Dependence of peripheral blood lymphocyte subpopulations on causative microorganisms able to produce superantigens

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**Key words:** Staphylococcus aureus, Streptococcus pyogenes, T cells, grampositive cocci, superantigen, lymphocyte immunophenotyping.

**Summary.** A retrospective study of 176 immunologically tested patients admitted to Kaunas Medical University Clinics during the 1997-2000 years period was performed. All of the patients had a positive bacteriological culture result confirming an infectious etiology of the disease. Our results showed that a majority of immunological parameters were dependent on such non-specific factors as intensity and localization of the inflammatory process, an overall functional status of the patient, and the number of the disease exacerbation episodes during the last year before admission. In contrast to this, the absolute number of CD4 lymphocytes, the relative amount of HLA-DR positive lymphocytes and the index of neutrophil latex phagocytosis were exceptionally dependent on the species of the causative microorganism, in particular the superantigen producing cocci. In this case the HLA-DR positive lymphocyte amount and the neutrophil phagocytosis index were significantly higher. In addition, the CD4/CD8 lymphocyte ratio (the immunoregulatory index) was significantly lower in this group.

As much as those findings are concordant with the signs of excessive immune activation we conclude that they reflect a possible superantigenic action of the disease causing bacteria. Therefore, a need for immunomodulating therapy during the infections caused by species able to produce superantigens is confirmed.

**Introduction**

It has been widely described that superantigen producing microorganisms such as *Staphylococcus aureus* or *Streptococcus pyogenes* cause a clonal expansion of T lymphocytes. This means a non-specific activation of considerable amounts of T lymphocytes bearing certain T cell receptor (TCR) Vβ families (1). These amounts are much higher than those obtained by means of conventional antigenic stimulation. It has been proposed that superantigenic stimulation is the most common cause of staphylococcal and streptococcal sepsis (2) as well as certain autoimmune diseases especially systemic vasculitides (3). Superantigens are able to induce T cell anergy and apoptosis thus causing immunosuppression (1). Despite of importance of these mechanisms there are few data about their impact on some practically important routine immunological tests such as peripheral blood lymphocyte immunophenotyping. This test can provide an information about (4):

1. A suspected immunodeficient state of an organism. Absolute amounts of immune cells are of importance in this respect.
2. The phase and intensity of the inflammatory process, the adequacy of the immune response, antigen persistence, reserve capacity of the immune system, and patient prognosis. Relative amounts of immune cells are important.

The most important superantigen-producing microorganism is *S. aureus*. Rats after the staphylococcal enterotoxin B (SEB) injection had increased amounts of CD4+ (T helper) cells during the first days after bacterial inoculation (5). After the same procedure in monkeys CD4+ cells decreased and CD8+ (T suppressor/cytotoxic) cells increased during the same period of time (6). The low amounts of CD4+ cells persisted thereafter (6).

In human patients with bacterial endocarditis, the CD4+/CD8+ ratio is substantially higher in case of *S. aureus* as a causative agent in comparison with unable to produce superantigens *Klebsiella*...
pneumoniae, Escherichia coli and Staphylococcus epidermidis (7). In case of repetitive S. aureus infections, the widespread phenomenon of staphylococcal sensitization has a marked decrease of CD4+ cells and an increase of CD8+ cells and B lymphocytes in peripheral blood (8). The specific immunotherapy with staphylococcal antigens restores the natural balance (9). During the glomerulonephritis caused by methicillin-resistant S. aureus (MRSA) a marked increase of DR+ T lymphocytes (activated T cells) was noticed (10). On the other hand, non-producing-superantigen microorganisms and viruses also cause certain peripheral blood lymphocyte subpopulation shifts. In case of syphilis, the relative amounts of CD4+ cells were decreased while those of CD8+ cells increased (11). The absolute amounts of both CD4+ and CD8+ cell decreased in case of shigellosis (12).

These findings are fragmental and episodic. The question of determining the exact impact of microbial and non-microbial factors such as the inflammatory process itself, its localization, the overall status of the patient, and the repetitive infections on each immune cell population remains unresolved. In addition, there is a huge disproportion between scarce clinical and abundant experimental studies. There is a great need for validation of a transfer of experimental data to a clinical setting.

**Aim of the study** – to study retrospectively changes in immune system parameters in patients with diseases of confirmed infectious etiology and to determine the dependence of these parameters on non-microbial and microbial factors focusing on ability of the causative agents for superantigen production.

**Patients and methods**

176 immunologically tested patients admitted to Kaunas Medical University Clinics during the 1997-2000 years period were studied. All of them had various diseases of infectious origin confirmed by bacterial culture. Cases of suspected underlying primary and secondary immunodeficiencies as well as allergic and autoimmune conditions were dismissed.

**Definition of patient groups**

Patients were grouped by intensity of inflammation, the overall functional status of the patient, exacerbation/partial remission, number of disease exacerbations during the last year before admission, the localization of the infection, and by characteristics of the causative microorganisms such as belonging to certain species, group and their ability to produce superantigens. Inflammation was considered of high intensity when during 3 days period before or after immune testing there were found at least 2 of following 4 criteria: fever ≥38.5°C; erythrocyte sedimentation rate (ESR) ≥50 mm/h; C reactive protein (CRP) >48 mg/l; >5 non-mature cells in leukocyte formula. Inflammation was considered of medium intensity when there were found during the same period at least 2 of the 4 following criteria during the same period: fever higher than 37.5°C and below 38.5°C; ESR higher than normal and lower than 50 mm/h; CRP ranging from 12 to 48 mg/l; 3-5 non-mature cells in leukocyte formula; or 1 sign of high and 1 of medium intensity inflammation. In absence of sufficient number of the criteria, the inflammation was considered of low intensity. The overall status of a patient was considered as easy if during 3 days period before or after the immune testing there was found high-grade functional disturbance at least in one organ or their system according to universally accepted criteria; or medium-grade functional disturbance at least in two organs or their systems. In case of medium-grade functional disturbance at least in one organ or their system or low-grade functional disturbance at least in two organs or their systems, the status of the patient was considered as medium disturbed. In absence of sufficient number of the criteria the status was considered as easily disturbed. A partial remission was considered if during the 2 weeks period before immune testing the intensity of inflammation had converted from high or medium to easy. In other cases, an exacerbation was considered. There was separated a group of patients who had 2 or more episodes of the disease in the same localization during the 1 year period before immune testing. There were groups of patients separated according to the localization of the disease: upper respiratory tract; lungs; kidneys and urinary tract; skin or mucosal layer; mixed localization; septicemia.

Patients also were grouped by microbial species found in bacterial culture. For the sake of statistical analysis, bigger groups of the causative agents were defined as well: S. aureus; S. pyogenes; other streptococci; other gram (+) bacteria not belonging to S. aureus or S. pyogenes; gram (-) bacteria; non-bacterial agents; combinations of causative agents having S. aureus or S. pyogenes; other combinations. Finally, two biggest groups were separated for mutual comparison: microorganisms able to produce superantigens (S. aureus, S. pyogenes or mixed...
culture including these bacteria); and those not able for superantigen production.

**Definition of parametrical data**

The list of immune tested peripheral blood cells included relative and absolute counts of: total lymphocyte population; CD3+ lymphocyte (T cells); CD4+ lymphocyte (T helper cells); CD8+ lymphocyte (T supressor/cytotoxic cells); CD4+/CD8+ ratio; CD16+ lymphocyte (NK or natural killer cells); CD19+ lymphocyte (B cells); DR+ lymphocyte (HLA II class positive lymphocytes); a difference of DR+ and CD19 lymphocyte. Cell counts were performed by flow cytometry using the FACSCalibur cytometer and Simultest IMK-Lymphocyte or Simultest IMK Plus kits (Becton Dickinson, Mountain View, CA) as described (13). Due to the phenomenon of physiological lymphocytosis (14) relative and absolute lymphocyte counts as well as absolute lymphocyte subpopulation counts were analysed separately to patients up to 5 years old and older. A neutrophil latex phagocytosis index was determined as described elsewhere (15). The method of radial immunodiffusion (Mancini) (16) was employed to obtain immunoglobulin G, A and M class values. Because of high age dispersion among the investigated patients a ratio of the immunoglobulin and the lowest, mean or highest value of the normal age range (17) was obtained for further analysis.

**Statistical analysis**

The statistical analysis was performed using the SpSS for Windows 10.1 program. Cross-tabulation of categorical parameters defining patient groups was performed by Fisher’s exact $P^2$-test. Because of failure of dispersion equality and normal distribution hypotheses in a considerable part of parameters, the non-parametric Mann-Whitney and Kruskal–Wallis tests were selected for immune parameter analysis. The ANOVA test was selected for evaluation of categorical parameters mutual influence to parametrical data. Values were expressed as means ± confidence interval limits. P values less than 0,05 were considered as significant.

**Results and discussion**

Cross-tabulation data are presented in table 1. As we can see, such parameters as intensity of inflammation, the overall status of the patient, the number of disease episodes and the localization of the inflammatory process were strongly cross-linked. The cross-linkage among species or group of the microorganism and aforementioned parameters was

**Table 1. The results of the cross-tabulation of categorical data (p values).**

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Gender</th>
<th>Localization</th>
<th>Exacerbation</th>
<th>No of exacerbations</th>
<th>Overall status</th>
<th>Inflammation level</th>
<th>Microbial species</th>
<th>Microbial group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>n. s.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Localization</strong></td>
<td>&lt;0.001</td>
<td>n. s.</td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Exacerbation</strong></td>
<td>n. s.</td>
<td>n. s.</td>
<td></td>
<td></td>
<td>0.019</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
</tr>
<tr>
<td><strong>No of exacerbations</strong></td>
<td>&lt;0.001</td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>0.019</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Overall status</strong></td>
<td>&lt;0.001</td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Inflammation level</strong></td>
<td>n. s.</td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Microbial species</strong></td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>&lt;0.001</td>
<td>n. s.</td>
<td>0.002</td>
<td>0.001</td>
<td>n. s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Microbial group</strong></td>
<td>&lt;0.001</td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>n. s.</td>
<td>0.001</td>
<td>0.001</td>
<td>n. s.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n. s. – not significant.

also present although the contribution of different microbial groups was unequal. In particular, there was strong inverse relation of \textit{S. aureus}, \textit{S. pyogenes} and \textit{Haemophilus influenzae} to the status of the patient and the level of the inflammation (there was a predominance of small disturbances in clinical picture and disease localization in upper respiratory tract and skin, although 26% of all septicemia cases were caused by \textit{S. aureus}). On the other hand, other gram-negative microorganisms predominated in cases of more severe disease.

When analysing results of immune testing (table 2), it could be seen that most of them were dependent on aforementioned clinical parameters. This kind of influence was noticed long ago (4). The reasons for that are close integration of the immune and other organ systems, ubiquity of immune components and mechanisms, and big compensatory reserves of the immune system (4). For this reason, only those parameters exclusively dependent on microbial characteristics (the absolute amount of CD4+ cells, the relative amount of DR+ lymphocytes, the neutrophil phagocytosis index, as well as the ratio of IgM to its lowest normal age range) were selected.

CD4+/CD8+ ratio was also added to the analysed parameters group because it was the only parameter strongly dependent on all microbial characteristics including ability for superantigen production and only one non-microbial characteristic (localization). This made applying of the ANOVA test possible where localization was plotted against CD4+/CD8+ ratio as a dependent variable. When the CD4+/CD8+ dependence on disease localization was eliminated by means of this its dependence on microbial ability for superantigen production remained highly statistically significant (p<0.001).

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**Table 2. Dependence of immune testing results on selected clinical characteristics (p value).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Localization</th>
<th>No of exacerbations</th>
<th>Overall status</th>
<th>Inflammation</th>
<th>Microbial species</th>
<th>Microbial group</th>
<th>SuperAg + or -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte (abs.)</td>
<td>n. s.</td>
<td>n. s.</td>
<td>0.021</td>
<td>0.036</td>
<td>0.019</td>
<td>0.032</td>
<td>n. s.</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.002</td>
<td>n. s.</td>
</tr>
<tr>
<td>Lymphocyte (abs.)</td>
<td>0.048</td>
<td>0.011</td>
<td>0.007</td>
<td>0.016</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
</tr>
<tr>
<td>CD3+ lymph. (abs.)</td>
<td>n. s.</td>
<td>0.006</td>
<td>0.032</td>
<td>n. s.</td>
<td>0.02</td>
<td>n. s.</td>
<td>n. s.</td>
</tr>
<tr>
<td>CD4+ lymph. (%)</td>
<td>0.003</td>
<td>n. s.</td>
<td>0.045</td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4+ lymph. (abs.)</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>0.016</td>
<td>n. s.</td>
<td>n. s.</td>
</tr>
<tr>
<td>CD8+ lymph. (%)</td>
<td>0.045</td>
<td>0.04</td>
<td>n. s.</td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD8+ lymph. (abs.)</td>
<td>0.009</td>
<td>0.001</td>
<td>0.002</td>
<td>0.014</td>
<td>0.003</td>
<td>0.023</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4+/CD8+</td>
<td>&lt;0.001</td>
<td>n. s.</td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD16+ lymph. (%)</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>0.032</td>
<td>n. s.</td>
<td>0.031</td>
<td>n. s.</td>
</tr>
<tr>
<td>CD16+ lymph. (abs.)</td>
<td>0.027</td>
<td>n. s.</td>
<td>0.002</td>
<td>0.012</td>
<td>n. s.</td>
<td>0.019</td>
<td>0.005</td>
</tr>
<tr>
<td>CD19+ lymph. (%)</td>
<td>n. s.</td>
<td>0.027</td>
<td>n. s.</td>
<td>0.009</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
</tr>
<tr>
<td>CD19+ lymph. (abs.)</td>
<td>0.027</td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.048</td>
<td>n. s.</td>
<td>n. s.</td>
</tr>
<tr>
<td>DR+ lymph. (%)</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>n. s.</td>
<td>&lt;0.001</td>
<td>n. s.</td>
</tr>
<tr>
<td>DR+ lymph. (abs.)</td>
<td>0.008</td>
<td>n. s.</td>
<td>n. s.</td>
<td>0.013</td>
<td>n. s.</td>
<td>n. s.</td>
<td>0.017</td>
</tr>
<tr>
<td>DR+/CD19+ (%)</td>
<td>n. s.</td>
<td>0.049</td>
<td>n. s.</td>
<td>0.006</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
</tr>
<tr>
<td>DR+/CD19+ (abs.)</td>
<td>n. s.</td>
<td>0.001</td>
<td>0.024</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
</tr>
<tr>
<td>Phagocytosis index</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
</tr>
<tr>
<td>IgA/lowest (%)</td>
<td>n. s.</td>
<td>0.014</td>
<td>0.025</td>
<td>0.001</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
</tr>
<tr>
<td>IgA/highest (%)</td>
<td>n. s.</td>
<td>0.031</td>
<td>n. s.</td>
<td>0.008</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
</tr>
<tr>
<td>IgM/lowest (%)</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>0.047</td>
<td>n. s.</td>
</tr>
<tr>
<td>IgM/mean (%)</td>
<td>n. s.</td>
<td>0.031</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
</tr>
<tr>
<td>IgM/highest (%)</td>
<td>n. s.</td>
<td>0.037</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
<td>n. s.</td>
</tr>
</tbody>
</table>

Abs., absolute count; n. s., not significant; %., percental (relative) count. Super Ag + or -, ability or inability of the microorganism for superantigen production.
The values of these selected immune parameters in various microbial groups are presented in Table 3.

As we can see, the relative DR+ lymphocyte count and neutrophil phagocytosis index were higher in *S. aureus* and able for superantigen production microorganism groups. It has been known that both *in vitro* and experimentally *in vivo* superantigen-stimulated T lymphocyte clones express HLA-DR molecules (18). This expression reflects the late phase of T lymphocyte activation (19). Despite of constitutional expression of HLA-DR+ on a considerable proportion of B lymphocytes (19) the total amount of HLA-DR+ B lymphocytes is unlikely to increase because of superantigen-induced direct or indirect (by superantigen-activated cytotoxic CD8+ cell-mediated) lysis of B lymphocytes (20). This could explain the lower IgM level in *S. aureus* group (Table 3). Other important fact showing the major activated T cell impact on DR+ lymphocyte increase is an elevated relative amount of HLA-DR+ T lymphocytes in patients with active psoriasis (21) and methicillin-resistant *S. aureus* induced glomerulonephritis (10) – superantigen-mediated diseases (1). Statistically significant increase of neutrophil phagocytosis index values in all superantigen-producing microorganism groups could be explained by suppression of neutrophil apoptosis and their stimulation due to the superantigenic action – phenomena observed *in vitro* (22). Increased levels of proinflammatory cytokines due to superantigen stimulated T lymphocyte and neutrophil action could be responsible for the changes in CD16+ lymphocyte level among the superantigen-producing bacteria (Table 2). The dependence of these cell level on disease and inflammation severity also confirms the indirectness of superantigenic action in this case.

The marked decrease of CD4+/CD8+ ratio in all able-for-superantigen-production microorganism groups is a confirmation of experimentally obtained results in the clinical setting. It was established that under the staphylococcal superantigen action *in vivo* CD4+ lymphocytes bearing superantigen-specific TCR V$_5^s$ families migrated to the tissues and activated other V$_5^s$ families expressing cells (23). The clonal local CD4+ lymphocyte expansion quantitatively exceeding CD8+ lymphocyte expansion was observed during the course of experimental *S. aureus* nephritis (24).

### Table 3. The selected immune testing results in various causative agent groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Microorganisms</th>
<th><em>S. aureus</em></th>
<th>CN staph.</th>
<th><em>S. pyogenes</em></th>
<th>Other. str.</th>
<th>OtherGram.(+)</th>
<th>Gram (–)</th>
<th>Sag (+)</th>
<th>Sag (–)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ lymph. (abs., x10^9/l)</td>
<td></td>
<td>0.71±0.09$^a$ (n=41)</td>
<td>1.22±0.34 (n=6)</td>
<td>0.96±0.27$^b$ (n=11)</td>
<td>0.65±0.17 (n=15)</td>
<td>0.83±0.19 (n=22)</td>
<td>0.75±0.12 (n=26)</td>
<td>0.77±0.09 (n=57)</td>
<td>0.81±0.1 (n=64)</td>
</tr>
<tr>
<td>CD4+/CD8+ (%)</td>
<td></td>
<td>1.16±0.08$^{ae}$ (n=52)</td>
<td>2.05±0.4 (n=9)</td>
<td>1.23±0.08$^{ae}$ (n=18)</td>
<td>1.77±0.22 (n=16)</td>
<td>1.85±0.19 (n=33)</td>
<td>2.01±0.21 (n=39)</td>
<td>1.18±0.06$^e$ (n=76)</td>
<td>1.89±0.12 (n=100)</td>
</tr>
<tr>
<td>DR+ lymph. (%)</td>
<td></td>
<td>14.9±2.9$^a$ (n=17)</td>
<td>5.0±2.0 (n=2)</td>
<td>12.5±2.1 (n=12)</td>
<td>11.3±2.4 (n=6)</td>
<td>10.6±3.9 (n=9)</td>
<td>9.8±1.9 (n=16)</td>
<td>13.3±2.5$^e$ (n=32)</td>
<td>9.4±1.5 (n=34)</td>
</tr>
<tr>
<td>Phagocytosis index (%)</td>
<td></td>
<td>58.0±2.6$^{ae}$ (n=52)</td>
<td>52.6±4.4 (n=12)</td>
<td>58.0±4.0 (n=18)</td>
<td>51.6±5.2 (n=21)</td>
<td>52.2±3.6 (n=34)</td>
<td>53.4±2.0 (n=41)</td>
<td>58.1±2.1$^e$ (n=76)</td>
<td>53.9±1.7 (n=100)</td>
</tr>
<tr>
<td>IgM/lowest (%)</td>
<td></td>
<td>266.0±58.8$^a$ (n=37)</td>
<td>395.6±145.2 (n=10)</td>
<td>360.8±123.2 (n=11)</td>
<td>300.8±77.7 (n=15)</td>
<td>330.2±74.0 (n=26)</td>
<td>362.2±100.6 (n=33)</td>
<td>304.1±53.3 (n=52)</td>
<td>325.6±53.5 (n=74)</td>
</tr>
</tbody>
</table>

Pastabos: CN staph., coagulase-negative staphylococci; Gram (+), grampositive; Gram (–), gramnegative; Sag (+), microorganisms able to produce superantigens; Sag (–), microorganisms unable to produce superantigens; n, number of measurements; abs., absolute count.

$^a$ – p < 0.05 compared to the coagulase-negative staphylococci group.

$^b$ – p < 0.05 compared to the other streptococci group.

$^c$ – p < 0.001 compared to the coagulase-negative staphylococci group.

$^d$ – p < 0.001 compared to the other streptococci group.

$^e$ – p < 0.001 compared to the other grampositive microorganism group.

$^f$ – p < 0.001 compared to the gramnegative microorganism group.

$^g$ – p < 0.001 compared to the non-producing-superantigen microorganism group.

$^h$ – p < 0.05 compared to the gramnegative microorganism group.

$^i$ – p < 0.05 compared to the other grampositive microorganism group.

**Vytis Verba, Solveiga Gudžinskiene**

Dependence of peripheral blood lymphocyte subpopulations

...phenomenon of CD4+ lymphocyte deletion during chronic superantigen exposition due to its action itself (25) or due to superantigen-stimulated CD8+ lymphocytes was observed (26). However, on the contrary to the CD4+ cells, the number of CD8+ lymphocytes in peripheral blood had a tendency to increase under chronic superantigen exposition (6). So, a substantial body of data supports the statement that the delayed type hypersensitivity phenomena during staphylococcal sensitization could be of superantigenic but not of conventional antigenic genesis as previously (8, 9) thought.

What could be the practical benefits of our investigation? Defining of superantigen role during the course of diseases caused by Staphylococcus aureus and Streptococcus pyogenes could promote research targeted at the search for superantigen-neutralizing immunomodulatory treatment. Another important consequence could be a broadening of the list of indications for the immunomodulatory treatment. The polyvalent donoric immunoglobulin preparations especially enriched with IgM and IgA remain the superantigenic action-targeted drugs of choice in case of Staphylococcus aureus or Streptococcus pyogenes-mediated septic and toxic shock (27). However, limited possibilities of this treatment could force to investigate in a clinical setting some experimentally created treatment modalities. Among them are applying of synthetic MHC class II-binding peptides (28), non-toxic superantigenic molecules-mutants (29) as well as vaccination with modified preparations of most common bacterial superantigens (30).

**Conclusions**

1. In case of diseases of an infectious origin, the most of the routine immune testing parameters were dependent on non-specific clinical factors such as intensity and localization of inflammation, severity of the disease, and number of recent disease episodes.

2. The absolute CD4+ lymphocyte (T helper), the relative DR+ lymphocyte count in peripheral blood and the neutrophil latex phagocytosis index were dependent exclusively on the type of a disease causing agent. In case of infection caused by able-to-produce-superantigen microorganisms an increased relative DR+ lymphocyte count and a higher neutrophil latex phagocytosis index as well as a lower CD4+/CD8+ lymphocyte ratio (the immunoregulatory index) were observed.

3. The excessive immune activation concordant changes in able-to-produce-superantigen microorganism groups allows broadening of the spectrum of indications for immunomodulatory treatment of Staphylococcus aureus and Streptococcus pyogenes infections, and validates the need for new treatment modalities research in the clinical setting.

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**Gebančių produkuoti superantigenus infekcinių agentų įtaka periferinio kraujo limfocitų subpopuliacijų pokyčiai**

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**Raktazodžiai:** Staphylococcus aureus, Streptococcus pyogenes, superantigenai, T limfocitai, HLA-DR+ limfocitai, imunoregulatorinės indeksas, limfocitų subpopuliacijos, fagocitozės indeksas.


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