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AN OVERVIEW OF HUMAN HISTOCOMPATIBILITY (HLA) SYSTEM

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INTRODUCTION

Histocompatibility in human transplantation is mainly related to the basis for the selection of compatible donors in ths ABO and histocompatibility antigens (HLA) (1-3). Both antigens are found on lymphocytes, granulocytes and platelets, and widespread in tissues (4-6). The rejections of skin graft and kidney transplantation are associated with HLA incompatibility (7-15). HLA system is revealed to be the genetic markers of certain diseases (16-29). The present communication deals with a review of background, genetic, histocompatibility testing and applications of HLA system.

BACKGROUND

The first of histocompatibility system was detected in mice by Medawar, and caled H-2 system, The histocompatibility systems in other species, i.e., H-1 (Ag. B) in rat. B in chicken, Ch-L-A in chimpanzee and DL-A in canine were subsequently documented (30-33). The first HLA antigen leukocytes was identified by Dausset, called "Mac" antigen (34), and now generally known as HLA-A2. HLA antigens were further reported by many investigators, i.e., van Rood. et al. (4^a, 4^b, 5^a, 5^b, 6^a, 6^b, 6^c), Shulman, et al. (Pl Gr Ly B^l, Pl Gr Ly C^l) and Payne, et al. (LA-1, LA-2, LA-3) (35-37). Human histocompatibility system was called LA by Payne, Du by Amos, Hu-1 by Dausset, FOUR by van Rood, and HL-A by the world Health Organization (WHO). At present, HL-A system, the major histocompatibility system, is recommended as HLA system, after

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the Sixth Workshop of WHO and the International Union of Immunological Societies in Arthus in July, 1975 (38)

CHARACTERIZATION OF HLA ANTIGENS

HLA antigens have been solubilized by exposure to low intensity sound, by proteolytic digestion, by organic solvent or detergent extraction and simple salt (39, 41-47). Transplantation antigens are glycoprotein and composed of two polypeptide chains. HLA alloantigenic determinant is responsible for the protein portion at the chain of 31,000 M.W. Another chain of 11,800 M.W. is similar or identical to B-2 macroglobulin. Carbohydrate possibly plays a role in the determination of some HLA specificity eventhough sialic acid is not essential for HLA alloantigenic activity (48-52).

GENETIC OF HLA

Understanding of a major histocompatibility antigen is realizing its great complexity which is composed of the series of many closedly linked genes. The genetic loci have been assigned to the autosomal chromosome C6 by somatic cell hybridization using IPO-B marker (53), and by family studies (54) which they showed linkage between HLA and phosphoglucomutase (PGM3) on the basis of detection of HLA components are mainly concerned to serological defined (SD) and lymphocyte defined (LD). The SD specificities of the HLA system mainly based on the lymphocytotoxicity testing to be assigned to two separated loci (first or LA, second or FOUR). The LA and FOUR are currently called A and B loci, respectively (38). AJ (C) locus can be considered to be the third locus. LD locus to be responsible for MLC is recognized to be the fourth (D) locus. Certainly, LD antigens have high linkage disequilibrium (Δ) association with HLA-B7 or HLA-B8 of SD determinants (55). Each locus of LA and FOUR can be represented by more than 20 different antigens (Table 1). The inheritance of the LA and FOUR antigens transmitted by the same parental chromosome is called the haplotype. A deriving of haplotype

combination is the result of crossing-over at mitosis which the frequency is determined around 0.467% (56).

GENETIC ANALYSIS

If there are I specificities in the LA series and k in the FOUR series, the numbers of haplotypes, genotypes, and phenotypes. are Ik, $\frac{1}{2}Ik\,(Ik+1)$ and $\left[I+\frac{1}{2}(I-1)(I-2)\right]\left[k+\frac{1}{2}(k-1)(k-2)\right]$ respectively.

A constant gene frequencies in the population are maintained followed to the Hardy–Weinberg law, the frequency of the gene (p) of an antigenic specificity is given by: $p=1-\sqrt{1-f}$, where f is the frequency of the positive individuals. On the other hand, the calculation of p is determined by the method of maximum likelihood (57). Estimated haplotype frequencies (xij.) (58) can be expressed from population phenotype frequencies in the form: $Xij = Dij + piPj$, where pi and Pj are the allele frequencies and Dij is the gametic associations (the linkage disequilibrium parameter or Δ). Dij value is estimated by Dij $=\sqrt{\frac{d}{n}}-\sqrt{\frac{(b+d)(c+d)}{n\quad n}}$, where $n = a+b+c+d$, and the corresponding frequencies are represented in table:

		LA Series i+	LA Series i-
FOUR Series	j +	a	b
	j −	c	d

The variance of estimated Dij can be approximated by the form:

$$V(\Delta)_{ss} \quad \frac{(a+b)(a+c)}{4n^3}$$

or $$V(\Delta)_{ss} \quad \frac{I}{4n} \quad fiFj$$

where fi and Fj are the frequencies of individual positive to the i-th and i−th specificities respectively. The standard error of Xij (74) is given by $S=\sqrt{f/4n}$; where f = relative frequency and n = number of families.

HISTOCOMPATIBILITY TESTING

An adequate matching of histocompatibility antigen between prospective donors and recipients is necessary for successful organ transplantation. Several methods of detection which can indicate degrees of histocompatibility are available both in vitro and in vivo. A

suitable method should be highly reproducible, simple, unsophisticated and less time-consuming.

I. LEUKOAGGLUTINATION

The first HLA antigen is recognized by leukoagglutination (34). The leukocyte suspension is performed by using detibrinated blood or collecting blood into ethylene diamine tetraacetic acid (EDTA). Red blood cells are removed by sedimentation, centrifugation, or sedimentation by dextran or polyvinylpyrrolidone (PVP). Platelets are eliminated by differential centrifugation. Using EDTA blood, the pH of blood and the concentration of EDTA are critical (59). The lenkocytes from defibrinated blood is rarely agglutinated. The viability of polymorphonuclear cells (PMN) is important in leukoagglutination. The sera prior to testing is inactivated at 56 °C for destroying the inhibitor. In microtechnic (59). acetic acid is added to lyse red blood cells in order to prevent the interference of the microscopic reading. Recently, the capillary leuko-agglutination (60) is developed and may be more effective.

II. LYMPHOCYTOTOXICITY

The lymphocytotoxicity testing is dependent upon the permeabilily of lymphocytes after Incubation with antibody and complement. The lymphocytes suspension is separated by the PMN attachment to nylon, grass, or cotton (61,62); phagocytic property of PMN to iron particles; isopynic solution of Ficoll-Hypaque, and Ficoll-Triosil (63-70). Recently the simple method for lymphocytes separation is the Ficoll-Hypaque-Sucrose technique method (71). Rabbit or human mixed with rabbit complement is immediately added or after preincubation (59). An excess antibody should be removed. Indicator systems for qualitative and/or quantitative lymphocytes dead cells are used In different ways.

Dye exclusion method is based on the ability of intact lymphocytes to exclude dye such as trypan blue, eosin-Y and Negrosin while dead cells take up the dyes. The dead cells

can also be identified under phase contrast microscope. and ^{51}Cr released method (59,72,73,75).

The sensitivity of the lymphocytotoxicity testing is enhanced by using frozen cells, synergism of sublytic antibody and the lymphocytes treated by proteolytic enzymes such as trypsin, papain and Ficin, chemicals. 2-Aminoethylisothiouronium bromide hydrobromide (AET), and cystein (76–82).

Many factors can affect antigen–antibody reaction including complement source, different ionic strength, ditferent in affinity, serum inhibitor, viability and purity of lymphocytes suspension, sialic acid covering of antigenic site and complement sensitivity are important (83–85).

III. COMPLEMENT FIXATION

The presence of HLA antigen on platelets was first recognized by Shulman et al. through complement fixation test which antigen was described as the P! Gr Ly^{B1}(86). The variant methods were developed by Nymand et al. (87), and Svejgaard et al. (88). Platelets suspension is prepared by a differential centrifugation of blood collected in sodium citrate or EDTA as disodium salt. In sterile condition, the platelets suspension obtained could be stored at $4^{o}C$ for 4 to 5 days.

If 0.1% sodium azide is added to the platelets suspension and stored at $4^{o}C$, the preparation is stable for 10 to 12 months. Platelets complement fixation test is 4 times less sensitive than lymphocytotoxic testing.

IV. MIXED LEUKOCYTE CULTURE (MLC)

The MLC test is a method to determine the compatibility of donor and recipient. One-way stimulation mehod of Bach and Voynow (89) is performed by the treatment of the stimulating cell population with Mitomycin C. A mixture of stimulating and responding cells is incubated at least for 72 hours of culture. An assay is finally performed by counting incorporated radioactive thymidine and/or examining the morphology of blastic transformation under light microscope (90,91). The MLC test is being used

for detection of HLA identity, especially in the bone marrow transplantation.

SOURCE OF ANTISERA

The HLA antibodies are found in multiparous women, polytransfused patients, transplanted patients, volunteer recipient of skin graft or leukocyte injection and/or alloimmunized chimpanzee. The monospecific antisera is found in 1.0% of multiparous women with leukocyte antibody.

The identification of HLA antibody is analyzed by 2 x 2 table (Table 2).

Table 2. The 2 x 2 table

		First serum +	First serum −
Second Serum	+	a	b
	-	c	d

If b = c = 0, the sera are identical

If b = 0, serum 2 is contained within serum 1, and vice versa if c = 0

If the sera show a significant positive association ($ad - bc > 0$, or $ad/bc > 1$), they may show one or more antibodies.

The correlation coefficients is measured by (37,97).

$$P = \frac{(ad - bc)}{\sqrt{(a + b)(c + d)(a + c)(b + d)}}$$

where $n = a + b + c + d$

APPLICATION OF HLA TYPING

It now appears that the HLA system is also important in other fields other than in the area of organ transplantation. In fact, the HLA system is involved in pregnancy, transfusion of white blood cells and platelets, association with diseases, paternity and anthropology.

I. TRANSPLANTATION

Antileukocyte antibodies was present after skin grafting. Nevertheless the observations that the survival times of skin graft well match for HLA and ABO were significently longer than poor ones. The survival time of one locus incompatibility skin graft was 13.37 ± 8.87 days while the two loci incompatibity was 11,51 ± 2.31 days ($p < 0.001$) (92). Dausset and Horst reported that the survival times

of HLA identical sibling, ½ identical and unrelated were 96, 71 and 47% at 2 years, respectively (93). Tissue typing is of clinical significance in kidney transplantation when donors and recipients have two HLA antigens of B (FOUR) locus in common. At present, transplantation with cadaverous kidney is frequently overemphasized because of ethical problems in removing kidney from living donors.

II. TRANSFUSION

About 2% of all blood transfusion (94), the febrile reaction is frequently due to HLA antibodies of recipients direct against HLA antigens of granulocyte donors and it causes destruction of cells and liberation of pyrogens. In modern blood transfusion therapy, infusion of platelet concentrated to arrest bleeding in thrombocytopenic patients is a common practice. Multiple transfusion in these cases will eventually develop multispecific HLA antibodies. Finally, HLA typing of platelets becomes necessary for selecting donors for thrombophoresis.

III. ASSOCIATION BETWEEN HLA AND DISEASES

The correlation between the specific HLA antigens or phenotypes and the development of disease has been investigated. Many reports suggested an association between HLA-Bw15 and juvenile diabetes. A highly significant increase of HLA-B8 in juvenile diabetes mellitus and w15 in insulin-dependent diabetics have been documented (95,96). HLA-B8 was also increased in Grave's disease (98). HLA-B27 has strong association with ankylosing spondylitis (99). Patel suggested that HLA-B7 associated with breast cancer but was not confirmed by Cordon (100). Increased incidence of HLA-A2, B12 and HLA-A1, B8 haplotypes have been shown in leukemia and HLA-A1, B8 in asthma (101). The mechanism of association between HLA and disease may be due to the possible receptor role of HLA antigens or the molecular mimicry between HLA antigens and viruses, and/or the immune response (Ir) gene linked to HLA complex. The LD determinants have

been revealed to exhibit a stronger association with diseases than SD determinants (102). HLA typing pay also be help in diagnosis of certain diseases such as, in differentiating Reiter's disease from gonococcal arthritis (103); in prognosis that children with HLA-A9 seem to be more resistant to leukemia (104); and in therapeutic approach by which myasthenia gravis patients with HLA-B8 are the good candidates for early thymectomy.

IV ANTHROPOLOGY

HLA antigens may be used in population genetic studies (40,81,82,105, 106), i.e., the frequencies of HLA antigens of haplotypes varies from one race to another, genetic drift and genetic distance. HLA-A1 and HLA-B8 are very common in Caucasians but are very low in Orientals, Negroes, South American Indians and Australasian Aborigine population. HLA-A9 has a higher frequencies in Thais, the New Guinea natives and in Eskimos than in Caucasian. HLA-A3 and B7 was a very low incidence in Orientals. The gametic association of HLA-A1 and HLA-B8 was associated is Caucasian and Negroes while a certain association was no significant in Thais.

ABSTRACT

An introductory paper of HLA antigens is presented in the form of the method of histocompatibility testing, genetics, its history, general study and its application in transplantation, blood transfusion, and the study of diseases. One hundred and six references have been included, and the recent nomenclature is also described.

Table 1 Recognized HLA Antigens

LA (first locas)	FOUR (second locus)	AJ (third locus)	D locus
HLA-A1	HLA-B5	HLA-Cw1	HLA-Dw1
HLA-A2	HLA-B7	HLA-Cw1	HLA-Dw2

HLA-A3	HLA-B8	HLA-Cw3	HLA-Dw3
HLA-A9	HLA-B12	HLA-Cw4	HLA-Dw4
HLA-A10	HLA-B13	HLA-Cw5	HLA-Dw5
HLA-A11	HLA-B14		HLA-Dw6
HLA-A28	HLA-B18		
HLA-A29	HLA-B27		
HLA-Aw19	HLA-Bw15		
HLA-Aw23	HLA-Bw16		
HLA-Aw24	HLA-Bw17		
HLA-Aw25	HLA-Bw21		
HLA-Aw26	HLA-Bw22		
HLA-Aw30	HLA-Bw35		
HLA-Aw31	HLA-Bw37		
HLA-Aw32	HLA-Bw38		
HLA-Aw33	HLA-Bw39		
HLA-Aw34	HLA-Bw40		
HLA-Aw36	HLA-Bw41		
HLA-Aw43	HLA-Bw42		

ย่อเรื่อง

ได้รวบรวมรายงานจากผลการศึกษา HL—A antigen ซึ่งเป็น antigen ที่อยู่บนเม็ดโลหิตขาว เพลตเลต และตามเนื้อเยื่อต่าง ๆ ซึ่งเกี่ยวข้องกับการเปลี่ยนถ่ายอวัยวะ และได้รวบรวมถึงวิธีการทดสอบต่าง ๆ การศึกษาทางกรรมพันธุ์ ประวัติ ตลอดจนการนำไปประยุกต์ในการให้เลือด รวมทั้งการศึกษาถึงความสัมพันธ์ของ HL—Antigen กับโรคต่าง ๆ และมนุษยวิทยา รายงานนี้ได้รวบรวมเอกสารอ้างอิงถึง 106 รายงาน

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