Nagoya J. Med. Sci. 43. 65 ~ 70, 1981

BLADDER CANCER AND HLA ANTIGENS

KOJI OBATA*[1], YOSHIYUKI OHNO*[2], TATSURO MURASE*[3], KUNIO AOKI*[2], and KIMIYOSHI TSUJI*[4]

*[1] *Department of Urology, Nagoya Second Red Cross Hospital*
*[2] *Department of Preventive Medicine, Nagoya University School of Medicine*
*[3] *Department of Urology, Nagoya University School of Medicine*
*[4] *Blood and Tissue Typing Center, Tokai University School of Medicine*

ABSTRACT

Geno- and phenotypes in HLA antigen were compared between 86 bladder cancer patients and 95 healthy contorls. Lower frequencies of HLA-A10 and HLA-B7 and higher frequency of HLA-B12 were found in bladder cancer patients without statistical significance, as compared to the healthy controls. HLA-AW19 and HLA-B40 were commonly found in two cases of bladder cancer in a family of 6 members. Since two other members of the family had the same HLA antigen of HLA-AW19 and HLA-B40, these antigens are assumed not to be cancer-specific, but to be familial in nature. Further investigations are undoubtedly required selecting the controls by virtue of some defined geographic relationship to the patients.

INTRODUCTION

Cancer of the urinary bladder is a relatively infrequent neoplasm, accounting for 2,078 deaths per annum in 1978 in Japan, compared with 49,564 deaths from stomach cancer, 18,530 deaths from lung cancer and 7,116 deaths from colon cancer.[1)]

Bladder cancer is, however, one of the few malignant neoplasms in which the role of the environmental agents has been clearly shown to be important.

Risk factors incriminated in regard to human bladder cancer are occupational exposures, use of tobacco, infection with Schistosoma haematobium, and possibly such variables as coffee drinking, use of artificial sweeteners, analgesics abuse and urine stasis.[2)] Bracken fern is also pressumed to be potentially hazardous.[3)] Some of the endogenously produced metabolites of tryptophan-nicotinic acid pathway are also assumed to be responsible for some of human bladder cancers with no apparent industiral exposures to chemical carcinogens.[4)] Less dietary intake of vitamin A is recently supposed to increase the risk of bladder cancer.[5)]

In contrast to the environmental factors in bladder cancer, genetic inheritance of this cancer has been rarely assumed, since only a few reports on familial aggregation of bladder cancer have been documented.[6,7,8)] Our investigations on bladder cancer mortality statistics in Japan[9,10)] and in the selected countries in the world[11)], however, revealed the possible existence of a certain segment of the population which is predisposed to bladder cancer deaths. Epidemiological findings supporting this concept include the almost identical risks of bladder cancer deaths for the birth cohorts born after around 1900 in both sexes, very similar increase gradients of age specific death rates, virtually unchanged trends in the age-adjusted mortality rates in recent decades, unaltered magnitude of international variations in the mortality and its sex ratio, and consistent order of the mortality ranking in an international

小幡浩司・大野良之・村瀬達良・青木国雄・辻　公美
Received for Publication August 5, 1980

comparison. It is accordingly of significance as a further step of the investigation to identify a segment of the population which is predisposed to succumb to bladder cancer. One of the most promising approaches to the host predisposition is currently believed to be the evaluation of HLA system.

With this epidemiological background in mind, this paper is prepared to present the preliminary results of our analysis on HLA antigens for bladder cancer patients observed in Nagoya, Japan.

MATERIALS AND METHODS

HLA-typing was carried out at the Blood and Tissue Typing Center, Tokai University for the blood samples obtained from 84 bladder cancer patients diagnosed at four major hospitals in Nagoya for the period of October 1977–May 1978. A family of 6 members, which includes two bladder cancer patients, was also analysed for HLA antigens.

Serotypes of 95 healthy subjects served as the controls, and were typed at the above center for the same period of 8 months.

The procedures of HLA-typing were reported in detail elsewhere.[12]

HLA antigens determined are as follows.

HLA-A: A1, A2, A3, A9, AW23, AW24, A10, A25, A26, A11, A28, A29, AW19, AW30, AW31, AW32, AW33, AW34, and AW36

HLA-B: B5, BW51, BW52, B7, B8, B12, B13, B14, B15, BW16, BW17, BW22, BW35, B37, B40, BW40.1, BW40.2, BW48, and BW54

Among HLA-A antigens, A9, AW23 and AW24 are inclusively grouped as 'A9', A10, AW25, and AW26 as 'A10', and A19, AW30, AW31, AW32 and AW33 as 'AW19'. Among HLA-B antigens, B5, BW51 and BW52 are grouped as 'B5', BW22 and BW54 as 'BW22', and B40, BW40.1, BW40.2 and BW48 as 'B40'.

Phenotype (PF), genotype (GF) and standard error (SE) are calculated by the following formulas.

$$PF = \frac{\text{each gene frequency}}{\text{total gene frequency}} \times 100$$

$$GF = \left(1 - \sqrt{1 - \frac{PF}{100}}\right) \times 100$$

$$SE = \frac{\sqrt{\text{total gene frequency} \times \frac{PF}{100}}}{2 \times \text{total gene frequency}} \times 100$$

The overall differences in geno- and phenotype frequency between bladder cancer patients and controls are examined by chi-square test with Yate's correction; statistical significance being determined by the corrected p-value.[13]

RESULTS

Table 1 and 2 detail the percentage frequency of pheno- and genotypes and standard errors

Table 1. HLA-A: Phenotype and Gene Frequencies in Bladder Cancer

Antigen	Bladder Cancer (N = 86)			Controls (N = 95)		
	PF%	GF%	SE%	PF%	GF%	SE%
A1	3.5	1.8	1.0	1.1	0.6	0.5
A2	37.2	20.8	3.2	46.3	26.7	3.5
A3	0.0	0.0	0.0	0.0	0.0	0.0
A9	62.8	39.6	4.2	48.4	28.2	3.6
A10	12.8	6.6	1.9	29.8	16.0	2.8
A11	16.3	8.5	2.2	15.8	8.2	2.0
A28	0.0	0.0	0.0	0.0	0.0	0.0
A29	0.0	0.0	0.0	0.0	0.0	0.0
AW19	18.6	9.8	2.3	27.4	14.8	2.7
AW34	0.0	0.0	0.0	0.0	0.0	0.0
AW36	0.0	0.0	0.0	0.0	0.0	0.0
AW43	0.0	0.0	0.0	0.0	0.0	0.0
Blank	48.6	28.4	3.8	31.6	17.3	2.9

Table 2. HLA-B: Phenotype and Gene Frequencies in Bladder Cancer

Antigen	Bladder Cancer (N = 86)			Controls (N = 95)		
	PF%	GF%	SE%	PF%	GF%	SE%
B5	33.7	18.6	3.1	34.7	19.2	3.0
B7	4.7	2.4	1.2	10.5	5.4	1.7
B12	26.7	14.4	2.8	15.8	8.2	2.0
B13	3.5	1.8	1.0	1.1	0.6	0.5
B15	14.0	7.3	2.0	21.1	11.2	2.3
BW16	4.7	2.4	1.2	8.4	4.3	1.5
B17	1.2	0.6	0.6	0.0	0.0	0.0
BW22	27.9	15.1	2.8	18.9	9.5	2.2
BW35	12.8	6.6	1.9	17.8	9.3	2.2
B37	3.5	1.8	1.0	1.1	0.6	0.5
B40	36.0	20.0	3.2	47.4	27.5	3.5
Blank	31.4	17.2	3.0	23.2	12.4	2.5

for 86 patients and 95 controls. Among HLA-A antigens, the frequency of A10 was 12.8% and 29.8% in bladder cancer patients and the controls, respectively; the difference being significant at a probability of less than $p = 0.01$, but being determined not significant by the corrected p-value. Among HLA-B antignes, B12 was found in 26.7% of the patients and 15.8% of the controls, and B7 in 4.7% and 10.5%, correspondingly. The differences were, however, below the significance level of $p = 0.05$. The overall differences in the frequency distribution of HLA-A and HLA-B antigens between the patients and the controls were determined not to be significant by chi-square test with Yate's correction.

Figure 1 shows the HLA-genotypes of 6 members in Y's family. Two members with bladder

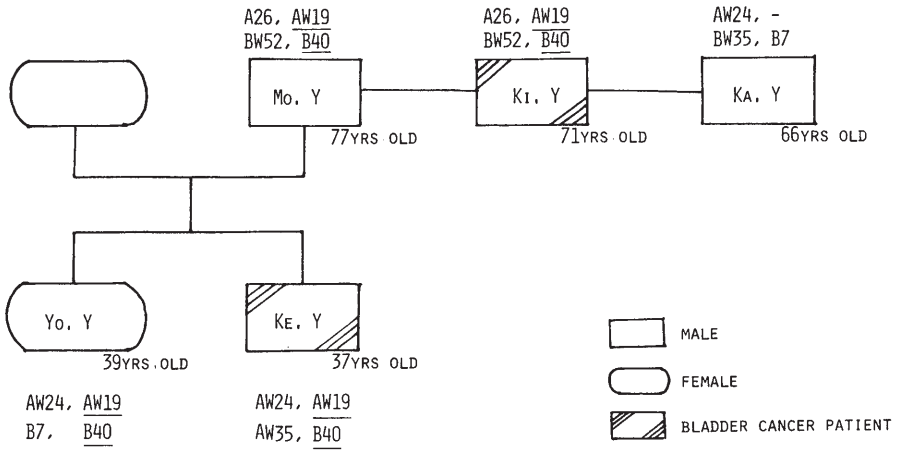

Figure 1. HLA Genotype in Y's Family

cancer (KI.Y and KE.Y) had HLA-AW19 and HLA-B40. Two other members of the family (MO.Y and YO.Y) also had the same genotype of AW19 and B40. From this observation in Y's family, HLA–AW19 and B40 are assumed not to be cancer-specific, but to be familial in nature.

DISCUSSION

Incidence of bladder cancer is comparatively high in Western countries and low in Japan[14,15], though the magnitude of international variation in incidence and mortality is not so large, as compared to that of cancers of such sites as the esophagus, lung, stomach and colon[16,17]. Ethnic difference in cancer incidence and mortality has long been presumed to be ascribable, in part, to the ethnic differences in combination of genes.

If cancer-specific HLA antigens would have been identified, the contributions to cancer prevention and treatment would certainly have been promising, through early detection and management of a certain segment of the population.

An approach to immunogenetic backgrounds in human malignant neoplasms is presumed to be initiated by Amiel and Kourisky in 1967.[18] Since then, the investigations have been devotedly focused on the genotype and haplotype antigens on Locus A and B of the haematologic diseases and cancers of such sites as the buccal cavity, tongue, esophagus, stomach, intestine, rectum, lung, kidney, prostate, breast, uterus and ovary.[18]

The relationship between bladder cancer and HLA antigens was analysed on 139 Caucasian patients in 1973 by Takasugi *et al.*,[19] who reported the low frequency of HLA-BW35 without statistical significance.

In 1975, Terasaki and Micky[20] observed the significantly higher frequencies of HLA-A2 and HLA-BW21 among 217 Caucasian patients with bladder cancer (corrected p-value less than 0.05).

In Japan, a few studies have been undertaken on the relationship between HLA antigens

and cancers. Tsuji[21] demonstrated the significantly higher frequency of HLA-B18 and the lower frequencies of HLA-B5 and HLA-BW35 among 87 stomach cancer patients, as compared with the healthy controls. To the best of the authors knowledge, however, HLA antigens specific for bladder cancer have not been reported in Japan. Our investigation of 86 Japanese patients with bladder cancer demonstrated the higher frequency of HLA-B12 and the lower frequencies of HLA-A10 and HLA-B7 as compared to the healthy controls; the differences being determined to be insignificant by corrected p-values. Of interest, however, is that the lower frequency of HLA-BW35 reported by Takasugi *et al.*[19] is also observed in our series.

Methodologically, the studies of linking HLA antigens to the diseases are undertaken by comparing the frequencies of antigens between the patients and the controls. It is quite true in this particular type of analysis that the ethnic relations and kinship should carefully be taken into consideration.

The Japanese are ethnically considered to be homogeneous, but some ethnic heterogeneity is claimed.[21] According to Yasuda,[22] for instance, the area-specific antigens are HLA-AW29 and HLA-B12 in Tokyo, and HLA-A2 and HLA-B5 in Nagoya.

Eight studies conducted in different institutions in Japan, in which common antigens and techniques are not always used, demonstrated somewhat diverse frequencies of certain phenotypes[23]; the frequency of HLA-B12 ranging from 7% to 17%, HLA-B7 from 8% to 18%, and HLA-A10 from 5% to 18%. These differences in the frequency are supposedly attributed to the differences in the antisera used for HLA typing.

In our study, the controls were sampled from the healthy inhabitants in the Kanto district, assuming that the Japanese are ethnically homogeneous. Selection of the controls from the Kanto district also required using the common antisera for typing at the Blood and Tissue Typing Center, Tokai University. Therefore, the regional differences in phenotypes between the Kanto district and the Nagoya area, if any, might possibly distort our results to some extent.

Consequently, in this respect, we ought to plan the population-based comparison with a well designed framework; selecting the controls by virtue of some defined geographic relationship to the patients.

HLA antigens are currently receiving much attention for their possible linkage to the prognosis rather than as a etiological factor in malignant neoplasms. The patients with acute lymphoblastic leukemia, for instance, are reported to experience relatively longer remissions when they have HLA-A9, though this particular antigen is known to be infrequently found in the patients with this disease[24,25].

Among the numerous cancer patients with catastrophic prognosis, there definitely exist some patients whose cancer is spontaneously healed. The tolerance threshold for radiation therapy and chemotherapeutics is known to be somewhat different from one patient to another. Since these facts are closely related to the ultimate goal of cancer therapy, then in future the investigations on HLA antigens could give a clue to the inherent resistance to cancer itself.

REFERENCES

1) Ministry of Health and Welfare, Department of Health and Welfare Statistics and Information., Vital Statistics 1978, Japan. Vol. 2, 1979.
2) Morrison, A.S. and Cole, P.: Epidemiology of bladder cancer. *Urologic Clinics of North America* **3**, 13—29, 1976.

3) Pamukcu, A.M., Ertürk, E., Yalçiner, S. Milli, U. and Bryan, G.T.: Carcinogenic and mutagenic activities of milk from cows fed braken fern (Pteridium aquilinum). *Cancer Res.* **38**, 1556—1560, 1978.
4) Rose, D.P.: Aspects of tryptophan metabolism in health and disease; A review. *J. Clin. Path.* **25**, 17—25, 1972.
5) Mettlin, C. and Graham, S.: Dietary risk factors in human bladder cancer. *Am. J. Epidemiol.* **110**, 255—263, 1979.
6) Fraumeni, J.F.Jr. and Thomas, L.B.: Malignant bladder tumors in a man and his three sons. *JAMA* **201**, 507—509, 1967.
7) McCullough, D.L., Lamm, D.L., McLaughlin, A.P.III. and Gittes, R.F.: Familial transitional cell carcinoma of the bladder. *J. Urol.* **113**, 629—635, 1975.
8) Sharma, S.K., Bapna, B.C. and Singh, S.M.: Familial profile of transitional carcinoma. *Brit. J. Urol.* **48**, 442, 1976.
9) Ohno, Y. and Aoki, K.: Epidemiology of bladder cancer deaths in Japan. *Gann* **68**, 715—729, 1977.
10) Ohno, Y., Aoki, K., Shimizu, H. and Tominaga, S.: Geographic epidemiology of bladder cancer deaths in Japan. *Acta Urol. Japonica* **25**, 121—132, 1979 (in Japanese with English abstract).
11) Ohno, Y. and Aoki, K.: Epidemiology of urinary bladder cancer: International variation in mortality. *Nagoya J. med. Sci.* **43**, 41—64, 1980.
12) Tsuji, K., Nose, Y. and Inoue, H.: Technique of tissue typing. *Clinic All Round* **26**, 2955—2962, 1977 (in Japanese).
13) Bodmer, W.F.: Genetic control of immunoresponsiveness., ed. H.O. McDevitto and M. Landy, pp. 338, Academic Press, New York, London, 1972.
14) Morrison, A.S. and Cole, P.: Epidemiology of urologic cancers. In principles and manegement of urologic cancer, pp. 1—27, Edited by N. Javadpour, Williams and Wilkins, Baltimore, 1979.
15) Miyakawa, M.: Epidemiology of bladder cancer. *Jap. J. Clin. Med.* **34**, 3104—3108, 1976 (in Japanese).
16) Segi, M. and Kurihara, M.: Cancer mortality for selected sites in 24 countries. No. 6 (1966—1967), Japan Cancer Society, Tokyo, 1972.
17) Doll, R.: Epidemiology of cancer: Current perspectives. *Amer. J. Epidemiol.* **104**, 396—404, 1976.
18) Tsuji, K. and Inoue, H.: Human major histo-compatibility complex and malignancy. *Saishin-Igaku* **32**, 1292—1302, 1977 (in Japanese).
19) Takasugi, M., Terasaki, P.I., Henderson, B., *et al.*: HL-A antigens in solid tumors. *Cancer Res.* **33**, 648—650, 1073.
20) Terasaki, P.I. and Mickey, M.R.: HL-A haplotypes of 32 diseases. *Transplant. Rev.* **22**, 105—119, 1975.
21) Tsuji, K.: Current perspectives of HLA in clinical medicine. *Rinshyo-Ketsueki,* **18**, 564—574, 1977 (in Japanese).
22) Yasuda, N., Tsuji, K., Aizawa, M., *et al.*: HLA antigens in Japanese populations. *Am. J. Hum. Genet.* **23**, 390—399, 1976.
23) Ohkochi, H., Nomoto, K. and Toyoda, S.: HLA antigens in Japan-problems of antisera. *Jap. J. Transplantation* **12**, 1—3, 1977 (in Japanese).
24) Rogentine, G.N.Jr., Yankee, R.A., Gart, J.J., Nam. J. and Trapani, R.J.: HL-A antigens and disease. Acute lymphocytic leukemia. *J. Clin. Invest.* **51**, 2420—2428, 1972.
25) Lowler, S.D., Klouda, P.T., Smith, P.G., *et al.*: Survival and the HL-A system in acute lymphoblastic leukemia. *Brit. Med. J.* **1**, 547—548, 1974.