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IL-10 Does Not Affect Oxidative Burst and Expression of Selected Surface Antigen on Human Blood Phagocytes in vitro

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Summary

Cytokines play a major role in the control of inflammatory responses, participate in the regulation of blood phagocyte activities and as such are used for immunomodulatory therapy. In the present study, the influence of IL-10 on human blood phagocyte activity in the presence/absence of IL-6, IL-8 and TNF-α was tested *in vitro*. Our research analyzed the effects of cytokines on the production of reactive oxygen species measured by chemiluminescence and flow cytometry, and on the expression of surface molecules (CD11b, CD15, CD62L, CD31) measured by flow cytometry. IL-10 had no inhibitory effect on reactive oxygen species production and the expression of any examined adhesion molecule by resting or stimulated blood phagocytes within 3 h of incubation. Conversely, TNF-α, IL-6, and IL-8 increased reactive oxygen species production and the expression of CD11b and CD15 on both neutrophils and monocytes and decreased the expression of CD62L. These priming effects of the tested pro-inflammatory cytokines were not affected by IL-10. The obtained results suggest that IL-10 does not directly control blood phagocyte activation. These results also provide better information about the contribution of IL-6, IL-8 and TNF-α to the regulation of blood phagocyte-mediated inflammatory processes.

Key words
Interleukins • TNF-α • Leukocytes • Oxidative burst • Surface molecules

Introduction

Polymorphonuclear leukocytes (PMNL) and monocytes are the most important blood phagocytes that play a key role in the non-specific immune defense of the body. Phagocytosis is accompanied by an oxidative burst, a process in which a significant increase in the production of reactive oxygen species (ROS) occurs (Drábiková *et al.* 2000). However, an overproduction of ROS evokes tissue inflammation and injury (Land *et al.* 1994, Lojek *et al.* 1998, Huber *et al.* 2000, Hamar *et al.* 2003). Cytokines may play a critical role in triggering this systemic inflammatory response. A sharp increase in the plasma concentration of both pro- and anti-inflammatory cytokines was observed during inflammatory pathological states including septic shock (Huber *et al.* 2000), organ transplantation (Lang *et al.* 1996, Wan *et al.* 1997, Kubala *et al.* 2001, 2002) and many others.

IL-10 is referred to as a cytokine with anti-inflammatory activity. It is produced by the CD4+/TH1

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and CD4+/TH2 subsets of lymphocytes, B lymphocytes and macrophages/monocytes (Wakkach *et al.* 2000). IL-10 inhibits the activation and proliferation of T lymphocytes, downregulates the production of pro-inflammatory cytokines and the expression of various cell surface antigens associated with the induction of inflammation (Wakkach *et al.* 2000). IL-10 exerts direct anti-inflammatory and immunosuppressive effects on various cell types (Raychaudhuri *et al.* 2000, Roilides *et al.* 2000). Previously, IL-10 was reported as a potent inhibitor of phagocyte H_2O_2 production (Bogdan *et al.* 1991), nitric oxide synthesis (Gazzinelli *et al.* 1992) and microbicidal activity (Silva *et al.* 1992). These activities of IL-10, together with its capacity to reduce the production of pro-inflammatory cytokines and chemokines, indicate that IL-10 is a potent immunosuppressant *in vitro* (de Vries 1995, Yano *et al.* 1995). Therefore, IL-10 appeared to be a promising agent for the therapeutic treatment of acute inflammations (Huber *et al.* 2000). However, its direct effect on phagocytes is still a matter of discussion. Conversely, cytokines such as IL-6, IL-8 and TNF-α are potent pro-inflammatory mediators with a wide range of biological activities (Pirenne *et al.* 1994). They have been shown to activate blood phagocyte chemotaxis, the expression of various adhesion molecules with consequent adhesion to endothelial cells, infiltration into tissues and the release of ROS, proteolytic enzymes and other bioactive substances (Pirenne *et al.* 1994).

The present study was designed to determine the effect of IL-10 on blood phagocyte activity: ROS production and the expression of surface antigens involved in phagocyte adhesion and phagocytosis, namely the complement receptor 3 (CD11b), Lewis-X (CD15), L-selectin (CD62L) and platelet-endothelial cell adhesion molecule (CD31). The combined effects of IL-10 and pro-inflammatory cytokines (IL-6, IL-8, TNF-α) were also tested at various times of incubation.

Material and Methods

Reagents

Recombinant human (rh) IL-10, IL-6, IL-8, and TNF-α were obtained from R&D Systems (USA). Phorbol myristate acetate (PMA), zymosan A from *saccharomyces cerevisiae* and luminol were obtained from Sigma-Aldrich (USA). Lysing solution Cal-Lyse, fluorescein isothiocyanate (FITC)-labeled anti-human CD62L murine monoclonal antibody, FITC-labeled anti-human CD15 murine monoclonal antibody, phycoerythrin (PE)-labeled anti-human CD11b murine monoclonal antibody, PE-labeled anti-human CD31 murine monoclonal antibody and appropriate control isotype murine antibodies were purchased from Caltag Laboratories (USA). Phycoerythrincyanin 5.1 (PC5)-labeled anti-human CD14 murine monoclonal antibody was purchased from Immunotech (USA). Dihydrorhodamine-123 (DHR-123) was purchased from Molecular Probes (USA). All other chemicals were purchased in the highest grades from local distributors.

Blood sampling and cytokine reconstitution

Heparinized blood samples (50 IU/ml) were obtained from five healthy volunteers having given informed consent. Blood was diluted ten times with Hanks balanced salt solution (HBSS). The recombinant human cytokines were reconstituted to a concentration of 10 μg/ml in HBSS containing 5 % bovine serum albumin. These cytokine solutions were further diluted with HBSS to obtain stock solutions. The final concentration of bovine serum albumin did not exceed 0.001 %.

Experimental protocol

Four experimental protocols were used in this study. Protocol A – diluted blood samples were incubated with various concentrations (400, 800, 1200 and 1600 pg/ml) of IL-10, IL-6, IL-8 or TNF-α at 37 °C for 15 min or 2 h prior to the measurement of ROS production by luminol-enhanced chemiluminescence (CL). Protocol B – blood samples were incubated with 1200 pg/ml of IL-10, IL-6, IL-8 or TNF-α at 37 °C for 2 h prior to the determination of selected surface antigens and intracellular ROS production using a flow cytometer. Protocols C and D were used to test the effects of IL-10 on the pro-inflammatory cytokine induced changes in both total and intracellular ROS production as well as surface antigen expression. Protocol C – blood samples were pre-incubated with IL-10 (1200 pg/ml) for 1 h prior to 15 min or 2 h of incubation with pro-inflammatory cytokines (1200 pg/ml). Protocol D – blood samples were simultaneously incubated with IL-10 (1200 pg/ml) and pro-inflammatory cytokines (1200 pg/ml). HBSS was used instead of cytokines as a control. The viability of leukocytes was determined by trypan blue exclusion after a hypotonic lysis of a part of each sample at the end of the incubations. The leukocyte viability of any sample did not decrease below 95 %.

Measurement of blood phagocyte oxidative burst

Two different methods for ROS detection were used to obtain comprehensive information about the oxidative burst of phagocytes.

Chemiluminescence determination of ROS

Chemiluminescence of the diluted blood was measured using a microplate luminometer LM-01T (Immunotech, Czech Republic). The principle of the method is based on luminol interaction with phagocyte-derived free radicals, which results in large measurable amounts of light. Briefly, the reaction mixture consisted of 100 µl of diluted blood, 1 mM luminol (stock solution of 10 mM luminol in 0.2 M borate buffer) and one of the activators (PMA – 0.4 µM or OZP – 0.25 mg/ml). The total reaction volume of 130 µl was adjusted with HBSS. The assays were run in duplicates. Spontaneous CL measurements in samples containing 100 µl of diluted blood and all other substances, but none of the activators, were included in each assay. The CL emission was followed for 90 min at 37 ^{o}C. The integral value of the CL reaction, which represents the total ROS production by blood phagocytes was evaluated.

Flow cytometric determination of ROS

Diluted blood samples (100 µl) were incubated with 10 µM DHR-123 (stock solution 10 mM DHR in dimethyl sulphoxide) and an anti-CD14 monoclonal antibody (2 µl). The tubes were shaken gently and incubated in the dark at 37 ^{o}C for 20 min. Then either PMA (0.4 µM) or OZP (0.25 mg/ml), or HBSS was added and the cell suspensions were incubated in the dark at 37 ^{o}C for further 20 min. At the end of the incubation period samples were fixed with Cal-lyse. The red blood cells were lysed by distilled water. The remaining cells were resuspended in PBS, placed on ice and analyzed within 2 h on the flow cytometer similarly to the analysis of surface antigens.

Determination of the cell surface expression of adhesion molecules

The measurements were performed according to the manufacturer's protocol (Caltag Laboratories, USA) for unfixed whole blood. Briefly, 100 µl of diluted blood was incubated in plastic tubes (Falcon, USA) with anti-CD11b, anti-CD15, anti-CD62L and anti-CD31 monoclonal antibodies (two antibodies per vial, 5 µl of each). Blood (100 µl) was incubated with FITC- or PE-conjugated murine immunoglobulins of the same isotype were used as the negative controls. Each tube also contained anti-CD14 monoclonal antibody (2 µl) for the discrimination of monocytes. Samples were incubated at room temperature for 15 min and then fixed with Cal-lyse. The red blood cells were lysed with distilled water. The remaining cells were resuspended in PBS, placed on ice and analyzed within 2 h by a flow cytometer FACSCalibur (Becton Dickinson, USA). Blood granulocytes and monocytes were selected on the basis of their typical scattering characteristics and their different expression of CD14 antigen. The median fluorescence intensity (MFI) was determined and corrected for unspecific staining by subtracting the fluorescence of cells stained with the control antibody (isotype control).

Statistical analysis

The results were analyzed by Student t-test for independent or dependent samples and significances were verified by the non-parametric Mann-Whitney U-test or Wilcoxon test using Statistica for Windows 5.0 (Statsoft, USA). The differences of $p<0.05$ were regarded as statistically significant. All values are reported as the means of five different experiments ± S.E.M.

Results

Influence of cytokines on spontaneous ROS production measured by chemiluminescence

No significant differences in the spontaneous ROS production were found among control samples and samples incubated with IL-10, IL-6 or IL-8 at any of the concentrations used either after 15 min or 2 h of incubation (Fig. 1). TNF-α significantly increased the spontaneous ROS production when compared with the controls at all concentrations used after 15 min of incubation (Fig. 1A). The effect of TNF-α after 2 h of incubation was not significant (Fig. 1B).

Influence of cytokines on activated ROS production measured by chemiluminescence

After 15 min of incubation, PMA-activated ROS production was significantly increased by IL-6 at a concentration of 1600 pg/ml and by TNF-α at all tested concentrations when compared with control samples (Fig. 1A). However, IL-6 at concentrations of 800, 1200 and 1600 pg/ml, IL-8 at a concentration of 1600 pg/ml and TNF-α at all tested concentrations significantly increased PMA-activated ROS production after 2 h of incubation (Fig. 1B). OZP-activated ROS production was

significantly increased by IL-6 at a concentration of 1600 pg/ml, by IL-8 at concentrations of 1200 and 1600 pg/ml and by TNF-α at all tested concentrations when compared with the controls after 15 min of incubation (Fig. 1A). On the other hand, OZP-activated ROS production was significantly enhanced by all the tested concentrations of IL-6, IL-8 and TNF-α with the exception of IL-6 and IL-8 at concentrations of 400 pg/ml after 2 h of incubation. IL-10 at concentrations of 1200 and 1600 pg/ml significantly increased OZP-activated ROS production after 2 h of incubation (Fig. 1B).

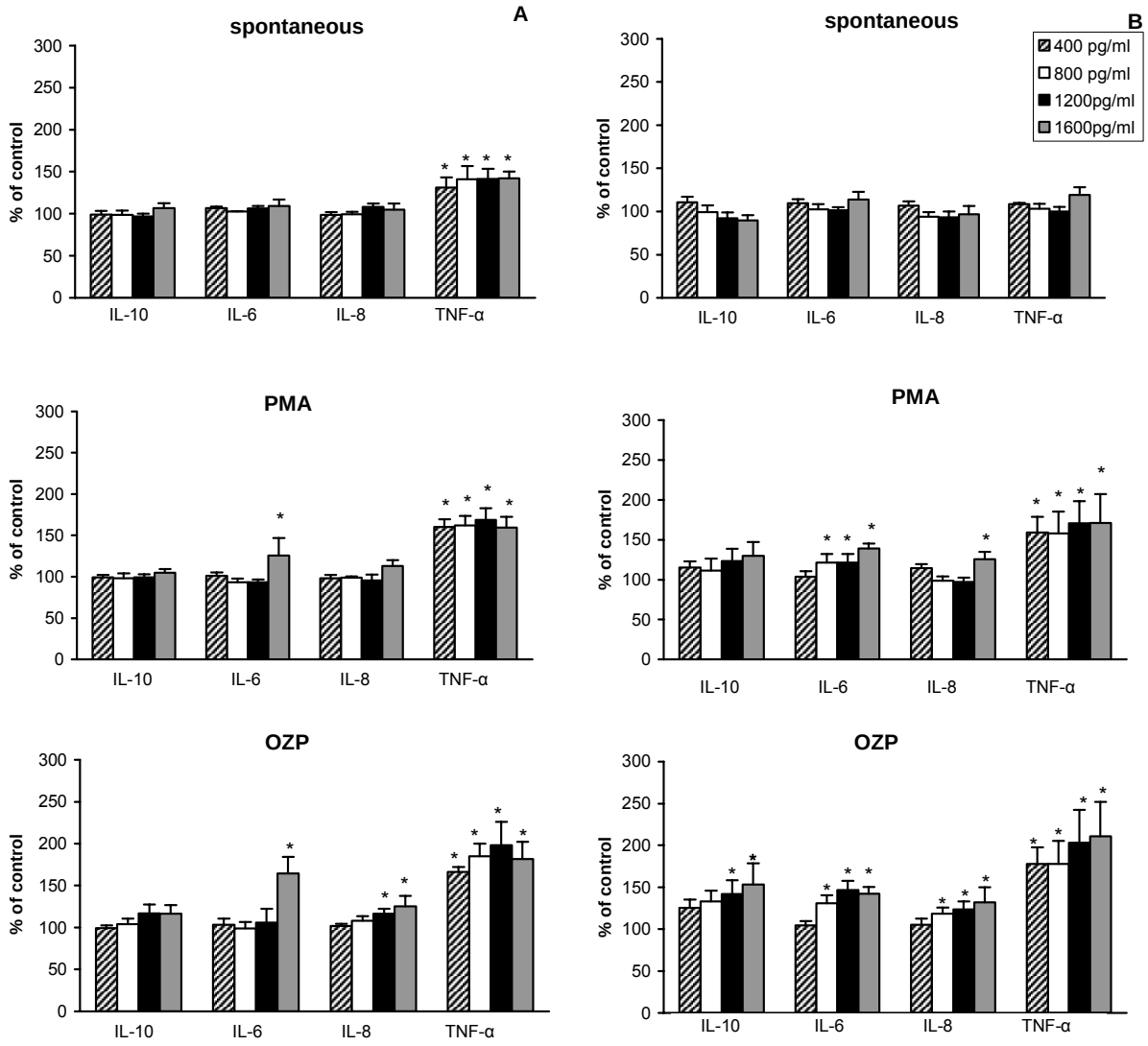

Fig. 1. Effects of IL-10, IL-6, IL-8 and TNF-α at concentrations of 400, 800, 1200 and 1600 pg/ml on spontaneous and PMA- or OZP-activated ROS production by blood phagocytes after 15 min (A) and 2 h (B) of incubation. Results are expressed as percentages of controls. The asterisk indicates significant ($p<0.05$) difference vs. control incubated in the absence of cytokine (n=5).

Influence of cytokines on ROS production measured by DHR-123

IL-10 and IL-8 did not significantly affect either spontaneous or activated intracellular ROS production by neutrophils or monocytes. Incubation of blood samples with IL-6 significantly increased spontaneous and OZP-activated ROS production by neutrophils and PMA-activated ROS production by monocytes (Table 1). TNF-α increased significantly spontaneous and PMA- or OZP-activated ROS production by both neutrophils and monocytes when compared with the controls.

Influence of cytokines on the expression of adhesion molecules

IL-10 did not significantly affect the expression of any of the studied surface molecules (CD11b, CD15,

CD62L and CD31) (Fig. 2). IL-6 and TNF-α induced significant increases in CD11b and CD15 expression and a decrease in CD62L expression on both neutrophils and monocytes when compared with control samples. IL-8 significantly increased the expression of CD11b on neutrophils and CD15 on both neutrophils and monocytes. IL-8 did not affect the expression of CD62L. The expression of CD31 was not significantly affected by any of the tested cytokines.

Table 1. The influence of IL-10 (1200 pg/ml) on spontaneous and PMA- or OZP-activated ROS production by neutrophils and monocytes measured by DHR-123. Blood samples were simultaneously incubated with IL-10 and with IL-6, IL-8 or TNF-α (1200 pg/ml) for 2 h or preincubated with IL-10 for 1 h before a 2 h incubation with IL-6, IL-8 or TNF-α (1200 pg/ml).

		NEUTROPHILS			MONOCYTES		
		Individual cytokines	**Simultaneous incubation with IL-10**	**Pre-incubation with IL-10**	**Individual cytokines**	**Simultaneous incubation with IL-10**	**Pre-incubation with IL-10**
Spontaneous	*IL-10*	101±6	–	–	96±9	–	–
	IL-6	125±11*	114±14	116±4*	119±8*	117±8	118±3*
	IL-8	101±8	108±11	98±3	94±11	103±4	104±7
	TNF-α	135±18*	137±14*	150±27*	145±19*	155±25*	151±18*
	–	–	–	99±11	–	–	97±5
PMA-activated	*IL-10*	109±13	–	–	103±5	–	–
	IL-6	112±4	126±18*	110±7	127±11*	128±7*	117±5*
	IL-8	102±6	114±25	111±7	96±7	104±6	104±4
	TNF-α	122±18*	133±21*	129±14*	119±6*	121±9*	128±18*
	–	–	–	110±12	–	–	89±17
OZP-activated	*IL-10*	114±26	–	–	103±5	–	–
	IL-6	124±16*	118±6*	129±10*	113±5*	107±10	109±15
	IL-8	114±16	115±12	112±12	103±7	110±13	108±4
	TNF-α	126±14 *	120±15*	122±11*	119±6*	125±14*	126±14*
	–	–	–	96±11	–	–	91±8

Results are expressed as percentages of controls incubated without any cytokine. The asterisk indicates significant ($p<0.05$) difference between controls and samples incubated simultaneously with IL-10 or preincubated with IL-10 for 1 h (n=5).

Influence of simultaneous incubation of IL-10 with individual pro-inflammatory cytokines

Simultaneous incubation of blood with IL-10 along with IL-6 or TNF-α did not significantly influence either spontaneous or PMA- or OZP-activated ROS production measured by CL when compared with analogous samples incubated without IL-10 at both 15 min and 2 h time intervals (Table 2). A simultaneous incubation of blood with IL-10 and IL-8 did not influence ROS production when compared with analogous samples incubated without IL-10 with the exception of spontaneous (significant increase) and OZP-activated (significant decrease) ROS production after 2 h of incubation (Table 2).

Simultaneous incubation of blood samples with IL-10 (1200 pg/ml) and any of the pro-inflammatory cytokines did not significantly affect either spontaneous or PMA- or OZP-activated ROS production by neutrophils or monocytes measured by DHR-123 when compared with analogous samples incubated without IL-10 (Table 1).

Simultaneous incubation of blood with IL-10 (1200 pg/ml) and any of the pro-inflammatory cytokines did not significantly affect the expression of any evaluated surface molecules on either neutrophils or monocytes when compared with analogous samples without IL-10 (data not shown).

Influence of preincubation with IL-10 on the priming effect of pro-inflammatory cytokines

Preincubation of blood with IL-10 for 1 h before 15 min or 2 h incubation with individual pro-

inflammatory cytokines did not have any influence on either spontaneous or activated ROS production measured by CL when compared with analogous samples without IL-10 pre-incubation (Table 2). Similarly, preincubation of blood with IL-10 for 1 h before incubation with individual pro-inflammatory cytokines did not significantly affect either spontaneous or PMA- or OZP-activated intracellular ROS production by neutrophils or monocytes as measured by DHR-123, when compared with analogous samples without IL-10 preincubation (Table 1). The expression of any evaluated surface molecules either on neutrophils or monocytes was not significantly affected when compared with analogous samples without IL-10 preincubation (data not shown).

Table 2. The influence of IL-10 (1200 pg/ml) on spontaneous and PMA- or OZP-activated ROS production by blood phagocytes measured by luminol-enhanced chemiluminescence. Blood samples were simultaneously incubated with IL-10 and with IL-6, IL-8 or TNF-α (1200 pg/ml) for 15 min or 2 h or preincubated with IL-10 for 1 h before 15 min or 2 h of incubation with IL-6, IL-8 or TNF-α (1200 pg/ml).

		Without IL-10	Simultaneous incubation with IL-10	Pre-incubation with IL-10	Without IL-10	Simultaneous incubation with IL-10	Pre-incubation with IL-10
		15 min incubation			2 h incubation		
Spontaneous	*IL-6*	106±3	99±6	105±3	101±3	91±11	111±17
	IL-8	107±4	98±2	99±1	93±6	111±6†	86±15
	TNF-α	141±1*	137±14*	166±25*	100±5	95±8	112±18
	–	–	–	101±1	–	–	94± 8
PMA-activated	*IL-6*	93±3	91±1*	103±4	121±10*	129±13*	117±15
	IL-8	95±7	94±8	118±3*	96±5	99±4	107±15
	TNF-α	168±14*	148±13*	170±6*	170±27*	174±31*	168±41 *
	–	–	–	102±7	–	–	96±9
OZP-activated	*IL-6*	105±16	100±5	110±6	146±10*	150±21*	139±18 *
	IL-8	116±5*	102±4	118±4*	123±9*	92±6†	121±16 *
	TNF-α	197±28*	192±22*	177±19*	203±39*	204±57*	206±54*
	–	–	–	116±5*	–	–	90±9

Results are expressed as percentages of controls incubated without any cytokine. The cross indicates significant ($p<0.05$) difference between samples without IL-10 and analogous samples simultaneously incubated or preincubated with IL-10 (n=5). For other symbols and explanations see Table 1.

Discussion

IL-10 is classified as an anti-inflammatory cytokine (Vicioso *et al.* 1998). However, its effect on phagocytes, which belong to the key components of the inflammatory response, is still a matter of discussion. In the present study, the influence of IL-10 on human blood phagocytes was tested by monitoring ROS production and the expression of several adhesion molecules on neutrophils and monocytes in the presence and in the absence of selected pro-inflammatory cytokines. In our experimental conditions, IL-10 did not have any inhibitory effect on ROS production by blood phagocytes. On the contrary, IL-10 at concentrations of 1200 and 1600 pg/ml significantly enhanced the activated ROS production after 2 h of incubation which agrees with our previous observations (unpublished data). Bussolati *et al.* (1997) found that IL-10 directly stimulated an early production of superoxide by monocytes and to a slight extent by PMNL, but inhibited the subsequent phagocytosis and the ability of these cells to respond to fMLP. Roilides *et al.* (2000) found that IL-10 (2-100 ng/ml) did not have any significant effect on the activated oxidative burst of PMNL after 1 h of incubation. Our results also show that the expression of neutrophil and monocyte surface antigens CD11b, CD15, CD31 and CD62L (Kubala *et al.* 2002ab) is not modified by IL-10. Similarly Vicioso *et al.* (1998) did not observe any effect of IL-10 (10 ng/ml) on CD11b/CD18 expression on PMNL and monocytes after 30 min of incubation. In

contrast, some authors have shown that IL-10 downregulated the expression of CD11b (Laichalk *et al.* 1996), chemotaxis (Vicioso *et al.* 1998) and the ability of phagocytosis (Laichalk *et al.* 1996).

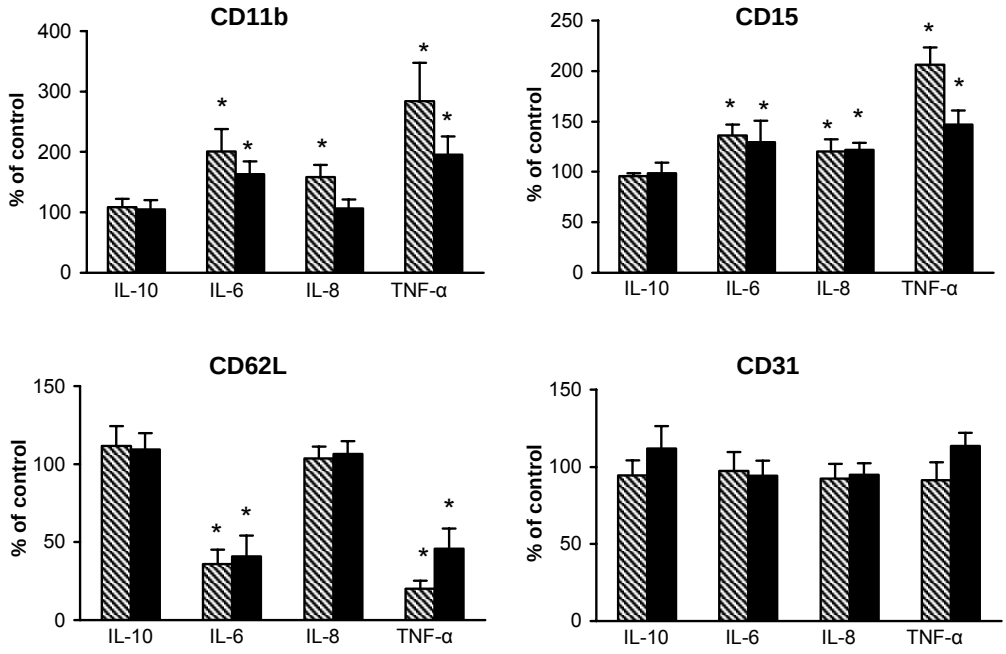

Fig. 2. Effects of IL-10, IL-6, IL-8 and TNF-α at a concentration of 1200 pg/ml on the expression of surface molecules (CD11b, CD15, CD62L and CD31) on neutrophils (hatched bars) and monocytes (full bars) after 2 h of incubation. Results are expressed as percentages of controls. The asterisk indicates significant ($p<0.05$) difference vs. control incubated in the absence of cytokine ($n=5$).

Furthermore, the effects of IL-10 in the presence of individual pro-inflammatory cytokines were analyzed in our study. Each of the tested pro-inflammatory cytokines alone induced a strong increase in phagocyte ROS production. The influence of TNF-α was the most effective at all the concentrations used as early as after 15 min of incubation. Other authors also found that IL-6, IL-8 and TNF-α enhanced the cytotoxicity and primed the oxidative burst of PMNL (Borish *et al.* 1989, Elbim *et al.* 1994, Elbim and Gougerot-Pocidalo 1996, Niwa *et al.* 1996, Vondráček 1997). Khwaja *et al.* (1992) observed a fast response of blood phagocytes to TNF-α stimulation after 10-20 min. Finally, in agreement with the literature (Baggiolini *et al.* 1994, Asman *et al.* 1996), IL-6, IL-8 and TNF-α increased the expression of CD11b and CD15 and decreased the expression of CD62L on both neutrophils and monocytes. Surprisingly, IL-10 did not have any influence on the priming effect of IL-6, IL-8, TNF-α on phagocyte ROS production and the expression of adhesion molecules during simultaneous incubation. Our results are in accord with Reglier-Pouplet *et al.* (1998) who showed that IL-10 did not have a direct effect on production of H_2O_2 and did not modulate priming effect of TNF-α on the response to fMLP. The only exception was that IL-10 abolished the stimulating effect of IL-8 on OZP-activated ROS production. It was also observed that preincubation with IL-10 did not affect the changes induced by IL-6, IL-8 or TNF-α. Ωε also confirmed that IL-10 did not have an inhibitory effect on phagocyte activity *in vitro* at the tested concentrations. The influence of IL-10 on the activation of blood phagocytes *in vitro* could be affected by several factors. The effects of cytokines are dose-dependent so that the selection of tested concentrations is very important for *in vitro* experiments (Laichalk *et al.* 1996, Capsoni *et al.* 1997). The concentrations of cytokines used in our study were selected on the basis of previous studies (Kubala *et al.* 2001, 2002ab) and other clinical studies on patients in various pathophysiological situations when the highest plasma level of any tested cytokine did not exceed 2 ng/ml (Lang *et al.* 1996). When high concentrations (10-100 ng/ml) of IL-10 were used for *in vitro* experiments, IL-10 downregulated the generation of ROS by blood phagocytes (Bogdan *et al.* 1991, Laichalk *et al.* 1996),

slightly augmented phagocytosis by monocytes and decreased phagocytosis by neutrophils (Buchwald *et al.* 1999). ICAM-1 expression on monocytes was markedly enhanced by IL-10 in the concentration range of 1 to 100 ng/ml, while the expression on neutrophils was not affected (Buchwald *et al.* 1999). Surface expression of IL-10 receptor on blood phagocytes is another important factor affecting the influence of IL-10 on phagocyte activity. It was reported that expression of IL-10 receptors on the surface of resting blood granulocytes was low and was not sufficient to induce a response of these cells to IL-10 (Elbim *et al.* 2001). These authors observed that a preincubation of cells with TNF-α for 10-40 min induced an increase of the expression of IL-10 receptor and consequently induced the responsiveness of cells to IL-10 which was expressed by a lower production of superoxide.

Another explanation for the absence of anti-inflammatory activity of IL-10 could be a short incubation of diluted blood with cytokines in our study. Bogdan *et al.* (1991) found that IL-10 reduced the generation of ROS when macrophages were preincubated with IL-10 for 48 h and then stimulated by PMA. Similarly, the incubation of monocytes or PMNL with IL-10 for 24 h before stimulating these cells with fMLP decreased superoxide production (Bussolati *et al.* 1997). Incubation of neutrophils with IL-10 for 18 h down-regulated their capacity to produce a superoxide anion, but conversely, shorter incubation times with IL-10, from 30 min to 5 h, were ineffective (Capsoni *et al.* 1997). It seems that the anti-inflammatory properties of IL-10 could be observed mainly after long incubation times of blood phagocytes with IL-10. However, a massive increase of IL-10 occurs *in vivo* at the same time as a massive increase of pro-inflammatory cytokines which cause phagocyte activation at time intervals up to several hours. Therefore, if an increase of IL-10 is protective against damage caused by activated phagocytes, as has been suggested by several authors (Kubala *et al.* 2001, Wan *et al.* 1997), it has to affect the phagocytes' activity at these time intervals. Therefore, the incubation times in our experiments did not exceed three hours.

Most studies have been performed on PMNL or monocytes isolated from blood by various procedures, which may modulate ROS production (Číž and Lojek 1997), surface receptor expression (Zahler *et al.* 1997), and thus alter the cell response. Therefore, in this study we measured the activation of blood phagocytes in diluted blood in order to avoid PMNL and monocyte activation and to simulate the *in vivo* situation more closely.

In conclusion, pro-inflammatory effects of IL-6, IL-8 and TNF-α expressed as an increased ROS production and the enhanced expression of adhesion molecules by neutrophils and monocytes were observed *in vitro*. These effects were dependent on cytokine concentrations and the time of incubation of blood with cytokines. On contrary, our results show that IL-10 does not have any direct anti-inflammatory activity towards blood phagocytes. The properties of IL-10 described in our study should be taken into consideration in therapeutic strategies where some anti-inflammatory benefit of IL-10 is expected.

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