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Evaluation of leukocyte chemotactic function in patients with chronic sinusitis*

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SUMMARY

In this study the chemotactic activities of neutrophils and monocytes were evaluated in patients with chronic sinusitis as compared to a control group. Leukocyte chemotaxis was measured with a micropore filter method in blood samples taken from 26 patients with chronic sinusitis and 10 volunteers. A statistically significant impairment of leukocyte chemotaxis capacity in patients with chronic sinusitis was found. It is concluded that a functional disorder of leukocytes may play a role in the progression of the disease into a chronic state.

Key words: chronic sinusitis, leukocyte chemotactic function, neutrophils, monocytes

INTRODUCTION

Polymorphonuclear and mononuclear leukocytes play an important role in the immune system. These cells destroy micro-organisms by phagocytosis. Chemotaxis, the first step in this process, is the movement of a cell toward the source of a chemo-attractive substance (Howard, 1988; Wilkinson and Haston, 1988). Neutrophil chemotaxis is especially important in bacterial infections. Any condition depressing neutrophil functions may result in serious infections (Brown and Gallin, 1988). Chronic sinusitis is a common condition which occurs mostly in the maxillary sinus. It usually follows incomplete resolution of acute sinusitis. There are many factors which contribute to the chronic state of the disease. Mechanical or mucosal factors (i.e., septal deviation, nasal polyposis or allergic rhinitis with impaired drainage and reduced ventilation of the paranasal cavities) are known to increase the risk of long-standing inflammatory processes (Melen et al., 1986).

Although many investigations have been performed on the clinical, bacteriological and pathophysiological aspects of chronic sinusitis, only one report on monocyte chemotaxis in patients with chronic sinusitis has been published (Van de Plassche et al., 1988).

The aim of this study was to evaluate the *in vitro* chemotactic functions of peripheral blood neutrophils and monocytes in patients with chronic purulent sinusitis.

MATERIAL AND METHOD

Subjects

The patient group comprised of 26 patients, 14 males and 12 females, who were diagnosed as having chronic maxillary sinusitis at the Otorhinolaryngology Department of Hacettepe University Medical School. Their ages ranged from 14 to 52 years (mean: 23.3 years). Laboratory studies were performed in the research laboratory of the Paediatric Haematology Unit of the same faculty. All patients who were included in this study satisfied the following criteria:

(1) nasal congestion or oedema, nasal or postnasal purulent discharge, radiologically cloudiness of sinuses or mucosal thickness of >5 mm, and no relief of these findings with a minimum of three courses of conservative treatment consisting of amoxycillin and clemastine suspensions for 10 days, and xylometazoline nose drops for five days.
(2) No co-existing congenital or systemic diseases.
(3) No patients with allergic history, allergic findings or with eosinophilia in the peripheral blood count.
(4) No medication for sinusitis in the previous month.
(5) The patients did not have any intervention (e.g. blood transfusions, general anaesthesia, steroid intake, radiotherapy, et cetera).
(6) Normal peripheral blood findings.

Ten completely healthy individuals (hospital staff members) were selected as the control group. Their ages ranged from 23 to 40 years (mean 29.3 years); of the healthy subjects, six were males and two were females.

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Measurement of chemotaxis

Neutrophil and mononuclear leukocytes were isolated from venous blood according Boyum's method (Boyum, 1968; Recalde, 1984). This techique provided approximately 36% non-specific esterase (NSE)-positive cells present in the Ficoll-Hypaque interface as stained according to the technique described by Yam et al. (1970). Chemotactic responsiveness towards 2% casein was determined with the use of a modified Boyden-Chamber technique using 3-μm (Sartorius SM 11301-0706) and 8-μm (Sartorius SM-11301-090G) cellulose-nitrate micropore filters for neutrophils and monocytes, respectively. The upper chamber, consisting of a LP3 tube top, contained 0.3 ml of the cell suspension in standard minimal essential medium (MEM) with 2×10^6 cells/ml. The lower chamber contained 2% casein in MEM for chemotaxis and MEM only for random migration. The upper and lower chambers were separated by a 3-μm or 8-μm millipore filter. After incubation for 60 min at 37°C the filters were fixed in propanediol and stained with haematoxylin. Filters were cleared in cedar wood oil, mounted on glass slides and examined with a bright-field microscope at a magnification of ×40. The distance of migration was recorded using the "leading front" technique (Zigmond and Hirsch, 1973) and expressed in μm. Blood samples of one or two patients and one control were tested in each experiment. Statistical analysis was performed and the difference between the patient and the control group's results were tested by the t-test for unpaired groups.

RESULTS

Neutrophils from patients with chronic sinusitis exhibited significant impairment of random migration and casein-stimulated chemotaxis *in vitro* (Table 1). Mean neutrophil random migration for 26 patients was 17.38±2.60 μm as compared to 23.62±3.55 μm for 10 controls ($t=4.48$; $p<0.005$). In response to 2% casein, mean values of neutrophil chemotaxis were 57.28±12.86 μm and 68.06±10.58 μm, respectively ($t=2.26$; $p<0.05$).

Table 1. The relationship between neutrophil random migration (RM) and chemotaxis (CTX) values of the patient and control groups.

group	RM	CTX
patient	17.39±2.60	57.28±12.86
control	23.62±3.55	68.06±10.58
	$p<0.05$	$p<0.05$

Table 2. The relationship between monocyte random migration (RM) and chemotaxis (CTX) values of the patient and the control groups.

group	RM	CTX
patient	14.19±1.47	31.21±8.82
control	20.00±2.70	45.15±10.06
	$p<0.05$	$p<0.05$

Chemotaxis and random migration values of monocytes in the patient group showed statistically significant differences compared to the same parameters of the control group (Table 2). Mean monocyte random migration for 26 patients was 14.19±1.47 μm compared to 20.00±2.70 μm for 10 controls ($t=6.42$; $p<0.05$). Mean monocyt chemotaxis was 31.21±8.82 μm and 45.15±10.06 μm, respectively ($t=3.84$; $p<0.05$).

DISCUSSION

In this study, leukocyte chemotactic functions in patients with chronic maxillary sinusitis have been investigated. Leukocyte chemotactic activity may be depressed in some chronic and recurrent infections (Howard, 1988; Miller, 1975). The cause of this decline may be the presence of inhibitory substances originating from the patient's serum or a defect in lymphokine release due to disturbance of T-cell functions (Smith et al., 1972; Clark et al., 1973; Miller, 1975). It is known that during the course of recurrent infections, such as lower respiratory tract infections, neutrophil chemotaxis is decreased (Smith et al., 1972; Soriano et al, 1973; Cazolla et al., 1989). Atopic dermatitis (Furukawa and Altman, 1978), AIDS (Smith et al., 1984), and various other malignancies (Walter et al., 1986) may cause an impairment in monocyte function.

There are very few studies in the literature in which the various aspects of the immune system have been investigated in patients with chronic upper respiratory tract (URT) disease, especially sinusitis. Drexhage et al. (1983) have reported that there are certain abnormalities in the cellular immune system (especially against *Haemophilus influenzae*) of patients with chronic purulent infections of URT. In their study, all patients have chronic recurrent purulent infections of the URT colonized by predominantly *Haemophilus influenzae* and *Streptococci.*

Toppozada (1988) reported that there was a partial impairment of cellular immunity in patients with chronic maxillary sinusitis. In his study, the lymphoblastic transformation was higher in control *Streptococcus*-free patients than in patients with chronic maxillary sinusitis. He also found the same findings in a previous study that had been done in patients with chronic streptococcal tonsillitis (Toppozada, 1988). Thus, deficiency of the cellular immune response can be a contributory factor to deterioration of sinusitis. It may also tend to become chronic.

Van de Plassche et al. (1986) have also investigated the role of cell-mediated immunity to commensal micro-organisms in patients suffering from unexplained chronic purulent rhinosinusitis. They have found micro-organism-specific T-cell defects in a significant number of these patients. They also reported that the chemotactic activity of monocytes was defective in about 60% of patients with chronic purulent rhinosinusitis (Van de Plassche et al., 1988). A concordance between the presence of decreased monocyte chemotaxis and defective T-cell function was established.

The presence of factors in serum capable of inhibiting the function of both lymphocytes and monocytes has been reported, particularly in malignancies (Tan et al., 1986). Factors of $M_r<25$ kD capable of inhibiting Interleukine-2 production and mono-

cyte chemotactic responsiveness were detected in serum of patients with purulent chronic rhinosinusitis (Van de Plassche et al., 1988). These factors appeared to share structural homology with the feline and murine retroviral transmembrane protein p15E, as could be shown by absorption studies using monoclonal antibodies to this immunosuppressive viral protein.

As it is defined in our patients, many of these patients clearly indicate that they had suffered from a virus infection just prior to the onset of their chronic upper respiratory tract complaints. The origin of the chemotactic inhibitory substances (p15E-related factors) in chronic sinusitis is speculative. Its presence might be related to an exogenous infection with an as yet unknown retrovirus possessing envelope substances that share structural homology with p15E. On the other hand, it has been reported that p15E-like factors can be endogenously produced by lymphocytes, monocytes and squamous epithelial cells, overlaying areas of inflammation, and in normal thymic epithelial cells (Van de Plassche et al., 1988).

In our study, we demonstrated that neutrophil and monocyte chemotaxis and random migration ability is lowered significantly in patients with chonic sinusitis when compared to the control group. It seems likely that some toxic substances which may emerge from the interaction of infectious agents and the target tissue in the sinus mucosa, may produce a chemotactic inhibitory action on neutrophils and monocytes. In addition, in chronic sinusitis there may be a suppressive action of these substances in the cellular immunity system towards specific microorganisms.

Tas et al. (1990) reported the beneficial effects of the thymic hormone preparation, thymostimulin, in patients with defects in cell-mediated immunity and chronic purulent rhinosinusitis. According to their findings the clinical improvements were accompanied by a better performance of the cell-mediated immune system.

Chronic sinusitis is a frequently diagnosed disorder, but some of the patients with this illness are not always cured with medical and surgical therapy. Detailed studies on chronic sinusitis have helped to determine many local and systemic factors which contribute to the emergence and development of the illness. Especially the cellular immune system and bactericidal functions of phagocytes, including chemotaxis, have not been investigated sufficiently in patients with chronic sinusitis, and important clues have been found in the few studies on this topic.

In our study, we found a statistically important impairment of leukocyte chemotactic ability in patients with chronic sinusitis. It may play a role in the progression of the disease into a chronic state in patients with sinusitis. However, this is just one aspect of the cellular immunity system and in order to be more precise we have to investigate its other functions. In this study we have taken into account only the systemic effects. Although the immunological behaviour of leukocytes of blood and tissues are the same (Howard, 1988), no study has been performed on the local effect of bacterial flora in rhinorrhoea and chemotaxis. We think this new investigation will further our knowledge on chronic sinusitis.

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