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Serum macrophage-derived chemokine/CCL22 levels are associated with glioma risk, CD4 T cell lymphopenia and survival time

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Abstract

Defects in antigen presenting cell function have been implicated in glioma immunosuppression. We measured peripheral CCL22, a dendritic cell/macrophage derived T cell trafficking chemokine, in sera from 1,208 glioma cases and 976 controls to assess whether it might provide a biomarker of glioma risk, survival, and immune dysfunction. Cluster models were used to examine the relationship between CCL22 and glioma risk. Patient survival was assessed using Cox regression models. We also examined the relationship between CCL22 levels and CD4 cell counts, as well as allergy history and IgE levels. CCL22 levels were significantly lower among glioma cases compared to controls (Mean±SEM: 1.23±0.03 ng/mL in cases versus 1.60±0.03 ng/mL in controls, $P<0.0001$), and this difference remained significant even after controlling for other covariates in the cluster models (highest quartile versus lowest Odds Ratio=0.21, $P<0.0001$). CD4 cell counts were positively correlated with CCL22 in glioma cases (Spearman $r^2=0.51$, $P<0.01$) and were significantly lower in cases compared with controls. Higher CCL22 levels were associated with longer survival in all cases combined and in GBM cases (hazard ratio_{allcases}=0.81; 95% CI: 0.72–0.91, $P=0.0003$). CCL22 levels were not associated with IgE level or self-reported allergies. Circulating CCL22 levels are related to both glioma risk and survival duration independent of age, histology, grade and *IDH* mutation status. CCL22 should be considered a marker of immune status with potential prognostic value.

Keywords

glioblastoma; T cells; CCL22; cytokines; survival

Introduction

Gliomas are the most common form of primary brain tumor with an incidence rate of approximately 6.5 individuals diagnosed per 100,000 in the United States¹. Traditional histopathological criteria, age, and some tumor markers are currently used to assess glioma patient prognosis^{2, 3, 8, 9}. Survival for patients diagnosed with glioblastomas (World Health Organization (WHO) grade IV) is poor with a median survival of 14.6 months⁴. The median survival of grade III astrocytoma patients is in the range of 2 to 3 years while those with grade III oligodendrogliomas have a median survival of 3 to 6 years⁴. Patients who are younger at diagnosis and who have IDH mutated tumors also have a more favorable survival⁴. The standard therapies for high grade glioma, including surgery, temozolomide (TMZ) chemotherapy and radiation, have been shown to improve survival, although modestly⁴. Important molecular subsets of glioma also are associated with heritable risk⁵⁻⁷. It has also been recognized for many years that glioma patients, particularly GBM patients, show peripheral immune defects affecting T cells^{10, 11}. Lymphocyte counts (particularly CD4 cells) in glioma patients are reduced and T-cell function is also suppressed, with impaired proliferation in response to IL2 and nonspecific mitogens¹². A study of patients with high-grade gliomas (grades III and IV) treated with radiation and TMZ showed that after 2 months of treatment about 40% of patients had CD4 blood counts less than 200 cells/ μ L¹³. These patients continued to have low CD4 counts for the full year of follow-up and exhibited early mortality from tumor progression¹³. Other studies also have reported that low CD4 counts were associated with poorer glioma survival times^{14, 15}. More generally, CD4 lymphopenia has been shown to be a grave prognostic indicator in multiple types of cancers¹⁶⁻²¹. Although the etiology of depressed T cell immunity in glioma is complex; exposures to therapeutic steroids, chemotherapy and ionizing radiation as well as tumor generated immune suppression are thought to be involved^{12, 13}. One important aspect of glioma related suppression of the immune response is the effect that tumors may have on professional antigen presenting cells (APC) and their crosstalk with T cells^{15, 22, 23}. For example, circulating peripheral blood monocytes in patients with GBM have increased expression of the suppressive B7-H1 protein and promote T cell apoptosis²⁴; this suppressive phenotype was induced by exposing monocytes to glioma cell lines and supernatants. Glioma cells exposed to direct contact with blood monocytes were shown in an earlier study to induce a suppressive phenotype²⁵. A crucial link between macrophage/dendritic cells (DC) and T cell immunity is the macrophage-derived chemokine CCL22²⁶. CCL22 is a CC type chemokine and a potent chemoattractant for CD4 and CD8 T cells, as well as for DCs expressing the CCL22 receptor CCR4. It is involved in chronic inflammation mediated by the continuous homing of DCs and lymphocytes^{27, 28}. In this study, we hypothesized that the chemokine CCL22 may be a biomarker of macrophage/DC compartment of immune suppression and provide relevant information about anti-tumor responses elicited by the host that may be negatively impacted by tumor cells or immunosuppressive treatments. We show that reduced levels of serum CCL22 are common in glioma patients and associated with low CD4 cell counts and shorter survival times. This suggests CCL22 may be a useful marker of a suppressed immune function.

Material and methods

Study participants and interview

All cases were adults with newly diagnosed histologically confirmed glioma (International Classification of Disease for Oncology, morphology codes 9380–9481). Population-based cases residing in the six Bay Area counties were ascertained using the Cancer Prevention Institute of California's early case ascertainment system during three recruitment periods: May 1997 to August 1999, November 2001 to September 2005, and September 2006 to September 2009. Clinic-based cases diagnosed between 2002–2006, 2006–2010, and 2009–2012 were recruited from the UCSF Neuro-oncology Clinic, regardless of place of residence. Cases enrolled in the 2009–2012 clinic-based recruitment were eligible to participate if they were 18 years or older at diagnosis, and cases recruited in the earlier years of the study were eligible to participate if they were 20 years and older at diagnosis.

Population-based controls 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 group, gender and ethnicity. Clinic-based controls age 18 years and older were recruited from the UCSF phlebotomy clinic between 2010–2012 and frequency matched to clinic-based cases on age group, gender and ethnicity. Informed consent was obtained from all study participants and study methods were approved by the Committee on Human Research at the University of California, San Francisco.

Pathological material was retrieved, when possible, for all resected brain cancers and reviewed and classified by one of two neuropathologists (Kenneth Aldape, MD Anderson, Houston, TX and Tarik Tihan, UCSF, San Francisco, CA). Blood and serum samples were usually collected at the time of interview. Allergy history data were collected in tabular form as described in detail in our earlier report^{37, 38}. All participants who provided a blood sample were administered an additional questionnaire at the time of blood draw about current and recent medications and treatments. *IDH* mutation was measured in cases with tumor tissue available as previously described⁹.

Measurement of serum CCL22 levels

The Luminex assay was developed using a standard sandwich capture format. Capture antibody to human CCL22 (DY336, Part 840498, R&D system, Minneapolis) was coupled to magnetic Luminex microspheres by using a two-step carbodiimide reaction. Serum samples were diluted 1:100 in sample diluent, which was a mixture of PBS, 10% (vol/vol) fetal bovine serum, and 2.5% (vol/vol) CBS-K (Millipore Corporation, Hayward, CA). The solution was then incubated at room temperature for 1 hour on a shaker. A standard curve was created by diluting human CCL22 (DY336, Part 840500, R&D system, Minneapolis) using the same sample diluent. The CCL22 standards or participant serum samples and coupled CCL22 microspheres were then incubated for 2 hours at room temperature on a shaker using a 96-well flat bottom plate (Bio-Rad Laboratories, Inc., Hercules, CA) and subsequently washed with the wash buffer (Bio-Rad Laboratories, Inc.). This step was followed by the addition of 25 μ L of 1:200 diluted (45ng/mL) biotinylated anti-human CCL22 antibody (DY336, Part 840499, R&D system, Minneapolis) to each well and the

incubation of the mixture at room temperature for 30 minutes on a shaker. The solution was then washed and treated with 50 μ L of streptavidin-conjugated R-phycoerythrin 1:100 diluted stock (Bio-Rad Laboratories, Inc.). After a 10 minutes incubation and final wash, the microspheres were resuspended in 105 μ L of assay buffer (Bio-Rad Laboratories, Inc.). The amount of CCL22 bound to the microspheres by this antibody sandwich technique was determined by the median fluorescence intensity (MFI) of the reporter molecules, 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. A single serum sample from a person without a brain tumor was repeated on some of the assay plates (13 out of 24). We performed standard addition experiments that yielded a recovery rate of 80%.

Flourescence-activated cell sorting (FACS) analysis of CD4 cells levels and IgE analysis

We measured CD4 cell levels on 47 clinic-based cases and 146 clinic-based controls recruited from the 2009–2012 clinic-based series of the study. The blood samples were directly stained with anti-human CD4 APC antibody (eBioscience, San Diego, cat #17-0048-41), anti-human CD45 PerCP-Cy5.5 antibody (eBioscience, San Diego, cat #45-0459-41), and anti-human CD3 FITC antibody (eBioscience, San Diego, cat #11-0038-41). CD45 and CD3 were added to provide reference for CD4 values. After staining the blood was incubated for 20 minutes in the dark at 4°C. Cal-Lyse Solution (Invitrogen, Camarillo, cat #GAS-010) was then added to lyse red blood cells and fix the cells. Flow cytometry counting beads were added for absolute quantification of CD4 cells. Flow cytometry was performed within 48 hours of blood draw on the FACSCalibur (Becton Dickinson, San Jose) flow cytometer using Cell-Quest (Becton Dickinson, San Jose) software. Analysis of flow data was done using Flowjo software (TreeStar Inc, Ashland).

IgE levels were assessed using Pharmacia Diagnostics UniCAP fluorescent “sandwich” assay as described previously²⁹. IgE levels were determined using serum derived from the same blood draw as used for the CCL22 analysis. Total IgE was determined by measuring fluorescence against the standard curve with known quantity inputs as previously described³⁰.

Statistical methods

Statistical analyses were conducted using SAS v9.3 (SAS Institute, Cary NC). Odds ratios for glioma cases versus controls were computed using a multivariable cluster analysis model (Proc Genmod) which controlled for variation within the CCL22 batches. Models were adjusted for age, gender, ethnicity (white/nonwhite), education (college education yes/no) and smoking history (ever/never). Analyses were conducted separately for all gliomas and by histological subtype (GBM versus non-GBM). For the case/control comparisons, CCL22 values were categorized into quartiles based on the distribution among controls. 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 cut points (IgE > 100 kU/L = “elevated”, 25–100 kU/L = “borderline” and < 25 kU/L = “normal”). Cox proportional hazard models were run to evaluate the association between CCL22 levels

and survival in glioma cases. Survival models controlled for age at diagnosis, race (white/non-white), gender, smoking history (ever/never), college degree, number of days between diagnosis and blood draw (continuous), Dexamethasone use at blood draw, and first course of treatment (Temozolomide or other chemotherapy use, radiation, and extent of surgery (biopsy only vs. any resection)). Survival was measured by calculating the total number of days between the date of diagnosis and date of death (if deceased) or last follow-up (if alive or lost to follow-up). Cases who were alive at the last follow-up or were lost to follow-up were treated as censored in the analysis. For the survival models, we compared the upper three quartiles of CCL22 to the lowest quartile since the survival experience for cases in the upper three quartiles was very similar (Supplemental Figure 1).

Results

Our study consisted of 1208 newly diagnosed glioma cases and 976 controls; cases and controls were comparable in their ethnicity and level of education (Table 1). However, cases were more likely than controls to be male, non-smokers, and less likely to report a history of allergies. Cases were also younger on average (median=51 years old) than controls (median=56 years old). Glioblastomas (GBM) were the most common histological subtype of brain tumor, followed by anaplastic astrocytomas, grade 2 astrocytomas, and oligodendrogliomas. More than three quarters of GBM cases (>82%) received the current standard of care treatment as a first course of treatment, which includes TMZ, radiation, and resection. Among GBM patients, 7% had *IDH* mutant tumors whereas for non-GBM patients, 74% of tumors were *IDH* mutant (Supplemental Table 1).

Serum CCL22 levels are depressed in glioma cases compared to controls

The overall concentration of CCL22 was statistically significantly lower in glioma cases compared to controls (mean±SEM: 1.23±0.03 ng/mL versus 1.60±0.03 ng/mL respectively, $P<0.0001$, Table 2). The results from the cluster models showed that after controlling for covariates, CCL22 levels were statistically significantly lower in cases than controls (highest versus lowest quartile OR: 0.21, 95% CI: 0.14–0.30, $P<0.0001$). Results were consistent for both GBM and non-GBM cases, but the magnitude of the effect was greater for GBM cases. In particular, for GBM cases versus controls, the OR for the highest versus lowest quartile was 0.16 (95% CI=0.10–0.24, $p<0.0001$); while in the non-GBM comparison it was 0.30 (95% CI=0.20–0.41, $p<0.001$) (Table 2). However, further analyses among only the non-GBM cases revealed that the lowest CCL22 values were among the grade III astrocytomas. No overall difference in CCL22 concentrations was noted among grade II oligodendroglioma or oligoastrocytoma cases compared to controls (Supplemental Table 1).

Low CCL22 levels are associated with shorter survival

We found that lower CCL22 levels were associated with shorter median survival days among all cases (Table 3). Cox regression models using continuous log transformed CCL22 values showed a significantly lower risk of death as CCL22 levels increased in all cases as well as in the GBM only case group ($HR_{allcases}=0.81$; 95% CI: 0.72–0.91, $P<0.001$) (Table 3). Cox regression models comparing the upper three quartiles of CCL22 to the lowest quartile in all cases also showed a significantly lower risk of death in cases with higher

CCL22 levels (highest quartiles vs. lowest quartile $HR_{\text{allcases}}=0.80$; 95% CI:0.69–0.93, $P=0.003$). However, the highest quartiles versus lowest quartile comparison did not show statistical significance in models where GBM and non-GBM cases were run separately. The Kaplan Meier (KM) curves comparing the survival of cases in the upper three quartiles (Q2–Q4) of CCL22 to those in the lowest quartile (Q1) are shown in Figure 1. The KM curves show that survival in the Q2–Q4 group (with higher CCL22 levels) is better than that in the Q1 group (with lower CCL22 levels) for all cases as well as the GBM and non-GBM case groups (Figure 1: A, B, and C).

We found that in GBM cases, the CCL22 levels were higher in cases treated with radiation before blood draw ($p=0.04$), but we did not find any significant differences in CCL22 levels when comparing cases who took chemotherapy prior to blood draw to those who did not (Supplemental Table 6). However, cases taking dexamethasone use at blood draw had significantly lower CCL22 levels than cases not taking dexamethasone at the time of blood draw. Dexamethasone use, along with first course of treatment, demographic, and other factors were adjusted for in the Cox regression models that show an association between CCL22 levels and overall survival in GBMs.

Serum CCL22 levels are associated with CD3 and CD4 cells in glioma cases

For the 47 cases and 146 controls with FACS analysis data, we compared the CCL22 levels with CD3 (pan-T cell marker), CD4 and CD45 cell counts as well as the ratio of CD3/CD45 and CD4/CD45. There were no observed differences in total CD45 cells (total leukocytes) among cases and controls. However, we observed a significant positive correlation between CCL22 levels and both CD3 and CD4 counts in cases (Table 4). The correlations between CCL22 and CD3 and CD4 were stronger in cases than in controls ($p<0.01$ in cases and $p\geq 0.10$ in controls for both CD3 and CD4). The mean absolute CD4 cell counts in glioma cases also were markedly lower than that in controls (659 cells/ μL in all cases and 392 in GBM cases versus 813 in controls, $p=0.04$). Statistically significant case/control differences were also observed for the ratios of CD3/CD45 and CD4/CD45, with cases having lower ratios ($p<0.01$ for each).

When we grouped the CD4 counts into three clinically meaningful categories (<200 , 200–499, 500+ CD4 cells/ μL), we found that in cases, the mean value of CCL22 increased as CD4 levels increased (Figure 2). Cases with the lowest levels of CD4 cells (<200) had a mean serum CCL22 level of 0.57 ng/mL, whereas cases with CD4 counts ≥ 500 had a mean CCL22 level of 1.20 ng/mL ($p=0.002$ for difference in means between the CD4 groups). However, we did not observe differences in CCL22 levels among the three CD4 groups in controls ($p=0.65$ for difference in mean CCL22 levels across the CD4 groups). In addition, we found that the CD4 counts of cases recently treated with radiation or chemotherapy were significantly lower than cases who had not yet received treatment ($P<0.001$, Supplementary Table 4b).

CCL22 and Allergies/IgE

We also examined whether CCL22 levels were associated with reported allergy history or measured serum IgE levels. Null relationships were observed between CCL22 concentration

and any reported allergy, total number of allergies reported, or IgE measurements in both cases and controls.

Discussion

Although systemic defects in APC function have been observed in glioma patients^{15, 22, 23}, there are no established biomarkers for assessing this dimension of the immune response for clinical prognostication. Here we focused on serum CCL22 as a simple protein assay applicable to routinely collected blood sera. A strong rationale for considering CCL22 as a biomarker of APC function is that DCs and monocyte derived macrophages are the most potent producers of CCL22 in vitro and in vivo³¹. The maturation of monocyte precursors into DCs with a functional APC phenotype leads to a many-fold increased expression and secretion of CCL22³¹. Given our current observation of suppressed CCL22 levels in glioma patients and its association with CD4 lymphopenia, it is reasonable to propose that this reflects depressed DC function and T cell trafficking.

We did not find a significant association between chemotherapy taken before blood draw and CCL22 levels, but found that GBM cases who had radiation before blood draw had higher CCL22 levels than those who had not yet had radiation. We explored the time elapsed since the last dose of radiation or chemotherapy and the day of the blood draw but found no correlation between time since last dose of either radiation or chemotherapy and CCL22 concentrations. Medications such as dexamethasone may also influence CCL22 expression^{32, 33} and in our data current dexamethasone exposure was associated with significantly lower CCL22 levels among glioma cases.

We found a strong correlation between CCL22 values and CD4 counts in cases. We also found that cases who received chemotherapy or radiation before blood draw had significantly lower CD4 counts than cases who had not yet received these treatments. Prospective studies during TMZ and radiation therapy indicate an inhibitory effect on CD4 and B cells followed by a recovery phase in some but not all exposed patients. Prolonged lymphopenia has been associated with the patient's ability to generate appropriate cytokine responses including the production of IL7 and IL15³⁴. Perhaps chemokine directed cell trafficking as marked by CCL22 also fails to recover in some patients and could explain our observed CD4 lymphopenia. It is interesting that another CCR4 responsive chemokine, CCL2, was found to be depressed in sera of GBM patients along with CD4 cells, and both appeared to correlate with increases in a CD14+ HLA-DR- subtype of blood monocyte³⁵. Our earlier study showed there were similar percentages of cases and controls positive for antibodies to three herpes viruses (herpes simplex virus, cytomegalovirus, and Epstein-Barr virus)³⁶. In addition, circulating CD14 levels in our past study³⁷ were significantly higher in cases compared with controls. Furthermore, in our current study we did not observe decreased total leukocyte counts in glioma cases versus controls. These observations argue against the hypothesis that lower CCL22 levels in glioma patients might be primarily the result of nonspecific effects of advanced tumors or therapies. Taken together, these observations implicate monocyte and DC derived trafficking molecules with suppression of T cell immunity. Further study is required to identify whether suppression of CCL22 is an

intrinsic patient related defect, or a tumor driven effect or some combinations of all these possibilities.

IDH mutation, 1p19q codeletion, and MGMT methylation are tumor markers that have been shown to be associated with improved prognosis in glioma patients⁵⁰. We controlled for the effect of IDH mutation in most patients and 1p19q codeletion in patients with oligodendroglial tumors, but were not able to examine MGMT methylation. However, MGMT methylation is highly correlated with IDH status and we have IDH mutation results for most cases.

The present study is, to our knowledge, the first to assess the relationship of peripheral CCL22 levels in glioma survival, which can be more easily applied as a routine assay for prognosis than most tumor markers. Our findings are consistent with two cohort studies on metastatic breast cancer showing that low CCL22 levels were associated both with low CD4 counts and reduced overall survival¹⁶. This raises the possibility that in the context of radio-chemotherapy for cancer, CCL22 might be considered an indicator of DC related immune suppression. However, the association of low CCL22 and abnormal CD4 counts was not observed in our control group, even though a small number of subjects demonstrated <200 CD4 cells/ μ L. In these exceptional control patients, a history of HIV, hepatitis or autoimmune disorders were common though their CCL22 values were normal. The specificity of chemokine measurements as indicators of DC function in different clinical populations requires further study.

Abnormalities in T cell immunity are most extreme in high grade (grades III and IV) glioma compared with lower grades. Our observations parallel this grade dependence in the sense that CCL22 concentrations were not significantly depressed in grade II glioma irrespective of histological subtype. There were individual exceptions, however, with some grade II patients demonstrating very low CCL22 and CD4 counts. The survival curve among non-GBM patients demonstrated a survival advantage among patients with higher CCL22 levels although the sample size of non-GBM cases was limited. Tumor grade and histology subtype analyses revealed that patients with grade III astrocytoma had the lowest CCL22 concentrations compared to those with the grade II oligodendroglioma, grade II astrocytoma, and oligoastrocytoma. Further work will be necessary to assess whether specific morphologies drive the survival associations in non-GBM patients and whether CCL22 may be useful in identifying lower grade glioma patients at high risk for early progression.

We, and others, have shown that T helper 2 (a subset of CD4 cells) related phenotypes such as atopic allergy are underrepresented in glioma patients^{38, 39}. Atopic conditions have been linked to CCL22⁴⁰. One study⁴¹ showed that increased cord blood CCL22 levels were associated with development of allergic sensitization and asthma; CCL22 increases preceded allergy development during the first 6 years of life. Another group demonstrated that cord blood CCL22 levels were associated with elevated total IgE levels during preschool age⁴². Our analyses did not reveal any associations of allergy history or serum IgE concentrations with CCL22 among cases or controls. Previous reports linking serum CCL22 with atopic conditions concerned children and it is not known whether circulating levels in adulthood are expected to signal greater atopic risk. It is also possible that the repertoire of cells

responsive to CCL22 is broader than just CD4 Th2 cells. Targeted knock down of CCL22 in DCs affected CD8 cytotoxic cells as well as CD4 and CD25+ Foxp3+ regulatory cells⁴³. Finally, a limitation of our studies in cases is that serum samples were obtained after glioma diagnosis and it would be very interesting to know if CCL22 depression precedes diagnosis and may therefore reflect a preexisting alteration in macrophage/DC function.

Our study focused on a peripheral marker of immune suppression and we did not evaluate the potential relationship between serum CCL22 and production of this chemokine by the tumor. CCL22 and CCL2 are implicated in the trafficking of T cells including T regs into the tumor microenvironment⁴⁴. IFN- γ , IL1 β and TNF- α will favor CCL22 overproduction by tumor cells, which promotes the recruitment of CCR4+ blood T reg that favors the development of a tolerogenic environment⁴⁵. Trafficking and accumulation of T regs at the tumor site may have a negative effect on patient survival⁴⁶. However, not all studies support this view⁴⁷ and the issue is complicated by the strong correlation of immune infiltrates with WHO glioma grade. In any case, it is not clear what effect local tumor related production of CCL22 would have on the peripheral blood concentration. Both CCL22 and CCL2, the primary chemokines directing T regs, are markedly depressed in sera of glioma patients, at least following tumor resection. It will be necessary to characterize the T cell infiltrate in glioma tissues and assess the peripheral chemokine profile in the same patients to address whether the blood parameter reflects the T cell immune response of the tumor microenvironment⁴⁸.

In conclusion, our observations combined with previous work suggest that monocyte and DC produced CCL22 and, possibly, CCL2 may serve as markers in glioma patients that signal patients at risk of prolonged CD4 cell suppression and poorer survival. New strategies to augment antitumor immunity⁴⁹ can be adversely affected by persistent defects in APC cells and CCL22 may prove a useful marker to assess APC function in patients receiving immunotherapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DC	dendritic cell
FACS	fluorescence-activated cell sorting
GBM	glioblastoma multiforme
CCL22	C-C motif chemokine 22
MDC	macrophage derived chemokine
APC	antigen presenting cell
IDH	isocitrate dehydrogenase
TMZ	temozolomide

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Description of the novelty and impact of the work

Here, we present the novel observation that depressed levels of CCL22 are common in glioma patients. We further have found that these depressed CCL22 levels are associated with low CD4 cell counts and poorer survival times. Thus, CCL22 could be a useful biomarker reflecting the suppressed immunity of glioma patients. It may also serve as a significant prognostic factor of gliomas and a possible marker of cancer associated suppression of immune function.

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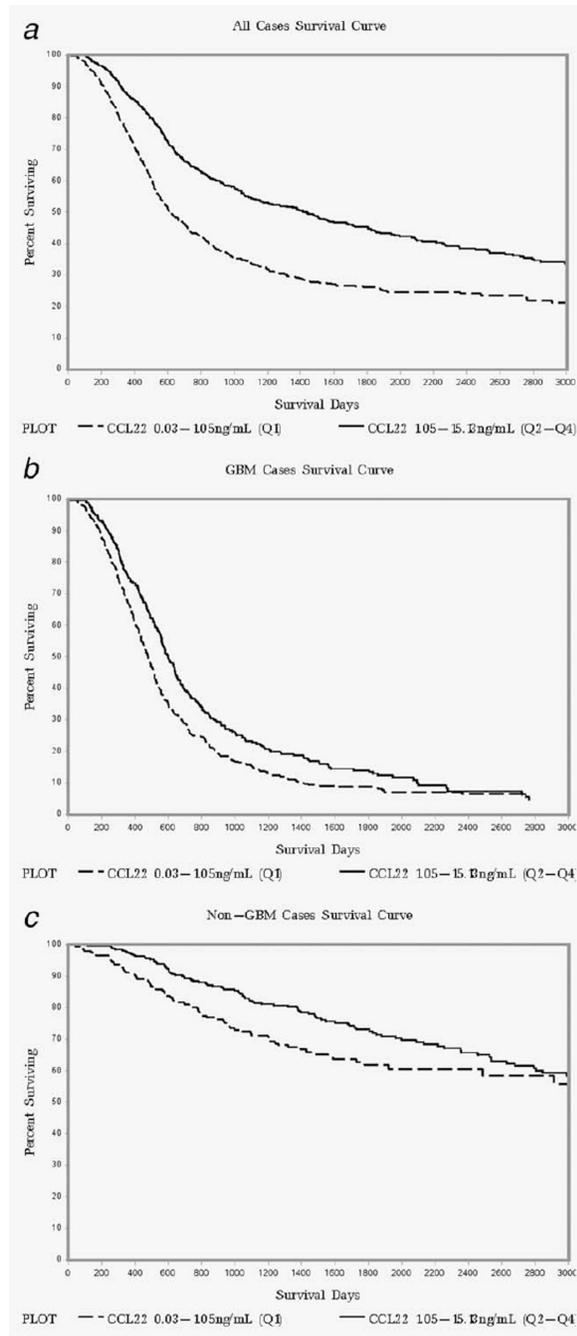


Figure 1. Kaplan-Meier survival curves stratified by CCL22 levels (Quartile 1 vs. Quartiles 2-4) in all glioma cases (a); GBM cases (b) and non-GBM cases (c), UCSF Adult Glioma Study 1997-2012.

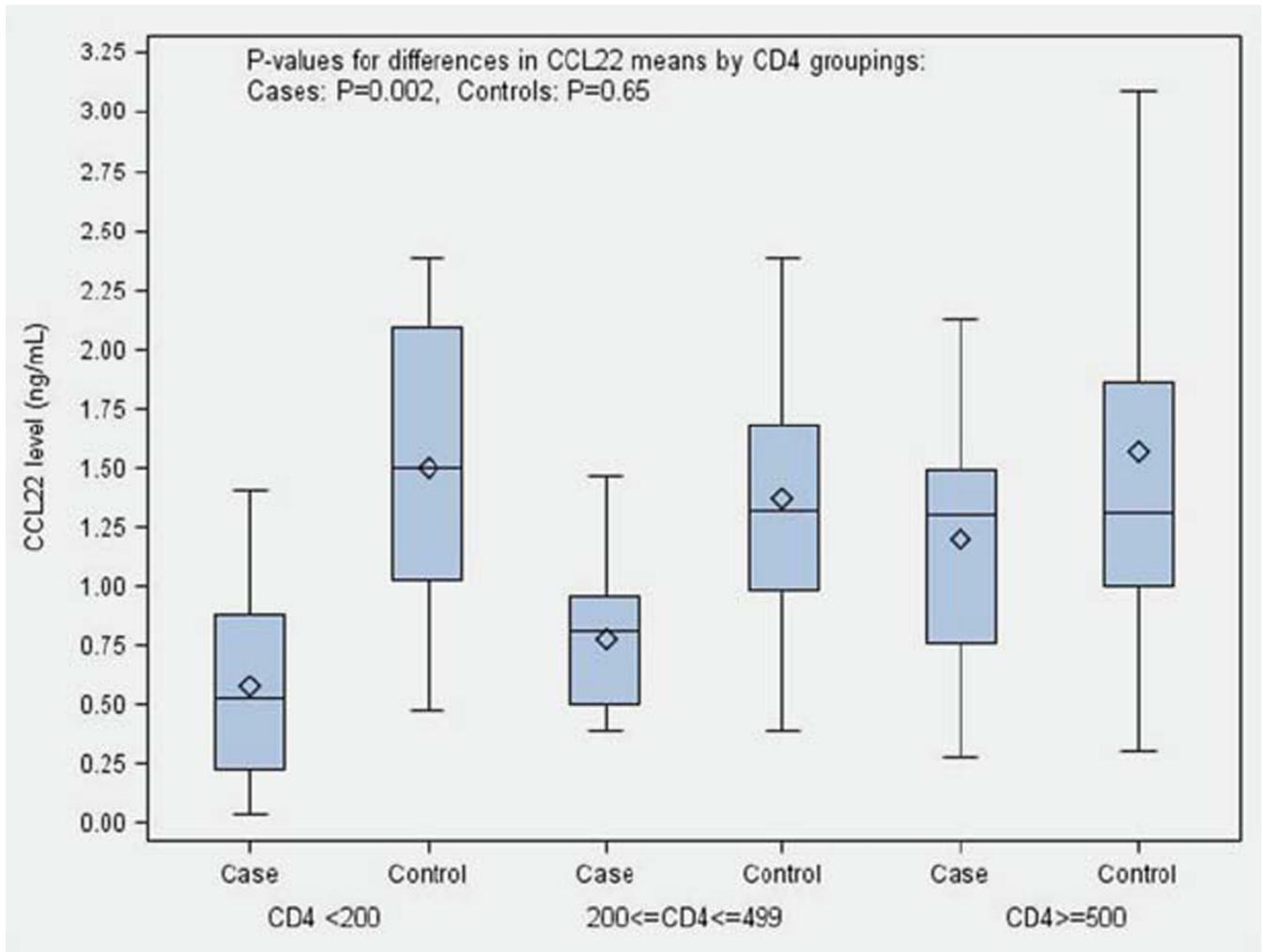


Figure 2.

CCL22 levels by case/control status within CD4 groupings. Samples were divided into three groups according to CD4 counts/ μ L (<200, 200–499, \geq 500), CCL22 levels (ng/mL) were compared in cases and controls of each group, UCSF Adult Glioma Study 2010–2012.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 1 Characteristics of Participants with sCCL22 Results (n=2184), UCSF Adult Glioma Study (1997–2012)

Outcome	Category	Cases (n=1208)		Controls (n=976)	
		#	%	#	%
Histology	GBM	681	56.4		NA
	Non-GBM	527	43.6		
IDH1 Status	Negative	585	70.5		
	Positive	245	29.5		NA
	Unknown/Not Done*	378	NA		
1p19q Co-Deletion Status (Oligodendroglial cases only)	No 1p/19q codeletion	31	27.7		
	1p/19q codeletion	81	72.3		NA
	No 1p/19q data	52	NA		
Gender	Male	723	59.9	498	51.0
	Female	485	40.1	478	49.0
Ethnicity	White	999	82.7	743	76.1
	Non-White	207	17.1	233	23.9
	Unknown	2	0.2	0	0.0
Recruitment Series (Year of diagnosis for cases, year of interview for controls)	1997–2000	70	5.8	110	11.3
	2001–2005	455	37.7	278	28.5
	2006–2010	546	45.2	374	38.3
	2009–2012	137	11.3	214	21.9
Used Temozolomide***	Yes**	860	71.2		
	No	334	27.6		NA
	Unknown	14	1.2		
Radiation Therapy***	Yes	949	78.6		
	No	254	21.0		NA
	Unknown	5	0.4		

Outcome	Category	Cases (n=1208)		Controls (n=976)	
		#	%	#	%
Surgery ^{***}	Biopsy Only	156	12.9		
	Resection	1051	87.0	NA	NA
	Unknown	1	0.1		
Any Allergy Reported	Yes	882	73.0	779	79.8
	No	320	26.5	190	19.5
	Unknown	6	0.5	7	0.7
Smoking History	Ever Smoker	516	42.7	513	52.6
	Never Smoker	685	56.7	462	47.3
	Unknown	7	0.6	1	0.1
Education Level	College Degree	667	55.2	536	54.9
	No College Degree	537	44.5	439	45.0
	Unknown	4	0.3	1	0.1
Age	Median	51		56	
Days Between Dx and Blood Draw	Median	96		NA	

* *IDH* has not been done yet or we do not have adequate tissue for *IDH* analysis.

** includes recommended or likely used

NA=not applicable

*** Only temozolomide, radiation and surgery given during the first course of treatment are included.

Table 2 Comparisons of sCCL22 Levels in Glioma Cases and Controls, UCSF Adult Glioma Study, 1997–2012

Value	Category	Cases		Controls		t-test p-value**	Trend Test	
		n	Mean ± SEM	n	Mean ± SEM			
sCCL22 ng/mL	All	1208	1.23 ± 0.03	976	1.60 ± 0.03	<0.0001		
Value	Category	n	Value (Quartile for Controls)	%	%	OR* (95% CI)	OR p-value	
sCCL22 ng/mL Values	All	607	0.03–1.05 (Q1)	50.2%	25%	1.00		
		247	1.05–1.40 (Q2)	20.4%	25%	0.28 (0.25–0.31)	<0.0001	
		187	1.40–1.87 (Q3)	15.5%	25%	0.24 (0.19–0.29)	<0.0001	
		167	1.87–15.13 (Q4)	13.8%	25%	0.21 (0.14–0.30)	<0.0001	
	GBM	404	0.03–1.05 (Q1)	59.3%	25%	1.00		
		116	1.05–1.40 (Q2)	17.0%	25%	0.23 (0.19–0.27)	<0.0001	
		87	1.40–1.87 (Q3)	12.8%	25%	0.18 (0.14–0.24)	<0.0001	
		74	1.87–15.13 (Q4)	10.9%	25%	0.16 (0.10–0.24)	<0.0001	
	Non-GBM	203	0.03–1.05 (Q1)	38.5%	25%	1.00		
		131	1.05–1.40 (Q2)	24.9%	25%	0.38 (0.33–0.43)	<0.0001	
		100	1.40–1.87 (Q3)	19.0%	25%	0.34 (0.27–0.41)	<0.0001	
		93	1.87–15.13 (Q4)	17.6%	25%	0.30 (0.20–0.41)	0.0009	
Log CCL22	All	1208	NA (continuous log CCL22)	NA	976	0.24 (0.17–0.33)	<0.0001	NA

* Cluster analysis was performed to control for variation within each batch. Models were adjusted for batch number, age (continuous), race (white/non-white), gender, education (college vs no college), and smoking (ever vs never).

** T-test comparing sCCL22 means between cases and controls.

SE=Standard error

Table 3 Relationship Between sCCL22 and Survival, Cox Regressions Stratified by Histology, UCSF Adult Glioma Study, 1997–2012

Variable	Category	All Cases				
		#	# Deceased	MS ^{**}	Hazard Ratio [*]	P-Value
Log CCL22	NA	1208	798	873	0.81 (0.72–0.91)	0.0003
sCCL22 value (ng/mL) ^{***}	0.03–1.05 (Q1)	607	448	611	Referent	NA
	1.05–15.13 (Q2 – Q4)	601	350	1446	0.80 (0.69–0.93)	0.003
GBM Cases						
Variable	Category	#	# Deceased	MS ^{**}	Hazard Ratio [*]	P-Value
Log CCL22	NA	681	614	520	0.79 (0.69–0.91)	0.001
sCCL22 value (ng/mL) ^{***}	0.03–1.05 (Q1)	404	370	479	Referent	NA
	1.05–15.13 (Q2 – Q4)	277	244	598	0.85 (0.72–1.01)	0.06
Non-GBM Cases						
Variable	Category	#	# Deceased	75% survival ^{***}	Hazard Ratio [*]	P-Value
Log CCL22	NA	527	184	1380	0.90 (0.70–1.15)	0.41
sCCL22 value (ng/mL) ^{***}	0.03–1.05 (Q1)	203	78	948	Referent	NA
	1.05–15.13 (Q2 – Q4)	324	106	1687	0.84 (0.61–1.15)	0.28

* The following variables were controlled for in the models: age, gender, race (white/non-white), 1st course of treatment (radiation, surgery, temodar, other chemo), dexamethasone use at blood draw, smoking (ever/never), college degree (yes/no), blooddays (days between dx and blood draw).

** MS=Median survival days. For non-GBM cases, median survival was not available due to high % censored observations so the cutoff where 75% of the cases were still alive was used instead of the median.

*** Quartiles were based on only sCCL22 values in controls.

Note: We did not include *IDH* status in the final survival models since it was not available for 378 cases. However, when we included *IDH* in the models we found similar results (Log CCL22 HR and 95% CI for all cases=0.88 (0.76–1.03), GBM cases=0.81 (0.68–0.95), and Non-GBM cases=1.11 (0.77–1.60)). We also did not include 1p/19q co-deletion status as we only had this on 112 of the 165

oligodendroglial cases. However, we ran the survival models for the 112 cases with and without 1p/19q in the model and found similar results (Without 1p/19q: HR=0.40 (0.13–1.26), With 1p/19q: HR=0.47 (0.15–1.46)).

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Table 4

CD3, CD4, and total leukocytes (CD45) cell counts (by fluorescence-activated cell sorting) and correlations of these values with sCCL22 levels, UCSF Adult Glioma Study, 2010–2012

Variable	Case Status	#	Median	Mean	St Dev	All Cases vs All Controls T-test p-value**	Correlation Coeff with sCCL22*	P-value (Correlation)
CD3 (Tcells) Absolute count/mL	All Cases and Controls	193	1225	1354	734		0.29	<0.01
	Cases Only	47	938	1232	1044		0.53	<0.01
	Non-GBM Cases Only	21	1931	1758	1065		0.41	0.06
	GBM Cases Only	26	511	808	825		0.50	<0.01
	Controls Only	146	1294	1392	601	0.19	0.13	0.13
CD4 (T-helper cells) Absolute count/mL	All Cases and Controls	193	716	775	459		0.30	<0.01
	Cases Only	47	486	659	597		0.51	<0.01
	Non-GBM Cases Only	21	758	989	708		0.36	0.11
	GBM Cases Only	26	275	392	298		0.47	0.02
	Controls Only	146	754	813	399	0.04	0.14	0.10
CD45 Total leukocytes/mL	All Cases and Controls	193	6949	7557	3252		0.08	0.29
	Cases Only	47	7827	8358	4634		-0.03	0.85
	Non-GBM Cases Only	21	8580	9301	4333		-0.09	0.68
	GBM Cases Only	26	6312	7597	4812		-0.18	0.38
	Controls Only	146	6874	7299	2630	0.05	0.11	0.17
CD3/CD45 Ratio	All Cases and Controls	193	0.19	0.19	0.08		0.21	<0.01
	Cases Only	47	0.13	0.15	0.09		0.61	<0.01
	Non-GBM Cases Only	21	0.23	0.19	0.08		0.52	0.02
	GBM Cases Only	26	0.10	0.11	0.08		0.53	0.01
	Controls Only	146	0.20	0.20	0.08	<0.01	-0.004	0.96
CD4/CD45 Ratio	All Cases and Controls	193	0.10	0.11	0.05		0.21	<0.01
	Cases Only	47	0.07	0.08	0.05		0.57	<0.01
	Non-GBM Cases Only	21	0.12	0.11	0.05		0.48	0.03
	GBM Cases Only	26	0.06	0.06	0.04		0.53	<0.01

Variable	Case Status	#	Median	Mean	St Dev	All Cases vs All Controls T-test p-value**	Correlation Coeff with sCCL22*	P-value (Correlation)
	Controls Only	146	0.11	0.12	0.05	<0.01	0.005	0.96

* Correlation between log transformed CCL22 and specified FACS variable (Spearman Rank Correlation).

** Comparing mean FACS values between cases and controls.