Immunologic Research (2019) 67:348–357
https://doi.org/10.1007/s12026-019-09106-7

ORIGINAL ARTICLE

The sialoglycan-Siglec-E checkpoint axis in dexamethasone-induced immune subversion in glioma-microglia transwell co-culture system

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Published online: 18 November 2019

Abstract

Dexamethasone (Dex) is considered as the main steroid routinely used in the standard therapy of brain tumor-induced edema. Strong immunosuppressive effects of Dex on effector systems of the immune system affect the patients' antitumor immunity and may thereby worsen the prognosis. Siglecs and their interacting sialoglycans have been described as a novel glyco-immune checkpoint axis that promotes cancer immune evasion. Despite the aberrant glycosylation in cancer is described, mechanisms involved in regulation of immune checkpoints in gliomas are not fully understood. The aim of this study was to investigate the effect of Dex on the Siglec-sialic acid interplay and determine its significance in immune inversion in monocultured and co-cultured microglia and glioma cells. Both monocultured and co-cultured in transwell system embryonic stem cell-derived microglia (ESdM) and glioma GL261 cells were exposed to Dex. Cell viability, immune inversion markers, and interaction between sialic acid and Siglec-E were detected by flow cytometry. Cell invasion was analyzed by scratch-wound migration assay using inverted phase-contrast microscopy. Exposure to Dex led to significant changes in IL-1β, IL-10, Iba-1, and Siglec-E in co-cultured microglia compared to naïve or monocultured cells. These alterations were accompanied by increased α2.8-sialylation and Siglec-E fusion protein binding to co-cultured glioma cell membranes. This study suggests that the interplay between sialic acids and Siglecs is a sensitive immune checkpoint axis and may be crucial for Dex-induced dampening of antitumor immunity. The targeting of sialic acid-Siglec glyco-immune checkpoint can be a novel therapeutic method in glioma therapy.

Keywords Sialic acid · Siglec · Microglia · Glioma · Immunosurveillance

Introduction

Gliomas are the most frequent primary intracranial malignancies characterized by extensive cellular and molecular heterogeneity which provides aggressive diffuse infiltration, high biological activity, and failure of targeted pharmacological standard strategies, leading to fatal outcome within approximately 16 months after clinical trial [1]. The progression of glial tumors is closely associated with impaired immune function of tumor microenvironment (TME) and attributed to the cross talk between tumor and immune cells, as well as altered expression of genes and related proteins involved in biological recognition, signaling, and secretive activity [2, 3]. In negative regulation of antitumor, immunity participates specific cell membrane receptors that perform their inhibitory function through interaction with their suitable ligands. There is increasing evidence that aberrant sialylation of cell surface glycocalyx is a crucial regulator of cancer immunogenicity and results in evasion of effective immune attack [4, 5]. Sialic acids are nine carbon ketosugars linked through α2.3, α2.6, and α2.8 glycosidic linkage to the nonreducing end of sugar chains in glycoconjugates. The terminal position of sialic acid in these structures determinates its implication in cell–cell and cell-extracellular matrix (ECM) interactions during immune recognition processes. The different linkage-specific sialoglycans are selectively recognized by sialic acid-binding immunoglobulin like lectins (Siglecs) family members that transduce activatory or suppressive signaling in immune cells [6]. It has been shown that the interplay between CD33-related Siglecs and tumoral sialic acids can inhibit antitumor

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function of natural killer cells (NK cells), facilitates myeloid cell-mediated cancer progression, and induces a tumor-related macrophage phenotype [7]. Despite sialic acid–Siglec pathway has been defined as a new inhibitory immune checkpoint, the involvement of Siglecs in immune surveillance in gliomas was not extensively explored. Additionally, there are increasing evidences that numerous chemical compounds, including standard therapy drugs, interfere with Siglec-mediated immunity and modulate sialylation pattern of cell membrane glycocalyx [8–11]. According to the guidelines for glioma therapy, dexamethasone (Dex) is considered as the main steroid routinely used in the management of tumor-induced edema [1]. Despite the significant clinical potential, safe use of Dex, in particularly high clinical doses, in individual patients with glioma remains unconvincing. As shown in The Cancer Genome Atlas (TCGA), dexamethasone-associated genes expression, e.g., CDC25C, CDCA8, CDC20, PRC1, and PLK1, correlates with worse predictions and shorter survival in patients with glioblastoma [12, 13]. Moreover, Dex decreases the effectiveness of radiotherapy and/or temozolomide (TMZ) treatment as has been demonstrated in retrospective clinical studies by German Glioma Network and European Organisation for Research and Treatment of Cancer/Clinical Trials Group [12, 14, 15]. While the mechanisms of therapeutic action of glucocorticoids and their modulating effects on cell biology are well-known, the non-genomic mechanisms underlying cancer immune evasion are not fully understood. Here we present evidence that Dex-induced changes in Siglec-sialic acid interplay may participate in the mechanism of immune inversion. In this study, glioma and microglial cells were grown in the transwell co-culture system or monocultures and exposed to high concentration of Dex. The influence of Dex on expression of α2.8-linked sialic acid and binding of their appropriate receptor, Siglec-E, was studied in glioma GL261 cell line, which is frequently tested in experimental models of local and systemic immune interaction in glioblastoma multiforme. The markers of immune inversion were evaluated in embryonic stem cell-derived microglia (ESdM), which has most characteristics of primary microglia but not oncologically transformed cells [16].

Materials and methods

Cell cultures, co-cultures, and treatment

Mouse glioma GL261 cells (ACC802, DSMZ Germany) were grown in Dulbecco's modified Eagle's medium/F12 supplemented with 10% heat-inactivated fetal bovine serum (all Gibco; Life Technologies, Germany) and 100 μg/ml penicillin/streptomycin (Merck, Germany). ESdM were kindly provided by Prof. Harald Neumann (University of Bonn) and cultured in Dulbecco's modified Eagle's medium/F12 with 1% N2 supplement, 0.48 mM L-Glutamine, 100 μg/ml penicillin/streptomycin (all Gibco; Life Technologies, Germany), and 15.3 μg/ ml D-glucose (Merck, Germany). Cells were incubated in 37 °C in a humidified atmosphere containing 5% CO_2. Both GL261 cells and ESdM with a passage number between 5 and 15 were used in the experiments. For co-culture studies, the transwell culture system was used. Naïve GL261 cells were cultured in six well plates to reach confluency of 80%, and naïve ESdM cells were added to the inserts which were placed in upper parts of culture dishes. Cells were co-cultured at a ratio 1:3 (microglia/glioma), but the pores of insert membrane at large of 0.4 μm did not permit to physical interaction. Both monocultured and co-cultured cells were exposed to Dex (Dexaven, PharmaSwiss, Czech Republic) concentration of 10 μM, which was defined as high clinical level [17, 18].

Cell cycle

After 24 hours of Dex exposure, monocultured and co-cultured GL261 and ESdM cells were carefully collected by scraping, fixed in ice-cold 70% methanol, treated with ribonuclease, and stained with propidium iodide (PI, 50 μg/ml) for DNA quantification. The distribution of PI fluorescence in naïve and Dex-treated was quantified using flow cytometry by their distribution in G0/G1, S, and G2/M phases. The S + G2/M population was quantified as proliferating cells.

Scratch wound assay

To detect the effect of co-cultured microglia and Dex-treatment on glioma motility, scratch wound assay was employed. GL261 cells were monocultured or co-cultured with microglia cells in transwell system on 6-well plate. At approximately 80% confluency, using a 10 μl pipette tip, a vertical scratch wound was made through the center of each well plate, and 10 μM Dex was added. After 24 hours, cells were examined by inverted microscope (Motic AE31) to determine the area of the wound. The images were captured at 0 h and 24 h of exposure to Dex and analyzed for cell-free area in the scratch wound by the ImageJ software.

Assessment of α2.8-linked sialic acid expression and binding of Siglec-E/Fc fusion protein to glioma cells

Polysialic acid (PSA) is a polymer of α2.8-linked sialic acid, which is attached to the neural cell adhesion molecules (NCAM) during posttranslational modification. To estimate the level of α2.8-sialylation, GL261 cells were analyzed by flow cytometry after incubation with primary PSA–NCAM antibody (Merck, 2 μg/ml) for 30 min at 4 °C and staining with appropriate secondary, isotype-specific FITC-conjugated antibody (Abcam, 2 μg/ml). The expression of PSA–NCAM was determined according to the corresponding mouse IgM

isotype control antibody used as negative control (Abcam, 2 μg/ml). To investigate the binding of Siglec-E receptor protein to glioma cells, the naïve and Dex-treated cells were incubated with mouse Siglec-E/Fc fusion protein (R&D Systems, 1 μg/ml), which recognizes predominantly α2.8-linked sialic acids, and then stained with Cy3-conjugated IgG secondary antibody (Jackson ImmunoResearch, 2 μg/ml). Samples were analyzed by flow cytometry, and the intensity of fusion protein binding was determined according to the negative control group stained with the secondary antibody alone. To verify sialic acid-dependent binding of Siglec-E, the α2.3-, α2.6-, and α2.8-glycosidic linkages of terminal sialic residues were hydrolyzed by α-neuraminidase. Briefly, the growing GL261 cells were incubated with 100 U/ml α-neuraminidase (from *Clostridium perfringens*, New England Biolabs) for 24 hours at 37 °C. After 24 hours, cells were washed three times with PBS and then incubated with recombinant Siglec-E/Fc fusion protein as a positive control.

Determination of intracellular expression of Iba-1, IL-1β, and IL-10 in microglia

The Iba-1 is a calcium-binding protein known to be restricted by expression in microglia/macrophages and involved with the function of activated cells. Monocultured or co-cultured naïve and Dex-treated microglia were scraped, diluted to 10^5 per sample, and incubated with Iba-1 (Abcam, 5 μg/ml) monoclonal for 30 min at 4 °C. To confirm activation status of microglia, intracellular interleukins were studied using specific primary antibodies to IL-1β (Abcam, 2 μg/ml) and IL-10 (Abcam, 4 μg/ml). To facilitate intracellular staining, cells were permeabilized with 0.1% Triton X-100. Cells were washed with phosphate-buffered saline and then stained with appropriate FITC-conjugated secondary antibody (Merck, 4 μg/ml) and analyzed on Becton Dickinson flow cytometry system.

Statistical analysis

For each group, a minimum of 3–5 independent experiments were performed. The statistical analysis was carried out with statistic package Statistica 6.0 using one-way ANOVA followed by Bonferroni posttest. Results are expressed as mean ± SEM. Statistical differences were deemed at $p < 0.05$.

Results

Effect of dexamethasone on glioma cells growth and invasion in monoculture and transwell co-culture system

The cultivation with ESdM in transwell co-culture system changed the distribution of glioma cells along G_0/G_1 and $S + G_2/M$ cycle phases. The number of GL261 cells within G_0/G_1 was significantly reduced, from 61.7% of naïve cell population in monoculture to 50.3% of cells co-cultured with ESdM. Correspondingly, the percentage of cells within $S + G_2M$ phase was enhanced from 38.2% in control glioma cells in monoculture to 49.7% cells in co-culture system. Both glioma and microglia displayed changes in cell cycle phases distribution in response to Dex, but these effects were weak and not statistically significant in monoculture and co-culture (Fig. 1A, B, C). In additional quantification of Dex effects on GL261 proliferation, we evaluated the migration behavior by measuring the closure of provoked scratch in time. After 24 hours, the relative wound width was significantly decreased by 31 ± 3.4% and 42 ± 1.9% in both monocultured and co-cultured GL261 cells, respectively (Fig. 2A, B). In presence of 10 μM Dex, the wound width in monoculture and co-culture was reduced by 12 ± 2.6% and 17 ± 1.9%, respectively, as compared to control cells (100%). As shown in Fig. 2C, naïve GL261 cells in non-treated monocultures and co-cultures displayed site-specific orientation (black arrows) in the border of the wound closely linked to their elevated invasive and proliferative potential.

Dexamethasone modulates cell membrane α2.8-sialylation and Siglec-E/Fc fusion protein binding to glioma cells

The GL261 cells were incubated with Siglec-E/Fc fusion protein to establish their recognizing capacity of changes in the cell surface glycocalyx. The analysis of Siglec-E/Fc protein binding cells, expressed as a percentage of population, evidenced differences between groups. Both monocultured and co-cultured glioma cells showed high potential to interact with recombinant extracellular domain of Siglec-E receptor (72 ± 4.9% and 41 ± 5.1%, respectively). Interestingly, this feature was notable decreased in co-cultured glioma cells when compared to monoculture ($p < 0.05$). After enzymatic desialylation, the binding of Siglec-E/Fc fusion protein was significantly abrogated in both cell culture systems (monoculture, 18 ± 7.2%; co-culture, 22.5 ± 4.1%). When GL261 cells were exposed to Dex, the affinity of Siglec-E/Fc protein tended to be reduced in monoculture (60.8 ± 4.5% vs. 72 ± 4.9% unstimulated cells) but increased in co-cultures (58 ± 7.7% vs. 41 ± 5.1% unstimulated cells) (Figs. 3A, B). This result was not consistent with sialylation level. The flow cytometric determination of α2.8-sialic acid attached to NCAMs (PSA–NCAMs) revealed significantly elevated mean relative fluorescence of PSA–NCAM positive glioma cells exposed to 10 μM Dex, in both monoculture and co-culture (monoculture, 123 ± 4.7% vs. 100% control; co-culture, 144 ± 7% vs. 100% control) (Fig. 4A).

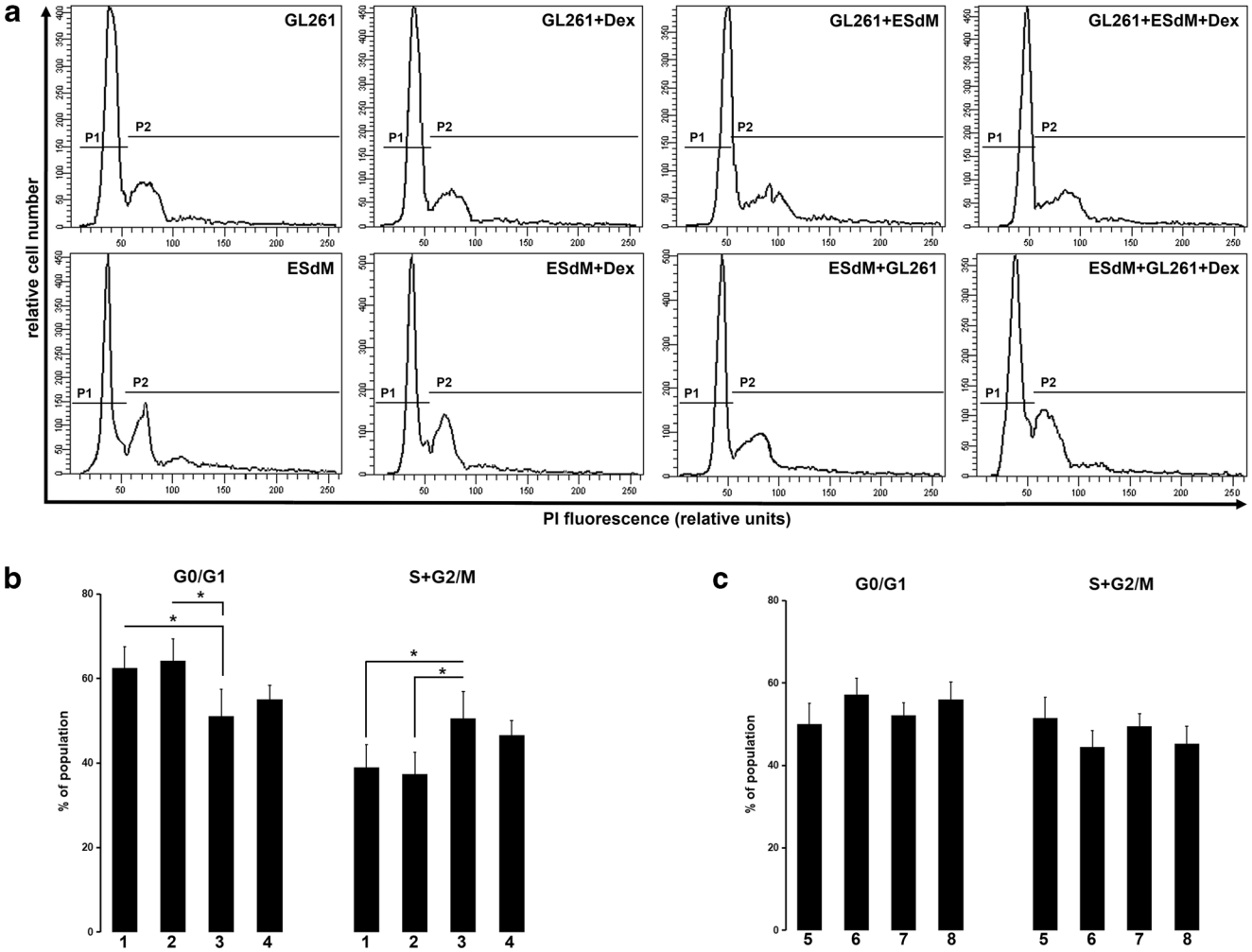

Fig. 1 Effect of Dex on the cell cycle phase distribution of GL261 and ESdM cells. (A) Representative histograms and (B, C) corresponding bar graphs were derived from 10000 cells and present the percentage of population within the G_0/G_1 (P1) and S + G_2/M (P2) phases of cell cycle. Each column presents a mean ± SEM of 3 independent experiments; *p < 0,05 vs. monoculture. *1*-GL261; *2*-GL261 + Dex; *3*-GL261 + ESdM; *4*-GL261 + ESdM+Dex; *5*-ESdM; *6*-ESdM+Dex; *7*-ESdM+GL261; *8*-ESdM+GL261 + Dex

The immune activity of microglia in mono- and co-cultures stimulated by dexamethasone

As shown in Fig. 4B, ESdM cells expressed Siglec-E protein in the cell membrane. In monocultured cells, the Siglec-E expression was slightly elevated in response to 10 μM Dex (114 ± 5.3% vs. 100% control; $p > 0.05$). In co-cultures, the mean expression of Siglec-E significantly increased to 126 ± 4.7% as compared to naïve cells (100% control). The alterations in Siglec-E expression were accompanied by changes in functional status of ESdM cells evaluated through measurement of intracellular cytokine levels. Statistical analysis of IL-1β, IL-10, and microglial activation marker Iba-1, expressed as percent of control, evidenced differences between control and Dex-treated groups (Fig. 5). In co-cultured naïve ESdM cells, the level of IL-1β tended to be reduced; however, these changes were statistically insignificant (92 ± 2.1% vs. 100% control). Dex at concentration of 10 μM significantly decreased intracellular IL-1β in monocultured and co-cultured cells (50.8 ± 3.1% vs. control 100%, 36 ± 3.3% vs. control 100%, respectively) (Fig. 5A). In opposite, the population of ESdM cells expressing IL-10 was increased in both Dex-exposed monocultures and co-cocultures (914 ± 44.8% vs. 100% control, 1515 ± 90.6% vs. 100% control, $p < 0.05$, respectively) (Fig. 5B). In naïve co-culture, the expression of Iba-1 tended to be reduced by 7% (93 ± 3% vs. 100% control; $p > 0.05$) and significantly decreased in response to Dex (54 ± 5.5% vs. 100% control) as shown in Fig. 5C.

Discussion

The therapy with Dex is part of the treatment that is a standard care for brain tumors [19]. Despite well-defined clinical benefits, Dex-based glioma management includes multiple pitfalls in the CNS (central nervous system) ranging from drugs

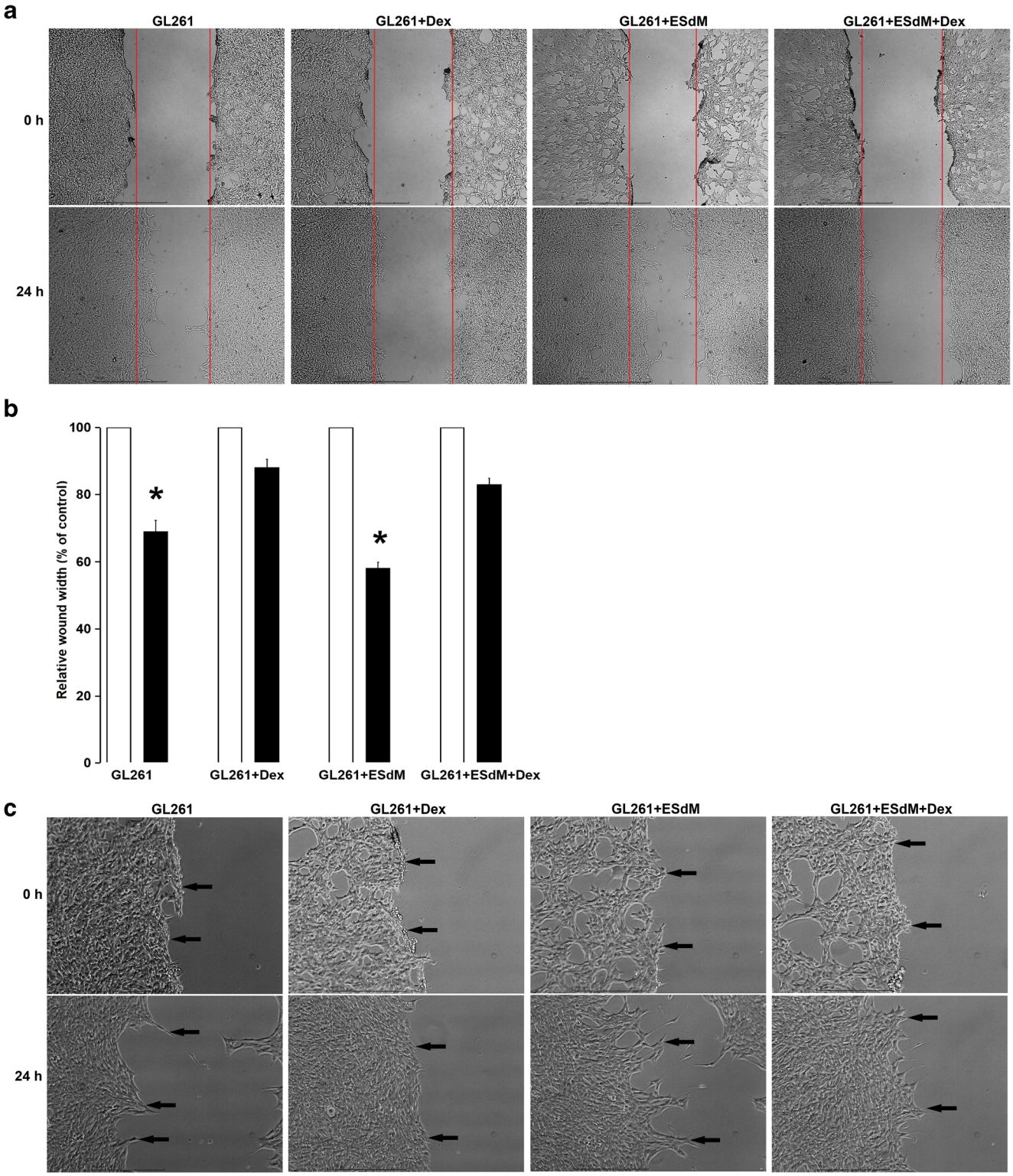

Fig. 2 Wound healing assay in GL261 cells grown in monocultures or co-cultures treated with Dex. (A) Representative inverted phase-contrast microscope images showing the scratch (wound) at time 0 h and 24 h with/without exposure to Dex. Magnification 100 ×. (B) Quantitative analysis of the percentage of wound healing in GL261 cells after treatment with Dex. The values were normalized by the wound width at the same area of the scratch at time 0 h. Each column presents the relative mean value ± SEM of 3 independent experiments. Data are presented as a percentage of control group (time 0 h; 100%); *p < 0,05 vs. control. (C) Representative inverted phase-contrast microscope images showing the invasion of monocultured and co-cultured GL261 cells at time 0 h and 24 h with/without exposure to Dex. Magnification 200 ×

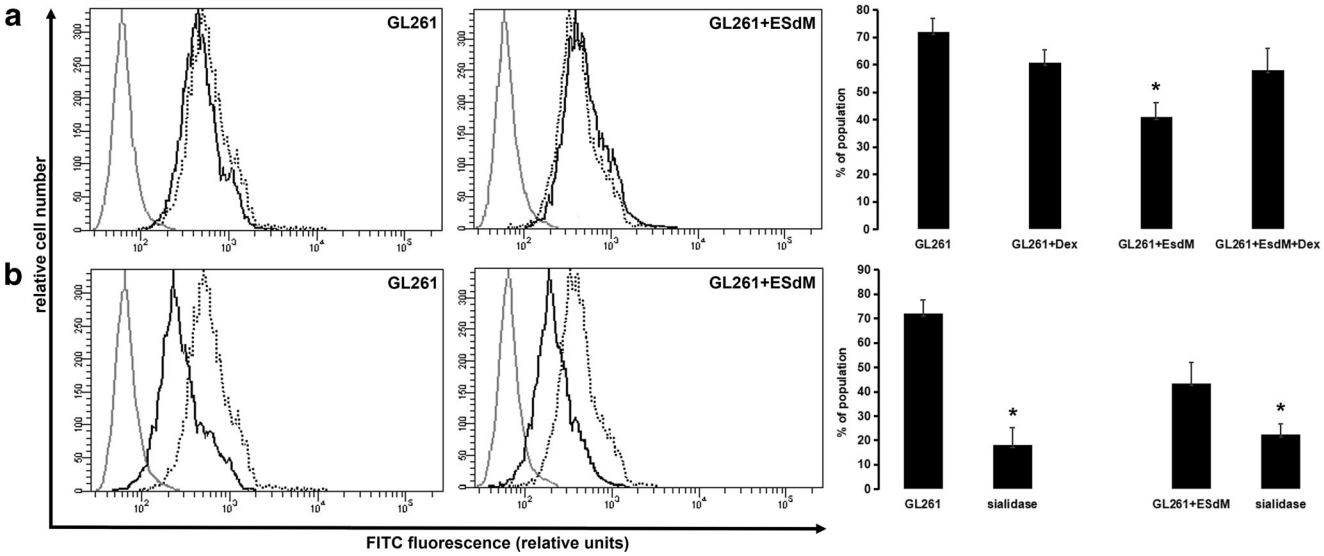

Fig. 3 Effect of Dex on Siglec-E/Fc fusion protein binding to GL261 cells grown in monocultures or co-cultures. (A) Representative histograms and corresponding bar graphs were obtained from flow cytometric analysis of 10000 cells and show isotype control (grey line), control cells (dropped line), and cells exposed to Dex (black line). Each column presents mean ± SEM of 3–5 independent experiments. (B) The histograms and appropriate bar graphs showing cells treated with α-neuraminidase were used as positive control (light grey line), control cells (dropped line), and α-neuraminidase-treated cells (black line). Data are presented as a percentage of analyzed population; *p < 0,05 vs. respective unstimulated cell line

activity-decreasing pharmacological interactions to effects on immune surveillance in the brain [12]. Our previous results on the effects of Dex on immunogenic potential of glioma cells suggest that these features are related to the cell membrane sialylation [11]. It was in agreement with the clinical observation that low immunogenicity of malignant cells correlates with impaired immune control systems and shorter patient's survival [20, 21]. Polysialic acid, a polymer of α2.8-linked sialic acids, has been described as a crucial cell membrane compound that is functionally involved in cell–cell and cell-matrix adhesion and thereby promotes migration, invasion, and metastasis of the numbers of cancers, including brain tumors [22–24]. Given the capacity binding to immune receptors, such as Siglecs, sialic acid is a potential participant in immunoregulation and promotes immune evasion by cancer cells. Siglec-E and its human homolog Siglec-9 were found on cancer cells and mediate multiple alterations in tissue proteomics composition that are essential in functional mechanisms

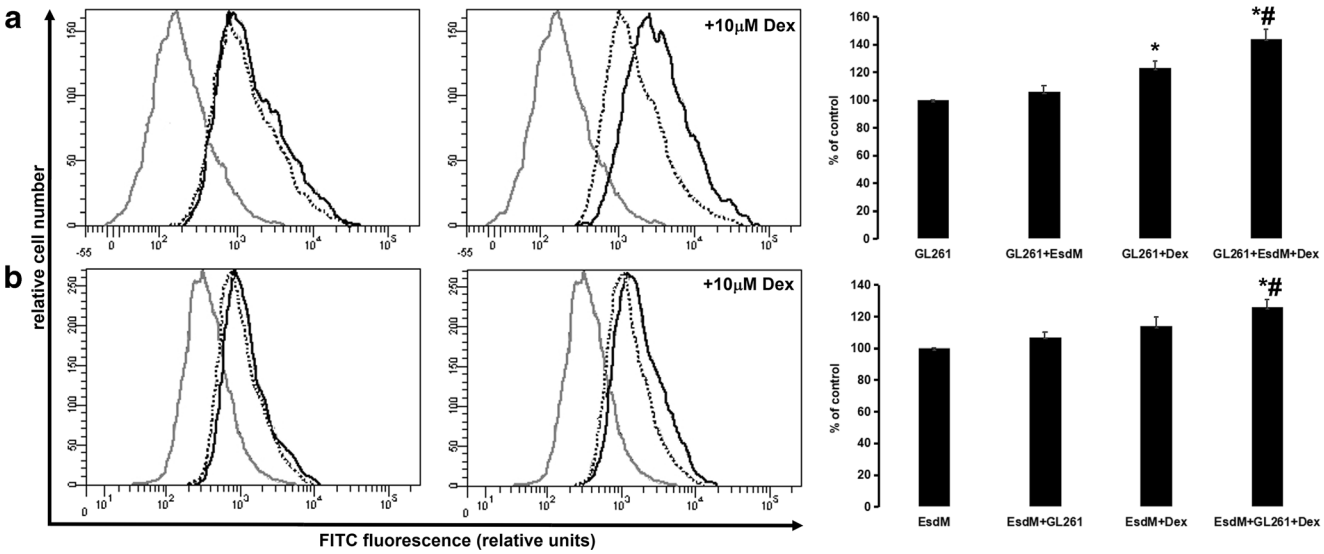

Fig. 4 Expression of PSA–NCAM in GL261 (A) and Siglec-E (B) in ESdM cells grown in monocultures or co-cultures and exposed to Dex. Representative histograms were derived from analysis of 10000 cells and show isotype control (grey line), monocultured cells (dropped line), and co-cultured cells (black line). Each column in corresponding bar graph presents mean ± SEM of 3–5 independent experiments. Data are presented as a percentage of control group (100%); *p < 0,05 vs. GL261; #p < 0,05 vs. GL261 + ESdM

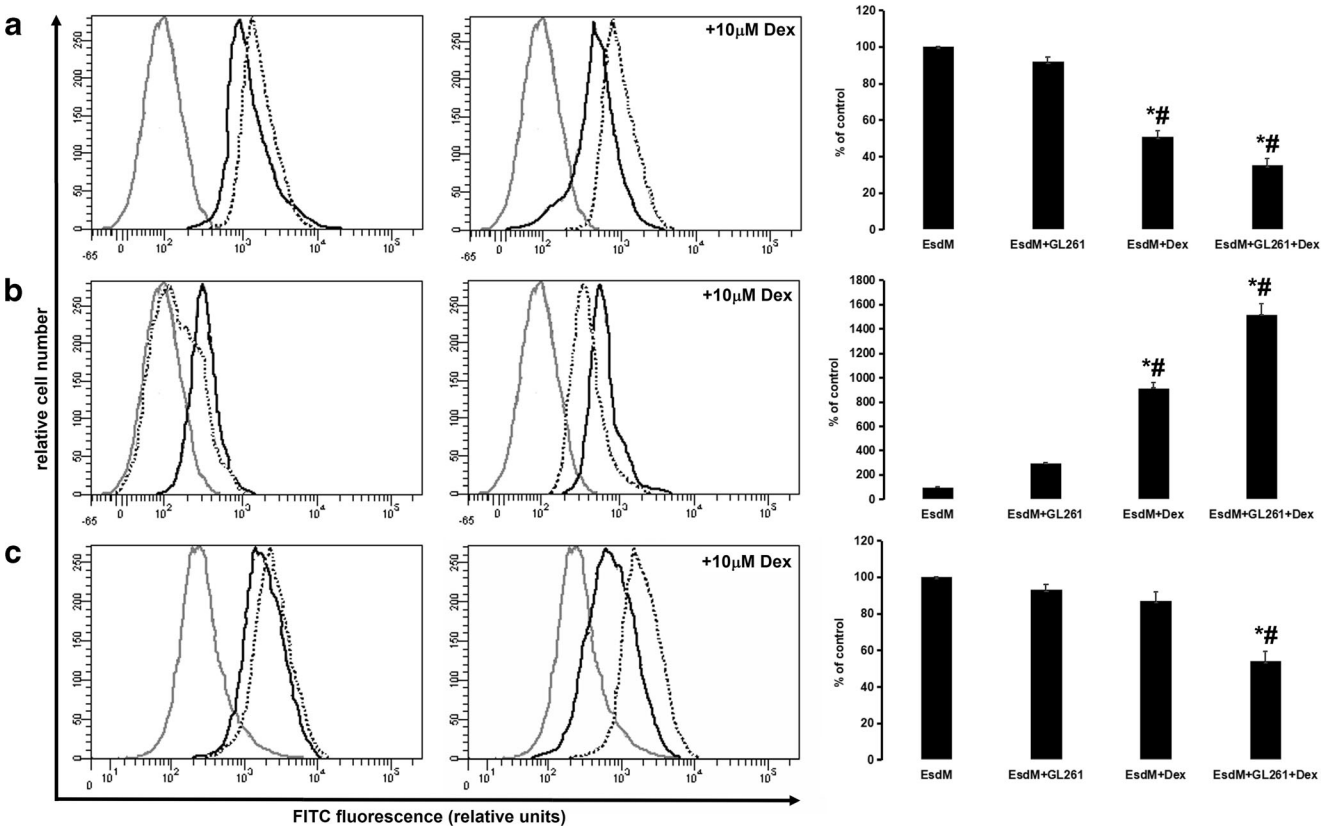

Fig. 5 Flow cytometric analysis of (A) IL-1β, (B) IL-10, and (C) Iba-1 ESdM cells grown in monocultures or co-cultures and exposed to Dex. Representative histograms and corresponding bar graphs were derived from analysis of 10000 cells and show isotype control (grey line), monocultured cells (dropped line), and co-cultured cells (black line). Each column presents mean ± SEM of 3–5 independent experiments. Data are presented as a percentage of control group (100%); *$p < 0,05$ vs. ESdM; $^{\#}p < 0,05$ vs. ESdM+GL261

of host immune defense [25–27]. Similarly, the upregulated expression of Siglec-15 was found on human tumor-infiltrating macrophages but not in normal tissues, and the interplay between Siglec-11 and α2.8-linked sialic acid in the brain was described as inhibitory mechanism of immune response [28–31]. The several retrospective analysis reports that high-dose dexamethasone therapy is closely linked to decreased survival of patients with glioma, perhaps through a strong inhibition of immune functions [12, 15, 32]. We have used these findings to establish the effect of high dose of Dex on microglia–glioma interplay focusing on Siglec-E and sialic acid expression. In response to Dex, GL261 cells showed elevated expression of α2.8-linked polysialic acid. Despite glucocorticosteroids were shown as inhibitors of NCAM's polysialylation in rodent hippocampal neurons, and brain atrophy inducers, there are increasing evidences that protein glycosylation in malignant cells is dramatically altered by these agents and feature with an increase in sialic acid moieties [33–36]. However, Dex inhibited cell growth but extended viability which was related to altered sialic acids and might correspond to high biological activity of glioma cells. Additionally, the present study revealed the dependence of Siglec-E expression on the anti-inflammatory therapy. It was in line to previous studies concerning altered Siglecs expression during clinical management with glucocorticosteroids. The published data suggest that corticosteroids, depending on the distribution of Siglecs, may cause opposite effects through altered inhibitory and/or activatory signaling pathways [10, 37, 38]. In activatory Siglec-14 overexpressing patients, the glucocorticoid-based therapies provoke adverse modulation of immune response, whereas the inhibitory signaling through ITIM-coupled (immunoreceptor tyrosine-based inhibition motif) Siglecs, such as murine Siglec-E or human Siglec-9, may promote immune evasion in cancer. Despite this molecular mechanism is not fully understood, the participation of inhibitory Siglecs in limiting patient's capacity to develop effective immune response is highly possible. The differences in effects of Dex on sialic acid-Siglec-E checkpoint in monocultured and co-cultured cells are a major finding in this study. The quantitative flow cytometric analysis detected selective upregulation of PSA–NCAM and Siglec-E on co-cultured glioma and microglial cells, respectively. Given the experiments were performed in transwell system, the alteration in PSA–NCAM and Siglec-E was independent

of cellular physical interactions but regulated by dexamethasone and/or soluble-specific glioma- and microglia-derived mediators which are crucial in the regulation of diversity of macrophage/microglia phenotype. The estimation of sialoglycan-Siglec-E axis and Siglec-related changes in the activity of microglia obtained from mixed glioma-microglia monolayer cultures may be an interesting observation in the future. The engagement of blocking antibodies, ligand/receptor knockdown, and modulators of sialylation might be helpful in the evaluation of the involvement of sialic and-Siglec checkpoints in the immune behavior of the glioma-microglia direct interaction. The performed Siglec-E Fc Chimera-binding analysis reflects only the possible participation of Siglec-E in the immune regulation of the cross talk between glioma and microglia exposed to Dex. Macrophages and microglia exhibit a high phenotypic plasticity which is changed in response to environmental stimuli. On the other hand, proinflammatory (M1) or immunosuppressive (M2) phenotype is related to the type, stages, and severity of pathology [39–41]. Many studies have shown that in neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease, M1 microglial cells predominate in injury areas and mediate chronic neuroinflammation, whereas M2 microglia are suppressed [42]. In contrast, malignant tumor-infiltrating macrophages, called tumor-associated macrophages (TAMs), display predominantly M2 phenotype, which favor tumor cell growth, survival, and metastasis [43]. Furthermore, macrophages regulate M1/M2 phenotype in response to pharmacological stimulation. Tedesco et al. have demonstrated that macrophages acquire the immunosuppressive M2 phenotype after Dex treatment [44]. In the present study, the exposure of microglia to 10 μM Dex increased intracellular expression of IL10 but limited IL1β and Iba-1. This effect was intensified in presence of glioma cells and accompanied by alteration in Siglec-E expression and cell membrane α2.8-sialylation. A growing body of evidence suggests that the interplay between inhibitory Siglecs and sialylated malignant cells induces a TAMs-like phenotype and thereby disturbs immune surveillance in the tumor microenvironment [45]. Beatson et al. have demonstrated that sialoepitopes of mucin MUC1 isolated from CHO cells exert suppressive effects in cultured primary monocytes through the engagement of Siglec-9 [25]. On the other hand, the contrary effects were observed in adenocarcinoma A549 cell line, colon cancer LS180 cells, and chemically induced sarcoma. According to Läubli et al. inhibitory Siglecs in these cancers limit the M2-polarized phenotype [26]. The presented differences could be closely related to cancer cell type and glycosylation pattern of plasma membrane. The participation of Siglec-sialic acid interplay in glioma progression is poorly understood. Previous studies have shown that glioblastoma multiforme (GBM) biopsies contain up to 50% of TAMs which display immunosuppressive phenotype [46, 47]. In these tumors, α2.8-sialylation of NCAMs was strongly correlated with progressive potential and worse prognosis [22, 23]. Furthermore, myeloid-derived suppressor cells (MDSC) isolated from fresh glioma tissue express Siglec-3, Siglec-5, Siglec-7, and Siglec-9 at a gene and protein level [46, 48]. To simulate the interplay between Siglec-E and sialyl epitopes in naïve and Dex-treated cells, we analyzed the affinity of Siglec-E/Fc protein to GL261 cells. Despite Dex-induced sialylation was confirmed, the binding of Siglec-E Fc/Fc protein was reduced in monocultured glioma cells. Surprisingly, this process was strongly reduced in co-cultured cells but increased in response to Dex. Better understanding of these processes and further studies on their mechanisms are still needed.

Conclusions

The present data suggest that Dex-based therapy, as a part of antitumor management, can have harmful impact on antitumor host immunity through the interplay between ITIM-coupled Siglecs and sialoglycans. The proposed hypothesis may help to determine the role of glucocorticoids in the immobilization of microglia in the glial tumors and reflect the need to activate immune surveillance by altering the function of Siglec receptors and their sialylated receptor ligands. The consideration of Siglec receptors in the regulation of M1/M2 phenotypes within brain tumor therapy may reduce Dex-related risk factors and improve the survival prognosis. In the future, it may be an important therapeutic direction of immunosuppression associated with cancer.

Funding information This work was funded by grant from Medical University of Bialystok (N/ST/ZB/18/001/1166) and grant from National Science Centre (2017/01/X/NZ3/01493).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

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