HLA System in Japanese Patients with Diabetes Mellitus

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SUMMARY

Seventy-eight Japanese diabetics were HLA-typed, with special reference to age at onset, insulin dependency, and family history.

HLA-A9, B5, and BW40 were increased, but A1, A3, and B8, which are found frequently among Caucasians, were almost absent among Japanese healthy controls as well as diabetics.

J-1, a Japanese specific subclass of BW22, was significantly increased in juvenile-onset diabetics as compared with controls or diabetics with late onset. J-1 was also increased in the diabetics with insulin dependency and/or positive family history. But the association of J-1 with juvenile-onset diabetes mellitus was found to be the strongest. A tendency to a decrease in B5 was also observed in Japanese diabetics with juvenile onset, but this did not reach statistical significance as far as corrected *P* was concerned. These results showed that genetic markers for diabetes mellitus, especially that with juvenile onset, were different among Japanese from those found among Caucasians.

There is ample evidence to indicate a race specificity in HLA phenotypes in diabetics as well as in controls.

These findings also strongly suggest that juvenile-onset diabetes mellitus is a disease entity in itself and different from late-onset diabetes mellitus in origin and pathogenesis. DIABETES *26*:591-95, June, 1977.

The experimental studies on the H-2 system in the mouse revealed that genes controlling specific immune responses[1] and susceptibility to virus-induced diseases[2] are present in the region of the major histocompatibility loci. Clinically there also are some indications of a relation between HLA and diseases, including neoplastic diseases, viral infections, and conditions with abnormal immune responses.[13]

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Diabetes mellitus is a genetically predisposed disorder of metabolism. But the importance of environmental factors is also obvious. In this regard Craighead and McLane reported the induction of glucose intolerance by encephalomyocarditis virus in the mouse.[4] Since their report, clinical evidence suggesting that viral infection may have a role in the pathogenesis of diabetes mellitus has been accumulated. Abnormalities in immune responses were also reported in the patients with diabetes mellitus.[5-10]

It seems to be important as well as of interest to investigate the relations between the HLA system and diabetes mellitus, because both may relate to heredity, virus infection, and immune responses.

The increased frequency of HLA-B8 and/or BW15 in Caucasian diabetics with juvenile-onset diabetes or insulin dependency was reported by Singal and Blajchman,[11] Nerup et al.,[12] Cudworth and Woodrow,[13] and Nelson et al.[14] These findings seem to be well established as far as Caucasian patients are concerned. But another important characteristic of the HLA system is race specificity.[15] This implies that HLA antigens associated with diabetes mellitus among Japanese might be different from those found among Caucasians.

In this report the results of the studies on the HLA system among Japanese diabetics will be presented.

PATIENTS AND METHODS

Seventy-eight Japanese patients with diabetes mellitus were HLA-typed by Terasaki's method.[16] Fifty-seven healthy controls were also HLA-typed. None of them was related to each other. All patients and healthy controls were typed for the following nineteen antigens, A1, A2, A3, A9, A10, B5, B7, B8, B12, B13, B14, BW16, BW17, BW21, BW22, BW27, BW35, BW37, and BW40. J-1, a Japanese specific

subclass of BW22,[17,18] was also included.

Patients developing clinical disease before the age of 30 are regarded as juvenile-onset, and those who developed the disease after this age as late-onset.[13] Of the diabetics examined, 20 patients had juvenile-onset. The remaining 58 were diabetics with late onset. Twenty-eight patients were on insulin therapy and 47 never required insulin. The remaining three patients were excluded from the analysis related to insulin dependency because they had a history of insulin treatment of only short duration, which makes it difficult to determine whether they are insulin-dependent or nondependent. Sixteen patients had a family history of diabetes mellitus and 62 had no such family history. Thirty-nine patients were diabetics with late onset who had neither insulin dependency nor family history.

The results of the statistical analyses were expressed as "corrected P,"[19] $P\chi 19$, to correct for the number of antigens being investigated in this study.

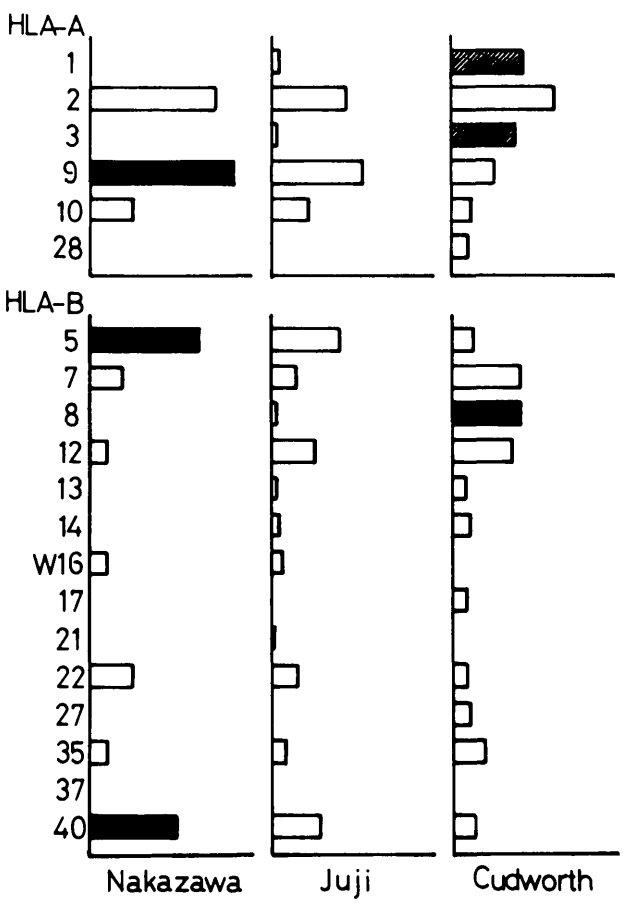

FIG. 1. Race specificity in phenotype frequency of HLA.

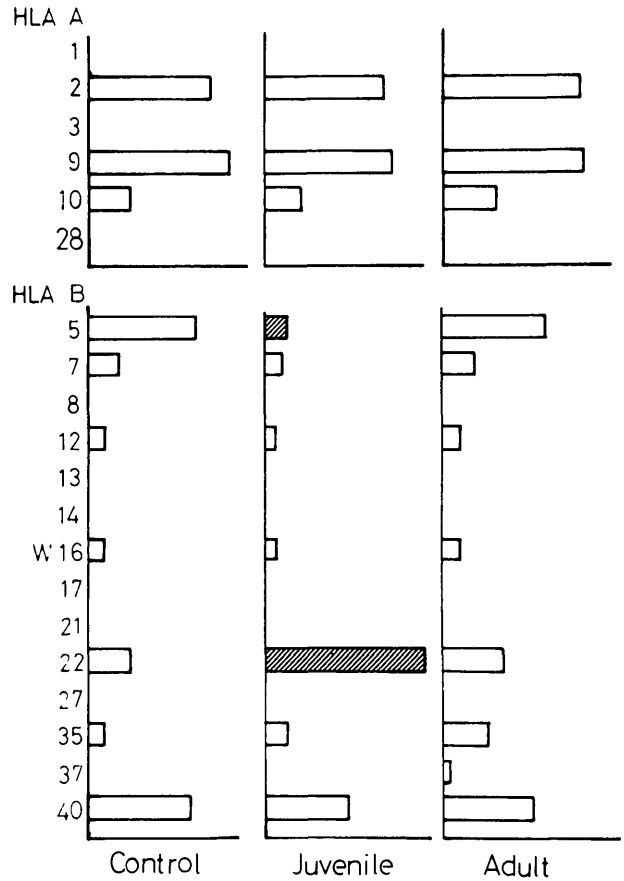

FIG. 2. Phenotype frequency of HLA related to age at onset in Japanese patients with diabetes mellitus.

RESULTS

Figure 1 shows the phenotype frequency of HLA antigens in the controls. The left column shows our results found in Japanese, and the middle one presents the results reported by Juji also in Japanese controls. Both results are found to converge. The right column shows the phenotype frequency in Caucasians as reported by Cudworth and Woodrow. As clearly shown, HLA-A9, B5, and BW40 are increased among Japanese, but A1, A3, and B8, which are found frequently among Caucasians, are almost absent among Japanese. This is an example of race specificity in the HLA system.

Figure 2 shows the phenotype frequency of HLA antigens in Japanese diabetics. Because an increase of J-1 and a decrease of B5 seem to be apparent in diabetics with juvenile onset, further statistical analyses were carried out on these two antigens.

Among 42 subjects with positive reaction to anti-BW22 sera, 36 reacted positively to anti-J-1 sera (table 1).

TABLE 1

J-1, a Japanese specific subclass of BW22, in Japanese diabetics related to age at onset, insulin dependency, and family history

		Age at onset		Insulin dependency		Family history		Late onset, insulin-independent, no family history
	Control	Juvenile	Late	(+)	(−)	(+)	(−)	
(+)	10 (12)	13 (15)	13 (15)	14 (16)	13 (15)	9 (9)	22 (22)	8 (10)
(−)	45	5	43	12	32	7	40	29
χ^{2}*		18.35	0.28	16.43	4.97	9.16	4.39	0.17
Corrected P*		0.01	N.S.	0.02	N.S.	0.02	N.S.	N.S.
		└ Corrected P ┘		└ N.S. ┘		└ N.S. ┘		
		0.02						

( ): Numbers of the patients who positively reacted to anti-BW22-sera.
N.S.: Not significant.
χ^{2}* and corrected P* show the values as compared with controls.

As clearly shown in table 1, J-1 was significantly increased in juvenile-onset diabetics as compared with healthy controls (corrected $P<0.01$) or with the patients with late onset (corrected $P < 0.02$). There was, however, no significant difference between the patients with late onset and the controls. The frequency of J-1 in the patients with insulin dependency was also significantly higher than that in the controls (corrected $P < 0.02$). But there was no significant difference in the frequency of J-1 between the insulin-independent diabetics and the controls or the patients with insulin dependency as far as corrected P was concerned. The increased frequency of J-1 in the patients with a positive family history was also statistically significant as compared with the controls (corrected $P < 0.02$). But again there was no significant difference between the patients with no family history and the controls or the patients with a positive family history. The frequency of J-1 in the patients with late onset who had neither insulin dependency nor family history showed no significant difference as compared with controls.

These findings suggest that an increase of J-1 is associated with juvenile-onset, insulin dependency, or positive family history among Japanese diabetics. But the strongest association with J-1 seems to be found in juvenile-onset diabetics.

HLA-B5 was detected in only two out of 20 cases of the patients with juvenile onset, while this antigen was detected in 28 out of 58 controls. But this difference ($0.002 < P < 0.005$) does not reach a significant level as far as corrected P is concerned (corrected $P <$ 0.095).

DISCUSSION

Among Caucasians, HLA-B8 is frequently observed in healthy controls as well as in patients with diabetes mellitus with juvenile onset or insulin dependency,[11-14] Graves' disease,[20] and systemic lupus erythematosus.[19] The results presented here show an absence of B8 among Japanese healthy controls. This race specificity warrants studies of HLA phenotypes in different races.

Previously we reported that HLA-BW22 is significantly more frequent in Japanese diabetics with juvenile onset, insulin dependency, and/or a positive family history.[21]

But it is well recognized that some antigens supposed to be homogenous in Caucasians are heterogenous in non-Caucasians. Batchelor et al.[22] pointed out that BW22 is a complex antigen in the Fijian population. Miyajima et al.[23] also stated that BW22 was found to be subdivided into three components, a, b, and c, in the Japanese population, and at least two of them, BW22a and BW22c, are Japanese (or oriental) specific new antigens. In 1973 Juji et al. already reported the existence of a possible new Japanese specific HLA antigen, J-1. Recently Juji et al.[18] carried out an absorption study on cross-reactivity between BW22 and J-1 antigens. They concluded that the BW22 might consist of at least two classes of antigenic components. One of them is a major component expressed on all BW22- and J-1-containing lymphocytes. The other class is further subdivided into at least two variants, one expressed on J-1 cells and one expressed on narrow BW22 cells.

These findings prompted us to investigate the relations between J-1 and Japanese patients with diabetes mellitus.

Our results presented here showed that a large portion of BW22 was J-1. Furthermore, the association of J-1 with juvenile-onset diabetes mellitus was found to be the strongest. This increase of J-1 in Japanese diabetics with juvenile onset is different from that reported in Caucasian diabetics, in which B8 is in-

creased. These results are also the expression of race specificity in HLA.

Another point in our data is that among Japanese the patients with diabetes mellitus have a different HLA marker from those found in the patients with Graves' disease. Konishi et al.[24] reported that the only antigen showing an increase in Japanese patients with Graves' disease was BW35, which did not increase in Japanese diabetics. Among Japanese, Graves' disease and diabetes mellitus do not share the same antigen as an immunogenetic marker, while among Caucasians an increase of B8 is a common characteristic in Graves' disease and diabetes mellitus with juvenile onset.

The significance of a tendency to decreased frequency of HLA-B5 in diabetics with juvenile onset is rather difficult to interpret at present. To our knowledge no report about a decrease of any antigens in patients with diabetes mellitus has been presented so far. There are, however, some reports dealing with a negative association between HLA and diseases.[25] Generally speaking, decreased frequencies of HLA antigens are less pronounced and most likely secondary to those increased. The negative association for locus A antigens can be explained, in part, in terms of the linkage disequilibrium between A and B locus antigens.[3] But another possibility, that an antigen that shows a decrease might suppress diseases, was proposed by Bach.[26] This interesting possibility does not seem to be completely excluded. As previously stated, among Japanese there is no common characteristic shared by diabetes mellitus with juvenile onset and Graves' diseases as far as a positive association between HLA and diseases is concerned. In Japanese patients with Graves' disease a decreased frequency in B5 was significant with the value of corrected P less than 0.02.[27] Thus, it is worth mentioning that a decreased frequency in B5 seems to be a common characteristic in HLA phenotype among Japanese patients with Graves' disease and diabetes mellitus of juvenile onset, in which antithyroidal autoantibodies are frequently observed.[5] More abundant data, however, may be needed to establish the significance of this negative association.

Our results presented here showed that juvenile-onset diabetes mellitus among Japanese was characterized by a positive association with J-1, a Japanese specific subclass of BW22, and a possible negative association with B5. These findings suggest that juvenile-onset diabetes mellitus is a disease entity in itself and different from adult-onset diabetes mellitus in origin and pathogenesis.

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REFERENCES

[1]Snell, G. D.: The H-2 locus of the mouse: Observations and speculations concerning its comparative genetics and its polymorphism. Folia Biol. (Praha) *14*:335-58, 1968.

[2]Lilly, F., Boyse, E. A., and Old, L. J.: Genetic basis of susceptibility to viral leukaemogenesis. Lancet *2*:1207-09, 1964.

[3]Vladutiu, A. O., and Rose, N. R.: HL-A antigens. Association with disease. Immunogenetics *1*:305-28, 1974.

[4]Craighead, J. E., and McLane, M. F.: Diabetes mellitus: Induction in mice by encephalomyocarditis virus. Science *162*: 913-14, 1968.

[5]Whittingham, S., Mathews, J. D., Mackay, I. R., Stocks, A. E., Ungar, B., and Martin, F. I. R.: Diabetes mellitus, autoimmunity and ageing. Lancet *1*:763-66, 1971.

[6]MacCuish, A. C., Barnes, E. W., Irvine, W. J., and Duncan, L. J. P.: Antibodies to pancreatic islet cells in insulin-dependent diabetics with coexistent autoimmune disease. Lancet *1*:1529-31, 1974.

[7]Egeberg, J., and Poulsen, J. E.: Antipancreatic cellular hypersensitivity in diabetes mellitus. Diabetes *20*:424-27, 1974.

[8]Ragab, A. H., Hazlett, B., and Cowan, D. H.: Response of peripheral lymphocytes from patients with diabetes mellitus to phytohemagglutinin and candida albicans antigen. Diabetes *21*:906-07, 1972.

[9]Delespesse, G., Duchateau, J., Bastenie, P. A., Lauvaux, J. P., Collet, H., and Govaerts, A.: Cell-mediated immunity in diabetes mellitus. Clin. Exp. Immunol. *18*:461-67, 1974.

[10]MacCuish, A. C., Jordan, J., Campbell, C. J., Duncan, L. J. P., and Irvine, W. J.: Cell-mediated immunity in diabetes mellitus. Leukocyte transformation by insulin and insulin fragments in insulin-treated and newly diagnosed diabetics. Diabetes *24*:36-43, 1975.

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

[12]Nerup, J., Platz, P., Andersen, O. O., Christy, M., Lyngsoe, J., Poulsen, J. E., Ryder, L. P., Nielsen, L. S., Thomsen, M., and Svejgaard, A.: HL-A antigens and diabetes mellitus. Lancet *2*:864-66, 1974.

[13]Cudworth, A. C., and Woodrow, J. C.: HL-A system and diabetes mellitus. Diabetes *24*:345-49, 1975.

[14]Nelson, P. G., Pyke, D. A., Cudworth, A. G., Woodrow, J. C., and Batchelor, J. R.: Histocompatibility antigens in diabetic identical twins. Lancet *2*:193-94, 1975.

[15]Albert, E. C., Mickay, M. R., McNicholas, A. C., and Terasaki, P. I.: Seven new HL-A specificities and their distribution in three races. *In* Histocompatibility Testing, Terasaki, P. I., Ed. Copenhagen, Munksgaard, 1970, pp. 221-30.

[16]Terasaki, P. I., McClelland, J., Park, M. S., and McCurdy, B.: Microdroplet lymphocytotoxicity test. *In* Manuals of Tissue Typing and Techniques, Ray, J. G., Jr., Hare, D. B., Pedersen, P. D., and Kayhoe, D. E., Eds. Bethesda, TIB, NIAID, NIH, 1974, pp. 67-72.

[17]Juji, T., Hagino, Y., Tamura, M., and Okochi, K.: A possible new Japanese specific HL-A antigen (J-1). Jpn. J. Exp. Med. *43*:447-49, 1973.

[18]Juji, T., Hagino, Y., Tamura, M., Ohtawa, T., Toyama, H., Shibata, Y., Schreuder, I., and Rood van, J. J.: Absorption study on crossreactivity between W22 and J-1 antigens. *In* Histocompatibility Testing 1975. Kissmyer-Nielsen, F., Ed. Copenhagen, Munksgaard, 1976.

[19]Grumet, F. C., Coukell, A., Bodmer, J. G., Bodmer, W. F., and McDevitt, H. C.: Histocompatibility (HL-A) antigens associated with systemic lupus erythematosus. N. Engl. J. Med. *285*:193-96, 1971.

[20]Grumet, F. C., Konishi, J., Payne, R. O., and Kriss, J. P.: Association of Graves' disease with HL-A8. Clin. Res. *21*:493, 1973.

[21]Kawa, A., Nakazawa, M., Sakaguchi, S., Kono, Y., Hazeki, H., and Kanehisa, T.: Human leukocyte antigen (HLA) in Japanese diabetics (A preliminary report). Tonyobyo (J. Jap. Diab. Soc.: Japanese with English abstract) *19*:404-07, 1976.

[22]Batchelor, J. R., Morris, P. J., Wolford, R. L., Dumble, L., Law, W., Kird, R., and Case, J.: Studies of HL-A in Fijian population. *In* Histocompatibility Testing 1972. Copenhagen, Munksgaard, 1973, pp. 283-86.

[23]Miyajima, T., Amemiya, H., Iwasaki, Y., Yokoyama, T., Kashiwabara, H., Hashizume, T., and Taguri, M.: HL-A system in the Japanese population 1. Tissue Antigens 6:93-100, 1975.

[24]Konishi, J., Torizuka, K., Mori, T., Grumet, F. C., Payne, R. O., and Kriss, J. P.: HL-A antigens in Japanese patients with Graves' disease or Hashimoto's thyroiditis. Igaku No Ayumi (Progress in Medicine: Japanese) *96*:13-15, 1976.

[25]Jersild, C., Svejgaard, A., Fog, T., and Ammitzball, T.: HL-A antigens and disease. I. Multiple sclerosis. Tissue Antigens *3*:243-50, 1973.

[26]Bach, F. H.: Disease and the HL-A histocompatibility system. Ann. Intern. Med. *75*:962, 1971.

[27]Nakazawa, M., Kawa, A., Nakamura, S., Sakaguchi, S., Okamoto, O., Kawabata, T., Maeda, Y., Ariyama, T., Kamisaki, T., and Inamori, Y.: HLA system in Japanese patients with Graves' disease. Igaku No Ayumi (Progress in Medicine: Japanese) *100*:567-68, 1977.