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Distribution of the HL-A antigens in Korean population

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1. INTRODUCTION

Since the HL-A system was defined as the major histocompatibility locus in man[9], leukocyte typing has made several important contributions to human biology, some of which are reviewed below.

First of all, it has been demonstrated that there is a significant relationship between tissue types and transplantation.[101] The correlation between the compatibility of the HL-A antigens of donor and recipient and the clinical result of human kidney transplantation was shown as early as 1965 by Terasaki and coworkers.[90, 103] Subsequent observations, that as the number of matched HL-A antigens increases the chance for a good clinical result also increases, have been confirmed in a number of reports on the basis of a progressively increasing amount of data.[11, 34, 47, 53, 73, 97] Besides ABO blood grouping and mixed lymphocyte culture, HL-A typing has now become the essential part of histocompatibility matching in clinical transplantation.[5, 17, 32, 86]

Secondly, the HL-A system has been found to be associated with anomalous clinical outcomes. Reports indicate that antibodies to leukocytes play a role in febrile reactions and thrombocytopenia in blood transfusions.[49]

Thirdly, it has been suspected to be associated with disease, based on the statistical analysis of the distribution of HL-A antigens in normal populations compared to patients. Certain HL-A antigens have been found to be predominant or deficient in patients with Hodgkin's disease,[12, 21, 52, 67, 68, 92, 110] leukemia[58, 76, 105, 107] choriocarcinoma and trophoblastic neoplasia,[59, 60, 66, 79] malignant melanoma,[19] cancer of the breast,[71] systemic lupus erythematosus,[41] psoriasis,[80, 108] ankylosing spondylitis,[81] adult celiac disease,[84] multiple myeloma,[12] multiple sclerosis,[69] chronic glomerulonephritis,[72] diabetes mellitus,[82] and leprosy.[31] It seems that these histocompatibility antigens have influence on individual susceptibility to certain disease.[33, 93, 102]

Finally, it is now well established that there are striking variations in the frequency of HL-A antigens in different ethnic groups,[1-3, 7, 16, 26, 29, 39, 42-45, 48, 78, 85, 95, 109] and it has been uncovered a genetically complex polymorphism,[25] due to its importance for the recognition of self, can be suspected of being a rather important parameter of the evolution of human populations.[78]

Current evaluation of the population data indicates most of the genes for HL-A antigens are defined in Caucasians,[1, 2, 3] Scandinavians,[85] Japanese,[48, 109] Mexicans,[39] Eskimos[29] and some African tribes.[42, 43]

Dausset et al.,[27] Albert et al.[1] and Singal et al.[83] had separately performed the typings for HL-A antigens in Oriental populations, which include various races originated from Asia, but there appeared no data concerning the genuine Koreans. Although there was a study on HL-A typing in Korea by Lee,[61] it was based on the leukoagglutination technique, and limited in number of cases surveyed and in number of antisera tested.

In Korea, renal transplantations were undertaken in several successive cases in recent years, and it has been urgently needed to perform a serotyping of HL-A system for better donor-recipient matching. Recent availability of specific antisera from NIH Serum Bank prompted this study to determine the incidence of HL-A antigens in normal Korean population.

2. MATERIALS AND METHODS

1) Nomenclature

The nomenclature of the antigens of the HL-A system was initially confusing since each laboratory used different designations for similar antisera. A nomenclature committee was established in 1967 under the auspices of the World Health Organization.[4, 5, 44, 45] Antigens which the committee agrees to be reproducibly detected are accepted and designated by "HL-A" or "A" followed by a number, or by this number only. Antigens not as yet accepted are assigned a Workshop number "W" followed by a number. The following specificities have adopted an international designation or provisional designation at Histocompatibility Workshop 1970, Munksgaard and a preliminary report from the fifth International Histocompatibility Workshop.[4, 45]

First segregant series (LA series); HL-A1, 2, 3, 9, 10, 11, 28, W19, W23, W24, W25, W26, W28, W29, W30, W31, W32.

Second segregant series (4 or 7 series); HL-A5, 7, 8, 12, 13, 14, 27, W5, W10, W15, W18, W21, W22.

2) Materials

A total of 196 Korean unrelated individuals were included in the study. The subjects were mostly normal healthy persons, consisting mainly of commercial blood donors, staff members of the hospitals, and out-patient clinic visitors for health certificates, of either sex. They were almostly inhabitants of Seoul city and neighbouring districts.

3) Methods

HL-A antigens were detected by testing purified lymphocytes against specific antisera. The reaction was detected by cytotoxicity that is if lymphocytes of a given person were killed by a given sera, his cells were assumed to have that antigen.

A. Preparation of Reagents

1. Ficoll-Isopaque mixture

Ficoll is a high molecular weight sugar available in plastic bottles (Mw. ca. 400,000; Pharmacia, Uppsala, Sweden). Isopaque is an organic iodide solution supplied in 20 ml ampules for use in radiography, with usual concentration of 60 or 75 per cent. The final mixture consisted of 10 parts 33.9 per cent Isopaque and 24 parts 9 per cent Ficoll.

Practically for a 20 ml ampule of 75 per cent Isopaque, 9.556g of Ficoll and 130.4 ml of distilled water was required to be 1.077g/ml in its final fluid density. This density gradient solution was stored at 4°C until use.

2. Complement

From a large pool of normal rabbits(usually 10 in number), whole blood was collected by cardiac puncture. The blood was spun at 2,000 rpm for 10 minutes. The supernatant was drawn and spun at 4,500 rpm at 0°C for 30 minutes in refrigerated centrifuge. The supernatant was poured off into small aliquots and stored at -70°C to dispense daily without refreezing.

3. Trypan blue dye

Two per cent solution of trypan blue standard preparation was diluted freshly with saline to obtain 1 per cent solution for daily use.

4. Formalin

Concentrated alkaline solution was added dropwise to formalin to adjust pH of 7.2-7.4. This reagent was stored in a brown bottle at room temperature.

5. Antisera

Standand antisera tested in this study were supplied from the National Institutes of Health Bank, Bethesda, Maryland, U.S.A. and as follows, totaling 29 in number.

Table 1. Lists of antisera tested.

Specificity	Serum name	Number
HL-A1	77.17; Caines*; 386*	3
HL-A2	8*; TO-07-02; De La Rie Weer*	3
HL-A3	Denning C; Caines*	2
HL-A9	Geer*; 102*; Voorbij*	3
HL-A10	Eva*; Aldridge	2
HL-A11	Chappell*	1
HL-A5	Byron; Chappell*; Jonkman; Voorbij*;	4
HL-A7	McCutchen; Hensing Molen; Smits*; Zand*	4
HL-A8	648598*; Cronin; Willet; Smits*; Zand*	5
HL-A12	Wallace; HOPM; 102*; Voorbij*	4
HL-A13	Blackham*; Elam*; 102*; Voorbij*	4
HL-A14	648598*	1
HL-A27	Bavel Fasel*;	1
W5	Hainault*; 102*; Voorbij*	3
W10	Vereen*	1
W22	Bavel Fasel*	1

* Duospecific or polyspecific serum.

Most antigens were detected by two or more sera and the above specificities listed in the left column were chiefly analyzed. All antisera were dispensed into 60-well micro-droplet testing trays (Falcon Microtest Tissue Culture Plate No. 3034, Scientific Products). To each well 0.005 ml of mineral oil was added to prevent evaporation with a single needle multiple repeating dispenser (250 lambda, Hamilton Company Inc.) and then sera were distributed 0.001 ml to each well with 50 lambda Hamilton syringe. These preloaded trays were stored in a -70°C freezer until use.

B. Lymphocyte Isolation

Starting as early as possible after bleeding, the process of isolation was usually finished within 6 hours of bleeding. Purified lymphocytes were isolated from heparinized whole blood by centrifugation over density gradient solution. The end result was a suspension of lymphocytes free of contaminating red cells, granulocytes and platelets. This separation technique was based on Boyum's method with slight modifications.[15, 88, 89, 91]

1. The heparinized whole blood of usually 2 ml was layered gently over the Ficoll-Isopaque mixture of 1 ml.

2. The mixture was centrifuged at 3,000 rpm for 10 minutes at room temperature, to separate the lymphocytes from polys, platelets and red blood cells.

3. The white layer of lymphocytes was aspirated with Pasteur pipette from interface between the supernatant and the density gradient separation fluid.

4. The lymphocyte pellet was washed two times with normal Hank's solution by spinning at 800-900 rpm for two minutes.

5. Discarding the supernatant, the pellet was mixed with normal Hank's solution to count the cells.

6. The final cell suspension was adjusted to be about 2-4 $x10^6$ cells per mililiter in diluent.

C. Typing Method

The lymphocytotoxicity test was applied

using two stage procedure, according to the modified method of Terasaki and McClelland as recommended by the National Institutes of Health. [87, 88, 89]

1. After thawing the sera, 0.001 ml of cell suspension was placed to each well, mixed thoroughly on the Yankee Rotator, and incubated for 30 minutes at room temperature.

2. Five microliter of rabbit complement was added to the cell-sera mixture and after 60 minutes incubation at room temperature, 0.005 ml of trypan blue was gently dropped.

3. Following another 30 minutes incubation at room temperature, 0.005 ml of formalin was added.

4. The excess supernatant fluid above the cells was removed by flicking off or by absorption on filter-papers.

5. The concentration of the dye in the well was diluted by adding normal saline to each well.

6. Cover glass (50×75 mm) was lowered on the wells just before reading in order to flatten the top of the droplet.

D. Microscopic Evaluation

Using the ordinary microscope with 10× objective, the results of the 30 reactions for each person were read. One out of these 30 reactions was control. Small and refractile cells were considered to be living lymphocytes whereas larger and stained cells with trypan blue were counted as dead ones. The following numeric code was chosen to read and record the result of cytotoxicity reactions.

Table 2. Numeric code for cytotoxicity test.

0: Invalid.
1: Negative·····same viability as control.
2: Negative·····0-19% dead lymphocytes.
4: Weak positive ···20-39% dead lymphocytes.
6: Positive ·····40-79% dead lymphocytes.
8: Strong positive···80-100% dead lymphocytes.

E. Calculations

Gene frequencies (GF) were estimated from the phenotype distributions (PF) in unrelated individuals using the following formula, according to Hardy-Weinberg law as in other studies. [2, 3, 29, 109]

$$GF=1-(1-PF)^{\frac{1}{2}} \cdots\cdots \text{equation 1.}$$

Haplotype frequencies were calculated from the gene frequencies and the phenotype data as outlined by Mattiuz et al. [63]

$$X_{ij}=D_{ij}+p_iP_j \cdots\cdots \text{equation 2.}$$

Where X_{ij} is the haplotype frequency corresponding to the i-th specificity at the first series and the j-th at the second, p_i and P_j are the allele frequencies corresponding to i and j repectively, D_{ij} is the gametic association between i and j estimated from the corresponding 2×2 table. Namely, if a, b, c, d are the respective frequencies of the ++, +−, −+ and −− phenotypes, then the corresponding D is estimated by

$$D=\left(\frac{d}{n}\right)^{\frac{1}{2}}-\left(\frac{b+d}{n}\times\frac{c+d}{n}\right)^{\frac{1}{2}} \cdots \text{equation 3.}$$

Where $n=a+b+c+d$.

3. RESULTS

1) Results of lymphocyte isolation

Because it is essential to acquire a pure lymphocyte suspension for the test and reading, the final cell preparations were examined by smear and Wright staining to know the composition of the differential cell types. Table 3 shows the result of cell purification obtained from 15 individuals who were sampled randomly out of 196 population group of the present study.

The erythrodcyte contamination ranged from 0 to 5.5 per cent with a mean of 2.0 per cent. The degree of granulocyte contamination in the individuals ranged from 2.8 to 8.0 per cent with a mean of 5.3%. Platelets were negligible to count in number. Thus lymphocytes with

Table 3. Result of lymphocyte isolation.

Subject	Erythrocytes (%)	Granulocytes (%)	Lymphocytes (%)
1	1.0	7.7	91.3
2	0.3	6.5	93.2
3	1.5	4.8	93.7
4	3.2	5.0	91.8
5	0.5	3.0	96.5
6	5.3	6.5	88.2
7	4.5	6.6	88.9
8	2.3	5.0	92.7
9	1.3	2.9	95.8
10	0	2.8	97.2
11	2.7	6.7	90.6
12	5.5	8.0	86.5
13	1.2	6.9	91.9
14	0.5	5.0	94.5
15	0.4	2.9	96.7
Mean	2.0	5.3	92.7

relative high degree of purity could be isolated from the process.

2) Result of cytotoxic reactions

The degree of cytotoxic reactions were analyzed in ten typing plates, containing 20 cell panels and 600 wells, of the last 20 samples. Table 4 shows the proportions of degree of reactions, classified according to the numeric code.

Table 4. Degree of cytotoxic reactions.

Code	per cent of dead lymphocytes	No. of wells	% (600)
1	Same viability as control.	494	82.4
2	0—19%	26	4.3
4	20—39%	29	4.8
6	40—70%	36	6.0
8	80—100%	15	2.5

The table revealed that 14 % (15 out of 106) of the wells, which contain more dead cells than control, resulted in strong positive reactions, 27% in weak positive and 25% in negative reactions.

3) Result of calculations

A population of 196 unrelated Koreans was tested for a total of 16 specificities, 6 of these belonging to the first segregant series and 10 belonging to the second series. Of this sample, forty one results were considered to be invalid due to the reactions with antisera specific for more than two antigens in either the first or second series, or other unreasonable findings in their final results. The detection rate of antigens from each individual in the remaining sample of 155 persons is presented in Table 5.

Table 5. Detection rate of antigens.

No. of antigens detected from one person	No. of cases	% (of 155)
4	60	38.7
3	54	34.9
2	27	17.4
1	7	4.5
0	7	4.5

Less than 40% of the population have a "full-house" of four different antigens. In other words, over 60% of the cases may have an antigen unidentified by the tested sera in this study, or some of them may have the same antigens expressed twice.

It is also remarkable that one quarter of the cells tested have two or less antigens or blank at all.

The population for the different HL-A antigens are given in Table 6. From this table, it can be seen that there is a rather even distribution of antigens throughout the first and second series. However, it is notable that HL-

Table 6. Incidence of HL-A antigens in Koreans.

Segregant series	Antigen	No. of positive	% (of 155)
First	HL-A1	27	17.4
	HL-A2	64	41.3
	HL-A3	38	24.5
	HL-A9	29	18.7
	HL-A10	42	27.1
	HL-A11	16	10.3
Second	HL-A5	28	18.0
	HL-A7	36	23.2
	HL-A8	18	11.6
	HL-A12	46	29.7
	HL-A13	64	41.3
	HL-A14	2	1.3
	HL-A27	1	0.6
	W5	25	16.1
	W10	20	12.9
	W22	4	2.6

A2 (41.3%) and HL-A10 (27.1%) from the first series and HL-A13 (41.3%) and HL-A12 (29.7%) from the second occur quite frequently. The frequencies of HL-Al and 9 are close together to each other, which should be beared in mind for later discussion.

Table 7 shows the gene frequencies of the HL-A antigens in the present sample. This was calculated from phenotype data by equation 1. In this sample of Korean population, about 24% from the first and 15% of genes from the second can not be defined with sera available to our laboratory presently.

Table 8 and 9 shows the phenotype distribution of the HL-A antigens in the first and second series respectively. In the first series, HL-A2/10 was found in 25 individuals, HL-A2/blank in 18, HL-A10/blank in 15 and unidentified phenotypes in 14. In the second series,

Table 7. Gene frequencies of the HL-A antigens.

Segregant series	Gene	Frequency
First	HL-A1	0.091
	HL-A2	0.234
	HL-A3	0.131
	HL-A9	0.098
	HL-A10	0.146
	HL-A11	0.059
	Total	0.759
Second	HL-A5	0.095
	HL-A7	0.124
	HL-A8	0.060
	HL-A12	0.161
	HL-A13	0.234
	HL-A14	0.006
	HL-A27	0.003
	W5	0.084
	W10	0.066
	W22	0.013
	Total	0.849

the most frequent phenotypes are HL-A12/13, detectable in 19 individuals, HL-A13/blank in 12, and blank alleles in 24. It is noted that total blank alleles far exceeds any other single phenotypes throughout the system.

The correlations between individual antigens are outlined in Table 10. This tabulation was made by direct counting the associated antigens from each phenotypes and filling the corresponding row and column. The most frequent associations were present between HL-A2/13 (45 cases) and HL-A2/10 (25 cases).

Haplotype frequencies were estimated according to the equations 2 and 3, from the gene frequencies in Table 7 and phenotype correlations in Table 10, and are given in Table 11.

The frequencies are represented in the form

Table 8. Phenotype distribution in first segregant series.

Phenotype	No. observed
1	10
1, 2	1
1, 3	8
1, 9	4
1, 10	4
1, 11	0
2	18
2, 3	9
2, 9	8
2, 10	25
2, 11	3
3	5
3, 9	5
3, 10	3
3, 11	5
9	5
9, 10	0
9, 11	3
10	15
10, 11	5
11	0
Blank	14

Table 9. Phenotype distribution in second segregant series.

Phenotype*	No. observed
5, 7	5
5, 12	11
5, W5	4
5, W10	4
5, W22	0
7	5
7, 12	1
7, 13	16
7, W5	4
8, 12	3
8, 13	5
8, 14	1
8, 27	1
8, W10	4
12	4
12, 13	19
12, W5	5
13	12
13, W5	8
13, W10	3
14	1
W5, W22	4
W10	5
Blank	24

* Other possible phenotypes were not observed.

of rectangular matrix whose row and column are the specificities belonging to the two series. From this table, it is considered that HL-A2-13, 2-12, 2-7, 10-13, 3-12, and 9-13 are common haplotypes in Koreans.

4. DISCUSSION

There are numerous satisfactory techniques for obtaining lymphocytes from the blood of healthy donors, as shown in Table 12.

Every method listed in the table has its own merits and drawbacks, but the most important factors in the isolation of lymphocytes are speed, simplicity, sterility, avoiding injury to cells, ability to prepare from either fresh, stored or cadaver blood, reasonable costs and equipments and finally its purity. Density gradient centrifugation method using Ficoll-Isopaque mixture seems to be particularly suitable for overcoming these problems,[15, 35, 50, 70, 88, 91, 94] and so was undertaken in the present study.

Table 10. Correlations between individual antigens.

	1	2	3	9	10	11	5	7	8	12	13	14	27	W5	W10	W22
HL-A1	*	1	8	4	4	0	4	7	4	17	13	1	0	8	2	0
HL-A2		*	9	8	25	3	12	15	0	21	45	0	0	0	13	0
HL-A3			*	8	3	5	5	5	4	17	4	0	0	8	5	4
HL-A9				*	0	3	0	4	5	13	14	0	0	0	4	0
HL-A10					*	5	0	0	8	4	8	0	0	3	2	3
HL-A11						*	0	0	3	5	9	0	0	5	0	6
HL-A5							*	5	0	11	0	0	0	4	4	0
HL-A7								*	0	1	16	0	0	4	0	0
HL-A8									*	3	5	1	1	0	4	0
HL-A12										*	19	0	0	5	0	0
HL-A13											*	0	0	8	3	0
HL-A14												*	0	0	0	0
HL-A27													*	0	0	0
W5														*	0	4
W10															*	0

Table 11. Haplotype frequencies for Korean population. (1,000)

	HL-A5	HL-A7	HL-A8	HL-A12	HL-A13	HL-A14	HL-A27	W5	W10	W22	Blank II
HL-A1	8.1	12.3	6.2	21.0	23.4	1.1	0.1	11.6	3.6	−1.0	6.2
HL-A2	33.8	31.9	12.1	34.0	75.7	0.6	0.5	−7.4	20.6	−1.0	37.0
HL-A3	11.7	13.1	8.1	27.2	16.5	0.5	0.1	3.8	11.4	4.1	25.3
HL-A9	3.6	10.0	8.0	18.2	23.5	0.3	0	3.2	6.3	−0.5	25.4
HL-A10	9.5	13.1	15.0	17.1	31.1	0.6	0.1	2.2	6.2	11.6	33.5
HL-A11	2.9	4.1	5.2	9.1	16.6	0.2	0	8.2	1.1	9.6	8.0
Blank I	36.7	39.3	16.9	32.1	52.4	3.1	2.6	26.5	17.9	0	24.1

Table 12. Lymphocyte isolation techniques.

1. Method employing a plasmagel-nylon fiber column technique, modified from Walford et al. [36, 104]
2. Albumin-flotation method of Kissmeyer-Nielsen and Kjerbye. [51, 27]
3. Hypotonic lysis technique of Terasaki. [50, 87]
4. Dausset's technique, using high molecular weight Dextran. [24, 50]
5. Patel's technique using Thrombin. [74]
6. Boyum's method using a mixture of Ficoll-Isopaque as a density gradient solution. [15]

By this technique, three distinct steps involved in other methods in order to sediment the red cells, remove the granulocytes and eliminate the remaining erythrocytes are avoided, [46] and finally pure lymphocyte suspension could be obtained as recorded in Table 3.

Various methods of tissue typing are in use currently, [13, 17, 25, 36, 50, 57, 77, 88, 96, 104] as shown in Table 13. The lymphocytotoxicity technique, originally described by Gorer and Gorman, [38] was adapted to human studies by Terasaki and McClelland, [87] Walford et al., [106] and Engelfriet and Britten. [30] This cytotoxicity method, based on the cell killing effect of complement in the presence of specific antibody, is the primary one used for clinical tissue typings. The cells usually employed in the test are purified lymphocytes. The most frequently used technique among the modifications of the cytotoxicity methods is that of Terasaki[88] which was adopted in the present study. It is rapid,

reproducible and relatively simple to perform. Recently the substitution of trypan blue for eosin as a vital dye, and the use of a two-step procedure with a buffer wash in order to eliminate anticomplementary factors replaced some steps of this technique.[57, 96]

Table 13. Current methods for histocompatibility testing.

1. Cytotoxicity
Microdroplet lymphocytotoxicity
Fluorochromasia
^{51}Cr release
2. One-way mixed lymphocyte culture
3. Complement fixation
4. Agglutination
EDTA
Mixed agglutination
Defibrinated blood
5. Immune adherence

Another increasingly popular cytotoxicity technique, fluorochromasia, utilizes the dye fluorescein diacetate(FDA).[13] It has been particularly useful in typing kidney cells in addition to lymphocytes.[77]

The release of ^{51}Cr from lymphocytes has been used to evaluate in a semiquantitative way the effectiveness of methods for purifying HL-A antigens, which remove the activity of corresponding HL-A antisera.[62]

In the mixed lymphocyte culture test,[6, 10, 37] stimulation is measured by cellular blast transformation, increased uptake of tritiated thymidine or both. It has been shown that the reaction of MLC correlate with histocompatibility as determined by cytotoxicity and by skin graft survival. The antigens measured by ML C reaction include the HL-A antigen and an additional series of antigens, not appreciated with any current serological technique.

Agglutination has been shown to detect some antigens appreciated not at all or only in part with other methods(4a, 4b, 5a, 5b, and 9b), but whose importance to allograft survival is not yet clear.[97] The platelet complement fixation technique has now been adapted to micromethod and semiautomated, so that when greater amounts of the scarce platelet typing sera are available, this test may become more prevalent.[20] The technique of immune adherence has the attraction of increased sensitivity especially apparent in detecting degree of presensitization in potential recipients.[17]

The microlymphocytotoxicity test used in this study revealed to be excellent method of typing as was shown in Table 4. It was said to be also useful in detection of antigens with low frequency and to have low degree of cross reactions[5].

Turning the focus of discussion to the main issue of HL-A system, a brief history of the system should be commented. In a critical series of experiments in 1944, Sir Peter Medawar[65] showed that accelerated rejections of rabbit skin grafts occurred when the recipient was pre-immunized with donor leukocytes. This indicated the major histocompatibility antigens were present not only on the cells of skin grafts but also on the leukocytes.

Antibodies to human leukocytes were described by Doan[28] in 1928, but the present extensive studies stem from the work of Dausset in 1954 who reported leukoagglutinating antibodies in 90 per cent of patients who had received multiple transfusions.[23] In 1958 Payne and Rolfs,[75] and van Rood et al.[98] reported the presence of antibodies to leukocytes in the sera of multiparous women. The antibodies formed during pregnancy are directed against paternal histocompatibility antigens present in the foetus. These description of leukocyte antibodies in man provided the starting point for the serological identification of the antigens with

specific antibodies. These antibodies, present in "typing sera", can be produced in the course of normal pregnancy, after blood transfusions, following rejection of kidney or skin allograft, or after intentional immunization of a volunteer with foreign leukocytes.[17]

Since the first description of a leukocyte antigen in man by Dausset[22] in 1958, a large number of antigens was described and was shown to belong to a single system, which was first called Hu-1,[9,27] and now is known as HL-A.[100]

The HL-A system is probably the major histocompatibility system in man and is comparable to the H-2 system in the mouse, the AgB system in the rat, the B system in the chicken, the DL-A system in the dog, and the H-I system in the rabbit.[32,40,49]

As a unique type of biological marker, determinants of the HL-A system are located on the cell membrane surface, and present on all nuclated cells tested thus far, although quantitative differences in their concentration may occur.[17,55,102] Practically, peripheral lymphocytes have been used as an easily accesible source for detection of these antigens, since their antigenic representation appears to be more complete.[5]

The chemical composition of these antigens is coming under intense investigation.[63] A molecular weight of 57,000 and 35,000 was estimated for the specificities. The exact composition of the antigens and cellular location of the true antigenic material is still unsettled.

The main characteristics of the HL-A antigenic system is its extreme polymorphism, as the number of possible phenotypes, even today, exceeds 10,000 and the number of possible genotypes exceeds 20,000, probably these figures are far below the actual truth.[23]

As a result of extensive family studies conducted during the Fourth Histocompatibility Testing Workshop,[4] it was agreed that the specificities could be arranged in the form of two segregant series called the first (or LA) and second (7 or 4) series. Each series appears to exist in various allelic forms. The number of alleles is already very large, although genes of each series are not all serologically recognized.

It has also been shown that an individual in whom his antigens are detected could have a total of four, so-called the "full-house" or saturated phenotype of histocompatibility antigens. No more than two of these antigens could arise from the first series, and no more than two from the second series.[8,54] Therefore, it became evident that these two series are controlled by two sets of mutually exclusive alleles at two closely linked loci.[17,32] The gamet from each parent, with half the chromosome number (haploid number) contributes two of the four antigens in the offsprings. This two antigen unit of inheritance, of haplotype, consists of one antigen from each segregant series.[2,3,8,26]

Reviewing the result of calculations in the present study, detection rate of antigens from each individual was relatively low, compared to other studies performed in other population groups.[2,29] In Eskimos,[29] two antigens only were detected on 26% of the cells, three only on 46%, and four on 28%, totaling 100% detection rate of at least two antigens. As evidently can be seen from gene frequency, there are as yet many antigens that are not detectable with available sera in Korean population.

In the Caucasians and Negro population,[2] about 98 and 88 per cent, respectively, of the genes for the first series of the antigens, and 89 and 78 per cent, respectively, of those for the second are known by the current population

Table 14. Comparision of frequencies of HL-A antigens in various races.

Population Investigator %(no. tested)	Caucasia-ns Albert (1028)	Danes Svejgaard (457)	Negro Albert (280)	Indian Hammond (147)	Mexican Gorodezky (200)	Eskimos Dossetor (?)	Orient. Albert (156)	Japan. Yokoyama (93)	Korean Hong & Kim(155)
HL-A1	26.1	29.2	6.8	27	29.0	0	0	1.0	17.4
HL-A2	50.0	57.6	36.0	31	68.0	50.6	44	41.9	41.3
HL-A3	24.8	27.2	14.6	15	44.5	0.3	2	6.5	24.5
HL-A9	21.8	28.0	29.0	16	23.5	87.7	61	59.1	18.7
HL-A10	11.6	—	10.4	23	—	0	15	19.4	27.0
HL-A11	12.9	36.4	5.0	25	—	—	17	—	10.3
W19	8.1	—	3.5	10	—	—	—	—	—
W28	—	62.5	—	12	—	—	—	—	—
HL-A5	11.4	11.0	11.7	37	35.5	27.4	33	31.2	18.0
HL-A7	23.4	31.5	16.7	13	16.0	19.3	6	18.3	23.2
HL-A8	18.0	18.6	6.4	5	24.0	0	0	4.3	11.6
HL-A12	27.1	28.7	20.3	10	—	0	15	15.1	29.7
HL-A13	3.8	5.8	0.3	7	—	0	—	11.8	41.3
W5	14.6	24.1	28.6	34	—	10.7	21	—	10.7
W10	12.5	16.8	7.9	34	—	80.1	34	—	12.9
W15	10.3	—	1.4	17	—	—	16	—	—
W18	9.2	—	8.9	—	—	—	15	—	—
W27	9.1	7.8	3.9	1	—	6.3	6	—	—

data. Initial study of the Bantu tribe,[43] in South Africa, showed that nearly 35% of the people had no antigens at the first locus detectable, but it was later found that almost all of the antigens, the Bantu posses, can be defined by the sera of the Bantu women.[42]

Accordingly it can be said that the Bantu had antigens that are unknown or rare in Caucasians, and the antibodies against such antigens were present in Bantu women. Therefore, it is necessary to prepare standard antisera for Koreans, from Korean women or available sources in near future to fill the blank alleles in this study.

Recent studies show, as repeatedly described, variation in the frequency of HL-A antigens in different racial groups. (Table. 14)

In a large and heterogenous sample of Caucasians, Albert et al.[2] found a high incidence of HL-A1(26.1%) and HL-A2(50.0%), and a relatively low incidence of HL-A13(3.7%).

Svejgaard et al.[85] observed in Danes and Norwegians that the most frequent antigens are W28, HL-A2, and HL-A7 in descending order. In Eskimos,[29] available data establish that the portion of the major histocompatibility system expressed by HL-A antigens is markedly restricted in polymorphism.

Ting et al,[95] examined Chinese, Malays and Indians in Singapore and found that, compared to the Caucasians, there is a marked variation in HL-A distribution. In the Chinese sample, there was a low incidence of HL-A1, 10, 7, and 8 and virtually no HL-A3.

These results support the findings of Singal et al.,[83] who found HL-A1 and HL-A8 virtually absent in a small Oriental sample in Los Angeles, and Albert et al.,[1] who also found a low incidence of HL-A1, as well as 3, 7 and 8 in 156 samples of various Oriental races.

In Japanese, there are certain discrepancies between studies according to investigators who performed serotyping in different times and at different places. Yokoyama[109] reported the in-

cidence of HL-A1, 3 and 8 to be low (1.1, 6.5, 4.3% respectively) from the Japanese population in Hawaii, but Ishibashi et al. showed a significantly different incidence of these antigens (11, 29, 7% respectively) from national survey in Japan. This discrepancy between the studies may be attributed to the sample size and availability of specific antisera tested.

In the present study of Korean population, it can be found that the incidence of HL-A antigens was evenly distributed and comparable to that of Scandinavians,[85] Negroes[2] or Japanese group of Ishibashi.[48] However, the result of the study evidently showed that HL-A1, 3, 8 and 13 behave as antigens of high frequency in Koreans, which are considered to have different pattern of antigen distribution from Japanese or other Orientals.

Ting et al.,[95] Bodmer et al.[14] and Yokoyama[109] suggested the HL-A1 as a "Caucasian antigen" on the ground of an absence of HL-A1 in Orientals, Babinga pygmies and Japanese they observed. But in Korean population, the incidence of this antigen is relatively high(17.4 %) and is located midway between the frequency of Caucasians and non-Caucasian populations.

HL-A13, one of the highest incidence in Koreans, is never found frequently in other populations[1, 2, 29, 85, 109] and is considered to be unique to the Koreans. Another strikingly different phenomenon can be noted in HL-A9, which occurs very frequently in Orientals (60 %)[1] and Eskimos(87.7%).[29] This antigen was observed rather infrequently in Koreans (18.7 %) and in Caucasians (21.8%)[1, 2, 3] in contrast to above figures.

In overall, it is probably safe to say that the distribution of HL-A antigens in Koreans differs significantly from those of any other populations reported in the literatures to date.

Another point of view may be elicited from data that there are many instances of undefined antigens, which can raise the conclusion that "a serum that behave perfectly in the population group against which it was originally characterized may perform quite differently."

In general, gene frequencies can be determined by three different methods;[3, 63]

1. using the square root formula of equation 1.
2. using Mickey's allele-fitting minimum chi-square program, and
3. by gene counting the total haplotypes from large number of families.

The results obtained with these three methods have been confirmed to be virtually identical.[3, 26, 63, 85]

In this study, the author estimated the frequency using the square root formula, because the other methods were unavailable. The pattern of gene frequency in Koreans is also significantly different from those of other populations.

Because the haplotype is the unit of inheritance for HL-A antigens, it is important to determine not only gene frequency but also haplotype frequency. This can be done by simple counting of haplotypes deduced from lage number of families[2, 3, 26, 85] or it can be calculated from a large sample of random phenotype data as outlined by Mickey or by Mattiuz at al.[2, 3, 4, 63] The frequencies determined by either methods also did not show any significant differences.[2, 3, 4, 26, 63] In the present investigation, it was estimated from the method of Mattiuz et al.

There are many reports that certain haplotypes are found with unexpected frequency in certain population. In Caucasians[2] the three

most frequent haplotypes are HL-A1-8, 2-12 and 3-7, and in Negroes[2] W28-W17, W19-W17 and HL-A9-12. In Koreans HL-A2-13, 2-12, 2-5, 2-7, 10-13, 3-12 and 9-13 were considered to be common types. There appeared no data concerning the haplotypes in Oriental population in large scale.

Reviewing the overall results, the data collected in the present study may not reveal the real status of the HL-A system in Koreans and must regard as tentative one, firstly because the sample size surveyed is rather limited to detect polymorphic antigens fully, secondly because the antisera tested is inadequate in number and specificities, thirdly because precise genotypes and haplotypes were not observed from family study, and finally because preparations of standard antisera from Korean peoples were not applied in this study.

However it can be firmly said that accurate and standardized methods for HL-A typing were established through this study, in which speedy and simple technique of lymphocyte isolation with high degree of purity, and rapid, reproducible and reliable method of cytotoxicity test were achieved as a prime goal.

It is recommended through this study to survey larger number of samples from the population, deduce the gene frequency and haplotype frequency from family study and prepare standard antisera from Koreans in order to grasp the real status of HL-A system in Koreans.

5. SUMMARY AND CONCLUSION

The HL-A system lymphocyte antigens were detected and analyzed in 155 normal unrelated Korean peoples, using the isolation technique of lymphocyte with Ficoll-Isopaque mixture and microlymphocytotoxicity tests.

The results obtained in this study are as follows:

1. The HL-A antigens are rather evenly scattered in its distribution throughout the first and second series, which seems to be quite different from any other population groups. In Koreans, therefore, there is evidence of diversity in the polymorphism of those regions of the major histocompatibility complex expressed by antigen.

2. The most frequent antigens are HL-A2, 13, 12 and 10 in the decreasing order of frequency. HL-A9 occurs rather infrequently compared to other Oriental populations and HL-A1, which has been recognized to be absent from Orientals, occurs in about 17.4% of the population surveyed. The most frequent haplotypes were HL-A2-13, 2-12, 2-5, 10-13, 3-12, and 9-13.

3. There is a large proportion of blank alleles, 24% and 15%, respectively from the first and second series, which seems to be related to having used not only limited number of antisera, to testing limited number of panels, to the probable existence of unknown specificities in our native population, and to the presence of still undefined or other recently defined antigens.

4. It is certainly suggested that the standard and accurate method of HL-A typing is established through this study, which made possible to perform better histocompatibility matchings for clinical transplantations, to investigate relationships between various diseases and HL-A antigens, and to evaluate further parameter of the evolution of human beings in Korea.

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=초 록=

한국인의 HL-A 항원분포에 관한 연구

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1944년 Sir Peter Medawar가 가토피부이식 실험을 통하여 백혈구에 조직적합성 항원이 있음을 지적한 것을 효시로, 1954년 Dausset가 인체에서 백혈구항체에 대하여 기술하고 계속하여 혈청학적 연구를 진행시켜 1958년 비로소 인체 백혈구 항원을 발견하였다.

그 후 다수의 항원이 발견 보고되면서 현재 HL-A라고 명명되는 단일체계에 속한다는 것이 알려지고 HL-A 체계는 다시 두개의 격리계열(Segregant series)로 구성되고 있음이 확인되었다.

이와 같이 인체 백혈구 항원체계가 가장 중요한 조직적합성인자(Major histocompatibility locus)로 밝혀진 이래 인체 장기이식분야에서의 각광은 물론 이들 항원의 각종 질환과의 연관성 및 인종에 따라 항원 분포상황의 다양성등으로 많은 학자들에 의해서 이 방면의 연구가 활발히 진척되고 있으며 각 연구소의 업적들이 국제적 협조하에 비교 검토되고 있다.

국내에서는 아직도 이 방면의 연구가 미비하던 차에 금번 NIH로부터 인체 백혈구 표준항혈청을 공급받을수 있게 되어 저자는 우선 정상한국인의 백혈구 항원분포를 조사하였다.

서로 인척관계가 없는 정상한국인 196명으로부터 채혈을하여 다음에 열거하는 백혈구 항원의 분포상황에 관한 실험을 실시하였다.

제1격리계열(First segregant series):HL-A 1, 2, 3, 9, 10 및 11.

제2격리계열(Second segregant series):HL-A 5, 7, 8, 12, 13, 14, 27, W5, W10 및 W22.

임파구분리는 Ficoll-Isopaque 혼합액을 이용한 Boyum씨의 개량방법을 사용하였으며 한 항원에 대하여 두가지 이상의 혈청이 대체로 시험되었다.

전실험을 통하여 Terasaki씨의 미량임파구 독성검사가 항원검출에 사용되었으며 판정은 Trypan blue 시약 염색으로 생존 및 사멸된 임파구수를 계산함으로써 반응의 음양성을 가렸다.

이상의 실험으로 한국인 HL-A 항원의 Phenotype, Genotype 및 Haplotype의 빈도를 산출하여 그 결과를 지금까지 보고된 타인종의 빈도와 비교 검토하였다.

1) 한국인의 HL-A 항원의 분포는 전체계를 통하여 비교적 널리 분산되어 있어 이 체계의 복잡성이 다시금 확인되었으며 이와 비슷한 분포를 지닌 인종은 문헌상 찾아 볼 수 없었다.

2) 제일 흔한 항원으로 HL-A 2, 13, 12 및 10의 순서이고, 동양인에게 제일 높은 빈도를 보인 것으로 알려진 HL-A 9는 비교적 낮은 수치를 나타냈으며 또한 동양인에게 존재하지 않는 것으로 보고된 HL-A1이 검출되고 있다는 점이 특이하였다.

3) 제1 및 제2 격리계열의 각각 24% 및 15%에서 항원 검출이 안되었는데 시험에 사용된 표준항혈청의 수 및 종류, 동원된 모집단의 크기, 현재까지 발견치 못하고 있는 항원이나 우리 민족에게만 고유하게 국한되어 나타날 수 있는 항원의 존재가능성 등에 기인되리라 사료된다.

4) 본 실험을 통하여 비교적 정확하고 표준화된 HL-A 항원 검출법이 확립된 것으로 확신하는 바 앞으로 인체 장기이식시의 조직적합성 검사, HL-A 항원의 각종 질환과의 연관성, 가계연구 및 한국인 자체의 표준항혈청 준비에 크게 기여될 것으로 믿는다.

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