

Quantities of Receptor Molecules for Colony Stimulating Factors on Leukocytes in Measles

You Jeong Kim¹, So-Young Kim¹, Young-Yoo Kim¹, Jong-Wan Kim¹, Hyun-Hee Kim¹, Jehoon Lee², Kyungja Han², and Wonbae Lee¹

Departments of ¹Pediatrics & ²Clinical Pathology, Catholic University Medical College, Seoul, Korea.

We analyzed the comparative amounts of granulocyte-colony stimulating factor (G-CSF) and granulocyte macrophage CSF (GM-CSF) receptors expressed on neutrophils and monocytes in measles patients to investigate the role of these CSFs in the development of leukopenia including neutropenia and monocytopenia in measles. EDTA-anticoagulated peripheral blood of 19 measles patients, 10 children with other infections showing leukopenia and 16 children with normal complete blood cell counts (CBC)s were analyzed using flow cytometry and QuantiBRITE. The leukocyte ($5260 \pm 2030/\mu\text{L}$ vs. $9900 \pm 2680/\mu\text{L}$, $p=0.000$), neutrophil ($2580 \pm 960/\mu\text{L}$ vs. $4250 \pm 2750/\mu\text{L}$, $p=0.024$) and the lymphocyte counts of measles patients ($1810 \pm 1430/\mu\text{L}$ vs. $4530 \pm 3450/\mu\text{L}$, $p=0.006$) were lower than in the normal controls. The neutrophils of measles patients expressed similar amounts of G-CSF (1858 ± 355) as normal children (1764 ± 477 , $p=0.564$) and leukopenic patients (1773 ± 673 , $p=0.713$), but lower levels of GM-CSF (535 ± 118) than normal children (957 ± 344 , $p=0.000$) and leukopenic patients (832 ± 294 , $p=0.002$). The monocytes of measles patients expressed similar amounts of G-CSF (916 ± 336) and GM-CSF (3718 ± 906) as normal children (1013 ± 391 & 4125 ± 2645 , $p>0.05$) but less than leukopenic patients (1454 ± 398 & 5388 ± 806 , $p>0.05$). The neutrophil and monocyte counts of measles patients did not correlate with the amount of G-CSF or GM-CSF expressed on neutrophils or monocytes ($p>0.05$), but in the normal children, the monocyte count correlated with the levels of GM-CSF on monocytes ($r=0.951$, $p=0.049$).

In conclusion, neutropenia is one of the more important characteristics of measles patients, which could be due to the decreased GM-CSF expression on neutrophils. However, the monocytopenia found in measles patients is not due to the

decreased expression of CSF on the monocytes.

Key Words: Granulocyte-colony stimulating factor, granulocyte macrophage-colony stimulating factor, G-CSF receptor, GM-CSF receptor, leukocyte, measles

INTRODUCTION

Measles is acquired as an infection of the respiratory tract, and principally damages the surface mucosal lining cells.¹ The use of live attenuated vaccines dramatically decreases the incidence and epidemiology of the disease. Where infant measles immunization is widely practiced, the disease has become rare. The last Korean outbreak occurred in 2000 to 2001.²

Measles virus infection causes profound immunosuppression, which makes measles patients susceptible to secondary infections, and accounts for high morbidity and mortality. This immunosuppression is primarily due to severe lymphopenia and monocytopenia.^{3,4} Although some researchers have found neutropenia in measles patients, and neutropenia is known to increase the incidence of bacterial infection, changes in the number of neutrophils in measles patients have not been well studied.

Granulocyte-colony stimulating factor (G-CSF) and granulocyte macrophage CSF (GM-CSF) are the principal hematopoietic growth factors, which regulate the production, differentiation and function of granulocytes.⁵ The biological actions of these CSFs are mediated through interactions with their receptors.⁶ Therefore, we determined the comparative amounts of these receptors on neutrophils and monocytes in measles patients to

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Reprint address: requests to Dr. Wonbae Lee, Department of Pediatrics, Catholic University Medical College, Holy Family Hospital, Wonmi-gu Sosa-dong 2, Pucheon, Kyunggi 420-717, Korea. Tel: 82-32-340-1683, Fax: 82-32-340-2255, E-mail: lwbb@hfh.cuk.ac.kr

investigate the role of these CSF receptors in developing leukopenia, including neutropenia and monocytopenia, in measles.

MATERIALS AND METHODS

Patients

EDTA-anticoagulated peripheral blood of 19 measles patients, 10 children with some other infection showing leukopenia and 16 children with a normal complete blood cell count (CBC) were analyzed. Measles was diagnosed according to the CDC criteria. Blood was collected within 3 days of the appearance of a rash. All patients and parents were informed and agreed to the use of the blood samples remaining after CBC for this study. All samples were analyzed within 4 hours of collection, and samples were kept at room temperature (18 to 20°C) before analysis.

Analyses of leukocyte count and differential count

EDTA-anticoagulated blood samples were used for this analysis. Leukocyte counting was performed using an automatic blood cell analyzer (Coulter STKS, Coulter Co., Miami, USA). For differential counting leukocytes, wedge smear preparations from EDTA-anticoagulated blood samples were prepared and stained with Wright's stain. Absolute neutrophil, lymphocyte and monocyte counts were calculated from leukocyte counts and percentage of each leukocyte was also calculated.

Quantitative analysis of G-CSFr and GM-CSFr

Immunofluorescence analyses were also performed using EDTA-anticoagulated whole blood. A phycoerythrin (PE) conjugated fluorescence quantification kit (QuantiBRITE, Becton Dickinson, San Jose, CA, U.S.A.) and QuantiQuest program were used for comparative fluorescence quantification. PE conjugated anti-CSF receptors were purchased from Serotec (UK) and isotypic control was purchased from Becton-Dickinson (San Diego, CA). All blood samples were stained with anti-G-CSFr (anti-CD114), anti-GM-CSFr

(anti-CD 116) and with the negative isotypic control antibodies. After incubation in the dark for 20 minutes, the erythrocytes were lysed by incubation in lysing solution (Beckton Dickinson). Fluorescence intensity was analyzed by flow cytometry (FACSC alibur, Beckton Dickinson) using CELLQuest software. The flow cytometer was calibrated twice a week using CaliBRITE™ beads (Becton Dickinson) and Auto-comp software monthly. Markers were set using isotypic control sera, so that less than 1% of the cells stained positively. Results were recorded as the geometric means of gated cells. The mean number of bound PE molecules per cell was calculated using the QuantiBRITE and Quanti Quest program.

Statistics

All statistical analyses were performed using SPSS software. Data are expressed as means (SD). Comparisons of results for each group of patients were made using the unpaired student-t test. To evaluate the correlation between the amount of CSFr and the leukocyte count, the Pearson correlation test was used. A p value ≤ 0.05 was considered statistically significant.

RESULTS

Results of leukocyte counting and differential counting

The leukocyte count of measles patients ($5260 \pm 2030/\mu\text{L}$) was lower than that of the normal controls ($9900 \pm 2680/\mu\text{L}$, $p=0.000$), similarly, the neutrophil count of measles patients ($2580 \pm 960/\mu\text{L}$) was lower than that of normal controls ($4250 \pm 2750/\mu\text{L}$, $p=0.024$). Lymphocyte counts of measles patients ($1810 \pm 1430/\mu\text{L}$) were also lower than normal controls ($4530 \pm 3450/\mu\text{L}$, $p=0.006$). Monocyte counts of measles patients ($510 \pm 330/\mu\text{L}$) were less than those of the normal controls, but this was not significant ($910 \pm 710/\mu\text{L}$, $p=0.054$) (Table 1).

Neutrophil, lymphocyte and monocyte counts were positively related to the total leukocyte count ($p < 0.05$).

Table 1. Comparison of the Leukocyte Counts of Measles Patients and Normal Children

	WBC (/uL)	Neutrophil (/uL)	Lymphocyte (/uL)	Monocyte (/uL)
Measles (n=19)	5260 ± 2030	2580 ± 960	1810 ± 1430	510 ± 330
Normal (n=16)	9900 ± 2680	4250 ± 2750	4530 ± 3450	910 ± 710
P value	0.000*	0.024*	0.006*	0.054

*statistically significant difference.

Table 2. Comparison of Receptors for Colony Stimulating Factors on the Leukocytes of Measles Patients and Normal Children

	G-CSFr on neutrophil (PE molecule/cell)	GM-CSFr on neutrophil (PE molecule/cell)	G-CSFr on monocyte (PE molecule/cell)	GM-CSFr on monocyte (PE molecule/cell)
Measles (n=19)	1848 ± 355	534 ± 117* [†]	916 ± 336 [†]	3718 ± 906 [†]
Leukopenia (n=10)	1773 ± 673	832 ± 294	1454 ± 398	5388 ± 806
Normal (n=16)	1763 ± 477	957 ± 344	1013 ± 391	4125 ± 2645

*statistically significant between measles patients and normal children.

[†]statistically significant between measles patients and leukopenia patients other than measles.

Quantity of G-CSFr and GM-CSFr

G-CSFr was expressed on neutrophils to the greatest extent and this was followed by monocytes expression in both patient groups and in normal children (Table 2). Lymphocytes did not express G-CSFr. On the other hand GM-CSFr was expressed more on monocytes than neutrophils in all patients groups and in normal children (Table 2). Neutrophils expressed more G-CSFr than GM-CSFr in all cases, and expressed G-CSFr in proportion to GM-CSFr ($r=0.595$, $p=0.007$). Monocytes expressed more GM-CSFr than G-CSFr, but the quantity of G-CSFr on monocytes was not related to the quantity of GM-CSFr ($p=0.432$) (Table 2).

The neutrophils of measles patients expressed similar amount of G-CSFr (1848 ± 355) as those of normal children (1764 ± 477 , $p=0.564$) and leukopenic patients (1773 ± 673 , $p=0.713$). The neutrophils of measles patients expressed lower amounts of GM-CSFr (534 ± 117) than those of normal children (957 ± 344 , $p=0.000$) and those of leukopenic patients (832 ± 294 , $p=0.002$). The neutrophil counts of measles patients and normal children did not correlate with the amount of G-CSFr or GM-CSFr expressed on neutrophils or monocytes ($p>0.05$).

The monocytes of measles patients expressed similar amount of G-CSFr (916 ± 336) as those of

normal children (1013 ± 391 , $p=0.550$) and less than that of leukopenic patients (1454 ± 398 , $p=0.019$). The monocytes of measles patients expressed similar amounts of GM-CSFr (3718 ± 906) as those of normal children (4125 ± 2645 , $p=0.574$) but less than those of leukopenic patients (5388 ± 806 , $p=0.004$) (Table 2). The monocyte count of measles patients did not correlated with the amount of expressed G-CSFr or GM-CSFr expressed on monocytes ($p>0.05$), but in the normal children, the monocyte count correlated with both G-CSFr and GM-CSFr on monocytes ($r=0.951$, $p=0.049$).

DISCUSSION

Variations in the antigenicity of the measles virus have not reduced the effectiveness of the immunity induced by measles vaccine. In 2000 to 2001, there was an outbreak of measles in Korea mainly in nonvaccinated children.² Leukopenia due to lymphopenia is one of the characteristic hematologic changes in measles. However, neutrophil alterations not been well studied. In the present study, measles patients showed leukopenia due to neutropenia as well as lymphopenia and monocytopenia. These results suggest that the morbidity and mortality of mea-

sles could be increased by an increased susceptibility to bacterial infections due to neutropenia in addition to lymphopenia. The mechanisms of lymphopenia in measles have been widely studied.^{3,7,8} Recently, apoptosis of noninfected lymphocytes causing lymphopenia was reported in measles.³ However, the mechanism of neutropenia in measles has not been studied. Neutrophils perform a key role and protect the body from bacterial infections, and are produced in the bone marrow under the stimulation of several cytokines.^{9,10} Among these, G-CSF and GM-CSF are the principal hematopoietic growth factors, which regulate the production, differentiation and function of granulocytes.⁵ Moreover, the biological actions of these CSFs are mediated through interactions with their receptors.

In this study, the counts of neutrophils, lymphocytes and monocytes were related to the total leukocyte count ($p < 0.05$). G-CSFr was expressed in the largest quantity on neutrophils and this was followed by monocytes, as reported previously.¹¹ G-CSFr was also expressed in the largest quantity on neutrophils followed by monocytes in measles patients. GM-CSFr was expressed more on monocytes than neutrophils in measles patients and in normal children. Neutrophils expressed more G-CSFr than GM-CSFr in all cases, and expressed G-CSFr in proportion to GM-CSFr. Monocytes expressed more GM-CSFr than G-CSFr, but the quantity of G-CSFr on monocytes was not related to the quantity of GM-CSFr ($p = 0.432$). These results suggest that the comparative distribution of these GM-CSFr in measles patients is independent of leukocyte type.¹¹

The neutrophils and monocytes of the measles patients were found to express similar amount of G-CSFr as those of normal children and those of leukopenic patients. However, the neutrophils of measles patients expressed less GM-CSFr than those of normal children and those of leukopenic patients. G-CSFs are expressed on myeloid cells from a very early stage of differentiation and their level of expression increases as cell maturation progresses.¹² However, the amount of GM-CSFr on the neutrophils has not been studied previously. Moreover, immature neutrophils are not found in measles patients. Therefore, decreased GM-CSFr expression on neutro-

phils could be a disease related alteration, rather than a reactive change associated with a left shift of granulocytic maturation resulted in immature neutrophils in the peripheral blood. The quantity of CSFr on leukocytes is not constant, but varies according to the patients' condition.¹¹ All measles patients were sampled within 3 days of rash appearance. This period is termed the secondary viremia period,¹³ and during this period, the measles virus carried within leukocytes and more than 5% of which may be infected.¹⁴⁻¹⁶ However, lymphopenia is caused more so by the apoptosis of noninfected lymphocytes than by the direct infection of cells by the measles virus. Although it is not certain that decreased GM-CSFr expression on neutrophils is associated with viral infection of cells, decreased GM-CSFr expression could cause decreased granulocytic proliferation in the bone marrow and neutropenia in the peripheral blood. The monocyte count of measles patients was less than that of normal controls, though this statistically significant. However, the monocytes of measles patients expressed similar amounts of GM-CSFr as those of normal children and of leukopenic patients. Moreover, the monocyte count of measles patients was not correlated with the amount of G-CSFr or GM-CSFr expressed on the monocytes, though in normal children, it was correlated with both G-CSFr and GM-CSFr on the monocytes. Therefore, we suggest that monocytopenia could be induced by mechanisms other than altered CSFr expression. It is not certain that monocytes in the peripheral blood of measles show the increased expression of leukocyte function-associated antigen-1 (LFA-1) like macrophages.^{17,18} Increased LFA-1 expression promotes adherence to endothelial cells and transendothelial migration into the surrounding tissues and this could result in monocytopenia.

In conclusion, neutropenia is one of the more important characteristics of measles patients and could be due to the decreased expression of GM-CSFr on neutrophils. The monocytopenia found in measles patients is another hematologic characteristic of measles patients, but is not due to the reduced expression of CSFr on monocytes.

REFERENCES

1. Joklik WK, Willett HP, Amos DB, Wilfert CM. Measles and subacute sclerosing panencephalitis, In: Zinsser Microbiology. 19th ed. Prentice-Hall International Inc.; 1988. p.835-7.
2. Yang BK. Survey of immunity for measles in children in Korea in 2000. *J Pediatr Infect (Korea)* 2001;8:11-6.
3. Okada H, Kobune F, Sato TA, Kohama T, Takeuchi Y, Abe T, et al. Extensive lymphopenia due to apoptosis of uninfected lymphocytes in acute measles patients. *Arch Virol* 2000;145:905-20.
4. Okada H, Sato TA, Katayama A, Higuchi K, Shichijo K, Tsuchiya T, et al. Comparative analysis of host responses related to immunosuppression between measles patients and vaccine recipients with live attenuated measles vaccines. *Arch Virol* 2001;146:859-74.
5. Anderlini P, Przepiorka D, Champlin R, Korbling M. Biologic and clinical effects of granulocyte colony-stimulating factor in normal individuals. *Blood* 1996;88:2819-25.
6. Iqbal MP, Burney IA, Sultana F, Mehboobali N, Siddiqui T. Increased levels of multiple forms of dehydrofolate reductase in peripheral leukocytes of cancer patients receiving haematopoietic colony-stimulating factors; interim analysis. *Exp Mol Med* 2000; 32:84-7.
7. Tatsuo H, Ono N, Yanagi Y. Morbilliviruses use signaling lymphocyte activation molecules (CD150) as cellular receptors. *J Virol* 2001;75:5842-50.
8. Nanan R, Chittka B, Hadam M, Kreth HW. Measles virus infection causes transient depletion of activated T cells from peripheral circulation. *J Clin Virol* 1999;12: 201-10.
9. Nemunaitis J. A comparative review of colony-stimulating factors. *Drugs* 1997;54:709-29.
10. Dempke W, Von Poblozki A, Grothey A, Schmoll HJ. Human hematopoietic growth factors: old lessons and perspectives. *Anticancer Res* 2000;20:5155-64.
11. Lee KY, Suh B, Kim JW, Lee W, Kim S, Kim Y, et al. Varying expression levels of colony stimulating factor receptors in disease states and different leukocytes. *Exp Mol Med* 2000;32:210-5.
12. Shinjo K, Takeshita A, Ohnishi K, Ohno R. Granulocyte colony-stimulating factor receptor at various differentiation stages of normal and leukemic hematopoietic cells. *Leuk Lymphoma* 1997;25:37-46.
13. Anderson JF, Goldberger J. The period of infectivity of the blood in measles. *JAMA* 1911;62:113-20.
14. Ruckle G, Rogers KD. Studies with measles virus.II. Isolation of virus and immunologic studies in persons who have had the natural disease. *J Immunol* 1957;78: 341-53.
15. Papp K. Fixation du virus morbilleux aux leucocytes du sang des la perioded' incubation de la maladie. *Bull Acad Natl Med* 1937;117:46-54.
16. Forthal DN, Aarnaes S, Blanding J, de la Mazal L, Tilles JG. Degree and length of viremia in adults with measles. *J Infect Dis* 1992;166:421-4.
17. Attibele N, Wyde PR, Trial J. Measles virus-induced changes in leukocyte function antigen I expression and leukocyte aggregation: possible role in measles virus pathogenesis. *J Virol* 1993;67:1075-9.
18. Nagendra AR, Smith CW, Wyde PR. Evidence that measles virus hemagglutinin initiates modulation of leukocyte function-associated antigen I expression. *J Virol* 1995;69:4357-63.