

Volume 12, issue 1, March 2001 | Articles originaux

Inducible nitric oxide synthase attenuates chronic colitis in human histocompatibility antigen HLA-B27/human b2 microglobulin transgenic rats.

Abstract

Rats transgenic for HLA-B27/human b2-microglobulin develop a spontaneous multisystem inflammatory disorder that closely mimicks human spondyloarthropathies. Prominent features of this disorder are gut inflammation that predominates in the colon, and arthritis. Several mediators such as IFN-g, IL-

1b, TNF-a, and inducible nitric oxide synthase (iNOS) have been found increased in the inflamed colonic mucosa. In the colon of HLA-B27 transgenic rats, iNOS is predominantly expressed by epithelial cells, and iNOS transcripts are detected in the hip cartilage of those rats, but not in nontransgenic littermates. The role of iNOS in this disorder was evaluated by administering the corticosteroid dexamethasone, or the NOS inhibitor L-N6-(1-iminoethyl)lysine (L-NIL) to HLA-B27 transgenic rats with established disease. Treatment with dexamethasone attenuated some aspects of gut inflammation, although it had no effect on iNOS expression. In contrast, treatment with L-NIL effectively inhibited iNOS activity, and resulted in an increase in colitis. Cytokine transcripts in the colon were modified by these treatments: IFN-g and IL-1b were decreased after dexamethasone treatment, whereas administration of L-NIL resulted in decreased IFN-g, and TNF-a. A trend towards increased IL-1b expression was observed which could have contributed to the L-NIL pro-inflammatory effect. These results suggest that iNOS exerts a protective effect on colitis, in the inflammatory disorder of HLA-B27 transgenic rats.

Full text

INTRODUCTION

The HLA-B27 class 1 major histocompatibility complex allele is strongly associated with the spondyloarthropathies, a group of human disorders characterized by the combination of osteoarticular, skin and gut inflammatory manifestations [1]. Several lines of rats transgenic for HLA-B27 and human *beta*$_2$-microglobulin develop a spontaneous multisystem inflammatory disease that strikingly resembles human spondyloarthropathies, by combining arthritis with colitis and psoriatic skin lesions [2]. The immune system is critically involved in the pathogenesis of this disease in HLA-B27 transgenic rats, which is transmittable to nontransgenic rats by bone marrow graft, and requires the presence of mature T cells for its development [3, 4]. Based on these findings, it is hypothesized that disease in HLA-B27 transgenic rats arises as a consequence of interaction between an antigen-presenting cell expressing high levels of HLA-B27, and peripheral T lymphocytes [3, 4]. The presence of a normal gut flora is also required for the gut and the joint disease to develop [2].

Mediators implicated in the pathogenesis in the HLA-B27 transgenic rat model have been most extensively studied in the gut mucosa. The profile of cytokines

expressed in the inflammed colonic mucosa is characterized by a prominent early increase in IFN-gamma and IL-2, suggesting a Th1-mediated predominant response [2]. In established disease, pro-inflammatory mediators such as IL-1alpha, IL-1beta, TNF-alpha, macrophage inflammatory protein 2, and myeloperoxidase (MPO) are also expressed in the inflammed colonic mucosa [5, 6]. Elevated plasma levels of nitrite/nitrate are consistent with an increased production of nitric oxide (NO) in this model [7] and the inducible nitric oxide synthase isoform (iNOS) was detected in the colonic mucosa of affected B27 transgenic rats by reverse transcriptase-polymerase chain reaction (RT-PCR) and by enzymatic activity assay [5, 6].

We previously reported that administration of murine recombinant IL-10 to HLA-B27 transgenic rats with established disease had no influence on the clinical or histopathological course of the disease. However, IL-10 efficiently inhibited production of IFN-gamma, TNF-alpha, and IL-1beta in the gut mucosa, and conversely increased colonic iNOS expression (5; M. Breban and D. Lamarque, unpublished results). This result suggested that iNOS could play a pathogenic role in this model, by the production of NO-derived injurious products such as peroxynitrites, or by altering the balance between pro- and anti-inflammatory cytokine production [8, 9]. In the present study, we tested this hypothesis by administering to HLA-B27 transgenic rats with established disease, dexamethasone, which can prevent the expression of iNOS [10, 11], and the iNOS inhibitor, L-N^6-(1-iminoethyl)lysine (L-NIL) [12], considered to offer selectivity for the iNOS isoform.

MATERIALS AND METHODS

Rats. The HLA-B27 transgenic rat line 33-3, on a Fisher (F344) background [2], was maintained by breeding 33-3 females with nontransgenic F344 males, and typing offsprings for the B27/h*beta*$_2$m transgene by PCR amplification of tail genomic DNA with an HLA-B27 specific pair of primers RT11 and RT12 [13]. All rats were maintained under conventional conditions at Cochin University Hospital animal facility.

Treatment. Dexamethasone was administered s.c. daily (study 1). L-NIL (Alexis, Paris, France) was administered s.c. twice daily (study 2). Sex- and age-matched rats of the 33-3 line with overt disease (6 rats per group, 11-39 weeks old: study

1; 8 rats per group, 17-21 weeks old: study 2), and control F344 littermates (4 rats per group in each study) received either active compound or vehicle.

Clinical evaluation. Rats were scored three times each week for diarrhea and arthritis, on a scale ranging from 0 (normal) to 4 for each of these symptoms, by the same examiner unaware of the treatment. The average daily total of these scores was calculated each week.

Macroscopic and histological evaluation of organs. After completion of treatment, rats were sacrificed. Mesenteric lymph nodes (MLN) were weighed and colon weight/length was measured. Tissue samples from the caecum, proximal colon and distal colon, were fixed in formalin, embedded in paraffin, sectioned, stained with hematoxylin, eosin, safran, and coded for blind examination. Sections were scored for ulceration density (absent: 0; covering < 1/3 of examined area: 1; covering 1/3 to 2/3 of examined area: 2; covering > 2/3 of examined area: 3), ulceration depth (limited to mucosa: 1; reaching submucosa: 2; reaching muscularis propria: 3) and extent of polymorphonuclear and mononuclear cells infiltration in the chorion (absent: 0; limited to mucosa: 1; reaching submucosa: 2; reaching muscularis propria: 3). The global histological score was the sum of the values obtained for the caecum, the proximal colon, and the distal colon per rat.

Immunohistochemistry. An antigen retrieval method was performed on 5 mum thick, deparaffinized sections of colon, using micro-wave oven heating (3 x 5 min) in 6 mM citrate buffer pH 6. Sections were then incubated for 10 min in 3% hydrogen peroxide to block endogenous peroxidase activity, followed by 1 hour with anti-iNOs monoclonal antibody, dilution 1/40 (Transduction Laboratories, Lexington, UK). A three-step indirect process based on avidin-biotinylated peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA, USA) and 3,3-diaminobenzidine (Sigma, St-Quentin Fallavier, France) as chromogen, was applied to reveal as a brown precipitate the positive reaction. Negative controls were obtained by omitting the primary antibody.

Western immunoblot analysis. The whole colonic mucosa or colonic epithelial cells, isolated by the standard method [14], were homogenized in buffer (2 mM Tris 7-9, 50 mM mannitol, 100 muM phenylmethylsulfonyl, 2 muM leupeptin, 0.5 mu/ml aprotinin, 0.5% triton X100). Spleen homogenate from rat challenged i.v. with *Escherichia coli* lipopolysaccharide (LPS serotype O111:B4; Sigma) was used

as a positive control for iNOS. Homogenates were sonicated twice for 10 sec on ice and spun (21,000 *g* at 4° C, 15 min). Aliquots of 20 mug of total cellular protein were denatured by boiling v/v with 20 mM Tris 7-9, 2 mM EDTA, 2% SDS, 10% beta-mercaptoethanol, 20% glycerol. Samples were electrophoresed on 7.5% SDS-polyacrylamide gel, and transferred to nitrocellulose membrane (Amersham, Little Chalfont, UK). After blocking with PBS (pH 7.4), 0.25% tween 20 (v/v) and 5% non-fat dried milk, the membrane was probed with anti-iNOS polyclonal antibody (sc-651; Santa Cruz, Santa Cruz, CA, USA) for 1 hour, washed with PBS-tween 20 and then incubated with horseradish peroxidase-conjugated second antibody for 1 hour at room temperature. Membranes were developed, using an enhanced chemiluminescence system (Amersham) and exposed to Hyperfilm (Amersham). Films were scanned on a GS-700 Imaging Densitometer (Bio-Rad, Hercules, USA) and analysed using the Molecular Analyst Software (Bio-Rad).

MPO assay. MPO activity was determined as described, with minor modifications [15]. Segments of colon were homogenized in ice-cold phosphate buffer containing 0.5% hexadecyltrimethylammonium-bromide, freeze-thawed three times and centrifuged (10,000 *g*, 15 min, 4° C). Supernatant was mixed with phosphate buffer containing 0.167 mg/ml of O-adenosine dihydrochloride and 5 x 10^{-4}% hydrogen peroxide and assayed spectrophotometrically (500 nm). MPO activity was expressed in mU/g of tissue.

Determination of iNOS enzymatic activity. Activity of iNOS was estimated by measuring the conversion of L-[^{14}C]-arginine monohydrochloride to L-[^{14}C]-citrulline, based on a previously described method [16]. Briefly, a colon sample was homogenized in HEPES buffer containing protease inhibitors. After centrifugation (10,000 *g*, 30 min, 4° C), an aliquot of supernatant was added to prewarmed buffer containing (final concentration) potassium phosphate (50 mM;
pH = 7.4), valine (50 mM), $MgCl_2$ (1 mM), $CaCl_2$ (200 muM), DL-DTT (1 mM), L-citrulline (1 mM), NADPH (0.3 mM), FAD (3 muM), FMN (3 muM), BH4 (3 muM), L-[^{14}C]-arginine monohydrochloride (15.5 nM; Amersham, Les Ulis, France) and incubated for 10 min at 37° C. The incubation was terminated by the binding of arginine, by addition of a 1:1 suspension of Dowex (AG 50W-8; Sigma) in water. Supernatant was taken for scintillation counting. Activity of iNOS was that which was inhibited by *in vitro* incubation with the NO synthase inhibitor N^G-

monomethyl-L-arginine (300 muM; Sigma) but not with EGTA (1 mM), and was expressed as pmol.min^{-1}.mg^{-1} protein [17].

RT-PCR. Total RNA was isolated with the TRI Reagent (Molecular Research Center, Cincinnati, OH, USA) and reverse transcribed into complementary DNA (cDNA) using AMV reverse transcriptase. Amplification was carried out in a 100 mul volume containing 100 ng cDNA, 125 muM of each 2'-deoxynucleoside 5'-triphosphate, 2 U of Taq DNA polymerase, 50 ng of each primer and 10 mul of 10 X PCR buffer. Primer sequences and the annealing temperature used for beta-actin, CD3, IL-1beta, TNF-alpha, and IFN-gamma were as described [5]; other primers were: IL-10 sense 5'-TGC CTT CAG TCA AGT GAA GAC T-3', antisense 5'-AAA CTC ATT CAT GGC CTT GTA-3', Tm = 55° C; iNOS sense 5'-CAA TCC ACA ACT CGC TCC AA-3', antisense 5'-GCT ACA CTT CCA ACT CAA CA-3', Tm = 57° C. Amplification conditions consisted of 3 min at 92° C followed by 35 cycles of denaturation at 94° C for 30 sec, annealing for 30 sec, extension at 72° C for 40 sec, and by final elongation at 72° C for 10 min. Fifty microliters of PCR product were electrophoresed on agarose gel. Negative pictures of the gels were taken for quantitation with a Personal Densitometer and the Image Quant 3.3 software (Molecular Dynamics, Sunnyvale, CA, USA). Data are expressed in arbitrary units, as the ratio between optical density for the specific cDNA and the one for beta-actin on the same sample.

Statistics. The data are expressed as mean ± SEM, unless otherwise stated. The statistical significance of differences was tested with the Mann Withney non-parametric U test. *P* values less than 0.05 were considered significant.

RESULTS

Localization of iNOS expression in HLA-B27 transgenic rats. Expression of iNOS was reproducibly detected by Western Blot analysis (Figure 1), and by immunohistochemistry (Figure 2) in the whole colon of HLA-B27 transgenic rats with established colitis, but not in the colon of nontransgenic F344 littermates. Immunohistochemistry performed on the colon of 33-3 rats, demonstrated the predominance of iNOS expression in the epithelium over the chorion (Figure 2). This result was consistent with the strong detection of iNOS in isolated colonic epithelial cells from 33-3 rats (Figure 1). In addition, RT-PCR analysis of several tissues was performed in HLA-B27 transgenic rats in parallel with nontransgenic littermates. These experiments demonstrated the presence of iNOS mRNA in

the hip cartilage of 7 out of 10 HLA-B27 transgenic rats studied, in addition to the colon (data not shown), but not in this tissue from nontransgenic littermates, nor in any the following tissues from affected 33-3 or from nontransgenic F344 rats that were examined: stomach, ileum, heart, eye, synovial tissue from arthritic and nonarthritic knees (specimens from 2 to 5 rats for each tissue were examined; data not shown).

Dexamethasone improves spontaneous disease in HLA-B27 transgenis rat but fails to inhibit iNOS expression. In study 1, groups of 33-3 rats and non-transgenic littermates were treated for 4 weeks with either dexamethasone (0.25 $mg.kg^{-1}.day^{-1}$), or vehicle. None of the 33-3 rats used in this experiment had clinical arthritis, but all had chronic diarrhea. Administration of dexamethasone decreased diarrhea in 33-3 rats (Table 1, study 1). Consistent with this observation, gross examination of MLN and the colon at autopsy demonstrated reduced MLN hyperplasia, and weight/length ratio of the colon in dexamethasone-treated 33-3 rats (Table 2, study 1). MLN weight was also reduced in dexamethasone-treated nontransgenic F344 rats. Histological examination of the colonic mucosa revealed chronic inflammation in 33-3 rats. However, the histological score of lesions was unchanged in dexamethasone-treated 33-3 rats, as compared to vehicle-treated rats (Table 2, study 1). An inhibitory effect of dexamethasone was detected in the colon on several markers linked to gut inflammation, *i.e.* MPO enzymatic activity (Table 3, study 1), and IFN-gamma, IL-1beta and CD3 transcripts evaluated by semi-quantitative RT-PCR (Figure 3A). However, neither iNOS enzymatic activity (Table 3, study 1), nor iNOS mRNA expression (Figure 3A), examined in the colonic mucosa were modified in dexamethasone-treated 33-3 rats.

L-NIL decreases iNOS activity, and aggravates the spontaneous inflammatory disease of HLA-B27 trasngenic rat. We further addressed the role played by iNOS in the disease process of HLA-B27 transgenic rat, by administering L-NIL (10 $mg.kg^{-1}.day^{-1}$) or vehicle for 3 weeks to 33-3 rats with established disease (study 2). In this experiment, both diarrhea and arthritis were present. Administration of L-NIL had no detectable effect on diarrhea (Table 1, study 2); arthritis was exacerbated in the L-NIL-treated group, as compared to the vehicle-treated group although the difference was not statistically significant (Table 1, study 2). Evidence for a detrimental effect of L-NIL on the course of inflammatory disease in 33-3 rats was further provided by gross organ examination: MLN hyperplasia was increased in L-NIL-treated 33-3 rats, as

compared to vehicle-treated group, whereas the colon weight/length ratio was no different between the two groups (Table 1, study 2). Finally, histological evaluation of the colon demonstrated increased lesions in L-NIL-treated 33-3 rats, as compared to the vehicle-treated group (Table 2, study 2). Variations in MLN weight, histological score, MPO and iNOS activity of vehicle-treated 33-3 groups between study 1 and 2 (Tables 1 and 2) merely reflects the difference in the overall degree of gut inflammation between the 2 studies.

Influence of L-NIL treatment on cytokine production in the colon of B27-transgenic rats. Inhibition of iNOS enzymatic activity by L-NIL was confirmed in the colon, whereas MPO activity was not significatively modified by L-NIL treatment (Table 3, study 2). RT-PCR analysis detected comparable levels of CD3, iNOS, and IL-10 transcripts in the colonic mucosa of 33-3 rats treated with L-NIL or with vehicle (Figure 3B, and data not shown). As regards cytokine transcripts, a significant decrease was observed for IFN-gamma, and TNF-alpha in L-NIL-treated 33-3 rats, in comparison to vehicle-treated group (Figure 3B). In contrast, IL-1beta mRNA transcripts were increased in the L-NIL-treated group, as compared to vehicle-treated 33-3 rats, but this difference did not reach statistical significance. No consistent difference in pathology or MPO activity was detected between a subgroup of 4 rats with high IL-1beta transcript values and the 3 remaining rats with lower values, among L-NIL-treated 33-3 rats (data not shown).

DISCUSSION

Expression of iNOS was detected in two prominent target organs of HLA-B27 transgenic rat inflammatory disorder, namely the colonic mucosa and joint. Induction of iNOS was reproducibly detected in the hip cartilage of 33-3 rats, even though arthritis was not observed at this site. In contrast, iNOS was not detected in the synovium, even from arthritic joints, which may suggest that chondrocytes are specifically triggered to produce iNOS in this model. Conceivably, a common mechanism may result in iNOS expression in both gut and joint, since inflammation of these two organs is closely linked in the HLA-B27 transgenic rat [2]. In the colonic mucosa, iNOS expression was predominantly localized in the epithelial cells, with only limited expression in the inflammatory cells infiltrating the lesions. Among possible triggers of iNOS expression, cytokines produced locally in the setting of chronic inflammation and luminal bacterial products, such as LPS, are likely candidates [18, 19].

We examined *in vivo* the role played by iNOS in the HLA-B27 transgenic rat model by administering dexamethasone, which may prevent the expression of iNOS [20] and the putative iNOS inhibitor, L-NIL. Dexamethasone treatment improved chronic diarrhea, and reduced MLN hyperplasia, and colon weight/length ratio in 33-3 rats with established disease. It also decreased MLN weight and MPO activity in the colon of nontransgenic control F344 rats. All these changes are suggestive of an anti-inflammatory effect. Nevertheless the efficacy of dexamethasone on 33-3 gut lesions was not confirmed when examined histologically. We also observed a lack of influence of dexamethasone on iNOS expression. This latter result is consistent with a study carried out in human inflammatory bowel disease [21], although it is at variance with reports showing inhibition by corticosteroids of cytokine- and LPS-mediated iNOS induction [11, 14]. Such inhibitory effects of dexamethasone are thought to involve inhibition of nuclear factor-kappaB (NF-kappaB) activation, which is important for induction of the iNOS gene [22]. However, inhibition of NF-kappaB by dexamethasone is not uniformly observed [23], and dexamethasone-insensitive pathways of iNOS induction, other than NF-kappaB have been described in epithelial cells [24]. In the present study, the decrease of CD3 and cytokine transcripts in colon, observed after dexamethasone treatment, was not associated with a striking inhibition of gut inflammation. This result is consistent with our previous work, in which administration of IL-10 to 33-3 rats failed to improve colonic inflammation, although it did reduce CD3, IFN-gamma, TNF-alpha, and IL-1beta, and conversely increased iNOS expression in the colon (5; M. Breban and D. Lamarque, unpublished results). Altogether, these results argue for a dispensable role of this set of pro-inflammatory cytokines in perpetuating the disease process and in the induction of iNOS. It is likely that other mediators are critically involved in the sustained colonic inflammation of HLA-B27 transgenic rats. However, since dexamethasone had no effect on iNOS, this study could not properly address the role of iNOS in this model of colitis.

In a further study, L-NIL was used as a selective inhibitor of iNOS [12]. Enzymatic inhibition of iNOS was achieved with this compound. L-NIL-treatment was associated with aggravation of several markers of disease severity, *i.e.* arthritis, MLN hyperplasia, and colonic histological inflammation. Another noticeable effect of L-NIL administration was a decrease of several cytokine transcripts in the colon, *i.e.* IFN-gamma and TNF-alpha. However, a trend towards increased IL-1beta was conversely observed in the same tissue. Hence, this result suggests that NO produced by epithelial cells in the inflammed colonic mucosa results in

either stimulatory (IFN-gamma, TNF-alpha) or inhibitory (IL-1beta) effects on cytokine production. Depending on the site of production, the amount produced, and the targets within the local environment, NO has indeed been shown to display opposite effects, including those on cytokine production [25-27]. In the colitis of HLA-B27 transgenic rats, it is not yet known which cells produce specific cytokines, and NO may exert an effect on different target cells, such as T cells, macrophages or epithelial cells, resulting in distinct variations of cytokine transcripts. In any case, stimulation of IL-1beta transcription may provide an explanation for the damaging consequence of iNOS inhibition in the present model. In a study by Aiko and colleagues, two other NO synthase inhibitors, *i.e.* aminoguanidine and L-NAME, were used in this HLA-B27 transgenic rat model [28]. The major effects of NO synthase inhibitors reported by these authors were decreased MPO activity in the colonic mucosa and decreased bowel mucosal permeability. Increased colonic lesions were not described. However, no quantitative histological analysis of colonic ulceration and infiltration by inflammatory cells was performed. Hence, differences with the present study could be related to different inhibitory activity of the inhibitors used in their study, as compared to L-NIL, or to a differences in study protocols.

Induction of iNOS has been described in a variety of inflammatory diseases, in humans and in animal models, including colitis, arthritis and psoriasis which are features of the HLA-B27 transgenic rat model [25, 29, 30]. Interestingly, expression of iNOS in human ulcerative colitis was shown to predominate in epithelial cells rather than in infiltrating leukocytes of the colonic mucosa, a finding similar to our observation in HLA-B27 transgenic rat [31]. Investigating the potential implication of iNOS induction in the pathogenic mechanism of this model is of considerable interest, since iNOS has been shown to exert both protective and detrimental effects on several inflammatory processes, including chronic gut inflammatory situations and arthritis [9, 32]. NO from iNOS seems to contribute to tissue destruction in acute inflammatory disease through the generation of its cytotoxic derivative peroxynitrite, when produced by macrophages and neutrophils in combination with oxygen free radicals [33]. Such a consequence is less likely to happen if NO production takes place in epithelial cells, such as in chronic colitis of HLA-B27 transgenic rat. Local production of NO in the epithelium may rather display antibacterial effects that could be beneficial in this model, in which gut bacterial flora is critically involved in the induction of both gut and joint inflammation [2]. NO may also display immunoregulatory activities which are mediated by the modulation of cytokine

production and apoptosis of effector T cells, resulting in a decrease of Th1-dependant autoimmune diseases [9]. Regarding a putative immunoregulatory mechanism, it is worth noting that IL-1beta, which has been repeatedly implicated in the pathogenesis of IBD was rather increased in the colon after L-NIL treatment [34-36].

CONCLUSION

Our results provide a strong suggestion that iNOS is protective, at least in colitis and presumably in arthritis in the HLA-B27 transgenic rat model. To our knowledge, this is the first evidence that NO is protective in a spontaneous animal model of chronic colitis or arthritis. It will be important to consider this result when discussing the therapeutic prospect of selective iNOS inhibitors in chronic inflammatory disorders [25, 37].

Acknowledgments. The technical contribution of Christine Girardot and Laurence Momeux is gratefully acknowledged.

References

1. Moll J M, Haslock I, Macrae I, Wright V. 1974. Associations between ankylosing spondylitis, psoriatic arthritis, Reiter's disease, the intestinal arthropathies, and Behçet's syndrome. (Review) *Medicine* (Baltimore) 53: 343.

2. Taurog J D, Maika S, Satumtira N, Dorris M L, McLean I L, Yanagisawa H, Sayad A, Stagg A J, Fox G M, O'Brien A L, Rehman M, Zhou M, Weiner A L, Splawski J B, Richardson J A, Hammer R E. 1999. Inflammatory disease in HLA-B27 transgenic rats. (Review) *Immunol. Rev.* 169: 209.

3. Breban M, Hammer R E, Richardson J A, Taurog J D. 1993. Transfer of the inflammatory disease of HLA-B27 transgenic rats by bone marrow engraftment. *J. Exp. Med.* 178: 1607.

4. Breban M, Fernandez-Sueiro J L, Richardson J A, Hadavand R R, Maika S D, Hammer R E, Taurog J D. 1996. T cells, but not thymic exposure to HLA-B27, are required for the inflammatory disease of HLA-B27 transgenic rats. *J. Immunol.* 156: 794.

5. Bertrand V, Quere S, Guimbaud R, Sogni P, Chauvelot-Moachon L, Tulliez M, Lamarque D, Charreire J, Giroud J P, Couturier D, Chaussade S, Breban M. 1998. Effects of murine recombinant IL-10 on the inflammatory disease of rat transgenic for HLA-B27 and human beta2-microglobulin. *Eur. Cytokine Netw.* 9: 161.

6. Breban M. 1998. Animal models and *in vitro* models for the study of aetiopathogenesis of spondyloarthropathies. (Review) *Bailliere Clin. Rheumatol.* 12: 611.

7. Aiko S, Grisham M B. 1995. Spontaneous intestinal inflammation and nitric oxide metabolism in HLA-B27 transgenic rats of spondylopathies. (Review) *Gastroenterology* 109: 142.

8. Nathan C. 1997. Inducible nitric oxide synthase: what difference does it make? (Review) *J. Clin. Invest.* 100: 2417.

9. Kolb H, Kolb-Bachofen V. 1998. Nitric oxide in autoimmune disease: cytotoxic or regulatory mediator. (Review) *Immunol. Today* 19: 556.

10. Radomski M W, Palmer R M, Moncada S. 1990. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc. Natl. Acad. Sci. USA* 87: 10043.

11. Geller D A, Nussler A K, Di Silvio M, Lowenstein C J, Shapiro R A, Wang S C, Simmons R L, Billiar T R. 1993. Cytokines, endotoxin, and glucocorticoids regulate the expression of inducible nitric oxide synthase in hepatocytes. *Proc. Natl. Acad. Sci. USA* 90: 522.

12. Moore W M, Webber R K, Jerome G M, Tjoeng F S, Misko T P, Currie M G. 1994. L-N^6-(1-iminoethyl)lysine: a selective inhibitor of inducible nitric oxide synthase. *J. Med. Chem.* 37: 3886.

13. Del Porto P, D'Amato M, Fiorillo M T, Tuosto L, Piccolella E, Sorrentino R. 1994. Identification of a novel HLA-B27 subtype by retriction analysis of a cytotoxic gammadelta T cell clone. *J. Immunol.* 153: 3093.

14. Tepperman B L, Brown J F, Korolkiewicz R, Whittle B J. 1994. Nitric oxide synthase activity, viability and cyclic GMP levels in rat colonic epithelial cells: effect of endotoxin challenge. *J. Pharmacol. Exp. Ther.* 271: 1477.

15. Bradley P P, Priebat D A, Christensen R D, Rothstein G. 1982. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J. Invest. Dermatol.* 78: 206.

16. Knowles R G, Merrett M, Salter M, Moncada S. 1990. Differential induction of brain, lung and liver nitric oxide synthase by endotoxin in the rat. *Biochem. J.* 270: 833.

17. Salter M, Knowles R G, Moncada S. 1991. Widespread tissue distribution, species distribution and changes in activity of Ca(2$^+$)-dependent and Ca(2$^+$)-independent nitric oxide synthases. *FEBS Lett.* 291: 145.

18. Moncada S, Higgs E A. 1995. Molecular mechanisms and therapeutic strategies related to nitric oxide. (Review) *FASEB J.* 9: 1319.

19. Fang F C. 1997. Perspectives series: host/pathogen interactions. Mechanisms of nitric oxide-related antimicrobial activity. (Review) *J. Clin. Invest.* 99: 2818.

20. Fernandez M, Garcia-Pagan J C, Casadevall M, Bernadich C, Piera C, Whittle B J, Pique J M, Bosch J, Rodes J. 1995. Evidence against a role for inducible nitric oxide synthase in the hyperdynamic circulation of portal-hypertensive rats. *Gastroenterology* 108: 1487.

21. Leonard N, Bishop A E, Polak J M, Talbot I C. 1998. Expression of nitric oxide synthase in inflammatory bowel disease is not affected by corticosteroid treatment. *J. Clin. Pathol.* 51: 750.

22. De Vera M E, Taylor B S, Wang Q, Shapiro R A, Billiar T R, Geller D A. 1997. Dexamethasone suppresses iNOS gene expression by upregulating I-kappa B alpha and inhibiting NF-kappaB. *Am. J. Physiol.* 273: G1290.

23. Newton R, Hart L A, Stevens D A, Bergmann M, Donnelly L E, Adcock I M, Barnes P J. 1998. Effect of dexamethasone on interleukin-1beta-(IL-1beta)-

induced nuclear factor-kappaB (NF-kappaB) and kappaB-dependent transcription in epithelial cells. *Eur. J. Biochem.* 15: 81.

24. Kleinert H, Wallerath T, Fritz G, Ihrig-Biedert I, Rodriguez-Pascual F, Geller D A, Forstermann U. 1998. Cytokine induction of NO synthase II in human DLD-1 cells: roles of the JAK-STAT, AP-1 and NF-kappaB-signaling pathways. *Br. J. Pharmacol.* 125: 193.

25. Clancy R M, Amin A R, Abramson S B. 1998. The role of nitric oxide in inflammation and immunity. (Review) *Arthritis Rheum.* 41: 1141.

26. Yan L, Wang S, Rafferty S P, Wesley R A, Danner R L. 1997. Endogenously produced nitric oxide increases tumor necrosis factor-alpha production in transfected human U937 cells. *Blood* 90: 1160.

27. Sinha B, Eigler A, Baumann K H, Greten T F, Moeller J, Endres S. 1998. Nitric oxide downregulates tumour necrosis factor mRNA in RAW 264.7 cells. *Res. Immunol.* 149: 139.

28. Aiko S, Fuseler J, Grisham M B. 1998. Effects of nitric oxide synthase inhibition or sulfasalazine on the spontaneous colitis observed in HLA-B27 transgenic rats. *J. Pharmacol. Exp. Ther.* 284: 722.

29. Boughton-Smith N K, Evans S M, Howkey C J, Cole A T, Balsitis M, Whittle B J, Moncada S. 1993. Nitric oxyde synthase activity in ulcerative colitis and Crohn's disease. *Lancet* 342: 338.

30. Bruch-Gerharz D, Fehsel K, Suschek C, Michel G, Ruzicka T, Kolb-Bachofen V. 1996. A proinflammatory activity of interleukin-8 in human skin: expression of the inducible nitric oxide synthase in psoriatic lesions and cultured keratinocytes. *J. Exp. Med.* 184: 2007.

31. Kolios G, Rooney N, Murphy C T, Robertson D A, Westwick J. 1998. Expression of inducible nitric oxide synthase activity in human colon epithelial cells: modulation by T lymphocyte derived cytokines. *Gut* 43: 56.

32. McCafferty D M, Mudgett J S, Swain M G, Kubes P. 1997. Inducible nitric oxide synthase plays a critical role in resolving intestinal inflammation.

Gastroenterology 112: 1022.

33. Rachmilewitz D, Stamler J S, Karmeli F, Mullins M E, Singel D J, Loscalzo J, Xavier R J, Podolsky D K. 1993. Peroxynitrite-induced rat colitis: a new model of colonic inflammation. *Gastroenterology* 105: 1681.

34. Nakamura M, Saito H, Kasanuki J, Tamura Y, Yoshida S. 1992. Cytokine production in patients with inflammatory bowel disease. *Gut* 33: 933.

35. Reinecker H C, Steffen M, Witthoeft T, Pflueger I, Schreiber S, MacDermott R P, Raedler A. 1993. Enhanced secretion of tumour necrosis factor-alpha, IL-6, and IL-1beta by isolated lamina propria mononuclear cells from patients with ulcerative colitis and Crohn's disease. *Clin. Exp. Immunol.* 94: 174.

36. Gilberts E C, Greenstein A J, Katsel P, Harpaz N, Greenstein R J. 1994. Molecular evidence for two forms of Crohn disease. *Proc. Natl. Acad. Sci. USA* 91: 12721.

37. Guslandi M. 1998. Nitric oxide and inflammatory bowel diseases. (Review) *Eur. J. Clin. Invest.* 28: 904.

Figures

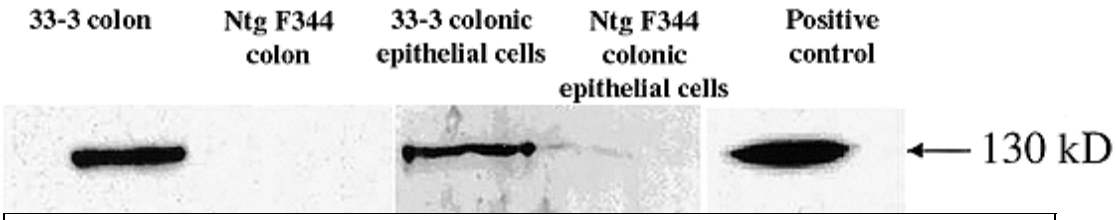


Figure 1

Western-blot analysis of iNOS in the whole colon and in isolated colonic epithelial cells from the HLA-B27 transgenic rat of the 33-3 line with established disease, and from a non-transgenic (Ntg) F344 littermate.

Spleen homogenate from rat challenged i.v. with LPS was used as a positive control of iNOS expression. This result is representative of 4 separate experiments.

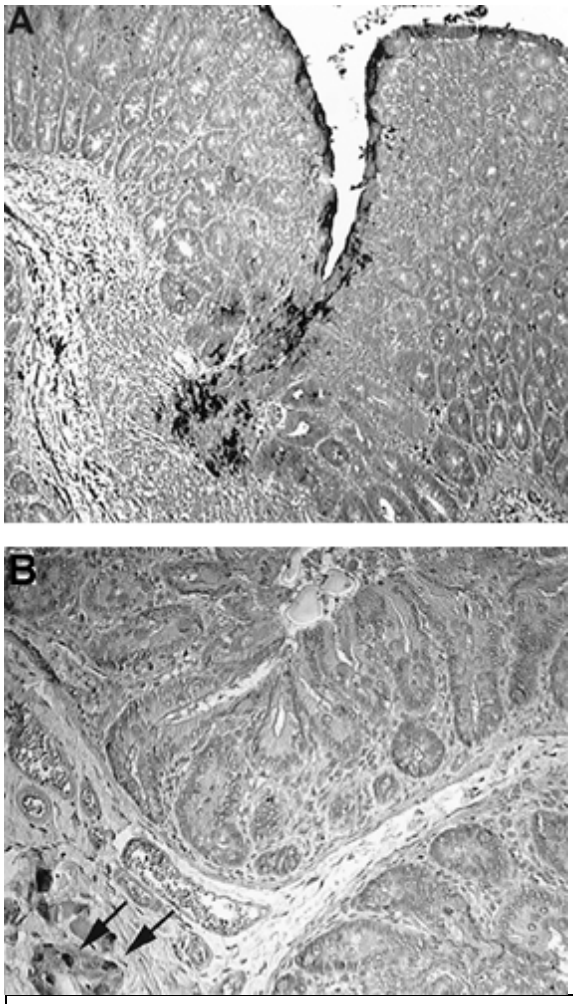


Figure 2

Detection of iNOS by immunohistochemistry performed on the colonic mucosa.

A: colon from an HLA-B27 transgenic rat of the 33-3 line with established disease. The section shows chronic colitis with ulceration, cryptic abcess and marked infiltration of inflammatory cells, and immunohistochemical localisation of iNOS as diffuse, dark staining, localized mainly in the apical epithelium (original

magnification x 40). Positive detection of iNOS was observed in colon from 8 different 33-3 rats examined. **B:** normal colon from non-transgenic F344 littermate. The section shows no epithelial cells or lamina propria staining (original magnification x 100). A positive staining of ganglionic cells from Meissner's plexus can be noted (arrows).

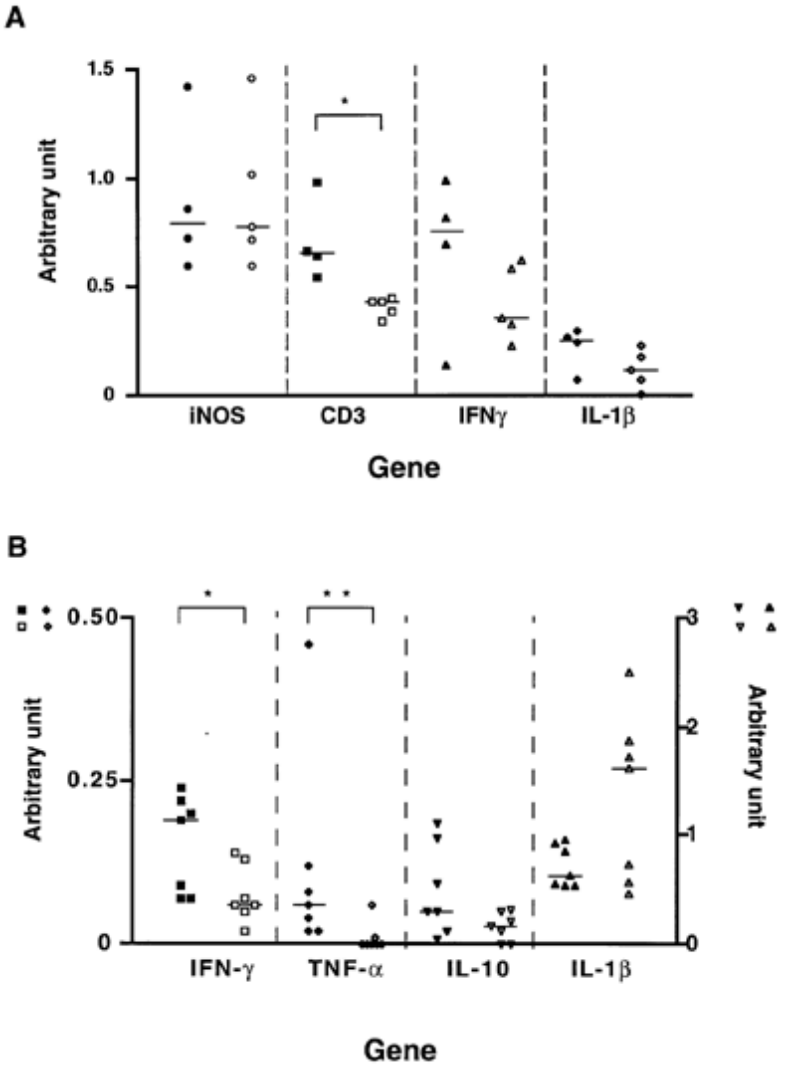


Figure 3

Semi-quantitative RT-PCR evaluation of iNOS and cytokines transcripts in the colonic mucosa from HLA-

B27 transgenic rats. Individual rat values and medians are shown.

A: *study 1:* iNOS, CD3, IFN-gamma, and IL-1beta transcripts in the colonic mucosa from vehicle-treated B27 transgenic rats (close symbols), and from dexamethasone-treated B27 transgenic rats (open symbols). * $P < 0.05$, dexamethasone- *vs* vehicle-treated groups. In the colon of dexamethasone- (n = 4) or vehicle-treated (n = 2) nontransgenic F344 littermates, trancripts for iNOS, IFN-gamma or IL-1beta were not detected and transcripts for CD3 were negative or ¾ 10-fold below the level of detection achieved in B27 transgenic rats. **B:** *study 2:* IFN-gamma, TNF-alpha (left scale), and
IL-10, IL-1beta (right scale) transcripts in the colonic mucosa from vehicle-treated (close symbols), and from L-NIL-treated B27 transgenic rats (open symbols). * $P < 0.05$, L-NIL- *vs* vehicle-treated group. ** $P < 0.01$, L-NIL- *vs* vehicle-treated group. In the colon of L-NIL- (n = 2) or vehicle-treated (n = 3) nontransgenic F344 littermates, trancripts for IFN-gamma, TNF-alpha, or IL-1beta were not detected, and transcripts for IL-10 were either negative or < 7-fold below the average level of detection in vehicle-treated B27 transgenic rats.

Table 1
Effect of dexamethasone (study 1) and L-NIL (study 2) on the scores of diarrhea and arthritis in HLA-B27 transgenic rats

	Week of treatment				
Group of rats	0	1	2	3	4
Study 1					
Score of diarrhea					
33-3: vehicle (n = 6)	1.7 ± 0.5	2 ± 0.6	2.3 ± 0.6	2.5 ± 0.8	2.9 ± 0.8
33-3: dexamethasone (n = 6)	2.3 ± 0.3	2.5 ± 0.4	2.1 ± 0.4[a]	1.6 ± 0.2[b]	1.3 ± 0.5[a]
Study 2					
Score of diarrhea					
33-3: vehicle (n = 8)	1.6 ± 0.3	2 ± 0.2	1.9 ± 0.1	2 ± 0.1	
33-3: L-NIL (n = 8)	2.3 ± 0.2	2.4 ± 0.2	2.3 ± 0.2	2.1 ± 0.2	
Arthritic score					
33-3: vehicle (n = 8)	0.5 ± 0.5	1.1 ± 0.7	1 ± 0.6	0.9 ± 0.5	
33-3: L-NIL (n = 8)	0.8 ± 0.4	1.6 ± 0.5	2 ± 0.5	2 ± 0.6	

Study 1: HLA-B27 transgenic rats of the 33-3 line with established disease received dexamethasone (0.25 mg.kg^{-1}.day^{-1}) or vehicle for 4 weeks. Dexamethasone- and vehicle-treated nontransgenic F344 littermates had no diarrhea. [a] $P = 0.015$ dexamethasone *vs* vehicle: week 2 and week 4 minus week 0. [b] $P < 0.01$ dexamethasone *vs* vehicle: week 3 minus week 0.
Study 2: HLA-B27 transgenic rats of the 33-3 line with established disease received L-NIL (10 mg.kg^{-1}.day^{-1}) or vehicle for 3 week. L-NIL- and vehicle-treated nontransgenic F344 littermates had no detectable diarrhea or arthritis.

Table 2
Effect of dexamethasone (study 1) and L-NIL (study 2) on MLN weight, colon weight/length and quantitative histological score of HLA-B27 transgenic rat colon

Group of rats	MLN wt (mg)	Colon wt/length (mg/cm)	Histologic score
Study 1			
33-3: vehicle (n = 6)	1,180 ± 184	239 ± 31	16.5 ± 1
33-3: dexamethasone (n = 5)	413 ± 88[a]	158 ± 12[b]	17 ± 1.1
Ntg: vehicle (n = 4)	126 ± 26	98 ± 19	0
Ntg: dexamethasone (n = 4)	27 ± 5[c]	90 ± 1	0
Study 2			
33-3: vehicle (n = 8)	863 ± 49	217 ± 22	8.3 ± 1.5
33-3: L-NIL (n = 8)	1055 ± 69[d]	224 ± 18	11.5[e] ± 0.4
Ntg: vehicle (n = 4)	188 ± 34	99 ± 9	1.9 ± 1.5
Ntg: L-NIL (n = 4)	163 ± 36	117 ± 3	0

HLA-B27 transgenic rats of the 33-3 line and nontransgenic (Ntg) F344 rats were treated with dexamethasone (0.25 mg.kg^{-1}.day^{-1}) or vehicle for 4 weeks (study 1), or with L-NIL (10 mg.kg^{-1}.day^{-1}) or vehicle for 3 weeks (study 2). MLN weight and weight/length of colon were measured at sacrifice.
Study 1: 33-3: dexamethasone *vs* vehicle; [a] $P < 0.005$; [b] $P < 0.04$
Ntg: dexamethasone *vs* vehicle; [c] $P < 0.03$
Study 2: 33-3: L-NIL *vs* vehicle: [d] $P < 0.04$; [e] $P < 0.03$.

Table 3
Effect of dexamethasone (study 1) and L-NIL (study 2) on MPO and iNOS enzymatic activities in the colon of HLA-B27 transgenic rats

Group of rats	MPO (mU/g of colon)	iNOS (pmol. $min^{-1}.mg^{-1}$ protein)
Study 1		
33-3: vehicle (n = 6)	1,451 ± 455	2.2 ± 0.6
33-3: dexamethasone (n = 5)	475 ± 112	2.2 ± 0.5
Ntg: vehicle (n = 4)	690 ± 126	0
Ntg: dexamethasone (n = 4)	76 ± 25[a]	0
Study 2		
33-3: vehicle (n = 8)	477 ± 113	1.4 ± 0.4
33-3: L-NIL (n = 6)	649 ± 212	0.4 ± 0.1[b]
Ntg: vehicle (n = 4)	319 ± 70	0.2 ± 0.1
Ntg: L-NIL (n = 4)	351 ± 105	0.1 ± 0.2

HLA-B27 transgenic rats of the 33-3 line and nontransgenic (Ntg) F344 rats were treated with dexamethasone (0.25 $mg.kg^{-1}.day^{-1}$) or vehicle for 4 weeks (study 1), or with L-NIL (10 $mg.kg^{-1}.day^{-1}$) or vehicle for 3 weeks (study 2). MPO and iNOS enzymatic activity were measured in samples of colon harvested at sacrifice.

[a] $P < 0.05$, Ntg: dexamethasone *vs* vehicle *(study 1)*.

[b] $P = 0.05$, 33-3: L-NIL *vs* vehicle *(study 2)*.

Authors

H.S. Blanchard, E. Dernis-Labous, D. Lamarque, J. Tran van Nhieu, Z. Szepes, J.F. Fléjou, E. Wollman, B.J.R. Whittle, M. Breban

Institut de Rhumatologie, Hôpital Cochin, 27, rue du Faubourg Saint-Jacques, 75014, Paris, France.

In this issue

Regulation of interferon-g receptor (INF-gR) chains: a peculiar way to rule the life and death of human lymphocytes.

P. Bernabei, A. Allione, L. Rigamonti, M. Bosticardo, G. Losana, I. Borghi, G. Forni, F. Novelli

human, IFN-g, IFN-g receptor, signal transduction, cellular proliferation, apoptosis.

The interleukin-6 cytokine system in embryonic development, embryo-maternal interactions and cardiogenesis.

P. Seiler, G. Plenz, M.C. Deng
interleukin-6, embryo, blastocyst, endometrium, heart, gp130.

Role of cytokines in the modulation of nitric oxide production by cyclic AMP.

Z. Zidek
cyclic AMP, prostaglandins, nitric oxide, interleukin-10, tumor necrosis factor-a, interferon-g.

The 3' untranslated region of tumor necrosis factor-a is highly conserved in idiopathic pulmonary fibrosis (IPF).

R.W. Freeburn, H. Kendall, L. Dobson, J. Egan, N.J. Simler, A.B. Millar
TNF-a, idiopathic pulmonary fibrosis, polymorphism.

Endogenous interferon-g impairs bacterial clearance from lungs during Pseudomonas aeruginosa pneumonia.

M.J. Schultz, A.W. Rijneveld, P. Speelman, S.J.H. van Deventer, T. van der Poll
interferon-g, cytokines, host defense, Pseudomonas aeruginosa, pneumonia.

Development of allergic contact dermatitis requires activation of both tumor necrosis factor-receptors.

F.M. Becke, T. Hehlgans, G. Brockhoff, D.N. Männel
allergic contact dermatitis, p75TNF receptor, p55TNF receptor, Langerhans cells, dendritic cells.

Transforming growth factor beta-1 and interferon-alpha in the aids dementia complex: possible relationship with cerebral viral load?

O. Perrella, P.B. Carreiri, A. Perrella, C. Sbreglia, F. Gorga, D. Guarnaccia, G. Tarantino
transforming growth factor-beta1, HIV-RNA, AIDS-dementia complex, interferon-alpha.

Interferon-beta (INF-b) antibodies in interferon-b1a- and interferon-b1b-treated multiple sclerosis patients.

Prevalence, kinetics, cross-reactivity, and factors enhancing interferon-b immunogenicity in vivo.

P. Perini, A. Facchinetti, P. Bulian, A.R. Massaro, D. De Pascalis, A. Bertolotto, G. Biasi, P. Gallo
interferon-beta, natural autoantibodies, multiple sclerosis.

The promoter polymorphism of the interleukin-6 gene regulates interleukin-6 production in neonates but not in adults.

S. Kilpinen, J. Hulkkonen, X.Y. Wang, M. Hurme
interleukin-6, cord blood, gene polymorphism, inflammatory mediators.

The hepatitis C virus (HCV) induces a long-term increase in interleukin-10 production by human CD4+ T cells (H9).

O. Delpuech, D. Buffello-Le Guillou, E. Rubinstein, C. Féray, M.A. Petit
HCV-RNA, interleukin-10, H9 T cells, CD81, capsid mutation, protein kinase C.

Retrovirus-mediated gene transfer of the cytokine genes interleukin-1b and tumor necrosis factor-a into human neuroblastoma cells: consequences for cell line behavior and immunomodulatory properties.

C. Coze, T. Leimig, M.T. Jimeno, P. Mannoni
neuroblastoma, immunotherapy, gene therapy, cytokine, IL-1b, TNF-a.

Characterisation of cytokine mRNA expression in tumour-infiltrating mononuclear cells and tumour cells freshly isolated from human colorectal carcinomas.

A. Csiszar, T. Szentes, B. Haraszti, W. Zou, D. Emilie, G. Petranyi, E. Pocsik
colorectal cancer, tumour-infiltrating lymphocytes, cytokines, gene expression, RT-PCR.

Analysis of interleukin-18, interleukin-1 converting enzyme (ICE) and interleukin-18-related cytokines in Crohnís disease lesions.

F. Pagès, V. Lazar, A. Berger, C. Danel, S. Lebel-Binay, F. Zinzindohoué, P. Desreumaux, C. Cellier, N. Thiounn, D. Bellet, P.H. Cugnenc, W.H. Fridman
intestinal mucosa, Crohn's disease, interleukin-18, caspase-1/ICE, immunohistochemistry, reverse transcription polymerase chain reaction.

Daily production of human tumor necrosis factor in lipopolysaccharide (LPS)-stimulated ex vivo blood culture assays.

J.C. Aguillon, A. Escobar, V. Ferreira, A. Aguirre, L. Ferreira, M.C. Molina, A. Ferreira
TNF, cytokine, LPS.

Inducible nitric oxide synthase attenuates chronic colitis in human histocompatibility antigen HLA-B27/human b2 microglobulin transgenic rats.

H.S. Blanchard, E. Dernis-Labous, D. Lamarque, J. Tran van Nhieu, Z. Szepes, J.F. Fléjou, E. Wollman, B.J.R. Whittle, M. Breban
rodent, inflammation, nitric oxide, cytokines, in vivo animal models.

Up-regulation of mucin secretion in HT29-MTX cells by the pro-inflammatory cytokines tumor necrosis factor-a and interleukin-6.

M.G. Smirnova, S.L. Kiselev, J.P. Birchall, J.P. Pearson
MUC5AC, MUC5B, mucus-secreting cell line HT29-MTX.

A Jurkat T cell variant resistant to death receptor-induced apoptosis. Correlation with heat shock protein 27 and 70 levels.

J.E. Ricci, L. Maulon, V. Battaglione-Hofman, C. Bertolotto, F. Luciano, B. Mari, P. Hofman, P. Auberger
apoptosis, caspases, Hsp27, Hsp70, T lymphocytes.

Increased cell surface expression of histocompatibility class II IAd in c-FOS transfected clones of the P388D1 murine macrophage cell line.

Y. Le Garrec, E. Rouer, F. Conti, P. Michel, A. Pompidou
MHC class II antigens, murine macrophages, c-Fos.

Transforming growth factor-b inhibits interleukin-10 synthesis by human monocytic cells.

S. Suberville, A. Bellocq, I. Peguillet, O. Lantz, P. Stordeur, B. Fouqueray, L. Baud
transforming growth factor-b1, interleukin-10, U. 937 cells.

Evidence for tumor necrosis factor receptors (TNFRs) in human MRC5 fibroblast cells.

D. Derouich-Guergour, H. Pelloux, D. Aldebert, P. Demenge, P. Ambroise-Thomas
MRC5, fibroblasts, TNF receptor, RT-PCR, binding, flow cytometry.

Interleukin-9 induces 24P3 lipocalin gene expression in murine T cell lymphomas.

C. Orabona, L. Dumoutier, J.C. Renauld
cytokines, lipocalin, IL-9, 24P3.

Contrasting effect of interleukin-4 on the expression of cytosolic phospholipase A2, 5-lipoxygenase and 5-lipoxygenase-activating protein in peritoneal macrophages from control and ovalbumin-sensitized rats.

L. Escoubet-Lozach, C.F. M'Rini, A. Rey, M. Béraud, J.C. Lepert, J. Bernard, J.R. Mathieu, B. Pipy
cytokines, lipid mediators, macrophages, rat.

Fibrinogen cooperates with cytokines to induce interleukin-6 receptor mRNA expression in human hematopoietic CD34+ progenitors.

A. Hatzfeld, B. Panterne, N.O. Fortunel, M.N. Monier, J.A. Hatzfeld
human CD34+ cells, fibrinogen, competence factor, IL-6 receptor.

Balance between autocrine interleukin-1b and caspases defines life versus death of polymorphonuclear cells.

P. Bossù, E. del Grosso, M.P. Cesaroni, G. Maurizi, N. Balestro, A. Stoppacciaro, E. del Giudice, P. Ruggiero, D. Boraschi
apoptosis, interleukin-1, caspases, polymorphonuclear leukocytes, ICE.

Rat interleukin-10: production and characterisation of biologically active protein in a recombinant bacterial expression system.

V. Ball, S. Vigues, C.K. Gee, S. Poole, A.F. Bristow
IL-10, recombinant, rat, cytokines.

Les éditions JLE

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