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# HLA Antigens in Patients with Germ Cell Cancers of the Testis[1]

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## ABSTRACT

The expression of HLA-A, -B, -C, and -DR antigens has been analyzed in 145 unrelated Caucasian patients with germ cell tumors of the testis. Eighteen of these patients had pure seminoma, while the remaining patients had nonseminomatous tumors with embryonal carcinoma, teratocarcinoma, choriocarcinoma, and/or yolk sac components, with or without seminoma. Increases were noted in the frequencies of Aw33, B5, DR5, and DRw6 among the patients with pure seminoma, A3 and B7 among the patients with embryonal carcinoma with or without seminoma, and Aw32 among the patients with yolk sac tumor components. A decrease in the frequency of HLA-DR3 was noted in all patient subgroups, although none of these differences were statistically significant after correction for the number of antigens tested. HLA typing results for three affected brothers of patients indicate that, in each family, the affected sibling pair share at least one HLA haplotype. The etiological and prognostic significance of this finding and of the increases in a few HLA antigen frequencies in particular patient groups and the overall decrease in DR3 remain to be determined.

## INTRODUCTION

Initial interest in studies of HLA-associated risk factors in human cancer was derived from the well-established role of H-2-linked factors in leukemogenesis in mice (7). Although only a few such associations have been found in humans [reviewed by Ryder *et al.* (11)], this may in part reflect the heterogeneity of human cancer and of human populations, and the search has continued. Testicular carcinoma and HLA has been the subject of 3 earlier studies (1, 3, 8), but these have yielded inconsistent results. The present study was undertaken to try to resolve differences between those earlier studies and to investigate the possible role of the HLA-DR antigens which had not been considered by the other authors.

## MATERIALS AND METHODS

**Patients.** Two different groups of Caucasian patients with germ cell tumors of the testis treated at the Memorial Sloan-Kettering Cancer Center were studied. The first group consisted of 75 patients tested in the period between May 1978 and December 1979 while the second group consisted of an additional 69 patients tested between October 1980 and February 1981. Histological diagnosis was established in all patients by the Pathology Department of Memorial Hospital. Classification categories included pure seminoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, yolk sac tumors, and mixtures of 2 or more of these components. Patients with Leydig cell tumors, squamous cell carcinomas, adenocarcinomas, and other non-germ cell tumors of the testis were excluded from the study. The patient groups included individuals with all stages of disease. Three brothers of patients who were also affected with germ cell tumors of the testis were also studied.

**HLA Studies.** HLA-A, -B, and -C locus typing was performed by the standard 2-stage microcytotoxicity technique using a large battery of highly selected typing antisera. One hundred five of the 143 patients were also typed for the HLA-DR locus antigens using the nylon column method for enrichment of test B-cell (2) and prolonged incubation times in the cytotoxicity test. DR typing reagents for the first patient group collectively defined the antigens HLA-DR1, 2, 3, 4, 5, w6, 7, and w8, while sera defining the antigens DRw9 and DRw10 were included at the time the second group was studied. Two different groups of healthy metropolitan area Caucasians who were HLA typed, respectively, during the same time periods as the 2 patient groups served as controls. These controls were verified to have essentially identical distributions of original geographical family origins (*e.g.*, percentage of Italian descent, etc.) as the patients. Because of slight differences in reagents and control antigen frequencies obtained for the 2 groups, the patient groups were analyzed separately as well as combined in comparison with the combined controls. The significance of antigen frequency deviations was calculated using the $\chi^2$ test with Yate's correction.

## RESULTS

Summary results for the analysis of HLA-A, -B, and -DR antigen frequencies in the 2 groups of patients with germ cell tumors of the testis are shown in Tables 1 and 2. Although the patients were typed for the subtype "splits" of the broad HLA antigens shown in the table (*e.g.*, the Bw62 and Bw63 splits of B15), only the broad-antigen frequency data are shown in the table because the distribution of subtypes did not differ from the controls in any of these instances. Since there were no major differences between the 2 groups of patients and between the 2 groups of controls with regard to HLA-A and -B antigen frequencies, only the combined data are shown (Table 1). The HLA-DR data, however, are shown for both patient groups, both in comparison with the respective control groups and combined in comparison with the combined controls (Table 2). Data for the patients include antigen frequencies for the total patient groups and for the patient subgroups: pure seminoma, nonseminoma, embryonal carcinoma with or without seminoma, and teratocarcinoma, choriocarcinoma, and yolk sac tumor components with or without other components.

The results for the HLA-A and -B antigen frequency analysis indicate (Table 1) a trend toward a decrease in the antigen B8 among both seminoma and nonseminomatous patient groups. In addition, the pure seminoma patient subgroup has significant increases in Aw33 and B5. Six of the 8 seminoma patients with B5 (75%) had the Bw51-type split which is essentially the same proportion as that found among our local Caucasian controls (77%). (The increase in Bw51 is also significant.) Among the patients with nonseminomatous tumors, the embryonal carcinoma patient subgroup has significant increases in A3 and B7 and the yolk sac tumor subgroup has a significant increase in Aw32. None of these differences is significant after correction for the number of antigens tested. Data for the HLA-C locus

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Table 1

*HLA antigen frequencies in germ cell testicular carcinoma*

| Antigen | Phenotype frequency (%)[a] Caucasian controls (388)[b] | Total patients (145) | Pure seminoma (18) | Nonseminoma (125) | Embryonal ± seminoma (35) | Teratocarcinoma (69) | Choriocarcinoma (29) | Yolk sac tumors (10) |
|---|---|---|---|---|---|---|---|---|
| A1 | 29 | 22 | 22 | 22 | 17 | 26 | 28 | 30 |
| A2 | 40 | 48 | 39 | 50 | 51 | 49 | 52 | 30 |
| A3 | 20 | 25 | 33 | 24 | 37[c] | 16 | 21 | 20 |
| A9(w23 + w24) | 23 | 19 | 22 | 18 | 23 | 19 | 21 | 0 |
| Aw23 | 5 | 5 | 11 | 4 | 9 | 3 | 3 | 0 |
| Aw24 | 14 | 13 | 11 | 13 | 9 | 17 | 14 | 0 |
| A10(25 + 26) | 23 | 15 | 22 | 14 | 17 | 17 | 10 | 10 |
| A25 | 6 | 3 | 0 | 3 | 6 | 3 | 0 | 0 |
| A26 | 14 | 13 | 22 | 12 | 11 | 14 | 10 | 10 |
| A11 | 10 | 15 | 17 | 15 | 9 | 19 | 17 | 30 |
| A28 | 10 | 13 | 11 | 13 | 11 | 13 | 14 | 20 |
| A29 | 7 | 8 | 0 | 9 | 14 | 9 | 3 | 10 |
| Aw30 | 6 | 6 | 0 | 6 | 9 | 4 | 0 | 0 |
| Aw31 | 4 | 4 | 6 | 5 | 0 | 6 | 14 | 10 |
| Aw32 | 6 | 7 | 0 | 8 | 0 | 9 | 3 | 40[c] |
| Aw33 | 1 | 3 | 17[c] | 1 | 0 | 0 | 3 | 0 |
| B5(w51 + w52) | 11 | 16 | 44[c] | 12 | 9 | 14 | 10 | 0 |
| B7 | 16 | 22 | 22 | 22 | 31[c] | 19 | 24 | 20 |
| B8 | 15 | 10 | 6 | 11[d] | 11 | 13 | 10 | 10 |
| B12(w44 + w45) | 21 | 22 | 11 | 24 | 23 | 26 | 21 | 20 |
| B13 | 7 | 5 | 0 | 6 | 6 | 6 | 7 | 0 |
| B14 | 12 | 15 | 22 | 14 | 9 | 16 | 24 | 10 |
| B15(w62 + w63) | 8 | 6 | 6 | 6 | 9 | 6 | 0 | 0 |
| Bw16(w38 + w39) | 16 | 9 | 11 | 9 | 3 | 12 | 7 | 20 |
| B17(w57 + w58) | 12 | 7 | 0 | 8 | 6 | 10 | 10 | 20 |
| B18 | 10 | 8 | 6 | 9 | 14 | 7 | 3 | 10 |
| Bw21(w49 + w50) | 7 | 11 | 6 | 12 | 9 | 16 | 14 | 0 |
| Bw22(w55 + w56) | 5 | 4 | 6 | 4 | 3 | 4 | 7 | 20 |
| B27 | 6 | 7 | 11 | 6 | 6 | 7 | 3 | 0 |
| Bw35 | 23 | 27 | 17 | 29 | 29 | 26 | 21 | 30 |
| B37 | 2 | 1 | 6 | 1 | 0 | 1 | 3 | 0 |
| B40(w60 + w61) | 8 | 8 | 11 | 8 | 9 | 7 | 14 | 10 |
| Bw41 | 2 | 2 | 11 | 1 | 6 | 1 | 0 | 0 |
| Bw47 | 0 | 1 | 0 | 2 | 0 | 3 | 0 | 0 |

[a] Data shown are the combined HLA antigen frequencies for the 2 groups of patients and controls.
[b] Numbers in parentheses, number of individuals.
[c] These phenotype frequencies were significantly different ($p < 0.05$) from the control values.
[d] Data reported by Majsky *et al.* (8) also indicate a trend for a decrease in B8 among patients with nonseminomatous germ cell tumors [2 of 22 patients (9%) were B8 positive in comparison with 51 of 301 controls (17%)]; however, the decrease is not statistically significant, even if data from the 2 studies are combined.

Table 2

*HLA-DR antigen frequencies in germ cell testicular carcinoma*

| | Phenotype frequency (%) Caucasian controls | | | Total patients | | | Pure seminoma | | | Nonseminoma-tous | | | Embryonal ± seminoma | | | Teratocarcinoma | | | Choriocarcinoma | | | Yolk sac tumors | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antigen | (100)[a] | (76) | (176) | (37) | (68) | (105) | (1)[b] | (12) | (13) | (36) | (56) | (92) | (11) | (11) | (22) | (20) | (35) | (55) | (4) | (17) | (21) | (1)[b] | (6) | (7) |
| DR1 | 17 | 18 | 18 | 14 | 15 | 14 | | 25 | 23 | 14 | 13 | 13 | 27 | 0 | 14 | 10 | 14 | 13 | 0 | 18 | 14 | | 17 | 14 |
| DR2 | 30 | 18 | 25 | 22 | 25 | 24 | | 33 | 38 | 19 | 23 | 22 | 18 | 36 | 27 | 20 | 17 | 18 | 0 | 18 | 14 | | 17 | 14 |
| DR3 | 24 | 18 | 22 | 11 | 12 | 11[c] | | 0 | 0 | 11 | 14 | 13 | 9 | 27 | 18 | 15 | 11 | 13 | 0 | 18 | 14 | | 33 | 29 |
| DR4 | 33 | 32 | 32 | 49 | 22 | 31 | | 0 | 8 | 47 | 27 | 35 | 73[c] | 18 | 45 | 40 | 31 | 35 | 25 | 12 | 14 | | 50 | 43 |
| DR5 | 18 | 24 | 20 | 14 | 32 | 26 | | 58 | 54[c] | 14 | 27 | 22 | 18 | 9 | 14 | 15 | 26 | 22 | 0 | 24 | 19 | | 50 | 43 |
| DRw6 (+6Y) | 10 | 22 | 15 | 16 | 34 | 28 | | 50 | 46[c] | 17 | 30 | 25 | 9 | 27 | 18 | 20 | 40 | 33 | 25 | 29 | 29 | | 17 | 29 |
| DR7 | 25 | 32 | 28 | 35 | 31 | 32 | | 25 | 23 | 36 | 32 | 34 | 18 | 55 | 36 | 40 | 29 | 33 | 75 | 35 | 43 | | 17 | 29 |
| DRw8 | 0 | 1 | 1 | 0 | 3 | 2 | | 0 | 0 | 0 | 4 | 2 | 0 | 0 | 0 | 0 | 6 | 4 | 0 | 0 | 0 | | 0 | 0 |
| DRw9 | ND[d] | 4 | ND | ND | 3 | ND | | 0 | ND | ND | 4 | ND | ND | 0 | ND | ND | 3 | ND | ND | 0 | ND | | 0 | ND |
| DRw10 | ND | 0 | ND | ND | 0 | ND | | 0 | ND | ND | 0 | ND | ND | 0 | ND | ND | 0 | ND | ND | 0 | ND | | 0 | ND |

[a] Numbers in parentheses, number of individuals in Group 1, Group 2, and the combined study groups, respectively.
[b] Antigen frequencies were not calculated in these groups because of the very limited number of patients.
[c] These phenotype frequencies were significantly different ($p < 0.05$) from those of the relevant Caucasian control group.
[d] ND, not done.

antigens (not shown) did not reveal any significant deviations from the control groups.

Results for the HLA-DR antigen frequency analysis indicate (Table 2) a significant decrease in HLA-DR3 in the total patient group and a significant increase in DR5 and DRw6 among the patients with pure seminoma. Although the first group of patients with embryonal carcinoma showed a significant increase in DR4, this trend was not found among the second group of

Table 3

*Familial cases of germ cell testicular carcinoma*

| Family | Individual | Relationship | HLA typing data[a] | Histology of tumor |
|---|---|---|---|---|
| 1 | D. R. | Twin brother | *A2, B12/A28, B12* | Nonseminomatous (embryonal) |
| | T. R. | Twin brother | *A2, B12/A28, B12* | Nonseminomatous (embryonal + choriocarcinoma + seminoma) |
| 2 | W. W. | Brother | A2, B12/*A3, B7* | Nonseminomatous (embryonal) |
| | C. W. | Brother | *A28, B14/A3, B7* | Nonseminomatous |
| | A. W. | Sister | *A28, B14/A3, B7* | Cervical carcinoma |
| 3 | M. N. | Brother | *A3, Bw35*/Aw24, B7 | Nonseminomatous (embryonal, teratoma, seminoma) |
| | R. N. | Brother | *A3, Bw35*/A28, B12 | Nonseminomatous (embryonal, teratoma) |

[a] HLA antigens in these individuals are expressed in genotype form, and haplotypes that are shared between the affected members of the same family are in italic.

patients with embryonal carcinoma. None of the observed deviations in DR frequencies were significant after correction for the number of antigens tested.

HLA typing results for 3 affected brothers of patients in the first group are shown in Table 3. (Since only one of these individuals was typed for DR antigens, DR data are not included in the table.) One brother was an identical twin of a patient and, as in previous studies (10), showed a similar age of disease onset. Tumors for all 3 brother pairs showed similar histological patterns but were not completely identical. In each of these families, the 2 brothers share at least one HLA haplotype. Since the expected level of haplotype sharing is 75%, this is not statistically significant. However, it is interesting to note that 2 of the families' shared haplotypes include the HLA antigen A3, which is significantly increased in patients with embryonal carcinoma (with or without seminoma). In one of the 3 families (Family 2), a sister with cervical carcinoma was also found to share one HLA haplotype with the 2 affected brothers and to be completely HLA identical to one of these brothers.

## DISCUSSION

Earlier studies of HLA antigen frequencies in patients with germ cell tumors of the testis were reported by Majsky *et al.* (8), Carr and Bach (1), and DeWolf *et al.* (3). Majsky *et al.* (8) found increases in A3, A10, B14, B40, and, especially, Bw35 among 40 patients with seminoma and increases in B18, Bw21, and B40, and, especially, A10 among 20 patients with nonseminomatous germ cell tumors. They also reported trends showing decreases in A2 among patients with seminoma and decreases in B8 and B17 among patients with nonseminomatous tumors. In contrast, DeWolf *et al.* (3) failed to note any significant deviations in HLA-A or -B antigen frequencies among subgroups of 61 patients with germ cell tumors of the testis although they did report a significant increase in the mixed lymphocyte culture-defined HLA-D antigen HLA-Dw7 among 26 patients with teratocarcinoma. Carr and Bach (1) reported a significant increase in Aw24 among a small group of 9 patients with metastatic teratocarcinoma.

The present study fails to confirm any of the previous reported trends for HLA-A or -B with the exception of the decrease in B8 which, however, is not statistically significant even when data from the study by Majsky *et al.* (8) and our data are combined (Table 1, Footnote *d*). The increase in A3 in the present study is found among a histologically different group of patients. Since the data reported here represent larger groups of nonseminoma patients than those studied previously, we can conclude that most of those earlier results may have been artifacts of the small number of patients and the relatively large number of different HLA antigens investigated. Similarly, our own findings of significant increases in Aw33 and B5 in seminoma, A3 and B7 in embryonal carcinoma, and Aw32 in yolk sac tumors are not significant after correction for the number of antigens tested and would need to be confirmed by another study before they could be considered valid. Although data from the present study were not analyzed in relation to disease stage and thus cannot be compared with results from the study of Carr and Bach (1), it is difficult, in any case, to understand why a particular HLA antigen would be increased selectively among patients with metastatic teratocarcinoma since all patients with teratocarcinoma develop metastatic disease if they are not treated.

None of the earlier reports included DR typing results, and since our own findings of a significant overall decrease in DR3 and significant increases in DR5 and DRw6 among patients with pure seminoma lose significance after correction for the number of antigens tested, these results must await confirmation by further studies before they can be considered valid. It is important to note, in this connection, that our own finding of a significant increase in DR4 among patients with embryonal carcinoma was not confirmed in the second patient group (Table 2). Since the geographical family origins of both these New York area patient groups were essentially the same as those of the controls, this change probably reflects the fact that significant HLA-disease associations are often found purely from chance, especially in small groups, because of the relatively large number of factors tested, and indicates that caution must be used in interpreting these kinds of results. It is nevertheless interesting to comment on the trend toward a decrease in B8 and the significant decrease in DR3 found in the total patient group (Tables 1 and 2) since increases in both these antigens are found among patients with a large variety of autoimmune diseases, such as chronic active hepatitis, juvenile diabetes, and idiopathic Addison's disease (11) while striking decreases in these antigens have been found among patients with the HLA-linked disease 21-hydroxylase deficiency (5, 9). It has often been suggested that the B8 and DR3 associations with autoimmune diseases result from a hyperimmune response, and it is possible that the relatively low frequency of these antigens among the patients with germ cell tumors of the

testis reflects, in part, the ability of individuals with B8 and/or DR3 to be more immunologically resistant to these types of neoplasm. It is also possible that effects of B8- and DR3-linked factors that appear to influence clinical expression of 21-hydroxylase deficiency (9) may also affect the psychoendocrine environment as it may contribute to resistance to onset of these tumors. In this connection, it is interesting to note that 2 patients with embryonal-teratocarcinoma mixed tumors have the HLA antigen Bw47 which, perhaps as the result of a "founder effect," is found in significantly high frequency among patients with 21-hydroxylase deficiency (5). This antigen is found so infrequently among healthy individuals that it has been suggested that most of such individuals may be heterozygous carriers of the relatively common 21-OH-def allele. It seems possible, therefore, that one or both of these 2 patients may also be heterozygous for 21-hydroxylase deficiency and thereby susceptible to subclinical alterations in their 17-hydroxyprogesterone and testosterone levels. The relative absence of B8 and DR3 expression and the presence of 2 patients with Bw47 thus both suggest that HLA-linked endocrine factors may play a role in the etiology of germ cell tumors of the testis.

Although no other studies have reported DR typing data for patients with testicular tumors, the study by DeWolf *et al.* (3) reported a significant increase in Dw7 in patients with teratocarcinoma. Since the DR antigen DR7 is found in association with at least 2 other mixed lymphocyte culture-defined HLA-D antigens, Dw11 and DB1 (4), it is not possible to compare our results directly, although it might have been expected that an increase in Dw7 would be reflected in an increase in DR7. The study by DeWolf *et al.* (3) thus would require confirmation by a second study in which HLA-D typing is utilized.

The role of HLA and/or other genetic factors in the etiology of germ cell testis tumors had been suggested previously by the existence of familial case (6, 10) and by the relatively high incidence of these tumors among Caucasian males in comparison with black or Oriental males living in the same geographical areas. Although only 3 brother pairs were tested in the present study, HLA results would be consistent with the presence of a dominantly expressed HLA-linked susceptibility factor since all 3 pairs shared at least one HLA haplotype. It is also interesting that the histological types were similar in all cases. Although some of the previously studied brother pairs did not have histologically similar tumors (10), HLA data were not available for these studies. Many additional brother pairs would have to be studied, however, before the existence of such a hypothetical HLA-linked susceptibility factor could be proved. The present data suggest, however, that further studies of multiple-case families and individual patients with particular HLA antigens, such as Bw47, may provide clues to the etiology of some germ cell tumors of the testis.

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