Positive and Negative Associations of Human Leukocyte Antigen Variants with the Onset and Prognosis of Adult Glioblastoma Multiforme

Jianming Tang,1 Wenshuo Shao,2 M. Tevfik Dorak,2 Yufeng Li,1 Rei Miike,3 Elena Lobashevsky,7 John K. Wiencke,3 Margaret Wrensch,3 Richard A. Kaslow,1,2 and Charles S. Cobbs4

Departments of 1Medicine, 2Epidemiology, and 3Surgery, University of Alabama at Birmingham, Birmingham, Alabama; and 4Department of Neurological Surgery, University of California, San Francisco, California

Abstract

Associations of genetic factors with malignant gliomas have been modest. We examined the relationships of human leukocyte antigen (HLA) and related polymorphisms to glioblastoma multiforme in adult Caucasians (non–Hispanic Whites) from the San Francisco Bay area. For 155 glioblastoma multiforme patients and 157 control subjects closely matched by ethnicity, age, and gender, PCR-based techniques resolved alleles at HLA-A, -B, -C, -DRB1 loci along with short tandem repeat polymorphisms of MICA exon 5 and TNFβ. By multivariable logistic regression, B*13 and the B*07-Cw*01 haplotype were positively associated with glioblastoma multiforme (P = 0.01 and <0.001, respectively), whereas Cw*01 was the only variant showing a negative association (P = 0.05). Among glioblastoma multiforme patients, progression to death after diagnosis was slower in those with A*32 (relative hazard, 0.45; P < 0.01) and faster in those with B*55 (relative hazard, 2.27; P < 0.01). Thus, both the occurrence and the prognosis of glioblastoma multiforme could be associated with specific but different HLA genotypes. B*07 and the B*07-Cw*07 haplotype are much more common in Caucasians than other ethnic groups in the U.S., which may partially explain the higher incidence of glioblastoma multiforme in Caucasians. (Cancer Epidemiol Biomarkers Prev 2005;14(8):2040–4)

Introduction

Glioblastoma multiforme (also known as WHO II grade IV glioma) is the most common and most severe form of primary malignant brain tumor (1-3). Rare heritable syndromes and high-dose therapeutic radiation have been unequivocally established as the causes of certain glioblastoma multiformes (2, 4); other studies have suggested additional roles for immunologic factors in mediating this molecularly heterogeneous and often rapidly fatal neoplasm (5-8). Ethnic and gender differences in glioblastoma multiforme incidence are also well-documented, with age-adjusted rates of glioblastoma multiforme in the U.S. being 2.5 times higher in Whites (Caucasians) than in blacks (African-Americans). We suspected that a larger study of well-categorized glioblastoma multiforme cases and controls, when closely matched by ethnicity, age, and gender, might reveal the importance of HLA molecules in modulating immune responses to infectious agents. Indeed, specific HLA class II haplotypes (e.g., DRB1*1501-DQB1*0602) may differentially influence the risk of cervical neoplasia by directing immune response to HPV16-specific epitopes (34-36). Similarly, HLA-mediated mechanisms may also operate in the pathogenesis of glioblastoma multiforme as human cytomegalovirus infection may facilitate adult gliomas (37, 38).

Several studies have already explored associations between HLA polymorphisms and risk of malignant glioma and related diseases in small cohorts of patients and controls (39-41). HLA and microsatellite typing was first done in two cohorts (n = 42-58) of Japanese and Italian patients (39, 40). In a recent, somewhat larger, study based on molecular HLA typing in 65 glioma cases and 157 controls from Germany (41), the cases showed higher frequencies of A*25, B*18, B*27 and DRB1*15, and a lower frequency of DRB1*07 than controls (41). Differences in results between the Japanese and German studies were quite striking, but so are HLA profiles between Asians and Europeans. We suspected that a larger study of well-categorized glioblastoma multiforme cases and controls, when closely matched by sex, age, ethnicity, and geography, could clarify whether polymorphism at the HLA loci might account for any of the predilection to this neoplasm. Our observations do suggest that variations at class I loci are associated with incidence and variable prognosis of glioblastoma multiforme.

Materials and Methods

Study Subjects. Subjects for this study came from a population-based case-control study of adult glioma in the

Received 3/2/05; revised 4/26/05; accepted 5/26/05.
Grant support: Grants CA097247, CA52689, and CA097257 from the National Cancer Institute.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Note: M.T. Dorak is currently at Sir James Spence Institute of Child Health, University of Newcastle, Newcastle, NE1 4LP, United Kingdom.

Request for reprints: Richard A. Kaslow, Program in Epidemiology of Infection and Immunity, Schools of Medicine and Public Health, University of Alabama at Birmingham, 1665 University Boulevard, RPHB Room 202A, Birmingham, AL 35294-0022.

Phone: 205-934-8665; E-mail: rkaslow@uab.edu
Copyright © 2005 American Association for Cancer Research. doi:10.1158/1055-9965.EPI-05-0136
San Francisco Bay area (7, 12, 42, 43). Briefly, malignant glioma patients were eligible for the study if: (a) age 20 or older; (b) residence (at time of histologically diagnosed glioma) in Alameda, Contra Costa, Marin, San Mateo, San Francisco, or Santa Clara counties; and (c) date of diagnosis from August 1991 to April 1994 (series 1) or May 1997 to August 1999 (series 2; refs. 7, 12, 42, 43). Patients were ascertained within a median of 7 weeks of initial diagnosis using Northern California Cancer Center’s Rapid Case Ascertainment program (7, 42).

For the same study, healthy control subjects were identified and interviewed through random-digit dialing until they had the 1:1 match with the cases by age (±1), gender and ethnicity (7). From the total of 159 glioma cases reviewed by Richard Davis (Department of Pathology, University of California, San Francisco, San Francisco, CA; for series 1) and Kenneth Aldape (Department of Pathology and Brain Tumor Center, M.D. Anderson Cancer Center, Houston, TX; for series 2), 157 with WHO grade IV glioma (glioblastoma multiforme) and 157 controls best matched to cases for age and gender were available for this substudy based on two criteria: being Caucasian (self-identified as White, non-Hispanic) and having sufficient blood specimen for DNA extraction. The original research and this follow-up study conformed to human experimentation guidelines set forth by the U.S. Department of Health and Human Services. Procedures for informed consent and data analyses were approved by institutional review boards at all local and sponsoring organizations.

**Whole Genome Amplification and Molecular Genotyping.** Genomic DNA was extracted by automated, organic procedures from heparinized blood samples stored at −20°C. Phi29 DNA polymerase and random hexamers were used for whole genome amplification, following protocols recommended by the manufacturer (Amersham Biosciences, Piscataway, NJ), as described in detail elsewhere (44). A combination of PCR-based techniques, including solid-phase sequencing, PCR with sequence-specific primers and automated, reference strand-mediated conformation analyses, resolved individual alleles from the classical HLA genes HLA-A, -B, -C, and -DRB1 at chromosome 6p21.3 (44, 45). Alleles of the TNFβ microsatellite [short tandem repeat (STR) sequence] and of another STR in exon 5 of the MHC class I-like chain A (MICA) gene were resolved through PCR amplification and automated, denaturing gel electrophoresis (44).

**Statistical Analysis.** Statistical Analysis Software, version 9.0 (SAS Institute, Cary, NC) was used for descriptive statistics and comparative analyses. First, the distributions (n and %) of genetic variants (alleles and haplotypes) were tabulated by direct counting, with the numbers of chromosomes (2N) as the denominators. Overall differences in allele frequencies between glioblastoma multiforme cases and controls were assessed in global tests (6,250,000 random simulations; ref. 46); P ≤ 0.05 signified genetic heterogeneity at a given locus. Second, associations were analyzed for individual alleles found in at least 10 subjects and odds ratio (OR) for glioblastoma multiforme with 95% confidence intervals (CI) were calculated by logistic regression, using the numbers of subjects (N) as the denominators. Initial attention was paid to all genetic variants with OR >1.5 or <0.7. Those in tight linkage disequilibrium were evaluated in stratified analyses in order to separate primary from secondary contributions. A P value ≤ 0.05 was accepted as a screening threshold for inclusion in further analysis. Third, independent associations of genetic markers with glioblastoma multiforme were defined by multivariable logistic regression models including age and gender to adjust for residual differences in those variables. A backward, stepwise selection procedure generated the most parsimonious model. Factors with an adjusted (multivariable) P value ≤ 0.05 were accepted as the putative contributors or markers. Fourth, survival patterns for patients with different genotypes were compared in Kaplan-Meier plots. Estimates of hazards ratios for death and 95% CI were based on the Cox proportional hazards model. The P values shown throughout the following section were not corrected for multiple comparisons. Instead, observed associations were tested for internal and external consistencies.

**Results**

PCR-based genotyping was successful for all six target loci (HLA-A, -B, -C, -DRB1, MICA exon 5 STR, and TNFβ) in 153 (97%) out of 157 Caucasian glioblastoma multiforme cases and in 154 (98%) of 157 control subjects. Two cases (1.5%) had to be excluded for their lack of reliable genotyping results at multiple loci, whereas two more cases and three controls could be analyzed for five of the six target loci.

The 155 cases and 157 controls included in genetic association analyses had respective ages (mean ± SE) of 58.4 ± 0.9 and 58.2 ± 1.0 years and respective sex ratios (M/F) of 1.72 (98/57) and 1.62 (97/60) (P > 0.50 for both comparisons). These minor differences in age and gender were treated as covariates in all subsequent comparative analyses, especially because two glioblastoma multiforme cases were not available for such analyses.


The allelic and genotypic profiles at all six loci conformed to Hardy-Weinberg equilibrium and they also closely resembled those observed in general Caucasian populations in the U.S. (47-49) and Europe (50). Patterns of linkage disequilibrium frequently seen in Caucasians were also confirmed (data available from J. Tang): 18 common B-C haplotypes (B*07-Cw*07, B*08-Cw*07, B*14-Cw*08, B*15-Cw*03, B*18-Cw*05, B*18-Cw*07, B*27-Cw*02, B*35-Cw*04, B*37-Cw*06, B*38-Cw*12, B*40-Cw*03, B*44-Cw*05, B*44-Cw*07, B*44-Cw*16, B*49-Cw*07, B*51-Cw*15, B*55-Cw*03 and B*57-Cw*06) could be assigned. The TNFβ microsatellite [short tandem repeat (STR) sequence] and of another STR in exon 5 of the MHC class I-like chain A (MICA) gene were resolved through PCR amplification and automated, denaturing gel electrophoresis (44).

Downloaded from cebp.aacjournals.org on October 13, 2017. © 2005 American Association for Cancer Research.
multiforme cases (21.3%) compared with controls (14.6%; OR, 1.56; 95% CI, 0.88-2.53; P = 0.13); this relationship was somewhat consistent with a positive association seen in Italians.

Positive and Negative Associations of HLA Variants with the Occurrence of Adult Glioblastoma Multiforme: Univariate and Multivariable Analyses. At nominal, univariate P value of ≤ 0.05, five HLA markers showed differential distribution between glioblastoma multiforme patients and controls (Table 2). Individual variants (B*07 and B*13) positively associated with glioblastoma multiforme had univariate ORs ranging from 2.56 to 5.34. The relationship of B*07 to glioblastoma multiforme was less strong than that of the common B*07-Cw*07 haplotype (univariate OR, 2.65; 95% CI, 1.49-4.71). Analyses of B*07 without Cw*07 (very rare), Cw*07 alone (OR, 1.47; P = 0.09) and Cw*07 without B*07 (OR < 1.20; P > 0.50) confirmed the primary effect of the B*07-Cw*07 haplotype. On the other hand, it was less clear whether the B*13-Cw*06 haplotype association (OR, 4.78; P = 0.035) was more likely due to B*13 alone because only a single B*13-positive subject did not have Cw*06; however, neither Cw*06 overall (OR, 1.49; P = 0.20) nor Cw*06 without B*13 (OR < 1.20; P > 0.50) was associated with glioblastoma multiforme. Meanwhile, only Cw*01 showed a statistically significant negative association with glioblastoma multiforme (OR, 0.34; 95% CI, 0.12-0.97; Table 2). Statistical adjustments for age and gender did not alter the univariate estimates of ORs and P values in any significant way (data available from J. Tang).

In multivariable analysis, three HLA markers (B*07-Cw*07, B*13, and Cw*01) most likely accounting for the above observations were all independently associated with glioblastoma multiforme (Table 3, model 1). These associations did not change appreciably after further adjustment for age and gender (Table 3, model 2). In short, the B*07-Cw*07 haplotype and B*13 were independently associated with glioblastoma multiforme (adjusted OR, 2.78 and 7.53; 95% CI, 1.55-4.98 and 1.56-36.31 respectively), whereas Cw*01 was negatively associated (OR, 0.33; 95% CI, 0.11-0.99). Association of the B*07-Cw*07 haplotype with glioblastoma multiforme was the only one that could withstand Bonferroni correction for multiple comparisons: the P value became <0.02 when multiplied by 18 (the number of major haplotypes tested).

Survival Analyses of Glioblastoma Multiforme Cases Alone. The 155 glioblastoma multiforme patients had a median survival of 13 months (interquartile range, 8-18 months). In Kaplan-Meier analyses, 9 A*32- and 12 B*55-positive glioblastoma multiforme patients differed from those carrying other alleles in their progression to death after diagnosis (log-rank and Wilcoxon P < 0.01 for both). In a combined analyses with all other patients as the referent group (Fig. 1), the relative hazards for death and 95% CIs were 0.40 (0.20-0.79) for A*32 and 2.26 (1.23-4.15) for B*55 (P < 0.01 for both). No patient had the combination of A*32 and B*55. Statistical adjustments for residual differences in age and gender did not alter the relationships (adjusted relative hazards, 0.43; P = 0.018 for A*32 and relative hazards, 2.28; P = 0.008 for B*55). Several other variants, including B*41-Cw*17, B*51-Cw*12, B*57-Cw*07, and DRB1*14 also showed trends toward association with disease prognosis (univariate P ≤ 0.05), but in each case, either too few subjects were available to permit strong inference or the association disappeared in multivariable analyses (data not shown).

Alternative Association Analyses. Several alternative analyses were also done. First, a number of HLA-A, -B, and -DRB1 alleles resolved to four digits (e.g., B*4402, *4403, DRB1*1101, *1102, *1301, and *1302) were common enough for separate comparisons. Second, overall homozygosity at each locus or homozygosity of individual variants was compared between glioblastoma multiforme patients and controls. Third, HLA-A and HLA-B alleles were analyzed according to their known supertypes that share similarities in peptide-binding sites.
Human MHC (HLA) genes are the most polymorphic within the MHC region. The MICA gene is evaluated by counting individuals with combinations of all alleles to summarize these analyses, particularly in patients and controls stratified by gender. To explore or refine the nature and extent of multigenic influences, analyses of HLA and related variants (alleles, haplotypes) defining these loci have been presumably maintained primarily through balancing selection by a variety of mechanisms (51, 52). Fourth, HLA-DRB1 alleles were grouped into five lineages (DR1, DR51, DR52, DR53, and DR8) corresponding to local linkage disequilibrium (and gene contents) with the neighboring genes HLA-DRB3, -DRB4, and -DRB5 (27, 53). Fifth, common extended haplotypes from HLA-A to DRB1 (e.g., A*01-Cw*07-DRB1*03) were evaluated by counting individuals with combinations of all alleles known to form extended haplotypes across the HLA blocks. Sixth, pairs (diplotypes) of HLA (e.g., B*07/B*08 and B*44), MICA exon 5 (e.g., A4/A5 and A4/A9) and TNFb alleles (e.g., b1/b3 and b1/b4) were treated as unique entities for comparisons. Seventh, association analyses were done separately in patients and controls stratified by gender. To summarize these analyses, HLA-DRB1 homozygosity (identity of paternal and maternal alleles at the four-digit level) showed a trend toward a negative association with glioblastoma multiforme (OR, 0.49; 95% CI, 0.23-1.02; P = 0.06), but this relationship diminished in multivariable analysis. Among the extended haplotypes, A*03-Cw*07-B*07-DRB1*15 had a slightly higher frequency in cases (univariate OR, 1.63; 95% CI, 0.69-3.89; P = 0.27). In every other comparison, the P value was >0.30. Finally, stratified analyses did not reveal any additional gender-specific associations.

**Discussion**

Human MHC (HLA) genes are the most polymorphic within the human genome (27, 54). The large number of genotypes (alleles and haplotypes) defining these loci have been presumably maintained primarily through balancing selection by a variety of human diseases. Recent discoveries (37, 38) that human cytomegalovirus infection might play some role in the pathogenesis of adult gliomas make HLA genes highly relevant as contributors to the observed effect. First, HLA-B like locus, along with other loci centromeric to HLA-B, as likely contributors to the observed effect. In global tests, the overall distribution of HLA-B locus alleles showed statistically significant heterogeneity between our glioblastoma multiforme patients and controls, whereas statistically significant associations of individual alleles were likewise confined to those at the adjacent HLA-B and HLA-C loci. The positive association of the B*07-Cw*07 haplotype with glioblastoma multiforme was of particular interest because this association remained statistically significant after correction for the number of major haplotypes tested in our study and both B*07 and Cw*07 were also enriched in certain German patients with glioblastoma multiforme (41). Other HLA variants at loci on either side of the HLA-B to HLA-C segment, i.e., A*03, DRB1*15 and MICA exon 5 allele A5.1, which are known to form extended haplotypes with B*07-Cw*07, did not show appreciable relationships with glioblastoma multiforme, suggesting that the association of B*07-Cw*07 did not arise from apparent linkage disequilibrium with variants at the other genotyped loci.

The biological significance of the association with the B*07-Cw*07 haplotype is not obvious. Because the majority of genes in the central MHC region participate in CTL response, natural killer function, and inflammation (27), the association most likely reflects involvement of some combination of those functions. Increased presence of B*07-Cw*07 among Caucasian patients with glioblastoma multiforme compared with controls may also help explain the higher incidence in persons of European ancestry (1, 11). The higher frequency of this haplotype in Caucasians than other ethnic groups in the U.S. has been well-established (47).

The two HLA variants (A*32 and B*55) associated with the prognosis of glioblastoma multiforme were relatively uncommon in the study population. In the absence of a clear biological mechanism for the involvement of either, these modest associations should be interpreted just as cautiously as others reported in small cohorts (39-41). Among the eight markers already proposed by other investigators, only TNFb allele 4 (b4) homozgyosity showed a slight trend toward positive association with adult glioblastoma multiforme in our investigation. TNFb microsatellite alleles located in the central MHC region frequently mark common haplotypes that contain single nucleotide polymorphisms at the loci encoding tumor necrosis factor-α, lymphotoxin-α, and lymphotoxin-β. A formal evaluation of functional single nucleotide polymorphisms at these and other related loci may prove informative.

Genes in the HLA region are not the only ones of interest for glioblastoma multiforme. For example, several markers in genes participating in various pathways not immediately related to immune responses (e.g., CYP2E1, GSTP1, and GSTT1) have been implicated in malignant glioma (15, 23, 41, 55). Although differences in the application of histopathologic classification systems and selection of particular patients for genotyping may preclude direct comparison of research findings, availability of glioblastoma multiforme patients and controls with defined HLA genotypes should provide a useful foundation on which to further explore or refine the nature and extent of multigenic influences on malignant brain tumors.

**Acknowledgments**

This work was conducted as part of the Brain Specialized Programs of Research Excellence projects at UAB and UCSF. We thank Richard Davis (MD) and Kenneth Aldape (MD), for neuropathology review of series 1 and 2 cases, respectively; Joe Patoka, for DNA extraction and inventory; Michelle Meghahadi, for data management; Chengbin Wang and Aleksandr Lazaryan, for assistance with preliminary data analyses. This work was supported in part by Cancer Research.  

Cancer Epidemiology, Biomarkers & Prevention 2005;14(8). August 2005
Positive and Negative Associations of Human Leukocyte Antigen Variants with the Onset and Prognosis of Adult Glioblastoma Multiforme


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/14/8/2040

Cited articles
This article cites 53 articles, 10 of which you can access for free at:
http://cebp.aacrjournals.org/content/14/8/2040.full#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
http://cebp.aacrjournals.org/content/14/8/2040.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.