

Volume 12, issue 4, December 2001 | Articles originaux

Absence of endogeneous interleukin-10 enhances the evolution of murine type-II collagen-induced arthritis

Abstract

Interleukin-10 (IL-10) exerts a wide spectrum of regulatory activities in the immune and inflammatory response. The aim of this study was to investigate the role of endogenous IL-10 in the modulation of the inflammatory response in mice subjected to collagen-induced arthritis. Collagen-induced arthritis (CIA) was induced in mice lacking the gene for IL-10 (IL-10 "knock-out", IL-10KO) and in wild-type control (IL-10WT) mice by an intradermal injection of 100 mul of the emulsion (containing 100 mug of bovine type II collagen) (CII) and complete

Freund's adjuvant (CFA) at the base of the tail. On day 21, a second injection of CII in CFA was administered. IL-10 wild type (WT) mice developed an erosive, hind paw arthritis when immunised with CII in CFA. Macroscopic clinical evidence of CIA first appeared as peri-articular erythema and oedema in the hind paws. The incidence of CIA was 100% by day 27 in the CII-challenged IL-10WT. The severity of CIA progressed over a 35-day period, with radiographic evaluation revealing focal resorption of bone. The histopathology of CIA included erosion of the cartilage at the joint margins. IL-10KO mice experienced higher rates of clinical signs and more severe knee and paw injury as compared to IL-10WT. The degree of oxidative and nitrosative damage was significantly higher in IL-10KO mice than in wild-type littermates, as indicated by elevated malondialdehyde levels and formation of nitrotyrosine and poly (ADP-ribose) synthetase (PARS). Plasma levels of the proinflammatory cytokines, tumour necrosis factor, interleukin-1beta and interleukin-6 were also greatly enhanced in comparison to wild-type mice. These data demonstrate that endogenous IL-10 exerts an anti-inflammatory role during chronic inflammation and tissue damage associated with collagen-induced arthritis, possibly by regulating neutrophil recruitment, and the subsequent cytokine and oxidant generation.

Full text

ABBREVIATIONS

NO: nitric oxide

NOS: nitric oxide synthase

ecNOS: constitutive endothelial nitric oxide synthase

iNOS: inducible nitric oxide synthase

PARS: poly (ADP-ribose) synthetase

MPO: myeloperoxidase

PMN: polymorphonuclear cell

PBS: phosphate-buffered saline

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterised by the sequestration of various leukocyte subpopulations within both the developing pannus and synovial space. The chronic nature of this disease results in multiple joint inflammation with subsequent destruction of joint cartilage and erosion of bone. While this disease has a worldwide distribution, its pathogenesis is not clearly understood [1]. Type II collagen-induced arthritis (CIA) in the mouse has proven to be a useful model of RA, as it possesses many of the cell and humoral immunity characteristics found in human RA [2, 3]. The pathogenesis of CIA is dependent upon the host's response to type II collagen challenge and the subsequent generation of antibodies that recognises collagen-rich joint tissue [2, 3]. The chronic activities initiated by immune complexes trigger a variety of cell-mediated and humoral events. Moreover, the recruitment and activation of neutrophils, macrophages, and lymphocytes into joint tissues, and the formation of the pannus are hallmarks of the pathogenesis of both CIA and human RA. While the contribution of inflammatory leukocytes to the progression of experimental arthritis and human RA is unquestioned, the mechanisms whereby these leukocytes are recruited to the inflamed joint are still not fully known. Recently, several chemotactic cytokines (chemokines), which demonstrate a high degree of specificity for the movement of leukocyte subpopulations, have been isolated and cloned. These chemokines include, MIP-1alpha, MIP-1beta, RANTES, MCP-1, MCP-2, IL-8, and IP-10. The chemokines are chemotactic for neutrophils and for mononuclear cells. Recently, it has been demonstrated that IL-8, MIP-1alpha, MIP-1beta, and RANTES are differentially chemotactic for lymphocyte subsets [4-7]. Chemokines may play prominent roles in RA, as neutrophil and mononuclear cell elicitation and activation are prevalent in this disease.

While a number of pro-inflammatory cytokines have been studied in both human RA and murine CIA, relatively little is known regarding the production of immunomodulating cytokines, such as interleukin-10 (IL-10), during the development of this disease. Originally, IL-10 was described as a cytokine synthesis inhibitory factor produced by murine Th2 cell clones that could inhibit the synthesis of interferon-gamma by Th1 clones [8-11]. Recent studies have demonstrated that IL-10 can inhibit the synthesis of the major pro-inflammatory

cytokines and chemokines, upregulate humoral immune responses and attenuate cell-mediated immune reactions [12-16]. Interestingly, because of its properties, IL-10 has the ability to modulate several infectious, immune and inflammatory diseases. In RA, IL-10 is produced not only by Th2 cells (as originally found in mice), but also by a variety of other cell types, including Th l cells, B cells, and in particular monocytes and macrophages [17-19]. IL-10 has a broad range of activities on T cells, monocyte/ macrophages, B cells, and other cell types in patients with RA. The effects of IL-10 have been studied on isolated cell populations as well as mixed cell populations such as SF and PBMC, and ST explants [20]. Although IL-10 is produced in substantial amounts in the RA joint, these levels are clearly insufficient to control inflammation [21]. Notwithstanding the fact that several studies involving neutralization of IL-10 have demonstrated a suppressive role of endogenous IL-10, since this process led to an increase in levels of IL-10, TNF-alpha and granulocyte-macrophage colony-stimulating factor (GM-CSF) [21]. Administration of IL-10 to RA synovium cultures was shown to suppress production of TNF-alpha, IL-10, and IL-6 [21, 22], although stimulation of IL-10 and IL-6 production by IL-10 has also been reported [22, 23]. In addition to the data on *in vitro* effects on RA inflammatory activity, results of studies on experimental models of arthritis demonstrate the beneficial effects of IL-10. Aggrecan-induced arthritis in BALB/c mice was down-regulated by IL-10, in association with inhibition of the Th l response and of TNF-alpha and IL-6 production in the joints and stimulation of peripheral Th2 activity (IL-4 and IL-10 production) [24]. Administration of IL-10 to DBA/1 Lac/J mice, as well as to DA rats, led to a similar suppression of CIA [25, 29]. Also, injection of mice intravenously and intra-articularly with adenoviral vectors expressing viral IL-10, prevented induction of CIA and caused suppression of established CIA [27, 28]. Furthermore, inhibitory effects on streptococcal cell wall-induced arthritis have been observed [30]. Thus, IL-10 appears to possess certain immune-regulatory activities during inflammatory/immune responses. The purpose of this study was to investigate the role of endogenous IL-10 in a murine model of collagen-induced arthritis. To address this question, the release of the pro-inflammatory cytokines and the neutrophil infiltration were evaluated. Nitrotyrosine formation, and plasma levels of malondialdehyde were determined as indices of nitrosative and oxidative stress, respectively. Furthermore, we investigated whether genetic absence of IL-10 affects the joint expression of PARS and COX-2. We observed that absence of the IL-10 gene exaggerated the joint injury induced by collagen, whereas maintenance of endogenous IL-10 production significantly attenuated the injury, indicating that the cytokine may mediate important features of acute inflammation.

MATERIALS AND METHODS

Animals

C57BLJ10 mice (4-5 weeks old, 20-22 g), with a targeted disruption of the IL-10 gene (IL-10KO) and littermate wild-type controls (IL-10WT) were purchased from Jackson Laboratories (Harlan Nossan, Italy). The animals were housed in a controlled environment and provided with a standard rodent diet and water. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purposes (D.M. 116192), as well as with the EEC regulations (O.J. of E.C. L 358/1 12/18/1986).

Induction of collagen-induced arthritis

Bovine type II collagen (CII) was dissolved in 0.01 M acetic acid at a concentration of 2 mg/ml, by stirring overnight at 4° C. Dissolved CII was frozen at - 70° C until use. Complete Freund's adjuvant (CFA) was prepared by the addition of *Mycobacterium tuberculosis* H37Ra at a concentration of 2 mg/ml. Before injection, CII was emulsified with an equal volume of CFA. Collagen-induced arthritis was induced as previously described [31]. On day 1, mice were injected intradermally at the base of the tail with 100 mul of the emulsion (containing 100 mug of CII). On day 21, a second injection of CII in CFA was administered.

Clinical assessment of CIA

Mice were evaluated daily for arthritis using a macroscopic scoring system: 0 = no signs of arthritis; 1 = swelling and/or redness of the paw or one digit; 2 = two joints involved; 3 = more than two joints involved; and 4 = severe arthritis of the entire paw and digits [32]. The arthritic index for each mouse was calculated by adding the four scores for the individual paws. Clinical severity was also determined by quantifying the change in the paw volume using plethysmometry (model 7140; Ugo Basile).

Assessment of arthritis damage

On day 35, animals were sacrificed under anaesthesia, and paws and knees were removed and fixed for histological examination, which was done by an

investigator blind to the treatment regime. The following morphological criteria were considered: score 0, no damage; score 1, oedema; score 2, inflammatory cell presence; score 3, bone resorption.

Histological examination

For microscopic histological evaluation, paws and knees were removed and fixed in 10% formalin. The paws were then trimmed, placed in a decalcifying solution for 24 hours, embedded in paraffin, sectioned at 5 mum, stained with haematoxylin/eosin and studied using light microscopy (Dialux 22 Leitz).

Radiography

The mice were anaesthetised with sodium pentobarbital (45 mg/kg, i.p.). They were placed on a radiographic box at a distance of 90 cm from the x-ray source. Radiographic analysis of normal and arthritic hind paws was performed by x-ray machine (Philips X12 Germany), with a 40 kW exposure for 0.01 sec. An investigator blind to the treatment regime performed the radiographic scoring. The following radiograph criteria were considered: score 0, no bone damage; score 1, tissue swelling and oedema; score 2 joint erosion; 3, bone erosion and osteophyte formation.

Immunohistochemical localisation of nitrotyrosine, PARS and COX-2

Tyrosine nitration, an index of the nitrosylation of proteins by peroxynitrite and/or oxygen-derived free radicals, was determined by immunohistochemistry as previously described [33]. On day 35, the joints organs were trimmed, placed in decalcifying solution for 24 hours, and 8 µm sections were prepared from paraffin-embedded tissues. After deparaffinization, endogenous peroxidase was quenched with 0.3% H_2O_2 in 60% methanol for 30 min. The sections were permeabilized with 0.1% Triton X-100 in PBS for 20 min. Non-specific adsorption was minimised by incubating the section in 2% normal goat serum in phosphate-buffered saline for 20 min. Endogenous biotin or avidin binding sites were blocked by sequential incubation for 15 min with avidin and biotin. The sections were then incubated overnight with primary anti-nitrotyrosine antibody (1:1,000), anti-COX-2 antibody or anti-poly (ADP-Ribose) (PAR) antibody (1:500), or with control solutions. Controls included buffer alone or non-specific purified rabbit IgG. Specific labelling was detected with a biotin-conjugated goat

anti-rabbit IgG (for nitrotyrosine) or with a biotin-conjugated goat anti-rabbit IgG (for PAR and for COX-2) and avidin-biotin peroxidase complex.

Malondialdehyde (MDA) measurement

Plasma malondialdehyde (MDA) levels were determined as an indicator of lipid peroxidation [34]. An aliquot (100 mul) of the plasma collected at the specified time was added to a reaction mixture containing 200 mul of 8.1% SDS, 1,500 mul of 20% acetic acid (pH 3.5), 1,500 mul of 0.8% thiobarbituric acid and 700 mul distilled water. Samples were then heated for 1 hour at 95° C and centrifuged at 3,000 **x** g for 10 min. The absorbance of the supernatant was measured by spectrophotometry at 650 nm.

Measurement of cytokines

TNF-alpha, IL-6, IL-1beta and IL-10 levels were evaluated in the plasma from CIA mice as previously described [29]. The assay was carried out using a colorimetric commercial ELISA kit (Calbiochem-Novabiochem Corporation, Milan, Italy) with a lower detection limit of 10 pg/ml.

Measurement of chemokines

Murine chemokines (MIP-1alpha and MIP-2) were evaluated in the aqueous joint extracts. Briefly, joint tissues were prepared by first removing the skin and separating the limb below the ankle joint. Joint tissues were homogenised on ice in 3 ml lysis buffer (PBS containing: 2 mM PMSF, and 1 mg/ml [final concentration], each of aprotinin, antipain, leupeptin, and pepstatin A) using a Polytron (Brinkinarm Instruments, Westbury, NY, USA). The homogenised tissues were then centrifuged at 2,000 g for 10 min. Supernatants were sterilised with a millipore filter (0.2 mum) and stored at - 80° C until analysed. The extracts usually contained 0.2-1.5 mg protein/ml, as measured by a protein assay kit (Pierce Chemical Co., Rockford, IL, USA). The levels of MIP-1alpha and MIP-2 were quantified using a modification of a double ligand method, as previously described [36]. Briefly, flat-bottomed, 96-well microtiter plates were coated with 50 mul/well of rabbit anti-cytokine antibodies (1 mug/ml in 0.6 mol/litre NaCl, 0.26 mol/litre H_3BO_4 and 0.08 N NaOH, pH 9.6) for 16 hours at 4° C, and then washed with PBS, pH 7.5, 0.05% Tween 20 (wash buffer). Nonspecific binding sites on microtiter plates were blocked with 2% BSA in PBS and

incubated for 90 min at 37° C. Plates were rinsed four times with washing buffer, and diluted aqueous joint samples (50 mul) were added, followed by incubation for 1 hour at 37° C. After washing of plates, chromogen substrate was added. The plates were incubated at room temperature to the desired extinction, and the reaction terminated with 50 mul/well of 3 M H_3SO_4 solution, and were read at 490 nm in an ELISA reader. This ELISA method consistently had a sensitivity limit of ~ 30 pg/ml.

Myeloperoxidase (MPO) assay

Neutrophil infiltration into the inflamed joints was indirectly quantified using an MPO assay, as previously described for neutrophil elicitation [37]. Tissue was prepared as described above and placed in a 50 mM phosphate buffer (pH = 6.0) with 5% hexadecyltrimethyl ammonium bromide (Sigma Chemical Co.). Joint tissues were homogenised, sonicated, and centrifuged at 12,000 g for 15 min at 4° C. Supernatants were assayed for MPO activity using a spectrophotometric reaction with O-dianisidine hydrochloride (Sigma Chemical Co.) at 460 nm.

Materials

Unless otherwise stated, all compounds were obtained from Sigma-Aldrich Company (Milan, Italy). Biotin-blocking kit, biotin-conjugated goat anti-rabbit IgG, primary anti-nitrotyrosine, anti-poly(ADP-ribose), anti-COX-2 antibodies and avidin-biotin peroxidase complex were obtained from DBA (Milan, Italy). All other chemicals were of the highest commercial grade available. All stock solutions were prepared in nonpyrogenic saline (0.9% NaCl; Baxter Healthcare Ltd., Thetford, Norfolk, U.K.).

Data analysis

All values in the figures and text are expressed as mean ± standard error (s.e.m.) of the mean of *n* observations. For the *in vivo* studies, *n* represents the number of animals studied. In the experiments involving histology or immunohistochemistry, the figures shown are representative of at least three experiments performed on different experimental days. Data sets were examined by one- or two-way analysis of variance, and individual group means were then compared with Student's unpaired t test. For the arthritis studies, the

Mann-Whitney U test (two-tailed, independent) was used to compare medians of the arthritic indices [38]. A *p*-value less than 0.05 was considered significant.

RESULTS

Absence of IL-10 increases joint injury during experimental arthritis

To imitate the clinical scenario of RA, mice were subjected to collagen-induced arthritis. CIA developed rapidly in mice immunised with CII, and clinical signs (periarticular erythema and oedema) of the disease first appeared in mice hind paws between 24 and 26 days post-challenge (Figure 1A) leading to a 100% incidence of CIA at day 30. Hind paw erythema and swelling increased in frequency and severity in a time-dependent mode with maximum arthritis indices of approximately 8 observed between 29 to 35 days post-immunisation (Figure 1B) in IL-10WT mice. IL-10KO mice demonstrated a significant acceleration of joint inflammation, represented by an earlier appearance in the incidence of arthritis, and a greater arthritis index as compared to wild-type mice (Figure 1A, B).

There was no macroscopic evidence of either hind paw erythema or oedema in the sham group of mice (data not shown).

The rate and the absolute gain in body weight were comparable in normal mice and CII-immunised mice for the first week (Figure 1C). Beginning on day 25, the CII-challenged IL-10WT mice gained significantly less weight than the normal mice, and this trend continued through to day 35. The absence of endogenous IL-10 correlated with a significant increase of the weight loss caused by immunisation with CII (when compared to the respective WT mice) (Figure 1C).

The data in Figure 1D demonstrate a time-dependent increase in hind paw volume (each value represents the mean of both hind paws) in IL-10KO mice immunised with CII. The presence of IL-10 significantly attenuated hind paw swelling when compared to IL-10KO mice (Figure 1D). No increase in hind paw volume over time was observed in the sham mice group (data not shown).

The histological evaluation (at day 35) of the paws from IL-10KO mice revealed signs of severe arthritis, with bone erosion. In addition, severe or moderate necrosis was observed (Figure 2B, see Figure 3A for damage score). The bone

erosion and the necrosis was more pronounced in the joints from IL-10KO mice (Figures 2C, 3A). A radiographic examination of hind paws from IL-10KO mice at 35 days post-CII immunisation revealed bone erosion (Figure 4C; see Figure 3B for radiograph score. The presence of IL-10 significantly attenuated the degree of bone resorption (Figures 4B, 3B). There was no evidence of pathology in the sham mice (Figures 2A, 4A).

Chemokine expression and neutrophil infiltration is increased in IL-10KO mice

The above histological pattern of joint pathology appeared to be correlated with the influx of leukocytes into the joint, joint space, and surrounding tissue. Therefore, we initiated studies to assess the role of IL-10 in the expression of chemokines into the inflamed joints during the development of CIA. As shown in Figure 5, the expression pattern of joint MIP-1alpha, and MIP-2 was assessed by ELISA and was found to correlate with the development of arthritis. MIP-lalpha and MIP-2 were significantly increased at 35 day after CII immunisation in the joint from IL-10WT mice (Figure 5) MIP-1alpha and MIP-2 levels in IL-10KO mice at day-35 were significantly increased in comparison to WT mice (Figure 5). Therefore, we next evaluated the neutrophil infiltration. Assessment of neutrophil infiltration in the inflamed joint tissue was performed by measurement of the activity of myeloperoxidase, an enzyme specific to granulocyte lysosomes and, therefore, directly correlated to the number of neutrophils. Myeloperoxidase activity was significantly elevated at 35 days after CII immunisation, in IL-10WT mice (Figure 6A). In IL-10KO mice, myeloperoxidase activity was markedly increased in comparison to those of IL-10WT animals (Figure 6A).

Absence of endogenous IL-10 favours lipid peroxidation and nitrotyrosine formation

The release of free radicals and oxidant molecules during the chronic inflammation has been suggested to contribute significantly to tissue injury [39]. At day 35, all IL-10WT arthritic mice animals exhibited a substantial increase in the plasma MDA levels indicative of lipid peroxidation (Figure 6B). Furthermore, a positive staining for nitrotyrosine, a marker of nitrosative injury, was found in the joint of IL-10WT mice (Figure 7A). Targeted disruption of the IL-10 gene in mice subjected to collagen-induced arthritis exaggerated the

formation of malondialdehyde (Figure 6B) and nitrotyrosine (Figure 7B), thus indicating the occurrence of a more severe oxidant-induced damage.

*Endogenous IL-10 modulates production of TNF-*alpha, *IL-1*beta *and IL-6 during experimental arthritis*

To test whether the endogenous IL-10 may modulate the inflammatory process through the regulation of cytokine secretion, we analysed the plasma levels of pro-inflammatory cytokines TNF-alpha, IL-1beta and IL-6 in IL-10-KO and wild-type mice. A substantial increase in TNF-alpha, IL-1beta, IL-6 and IL-10 production was found in IL-10WT mice at 35 days after CII immunisation (Figure 8). Levels of TNF-alpha, IL-1beta and IL-6 were significantly higher in IL-10-deficient mice in comparison to those of IL-10WT animals (Figure 8).

PARS activation and COX-2 expression during CIA is increased in the absence of a functional gene for IL-10

To investigate the cellular mechanisms, by which endogenous IL-10 may attenuate joint injury, we evaluated the activation of PARS and the expression of COX-2, two important mediators involved in the inflammatory process [40]. Immunohistochemical analysis of joint sections obtained from IL-10WT mice treated with collagen type II revealed a positive staining for PARS (Figure 9A) and COX-2 (Figure 9C). A significant increase in positive staining for PARS (Figure 9B) and COX-2 (Figure 9D) was found in the joint of CIA-treated IL-10KO mice. There was no staining for either COX-2 or PARS in joints obtained from the sham group of mice (data not shown).

DISCUSSION

Our data demonstrate that mice with a targeted deletion of the IL-10 gene are significantly more vulnerable to pathological changes in the joint associated with CIA, as compared to wild-type controls. Thus, these results suggest that the presence of a functional IL-10 gene is a major requirement to limit the magnitude and duration of CIA. Furthermore, our data provide the first evidence that the oxidative stress and PARS pathway, activated during experimental arthritis, is regulated by the endogenous secretion of IL-10.

IL-10 is a potent anti-inflammatory cytokine, which has been shown to activate a diverse array of immunomodulatory responses. To prove the crucial role of IL-10 in controlling the inflammatory process, previous experimental studies have depended on *in vivo* administration of exogenous IL-10. There is ample evidence that IL-10 is an important down-regulator of a number of macrophage functions, including the production of TNF-alpha and IL-1beta. IL-10 is abundantly present in joints with active RA [40], and *in vitro* studies with isolated synovial tissue revealed that TNF-alpha and IL-1beta production was markedly enhanced after anti-IL-10 treatment, whereas additional exogenous IL-10 was able to still further suppress this cytokine production [41, 42]. IL-4, but not IL-10, has been shown to enhance the production of IL-1beta by RA synovial cells [43]. In our study, using genetically engineered mice, we have also demonstrated that the endogenous production of IL-10 has a notable impact in determining the outcome of joint injury in experimental CIA. Interestingly, IL-10 seems to play an obligate role during the development of CIA, as demonstrated by the fact that mice lacking a functional gene for IL-10 exhibited a high arthritis score as soon as 22-26 days after collagen immunisation.

Rheumatoid arthritis is one of the most common inflammatory joint diseases, having a world-wide distribution. In spite of a large research effort, the pathogenesis of this disease is not entirely clear. However, it is known that the progression of the disease is characterised by the presence of inflammatory cells in both the granuloma-like pannus and the joint fluid, followed by cartilage destruction and bone erosion. Interestingly, the active inflammatory stage of arthritis shares a number of common histological features of chronic inflammation, including the organised focal accumulation of mononuclear cells in the developing pannus, proliferation of fibroblast-like synovial cells, and injury to the surrounding tissue. While the proliferation of synovial cells and the infiltration of leukocytes are fundamental events in the development of joint inflammation, it is difficult to examine the mediators important to the initiation and maintenance of this pathological cascade in human arthritis. Therefore, it is necessary to establish and characterise experimental animal models to assess cellular and molecular events that contribute to the pathogenesis of joint inflammation. Interestingly, type-II collagen-induced arthritis in the mouse has proven to be a useful model, as it possesses many of the cellular and humoral immune events found in human rheumatoid arthritis.

While T cell and antibody responses against type II collagen are a crucial event for the initiation of CIA [2, 43], it has been demonstrated that several cytokines also appear to direct cell-to-cell communication in a cascade fashion during the progression of CIA. These cytokines include: IL-1 [44-46], TNF-alpha [47-49], IL-6 [50], TGF-beta [49], and IFN-gamma [51, 52]. The data presented in this report confirm that the cytokines (IL-1 and TNF-alpha), as well as the chemokines (MIP-1alpha and MIP-2) are expressed at the sites of inflamed joints and that these cytokines probably contribute in different capacities to the evolution of chronic joint inflammation. A number of recent studies have demonstrated that the recruitment of cells into the area of inflammation may be mediated not only by C5a, leukotrienes, platelet-activating factor, or bacterial-derived peptides, but also by a novel group of small proteins with relatively specific chemotactic activity for leukocyte subpopulations. This group includes MIP-1alpha, MIP-1beta, RANTES, MCPA, MCP-2, MCP-3, and 1-309. Recently, it has been demonstrated that MCP-1, MIP-1alpha, MIP-1beta, and RANTES are differentially chemotactic for lymphocyte subsets [4-7]. Since the recruitment of neutrophils, macrophages, and lymphocytes into joint tissue are hallmarks of both CIA and human rheumatoid arthritis, it is important to determine the contribution of chemokines in the progression of this experimental model of human rheumatoid arthritis. Recently it has been demonstrated that several chemokines, including IL-8, MCP-1, RANTES, and MIP-1alpha, are expressed in tissue from the inflamed joints in human rheumatoid arthritis [53-59]. While chemokines and other cytokines are known to play a pro-inflammatory role in the development of chronic inflammation, it has been reported that IL-10 may play an important regulatory role during the initiation and maintenance of inflammation [60, 61]. Many *in vivo* studies suggest that IL-10 can block the expression of pro-inflammatory cytokines, including IL-1 and TNF-alpha, which would corroborate a number of *in vitro* studies [13, 14, 63]. It has also been reported that IL-10 can inhibit the production and expression of chemokines, including IL-8, MIP-1alpha, and MIP-1beta in human monocytes and neutrophils [15, 16, 63]. Furthermore, IL-10 appears to increase the release of cytokine-modulating proteins, such as soluble TNF-receptor, and interleukin-1 receptor antagonist protein [21, 64]. Interestingly, using genetically engineered mice, we have also demonstrated that the endogenous production of IL-10 may regulate the release of these pro-inflammatory cytokines. Although the above studies demonstrate that endogenous IL-10 may act as a protective cytokine during the evolution of an inflammatory reaction and IL-10 is expressed by human synovium [65, 66], the contribution of chemokines and IL-10 to the arthritic response is not clear. However, it appears that the balance of pro-inflammatory cytokines,

such as chemokines, and anti-inflammatory cytokines, such as IL-10, may dictate the magnitude of the arthritis response.

Other studies have also demonstrated that IL-10 has a homeostatic role on leukocyte-endothelial cell interactions in response to endotoxin through regulation of endothelial adhesion molecules [67, 68]. Similarly, it has recently been demonstrated that endogenous IL-10 protects the ischaemic and reperfused myocardium through the suppression of ICAM-I expression and neutrophil recruitment [69]. In the present study, the neutrophil infiltration which took place to lesser degree in wild-type mice correlated well with the moderation of joint tissue damage. Furthermore, we found that the joint damage induced by CIA in IL-10-deficient mice was associated with high levels of plasma thiobarbituric acid-reactant malondialdehyde, which is considered a good indicator of lipid peroxidation [34, 71]. An intense immunostaining of nitrotyrosine formation also suggested that a structural alteration of the joint had occurred, most probably due to the formation of highly reactive nitrogen-derivatives. Recent evidence indicates in fact, that several chemical reactions, involving nitrite, peroxynitrite, hypochlorous acid and peroxidases can induce tyrosine nitration and may contribute to tissue damage [72-75].

There is a large amount of evidence that the production of ROS such as hydrogen peroxide, superoxide and hydroxyl radicals at the site of inflammation contributes to tissue damage [76-78]. Inhibitors of NOS activity reduce the development of arthritis, and these findings support a role for NO in the pathophysiology associated with this model of inflammation [79, 80]. In addition to NO, peroxynitrite is also generated in collagen-induced arthritis [32]. The biological activity and decomposition of peroxynitrite is very much dependent on the cellular or chemical environment (presence of proteins, thiols, glucose, the ratio of NO and superoxide, carbon dioxide levels and other factors), and these factors influence its toxic potential [81, 82]. ROS and peroxynitrite produce cellular injury and necrosis *via* several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage. ROS produce strand breaks in DNA which triggers energy-consuming DNA repair mechanisms and activates the nuclear enzyme PARS resulting in the depletion of its substrate NAD *in vitro* and a reduction in the rate of glycolysis. As NAD functions as a cofactor in glycolysis and the tricarboxylic acid cycle, NAD depletion leads to a rapid fall in intracellular ATP. This process has been termed *"the PARS suicide hypothesis"* [83-85]. There is recent evidence that the

activation of PARS may also play an important role in inflammation [32, 83, 84]. We demonstrate here that endogenous IL-10 modulated the activation of PARS during collagen-induced arthritis in the joint. Thus, we propose that the anti-inflammatory effects of IL-10 may be, at least in part, due to the prevention of the activation of PARS.

Several cellular mechanisms, including the mode of gene regulation and signal transduction, may account for the role of IL-10 in the modulation of joint injury. *In vitro* and *in vivo* studies have reported that exogenously administered IL-10 inhibited NF-kappaB activation, thus suppressing the pro-inflammatory cytokine production in human monocyte [85], the development of immune complex-induced lung injury in rats and hepatic ischaemia and reperfusion in mice [86]. NF-kappaB has been shown to activate, *via* transcription, the genes encoding pro-inflammatory cytokines (TNF-alpha, IL-Ibeta and IL-12), cell adhesion molecules (VCAM-1 and ICAM-1), inducible nitric oxide synthase (iNOS) and cyclo-oxygenase-2 (COX-2). Several studies also support the conclusion that pro-inflammatory cytokines, cell adhesion molecules and NO and prostaglandin E_2 (PGE_2) derived from iNOS and COX-2, respectively, play important roles in the pathogenesis of acute and chronic inflammation.

CONCLUSION

In the current study, we have obtained evidence that expression of COX-2 is reduced in the joint tissue of wild-type mice when compared to IL-10KO mice. This suggests that endogenous IL-10 may also target the COX-2 signalling pathway. Taken together, our studies demonstrate that chronic joint inflammation is a multi-factorial response, which is dependent upon both pro-inflammatory chemokines, such as MIP-1alpha and MIP-2, as well as regulatory cytokines, such as IL-10. This latter cytokine appears to be particularly important as a modulating cytokine during the progression of experimental arthritis and may play a similar role during the pathogenesis of autoimune responses in rheumatoid arthritis.

Acknowledgements. This study was supported by a grant from Consiglio Nazionale delle ricerche. The authors would like to thank Giovanni Pergolizzi and Carmelo La Spada for their excellent technical assistance during this study, Mrs Caterina Cutrona for secretarial assistance and Miss Valentina Malvagni for editorial assistance with the manuscript.

References

1. Harris ED Jr. 1990. Rheumatoid arthritis. Pathophysiology and implication for therapy. (Review) *N. Engl. J. Med.* 322: 1277.

2. Stuart J M, Townes A S, Kang A H. 1984. Collagen autoimmune arthritis. (Review) *Annu. Rev. Immunol.* 2: 199.

3. Holmdahl R, Andersson M, Goldschmidt T J, Gustafsson K, Jansson L, Mo J A. 1990. Type II collagen autoimmunity in animals and provocations leading to arthritis. (Review) *Immunol. Rev.* 118: 193.

4. Larsen C G, Anderson A O, Appella E, Oppenheim J J, Matsushima K. 1989. The neutrophil activating protein (NAP-1) is also chemotactic for T lymphocytes. *Science* 243: 1464.

5. Schall T J, Bacon K, Toy K J, Goeddel D V. 1990. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. *Nature* 347: 669.

6. Tanaka Y, Adams D H, Hubscher S, Hirano H, Siebenlist U, Shaw S. 1993. T-cell adhesion induced by proteoglican-immobilized cytokine MIP-1beta. *Nature* 361: 79.

7. Taub D D, Conlon K, Lloyd A R, Oppenheim J J, Kelvin D J. 1993. Preferential migration of activated $CD4^+$ and $CD8^+$ T cells in response to MIP-1alpha and MIP-1beta. *Science* 260: 355.

8. Fiorentino D F, Bond M W, Mosmann T R. 1989. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med.* 170: 2081.

9. Hsu D H, de Waal Malefyt R, Fiorentino D F, Dang M N, Viera P, de Vries J, Spits H, Mosmann T R, Moore K W. 1990. Expression of interleukin-10 activity by Epstein-Barr virus protein BCRF1. *Science* 250: 830.

10. Moore K W, Vieira P, Fiorentino D F, Trounstine M L, Khan T A, Mosmann TR. 1990. Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCRF1. *Science* 248: 1230.

11. Vieira P, de Waal Malefyt R, Dang M N, Johnson K E, Kastelein R, Fiorentino D F, de Vries J E, Roncarolo M G, Mosmann T R, Moore K W. 1991. Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: homology to Epstein-Barr virus open reading frame BCRF1. *Proc. Natl. Acad. Sci. USA* 88: 1172.

12. de Waal Malefyt R, Abrams J, Bennett T B, Figdor G, de Vries E. 1991. Interleukin-10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med.* 174: 1209.

13. Bogdan C, Vodovotz Y, Nathan C. 1991. Macrophage deactivation by IL-10. *J. Exp. Med.* 174: 1549.

14. Fiorentino D F, Zlotnik A, Mosmann T R, Howard M, O'Garra A. 1991. IL-10 inhibits cytokine production by activated macrophages. *J. Immunol.* 147: 3815.

15. Cassatella M A, Meda L, Bonora S, Caska M, Constantin G. 1993. Interleukin-10 (IL-10) inhibits the release of proinflammatory cytokines from human polymorphonuclear leukocytes. Evidence for an autocrine role of tumour necrosis factor and IL-1beta in mediating the production of IL-8 triggered by lipopolysaccharide. *J. Exp. Med.* 178: 2207.

16. Kasama T, Strieter R M, Lukacs N W, Burdick M D, Kunkel S L. 1994. Regulation of neutrophil-derived chemokine expression by IL-10. *J. Immunol.* 152: 3559.

17. Mosmann T R, Cherwinski H, Bond M W, Giedlin M A, Coffman R L. 1986. Two types of murine helper T cell clone I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 136: 2348.

18. Llorente L, Richaud- Patin Y, Fior R, Alcocer-Varela J, Wijdens J, Fourrier BM, Galanaud P, Emilie D. 1994. *In vivo* production of interleukin-10 by non-T cells in rheumatoid arthritis, Sjogren's syndrome, and systemic lupus erythematosus. A potential mechanism of B lymphocyte hypereactivity and autoimmunity. *Arthritis Rheum.* 37: 1647.

19. Yudoh K, Matsuno H, Nakazawa F, Yonezawa T, Kimura T. 2000. Reduced expression of the regulatory $CD4^+$ T cell subset is related to Th1/Th2 balance and disease severity in rheumatoid arthritis. *Arthritis Rheum.* 43: 617.

20. van Roon J A, Lafeber F P, Bijlsma J W. 2001. Synergistic activity of interleukin-4 and interleukin-10 in suppression of inflammation and joint destruction in rheumatoid arthritis. *Arthritis Rheum.* 44: 3.

21. Katsikis P D, Chu C Q, Brennan F M, Maini R N, Felmann M. 1994. Immunoregulatory role of interleukin-10 in rheumatoid arthritis. *J. Exp. Med.* 179: 1517.

22. Chomarat P, Vannier E, Dechanet J, Rissoan M C, Banchereau J, Dinarello C A, Miossec P. 1995. Balance of IL-1 receptor antagonist/IL-1 beta in rheumatoid synovium and its regulation by IL-4 and IL-10. *J. Immunol.* 154: 1432.

23. Chomarat P, Banchereau J, Miossec P. 1995. Differential effects of interleukin-10 and 4 on the production of interleukin-6 by blood and synovium monocytes in rheumatoid arthritis. *Arthritis Rheum.* 38: 1046.

24. Finnegan A, Mikecz K, Tao P, Glant T T. 1999. Proteoglycan (aggrecan)-induced arthritis in BALB/c mice is a Th1-type disease regulated by Th2 cytokines. *J. Immunol.* 163: 5383.

25. Joosten L A, Lubberts E, Durez P, Helsen M M, Jacobs M J, Goldman M, van den Berg W B. 1997. Role of interleukin-4 and interleukin-10 in murine collagen-induced arthritis. Protective effect of interleukin-4 and interleukin-10 treatment on cartilage destruction. *Arthritis Rheum.* 40: 249.

26. Persson S, Mikulowska A, Narula S, O'Garra A, Holmdahl R. 1996. Interleukin-10 suppresses the development of collagen type II-induced arthritis and ameliorates sustained arthritis in rats. *Scand. J. Immunol.* 44: 607.

27. Apparailly F, Verwaerde C, Jaquet C, Auriault C, Sany J, Jorgensen C. 1998. Adenovirus-mediated transfer of viral IL-10 gene inhibits murine collagen-induced arthritis. *J. Immunol.* 160: 5213.

28. Ma Y, Thornton S, Duwel L E, Boivin G P, Giannini E H, Leiden J M, Bluestone J A, Hirsch R. 1998. Inhibition of collagen-induced arthritis in mice by viral IL-10 gene transfer. *J. Immunol.* 161: 1516.

29. Walmsley M, Katsikis P D, Abney E, Parry S, William R O, Maini R N, Feldmann M. 1996. Interleukin-10 inhibition of the progresion of established collagen-induced arthritis. *Arthritis Rheum.* 39: 495.

30. Lubberts E, Joosten L A, Helsen M M, van den Berg W B. 1998. Regulatory role of interleukin-10 in joint inflammation and cartilage destruction in murine streptococcal cell wall (SCW) arthritis. More therapeutic benefit with IL-4/IL-10 combination than IL-10 treatment alone. *Cytokine* 10: 361.

31. Campbell I K, Hamilton J A, Wicks I P. 2000. Collagen-induced arthritis in C57BL/6 (H-2b) mice: new insights into an important disease model of rheumatoid arthritis. *Eur. J. Immunol.* 30: 1568.

32. Szabo C, Virag L, Cuzzocrea S, Scott G S, Hake P, O'Connor M, Zingarelli B, Salzman A, Kun E. 1998. Protection against peroxynitrite-induced fibroblast injury and arthritis development by inhibition of poly (ADP-ribose) synthase. *Proc. Natl. Acad. Sci. USA* 9: 3867.

33. Cuzzocrea S, Zingarelli B, Costantino G, Caputi A P. 1999. Beneficial effects of Mn(III)tetrakis (4-benzoic acid) porphyrin (MnTBAP), a superoxide dismutase mimetic, in carrageenan-induced pleurisy. *Free Rad. Biol. Med.* 26: 25.

34. Ohkawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95: 351.

35. Cuzzocrea S, Mazzon E, Bevilaqua C, Costantino G, Britti D, Mazzullo G, De Sarro A, Caputi A P. 2000. Cloricromene, a coumarine derivative, protects against collagen-induced arthritis in Lewis rats. *Br. J. Pharmacol.* 131: 1399.

36. Kasama T, Strieter R M, Lukacs N W, Burdick M D, Kunke S L. 1994. Regulation of neutrophil-derived chemokine expression by IL-10. *J. Immunol.* 152: 3559.

37. Goldblum S E, Wu K M, Jay M. 1985. Lung myeloperoxidase as a measure of pulmonary leukostasis in rabbits. *J. Appl. Physiol.* 59: 1978.

38. Youn Y K, La Londe C, Demling R. 1991. Use of antioxidant therapy in shock and trauma. (Review) *Circ. Shock* 35: 245.

39. Cuzzocrea S, Riley D P, Caputi A P, Salvemini D. 2001. Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. (Review) *Pharmacol. Rev.* 53: 135.

40. Cuzzocrea S, McDonald M C, Mazzon E, Siriwardena D, Calabro G, Britti D, Mazzullo G, De Sarro A, Caputi A P, Thiemermann C. 2000. The tyrosine kinase inhibitor tyrphostin AG126 reduces the development of acute and chronic inflammation. *Am. J. Pathol.* 157: 145.

41. Kasama T, Strieter R M, Lukas N W, Lincoln P M, Burdick M D, Kunkel S L. 1995. Interleukin-10 expression and chemochine regulation during the evolution of murine type II collagen-induced arthritis. *J. Clin. Invest.* 95: 2868.

42. Morita Y, Yamamura M, Kawashima M, Aita T, Harada S, Okamoto H, Inoue H, Makino H. 2001. Differential *in vitro* effects of IL-4, IL-10, and IL-13 on proinflammatory cytokine production and fibroblast proliferation in rheumatoid synovium. *Rheumatol. Int.* 20: 49.

43. Holmdahl R, Mo J, Nordling C, Larsson P, Jansson L, Goldschmidt T, Andersson M, Klareskog L. 1989. Collagen induced arthritis: an experimental model for rheumatoid arthritis with involvement of both DTH and immune complex mediated mechanisms. (Review) *Clin. Exp. Rheumatol.* 7 (Suppl): S51.

44. Hom J T, Bendele A M, Carlson D G. 1988. *In vivo* administration with IL-1 accelerates the development of collagen induced arthritis in mice. *J. Immunol.* 141: 834.

45. Hom J T, Gliszcynski V L, Cole H W, Bendele A M. 1991. Interleukin-1 mediated acceleration of type II collagen-induced arthritis: effects of anti-inflammatory or anti-arthritic drugs. *Agents Actions* 33: 300.

46. Hom J T, Cole H, Estridge T, Gliszcynski V L. 1992. Interleukin-1 enhances the development of type II collalegen-induced arthritis only in susceptible and not in resistant mice. *Clin. Immunol. Immunopathol.* 62: 56.

47. Williams R O, Feldmann M, Maini R N. 1992. Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. *Proc. Natl. Acad. Sci. USA* 89: 9784.

48. Piguet P F, Grau G E, Vesin C, Loetscher H, Gentz R, Lesslauter W. 1992. Evolution of collagen induced arthritis in mice is arrested by treatment with anti-tumour necrosis factor (TNF) antibody or a recombinant soluble TNF-receptor. *Immunology* 77: 510.

49. Thorbecke G J, Shah R, Leu C H, Kuruvilla A P, Hardison A M, Palladino M A. 1992. Involvment of endogenous tumor necrosis factor alpha and transforming growth factor beta during induction of collagen II arthritis in mice. *Proc. Natl. Acad. Sci. USA* 89: 7375.

50. Takai Y, Seki N, Senoh H, Yokota T, Lee F, Hamaoka T, Fujiwara H. 1989. Enhanced production of interleukin-6 in mice with type II collagen-induced arthritis. *Arthritis Rheum.* 32: 594.

51. Cooper S M, Siram S, Ranges G E. 1988. Suppression of murine collagen-induced arthritis with monoclonal anti-Ia antibodies and augmentation with IFN-gamma. *J. Immunol.* 141: 1958.

52. Mauritz N J, Holmdahl R, Jonsson R, van der Meide P H, Scheynius A, Klareskog L. 1988. Treatment with gamma-interferon trigger the onset of collageen arthritis in mice. *Arthritis Rheum.* 31: 1297.

53. Brennan F M, Zachariae C O, Chantry D, Larsen C G, Turner M, Maini R N, Matsushima K, Feldmann M. 1990. Detection of interleukin-8 biological activity in synovial fluids from patients with rheumatoid arthritis and production of interleukin-8 mRNA by isolated synovial cells. *Eur. J. Immunol.* 20: 2141.

54. Koch A E, Kunkel S L, Burrows J C, Evanoff H L, Haines G K, Pope R M, Strieter R M. 1991. Synovial tissue macrophage as a source of the chemotactic cytokine IL-8. *J. Immunol.* 147: 2187.

55. Seitz M, Dewald B, Gerber N, Baggiolini M. 1991. Enhanced production of neutrophil-activating peptide-1/interleukine-8 in rheumatoid arthritis. *J. Clin. Invest.* 87: 463.

56. Villiger P M, Terkeltaub R, Lotz M. 1992. Production of monocyte chemoattractant protein-1 by inflamed synovial tissue and cultured synoviocytes. *J. Immunol.* 149: 722.

57. Koch A E, Kunkel S L, Harlow L A, Johnson B, Evanoff H L, Haines G K, Burdick M D, Pope R M, Strieter R M. 1992. Enhanced production of monocyte chemoattractant protein-1 in rheumatoid arthritis. *J. Clin. Invest.* 90: 772.

58. Rathanaswami P, Hachicha M, Sadick M, Schall T J, McColl S R. 1993. Expresion of the cytokine RANTES in human rheumatoid synovial fibroblasts. Differential regulation of RANTES and interleukin-8 genes by inflammatory cytokines *J. Biol. Chem.* 268: 5834.

59. Koch A E, Kunkel S L, Harlow L A, Mazarakis D D, Haines G K, Burdick M D, Pope R M, Strieter R M. 1994. Macrophage inflammatory protein-1 alpha. A novel chemotactic cytokine for macrophages in rheumatoid arthritis. *J. Clin. Invest.* 93: 921.

60. Gerard C, Bruyns C, Marchant A, Abramowicz D, Vandenabeele P, Delvaux A, Fiers W, Goldman M, Velu T. 1993. Interleukin-10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. *J. Exp. Med.* 177: 547.

61. Mulligan M S, Jones M L, Vaporciyan A A, Howard M C, Ward P A. 1993. Protective effects of IL-4 and IL-10 against immune complex-induced lung injury. *J. Immunol.* 151: 5666.

62. Marchant A, Deviere J, Byl B, De Groote D, Vincent J L, Goldman M. 1994. Interleukin-10 production during septicaemia. *Lancet* 343: 707.

63. Leeuwenberg J F, Jeunhomme T M, Buurman W A. 1994. Slow release of soluble TNF-receptors by monocytes *in vitro.* 152: 4036.

64. Cassatella M A, Meda L, Gasperini F, Calzetti F, Bonora S. 1994. Interleukin-10 (IL-10) upregulates IL-1 receptors antagonist production from lipopolysaccharide-stimulated human polymorphonuclear leukocytes by delaying mRNA degradation. *J. Exp. Med.* 179: 1695.

65. Quayle A J, Chomarat P, Miossec P, Kjeldsen Kragh J, Forre O, Natvig J B. 1993. Rheumatoid inflammatory T cell clones express mostly Th1 but also Th2 and mixed (Th0 like) cytokine patterns. *Scand. J. Immunol.* 38: 75.

66. Santucci L, Fiorucci S, Chiorean M, Brunori P M, di Mateo F M, Sidoni A, Migliorati G, Morelli A. 1996. Interleukin-10 reduces lethality and hepatic injury induced by lipopolysaccharide in galactosamine-sensitized mice. *Gastroenterology* 111: 736.

67. Hickey M J, Issekutz A C, Reinhardt P H, Fedorak R N, Kubes P. 1998. Endogenous interleukin-10 regulates hemodynamic parameters, leukocyte-endothelial cell interactions, and microvascular permeability during endotoxemia. *Circ. Res.* 83: 1124.

68. Yang Z, Zingarelli B, Szabo C. 2000. Crucial role of endogenous interleukin-10 production in myocardial ischemia/reperfusion injury. *Circulation* 101: 1019.

69. Zingarelli B, Szabo C, Salzman A L. 1999. Blockade of poly (ADP-ribose) syntethase inhibits neutrophil recruitment, oxidant generation, and mucosal injury in murine colitis. *Gastroenterology* 116: 335.

70. Beckman J S. 1996. Oxidative damage and tyrosine nitration from peroxynitrite. (Review) *Chem. Res. Toxicol.* 9: 836.

71. Eiserich J P, Cross C E, Jones A D, Halliwell B, van der Vlieta A. 1996. Formation of nitrating and chlorinating species by reaction of nitrite with hypochlorous acid. A novel mechanism for nitric oxide-mediated protein modification. *J. Biol. Chem.* 271: 19199.

72. Eiserich J P, Hristova M, Cross C E, Jones A D, Freeman B A, Halliwell B, van der Vliet A. 1998. Formation of nitric oxidants derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature* 391: 393.

73. Halliwell B. 1997. What nitrates tyrosine? Is nitrotyrosine specific as a biomarker of peroxynitrite formation *in vivo*? *Febs Lett.* 411: 157.

74. Cuzzocrea S, Zingarelli B, Hake P, Salzman A L, Szabó C. 1998. Anti-inflammatory effects of mercaptoethylguanidine, a combined inhibitor of nitric

oxide synthase and peroxynitrite scavenger, in carrageenan-induced models of inflammation. *Free Rad. Biol. Med.* 24: 450.

75. Cuzzocrea S, Costantino G, Mazzon E, Caputi A P. 1999. Beneficial effects of raxofelast (IRFI 016), a new hydrophilic vitamin E-like antioxidant, in carrageenan-induced pleurisy. *Br. J. Pharmacol.* 126: 407.

76. Salvemini D, Wang Z Q, Stern M K, Currie M G, Misko T P. 1998. Peroxynitrite decomposition catalysts: therapeutics for peroxynitrite-mediated pathology. *Proc. Natl. Acad. Sci. USA* 95: 2659.

77. Ialenti A, Moncada S, Di Rosa M. 1993. Modulation of adjuvant arthritis by endogenous nitric oxide. *Br. J. Pharmacol.* 110: 701.

78. Holmdahl R, Klareskog L, Rubin K, Larsson E, Wigzell H. 1985. T lymphocytes in collagen II-induced arthritis in mice. Characterization of arthritogenic collagen II-specific T cell lines and clones. *Scand. J. Immunol.* 22: 295.

79. Brahn E, Banquerigo M L, Firestein G S, Boyle D L, Salzman A L, Szabo C. 1998. Collagen induced arthritis: reversal by mercaptoethylguanidine, a novel antiinflammatory agent with a combined mechanism of action. *J. Rheumatol.* 25: 1785.

80. Beckman J S, Beckman T W, Chen J, Marshall P A, Freeman B A. 1990. Apparent hydroxyl radical production by peroxynitrite: implication for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA* 87: 1620.

81. Cuzzocrea S, Caputi A P, Zingarelli B. 1998. Peroxynitrite-mediated DNA strand breakage activates poly (ADP-ribose) synthetase and causes cellular energy depletion in carrageenan-induced pleurisy. *Immunology* 93: 96.

82. Eiserich J P, Hristova M, Cross C E, Jones A D, Freeman B A, Halliwell B, van der Vliet A. 1998. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature* 391: 393.

83. Szabó C, Lim L H, Cuzzocrea S, Getting S J, Zingarelli B, Flower R J, Salzman A L, Perretti M. 1997. Inhibition of poly (ADP-ribose) synthetase and attenuates

neutrophil recruitment and exerts anti-inflammatory effects. *J. Exp. Med.* 186: 1041.

84. Wang P, Wu P, Siegel M I, Egan R W, Billah M M. 1995. Interleukin (IL)-10 inhibits nuclear factor kappaB (NF-kappaB) activation in human monocytes. IL-10 and IL-4 suppres cytokine synthesis by different mechanism. *J. Biol. Chem.* 270: 9558.

85. Lentsch A B, Shanley T P, Sarma V, Ward P A. 1997. *In vivo* suppresion of NF-kappaB and preservation of IkappaBalpha by interleukin-10 and interleukin-13. *J. Clin. Invest.* 100: 2443.

86. Yoshidome H, Kato A, Edwards M J, Lentsch A B. 1999. Interleukine-10 suppresses hepatic ischemia/reperfusion injury in mice: implications of a central role for nuclear factor kappaB. *Hepatology* 30: 203.

Figures

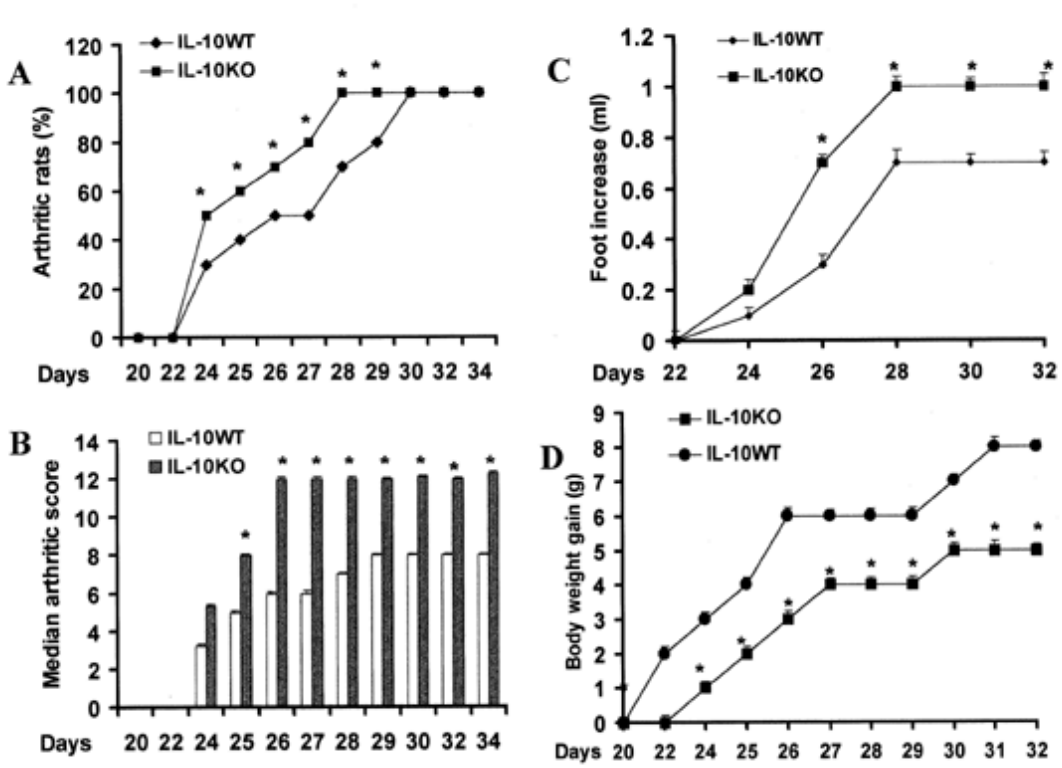


Figure 1
Effect of the absence of IL-10 on the onset of collagen-induced arthritis, secondary lesions and body weight gain. The percentage of arthritic mice (*i.e.* those showing

clinical scores of arthritis > 1) are represented **(A)**. Hind paw erythema and swelling increased in frequency and severity in a time-dependent manner **(B)**. Swelling in hind paws **(C)** over time was measured at 2 day intervals. Beginning on day 25, the collagen-challenged mice gained significantly less weight than the normal mice, and this trend continued through to day 35 **(D)**. IL-10KO mice demonstrated an early appearance in the incidence of arthritis, greater arthritic score, a greater weight loss, as well as a more pronounced paw oedema. Values are means ± SEM of 10 animals for each group. **P* < 0.01 represents a significant increase of the various parameters in the group in which IL-10 was absent.

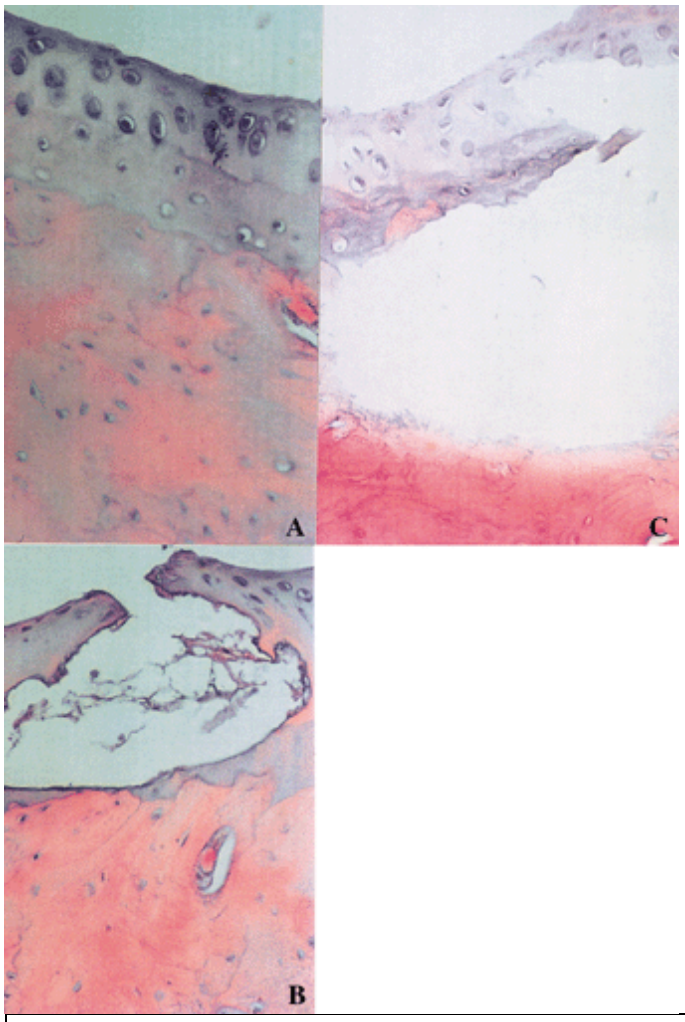


Figure 2
Morphological changes in CIA. A representative

haematoxylin/eosin-stained section of joint was examined by light microscopy in normal control animals **(A)**, in collagen-immunised IL-10 wild-type mice **(B)** and in collagen-immunised IL-10KO mice **(C)**. Original magnification: **x** 180. Figure is representative of at least 3 experiments performed on different experimental days.

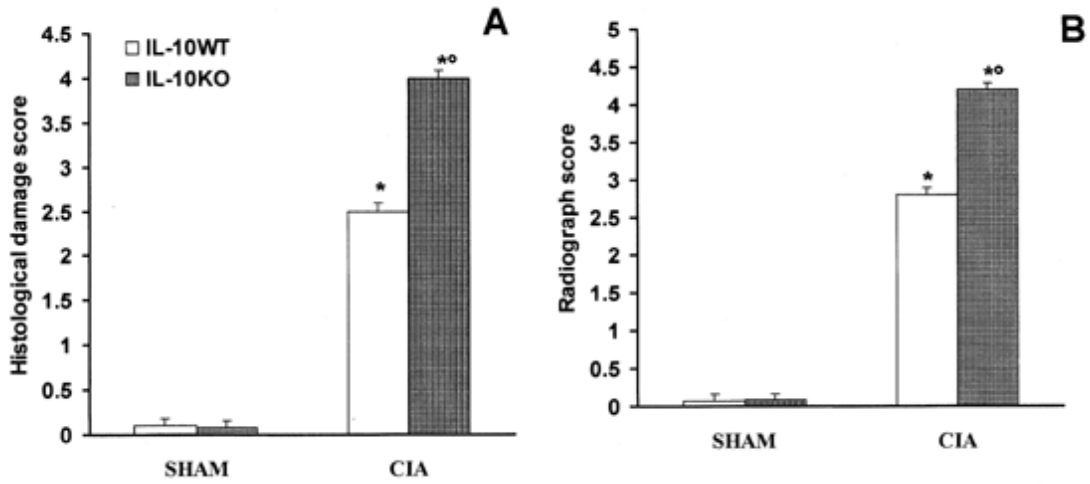


Figure 3

Effect of the absence of IL-10 on the histological damage score (A)**, and radiograph score** (B)**.** Values are means ± s.e. means of 10 animals for each group. * $P < 0.01$ *versus* vehicle. ° $P < 0.01$ represents a significant increase of the various parameters in the group in which IL-10 was absent.

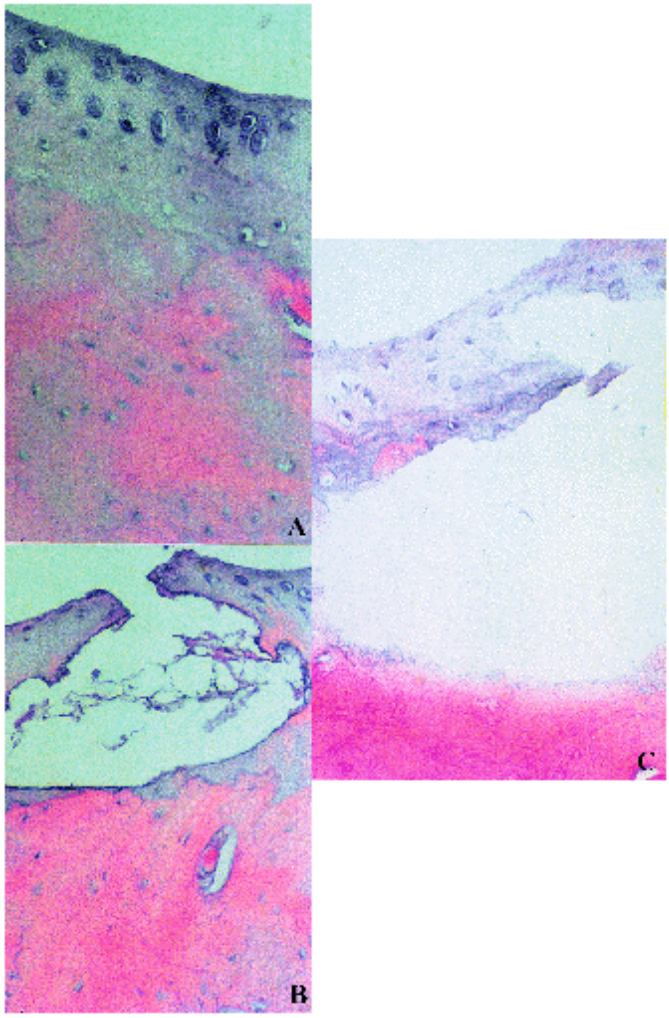


Figure 4

Radiographic progression of CIA in the tibiotarsal joint of mice with CIA. There is no evidence of pathology in the tibiotarsal joints of normal mice **(A)**. The hind paws from CII-immunised (35 days) IL-10WT mice demonstrated bone resorption (see arrow) **(B)**. The absence of IL-10 enhanced the bone erosion (arrow) **(C)**. Figure is representative of at least 3 experiments performed on different experimental days.

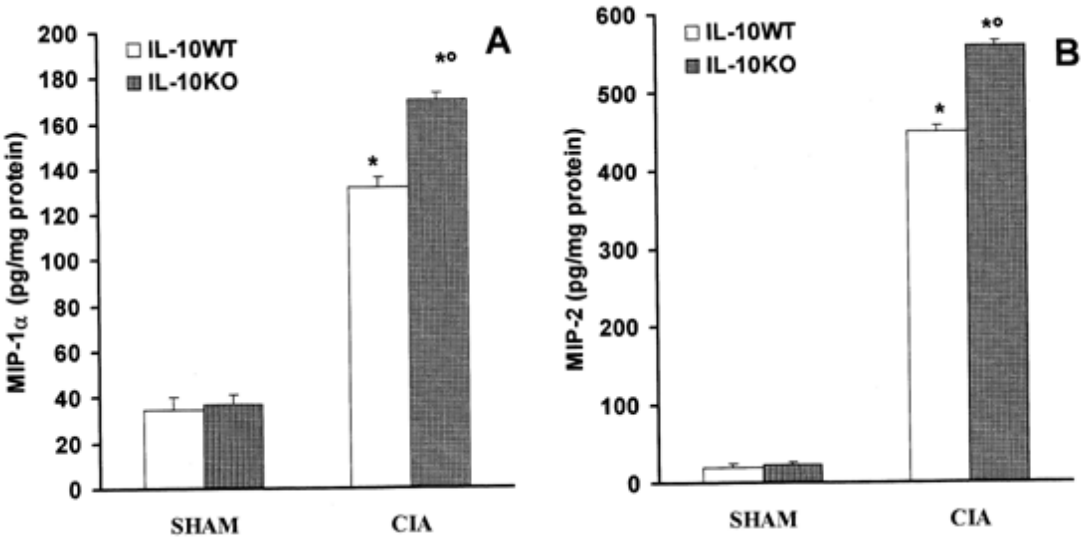


Figure 5
Effect of the absence of IL-10 on MIP-Ialpha (A), and MIP-2 (B). Values are means ± s.e. means of 10 animals for each group. * $P < 0.01$ *versus* vehicle. ° $P < 0.01$ represents a significant increase of the various parameters in the group in which IL-10 was absent.

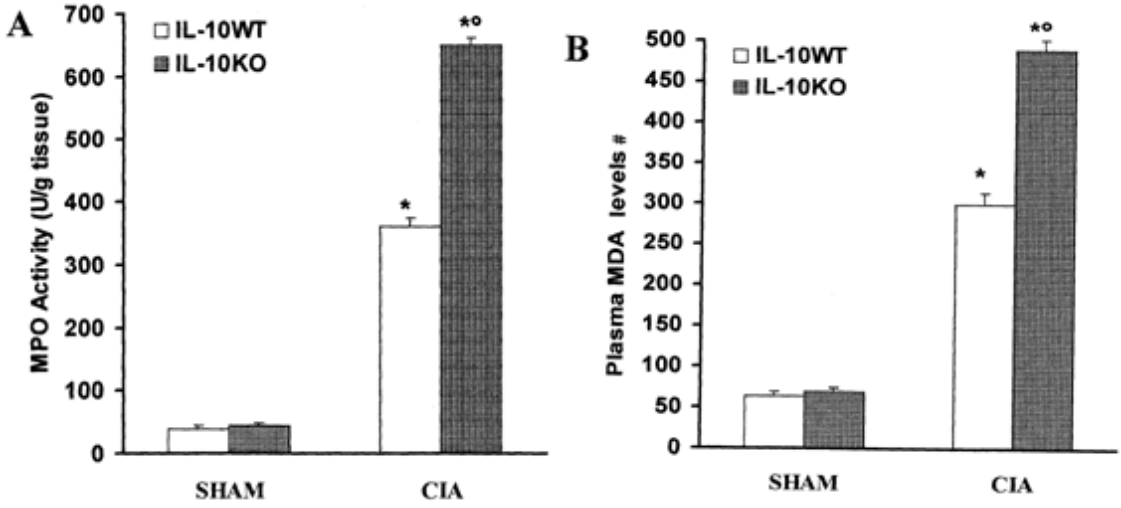


Figure 6
Effect of the absence of IL-10 on MPO activity (A), and MDA levels (B). Values are means ± s.e. means of 10 animals for each group. *$P < 0.01$ *versus* vehicle. °$P < 0.01$

represents a significant increase of the various parameters in the group in which IL-10 was absent.

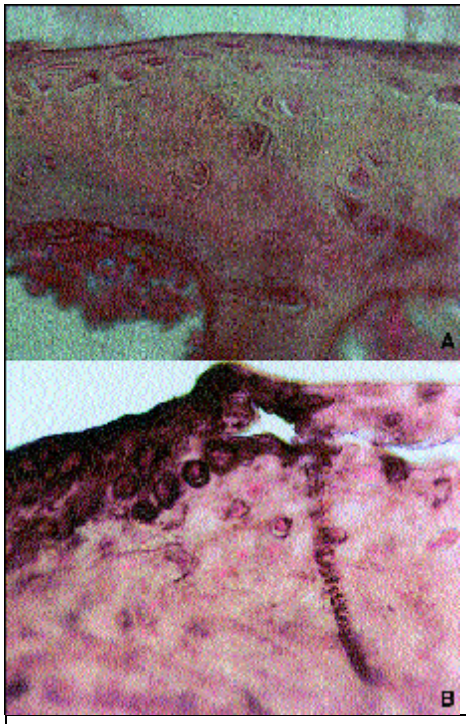


Figure 7
Positive nitrotyrosine immunostaining was observed in the paw of IL-10WT mice at 35 days of collagen-induced arthritis (A). A marked increase in nitrotyrosine staining is evident in the paws from IL-10KO mice **(B)**. Original magnification: **x** 200. Figure is representative of at least 3 experiments performed on different experimental days.

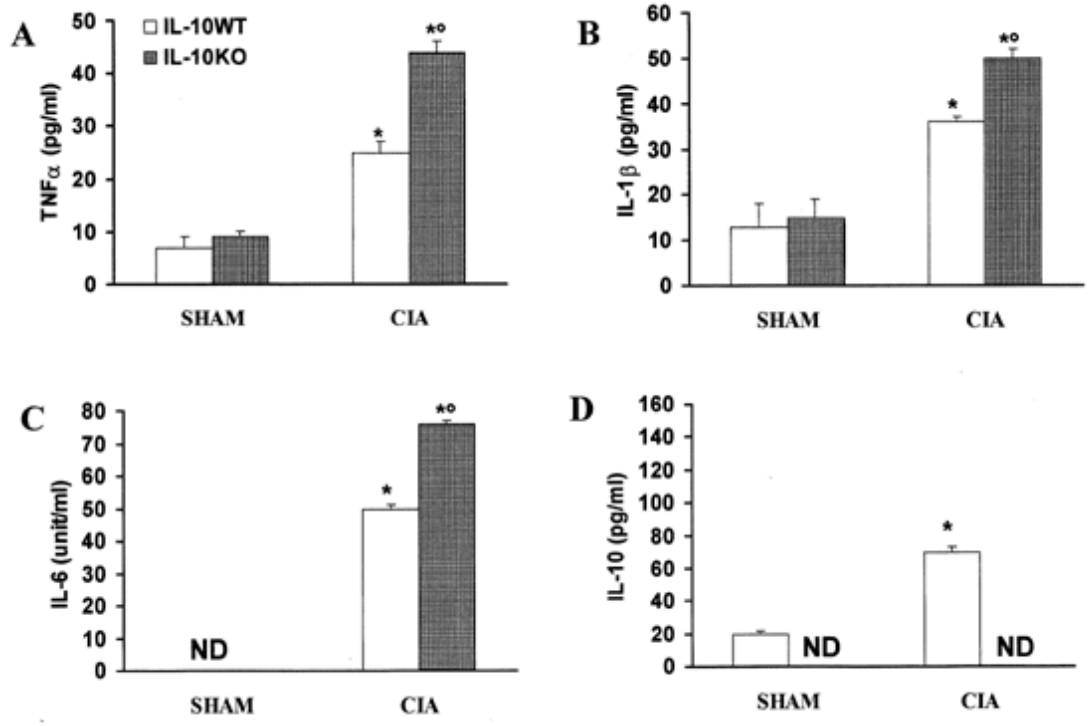


Figure. 8

Plasma TNF-alpha, IL-1beta, IL-6 and IL-10 production at 35 days after collagen immunisation. TNF-alpha **(A)**; IL-1beta **(B)** IL-6 **(C)** and IL-10 **(D)** plasma levels. IL-10WT mice show a significant production of cytokines at 35 days after CIA. Cytokine levels were significantly enhanced in IL-10KO mice. No IL-10 production was found in plasma from IL-10KO mice. Data are means ± SEM of 10 animals for each group. *$P < 0.01$ *versus* vehicle. °$P < 0.01$ represents a significant increase of the various parameters in the group in which IL-10 was absent.

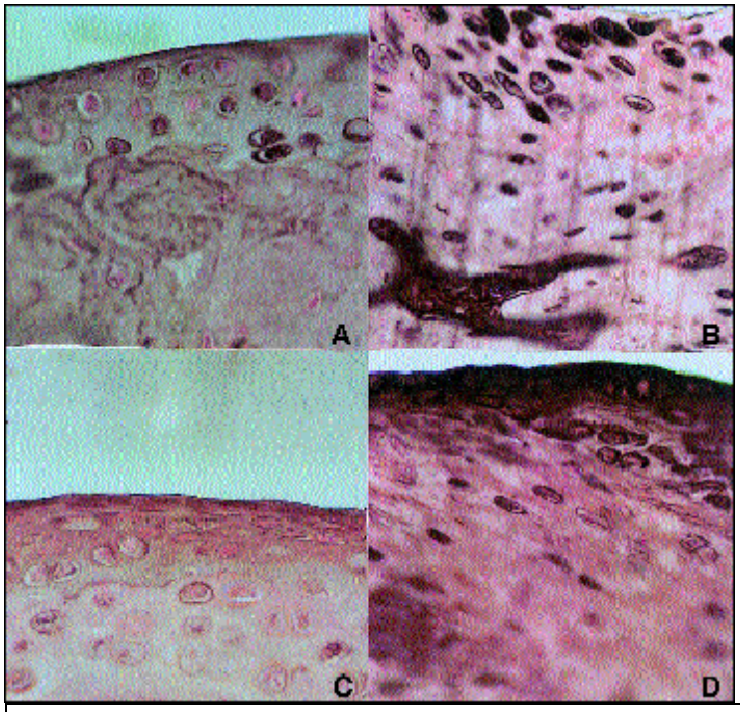


Figure 9
Effect of the absence IL-10 on PARS and COX-2 immunostaining. Positive immunostaining for PARS and COX-2 was observed in the paw of IL-WT mice after 35 days of collagen-induced arthritis **(A, C)**. A marked increase in iNOS and COX-2 staining is evident in the paws in IL-10KO arthritic mice **(B, D)**. Original magnification: **x** 180. Figure is representative of at least 3 experiments performed on different experimental days.

Authors

Salvatore Cuzzocrea, Emanuela Mazzon, Laura Dugo, Ivana Serraino, Domenico Britti, Massimo De Maio, Achille P. Caputi

Institute of Pharmacology, School of Medicine, University of Messina, Torre Biologica, Policlinico Universitario Via C. Valeria, Gazzi, 98100 Messina Italy.

In this issue

Monocyte chemotactic protein-3

Patricia Menten, Anja Wuyts, Jo Van Damme
MCP-3, induction, diseases, structure, receptors.

Adenoviral supply of active transforming growth factor-beta1 (TGF-beta1) did not prevent lethality in transforming growth factor-beta1-knockout embryos

Hedia Chagraoui, Stéphane Giraudier, William Vainchenker, Françoise Wendling
TGF-beta1, cytokine, knockout mice, gene therapy, adenovirus.

Absence of endogeneous interleukin-10 enhances the evolution of murine type-II collagen-induced arthritis

Salvatore Cuzzocrea, Emanuela Mazzon, Laura Dugo, Ivana Serraino, Domenico Britti, Massimo De Maio, Achille P. Caputi
collagen, inflammation, nitric oxide, peroxynitrite, superoxide dismutase, IL-10.

Reduced adrenal response and increased mortality after systemic Klebsiella pneumoniae infection in interleukin-6-deficient mice

Frank H.J. van Enckevort, C.G.J. Sweep, Paul N. Span, Mihai G. Netea, Ad R.M.M. Hermus, Bart Jan Kullberg
corticosterone, cytokine, IL-6-deficient mice, granulocyte recruitment, survival.

Gamma-interferon induces apoptosis of the B lymphoma WEHI-279 cell line through a CD95/CD95L-independent mechanism

K.-E. Ben Jilani, K. Akarid, D. Arnoult, F. Petit, E. Baert, J.-P. Gaillard, J.-C. Ameisen, J. Estaquier
IFN-g, B cells, apoptosis, CD95, caspases, cytokines.

IFN-beta stimulates the production of beta-chemokines in human peripheral blood monocytes. Importance of macrophage differentiation

Laura Fantuzzi, Irene Canini, Filippo Belardelli, Sandra Gessani
MCP-1, MIP-1alpha, MIP-1beta, IFN-beta, monocytes/macrophages, differentiation.

Relationship between cell kinetics and apoptotic effects in TNF-alpha and IFN-gamma-treated human tumour cell

lines

Heinz Baisch
apoptosis, c-Myc, cell cycle, cytokines, dual pathways.

L-Buthionine-(S,R)-sulfoximine, an irreversible inhibitor of gamma-glutamylcysteine synthetase, augments LPS-mediated pro-inflammatory cytokine biosynthesis: evidence for the implication of an IkappaB-alpha/NF-kappaB insensitive pathway

John J. Haddad
antioxidant, chemioxyexcitation, cytokines, glutathione disequilibrium, pharmacotherapy, reactive oxygen species, redox/thiol regulation.

Endotoxin regulates the maturation of sterol regulatory element binding protein-1 through the induction of cytokines

Luisa Diomede, Diego Albani, Marco Bianchi, Mario Salmona
SREBP-1, LPS, HepG2, liver, mice.

Regulation of Staphylococcus aureus-mediated activation of interleukin-18 in peripheral blood mononuclear cells

Ulrich Böcker, Tobias Manigold, Joanna M. Watson, Manfred V. Singer, Siegbert Rossol
human, cytokine, bacterial antigen, CD14, signal transduction.

Interleukine-6 (IL-6) synthesis and gp130 expression by human pericardium

Emmanuelle Menet, Pierre Corbi, Cécile Ancey, Franck Morel, Adriana Delwail, Martine Garcia, Anne-Marie Osta, John Wijdenes, Daniel Potreau, Jean-Claude Lecron
IL-6, gp130, pericardium.

Plasmodium falciparum liver-stage antigen-1 peptide-specific interferon-gamma responses are not suppressed during uncomplicated malaria in African children

Adrian J.F. Luty, Martina Bongartz, Philipp Rezbach, Jean-Francois Faucher, Michael R. Hollingdale, Peter G. Kremsner
malaria, cytokine, interferon-gamma, liver.

Immunoexpressions of p21, Rb, mcl-1 and bad gene products in normal, hyperplastic and carcinomatous human prostates

Mar Royuela, María Isabel Arenas, Fermín R. Bethencourt, Manuel Sánchez-Chapado, Benito Fraile, Ricardo Paniagua
p21, Rb, mcl-1, bad, human prostate, benign prostatic hyperplasia, prostatic carcinoma.

Recombinant human interleukin-1 beta: new possibilities for the prophylaxis and correction of toxic myelodepression in patients with malignant tumors I. Phase I-II clinical trials of recombinant human interleukin-1 beta as a leukopoiesis stimulator in cancer patients receiving combination chemotherapy

M.L. Gershanovich, L.V. Filatova, S.A. Ketlinsky, A.S. Simbirtsev
human recombinant interleukin-1 beta, myelopoiesis, myelodepression, cancer chemotherapy.

Recombinant human interleukin-1 beta: new possibilities for the prophylaxis and correction of toxic myelodepression in patients with malignant tumors II. Phase II study of the protective effect of recombinant human interleukin-1 beta on myelodepression induced by chemotherapy in cancer patients

M.L. Gershanovich, L.V. Filatova, S.A. Ketlinsky, A.S. Simbirtsev
human recombinant interleukin-1 beta, leukopoiesis, myelodepression, cancer chemotherapy.

French society of immunology, cytokines

Eric Lelièvre, Isabelle Corre

30 rue Berthollet, Bât. A,
94110 Arcueil, France

+ 33 (0)1 46 73 06 60

contact@jle.com

Publishing Policy

Books and e-books

Want to publish ?

Become a partner