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# The combination of the interleukin-1alpha (IL-1alpha-889) genotype and the interleukin-10 (IL-10 ATA) haplotype is associated with increased interleukin-10 (IL-10) plasma levels in healthy individuals

## Abstract

Family studies have demonstrated striking differences between individuals in their ability to produce IL-10 following lipopolysaccharide (LPS) stimulation of

whole blood cultures in vitro, suggesting that differences in IL-10 production involve a considerable hereditary component. The first aim of this study was to analyse the possible effect of IL-10 genotypes and haplotypes on IL-10 plasma levels in a healthy Finnish population. As previous reports have demonstrated that endogenously produced IL-1 induces LPS-stimulated IL-10 production and that IL-10 inhibits synthesis of IL-1 in human monocytes, it is apparent that these two cytokines form an autoregulatory feedback loop. Secondly, we were interested whether any relationship could be found between IL-10 and IL-1beta in vivo. To examine this, the influence of IL-1alpha -889, IL-1beta -511 and IL-1Ra VNTR genotypes and IL-10 genotypes/haplotypes (ACC, GCC and ATA) on IL-10 plasma levels, and a putative correlation between IL-10 and IL-1alpha plasma levels were analysed. Four hundred adult blood samples were obtained from the Finnish Red Cross Blood Transfusion Centre, Tampere. The IL-10, IL-1alpha, IL-1beta and IL-1Ra gene polymorphisms were analysed using PCR. IL-1beta and IL-10 plasma levels were measured using an ELISA method. Our results indicated that increased IL-10 plasma levels were associated with the ATA haplotype ($p = 0.03$) and, surprisingly, with the IL-1alpha allele 2 carrier status ($p = 0.02$) in healthy individuals. This IL-1alpha 2+/ATA+ combination was found in 93 subjects out of 400 analysed (23%) and was associated with significantly high IL-10 plasma levels ($p = 0.002$). When individuals were classified into three groups, with no detectable IL-10 plasma levels ($n = 145$), with moderate levels ($n = 152$) and with high levels ($n = 100$) of IL-10, the IL-1alpha2+/ATA+ combination was more likely present among those with high levels than among those with undetectable levels of IL-10 (OR = 3.3, 95% CI 1.8 - 6.0, $p < 0.001$) or those with moderate levels of IL-10 (OR = 2.0, 95% CI 1.2 - 3.6, $p = 0.012$). Besides the observed association between IL-1alpha genotype and IL-10 levels, a moderate correlation was found between IL-10 and IL-1beta levels ($r = 0.6$, $p = 0.01$) among IL-10 producers ($n = 252$). The present findings suggest that the genotype combination of IL-1alpha 2+/ATA+ has a regulatory effect on basal IL-10 levels and that among individuals with measurable IL-10 plasma levels, IL-1beta and IL-10 basal levels correlate. Until now, data on the feedback loop between IL-1 and IL-10 cytokines have been based on studies in vitro, but now our results suggest that this relationship may also exist in vivo.

## Full text

### INTRODUCTION

Interleukin-10 is an 18 kDa, homodimeric cytokine which has strong immunosuppressive and anti-inflammatory activities in man. IL-10 has been shown to inhibit proliferation and IL-2 production in activated $CD4^+$ T cells [1], and is also able to induce a long-term antigen-specific anergic state in these cells [2]. The effects of IL-10 on human monocytes include inhibition of lipopolysaccharide-induced production of proinflammatory cytokines [3] and down-regulation of expression of costimulatory and cell adhesion molecules [4], as well as MHC class II molecules [5]. IL-10 has also some proinflammatory activity since it promotes activation and differentiation of B cells and induces immunoglobulin synthesis and autoantibody production [6]. Activated T cells, B cells and monocytes are cellular sources of IL-10.

Family studies have demonstrated striking differences between individuals in their ability to produce IL-10 following lipopolysaccharide (LPS) stimulation of whole blood cultures *in vitro* suggesting that differences in IL-10 production involve a considerable hereditary component [7]. The gene encoding IL-10 has been mapped to chromosome 1 [8], and several research groups have demonstrated that both microsatellites and single nucleotide polymorphisms (SNP) exist within this gene [9-12]. Three single-base-exchange polymorphisms in the promoter of the IL-10 gene at positions -1082 (G => A), -819 (C => T) and -592 (C => A) are in strong linkage disequilibrium, forming three haplotypes common in Caucasian populations; GCC, ACC and ATA [13]. Furthermore, the haplotype GTA is a possible, but extremely rare combination in Caucasians, 0.6% in Dutch individuals [13].

To find out the functional significance of the IL-10 haplotypes, both in *in vivo* disease associations and in *in vitro* gene expression, studies were performed [14]. It is thought that the reported SNPs in the promoter of the IL-10 gene occur within important regulatory regions, which may alter the structure of transcription factor binding sites. These potential transcription factors are still poorly defined. The SNP at position -1082 lies within an ETS-like recognition site and may therefore affect the binding of this factor. The SNP at -819 exists within a putative positive regulatory region, and the SNP at -592 occurs within a possible STAT3 binding site and a negative regulatory region [15].

IL-1 is a multifunctional and potent proinflammatory cytokine, which is mainly produced by macrophages. Although there are several similarities between IL-1alpha and IL-1beta molecules, differences are remarkable when studying

regulation of gene expression, translation, secretion and activation [16]. Based on these differences, it is thought that IL-1beta is a systemic, hormone-like mediator intended to be released from cells, whereas IL-1alpha is principally a regulator of intracellular events and mediator of local inflammation [16]. The genes for IL-1 are located on chromosome 2 and are polymorphic [17-19]. Our recent data suggest that the rare allele combination of IL-1alpha -889 2.2 + IL-1beta -511 allele 2 carrier status, has a regulatory effect on basal IL-1beta production in healthy individuals [20].

Our study aimed to analyse the possible effect of the IL-10 genotypes and the haplotypes on IL-10 plasma levels in healthy blood donors. As previous reports have demonstrated that endogenously produced IL-1 induces LPS-stimulated IL-10 production [21], and that IL-10 inhibits synthesis of IL-1 in human monocytes [3, 22], it is apparent that these two cytokines form an autoregulatory feedback loop. Thus, we were interested to find out whether any relationship could be found between IL-10 and IL-1beta *in vivo*. To examine this, the influence of IL-1alpha -889, IL-1beta -511 and IL-1RA VNTR (variable number of tandem repeats) genotypes on IL-10 plasma levels and a putative correlation between IL-10 and IL-1beta were investigated.

## METHODS

### *Subjects*

Four hundred adult blood samples were obtained from the Finnish Red Cross Blood Transfusion Centre, Tampere. The donors were adults (18-60 years old) and they had had no blood-transmitted diseases or any other signs of infection during a 2-week period prior to the blood donation. Samples were collected into plastic tubes treated with citrate. From the blood samples, plasma was separated, compartmentalised and stored at - 20° C until further use. The approval for human blood use was given by the ethics board of the Finnish Red Cross Blood Transfusion Centre.

### *Measurement of IL-10 and IL-1beta plasma levels*

IL-10 plasma levels (n = 397) were measured using an enzyme-linked immunosorbent assay (ELISA; CLB, Pelikine Compact human IL-10 ELISA kit, Amsterdam, The Netherlands). The sensitivity of the assay was 1 pg/ml and the

assay was performed according to the manufacturer's instructions. The manufacturer reports an intra-assay coefficient of variation of less than 10% and an inter-assay coefficient of variation of less than 10%. Plasma IL-1beta levels were measured as previously described [20].

### *Analysis of IL-10, IL-1alpha, IL-1beta and IL-1Ra gene polymorphisms*

Genomic DNA was isolated from the mononuclear cells using the salting out method [23]. The region of the IL-10 promoter, from -1120 to -533, was amplified by PCR, and single polymorphic sites (G/A at -1082, C/T at -819 and C/A at -592) were analysed by restriction fragment length polymorphism (RFLP) analysis [24-26]. The validity of these RFLP-based methodologies was confirmed by additionally analysing 70 samples with cycle sequencing. The -889 C = › T polymorphism of the IL-1beta gene, the -511 C = › T polymorphism of the IL-1beta gene and the penta-allelic VNTR in the second intron of the IL-1RA gene were analysed as previously described [17-19].

### *Statistical analysis*

The Kruskal-Wallis test was used to test for significant associations between IL-10 plasma levels and IL-10 genotypes or IL-1alpha genotypes (Table 1). The Mann-Whitney U-test was used to test for significant associations between IL-10 plasma levels and the carrier status of IL-1alpha allele 2 or the IL-10 haplotypes (Table 1). The combined effect of the carrier status of IL-1alpha allele 2 and the IL-10 ATA haplotype on plasma IL-10 levels was analysed using the Kruskal-Wallis and the Mann-Whitney U-test (Figure 1). As IL-10 and IL-1beta concentrations were not normally distributed, the values were square-root transformed before Pearson's correlation coefficient test. The frequencies of the IL-1alpha $2^+$/$ATA^+$ combination were compared to those of all other combinations in nonproducers, moderate producers and high producers of IL-10 using the chi-square test (Table 2). The *odds ratios* were calculated using the logistic regression analysis. Statistical significance was considered to be at the 0.05 level. Statistical calculations were performed using SPSS for Windows, version 10.1 (SPSS Inc., Chicago, Illinois, USA).

## RESULTS

The data shown in Table 1 and our previous results [20, 27] demonstrate the allele frequencies of IL-1alpha, IL-1beta and IL-1Ra, and the genotype and haplotype frequencies of IL-10. The median IL-10 plasma value was 1.52 pg/ml (25% and 75% quartiles were 0-3.00 pg/ml) and in 252 of 397 samples (63%) concentrations were above the detection limit of the assay.

An analysis was carried out to ascertain if there was a genetic association between different genotypes and IL-10 plasma levels. The IL-10 genotype status by itself did not have any effect on IL-10 levels ($p = 0.10$, Table 1). However, when levels were compared according to the IL-10 haplotypes, it was obvious, that the ATA carriers had significantly higher IL-10 plasma levels than the noncarriers ($p = 0.03$, Table 1). The ACC or GCC carrier status did not have any effect on plasma IL-10 ($p = 0.38$ and $p = 0.22$, respectively). It was also noticed that the IL-1alpha 2.2 and 1.2 genotypes had significantly increased IL-10 plasma levels than the genotype 1.1 ($p = 0.05$, Table 1). This finding was also confirmed when a comparison was made according to the carrier status. The carriers of allele 2 secreted significantly more IL-10 than the noncarriers ($p = 0.02$, Table 1). Neither the genotype of IL-1beta or the genotype of IL-1Ra had an effect on IL-10 levels ($p = 0.50$ or $p = 0.51$, data not shown).

Since both the carriers of IL-1alpha -889 allele 2 and the carriers of IL-10 ATA haplotype had increased IL-10 plasma levels, we wanted to analyse a possible influence of the allele combination on IL-10 levels. This combination was found in 93 subjects out of 400 analysed (23%) (Figure 1) and associated with significantly high IL-10 plasma levels ($p = 0.002$, the Kruskal-Wallis test). This finding was also confirmed when comparisons were made between different combinations using the Mann-Whitney U-test; IL-1alpha $2^+$/$ATA^+$ *versus* IL-1alpha 2-/ATA- ($p = 0.002$), IL-1alpha $2^+$/$ATA^+$ *versus* IL-1alpha $2^+$/$ATA^-$ ($p = 0.002$) and IL-1alpha $2^+$/ALPHA/$ALPHA^+$ *versus* IL-1alpha $2^-$/$ATA^+$ ($p = 0.003$).

To clarify the significance of genotype combinations for IL-10 plasma levels, individuals were grouped into three groups according to the 25 and 75% quartiles of IL-10 values; nonproducers (0.00-0.00 pg/ml, n = 145), moderate producers (1.20-2.97 pg/ml, n = 152) and high producers (3.00-118.00 pg/ml, n = 100). It was evident that among high producers, the frequency of the IL-1alpha $2^+$/$ATA^+$ genotype combination was clearly increased as compared to nonproducers and moderate producers ($p < 0.001$); 37/100 (37%) *versus* 22/145 (15.2%) or 34/152 (22.4%), respectively (Table 2). Accordingly, the IL-1alpha

$2^+$/$ATA^+$ combination was more likely present among high producers than among nonproducers of IL-10 (OR = 3.3, 95% CI 1.8-6.0, p < 0.001) or moderate producers of IL-10 (OR = 2.0, 95% CI 1.2-3.6, p = 0.012). Here, the term "producer of IL-10" is used although basal plasma levels have been measured and, thus, rather reflect both production and degradation of systemic IL-10.

Several *in vitro* studies [3, 22] have shown that IL-10 is a potent inhibitor of IL-1beta synthesis and, on the other hand, IL-1beta can induce synthesis of IL-10 [21]. To analyse the possible relationship between these cytokines *in vivo* the study population was divided into two subgroups: IL-10 nonproducers and IL-10 producers, whose IL-10 levels were 1.20 pg/ml-118.00 pg/ml. This categorisation was necessary since systemic levels of IL-10 were clearly lower than those of IL-1beta; the respective median values with quartiles were 1.52 pg/ml (0.00-3.00 pg/ml) and 5.78 pg/ml (2.20-13.60 pg/ml). Due to this difference in systemic cytokine values and also the difference in detection limits of the cytokine assays, 37% of IL-10 levels were undetectable compared to 2% of IL-1beta levels. Thus, the correlation analysis was restricted to IL-10 producers and a moderate correlation was found in this group between IL-10 and IL-1beta levels; r = 0.6 (p = 0.01). This result could indicate that production of IL-1beta may induce synthesis of IL-10 *in vivo* and that these levels may be genetically determined.

## DISCUSSION

This report indicates that increased IL-10 plasma levels are associated with the ATA haplotype and, surprisingly, the IL-1alpha allele 2 carrier status in healthy individuals. The genetic effect on IL-10 levels *in vivo* is more distinct when both of these markers are present at the same time; in our study 23% had this combination (Figure 1). In line with this, the IL-1alpha $2^+$/$ATA^+$ combination was more likely present among high producers than among nonproducers or moderate producers of IL-10.

Although *in vitro* studies may be critically influenced by several factors such as cell line and cell stimulus used in the assays, the previous *in vitro* studies on the IL-10 promoter region and the IL-10 haplotypes have quite uniformly demonstrated the association between the ATA haplotype and low IL-10 production capacity. The presence of the ATA or the ACC haplotype has been associated with low IL-10 production after stimulation of lymphocytes [12], and also, decreased IL-10 secretion for the ATA haplotype was found after

stimulation of peripheral blood mononuclear cells [26]. Crawley *et al.* reported that LPS-stimulated IL-10 production in whole blood cultures was the lowest in those with the ATA/ATA genotype and that the relative transcriptional activity of the ATA construct was significantly weaker than that of the GCC construct in the monocytic cell line [28]. Conversely, the presence of an A at position -1082 related to higher IL-10 production in LPS-stimulated whole blood cultures from 158 donors [29]. We also showed, for the first time, the association between the ATA haplotype and *in vivo* IL-10 plasma levels, but in contrast to previous data, this haplotype association was with high IL-10 levels. It could be argued whether these *in vitro* culture conditions mimic those present *in vivo* and in plasma. However, both present and previous findings suggest that these polymorphisms may have functional effects on IL-10 transcription. Accordingly, IL-10 promoter deletion studies have found regions of the IL-10 promoter between 1200 and 1000 and 750 and 350, which decrease reporter gene activity [15].

There are a few disease association studies concerning the ATA haplotype. The increased frequency of the ACC or the ATA haplotype has been found in patients with rheumatoid arthritis who are positive for IgA rheumatoid factor but negative for IgG rheumatoid factor, although numbers for this patient subgroup were small and thus the significance of these results is limited [30]. Also the ATA haplotype has been associated with disease severity in children with oligoarticular-onset juvenile rheumatoid arthritis that progressed to polyarthritis [28], and with severe asthma [31]. In a Chinese population, an association between lupus nephritis and this haplotype has been described [25]. Interestingly, the ATA haplotype is much more common in the Chinese populations than in Caucasian populations, but the significance of this finding is still unclear. Our frequency of haplotypes did not differ significantly from those published earlier in other Caucasian populations [12, 27, 28].

In addition to the ATA haplotype, the IL-1alpha genotype has an effect on IL-10 plasma levels; this genotype alone had an effect, but this effect was more significant when both markers were present. Although the molecular mechanism between the IL-1alpha genotype and IL-10 levels is not clear, we assume that the missing link between these factors could be IL-1. Our group has shown that in healthy individuals, a rare allele combination, IL-1alpha -889 2.2/IL-1beta -511 $2^{+}$, of the interleukin-1 gene complex associates with high interleukin-1beta plasma levels [20] and previous *in vitro* studies suggest that IL-1 is able to induce IL-10 production in human monocytes [21]. Therefore, we suggest that

the observed IL-1alpha genotype effect on IL-10 levels reflects the effect of this genotype on IL-1beta levels, which in turn may induce plasma IL-10 levels. The combination of IL-1alpha 2.2/IL-1beta $2^+$, previously related to IL-1beta levels, did not associate with plasma IL-10 levels (unpublished observation). Until now data on the feedback loop between IL-1 and IL-10 has been based on *in vitro* studies, but now our results suggest that this relationship may also exist *in vivo*. Besides the observed association between the IL-1alpha genotype and IL-10 levels, a moderate correlation was found between IL-10 and IL-1beta levels among IL-10 producers.

CONCLUSION

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# Figures

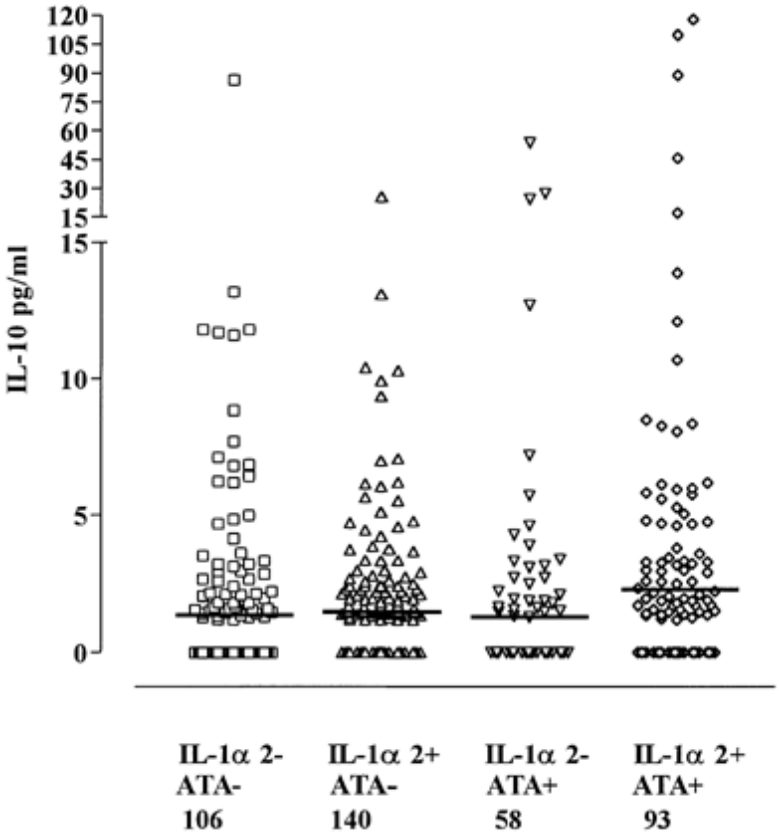



**Figure 1**

**IL-10 plasma levels in 397 healthy individuals grouped according to their carrier status for IL-1alpha -889 allele 2 and the IL-10 ATA haplotype.** Each dot represents the value of one sample, and bars indicate group medians. The combination of IL-alpha $2^+$/$ATA^+$ was found in 93 subjects out of 400 analysed (23%) and was associated with significantly high IL-10 plasma levels (p = 0.002, the Kruskal-Wallis test). The respective group medians with 25 and 75% quartiles were 1.40 pg/ml (0.00-2.89), 1.48 pg/ml (0.00-2.48), 1.32 pg/ml (0.00-2.73) and 2.11 pg/ml (1.22-4.73).

**Table 1**
**The influence of the IL-1α -889 genotypes and the IL-10 genotypes/haplotypes on IL-10 plasma levels in 400 healthy individuals**

| | n | IL-10 pg/ml Median (25%-75% quartiles) | p-value |
|---|---|---|---|
| IL-1α genotype | | | |
| 1.1 | 167 | 1.3 (0.0-2.7) | p = 0.05[2] |
| 1.2 | 201 | 1.7 (0.0-3.1) | |
| 2.2 | 32 | 1.8 (0.0-5.8) | |
| 1.1 | 167 | 1.3 (0.0.-2.7) | p = 0.02[1] |
| 1.2 + 2.2 | 233 | 1.7 (0.0-3.3) | |
| IL-10 genotype | | | |
| ATA/ATA | 25 | 1.6 (0.0-3.1) | p = 0.10[2] |
| ATA/ACC | 65 | 2.0 (0.0-4.8) | |
| ATA/GCC | 62 | 1.5 (0.0-3.0) | |
| ACC/GCC | 120 | 1.5 (0.0-3.0) | |
| ACC/ACC | 45 | 1.4 (0.0-2.2) | |
| GCC/GCC | 83 | 1.5 (0.0-2.3) | |
| IL-10 haplotype | | | |
| ATA$^+$ | 152 | 1.8 (0.0-3.5) | p = 0.03[1] |
| ATA$^-$ | 248 | 1.5 (0.0-2.6) | |
| ACC$^+$ | 230 | 1.6 (0.0-3.4) | p = 0.38[1] |
| ACC$^-$ | 170 | 1.5 (0.0-2.7) | |
| GCC$^+$ | 265 | 1.5 (0.0-2.9) | p = 0.22[1] |
| GCC$^-$ | 135 | 1.7 (0.0-3.5) | |

[1] Mann-Whitney-test.
[2] Kruskal-Wallis-test.

**Table 2**
**The frequency comparison between the IL-1α 2$^+$/ATA$^+$ genotype combination and other possible genotype combinations in nonproducers, moderate producers and high producers of plasma IL-10**

| | IL-10 plasma levels | | |
|---|---|---|---|
| Genotype combination | 0.00 – 0.00 pg/ml n = 145 % | 1.20 – 2.97 pg/ml n = 152 % | 3.00 – 118.00 pg/ml n = 100 % |
| IL-1α 2$^+$/ATA$^+$ | 15.2 | 22.4 | 37.0 |
| IL-1α 2$^-$/ATA$^-$<br>IL-1α 2$^+$/ATA$^-$<br>IL-1α 2$^-$/ATA$^+$ | 84.8 | 77.6 | 63.0 |

$p < 0.001$, $\chi^2$-test, df = 2.

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### Interleukin-10 and cAMP-elevating agents cooperate to induce suppressor of cytokine signaling-3 via a protein kinase A-independent signal

Sara Gasperini, Luca Crepaldi, Federica Calzetti, Luana Gatto, Chiara Berlato, Flavia Bazzoni, Akihiko Yoshimura, Marco A. Cassatella
SOCS-3, PGE, IL-10, PBMC, neutrophils, PKA.

## Different patterns of interleukin-1alpha and interleukin-1beta expression in organs of normal young and old mice

Moshe Hacham, Shmuel Argov, Rosalyn M. White, Shraga Segal, Ron N. Apte
IL-1alpha, IL-1beta, organ cytokine expression, young/old age, homeostasis.

## The combination of the interleukin-1alpha (IL-1alpha-889) genotype and the interleukin-10 (IL-10 ATA) haplotype is associated with increased interleukin-10 (IL-10) plasma levels in healthy individuals

Sanna Kilpinen, Heini Huhtala, Mikko Hurme
interleukin-1, interleukin-10, gene polymorphism, plasma.

## The interleukin-6 G(-174)C polymorphism and the ex vivo interleukin-6 response to endotoxin in severely injured blunt trauma patients

Michael Heesen, Udo Obertacke, F. Ulrich Schade, Brunhilde Bloemeke, Matthias Majetschak
interleukin-6, endotoxin, genetic polymorphism, polymerase chain reaction, whole blood, critical illness.

## Comparison of leptin- and interleukin-6-regulated expression of the rPAP gene family: evidence for differential co-regulatory signals

Daniël Broekaert, Sven Eyckerman, Delphine Lavens, Annick Verhee, Wim Waelput, Joël Vandekerckhove, Jan Tavernier
forskolin, IL-6, leptin, rPAP/Reg gene family, signal transduction.

## Mice lacking alpha2-macroglobulin show an increased host defense against Gram-negative bacterial sepsis, but are more susceptible to endotoxic shock

Tino Hochepied, Fred Van Leuven, Claude Libert
TNF, shock, acute phase, inflammation, alpha2-macroglobulin.

## Tumor necrosis factor-alpha mRNA stability in human peripheral blood cells after lipopolysaccharide stimulation

Lucile Baseggio, Carole Charlot, Jacques Bienvenu, Pascale Felman, Gilles Salles
tumor necrosis factor, mRNA stability, lipopolysaccharide, tristetraprolin.

## Caspase-1 levels in biological fluids from patients with multiple sclerosis and from patients with other neurological and non-neurological diseases

Diego Franciotta, Gianvito Martino, Elisabetta Zardini, Roberto Furlan, Roberto Bergamaschi, Maira Gironi, Alessandra Bergami, Giovanna Angelini, Fabrizio De Benedetti, Patrizia Pignatti, Gianna Moscato, Vittorio Cosi
asthma, cerebrospinal fluid, juvenile idiopathic arthritis, sputum, synovial fluid, THP-1 cells.

## Divergent effects of tumor necrosis factor-alpha and lymphotoxin-alpha on lethal endotoxemia and infection with live Salmonella typhimurium in mice

Edi Dharmana, Monique Keuter, Mihai G. Netea, Ineke C.M.M. Verschueren, Bart Jan Kullberg
tumor necrosis factor, lymphotoxin, salmonella typhimunium, knock-out mice.

## Th1/Th2 cytokine balance and nitric oxide in cerebrospinal fluid and serum from patients with multiple sclerosis

María del Carmen Rodríguez-Sáinz, Silvia Sánchez-Ramón, Clara de Andrés, Margarita Rodríguez-Mahou, M Angeles Muñoz-Fernández
cytokine, nitric oxide, multiple sclerosis, cerebrospinal fluid, oligoclonal bands, Igs.

## Retinoids, cisplatin and interferon-alpha in recurrent or metastatic cervical squamous cell carcinoma: clinical results of 2 phase II trials

Anne-Chantal Braud, Laurence Gonzague, François Bertucci, Dominique Genre, Jacques Camerlo, Gwenaelle Gravis, Anthony Goncalves, Vincent Moutardier, Frédéric Viret, Dominique Maraninchi, Patrice Viens
all-trans-retinoic acid, 13 Cis retinoic acid, interferon-alpha, cervical squamous cell carcinoma.

## Circulating macrophage colony stimulating factor as a marker of tumour progression

Raymond S. McDermott, Laurent Deneux, Véronique Mosseri, Jacques Védrenne, Krishna Clough, Alain Fourquet, José Rodriguez, Jean-Marc Cosset, Xavier Sastre, Philippe Beuzeboc, Pierre Pouillart, Susy M. Scholl
CSF-1, tumour progression, metastasis, de-differentiation.

# The effect of interleukin-1beta (IL-1beta) and tumor necrosis factor-alpha (TNF-alpha) on granulocyte macrophage-colony stimulating factor (GM-CSF) production by neuronal precursor cells

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IL-1beta, TNF-alpha, GM-CSF, neuron, mRNA, protein.

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