Juvenile-onset Diabetes: HLA-A, -B, -C, and -DR Alloantigens

H. SOLOW, R. HIDALGO, AND D. P. SINGAL

SUMMARY

We studied the distribution of HLA-A, -B, and -C antigens in 94 juvenile-onset diabetic patients and of HLA-DR antigens in 62 of these patients. The frequencies for HLA-B15, -B40, and -Cw3 were significantly increased in the patient group as compared with the control group. With respect to the B-cell specificities, DRw4 was significantly increased in the patients. Analysis of the data to detect the possible presence of primary and secondary associations between HLA alleles and diabetogenic gene(s) indicated that DRw4 possessed a primary association with the diabetogenic gene(s). As a result, B15, B40, and Cw3 possessed secondary associations. DIABETES *28*:1–4, January 1979.

Juvenile-onset diabetes is a disease of dramatic onset and, often, fatal outcome. It results from disturbances in the normal insulin and carbohydrate metabolism.[1] Several hypotheses have been proposed to explain the mechanism(s) of abnormal insulin metabolism; for example, viral infection and autoimmune processes could impair insulin production by destroying the islet cells of the pancreas.[2] The latter have been suggested by the demonstration of autoantibodies reactive with islet cells.[3]

The involvement of the major histocompatibility complex (MHC) in controlling immunologic responses has been considered. Evidence obtained in the mouse MHC, the H-2 gene complex, suggested that an area referred to as the immune (I) region is involved in the immunologic responses of the mouse to single synthetic antigens.[4] Also, the H-2^k phenotype, in the mouse, is positively associated with susceptibility to Gross leukemia virus and negatively to Tennant leukemia virus.[5] In recent years evidence has accumulated that the immune response to ordinary complex antigens and the susceptibility of laboratory animals to experimentally induced diseases, such as allergic thyroiditis[6] or allergic encephalomyelitis,[7] is also under the control of genes closely linked with the histocompatibility locus. The genes thought to control these responses have been labeled immune-response (Ir) genes and their products, immune-associated (Ia) antigens.

The human counterpart to the mouse Ia antigens has been studied independently by many workers.[8] Recently, the locus controlling the human Ia antigens was defined as the HLA-DR locus, and seven alleles were described: DRw1, DRw2, DRw3, DRw4, DRw5, DRw6, and DRw7.[9] The DR antigens have been serologically detected on B lymphocytes, macrophages, sperm, and epithelial tissue.[10–12] Functionally the DR antigens have been hypothesized to be: antigen receptors, cell-cell interaction components, or self-recognition determinants.[13]

The HLA-DR locus is a part of the HLA supergene, which is located on the short arm of chromosome 6.[14] At the present time, four other highly polymorphic loci (A, B, C, and D) are recognized.[15] These loci, although closely linked, are distinct from each other. The gene products of the A, B, and C loci are glycoprotein structures that are serologically detectable in a cytotoxicity assay. The D-locus gene products control lymphocyte responses, which are assayed in vitro by measuring the proliferative response of one person's lymphocytes to those of a second person incompatible at that locus. This procedure is called a mixed lymphocyte culture test (MLC). Recent advances have suggested that the D-locus gene products are also serologically detectable.[16]

During the past few years the studies of the HLA supergene have demonstrated an association between antigens HLA-B8, -B15, -Cw3, and -Dw3 and juvenile-onset diabetes (JOD).[17–22] The association of B-cell alloantigens defined in our laboratory and JOD has been previously reported.[22] B-cell group 4 was increased and group 7 was decreased in the patients as compared with control subjects.

Presently we report the results of typing for the HLA-DR antigens in a larger series of Caucasian, juvenile-onset insulin-dependent diabetic patients than previously. Also, the

From the Department of Pathology, McMaster University, Hamilton, Ontario, Canada.

Accepted for publication 31 July 1978.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/28/1/1/349888/28-1-1.pdf by guest on 24 May 2024

TABLE 1
Frequency of HLA-B15, -B40, -B8, -B7, -Cw3, and -Cw4 in normal and juvenile-onset diabetic patient populations

HLA antigen	Patients n = 94 (%)	Controls n = 200 (%)	χ^2	Corrected P	RR*
B15	30	14	9.34	0.035	2.61
B40	22	10	11.77	0.012	2.58
Cw3	28	14	7.07	0.047	2.34
Cw4	21	11	6.39	NS	2.18
B8	29	22	1.50	NS	1.47
B7	18	29	3.15	NS	0.55

* Value for relative risk.

DR antigen specificities were defined on the basis of cytotoxicity using the antisera of the Seventh International Histocompatibility Workshop. We found the incidence of HLA-DRw4 to be increased significantly in the diabetic group. DRw3 showed an increase in the diabetics and DRw2 was decreased in the diabetics; however, neither difference was statistically significant. In also studying the HLA-A, -B, and -C alloantigen frequencies, we found B15, B40, and Cw3 to be significantly increased in the patients. Further analyses of our data showed that the primary association of the MHC alleles with JOD was with HLA-DRw4 and that the associations with B15, B40, and Cw3 were secondary.

MATERIALS AND METHODS

Ninety-four unrelated Caucasians with juvenile-onset, insulin-dependent diabetes were typed for HLA-A, -B, and -C antigens. Sixty-two of these patients were also typed for HLA-DR antigens. The results were compared with 200 unrelated adult Caucasians typed for HLA-A, -B, and -C antigens and with 54 subjects of the control population typed for HLA-DR specificities.

The HLA-A, -B, and -C typing was performed on peripheral blood lymphocytes by the microdroplet lymphocyte cytotoxicity test.[23] The patients and the controls were typed for the following HLA-A, -B, and -C antigens: A1, A2, A3, A9, Aw23(9), Aw24(9), A10, A25(10), A26(10), A11, A19, A28, A29, Aw30, Aw31, Aw32, B5, B7, B8, B12, B13, B14, B15, B17, B18, B27, B37, B40, Bw16, Bw38(16), Bw39(16), Bw21, Bw22, Bw35, Cw1, Cw2, Cw3, Cw4, and Cw5.

The HLA-DR typing was performed on a B-cell–enriched lymphocyte population we have previously described.[24] DR specificities were defined on the basis of reactivity with sera in the Seventh International Histocompatibility Workshop.

The frequency distributions of the HLA-A, -B, -C, and -DR antigens in diabetics were compared with those in control subjects. An approximate test of significance was used to test the differences.[25] The data were analyzed for linkage disequilibrium between alleles of the DR locus with those of the B and C loci. The correlation coefficient values (r) were calculated using the method referred to by Colton.[25] To test the possibility of primary and secondary associations of the HLA-B, -C, and -DR alleles with JOD the two populations were subdivided into groups of antigen-positive and antigen-negative subgroups. The frequency distributions of the remaining antigens were subsequently analyzed in these subgroups using the method described by Woolf.[26]

RESULTS

The frequencies of the HLA-A, -B, and -C antigens in JOD were compared with those in the control population. The frequencies for some of these antigens in these two populations are given in Table 1. The incidence of B15 was significantly increased ($X^2 = 9.34$; corrected $P = 0.035$; relative risk (RR) = 2.61) in the JOD group (30%) as compared with the controls (14%). Similarly, the incidence of Cw3 was significantly higher ($X^2 = 7.07$; corrected $P = 0.047$; RR = 2.34) in the patients (28%) than in the control subjects (14%). The incidences of B8 and Cw4 were higher in the patients than in the controls; the increases, however, were not statistically significant. Similarly, the decrease in the incidence of B7 in the JOD group was not significant. In addition, the present data show a significant increase ($X^2 = 11.77$, corrected $P = 0.012$; RR = 2.58) in the frequency of B40 in the patients (22%) as compared with the controls (10%). No difference in the incidence of B18 was observed in JOD (10%) and controls (9%). The remaining HLA-A, -B, and -C alleles did not demonstrate any significant differences in their frequencies between the patient and the control populations.

The incidences of HLA-DR specificities in JOD and control populations are given in Table 2. The incidence of DRw4 was significantly increased ($X^2 = 7.53$, corrected $P = 0.043$; RR = 3.47) in the diabetics (44%) as compared with control subjects (18%). However, an increase in the frequency of DRw3 and a decrease in the incidence of DRw2 in the patients were not statistically significant.

The existence of linkage disequilibrium between HLA-B and -C alleles and HLA-DR specificities has been reported.[9] In the present data we found a strong association between B8 and DRw3 and between B7 and DRw2 in the control group (Table 3). These associations were not observed in the diabetics. On the other hand, an association between

TABLE 2
Frequency of HLA-DR antigens in normal and juvenile-onset diabetic patient populations

Antigen	Patients n = 62 (%)	Controls n = 54 (%)	χ^2	Corrected P	RR
DRw1	18	13	3.80	NS	1.47
DRw2	18	38	5.12	NS	0.35
DRw3	39	18	5.00	NS	2.84
DRw4	44	18	7.53	0.043	3.47
DRw5	5	9	0.92	NS	0.05
DRw6	21	32	1.48	NS	0.55
DRw7	18	27	1.05	NS	0.58

RR, relative risk.

TABLE 3
Association between HLA-DR and HLA-B and -C specificities in normal and in juvenile-onset diabetic patient populations*

		Patients	Controls
HLA-DRw2	HLA-B7	0.17	0.37
HLA-DRw3	HLA-B8	0.19	0.63
HLA-DRw4	HLA-Cw3	0.32	0.30

* The data are expressed as correlation coefficient (r) values.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/28/1/1/349888/28-1-1.pdf by guest on 24 May 2024

Cw3 and DRw4 was observed in both groups. Also, B15 and Cw3 showed linkage disequilibrium in both populations (JOD, $r = 0.25$; controls, $r = 0.51$).

Alleles of the gene loci in the HLA region are now well recognized to be associated with JOD. The data in the present report raise important questions. One of these questions is which alleles of the HLA loci are most strongly associated with juvenile diabetes. Therefore, we attempted to characterize our results by Woolf's analysis, as has been done for diabetes and celiac disease.[27] Table 4 compares the strength of the association of B40, Cw3, and DRw4 with JOD. The two groups of subjects were divided into B15-positive and B15-negative subgroups, and the incidences of B40, Cw3, and DRw4 were subsequently tabulated. It was observed that DRw4 was significantly associated with the diabetogenic gene(s), whereas B40 and Cw3 were not. Both B15 and DRw4 were significantly associated with the diabetogenic gene(s) when the two groups were divided into B40-positive and B40-negative subgroups and the incidences of B15, Cw3, and DRw4 were evaluated (Table 5). DRw4 was again significantly associated with the diabetogenic gene(s) when the two populations were first subdivided into Cw3-positive and Cw3-negative subpopulations and the B15, B40, and DRw4 frequencies subsequently tabulated (Table 6). Neither B15 nor B40 was significantly associated with the diabetogenic gene(s) in this analysis. When similar calculations were performed by first dividing the two populations into DRw4-positive and DRw4-negative groups and then tabulating the incidences of B15, B40, and Cw3, only B15 was significant (Table 7).

TABLE 4
Chi-square test for an association between B40, Cw3, or DRw4 and juvenile-onset diabetes after having divided the two populations into B15-positive and B15-negative subgroups

Antigens	Chi-square	Homogeneity Chi-square
B40	NS	0.1365 ($P < 0.7114$)
Cw3	NS	0.7118 ($P < 0.4009$)
DRw4	6.99 ($P < 0.0083$)	0.0631 ($P < 0.8026$)

DISCUSSION

The present data support earlier observations of an association of HLA-B15 and -Cw3 with juvenile-onset diabetes.[17–22,28,29] In relation to B8 although the data suggest a slight increase in its frequency in the patient population, the difference found was not statistically significant. Similarly the "protective association" of B7 with the absence of JOD as reported by Ludwig et al.[21] is not present in our data. In addition, the present data show a significant increase in the frequency of B40 in the patients as compared with controls.

The focus of our current work was the human B lymphocyte alloantigen locus, HLA-DR. The data presented here show a significantly higher incidence of HLA-DRw4 in the patients. Also the data show an increase in the incidence of DRw3 and a decrease in the incidence of DRw2 in the patients.

TABLE 5
Chi-square test for an association between B15, Cw3, or DRw4 and juvenile-onset diabetes after having divided the patients and the controls into B40-positive and B40-negative subgroups

Antigen	Chi-square	Homogeneity chi-square
B15	5.479 ($P < 0.0193$)	0.1365 ($P < 0.7114$)
Cw3	NS	0.3450 ($P < 0.5552$)
DRw4	7.1889 ($P < 0.0042$)	0.0783 ($P < 0.7795$)

These data are in general agreement with our earlier observations[22,28] and those of other workers.[32–37] We reported a decrease in the frequency of locally defined B cell group 7, UK II, and Thorsby 2 (all comparable to DRw2) and an increase in the frequency of locally defined B cell group 4 (comparable to DRw4 and DRw7) and UK III (comparable to DRw3) in the JOD patients.

The presence of linkage disequilibrium between certain alleles of the HLA supergene have been reported[9] and may account for either a primary or a secondary association of HLA alleles with a disease. It is known that HLA-B15, -Cw3, and -DRw4 are in linkage disequilibrium and so are B40 and Cw3.[9,30] Analysis of the present data by Woolf's method[26] indicates that DRw4 possesses the strongest association with JOD. It could be interpreted to mean that DRw4 has the primary association with the diabetogenic gene(s) and the associations of B15, B40, and Cw3 with the diabetogenic gene(s) are secondary.

The numerous reports of HLA associations with JOD can be divided into two groups. On one side are those who feel that JOD is associated with a single recessive gene and that any HLA association(s) found is with this recessive gene.[31] On the other side are those that feel there are two diabetogenic genes and two different HLA associations, one with B8, Dw3, and DRw3 and the other with B15, CW3, and DRw4. The present data extend the two-gene-hypothesis concept in the etiology of juvenile-onset diabetes[19] in that the one diabetogenic gene is associated with DRw4 and the second with Dw3, and the latter possibly more strongly with DRw3.

It seems likely from the evidence presented here that a major factor determining susceptibility to juvenile-onset diabetes is the presence of a diabetogenic gene or genes at

TABLE 6
Chi-square test for an association between B15, B40, or DRw4 and juvenile-onset diabetes after having divided the patients and controls into Cw3-positive and Cw3-negative subgroups

Antigen	Chi-square	Homogeneity Chi-square
B15	NS	0.7118 ($P < 0.3789$)
B40	NS	0.3450 ($P < 0.5552$)
DRw4	5.94 ($P < 0.0147$)	0.075 ($P < 0.7872$)

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/28/1/1/349888/28-1-1.pdf by guest on 24 May 2024

TABLE 7
Chi-square test for an association between B15, B40, or Cw3 and juvenile-onset diabetes after having divided the patients and controls into DRw4-positive and DRw4-negative subgroups

Antigen	Chi-square	Homogeneity Chi-square
B15	3.85 ($P < 0.05$)	0.0631 ($P < 0.8026$)
B40	NS	0.0783 ($P < 0.7795$)
Cw3	NS	0.0796 ($P < 0.7795$)

a locus closely linked to the HLA chromosomal region. The mechanism of the association between HLA and insulin-dependent diabetes remains speculative and has been discussed elsewhere.[19]

ACKNOWLEDGMENTS

We would like to express our gratitude to Drs. M. A. Blajchman and W. Spaulding (McMaster University Medical Centre) and M. C. Peterson (Henderson General Hospital) for providing the blood samples of the patients. We also thank Mr. N. Naipaul and Mrs. S. Joseph for their technical assistance and Miss M. Fuhringer for her secretarial assistance.

REFERENCES

[1] Bondy, P. K.: Diabetes mellitus: Disorders of carbohydrate metabolism. *In* Cecil-Loeb Textbook of Medicine, Beeson, P., and McDermott, W., Eds. Philadelphia, W. B. Saunders, 1968, p. 1175.

[2] Cudworth, A., White, G., Woodrow, J., et al.: Aetiology of juvenile-onset diabetes. Lancet *1*:385–88, 1977.

[3] Irvine, W., McCallum, C., Gray, R., et al.: Pancreatic islet cell antibodies in diabetes mellitus correlated with duration and type of diabetes, coexistent autoimmune disease, and HLA type. Diabetes *26*:138–47, 1977.

[4] Benacerraf, B., and McDevitt, H.: Histocompatibility-linked immune response genes. Science (Wash. D.C.) *175*:273–79, 1972.

[5] Tennant, J. R.: Susceptibility and resistance to viral leukogenesis in the mouse. II. Response to the virus relative to histocompatibility factors carried by the prospective host. J. Natl. Cancer Inst. *34*:633–41, 1965.

[6] Vladuliu, A. O., and Rose, N. R.: Autoimmune murine thyroiditis: relationship to histocompatibility (H-2) type. Science (Wash. D.C.) *174*:1137–39, 1971.

[7] Gasser, D., Newbin, C., Palm, J., et al.: Genetic control of susceptibility to experimental allergic encephalomyelitis in rats. Science (Wash. D.C.) *181*:872–73, 1973.

[8] Arbeit, R., Sachs, D., Amos, B., et al.: Human lymphocyte alloantigen(s) similar to murine Ir region-associated (Ia) antigens. J. Immunol. *115*: 1173–75, 1975.

[9] Bodmer, W. F.: Joint report of the Seventh International Histocompatibility Workshop. *In* Histocompatibility Testing 1977. Bodmer, W. F., Batchelor, J. R., Bodmer, J. G., et al., Eds. Copenhagen, Munksgaard, 1978, pp. 351–57.

[10] Winchester, R., Fu, S., Wernet, P., et al.: Recognition by pregnancy serums of non-HLA alloantigens selectively expressed on B lymphocytes. J. Exp. Med. *141*:924–29, 1975.

[11] Rowden, G., Lewis, M., and Sullivan, A.: Ia antigen expression on human epidermal Langerhans cells. Nature (London) *268*:247–48, 1977.

[12] Kamoun, M., Wiels, J., Lepage, V., et al.: Presence of Ia equivalent on human spermatozoa detected by specific reagents prepared by the absorption-elution technique on B cell lymphoid lines or PHA stimulated cells. Tissue Antigens *10*:245, 1977. (Abstr.)

[13] Rosenberg, L., and Kidd, K.: HLA and disease susceptibility: a primer. N. Engl. J. Med. *297*:864–66, 1977.

[14] Francke, U., and Pellegrino, M.: Assignment of the major histocompatibility complex to a region of the short arm of human chromosome 6. Proc. Natl. Acad. Sci. U.S.A. *74*:1147–51, 1977.

[15] Nomenclature for Factors of the HLA System. Tissue Antigens *11*: 81–86, 1978.

[16] van Rood, J., van Leeuwen, A., Termijtelen, A., et al.: Recognition of HLA-D by serology. Tissue Antigens *10*:125, 1977 (Abstr.)

[17] Singal, D. P., and Blajchman, M.: Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. Diabetes *22*:429–32, 1973.

[18] Nerup, J., Platz, P., Ortved-Anderson, O., et al.: HLA antigens and diabetes mellitus. Lancet *2*:864–66, 1974.

[19] Thomsen, M., Platz, P., Ortved-Anderson, O., et al.: MLC typing in juvenile diabetes and idiopathic Addison's disease. Transplant. Rev. *22*:125–47, 1975.

[20] Cudworth, A., and Woodrow, J.: HLA system and diabetes mellitus. Diabetes *24*:345–49, 1975.

[21] Ludwig, H., Schernthaner, G., and Mayr, W.: Is HLA-B7 a marker associated with a protective gene in juvenile-onset diabetes mellitus? N. Engl. J. Med. *294*:1066, 1976.

[22] Solow, H., Hidalgo, R., Blajchman, M., et al.: HLA-A, B, C and B lymphocyte alloantigens in insulin dependent diabetes. Transplant. Proc. *9*:1859–61, 1977.

[23] Mittal, K., Mickey, M., Singal, D., et al.: Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. Transplantation *6*:913–27, 1968.

[24] Singal, D., Naipaul, N., and Hidalgo, R.: *In* NIAID Manual of Tissue Typing Techniques, Ray, J., Hare, D., Pederson, P., et al.., DHEW publication no. (N.I.H.) 76-545, 1976, pp. 126–29.

[25] Colton, T.: Statistics in Medicine, Boston, Little, Brown & Co., 1974.

[26] Woolf, B.: On estimating the relation between blood group and disease. Ann. Human Genet. *19*:251–53, 1955.

[27] Keuning, J., Pena, A., van Hooff, J., et al.: HLA-DW3 associated with coeliac disease. Lancet *1*:506–08, 1976.

[28] Solow, H., Hidalgo, R., and Singal, D.: Insulin dependent diabetes: HLA-A, B, C and B cell alloantigens. Tissue Antigens *10*:195, 1977. (Abstr.)

[29] Schernthaner, G., Ludwig, H., and Mayr, W.: Juvenile diabetes mellitus: HLA-antigen frequencies dependent on the age of onset of the disease. J. Immunogenet. *3*:117–21, 1976.

[30] Bodmer, J., Curtoni, E., van Leeuwen, A., et al.: The A B C of HLA. *In* Histocompatibility Testing 1975, Kissmeyer-Nielsen, F., Ed. 1975, pp. 21–29.

[31] Rubinstein, P., Suciu-Foca, N., and Nicholson, J.: Genetics of juvenile diabetes mellitus. A recessive gene closely linked to HLA-D and with 50 per cent penetrance. N. Engl. J. Med. *297*:1036–40, 1977.

[32] Mayr, W. R., Schernthaner, G., Ludwig, H., et al.: Ia type alloantigens in insulin-dependent diabetes mellitus. Tissue Antigens *10*:194, 1977. (Abstr.)

[33] Jeannet, M., Raffoux, C., de Meerlosse, P., et al.: HLA and Ia-like antigens in juvenile-onset diabetes. Tissue Antigens *10*:196, 1977. (Abstr.)

[34] Cattaneo, R., Busetto, F., Mazzoli, M., et al.: Associations between A, B, C and Ia antigens with juvenile onset diabetes (JOD). Tissue Antigens *10*:196, 1977. (Abstr.)

[35] Bodmer, J., Mann, J., Hill, A., et al.: The association of Ia antigens with juvenile onset diabetes and rheumatoid arthritis. Tissue Antigens *10*: 197, 1977. (Abstr.)

[36] Suciu-Foca, N., Rubenstein, P., Susinno, E., et al.: Ia typing of J.D.M. families. Tissue Antigens *10*:199, 1977. (Abstr.)

[37] Garovay, M. R., Carpenter, C., Reddish, M., et al.: "Ia" specificities associated with juvenile diabetes mellitus. Tissue Antigens *10*:200, 1977. (Abstr.)

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/28/1/1/349888/28-1-1.pdf by guest on 24 May 2024