Serum IgG-induced microglial activation enhances neuronal cytolysis via the NO/sGC/PKG pathway in children with opsoclonus-myoclonus syndrome and neuroblastoma

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Abstract

Background

Opsoclonus-myoclonus syndrome (OMS) is a rare neurological disease. Some children with OMS also have neuroblastoma (NB). We and others have previously documented that serum IgG from children with OMS and NB induces neuronal cytolysis via several signaling pathways. However, mechanisms underlying OMS remain unclear. Here we investigated whether nitric oxide (NO) from activated microglias and its cascade contribute to neuronal cytolysis in pediatric OMS.

Methods

The activation of cultured cerebral cortical and cerebellar microglias incubated with sera or IgG isolated from sera of children with OMS and NB was measured by the expression of the activation marker, cytokines and NO. Neuronal cytolysis was determined after exposing to IgG-treated microglia conditioned media. Using inhibitors and activators, the effects of NO synthesis and its intracellular cascade, namely soluble guanylyl cyclase (sGC) and protein kinase G (PKG), on neuronal cytolysis were evaluated.

Results

Incubation with sera or IgG from children with OMS and NB increased the activation of cerebral cortical and cerebellar microglias, but not the activation of astrocytes or the cytolysis of glial cells. Moreover, the cytolysis of neurons was elevated by conditioned media from microglias incubated with IgG from children with OMS and NB, which was relieved by the inhibitors of NO signaling. By contrast, neuronal cytolysis was exacerbated by the activators of NO signaling but not proinflammatory cytokines. Neuronal cytolysis was suppressed by pretreatment with the microglial inhibitor minocycline, a clinically tested drug. Finally, increased microglial activation did not depend on the Fab fragment of serum IgG.
Conclusions

Serum IgG from children with OMS and NB potentiates microglial activation, which induces neuronal cytolysis through the NO/sGC/PKG pathway, suggesting an applicability of microglial inhibitor as a therapeutic candidate.

Background

Opsoclonus-myoclonus syndrome (OMS) is a rare but devastating neurological disease, characterized by opsoclonus, myoclonus and ataxia. Most patients suffer from persistent deficits in cognition, neurology and behavior. Some children with OMS also have neuroblastoma (NB), although varied percentages have been reported [1-5]. Previously, we have documented that the insulin-like growth factor 1 (IGF-1)/phosphoinositide 3-kinase cascade alleviates neuronal cytolysis induced by serum IgG from children with OMS and NB [6], others also reported that the phosphorylation of extracellular signal-regulated kinase contributes to neuronal cytolysis in pediatric OMS [7]. Yet, the cellular and molecular mechanisms associated with neuronal cytolysis underlying pediatric OMS remain unclear.

Microglias are major immune effectors in the central nervous system (CNS) and have an important physiological function in inflammation [8]. Notably, patients with pediatric OMS exhibit increased expression of a microglial marker and proinflammatory cytokines in cerebrospinal fluid (CSF) and some children with OMS are post-infectious [10, 11]. Furthermore, serum IgG or autoantibody existed in patients enhances microglial activation in Parkinson disease (PD) [12], amyotrophic lateral sclerosis (ALS) [13] and systemic lupus erythematosus (SLE) [14-16]. Thus, it is reasonable to hypothesize that serum IgG from children with OMS and NB may impact the activation of microglia.{Heck, 1999 #57}

Activated microglias release various neurotoxic molecules [8, 17], causing the loss of neurons in neurodegenerative diseases [18-20]. In patients with pediatric OMS and NB, we
and others have revealed that sera or IgG purified from sera causes cytolysis in cultured cerebellar and cerebral neurons [6, 7]. Additionally, activated microglias can synthesize and release nitric oxide (NO), which can activate the downstream soluble guanylyl cyclase (sGC) and protein kinase G (PKG) in neurons, thus exerting neuronal death by mitochondrial dysfunction [21, 22] and increased neuronal susceptibility to mitochondrial dysfunction [23]. However, whether the cytolysis of neurons can be induced by activated microglias in children with OMS and NB via the NO/sGC/PKG cascade is still unknown. Here, we found that sera or serum IgG from children with OMS and NB induces the activation of cultured cerebral cortical and cerebellar microglias, but not astrocytes. Neuronal cytolysis is exerted by conditioned media from microglias treated with IgG from children with OMS and NB. Furthermore, the cytolysis of neurons is alleviated by the inhibitors of NO signaling, whereas it is exacerbated by the activators of NO signaling, which is blocked by the microglia inhibitor minocycline. Finally, increased microglial activation may depend on the Fc fragment of serum IgG rather than the Fab fragment. Our results suggest that serum IgG from children with OMS and NB increases the activation of cultured microglias, leading to the upregulation of NO, which subsequently activates sGC and PKG in neurons, thereby inducing neuronal cytolysis. These results suggested that the microglial inhibitor, such as minocycline, may serve as a plausible therapeutic candidate.

Methods

Subjects

Enrolled participants

This project was reviewed and approved by the Ethics Committees of Beijing Children's Hospital, Capital Medical University (No. IEC-C-028-A10-V.05). Parents of participants signed written informed consent. Children were enrolled between January 2015 and December 2019 from Beijing Children’s Hospital using internationally accepted diagnostic
criteria for OMS [24] and they were Han Chinese. Ten children with OMS and NB were collected (OMS+NB). Control groups were 20 children with NB without OMS (NB), 10 age and sex-matched healthy children (healthy control), 10 children with juvenile idiopathic arthritis (JIA) to reveal whether the effects on microglia are common to all IgG-related diseases, and 6 children with anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis to investigate whether the effects on microglia are common to all autoantibody-mediated disorders of the CNS. Two individuals in the OMS+NB group, 3 individuals in the NB group, and all the individuals in the anti-NMDAR encephalitis were newly collected, while the rest subjects were previously described in our another study [6].

**Clinical information**

Demographic and clinical information is shown in Table 1. The age and gender of 5 groups, as well as tumor stage of the NB and OMS+NB groups were not statistically different. The age of subjects was at the time of our study, thus it may be older than the age of onset. Although a higher female sex ratio of approximately 1.2 was already evident in toddlers with OMS [2-4], only 10 children with OMS and NB in our study may not enough to reflect the gender difference, other literatures with a small sample size also showed much lower (2 female/3 male) [25] or higher ratios (9 female/2 male, 11 female/4 male) [7, 26]. Moreover, the age and gender of subjects may be affected by whether timely visiting to hospitals, misdiagnosis as other diseases before, genetic background and whether willing to enroll in the study. All the children in the JIA group had systemic onset JIA.

Electroencephalogram (EEG) results of OMS patients were clinically normal, while EEG results of all the children with anti-NMDAR encephalitis were abnormal. All the blood samples of the NB and OMS+NB groups were recruited before surgery for NB, and most of blood samples were recruited before any treatment. After collection, sera were stored at −80 °C.
According to criteria for evaluating OMS [1], the degree of ataxia, opsoclonus, ataxia/gait, ataxia/stance and sleep/mood disturbance of patients with OMS was graded from 0 to 3, and summarized into OMS score in Table 2. The frequency of symptom was similar with a previous report [4].

**IgG purified from sera and the Fab fragment preparation**

As we previously used [6], 100 μl protein G agaroses (Thermo Fisher Scientific, California, USA) were applied to sera. The IgG-free fraction in supernatants was collected after centrifugation at 10000 g for 10 min. The IgG fraction was eluted by 0.1 M glycine-HCl (pH 2.7) after washing with 0.01 M phosphate buffer saline (PBS), and then neutralization buffer was added. The BCA assay (Pierce, Rockford, USA) was applied to determine IgG concentration. The Fab fragment of IgG was prepared by enzymatic digestion [16]. Pepsin (Sigma-Aldrich, St. Louis, MO, USA) was mixed with IgG at a ratio of 1:20, and then incubated at 37 °C for 6 h. Adjusting the solution pH to 7.4 to stop the digestion. HiTrap Protein G HP columns (GE Healthcare, Freiburg, Germany) and Amicon Ultra-15 centrifugal filters (Merck Millipore, Massachusetts, USA) were used to obtain the Fab fragment.

**Primary culture of neurons**

In order to avoid the direct effects of serum IgG on neurons [6], instead of co-culture of microglias and neurons and treating both kinds of cells with IgG at the same time, here we cultured microglias and neurons separately. IgG from children was added into the culture media of microglias to collect conditioned media, and then conditioned media were filtered with protein G agarose to get the IgG-free fraction. Finally, culture media of neurons were replaced with IgG-free conditioned media from microglias. This method has been used before [27].

For primary culture of neurons, the cerebral cortex and cerebellum of 16 to 18 days old Sprague Dawley rat embryos were provided by the Department of Experimental Animal
Sciences, Capital Medical University (Beijing, China) and methods were the same as those in our previous report [6]. Notably, 24 h after seeding neurons, cytosine arabinoside at 10 μM was added to suppress glial proliferation and prepare neuron-enriched cultures.

**Primary cultures of microglias or astrocytes separately**

**Monoculture**

Primary cultures of microglias [28] or astrocytes [29] were prepared as previously described. Sprague Dawley rats at postnatal day 1 were used. The cerebral cortex and cerebellum were cut and digested in 0.1% trypsin at 37°C for 20 min, and then tissues were triturated gently. After filtered through a 30 μm cell drainer (BD Biosciences Discovery Labware, Bedford, MA, USA), cell suspensions were centrifuged at 1500 rpm for 5 min and resuspended in Dulbecco’s modified Eagle medium (Life Technologies, Rockville, MD, USA) with 10% inactivated low-endotoxin fetal bovine serum. For primary culture of microglias, mixed glial cultures were shaken after seeding onto lysine-coated dishes for 7 days at 200 rpm for 2 h. After pelleting, floating cells were subcultured at $3.0 \times 10^5$ cells/2000 μl medium/well in 6-well plates or at $1.0 \times 10^4$ cells/200 μl medium/well in 96-well plates. One day after subculturing, medium was fully replaced by macrophage serum-free medium, and cells were used 2 days later. Microglias to examine cluster of differentiation 11b (CD11b), a marker of microglial activation, and conditioned media to detect cytokines or neuronal cytolysis were got from the same well.

For primary culture of astrocytes, mixed glial cultures were shaken after seeding onto dishes for 10 days at 200 rpm for 2 h, trypsinized (0.05%) and subcultured at $3.0 \times 10^5$ cells/2000 μl medium/well in 6-well plates with glial culture medium. One day after subculturing, plates were manually shaken for 1 min and medium was fully replaced with glial culture medium. Cells were used 7-10 days later.
Incubation with sera or IgG

As we used previously [6], the culture medium of microglia or astrocytes was replaced with a fresh medium containing sera or IgG from children (6-well plates in a 2000-μl volume: 40 μl sera of children, 1:50 diluted; 100-200 μg IgG; 1000-1400 μg IgG-free fraction; 96-well plates in a 200-μl volume: 4 μl sera of children, 1:50 diluted; 10-20 μg IgG) and incubated for 48 h before performing ELISA tests or getting conditioned media.

To explore whether increased microglial activation are simply induced by higher concentration of IgG, commercially available human IgG (Sigma-Aldrich) was dissolved in sterile normal saline and was added into each well with medium (10 μg/well) at the final concentration of 0.1 μg/μl, the largest concentration in the OMS+NB group. Each serum or IgG of an individual parallelly treated 3 wells of glial cells.

Inhibitors, activators and recombinant cytokines

To investigate the role of NO and its intracellular cascade in neuronal cytolysis, the NO synthesis inhibitor 7-nitroindazole (7-NINA) was added into the media of microglia 30 min before treatment with the serum IgG fraction, whereas the sGC inhibitor 1H-1,2,4 oxadiazolo-4,3-a quinoxalin-1-one (ODQ) or thePKG inhibitor Rp-8Br-PET-cGMP (all from Tocris Biosciences, Bristol, UK) was added into the culture media of neurons 30 min before the replacement of conditioned media from microglia. While 7-NINA or Rp-8Br-PET-cGMP was dissolved in normal saline and added into the medium to make a final concentration of 10 μM or 1 μM, ODQ was dissolved in 5% dimethyl sulfoxide (DMSO, Sigma-Aldrich) at a final concentration of 10 μM. Moreover, pharmacological activators of the NO signaling pathway were also tested. The NO-donor S-nitroso-N-acetylpenicillamine (SNAP) was added into the media of microglia 30 min before treatment of IgG from children, while the sGC activator 3-5-hydroxymethyl-2-furyl-1-benzyl-indazole (YC-1) or the PKG activator 8Br-cGMP (all from Tocris Biosciences, Bristol, UK) was added into the culture media of
neurons 30 min before the replacement of IgG-treated microglia conditioned media.

Whereas SNAP or YC-1 was dissolved in 5% DMSO and added into the medium at 100 µM or 1µM, 8Br-cGMP was dissolved in normal saline at a final concentration of 1 µM. The final concentration of DMSO was 0.01%. Additionally, minocycline (Sigma-Aldrich), which inhibits microglial activation, was given 30 min before SNAP or IgG from children, which was freshly dissolved to 20 µM in normal saline. Finally, recombinant IL-1β, IL-6, TNF-α or MCP-1 (all from R & D systems, Minneapolis, USA) was used 30 min before the replacement of neuronal medium. Cytokines were dissolved in 0.01 M PBS containing 0.1% bovine serum albumin (BSA) and were added into the culture medium of neurons at 100pg/ml, 100 pg/ml, 80 ng/ml and 20 ng/ml respectively. The concentrations of inhibitors, activators and recombinant cytokines were selected based on previous studies [23, 30].

Enzyme-linked immunosorbent assay (ELISA)

Cultured microglias or astrocytes were lysed [31] and commercially available ELISA kits were applied to explore the expression of CD11b (Cloud-Clone Corp., Houston, TX, USA) and glial fibrillary acidic protein (GFAP) (Abcam, Cambridge, MA, USA) following manufacturer’s instructions. The concentrations of CD11b and GFAP were normalized to the amount of total protein in rat microglias and astrocytes. The level of total protein was detected at the same time as ELISA using the BCA protein assay kit. Moreover, the concentrations of proinflammatory cytokines IL-1β (Bio-Swamp, Wuhan, China), IL-6 (Promocell, Heidelberg, Germany), TNF-α (Abcam, Cambridge, MA, USA) and MCP-1 (Abcam, Cambridge, MA, USA) in the culture medium of microglias were also tested by commercially available ELISA kits.

Nitrite assay

Nitrite is a product resulting from the reaction of NO with molecular oxygen, which were
measured in rat microglia by adding 50 μl of cell culture media with an equal volume of Griess reagent (0.1% naphtyletylenediamine dihydrochloride, 1% sulphanilamide, 2.5% phosphoric acid, Sigma-Aldrich) in 96-well plates. The absorbance at 550 nm was determined. Standard curves with sodium nitrite (NaNO₂, Sigma-Aldrich) were generated to calculate the concentrations of nitrite [28].

**Cytolysis assay**

Cytoplasmic lactate dehydrogenase (LDH) was used as an indicator of plasma membrane-damaged cells, measuring by the cytotoxicity detection kit (Roche, Indianapolis, USA) [6]. Cells were seeded onto 96-well plates at 1.0×10⁴ cells/200 μl medium/well. After cultured for 16 h, the medium of neurons was replaced with 200 μl conditioned media from microglia treated with IgG, and further incubated for 48 h. An equal volume of neuron culture supernatants and reaction mixture was mixed followed by incubation for 30 min. LDH was detected by the absorbance at 490 nm. Maximum LDH release was induced by 1% Triton X-100, while background control was untreated neurons. The percentage of specific cytolysis was determined using the following formula: [{(experimental value-background control)}/(maximum value-background control)]×100.

**Statistical analysis**

All the data were expressed as mean ± SEM. Statistical analyses were carried out using GraphPad Prism version 6.0 software for Windows (GraphPad Software Inc., San Diego, USA). One-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test was used for multiple analyzation. The statistically significant level was considered at p <0.05.

**Results**

The activation of cerebral cortical and cerebellar microglia increased by sera or
**IgG from children with OMS and NB**

Besides cerebellum, emerging evidence has shown that the cerebral cortex has structural and functional changes in OMS patients, for most OMS patients have neurological handicaps in cerebral functions, such as deficits in attention, memory and language [4, 32]. Additionally, brain imaging of OMS patients shows changes in the cerebrum. Cerebral cortical thickness is reduced across the motor and visual areas in patients with pediatric OMS [33]. A patient with OMS revealed significant nodular enhancing lesions at gray-white junction of bilateral cerebral hemispheres by magnetic resonance imaging [34]. Another patient showed decreased metabolism in the bilateral occipital lobes and increased functional connectivity, including the primary and motion-sensitive visual cortex [35]. Therefore, both rat cerebral cortical and cerebellar microglias were exposed to sera or the IgG fraction isolated from sera of children with OMS and NB.

The expression of CD11b, a marker of microglial activation, was upregulated in cerebral cortical microglias incubated with sera from children with OMS and NB (3.41 ± 0.32 ng/mg total protein OMS+NB, 0.99 ± 0.09 ng/mg total protein NB, 1.09 ± 0.12 ng/mg total protein Healthy control, \( p < 0.001 \) vs NB, \( p < 0.001 \) vs Healthy control), whereas CD11b concentration was not statistically changed by sera of children with only NB at least under our experimental conditions (Fig. 1a). Moreover, IgG isolated from sera upregulated CD11b expression from 0.91 ± 0.10 ng/mg total protein in the NB group and 0.88 ± 0.07 ng/mg total protein in the healthy control group to 2.95 ± 0.23 ng/mg total protein in the OMS+NB group (\( p < 0.001 \) vs NB, \( p < 0.001 \) vs Healthy control, Fig. 1c). With respect to cerebellar microglias, the concentration of CD11b was also increased after incubation with sera or IgG from the OMS+NB group compared with the NB or healthy control group (Fig. 1b, d). However, neither commercially available human IgG nor IgG from children with JIA or anti-NMDAR encephalitis had a significant impact on the concentration of CD11b (Fig.
suggesting that upregulation of CD11b induced by serum IgG from children with OMS and NB is not simply induced by increased dose of IgG, is not common to all diseases with IgG, and is not common to all autoantibody-mediated disorders of the CNS. In addition, no alteration of CD11b concentration was observed after treatment with the IgG-free fraction (Fig. 1e, f), which suggested that the upregulation of CD11b induced by sera mainly depends on the IgG fraction.

To further investigate the effects of serum IgG from children with OMS and NB on microglial activation, the concentrations of proinflammatory cytokines (including IL-1β, IL-6, TNF-α and MCP-1) and NO in the media of cerebral cortical and cerebellar microglias were detected by ELISA. The releases of IL-1β, IL-6, TNF-α and MCP-1 from cerebral cortical microglias exposed to OMS+NB sera were elevated (IL-1β: 105.4 ± 10.48 pg/ml OMS+NB, 43.99 ± 4.29 pg/ml NB, 39.83 ± 5.64 pg/ml Healthy control, p< 0.001 vs NB, p< 0.001 vs Healthy control; IL-6: 107.8 ± 11.20 pg/ml OMS+NB, 61.61 ± 3.67 pg/ml NB, 49.11 ± 5.53 pg/ml Healthy control, p< 0.01 vs NB, p< 0.001 vs Healthy control; TNF-α: 169.1 ± 14.32 pg/ml OMS+NB, 100.3 ± 9.15 pg/ml NB, 90.24 ± 6.76 pg/ml Healthy control, p< 0.001 vs NB, p< 0.001 vs Healthy control; MCP-1: 294.1 ± 9.30 pg/ml OMS+NB, 181.7 ± 7.93 pg/ml NB, 184.7 ± 9.59 pg/ml Healthy control, p< 0.001 vs NB, p< 0.001 vs Healthy control), whereas incubation with NB sera had no such effect (Fig. 2a1).

Furthermore, the levels of IL-1β, IL-6, TNF-α and MCP-1 secreted from cerebral cortical microglias were increased to 109.6 ± 12.09 pg/ml, 133.2 ± 11.28 pg/ml, 225.3 ± 9.84 pg/ml and 324.2 ± 6.68 pg/ml after incubation with OMS+NB IgG, compared with NB IgG or healthy control IgG (p< 0.001 vs NB, p< 0.001 vs Healthy control, Fig. 2b1). In cerebellar microglias, similar impacts of sera and IgG were observed (Fig. 2c1, d1). Consistent with the results of cytokines, NO expression was also improved in the media of cerebral cortical (Fig. 2a2, b2) and cerebellar microglias (Fig. 2c2, d2) treated with OMS+NB sera.
or IgG. Together with the aforementioned CD11b expression, these results suggested that the activation of cultured cerebral cortical and cerebellar microglias was upregulated by serum IgG from children with OMS and NB.

In order to explore whether serum IgG-enhanced activation is specific to microglias or common to glial cells in the CNS, we detected the activation of cerebral cortical and cerebellar astrocytes. Using the marker of astrocytic activation, we found that the expression of GFAP was not significantly changed in cerebral cortical (Fig. 3a, c) and cerebellar astrocytes (Fig. 3b, d) incubated with sera or IgG from the OMS+NB group compared with the NB group and healthy control group at least under our experimental conditions, suggesting that the enhancement of activation induced by IgG from children with OMS and NB is specific to microglias, but not astrocytes.

Previously we have revealed that serum IgG from children with OMS and NB induces cytolysis in cultured neurons [6], whether serum IgG impacts the cytolysis of microglias or astrocytes needs further study. We observed that neither the cytolysis of cerebral cortical microglias (Fig. 4a, c) and cerebellar microglias (Fig. 4b, d) treated with OMS+NB sera or IgG, nor the cytolysis of cerebral cortical astrocytes (Fig. 4e, g) and cerebellar astrocytes (Fig. 4f, h) treated with OMS+NB sera or IgG was statistically changed, which suggested that preincubation with sera or IgG from children with OMS and NB specially upregulates microglial activation, rather than the cytolysis of microglias and astrocytes at least under our experimental conditions.

**The cytolysis of neurons induced by conditioned media from microglias treated with OMS+NB IgG**

It has been established that activated microglias contribute to neuron death by secreting various neurotoxic molecules in multiple disorders [8, 17] and serum IgG from patients with OMS and NB enhances neuronal death [6, 7, 36], we therefore explored whether
neuronal cytolysis is enhanced by microglial activation in OMS. After getting rid of the remaining IgG, serum IgG-treated microglia conditioned media were collected and replaced the culture media of neurons. As expected, incubation with conditioned media from cerebral cortical and cerebellar microglias increased the cytolysis of neurons in the same brain regions (Cerebral cortical neuron: 31.95 ± 1.09% OMS+NB, 13.45 ± 1.12% NB, 14.53 ± 1.08% Healthy control; Cerebellar neuron: 31.21 ± 1.25% OMS+NB, 10.50 ± 1.27% NB, 12.75 ± 1.34% Healthy control; p < 0.001 vs NB, p < 0.001 vs Healthy control, Figure 5a, b). Moreover, conditioned media from cerebral cortical microglias to cerebellar neurons or the exchanged situation had similar results (Cerebral cortical neuron: 25.67 ± 2.71% OMS+NB, 9.00 ± 1.21% NB, 9.85 ± 1.95% Healthy control; Cerebellar neuron: 27.24 ± 2.55% OMS+NB, 11.79 ± 1.06% NB, 10.03 ± 2.86% Healthy control; p < 0.001 vs NB, p < 0.001 vs Healthy control, Fig. 5c, d). In contrast, we observed no alteration in the cytolysis of neurons treated with conditional media from astrocytes (Fig. 5e, f). Taken together, these results suggested that conditioned media from cerebral cortical or cerebellar microglias rather than astrocytes induce the cytolysis of neurons.

**The effects of inhibitors or activators of NO/sGC/PKG pathway on conditioned media-induced neuronal cytolysis**

To investigate the mechanisms of cytolysis induced by conditioned media from microglias treated with OMS+NB IgG, we focus on the NO/sGC/PKG pathway, for its role in neuronal death is well documented [21-23] and the production of NO from microglias was raised by OMS+NB IgG (Fig. 2a2-d2). The cytolysis of cerebral cortical and cerebellar neurons induced by conditioned media was alleviated by pretreatment with the NO synthesis inhibitor 7-NINA before OMS+NB IgG to microglias (Cerebral cortical neuron: 6.50± 1.46% 7-NINA, OMS+NB vs 26.91 ± 1.45% Saline, OMS+NB, p <0.001; 26.91 ± 1.45% Saline, OMS+NB vs 8.36 ± 1.10% Saline, NB, p <0.001; Cerebellar neuron: 11.30 ± 3.14% 7-NINA,
OMS+NB vs 28.72 ± 2.43% Saline, OMS+NB, p <0.001; 28.72 ± 2.43% Saline, OMS+NB vs 11.50 ± 1.83% Saline, NB, p <0.001; Fig. 6a, b). Similar to 7-NINA, pretreatment with the sGC inhibitor ODQ or the PKG inhibitor Rp-8Br-PET-cGMP ameliorated the cytolysis of cerebral cortical and cerebellar neurons (Fig. 6c-f).

Moreover, the activators of NO synthesis and NO-activated intracellular pathway were also examined. The cytolysis of cerebral cortical neurons was exaggerated after incubation with conditioned media from microglias pretreated with the NO-donor SNAP 30 min before OMS+NB IgG (45.05 ± 1.74% SNAP, OMS+NB vs 26.09 ± 2.08% DMSO, OMS+NB, p <0.001; 26.09 ± 2.08% DMSO, OMS+NB vs 7.12 ± 0.98% DMSO, NB, p <0.001; Fig. 7a), and the cytolysis of cerebellar neurons was exacerbated by treatment with SNAP before OMS+NB IgG (51.51 ± 2.55% SNAP, OMS+NB vs 35.27 ± 1.87% DMSO, OMS+NB, p <0.001; 35.27 ± 1.87% DMSO, OMS+NB vs 8.06 ± 1.21% DMSO, NB, p <0.001; Fig. 7b). Pretreatment with the activator of sGC or PKG, namely YC-1 or 8Br-cGMP, also exacerbated cerebral cortical and cerebellar neuronal cytolysis induced by conditioned media from microglias stimulated with OMS+NB IgG (Fig. 7c-f). In addition, the effects of these above-mentioned activators were at least partly blocked by pretreatment with minocycline, an inhibitor of microglia, in cerebral cortical and cerebellar neurons exposed to OMS+NB IgG-treated microglia conditioned media (Fig. 7a-f). Together with the data of inhibitors, these results suggested that the NO/sGC/PKG cascade plays a vital role in neuronal cytolysis induced by conditioned media from microglias treated with serum IgG from children with OMS and NB, which depends on the activation of microglias.

The expression of proinflammatory cytokines from microglias incubated with serum IgG from OMS+NB children (Fig. 2a1-d1) and in CSF of OMS children [37] were upregulated, which raises the possibility that cytokines may have similar effects on neuronal cytolysis. Unexpectedly, our results showed that the cytolysis of cerebral cortical and cerebellar
neurons induced by conditioned media from microglias preincubated with OMS+NB IgG was not influenced by IL-1β (Cerebral cortical neuron: 32.89 ± 1.70% IL-1β, OMS+NB vs 33.23 ± 1.37% 0.1% BSA, OMS+NB, p >0.05; 33.23 ± 1.37% 0.1% BSA, OMS+NB vs 10.65± 1.76% 0.1% BSA, NB, p <0.001; Cerebellar neuron: 27.55 ± 2.32% IL-1β, OMS+NB vs 31.26 ± 1.88% 0.1% BSA, OMS+NB, p >0.05; 31.26 ± 1.88% 0.1% BSA, OMS+NB vs 11.48 ± 1.39% 0.1% BSA, NB, p <0.001; Fig. 8a, b). Also, enhanced cytolysis of cerebral cortical and cerebellar neurons was not affected by IL-6, TNF-α or MCP-1 (Fig. 8c-h). These results suggested that the application of cytokines does not have similar effects as NO on the cytolysis of neurons induced by IgG-treated microglia conditioned media.

The activation of cerebral cortical and cerebellar microglias by the Fc fragment of serum IgG rather than the Fab fragment

IgG contains the Fab fragment and the Fc fragment. The Fab fragment combines with the targeted antigens, while the Fc fragment interacts with Fcγ receptor (FcγR), which can be expressed on the surface of microglia and other immune effector cells, mediating immune reactions in the brain. In order to explore whether increased microglial activation depends on the Fab fragment or the Fc fragment of serum IgG, we detected the effects of the Fab fragment on the concentrations of CD11b and NO in cerebral cortical and cerebellar microglias. The results showed that the expression of CD11b and the release of NO was not significantly changed in cerebral cortical and cerebellar microglias incubated with the Fab fragment from the OMS+NB group compared with the NB group and healthy control group (Fig. 9a, b). Consistently, the release of NO was not significantly changed in cerebral cortical and cerebellar microglias (Fig. 9c, d). These results suggesting that microglial activation induced by IgG from children with OMS and NB may depend on the Fc fragment of IgG, but not the Fab fragment.

Discussion
Sera and the IgG fraction from children with OMS and NB give rise to the activation of cultured cerebral cortical and cerebellar microglias.

Neuroinflammation in the CNS is a common clinical feature in children with OMS, which is the dominant physiological function of microglias and astrocytes [9]. Moreover, the expression of microglial marker soluble CD14 and proinflammatory cytokines is enhanced in CSF from patients with pediatric OMS [10, 11]. Additionally, serum IgG from patients or autoantibody existed in patients directly enhances microglial activation in PD [12], ALS [13] and SLE [14-16], or initially binds with astrocytes or neurons and further indirectly affects microglial activation [38, 39]. Autoantibodies are also detectable in serum and CSF of children with OMS [25, 26] and may be contained in serum IgG from children with OMS and NB in our study, although we did not identify these autoantibodies and autoantibodies include IgM besides IgG. Thus, we hypothesized that serum IgG from children with OMS and NB may impact the activation of microglias. As expected, our results revealed that sera or IgG from children with OMS and NB upregulated the activation of cultured cerebral cortical and cerebellar microglias.

In contrast, commercial available human IgG, or IgG from children with JIA or anti-NMDAR encephalitis had no such effect, suggesting that upregulation of microglial activation induced by serum IgG from children with OMS and NB is not simply induced by increased dose of IgG, is not common to all IgG-related diseases, and is not common to all autoantibody-mediated disorders of the CNS, indicating specific changes in pediatric OMS at least to some degree. Consistently, we and others found that IgG-induced neuronal cytolysis occurs in pediatric OMS rather than adult OMS [6, 7]. Notably, increased microglial activation is not specific to only OMS, since previous literatures have documented that serum IgG from patients with PD [12] or ALS [13] enhances the activation of microglia and the production of NO, and serum IgG from patients with SLE
induced behavioral changes are mediated by microglial activation [14-16]. Moreover, several autoantibodies found in patients with OMS [40-43] also exist in other diseases, such as autoantibody against glycine receptor in progressive encephalomyelitis with rigidity and myoclonus [42, 44] or autoantibody against glutamic acid decarboxylase in stiff-person syndrome [44].

Unlike microglial activation, astrocytic activation by serum IgG from children with OMS and NB was not observed by us, indicating that microglia reactivity may be a special mechanism. This notion is supported by studies demonstrating that human immunodeficiency virus infection increases microglial activation but not astrocytic activation [14], and administration of AMD3100 alleviates the pathology of ALS by decreasing microglial activation without affection of astrocytes [17]. However, we cannot completely exclude the role of astrocytes, the activation of astrocytes may be improved by conditioned media from microglia treated with serum IgG from OMS patients, rather than direct stimulation by serum IgG. Furthermore, distinct from the upregulated cytolysis of neurons [6], we observed that the cytolysis of microglia and astrocytes by serum IgG from children with OMS and NB was not changed.

Some cases of pediatric OMS are paraneoplastic and are associated with NB, although varied percentages have been reported [1-5], the remaining cases are believed to be post-infectious or resulted from NB that has regressed prior to onset of symptoms [49]. In adults, patients with non-paraneoplastic OMS have better outcomes with fewer relapses [42], while in children, no significant difference in viral-like prodrome and neurological outcome is noted between paraneoplastic and non-paraneoplastic OMS [1, 50]. However, whether there is a distinction of paraneoplastic and non-paraneoplastic childhood OMS is still unclear. Our present and previous studies showed that microglial activation or neuronal cytolysis can be induced by serum IgG from children with OMS and NB, but not
children with only NB [6], indicating that the IgG fraction may be involved in the pathogenesis of OMS rather than NB. However, we did not obtain sera from children with OMS and without NB, therefore we cannot completely exclude the possibility that the cytotoxicity induced by the sera IgG fraction from children with OMS and NB may be synergistic effects of OMS and NB.

**The NO/sGC/PKG pathway takes part in neuronal cytolysis induced by conditioned media from microglias treated with IgG from children with OMS and NB.**

A growing body of evidence implicates that microglias in the active state can release various neurotoxic molecules [8, 17], causing the loss of neurons in neurodegenerative diseases, including Alzheimer’s disease [18], ALS [20] and retinal degeneration [19]. Moreover, we and others have previously demonstrated that sera or IgG from patients with pediatric OMS and NB induces the cytolysis of cerebellar granular and cerebral cortical neurons [6, 7]. Consistently, here our results showed that neuronal cytolysis was elevated by conditional media from microglias treated with IgG from children with OMS and NB.

Notably, conditioned media from microglias were applied to cultured neurons instead of co-culture of microglias and neurons, and conditioned media were used to cultured neurons after IgG were filtered out, to avoid the direct effects of serum IgG on neurons [6].

NO, synthesized and released by activated microglias, can activate sGC and PKG in neurons, leading to neuronal death by mitochondrial dysfunction [21, 22] and increased neuronal susceptibility to mitochondrial dysfunction [23], similar with its function in pancreatic tissues [45]. On the other hand, exposure to NO has anti-apoptotic function through sGC and PKG in both neuronal and myocardial tissues [46, 47], the opposite influences of NO intracellular signal may be caused by the way (transient or sustained) and concentration of NO, as well as cell type. In the present study, our results showed the
NO/sGC/PKG cascade was a positive regulator of neuronal cytolysis induced by conditioned media from microglias treated with IgG from children with OMS and NB. Of cause, other factors secreted by microglias may also participate in the cytolysis of neurons, for example, IGF-1 involved in neuronal cytolysis induced by serum IgG from children with OMS and NB [6] can be secreted from microglia. Interestingly, our results showed that neuronal cytolysis induced by conditioned media from microglias via NO was almost completely attenuated by the inhibitor of microglia, minocycline. Minocycline has been clinically used for treating infections as an antibiotic, providing a systemic anti-inflammatory effect. Moreover, it produces the anti-inflammatory response in the CNS via microglias and has neuroprotective properties in neurodegenerative diseases, mental illnesses and others [48-52]. In particular, minocycline delays motor alterations, inflammation and apoptosis in experimental models of PD and ALS [52]. Although there are controversies about its efficacy, the relative safety and tolerability of minocycline have led to the launching of various clinical trials, for instance, it may be a possible treatment for patients with acute ischemic stroke [48, 49]. Taken together, the administration of minocycline might be an efficient way to treat pediatric OMS. Although we found that proinflammatory cytokines, namely IL-1β, IL-6, TNF-α and MCP-1, were elevated in the media of microglias treated with IgG from patients with pediatric OMS and NB, and others reported that IL-6 in CSF of untreated OMS patients and IL-1 receptor antagonist in CSF of intravenous immune globulin-treated OMS patients are higher [37], the elevated cytolysis of cerebral cortical and cerebellar neurons induced by conditioned media from microglias preincubated with serum IgG was not influenced by these proinflammatory cytokines, indicating a specific effect of the NO pathway on neuronal cytolysis induced by microglial activation. Possible explanations are that
cytokines secreted from microglias may take part in other functions rather than neuronal cytolysis, such as the recruitment of immune cells [8], and cytokines enhanced in patients with OMS may be produced by other cells, not microglias.

**Microglial activation induced by serum IgG from children with OMS and NB may be through the Fc fragment of IgG.**

FcyR interacts with the Fc fragment of IgG and is expressed on the surface of microglias, releasing of proinflammation cytokines and mediating immune reactions. Several previous studies have documented that IgG combines with FcyR to activate microglias, thus taking part in many diseases. For example, serum IgG from patients with PD significantly induces microglial activation via FcyR in the microglia-supplemented neuronal cultures [12]. Also, in an in vitro PD model, neuron-derived IgG activates microglias through FcyR [53]. Moreover, autoantibodies can activate microglias through FcyR underlie the pathogenesis of autoimmune diseases. For instance, the expression of FcyR crucially contributes to autoantibody-induced tissue injury in experimental epidermolysis bullosa acquisita, an organ-specific autoimmune disease [54]. Consistently, our results showed that increased microglial activation induced by serum IgG from children with OMS and NB may be through the Fc fragment of IgG rather than the Fab fragment. Of interest to note is that we found the activation of microglias was not affected by treatment with commercial available human IgG, IgG from children with JIA or anti-NMDAR encephalitis, consistent with previous findings that IgG combined with microglial FcyR can secrete different molecules, either proinflammatory or anti-inflammatory, depends on the origin and content of IgG [12, 53]. Taken together, it is reasonable to speculate that FcyR may be involved in enhanced microglial activation triggered by the Fc fragment of serum IgG from children with OMS and NB.

**Conclusions**
In the present study (Fig. 10), we demonstrated that incubation with sera or the IgG fraction from children with OMS and NB upregulates the activation of cultured cerebral cortical and cerebellar microglias. Furthermore, neuronal cytolysis is exerted by incubation with conditioned media from microglia treated with IgG from children with OMS and NB. In addition, neuronal cytolysis is relieved by the inhibitors of NO/sGC/PKG signaling, whereas neuronal cytolysis is exacerbated by the activators of this pathway, which is blocked by the microglial inhibitor. Finally, increased microglial activation may depend on the Fc fragment of serum IgG rather than the Fab fragment. Our data provides solid evidence that the activation of cultured microglia and NO signaling induced by serum IgG from children with OMS and NB leads to an increased cytolysis of neurons, suggesting that the inhibitor of microglia, such as minocycline, may serve as a plausible therapeutic candidate for pediatric OMS.

Abbreviations

ALS, amyotrophic lateral sclerosis; ANOVA, analysis of variance; BSA, bovine serum albumin; CD11b, cluster of differentiation 11b; CNS, central nervous system; CSF, cerebrospinal fluid; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; GFAP, glial fibrillary acidic protein; IGF-1, insulin-like growth factor 1; IL-1β, interleukin-1β; IL-6, interleukin-6; JIA, juvenile idiopathic arthritis; LDH, lactate dehydrogenase; MCP-1, monocyte chemoattractant protein-1; NB, neuroblastoma; NMDA, N-methyl-D-aspartate; NO, nitric oxide; ODQ, 1H-1,2,4-oxadiazolo-4,3-a quinoxalin-1-one; OMS, opsoclonus-myoclonus syndrome; PBS, phosphate buffer saline; PD, Parkinson disease; PKG, protein kinase G; sGC, soluble guanylyl cyclase; SLE, systemic lupus erythematosus; SNAP, S-nitroso-N-acetylpenicillamine; TNF-α, tumor necrosis factor-α; YC-1, 3-5-hydroxymethyl-2-furyl-1-benzyl-indazole; 7-NINA, 7-nitroindazole.
Declarations

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
XD carried out ELISA tests and participated in the design of the study, drafted the manuscript. WY performed the purification of IgG and cytolysis studies. QR carried out primary culture of glial cells and neurons. JH and SY participated in the statistical analysis. JW and XW contributed to the design of the study and the revision of the data. HW conceived the study and participated in the analysis of the data and the draft of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Ethic approval was granted by the Ethics Committees of Beijing Children's Hospital, Capital Medical University (No. IEC-C-028-A10-V.05). Parents of participants all signed written informed consent. All animals were handled according to the National Institutes of Health Guide for the Care and Use of laboratory animals (NIH Publication No. 8023, revised 1978) in accordance with protocols approved by the Animal Care and Use Committees of Capital Medical University (No. AEEI-2018-034).

Consent for publication
Not applicable

**Competing interests**

The authors declare that they have no competing interests.

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### Tables

**Table 1. Demographic and clinical data**

|                          | n   | Age enrolled in our study (month) | Gender (female/male) | Tumor stage | Treatment before serum collection |
|--------------------------|-----|----------------------------------|----------------------|-------------|-----------------------------------|
| Healthy children         | 10  | 41.9 ± 1.7                       | 5/5                  | I: 4        | 13 children: no treatment         |
| NB                       | 20  | 44.5 ± 6.4                       | 9/11                 | II: 9       | 7 children: chemotherapy, receive vincristine, cyclophosphamide, cisplatin |
| OMS+NB                   | 10  | 35.6 ± 9.9                       | 5/5                  | I: 3        | 8 children: no treatment          |
|                          |     |                                  |                      | II: 4       | 1 child: steroid                   |
|                          |     |                                  |                      | III: 3      | 1 child: intravenous immune globulin |
|                          |     |                                  |                      | IV: 0       |                                    |
| Juvenile idiopathic arthritis (JIA) | 10  | 40.8 ± 10.5                      | 6/4                  | ——          | 6 children: no treatment          |
|                          |     |                                  |                      | ——          | 3 children: nonsteroidal anti-inf |
|                          |     |                                  |                      | ——          | 1 child: received steroid          |
| Anti-NDMAR encephalitis  | 6   | 54.3 ± 9.9                       | 4/2                  | ——          | 2 children: no treatment          |
|                          |     |                                  |                      | ——          | 4 children: steroid and intraven  |

**Table 2. OMS scores of children with OMS and NB at the time of the blood draw**

| Symptom                  | Grade 0 | Number of children | %   |
|--------------------------|---------|--------------------|-----|
| Opsoclonus               | 1       | 4                  | 90  |
| Myoclonus                | 3       | 5                  | 70  |
| Ataxia/gait              | 4       | 4                  | 60  |
| Ataxia/stance            | 6       | 4                  | 40  |
| Mood/sleep disturbance   | 5       | 5                  | 50  |

**Figures**
CD11b expression in cerebral cortical and cerebellar microglias incubated with OMS+NB sera or IgG. Note that the concentration of CD11b, a marker of microglial activation, was upregulated in cerebral cortical microglias (a) and
cerebellar microglias (b) incubated with sera from children with OMS and NB (OMS+NB) compared with that of the NB or healthy control group. The IgG fraction (c, d) in the OMS+NB group also elevated the level of CD11b, but not the IgG-free fraction (e, f). No alteration was observed in the expression of CD11b after treatment with commercially available human IgG, the IgG fraction from children with juvenile idiopathic arthritis (JIA) or anti-NMDAR encephalitis (c, d).

***p < 0.001, one-way ANOVA, n=10 (Health control), n=20 (NB), n=10 (OMS+NB), n=15 (IgG), n=10 (JIA), n=6 (anti-NMDAR encephalitis).
Cerebral cortical microglia

A1

Sera

Level of cytokines (pg/ml)

IL-1β, IL-6, TNF-α, MCP-1

Healthy control, NB, OMS+NB

A2

Nitrite (µM)

B1

IgG from sera

Level of cytokines (pg/ml)

IL-1β, IL-6, TNF-α, MCP-1

B2

Nitrite (µM)

Cerebellar microglia

C1

Sera

Level of cytokines (pg/ml)

IL-1β, IL-6, TNF-α, MCP-1

C2

Nitrite (µM)

D1

IgG from sera

Level of cytokines (pg/ml)

IL-1β, IL-6, TNF-α, MCP-1

D2

Nitrite (µM)

Figure 2
Proinflammatory cytokines and NO levels from cerebral cortical and cerebellar microglias incubated with OMS+NB sera or IgG. Note that the concentrations of IL-1β, IL-6, TNF-α and MCP-1 were raised in the media of cerebral cortical microglias (a1, b1) and cerebellar microglias (c1, d1) treated with sera or IgG in the OMS+NB group, consistently the expression of nitric oxide (NO) was also enhanced in the media of cerebral cortical microglias (a2, b2) and cerebellar microglias (c2, d2) treated with sera or IgG in the OMS+NB group, whereas incubation with sera or IgG from children with only NB had no such effect (a-d).

**p < 0.01, ***p < 0.001, one-way ANOVA, n=10 (Health control), n=20 (NB), n=10 (OMS+NB).
GFAP concentration in cerebral cortical and cerebellar astrocytes incubated with OMS+NB sera or IgG. Note that the expression of GFAP, a marker of astrocytic activation, was not significantly changed in cerebral cortical and cerebellar astrocytes incubated with sera (a, b) or IgG (c, d) from children with OMS and NB compared with that of NB patients and healthy control at least under our experimental design. p > 0.05, one-way ANOVA, n=10 (Health control), n=20 (NB), n=10 (OMS+NB).
Figure 4
The cytolysis of cerebral cortical and cerebellar glial cells incubated with OMS+NB sera or IgG. Note that neither the cytolysis of cerebral cortical and cerebellar microglia treated with sera (a, b) or IgG (c, d) from patients with pediatric OMS and NB, nor the cytolysis of cerebral cortical and cerebellar astrocytes treated with sera (e, f) or IgG (g, h) from patients with pediatric OMS and NB was statistically influenced at least under our experimental design. p > 0.05, one-way ANOVA, n=10 (Health control), n=20 (NB), n=10 (OMS+NB).
Neuronal cytolysis induced by conditioned media from cerebral cortical and cerebellar microglias. After microglias incubated with OMS+NB IgG, conditioned media were collected, got rid of the remaining IgG and replaced the culture media of neurons. Note that preincubation with conditioned media from cerebral cortical
and cerebellar microglia enhanced the cytolysis of neurons in the same brain regions (a, b) or exchanged brain regions (c, d), while no alteration was observed in the cytolysis of neurons incubated with conditional media from astrocytes (e, f). ***p < 0.001, one-way ANOVA, n=10 (Health control), n=20 (NB), n=10 (OMS+NB).
Neuronal cytolysis alleviated by pretreatment with pharmacological inhibitors of
the NO cascade. Note that the cytolysis of cerebral cortical neurons (a) and cerebellar neurons (b) induced by conditioned media from microglia treated with OMS+NB IgG was relieved by the NO synthesis inhibitor 7-NINA, the cytolysis of cerebral cortical neurons (c) and cerebellar neurons (d) was reduced by the sGC inhibitor ODQ, and the cytolysis of cerebral cortical neurons (e) and cerebellar neurons (f) was abrogated by Rp-8Br-PET-cGMP, an inhibitor of PKG. ***p < 0.001, one-way ANOVA, n=20 (Vehicle, NB; Inhibitor, NB), n=10 (Vehicle, OMS+NB; Inhibitor, OMS+NB).
Conditioned media from cerebral cortical microglia to neuron

- DMSO, NB
- SNAP, NB
- Saline, SNAP, OMS+NB
- Minocycline, SNAP, OMS+NB

Conditioned media from cerebellar microglia to neuron

- DMSO, OMS+NB
- SNAP, OMS+NB
- YC-1, OMS+NB
- Minocycline, YC-1, OMS+NB

Cytoysis (%)

A

B

C

D

E

F

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Figure 7

Neuronal cytolysis exaggerated by pretreatment with pharmacological activators of the NO pathway. Note that the cytolysis of cerebral cortical neurons (a) and cerebellar neurons (b) induced by conditioned media from microglias treated with OMS+NB IgG was exacerbated by the NO-donor SNAP, and the cytolysis of cerebral cortical neurons (c) and cerebellar neurons (d) was also up-regulated by YC-1, an activator of sGC. Similarly, the PKG activator 8Br-cGMP had exaggerated effects on the cytolysis of cerebral cortical neurons (e) and cerebellar neurons (f).

The effects of SNAP, YC-1 or 8Br-cGMP on rat neurons were suppressed by pretreatment with minocycline, an inhibitor of microglias (a-f). ***p < 0.001, one-way ANOVA, n=20 (Vehicle, NB; Activator, NB), n=10 (Vehicle, OMS+NB; Activator, OMS+NB; Saline, Activator, OMS+NB; Minocycline, Activator, OMS+NB).
Figure 8

The effects of preincubated with cytokines on neuronal cytolysis induced by conditioned media from microglia. Note that the cytolysis of cerebral cortical neurons (a) and cerebellar neurons (b) induced by conditioned media from microglia preincubated with OMS+NB IgG was not influenced by IL-1β, the cytolysis of cerebral cortical neurons (c) and cerebellar neurons (d) was also not changed by IL-6. Consistently, the cytolysis of cerebral cortical neurons (e) and cerebellar neurons (f) was not affected by TNF-α, and the cytolysis of cerebral cortical neurons (g) and cerebellar neurons (h) was not changed by MCP-1. p > 0.05; 0.1% BSA, OMS+NB vs cytokine, OMS+NB. ***p < 0.001; 0.1% BSA, NB vs cytokine, NB. One-way ANOVA, n=20 (0.1% BSA, NB; cytokine, NB), n=10 (0.1% BSA, OMS+NB; cytokine, OMS+NB).
Figure 9

Microglial activation induced by the Fab fragment of serum IgG. Note that expression of CD11b, a marker of microglial activation, and NO was not significantly changed in cerebral cortical microglias (a, b) and cerebellar microglias (c, d) incubated with the Fab fragment of IgG from children with OMS and NB compared with that of NB patients and healthy control. p > 0.05, one-way ANOVA, n=10 (Health control), n=20 (NB), n=10 (OMS+NB).
Schematic diagram illustrating the possible mechanisms of serum IgG-induced cytolysis of neurons. As we previously demonstrated, serum IgG from children with OMS and NB enhances the cytolysis of cultured cerebral cortical and cerebellar neurons directly. On the other hand, serum IgG also increases the activation of cultured microglias, leading to the upregulation of NO, which subsequently activate sGC and PKG in neurons, thereby inducing neuronal cytolysis. Although activated microglias may also secret IGF-1 (?), which alleviates the cytolysis of cultured neurons, the impact of the NO/sGC/PKG pathway may be more significant.
