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Activation of caspase-3 by interferon alpha causes interleukin-16 secretion but fails to modulate activation induced cell death

Abstract

Interferon alpha (IFN-alpha) is the mainstay in the treatment of chronic hepatitis C virus (HCV) infection. Interleukin-16 (IL-16) attracts CD4+ cells to sites of inflammation and plays a role in the interaction of dendritic cells, T cells and B cells. In this study, we show that IFN-alpha itself induces IL-16 secretion by peripheral blood lymphocytes (PBL) and enhances IL-16 secretion by anti-CD3

stimulated PBL. Pro-IL-16 is cleaved into its active form by caspase-3. IFN-alpha increases caspase-3 mRNA levels in activated T cells (ATC), as shown by Northern blot analysis, whereas IL-16 mRNA levels are not affected by IFN-alpha. IL-16 secretion into culture supernatants correlates tightly with intracellular caspase-3 activity in ATC. In our experiments addition of specific caspase inhibitors did not reduce the proportion of ATC undergoing Fas-mediated cell death, but completely blocked IFN-alpha-induced IL-16 secretion into culture supernatants. In conclusion, our results suggest that IFN-alpha activates caspase-3, thereby increasing secretion of IL-16, whereas IFN-alpha-enhanced Fas-mediated cell death in ATC is not caspase-dependent.

Full text

INTRODUCTION

Interferon alpha (IFN-alpha) is a highly pleiotropic cytokine that was initially described for its ability to interfere with viral replication [1]. It has been shown to be useful in a variety of diseases of diverse pathogenesis including chronic viral hepatitis, chronic myelogenous leukemia, hairy cell leukemia and papillomas [2]. Despite its clinical success, the detailed mechanisms of action have not yet been well defined. Besides its direct antiviral effects, IFN-alpha interacts with the cytokine cascade and therefore exerts many of its biological effects *via* regulation of various pro- and anti-inflammatory cytokines [3].

Interleukin-16 (IL-16) is a cytokine that is secreted by T cells [4], B cells [5], dendritic cells [6], eosinophils [7], mast cells [8] and bronchial epithelial cells [9]. It acts as a chemoattractant for $CD4^+$ cells [10] and leads to the expression of IL-2-receptors on $CD4^+$ T cells [11]. A role of IL-16 could be shown in several inflammatory diseases such as allergic asthma [12], rheumatoid arthritis [13], atopic dermatitis [14], ulcerative colitis and Crohn's disease [15, 16]. IL-16 is also likely to play an important role in the induction of a specific immune response, as it has been shown to be produced by dendritic cells, which are critical for priming of $CD4^+$ T helper cells [6]. Of considerable interest in the finding that IL-16 can suppress HIV-1 replication *in vitro* [17].

Caspases form a family of a least 14 proteases, which are critically involved in effecting apoptosis [18]. Several pathways, including different subsets of caspases, have been described in apoptosis, depending on cell type and the

trigger of apoptosis [19]. Caspases form a cascade of consecutively activated proteases which finally results in proteolytic cleavage of "death substrates", including structural proteins, enzymes, and DNA fragmentation [18]. Caspase-3 (CPP32) is a key molecule where several pathways converge. Besides its crucial role in apoptosis, it has recently been shown that caspase-3 is responsible for the activation of pro-IL-16 by cleavage of the bioactive 121-aa C-terminal residue from the biologically inactive IL-16-precursor [20].

In our study we addressed the question whether IL-16 is regulated by IFN-alpha in peripheral blood lymphocytes (PBL) and could therefore contribute to the biological activities of IFN-alpha. We investigated whether regulation of IL-16 by IFN-alpha occurs either at the level of mRNA expression or post-trancriptionally. Thereby we focused on caspase-3, which has been shown to be crucial for cleavage of pro-IL-16.

We investigated the influence of IFN-alpha on the expression of caspase-3 mRNA, the cleavage of the caspase-3 pro-enzyme and caspase-3-enzymatic activity. In additional experiments we addressed the question if IFN-alpha-induced cell death is caspase-3-mediated or if there is evidence for other, caspase-3-independent pathways.

METHODS

Reagents

Recombinant human (rh) IFN-alpha2b was obtained from Schering-Plough (AESCA, Traiskirchen, Austria). An anti-CD3 monoclonal antibody (CLB-T3/4.E) of IgE-isotype was purchased from CLB (Diagnostica, Vienna, Austria). Concanavalin A (ConA) was from Sigma Chemical Co. (Vienna, Austria). Fetal calf serum (FCS) was from GIBCO (Life Technologies, Schoeller Pharma, Vienna, Austria). Culture medium RPMI 1640, penicillin G and streptomycin were from Schoeller Pharma (Vienna, Austria). rhuIL-16 and mouse IgG2a anti-IL-16 mAb 14.1 were purchased from PharMingen (Hamburg, Germany). Biotinylated goat polyclonal IL-16 Ab and the immunoassay for measuring active caspase-3 were from R&D Systems (Biomedica, Vienna, Austria). IL-16 cDNA was prepared as described previously [5]. The caspase-3 cellular activity assay kit was from Calbiochem (Margaritella, Vienna, Austria). CPP-32 cDNA was kindly provided by Dr. Alnemri and Dr. Srinivasula (Kimmel Cancer Center at Jefferson, Philadelphia

PA). Caspase inhibitor Z-DEVD-FMK was purchased from R&D Systems (Biomedica, Vienna, Austria) and from BioVision Research Products (Palo Alto, CA, USA), Z-VAD-FMK was from R & D.

Isolation of PBL

Peripheral blood mononuclear cells (PBMC) were isolated from the heparinized blood of healthy volunteers by density gradient centrifugation through Ficoll-Hypaque (Sigma). The cells were washed four times in phosphate buffered saline (PBS, Biochrom KG, Berlin) and then depleted of monocytes by plastic adherence in 75 cm^2 culture flasks. The resulting PBL contained less than 3% monocytes as determined by FACS analysis. The indicated number of cells was used for stimulation of cytokine synthesis for subsequent IL-16 measurement by enzyme-linked immunosorbent assay (ELISA) and corresponding Northern hybridizations.

Generation of activated T cells (ATC) - ATC were obtained by culturing freshly isolated PBMC in RPMI with 5%FCS and ConA (5 µg/ml) for 24 hours. Cells were then thoroughly washed in PBS and further expanded in RPMI/5%FCS with 100 U/ml IL-2 for 4-6 days [21].

Stimulation of cytokine synthesis

In experiments with stimulation of cytokine synthesis by anti-CD3 antibodies, isolated PBL were cultured in polypropylene tubes at a density of 1×10^6 cells/ml in a final volume of 1 ml RPMI 1640 supplemented with 5% FCS and 100 U/ml penicillin/100 mug/ml streptomycin. PBL were incubated at 37° C in a humidified atmosphere containing 5% CO_2 for 24 hours. After a preincubation period of 15 min with 1,000 U/ml IFN-alpha, PBL were stimulated with anti-CD3. The anti-CD3 antibody was used in a soluble form at a final dilution of 1:4,000. IFN-alpha was used in a concentration of 1,000 U/ml. After incubation, cell cultures were centrifuged, supernatants were collected and stored at - 20° C until cytokine assessment by ELISA.

IL-16 ELISA - Sandwich ELISA was performed according to standard protocols with immobilized anti-IL-16 14.1 mAb (3 µg/ml in carbonate buffer) and biotinylated IL-16 pAb (200 ng/ml) as detection Ab. The secondary reagent

streptavidin-POD (Boehringer Mannheim) was used according to manufacturer's instructions.

Tetramethylbenzidine (BM Blue POD substrate, Boehringer Mannheim) was used as substrate and measured at 450 nm in an ELISA reader. The standard curve was prepared with rhuIL-16 in a concentration range from 0.03 to 2 ng/ml. The detection limit of the assay was 20 pg/ml.

Northern analysis - PBL (1×10^7) were suspended in RPMI 1640/5% FCS and cultured for 24 hours after stimulation with ConA (10 mug/ml) or anti-CD3-antibody (1:4,000) (with unstimulated controls) and addition of the indicated amounts of IFN-alpha 15 min in advance. The cells were washed once with PBS and then the total RNA was purified by the guanidinium-isothiocyanate phenol/chloroform extraction method using the RNA-Clean system (AGS GmbH, Heidelberg, Germany) following manufacturer's instructions. The amount of RNA was measured by spectrophotometry at 260 nm. Ten micrograms of total RNA were separated through 1% agarose/1% formaldehyde gels, and transferred to Nytran nylon membranes (Schleicher & Schuell, Vienna, Austria) and cross-linked by short wave UV exposure in a Stratalinker (Stratagene). Filters were hybridized with probes, labelled to high specific activity by the random primed method (Boehringer Mannheim, Vienna, Austria), washed under stringent conditions and developed. Control hybridisations were performed with the rat cDNA of the housekeeping gene glyceraldehyde-3-phosphatedehydrogenase (GAPDH).

Measurement of caspase-3-activity an immunoassay specific for active caspase-3, which has been described previously [22], has been used for determining active caspase-3. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for caspase-3 has been pre-coated onto a microplate. Active caspase-3-biotin-inhibitor, Standards and cell lysate samples containing covalently linked active caspase-3-biotin ZVKD are pipetted into the wells and any caspase-3 present is bound by the immobilized antibody. Inactive caspase-3 zymogen is not modified by the biotin-ZVKD-fmk inhibitor and therefore is not detected. Following a wash to remove any unbound substances, strepavidin conjugated to horseradish peroxidase is added to the wells and binds to the biotin on the inhibitor. Following a wash to remove any unbound Streptavidin-HRP reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that

turns yellow when the stop solution is added. The intensity of the color measured is in proportion to the amount of active caspase-3 bound in the initial step. The sample values are then read off the standard curve.

Assessment of cell death

The proportion of cells in advanced stages of activation induced cell death (AICD) was accessed by propidium iodide (PI) uptake. Analysis was performed on a FACScan (Becton Dickinson, Vienna, Austria) calibrated for optimal compensation [21].

Statistics

Data are presented as mean ± SEM. Statistical analysis was performed using the paired t-test. P values less than 0.05 were considered to be significant. Correlations were calculated using Pearson's correlation coefficient.

RESULTS

Regulation of IL-16 secretion by IFN-alpha

After incubation for 24 hours, supernatants of unstimulated PBL (1×10^6/ml) contained low levels of IL-16 measured by ELISA (mean ± SEM 80 ± 22 pg/ml, n = 10). Incubation with 1,000 U/ml IFN-alpha significantly increased IL-16 secretion of PBL (224 ± 43 pg/ml, n = 10, $p < 0.01$).

Regulation of anti-CD3-antibody stimulated PBL by IFN-alpha

Stimulation of PBL over 24 hours with anti-CD3 antibody strongly enhanced IL-16 secretion into culture supernatants. Costimulation with IFN-alpha further increased IL-16 production (unstimulated PBL: 74 ± 21 pg/ml; anti-CD3 stimulated PBL: 627 ± 159 pg/ml; costimulation with anti-CD3 and IFN-alpha: 954 ± 192 pg/ml; n = 5, $p < 0.01$ for anti-CD3 *vs.* anti CD3 + IFN-alpha (Figure 1A).

Dose-response-relationship of IL-16 secretion by anti-CD3 stimulated PBL and various IFN-alpha concentrations

PBL were co-stimulated with anti-CD3 and various concentrations of IFN-alpha for 24 hours. Even a concentration of 10 U/ml IFN-alpha significantly enhanced anti-CD3-stimulated IL-16 secretion into culture supernatants (unstimulated control: 159 ± 14 pg/ml; + anti-CD3: 613 ± 8 pg/ml; anti-CD3 + 10 U/ml IFN-alpha: 710 ± 56 pg/ml, $p < 0,01$ compared to anti-CD3 alone, $n = 6$, for details see Figure 1B).

IL-16 mRNA levels are not regulated by IFN-alpha in PBL

IL-16 mRNA was detected by Northern blot analysis in unstimulated PBL. However, neither stimulation with IFN-alpha, ConA, or anti-CD3 alone, nor costimulation with IFN-alpha + ConA or IFN-alpha + anti-CD3 regulated IL-16 mRNA levels (Figure 2).

Caspase-3 mRNA levels are regulated by IFN-alpha

In Northern blot analysis activated T cells (ATC) expressed caspase-3 mRNA. Stimulation with anti-CD3 or IFN-alpha for 12 hours increased mRNA levels. The highest levels were observed after costimulation with anti-CD3 + IFN-alpha (Figure 3a). Densitometric quantitation of mRNA levels revealed an increase of 54 ± 17% after stimulation with IFN-alpha and an increase of 38 ± 18% after stimulation with anti-CD3, compared to unstimulated controls. Costimulation with anti-CD3 + IFN-alpha resulted in an increase of caspase-3 mRNA levels up to 106 ± 10% (Figure 3b).

Detection of caspase-3 like activity in ATC

In cell lysates of ATC from 5 donors, caspase-3 like activity could be detected, using an enzymatic activity assay with DEVD-p-nitroaniline as specific colorimetric substrate that exhibits increased absorption upon cleavage. After stimulation with IFN-alpha and anti-CD3 + IFN-alpha, culture supernatants were collected for IL-16 quantitation and cells were lysed for determination of caspase-3 activity. There was a close correlation of IL-16 levels in the supernatants and caspase-3 activity (Pearson's correlation coefficient: $r = 0.90$, $p < 0.001$) (Figure 4). These experiments were repeated with a previously described immunoassay [22], which is specific for activated caspase-3. The results confirmed the data obtained with the enzymatic assay (data not shown).

Inhibition of IL-16 secretion, but not of activation-induced cell death (AICD) by the capsase-3 inhibitor Z-DEVD-FMK

ATC were stimulated with anti-CD3 for 24 hours. This stimulation significantly augmented the proportion of cells undergoing AICD, as measured by propidium iodide uptake (Figure 5). The proportion of dead cells was further increased by costimulation with IFN-alpha (% AICD control: 20 ± 3%, + IFN-alpha: 22 ± 3%, + anti-CD3: 42 ± 2%, + anti-CD3 + IFN-alpha: 46 ± 2%, n = 4, $p < 0.05$ for anti-CD3 *vs* anti-CD3 + IFN-alpha). The rate of AICD could not be diminished by preincubating cells with the caspase-3 inhibitor Z-DEVD-FMK (% AICD, + Z-DEVD-FMK: control: 22 ± 4%, + IFN-alpha: 23 ± 3% + anti-CD3: 41 ± 3% + anti-CD3 + IFN-alpha: 47 ± 4%, n = 4) (Figure 6A), or the pan-caspase inhibitor z-VAD-FMK, which inhibits caspase 1, 3, 5, 7, 8 and 9% AICD, control: 14 ± 1%, + IFN-alpha: 14 ± 2%, + anti-CD3: 34 ± 3%, + anti-CD3 + IFN-alpha: 43 ± 6%, + Z-VAD-FMK: control: 18 ± 3%, + IFN-alpha: 24 ± 1%, + anti-CD3: 41 ± 2%, + anti-CD3 + IFN-alpha: 44 ± 7%, n = 3) (Figure 6B). DEVD-FMK, however, completely blocked the secretion of IL-16 into culture supernatants, therefore indicating that it was able to permeate into cells and to block caspase-3. Addition of the caspase-3 inhibitor to cell cultures abrogated all caspase-3 activity as assessed by the specific enzymatic activity assay (data not shown). To avoid contamination with inhibitor from culture supernatants after cell lysis, cells were thoroughly washed prior to lysis. As a positive control, Jurkat cells were stimulated with anti-CD95. In this system blocking of caspases by z-VAD-FMK completely inhibited CD95-mediated cell death (control: 2.1%, + IFN-alpha: 3.1%, + anti-CD95: 13.7%, + anti-CD95 + IFN-alpha: 15.9%, + Z-VAD-FMK: control: 1.9%, + IFN-alpha: 1.9%, + anti-CD95: 2.5 %, = anti-CD95 + IFN-alpha: 2.4%) (Figure 6C).

DISCUSSION

IFN-alpha is a highly pleiotropic cytokine. Among the numerous described biological activities, the antiviral properties of IFN-alpha are the best known [1]. During the last decade many studies have accumulated evidence that the antiviral effect of IFN-alpha is not only due to the induction of an "antiviral state" in the target cells, but is partly mediated *via* its influence on immune cells and the cytokine cascade [22, 24].

IL-16 has a number of properties, which point to an important role of this cytokine in inflammatory processes. IL-16 is a potent chemoattractant for $CD4^+$

cells [10], enhances the expression of IL-2 receptor (CD25) on T cells [25] and stimulates the production of pro-inflammatory cytokines, such as IL-1beta, IL-6, IL-15 and TNF-alpha, by human monocytes [26]. Both studies in humans and data from animal models confirm this role of IL-16 as an important mediator of inflammation in diseases, such as asthma [12, 27, 28], sarcoidosis [29], ulcerative colitis and Crohn's disease [15, 16] Recent data suggest that IL-16 may not only be involved in inflammation but also in the induction of a specific immune response, since it has been shown to be secreted by dendritic cells and to be functional as chemoattractant for $CD4^+$ T cells [6]. It may therefore be important for enabling the interaction of T helper cells with dendritic cells, which is crucial for establishing an immune response.

Although IFN-alpha is successfully applied in chronic HCV infection [30], the mechanism of its antiviral action is not yet clearly understood. Our results show that IFN-alpha enhances the secretion of IL-16 by anti-CD3-stimulated PBL. It might be speculated that enhanced secretion of IL-16 could be part of the clinical activity of IFN-alpha in HCV infection. IFN-alpha could thereby enhance the recruitment of T helper cells and thus facilitate the T cell-dendritic cell interaction, resulting in an efficient immune response. IFN-alpha might therefore not only be an effective agent in the early, unspecific phases of the host's antiviral defense, but also an important link in the transition to a specific immune response. Experiments of IFN-alpha on IL-16 secretion show an effect to be affective already at a low concentration of 10 U/ml, a concentration which is easily achieved *in vivo* during IFN-alpha therapy [24].

IL-16 has been shown to be synthesized by T cells as a precursor molecule, which is cleaved upon stimulation, followed by secretion of the bioactive C-terminal residue [31]. Recently it has been reported that pro-IL-16 is cleaved by caspase-3 [20], which has previously been recognized as a key component in the apoptotic pathway [32-34]. This is in parallel to IL-1beta and IL-18, which have also been reported to be processed by a member of the caspase family, namely caspase-1 [35]. In our study we investigated, at which level IFN-alpha regulates IL-16 secretion and if there is an association of activation induced cell death and IL-16 secretion. In contrast to many other cytokines, IL-16 mRNA is not regulated by stimuli which induce IL-16 secretion into culture supernatants, such as anti-CD3 [36]. Since large amounts of preformed IL-16 are present in lymphocytes [37], and processing of pro-IL-16 is followed by IL-16 secretion, we hypothesized that regulation of IL-16 secretion by IFN-alpha might occur at the level of IL-16

cleavage by caspase-3. Northern blot analysis showed that caspase-3 mRNA is regulated in ATC after stimulation with IFN-alpha and anti-CD3 + IFN-alpha. Caspase-3 mRNA levels were increased after incubation with anti-CD3, and costimulation with IFN-alpha further increased RNA levels, indicating a regulatory effect of IFN-alpha on caspase-3 expression. In addition, we measured the enzymatic activity of caspase-3 in lysates of ATC and correlated the activity with IL-16 levels in the corresponding culture supernatants. We found a very close correlation of these parameters, suggesting that caspase-3 activity is pivotal in regulating IL-16 secretion. For these experiments we used ATC, because in PBL, levels of caspase-3 were too low to be measured by the method used. One might, however, object that activation of caspase-3 in ATC may not be a key component of regulation of IL-16 secretion by IFN-alpha, but simply reflect AICD in preactivated T cells. For this reason we performed further experiments, adding the irreversible caspase-3 inhibitor Z-DEVD-FMK to cell cultures prior to stimulation with IFN-alpha, anti-CD3 or anti-CD95. This treatment completely blocked IL-16 secretion by ATC, confirming the essential role of caspase-3 for processing and secretion of IL-16. However, the proportion of cells undergoing AICD after stimulation with anti-CD3 + IFN-alpha or anti-CD95 + IFN-alpha could not be reduced by pre- or co-incubation with caspase-3 inhibitor Z-DEVD-FMK or the pan-caspase inhibitor Z-VAD-FMK which inhibits the caspases 1, 3, 5, 7, 8 and 9. This means that cell death in ATC after stimulation with anti-CD3 and anti-CD95 is not caspase-3-dependent, and therefore elevation of caspase-3-activity is very likely not to be a feature of AICD but to be indeed a key regulator in IFN-alpha-induced IL-16 secretion. To prove the functionality of the used caspase inhibitors, the same experiments were performed with Jurkat cells (a broadly used model for Fas-mediated apoptosis in T cells [38], as a positive control [39, 40, 41, 42]. In these experiments, induction of AICD by CD95-ligation could be prevented by blocking caspases by Z-VAD-FMK.

CONCLUSION

In conclusion, our results show that IL-16 secretion by anti-CD3 stimulated PBL is enhanced by IFN-alpha. Furthermore, our studies suggest that IFN-alpha controls IL-16 secretion by regulating caspase-3 activity. Finally, there is some evidence that other signaling pathways than those using caspase-3 might be involved in anti-CD3-/anti-CD95-induced cell death in ATC.

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Figures

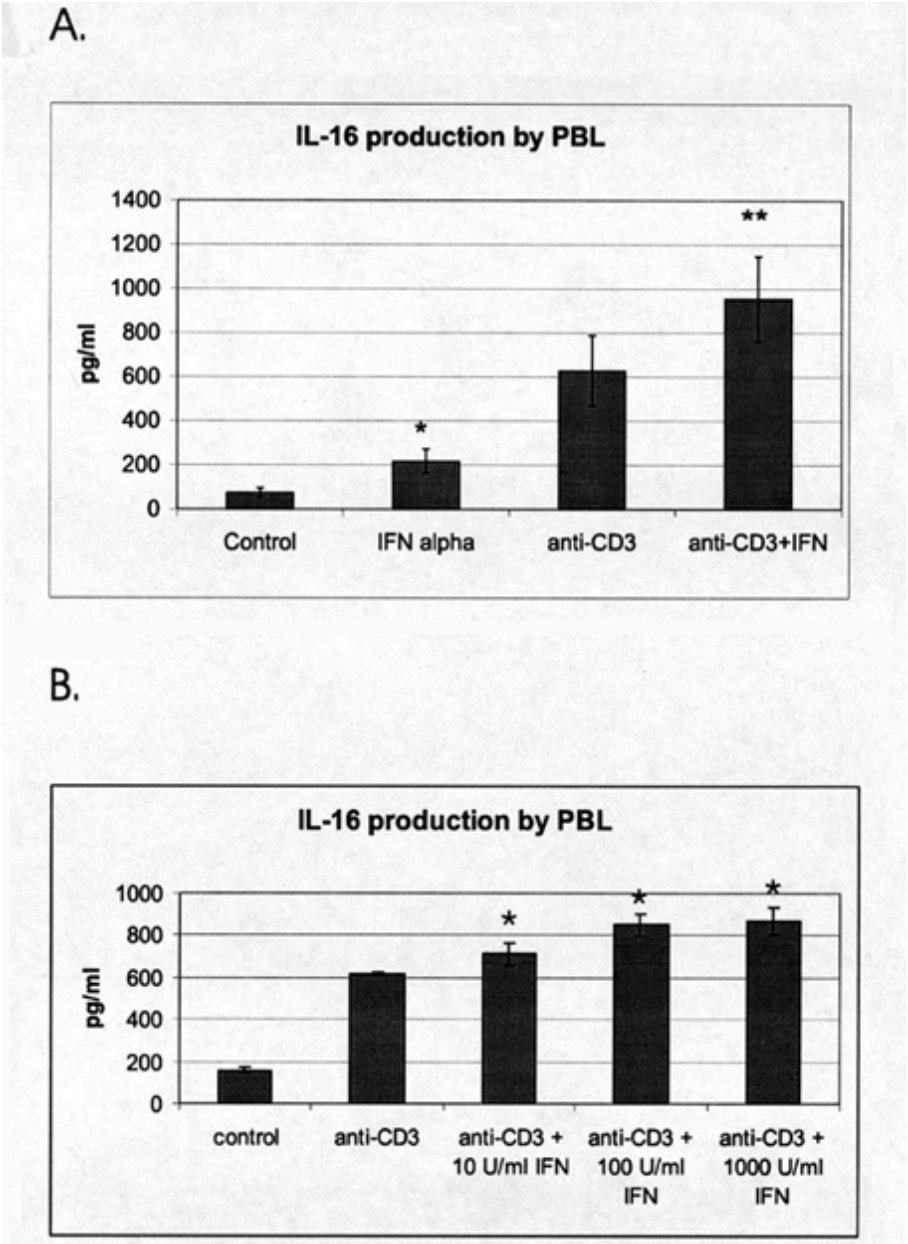


Figure 1

(A) Influence of IFN-alpha on IL-16 secretion by PBL.

Stimulation of PBL for 24 hours with activating anti-CD3 antibody induces IL-16 secretion into culture supernatants. Costimulation with IFN-alpha significantly enhances IL-16 production, compared with anti-CD3 stimulation alone (* $p < 0.01$ for stimulation with IFN-alpha *vs* control; ** $p < 0.01$ for stimulation with CD3 + IFN-alpha *vs* anti-CD3 alone, $n = 5$).

(B) Dose response relationship of IL-16 secretion by anti-CD3 stimulated PBL and different IFN-alpha-

concentrations.

PBL were stimulated with anti-CD3 and varying concentrations of IFN-alpha for 24 hours. Raising the concentration of IFN-alpha from 10 U/ml to 1,000 U/ml increased IL-16 secretion into culture supernatants (* $p < 0.01$ for stimulation with anti-CD3 + IFN-alpha *vs* anti-CD3 alone, $n = 6$).

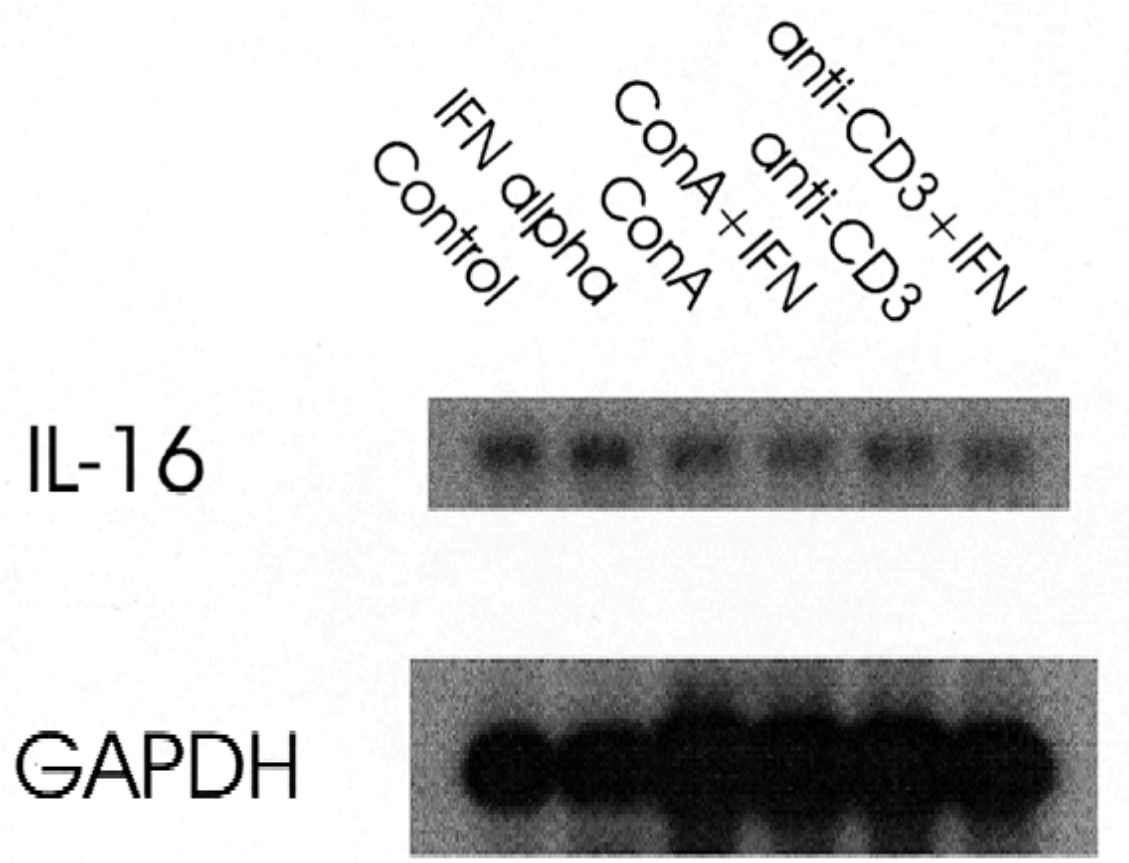


Figure 2
IL-16 mRNA-levels in PBMC.

IL-16 mRNA-levels in PBMC were not regulated by stimulation with IFN-alpha, ConA, anti-CD3 or ConA + IFN-alpha or anti-CD3 + IFN-alpha.

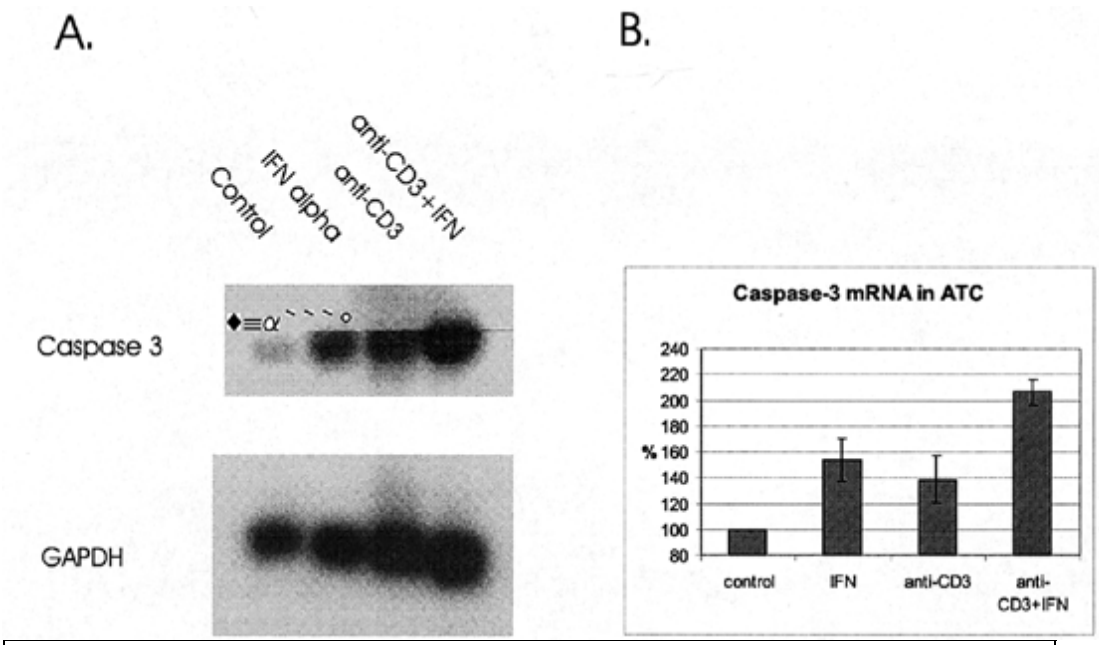


Figure 3
Caspase-3 mRNA-levels in activated T cells (ATC).

(A) IFN-alpha increased caspase-3 mRNA levels in ATC. Costimulation with anti-CD3 + IFN-alpha induced higher mRNA levels than stimulation with anti-CD3 alone. The Northern blot shown is representative of three separate experiments.

(B) Densitometric analysis is shown as the average caspase-3/GAPDH ratio ± SEM of three separate experiments.

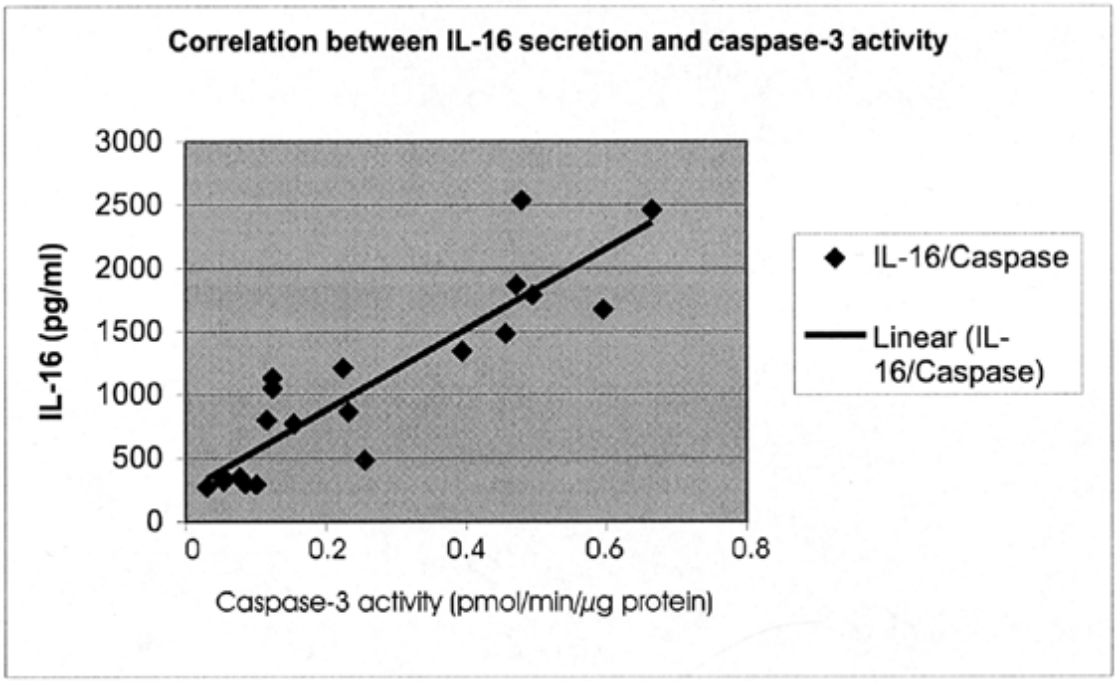


Figure 4
Correlation of IL-16 secretion and enzymatic activity of caspase-3 in activated T cells (ATC).

There was a close correlation between enzymatic activity of caspase-3 and IL-16 levels in culture supernatants of ATC (Pearsons's correlation coefficient: $r = 0.90$, $p < 0.001$).

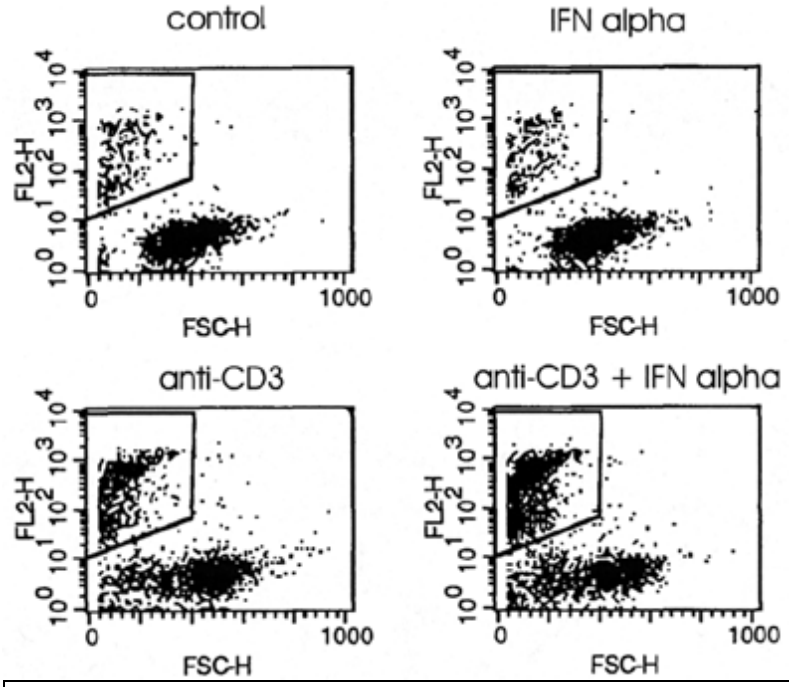
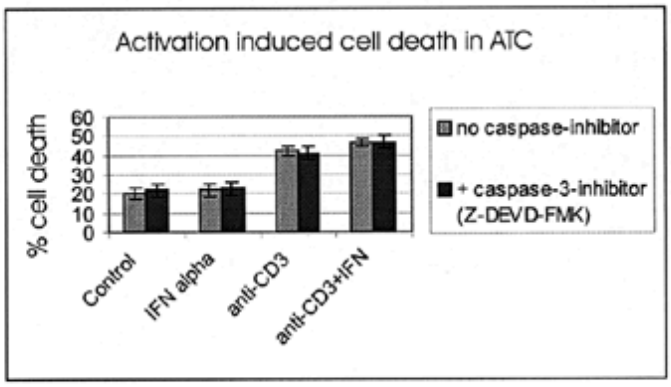


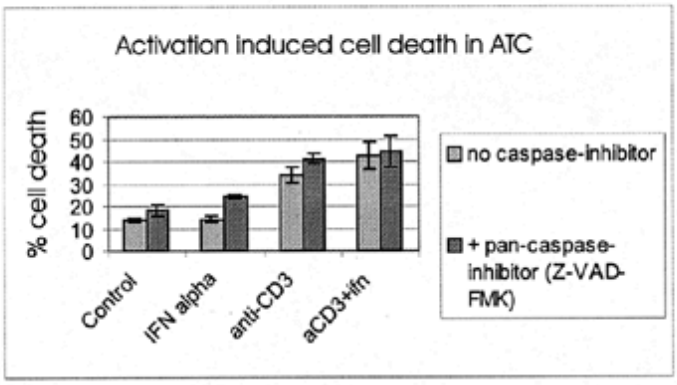
Figure 5
ATC undergoing activation induced cell death (AICD) after 24 hours of stimulation with IFN-alpha, anti-CD3 and anti-CD3 + IFN-alpha, measured by propidium iodide uptake.

Fig. 5 shows one representative experiment of a total of four experiments. The percentage of cells undergoing AICD is shown in the results section.

A.



B.



C.

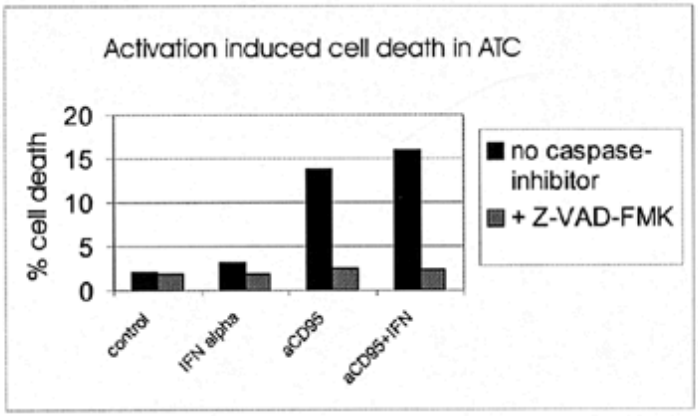


Figure 6

Caspase inhibitors do not inhibit anti-CD3/IFN-alpha induced AICD in ATC.

(A) ATC were stimulated with anti-CD3 ± IFN-alpha for 24 hours. Stimulation with anti-CD3 increased the proportion of cells undergoing AICD. Costimulation with IFN-alpha significantly enhanced AICD ($n = 4$, $p < 0.05$). Addition of the caspase-3 inhibitor Z-DEVD-FMK 1 hour before stimulation with anti-CD3 ± IFN-alpha did not reduce AICD.

(B) ATC were stimulated with anti-CD3 ± IFN-alpha for 24 hours. Stimulation with anti-CD3 increased the proportion of cells undergoing AICD. Costimulation with IFN-alpha further enhanced AICD ($n = 3$). Addition of the caspase-3 inhibitor Z-VAD-FMK 1 hour before stimulation with anti-CD3 ± IFN-alpha did not reduce AICD.

(C) Addition of the capsase-inhibitor Z-VAD-FMK prevented anti-CD95-induced AICD in Jurkat cells.

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In this issue

Virus-cell interactions: impact on cytokine production, immune evasion and tumor growth

T. H. Mogensen, S. R. Paludan
virus, cytokines, signal transduction, immune evasion, tumor growth.

Safety, pharmacokinetics and pharmacodynamics of recombinant human tumour necrosis factor-binding protein-1 (Onercept) injected by intravenous, intramuscular and subcutaneous routes into healthy volunteers

I. Trinchard-Lugan, Q. Ho-Nguyen, W.M. Bilham, M. Buraglio, A. Ythier, A. Munafo
recombinant human tumour necrosis factor-binding protein-1 (r-hTBP-1), safety, pharmacokinetics, pharmacodynamics.

Direct effects of tumor necrosis factor alpha (TNF-alpha) on murine skeletal muscle cell lines. Bimodal effects on protein metabolism

B. Alvarez, L. S. Quinn, S. Busquets, F. J. López-Soriano, J. M. Argilés
cytokines, myotubes, cachexia, protein turnover.

Receptor-like properties of the 26 kDa transmembrane form of TNF

A. Domonkos, A. Udvardy, L. László, T. Nagy, E. Duda
cytokine, TNF family, receptor, signaling, reverse-signaling.

Local administration of interleukin-1beta for the treatment of lung abscesses induces neutrophil activation and changes in proinflammation cytokine production

A. Simbirtsev, E. Variouchina, V. Konusova, A. Kotov, S. Ketlinsky, A. Salamatov, L. Bisenkov
recombinant human interleukin-1beta, lung inflammation, abscess, neutrophils, cytokines.

Effects of interleukin-2 therapy on the proliferation and differentiation of CD4/CD25 positive and CD4/CD25 negative cells in HIV+ patients

L. Caggiari, S. Zanussi, M. D'Andrea, M. T. Bortolin, C. Crepaldi, C. Caffau, P. de Paoli
cytokines, Ki67, intracellular IFN-gamma.

Tumor necrosis factor-alpha and nitric oxide in vertically HIV-1-infected children: implications for pathogenesis

J. González-Nicolás, S. Resino, J. L. Jiménez, S. Alvarez, M. Fresno, M. A. Muñoz-Fernández
tumor necrosis factor, nitric oxide, HIV-infection, immunological and virological markers.

Serum levels of angiogenic cytokines in systemic lupus erythematosus and their correlation with disease activity

E. Robak, A. Wozniacka, A. Sysa-Jedrzejowska, H. Stepien, T. Robak
systemic lupus erythematosus, disease activity, angiogenesis, platelets, hepatocyte growth factor, vascular endothelial growth factor, basic fibroblast growth factor, transforming growth factor beta.

Modulation of the allergen-induced human IgE response in Hu-SCID mice: inhibitory effect of human recombinant IFN-gamma and allergen-derived lipopeptide

C. Duez, H. Gras-Masse, H. Hammad, H. Akoum, A. Didierlaurent, C. André, A.-B. Tonnel, J. Pestel
SCID mouse, IgE, IFN-gamma, lipopeptide, house dust mite.

Cytokine-inducing activity of a proline-rich polypeptide complex (PRP) from ovine colostrum and its active nonapeptide fragment analogs

A. Zablocka, M. Janusz, K. Rybka, I. Wirkus-Romanowska, G. Kupryszewski, J. Lisowski
ovine colostrum, proline-rich polypeptide complex (PRP), nonapeptide (NP) and hexapeptide (HP) from PRP, oligomers of NP and HP, cytokines.

Quantitation of chemokines (MDC, TARC) expression in mucosa from Crohn's disease and ulcerative colitis

F. Jugde, M. Alizadeh, C. Boissier, D. Chantry, L. Siproudhis, S. Corbinais, E. Quelvennec, F. Dyard, J.-P. Campion, M. Gosselin, J.-F. Bretagne, G. Sémana, D. Heresbach
real-time TR-PCR, chemokines, Crohn’s disease, ulcerative colitis.

Activation of caspase-3 by interferon alpha causes interleukin-16 secretion but fails to modulate activation induced cell death

O. Ludwiczek, A. Kaser, R. O. Koch, W. Vogel, W. W. Cruikshank, H. Tilg
interleukin-16, interferon-alpha, caspase-3.

Cytokine deviation induced by intrathymic injection of staphylococcal enterotoxin B (SEB)

P. Goettelfinger, F. Lecerf, S. Berrih-Aknin, M. German-Fattal
SEB, intrathymic injection, anergy, cytokine deviation, quantitative RT-PCR.

Strain-dependent difference in inducible nitric oxide synthesis (iNOS) expression in rat pancreatic islets correlates with interferon regulating factor 1 (IRF-1) and heat shock protein 70 (HSP70) expression

J. Johannesen, F. Pociot, A. E. Karlsen, T. Mandrup-Poulsen, J. Nerup
type 1 diabetes, islets of Langerhans, beta-cells, cytokines, interleukin-1beta, interleukin-1beta receptor type I, nitric oxide, inducible nitric oxide synthase, heat shock protein 70, interferon regulating factor 1.

Neutrophil apoptosis in autoimmune Fas-defective MRL lpr/lpr mice

E. Del Giudice, A. Ciaramella, N. Balestro, D. Neumann, P. G. Romano, M. P. Cesaroni, G. Maurizi, P. Ruggiero, D. Boraschi, P. Bossù
neutrophils, apoptosis, autoimmunity.

A role for interferon-gamma in the hypermetabolic response to murine toxoplasmosis

D. Arsenijevic, F. de Bilbao, P. Giannakopoulos, L. Girardier, S. Samec, D. Richard
toxoplasma, hypermetabolism, reactive oxygen species, lipid peroxidation, UCP1, interferon-gamma.

Protection of mice from malaria after co-administration of recombinant mouse granulocyte-macrophage colony-stimulating factor and methionine-enkephalin

P. Pal Singh, S. Singh
Plasmodium berghei, protection, rmGM-CSF, met-enkephalin.

Semiautomated and simultaneous analysis of the interleukin-10 gene microsatellites IL-10G and IL-10R by fluorescence-based polymerase chain reaction reveals significant differences in allele distributions between Caucasians (Germany) and Africans (Gabon)

D. Kube, D. Schmidt, M. Mörmann, A.C. Uhlemann, J. Tomiuk, H. Tesch, P.G. Kremsner
microsatellites, interleukin-10 promoter, multiplex-PCR, Caucasian, African.

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