Association of HLA-DRw2 with autoimmune thrombocytopenic purpura.

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J Clin Invest. 1979;63(5):1085-1088. https://doi.org/10.1172/JCI109378.

Research Article

Peripheral blood lymphocytes from 38 patients with autoimmune thrombocytopenic purpura (AITP) were tested for HLA-A, -B, and -C alloantigens. Isolated B lymphocytes from 20 of these patients were tested for HLA-DRw (Ia) alloantigens. The profile of HLA alloantigens in the patients with AITP was significantly different from that of a matched control population. The most significant finding was the presence of the HLA-DRw2 alloantigen in 75% of patients as compared with 23% in the control population, P less than 0.001, relative risk 10.0 (A relative risk of 1 would indicate no association between the presence of the antigen and the disease.) The co-occurrence of either A3 and B7 (known to be in linkage disequilibrium with DRw2) or A26 and Bw38 was significantly increased as compared with the control population (P less than 0.001). Of the patients positive for DRw2, 47% had the association A26 and Bw38 as compared with the control population association incidence of 21% (P less than 0.1). Thus, in the patient population, A26-Bw38 appears to be a haplotype that is in linkage disequilibrium with DRw2 (as presumably is the case with A3-B7). These data indicate that a predisposition to AITP is inherited with a DRw2 gene of the major histocompatibility system.

RAPID

PUBLICATIONS

Association of HLA-DRw2 with Autoimmune Thrombocytopenic Purpura

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ABSTRACT Peripheral blood lymphocytes from 38 patients with autoimmune thrombocytopenic purpura (AITP) were tested for HLA-A, -B, and -C alloantigens. Isolated B lymphocytes from 20 of these patients were tested for HLA-DRw (Ia) alloantigens. The profile of HLA alloantigens in the patients with AITP was significantly different from that of a matched control population. The most significant finding was the presence of the HLA-DRw2 alloantigen in 75% of patients as compared with 23% in the control population, $P < 0.001$, relative risk 10.0. (A relative risk of 1 would indicate no association between the presence of the antigen and the disease.) The co-occurrence of either A3 and B7 (known to be in linkage disequilibrium with DRw2) or A26 and Bw38 was significantly increased as compared with the control population ($P < 0.001$). Of the patients positive for DRw2, 47% had the association A26 and Bw38 as compared with the control population association incidence of 21% ($P < 0.1$). Thus, in the patient population, A26-Bw38 appears to be a haplotype that is in linkage disequilibrium with DRw2 (as presumably is the case with A3-B7). These data indicate that a predisposition to AITP is inherited with a DRw2 gene of the major histocompatibility system.

INTRODUCTION

In the past several years it has become increasingly apparent that idiopathic thrombocytopenic purpura (1) is an autoimmune disorder (2, 3). Although genetic factors have been shown to influence the predisposition to certain diseases with autoimmune features such as systemic lupus erythematosus (SLE)[1] and rheumatoid arthritis (4), little is known concerning the genetic predisposition to autoimmune thrombocytopenic purpura (AITP). Of the numerous cases of hereditary thrombocytopenia which have been reported (5), four families (6, 7) appear to have criteria compatible with the diagnosis of AITP (however none had antiplatelet-antibody studies).

The association of particular alleles of the histocompatibility loci with certain diseases provides a method of delineating the role of immunogenetic factors in disease susceptibility, as well as an approach to investigating relationships among various diseases. Attention was initially focused on the major histocompatibility complex genes of the HLA-A and -B series. More recent studies have revealed the presence of genes distinct from the HLA-A, -B, and -C series (4), which determine cell surface antigens found selectively on B lymphocytes and macrophages (8). These B-cell surface antigens have been termed "I region-associated anti-

Dr. Winchester is the recipient of Research Career Development Award AI 00216. Dr. Gibofsky is the recipient of National Research Service Award AM 05766.
Received for publication 22 January 1979 and in revised form 26 February 1979.

[1] *Abbreviations used in this paper*: AITP, autoimmune thrombocytopenic purpura; Ia, I region-associated antigens; RR, relative risk(s); SLE, systemic lupus erythematosus.

gens" (Ia) (as in the mouse system) and certain ones are closely associated with the primary determinants involved in the stimulation of the mixed lymphocyte culture reaction which map at the locus designated HLA-D. A number of human B-lymphocyte alloantigens have been defined that are highly associated with the HLA-D specificities and have therefore been designated HLA-DR for HLA-D "related" (9). Of particular interest is the recent recognition that HLA-DR alloantigens reveal an association with certain diseases in which no or only minimal association was found with specific HLA-A and -B alloantigens such as SLE (10, 11) and rheumatoid arthritis (11, 12).

The purpose of this study was to define the HLA-A, -B, -C, and the newly recognized B-cell alloantigens (HLA-DR) in patients with AITP in order to determine whether this disease has a genetic predisposition. Such a predisposition has been found associated with the presence of the HLA-DRw2 alloantigen. Individuals typing for this alloantigen have a relative risk of 10 for acquiring AITP.

METHODS

Patient and control population. 38 patients with classic chronic AITP from the New York area were studied. 29 patients (76%) were female. All patients had isolated thrombocytopenia, increased megakaryocytes in the bone marrow, negative antinuclear antibody titers, and lacked splenomegaly. All but two patients responded to steroids and(or) splenectomy. 55% of the patients in the total group were Ashkenazi Jews. All patients were compared with a control group which was matched for ethnic origin. Controls were matched for the 55% incidence of Ashkenazi Jews in the patient group by weighting the average incidence of non-Jewish Caucasians from a group of 450 controls and Ashkenazi Jews from a group of 160 controls with their respective incidence in the patient group for the HLA-A, -B, and -C alloantigens.

HLA-A, -B, and -C tissue typing. HLA-A, -B, and -C tissue typing was performed on lymphocytes by cytotoxicity methods previously described (13), with a panel of 118 different antisera, capable of detecting 16 different HLA-A alloantigens, 25 HLA-B alloantigens, and 4 HLA-C alloantigens. Lymphocytes were prepared from heparinized blood by Ficoll-(Pharmacia Fine Chemicals, Div. Pharmacia Inc., Piscataway, N. J.) Hypaque (Winthrop Laboratories, New York) sedimentation.

Ia alloantigen (DRw) determination. Ia alloantigen (DRw) determination was performed on B lymphocytes separated by depletion of thymus-derived (T) cells through rosette formation with neuraminidase-treated sheep erythrocytes (14, 15), employing a modified Amos (16) two-stage cytotoxic assay. Indirect immunofluorescence was performed on mononuclear cells cultured with pokeweed mitogen for 5–6 d (8, 15), Both assays were performed in parallel.

HLA-DRw antisera. Alloantisera specific for B-cell alloantigens were used after absorption with platelets and T-cell lymphoblastoid lines to remove any contaminating reactivity for HLA-A, -B, or -C antigens (15). The typing was performed with 60 different antisera capable of detecting 7 different HLA-DRw and related public specificities according to methods described (8, 11, 15). DRw specificities were assigned to individuals who reacted with approximately two-thirds or more of reagent sera-detecting DRw clusters. The sera were defined according to their pattern of reaction with reference HLA-D homozygous individuals who were typed in the Seventh International Histocompatibility Workshop (9). The assignment specificities included DRw1, DRw2, DRw3, DRw4, DRw5, DRw7, Ia4 × 10, and Ia4 × 7 × 10. The percentage of incidence of these alloantigens in 66 control subjects was DRw1 (16%), DRw2 (24%), DRw3 (29%), Ia4 × 7 × 10 (30%), and DRw5 (9%). The incidence of DRw2 in the Ashkenazi Jewish group was 22% and not significantly different from that of the non-Jewish Caucasian group (24%).

Statistical methods. Frequencies of HLA alloantigens in the patient and matched control populations were compared by Chi-square analysis with Yates' correction. Relative risk (RR) was determined from the formula RR = ([P+][C−])/([P−][C+]), where P is the number of patients and C the number of controls positive or negative for the specificity, according to Woolf (17).

RESULTS

Table I presents the data obtained from 20 patients tested for HLA-A, -B, -C, and HLA-DR alloantigens. The most striking observation noted was the high incidence of HLA-DRw2 found in 75% of the patients studied, when compared with an ethnically matched control population in which the incidence was 23% (Table II). This was statistically significant by X^2 analysis with Yates' correction ($P < 0.001$) and provided a RR of 10.0. (A RR of 1 would indicate no association between the presence of the antigen and the disease.) When the patients were analyzed with regard to ethnic origin, the incidence of DRw2 was 67% ($P < 0.05$) in the non-Jewish Caucasian group and 79% ($P < 0.01$) in the Ashkenazi Jewish group. Of particular interest was the apparent typing for a single allele, DRw2, in 10 of the 20 patients tested. Of possible significance was an associated decreased incidence of Ia4 × 7 × 10 which was 10% as compared with 30% for the control population, $P < 0.1$, with a RR of 0.25.

Whereas family studies to identify particular haplotypes are not yet available, the co-occurrence of either A3 and B7 or A26 and Bw38 was significantly increased as compared with the control population, respectively ($P < 0.001$), which suggests that they may constitute haplotypes (Table II). Linkage disequilibrium for A3-B7 and DRw2 has been reported (18). Of the 15 patients positive for DRw2, 47% had both A26 and Bw38 as compared with the matched control population incidence of 21% ($P < 0.1$). None of the five DRw2 negative patients had A26 or Bw38.

DISCUSSION

These data clearly indicate that immunogenetic determinants, mapping in the major histocompatibility complex, are related to the occurrence of AITP. The presence of the DRw2 alloantigen provides a 10-fold

TABLE I

*Representative HLA Alloantigens of the HLA-A, -B, -C, and -DRw Loci in 20 Patients with AITP**

Patient	Loci								
	A		B		C		DRw		
1‡	3	—§	7	—§	—‖		2		
2	2	3	7	W40	—		2		
3	3	11	7	W35	—		2		
4	3	—	7	—	—		2	3	
5	3	26	7	W38	—		2		
6	11	26	W38	W52	—		2		
7	W24	26	W38	W49	—		2		
8	11	26	W35	W38	W4		2	3	
9	26	28	14	W38	—		2		
10‡	W24	26	18	W38	—		2	3	
11	2	26	W38	W50	—	1	2		
12	W24	W31	W35	—	W4		2		
13	2	28	14	W51	—		2		
14‡	1	—	8	17	—		2	3	
15‡	2	—	7	W44	—		2		
16‡	2	W30	W35	—	W2				Ia4 × 7 × 10
17	3	29	14	W38	—	1		3	
18	1	3	8	W41	—			3	
19	2	3	5	13	—	1			Ia4 × 7 × 10
20‡	1	W24	18	W35	—	1			

* Peripheral blood lymphocytes were tested for HLA-A, -B, and -C alloantigens by cytotoxicity (13). Peripheral blood B lymphocytes were separated from T cells (14) and tested for Ia (DRw) alloantigens by cytotoxicity (15) and, in parallel, by indirect immunofluorescence (8).

‡ Non-Jewish population.

§ Only one alloantigen detected with the antisera employed.

‖ No alloantigen detectable for the HLA-C locus with the antisera employed.

greater risk of developing AITP. The apparent typing of a single DRw2 allele for 10 of the 15 patients with the DRw2 alloantigen suggests homozygosity for this alloantigen, or the presence of an allele which is undetectable with currently available typing sera. Two presumptive haplotypes, A26-Bw38 and A3-B7, were associated with the presence of DRw2. Linkage disequilibrium for A3-B7 and DRw2 has been previously reported (18). However, the association of A26-Bw38 with DRw2 has not been reported. It was infrequent

TABLE II

Incidence of HLA Alloantigens and Alloantigen Associations of the HLA-A, -B, and -DRw Loci in Patients with AITP

Alloantigens	Incidence	Matched controls*	Caucasian non-Jewish controls	Ashkenazi Jewish controls	RR	X^2 Analysis
	%	%	%	%		*P*
A3	29	17.5	18.0	17.0	1.92	<0.2
A26	34	18.4	11.6	24.0	2.69	<0.05
B7	26	16.4	18.0	15.1	1.79	<0.2
Bw38	24	12.2	5.0	18.0	2.27	<0.1
A3 and B7‡	18	4.0	6.6	1.9		<0.001
A26 and Bw38‡	21	6.0	1.1	9.9		<0.001
A26, Bw38, and DRw2‡	47	21.1	3.7	28.6		<0.1

* Controls were matched for the occurrence of Ashkenazi Jews in the patients as described in Methods.

‡ Represents frequency.

in the non-Jewish Caucasian control population where the incidence was 3.7% (and primarily found in individuals of mediterranean origin). The association was considerable in an Ashkenazi Jewish control population where the incidence was 29%, and still greater, but of borderline significance, in the patient population at risk, where the incidence was 47% ($P < 0.1$). Thus the presumptive haplotypes A3-B7-DRw2 and A26-Bw38-DRw2 are striking in frequency in this disorder and probably reflect the selection of a population at risk, sharing DRw2, as well as the indigenous New York population studied. Family studies will be required to definitively characterize the haplotypes.

It is of interest to compare the recent HLA-D alloantigens found in patients with SLE and rheumatoid arthritis with patients with AITP. Patients with SLE (10, 11) had a greater incidence of both HLA-DRw2 and DRw3, with RR of 3.9 and 6.5, respectively, as well as a decreased incidence of Ia4 × 7 × 10 (15); whereas patients with rheumatoid arthritis (10, 11) had an increased incidence of Ia4 × 7 × 10 with a RR of 9.1 (15), as compared with AITP patients with a greater incidence of HLA-DRw2 with a RR of 10 and decreased incidence of Ia4 × 7 × 10. These interesting differences in the three autoimmune disorders indicate that different genetic profiles may be important in the development of specific autoimmune disorders, and suggest a possible relationship between SLE and AITP. For example, Ia4 × 7 × 10 is decreased in both SLE and AITP, whereas it is increased in rheumatoid arthritis. Both SLE and AITP have an increased incidence of DRw2, with SLE having an increase in DRw3 as well as DRw2. Finally, it is clinically recognized that 3–16% of patients with AITP eventually develop SLE (19, 20). It is tempting to speculate that patients who do develop SLE will have a particular DRw profile; and that patients with SLE may be divided into different disease categories relating to their DRw profile.

ACKNOWLEDGMENTS

B-cell alloantisera used in this study were graciously supplied by Dr. Manuel E. Patarroyo, Hospital San Juan de Dios, Bogotà, Columbia.

This study was supported by grant HL-13336-09 of the National Heart and Lung Institute and grant CA 20107 of the National Cancer Institute, Bethesda, Md.

REFERENCES

1. Harrington, W. J., V. Minnich, J. W. Hollingsworth, and C. V. Moore. 1951. Demonstration of a thrombocytopenic factor in the blood of patients with thrombocytopenic purpura. *J. Lab. Clin. Med.* **38**: 1–10.
2. Karpatkin, S., N. Strick, M. B. Karpatkin, and G. W. Siskind. 1972. Cumulative experience in the detection of antiplatelet antibody in 234 patients with idiopathic thrombocytopenic purpura, systemic lupus erythematosus and other clinical disorders. *Am. J. Med.* **52**: 776–785.
3. Dixon, R. H., and W. Rosse. 1975. Platelet antibody in autoimmune thrombocytopenia. *Br. J. Haematol.* **31**: 129–134.
4. Sasuki, T., H. O. McDevitt, and F. C. Grumet. 1977. The association between genes in the major histocompatibility complex and disease susceptibility. *Annu. Rev. Med.* **28**: 425–452.
5. Murphy, S. 1972. Hereditary thrombocytopenia. *Clin. Hematol.* **1**: 359–368.
6. Stavem, P., M. Jeremic, P. F. Hjort, F. Wisloff, E. Vogt, R. Oyen, A. F. Abrahamsen, and A. Sovde. 1969. Hereditary thrombocytopenia with excessively prolonged bleeding time. *Scand. J. Haematol.* **6**: 250–261.
7. Stuart, M. J., R. H. Tomar, M. L. Miller, and P. R. Davey. 1978. Chronic idiopathic thrombocytopenic purpura. A familial immunodeficiency syndrome. *JAMA (J. Am. Med. Assoc.).* **239**: 939–942.
8. Winchester, R. J., S. M. Fu, P. Wernet, H. G. Kunkel, B. Dupont, and C. Jersild. 1975. Recognition by pregnancy serums of non-HLA alloantigens selectively expressed on B lymphocytes. *J. Exp. Med.* **141**: 924–929.
9. Proceedings of the Seventh International Histocompatibility Workshop 1977. Histocompatibility Testing. 1978. W. F. Bodmer and J. Bodmer, editors. Munksgaard, International Booksellers & Publishers Ltd., Copenhagen, Denmark.
10. Reinerstein, J. L., J. H. Kippel, A. H. Johnson, A. D. Steinberg, J. L. Decker, and D. L. Mann. 1978. B lymphocyte alloantigens associated with systemic lupus erythematosus. *N. Engl. J. Med.* **299**: 515–518.
11. Gibofsky, A., R. Winchester, J. Hansen, M. Patarroyo, B. Dupont, S. Paget, R. Lahita, J. Halper, M. Fotino, E. Yunis, and H. G. Kunkel. 1978. Contrasting patterns of newer histocompatibility determinants in patients with rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Rheum.* **21**: S133–137.
12. Stastny, P. 1976. Mixed lymphocyte cultures in rheumatoid arthritis. *J. Clin. Invest.* **57**: 1148–1157.
13. Terasaki, P. I., and J. D. McClelland. 1964. Microdroplet assay of human serum cytotoxins. *Nature (Lond.).* **204**: 998–1000.
14. Hoffman, T., and H. G. Kunkel. 1976. The E-rosette test. *In* In Vitro Methods in Cell Mediated and Tumor Immunity. B. R. Bloom and J. R. David, editors. Academic Press, Inc., New York.
15. Gibofsky, A., R. J. Winchester, M. Patarroyo, M. Fotino, and H. G. Kunkel. 1978. Disease associations of the Ia-like human alloantigens. Contrasting patterns in rheumatoid arthritis and systemic lupus erythematosus. *J. Exp. Med.* **148**: 1728–1732.
16. Amos, D. B., and P. Pool. 1976. HLA typing. Manual of Clinical Immunology. N. R. Rose and H. Friedman, editors. American Society for Microbiology, Washington, D. C. 797–804.
17. Woolf, B. 1955. On estimating the relation between blood groups and disease. *Ann. Hum. Genet.* **19**: 251–253.
18. Dupont, B., E. J. Yunis, R. Duquesnoy, M. Pollack, H. Noreen, J. A. Hanson, N. Reinsmoen, K. Annen, L. Greenberg, T. D. Lee, C. Whitsett, P. Antonelli, and D. Braun. 1977. HLA-D and Ia-like alloantigens in North American Caucasian (US Region 7). *In* Histocompatibility Testing 1977. Munksgaard, International Booksellers & Publishers Ltd., Copenhagen, Denmark. 599.
19. McClure, P. D. 1975. Idiopathic thrombocytopenic purpura in children. Diagnosis and management. *Pediatrics.* **55**: 68–74.
20. Rabinowitz, Y., and W. Dameshek. 1960. Systemic lupus erythematosus after idiopathic thrombocytopenic purpura. *Ann. Intern. Med.* **52**: 1–28.