HLA TYPING

A Technical Workshop

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PREFACE

THIS VOLUME is composed of papers prepared by speakers at the 1976 American Association of Blood Banks Workshop on Histocompatibility Testing. It has been five years since the last AABB publication on histocompatibility testing, the proceedings of the preconvention seminar in 1971. Like its predecessor, the present volume will serve as a general review of the subject for the blood banker. Since 1971, blood banks have become increasingly involved in the area of histocompatibility testing; but many of the potentials, and pitfalls, have yet to be realized.

Because of their background in immunogenetics, immunohematology, and transfusion practice, blood banks are logical places for histocompatibility labs. The techniques, while different from classical red cell immunohematology, are not difficult to master. The principal problem continues to be the lack of availability of an adequate supply of quality reagents. Blood banks and regional transfusion centers already are helping to solve this problem by developing vigorous antibody screening programs. These programs already have yielded substantial quantities of valuable typing reagents and, hopefully, the supply soon will be adequate to meet the needs of transfusion practice.

Histocompatibility testing remains a sophisticated body of knowledge, but its enormous potential for blood transfusion, transplantation, and immunobiology suggest that the field will continue to grow.

This volume should serve as a standard reference for blood bankers who wish to learn more about the field during the coming years.

WILLIAM V. MILLER, M. D.
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TABLE OF CONTENTS

Chapter I

HISTORY AND NOMENCLATURE OF THE HLA SYSTEM

Glenn E. Rodey

Introduction

ALL MAMMALIAN species in which transplantation immunity has been intensively studied have a series of closely-linked genetic loci which determine major histocompatibility factors, ie, surface antigens or receptors which are responsible for the recognition and elimination of foreign tissues. These regions are collectively termed major histocompatibility complexes (MHC). In mice the MHC is referred to as the H-2 complex, and in humans, as the HLA complex.

The importance of HLA complex matching in renal and bone marrow transplantation is now well established, as is the need for HLA-compatible platelets or granulocytes for transfusion into highly immunized, refractory patients. The biologic significance of HLA complex, however, extends far beyond the field of transfusion and transplantation, as will be noted by other speakers in this Workshop.

Characterization of this complex system is by no means complete. There remain unanswered questions concerning other, as yet undefined, genetic loci and alleles within the region as well as the general biological and clinical significance of the defined factors. Still, there is a measure of order in the system for students who currently enter this field. Pioneer investigators who studied leukoagglutinating antibodies in the 1950's could scarcely foresee the scope and complexity of their task, and red cell serologists will certainly appreciate the enormous problems which faced these investigators: Antisera were multispecific and of low titer, and testing procedures (leukoagglutination) were unreliable; further, there were several distinct but linked genetic loci, each coding for a large number of alleles. Indeed, loci in the HLA complex are the most extreme form of genetic polymorphism yet described in humans; finally, considerable cross-reactivity occurred between the products of alleles of the same locus, further frustrating efforts to define discrete allelic antigens. It is not surprising, therefore, that definition of the HLA complex as we understand it today has consumed the efforts of many investigators working for over 20 years.

Historical Review

Serologic Studies

The many studies which culminated in characterization of human histocompatibility factors had their origins in immunohematology. In the early 1950's, sera from a number of patients who had received multiple blood transfusions were found to contain antibodies which would agglutinate leukocytes. Initial interest in these leukoagglutinins stemmed from the possibility that they were autoantibodies which were capable of producing agranulocytosis.[1] Several investigators, however, clearly demonstrated that the leukoagglutinins did not agglutinate leukocytes from the serum donor, but were acquired by immunization through blood transfusion. Others[2,3] later showed that the antigens with which these leukoagglutinins combined were genetically determined alloantigens. By immunizing volunteers with leukocyte-rich cell suspensions from other donors, antibodies were detected which reacted only with limited numbers of the general population.[4] At about the same time, two lines of evidence indicated that the leukocyte antibodies and the corresponding antigens were clinically important. Brittingham[5] showed that leukoagglutinating antibodies were associated with nonhemolytic febrile transfusion reactions; and studies in mice[6,7] and subsequently in humans[8,9] indicated that these antigens were transplantation antigens, ie, sensitization to leukocyte antigens could cause accelerated rejection of skin grafts. These studies were also the first indication that the antigens detected by leukoagglutinins were expressed on other tissues as well.

Characterization of antigens in this new genetic system was difficult because the majority of antisera studied were multispecific. This is not surprising since the bulk of patients had received blood from several different donors. Resolution of this problem was greatly facilitated by the observation that leukoagglutinins also occurred in the sera of 20-30% of multiparous women.[10,11] Antibodies from these women tended to have more limited specificity because the antigen donor (immunizer) in most cases was restricted to the father of the fetus. Even then, definition of specific HLA alleles with oligospecific antisera was difficult. To facilitate this task, Jon van Rood[12] applied computer analysis techniques to the problem. He tested approximately 66 sera containing leukoagglutinating antibodies against a panel of cells derived from 100 random donors. Using two-by-two chi-square analysis, he compared reactivity of each serum to that of every other serum (2,145 compari-

sons). In this way he was able to identify several groups of sera which were detecting common specificities. In addition, one group of sera having high chi-square values with each other, but low values with other serum groups, suggested that products of allelic genes were being detected. Van Rood called this diallelic system 4, with 4^a and 4^b as alleles.

By the mid-1960's, many investigators all over the world had developed panels of leukocyte typing sera, and several allelic antigen systems were defined.[12,14] Because of inherent problems of reproductibility using leukoagglutination procedures and because investigators had assigned their own nomenclature to antisera, communication and comparisons were difficult. These problems were partially resolved by the introduction of lymphocytoxicity techniques in 1964[15-17] and by the establishment of international workshops.[18-23] It was soon apparent that many participants had antisera which were detecting common antigens. These workshops, which are collaborative efforts to advance the field of histocompatibility, have been held approximately every two years. They have provided standardized nomenclature and have been instrumental in advancing this field.

In 1965, Dausset and Ivanyi postulated that most of the leukocyte antigens detected were part of a single complex genetic system which was analogous to the H-2 histocompatibility system of mice, and the term, Hu-1, was proposed to denote the human complex.[24] Many of the antisera appeared to detect "broad" antigens, whereas other antisera of narrower specificity detected antigens which were included within the broader specificities. The "inclusion phenomena" complicated the definition of specific alleles and was subsequently shown to be due primarily to oligospecific antisera. In addition, many so-called broad antisera were detecting antigenic determinants which were shared in common by the products of several alleles (crossreactivity). Discrete antigens eventually were detected when monospecific antisera were found which reacted only with antigenic determinants unique to the specific antigen. This process of "splitting" previously recognized antigens is still going on. For example, HLA-A9 and A10, BW16 and BW22 have been recently split.

In 1967, a nomenclature committee was formed under the auspices of the World Health Organization (W.H.O.). When certain alleles were recognized by the majority of laboratories, they received a standardized nomenclature, HL-A (human leukocyte antigens, of the A complex).

By this time, it was generally accepted that the HL-A antigens were coded for by two closely linked loci, termed the LA and FOUR loci.[25-27] With rare exceptions, antigens could be assigned to one of the two loci, based upon large population studies and segregation analysis within families. Later, antigens which had not received official W.H.O. nomenclature were, by general agreement, assigned a common workshop number which was preceded by the letter, W.

In 1970, Thorsby et al[28] described an antiserum, AJ, which by family studies, appeared to detect a new specificity associated with the HLA complex but was not an allele of either the first or second locus. He proposed a third locus, but the concept of a third locus was not widely accepted, in part, because the serum AJ seemed to be detecting a crossreactive variant of the W27 antigen. In a series of elegant studies using absorption and antigen capping techniques however, this group clearly separated AJ and W27.[29] In retrospect, the apparent cross-reactivity was due to significant linkage disequilibrium between W27 and AJ (50% of BW27-positive individuals are also AJ-positive). Subsequently, five presumptive third locus specificities were identified serologically.

Role in Transfusion Therapy

While the use of HLA testing for defining donor-recipient compatibility in transplant programs was well underway by the late 1960's, similar compatibility testing was not being done for transfusion purposes. In 1969, howeer, Yankee and his colleagues[30] showed that certain patients who were refractory to platelets due to sensitization by prior transfusions, could be supported with platelets obtained from HLA-compatible donors. Subsequent studies confirmed this and it was established that many, if not most cases of diminished survival of transfused platelets and more recently, granulocytes, are due to alloimmunization to HLA antigens.

Mixed Leukocyte Cultures

In 1962, a major technique was developed which led to the identification of another major locus in the HLA complex.[31] It was observed that when peripheral blood lymphocytes from genetically dissimilar donors were cocultured *in vitro,* each population was mutually "activated." Activation was manifest morphologically by transformation of small lymphocytes into large lymphoblastic cells (blast transformation)

and metabolically by increased turnover of membrane lipids, increased protein, RNA and DNA synthesis. Cells so activated eventually underwent a number of cell divisions. The technique, termed the mixed leukocyte reaction (MLR), was later modified so that the activation of an individual cell population could be analyzed separately by treating one population with mitomycin-C, or x-irradiation to inhibit DNA synthesis.[32] The untreated cells were termed the responder and the treated cells, the stimulator populations. Cellular factors responsible for this response also appeared to be genetically determined and studies were quickly performed to see whether the responses was determined by differences in HLA antigens.

It should be noted that few HLA antigens were identified in the mid 1960's, so that "HLA-identical" individuals could only be found, by inference, among siblings. In such family studies, virtually no MLR stimulation occurred in cultures performed between siblings who were HLA identical; whereas stimulation invariably occurred between non-identical siblings. It was assumed, therefore, that HLA antigen differences determined MLR reactivity. In the 1970's, however, a family study was presented in which lymphocytes from two HLA-identical siblings stimulated strongly in MLR.[33] The investigators postulated that this discrepancy was due to a genetic recombination within the HLA system in one sibling and they proposed that the MLR reaction was determined by a genetic locus which was distinct from, but closely linked to the second HLA locus.

Subsequent family studies in which recombinant members were found confirmed this concept. Further, several investigators by then had accumulated a number of HLA-identical but unrelated individuals and found that 90% of these people stimulated strongly in MLR,[34] strongly supporting the idea that the proliferative response in MLR was determined by a distinct locus within the HLA complex. With few exceptions, MLR's performed between unrelated individuals, irrespective of HLA type, were mutually stimulatory, indicating that the number of alleles determined by this new locus would be as complex as the serologically-defined alleles. Since the products of this locus could not be detected serologically, but only by MLR, characterization of specific alleles was virtually impossible without cells which were homozygous for the MLR or LD (lymphocyte defined) locus. Such cells were eventually found in two populations. First, van Rood and colleagues HLA-typed the offspring of first cousin marriages, where 1 in 16 off-

spring were expected to be homozygous for the entire HLA complex.[35] Second, it was found that a high degree of linkage disequilibrium existed between the FOUR or second locus antigens and some MLR locus receptors so that a number of individuals who were homozygous for the FOUR locus were also homozygous for the corresponding MLR locus. These difficult studies culminated in the definition of at least six MLR alleles, which will be discussed later in this Workshop.

Summary

We have reviewed some of the historical events which led to the partial characterization of the HLA complex. There is insufficient time to describe other, equally important observations such as the placement of the HLA complex on chromosome 6 using somatic cell hybridization studies; the recognition that complement components C2, C4, C8, and factor B are controlled by genes within the HLA complex; and the possibility that the HLA complex contains immune response genes analogous to those found in the mouse H-2 complex.

In summary, the HLA complex, as currently defined, is located on chromosome 6 and contains four defined genetic loci which are inherited as autosomal condominant genes. Three loci can be defined by serologic techniques, using specific HLA antisera and the fourth, by MLR. Each locus is characterized by marked polymorphism, crossreactivity between alleles and a high degree of linkage disequilibrium. Many other loci exist within this complex, but have not yet been recognized or need further characterization.

Nomenclature

The first step toward development of a standard nomenclature was taken in 1967 when a committee was formed under the auspices of the World Health Organization (W.H.O.). After considerable debate, the committee decided upon the term "HL-A" to indicate the major system of leukocyte antigens. Alleles originally named were HL-A1, 2, 3, 5, 7, 8, and 9. HL-A10, 11, 12, and 13 were subsequently defined in the 1970 Workshop. Additionally, other antigens which were in all probability distinct alleles, received W numbers; the prefix, W, referring to "Workshop" sera. Local nomenclature was continued for certain antigens which needed further characterization.

HL-A nomenclature had several disadvantages, the major one being that it did not distinguish the locus within the complex to which a given

allele belonged. Also, certain overlap in numbers occurred which was confusing, notably HL-A5 and W5, HL-A10 and W10. More important, there was no provision for the newly defined third locus and MLR locus.

In 1975, the nomenclature was revised in order to provide a more flexible system for naming new genetic loci as they became recognized and to alleviate the problems mentioned above. The changes are schematically summarized in Figure 1. Alleles are assigned specific

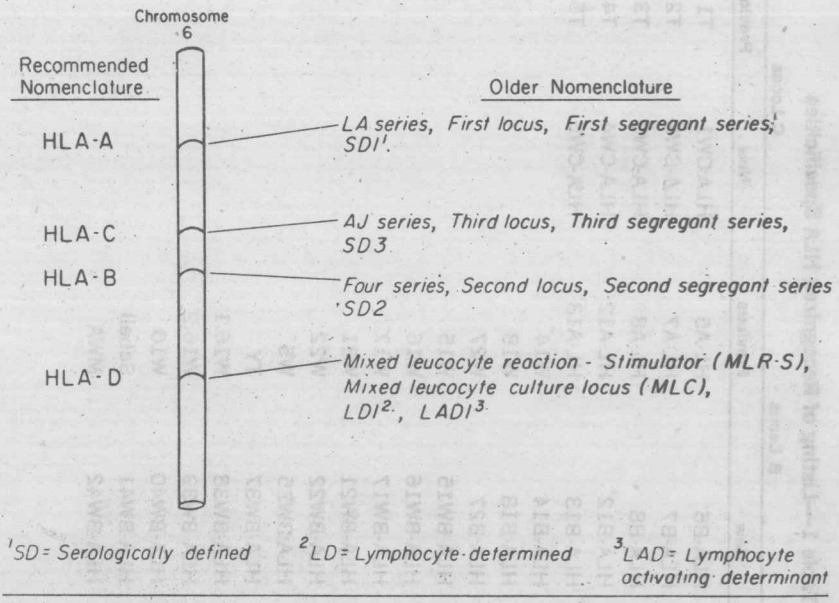


Fig. 1. Nomenclature of defined loci within the HLA complex.

numbers prefixed by letters which identify the locus; and the term HL-A, is changed to HLA (no hyphen). For example, HL-A1, an allele of the first segregant series (LA) is now written HLA-A1, which identifies the major complex (HLA), the locus (A), and the specific allele (1).

Tentative HLA specificities can also be assigned a temporary number which is indicated by an additional prefix, W (designates Workshop number), eg, HLA-BW15. The W specificities are, in all probability, distinct alleles, but they require further definition and confirmation.

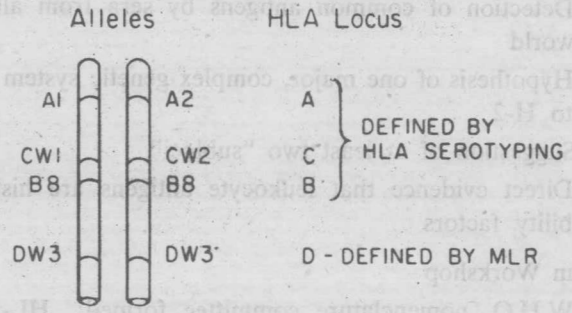
Table 1—Listing of Recognized HLA Specificities

A Locus		B Locus		C Locus		D Locus	
New	Previous	New	Previous	New	Previous	New	Previous
HLA-A1	HL-A1	HLA-B5	HL-A5	HLA-CW1	T1	HLA-DW1	LD 101
HLA-A2	HL-A2	HLA-B7	HL-A7	HLA-CW2	T2	HLA-DW2	LD 102
HLA-A3	HL-A3	HLA-B8	HL-A8	HLA-CW3	T3	HLA-DW3	LD 103
HLA-A9	HL-A9	HLA-B12	HL-A12	HLA-CW4	T4	HLA-DW4	LD 104
HLA-A10	HL-A10	HLA-B13	HL-A13	HLA-CW5	T5	HLA-DW5	LD 105
HLA-A11	HL-A11	HLA-B14	W14			HLA-DW6	LD 106
HLA-A28	W28	HLA-B18	W18				
HLA-A29	W29	HLA-B27	W27				
HLA-AW23	W23	HLA-BW15	W15				
HLA-AW24	W24	HLA-BW16	W16				
HLA-AW25	W25	HLA-BW17	W17				
HLA-AW26	W26	HLA-BW21	W21				
HLA-AW30	W30	HLA-BW22	W22				
HLA-AW31	W31	HLA-BW35	W5				
HLA-AW32	W32	HLA-BW37	TY				
HLA-AW33	W19.6	HLA-BW38	W16.1				
HLA-AW34	Malay 2	HLA-BW39	W16.2				
HLA-AW36	Mo	HLA-BW40	W10				
HLA-AW43	BK	HLA-BW41	Sabell				
		HLA-BW42	MWA				

When there is general agreement that such specificities are the product of distinct alleles, the W number is dropped, ie, HLA-BW15 would become HLA-B15. A listing of currently recognized specificities is given in Table 1. Older nomenclature is also listed for comparison.

Since the expression of HLA antigens is autosomal codominant, a total of six serologically detectable antigens can be identified on lymphocytes from normal individuals (3 loci each coding for 2 antigens). The antigen specificities which are detected by typing the individual's cells constitutes the *phenotype* (expressed antigens). In many cases, less than six antigens are detected. This may be due to the presence in the individual of an unknown allele which has not been previously defined (or for which good antisera are not available); or the individual may be homozygous for one or more antigens. (Current techniques do not distinguish quantity of antigens on the cell surface; therefore, a heterozygous and homozygous allele would type similarly and the homozygote would appear to have a "blank," ie, only one detectable antigen for that locus.) To identify homozygosity, family studies are required which permit determination of the *genotype* (the genetic profile) and *haplotypes* (the assignment of specific antigens to individual chromosomes). Figure 2 illustrates the use of these terms.

DEFINING PHENOTYPE, GENOTYPE AND HAPLOTYPES



Phenotype: A1, 2, B8, -, CW1, 2, DW3, -

Genotype: A1, 2, B8, 8, CW1, 2, DW3, 3

Haplotypes A1, B8, CW1, DW3/A2, B8, CW2, DW3

Fig. 2. Defining phenotype, genotype and haplotypes.

Summary of Historical Development in Analyses of the HLA Complex

1952—Leukoagglutinins in leukopenic patients

1957—Leukoagglutinins as major cause of febrile, nonhemolytic transfusion reactions

1958—a. Detection of leukoagglutinins in multiparous women

b. Determination that leukocyte antigens detected are alloantigens

1962—a. Development of mixed leukocyte reaction (MLR) techniques

b. Use of computer analysis to define common antigens and allelic antigens

c. Early evidence that leukocyte antigens are histocompatibility factors

1964—Introduction of lymphocytotoxicity techniques

1964—Duke Workshop

a. Comparison of techniques and standardization of methods

b. Analysis of sera

1965—Leiden Workshop

a. Comparisons of different antisera against common cell panel Detection of common antigens by sera from all over the world

b. Hypothesis of one major, complex genetic system analogous to H-2

c. Suggestion of at least two "subloci"

d. Direct evidence that leukocyte antigens are histocompatibility factors

1967—Turin Workshop

a. W.H.O. nomenclature committee formed: HL-A nomenclature

b. Evidence of one major genetic region containing at least two loci

c. Further alleles identified

d. Prospective studies for analysis of transplants and HL-A

e. Beginning studies of HLA and disease associations