Cancer Research

Circulating Levels of the Innate and Humoral Immune Regulators CD14 and CD23 Are Associated with Adult Glioma

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Abstract

Allergy history has been consistently inversely associated with glioma risk. Two serologic markers, soluble CD23 (sCD23) and soluble CD14 (sCD14), are part of the innate and adaptive humoral immune systems and modulate allergic responses in opposite directions, with sCD23 enhancing and sCD14 blunting inflammatory responses. We measured sCD23 and sCD14 in serum from blood that was drawn at a single time point from 1,079 glioma patients postdiagnosis and 736 healthy controls. Glioma was strongly associated with high sCD14 [highest versus lowest quartile odds ratio (OR), 3.94; 95% confidence interval (95% CI), 2.98–5.21] and low sCD23 (lowest versus highest quartile OR, 2.5; 95% CI, 1.89–3.23). Results were consistent across glioma histologic types and grades, but were strongest for glioblastoma. Whereas temozolomide treatment was not associated with either sCD14 or sCD23 levels among cases, those taking dexamethasone had somewhat lower sCD23 levels than those not taking dexamethasone. However, sCD23 was associated with case status regardless of dexamethasone treatment. These results augment the long-observed association between allergies and glioma and support a role for the innate and adaptive humoral functions of the immune system, in particular immunoregulatory proteins, in gliomagenesis. *Cancer Res; 70(19); 7534–42. ©2010 AACR.*

Introduction

The etiology of adult glioma is currently unknown. Two recent genome-wide association studies identified five susceptibility regions for glioma (1, 2). These are the only definitive risk factors for glioma apart from long-established associations with rare inherited cancer syndromes and relatively high-dose ionizing radiation exposures (3). Most epidemiologic studies that addressed immune factors have reported that adults with glioma are 1.5- to 4-fold less likely than controls to report a variety of allergies (4–6), which ranks the absence of allergies among the most consistent risk factors for glioma reported to date. However, mechanisms accounting for this association have been difficult to establish. We hypothesized that allergy may reflect a state of balance of immune functions that could influence antitumor reactions. In one previous analysis, we found an inverse relationship between postdiagnosis serum IgE, an indicator of atopic immune response, and glioma (7). However, in a second study, the inverse association was only apparent in temozolomide-treated patients and the analysis suggested that the lower levels of IgE in the patients could be related to temozolomide treatment (8). Although allergy-related IgE is an evidence of adaptive humoral immune response, allergy is a complex phenotype that involves both adaptive and innate branches of the immune system. Recent studies have shown clear interactions between the innate and adaptive branches of the immune system (9, 10). Here, we focused on two candidate serologic markers of immunoregulation that have been implicated in allergic immune responses; sCD23 (serum soluble CD23), the low-affinity IgE receptor, is potentially stimulatory for atopic immunity whereas the innate immune marker, sCD14 (serum soluble CD14), may be inhibitory.

CD23 is an important mediator of the allergic response and can function to enhance antigen presentation of IgE antigen complexes (11). Soluble CD23 exhibits clear proinflammatory properties (12, 13), mediates macrophage (14, 15) or lymphocyte (16) activation, and has been shown to be concentrated in inflamed brain tissues and cerebrospinal fluid of encephalitis patients (17).

CD14 is an important component of the innate immune Toll-like receptor system and gram-negative and gram-positive bacterial pattern recognition (18, 19). The soluble form, sCD14, can bind to different B-cell subsets and enhance IgG1 while suppressing IgE production (20). sCD14 inhibits the production of interleukin (IL)-2 and IL-4, thus contributing to reduced IgE isotype switching (21). An example that sCD14 plays a part in immune tolerance (22, 23) comes from a study of farmers' children; these children, exposed to high

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levels of endotoxin and other bacterial components as infants, have reduced risk of developing allergy and show relatively elevated levels of sCD14 (24). Membrane-bound CD14 is involved in Alzheimer's disease pathology (25), and sCD14 is found in cerebrospinal fluid where it may inhibit immune activation of glial cells and neurotoxicity (26). This shows that CD14 is relevant to the central nervous system.

Given the inverse relationship of glioma with allergy history and IgE levels, the positive association of sCD23 with IgE, and the immune tolerance role of sCD14, we evaluated the hypotheses that glioma cases would have higher levels of sCD14 and lower levels of sCD23 than controls.

Materials and Methods

Study participants

Population and clinic-based subject recruitment methods for the San Francisco Bay Area Adult Glioma Study have been described in detail elsewhere (5, 7, 8). Briefly, all cases were adults age 20 years or older with newly diagnosed histologically confirmed glioma (International Classification of Disease for Oncology, morphology codes 9380–9481). Population-based cases from six Bay Area counties were ascertained using the Northern California Cancer Center's rapid case ascertainment system from May 1997 to August 1999 (series 2) and from November 2001 to September 2005 (series 3). Additionally, cases with the same eligibility criteria diagnosed between 2002 and 2006 and seen at the UCSF Neuro-oncology Clinic were eligible to participate, regardless of place of residence; these are referred to as clinic-based cases. Pathologic material was retrieved, when possible, for all resected brain cancers and reviewed and classified by one of two neuropathologists [Kenneth Aldape, M.D. Anderson, Houston, TX, and Tarik Tihan, University of California San Francisco (UCSF), San Francisco, CA]. Blood and serum samples were usually collected at the time of interview. Controls ages 20 years or older from the same residential area as the population-based cases were identified using random-digit dialing and were frequency matched to population-based cases on age, gender, and ethnicity as previously described (8). Study methods were approved by the Committee on Human Research at UCSF, San Francisco, CA.

Interview

Interview methods for subjects, cases and controls, were described previously (7). For population-based subjects, the full process lasted ~2 hours and used a structured questionnaire and show cards to facilitate recall. Allergy history data were collected in tabular form as described in detail in our earlier report (8). Extensive data were also collected about family and personal medical history, including asthma and eczema, use of prescription and nonprescription medications, demographic factors, and lifestyle factors such as cigarette smoking and diet. Clinic-based patients were asked to answer a much shorter (about 30 minutes) questionnaire, which contained key elements of the longer questionnaire. All participants who provided a blood sample were administered an additional questionnaire about current and recent medications and treatments.

Measurements of sCD14 and sCD23

A single serum sample was collected and tested for each study subject. The serologic Luminex assays were developed using a standard sandwich capture format (27, 28). Briefly, monoclonal antibodies to human sCD14 (clone 55-3, BD Pharmingen) or sCD23 (clone 138633, R&D) were coupled to carboxylated Luminex microspheres by using a two-step carbodiimide reaction. Serum samples were diluted 1:100 in diluent for sCD14 and 1:10 for sCD23; the serum diluent was a mixture of PBS, 10% (vol/vol) fetal bovine serum, and 2.5% (vol/vol) CBS-K (Millipore Corporation). The solution was then incubated at room temperature for 1 hour on a shaker. A standard curve was created by diluting known concentrations of recombinant human sCD14 (Cellsciences) or sCD23 (R&D) using the standard serum diluent. The sCD14 and sCD23 standards or participant serum samples and coupled sCD14 or sCD23 microspheres were then incubated for 2 hours at room temperature on a shaker using a 96-well filter-bottomed plate (Bio-Rad Laboratories, Inc.) and subsequently washed with the wash buffer (Bio-Rad Laboratories, Inc.). This step was followed by the addition of 25 μL of 1:1,000 diluted biotinylated anti-human sCD14 antibody (clone 3-C39, BD Pharmingen) or anti-human sCD23 antibody (clone BAF123, R&D) to each well and then incubating the mixture at room temperature for 30 minutes on a shaker. The serum solution was then washed and treated with 50 μL of streptavidin-conjugated R-phycoerythrin 1:100 diluted stock (Bio-Rad Laboratories, Inc.). After a 10-minute incubation and final wash, the microspheres were resuspended in 125 μL of assay buffer (Bio-Rad Laboratories, Inc.). The amount of sCD14 or sCD23 bound to the microspheres by this antibody sandwich technique was determined by the median fluorescence intensity (MFI) of the reporter molecule, phycoerythrin, using the Bio-plex 200 plate reader system. The MFI of the unknown serum sample was then converted into a picograms-per-milliliter value based on the known concentrations of the standard curve by using a five-parameter (5PL) regression formula. Each sample was run with a replicate. The intraclass correlation coefficient for sCD14 and sCD23 was 0.92 and 0.93, respectively. A single serum sample from a person without a brain tumor was repeated on most of the assay plates (39 of 44), and the coefficient of variation between the assays was 11.5% for sCD14 and 18.3% for sCD23. We performed standard addition experiments that yielded recovery rates of 94% for sCD14 and 104% for sCD23.

IgE measurements

IgE levels were assessed using Pharmacia Diagnostics UniCAP fluorescent "sandwich" assay as described previously (29). IgE levels were determined using serum derived from the same blood draw as used for the current sCD14/sCD23 analysis. Total IgE was determined by measuring fluorescence against the standard curve with known quantity

inputs. The intraclass correlation for replicate samples of IgE was 0.99.

Statistical methods

Statistical analyses were conducted using SAS v9 (SAS Institute). Odds ratios (OR) for glioma cases versus controls were computed using unconditional logistic regression, adjusted for age, gender, ethnicity (white/nonwhite), education (college education yes/no), and smoking history (ever/never) because these characteristics were possible confounders of IgE levels and we thought that they also might confound other serologic measures. Analyses were conducted for all gliomas and by histologic subtype (using polytomous logistic regression) and treatment, e.g., temozolomide-treated versus not treated. Associations with continuous variables were determined using standard *t* tests to compare means between groups. For the case-control comparisons, sCD14 and sCD23 values were categorized into quartiles based on the distribution among controls. In addition to analyses of the main effects of sCD23 and sCD14 on glioma, we determined whether the association between sCD23 and glioma was modified by level of sCD14 (or vice versa). To increase power for these statistical interaction analyses, sCD14 was dichotomized based on main-effect results by combining the lowest three quartiles as the reference, whereas sCD23 was dichotomized by combining the three highest quartiles as the reference group. sCD23 was "reverse" coded to reflect the hypothesized inverse relationship between sCD23 and disease status and for ease of interpretation of the statistical interaction effects. Likelihood ratio tests were used to formally assess statistical interaction in nested adjusted unconditional logistic regression models with and without the cross-product term for the grouped sCD14 and sCD23 concentrations. Total IgE was analyzed both as a log-transformed continuous variable and for comparison with earlier studies, as a categorical variable with groups defined based on clinically relevant cutpoints (IgE >100 kU/L = "elevated," 25–100 kU/L = "borderline," and <25 kU/L = "normal").

For any analyses involving allergy history, only population-based subjects were included because the shorter questionnaire used for the clinic patients yielded a much lower prevalence of ever having allergies and numbers of allergies could not be as thoroughly quantified as it could be from the long questionnaire.

Results

Study population

We selected 1,079 cases and 737 controls with sufficient amounts of serum such that we would not deplete our biorepository. Serum analysis for sCD14 failed in one case and one control, whereas, for sCD23, it failed in 15 cases and 10 controls. Therefore, a total of 1,078 cases and 736 controls were included in the CD14 analysis and 1,064 cases and 736 controls in the sCD23 analysis. Of the 1,079 cases included in this analysis, 671 completed the full questionnaire and 471 completed the abbreviated clinic questionnaire.

Cases and controls were comparable in their ethnicity, college graduate and household income distributions, and total years of education. Controls were older than cases and more likely to be female. Controls also were more likely to have smoked cigarettes compared with cases (Table 1). Glioblastomas (GBM) were the most common histologic subtype of brain tumor, followed by anaplastic astrocytomas, astrocytomas, and oligodendrogliomas (Table 1).

sCD14 and sCD23 levels and glioma case-control comparisons

The overall concentration of sCD14 was statistically significantly higher among glioma cases compared with controls (mean ± SEM: 10.3 ± 0.13 μg/mL versus 8.1 ± 0.09 μg/mL, respectively, $P < 0.01$; Table 2). Glioma was strongly associated

Table 1. Age, gender, ethnicity, education, income, smoking history, and histology of participants with sCD14 or sCD23 results, San Francisco Bay Area Adult Glioma Study (1997–2006)

	Glioma cases (*n* = 1,079)	Controls (*n* = 737)
Mean age (y) ± SEM	50.6 ± 0.4	55.6 ± 0.6
% White	83	81
% Male	60	53
% College graduate	51	52
Mean education (y) ± SEM	15.2 ± 0.1	15.4 ± 0.1
Household income (USD/y; %)	%	%
≤$29,999	17	20
$30–49,999	17	17
$50–69,999	14	16
$70–99,999	17	18
$100,000+	31	28
Missing/refused	3	1
Smoking history (%)	%	%
Never smoked	52	45
Past smoker	36	42
Current smoker	12	13
Histology (%)	%	%
GBM	57	NA
Anaplastic astrocytoma	12	NA
Astrocytoma	8	NA
Anaplastic oligodendroglioma	4	NA
Oligodendroglioma	8	NA
Oligoastrocytoma	3	NA
Ependymoma	<1	NA
Juvenile pilocytic astrocytoma	2	NA
Medulloblastoma	1	NA
Other/unknown	4	NA
Astrocytoma NOS	<1	NA

Abbreviations: NOS, not otherwise specified; NA, not available.

Table 2. Comparisons of sCD14 and sCD23 levels in glioma cases and controls, San Francisco Bay Area Adult Glioma Study (1997–2006)

Value	Case category	Cases		Controls		*t* test *P**
		n	Mean ± SEM	*n*	Mean ± SEM	
sCD14	All	1,078	10.3 ± 0.13 μg/mL	736	8.1 ± 0.09 μg/mL	<0.01
sCD23	All	1,064	2.7 ± 0.08 ng/mL	727	3.6 ± 0.13 ng/mL	<0.01

Value	Category	Quartile	*n*	%	*n*	%	OR† (95% CI)	OR *P**	Test for trend, *P*
sCD14	All	1	174	16	184	25	1.00		
		2	130	12	184	25	0.79 (0.58–1.08)	0.14	
		3	195	18	184	25	1.21 (0.90–1.63)	0.21	
		4	579	54	184	25	3.94 (2.98–5.21)	<0.01	<0.01
	GBM	1	94	15	184	25	1.00		
		2	66	11	184	25	0.71 (0.49–1.04)	0.08	
		3	110	18	184	25	1.19 (0.84–1.69)	0.33	
		4	347	56	184	25	4.02 (2.93–5.50)	<0.01	<0.01
	Non-GBM	1	80	17	184	25	1.00		
		2	64	14	184	25	0.90 (0.60–1.36)	0.61	
		3	85	18	184	25	1.21 (0.81–1.79)	0.35	
		4	232	50	184	25	3.88 (2.73–5.53)	<0.01	<0.01
sCD23	All	1	437	41	183	25	1.00		
		2	221	21	181	25	0.52 (0.40–0.68)	<0.01	
		3	232	22	182	25	0.53 (0.41–0.69)	<0.01	
		4	174	16	181	25	0.40 (0.31–0.53)	<0.01	<0.01
	GBM	1	281	46	183	25	1.00		
		2	129	21	181	25	0.47 (0.35–0.63)	<0.01	
		3	117	19	182	25	0.41 (0.30–0.56)	<0.01	
		4	82	14	181	25	0.29 (0.21–0.41)	<0.01	<0.01
	Non-GBM	1	156	34	183	25	1.00		
		2	92	20	181	25	0.62 (0.44–0.88)	<0.01	
		3	115	25	182	25	0.75 (0.54–1.05)	0.09	
		4	92	20	181	25	0.62 (0.43–0.88)	<0.01	0.02

NOTE: sCD14 quartile cutoff values were as follows: (1) quartile 1 = 1.45 to 6.49 μg/mL, (2) quartile 2 = 6.50 to 7.65 μg/mL, (3) quartile 3 = 7.66 to 9.26 μg/mL, and (4) quartile 4 = 9.27 to 37.14 μg/mL. sCD23 quartile cutoff values were as follows: (1) quartile 1 = 0.03 to 1.57 ng/mL, (2) quartile 2 = 1.58 to 2.59 ng/mL, (3) quartile 3 = 2.61 to 4.41 ng/mL, and (4) quartile 4 = 4.43 to 38.72 ng/mL.
**t* test comparing sCD14/sCD23 means between cases and controls.
†ORs were adjusted for age (continuous), race (white/nonwhite), gender, education (college versus no college), and smoking (ever versus never). ORs for the GBM and non-GBM groups were estimated using polytomous logistic regression models.

with high sCD14 [highest versus lowest quartile OR, 3.94; 95% confidence interval (95% CI), 2.98–5.21]. Results were consistent for both GBMs and other glioma histologies (Table 2).

In contrast, the mean serum sCD23 was lower for cases (2.7 ± 0.08 ng/mL) compared with controls (3.6 ± 0.13 ng/mL; $P < 0.01$). Glioma was strongly associated with low sCD23 (lowest versus highest quartile OR, 2.5; 95% CI, 1.89–3.23). Although a similar pattern in ORs was observed for GBM and cases with other glioma histologies, the ORs were further from the null for those with GBM (Table 2).

Stratification of sCD14 and sCD23 levels by histologic diagnosis show that, irrespective of subtype, levels are different for cases and controls, although differences were most pronounced for GBM (Supplementary Table S1). The median number of days between diagnosis and blood draw for cases was 89 days. Among cases, time between diagnosis and blood draw was weakly negatively correlated with sCD14 concentration (Spearman $r^2 = -0.08$, $P < 0.01$), whereas no correlation was observed with sCD23 concentration and time between diagnosis and blood draw (Supplementary Table S2). We also did not find any statistically significant association between smoking history and sCD14 or sCD23 levels.

Temozolomide treatment and sCD14 and sCD23 levels

Among the GBM cases diagnosed in 2001 or later, 406 of the 505 patients received temozolomide treatment. There was no

significant difference of sCD14 levels in the temozolomide-treated group compared with the non–temozolomide-treated group (P = 0.6), with similar results in non-GBM patients (P = 0.2). In stratified analyses, there also was no evidence that history of temozolomide use altered the association of sCD14 and sCD23 with glioma (Supplementary Table S3). Glioma was associated with higher sCD14 quartiles in both groups (Supplementary Tables S3 and S4). Similarly, temozolomide not associated with sCD23 levels in glioma patients (Supplementary Tables S3 and S4). Temozolomide treatment schedules are typically cyclical—5 days on treatment followed by 23 days off. We assessed whether sCD14 or sCD32 levels were related to time since last dose of chemotherapy treatment among the 97 patients who were currently within a 28 day temozolomide cycle and found no correlation (data not shown).

Dexamethasone treatment and sCD14 and sCD23 levels

There was no difference in means or quartile distributions of sCD14 levels for GBM patients who reported taking dexamethasone at the time of blood draw versus those who did not (Supplementary Tables S5 and S6). In contrast, sCD23 levels in patients who reported dexamethasone use at the time of blood draw were statistically significantly lower than in patients who did not (Supplementary Table 5). Although dexamethasone status did not change the pattern of association between sCD23 and glioma (see Fig. 1), the case-control OR was lower for patients reporting dexamethasone use versus those who were not using dexamethasone (Supplementary Table S6; dexamethosone: OR, 0.25; 95% CI, 0.17–0.36 versus non-dexamethasone: OR, 0.53; 95% CI, 0.39–0.73). Apart from dexamethasone, there were no significant associations of sCD14 or sCD23 with other medications (Supplementary Tables S7a and b).

sCD14, sCD23, and IgE levels and allergy history

There were no statistically significant associations between sCD14 or sCD23 levels and IgE in controls (Supplementary Table S8). sCD23 levels in cases were lower among those with elevated IgE but not among controls. sCD14 concentration was not related to number of allergies, allergy type, or age at first allergy in either cases or controls (Supplementary Table S9a). However, both cases and controls who reported any allergies had higher sCD23 levels than those with no allergies, although the association was not statistically significant (P > 0.05; Supplementary Table S9b).

Joint association of sCD14 and sCD23 with glioma

The joint associations of sCD14 and sCD23 by histopathologic subtype and recent dexamethasone use are presented in Table 3. Results among all cases suggested that the association between sCD23 and GBM was modified by sCD14 level. Additional analyses conducted to assess the potential immune-related effect of dexamethasone on this relationship showed that the suggested statistical interaction between sCD14 and sCD23 in GBMs was limited to GBM patients

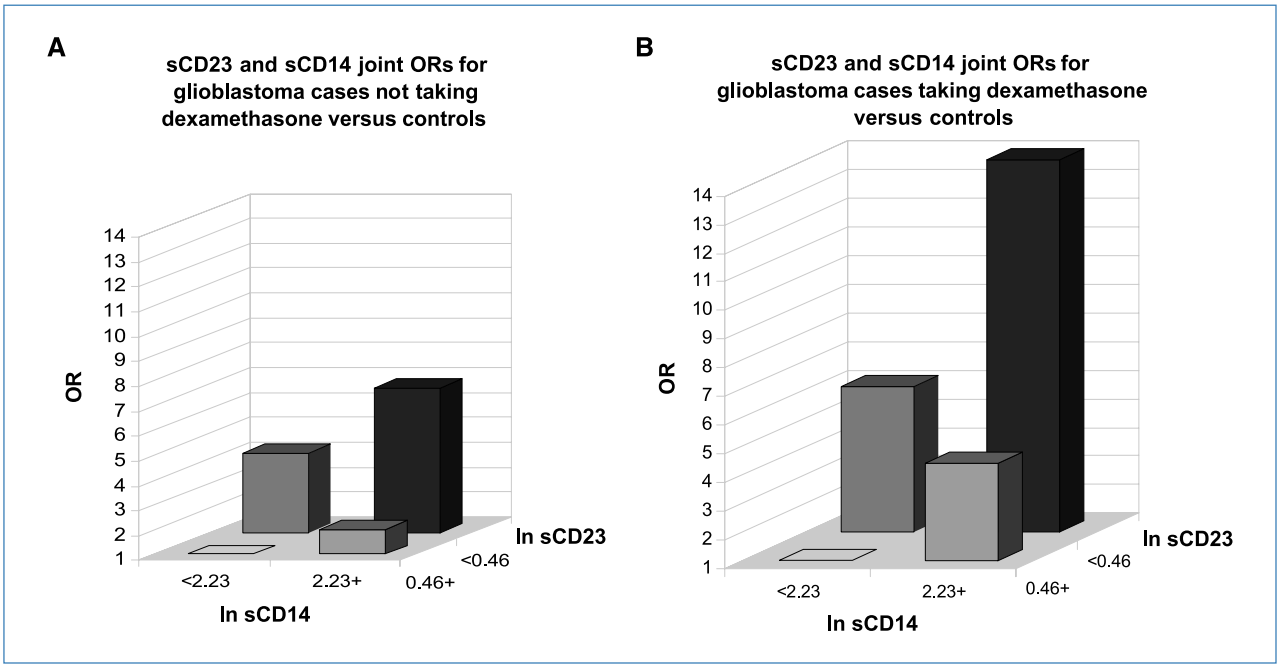

Figure 1. Analysis of joint OR of sCD23 and sCD14 in GBM patients taking or not taking dexamethasone. The patients were stratified into four groups by ln sCD14 and ln sCD23 values (ln sCD14 <2.23 or >2.23, ln sCD23 <0.46 or >0.46). A, in GBM patients who were not taking dexamethasone at the time of blood draw, the OR in the group with high sCD14 and low sCD23 was >6.8-fold higher than the group with the low sCD14 and high sCD23 values. B, in GBM patients who were taking dexamethosone, the OR in the high sCD14 and low sCD23 was >14-fold higher than those with the low sCD14 and high sCD23 values.

Table 3. Multiplicative interaction results, combined sCD14 and sCD23 levels (dichotomous groupings), stratified by histologic type and dexamethasone use, San Francisco Bay Area Adult Glioma Study participants (1997–2006)

Case grouping	Dichotomous grouping*	No.		OR† (95% CI)	Multiplicative *P*
		Cases	Controls		
All cases	Low sCD14/high sCD23	276	416	1.00	
	Low sCD14/low sCD23	221	133	2.6 (2.0–3.4)	
	High sCD14/high sCD23	344	126	4.7 (3.6–6.2)	
	High sCD14/low sCD23	222	51	7.2 (5.1–10.3)	0.03
Cases taking dexamethasone	Low sCD14/high sCD23	87	416	1.00	
	Low sCD14/low sCD23	113	133	4.1 (2.9–5.8)	
	High sCD14/high sCD23	136	126	5.8 (4.1–8.1)	
	High sCD14/low sCD23	115	51	11.8 (7.8–17.8)	0.01
Cases not taking dexamethasone	Low sCD14/high sCD23	189	416	1.00	
	Low sCD14/low sCD23	108	133	1.8 (1.3–2.5)	
	High sCD14/high sCD23	208	126	4.3 (3.2–5.7)	
	High sCD14/low sCD23	107	51	5.0 (3.4–7.4)	0.10
GBM cases	Low sCD14/high sCD23	134	416	1.00	
	Low sCD14/low sCD23	134	133	3.2 (2.3–4.3)	
	High sCD14/high sCD23	189	126	5.1 (3.7–6.9)	
	High sCD14/low sCD23	151	51	9.8 (6.7–14.3)	0.05
Non-GBM cases	Low sCD14/high sCD23	142	416	1.00	
	Low sCD14/low sCD23	86	133	1.9 (1.4–2.8)	
	High sCD14/high sCD23	155	126	4.5 (3.3–6.3)	
	High sCD14/low sCD23	71	51	4.7 (3.1–7.3)	0.04

NOTE: The stratified results were estimated using polytomous regression models.

*The cutoff point for dichotomizing the concentration of sCD14 was 9.30 μg/mL and for sCD23 was 1.59 ng/mL.

†ORs were estimated in logistic regression models adjusted for age (continuous), gender, race (white/nonwhite), education (college versus no college), and smoking (ever versus never).

who had recently used dexamethasone (Table 3; Fig. 1). Results also suggested statistical interaction between sCD23 and sCD14 among non-GBM patients. Similar to GBM, it seems that dexamethasone use may explain the observed associations (data not shown). Small numbers of exposed patients prohibited our ability to adequately assess the effect of dexamethasone use on the relationship between sCD14 and sCD23 and rarer histologic subtypes. The apparent interaction was thus simply an effect of dexamethasone on sCD23 and not sCD14 and therefore not a true interaction.

Discussion

Immunologic factors have long been hypothesized to play a role in glioma risk, and secretion of immunosuppressant molecules by high-grade gliomas themselves is well documented (30). Despite this, there are few serum biomarkers to evaluate the etiologic or prognostic immune aspects of this disease. Here, we present the first analysis of the immune regulatory proteins sCD23 and sCD14 in glioma patients and controls showing that sCD14 levels were higher and sCD23 levels were lower in glioma cases compared with controls.

First, we discuss sCD14 levels, which are higher in glioma cases than controls. Because measurements of sCD14 are taken after glioma diagnosis, we need to consider whether sCD14 levels were affected by the brain tumor or treatment. In these data, there were no differences among glioma patients for sCD14 levels by medications, radiation, or extent of resection. Although it is not easy to track the details of temozolomide administration in this study, there was no evidence that temozolomide exposure altered the association of sCD14 or sCD23 with glioma risk. Temozolomide may induce lymphopenia and affect patients' levels of white blood cell subsets (31); however, IgE levels were not altered during temozolomide treatment schedule (8). Similarly, we found no relationship of sCD14 or sCD23 with timing of blood draw within the temozolomide treatment schedule. sCD14 levels were also not associated with a history of allergies in either cases or controls. Alternatively, high sCD14 may reflect an individual's high level of current bacterial antigen exposure or potentially a greater sensitivity to this exposure (32). We had no record of infections within our case population but note that self-reported use of antibiotics was not associated with sCD14. In addition, a modest increase in sCD14 with

increasing age was observed in our controls but not in cases; age was included in all models assessing the association of sCD14 with glioma and hence was unlikely to have influenced case-control associations. It is possible that sCD14 levels reflect an individual's "set point" for the capacity of the innate immune system to recognize foreign or pathologic molecular patterns independent of allergy, as has been suggested in other studies. Several studies have shown that sCD14 levels are modified in the first years of life by exposure to microbial antigens (24, 33), leading to an adaptive and developmentally normal immune system that may establish a permanent physiologic sCD14 set point. Because this set point may persist later in adult life, much as other immune factors do, it could represent a brain tumor–susceptible phenotype or the effect of a tumor itself. These hypotheses will have to be tested in cohort studies. It has been suggested that sCD14 plays an immunomodulatory role in the normal central nervous system and seems to inhibit glial cell activation by interfering with lipopolysaccharide effects (26). In malignant brain tumors, CD14 accumulates in GBM but not among the lower-grade astrocytomas (34). This indicates that CD14 may modulate immunologic reactions in these tumors, contributing to their grim prognosis (34). The increased levels of sCD14 in brain tumor patients could be generated either by shedding the glycophosphatidylinositol (GPI) anchor from previously membranous CD14 or by increased CD14 transcription without the GPI anchor attachment (35); our data were unable to distinguish the source of sCD14. It is also possible that brain tumors themselves increase monocyte/macrophage activity locally with concomitant expression of CD14 and sCD14.

Although no known immunologic mechanism links sCD14 with gliomagenesis, sCD14 has the capacity to negatively affect T-lymphocyte activation and function by interacting directly with activated T cells. sCD14 inhibits T-cell proliferation and the production of IL-2 and IFN-γ (36). In addition, sCD14 is known to suppress B-cell development, leading to reduced IgE production. An antitumor role for IgE has been proposed for solid tumors (37). Given that sCD14 has been shown to suppress both T-cell and B-cell functions, a chronic high level of sCD14 may predispose to a permissive immune reaction with respect to glioma formation and participate in the known immunosuppressive serologic factors that promote T-cell and immune anergy in the brain, including transforming growth factor-β, IL-13 decoy receptor (IL$R\alpha$2), and prostaglandin E_2. Although disease or treatment-related factors might possibly affect sCD14 levels, neither temozolomide nor dexamethasone treatment seemed to influence sCD14 levels in this study. We observed a weak negative correlation between the time since diagnosis and blood draw. This modest effect would in fact lead to an underestimation of sCD14 in some cases and a lower OR of association with glioma.

Second, we consider sCD23 levels in relation to glioma. We found that the concentration of serum sCD23 was lower in glioma cases compared with controls. CD23 plays a critical role during immune response, including IgE synthesis, B- and T-cell differentiation, and the secretion of inflammation mediators by various human cells (38). These mechanisms could be important in gliomagenesis. Our group has previously shown that lack of allergy in this patient population is a consistent risk factor for glioma. Although the relationship was not strong in our data, we observed an association of high sCD23 with increased self-reported allergy history (Supplementary Table S9b). Thus, sCD23 may be a useful marker of upstream immunoregulation in glioma research. Finally, we note that production of sCD23 is dependent on the proteolytic cleavage of membrane CD23 by the metalloproteinase ADAM10. Lower amounts or activity of ADAM10 in glioma patients could be responsible for the decreased levels of sCD23. There are no studies of ADAM10 in human blood samples, although in glioma tissues the levels seem to be similar to that observed in normal brain (13, 14).

Consistent with other studies, sCD23 levels were lower in patients using dexamethasone than in those who were not using this glucocorticoid drug. Despite this association, sCD23 levels were lower in glioma cases than controls, both in patients who were and were not taking dexamethasone at or near the time of blood draw (Fig. 1). Thus, we believe that our results provide support for the concept that sCD23 levels are a marker of glioma risk and not merely a marker of glucocorticoid use.

Although we observed an interaction between sCD23 and sCD14 levels in serum, further analyses accounting for dexamethasone use indicate that the observed interaction was likely due to the underlying effects of dexamethasone use on immune function. This was suggested across all histologic subtypes. However, we have limited power to adequately assess the effect of dexamethasone in rare histologic subtypes (specific non-GBM types). The number and activation of B cells is known to contribute to sCD23 levels in peripheral blood (39). If an interaction between sCD14 and sCD23 exists, an explanation could be that high levels of sCD14 may suppress sCD23 by inhibiting B-cell proliferation. However, this hypothesis requires more supporting evidence and data from glioma patients who have not yet been treated with dexamethasone.

Although we expected to see a relationship between IgE levels and sCD23 and sCD14, we did not observe a consistent dose-response association of sCD14 with IgE, and only a marginal inverse association of sCD23 with IgE (Supplementary Table S9). In addition, there was no relationship between sCD14 and allergy, but we noticed that persons reporting allergies had higher levels of sCD23 than those reporting no allergy for both cases and controls. This is an interesting finding that implies that more allergies and higher sCD23 levels may be related because both have an inverse relationship with glioma. Another report on sCD14 in children with status asthmaticus showed that sCD14 levels were significantly higher during acute asthma attacks than at recovery, but there was no correlation between level of sCD14 or the change in sCD14 and the serum IgE concentration (40). It remains unclear whether allergies protect against tumors or whether immunosuppressive gliomas inhibit allergies (41). The original report on IgE

and glioma also reported that non-IgE–related allergies were inversely related to glioma, suggesting that IgE per se may not be on the causal pathway driving the association but, rather, some other related immune factors may be responsible (6).

In summary, we believe that the associations of sCD23 and sCD14 with glioma are very robust and unlikely to be due to chance because our study entailed a large sample size that included patients with various grades and histologic types of glioma. Our epidemiologic approach allowed us to investigate the potential role of factors such as age, gender, race/ethnicity, cigarette smoking, and treatments on our results. We conclude that sCD14 and sCD23 measurements may provide information on how the balance of immune functions within an individual can play a role in glioma risk.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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