Acta Genet Med Gemellol 44: 203-214 (1995)

Intrapair Differences of the Blood Cell Components and Lymphocyte Subsets in Monozygotic and Dizygotic Twins

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Abstract. We evaluated the relative influence of genetic and environmental factors on leukocytes, lymphocytes, neutrophils, monocytes, erythrocytes, reticulocytes, hemoglobin, platelets, T lymphocyte subsets, and NK marker cells in monozygotic (MZ) and dizygotic (DZ) twins. Peripheral blood components were measured in 154 healthy twin pairs (118 MZ pairs and 36 DZ pairs) and lymphocyte subsets in 48 healthy twin pairs (38 MZ pairs and 10 DZ pairs).

Platelets and erythrocytes showed higher intraclass correlations in the MZ pairs than in the DZ pairs in the males. However, in the females, the intraclass correlations for these components were similar between the MZ and DZ pairs. Neutrophils and monocytes showed low intraclass correlations in the MZ pairs. The absolute number of $CD4^+$ cells and the $CD4^+/CD8^+$ cell ratio showed high intraclass correlations regardless of age in the MZ pairs in both males and females. The intraclass correlation for the absolute number of $CD3^+$ cells was low in pairs aged $\geq$ 60 years in the MZ pairs. The absolute numbers of $CD16^+$ cells and $CD56^+$ cells showed high intraclass correlations regardless of age in the MZ pairs.

Key words: Twins, Platelet, Erythrocyte, T lymphocyte subset, NK cell, Age

INTRODUCTION

Studies on twins are imporant to analyze human responses involving both genetic and environmental factors. Since monozygotic (MZ) twins have the same genes but live in different environments, they provide useful data on the influence of environmental factors to epidemiologists.

In recent years, remarkable progress has been made in immunological studies. These immunological studies often use twins. Some studies have analyzed the serum concen-

trations of immunoglobulins in twins [5, 6, 8]. There are only a few studies in twins that analyzed lymphocyte subsets or other blood cell components from the genetic aspect. One of them reported marked intrapair differences in the CD8 T cell compartment between MZ twins but strict regulation of CD4 T cells expressing the Vß gene [4].

In this study, we evaluated the influence of genetic and environmental factors on leukocytes, lymphocytes, neutrophils, monocytes, erythrocytes, reticulocytes, hemoglobin, platelets, T lymphocyte subsets, and NK marker cells in MZ and dizygotic (DZ) twin pairs. The influence of environmental factors including age and lifestyle were analyzed in MZ twins, as well.

MATERIALS AND METHODS

Subjects

MZ and DZ twins were recruited from 2000 pairs in the Kinki University Adult Twin Registry [9, 10, 17, 18, 19]. The twin pairs in this registry were collected by midwife records, posters, publicity activities, and follow up of the subjects in the previous twin studies with cooperation of retired researchers. Among the twin pairs, 210 pairs volunteered to undergo comprehensive medical examination. Of the 210 pairs, 154 without history of allergic symptoms, diabetes mellitus, or rheumatism or abnormalities by routine blood examination, blood biochemical examination, or urinalysis were included in this study. $CD3^+$, $CD4^+$, and $CD8^+$ cells were measured in 48 pairs (38 MZ pairs and 10 DZ pairs). $CD16^+$ and $CD56^+$ cells were also measured in 32 (23 MZ and 9 DZ pairs) of the 48 pairs.

Twin zygosity was determined on the basis of the results of phenylthiocarbamide (PTC) taste blindness test and 9 blood type systems: ABO, Rh (C, c, D, E, e), MN (M, N), Lewis (Le^a, Le^b), P (P_1), Duffy (Fy^a, Fy^a), Kidd (Jk^a, Jk^b), Kell (k), and Diego (Di^a). There were 118 MZ pairs (67 male and 51 female pairs), and 36 DZ twins (24 male and 12 female pairs). The mean age ± standard deviation was 54.9 ± 11.9 years (range, 20-81 years) in all male MZ pairs, 49.4 ± 13.0 years (17-74 years) in all female MZ pairs, 57.7 ± 13.2 years (21-90 years) in all male DZ pairs, and 46.1 ± 12.2 years (31-73 years) in all female DZ pairs. In the pairs who underwent measurement of $CD3^+$, $CD4^+$, and $CD8^+$ cells, the mean age was 53.9 ± 13.2 years (28-69 years) in the male MZ pairs, 51.3 ± 10.5 years (34-74 years) in the female MZ pairs, and 53.1 ± 15.9 years (31-74 years) in the male and female DZ pairs. In the pairs who underwent measurement of $CD16^+$ and $CD56^+$ cells, the mean age was 53.1 ± 15.1 years (28-69 years) in the male MZ pairs, 43.3 ± 6.3 years (34-52 years) in the female MZ pairs, and 52.8 ± 16.8 years (31-74 years) in the male and female DZ pairs.

Methods

The subjects were told not to have breakfast on the day of examination. Blood samples (20 ml) were obtained via the brachial median vein. Blood cells were counted, and the

percentage of lymphocytes, neutrophils, and monocytes were determined by May-Giemsa staining. Reticulocytes were counted by the Brecher's method. Hemoglobin was measured by the oxyhemoglobin method.

Lymphocyte subsets were measured by flow cytometry [7, 12, 13, 14] using FITC-labeled monoclonal antibodies CD3 (OKT3), CD8 (OKT8), CD16 (OKNK) (Orth Pharmaceuticals), CD4 (Leu3a), and CD56 (Leul9) (Becton Dickinson). These antibodies react with mature T cells, cytotoxic/suppressor T cells, FcIgG receptors of NK cells, helper/inducer T cells, and NK cells and cytotoxic T cells, respectively. The absolute numbers of $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$ and $CD56^+$ cells were calculated as percentages of lymphocytes. The absolute numbers of these cells were used for analysis of lymphocyte subsets in this study on twins.

Data analysis

Intraclass variation, interclass variation, the variation ratio (interclass/intraclass variation), and the coefficient of intraclass variation were analyzed. The skewness and kurtosis of data distribution were tested by Fisher's cumulant method [3]. In variables not showing normal distribution of raw data, data were Log_{10} transformed. For analysis of variance, Snedecor's formula [16] was used. Eosinophils and basophils were excluded from analysis because the number of cells was small.

RESULTS

Intraclass Correlation Coefficient

Table 1 shows the mean values and the intraclass correlation for leukocytes, lymphocytes, neutrophils, monocytes, erythrocytes, reticulocytes, hemoglobin, and platelets. In the male MZ, erythrocytes showed the highest intraclass correlation (0.806) among the 8 variables. The intraclass correlations for erythrocytes and platelets were significantly higher in the male MZ pairs than in the male DZ pairs. In the female MZ pairs, hemoglobin showed the highest intraclass correlation (0.673).

Table 2 shows the mean absolute numbers and the intraclass correlations of $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, $CD56^+$ cells. The intraclass correlations for all variables were higher in the MZ pairs than in the DZ pairs. The absolute number of $CD56^+$ cells differed between the males and females (male, 0.820; female, 0.574).

Influence of Age

Table 3 shows the mean values and intraclass correlations for leukocytes, lymphocytes, neutrophils, monocytes, erythrocytes, reticulocytes, hemoglobin, and platelets according to age. The intraclass correlation for reticulocytes was significantly higher in the pairs aged < 40 years than in those aged 40-59 years or ≥ 60 years.

https://doi.org/10.1017/S0001566000001628 Published online by Cambridge University Press

Table 1 - Mean Values and Standard Deviations for Leukocytes, Lymphocytes, Neutrophils, Monocytes, Erythrocytes, Reticulocytes, Hemoglobin, and Thrombocytes in Healthy Twins

	Monozygotic			Dizygotic		
	Male 67 pairs Raw (log)	Female 51 pairs Raw (log)	M & F 118 pairs Raw (log)	Male 24 pairs Raw (log)	Female 12 pairs Raw (log)	M & F 36 pairs Raw (log)
Leukocytes (μ 1) – Mean	6125 ± 1566.3	5469 ± 1224.3	5842 ± 1462.5	5929 ± 1738.1	5592 ± 1465.5	5817 ± 1649.6
– Variance ratio	3.32 (3.54)	4.06 (4.86)	3.74 (4.16)	2.78 (2.49)	3.20	2.85 (2.72)
– Intraclass correlation	0.537 (0.559)**	0.605 (0.659)**	0.578 (0.612)**	0.472 (0.427)*	0.523	0.480 (0.462)**
Lymphocytes (μ1) – Mean	2099 ± 720.5	2039 ± 623.4	2073 ± 679.2	2022 ± 772.5	1852 ± 468.8	1965 ± 687.6
– Variance ratio	2.71 (2.77)	3.72 (3.54)	3.01	1.76	1.15	1.65
– Intraclass correlation	0.462 (0.469)**	0.576 (0.560)**	0.502**	0.276	0.070	0.245
Neutrophils (μ 1) – Mean	3486 ± 1236.3	3009 ± 976.6	3285 ± 1155.8	3424 ± 1499.5	3320 ± 1117.7	3388 ± 1369.1
– Variance ratio	1.79 (1.78)	3.63 (4.20)	2.30 (2.49)	3.67 (2.66)	3.57	3.55 (2.77)
– Intraclass correlation	0.283 (0.281)*	0.568 (0.615)**	0.395 (0.427)**	0.571 (0.454)*	0.562	0.561 (0.469)**
Monocytes (μ 1) – Mean	411 ± 201.0	371 ± 142.6	370 ± 183.6	398 ± 206.0	286 ± 205.8	361 ± 211.4
– Variance ratio	2.90 (2.60)	1.16 (1.31)	2.39 (2.20)	2.03	1.06 (1.23)	1.76 (1.99)
– Intraclass correlation	0.487 (0.444)**	0.073 (0.134)	0.409 (0.375)**	0.341	0.027 (0.102)	0.274 (0.332)
Erythrocytes (10^4/μ 1) – Mean	482 ± 47.6	431 ± 34.0	460 ± 49.2	472 ± 52.2	437 ± 52.6	460 ± 54.5
– Variance ratio	9.32	4.28 (3.68)	7.79 (6.87)	2.75	3.46	3.27
– Intraclass correlation	0.806** [1]	0.621 (0.573)**	0.773 (0.746)**	0.467*	0.551	0.531**
Reticulocytes (10^4/μ 1) – Mean	6.48 ± 2.39	5.32 ± 1.86	5.95 ± 2.24	6.71 + 3.39	4.79 ± 1.42	6.07 ± 3.01
– Variance ratio	2.32 (2.70)	2.45 (2.41)	2.61 (3.11)	1.63 (1.84)	0.89	1.78 (1.81)
– Intraclass correlation	0.397 (0.459)**	0.420 (0.414)**	0.445 (0.514)**	0.240 (0.297)	–0.059	0.280 (0.288)
Hemoglobin (g/dl) – Mean	15.2 ± 1.24	12.7 ± 1.39	14.2 ± 1.80	14.8 ± 1.85	13.0 ± 1.56	14.2 ± 1.95
– Variance ratio	6.22 (6.17)	4.98 (5.12)	11.42 (10.36)	4.36	2.68	4.86
– Intraclass correlation	0.723 (0.721)**	0.666 (0.673)**	0.839 (0.824)**	0.627**	0.457	0.659**
Thronbocytes (10^4/μ 1) – Mean	23.6 ± 6.23	25.2 ± 7.71	24.3 ± 6.94	22.8 ± 7.38	2.32 ± 8.18	22.9 ± 7.60
– Variance ratio	7.90	6.03 (4.57)	6.84 (5.74)	1.62	4.41 (4.52)	2.24 (2.33)
– Intraclass correlation	0.775** [2]	0.716 (0.641)**	0.745 (0.703)** [1]	0.235	0.630 (0.637)*	0.384 (0.399)*

* $p < 0.05$, ** $p < 0.01$, (intraclass correlation coefficient).

[1] $p < 0.05$, [2] $p < 0.01$, (comparison between MZ males and DZ males or between MZ females and DZ females).

Table 2 - Absolute Number of Lymphocyte Subsets in Healthy Twins

	Monozygotic			Dizygotic
	Male Raw (log)	Female Raw (log)	M & F Raw (log)	M & F Raw (log)
CD3		Total positive cells/μl		
Mean	1546 ± 610.9	1459 ± 383.1	1503 ± 508.4	1442 ± 546.6
Variance ratio	4.41 (4.06)	4.54	4.37 (4.24)	5.10 (3.85)
Intraclass correlation	0.630 (0.605)**	0.639**	0.628 (0.618)**	0.672 (0.588)
CD4				
Mean	971 ± 395.7	1020 ± 358.1	995 ± 375.7	983 ± 379.8
Variance ratio	7.17	8.38 (6.74)	7.50 (6.71)	3.85
Intraclass correlation	0.755**	0.787 (0.742)**	0.765 (0.741)**	0.588
CD8				
Mean	732 ± 377.0	505 ± 165.3	618 ± 310.9	514 ± 212.4
Variance ratio	3.78 (4.77)	2.58 (3.15)	4.15 (4.86)	3.64
Intraclass correlation	0.581 (0.654)**	0.442 (0.519)*	0.612 (0.659)**	0.569
CD4/CD8				
Mean	6.48 ± 2.39	5.32 ± 1.86	5.95 ± 2.24	6.07 ± 3.01
Variance ratio	6.63 (11.24)	7.88 (11.79)	8.21 (13.07)	5.50 (6.12)
Intraclass correlation	0.738 (0.837)**	0.775 (0.844)**	0.783 (0.858)**	0.692 (0.719)*
CD16				
Mean	722 ± 466.5	381 ± 169.8	589 ± 412.0	469 ± 201.7
Variance ratio	7.08 (8.01)	5.52	8.25 (7.88)	3.34
Intraclass correlation	0.752 (0.778)**	0.693*	0.784 (0.775)**	0.539
CD56				
Mean	645 ± 459.6	364 ± 191.7	535 ± 399.9	354 ± 186.6
Variance ratio	6.74 (10.09)	3.70	7.01 (7.34)	5.41
Intraclass correlation	0.742 (0.820)**	0.574*	0.750 (0.760)**	0.688*

Subjects analyzed for CD3, CD4, CD8 and CD4/CD8: 38 MZ pairs (19 male MZ and 19 female MZ) and 10 DZ pairs (4 male DZ and 6 female DZ).
Subjects analyzed for CD16 and CD56: 23 MZ pairs (14 male MZ and 9 female MZ) and 9 DZ pairs (3 male DZ and 6 female DZ).
* $p < 0.05$ ** $p < 0.01$.

Table 3 - Influence of Age on Leukocytes, Lymphoytes, Neutrophils, Monocytes, Erythrocytes, Reticulocytes, Hemoglobin and Thrombocytes in MZ twins

	Monozygotic twins		
	≤ 39 Raw (log) (n = 19)	40-59 Raw (log) (n = 65)	60 ≤ Raw (log) (n = 34)
Leukocytes (μ l)			
Mean	5561 ± 1524.8	6088 ± 1317.9	5528 ± 1616.1
Variance ratio	2.29 (2.85)	3.48	5.20 (5.54)
Intraclass correlation	0.393 (0.481)*	0.553**	0.677 (0.694)**
Lymphoytes (μ l)			
Mean	1982 ± 704.5	2155 ± 675.4	1964 ± 659.8
Variance ratio	3.79 (4.09)	2.76 (2.59)	3.06
Intraclass correlation	0.583 (0.607)**	0.468 (0.443)**	0.507**
Neutrophils (μ l)			
Mean	3089 ± 1125.0	3412 ± 1074.0	3155 ± 1311.0
Variance ratio	2.65 (2.49)	2.02 (2.15)	2.58 (2.98)
Intraclass correlation	0.452 (0.426)	0.339 (0.365)**	0.441 (0.498)**
Monocytes (μ l)			
Mean	299 ± 126.9	377 ± 193.5	400 ± 183.2
Variance ratio	1.82	2.71 (1.98)	1.77 (1.82)
Intraclass correlation	0.292	0.460 (0.330)**	0.279 (0.290)
Erythrocytes (10^4/μ l)			
Mean	477 ± 58.3	459 ± 46.0	451 ± 47.7
Variance ratio	27.24	5.03 (3.66)	9.84
Intraclass correlation	0.929**	0.668 (0.571)**[2]	0.815**
Reticulocytes (10^4/μ l)			
Mean	4.62 ± 1.41	6.03 ± 2.23	6.57 ± 2.33
Variance ratio	14.63	1.99 (1.98)	2.16
Intraclass correlation	0.872**	0.330 (0.330)**[2]	0.367*[2]
Hemoglobin (g/dl)			
Mean	14.1 ± 2.49	14.2 ± 1.75	14.2 ± 1.42
Variance ratio	15.63	10.83 (9.00)	9.40
Intraclass correlation	0.880**	0.831(0.800)**	0.808**
Thrombocytes (104/μ l)			
Mean	24.7 ± 8.52	25.0 ± 7.30	22.8 ± 4.86
Variance ratio	7.02 (3.47)	7.19 (6.09)	5.31
Intraclass correlation	0.751 (0.553)*	0.756 (0.718)**	0.683**

* $p < 0.05$, ** $p < 0.01$, (intraclass correlation coefficient).

[1] $p < 0.05$, [2] $p < 0.01$, (comparison between ≤ 39 years of age and other age groups).

https://doi.org/10.1017/S0001566000001628 Published online by Cambridge University Press

Table 4 shows the mean absolute numbers and intraclass correlations for $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, and $CD56^+$ cells according to age. The subjects were classified into two age groups (≥ 60 years, < 60 years) since the number of subjects for the analysis of these subsets was small. The intraclass correlation for $CD3^+$ cells was lower in the pairs aged ≥ 60 years than in those aged < 60 years. $CD4^+$ cells and the $CD4^+$/ $CD8^+$ cell ratio showed high intraclass correlations regardless of age.

Table 4 - Influence of Age on Absolute Numbers of Lymphocyte Subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, $CD56^+$ cells and CD4/CD8) in MZ Twins

	≤ 59 Raw (log)	60 ≤ Raw (log)
	Total positive cells/μ l	
CD3		
Mean	1541 ± 529.9	1419 ± 457.7
Variance ratio	6.14 (5.17)	2.17
Intraclass correlation	0.720 (0.676)**	0.368
CD4		
Mean	1010 ± 374.5	965 ± 384.4
Variance ratio	8.13	6.91
Intraclass correlation	0.781**	0.747**
CD8		
Mean	591 ± 261.5	677 ± 397.8
Variance ratio	6.36 (5.46)	3.10 (4.38)
Intraclass correlation	0.728 (0.690)**	0.512 (0.628)*
CD4/CD8		
Mean	1.90 ± 0.92	1.87 ± 1.31
Variance ratio	10.55 (16.78)	7.02 (11.17)
Intraclass correlation	0.827 (0.887)**	0.751 (0.836)**
CD16		
Mean	527 ± 292.0	730 ± 594.3
Variance ratio	8.12 (7.01)	8.54 (9.87)
Intraclass correlation	0.781 (0.750)**	0.790 (0.816)*
CD56		
Mean	452 ± 240.8	726 ± 599.1
Variance ratio	5.74 (5.96)	7.18 (11.95)
Intraclass correlation	0.703(0.713)**	0.756(0.846)*

Subjects analyzed for CD3, CD4, CD8 and CD4/CD8: 38 MZ pairs (26 MZ pairs aged ≤ 59 years and 12 MZ pairs aged 60 ≤ years).

Subjects analyzed for CD16 and CD56: 23 MZ pairs (16 MZ pairs aged ≤ 59 years and 7 MZ pairs aged 60 ≤ years).

* $p < 0.05$ ** $p < 0.01$.

https://doi.org/10.1017/S0001566000001628 Published online by Cambridge University Press

Influence of Lifestyle

Table 5 compares the mean values of leukocytes, lymphocytes, neutrophils, monocytes, erythrocytes, reticulocytes, hemoglobin, and platelets between MZ twins who showed intrapair differences in alcohol consumption, cigarette consumption, occupation, or body weight.

Of the 118 MZ pairs, 16 pair showed relatively marked intrapair differences in alcohol consumption, fulfilling the following criteria.

1. The mean daily alcohol consumption in one member of the pair was more than twice compared to that in the other.
2. The intrapair difference in alcohol consumption was at least 40 ml/day as pure ethyl alcohol.
3. The mean daily alcohol consumption was less than 100 ml of ethanol, and there were no symptoms of alcoholism.

The ethanol consumption was about 57 ml/day in the higher consumer group and about 10 ml/day in the lower consumer group. However, no significant difference was observed in any variable between the two groups.

Of the 118 MZ pairs, 15 pairs showed intrapair differences in cigarette consumption according to the following criteria.

1. One member of the pair smoked 20 cigarettes/day or more, and the other smoked no cigarette or smoked 5 cigarettes/day or less.
2. The intrapair difference in cigarette consumption was 20 cigarettes/day or more.

The higher consumer group smoked about 25 cigarettes/day, and the lower consumer group smoked almost no cigarettes. However, no significant difference was observed in any variable between the two groups.

It is common for Japanese to be engaged in a single occupation throughout their lives. The subjects were classified according to the occupation classification in the vital statistics according to occupations by the Japanese Ministry of Health and Social Welfare [11] into 11 groups (A, professional and technical workers; B, managers and officials; C, clerical and related workers; D, sales workers; E, agricultural, forestry and fisheries workers; F, mining workers; G, workers in transport and communications occupations; H, craftsmen, production process workers; I, protective service workers; J, service workers; K, workers not classifiable by occupation). In this study, A, B, and C were considered to be the 'light work' group, and E, F, and H to be the 'heavy work' group. In 17 of the 118 MZ pairs, one member was classified as the 'heavy work' group, and the other as the 'light work' group.

However, no significant difference was observed in any variable between the two groups.

Of the 118 MZ pairs, 32 pairs showed intrapair differences in body weight according to the following criteria.

1. The intrapair difference in body weight was ≥ 6 kg.
2. The intrapair difference in height was ≤ 2 cm.

Table 5 - Mean Values ± SD of Leukocytes. Lymphocytes, Neutrophils, Monocytes, Erythrocytes, Reticulocytes, Hemoglobin and Thrombocytes in Monozygotic Twins Discordant in Alcohol Consumption, Cigarette Consumption, Occupation and Body weight

	Leukocytes (μ 1)	Lymphocytes (μ 1)	Neutrophils (μ 10)	Monocytes (μ 1)	Erythrocytes (10^4/μ 1)	Reticulocytes (10^3/μ 1)	Hemoglobin (g/dl)	Thrombocytes (10^4/μ 1)
Alcohol consumption (n = 16)								
Low	6563 ± 1634.6	2310 ± 913.3	3462 ± 911.6	419 ± 181.2	484 ± 45.2	7.22 ± 2.50	15.3 ±1.23	24.4 ± 4.82
High	6388 ± 1218.7	2312 ± 652.1	3541 ± 1000.5	444 ± 178.1	470 ± 34.8	7.18 ± 2.73	15.0 ± 1.18	24.6 ± 5.66
Cigarette consumption (n = 15)								
Low	6167 ± 1472.9	2122 ± 1040.3	3458 ± 873.2	382 ± 165.3	480 + 58.0	6.63 ± 2.76	15.0 + 1.56	23.6 ± 5.80
High	6073 ± 1423.5	2354 ± 726.3	3531 ± 1520.8	413 ± 146.9	463 ± 59.7	5.50 ± 1.76	14.9 ± 1.80	22.5 ± 6.10
Occupation (n = 17)								
Light	6353 ± 1645.9	2375 ± 663.5	3435 ± 1480.7	402 ± 182.9	467 ± 54.1	7.23 ± 2.67	14.5 ± 1.68	23.4 ± 4.11
Heavy	5976 ± 1393.5	2135 ± 732.6	3349 ± 1206.9	374 ± 174.4	473 ± 53.7	6.31 ± 2.61	14.5 ± 1.49	23.0 ± 3.68
Body weight (n = 32)								
Light	5819 ± 1503.4	1975 ± 806.6	3326 ± 1211.2	361 ± 195.5	462 ± 60.6	5.55 ± 2.16*	14.3 ± 1.81	23.4 ± 5.73
Heavy	5884 ± 1383.6	2066 ± 642.6	3201 ± 976.7	424 ± 185.1	475 ± 53.6	7.11 ± 3.15	14.7 ± 1.70	24.6 ± 6.11

* $p < 0.05$

The body weight in the heavier group was 65.8 kg (± 12.1 kg), and that in the lighter group was 55.1 kg (± 9.4 kg). The mean intrapair difference in height was 0.2 cm. The reticulocyte count was significantly lower in the lighter group than in the heavier group ($p < 0.05$).

The association between the environmental factors and lymphocyte subsets could not be evaluated because of the small number of subjects examined. However, in 3 MZ pairs showing occupational differences, the absolute numbers of $CD16^+$ and $CD56^+$ cells were higher in twins in the 'heavy work' group than in those in the 'light work' group. The absolute numbers of $CD16^+$ cells in the 'heavy work' twin and 'light work' twin in the 3 pairs were 1024 and 724 (μl), 633 and 529, and 278 and 195, respectively. The absolute numbers of CD56+ cells in the 'heavy work' twin and 'light work' twin in the 3 pairs were 1546 and 876 (μl), 442 and 416, and 266 and 100, respectively.

DISCUSSION

The degree of genetic control of cells that differentiate from hematopoietic cells and the influence of environmental factors on these cells are still unclear. Studies on twins are useful to evaluate the influence of genetic and environmental factors.

Since number of DZ twins was low in this study, further studies are needed on the genetic contribution rate in additional cases. In this study, the intraclass correlation was primarily analyzed in MZ and DZ twins. The influence of environmental factors varied even among cells that differentiated from the same hematopoietic cells. Platelets and erythrocytes showed significantly high intraclass correlations in the male MZ pairs, suggesting marked genetic control. Anti-platelet antibodies such as CD9, CD41, CD42a, and CD42b were not evaluated in this study. These antibodies may also show high genetic contribution rates in the males. On the other hand, in the females, no difference was observed in the intraclass correlation for platelets or erythrocytes between the MZ and DZ pairs. This might be associated with menstruation in females.

On the other hand, the intraclass correlations for neutrophils and monocytes were relatively low. This finding suggests that the degree of changes in these cells is affected by environmental factors rather than genetic factors. Neither neutrophils or monocytes were associated with alcohol consumption, cigarette consumption, occupation, or body weight. Lymphocytes showed a moderate intraclass correlation in the MZ twins, suggesting that the degree of influence by genetic factors is similar to that by environmental factors.

T lymphocytes are roughly classified into $CD4^+$ cells and $CD8^+$ cells. The former bind to class II MHC molecules, the latter to class I MHC and regulate immunoresponses as helper / inducer T cells and suppressor / cytotoxic T cells, respectively. NK cells injure, in a non-restraint manner, autologous, isologous, homologous, and heterologous tumor cells, and their membrane surface markers are $CD3^-$, $CD4^-$, $CD8^-$, TCR^-, $CD16^+$, and $CD56^+$.

In this study, the intraclass correlations for the absolute numbers of $CD3^+$, $CD4^+$, and $CD8^+$ cells and the $CD4^+/CD8^+$ cell ratio were higher in the MZ pairs than in the DZ pairs. In particular, the intraclass correlation for $CD4^+$ cells was high in both male and female MZ pairs and was constant irrespective of age. These results indicate the marked

genetic influence on the absolute number of $CD4^+$ cells and also support the report that $CD4^+$ cells expressing Vβ gene of TCR in MZ pairs are strictly limited. The intraclass correlation for the absolute number of $CD8^+$ cells was slightly lower than that for $CD4^+$ cells. However, the $CD4^+/CD8^+$ cell ratio showed an even higher intraclass correlation both in the males and females regardless of age. This also indicates marked genetic regulation of $CD4^+/CD8^+$ cell ratio in healthy twins. On the other hand, the absolute number of $CD3^+$ cells showed a considerably low intraclass correlation in the pairs aged ≥ 60 years.

In general, the absolute number of cells decreases with age. It has been observed a decrease in the absolute numbers of $CD3^+$ cells and $CD8^+$ cells with age [7]. The absolute number of $CD3^+$ cells was slightly decreased in the twins aged ≥ 60 years in this study. Since the number of subjects was small in the present study, further studies are needed in additional cases on the association between the $CD3^+$ cells and aging.

The number of cells in NK cell subsets has been reported to be increased in aged subjects [1, 2, 15]. Our results are consistent with this finding. On the other hand, the absolute numbers of $CD16^+$ cells and $CD56^+$ cells showed high intraclass correlations in the MZ pairs and further higher correlations in the pairs aged ≥ 60 years than in those aged < 60 years. NK cells are derived from hematopoietic stem cells. However, their differentiation process remains unclear. The high intraclass correlation even in aged twins suggests only slight effects of age on NK cells and the differentiation of these cells under marked genetic control. The association between NK cells and environmental factors could not be evaluated because of the small number of subjects. However, in MZ pairs, the absolute number of NK cells was slightly higher in twins who were engaged in heavy work than in twins engaged in light work. This finding requires further investigation.

The intraclass correlations for the values of T lymphocyte subsets and NK cell subsets were lower in the DZ pairs than the MZ pairs but were relatively high even in the DZ pairs (0.5-0.7). In this study, healthy pairs were selected as subjects. Therefore, it is possible that there was selection bias in terms of genetic characters among siblings and environmental conditions. This should be taken into consideration when the results of this study are interpreted.

Acknowledgements: We express our deep gratitude to Prof. Tadahiko Shimizu at our department for instructions from the beginning of our study, and to Prof. Kazuo Hayakawa at Osaka University and Prof. Yasushi Ohba at the Department of Clinical Pathology of our university for cordial instructions. We also thank the staff at our department for cooperation in examination, the staff at the Central Laboratory of our university for cooperation in the measurement of lymphocyte subsets, and the twins who voluntarly underwent examination.

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