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Tumors are believed to emerge only when immune surveillance fails. We wished to ascertain whether the failure to inherit putative protective alleles of HLA class II genes is linked to the development of breast cancer. We molecularly typed HLA DPB1, DQB1, DRB1 and DRB3 alleles in 176 Caucasian women diagnosed with early onset breast cancer, and in 215 ethnically matched controls. HLA DQB*03032 was identified in 7% of controls but in no patients with early onset breast cancer ($p = 0.0001$). HLA DRB1*11 alleles were also significantly overrepresented ($p < 0.0001$) in controls (16.3%) as compared with patients with early onset breast cancer (3.5%). HLA DQB*03032 and HLA DRB1*11 alleles may have a protective role in human breast cancer.

The above results are in press and we are currently extending this study in a larger cohort to confirm that these HLA class II alleles are indeed protective and thus determine if future approaches to the therapy of breast cancer should be based on the reintroduction of protective alleles into antigen presenting cells.

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INTRODUCTION

While it is likely that genetic susceptibility plays a role in the development of most human cancers, evidence supporting this view has hitherto been obtained in only a small fraction of patients who typically carry germline mutations in tumor suppressor genes. In addition to the widely recognized role of acquired alterations in oncogenes and tumor suppressor genes, considerable evidence exists to suggest that the immune system might play a protective role in tumorigenesis. Although immune surveillance is believed to be involved in the elimination of tumors, immunotherapeutic approaches to human cancer have by and large proved unsuccessful. In this study we have used molecular approaches to type all HLA class II DP, DQ, and DR alleles in patients who were diagnosed with breast cancer before the age of 40, and in ethnically matched controls. The goal was to identify if any specific MHC class II allele is linked either positively or negatively in this disease.

BODY

We have typed all MHC class II alleles in 186 patients (all Caucasians under the age of 40) with early onset breast cancer and 233 ethnically matched controls using a total of 133 different Single Stranded Oligonucleotide probes. Our studies have revealed two very strong negative HLA class II associations with early onset breast cancer. HLA DQB*03032 was identified in 7% of controls but in no patients with early onset breast cancer ($p = 0.0001$). HLA DRB1*11 alleles were also significantly overrepresented ($p < 0.0001$) in controls (16.3%) as compared with patients with early onset breast cancer (3.5%). Both these negative associations remained extremely significant when corrections were made for the number of comparisons. HLA DQB*03032 and HLA DRB1*11 alleles may have a protective role in human breast cancer. These results are now in press in the Proceedings of the National Academy of Sciences U.S.A. (Appendix A).

One concern in any population based study is the role of ethnicity. The vast majority of the patients and controls originally collected for study were Caucasians. A few individuals in each of these groups were of Asian, Hispanic, or African-American origin and were excluded from the analysis. The patient group contained 17 Jewish subjects and 13 Jewish controls were studied. When the data was separately analyzed excluding Jewish patients and controls, the negative associations of DQB*03032 and the DRB1*11 in breast cancer remained highly significant (DQB*03032 $p = 0.0002$; DRB1*11 $p = 0.0006$). The numbers of Jewish patients and controls was insufficient for this sub-group to be analyzed separately in a statistically meaningful manner. None of the Jewish patients or controls inherited DQB*03032. It was nonetheless intriguing that 5 out of 13 Jewish controls (38.5%) and 0 out of 17 Jewish patients (0%) inherited DRB1*11 ($p = 0.0090$).

No studies on HLA class II alleles in breast cancer have been reported to date. We chose to study early onset breast cancer based on the presumption that genetic susceptibility would be more easily revealed in this subset of patients. Our control group, included individuals of both sexes. The most power would perhaps have been

achieved by selecting a cohort of ethnically matched elderly women with no history of cancer.

KEY RESEARCH ACCOMPLISHMENTS

- Complete molecular analyses of all MHC class II genes in patients with early onset breast cancer and controls has been performed
- Two resistance alleles have been identified in women with breast cancer. These represent potential "resistance" genes for breast cancer.

REPORTABLE OUTCOMES

1. Chaudhuri S, Cariappa A, Tang M, Bell D, Haber DA, Isselbacher KJ, Finkelstein D, Forcione D, Pillai S. 2000. Genetic susceptibility to breast cancer: HLA DQB*03032 and HLA DRB1*11 may represent protective alleles. Proc. Natl. Acad. Sci. USA. 2000 in press.

2. PROTECTIVE HLA CLASS II ALLELES IN THE PROGNOSIS AND THERAPY OF HUMAN CANCER. Pillai S, Cariappa A, Forcione D. (Patent applied for).

CONCLUSIONS

We conclude that there is an extremely significant immunogenetic component to breast cancer. Immune surveillance may be of critical importance in the genesis of breast cancer in young women. There are obvious and very important therapeutic implications which will be considered more fully if we are able to verify and fully establish our current findings.

REFERENCES

Chaudhuri S, Cariappa A, Tang M, Bell D, Haber DA, Isselbacher KJ, Finkelstein D, Forcione D, Pillai S. 2000. Genetic susceptibility to breast cancer: HLA DQB*03032 and HLA DRB1*11 may represent protective alleles. Proc. Natl. Acad. Sci. USA. 2000 in press.

Genetic susceptibility to breast cancer: HLA DQB*03032 and HLA DRB1*11 may represent protective alleles

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Contributed by Kurt J. Isselbacher, August 2, 2000

Tumors are believed to emerge only when immune surveillance fails. We wished to ascertain whether the failure to inherit putative protective alleles of HLA class II genes is linked to the development of breast cancer. We molecularly typed HLA DPB1, DQB1, DRB1, and DRB3 alleles in 176 Caucasian women diagnosed with early-onset breast cancer and in 215 ethnically matched controls. HLA DQB*03032 was identified in 7% of controls but in no patients with early-onset breast cancer ($P = 0.0001$). HLA DRB1*11 alleles were also significantly overrepresented ($P < 0.0001$) in controls (16.3%) as compared with patients with early-onset breast cancer (3.5%). HLA DQB*03032 and HLA DRB1*11 alleles may have a protective role in human breast cancer.

may have the potential to contribute genetically derived insights regarding the role of immune surveillance in cancer.

Methods

Patients. One hundred and eighty-six consecutive women with breast cancer diagnosed before the age of 40 at hospitals in Boston were included in the study (13). Information regarding ethnicity was obtained from each patient. Results from one hundred and seventy-six Caucasian patients are included in this report.

Controls. Two hundred and fifteen healthy Caucasians were included as controls. Ninety-three of these controls have been described earlier (14). The remainder included healthy volunteer blood donors at Massachusetts General Hospital. Information on ethnicity also was obtained from controls.

HLA Class II Typing. Genomic DNA was obtained from lymphoblastoid cell lines derived individually for each subject or from peripheral blood. Genotyping of DRB, DPB1, and DQB1 alleles was performed by using a PCR-SSO technique according to the protocols described in the 11th and 12th International HLA Workshops (15, 16). Details of the PCR and DNA hybridization conditions have been published earlier (14, 17). Briefly, PCRs were performed in a total volume of 200 μ l and included 100 ng of genomic DNA and a reaction mixture [200 pM of each primer, 0.2 mM of each deoxynucleotide triphosphate, 2 mM MgCl₂, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 0.001% (wt/vol) gelatin, and 2.5 units of *Taq* DNA polymerase (Fisher)]. Samples were denatured at 96°C for 6 min followed by 40 cycles of amplification. The annealing temperature for the generic DRB1 alleles was 55°C and 60°C, respectively, for DRB1 and DRB3 group-specific amplifications, 60°C for DP, and 55°C for DQ. After confirmation of the PCR products on a 1.5% agarose gel, up to 5 μ l of the product was spotted on nylon membrane filters. The filters were prehybridized overnight at 54°C in a buffer containing 3 M tetramethylammonium chloride, 50 mM Tris-HCl (pH 8.0), and 2 mM EDTA/5 \times Denhardt's solution/0.1% SDS/100 μ g/ml of salmon sperm DNA. SSO probes were 5' end-labeled by using [γ -³²P]ATP and T4 polynucleotide kinase. Hybridization was carried out at 54°C for 2 h. The filters were washed twice at room temperature for 15 min each in a solution containing 2 \times SSPE [standard saline phosphate/EDTA (0.18 M NaCl/10 mM phosphate, pH 7.4/1 mM EDTA)] and 0.1% SDS followed by three washes for 10 min at 58°C in a buffer containing 3 M

Although it is likely that genetic susceptibility plays a role in the development of most human cancers, evidence supporting this view hitherto has been obtained in only a small fraction of patients who typically carry germ-line mutations in tumor suppressor genes. In addition to the widely recognized role of acquired alterations in oncogenes and tumor suppressor genes, considerable evidence exists to suggest that the immune system might play a protective role in tumorigenesis. Although immune surveillance is believed to be involved in the elimination of tumors (1, 2), immunotherapeutic approaches to human cancer by and large have proved unsuccessful.

T cell responses are dependent on the inheritance of specific alleles of the highly polymorphic HLA class I and class II genes. Although weak associations of specific HLA alleles with tumors of viral origin have been described (3–10), the relevance of MHC polymorphisms to the broader category of spontaneous nonviral human tumors remains to be established. Somatic alterations in many tumors can contribute to the down-regulation of HLA class I gene expression in tumor cells (11). These alterations potentially could contribute to immune evasion and might represent a discrete event in the multistep paradigm of tumorigenesis. Although HLA class I genes are expressed in all cells, immune responses also require the presentation of antigenic peptides to T cells by HLA class II molecules. These heterodimers primarily are expressed by professional antigen-presenting cells such as macrophages, dendritic cells, and B lymphocytes. Somatic alterations in tumor cells cannot influence the expression of HLA class II genes in dendritic cells and other professional antigen-presenting cells. If, indeed, immune surveillance is important during tumorigenesis, certain individuals who inherit specific alleles of the highly polymorphic HLA class II DPB, DQB, or DRB genes might be resistant to specific types of cancers.

We reasoned that a detailed molecular analysis of HLA DPB, DQB, and DRB alleles in patients with breast cancer and ethnically matched controls might provide information on the potential existence of alleles that could confer susceptibility or resistance to this human cancer. Women with early-onset breast cancer (diagnosed at or before the age of 40) constitute a subset of the population at increased risk for genetic predisposition (12) and, hence, were chosen to test this hypothesis. Such an approach

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Table 1. DPB1 alleles in controls and breast cancer

Allele	Controls (n = 207)	Breast cancer (n = 157)	P value*
DPB1*0101	8.2% (17)	5.1% (8)	0.2981
DPB1*0201	16.4% (34)	22.3% (35)	0.1776
DPB1*0202	1.4% (3)	0.6% (1)	0.6371
DPB1*0301	17.4% (36)	17.2% (27)	1.0000
DPB1*0401	52.2% (108)	36.9% (58)	0.0042
DPB1*0402	25.6% (53)	21.0% (33)	0.3218
DPB1*0501	1.9% (4)	4.5% (7)	0.2184
DPB1*0601	1.0% (2)	4.5% (7)	0.0429
DPB1*0801	1.9% (4)	1.9% (3)	1.0000
DPB1*0901	0.0% (0)	1.3% (2)	0.1854
DPB1*1001	6.3% (13)	1.3% (2)	0.0172
DPB1*1101	1.9% (4)	3.2% (5)	0.5077
DPB1*1301	4.3% (9)	0.6% (1)	0.0479
DPB1*1401	3.4% (7)	2.5% (4)	0.7632
DPB1*1501	1.4% (3)	2.5% (4)	0.4704
DPB1*1601	1.9% (4)	1.3% (2)	0.7027?
DPB1*1701	1.9% (4)	3.8% (6)	0.3388
DPB1*1801	0.5% (1)	2.5% (4)	0.1700
DPB1*1901	0.5% (1)	0.6% (1)	1.0000
DPB1*2001	4.8% (10)	5.7% (9)	0.8130
DPB1*2201	0.0% (0)	0.6% (1)	0.4313
DPB1*2301	23.7% (49)	19.1% (30)	0.3077
DPB1*2401	1.9% (4)	1.9% (3)	1.0000
DPB1*2501	1.4% (3)	5.7% (9)	0.0350
DPB1*2601	1.0% (2)	3.2% (5)	0.1459
DPB1*2701	2.9% (6)	1.9% (3)	0.7374
DPB1*2801	0.0% (0)	0.6% (1)	0.4313
DPB1*2901	1.4% (3)	5.1% (8)	0.0620
DPB1*3101	1.9% (4)	0.0% (0)	0.1371
DPB1*3201	1.9% (4)	7.0% (11)	0.0297
DPB1*3301	0.5% (1)	5.1% (8)	0.0061
DPB1*3401	0.0% (0)	0.6% (1)	0.4313
DPB1*3501	1.4% (3)	1.3% (2)	1.0000

*Uncorrected two-tailed P value (Fisher's Exact Test).

tetramethylammonium chloride, 50 mM Tris-HCl, 2 mM EDTA, and 0.1% SDS. Each filter was exposed twice for autoradiography, once for 1-2 h, and subsequently for 14-16 h. Reactivity was graded visually, using a scale recommended by the 11th International HLA Workshop (15). Controls included previously typed samples.

Generic typing of DRB1 and DRB3 loci was performed by using a single pair of generic primers for PCR amplification followed by hybridization of filters with 29 different group-identifying SSOs. For group-specific DRB typing, genomic DNA was amplified with five different 5' primers specific for DRB1-DR1, DRB1-DR2, DRB1-DR4, the DRB1-DR52-associated group and the DRB3-DR52 group. The 3' primer in the above cases was the same as that used for generic DRB typing. A total of 50 different SSOs were used in the group-specific typing studies. Five SSOs were used for DRB1-DR1 (DRB1*0101-03), 12 were used for DRB1-DR2 (DRB1*1501-03, DRB1*1601-02), 9 were used for DRB1-DR4 (DRB1*0401-0411), 12 were used for the DRB1-DR52-associated group (DRB1*0301/02, DRB1*0801-0804, DRB1*1101-04, DRB1*1201/02, DRB1*1301-05, DRB1*1401-08), and 12 were used for the DRB3-DR52 group (DRB3*0101, DRB3*0201/0202 and DRB3*0301).

Generic primers were used for PCR amplification of DPB1 and DQB1 loci. Twenty-five SSOs were used to type 36 DPB1 alleles, and 20 SSOs were used for 17 DQB1 alleles. The use of multiple oligonucleotide probes facilitated definitive identification of negative and positive alleles.

Validation. Selected PCR products were subcloned (five sub-clones were picked after transformation with DNA from each ligation mixture), and individual clones were sequenced to confirm the validity of allele assignment by the PCR/SSO typing approach.

Statistical Analysis. Two-tailed uncorrected P values were reported by using Fisher's exact test for the analyses of HLA class II allele frequencies. The relative risk was calculated as an odds ratio by using the approximation of Woolf (18). P values were corrected for the number of comparisons essentially by using a modified Bonferroni correction as suggested by Sveigaard and Ryder (19). The number of alleles assayed from a specific PCR amplification reaction was used as the basis for the number of comparisons made. Alleles for which the combined frequency in patients and controls was less than 1 were not included in the number of comparisons. In the case of the DPB1 locus, the number of comparisons made was 33. Thus, the nominal level for comparison was $P = 0.0015$. In the case of the DQB1 locus, the number of comparisons made was 16 and the nominal level for comparison was $P = 0.0031$. In the case of the DRB1 locus, the number of comparisons was 31 and the nominal level for comparison was $P = 0.0016$. In the case of the DRB3 locus, the number of comparisons was 3 and the nominal level for comparison was $P = 0.0166$.

Results

We noted two strong, negative associations of HLA class II alleles with early-onset breast cancer, one with a DQB1 allele and another with a set of DRB1 alleles. A weaker, positive association was noted with a single DRB3 allele.

No strong negative or positive associations were noted for any DPB1 alleles in patients with breast cancer (Table 1). A weak negative association was seen for DPB1*0401 ($P = 0.0042$; corrected $P = 0.1386$, not significant), and a weak positive association was noted for DPB1*3301 ($P = 0.0061$; corrected $P = 0.2013$, not significant).

However, we found that 14 of 199 controls but none of 176 patients with breast cancer inherited the DQB*03032 allele ($P = 0.0001$) (Table 2). The relative risk was 0.0358. The corrected P value is 0.0016, which remains highly significant. No other DQB alleles were significantly over- or underrepresented in the control group.

Table 2. DQB1 alleles in controls and breast cancer

Allele	Controls (n = 199)	Breast cancer (n = 176)	P value*
DQB1*0201	30.7% (61)	39.8% (70)	0.0663
DQB1*0301	40.2% (80)	36.4% (64)	0.4584
DQB1*0302	28.1% (56)	20.5% (36)	0.0930
DQB1*03032	7.0% (14)	0.0% (0)	0.0001*
DQB1*0305	1.0% (2)	0.6% (1)	1.0000
DQB1*0401	0.5% (1)	3.4% (6)	0.0545
DQB1*0402	4.5% (9)	1.1% (2)	0.0670
DQB1*0501	17.1% (34)	21.6% (38)	0.2946
DQB1*0502	3.5% (7)	4.0% (7)	1.0000
DQB1*05031	6.5% (13)	3.4% (6)	0.2381
DQB1*05032	0.5% (1)	0.0% (0)	1.0000
DQB1*0504	0.0% (0)	0.0% (0)	—
DQB1*0601	2.0% (4)	5.7% (10)	0.0986
DQB1*0602	26.6% (53)	22.7% (40)	0.4035
DQB1*0603	10.0% (20)	10.2% (18)	1.0000
DQB1*0604	4.5% (9)	3.4% (6)	0.6105
DQB1*0605	1.0% (2)	4.5% (8)	0.0506

*Uncorrected two-tailed P value (Fisher's Exact Test).

*Significant after correction; nominal value for comparison, $P \leq 0.0031$.

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Table 3. DRB1 alleles in controls and breast cancer

Allele	Controls (n = 215)	Breast cancer (n = 173)	P value [†]
DR1			
*0101	11.6% (25)	13.3% (23)	0.6442
*0102	4.2% (9)	5.8% (10)	0.4875
*0103	2.8% (6)	5.8% (10)	0.1984
DR2			
*1501	27.9% (60)	20.2% (35)	0.0963
*1502	0.9% (2)	1.7% (3)	0.6597
*1503	0.9% (2)	0.6% (1)	1.0000
*1601	2.8% (6)	2.9% (5)	1.0000
*1602	1.4% (3)	1.2% (2)	1.0000
DR3			
*0301-02	20.5% (44)	30.1% (52)	0.0333
DR4			
*0401	6.5% (14)	4.0% (7)	0.3684
*0402	5.6% (12)	4.6% (8)	0.8183
*0403	1.4% (3)	0.0% (0)	0.2569
*0404	10.7% (23)	11.6% (20)	0.8711
*0405	1.9% (4)	0.6% (1)	0.3865
*0407	2.3% (5)	1.2% (2)	0.4682
*0408	7.4% (16)	6.4% (11)	0.8413
*0409	0.0% (0)	0.0% (0)	—
*0410	0.0% (0)	0.0% (0)	—
*0411	0.0% (0)	0.0% (0)	—
DR7			
*0701	19.5% (42)	24.9% (43)	0.2189
DR8			
*0801-04	4.2% (9)	6.4% (11)	0.3632
DR9			
*0901A/B	2.8% (6)	0.6% (1)	0.1370
DR10			
*1001	0.9% (2)	1.2% (2)	1.0000
DR11			
1101-04	16.3% (35)	3.5% (6)	<0.0001
DR12			
*1201-02	3.2% (7)	5.2% (9)	0.4425
DR13			
*1301	5.1% (11)	0.6% (1)	0.0147
*1302	10.2% (22)	12.1% (21)	0.6263
*1303	0.9% (2)	4.6% (8)	0.0268
*1304	0.0% (0)	0.0% (0)	—
*1305	0.0% (0)	0.6% (1)	0.4459
DR14			
*1401	5.1% (11)	1.2% (2)	0.0441
*1402	0.0% (0)	0.6% (1)	0.4459
*1403	6.5% (14)	7.5% (13)	0.6944
*1404	1.9% (4)	1.2% (2)	0.6961
*1405	2.8% (6)	5.8% (10)	0.1984
*1406	0.0% (0)	0.0% (0)	—
*1407	0.0% (0)	0.0% (0)	—
*1408	0.0% (0)	0.0% (0)	—

[†]Uncorrected two-tailed P value (Fisher's Exact Test).
^{*}Significant after correction; nominal value for comparison, $P \leq 0.0016$.

The only allele or group of alleles at the DRB1 locus that was significantly different between patients and controls was DRB1*11, which was found in 35 controls but in only 6 patients with breast cancer ($P < 0.0001$) (Table 3). The relative risk was 0.1846. The corrected P value for this negative association is < 0.0030 , which is also highly significant. At least 34 different DRB1*11 alleles have been described in recent years, some of which remain to be confirmed (20). The frequencies with which many of these newer alleles are seen in Caucasians has not been established, and it is likely that many of them will be extremely rare. This group of alleles deserves extremely thorough scrutiny in future studies.

DRB1*11 alleles are not in linkage disequilibrium with DQB*03032. In Caucasians, DQB*03032 is in very weak linkage disequilibrium with DRB1*0701, DRB1*0901, and DRB1*1602. It is clear from Table 3 that the negative association with DQB*03032 does not represent linkage disequilibrium with a known DRB1 gene.

More than half the patients with breast cancer (94 of a total of 171, 55%) and a substantial but smaller proportion of the controls (85 of 208, 40.9%) inherited DRB3*02 (Table 4). The P value for this positive association was 0.0072. The corrected P value was 0.0216, which remains significant.

T3

T4

Table 4. DRB3 alleles in controls and breast cancer

Allele	Controls (n = 208)	Breast cancer (n = 171)	P value [†]
DRB3			
*0101	24.5% (51)	25.1% (43)	0.9053
*0201/*0202	40.9% (85)	55.0% (94)	0.0072*
*0301	7.7% (16)	9.4% (16)	0.5823

[†]Uncorrected two-tailed P value (Fisher's Exact Test).
^{*}Significant after correction; nominal value for comparison, $P \leq 0.0166$.

One concern in any population-based study is the role of ethnicity. The vast majority of the patients and controls originally collected for study were Caucasians. A few individuals in each of these groups were of Asian, Hispanic, or African American origin and were excluded from the study. The patient group contained 17 Jewish subjects, and 13 Jewish controls were studied. When the data were analyzed separately excluding Jewish patients and controls, the negative associations of DQB*03032 and the DRB1*11 in breast cancer remained highly significant (DQB*03032, $P = 0.0002$; DRB1*11, $P = 0.0006$). The numbers of Jewish patients and controls were insufficient for this subgroup to be analyzed separately in a statistically meaningful manner. None of the Jewish patients or controls inherited DQB*03032. It was nonetheless intriguing that 5 of 13 Jewish controls (38.5%) and 0 of 17 Jewish patients (0%) inherited DRB1*11 ($P = 0.0090$).

Discussion

It has long been recognized that genetic susceptibility to cancer in part may be due to inherited variations in MHC genes. Inheritance of specific MHC class II genes may promote the generation of specific T cell help for the elimination of pathogens and, thus, may be correlated with resistance to tumors, particularly those linked to viral etiologies. An example of such an association is observed in the regression of cottontail rabbit papilloma virus-induced warts in rabbits that inherit a particular MHC class II DQ α restriction fragment length polymorphism (21).

We wanted to perform a study with sufficient power (in terms of numbers of subjects and the range of alleles examined) to ascertain whether protective HLA class II alleles could be identified in the context of a human cancer of presumed nonviral origin. Such alleles, if they exist, theoretically would fit the definition of being dominant tumor suppressor genes (10). No studies on HLA class II alleles in breast cancer have been reported to date. We chose to study early-onset breast cancer based on the presumption that genetic susceptibility would be revealed more easily in this subset of patients. Our control group included individuals of both sexes. The most power perhaps would have been achieved by selecting a cohort of ethnically matched elderly women with no history of cancer.

Our studies suggest that DQB*03032 and DRB1*11 alleles may represent resistance alleles for early-onset breast cancer. It is theoretically possible that DQB*03032 is in linkage disequilibrium with an unidentified growth-regulating gene, a polymorphic allele of which dominantly suppresses mammary tumorigenesis. If such a polymorphic-linked tumor suppressor gene exists, an allele of this gene would be expected to form an extended haplotype in conjunction with DQB*03032 and a specific linked DRB1 allele. The failure to note a negative association in breast cancer with any one of the three DRB1 alleles known to be in linkage disequilibrium with DQB*03032 in Caucasians argues against the theoretical possibility that DQB*03032 is in linkage disequilibrium with an unknown dominant tumor suppressor gene. The possible existence of such a linked dominant tumor suppressor, however, has not been ruled out.

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Although we have examined a relatively large cohort of women who developed breast cancer at or before the age of 40, clearly more extensive studies need to be conducted. It is possible that our results are meaningful only for early-onset breast cancer, and it remains to be seen whether similar negative associations will be revealed in studies on a more broadly selected group of patients. The negative association noted for DRB1*11 even in the very small subgroup of Jewish subjects is intriguing, and, clearly, a large study needs to be undertaken involving Jewish women with breast cancer and ethnically matched controls. Although, at the very outset, we suspected that negative associations of HLA class II genes might be observed in early-onset breast cancer, we had no *a priori* reason to focus on any specific allele. Our study, therefore, should be considered exploratory and requires to be confirmed by a study on a distinct set of patients and controls.

If, indeed, the negative associations described here are supported in subsequent independent studies, it would strengthen the view that yet to be identified protective mammary tumor-specific peptides lodge in the antigen-binding grooves of specific HLA class II heterodimers in resistant individuals. Peptides bound to DQB*03032 and DRB1*11 may be presented to T cells in resistant individuals. Typing of these HLA class II alleles may prove of prognostic value. The introduction of these specific alleles into hematopoietic stem cells or into dendritic cells in breast cancer patients eventually might be considered if their protective importance is confirmed.

A significant positive association was noted with DRB3*02 alleles, although these alleles were also frequently inherited by controls. The positive association of specific HLA class II alleles in any form of cancer may reflect the role of specific HLA class II molecules either in promoting chronic inflammation or in influencing the development of a hole in the T cell repertoire during thymic education. Although lymphocytic infiltration and fibrosis are seen frequently in human breast cancer, there is little

clinical evidence to suggest that breast cancer in women develops in a setting of chronic inflammation. In any individual, CD4⁺CD8⁺ double-positive thymocytes bearing T cell receptors capable of avidly recognizing self-MHC molecules are eliminated. This deletional process is important in maintaining self-tolerance but can be a two-edged sword. The presentation of self-peptides by specific breast cancer-associated HLA class II alleles may eliminate certain T cell clones that might have the potential to respond to specific tumor antigens.

The role of endocrine and genetic factors in the pathogenesis of breast cancer is widely appreciated. In recent years, considerable molecular knowledge has accrued on the genetic susceptibility to breast cancer. In a small subset of patients, germ-line mutations in tumor suppressor genes have been demonstrated. These genes include p53 (22, 23), BRCA1 (13, 24-26), and BRCA 2 (26); other susceptibility loci that remain to be characterized may also play a role.

Immune surveillance potentially could be directed against mutant self-proteins (27) or against proteins expressed in a highly tissue-specific manner in the tissue of origin of the tumor (28, 29). A third category of tumor antigen is represented by proteins that are poorly expressed during development, but are expressed at high levels in some tumors (30, 31), including breast carcinomas (32). Although immune surveillance might play a role in eliminating incipient breast malignancies, no direct evidence exists to support such a postulate. The confirmation of the existence of protective HLA class II alleles in any form of human cancer, including breast cancer, would lend genetic support to the concept of immune surveillance as a critical component involved in tumorigenesis.

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