitivity which was significantly attenuated by prior administration of anti-NGF antiserum.

6 Intraplantar TNFα (100–500 ng) also elevated at 6 but not 48 h the levels of IL-1β and NGF in the hindpaw.

7 A single injection of anti-TNFα antiserum, 1 h before the CFA, at a dose sufficient to reduce the effects of a 100 ng intraplantar injection of TNFα, significantly delayed the onset of the resultant inflammatory hyperalgesia and reduced IL-1β but not NGF levels measured at 24 h.

8 The elevation of TNFα in inflammation, by virtue of its capacity to induce IL-1β and NGF, may contribute to the initiation of inflammatory hyperalgesia.

Keywords: Pain; inflammation; hyperalgesia; nerve growth factor; interleukin-1β; tumour necrosis factor; cytokines; neurotrophins

Introduction

Inflammatory pain hypersensitivity is the consequence of alterations in transduction sensitivity of high threshold nociceptors (Treede & Cole, 1993; Reeh, 1994), activity-dependent changes in the excitability of spinal neurones (McMahon *et al*., 1993; Woolf, 1995) and phenotypic changes in sensory neurones innervating the inflamed tissue (Woolf, 1996; Neumann *et al*., 1996). These changes, both at the inflamed site and within the nervous system are initiated by a complex pattern of chemical signals interacting with the sensory fibre terminals. These signals originate from infective agents, damaged host cells or activated inflammatory cells. A major role is played by inflammatory mediators such as the kinin bradykinin, amines like histamine and 5-hydroxytryptamine, eicosanoids like prostaglandin E_2, purines like ATP, and ions like potassium and hydrogen, which appear collectively to alter the sensitivity of the transduction processes in the peripheral terminals of high threshold primary sensory neurones by a modulation of ion channels in a phosphorylation-dependent fashion (England *et al*., 1996; Gold *et al*., 1996). A second series of signals are those generated or initiated by inflammatory cells, the inflammatory cytokines (Arai *et al*., 1990). The cytokines are pro-inflammatory, acting on and between the inflammatory cells to induce some of the features of the inflammatory response and also mediate some of the systemic effects of inflammation such as fever or cachexia (Dinarello, 1991). Many of these effects are transcription-dependent (Gordon *et al*., 1992). The cytokines also appear to have a role in the sensory hypersensitivity associated with inflammation (Ferreira *et al*., 1988; 1993; Cunha *et al*., 1992; Watkins *et al*., 1994; 1995; Safieh-Garabedian *et al*., 1995). This may be in some cases direct, acting on cytokine receptors on neurones, or more commonly indirect, stimulating the release of agents that act on the neurone (Safieh-Garabedian *et al*., 1995).

Recently evidence has accumulated for a major role for the neurotrophin nerve growth factor (NGF) in mediating inflammatory hyperalgesia, partly as a result of its indirect action on sympathetic neurones (Andrew *et al*., 1995; Woolf *et al*., 1996) and by stimulation of mast cell degranulation (Horigome *et al*., 1994; Lewin *et al*., 1994; Woolf *et al*., 1996), but mainly as a result of its interaction with the high affinity NGF receptor trkA (Woolf *et al*., 1996) which is expressed on a subpopulation of sensory neurones (Averill *et al*., 1995). The level of NGF is elevated during inflammation (Donnerer *et al*., 1992; Woolf *et al*., 1994), NGF administration produces hyperalgesia (Lewin *et al*., 1993; Woolf *et al*., 1994; Della Seta *et al*., 1994; Petty *et al*., 1994) and anti-NGF or competing trkA-Ig fusion proteins substantially attenuate inflammatory hyperalgesia (Woolf *et al*., 1994; Lewin *et al*., 1994; McMahon *et al*., 1995).

The inflammatory elevation in NGF levels is induced to a substantial extent by interleukin-1β (Safieh-Garabedian *et al*., 1995). This cytokine, when injected into the skin, increases

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local NGF levels and systemic administration of a recombinant interleukin-1 (IL-1)-receptor antagonist reduces both inflammatory hyperalgesia and the elevated levels of NGF (Salfieh-Garabedian *et al*., 1995). Since tumour necrosis factor α (TNFα) usually precedes IL-1β in the inflammatory cytokine cascade (Vassalli, 1992), and because this cytokine upregulates NGF *in vitro* (Hattori *et al*., 1993) and has hyperalgesic actions (Cunha *et al*., 1992; Watkins *et al*., 1995), we have now investigated whether TNFα contributes, via IL-1β and NGF upregulation, to the establishment of inflammatory hyperalgesia.

Methods

All experiments were performed on adult male Sprague-Dawley rats (~200 g, supplied by U.C.L. Biological Services).

Inflammation

Inflammation was produced by the subcutaneous injection of 100 μl of complete Freund's Adjuvant (CFA; Sigma) into the plantar surface of the left hind paw under fluothane (ICI) anaesthesia (induction 4%, maintenance 2%). The CFA injection produced an area of localized erythema and oedema that did not disturb weight gain, grooming, the sleep-wake cycle or social interactions.

Behavioural tests

Mechanical hypersensitivity was assessed as the minimum force required to elicit a reproducible flexor withdrawal reflex on each of 3 applications to the dorsal surface of the toes, with a set of nylon monofilament Von Frey hairs (4.1 to 72 g), as described previously (Safieh-Garabedian *et al*., 1995). Thermal hypersensitivity was determined by the hot plate technique, the time for foot withdrawal on contact with a metal plate at 50°C was measured (Safieh-Garabedian *et al*., 1995).

Experimental procedures

Each experimental group comprised 4 or 5 animals. Behavioural measurements were made immediately before an intraplantar injection of either CFA or TNFα (preinjection, time 0) and 1, 3, 6, 24 and 48 h post injection in the case of TNFα and 3, 6, 24, 48, 120, 240 and 360 h post CFA. The animals were terminally anaesthetized with sodium pentobarbitone (Duphar) at 3, 6, 24, 48, 120 h and the entire hind paw skin, ipsilateral and contralateral to the inflammation removed. The tissue samples were weighed, frozen on dry ice, stored at −70°C and subsequently processed for TNFα, IL-1β and NGF determinations. Blood samples from naive and animals 24 h post CFA were collected, diluted 1 : 10 in phosphate buffered saline (PBS) containing; 0.4 M NaCl, 0.05% Tween 20, 0.5% bovine serum albumin, 0.1 mM phenylmethylsulphonyl fluoride, 0.1 mM benzethonium chloride, 10 mM EDTA and 20 KI ml^{-1} aprotinin (Sigma), allowed to stand on ice for 2 h, centrifuged and the supernatant collected for assay.

Drug administration

Recombinant murine TNFα (NIBSC preparation coded 88/532 200,000 iu mg^{-1}) was dissolved in saline and administered by intraplantar injection (100 μl) under fluothane (ICI) anaesthesia. Sheep anti-murine TNFα serum (Mahadevan *et al*., 1990) kindly provided by Dr T. Meager (Division of Immunobiology, NIBSC), was given i.p. one hour before the intraplantar CFA injection at a dose of 5 ml kg^{-1}. Sheep anti-murine NGF serum (Woolf *et al*., 1994), raised in collaboration with the Hannah Research Institute (Ayr, Scotland) was given i.p. one hour before the intraplantar TNFα injection (100 ng) at a dose of 5 ml kg^{-1}.

Assays

Before the NGF, IL-1β and TNFα assay, skin samples were homogenized in PBS. The supernatant was used to measure NGF, IL-1β and TNFα levels.

NGF assay

NGF was measured by using a two-site enzyme-linked immunoassay (ELISA), based on the method of Weskamp & Otten (1987), with modifications as described previously (Safieh-Garabedian *et al*., 1995). Polystyrene high binding 96 well microtitre plates (Nunc), were coated with a polyclonal rabbit anti-NGF (Safieh-Garabedian *et al*., 1995) in bicarbonate buffer (1/20000). After incubation at 4°C, washing with PBS containing 0.05% Tween 20 and 0.4 M NaCl and blocking with PBS-0.5% BSA for 1 h at 37°C, NGF standard (Promega) and samples (100μl of each) were added in duplicate to the plates and incubated overnight at 4°C. After washing, 100 μl of rat anti-NGF (1/20000) monoclonal antibody (23c4) (Weskamp & Otten, 1987) was added to each well and the plates incubated overnight at 4°C. Colour was developed by use of biotinylated rabbit anti-rat IgG (Zymed) and peroxidase conjugated streptavidin (Dako) with 3,3′,5,5-tetramethyl-benzidine (TMB) (Sigma) and optical density (OD) measured at 450 nm. Results are expressed as ng/hind paw, since the weight of the inflamed paw skin increased more than two fold.

IL-1β assay

This was measured by a two-site ELISA (Safieh-Garabedian *et al*., 1995). Immunoaffinity purified sheep polyclonal anti-rat IL-β antibodies (2 mg ml^{-1}) in 100 ml PBS buffer, was used to coat microtitre plates (Nunc Maxisorb). After incubation (4°C overnight) and washing of the plates in assay buffer (0.01 M phosphate, 0.05 M NaCl, 0.1% Tween 20, pH 7.2), 100 μl of standard recombinant rat IL-1β (a generous gift from Dr Robert Newton, DuPont-Merk, Wilmington, Delaware) or sample was added to each well and incubated overnight at 4°C. After washing, 100 μl of biotinylated, immunoaffinity purified sheep polyclonal anti-rat IL-1β antibodies (1/1000) with 1% normal sheep serum, were added to the plates and incubated for 1 h at room temperature. Colour was developed with avidin-peroxidase (Dako) as detailed above.

TNFα assay

TNFα was measured by use of a solid phase sandwich ELISA (Cytoscreen Immunoassay kit Serotec/Biosource International). Microtitre plates pre-coated with anti rat TNFα were allowed to warm to room temperature, 50 μl of standard recombinant rat TNFα or sample was added to the appropriate wells in duplicate and 50 μl of biotinylated anti TNFα was added to all the wells. These were incubated for 1.5 h at room temperature. After washing, colour was developed with streptavidin peroxidase conjugate and TMB, and the OD measured at 450 nm.

Statistical analysis

Data are presented as the mean ± s.e.mean. Differences between experimental groups and controls were analysed by Student's or Welch's *t* test and one-way analysis of variance (ANOVA) followed by Dunnet's Multiple Comparison test.

Results

CFA injection into the hindpaw produced by 3 h a substantial increase in thermal sensitivity (as measured by a reduction in the latency to withdrawal to a 50°C stimulus) and in mechanical sensitivity (as measured by the fall in threshold of the flexion

withdrawal reflex) (Figure 1). The elevated sensitivity remained present throughout the 15 days it was tested (Figure 1).

TNFα, NGF and IL-1β levels in the hindpaw

At the earliest time tested (3 h) the levels of TNFα, IL-1β and NGF were all significantly elevated over those found in non-inflamed skin (Figure 2). However, the temporal pattern of changes induced by the inflammation differed for each. In the case of TNFα, levels were elevated at 3 h from a basal level, rose substantially at 6 h, peaked at 24 h with a second rise at 5 days and the levels in the contralateral non-inflamed hindpaw paralleled, at a lower lever, those in the ipsilateral inflamed hindpaw. IL-1β showed the largest relative increase from its very low basal levels, with a peak increase at 6 h followed by a

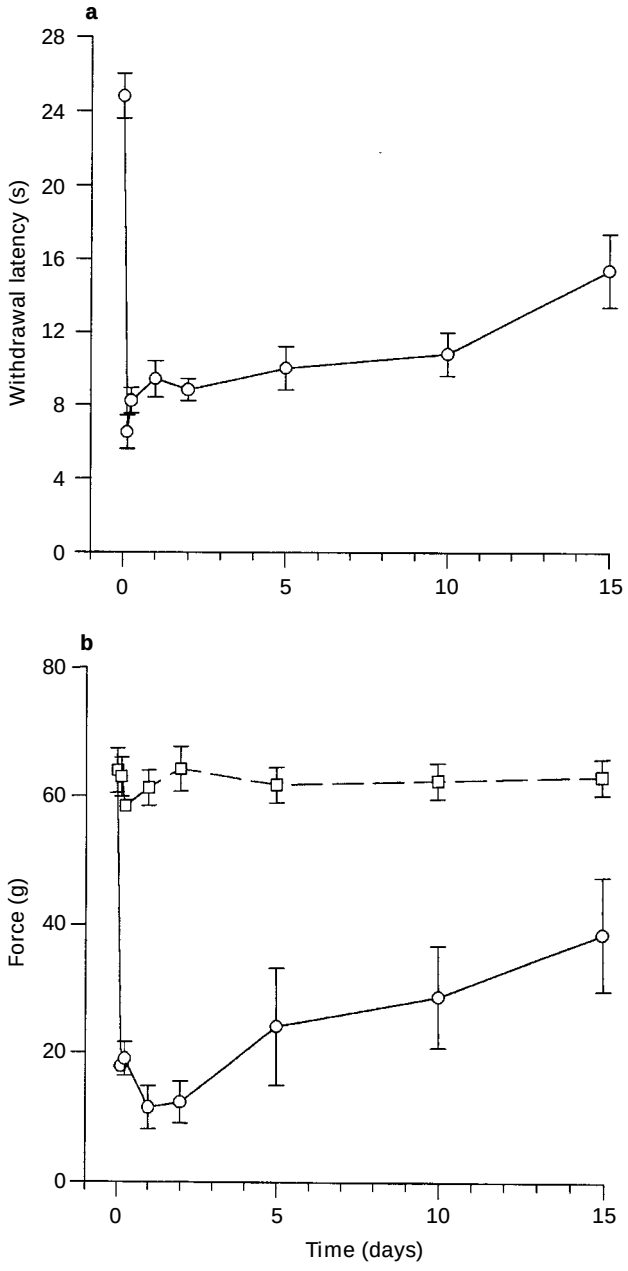

Figure 1 The changes in (a) thermal and (b) mechanical sensitivity produced after an intraplantar injection of complete Freund's adjuvant ($n=5$). (a) Thermal sensitivity was measured as the reduction in withdrawal latency in a standard hot plate test (50°C). (b) Mechanical sensitivity was measured as the reduction in force necessary to elicit a flexion withdrawal reflex with von Frey hairs. (○—○) Changes in sensitivity ipsilateral to the inflammation, (□- - -□) contralateral sensitivity. Values shown are mean and vertical lines indicate s.e.mean.

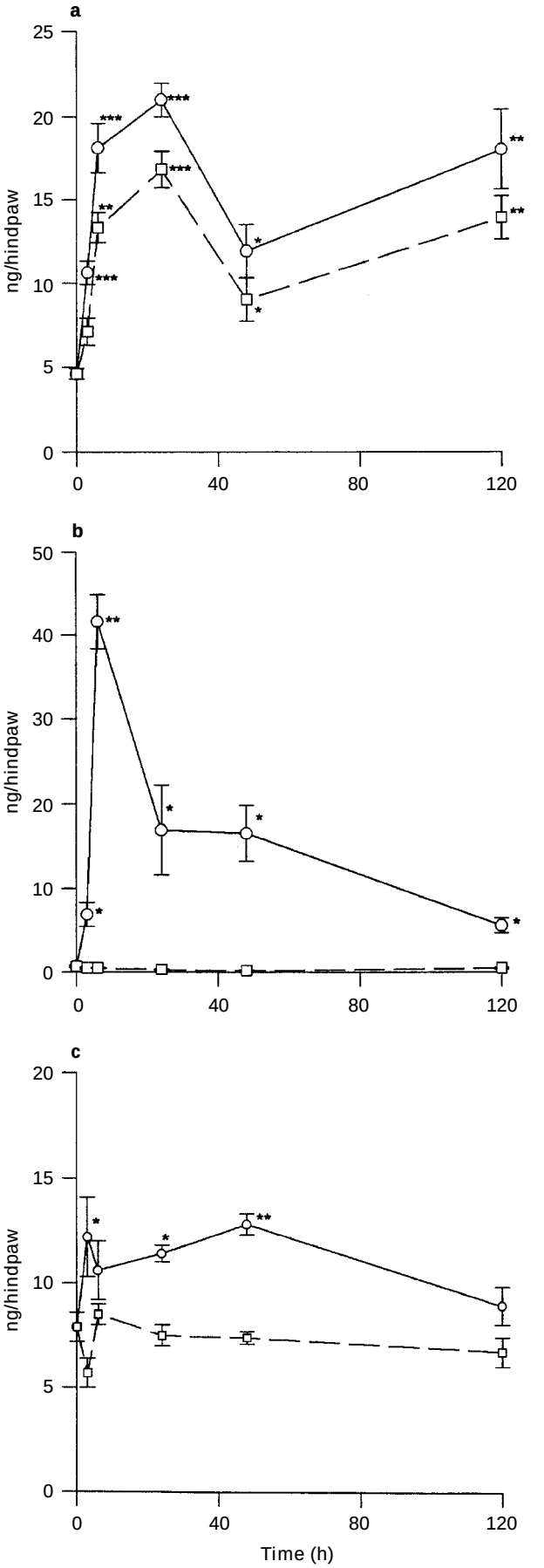

Figure 2 Changes in the levels of (a) TNFα, (b) IL-1β and (c) NGF in the hindpaw ipsilateral (○—○) and contralateral (□- - -□) to a CFA-induced inflammation, $n=4$. *$P<0.05$. **$P<0.01$, ***$P<0.001$ compared with non-inflamed skin. Values shown are mean and vertical lines indicate s.e.mean.

steady decline, although still at significantly increased levels (up to 5 days). No changes in IL-1β in the contralateral hindpaw were detected. The NGF levels showed the smallest relative change and after the early rise remained elevated at a new plateau level. TNFα levels just above the detection threshold of the assay were detected in serum of naive animals (150 pg ml^{-1}) with no change in the serum level 24 h post CFA injection.

Hyperalgesic activity of TNFα

Intraplantar injections of murine recombinant TNFα resulted in a transient (3–6 h) increase in thermal sensitivity first detected at a dose of 50 ng and reaching a plateau at >100 ng (Figure 3). A significant increase in mechanical sensitivity at 3 and 6 h postinjection was found with the 100 and 500 ng doses (Figure 3). This was associated with some erythema and minor swelling.

Effect of TNFα on IL-1β and NGF levels

Intraplantar TNFα at 100 and 500 ng elevated IL-1β and NGF levels in a dose-dependent fashion when measured 6 but not 48 h after the intraplantar injection (Figure 4). The IL-1β levels although significantly elevated, were considerably less than those achieved with CFA but the NGF increases were similar.

Anti-TNFα antiserum

The administration of 5 ml kg^{-1} anti-TNFα antiserum i.p., 1 h before CFA, significantly attenuated the development of thermal sensitivity at 3 h and effectively blocked the mechanical hypersensitivity at 3 and 6 h. No effect was present at 24 h (Figure 5). The anti-TNFα antiserum reduced the elevation in the levels of IL-1β normally found at 24 h post CFA but had no effect on NGF levels which remained significantly elevated above controls at this time point (Figure 6). Pretreatment with the anti-TNFα serum at this dose significantly attenuated the

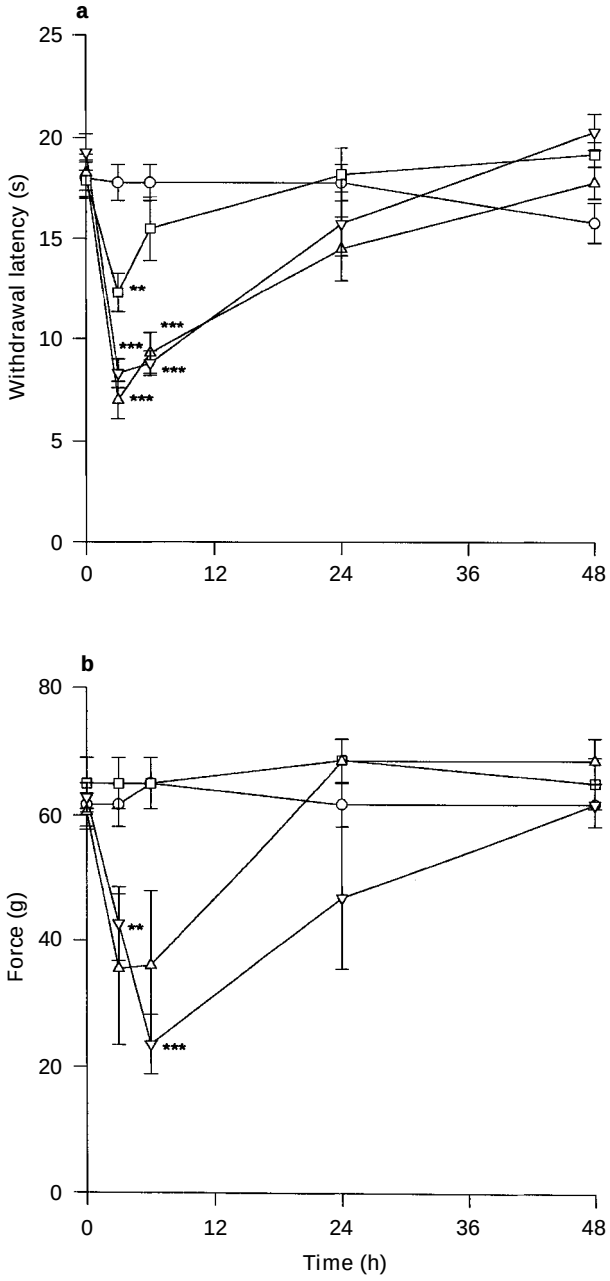

Figure 3 The effect of intraplantar murine TNFα on (a) thermal and (b) mechanical sensitivity. TNFα (○) 1 ng, (□) 50 ng, (△) 100 ng and (▽) 500 ng. Values shown are mean and vertical lines indicate s.e.mean, $n=4$ per dose. $^{**}P<0.01$, $^{***}P<0.001$ compared with basal sensitivity.

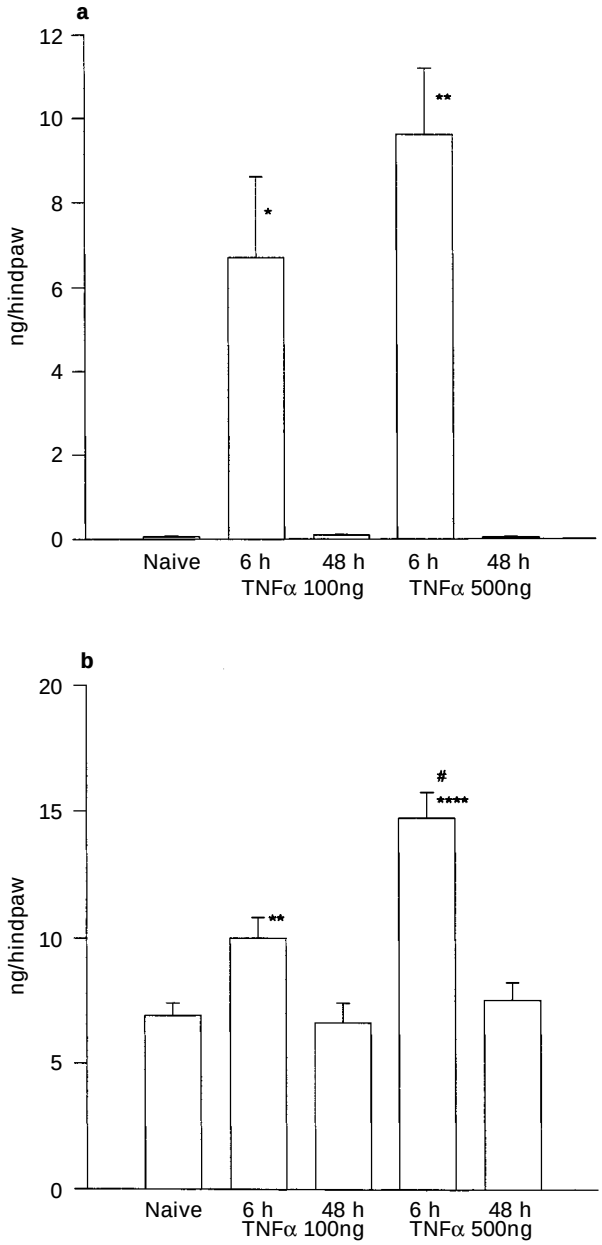

Figure 4 The effect of intraplantar murine rTNFα on (a) IL-1β and (b) NGF levels in the injected hindpaw. Values shown are mean±s.e.mean, $n=4$ per group. $^{*}P<0.05$, $^{**}P<0.01$, $^{****}P<0.0001$ compared with naive; $\#P<0.05$ 100 ng vs 500 ng.

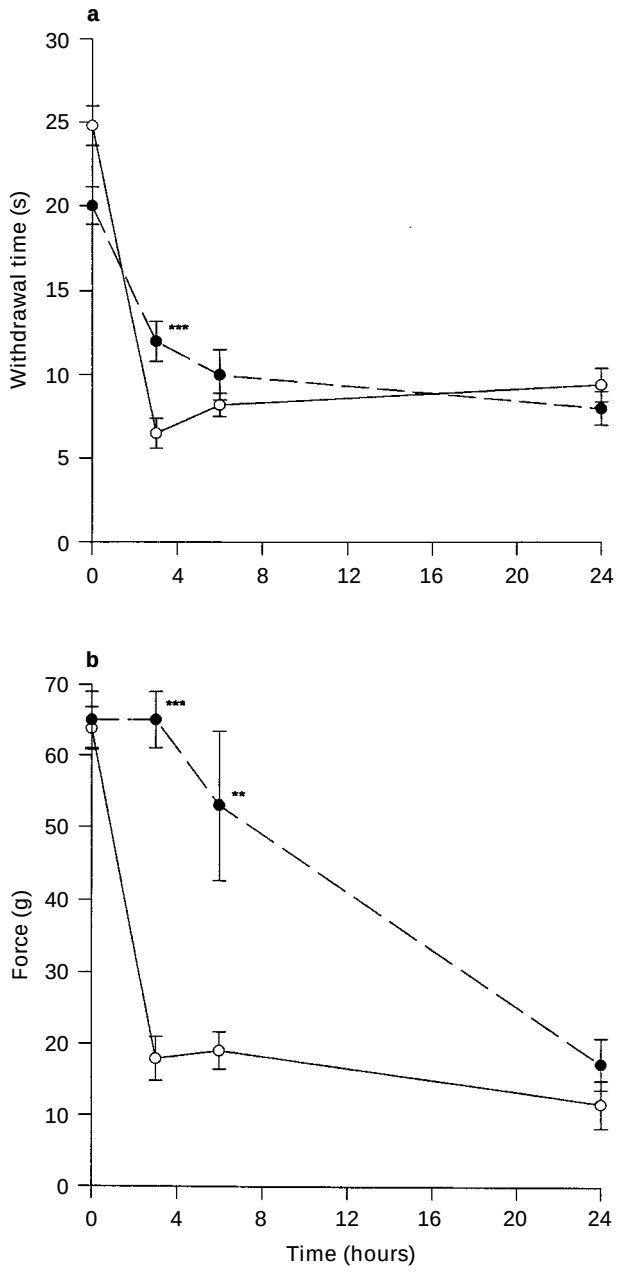

Figure 5 Anti-TNFα antiserum administered 1 h before the induction of inflammation with CFA (a) reduces thermal and (b) delays mechanical hypersensitivity. (○—○) Effect of CFA alone; (●- - -●) Effect of CFA + anti-TNFα antiserum. Values shown are mean and vertical lines indicate s.e.mean, $n=4$ per group. $^{**}P<0.01$, $^{***}P<0.001$ treated vs non treated.

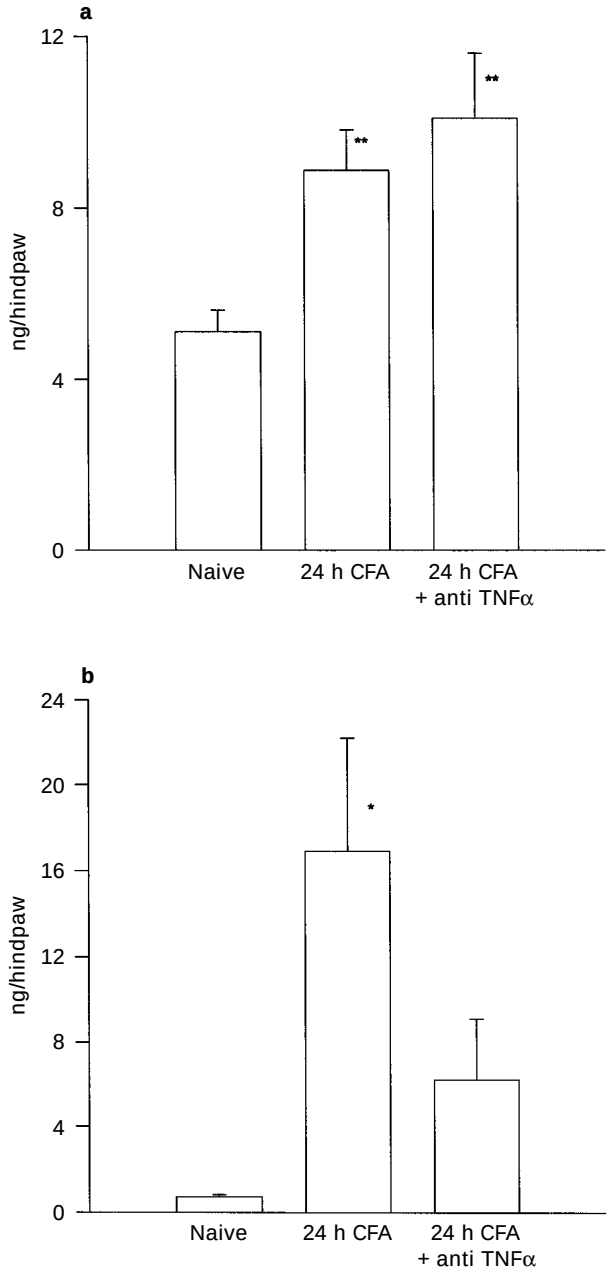

Figure 6 The effect of a single pretreatment with anti-TNFα antiserum on the CFA-induced increase in (a) NGF and (b) IL-1β found at 24 h post CFA. The anti-TNFα failed to prevent the elevation in NGF but reduced that of IL-1β. Values shown are mean ± s.e.,mean, $n=4$ per group. $^{**}P<0.01$ naive vs treated.

behavioural changes produced by intraplantar TNFα (100 ng) (mechanical sensitivity 1 h post TNFα alone 32.1 ± 8.8 g vs 61 ± 3.4 g for TNFα plus anti-TNFα ($n=4$, $P<0.05$); thermal sensitivity 1 h post TNFα alone 11 ± 2.1 s vs 19.3 ± 1.3 s for TNFα plus anti-TNFα ($n=4$, $P<0.05$)) and reduced the level of NGF induced at 24 h by intraplantar TNFα (12.9 ± 2.8 ng/hindpaw for TNFα alone, $n=4$, vs 7.8 ± 0.74 ng/hindpaw, $n=4$, for TNFα plus anti-TNFα).

Anti-NGF antiserum

To investigate whether the hyperalgesia produced by TNFα was NGF-dependent we pretreated with anti-NGF (5 ml kg^{-1}) 1 h before administration of intraplantar TNFα (100 ng). Figure 7 shows that the anti-NGF serum resulted in a significant reduction in both the thermal and mechanical hyperalgesia produced by TNFα.

Discussion

TNFα is a 17 kDa cytokine produced by a number of cell types including inflammatory cells (neurotrophils, activated lymphocytes, macrophages) and tissue cells (endothelial cells, smooth muscle cells, fibroblasts, basal keratinocytes (di Giovine *et al*., 1991; Vilcek & Lee, 1991; Vassalli, 1992; Zhang *et al*., 1995). This immunomodulating factor has multiple actions including cytolysis, mitogenesis, polymorphonuclear and lymphocyte recruitment as well as initiating a cascade of other cytokines most notably IL-6 and IL-1β (Issekutz *et al*., 1994; Lukacs *et al*., 1995; Zhang *et al*., 1995). Levels of TNFα increase in disease and immune states including endotoxic shock (Hasko *et al*., 1995), gout (di Giovine *et al*., 1991) and rheumatoid arthritis (Issekutz *et al*., 1994), contact hypersensitivity (Piguet *et al*., 1991), airway inflammation (Lukacs *et al*., 1995) and immune complex disease (Sekut *et al*., 1994). Neutraliza-

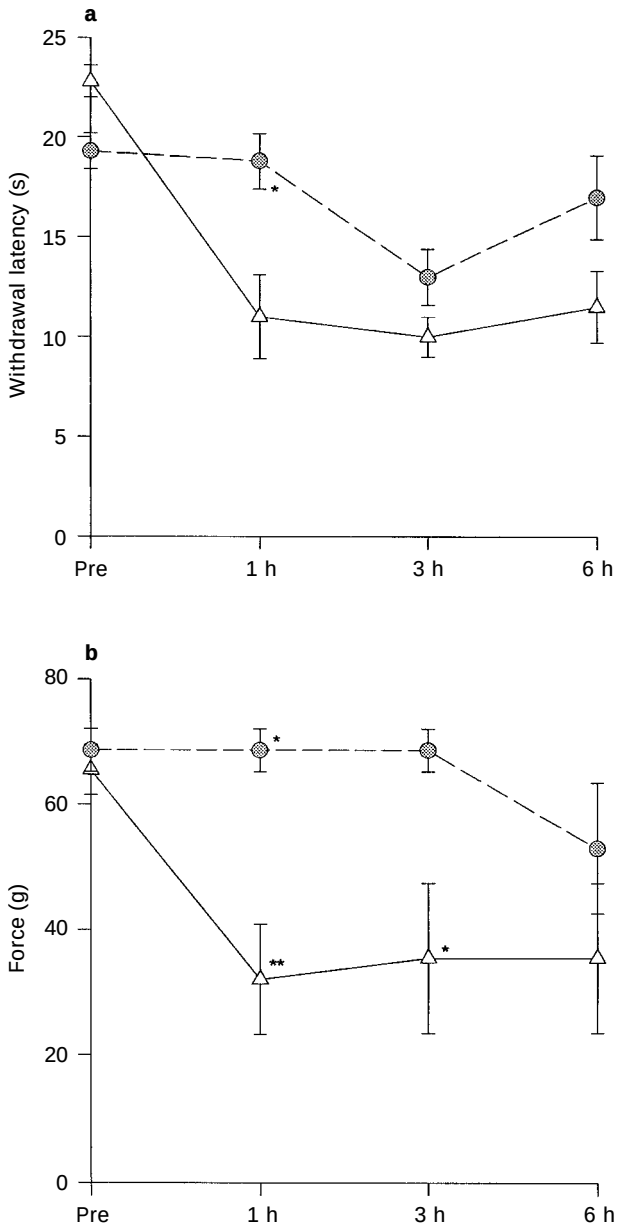

Figure 7 The effect of anti-NGF pretreatment on the hyperalgesia produced by intraplantar TNFα (100 ng). (△—△) The effect of TNFα administered on its own on (a) thermal and (b) mechanical sensitivity, (○- - -○) the effect of anti-NGF given 1 h before the TNFα injection. Values shown are mean and vertical lines indicate s.e.mean, $n=4$ per group. $*P<0.05$, $**P<0.01$ anti-NGF treated vs untreated.

tion of TNFα with antiserum or by competing with recombinant TNF receptor-fusion protein molecules has been shown to reduce the lethality of bacterial lipopolysaccharide (Lessalauer *et al*., 1991), turpentine-induced fever (Cooper *et al*., 1994) and inflammatory damage in rheumatoid (Rankin *et al*., 1995) and experimental arthritis (Williams *et al*., 1995). A specific role of TNFα in hyperalgesia has been suggested on the basis of the hypersensitivity produced by local (Cunha *et al*., 1992) or systemic administration (Watkins *et al*., 1995) and because of the anti-hyperalgesic action of anti-TNFα antibodies on bradykinin, lipopolysaccharide and carageenin-induced inflammation (Cunha *et al*., 1992; Ferreira *et al*., 1993).

We have now found that TNFα, like IL-1β and NGF, is upregulated in the rat hindpaw after the induction of localized peripheral inflammation with complete Freund's adjuvant. All are elevated by 3 h post CFA injection but the temporal profile of their response to the inflammation differs; IL-1β is greatly increased at an early stage (3–6 h) with a gradual decline from this peak over the ensuing 5 days. NGF levels show an early increase with maintenance at this increased level for at least the first 5 days of the inflammation, and TNFα rises at 3 h is high at 6 h, peaks at 24 h, dips at 48 h and then rises again. TNFα is unusual, moreover, in that the contralateral non-inflamed hindpaw also shows a significant elevation, implying systemic spread of the cytokine, although we found no elevation of TNFα levels in the serum 24 h post CFA injection. This suggests that the increased contralateral levels are not simply a reflection of increased circulating levels, the actual mechanism involved is, though, unknown. It may be secondary to systemic spread of some other signal molecule. That detectable basal levels of the cytokine were found in the skin in the absence of inflammation was unexpected and although it is unlikely, given the specificity of the assay, to reflect cross-reactivity with another protein, this cannot be excluded.

As shown previously (Cunha *et al*., 1992), we found that TNFα produces hyperalgesia when injected locally subcutaneously but the effect, like that of IL-1β (Safieh-Garabedian *et al*., 1995) or NGF (Woolf *et al*., 1996) was short-lived and required fairly large doses to elicit changes in sensitivity in our tests. However, these doses did result in the local elevation of IL-1β and NGF. The elevated level in NGF is likely to be mediated by the prior upregulation of IL-1β (Safieh-Garabedian *et al*., 1995), although in culture, TNFα and IL-1β interact synergistically to increase NGF production from non-neuronal cells (Hattori *et al*., 1993), implying a non-IL-1β-dependent action of TNFα on NGF production. TNFα appears, then, to be able to initiate a cytokine cascade, involving IL-1β, that leads to the upregulation of a potent sensitizing agent, NGF.

The preadministration of anti-NGF antibodies results in a very substantial reduction in CFA-induced hyperalgesia and in the phenotypic changes it produces in primary sensory neurones (Woolf *et al*., 1994; Neumann *et al*., 1996). Anti-NGF antiserum also reduces the hyperalgesic effects of both IL-1β (Safieh-Garabedian *et al*., 1995) and as found here, TNFα, showing that these two cytokines produce a substantial component of their sensitivity changes via NGF. NGF in turn produces hypersensitivity in part via the degranulation of mast cells and the activation of postganglionic sympathetic terminals and, in part, via a direct action on trkA expressing high threshold sensory terminals (Woolf *et al*., 1996). Systemic administration of the rIL-1 receptor antagonist (IL-1 RA), which blocks the action of IL-1β, reduces the early phase of CFA-induced hyperalgesia (Safieh-Garabedian *et al*., 1995). Anti-TNFα administration also delayed the onset of CFA-induced hyperalgesia with a maximal but incomplete effect on thermal sensitivity at 3 h, and a larger and longer lasting (6 h) effect on mechanical hypersensitivity. However, by 24 h the hyperalgesia was fully developed in spite of the anti-TNFα pretreatment. Anti-TNF administration resulted in a reduction in IL-1β but not in NGF levels when measured 24 h post induction of the inflammation. It was not possible to ascertain whether the short duration of the effects of the anti-TNFα on the behavioural hypersensitivity and the failure to reduce NGF levels at 24 h was because an inadequate amount of anti TNFα was administered or its action was too shortlasting. A further possibility is that IL-1β and NGF may be induced during inflammation in TNFα-dependent and -independent fashions. Therefore, while a linear cascade of signal molecules appears to operate to produce the transient hyperalgesic effects of the intraplantar administration of TNFα, the situation is more complex during inflammation when multiple events operating over a diverse time course occur.

The cytokine response to inflammation is extremely complex, involving upregulation of pro- and anti-inflammatory factors which act and interact on a broad number of cell types producing many transcription-independent and -dependent changes. In view of the wide range of inflammatory mediators that act on sensory nerve terminals, multiple opportunities exist to produce local changes in terminal sensitivity and hence

hyperalgesia. These will include degranulation of mast cells, induction of cyclo-oxygenase, activation of kininogins, interaction with postganglionic sympathetic terminals and upregulation of neuroactive growth factors such as NGF (Levine & Taiwo, 1994). The current evidence points to TNFα having a role in inducing the hyperalgesic response to inflammation and this is very likely to be the consequence of its induction of later acting intermediaries, particularly IL-1β and NGF. If TNFα were to be a suitable target for the development of novel analgesics, the extent to which IL-1β and NGF upregulation is contingent on prior TNFα upregulation needs to be established. The fact that in the present experiments TNFα levels increased in the non-inflamed hindpaw without any changes in IL-1β/NGF levels indicates either that a threshold must exist for it to exert its action in producing these factors or that TNFα acts in concert with some other factor(s) which are restricted to the site of the inflammation.

Unraveling the cytokine response to inflammation is likely to contribute not only to improved understanding and therefore therapeutic options for managing local inflammation and its systemic effect like fever, but also, by virtue of the cytokine-dependent production of neuroactive growth factors, on the intimate communication between inflamed tissue and neurones which can lead to functional and chemical changes which manifest as sensory hypersensitivity. A major effort needs to be directed at elucidating whether the cytokine involvement in inflammatory hyperalgesia is organised in series, initiated by a single early pro-inflammatory cytokine like TNFα which then initiates the release/induction of a specific intermediary, which in turn acts to release a further factor (i.e. a defined serial cascade), or in parallel, where many factors are simultaneously released which, acting either on different cell types or on the same cells, but via different signal transduction pathways, either directly or through intermediaries, can initiate similar changes in sensory hypersensitivity.

We thank the MRC, the EU (Biomed 4) and the International Human Frontiers Science Program for their financial support.

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(Received January 17, 1997
Accepted February 24, 1997)