Transient depletion of innate immunity in varicella infections in otherwise healthy children

Sağlıklı çocuklarda gelişen varisella enfeksiyonu sırasında bağışıklık sisteminde geçici baskılanma

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Abstract

Objective: Varicella is a common childhood infection and has a number of complications in the unvaccinated population. Perforin, found in natural killer cells, is important for the killing of virally infected cells. For this reason, the aim of this study was to determine natural killer cell count and activity, perforin expression, and Fas and soluble Fas ligand (sFas-L) levels in immunocompetent children with varicella infection and define any possible relations between the levels and varicella complications.

Material and Methods: Forty children were analyzed at diagnosis and on the 15th day of varicella infection. There was a significant difference in hemoglobin levels and leukocyte and platelet counts between days 0 and 15.

Results: Thirteen (32%) patients were found to be lymphopenic. Natural killer cell count and activity were significantly higher on day 15 when compared to values at diagnosis. The Fas-mediated apoptotic pathway was found to be active in acute varicella infection because Fas and sFas-L levels at diagnosis were higher than values on day 15.

Conclusion: These findings suggest that the Fas and Fas-L apoptotic pathway is active during the acute phase of the viral infection and that it becomes inactive by day 15, paralleling the hematologic recovery.

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Key words: Varicella, Fas-Fas ligand, natural killer cell, perforin expression

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Özet

Amaç: Varisella çocukluk çağının sık görülen bir enfeksiyonu olup, aşılanmamış grupta komplikasyonlara yol açabilmektedir. Bu çalışmanın amacı, diğer yönlerden sağlıklı çocuklarda varisella enfeksiyonu geliştiğinde NK hücre sayısı ve aktivitesi, perforin ekspresyonu, *Fas* ve *Fas* ligand seviyelerini belirlemek ve bu düzeylerle varisella komplikasyonları arasında olası bir ilişkiyi saptamaktır.

Yöntem ve Gereçler: Kırk hastanın varisella enfeksiyonu tanısı konulduğunda ve enfeksiyonun 15. gününde değerlendirmeleri yapılmıştır.

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Bulgular: Hastaların 0 ve 15. günlerdeki hemoglobin, lökosit ve trombosit değerlerinde anlamlı fark bulundu. NK hücre sayısı ve aktivitesi, tanıdaki değerlerle karşılaştırıldığında 15. günde daha yüksek bulunmuştur. Akut varicella enfeksiyonu sırasında *FAS* aracılıklı apoptotik yolağın aktif olduğu saptanmıştır.
Sonuç: Bu bulgular hematolojik düzelme ile parallel olarak 15. günde Fas aracılıklı yolağın akut dönemdeki artmış aktivitesinde azalmaya işaret etmektedir. *(Turk J Hematol 2009; 26: 12-6)*
Anahtar kelimeler: Varicella, fas-fas ligand, NK hücre, perforin

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Introduction

Varicella is a common childhood infection and can be complicated by secondary bacterial infection, pneumonia, acute cerebellar ataxia, meningoencephalitis, Reye's syndrome, purpura fulminans and hemophagocytic syndrome, especially in immunocompromised hosts [1]. After the host is infected by varicella zoster virus (VZV) via droplets, VZV completes 10 to 21 days of an incubation period in respiratory mucosa and reaches the lymph nodes and reticuloendothelial system after primary viremia. One of the primary defensive systems of the host against VZV is maintained by natural killer (NK) cells, as a part of innate immunity [2]. NK cells are involved in direct killing of virally infected cells via apoptosis with perforin-granzyme or Fas-Fas ligand pathways. If there is a defect in NK cells count or function, defense against some viral infections will be impaired and complications will appear [3]. Since varicella vaccination is not routinely applied in Turkey, epidemics can be observed in the wintertime and some children develop complications.

The aim of this study was to determine the changes in complete blood count (CBC), NK cell count and activity, perforin expression, and Fas and soluble Fas ligand (sFas-L) levels in immunocompetent children with VZV infection and define any possible relations between the levels and development of varicella complications.

Materials and Methods

Forty immunocompetent children without any underlying disease, between 1-16 years of age with VZV infection who presented to Hacettepe University İhsan Doğramacı Children's Hospital, Division of Pediatric Infection, within the first three days of the onset of symptoms were included in the study. Hemogram, peripheral blood smear, NK cell count, NK cell activity, perforin expression, Fas and sFas-L were examined at admission and after crusting of all lesions (day 15). Fas and sFas-L could be studied in 19 of the 40 children. The same laboratory examinations were also made among 39 healthy controls with no recent clinical infection. NK cell count, perforin expression and Fas measurements were made by flow cytometric analysis (FACScan, Becton Dickinson; San Jose, CA, USA). sFas-L was measured by ELISA. NK cell activity was measured by flow cytometry as K562 cell binding of NK cells. Liver function tests were analyzed in all patients at diagnosis and in the control group, whereas on day 15, liver functions were studied in only two patients with high transaminase levels at diagnosis. The normal NK cell count is 8-15%. Normal values for NK cell activity and perforin expression levels were compared with healthy controls. There was no statistically significant difference between the VZV and healthy control groups in terms of age and gender. All statistical analyses were performed using SPSS version 11.0 software (SPSS Inc., Texas, USA). Differences in the day 0 and 15 values were compared with paired samples test. The 0 day mean values of the patient group were compared to the mean values of control group by the aid of independent samples test. Fas L and ALT values were compared by Mann-Whitney U test.

The study was approved by Hacettepe University, Ethical Committee (approval number: 05/38). Informed consent was obtained from both VZV and control groups.

Results

Forty children, between 1 and 16 years old, who were within three days of onset of acute VZV infection and without any underlying disease, were included in the study. The mean age was 6.9±2.7 years (range 2-15) and the male/female ratio was 28/12.

White Blood Cell (WBC), Neutrophil, Lymphocyte and Platelet Counts

The laboratory data of the patients at diagnosis and on day 15 are demonstrated in Table 1. There was significant difference between WBC and platelet counts at diagnosis and on day 15 ($p=0.007$, $p<0.001$, respectively).

Five of all patients were anemic according to their age and gender normal values and on day 15, four of these five patients re-achieved normal hemoglobin levels. The fifth patient was diagnosed as having α-thalassemia trait.

Ten (25%) of the 40 patients were leukopenic at diagnosis, one of them being neutropenic as well. Thirteen (32%) patients were found to be lymphopenic. By day 15, it was observed that leukopenia recovered in all and lymphopenia recovered in all except one.

Platelet count below $150x10^9$/L was detected in two out of 40 patients ($83x10^9$/L and $110x10^9$/L) on day 0 and had recovered by day 15. On the other hand, platelet count below $200x10^9$/L was detected in 16 out of 40 patients and all recovered by day 15. None of the patients manifested purpura, ecchymosis or bleeding.

NK Cell Activity and Count, Perforin Expression, Fas and sFas-L Levels at Diagnosis and on Day 15

NK cell activity and count

Nineteen of 40 (47%) patients were found to have NK cell count below 10% at diagnosis, but only 2 of 40 patients were

found to have NK activity lower than the normal range. There was a positive correlation between day 0 NK cell count and perforin expression (r=0.76). Mean NK cell counts at diagnosis and on day 15 were 10.7±6.3% (2.3-36.3) and 12.8±7.0% (3.23-35.0), respectively, and the difference was found to be statistically significant (p=0.029). Mean NK cell activity at diagnosis was 51.5±20.3% (5-82) and on day 15 was 61.7±21.1% (10-100), and the difference was statistically significant (p=0.014).

Perforin expression

Perforin expression at diagnosis and on day 15 were 7.6±6.4% (0-30.1) and 9.6±6.9% (0.2-33.5), respectively, and the difference was significant (p=0.030).

Fas and sFas-L levels

Fas levels at diagnosis and on day 15 were 66.4±14.9% (29.1-89.4) and 45.7±12.6% (29.1-73.3), respectively, and sFas-L levels at diagnosis and on day 15 were 0.098±0.116 ng/ml (0-0.369) and 0.036±0.059 ng/ml (0-0.202), respectively. The differences between Fas and sFas-L levels on day 0 and 15 were statistically significant (p<0.001 and p=0.012, respectively).

NK Cell Activity and Count, Perforin Expression, Fas and sFas-L Levels in Patients and Controls

The laboratory data of the control group are given in Table 2. There was no statistically significant difference between the control group and values at diagnosis of the VZV group in terms of hemoglobin, absolute neutrophil and lymphocyte counts, NK cell number and sFas-L level, which were studied on the first day for comparison with the control group values. WBC and platelet count, NK activity, perforin expression and Fas level at diagnosis were lower in the VZV group than in the control group (p values 0.010, 0.0, 0.046, 0.013, 0.0, respectively).

On day 0, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were above normal range in two patients but normalized by day 15. ALT and AST levels of the VZV group at diagnosis were higher than in the control group (p=0.008, p=0.005, respectively).

On day 15, there were no significant differences between the control group and VZV group in hemoglobin, WBC and platelet counts, neutropenia, lymphopenia, liver function tests, NK cell counts, NK cell activity, perforin expression, and Fas and sFas-L levels.

Discussion

The defensive mechanisms against viral agents are composed of an earlier innate immunity and a later response of adaptive immunity. NK cells are crucial for the innate immunity response. Viral agents may cause alterations in the hematological parameters through direct myelosuppressive effect of the virus or immune response against the virus. The common viral infections associated with leukopenia are infectious hepatitis, infectious mononucleosis, rubella, measles, parvovirus B19 and influenza [4]. In addition, lymphopenia and lymphocytosis are not uncommon in the course of viral infections.

Table 1. Comparative data of the VZV-infected patients at diagnosis and on day 15

	At diagnosis	Day 15	p
White blood cell count (x10^9/L)	6.4±2.6	7.9±2.1	0.007
Platelet count (x10^9/L)	224±76	339±95	<0.001
Neutrophil (%)	51±15	46±11	0.11
Lymphocyte (%)	37±11	43±10	0.015
ALT (U/L) *	21	-**	-
AST (U/L)	37±12	-	-
NK cell count (%)	10.7±6.3	12.8±7.0	0.029
NK cell activity (%)	51.5±20.3	61.7±21.1	0.014
Perforin expression (%)	7.6±6.4	9.6±6.9	0.030
Fas (%)	66.4±14.9	45.7±12.6	<0.001
sFas-L (ng/ml)	0.09±0.12	0.04±0.06	0.012

*All values are mean±standard deviation, except ALT, which is median value
NK: Natural killer

** ALT and AST levels were not studied on day 15

Table 2. Comparative data of VZV group at diagnosis and control group

Parameter	At diagnosis	Control	p
White blood cell count (x10^9/L)	6.4±2.6	7.9±2.2	0.010
Platelet count (x10^9/L)	224±76	335±128	<0.001
Neutrophil (%)	51±15	50±12	NS*
Lymphocyte (%)	37±11	39±11	0.05
ALT (U/L)**	21	17	0.008
AST (U/L)	37±12	30±8	0.005
NK cell count (%)	10.7±6.3	12.5±5.5	NS
NK cell activity (%)	51.5±20.3	59±10.8	0.046
Perforin expression (%)	7.6±6.4	10.9±4.9	0.013
Fas (%)	66.4±14.8	42.4±15.4	<0.001
Fas-L (ng/ml)	0.073	0.08	NS

*NS: not statistically significant, p>0.05
**All values are mean±standard deviation, except ALT, which is median value
NK: natural killer

In our study, leukopenia occurred in 25% of the patients. Neutropenia was seen in one patient. It could be stated that varicella causes leukopenia in one-fourth of patients but does not cause neutropenia.

It was interesting that VZV caused lymphopenia in 32% of the patients. This finding revealed that varicella generated the defect in the immune system through a lymphopenic effect. Lymphopenia was present in 32% of the patients and recovered by the 15th day in all except one patient. In our study, relative lymphocytosis was observed in 30% of the patients. Okada et al. [5] demonstrated that lymphopenia in patients with measles developed due to the decrease in non-infected

lymphocyte count by apoptosis. It was previously reported that of 119 previously healthy children with VZV infection, one case of neutropenia and five cases of thrombocytopenia were observed [6]. Similarly, thrombocytopenia was reported in 8 among 178 immunocompetent children with VZV infection and also in varicella infections in adults [7,8].

It was previously reported that glycoprotein V was the target antigen in autoimmune thrombocytopenia in three varicella-infected children complicated by acute idiopathic thrombocytopenic purpura (ITP) [9,10]. Thrombocytopenia may also occur after immunization with viral vaccines. In a report of 23 patients, ITP developed after measles-rubella-rubeola immunization [11]. These reports indicate that platelets are affected after viral infections, like in varicella, and immunizations as well. The decrease in hemoglobin level, white blood cell and platelet count urged us to consider that VZV may cause trilineage myelosuppression.

As previously known, the most important immune cell limiting the varicella infection is NK cells directed to kill virally-infected cells. NK cells have specialized secretory lysosomes containing perforin and granzymes. Upon target cell recognition, secretory lysosomes polarize and release their contents at the immunological synapse. Secreted perforin lyses the lipid bilayer and polymerizes to form pores in the membranes of target cells and facilitate entry of granzymes that activate the apoptotic pathway in the target. If perforin is deficient or absent, cytotoxic T lymphocytes and NK cells can not develop their cytolytic effect on virus-infected cells [12,13].

In our study, we observed low NK activity with low numbers of NK cells in VZV infection, compared to the control group, and NK cell number and NK cell activity improved up to normal levels after recovery from the infection. NK cell count and activity were significantly higher on day 15 when compared to values at diagnosis. Although 47% of the patients were found to have NK number below 10% at diagnosis of varicella infection, only 5% patients were found to have NK activity lower than the normal range, indicating that VZV infection affects NK cell count more when compared to NK cell activity. Additionally, this result may explain why there were no severe complications in the present study.

It was previously documented that NK activity was reduced within seven days of onset in varicella and herpes zoster patients although NK cell counts were normal [14]. However, there was a decrease in NK cell count and activity between the 8^{th} and 14^{th} day. NK cell count and activity then recovered by the 14^{th} day of infection. These studies support our observations.

As is known, NK cell count and activity have been studied in hemophagocytic lymphohistiocytosis (HLH) patients. Imashuku et al. [15] suggested that there is a correlation between low NK activity and overt central nervous system (CNS) disease in familial HLH. Decrease in NK cell count and activity occur in secondary HLH cases, which are usually associated with viral infections. A previous study conducted in our department demonstrated that most of the secondary HLH patients were related to severe viral infections [16].

Although in some previous studies alteration in NK number and activity in severe or complicated viral infections were described, in our study, none of the patients with varicella were complicated by disseminated varicella, purpura fulminans or immune hemolytic anemia. Biron et al. [17] reported human NK cell deficiency in an adolescent female who presented with disseminated life-threatening varicella infection, who subsequently developed cytomegalovirus pneumonitis followed by aplastic anemia and died after bone marrow transplantation. The laboratory findings revealed that she had an intact adaptive immunity for varicella but lack of lymphocytes expressing CD56 or CD16 and lack of demonstrable NK cell cytotoxicity and antibody-dependent cell-mediated cytotoxicity.

Etzioni et al. [18] reported an infant with recurrent severe varicella infections. A 23-month-old girl was initially admitted to hospital with severe varicella infection including CNS involvement. Her personal history revealed that she suffered from typical chickenpox with definite contact history at seven months of age. She was treated with intravenous immunoglobulin (IVIG) and acyclovir but had irreversible brain damage. Six months later she had another episode of varicella and died after several days. Her laboratory findings revealed that adaptive immunity was normal but NK cell count was below 1% and NK cell activity was lower than in healthy controls.

It was previously demonstrated that NK cells were absent and up to 98% of the $CD8^+$ T cells were naive in five children with a life-threatening course of varicella who had no previous history of recurrent infections [19]. These cases describe that NK cells are the most important cells in the control of herpes group infections, including VZV.

It was previously described that perforin expression decreases in some illnesses, especially in viral infections. It was also demonstrated that NK cell activity and perforin expression decrease in systemic lupus erythematosus-complicated macrophage activation syndrome [20]. In the present study, there was no correlation between perforin expression and NK cell activity. However, there was a significant correlation between reduction in NK cell number and reduction in perforin expression. This may explain the absence of hemophagocytosis secondary to VZV infection, since it is well-known that in familial HLH patients, the decrease in NK cell activity has a more important role in the pathogenesis of disease compared to NK cell count [21].

Significant increase in Fas and sFas-L at diagnosis of VZV infection showed that the Fas-mediated apoptotic pathway was active, indicating the role of apoptotic pathways in the killing of virally infected cells. Elevated levels of sFas-L have also been observed in hepatitis B and hepatitis C infections, rheumatoid arthritis, Churg-Strauss syndrome and ankylosing spondylitis [22,23].

In conclusion, the alteration in hemoglobin level, WBC and platelet counts occurred in patients with varicella at the onset of illness. Both the innate and adaptive immune system may have a contribution in the defense against VZV infections. In our study, NK cell count, NK cell activity, perforin expression and Fas and sFas-L levels were found to be affected in acute VZV infection. The decrease in NK number may be related to the

increase in apoptosis of NK cells. The Fas-mediated apoptotic pathway has been thought to be active in removing virally-infected cells in acute VZV infection because Fas and sFas-L levels at diagnosis were higher than the values on day 15. These findings suggest that the Fas and Fas-L apoptotic pathway is active during the acute phase of the viral infection and becomes inactive by day 15, paralleling the hematologic recovery.

In future studies, NK cell count, NK cell activity, perforin expression, Fas, sFas-L and other factors in the apoptotic pathway should be investigated in patients who were severely complicated after VZV infection, in order to evaluate the contribution of these parameters to prognosis.

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