CD and HLA antigen expression by immunocompetent cells from peripheral blood and granuloma in patients with scleroma

LEONID PETROVICH TITOV and ZHANNA GEORGIEVNA SHABAN

Department of Microbiology and Immunology, Medical University, Minsk, Belarus

Abstract

T- and B- lymphocytes immunophenotype, expression of activation and monocyte's markers in patients with scleroma were studied. The dependence of these parameters upon the disease clinical form was also established. Monoclonal antibodies to CD3, CD4, CD8, pan-B (CD72), CD14, CD38 and HLA-Dr antigens, antibodies to surface immunoglobulins G, A, M were applied. The changes of immune status of patients with scleroma, which can cause the development of local mononuclear-infiltrative inflammation were revealed. Activation markers expression on peripheral blood and granuloma mononuclears, pan-B+ (CD72+) cells content and expression of surface Ig G, A, M were decreased, especially in patients with atrophic form of scleroma. CD8+ cells amount was increased, especially in patients with cicatrix and infiltrative forms. The increase of CD14+ cells content was also observed in the case of cicatrix scleroma form. The CD4/CD8 ratio, content of CD3+ and CD4+ lymphocytes were decreased. The increase of CD38+ cells number was noted in all scleroma forms, was especially marked in patients, suffering from infiltrative form of scleroma.

Key words: *scleroma, cells markers (CD3, CD4, CD8, CD72, CD14, CD38, HLA-Dr, sIgG, sIgA, sIgM)*

(Centr Eur J Immunol 2004; 29 (2): 44-50)

Introduction

The scleroma is a primary chronic infection disease of the upper respiratory tract of human. The etiologic agent of the scleroma is *Klebsiella rhinoscleromatis.* This pathogen is usually localized in cells. There are some endemic foci in the world: Central America, countries from the Torrid Zone, countries of Western and Eastern Europe [7, 13, 20, 27, 31, 37]. Isolated cases of the disease have also been reported in other areas. In the conditions of the decrease of socio-economic level of life and the increase of immigration in populations, the problem of the disease spread may become a major international issue [2, 3, 17, 25, 38]. The scleroma incidence rate is probably higher than reported due to the wide scope of the disease manifestations and to the complex diagnostics. Infiltrative forms of the disease are easily diagnosed. On the contrary, atrophic forms are frequently misdiagnosed as other diseases.

Prolonged interaction of an immune system with the pathogen determines DTH development in scleroma. It is morphologically expressed as granuloma formation. The granulomatous response contributes to the isolation of the microorganism and detection of its antigens from the surrounding tissues; it also slows down their spread and damaging effect on the host tissues. At the same time, the cellular reaction hampers microbe antigen resorption and inhibits immunologic reaction, which is not strong because of microbe low antigenicity. The granuloma formation provides the morphological basis for local immune processes which are central in the determination of scleroma. The activity of granulomatous response may vary from the maximum in the infiltrative form of scleroma to slightly pronounced in atrophic form. The mechanism of clinical manifestation development in this disease is still an open question. In classical cases (infiltrative forms) the

Correspondence: Titov Leonid, Head of Department of Microbiology and Immunology, Byelorussian State Medical University, Dzerzhinsky prospect, 83, Minsk, Belarus, 220116. Tel.: 80172-643050, 80172-755623, fax: 80172-643267, e-mail: leonidtitov@tut.by

disease can result in remission with the resorption of infiltrates, and atrophy or cicatrix formation in the focus. But there are also atrophic forms of the disease with the lack of infiltrates in the anamnesis. The number of such cases is increasing. These are just mild cases (they are frequently beyond a physician's special attention), that maintain the epidemic process [12, 15, 16, 17, 29].

The leading role of the peculiar features of individual reactivity is widely recognised in the development of allergic diseases. Perhaps, individual immunoreactivity predetermines scleroma course. The determination of types of immunoreactivity under different pathologic conditions is the focus of interest from the practical and theoretical viewpoints. But till now the information about functioning of T-cell mediated immunity is very limited. The granuloma development to a large extent depends upon the T-lymphocyte subsets reactivity, as the main source of cytokines for the macrophage stimulation [22, 23, 28, 33-36]. That's why studies of cells, which participate in DTH formation and manifestation in scleroma, draw keen scientific interest. Our investigations were targeted the phenotype of immunocompetent cells from peripheral blood and granuloma, as well as the expression of activation markers and surface immunoglobulins on B-lymphocytes in patients with scleroma.

Materials and methods

Subjects. 54 patients diagnosed of upper respiratory tract scleroma were examined (19 – male, 35 – female, aged 26-72). The period of illness in patients was 10-50 years with an average of 30 years. The atrophy of mucous in upper respiratory tract was noted in 15 patients, cicatrix changes in 21 patients, infiltrative changes in 18 patients. The immunophenotype of granuloma cells was investigated in 4 patients with the infiltrative form of scleroma. Forty healthy donors were selected as controls.

Cell preparation. Peripheral blood mononuclear cells were separated from heparinized blood samples using a Ficoll-verographine density gradient and washed twice in 0.9% PBS. Mononuclears consisted of lymphocytes (85-90%) and monocytes (10-15%). Cell viability was determined by blue exclusion. It constituted 90-95%.

Phenotype analysis of cells. The investigations were carried out with indirect immunofluorescence method. 40 mcL mouse monoclonal antibodies against T-cell markers: CD3, CD4, CD8; B-cell marker pan-B(CD72); monocyte marker CD14 (Research Immunology Institute, Moscow) and activation markers CD38 and HLA-Dr antigens (Oncology Centre, Moscow) were added to 100 mcL of mononuclear cells suspension ($1x10^6$ cells/mL). After incubation for for 45 minutes at 4^0C cells were washed twice in 1 mL RPMI-1640 medium (GIBCO) with 5% fetal calf serum (Research Institute of Immunology and Epidemiology, Minsk, Belarus) and treated with 20 mcL fluorescein-labeled $F(ab')_2$ fragment of rabbit immunoglobulin to mouse immunoglobulins (Oncology Centre, Moscow). The cells were suspended and incubated at $+4^0$C for 30 minutes. They were washed as described above and resuspended in 0.5 mL PBS with 0.5% paraformaldehyde for flow cytometry. The expression of surface immunoglobulins A, G, M, was studied by means of a direct immunofluorescence method with the application of luminescent rabbit antiglobulin antibodies (Research Institute by Gamaleya, Moscow). Stained lymphocytes were analysed by FACScan flow cytometer (Becton). There were 98% lymphocytes and 2% monocytes in the analyzed cell population. The absolute number of cell subpopulations was counted.

Statistics. The method of percentiles was applied in the statistic estimations [24]. The reliability of results was evaluated by Fisher method (P_F). Analyses were performed using STATGRAPH software as described in the text.

Results

Immunophenotype of peripheral blood T-lymphocytes. The relative content of CD3+ lymphocytes in patients with scleroma was 42.6 (36.6-65.6)% and it was lower as compared to the controls - 49.5 (26.85-68.05)% (P_F<.01). The CD4:CD8 ratio tended to decrease due to lowering of relative content of CD4 lymphocytes: 37.6 (27.2-45.7)% against 40.85 (29.2-53.9)% in controls (P_F<.01). Absolute CD8+ lymphocytes content in patients with scleroma was 0.7 (0.48-0.93)$x10^9$/L and was higher as compared to the controls 0.475 (0.36-0.66)$x10^9$/L (P_F <.05).

The relative CD3+ lymphocytes content in patients with atrophic scleroma was also still lower, than in controls. The percentage of CD3+ lymphocytes in patients with the infiltrative form was higher than in other groups of patients examined (P_F<.05 in atrophic form and P_F>.05 in cicatrix form). The absolute CD3+ lymphocyte content in atrophic and infiltrative forms was higher than in controls (table 1).

The relative CD4+ lymphocytes content in atrophic form turn seemed to be lower when compared to other groups of patients examined. The absolute CD4+ lymphocytes content in the infiltrative form of scleroma was higher than in controls (table 1) and higher than in patients with the cicatrix form (P_F<.01). The relative and absolute contents of CD8+ lymphocytes in patients with cicatrix and infiltrative forms of scleroma were higher both than in control group and group of patients with atrophic form (P_F<.01 in the two cases). The absolute number of CD8+ lymphocytes was also higher in atrophic scleroma in comparison with control parameters (table 1).

The median CD4:CD8 ratio in groups of patients tends to decrease in comparison with controls. Groups of patients with infiltrative and cicatrix scleroma forms were heterogeneous on this parameter (fig. 1).

Table 1. Immunologic phenotype of T-lymphocytes in patients with scleroma

Percent of cells	N	Percentiles			Stand. deviations	P_F*
		25	50	75		
CD3+						
controls	32	26,85	49,5	68,05	45,434	
atrophic form	10	36,6	42,2	49,6	14,512	>.05
cicatrix form	18	37	41,8	65,3	38,2	>.05
infiltrative form	15	35,8	54,4	66,5	53,961	<.01
CD4+						
controls	34	29,2	40,85	53,9	15,391	
atrophic form	13	22,6	32,3	44	30,132	>.05
cicatrix form	19	28,2	39,3	45,9	14,132	>.05
infiltrative form	17	34,1	42,7	50,9	14,258	>.05
CD8+						
controls	34	24,1	30,85	34,2	2,574	
atrophic form	13	24,8	27,4	29,1	1,217	>.05
cicatrix form	19	26,1	33,1	44,6	15,438	>.01
infiltrative form	17	26,1	31,2	38,5	7,767	<.01

*NOTE. *patients compared with control*

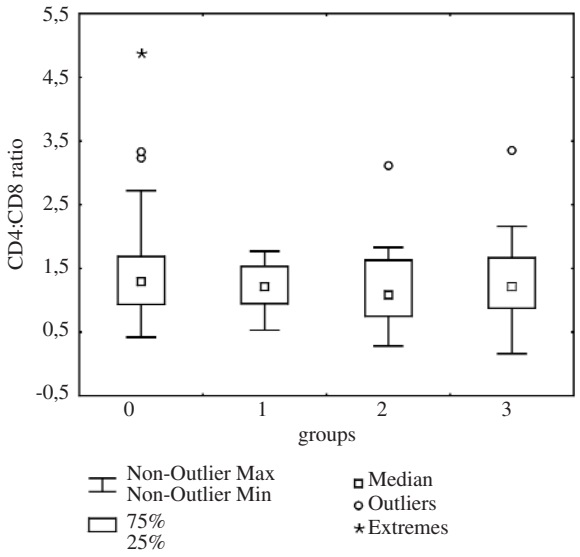

Fig. 1. CD4:CD8 ratio in patients with different clinical forms of scleroma: 0 – controls, 1 – atrophic form, 2 – cicatrix form, 3 – infiltrative form

Immunophenotype of peripheral blood B-lymphocytes. The relative B-lymphocyte content was 13.2 (10.1-21.7)% in patients with scleroma and it was lower, than in controls - 17.55 (11.0-25.3)% (P_F<.05). The percentage of B_u+ cells in patients with scleroma was lower than in controls, the relative content of B_γ+ and B_α+ cells was characterized by the same tendencies in their changes. The absolute content of B-lymphocytes, which express surface immunoglobulins of G, A, M isotypes was lower in patients with scleroma in comparison with the controls (table 2).

A certain decrease in the number of pan-B+ cells in median was determined in patients with all forms of scleroma as compared to the controls. This decrease was expressed the most in atrophic forms of the disease (table 2).

Expression of monocyte and activation markers. The relative content of CD14+ cells in patients with main clinical forms of scleroma showed the tendency to decrease in comparison with the controls (fig. 2). The relative and absolute contents of CD14+ cells on the level of percentiles 75-90 in patients with cicatrix form was higher than in patients with infiltrative scleroma (P_F<.05 in both cases).

The relative content of HLA-Dr+ cells in patients with scleroma was 18.8 (13.1-25.6)% and tended to decrease as compared to control 24.5 (17.1-31.3)% (P_F>.05). The decrease in HLA-Dr antigen expression was observed on the level of all percentiles.

In median the tendency to decrease of HLA-Dr antigen expression (in comparison with controls) was registered. This decrease was expressed the most in atrophic scleroma, and there was the highest variability of indexes within the group. The difference in HLA-Dr antigen expression by cells in patients with infiltrative and cicatrix forms were not significant (table 3). Only on the level of percentiles 84-90, the growth in the absolute count of HLA-Dr+ cells was determined in patients with infiltrative form as compared to cicatrix form and controls.

Table 2. Immunologic phenotype of B-lymphocytes in patients with scleroma

Percent of cells	N	Percentiles			Stand. deviations	P_F*
		25	50	75		
Pan-B+						
controls	34	11	18	25	5,159	
atrophic form	12	6	12	22	18,797	>.05
cicatrix form	19	11,2	15	24	7,275	>.05
infiltrative form	17	11,2	13	21	4,464	>.05
Bγ+						
controls		11,2	23,25	35,3	250,51	
scleroma patients	8	14,7	17,5	26,35	14,531	>.05
Bα+						
controls	8	19,2	27,1	35	107,67	
scleroma patients	7	8,4	18,4	19,3	14,507	>.05
Bμ+						
controls	8	10,2	29,65	49,1	6,526	
scleroma patients	8	7,35	12,2	17,9	11,917	<.01

*NOTE. *patients compared with control.*

The relative and absolute contents of CD38+ cells in patients with scleroma were 13.3 (8.9-25)% and 0.26 (0.18-0.56)x10^9/L and they were higher than in controls: 6.75 (4.2-8.9)% and 0.12 (0.08-0.18)x10^9/L (P_F<.01 in both cases). The relative and absolute contents of CD38+ cells were considerably higher in patients with all forms of scleroma than in controls (fig. 3).

Immunophenotype characteristics of scleroma granuloma cells. Immunophenotype characteristics of cells, isolated from infiltrates after bronchoscopy are presented in table 4. In two cases we were unable to isolate cells from tissues obtained from upper respiratory tract by bronchoscopy. Perhaps, in these cases cicatrices were extracted, i.e. connective tissues, that substituted infiltrates, in which mononuclear cells were absent.

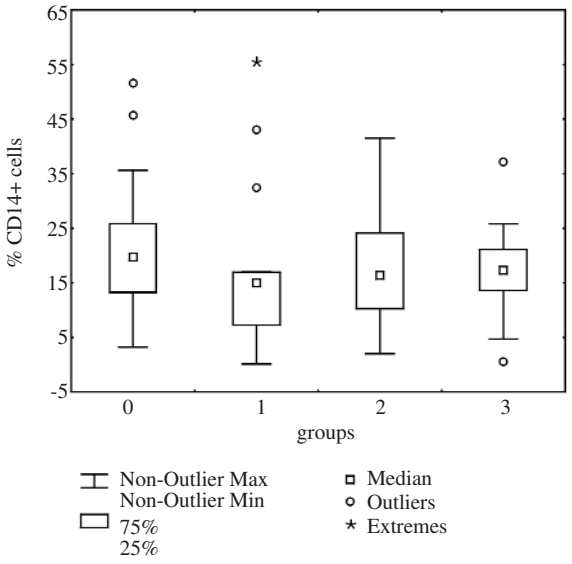

Fig. 2. Relative content of CD14+ cells in patients with different clinical forms of scleroma: 0 – controls, 1 – atrophic form, 2 – cicatrix form, 3 – infiltrative form

Discussion

It is well known that the cellular immunity damage may influence dramatically the course of the diseases with chronic inflammation process [1, 4, 8, 11, 14, 18, 19, 26]. As regards its general biologic essence, inflammation is a phenomenon of protective character, though acute hyperergy reactions and chronic inflammation often are accompanied by considerable tissue injuries and are not favourable for the host. It is a matter of cell-mediated pathology - DTH [7, 9, 25, 27, 36]. Though there are limited and often contradictive data on functions of separate cell-mediated immune mechanisms in patients with scleroma, immunologic and genetic mechanisms of resistance are assumed to determine the disease course [6, 30, 32, 33].

Genetic restriction of cell interactions in DTH response may be realized both on the level of single subpopulations of immunocompetent cells and on the level of cell interactions. MHC molecule expression on antigen-presenting cells (macrophages, dendrites cells) is a critical stage in the antigen identification and has special significance for proliferation and differentiation of immune cells, and for determination of profile

Table 3. HLA-Dr antigen expression in patients with scleroma

	atrophic form (n=8)	cicatrix form (n=15)	infiltrative form (n=15)	controls (n=34)
Percentiles		**HLA-Dr, %**		
25 percentile	8,55	15,9	15,6	17,1
50 percentile	14,3	21,4	18,1	24,5
75 percentile	19,25	29,5	26,2	31,3
Stand. deviations	12,258	10,59	6,433	5,087
P_F*	>.05	>.05	>.05	

*NOTE. *patients compared with control.*

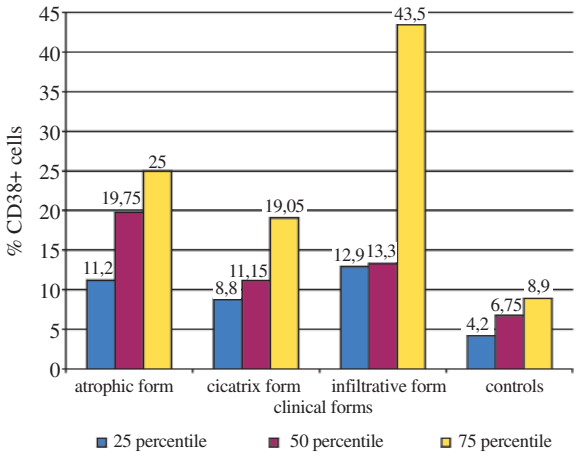

Fig. 3. CD38 antigen expression in patients with different clinical forms of scleroma; ** P_F <.01 in comparison with controls

of regulatory factors as well [22, 23]. As it was reported by other authors, HLA-Dr antigen is present on B-lymphocytes, monocytes, dendrites cells, activated T-lymphocytes, and is absent or slightly expressed on resting cells; cell stimulation leads to the increase of this antigen expression [34]. The expression of the late activation marker, HLA-Dr, on mononuclears in patients with scleroma is lowered.

The decrease of HLA-Dr antigen expression in the presence of primary chronic infectious inflammation process in respiratory tract in scleroma gives clear evidences for the pathogeneses defect in immune system on the stage of activation. Correlation analysis demonstrates, that in patients with scleroma HLA-Dr antigen is expressed mainly by B-cells.

T- and B-lymphocytes reticulates via lymph and blood between lymph nodes, spleen, Payer's patches and similar secondary lymphoid tissues (SLTs) that encounters between lymphocytes and antigens take place and immune reactivity develops. Antigens must get to SLTs from sites of pathogen entry. Klebsiella rhinoscleromatis is an intracellular pathogen. It is possible that bacteria localized in phagocytes-macrophages or dendrite cells (DC). DC are present in STLs and in most other tissues as a trace population and are thought to be involved in the initiation of T-cell response of all kinds [5]. DC act as tissue sentinels bringing antigens in processed form to the areas where T-cells congregate [10].

The immune response presupposes the co-operation of cells, which are characterized by a certain phenotype and fulfil their own functions. Cell integration in the process of immune response promotes the implementation of regulatory and effectors functions [23, 34]. It is well known that in the diseases with DTH formation, T-system immunity is often damaged. The intensity of DTH development is interconnected with severity of the disease and is regulated by T-suppressors and T-helpers, which are activated and inhibited to various degrees. In its turn, the degree of T-suppressors activation and T-helpers inhibition is connected with the antigen dosage. Course of disease depends as well on Th1 or Th2 prevalence [22, 36].

Table 4. Cell characteristics in sclerome granuloma

Examined patients	Cells phenotype, %						
	CD3	CD4	CD8	CD5	PAN-B	CD14	HLA-Dr
Patient K., 61 age	13,3	7,1	5,9	9,1	1,9	1,2	ND
Patient Ch., 72 age	0,4	ND	1,9	0,9	1,4	2,4	4,1
Patient Ch., 26 age	9,3	0,4	ND	14,9	ND	0,7	7,5
Patient L., 30 age	20,7	22,3	32,6	59,1	16,6	24,2	16,3

NOTE. ND - no determined

In scleroma immunopathogenesis, the increase of CD8+ lymphocytes number is a phenomenon of special importance. It is observed when the number of CD3+ and CD4+ lymphocytes decreases, and it determines the lowering of CD4:CD8 ratio. CD8+ cells subpopulation is recognized to be heterogeneous: it comprises cytotoxic, suppressor, NK cells. In patients with scleroma the relative and absolute content of CD38+ cells is increased. CD38 antigen is chiefly expressed by activated T-cells and also NK-cells, which realize lyses of target-cells, that are not restricted by MHC antigens. The growth of level of CD38 epitope expression by mononuclears in peripheral blood in scleroma patients reflects NK-cells mobilization, which is phylogenetically most ancient and universal non-specific cellular mechanism of protection, though the activity of these cells doesn't provide eliminating function. NK-cells recognize targets using several cell surface molecular receptors (CD2, NKR-P1) and due to a high density of the Fc receptor CD16 of IgG (FcR-III). They also receive inhibitory signals from MHC class I on potential target cells, transuded via killer inhibitory receptor on the NK cells. LGL possess carbohydrate-binding proteins, known as lectins, which can interact with certain carbohydrates normally expressed on cell surface. These interactions alone would trigger the LGL to kill the cell to which it binds. Thus, the increase of lymphocytes number of suppressor and cytotoxic functional subpopulations is observed. Alongside with the decrease of CD4+cells number it proves the formation of misbalance in the immunoregulatory mechanisms. Cellular immune reactions are not effective in this case as it is reported by other authors and confirmed by our results [36].

The relative and absolute number of CD14+ cells exhibits the tendency to decrease in patient with scleroma as compared to controls. CD14 antigen is a receptor for lipopolysaccaride (LPS) and mostly expressed on surface of mature monocytes and macrophages. It is an admitted fact, that CD14 expression goes down due to the effect of IL4, produced by T-helpers type 2 [21]. The basic IL4 effect is manifested in the expression of HLA-Dr molecules on B-cells and macrophages, stimulation of B-lymphocytes proliferation and isotype switching to IgG_1 and IgE. The lowering amount of pan-B+ (CD72+) cells is determined in patients with scleroma. CD72 antigen expressed on surface of progenitor B-cells, mature B-cells. It is a member of Ca^{++} dependent lectins superfamily (ligand for CD5). B-cells have antigen-binding molecules on their surface, these molecules play the role of primary antigen receptors. Switch over of synthesis of immunoglobulin heavy chains take place in the activated B-cell. Receptors for the antigen on the surface of immunocompetent cells serve as membrane-binding antibodies, and they are characterized by the same antigen specificity just like antibodies, secreted by the cell after its stimulation with a certain antigen. Our findings show that the expression of by G, A, M isotypes by B-lymphocytes is lowered in patients with scleroma. The decrease of B-cells number and surface immunoglobulins expression of in scleroma demonstrates, probably, that peripheral blood B-cells are on the late stages of differentiation.

Other authors in their independent investigations point out a wide range of variability of individual immunologic parameters in different forms of pathology. Because of this fact in studies on population immunity the attempts are made to do a discrete analysis of parameters, depending on various characteristics [1, 7, 12, 14, 27]. The analysis of immunologic parameters in all forms of scleroma (atrophic, cicatrix, infiltrative ones) is the subject of great scientific interest for us. The conformity of initiation and development of the process, mechanisms of changing cell phases in the focus of chronic inflammation - all these aspects remain to be further elucidated.

Mononuclears play a critical role in the induction of fibrogenesis. They produce the factor of fibroblasts stimulation, IL1, fibronectin, interferons and other growth-regulating molecules. Activated macrophages strengthen the attraction of fibroblasts into the region of inflammation and stimulate their proliferation [5, 34]. In patients with cicatrix form of scleroma the tendency for the increase of relative and absolute number of CD4+ cells in peripheral blood is established as compared to controls, and to the group of patients with infiltrative and particularly atrophic forms.

The relative and absolute number of CD38+ cells (activated T- and B-cells, NK-cells) is increased in all clinical forms of scleroma. This increase is most pronounced in patients with infiltrative form. Furthermore, the content of CD3+ lymphocytes is also higher in the patients with infiltrative scleroma and it reflects the activity of the local immune inflammatory process.

The lowest level of HLA-Dr expression is reported in patients with atrophic form of scleroma. The lowest CD4+lymphocyte content is also registered in this group. Besides, there is a direct link between the relative content of HLA-Dr+ and CD4+ ($r=0.72$; $p<.05$) as well as between the absolute content of HLA-Dr+ and CD4+ ($r=0.8$; $p<.05$). It gives evidences in favour of the functional co-operation of these cell types in the activation of T-cellular response.

Increased of CD8+ cells in infiltrative and cicatrix forms of scleroma, in comparison with atrophic form contributes, perhaps, to the restriction of pathology process spread. The increase in CD4:CD8 ratio is observed in patients with infiltrate form. The growth of this parameter, predetermined by the increase of CD4+ lymphocytes subpopulations can be considered as a sign of the development of autoimmune processes in some patients or increasing proportion of the regulatory T-cells – $CD4^+CD25^+$. These observations are supported by other researchers' findings. Amyloid-like protein was found on the walls of vessels and in the basic membrane in mucous samples from patients with rhinoscleroma. This protein seems to be a result of autoimmune reactions [14].

The decrease in pan-B+ (CD72+) cells content is most pronounced in atrophic form. The content of pan-

B+(CD72+) lymphocytes is higher in infiltrate form, and it reflects the activity of the local process.

Phenotypic characteristics of scleroma granuloma cells vary considerably in different patients. Probably, it is a feature of the opportunity in principle to reflects the prevalence of certain pathogenic mechanisms which are involved in formation of a particular local focus. It is likely to determine different clinical forms of the disease. It is a fact of special significance as well as currently observed decrease of infiltrative forms and increase of atrophic forms prevalence. The high variability of granuloma immune cells phenotype is registered alongside with great intragroup diversity of separate immunologic parameters in peripheral blood of patients with scleroma. But in our opinion, the low level of HLA-DR molecule expression on cells in scleroma granuloma decreases the efficacy of presentation of *K. rhinoscleromatis* antigens to lymphocytes.

References

1. Al Jahdali H, Bamefleh H, Memish Z, Al-Zuwayed M, Al Othman A (2001): Upper airway obstruction due to rhinoscleroma: case report. J Chemoter Suppl. 1: 69-72.
2. Ammar ME, Rosen A (2001): Rhinoscleroma mimicking nasal polyposis. Ann Otol Rhinol Laryngol 110 (3): 290-292.
3. Andraca R, Edson RS, Kern EB (1993): Rhinoscleroma: a growing concern in the United States? Mayo Clinic experience. Mayo Clin Proc 68: 1151-1157.
4. August C, Hustert B (1998): Nasal scleroma (rhinoscleroma): pathological and clinical results. Pathologe 19 (5): 384-387.
5. Austing JM (1996): New insight into the mobilization and phagocytic activity of dendritic cells. J Exp Med 483: 1287-1292.
6. Badia L, Lund VJ (2001): A case of rhinoscleroma treated with ciprofloxacin. J Laryngol Otol 115 (3): 220-222.
7. Bhargava D, Date A (2001): Palatal presentation of scleroma. J Laryngol Otol 115 (8): 679-680.
8. Bhat NA, East DM (1999): Paranasal sinus scleroma. J Otolaryngol 28 (4): 242-244.
9. Boggino HE, Borcowsky J, Xiao SY (2001): Pathologic quiz case. Arch Pathol Lab Med 125 (1): 159-160.
10. Bauchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YI, Pulendrau B, Palucka K (2000): Immunobiology of dendritic cells. Ann Rev Immunol 18: 767-811.
11. Canalis RF, Zamboni L (2001). An interpretation of the structurial changes responsible for the chronicity of rhinoscleroma. Laryngoscope 111 (6): 1020-1026.
12. Collis N, Stanley PR, Sharpe DT, Batman PA (1998): Rhinoscleroma: an interesting differential diagnosis. Br J Plast Surg 51 (1): 78-79.
13. Di Carlo R, D'Agostino L, Grandi E, Pastore A (2001): Rhinoscleroma with extramaxillary extension; report of a clinical case. Acta Otorhinolaryngol Ital 21 (4): 254-259.
14. Evrard I, Gruyer X, Desseb P, Francoios A, Marie JP, Dehesdin D, Andrieu-Guitrancourt J (1998): Spheno-ethmoidal rhinoscleroma. Report of a case and review of the literature. Ann Otolaryngol Chir Cervicofac 115 (2): 85-88.
15. Fajardo-Dolci G, Chavolla R, Lamadrid-Bautista E, Rizo-Alvarez J (1999): Laryngeal scleroma. 28 (4): 229-231.
16. Firdousi FA, Sadiq S, Kadar AA (2000): Rhinoscleroma. J Pak Med Assoc 50 (8): 276-277.
17. Hart CA, Rao SK (2000): Rhinoscleroma. J Med Microbiol 49 (5): 395-396.
18. Issing WJ, Bodllaj R (1999): Rhinoscleroma: a case report. Laryngorhinootologie 78 (4): 200-203.
19. Keschner D, Kelley TF, Wong BJ (1998): Transglottic scleroma. Am J Otolaryngol 19 (6): 407-411.
20. Konoval,chikov GD, Timoshenko PA (1999): Some aspects of scleroma problem. Vestn Otorhinolaryngol (4): 51-55.
21. Lanuer RP, Goyert SM, Geha RS, Verselli D (1990): Interleukin 4 down-regulates the expression of CD14 in normal human monocytes. Europ J Immunol 20 (11): 2375-2388.
22. MacLennan ICM, Gulbranson-Judge A, Toellnek K-M (1998): Th1 and Th2 activity rapidly developing in a single node. The Immunologist 6 (5): 179-181.
23. Mason D (1998): Antigen cross-reactivity: essential in the function of TCRs. The Immunologist 6 (6): 220-222.
24. McGill R., Turkey JW, Larsen WA (1978): Variations of box plots. The American Statistician 32 (1): 12-16.
25. Omerogli A, Weisenberg E, Baim HM, Rhone DP (2001): Pathologic quiz case. Arch Pathol Lab Med 125 (1): 157-158.
26. Pena J, Cicero R, Marin J, Ramirez M, Cruz S, Navarro F (2001): Laryngotracheal reconstruction in subglottic stenosis: an ancient problem still present. Otolaryngol Head Neck Surg 125 (4): 397-400.
27. Prince JS, Duchamel DR, Levin DL, Harrell JH, Friedman PJ (2003): Noneoplastiic lesions of the tracheobronchial wall: radiologic findings with bronchoscopic correlation. Radiographics 23 (1): 191.
28. Salmon M, Pilling D, Borthwick NJ, Akbar AN (1997): Inhibition of T-cell apoptosis – a mechanism for persistencew in chronic inflammation. The Immunologist 5 (3): 87-92.
29. Soni NK (1997): Scleroma of the larynx. J Lagyngol Otol 111 (5): 438-440.
30. Stock I, Wiedemann B (2001): Natural antibiotic susceptibility of Klebsiella pneumoniae, K. oxytoca, K. planticola, K. ornitinolytica and K. terrigena strains. J Med Microbiol 50 (5): 396-406.
31. Stougaard MJ, Vimpel T, Petersen K (1996): Rinoscleroma in eldery Danish woman. Ugeskr Laeger 158 (36): 5041-5043.
32. Sun Y, Sun W, Lu X (1998): Clinical analysis of 19 cases of scleroma respiratorum treated surgically. Lin Chuang Er Bi Yan Hou Ke Za Zhi 12 (7): 314-316.
33. Titov LP, Krylov IA, Ovcharenko TM. Immunogenetic studies of kliebsiliosis of respiratory organs. Proc. of I Byelorussian Immunologic Conference. Vitebsk 1991; 89-91.
34. Titov LP (1997): Introduction in immunology. Immunecompetent cells. Medicine 3: 34-35.
35. Titov LP (1997): Introduction in immunology. Molecules of immune system. Medicine 4: 32-35.
36. Titov LP. Mollecular mechanisms of T- and B-lymphocytes activation. In: Actual problems of infection pathology of human. Minsk 2001; 287-317.
37. Valor Garcia C, Castillo Serrano E, Martin del Guayo G, Bhathal Guede B, Garcia Fernandez E, Olalla Tabar A, Poch Broto J (1999): Rhinoscleroma. A case report. Acta Otorrinolaryngol Esp 50 (4): 321-323.
38. Yigla M, Ben-Iznak O, Hashman N, Lejbkowicz F (2000): Laryngotrachebronchial involvment in a patient with nonendemic rhinoscleroma. Chest 117 (6): 1795-1798.