Presence of identical B-cell clone in both cerebrospinal fluid and tumor tissue in a patient with Opsoclonus-myoclonus syndrome associated with neuroblastoma

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Abstract

Opsoclonus-myoclonus syndrome associated with neuroblastoma (OMS-NB) is a refractory paraneoplastic syndrome which often remain neurological sequelae, and detailed pathogenesis has remained elusive. We encountered a pediatric patient with OMS-NB treated by immunosuppressed therapy who showed anti-glutamate receptor δ2 antibody and increased B-cells in cerebrospinal fluid (CSF), and multiple lymphoid follicles containing abundant B-cells in tumor tissue. Unbiased B-cell receptor repertoire analysis revealed identical B-cell clone was identified as the dominant clone in both CSF and tumor tissue. These identical B-cell clone may contribute to the pathogenesis of OMS-NB. Our results could facilitate the establishment of pathogenesis-based treatment strategies for OMS-NB.

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Abbreviations:

OMS	Opsoclonus-myoclonus syndrome
OMS-NB	Opsoclonus-myoclonus syndrome associated with neuroblastoma
CSF	cerebrospinal fluid
GluRδ2	glutamate receptor δ2
IVIg	intravenous immunoglobulin
DEX	dexamethasone
BCR	B-cell receptor

ABSTRACT

Opsoclonus-myoclonus syndrome associated with neuroblastoma (OMS-NB) is a refractory paraneoplastic syndrome which often remain neurological sequelae, and detailed pathogenesis has remained elusive. We encountered a pediatric patient with OMS-NB treated by immunosuppressed therapy who showed anti-glutamate receptor δ2 antibody and increased B-cells in cerebrospinal fluid (CSF), and multiple lymphoid follicles containing abundant B-cells in tumor tissue. Unbiased B-cell receptor repertoire analysis revealed identical B-cell clone was identified as the dominant clone in both CSF and tumor tissue. These identical B-cell clone may contribute to the pathogenesis of OMS-NB. Our results could facilitate the establishment of pathogenesis-based treatment strategies for OMS-NB.

Introduction

Opsoclonus-myoclonus syndrome (OMS) associated with neuroblastoma (OMS-NB) is a refractory paraneoplastic syndrome presenting as opsoclonus, myoclonus, ataxia, dysarthria, behavioral and cognitive disorder. Although OMS-NB patients show good life prognosis [1], the neurological prognosis is poor. OMS-related neurological symptoms often remain even after tumor resection and inflict permanent neurological sequelae, including developmental, cognitive, and behavioral deficits, disturbing daily life in 30–70% of those patients. Some autoantibodies such as anti-glutamate receptor δ2 (GluRδ2) antibody and oligoclonal bands in cerebrospinal fluid (CSF) have been detected in OMS patients continuously, even after tumor resection.[2,3] Therefore, humoral autoimmunity has been considered the cause of OMS. Immunosuppressive therapies, including glucocorticoid and intravenous immunoglobulin (IVIg), have been reported as initial therapy[4], however pathogenesis-based treatment strategies have not yet been established.

We encountered a pediatric patient presenting with OMS-NB who showed an increased number of CD20-positive B cells in both CSF and neuroblastoma tissue. Unbiased B-cell receptor (BCR) repertoire analysis followed by next-generation sequencing revealed identical B-cell clone in both CSF and tumor tissue in our patient. This mechanism could facilitate the establishment of novel treatment strategies for OMS-NB.

Case

An 18-month-old Japanese boy without significant past history was admitted to our hospital with a 1-week history of limb tremor, opsoclonus and ataxia. He was unable to sit or walk without support. He had no history of vaccinations or infections within the preceding month. Computed tomography revealed a localized anterior sacral tumor and no lesions of the central nervous system. The anterior sacral tumor showed avid uptake of ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG). Blood testing revealed none of the autoantibodies reported in association with paraneoplastic neurological syndromes such as anti-Hu, anti-Ri, anti-Yo, anti-amphiphysin, anti-PNMA2, anti-CV2, anti-recoverin, anti-SOX1, anti-titin, anti-zic4, anti-GAD65 and anti-Tr.[5] CSF analysis revealed an increased number and ratio of CD20-positive B cells, at 0.66 cells/μl and 12%, respectively (reference B-cell ratio in CSF: 0.00–0.03%[6]). In addition, oligoclonal bands and anti-GluRδ2 antibodies were detected in CSF.[2,3] The anterior sacral tumor was resected completely and diagnosed pathologically as neuroblastoma without *MYCN* amplification, classified as very low risk group in the International Neuroblastoma Risk Group Classification. In addition, detailed pathological analysis revealed multiple lymphoid follicles containing abundant CD20-positive B cells in the neuroblastoma tissue (Fig. 1). The increased CD20-positive B cells, oligoclonal bands and anti-GluRδ2 antibodies in CSF, and multiple lymphoid follicles containing abundant CD20-positive B cells in neuroblastoma tissue led us to consider the possibility that humoral immunity may contribute to OMS.

OMS has been reported to persist after complete resection of neuroblastoma and to require immunosuppressive therapy after tumor resection. We started administration of monthly dexamethasone (DEX) (20 mg/m^2/day for 3 days) pulses and IVIg (1 g/kg/day) according to a previous report.[7] To assess the effectiveness of treatment, we monitored the severity of OMS symptoms using "OMS Rating Scales", comprising evaluations of six symptoms: stance, gait, arm function, opsoclonus, mood/behavior and speech (Supplementary Table S1).[8] Simultaneously, we monitored CD20-positive B cells and oligoclonal bands in CSF over time, as factors reported to reflect the disease activity of OMS. After starting immunosuppressive therapy, limb tremor, ataxia and opsoclonus gradually remitted. After three cycles, the patient was able to walk again without support. OMS Rating Scales score also improved to 2, from 8 at the first visit. Although oligoclonal bands in CSF had been detected persistently, the B-cell ratio in CSF declined in parallel with symptom improvement (Fig. 2).

Since significant improvements by DEX pulse and IVIg therapy and increased CD20-positive B cells in CSF and multiple lymphoid follicles with abundant CD20-positive B cells in tumor tissue were observed, we considered the existence of identical B-cell clones contributed to the pathogenesis of OMS in both CSF and tumor tissues. We therefore performed BCR repertoire analysis of B-cells in both CSF and tumor tissue using unbiased amplification of BCR genes followed by next-generation sequencing (Repertoire Genesis Inc., Osaka, Japan) as previously described.[9] Consequently, the existence of identical B-cell clone was confirmed in both CSF and tumor tissue (Supplemental Table S2). Intriguingly, the identical B-cell clone was the dominant clone in both CSF and tumor tissue. Moreover, various diversity indices including the Shannon Weaver index, inverse Simpson index, Pielou's evenness and Diversity Evenness 50 were applied to evaluate the diversity and clonality of B-cells in both CSF and tumor tissue. As a result, every diversity index confirmed that B-cells in CSF showed low diversity and B-cells in tumor tissue showed high diversity (Supplemental Table S3). These data suggested that low-diversity B-cell clones migrating into the central nervous system from tumor tissue may express autoantibodies such as anti-GluRδ2 antibodies, leading to the pathogenesis of OMS.

Discussion

In our case, multiple lymphoid follicles including abundant CD20-positive B cells in the tumor tissue of neuroblastoma[10], oligoclonal bands, anti-GluRδ2 antibodies and increased CD20-positive B cells in CSF[11] have been recognized, as previously reported. In addition, these findings in CSF declined in parallel with improvement of symptoms. Therefore, humoral immunity in CSF has been considered to contribute to the pathogenesis of OMS.[2] Based on humoral immunity pathogenesis theory, immunosuppressive therapies such as glucocorticoid and IVIg have been performed. However, the details of antibody-production mechanisms and the mechanisms underlying the effects of immunosuppressive strategies on OMS have yet to be elucidated.

Our study revealed that the most abundant B-cell clone in CSF, which has been reported to reflect the disease activity of OMS, also existed in tumor tissue as the most abundant clone. Since the dominant B-cell clone in microenvironment has been reported to reflect the existence of specific antigen targeted by specific humoral immune response [12,13], the low diversity of B-cell clones in CSF may suggest a specific humoral immune response to the central nervous system. Anti-GluRδ2 antibodies have also been reported to bind to cerebellar Purkinje cells.[3] Therefore, these findings may indicate that the B-cell clone sensitized in neuroblastoma tumor tissue migrates into the CSF and produces autoantibodies such as anti-GluRδ2 antibodies, contributing to the pathogenesis of OMS.

No pathogenesis-based treatment strategies for OMS have yet been established. However, considering that identical B-cell clone in CSF and tumor tissue contribute to the pathogenesis of OMS, rituximab may be a reasonable strategy to diminish identical B-cell clone. In the past, rituximab has been reported as a salvage therapy for refractory OMS patients showing no response to glucocorticoid and IVIg[14,15], however late administration of rituximab as salvage therapy has been reported as ineffective.[5] Recently, early administration of rituximab as the first-line treatment has been reported as an effective therapy against OMS.[6,16] It is conceivable that depletion of identical B-cell clone from both CSF and tumor tissue may lead to cure of OMS. Although our findings are based on a single case, early administration of rituximab might represent a reasonable strategy for OMS-NB.

Conclusion

Identical B-cell clone was identified as the dominant clone in both CSF and tumor tissue. The identical B-cell clone likely play an important role in the pathogenesis of OMS-NB.

Conflict of Interest Statement

We have no conflicts of interest to declare.

Acknowledgements

K.N. wrote the manuscript. M.T., Y.S., T.F., R.K., K.N., S.S., M.H. and R.A. provided patient care. H.I. and S.N. provided pathological analysis. Y.T. provided anti-glutamate receptor δ2 analysis. Y.I. was the principal investigator and takes primary responsibility for the paper. T.W. was the chief of the department and provided advice on this study.

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Figure Legends

FIGURE 1. Histological and immunohistochemical features of our patient with OMS-NB.

(A) Multiple lymphoid follicles (arrowhead) are present in the neuroblastoma tissue (hematoxylin and eosin staining; x4). (B) High magnification image of lymphoid follicle in neuroblastoma tissue (hematoxylin and eosin staining; x200). (C) Lymphoid follicle stained with CD3 monoclonal antibody. Sparse CD3-positive cells are visible in the germinal center, follicular mantle and interstitium (peroxidase staining; x100). (D) Lymphoid follicle stained with CD20 antibody. CD20-positive cells are concentrated in the germinal center and follicular mantle (peroxidase staining; x100). (E) Lymphoid follicle stained with CD21 monoclonal antibody. CD21-positive cells are concentrated in the germinal center (peroxidase staining; x100). OMS-NB, Opsoclonus-myoclonus syndrome associated with neuroblastoma.

FIGURE 2. DEX pulse and IVIg treatment effect on OMS Rating Scales and CSF data.

Severity of OMS symptoms was evaluated using OMS Rating Scales, as previously reported. [8] The OMS Rating Scales evaluate six symptoms: stance, gait, arm function, opsoclonus, mood/behavior and speech. Each symptom is scored from 0 to 3 for increasing severity, then the total score is evaluated. Our patient was too young to be speaking, therefore we evaluated five symptoms (excluding speech) for the OMS Rating Scales score. B-cell ratio in CSF means the ratio of B cells to total cells in CSF. DEX, dexamethasone; IVIg, intravenous immunoglobulin; OMS, Opsoclonus-myoclonus syndrome; CSF, cerebrospinal fluid.

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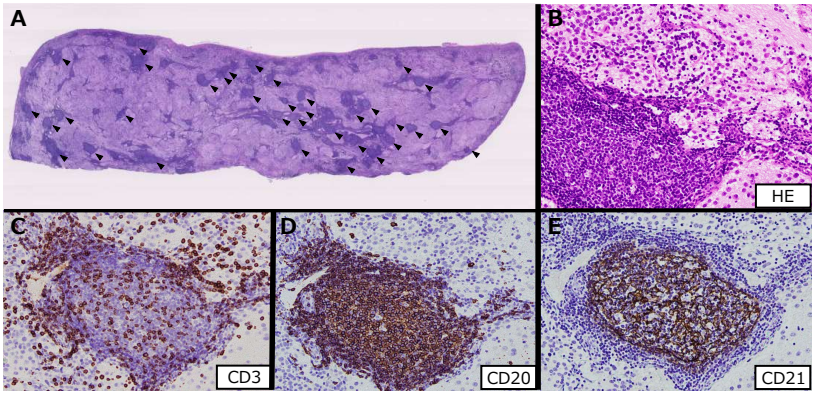

FIGURE 2. DEX pulse and IVIg treatment effect on OMS Rating Scales and CSF data.