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

## Abstract

Interleukin-1 (IL-1) is a potent immunostimulatory molecule with broad range biological activity that limits its systemic application in humans. Alternatively, IL-1 can be applied locally, directly to the inflammatory site for stimulation of local defense mechanisms, without activating an acute phase response. IL-1beta

preparations have been successfully used for local therapy of patients with purulent bacterial lung abscesses who were resistant to usual antibiotic treatment. IL-1beta was applied directly to the abscess cavity at a concentration of 10 ng/ml, once a day for 7 days. Before IL-1beta administration, local leukocyte functional activity was significantly reduced compared to the same activity in neutrophils isolated from the peripheral blood of the same patient. Local application of IL-1beta led to increases in adhesion, chemotaxis, oxygen radical production and phagocytosis of abscess fluid neutrophils. After local IL-1beta treatment, the concentration of IL-8 and TNF-alpha in the abscess fluids increased, while in nearly all patients levels of endogenous IL-1beta decreased. Immunocytochemical analysis showed that IL-1beta increased the numbers of cytokine-producing cells and induced proinflammatory cytokine production by neutrophilic granulocytes. According to the results obtained, the mechanism of the local immunostimulatory activity by IL-1beta is associated with the significant activation of neutrophilic granulocyte functions and changes in cytokine production at the inflammatory site. IL-1beta can be used as a highly effective immunotherapeutic drug when applied locally at adequate immunostimulatory dose levels.

## Full text

### INTRODUCTION

Interleukin-1 (IL-1) is one of the main mediators for the development of inflammation, and acute-phase and immune responses [1]. IL-1 is produced in response to invading pathogens and to tissue damage, and stimulates the development of the local inflammatory reaction directed towards the elimination of the pathogen and tissue regeneration. If local the inflammation is insufficient to block pathogen dissemination, the inflammatory reaction becomes systemic, IL-1 is produced in large amounts, it appears in the circulation and activates acute phase responses. Experimental studies have shown that recombinant IL-1 has a pleiotropic mode of biological activity and stimulates nearly all cells and tissues participating in the host defense response, including those of the central nervous, endocrine and hematopoietic systems [2].

Several attempts have been made to use both members of the IL-1 family (IL-1alpha and IL-1beta) for reconstitution of bone marrow in cancer patients after high-dose chemotherapy [3-5]. Systemic IL-1 administration in these trials had

clinical benefits, but unfortunately was significantly limited, due to various adverse effects which occur because of the proximity between therapeutically effective, and toxic doses [6].

Alternatively, IL-1 can be administered locally, directly to the inflammatory site, for stimulation of local defense mechanisms, without activating acute phase responses. Preparations of IL-1beta have been successfully used for local treatment in patients with bacterial lung abscesses who were resistant to the usual antibiotic therapy [7].

In the present report we study the mechanisms of IL-1beta immunostimulatory activity after local administration for the treatment of lung abscesses.

## MATERIALS AND METHODS

### *Patients*

Fifty-nine patients with pyogeneous bacterial lung abscesses which had developed after pneumonia were enrolled in this study. The age range of patients was from 19 to 71 years, 47 males (79.7%) and 12 females (20.3%). Patients were randomized to form two groups: 1 - local treatment with IL-1beta (32 patients), 2 - control group treated with conventional conservative therapy (27 patients). Before the beginning of the IL-1beta treatment, all patients were treated for 15-45 days with antibiotics (penicillin, linkomycin, gentamycin, metranidazole, aminoglycozides and 1st-2nd generations cephalosporins: Cefotaxim, Ceftazidime, Cefoperazone). Abscess cavities were drained and washed with saline and mucolytic enzymes. Unfortunately, this treatment was ineffective and gave no positive clinical changes.

All patients in this study had one lung abscess cavity located either in the left lung (39%) or in the right lung (61%). Exact abscess cavity location, size and perifocal infiltration of the lung tissue were determined by radiological investigation and radiological computer tomography. According to the microbiological investigations, the following pathogens were isolated from abscess cavities: *Staphillococcus* (23.7%), *Streptococcus* (22%), *E. coli* (37.3%), *Proteus* and anaerobic bacteria. Microbiological investigations of abscess fluids showed that in one third of patients, pure cultures of one

microbe were obtained and in two thirds - mixed cultures of microorganisms were detected.

Patients with multiple abscesses, with post-traumatic abscesses, and patients with other sites of infection such as pleural empyema or other pathologies were excluded from the study. Most patients had subfebrile body temperature, only 8 patients had a temperature of more then 38 °C, with chills, and 10 patients had normal body temperature.

### *IL-1*beta *administration*

Human recombinant IL-1beta [8], approved by the Russian National Pharmacological Committee for human use, was used for local treatment of 32 patients with pyogeneous bacterial lung abscesses at the Thoracic Surgery Clinic of the Military Medical Academy, St.Petersburg [7]. IL-1beta was administered directly to the abscess cavity through an Olympus bronchoscope instrumental channel, at a concentration of 10 ng/ml in physiological saline. Two to ten ml of IL-1beta solution (depending on the size of the abscess cavity) was administered, once a day for 7 days. Approximate abscess size was determined by radiological investigation. Just before local administration of IL-1beta, abscess cavities were washed with 30-50 ml of saline until all the pyogeneous material was removed and the washings became clear. A control group of patients received saline instead of IL-1beta solution. During the local treatment with IL-1beta, all patients continued conventional antibiotic treatment.

### *Sample preparation.*

Abscess fluid was obtained from the abscess cavity using a bronchoscope; before local treatment with IL-1beta, on days 3 and 5 during the IL-1beta treatment, just before the next administration, and then on days 10 and 20. Leukocytes were isolated by centrifugation in saline, and then in RPMI-1640 medium supplemented with 1% fetal calf serum, 2 mM L-glutamine, and 80 mug/ml gentamycin. Peripheral blood neutrophils were isolated on a Histopaque gradient [9]. Cell viability, determined using the trypan blue exclusion test was not less than 98% for blood neutrophils, and 80-90% for leukocytes isolated from abscess cavities.

### *Leukocyte functional activity*

The neutrophil chemotactic response to fMLP ($10^{-7}$ M) and recombinant human IL-8 (50 ng/ml) was studied in migration during an agarose assay [10]. One percent agarose (Sigma) in 199 medium, supplemented with 10% human pooled AB serum was used for these experiments.

Neutrophil phagocytosis was developed in Petri dishes using opsonized yeast cells [11], and expressed as phagocytic activity (percentage of phagocytes involved in phagocytosis) and phagocytic index (number of yeast cells engulfed by one phagocyte). Two hundred or more cells were counted microscopically. Spontaneous and 10 ng/ml phorbol myristate acetate (PMA)-induced neutrophil oxidative metabolism was studied using the tetrazolium nitroblue (NBT) reduction test [12] as described [8].

Neutrophil adhesion to the plastic surface was studied using spectrophotometry. Abscess fluid neutrophils were resuspended in Eagle's medium with 10% fetal bovine serum, at a concentration of $2 \times 10^6$ cells per ml in 96-well, flat-bottomed culture plates (Costar). Cells were incubated in the presence of 10 ng/ml PMA at 37 °C, in a $CO_2$-incubator for 1 hour, and then wells were washed 3 times with 0.9% NaCl to remove nonadherent cells. After this, plates were air dried, stained for 10 min with crystal violet in 30% methanol and washed with water. Fixed stain was dissolved with 2% SDS, and staining intensity was measured using a Bio-Rad 3550 microplate spectrophotometer at wavelength 595 nm.

### *Cytokine determination*

Quantitative determination of IL-1alpha, IL-1beta, IL-8 and TNF-alpha in abscess fluids and in sera of patients was performed using a home-made ELISA with monoclonal and polyclonal antibodies to human cytokines [13].

### *Cytology and immunocytochemistry*

Cell smears were fixed in 96 °C ethanol and stained with eosin-hematoxylin or Giemsa stain. The number of neutrophils, monocytes, lymphocytes and epithelial cells was determined after microscopic counting of no fewer than 200 cells in each smear.

For immunocytochemistry, cell smears were air dried and fixed in 4% paraformaldehyde. Immunocytochemical stainings were developed by conventional, indirect methods using firstly monoclonal antibodies to human cytokines and then biotinylated, anti-mouse antibodies and avidin-alkaline phosphatase complex [14, 15]. Smears were counterstained with hematoxylin. Three hundred cells were counted microscopically in each smear and cytokine positive cells were expressed as a percentage.

Statistical analysis was performed using a two tailed Student's t-test and Wilcoxon-Mann-Whitney's U-test.

## RESULTS

Local administration of IL-1beta applied application directly to the abscess cavities led to clearance of pathogens from the inflammatory site, a gradual decrease of leukocyte infiltration in surrounding tissues and a general decrease in local inflammation. Usual treatment with antibiotics was ineffective in these patients. Daily applications of IL-1beta for 7-10 days led to rapid clinical improvement that was accompanied by a reduction in the inflammatory process in the abscess cavities. X-ray examination 1 month after treatment showed that cavities had become smaller in size. The average in size decrease 1 month after the treatment was 3.8 cm in patients treated with IL-1beta compared to only 1.5 cm in the control group. After the IL-1beta treatment, abscess cavities had thin walls and almost no perifocal inflammatory infiltration was found in the surrounding lung tissue. Subsequent scar formation was observed within 1-2 months.

According to cytological analysis before IL-1beta administration, abscess fluids contained 90-94% neutrophilic granulocytes with small numbers of monocytes and lymphocytes. After IL-1beta administration, there was an increase in the monocyte count and a parallel relative decrease in granulocyte numbers (Figure 1).

In this study, we have analyzed the functional activity of leukocytes isolated from the abscess cavities of patients with a chronic inflammatory process, unsuccessfully treated with antibiotics for several days. According to results obtained before IL-1beta administration, leukocyte functional activity was significantly reduced compared to the same function in neutrophils isolated

from the peripheral blood of the same patient. However, the peripheral blood leukocyte functional activity of these patients as regards most parameters, did not differ from the control group of healthy donors (Table 1).

The functional activity of leukocytes isolated from the abscess cavities changed during the course of the IL-1beta administration. Migration to fMLP and to IL-8 began to increase on the 3rd day. It became significantly higher on day 5 and remained elevated on day 10 when IL-1beta treatment had already finished. Only on day 20 did leukocyte chemotaxis return to initial levels (Figure 2). Phagocytic activity increased as compared to the control level on day 3. It remained elevated on day 10, and then decreased, but by day 20 was still higher than the initial level (Figure 3). Neutrophil adhesion significantly increased on day 3 after the beginning of IL-1beta treatment, but then progressively decreased, and on day 20 was slightly lower than in the control group, however this was not statistically significant (Figure 4). Superoxide radical production by cells isolated from the abscess cavities and stimulated *in vitro* with PMA, increased on the 3rd day of the IL-1beta treatment but did not differ significantly from that of control group cells. However, termination of IL-1beta administration led to a decrease in PMA-induced superoxide production on day 10 that then reached initial levels, on day 20 (Figure 5). Superoxide production by unstimulated abscess fluid leukocytes did not significantly change during or after IL-1beta administration.

We simultaneously analyzed the changes in peripheral blood neutrophil counts and leukocyte functional activity in patients undergoing local IL-1beta treatment. No changes were found in the total peripheral blood leukocyte counts, in the composition of blood leukocyte subsets (especially neutrophilic granulocytes), or in the functional activity of peripheral blood leukocytes in the same assays, during and after local IL-1beta administration (data not shown). No signs of systemic inflammatory reactions were observed in any patient receiving local IL-1beta treatment (temperature elevation, flu-like symptoms, discomfort or other).

Abscess fluids obtained from patients before IL-1beta treatment contained proinflammatory cytokines: endogenous IL-1beta itself, TNF-alpha and IL-8. After local IL-1beta treatment, the concentration of IL-8 and TNF-alpha in the abscess fluids increased, while in nearly all patients endogenous IL-1beta production decreased (Figure 6).

Data on the immunocytochemical determination of some proinflammatory cytokines in abscess fluid cells from 12, randomly selected patients treated with IL-1beta, are summarized in Table 2. Using this technique, endogenous IL-1beta was only detected in the cytoplasm of macrophages, before and after administration of recombinant IL-1beta. IL-1alpha was detected in the cytoplasm of macrophages in half of the patients before treatment, but rarely in neutrophilic leukocytes. Already after the first IL-1beta administration into the abscess cavity, the number of neutrophils containing intracellular IL-1alpha increased significantly. This remained the same during the treatment, and also 3 days after treatment had finished. The number of IL-1alpha-positive cells began to decrease 7 days after the end of treatment. Intracellular IL-8 was detected mainly in macrophages before IL-1beta therapy. Only in a few patients it was found in neutrophils. After IL-1beta treatment, the incidence of IL-8 and the number of IL-8-producing cells in each patient increased. Increased TNF-alpha synthesis following local IL-1beta administration was identified both in macrophages and in neutrophils, and the numbers of TNF-alpha-producing cells changed significantly after IL-1beta treatment (Table 2).

## DISCUSSION

Clinical trials have shown that recombinant human IL-1beta can be used successfully for local treatment of lung abscesses when it is applied directly to the inflammatory site [7]. In this way, IL-1beta was administered at a dose of 10 ng/ml, which gave a high local concentration and allowed a strong immunostimulatory activity to develop. On the other hand, this dose was too small to give adverse systemic effects. This IL-1beta concentration was chosen as a result of our previous *in vitro* experiments with human recombinant IL-1beta, where it was shown that this dose was at the top of the dose-dependent curve, and that a further increase in the IL-1beta concentration did not give an increase in biological response [8]. The volume of IL-1beta solution (10 ng/ml) administered into the abscess cavity usually varied from 5 to 10 ml. So the maximal total dose of IL-1beta per patient did not exceed 50-100 ng. This means that even if all of the IL-1beta applied locally appeared in the systemic circulation, the dose would be approximately 1 ng per kg body weight. Such a small dose of IL-1 could not induce systemic reactions [6]. Local IL-1beta administration led to a rapid activation of defense reactions, elimination of pathogen and termination of the inflammatory reaction.

The main question of this investigation was how the local application of the proinflammatory cytokine IL-1beta, directly into the site of pyogeneous bacterial inflammation led to the resolution of the inflammation? The patients' spontaneous endogenous inflammatory reactions were insufficient to eliminate pathogens, even when high antibiotic doses were administered locally and systemically.

The curative effects of exogeneous IL-1beta probably result from the fact that IL-1beta significantly increases leukocyte functional activity, so that these cells can more effectively fight against infection. Local IL-1beta administration did not change the systemic defense reactions when the same functions were studied in peripheral blood.

Laboratory data have shown that administration of IL-1beta stimulated leukocyte activity and cytokine production, but this did not lead to an increase in inflammatory manifestations, cell infiltration, quantity of pus washed from the cavities, etc. even on the first days of treatment.

Neutrophilic leukocytes play the main role in the elimination of bacteria from the inflammatory site. Cytological analysis showed that the number of neutrophilic granulocytes in the abscess cavities was almost 90% of all cells, which is typical for active inflammation. Patients with abscesses were treated locally for about a week with antiseptics and enzymes, followed by washing of cavities with physiological saline to clear the abscess cavities before IL-1 administration. We believe that these procedures may explain why the viability of abscess cavity cells was relatively high.

Local administration of Il-1beta lead to an increase in adhesion, migration, free oxygen radical production and phagocytosis of abscess fluid neutrophils. All these functions were significantly less obvious in neutrophils from the abscess fluids compared to peripheral blood neutrophils from the same patient. This decrease may be connected to the functional paralysis of the neutrophils from the inflammatory site, which may be due to the chronic inflammation and depression of these functions. High dose antibiotics also can diminish leukocyte functional activity [16]. On the other hand, after an IL-1-induced elevation of functional activity, there was a decrease to the initial low levels that were seen prior to IL-1 treatment, in some cases levels were even lower than initial levels. Discontinuation of IL-1 administration or elimination of pathogen may have been

responsible for the observed decrease in activity. These low levels may be characteristic of leukocyte activity at the site of inflammation. Among the neutrophil functions studied, adhesion and superoxide radical production were the functions most rapidly stimulated by IL-1beta. Local IL-1beta administration stimulated migration of isolated leukocytes towards bacterial peptide fMLP and to recombinant IL-8. The IL-1beta-induced changes in migration towards fMLP were detected earlier than IL-8-induced changes. IL-1beta is known to increase chemotaxis of neutrophils stimulated with fMLP or IL-8 [17]. Pretreatment of neutrophils with IL-1beta was also shown to increase phagocytosis, production of myeloperoxidase and superoxide radicals [18, 19]. These functions are very important for clearance of pathogen from the inflammatory site.

The pure recombinant IL-1beta used in this study could not directly activate the neutrophilic granulocytes [8]. However, when injected into volunteers it was a potent stimulator of neutrophil functions [20]. This suggests that IL-1beta acts indirectly, probably through induction of other cytokines. IL-1beta is a potent stimulator of cytokine production by different cell types, particularly TNF-alpha [21]. Results obtained indicate that IL-1beta induced changes in proinflammatory cytokine production at the site of inflammation. The most profound changes were connected with the increase in IL-8 production, which is not only chemoattractant for leukocytes, but which can also activate them at the inflammatory site. IL-8 is known to be one of the most potent stimulatory molecules for various leukocyte functions including chemotaxis and respiratory burst [22, 23]. A significant increase in IL-8 production induced by IL-1beta at the inflammatory site may be responsible for the strong activation of all of the leukocyte functions studied. Probably not all of the stimulation can be connected to IL-8 induction. Other cytokines that were not studied in this paper, in particular some of the chemokine family, may be also involved in leukocyte activation.

We also detected an increase in levels of TNF-alpha which can also stimulate several neutrophil functions [17, 24]. Endogenous IL-1beta levels decreased after local administration of recombinant IL-1beta. However, exogenous IL-1beta can act synergistically with IL-8 by stimulation of neutrophil chemotaxis [25]. A decrease in endogenous IL-1beta concentration may appear due to the mechanism of negative feedback regulation of IL-1beta synthesis after administration of high doses of recombinant protein. This must be highly

specific for IL-1beta gene regulation because we observed a parallel increase in IL-1alpha synthesis by cells in the abscess cavities.

IL-1beta and IL-8 are probably the main markers of inflammation. High levels of these cytokines have been described in pleural fluid of patients with pleural empyemas, and their concentrations correlated with the intensity of the local inflammatory processes [26-28]. Before the start of the local IL-1beta administrations, we found high levels of endogenous IL-1beta in the abscess fluids. Nevertheless, inflammation was ongoing, and elimination of pathogen did not occur. High antibiotic doses were ineffective and did not change the clinical outcome in these patients. Local administration of recombinant IL-1beta in doses 100 times higher than that of endogenous IL-1beta levels, caused a push of the inflammation towards rapid and effective pathogen elimination. After this, inflammation decreased simultaneously with clinical improvement.

Immunocytochemical analysis revealed that different cells in the abscess cavities were involved in cytokine synthesis. Alveolar macrophages are considered to be the main source of cytokine production in the lung. Before IL-1beta treatment, macrophages were the main cells producing proinflammatory cytokines. After local IL-1beta administration, IL-1alpha and IL-8 were identified in the cytoplasm of macrophages and neutrophils. During recent years, IL-1beta-stimulated neutrophils have been shown to express mRNA and to synthesize different cytokines *in vitro* [29, 30]. When stimulated in a similar way, neutrophilic granulocytes produce much less cytokine than monocytes/macrophages. However, neutrophils are the main cell population at the inflammatory site and these cells can be an important source of cytokines at the local level.

According to the data presented, local IL-1beta administration can be used to treat bacterial inflammatory processes through its strong local immunostimulatory action. The mechanism of IL-1beta's local immunostimulatory activity is connected to the stimulation of neutrophilic granulocyte functional activity and proinflammatory cytokine production at the site of inflammation. Results obtained indicate that IL-1 may not only be a target in anticytokine strategies, but can also be used as a highly effective, immunotherapeutic drug when applied locally at adequate immunostimulatory dose levels.

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## Figures

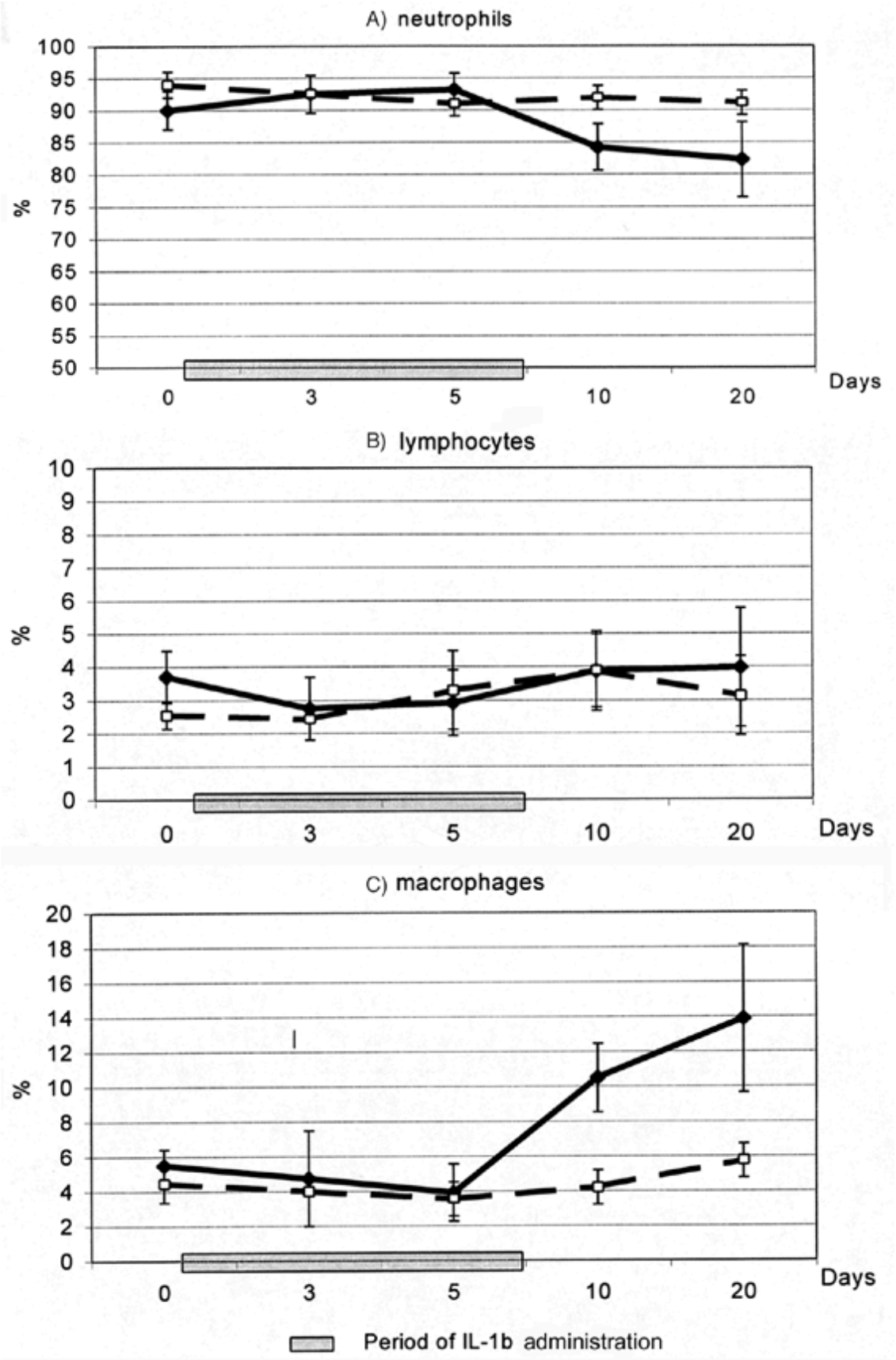



**Figure 1**
**Changes in abscess fluid cytology during local IL-1beta treatment.**

Cytological analysis of abscess fluid cells was performed before ("zero" point), during and after the course of local IL-1beta administration into abscess cavities. Plain line - neutrophils obtained from patients who had been treated with IL-1beta, dotted line - neutrophils obtained from patients during usual therapy (control group). Vertical bars represent S.D.

A: neutrophils, B: lymphocytes, C: macrophages.

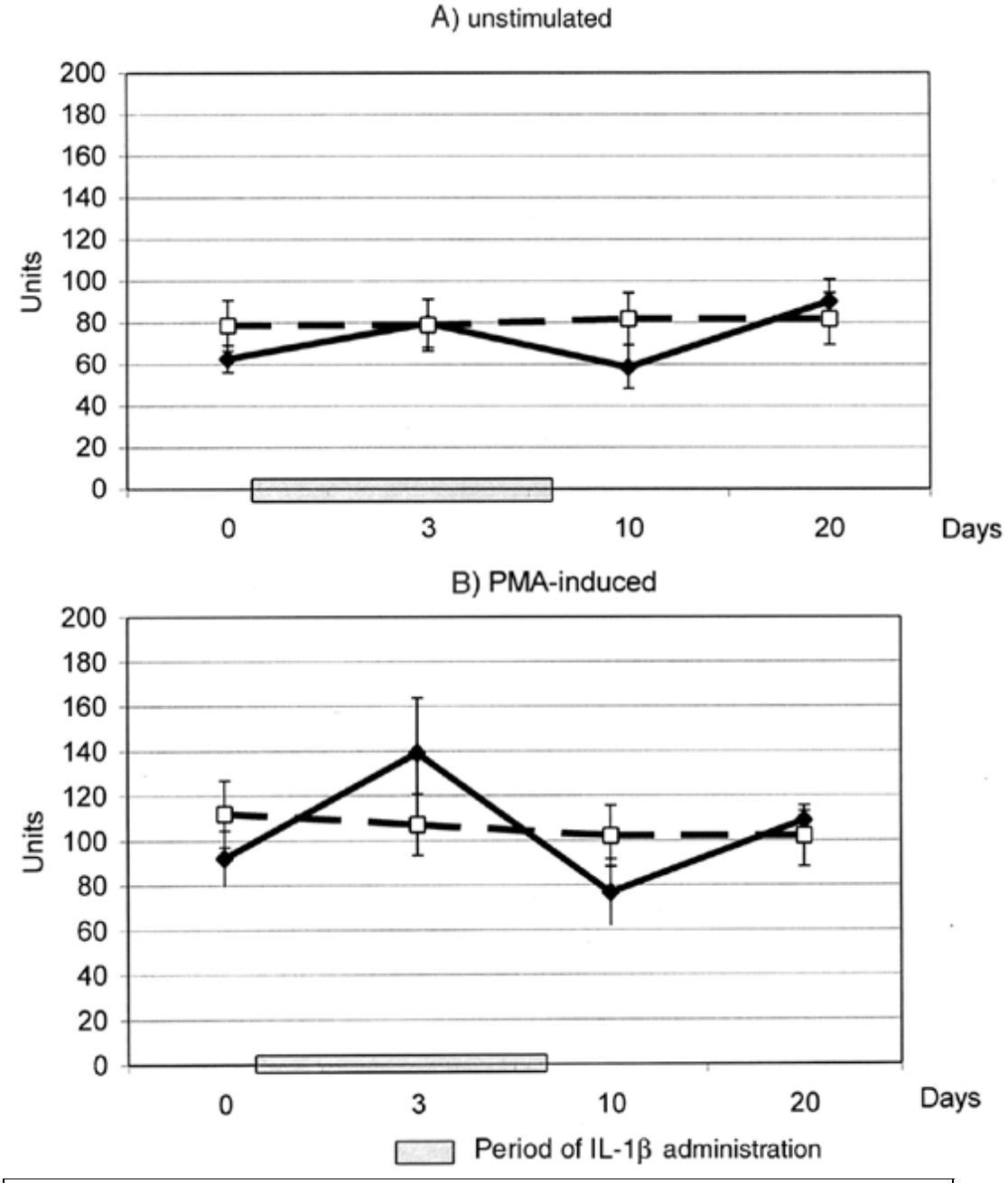


**Figure 2**
**Activation of neutrophil phagocytosis during local IL-1beta treatment.**

Abscess fluid-neutrophil phagocytosis with opsonized baker's yeast was studied before ("zero" point), during and after the course of local IL-1beta administration into abscess cavities. Plain line - neutrophils obtained from patients who had been treated with IL-1beta, dotted line - neutrophils obtained from patients during usual therapy (control group). Vertical bars represent S.D.

**A:** chemotaxis induced with fMLP ($10^{-7}$ M),

**B:** chemotaxis induced with rhIL-8 (50 ng/ml).

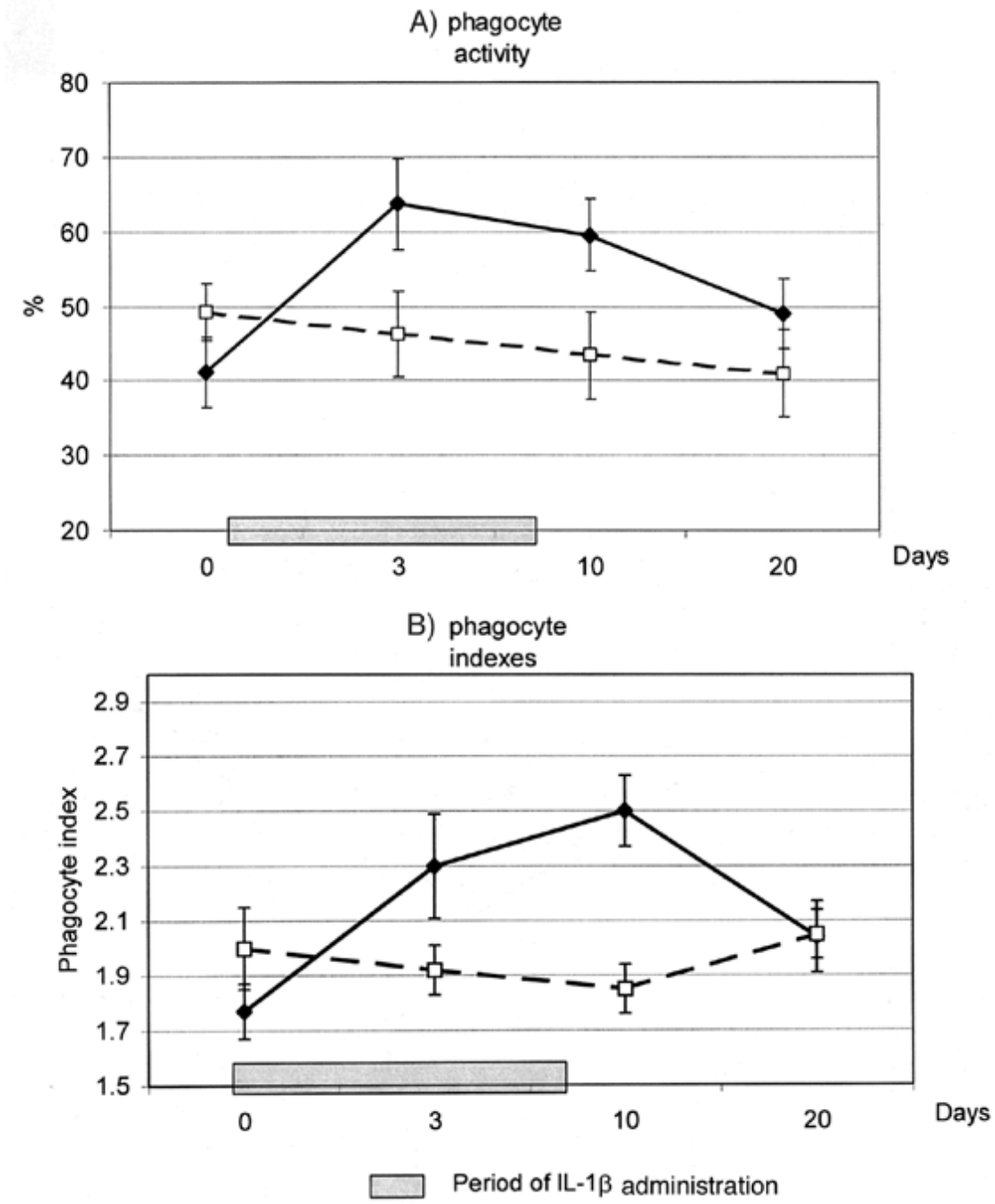

A) phagocyte activity
80
70
60
50
40
30
20
%
0
3
10
20
Days
B) phagocyte indexes
2.9
2.7
2.5
2.3
2.1
1.9
1.7
1.5
Phagocyte index
0
3
10
20
Days
Period of IL-1β administration

**Figure 3**
**Activation of neutrophil phagocytosis during local IL-1beta treatment.**

Abscess fluid-neutrophil phagocytosis with opsonized baker's yeast was studied before ("zero" point), during and after the course of local IL-1beta administration into abscess cavities. Plain line - neutrophils obtained from patients who had been treated with IL-1beta, dotted line - neutrophils obtained from patients during usual therapy (control group). Vertical bars represent S.D.

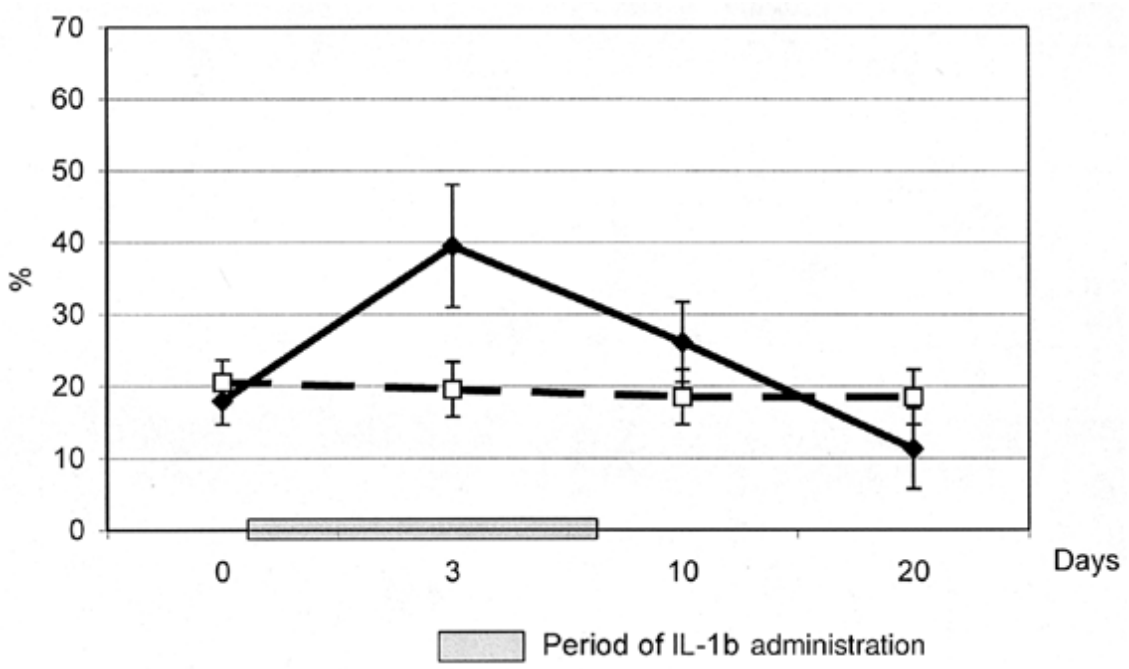



**Figure 4**
**Enhancement of neutrophil adhesion during local IL-1beta treatment.**

Abscess fluid-neutrophil adhesion to bottom of plastic wells was studied before ("zero" point), during and after the course of local IL-1beta administration into abscess cavities. Plain line of neutrophils obtained from patients

who had been treated with IL-1beta, dotted line - neutrophils obtained from patients in control group.

Vertical bars represent S.D.

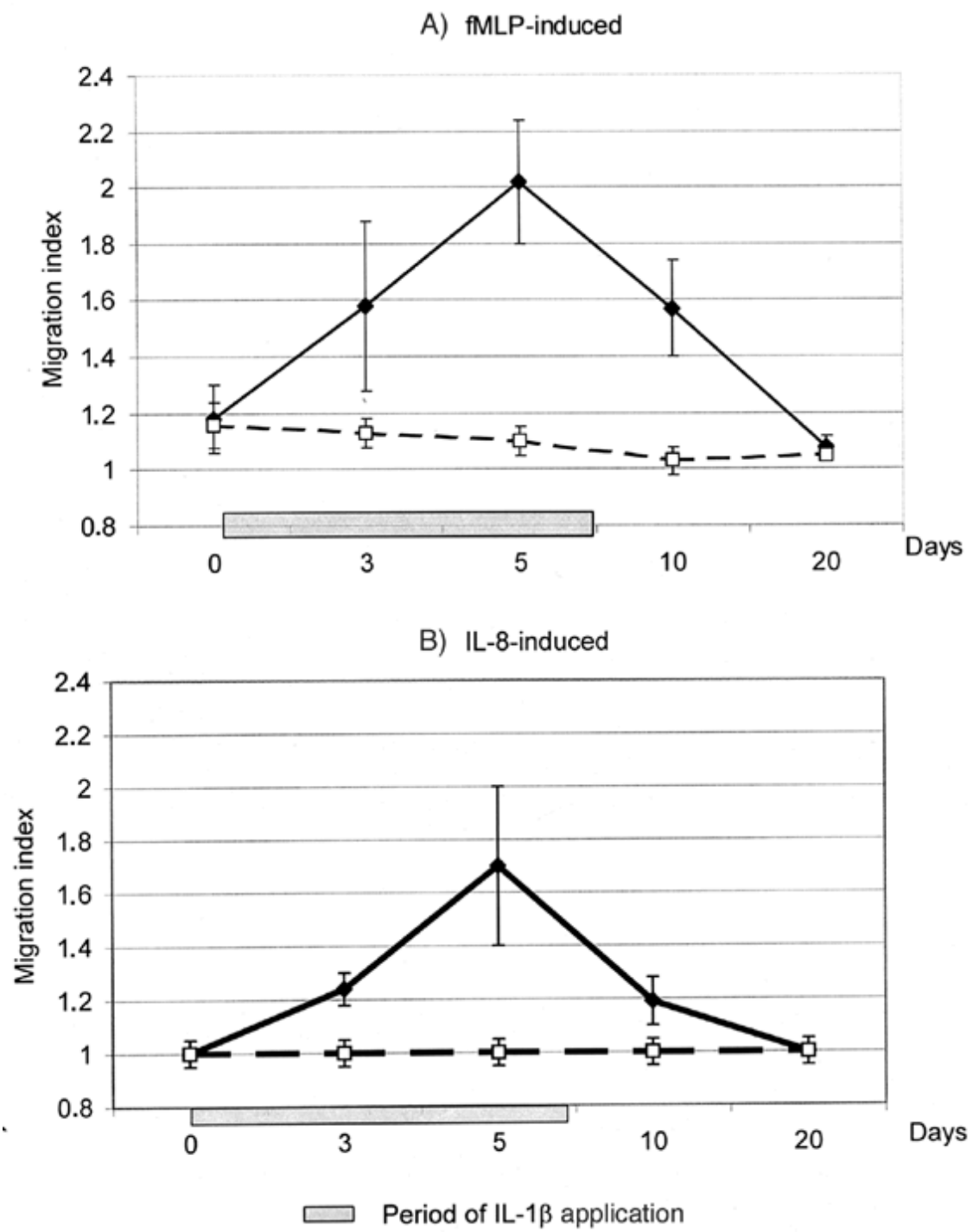



**Figure 5**

**Neutrophil superoxide production during local IL-1beta treatment.**

Abscess fluid-neutrophil superoxide production was studied with the NBT reduction test before ("zero" point), during and after the course of IL-1beta administration into abscess cavities. Plain line - neutrophils obtained from patients who had been treated with IL-1beta, dotted line - neutrophils obtained from patients in control group.

Vertical bars represent S.D.

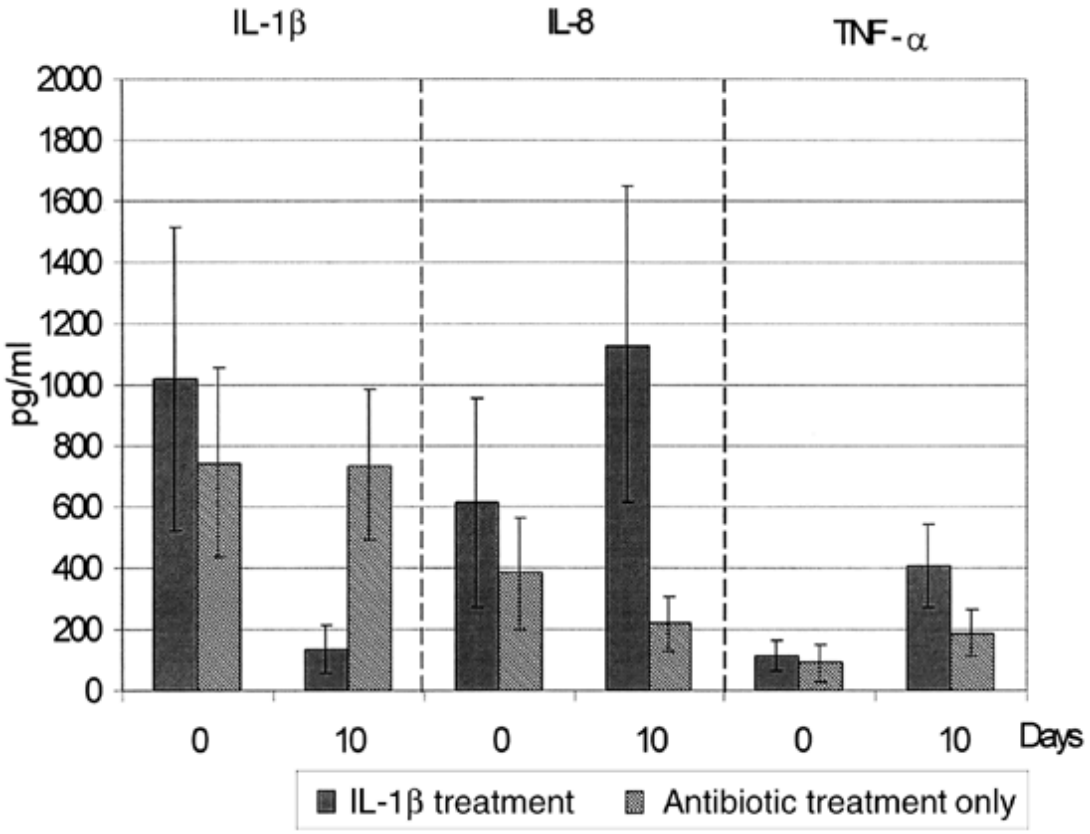



**Figure 6 during local IL-1beta treatment.**
Cytokine levels in abscess fluids were measured using ELISA, before ("zero" point) and after local IL-1beta treatment (10 days after the first IL-1beta administration). Asterisk shows that the difference between the data before therapy ("zero" point) is statistically significant, p

< 0.05. A cross shows that the difference from control mean value is statistically significant, p < 0.05.

**Table 1**
**Functional activities of peripheral blood neutrophils and abscess cavity neutrophils before local IL-1beta administration. Mean ± SD and range are shown (n = 32).**

| Groups of patients | Source of neutrophils | Chemotaxis migration indexes | | NBT-test, units | | | Adhesion % | Phagocytosis | |
|---|---|---|---|---|---|---|---|---|---|
| | | fMLP-induced | IL-8-induced | No stimulus | PMA-induced | Indexes | No stimulus | % | Phagocyte indexes |
| Patients with purulent lung abscesses | Peripheral blood | 2.2 ± 0.1 | 1.8 ± 0.1 | 115.0 ± 12.2 | 206.0 ± 21.7 | 1.8 ± 0.1 | 28.8 ± 2.5 | 70.7 ± 1.9 | 2.6 ± 0.2 |
| | Abscess cavities | 1.2 ± 0.1* | 1.0 ± 0* | 74.3 ± 7.8* | 104.0 ± 10.5* | 1.4 ± 0.1* | 18.1 ± 2.4* | 45.7 ± 2.9* | 1.9 ± 0.1* |
| Healthy volunteers | Peripheral blood | 2.6-2.8 | > 1.7 | 70.0-120.0 | 150.0-200.0 | > 1.2 | 30.0-40.0 | 60.0-80.0 | 2.0-3.0 |

*Differences between data for peripheral blood – and abscess cavity – neutrophil functions are significant, $p_t < 0.01$; $p_u < 0.001$. Normal range for data for donor's peripheral blood neutrophil function are shown in the line "healthy volunteers".

**Table 2**
**Influence of local IL-1beta application on changes in the numbers of cytokine-producing leukocytes in the abscess cavities. Data are expressed in percentage of positive cells among an appropriate cell type. Mean ± SD and ranges are shown (n = 12)**

| Cytokine | Cell type | Days | | |
|---|---|---|---|---|
| | | 0 | 3 | 10 |
| IL-1beta | Macrophages | 25.4 ± 11.2 (0-100) | 39.4 ± 12.7 (0-100) | 24.7 ± 7.6 (0-60.0) |
| | Neutrophils | 0 | 0 | 0 |
| IL-1alpha | Macrophages | 19.8 ± 9.4 (0-100) | 39.3 ± 11.0 (0-100) | 34.4 ± 7.4 (0-57.0) |
| | Neutrophils | 6.2 ± 4.7 (0-54.0) | 28.0 ± 8.5* (0-68.0) | 21.6 ± 8.8* (0-68.0) |
| IL-8 | Macrophages | 12.9 ± 6.2 (0-75.0) | 31.6 ± 13.5 (0-100) | 31.2 ± 8.5* (0-100) |
| | Neutrophils | 4.9 ± 3.1 (0-40.0) | 22.0 ± 8.7* (0-50.0) | 25.1 ± 6.1* (0-62.0) |
| TNF-alpha | Macrophages | 13.6 ± 9.8 (0-100) | 58.8 ± 18.9* (0-100) | 32.8 ± 13.9 (0-100) |
| | Neutrophils | 0 | 15.8 ± 9.7 (0-60.0) | 12.2 ± 6.4 (0-30.0) |

* Significant difference compared to day "0", p < 0,05.

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recombinant human interleukin-1beta, lung inflammation, abscess, neutrophils, cytokines.

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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.

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neutrophils, apoptosis, autoimmunity.

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## 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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