Correlation of Natural Killer (NK) Cell Activity, Antibody Dependent Cellular Cytotoxicity (ADCC), and Serum Zinc Level in Behçet's Disease

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***Background*: Zinc which is widely used to treat Behçet's disease, is known to be an important modulator in various aspects of immunity including cell mediated immunity (CMI). CMI is suspected of playing a major role in the pathogenesis of Behçet's disease.**

***Objective*: This study was done to clarify the relationship of CMI and zinc in Behcet's disease.**

***Methods*: Serum zinc level, NK cell activity, and ADCC were measured in 83 patients with Bchsçt's diseade. The results were analyzed using multiple regression analysis.**

***Results*: ADCC and serum zinc level were found to be two significant variables that affect NK cell activity positively and negatively, respectively.**

***Conclusion*: Serum zinc is presumed to exert inhibitory effect on NK cell activity but does not affect ADCC in Behçet's disease patients. (Ann Dermatol 6:(2) 152-156, 1994)**

Key Words: Behçet's disease, NK cell activity, ADCC, Serum zinc

Zinc is known to be a major factor in the regulation of the immune response. The effect of zinc on the immune system is universal in that it affects cell-mediated as well as humoral immunity[1]. Primary antibody response[2], T-cell number[3], lymphokine production[4], blastogenic response[5], monocyte migration[6], and phagocytosis[7] were all found to be decreased when the serum zinc level is low. However, ADCC and NK cell activity in relation to the serum zinc level showed a marked variation among different investigators[3,8,9].

The pathogenesis of Behçet's disease is still obscure but it is now accepted that impaired immunity plays a signficant role and NK cell activity was found to be signficantly lower in patients with clincally active Behçet's disease[10,11]. Although zinc is being used to treat Behçet's disease, its role in the pathogenesis has been given only minor consideration[12]. In view of the link between the zinc and the cell mediated immunity (CMI), which is believed to be impaired in Behçet's disease, we have evaluated the mutual relationship between the level of serum zinc, NK cell activity and ADCC in Behçet's disease patients to find out how serum level of zinc might affect the CMI.

Received Junuary 14, 1994.
Accepted for publication March 28, 1994.
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This work was supported by the Faculty Research Grant and the Research Instructor Fund of Yonsei University College of Medicine, 1992.

MATERIALS AND METHODS

Subjects: Serum samples were collected from 83 Behçet's disease patients with more than one active lesion. Their diagnoses were made according to the Shimizu criteria[13] at the Behçet's Disease Specialty Clinic at Severance Hospital, Yonsei U-

niversity College of Medicine, Seoul, Korea. The types of manifestations found in patients were 8 complete, 37 incomplete, 34 suspected, and 4 possible.

Separation of lymphocytes from peripheral blood: Heparinized peripheral blood was mixed, within 2 hours after collection, 1:1 with the RPMI 1640 media (Gibco, Grand Island N.Y.) containing 5,000 μg/ml of L-glutamine, 100 units/ml of penicillin and 100 μg/ml of streptomycin (incomplete media). Mononuclear interface cells were separated by Ficoll Hypaque solution (1.077 g/ml density, Pharmacia Fine Chemicals, Piscataway, N.J.) and then washed twice with the same RPMI 1640 medi

Measurement of serum zinc: Serum, 2 ml, was mixed with the same amount of distilled water and 1.4 N HCl. The mixture was left standing for 30 minutes at room temperature. The same amount of 20% trichloroacetic acid was added and left alone for 10 minutes under the same condition. It was then centrifuged at 2,000 rpm for 10 minutes and the protein-free supernatant was used for the measurement of the serum zinc level by the atomic absorption spectrometry(Shimadzu AA650, Kyoto, Japan) at spectral absorption wave length of 213.9 nm.

Measurement of NK cell activity: Lymphocytes collected by the above procedure were dissolved to a final concentration of 5 x 10^6 cells in 5 ml of RPMI 1640 media containing 10% heat-inactivated fetal calf serum (complete media). The cells were put in a plastic culture flask (25cm^2, Costar, Boston, MA) and incubated at 37°C in a 5% CO_2 incubator. After 24 hours of incubation, non-adherent cells were collected to use as main effector cells.

K562 cells[14] labled with ^{51}Cr were used as the target cells for the 4-hour-^{51}Cr release technique. K562 cells, numbering 1 x 10^6, were dissolved in 0.2 ml of RPMI 1640 media containing 100 μCi of Na_2CrO_4 (1 mCi/ml NEZ-0305, New England Nuclear, Boston, MA). The solution was incubated for one hour and 15 minutes in a 37°C shaking water bath. Main effector cells and target cells were dissolved in the complete media to a final concentration of 5×10^6 cells/ml and 1 x 10^5 cells/ml, respectively. Equal volumes of each media (100μl) containing the cells (main effector: target cells = 50:1) were mixed in a 96-well round-bottomed microtiter plate and incubated for 4 hours at 37°C in a 5% CO_2 incubator. After the incubation, 100μl of the supernatant was taken from each well and their radioactivity (cpm)) were measured by a gamma counter. NK cell activity was calculated according to the equation shown below.

$$\text{percent lysis} = \frac{\text{test release-spontaneous release}}{\text{maximum release - spontaneous release}} \times 100$$

Spontaneous release was measured by using 100μl of the complete media put into a well containing only the labeled target cells. Maximum release was measured by adding 10μl of 0.25% Triton X-100 and 100 μl of the complete media into the well containing the labeled target cells.

Measurement of ADCC: Main effector cells for the ADCC measurement were identically prepared as for the NK cell activity measurement. For the target cells, human NK cell-resistant mouse L1210 tumor cells[15] were used. Immunized serum against the L1210 tumor cells were made by injecting 3~5 x 10^7 L1210 cells into New Zealand white rabbits. After 6 weekly injections, serum was collected and stored at - 20°C. The immunized sera were heat-inactivated at 56°C for 30 minutes and diluted 1:400 for use. Measurement was carried out by the same method used for the measurement of NK cell activity except for an addition of immunized serum.

Analysis of the results: Serum zinc level, NK cell activity, and ADCC were set as variables zinc, NK and ADCC, respectively, and analyzed by the SPSS/PC+program installed in an IBM-compatible personal computer. Multiple regression analysis was done to identify their mutual relationships.

RESULTS

Distribution of serum zinc level in Behçet's disease patients in our study was so variable that no correlation could be identified between the level of serum zinc and the disease activity, clinical types, or serum zinc level in controls (data not shown). We could not confirm the difference of NK cell activity between patients and controls but the ADCC was significantly increased in patients (Table 1). The Kolmogorov-Smirnov test resulted in the 2-tailed probabilities of greater than 0.05 for all 3 variables (Table 2). Comparison of the NK cell activity and ADCC in patients according to their levels of serum zinc showed significantly increased NK cell

Table 1. NK cell activity and ADCC in Behçet's disease patients and normal

	Patients(n=83) (% cytotoxicity)	Normal controls(n=16) (%cytotoxicity)
NK	43.7±22.6	37.8±25.5
ADCC	65.8±16.4*	56.6±15.8

*p<0.01 when compared to normal controls

Values are mean ± s.d.

Table 2. Result of the Kolmogorov-Smirnov goodness of fit test

	2-tailed probability
NK	0.245
ADCC	0.281
Zinc	0.203

Norml distribution if 2-tailed probability>0.05

Table 3. NK cell activities in Behçet's disease patients with low and normal serum zinc levels

	Zn(μg%)	
	Zn≤80 (n=22)	100<Zn≤120 (n=17)
NK*	51.47±18.48	38.14±18.20
ADCC	68.06±14.59	63.59±21.98

*p<0.05 by t-test

Values are mean±s.d.

Table 4. Correlation among the variables

	NK	ADCC	Zinc
NK	1.000	–	–
ADCC	0.318*	1.000	–
Zinc	–0.289*	0.002	1.000

*p<0.01 when compared to NK cell activity

Table 5. Result of the regression analysis

Independent variables	Coefficient	S.E.	P-value
Zinc	–0.26001	0.09071	0.0053
ADCC	0.43862	0.13912	0.0023
F(Significant F)		9.06162(0.0003)	
R^2		0.18	

P: probability
S.E.: standard error
Constant: 40.20903

activity in patients with lowered serum zinc level (Table 3).

The correlation was measured to determine the linear association between the variables. Significant positive correlation was seen between the variables NK and ADCC, and negative correlation was seen between the NK and zinc (Table 4). NK was set as the dependent variable, and ADCC and zinc were set as the independent variables. Multiple regression analysis resulted in the R^2 value of 0.18470. Regression coefficients were -0.26001 for zinc and 0.43862 for ADCC with a constant of 40.20903. The regression equation derived from the above procedure is

NK cell activity = 40.20903 + 0.43862 × ADCC – 0.26001 × zinc

P values for the zinc and ADCC were 0.0053 and 0.0023, respectively. F value was 9.06162 with a significance of 0.0003 (Table 5).

DISCUSSION

Of the numerous efferent pathways of immunity, NK cell cytotoxicity and ADCC are the main known routes of cell mediated immunity (CMI) which induce tissue damage in autoimmune diseases[1]. In Behçet's disease, Kaneko et al.[10] reported that NK cell activity in patients with clinically-

active stage was significantly lower than those of normal controls and patients in the inactive stage. Hamzaoui et al.[11] also noted that in the clinically active stage of Behçet's disease, NK cell activity was significantly lowered than those of healthy controls and patients in the convalescent stage. In our study, however, the NK cell activity showed no significant difference between the patients and controls but the ADCC was significantly increased in patients.

Zinc affects almost every step in the immune response and numerous investigators reported decreased NK cell activity in the state of serum zinc deficiency. Fernandes et al.[3] noted decreased NK cell activity and normal ADCC in zinc-deprived mice. Chandra[8], on the other hand, noted significant increase in the NK cell activity and ADCC under the same conditions. Although the mechanism of action in unknown, zinc is widely used to treat Behçet's disease. We have analyzed the relationship between the level of serum zinc and the two parameters of CMI in order to clarify how serum zinc affects the CMI in Behçet's disease.

Kolmogorov-Smirnov test result showed that all 3 variables, NK, ADCC and zinc, were normally distributed, which enabled them to be analyzed by the parametric tests. Correlation coefficients between the NK and ADCC, and NK and zinc were 0.318 and -0.289, respectively. Their p values were both below 0.01 and imply that ADCC has significant positive correlation with the NK cell activity, and NK cell activity has significant negative correlation with the serum zinc level, respectively. Zinc and ADCC showed no correlation with each other.

Value of the R^2 from the multiple regression analysis shows that ADCC and zinc together explains about 18% of the variation in the NK cell activity. The value seems to be relatively low but is significant is the view that the source of the varialbes is human and many other factors not yet revealed are responsible for the rest of the variation. Anyway, from the regression analysis, ADCC and serum zinc were found to be two significant variables that affect NK cell activity positively and negatively, respectively. F value was calculated to be 9.06 with a significance of 0.0003 which implies that the derived regression equation is valid and is highly significant.

It can be deduced from this analysis that serum zinc has an inhibitory effect on NK cell activity but does not affect ADCC in Behçet's disease patients. Supplement of zinc by oral medication might result in the partially suppressed CMI which in turn leads to the alleviation of inflammation in Behçet's disease.

Acknowledgement

We would like to thank Dr. Chung Mo Nam of the Deparment of Preventive Medicine, Yonsei University College of Medicine for his excellent advice in statistical processing.

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