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The Major Histocompatibility Complex*†

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ABSTRACT

The major histocompatibility complex on the sixth chromosome controls expression of a complex series of cell surface antigens which comprise the human leukocyte antigen (HLA) system. These markers, beyond their importance in human organ transplantation, have been demonstrated to occur with an increased prevalence in certain disease states. The group of conditions showing the closest association with specific HLA antigens are the "spondyloarthropathies." These include ankylosing spondylitis (AS), Reiter's syndrome (RS), psoriatic arthritis (PsA), and the arthritis of inflammatory bowel disease (AIBD). Clinical and radiographic studies were made of 310 unrelated caucasoid patients with seronegative arthritis. HLA-A, B, C, and DR typing were performed using the microdroplet lymphocyte cytotoxicity test. Statistically increased prevalences of A26, B27, and Bw38 were observed, while B27 was associated with spinal involvement regardless of diagnosis (90 percent in AS $p < 0.0001$). Experiments found A26 (23 percent $p < 0.001$) and Bw38 (38 percent $p < 0.0001$) in patients with PsA. Spondyloarthritis patients with spinal involvement who lacked B27 frequently had B7. The HLA DR typing for seven specificities was carried out in 196 patients. It was found that DRw4 (52 percent $p < 0.03$) and DRw7 (39 percent $p < 0.04$) were increased in the PsA patients. This study further confirms the close association of HLA antigens and the spondylarthropathies.

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Introduction

The major histocompatibility complex (MHC) on the sixth chromosome in man controls the expression of the cell surface proteins known as human leukocyte antigens (HLA) and influences diverse biological functions. Some of these func-

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tions include humoral and cellular response to antigenic stimulation, graft rejection, complement activation, and possibly disease expression. In this review, brief consideration was made of the development of understanding of this complex system, biochemical and genetic aspects, and a consideration of the possible relationship of the MHC to the occurrence and manifestations of human disease.

The understanding of this complex in man has been greatly facilitated by studies in the mouse, rat, and other animals. Striking homology has been observed between man and animals. This review will emphasize the MHC in man; considerable information concerning the H-2 region in the mouse is available.[16,21]

Four principle regions denoted as A, B, C, D/DR are recognized. Debate continues concerning the relationship between the D locus and the DR (D related) locus. It seems likely the D and DR loci will be recognized as distinct areas. The antigens of the A, B, C, and DR loci are detected by serological means utilizing a lymphocytotoxicity assay, and the D locus antigens are determined by use of homozygous typing cells in a mixed lymphocyte reaction (MLC). The development of understanding of the MHC can best be separated into humoral and cellular observations. In the 1950's, antibodies to white cells were recognized to develop during pregnancy and after blood transfusion.[27] Detection of "leuko agglutination" after mixing of leukocytes and pregnancy sera was imprecise and technically demanding, but identity of these markers in homozygous twins[10] and increased prevalence of specific markers in families documented genetic control of the expression of the agglutination reaction.[28]

A major technical advance was the advent of the microlymphocytotoxicity assay which had a more precise end point, cell death, and conserved serological reagents.[31] The technique utilizes the placement of 50,000 lymphocytes into individual wells of a plastic microtitre plate. In each well are carefully screened and adsorbed antisera, each to a different HLA anitgen. After addition of screened rabbit complement and a counter stain, the wells are examined for the presence of cytolysis.

The recognition of the DR locus, distinct from the ABC loci, was delayed because these markers are principally expressed on B lymphocytes (monocytes, macrophages, and endothelial cells as well), but not on the more numerous T cells.[34] Anomalous cytotoxic typing reactions with lymphocytes from patients with chronic lymphatic leukemia with well characterized antisera was an early clue to the existance of B cell alloantigens. Antibodies interfering in the MLC were also described. Reliable separation of B and T cells with isolation from B cell membranes of glycoproteins distinct from those of the A, B, and C locus led to acceptance of the DR locus. Recently, the MB and MT systems have also been described.[22] Debate continues whether antisera recognizing these new markers are supertypic (because much overlap with DR exists) or distinct.

Despite the use of microtitre plates, the supply of precise reagents in serological testing is an ongoing problem. It is hoped the development of monoclonal antibodies to HLA antigens through hybridoma technology will solve this problem.

Concurrently with serological studies, it was recognized that mixture of lymphocytes from different individuals induced blastogenesis.[6] This sytem known as the two way MLC was subsequently modified by inactivating one donor's lymphocytes with mitomycin or radiation allowing precise recognition of which cells were responding and which cells were stimulating in the cultures[4] and the new locus was termed the D locus.[2,11] Use of lymphocytes from patients homozygous for D locus antigens aided precision in testing. Storage by freezing of viable lymphocytes is more difficult than storing antibodies

limiting the number of laboratories routinely doing D locus typing.

No discussion of these developments would be complete without mention of the eight international workshops held first in 1964 and most recently in 1980. The collaborative efforts involving massive worldwide exchange of antisera and cells are unique in the history of science.

These exchanges established an internationally accepted definition of reagents allowing comparisons of observations among laboratories particularly important for disease relation studies. Reagents under study prior to final acceptance are denoted by a "w" for workshop.

The biochemical composition of HLA antigens is quite similar in the mouse, rat, and man. Similarity is also noted to immunoglobulin molecules. The gene products of the A, B, and C locus are glycoprotein structures consisting of a polymorphic heavy chain with a molecular weight of 45,000 daltons and a constant light chain of 11,000 daltons known as B2 microglobulin.[17] DR antigens are composed of a 34,000 dalton alpha constant chain and a 29,000 dalton beta B polymorphic chain.[18] Certain of the antigens are quite similar in structure and said to share public determinants. The most important example is the B7 CREG or cross reacting group which includes B7, 22, 27, 40, and 44.

Genetics

Generally the MHC is inherited as a block in dominant fashion. Genetic recombination occurs in less than one percent of individuals but is useful in locating elements of the MHC on the sixth chromosome.[5] The distance between genes as determined by the frequency of recombination is expressed as centromorgans (Cm). For example it has been estimated the distance between HLA A and B is 0.8 to 1 Cm.[17] Another important genetic aspect in the MHC is the occurrence of linkage disequilibrium. This is defined as the occurrence of two HLA antigens together more or less frequently than would be predicted by chance. For example, A26 and BW 38 occurred together more often than their individual prevalences in the population multiplied together would predict. Some survival advantage of specific combinations of HLA antigens may be the explanation.

The cross products from a 2×2 table established from the prevalence of antigen positive and negative patients and control groups can be used to calculate relative risk RR = (ad/bc) of a patient with a specific antigen developing a specific disease.[34]

The term genetic linkage refers to inherited genetic diseases whose expression is controlled entirely genetically as determined by family studies. Examples of conditions genetically linked to the MHC include 21-hydroxylase deficiency resulting in congenital adrenal hyperplasia and deficiency of the second and fourth component of complement. The term association indicates a genetic predisposition to a disease which requires other non-genetic factors as well for expression. Most of the disease relationships to the MHC are associations.

The first association of the MHC and a human disease was reported in 1967 in Hodgkin's disease patients. Increased prevalence of an early specificity known as 4C was noted.[1] It was subsequently shown that the increased prevalence was related to a heightened frequency of B5 in these patients.[15] The weak nature of this association has rendered it controversial and not demonstrable in all series. The prognosis in Hodgkin's has also been related to the presence of HLA antigens.[14] This requires confirmation but indicates one possible usefulness in determining HLA phenotypes. The majority of HLA associated diseases involve malignancies, infectious diseases, immunologic, and rheumatic diseases.[24] In table I are indi-

cated the broad range of conditions that appear to have an HLA association.

Antigens in linkage disequilibrium may also have disease associations. One of the most commonly observed is A1, B8, and DRW 3. This combination is frequently observed in "autoimmune" conditions such as myasthenia gravis, chronic active hepatitis, systemic lupus erythematosus, and Graves' disease, among others. The prevalence of the antigens comprising the haplotype may be studied individually in population studies. The antigen with the highest prevalence and greatest relative risk in the disease population is most likely to be the antigen involved in the pathogenesis of the disease. For example, in the "autoimmune" diseases, the closest association is generally observed with DRW 3. This likelihood could be predicted from mouse studies which have documented the immune response (IR) region of the H-2 complex which appears to be homologous with the DR locus in man. Another aspect of the association of HLA and systemic lupus erythematosus (SLE) is the recognition of the occurrence of this disease in individuals who lack the second component of complement.[26] The apparent paradox because of complement involvement in SLE induced inflammation remains unexplained. A locus associations are unusual. The best example is the association of A3 and hemochromatosis.[8]

TABLE I

Diversity of Human Leukocyte Antigen

Disease	*Antigen*	*Patients (Percent)*	*Controls (Percent)*
Rheumatoid arthritis	DRW4	50-70	10-20
Systemic lupus erythematosus	DRW2	40-60	10-20
Sjogren's syndrome	B8	40-60	20-30
	DRW3	60-70	10-20
Multiple Sclerosis	B7	20-45	10-25
	DRW2	40-70	20-30
Ragweed allergy (R_a5)	B7	40-60	10-25
Psoriasis	B13	5-10	<5
	B17	10-35	5-10
	B37	15-20	<5
	B39	15-20	5-10
	CW6	40-60	5-15
Myasthenia gravis	B8	40-60	15-30
	DW3	25-40	10-20
Chronic active hepatitis	B8	20-70	15-30
	DR3	40-70	10-20
Graves' disease	B8	20-60	15-20
	DW3	30-50	10-20
Insulin dependent diabetes	DW4	40-60	10-20

In table II and III are indicated our results in HLA typing spondyloarthritis patients in the Tampa, Florida area. In our study, the frequency distribution of the HLA A, B, and C antigens, with the exception of A26, B27, Bw38, Cw1, and Cw2, did not differ substantially in the six groups of patients and unaffected controls (table II). The values for seronegative rheumatoid arthritis and osteoarthritis groups of patients were normal. In the ankylosing spondylitis group, B27 was present in 89.5 percent (77/86) of patients ($p < 0.0001$). Two of nine patients (22.2 percent) negative for B27 were Bw38 and another (11.1 percent) was positive for Bw39. Five patients in this group also had the B7-CREG antigens. Cwl was seen in 31.4 percent (27/86) of patients ($p < 0.002$), while Cw2 was observed in 46.5 percent ($p < 0.001$). In the group of patients with psoriatic arthritis, A 26 was found in 23.3 percent ($p < 0.0014$); B27 was found in 19.5 percent compared to 8 percent in control subjects, but this difference was not statistically significant. The Bw38 was observed in 37.8 percent ($p < 0.0001$). In Reiter's syndrome patients, B27 was seen in 88.8 percent ($p < 0.0001$) while Cw2 was also found elevated, 40.0 percent ($p < 0.0014$). Three of the five patients negative for B27 had B7-CREG antigens.

In patients with inflammatory bowel disease (IBD) and arthritis, B27 was present in 37.5 percent ($p < 0.01$). HLA-DR typing was performed in 196 patients. The frequency distribution of the HLA-DR specificities was normal in all groups of

TABLE II

Increased Frequencies of Human Leukocyte Antigen-A, B, and C Specificities Among Seronegative Spondyloarthropathies and Normal Controls

	Normal Group	*Ankylosing Spondylitis*	*Psoriatic Arthritis*	*Reiter's Syndrome*	*Inflammatory Bowel Disease*
Antigen	n=100 (percent)	n=86 (percent)	n=82 (percent)	n=45 (percent)	n=32 (percent)
Aw26	7	1.2	24.4*	2.2	3.1
B27	8	89.5†	19.5	88.8*	37.5
Bw38	6	5.8	37.8‡	0.0	0.0
Cw1	9	31.3§	18.2	6.6	15.6
Cw2	15	46.5ƒ	21.9	40.0¶	25.0

*Significantly different from controls ($p < 0.001$, and $p < 0.015$ when corrected for the fifteen specificities tested)

†Significantly different from controls ($p < 0.0001$, and $p < 0.0018$ when corrected for the eighteen specificities tested)

‡P value < 0.0001 compared to controls, and $p < 0.0018$ when corrected for the number of specificities tested

§P value < 0.002 compared to controls, and $p < 0.01$ when corrected for the number of specificities tested

ƒP value < 0.0001 compared to controls, and $p < 0.0006$ when corrected for the number of specificities tested

¶P value < 0.0014 compared to controls, and $p < 0.008$ when corrected for the number of specificities tested

patients except for the psoriatic arthritis group (table III). DRw4 was present in 52.9 percent ($p < 0.03$) compared to our controls, and $p < 0.003$ compared to the Seventh International Histocompatibility Workshop controls, corrected $p < 0.02$. The specificity of DRw7 was also found in 39 percent of patients (corrected $p < 0.04$).

Our data indicate that HLA-B27, Bw38, DRw4, and DRw7 antigens are distinctive markers for patients with seronegative spondyloarthritis. The B27 seems to be a highly specific genetic marker for patients with spinal involvement while Bw38 and DRw4 characterize patients with psoriatic arthritis.

TABLE III

Frequencies of Human Leukocyte Antigen-DR Specificities Among Patient Study Groups and Normal Controls

DRW Antigens	*Normal Groups*		*Osteo-arthritis*	*Seronegative Rheumatoid Arthritis*	*Ankylosing Spondylitis*	*Psoriatic Arthritis*	*Reiter's Syndrome*	*Inflammatory Bowel Disease*
	n=45 (percent)	n=495* (percent)	n=20 (percent)	n=20 (percent)	n=54 (percent)	n=51 (percent)	n=32 (percent)	n=14 (percent)
DRW1	13.3¶	11.4	12.0	15.0	11.1	9.8	12.5	7.1
DRW2	26.6	26.4	28.0	20.0	22.2	17.6	25.0	21.4
DRW3	17.7	22.2	20.0	25.0	27.7	15.6	21.8	21.4
DRW4	31.1	32.0	6.0	35.0	29.6	52.9†	31.3	35.7
DRW5	22.2	24.3	16.0	20.0	24.0	17.6	18.8	14.3
DRW6	24.4	24.5	24.0	25.0	25.9	15.6	28.1	28.6
DRW7	20.0	21.7	24.0	25.0	24.0	39.0‡	25.0	28.6
Blank	42.2	29.8	40.0	35.0	35.2	31.3	37.5	42.8

*Combined HLA-DR antigen frequencies of 495 controls during the Seventh International Histocompatibility Workshop, Oxford, England, 1977.

†P value < 0.03 compared to our own controls, $p < 0.003$ compared to Workshop controls ($p < 0.002$ when corrected for the seven specificities tested).

‡P value < 0.04 compared to our controls, and $p < 0.006$ compared to Workshop controls ($p < 0.04$ when corrected for the seven specificities tested).

¶Error

The closest association between disease expression and the MHC occurs in ankylosing spondylitis (AS). The discovery of the association between AS and B27 was made by two groups independently in 1973.[9,30] It is now clear that 90 to 100 percent of caucasoid individuals with AS will express B27. Black patients, however, have a comparatively decreased prevalence of B27 (50 percent) and an increased prevalence of B7 (30 percent) a related antigen.[19]

The B27 also is closely linked as well to the other forms of spondyloarthritis which include psoriatic arthritis (PsA), Reiter's syndrome (RS), and the arthritis of inflammatory bowel disease (AIBD). Generally a somewhat lower frequency of B27 (60 to 80 percent) is observed in these related spinal arthropathies.

Psoriasis and PsA are of particular interest for the variety of observed associations. Thus, B13, B17, B37, B39, or CW6 occur in approximately 10 to 50 percent of psoriasis without arthritis patients.[29,33] The B27 occurs in 60 to 80 percent of PsA patients who have spinal involvement[23] while BW38 is prevalent (40 percent) in PsA patients with peripheral arthritis.[3,12,25] Two groups[13,25] have found DRW4 in 50 percent of PsA patients, but one group has not.[20] This discrepancy may result from the heterogeneity of DRW4,[7] variation in the patient population studied, or technical factors within laboratories. Concerning the prevalence of B27 in RS and AIBD, our data expressed in table II are typical noting B27 in approximately 60 to 80 percent of patients with RS and AIBD. The DR locus associations do not seem to be important in AS, RS, and AIBD.

Presently studies are directed at resolving the question concerning how the MHC is related to disease expression. A number of suggestions have been made. These include "molecular mimicry" in which the structure of an environmental pathogen mimics that of the HLA antigen allowing the pathogen to induce disease. The HLA antigen may act as a receptor for the disease causing agent. The HLA antigen itself may not be important, but it may be closely linked to a yet undiscovered immune response or disease causing gene. Better understanding of complex relationships among the MHC, immune response, and disease expression may lead to precise genetic, immunological, or environmental manipulation to paliate or prevent many of today's diseases.

References

1. AMIEL, J. L.: Study of the leukocyte phenotypes in Hodgkin's disease. Histocompatibility Testing. Curtoni, E. S., Mattiuz, P. L., and Tosi, R. M., eds. Copenhagen, Munksgoard, 1967, 79–81.
2. AMOS, B. D. and BACH, F. H.: Phenotypic expressions of the major histocompatibility locus in man (HL-A) leukocyte. J. Exp. Med. *128*: 623–637, 1968.
3. ARNETT, F. C. and BIAS, W. B.: HLA-Bw38 in psoriatic arthritis relationship and implications for peripheral and axial involvement. Arthritis Rheum. *23*:64, 1980.
4. BACH, F. H. and VOYNOW, N. K.: One-way stimulation in mixed leukocyte cultures. Science *153*:545–547, 1966.
5. BACH, R. F. and VAN ROOD, J. J.: The major histocompatibility complex—genetics and biology. New Eng. J. Med. *295*:806–813, 1976.
6. BAIN, B., VAS, M. R., and LOWENSTEIN, L.: The development of large immature mononuclear cells in mixed leukocyte cultures. Blood *23*:108–116, 1954.
7. BIAS, W. B., HSU, S. H., POLLARD, K., ET AL: HLA-DR characterization of a Chippewa Indian subpopulation with a high prevalence of rheumatoid arthritis. Human Immunol. *2*:155–162, 1981.
8. BEAUMONT, C., SIMON, M., FAUCHET, R., ET AL: Serum Ferritin as a possible marker of the hemochromatosis allele. New Eng. J. Med. *301*:169–174, 1979.
9. BREWERTON, D. A., CAFFREY, M., HART, F. D., ET AL: Ankylosing spondylitis and HL-A 27. Lancet *1*:904–907, 1973.
10. DAUSSET, J. and BRECY, H.: Identical nature of the leukocyte antigens detectable in monozygotic twins by means of immune iso-leuko agglutinins. Nature *180*:1430–1432, 1957.
11. DUPONT, B., JERSILD, L., HANSEL, G., ET AL: Typing for MLC determinants by means of LD homozygous and LD heterozygous test cells. Transplant Proc. *5*:1543–1549, 1973.

12. ESPINOZA, L. R., VASEY, F. B., and OH, J. H.: Association between HLA Bw38 and peripheral psoriatic arthritis. Arthritis Rheum. *21*:72–75, 1978.
13. ESPINOZA, L. R., VASEY, F. B., GAYLORD, S., ET AL: Histocompatibility typing in sera negative spondyloarthritis: a survey. Seminars Arthritis Rheum. (in press).
14. FALK, J. and OSOBA, D.: HL-A antigens and survival in Hodgkin's disease. Lancet *2*:1118–1120, 1971.
15. FORBES, J. F. and MORRIS, P. J.: Leukocyte antigens in Hodgkin's disease. Lancet *2*:849–851, 1970.
16. GILL, T. J., CRAMER, D. V., and KUNZ, H. W.: The major histocompatibility complex: comparison in the mouse, man, and rat. Amer. J. Path. *90*:735, 1978.
17. GILL, T. J.: Structure and function of the major histocompatibility complex. Arch. Path. Lab. Med. *104*:559, 1980.
18. GOYERT, S., HUBERT, J. J., CURRY, R. A., and SILVER, J.: A novel molecule expressing HLA-DR antigenic determinants. Human Immunol. *1*:161–175, 1980.
19. KAHN, M. A., BRAUN, W. E., KUSHNER, I., ET AL: HLA-B27 in ankylosing spondylitis: differences in frequency and relative risk in American blacks and Caucasians. J. Rheumatol. (suppl 3) *4*:39–41, 1977.
20. KANTOR, S. M., HSU, S. H., BIAS, W. B., ET AL: Further evidence localizing disease susceptibility towards the HLA-B locus in psoriatic arthritis: results of HLA-Dw and DR testing. Arthritis Rheum (suppl) *24*:S119, 1981.
21. KLEIN, J.: The major histocompatibility complex of the mouse. Science *203*:516–521, 1979.
22. MARRARI, M., PROTELSCH, A, and DUQUESNOY, R. J.: HLA-DR, MB, and MT the three serological distinct systems of B cell alloantigens. Human Immunol. *1*:271–272, 1980.
23. METZGER, A., MORRIS, R., BLUESTONE, R., ET AL: HLA W27 in psoriatic arthropathy. Arthritis Rheum. *18*:111–115, 1975.
24. MORRIS, P. J.: Histocompatibility systems, immune responses and disease in man. Contemp. Top. Immunobiol. *3*:141–164, 1974.
25. MURRAY, D. L., MANN, L. N., DECKER, J., ET AL: Histocompatibility alloantigens in psoriasis and psoriatic arthritis. J. Clin. Invest. *66*:670–675, 1980.
26. OSTERLAND, C. K., ESPINOZA, L. R., PARKER, L. P., ET AL: Inherited C2 deficiency and systemic lupus erythematosus: studies on a family. Ann. Int. Med. *82*:323–328, 1975.
27. PAYNE, R.: Leukocyte, agglutinins in human sera. Arch. Intern. Med. *99*:587–606, 1957.
28. PAYNE, R. and HACKEL, E.: Inheritance of human leukocyte antigens. Amer. J. Human Genet. *13*:306–315, 1961.
29. RUSSELL, T. J., SCHULTES, L. M. and KUBAN, D. J.: Histocompatibility (HL-A) antigens associated with psoriasis. New Eng. J. Med. *287*:738–740, 1972.
30. SCHLOSSTEIN, L., TERASAKI, P. H., BLUESTONE, R., ET AL: High association of an HL-A antigen W27 with ankylosing spondylitis. New Eng. J. Med. *288*:704–706, 1973.
31. TERASAKI, P. H. and MCCLELLAND, J. D.: Antibody response to homografts VIII relation of mouse hemagglutinins and cytotoxins. J. Exp. Med. *117*:675–685, 1963.
32. TRUCCO, M., DEPETRIS, S., GAROTTA, G., ET AL: Quantitative analysis of cell structures by means of monoclonal antibodies. Human Immunol. *1*:233–243, 1980.
33. WHITE, S., NEWCOMER, V., MICKEY, M. R., ET AL: Disturbance of HL-A antigen frequency in psoriasis. New Eng. J. Med. *287*:740–743, 1972.
34. WINCHESTER, R. J. and KUNKEL, H. G.: The human Ia system. Adv. Immunol. *28*:221–292, 1979.