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High serum levels of Soluble Intercellular Adhesion Molecule-1 (sICAM-1) and -3 (sICAM-3), Interleukin-1β (IL-1β) and Tumor Necrosis Factor α (TNF-α) in the course of measles

The morbidity of measles remains high all over the world despite the introduction and wide use of well-tolerated and highly protective vaccine. This disease causes the death of more than 1,000000 persons each year. The problem exists mainly in poor Asian, African and South American populations but even in developed countries sporadic cases are seen and every few years small epidemics occur. The early and late complications of measles may be the reason for additional treatment, prolonged hospitalization and in some cases may even lead to death. Measles is a dangerous disease in the immunosuppression. But on the other hand, the infection itself produces significant immunosuppression that may predispose to secondary infection or exacerbate coexisting pathology. Measles-induced immunosuppression was first described in 1908 by von Pirquet, who observed a decrease in tuberculin skin reactivity in patients with measles. Several studies conducted during recent years have shown that measles virus causes the complicated immunologic abnormalities (12, 21).

Immune responses and inflammatory reactions are modulated by many factors, but cytokine system seems to be particularly of the great importance. Interleukin-1 (IL-1) plays an important role due to its wide biologic activity, including influence on the function of immune system cells. This cytokine is called lymphocyte-activating factor, as it increases mitogen-induced T cells activity. IL-1 induces T cells activation via the stimulation interleukin-2 secretion. On the other hand inflammatory reactions are enabled due to increased expression of intercellular adhesion molecules (ICAMs) (13, 18).

The other cytokine playing a key-role in human immune responses is tumor necrosis factor-α (TNF-α). Activating ICAM expression, it induces neutrophils, monocytes and lymphocytes adhesion to endothelial cells. Incubation of cultured human dendritic cells with TNFá results in an increased surface expression of ICAM-1 (4, 20). ICAM-1 (CD50), a member of the immunoglobulin supergene family, was the aim of many studies. It plays an important role in the process of antigen presentation and cooperation between lymphocytes B and T. Results of several studies showed that the expression of ICAM-1 is influenced by cytokines, such as IL-1, TNF and interferon (INF) gamma (8, 13, 18).

The intercellular adhesion molecule 3 (ICAM-3, CD50), another member of immunoglobulin supergene family, plays an important role in the early phase of antigen presentation to lymphocytes T. Montoya et al. has recently proved that ICAM-3 is essential in the initial scanning for specific antigens on the antigen-presenting cell (APC) surface by T cells and, therefore, in generating the immune response. It was demonstrated that ICAM-3 was expressed by resting T cells, and dendritic cells abundantly expressed specific ICAM-3 receptor, which can support primary immune responses (11, 17). Increased expression of ICAMs on the surface of immune system cells, including lymphocytes T,

is a valuable marker of their activation. It leads to the soluble form of ICAM (sICAM) appearance in serum, and elevated levels were found in several inflammatory and autoimmune diseases. The elevated level of sICAM-1 was observed in the course of *Plasmodium falciparum* and *Schistosoma mansoni* infection, in sepsis and opportunistic infections in AIDS (7, 15, 18, 19, 23).

The aim of the study was to assess the levels of sICAM-1 and sICAM-3 in serum of measles patients. At the same time we measured the levels of TNF-α and IL-1β, which are, as mentioned above, cytokines with proved influence on ICAMs.

MATERIAL AND METHODS

The study was conducted on 31 subjects, including 21 patients with measles (9 women and 12 men), aged 16–34 years, and 10 healthy individuals (5 women and 5 men), aged 19–24 years, who composed the control group. In the control group all the subjects underwent the routine physical examination and the laboratory evaluation. Patients with measles were hospitalized at the Department of Infectious Diseases, Medical University of Lublin, and they did not have any coexisting diseases. Clinical diagnosis of measles was based on history, physical examination and laboratory examination. It was confirmed by serological tests. Therapy was the same for all the patients and included antipyretic medications, mostly paracetamol or ibuprofen, antihistaminic drugs and anti-cough medications. The patients did not receive any antiviral or antimicrobial agents and corticosteroids. In our opinion the treatment did not have the potential to affect the expression of ICAMs and the cytokines.

In the control group blood samples were collected once, whereas in measles patients samples were collected twice: on the first day of hospitalization, which was the beginning of the rash phase (test 1) and on the tenth day of hospitalization, after disappearance of acute clinical symptoms and signs (test 2). All the patients and healthy volunteers of control group had been informed of the study and had signed an informed consent.

Both in the control group and measles patients, routine tests included morphology with leukocytes differentiation, bilirubin, total protein, alkaline phosphatase and alanine aminotransferase (ALT) levels. Simultaneously with routine tests, sICAM-1, sICAM-3, TNFα and IL-1β concentrations were measured. The levels of sICAM-1, sICAM-3, TNFα and IL-1β were determined with the following ELISA tests manufactured by Endogen (USA): Human ELISA sICAM-1, Human ELISA sICAM-3, Human ELISA TNFα and Human ELISA IL-1β used according to the manufacturer's instructions.

Statistical analysis. T-Student test was used for the assessment for interrelationships between variables.

RESULTS

The mean white blood cells count was lower in test 1 compared to the control group (4.09±1.64 k/μl vs. 7.61±1.82k/μl) and the difference was statistically significant (t= 5.21; $t_{0.05=}$2.045). The difference in white blood cells count between test 2 and the controls (6.46±2.35 k/ml vs. 7.61±1.82 k/μ) was insignificant (t= 1.32; $t_{0.05}$= 2.045). Leukocyte count in the first day of hospitalization was significantly lower than after disappearance of clinical symptoms (t= 3.67; $t_{0.05}$= 2.021) The same statistical significance was found analyzing the percentage of lymphocytes, which was lower in test 1 compare to the control group and test 2 (18.9±9.9% vs. 31.2±3.8% and 31.8±12.6% respectively). The activity of ALT was increased in 19 from 21 examined measles patients. The mean ALT level was 125±64 IU/l in test 1 and 65±29IU/l in test 2. Comparing to the control group the elevation of ALT level in test 2 was (t= 3.98; $t_{0.05}$= 2.045) whereas in test 1 was not (t= 1.77) statistically significant.

Table. 1. Leukocytes (WBC) count, percentage of lymphocytes and alanine aminotransferase (ALT) activity in patients with measles

	Control			Test 1			Test 2		
	Min-Max	M	SD	Min-Max	M	SD	Min-Max	M	SD
WBC count (k/μl)	4.7 ÷ 9.4	7.61	1.82	2.4 ÷ 7.7	4.09*	1.64	4.0 ÷ 11.8	6.46	2.35
Lymphocytes (%)	22.1 – 37.1	31.2	3.8	8.6 ÷ 35.6	18.9*	9.9	17.5 ÷ 47.5	31.8	12.6
ALT (IU/L)	17 – 31	27	5.4	33 ÷ 519	125	64	32 ÷ 142	65*	29

Min – minimum value, Max – maximum value, M – medium value, SD – standard deviation
* Statistical significancy $p<0.05$

There were no significant differences between tests 1 and 2. In every patient bilirubin, protein and alkaline phosphatase concentration were normal. In the control group sICAM-1 concentrations ranged from 149.0 ng/ml to 270.5 ng/ml (mean 204.9±41.5 ng/ml). In the patients group the mean concentration of sICAM-1 in test 1 was 384.2±64.4 ng/ml, whereas the mean level in test 2 was 353.0±56.8 ng/ml. These levels of sICAM-1 in both tests were significantly higher than in the control group for test 1: $t=7.772$, $t_{0.05}=2.045$; for test 2: $t=7.108$; $t_{0.05}=2.045$). The change between tests remained statistically insignificant ($t=1.625$, $t_{0.05}=2.021$).

The serum levels of sICAM-3 in the control group ranged from 39.9 ng/ml to 95.5 ng/ml (mean 68.3±17.9 ng/ml). In measles patients sICAM-3 concentrations were higher both in test 1 (mean 94.0±21.1 ng/ml) and in test 2 (mean 103.1±20.8 ng/ml). These values were significantly higher compared with that from controls (for test 1: $t=3.211$ and for test 2: $t=4.395$; $t_{0.05}=2.045$). There was no statistical significance of changes between tests ($t=1.374$; $t_{0.05}=2.021$).

Table. 2. Serum levels of sICAM-1, sICAM-3, IL-1β, TNF-α, in the control group and in measles patients

	Controls			Measles patients					
				Test 1			Test 2		
	Min-Max	M	SD	Min-Max	M	SD	Min-Max	M	SD
sICAM-1 (ng/ml)	149.0÷270.5	204.9	41.5	275÷726.5	384.2*	64.4	238÷601	353*	56.8
sICAM-3 (ng/ml)	39.9÷95.5	68.3	17.9	48.9÷131.9	94*	21.1	58.0÷135.1	103.1*	20.8
TNF-α (pg/ml)	0.0÷0.4	0.14	0.10	0.0÷0.9	0.32*	0.22	0.0÷1.3	0.51*	0.32
IL-1β (pg/ml)	0.0÷0.74	0.31	0.18	0.41÷1.61	1.14**	0.33	0.44÷2.02	1.26**	0.40

Min – minimum value, Max – maximum value, M – medium value, SD – standard deviation
* Statistical significancy $p<0.05$
** Statistical significancy $p<0.001$

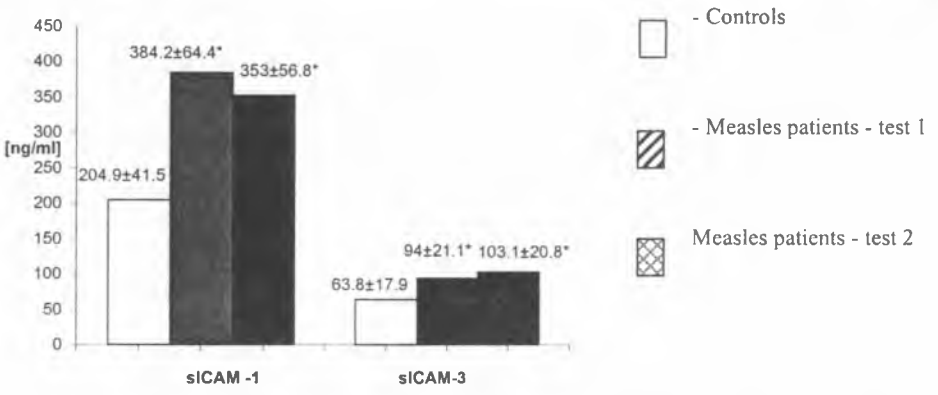

Fig. 1. Serum levels of sICAM-1, sICAM-3, in the control group and in measles patients
* statistical significancy $p<0.05$

In the control group IL-1β levels ranged from 0.0 pg/ml to 0.74 pg/ml (mean 0.31±0.31 pg/ml). In the study group IL-1β concentrations both in test 1 (mean 1.14±0.33 pg/ml) and in test 2 (mean 1.26±0.40 pg/ml) were significantly higher than in control group (for test 1: t=7.195, $t_{0.001}$=3.659; for test 2: t=6.663, $t_{0.001}$=3.659). Differences between these two tests were statistically insignificant (t=1.303;$t_{0.05}$=2.021).

In the control group TNFα levels ranged from 0.0 pg/ml to 0.4 pg/ml (mean 0.14±0.10 pg/ml). In measles patients TNFα levels ranged from 0.0 pg/ml (in 3 individuals) to 0.9 pg/ml (mean 0.32±0.22 pg/ml) in test 1, and in test 2 we observed significant elevation of the mean value, which was 0.51±0.32 pg/ml (t=2.188,$t_{0.05}$=2.021). TNFα mean concentration both in test 1 and in test 2 was significantly higher than in the control group (for test 1: t= 2.257, $t_{0.05}$ = 2.045; for test 2: t= 2.301, $t_{0.05}$ = 2.045).

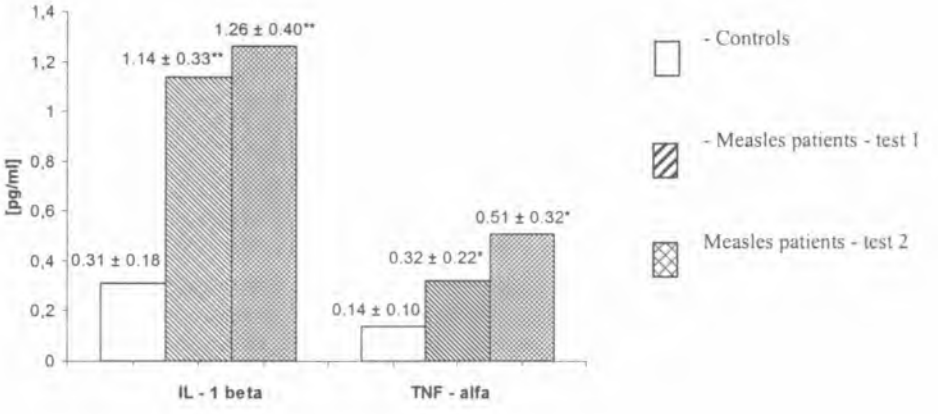

Fig. 2. Serum levels of IL-1β and TNF-α in the control group and in measles patients
* statistical significancy $p<0.05$; ** statistical significancy $p<0.001$

DISCUSSION

Studies of the measles pathogenesis and the mechanisms of immunosuppression are hampered by the lack of easily accessible animal model for measles virus infection. Nonhuman primates are susceptible to the infection, and typical clinical symptoms are most likely to develop in *Rhesus macaques* (2).

In this study we observed low lymphocytes count in the early rash phase of measles. The number of lymphocytes increased after acute clinical symptoms disappearing. Auwaerter et al. suggested that lymphopenia in the early stage of measles is closely associated with viremia and may be a consequence of measles virus-induced lymphocytes death (2).

Immune suppression associated with morbillivirus infection reflects mainly an impairment of cell-mediated responses. In several both *in vivo* and *in vitro* studies the inhibition of specific T cell proliferation was documented, but B lymphocytes seems to be affected as well (9, 12, 16, 26). Yanagi et al. have shown that measles virus inhibits mitogen-induced T cell proliferation, but does not directly suppress the T cell activation process inside the cell (26). Moreover, there are some findings that the virus induces increased activity of T lymphocytes (10, 14).

In spite of previously suggested hypothesis, in 1993, Esolen et al. proved that the primary leukocytes infected during measles are monocytes, while lymphocytes are generally not infected (6). Later, some investigators demonstrated the lymphotropism of measles virus, and suggested that the virus can replicate in and affect dendritic cells, which seems to be especially important for immune responses during measles (9, 21, 24). The virus replication in lymphocytes T and in monocytes induces cells apoptosis, particularly in syncytia. The results of some recent studies may suggest that these disturbances are present not only in T cells, but in APCs (e.g. lymphocytes B) as well. Immune suppression during measles may be at least partly a consequence of dendritic cell dysfunction, as they show both APC and cytotoxic activities (21, 24). A few proteins taking part in adhesion processes between leukocytes and endothelial cells have been identified recently. It is thought that adhesion molecules may play a key role in inflammatory reactions following infection (4, 13). It was proved they serve as receptors for parasites (e.g. plasmodia) and viruses (e.g. rhinoviruses) or for neoplastic cells and hematopoiesis precursors (4, 13, 23).

In the present study, we found increased sICAM-1 and sICAM-3 serum levels both at the beginning of rash phase of measles and when acute symptoms disappeared. Although in test 2 sICAM-1 serum levels decreased and sICAM-3 serum levels increased compare to test 1, these differences were not statistically significant. Elevated sICAM-1 and sICAM-3 serum levels in measles patients may be related to activation of antigen presenting process. ICAM-1 is present on the surface of lymphocytes B and as a ligand for lymphocyte function-associated protein (LFA)-1 participates in their activation and antigen presentation to lymphocytes T (4, 13, 25). The elevated levels of sICAM-1 may represent increased shedding of this molecule from a systemic inflammatory reaction and endothelial cell activation during acute phase of measles. There are some suggestions the elevated sICAM-1 concentration is not just a marker of different processes, but may play a role in modulation of immune reactions, particularly of cellular response (23).

In our study we found the increased sICAM-3 serum level, and it even grew during an observation period. Probably it is, at least partly, due to dendritic cells involvement in specific immune responses.

Maintaintenance of increased sICAM-1 and sICAM-3 serum levels in spite of acute clinical symptoms disappearing may suggest that both immunologic system activation and infectious inflammatory reaction do not end with an abatement of clinical manifestations. The study in leukocytes from infected macaques showed that the production of macrophage-derived IL-1β and TNF-α was

increased (2). In our study we observed a significant elevation of IL-1β and TNF-α serum concentration. Elevated levels of these proinflammatory cytokines may suggest increased Th1 lymphocytes activity. In addition, it may be one of the reasons for ICAM-1 and ICAM-3 high activity that is reflected by increased levels of soluble forms for adhesion molecules.

Measles virus infection naturally spreads over the organism and especially respiratory system and liver are involved. We have confirmed the liver damage showing an elevated activity of alanine aminotransferase. It can be speculated that both cytokine and ICAMs production may be local. That problem needs to be explained in further studes. Local production of these immune activation markers was recognized in different diseases of liver and respiratory tract, using modern immunoassays and histochemical methods (1, 5, 22).

The analysis of serum concentration of immune response markers does not let us answer all the questions about measles pathogenesis, but we believe it is likely to improve our understanding of immunopathogenesis mechanisms. These parameters are thought to be an objective determinant of immunologic and inflammatory reaction intensity and activity of infectious process.

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SUMMARY

Measles pathogenesis and the mechanisms of immunosuppression are still not fully explained. Immune suppression associated with morbillivirus infection reflects mainly an impairment of cell-mediated responses. In the present study serum levels of soluble intercellular adhesion molecule-1 (sICAM-1) and -3 (sICAM-3), tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were measured in measles patients. Significantly increased sICAM-1 and sICAM-3 serum levels both at the beginning of rash phase of measles and when acute symptoms disappeared were found. The elevated levels of sICAMs may represent increased shedding of these molecules from a systemic inflammatory reaction and endothelial cell activation during acute phase of measles as well as it may be related to the activation of antigen presenting process. In this study a significant elevation of IL-1β and TNF-α serum concentration was observed. Elevated levels of these proinflammatory cytokines may suggest increased Th1 lymphocytes activity. In addition, it may be one of the reasons of ICAM-1 and ICAM-3 high activity, that is reflected by increased levels of soluble forms of adhesion molecules.

Wzrost poziomu rozpuszczalnej Cząstki Adhezji Komórkowej-1 (sICAM-1) i -3 (sICAM-3), Interleukiny-1β (IL-1β) oraz Czynnika Martwicy Nowotworów α (TNF-α) w surowicy krwi w przebiegu odry

Patogeneza i mechanizmy immunosupresji w przebiegu odry nie zostały dotychczas w pełni poznane. Supresja odpowiedzi immunologicznej, wywołana zakażeniem morbilliwirusem odzwierciedla przede wszystkim upośledzenie mechanizmów typu komórkowego. W pracy przedstawiono ocenę poziomu rozpuszczalnej cząstki adhezji komórkowej-1 (sICAM-1) i -3 (sICAM-3), interleukiny-1β (IL-1β) oraz czynnika martwicy nowotworów α (TNF-α) w surowicy krwi w przebiegu odry. Stwierdzono statystycznie istotny wzrost sICAM-1 i sICAM-3 w surowicy krwi w początkowej fazie okresu wysypkowego oraz po ustąpieniu ostrych objawów klinicznych. Zwiększone stężenie sICAM może być związane z uwalnianiem cząstek w przebiegu układowej reakcji zapalnej oraz aktywacją komórek śródbłonka w ostrej fazie choroby. Należy brać pod uwagę również jego związek z procesem prezentacji antygenu. W badaniu obserwowano także istotny wzrost poziomu IL-1β i TNF-α. Podwyższenie poziomu cytokin prozapalnych może mieć związek zarówno z pobudzeniem limfocytów Th1, jak również zwiększeniem aktywności ICAM-1 i ICAM-3, czego odzwierciedleniem są wysokie stężenia ich form rozpuszczalnych.