# LETTER TO THE EDITOR

# CHANGES IN THE REACTIVITY OF THE IMMUNE SYSTEM OF CHILDREN AFTER THE INFLUENZA A INFECTION

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Viruses cause 80–90% of acute respiratory diseases in children (*1*). Influenza A virus belongs to the most serious etiological agents with significant clinical response of organism. The severity of influenza is a result of the interaction between the virus and the organism, depending on the status of the immune system. Children whose immune system is under the development represent a high-risk group with respect to influenza-mediated complications.

The presentation of the virus after the infection triggers release of cytokines and activation of immunologically competent cells (*2–4*). With the progreding infection the local early immune response continues to the overall response with expression of clinical symptoms. The parameters of humoral and cellular immunity are reflected in laboratory measurable changes (*5–8*).

The aim of this work was to follow the changes in the expression of selected CD markers on leukocytes in the venous blood of 35 children aged 0–18 years with confirmed influenza A infection. The criteria for including the children patients into this study were as follows: (i) clinical symptoms of viral infection with fever, (ii) influenza A infection confirmed by RT PCR, (iii) no complications in the course of infection, (iv) anamnestic exclusion of another disease until the last draw of venous blood (day 90), and (v) informed consent of child's parents to draw blood samples for immunological examination. Influenza A virus was detected in nasopharyngeal washes by RT-PCR using the SV Total RNA Isolation Kit (Promega). cDNA was amplified using specific primers FLA1 and FLA2 for Influenza A virus (*9*), and the products were detected by agarose gel electrophoresis. Expression of CD markers in peripheral blood was measured. On days 1 (acute manifestation of clinical symptoms), 14 (regression of clinical symptoms), and 90 (convalescence), cell numbers with CD markers were assayed by flow cytometry (Culter Beckman FC500) using monoclonal antibodies specific for individual CD markers (Immunotech), and leukocyte numbers were determined in a hematological analyzer (Celldyn 1200, Abbot). Statistical significance of differences was evaluated by the t-test.

The CD markers of leukocyte cells of 35 children were evaluated on selected days of the disease in comparison to physiological status (the table). In control healthy children, the CD marker values varied in broad range because their cell immune system is under development, not yet stabilized. Therefore we document here only those CD markers in which significant differences from physiological values were observed.

On days 1 and 14, the most significant change consisted of a more than 35-fold increase in the CD69 marker due to early activation of Ts/c-lymphocytes (CD8+), and a rise of the CD64 marker (high affinity receptor for Fc fragment of IgG) on neutrophils (Ne64+). On day 1, there was also

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| CD markers | Units | Reference limits[a] | Day of infection | | |
|---|---|---|---|---|---|
| | | | 1 | 14 | 90 |
| CD16 on Mo | % from Le | <0.0, 15.0> | **25.5** | [11.3] | [8.9] |
| | | | | P(1,14)=0.001 | P(1,90)=0.019 |
| CD64 on Ne | % from Le | <0.0, 3.0> | **27.4** | **12.3** | **26.5** |
| CD3 | No. of cells/µl of blood | <1369, 3032> | [1961] | [2521] | [2230] |
| | % from Ly | <58.0, 72.0> | [58.6] | [58.4] | [59.3] |
| CD4 | No. of cells/µl of blood | <849, 1959> | [1114] | [1483] | [1293] |
| | % from Ly | <34.8, 44.3> | [34.8] | [34.9] | [34.3] |
| | | | | | P(1,90)=0.030 |
| CD8 | No. of cells/µl of blood | <574, 1332> | [763] | [1038] | [936] |
| | % from Ly | <23.9, 33.0> | **22.3** | **23.4** | [24.9] |
| CD4+CD45RO+ | % z Th-Ly | <29.8, 45.3> | **29.1** | **49.6** | **18.5** |
| | | | | P(1,14)=0.011 | P(14,90)=0.004 |
| CD69 on CD4 | % from Th-Ly | <0.0, 1.0> | **2.4** | **1.5** | **[0.3]** |
| | | | | | P(14,90)=0.009 |
| CD69 on CD8 | % from Ts/c-Ly | <0.0, 1.0> | **37.7** | **35.8** | **1.5** |
| | | | | | P(1,90)<0.001 |
| | | | | | P(2,90)=0.025 |
| CD69 on CD19 | % from B-Ly | <0.0, 1.0> | **1.5** | **2.9** | [0.1] |
| | | | | | P(1,90)=0.026 |
| | | | | | P(14,90)=0.025 |
| CD69 on Mo | % from Mo | <0.0, 1.0> | **2.7** | **1.4** | [0.3] |
| | | | | | P(1,90)=0.007 |
| CD71 on CD4 | % from Th-Ly | <0.0, 3.5> | [2.9] | [3.1] | [1.8] |
| CD71 on CD8 | % from Ts/c-Ly | <0.0, 1.0> | **1.6** | **1.5** | [0.6] |
| CD71 on CD19 | % from B-Ly | <0.0, 1.0> | **4.0** | **6.1** | **4.1** |
| | | | | P(1,14)=0.002 | P(14,90)=0.025 |
| CD71 on Mo | % from Mo | <0.0, 1.0> | [0.7] | [0.2] | [0.1] |
| | | | | | P(1,90)=0.098 |

[a]Values within reference limits are given in square brackets, those out of limits are in bold.
P = significance level of the difference between values on given days. Le = leukocytes; Ly = lymphocytes; Th-Ly = T-helper inductor lymphocytes; Ts/c-Ly = T-suppressor-cytotoxic lymphocytes; B-Ly = B-lymphocytes.

observed an increase in the population of activated monocytes (Mo16+), which was manifested by increased markers CD16 (the low affinity receptor for Fc fragment of IgG) and CD69. The higher expression of CD69 on monocytes (CD16) persisted also on day 14. On days 1 and 14, the expression of the CD69 marker over physiological values was manifested also in the populations of Th- and B-lymphocytes. In this group of lymphocytes, also the expression of the proliferative marker CD71 (transferring receptor) was augmented. On the other hand, the relative numbers of Ts/c-lymphocytes (the CD8 marker) decreased on day 1 physiological levels. Significant differences were also observed in the level of cortical thymocytes (the CD4+CD45RO+marker): a decrease (day 1), an increase (day 14), and a decrease (day 90) in comparison to physiological values.

In the convalescence phase (day 90) of the disease all other followed parameters returned to physiological values, except the CD64 marker in activated neutrophils (Ne64+), the increase expression of which persisted. The rest of the followed parameters revealed values within physiological levels during the whole period of the disease (data not shown).

From these results it is apparent that the influenza infection activates the cytotoxic part of the lymphocyte system that is demonstrated by an increased number of cells expressing the markers CD3, CD8, and CD69, while the total number of the T-lymphocytes remains unchanged. Such an observation, made on a well-defined group of children that experienced influenza infection, has not yet been described.

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