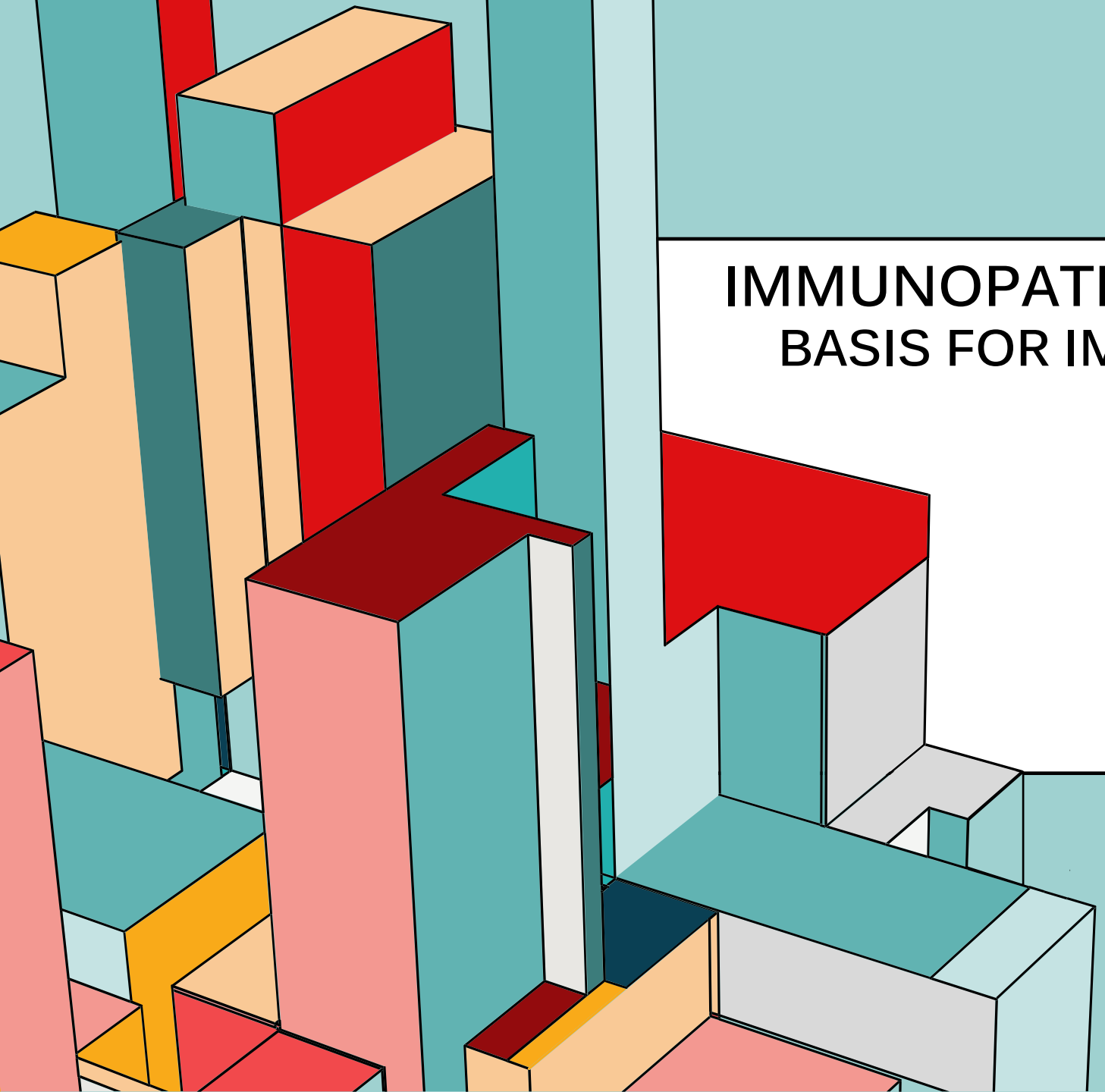

IMMUNOPATHOGENESIS OF OMS:
BASIS FOR IMMUNE DYSREGULATION

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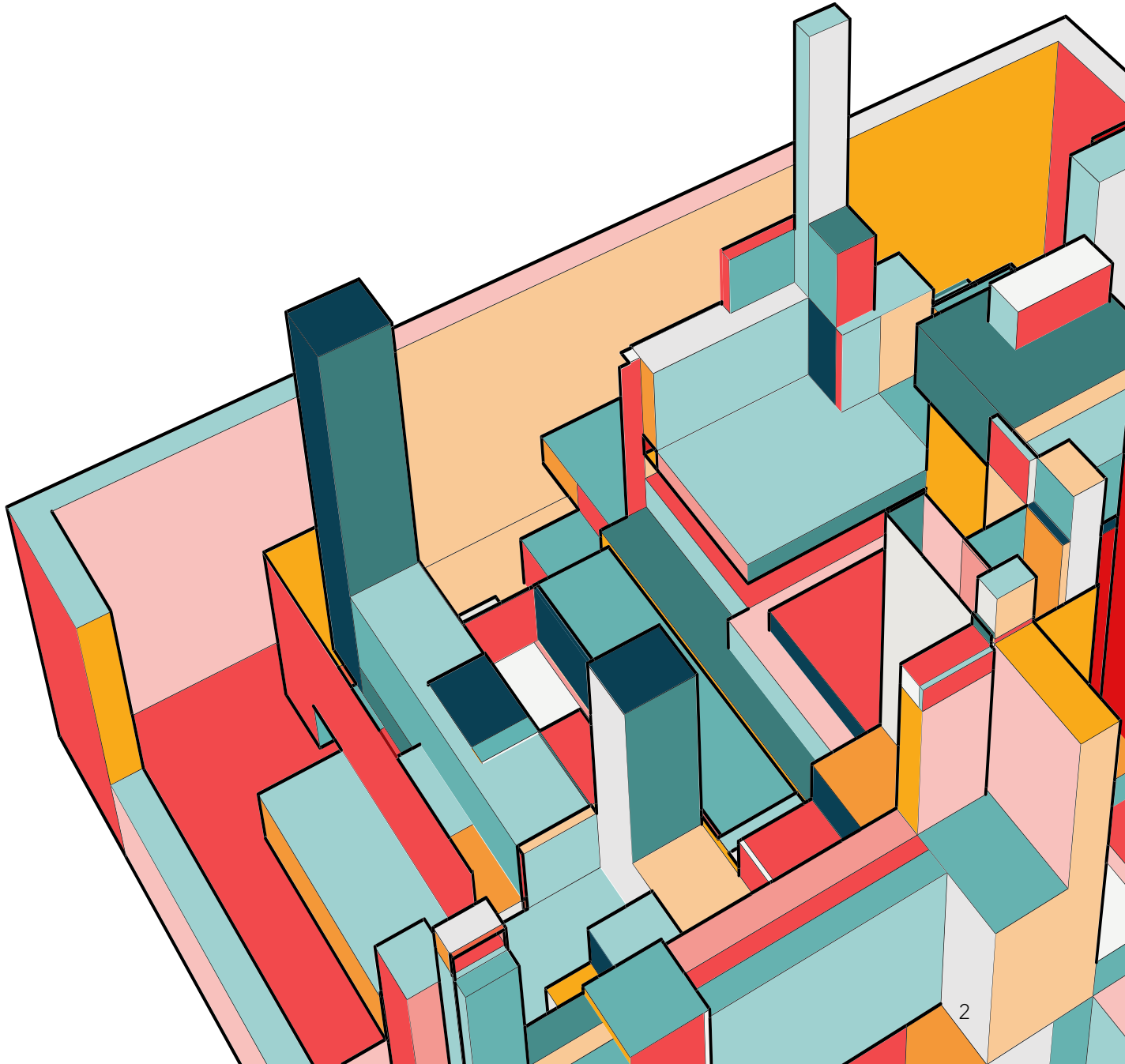
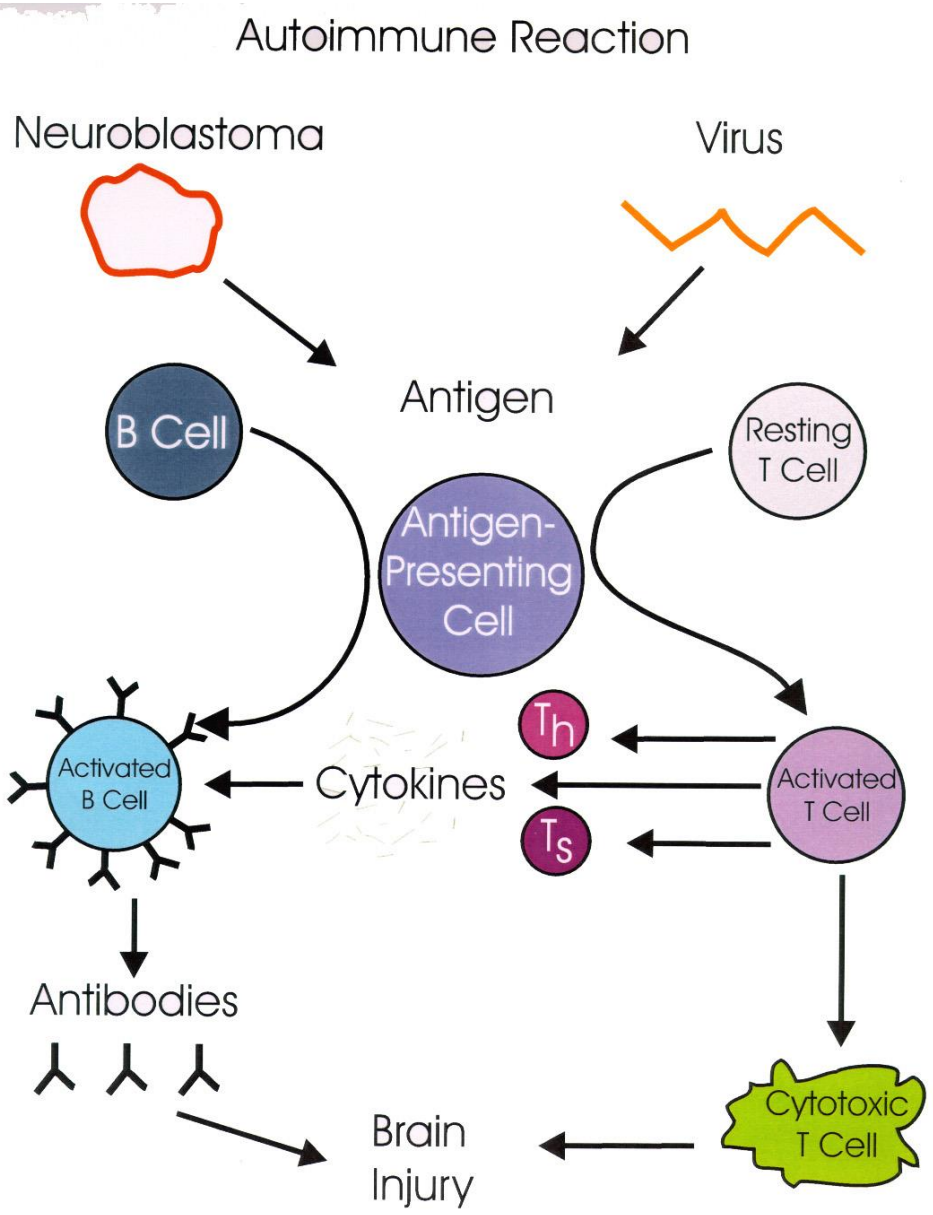
Children's Hospital LOS ANGELES

OVERVIEW

1. Overview and Cellular Cascades
2. B Cells and Immune Dysregulation
3. T Cells and Immune Regulation
4. Cytokine/Chemokine Signaling



THE BASICS


Autoimmune Reaction
Neuroblastoma
Virus
Antigen
B Cell
Resting T Cell
Antigen-Presenting Cell
Th
Activated B Cell
Cytokines
Activated T Cell
Ts
Antibodies
Cytotoxic T Cell
Brain Injury

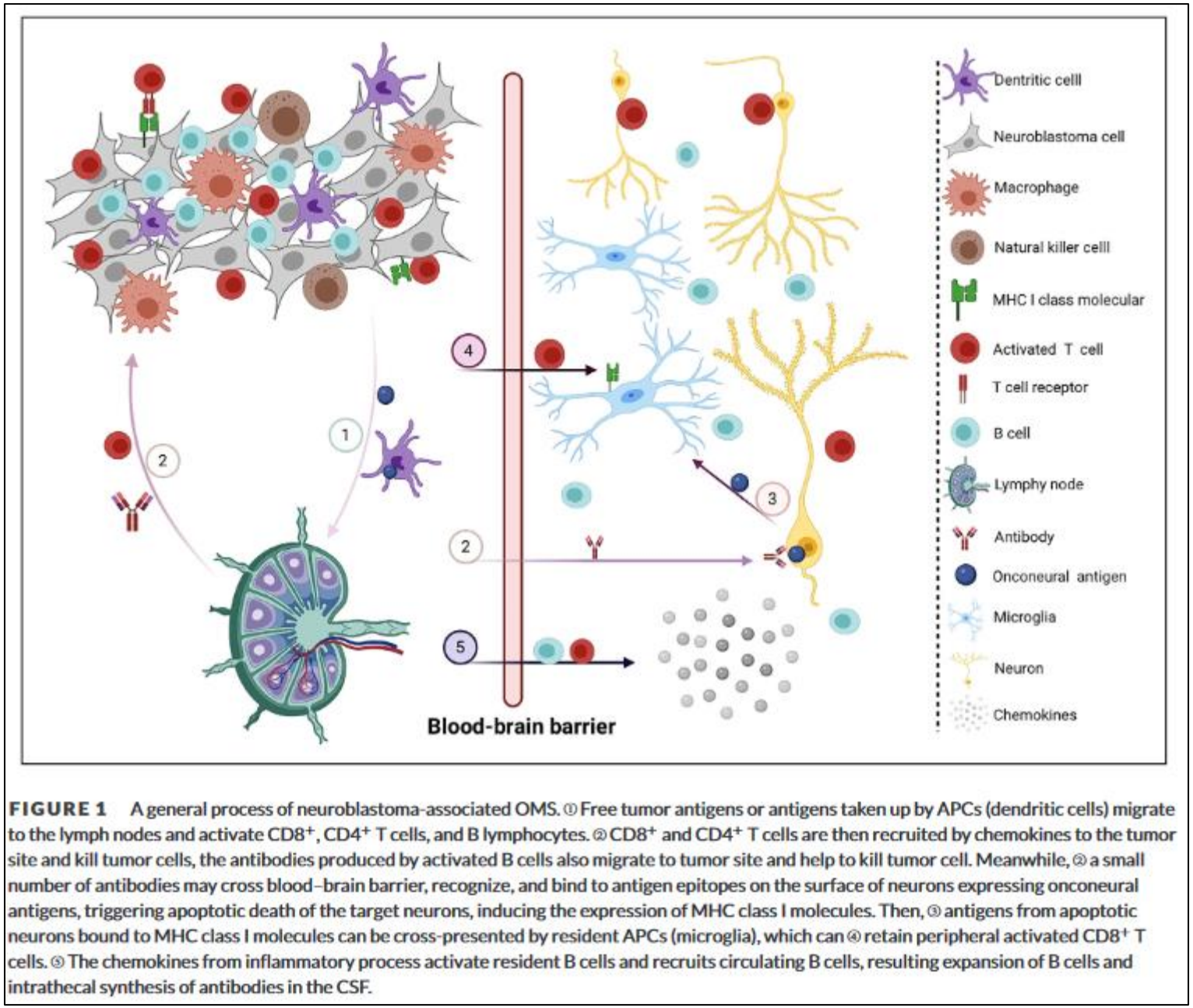
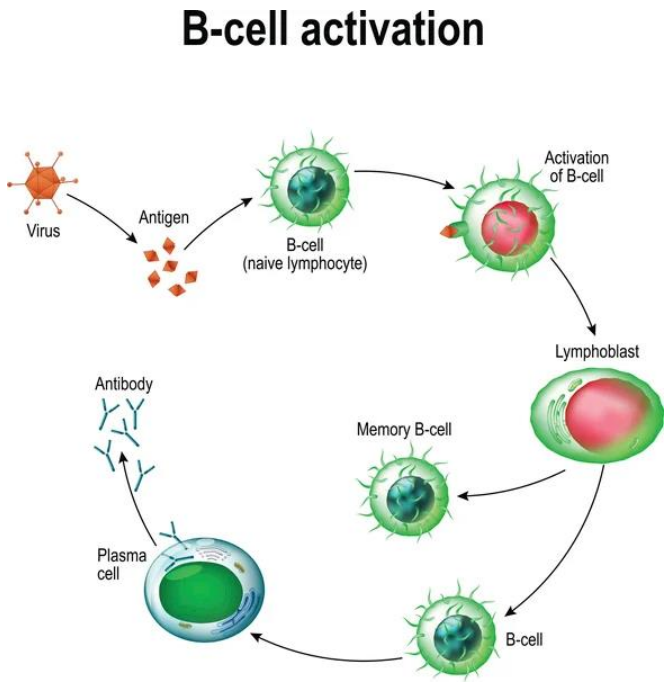


FIGURE 1 A general process of neuroblastoma-associated OMS. ① Free tumor antigens or antigens taken up by APCs (dendritic cells) migrate to the lymph nodes and activate $CD8^+$, $CD4^+$ T cells, and B lymphocytes. ② $CD8^+$ and $CD4^+$ T cells are then recruited by chemokines to the tumor site and kill tumor cells, the antibodies produced by activated B cells also migrate to tumor site and help to kill tumor cell. Meanwhile, ② a small number of antibodies may cross blood–brain barrier, recognize, and bind to antigen epitopes on the surface of neurons expressing onconeural antigens, triggering apoptotic death of the target neurons, inducing the expression of MHC class I molecules. Then, ③ antigens from apoptotic neurons bound to MHC class I molecules can be cross-presented by resident APCs (microglia), which can ④ retain peripheral activated $CD8^+$ T cells. ⑤ The chemokines from inflammatory process activate resident B cells and recruits circulating B cells, resulting expansion of B cells and intrathecal synthesis of antibodies in the CSF.

Du et al, *Ped Blood Cancer,* 2021

CELLULAR PROFILES



CSF of Individuals with Paraneoplastic and Non-Paraneoplastic OMS Have B-Cell Expansion with some T-cell aberrations as well

- Pranzatelli et al (2004) reported that in the CSF of 36 individuals with OMS (paraneoplastic and non-paraneoplastic) expansion of CD19+ B-cells, T-cells, and lower percentages of CD4+ T-cells and CD4/CD8 ratios was present in the setting of normal cellular profiles
- More severe disease was associated with greater expansion of these cell lines

Pranzatelli et al., *Neurology,* 2004

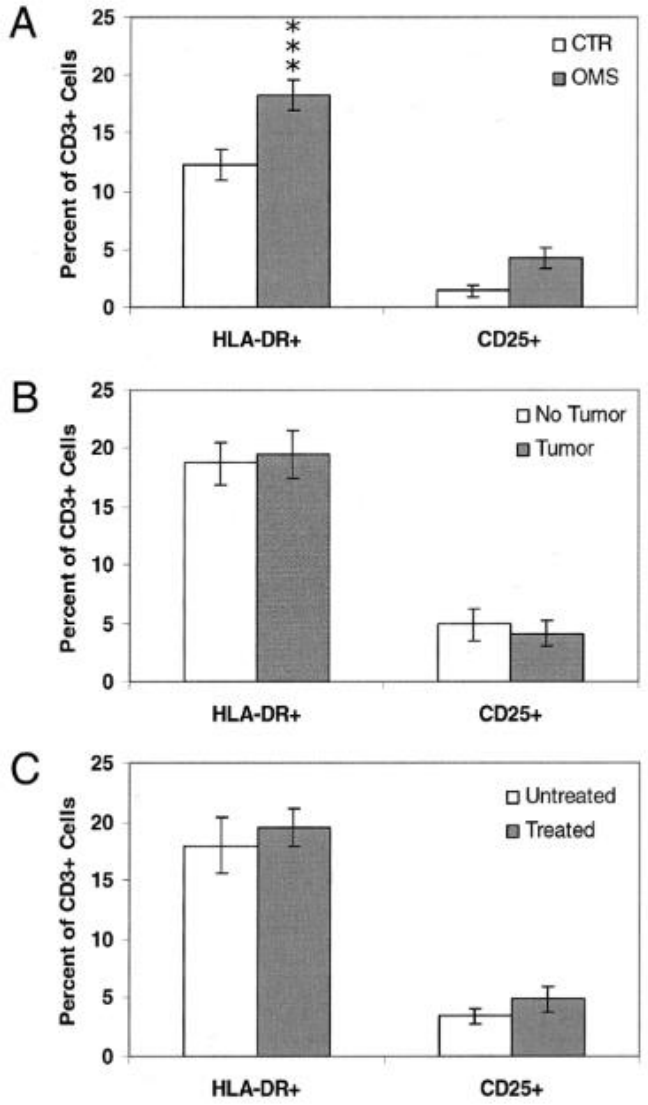


*Figure 2. CSF CD3⁺ T-cell activation markers in control subjects vs opsoclonus–myoclonus syndrome (OMS) (A), tumor vs no tumor (B), and untreated vs treated OMS (C). Data are means ± SEM. Asterisks signify statistical significance by t-tests: ***0.0001 ≤ p < 0.001. In OMS, 21 of 36 children had values above the upper confidence limit of the control group for human leukocyte antigen DR⁺ T-cells (up to 34%) but only 11 children for CD3⁺CD25⁺ cells (up to 25%).*

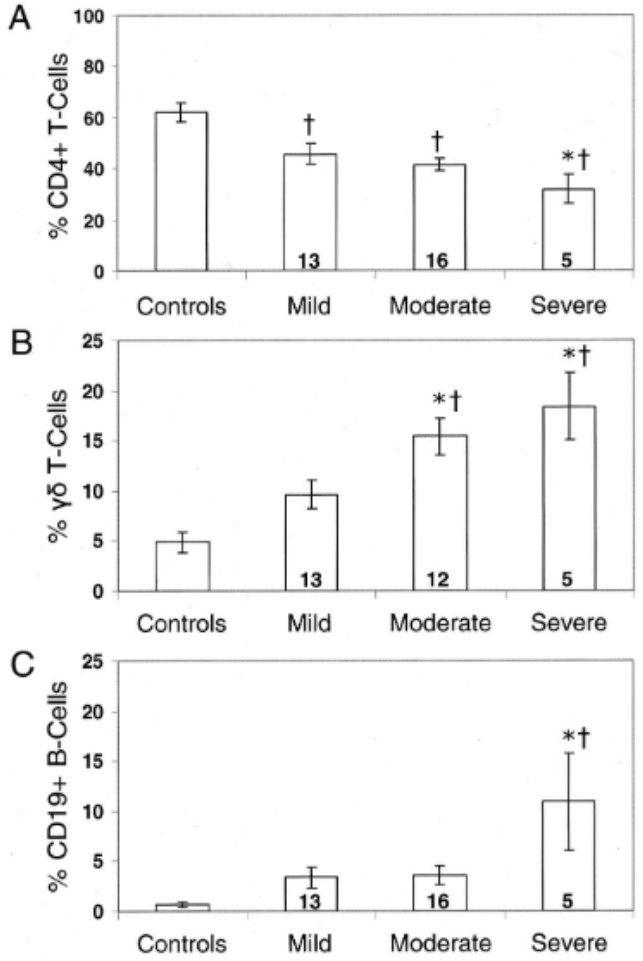


Figure 3. Relation between neurologic severity (total score) in opsoclonus–myoclonus syndrome (OMS) and percentage of CSF CD4⁺ T-cells (A), γδ T-cells (B), and CD19⁺ B-cells (C). Sample size is shown at the base of each column. Data are means ± SEM. Asterisks indicate statistically significant differences between OMS severity categories on Duncan test, p < 0.05. Dagger indicates significant differences between OMS and control subjects. Analysis of variance with linear trend analysis revealed that the more severely affected children (higher total score) had a lower percentage of CSF CD4⁺ T-cells (F = 20.6, p ≤ 0.0001). In contrast, the percentage of CSF γδ T-cells increased with severity (F = 25.6, p ≤ 0.0001), being nearly double in the severe category, and the percentage of CSF CD19⁺ B-cells was also higher (F = 21.2, p ≤ 0.0001).

Pranzatelli et al., *Neurology*, 2004

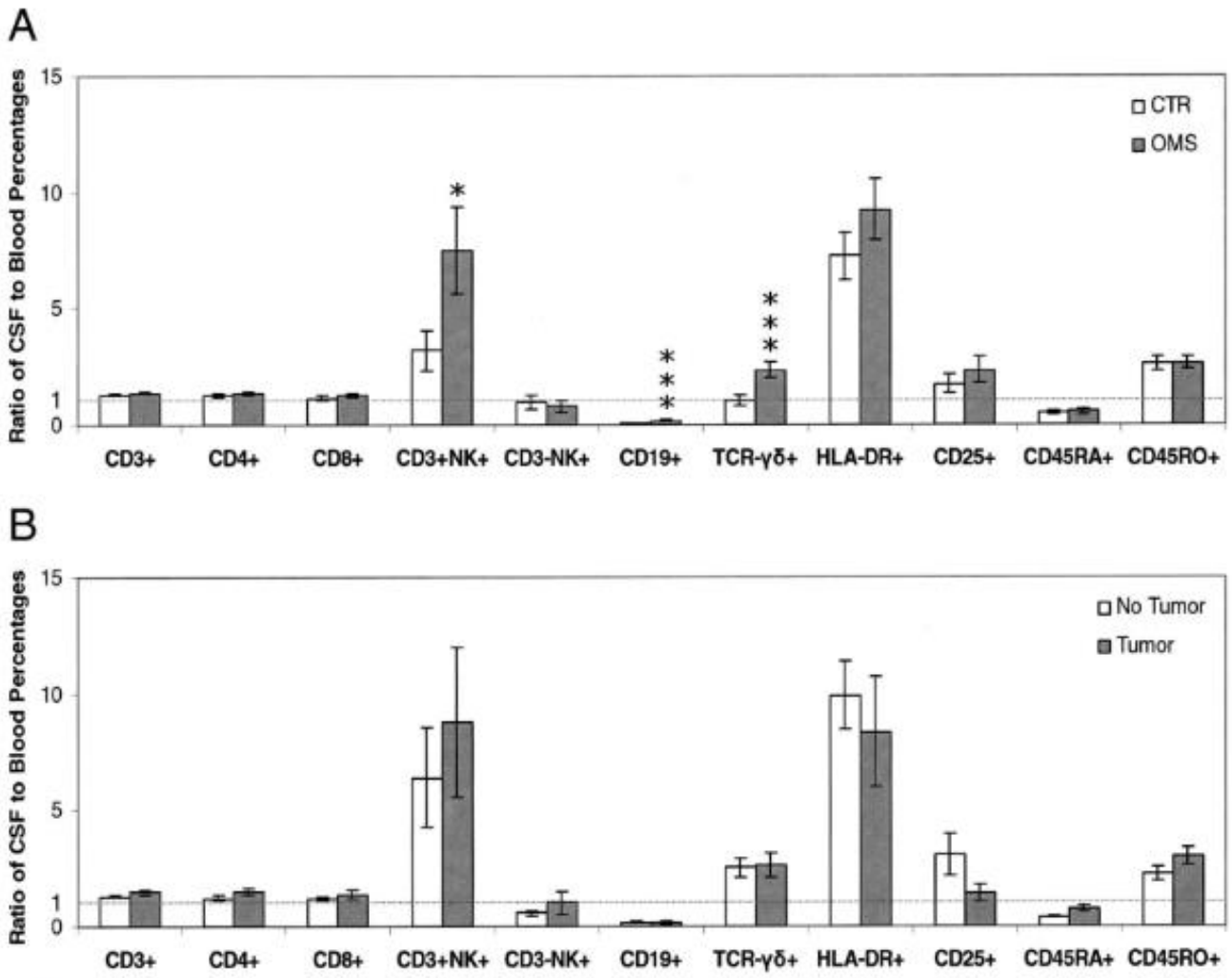


*Figure 4. CSF/blood lymphocyte ratios. (A) In opsoclonus–myoclonus syndrome (OMS), the ratios for γδ T-cells, $CD19^+$ B-cells, and $CD3^+CD16/56^+$ cells were significantly increased. The B-cell ratios were 0.05 ± 0.01 for controls and 0.16 ± 0.03 for OMS. (B) There were no statistically significant differences between the tumor and no-tumor groups. Data are means ± SEM. Asterisks signify statistical significance by t-tests: *0.01 ≤ p < 0.05, ***0.0001 ≤ p < 0.001.*

B-cell rations were 0.05 for controls and 0.16 for OMS

Importantly, no difference between tumor and non-tumor group

Pranzatelli et al., *Neurology,* 2004

TRIGGER? WHY THE CELL CHANGES

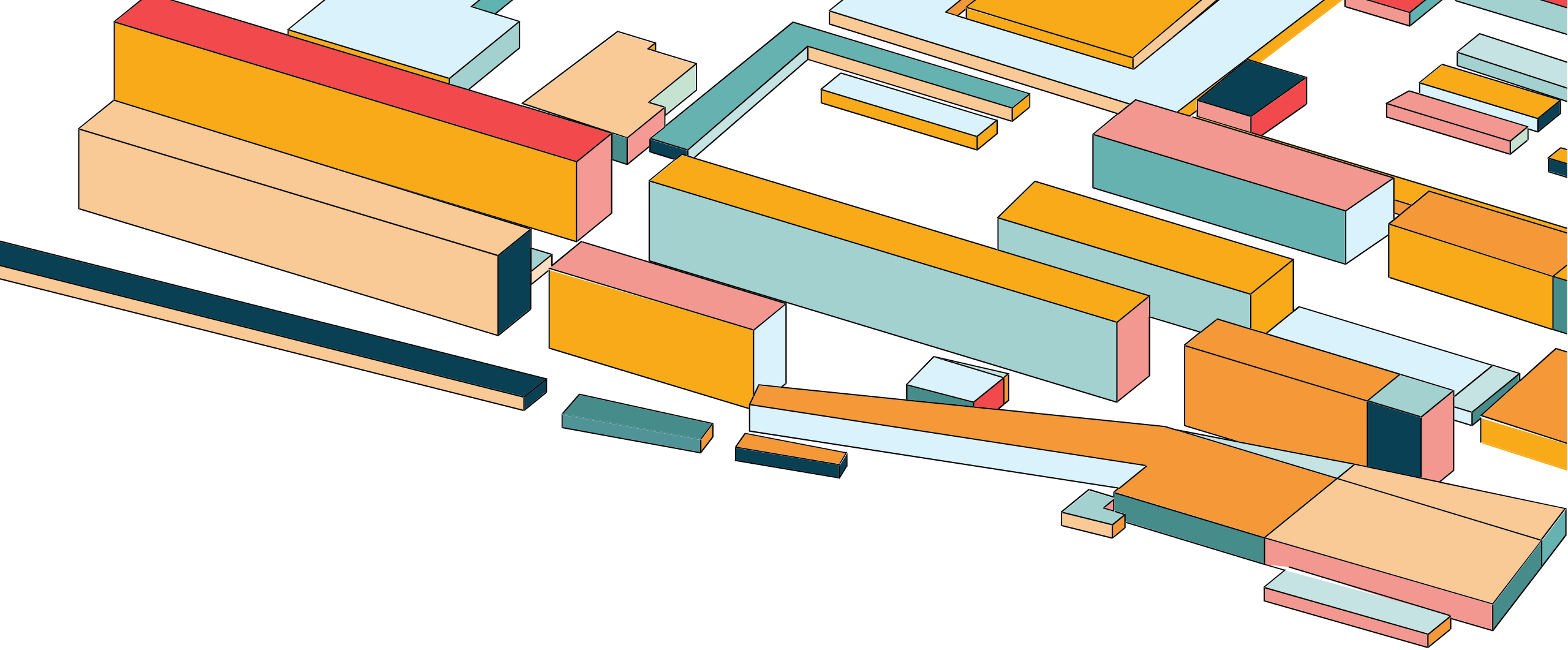
Genetics of NB?

- Hero et al. (2018) reported that nearly 52% of individuals with NB-associated OMS will have segmental chromosome alterations (such as gain of chromosomal arms)
- Hypothesis: certain genetic tumor variations may predispose to overexpression of pathologic surface antigen or overactive immunologic response

Genetics of Immune Dysregulation?

- Santoro et al (2021) reported that rates of personal and familial autoimmunity were elevated in both children with and without NB. Autoimmune diseases were largely T-cell driven.
- Hypothesis: a genetic/inheritable predilection towards immune dysregulation may differentiate those who get OMS and those who do not

Hero et al., *J Pediatr Hematol Oncol,* 2018; Santoro et al., *Neurol Neuroimmunol Neuroinflamm,* 2021



THE “B” TEAM

B-CELL EXPANSION: THE OG FINDINGS

Early Studies in OMS Revealed B-Cell Expansion and Intrathecal Synthesis of Oligoclonal Bands

- Pranzatelli et al (2004) identified B-cell populations in the CSF were expanded in more severe disease and subsequently reduced in population after treatment.
- Pranzatelli et al (2006) and (2018) identified intrathecal production of IgG causing oligoclonal banding
    - 58% of patients will have OCB

Immunotherapy Treats OMS

- Although an entire slide deck could be dedicated to treatment, the identification of this disease as 1) autoimmune and 2) with definitive biomarkers of B-cell mediated disease activity led to therapeutic interventions with immunotherapy.
- Clinical improvement, particularly with steroids, IVIg and B-cell depletion were proof of concept of this theory.

Pranzatelli et al., *Mov Disord,* 2004; Pranzatelli et al., *J Pediatr Hematol Oncol,* 2006; Pranzatelli et al., *J Neuroimmunol,* 2018

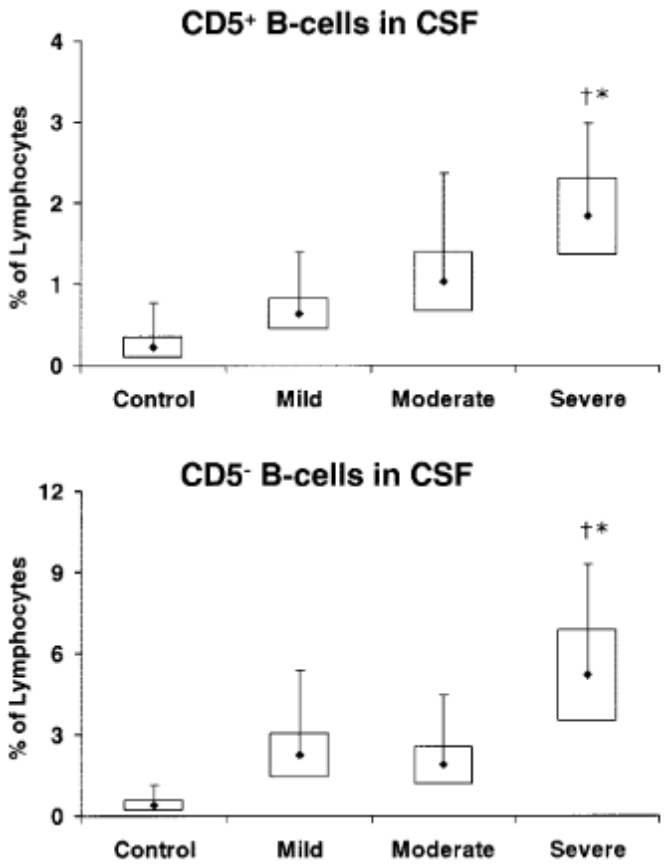


FIG. 3. Relation of neurological severity to percentage of $CD5^+$ (upper figure) and $CD5^-$ (lower figure) B-cell subsets in CSF. As an aid to clinical interpretation, neurological severity was defined as mild if the videotape total score was ≤12, moderate if 13–24, and severe if 25–36. Controls are shown for comparison. Asterisk indicates significant difference among severity categories, $P < 0.05$ by Duncan test. Cross signifies significant difference compared with controls. From left to right, n = 18, 16, 14, and 6.

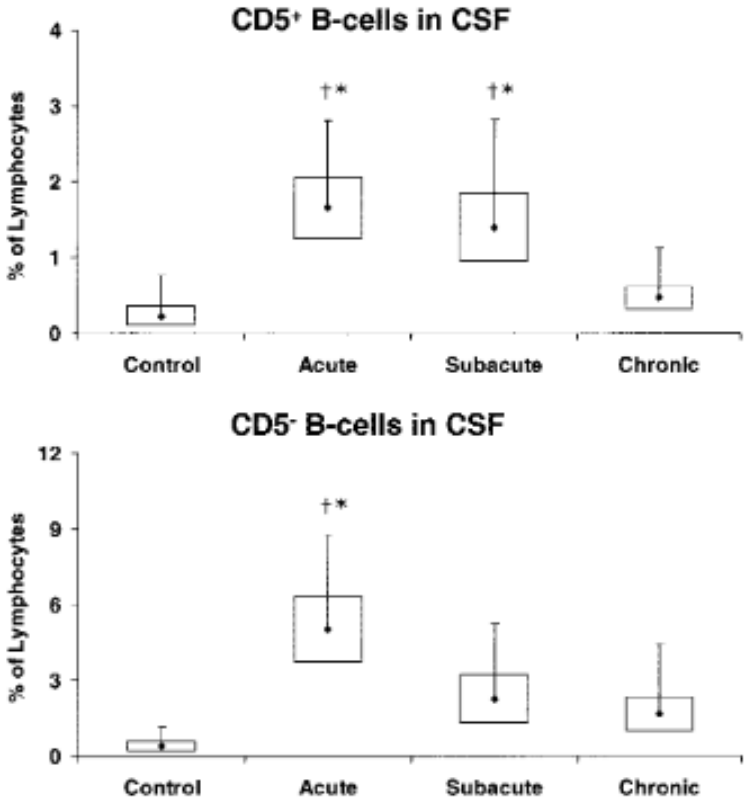


FIG. 4. Relation of syndrome duration to the percentage of CSF B-cell subsets. The syndrome duration category was defined as acute if ≤3 months, subacute if 3–12 months, and chronic if >1 year. Controls are shown for comparison. Asterisk indicates significant difference among duration categories, $P < 0.05$ by Duncan test. Cross signifies significant difference compared with controls. From left to right, n = 18, 8, 10, and 18. As a point of comparison, neurological severity (total score) also varied with the duration of the illness ($F_{2,51} = 4.6$; $P = 0.014$, ANOVA). Scores in the chronic phase (11.8 ± 8.8) were about one-half of those in the acute and subacute phases ($P < 0.05$, Duncan's test).

Pranzatelli et al., *Mov Disord,* 2004; Pranzatelli et al., *J Pediatr Hematol Oncol,* 2006; Pranzatelli et al., *J Neuroimmunol,* 2018

B-CELLS & ANTIBODIES

Surface-binding autoantibodies against cerebellar neurons

- Blaes et al (2005) reported that in 10/14 children with OMS had evidence of IgG binding against the surface of isolated rat cerebellar neurons.

Anti-neuroblastoma IgG

- Korfei et al (2005) reported that in 11 children with paraneoplastic OMS, anti-neuroblastoma antibody fractions yielded intracellular autoantigen binding in cerebellar cells.
- These IgG exhibit an anti-proliferative effect and cytotoxic effect on neuroblastoma cells.

Blaes et al., *Ann Neurol,* 2005; Korfei et al., *J Neuroimmunol,* 2005

Serum of individuals with OMS displayed IgG-mediated binding to cerebellar granular neurons with and without preincubation

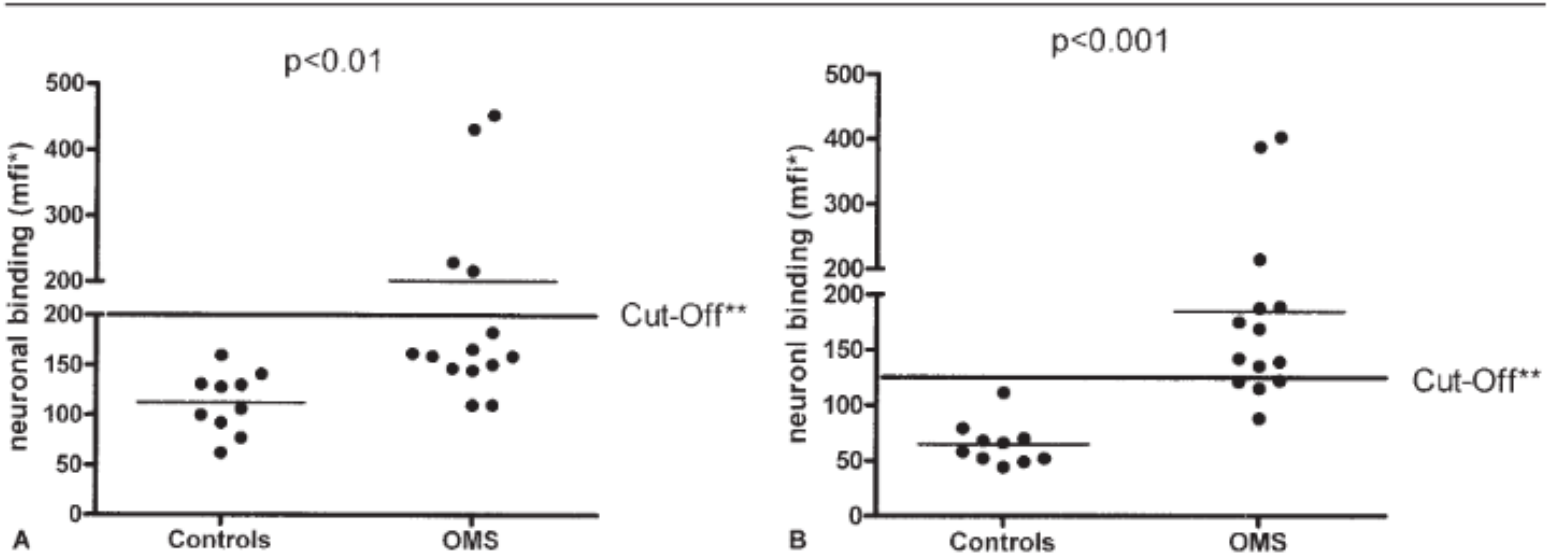


Fig 1. Binding of opsoclonus-myoclonus syndrome (OMS) and control sera to cerebellar granular neurons (A) without preincubation with HEK 293 cells and (B) after 24-hour preincubation with HEK 293 cells. Each value is the mean of two determinations from three independent experiments (standard deviation [SD] < 5%). Asterisk *denotes binding is expressed as mean fluorescence intensity (MFI);* double asterisk *denotes cutoff was defined as mean MFI of the control subjects + 3 SD.*

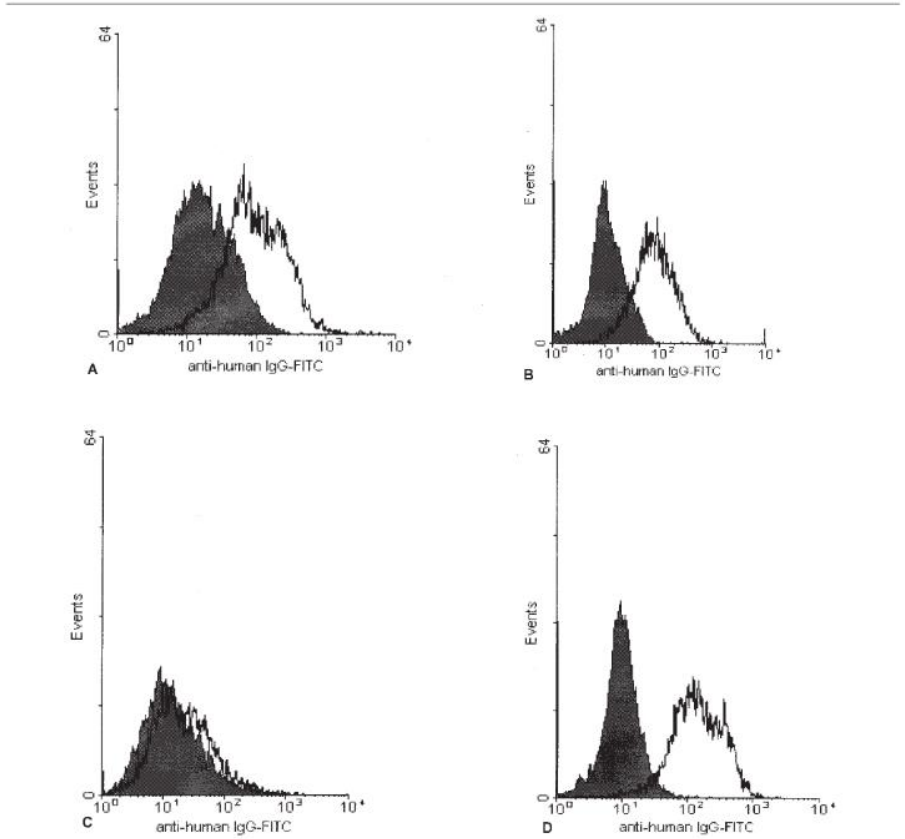


Fig 2. Preincubation experiment with an opsoclonus-myoclonus syndrome (OMS) serum (line graph) *and a healthy control* (filled graph) *before (A, B) and after 24-hour preincubation with HEK 293 cells (C, D). (A, C) Binding to HEK 293; (B, D) binding to cerebellar granular neurons (CGN). Binding to CGN is still detectable after removing the IgG binding to HEK 293 (D), whereas the binding to the HEK 293 has disappeared (C).*

Blaes et al., *Ann Neurol,* 2005

ANTIBODY KNOWLEDGE IMPROVES

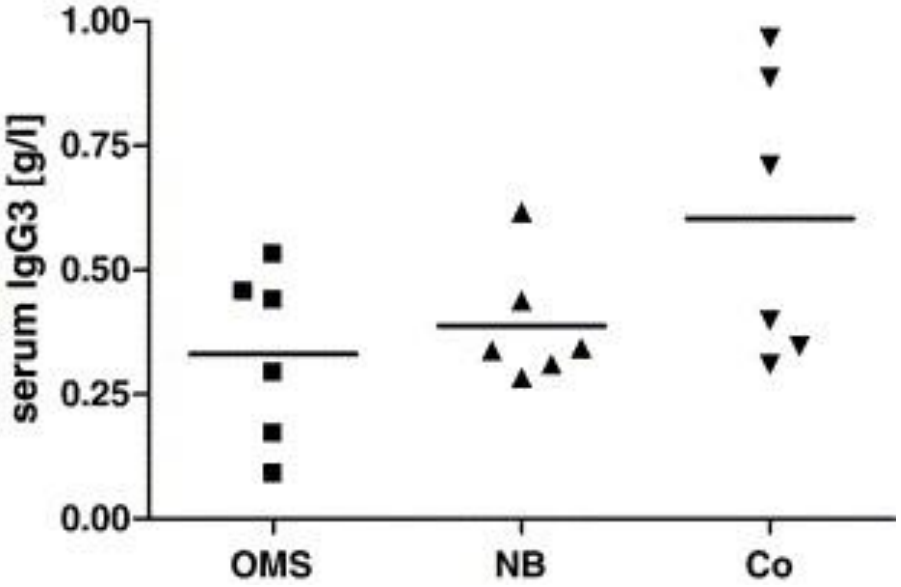
IgG-3 Mediated Process

- Beck et al. (2007) identified that in 16 individuals with paraneoplastic and non-paraneoplastic OMS, individuals displayed IgG3-mediated binding against both intracellular and surface bound antigens in the cerebellum of primates.
- Serum IgG3 was NOT elevated

Addition of Other Antibody Binding Sites

- Berridge et al., (2018) used mass spectrometry to identify 12 cell-surface proteins in rats where high affinity binding occurred in 4/6 OMS samples.
- GluD2 receptor was the most commonly identified target ***however*** IgG cerebellar reactivities after GluD2 absorption suggest that this was not the sole pathologic cause.
- Armangue et al (2016) reviewed a similarly large cohort and did NOT identify GluD2 Ab although glycine receptor antibodies were identified in adults with P-OMS

Beck et al., *J Neuroimmunol,* 2007; Berridge et al., *Neurology*, 2018; Armangue et al., *JAMA Neurol*, 2016



IgG3-driven binding in Perkinje cell cytoplasm without associated increase in serum levels

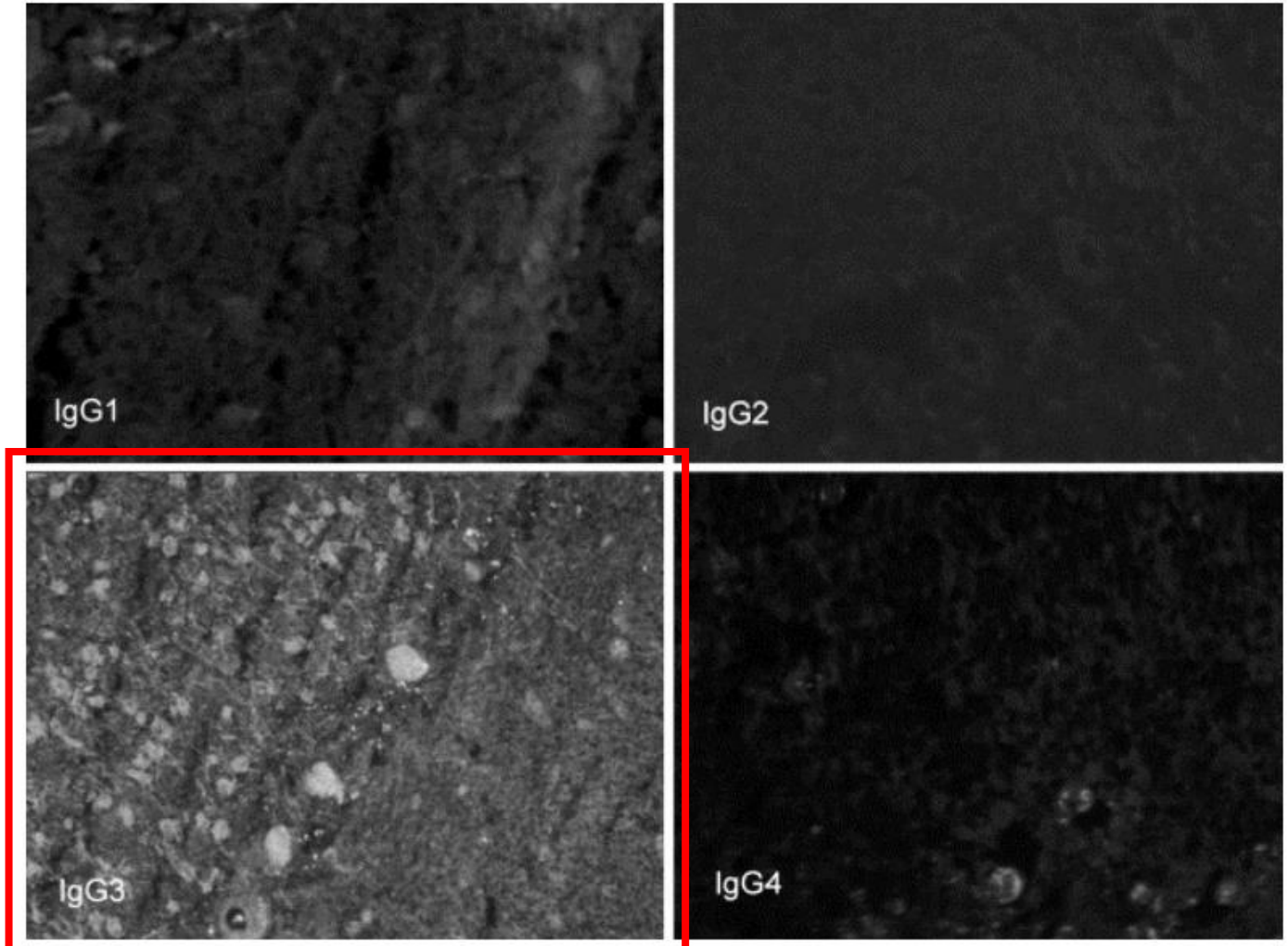


Fig. 1

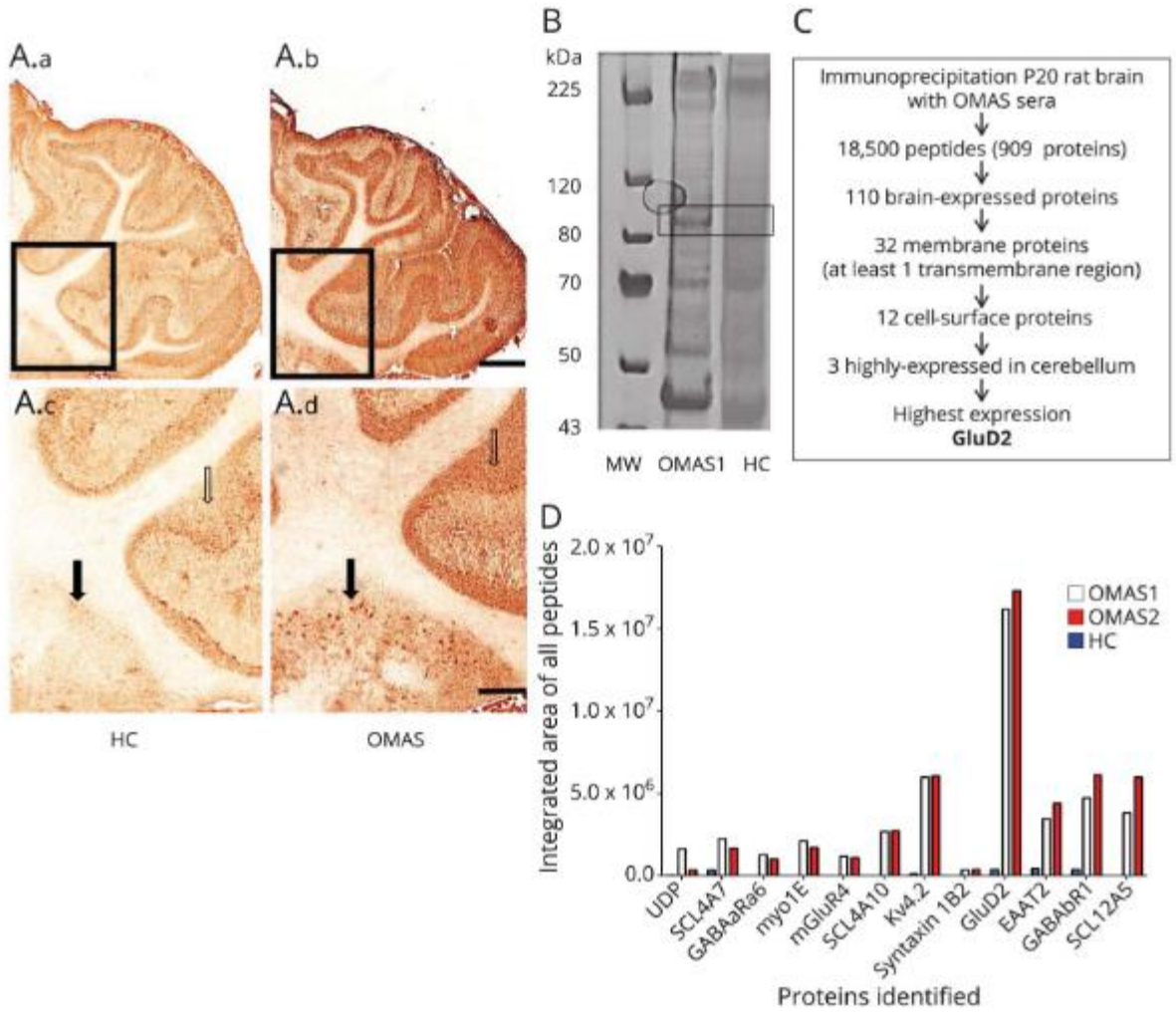
Primate cerebellum: immunofluorescence of anti-Purkinje cell autoantibody of an OMS patient. Staining of the Purkinje cell cytoplasm by IgG3, to a lesser extent IgG1, but not IgG2 or IgG4 (serum dilution 1/50, magnification × 200).

Beck et al., *J Neuroimmunol,* 2007

An important note…

Since preabsorption of OMS-IgG with GluR D2-transfected cells did reduce, but not eliminate, the surface-binding properties to cerebellar cells, GluR D2 may only be one autoantigen among others

Figure 1 Autoantigen identification in OMAS



(A) Serum immunoglobulin G (1:100) binding to rat cerebellar sections (12 µm) from HCs (A.a, A.c) or patients with OMAS (A.b, A.d). Strong staining is observed with OMAS sera in the granular layer (open arrow) and also in areas of the deep cerebellar nuclei (boxed area in upper panels, filled arrow in lower panels). Scale bars: 500 µm (A.a, A.b) and 200 µm (A.c, A.d). (B) Gel electrophoresis of postnatal day 20 rat cerebellum tissue immunoprecipitate. Eluted samples after immunoprecipitation with OMAS1 sera and pooled HC sera were run on a 4% to 12% sodium dodecyl sulfate precast gradient gel (WG1402; Invitrogen). (A) Unique band, approximate molecular weight of 100 to 110 kDa was seen exclusively in OMAS samples. The boxed area was excised for mass spectrometry. (C) Flow diagram for filters in mass spectrometry experiments. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository[31,32] with the dataset identifier PXD009578. (D) Identification of GluD2 as a putative autoantigen target in OMAS. Relative amounts of 12 surface-expressed neuronal proteins immunoprecipitated by 2 different OMAS sera (red/white bars) and not by HC (blue bars). For full description of the identified proteins, see data available from Dryad (table 2, doi.org/10.5061/dryad.tq61224). GluD2 = glutamate receptor δ2; HC = healthy control; MW = molecular weight; OMAS = opsoclonus myoclonus ataxia syndrome.

Berridge et al., Neurology, 2018

GLUD2 ANTIBODY….

Role of the GluD2 Receptor?

- In children with mutations of GRID2 (GluD2 gene), the phenotype is developmental delay, loss of acquired motor skills, ocular apraxia, cerebellar ataxia and cerebellar atrophy (Hill et al and Utine et al 2013)
- GluD2 is highly expressed in the dendritic spines of the Perkinje cells which go on to project into the vermis and the deep cerebellar nuclei.

But is it Rea

- GluD2 exp … g
  developm … ein
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- Is this a pe … ng
  neuroblast … ws for
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- BUT WAIT … 45
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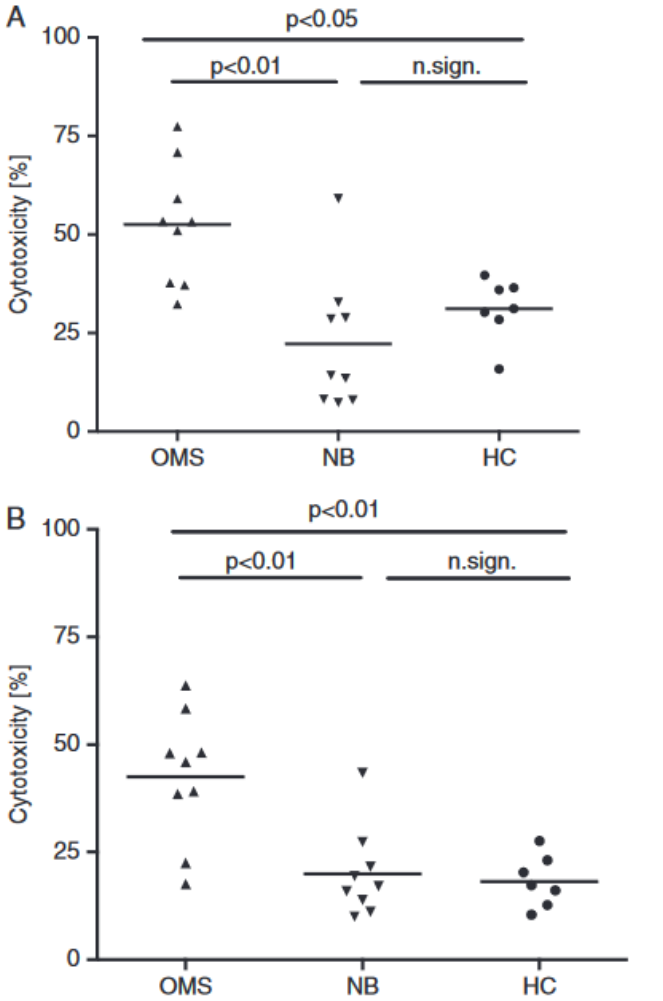


FIGURE 3. Incubation of the neuroblastoma (NB) cell lines NB-K (A) and SKN-AS (B) with immunoglobulin G (IgG) from opsoclonus-myoclonus syndrome (OMS), NB, or healthy controls (HC) and human-isolated natural killer (NK) cells. Human-isolated NK cells together with IgG from OMS patients induced significant cytotoxicity to both NB cell lines, compared with the NB and the HC groups ($P < 0.01$ for all, except OMS vs. HC in SKN-AS with $P < 0.05$). n. sign. indicates not significant.

Hill et al., *Neurology*, 2013; Utine et al., *J Child Neurol,* 2013; Petit-Pedrol et al., *Neurology,* 2021; Zar et al., *J Pediatr Hematol Oncol* 2021

SO IT IS GLUK2?

Three neuronal antigens identified in children with OMS of unknown etiology

- Petit-Pedrol (2021) identified that three patients had neuronal antigens identified although others had antibodies against glycine, HNK1, GluK2, and GAD65

- Landa et al (2021) reported on multiple patients with co-existent antibodies for GluK2 however the dominant phenotype was the other antibody in multiple cases.

TABLE 2. Clinical Features of Patients with GluK2 and Concurrent Antibodies

Case # Sex/age	Concurrent antibodies	Main syndrome	Brain MRI	CSF	Tumor	Follow-up, outcome
9 M/67	AMPAR, GluK2, 1, 3	Limbic encephalitis[a]	FLAIR/T2 increased signal in medial temporal lobes	WBC N Prot N	No	3 months, died in the acute phase of the disease (sepsis)
10 F/70	AMPAR, GluK2	Acute presentation of short-term memory loss, confused, disoriented	Normal	WBC N Prot 64	SCLC	Partial improvement, died of cancer 39 mo after symptom onset
11 F/44	AMPAR, CRMP5, GluK2, 1	Limbic encephalitis[a]	N/A	WBC 15 Prot, N	Thymoma	No improvement. Autopsy confirmed inflammatory infiltrates in both temporal lobes.
12 F/51	AMPAR, CRMP5, GluK2, 1	Acute confusion, memory loss, asymmetric weakness in arms and legs	N/A	WBC N Prot, N	Thymoma	N/A
13 F/41	AMPAR, GABAbR, CASPR2, GluK2, 1, 3	Limbic encephalitis[a]	FLAIR/T2 increased signal in medial temporal lobes	WBC 10 Prot, N	Metastatic thymoma	N/A
14 F/14	NMDAR, GluK2	Anti-NMDAR encephalitis[a]	Normal	WBC 63 Prot N	No	Substantial recovery; mild residual deficits in processing speed

[a]Patients with typical clinical manifestations of limbic or anti-NMDAR encephalitis.
AMPAR = alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CSF = cerebrospinal fluid; F = female; FLAIR = Fluid-attenuated inversion recovery; M = male; MRI = magnetic resonance imaging; N = normal; NMDAR = N-methyl-D-aspartate receptor; Prot = protein concentration (mg/dl); SCLC = small-cell lung carcinoma; WBC = white blood cell count (cells/µl).

B-CELL BIOMARKERS: SMOKE FROM FIRE?

B-Cell Activating Factor (BAFF) is a B-cell Modulating Cytokine Upregulated in OMS

- Expressed by astrocytes, dendritic cells, macrophages, and monocytes. Part of the TNF ligand superfamily.
- BAFF is elevated in OMS and declines with both ACTH and corticosteroids
- Fuhlbuer (2009) reported that while individuals with inflammatory CNS disease have BBB disruption, OMS did not, indicating that BAFF is produced intrathecally

A Proliferation Inducing Ligand (APRIL) is Another B-cell Modulating Cytokine Upregulated in OMS

- APRIL is a tumor necrosis factor ligand superfamily member. This protein signals apoptosis through interaction with a variety of other TNFRSF proteins and interactions with BAFF to increase local B-cell recruitment
- APRIL is increased by utilization of IVIg and Rituximab in OMS. The rationale for this is unclear as APRIL is thought to be pathologic in serum and therapies like IVIg improve the symptoms of OMS

Pranzatelli et al., *J Neuroinflammation* , 2013 Fuhlhuber et al., *J Neuroimmunol,* 2009

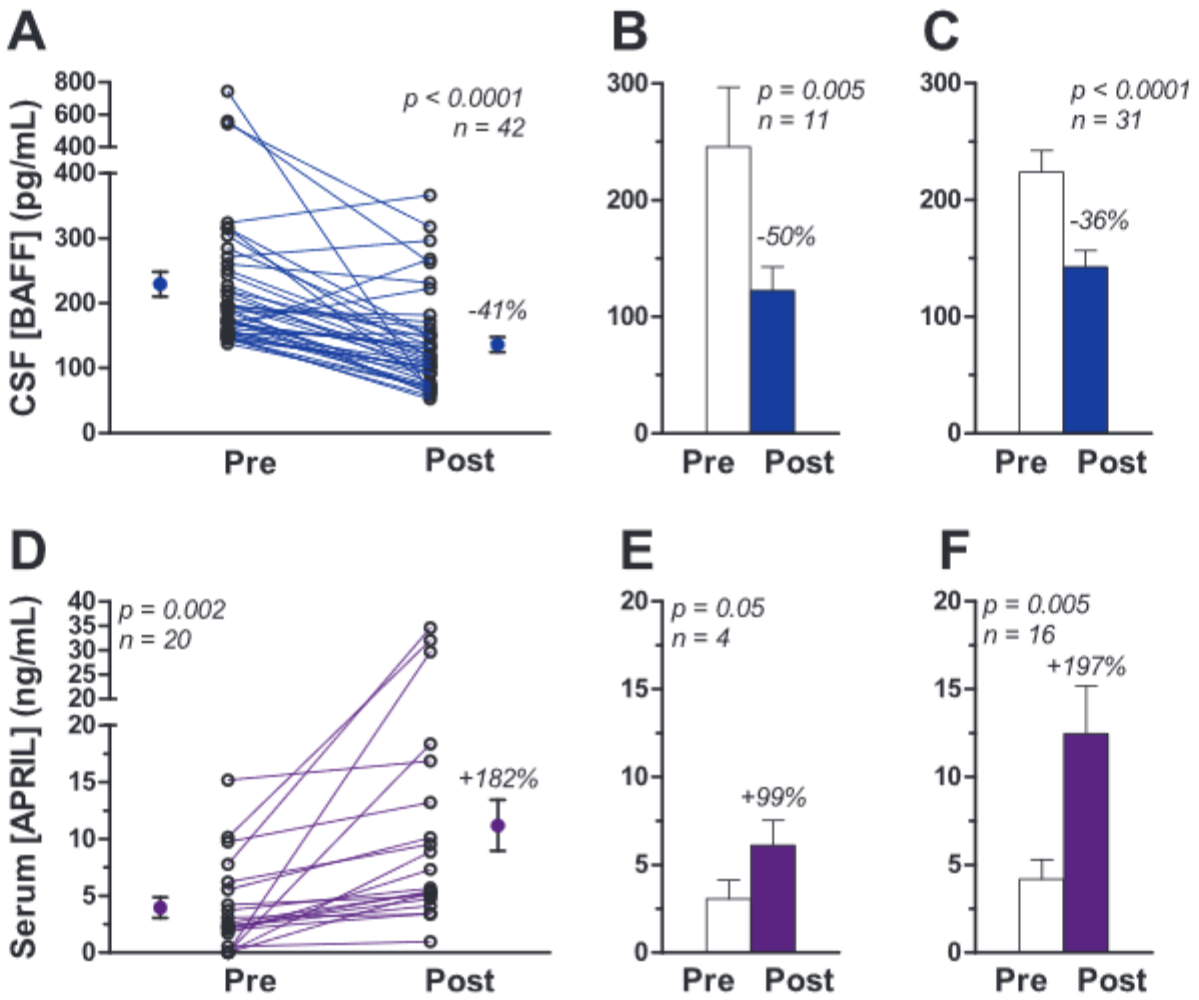
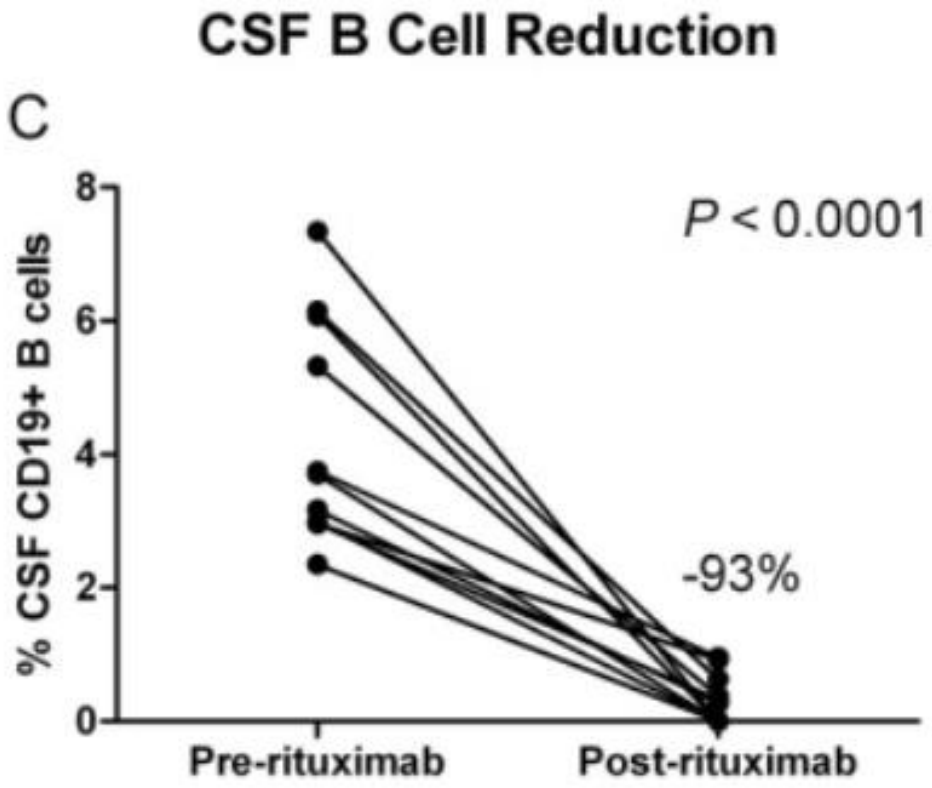


Figure 5 Longitudinal effects of immunotherapy on CSF BAFF and serum APRIL. (**A**) CSF BAFF pre- and post-treatment with ACTH-based combination immunotherapy. Group means with SEM and percent reduction are shown to either side of the line plot. (**B**) ACTH-based immunotherapy without rituximab. Comparisons were made by paired *t* tests. (**C**) ACTH-based immunotherapy with rituximab. Pre-treatment means in Figures B and C did not differ significantly; neither did post-treatment means. (**D**) Pre- and post-IVIg treatment serum APRIL. (**E**) Data from 1 g/kg IVIg dose. (**F**) Data from 2 g/kg IVIg dose. No significant differences were found between pre-treatment means or between post-treatment means in Figures E and F.

Pranzatelli et al., *J Neuroinflammation* , 2013



Pranzatelli et al., *Movement Disorder,* 2010

PERIPHERAL IGG MEDIATED NEURONAL CYTOLYSIS

Serum IgG in Paraneoplastic OMS Hijacks the NO/sGC/PKG Pathway

- Serum IgG in 10 children with paraneoplastic OMS were compared to healthy controls, neuroblastoma controls, JIA, and NMDARE
- The Fab segment of IgG did not induce microglial activation
- Microglial activation and cytolysis was inhibited by application of NO inhibition
- Destruction was **microglia-specific** with astrocytes being spare

These Cytoylitic Cascades Can Be Altered Through IGF-1/PI3K Manipulation

- PI3K expression is higher in serum of children with OMS
- Application of PI3K inhibition increases cytolysis in both cerebellar and cortical neurons. Application of a cell-permeable PI3K activator alleviated cytolysis similarly.
- Similar patterns were observed with IGF1- application (reduced cytolysis) and IGF-1 antagonism (increased cytolysis)

Ding et al., *Pediatr Res,* 2019; Ding et al., *J Neuroinflamm,* 2020

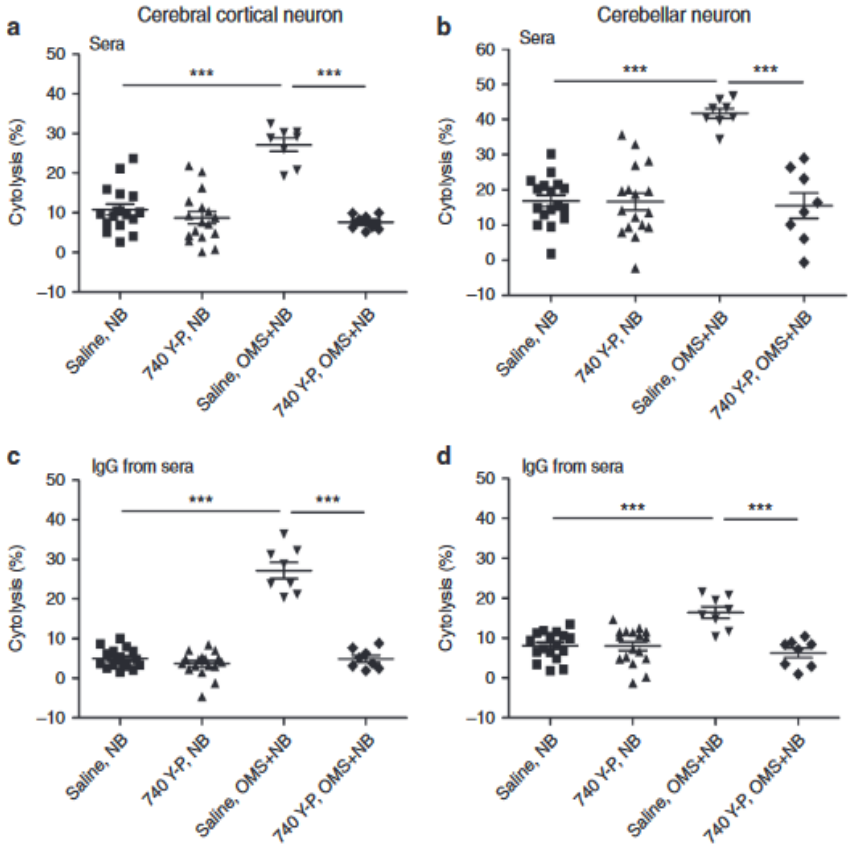


Fig. 6 Exposure to 740 Y-P, a cell-permeable phosphopeptide activator of PI3K, alleviates the cytolysis of cultured rat neurons induced by sera or IgG of children with OMS and NB. Note that the cytolysis of cerebral cortical **a** and cerebellar neurons **b** induced by sera in the OMS + NB group was blocked by 740 Y-P, and the cytolysis of cerebral cortical **c** and cerebellar neurons **d** induced by IgG in the OMS + NB group was attenuated by 740 Y-P, whereas 740 Y-P had no effect on cytolysis in neurons incubated with sera or IgG of NB patients. ***$P < 0.001$, one-way ANOVA, $n = 17$ (Saline, NB; 740 Y-P, NB), $n = 8$ (Saline, OMS + NB; 740 Y-P, OMS + NB)

PI3K activation decreases cytolysis

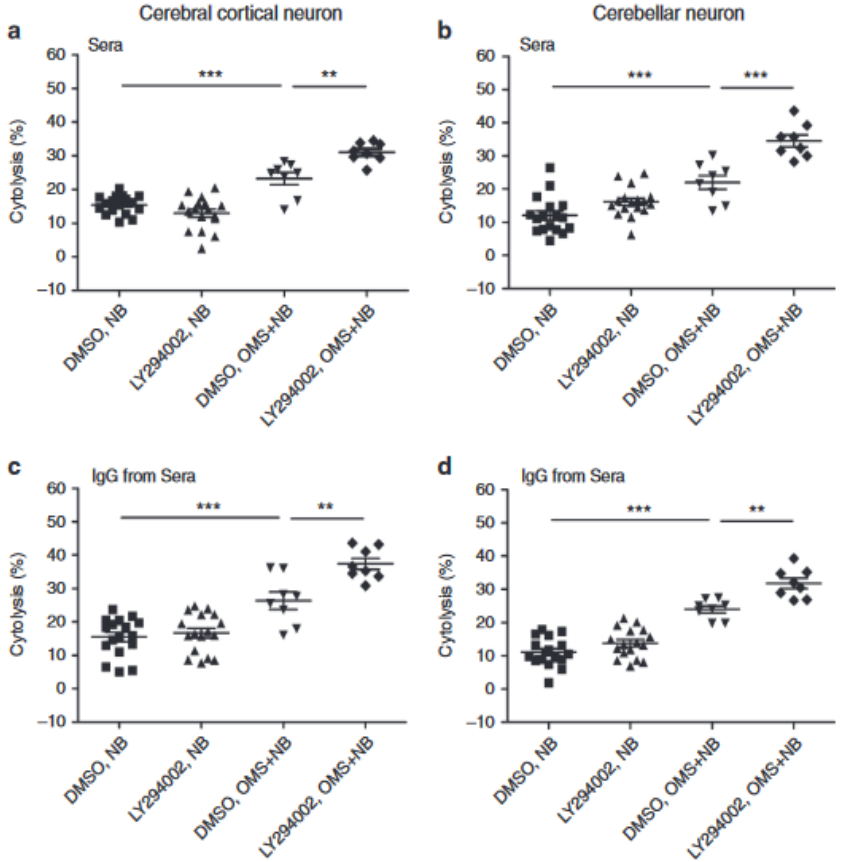


Fig. 5 The PI3K pharmacological inhibitor LY294002 aggravates the cytolysis of cultured neurons induced by sera or IgG from children with OMS and NB. Note that the cytolysis of cerebral cortical **a** and cerebellar neurons **b** induced by sera in the OMS + NB group was exaggerated by LY294002, and the cytolysis of cerebral cortical **c** and cerebellar neurons **d** induced by IgG in the OMS + NB group was aggravated by LY294002, whereas LY294002 had no effect on cytolysis in neurons incubated with sera or IgG of NB patients. **$P < 0.01$, ***$P < 0.001$, one-way ANOVA, $n = 17$ (DMSO, NB; LY294002, NB), $n = 8$ (DMSO, OMS + NB; LY294002, OMS + NB)

PI3K inhibition increases cytolysis

Ding et al., *Pediatr Res*, 2019

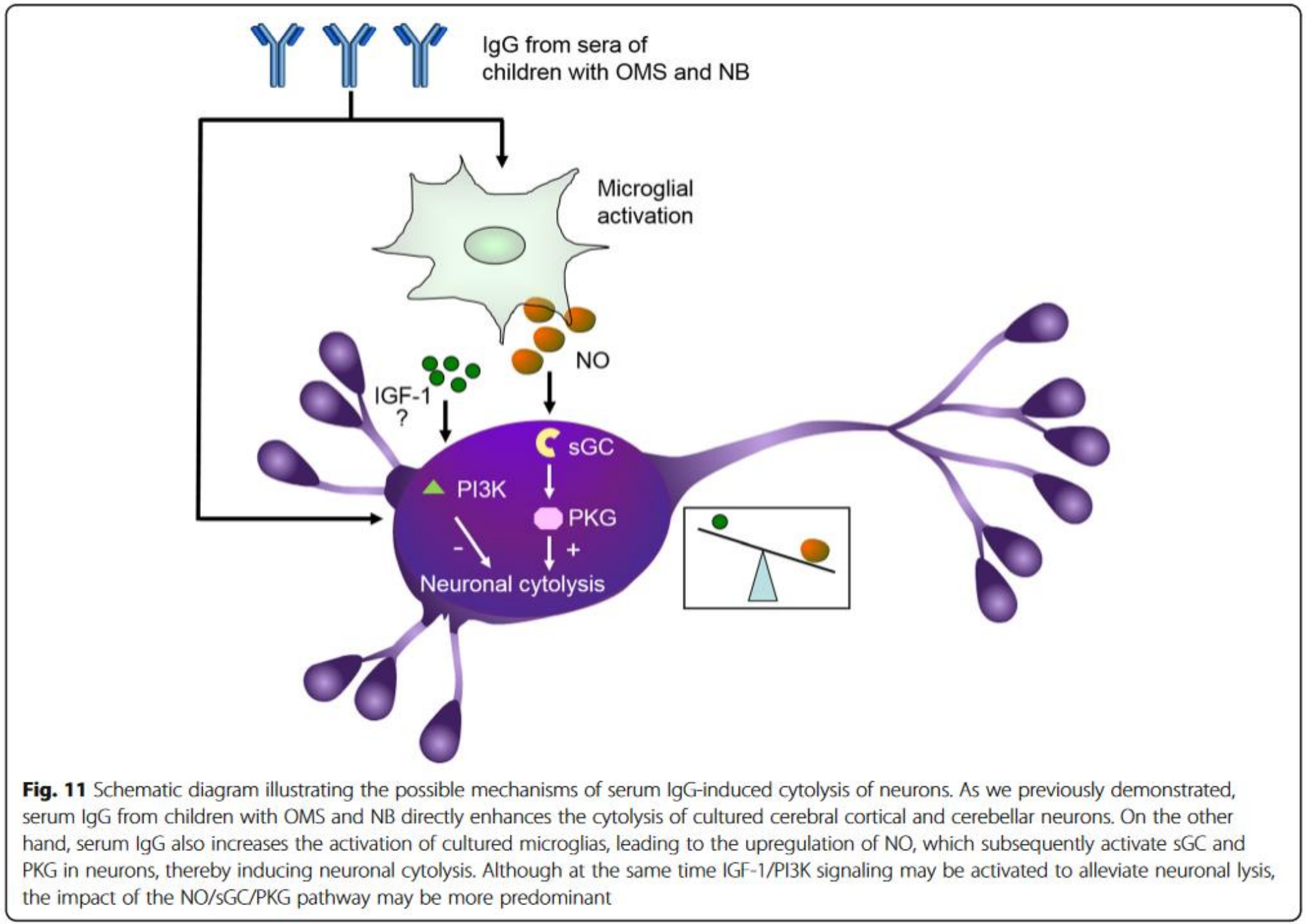
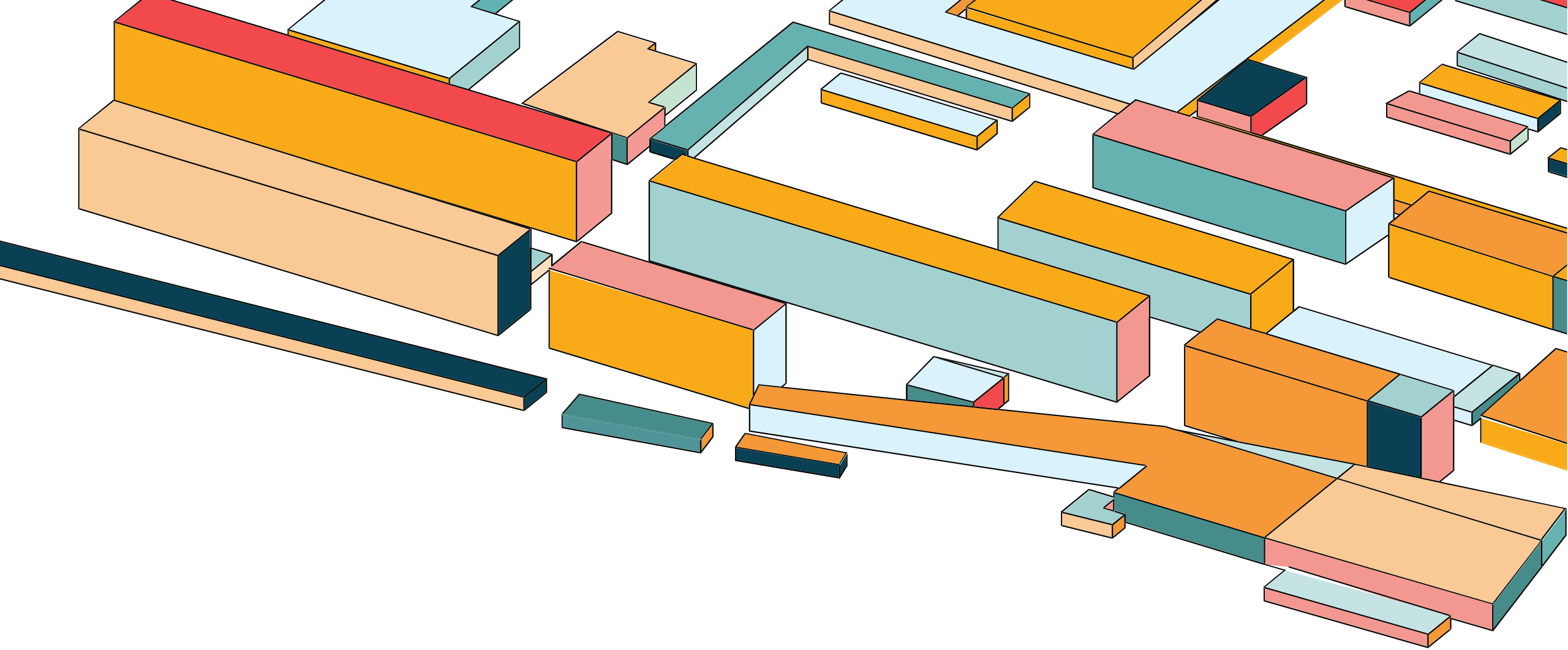


Fig. 11 Schematic diagram illustrating the possible mechanisms of serum IgG-induced cytolysis of neurons. As we previously demonstrated, serum IgG from children with OMS and NB directly enhances the cytolysis of cultured cerebral cortical and cerebellar neurons. On the other hand, serum IgG also increases the activation of cultured microglias, leading to the upregulation of NO, which subsequently activate sGC and PKG in neurons, thereby inducing neuronal cytolysis. Although at the same time IGF-1/PI3K signaling may be activated to alleviate neuronal lysis, the impact of the NO/sGC/PKG pathway may be more predominant



TIME FOR HIGH “T”

T-CELLS IN A B-CELL WORLD

OMS has Increased CD3/4+ Cell Populations in the CSF

- On the larger scale, increased T-cell activation and decreased helper T-cell populations are noted in OMS
- The reduced populations of T-helper cells has been observed in a variety of autoimmune disorders (CNS and non-CNS)
- It remains unclear if T-cells are recruited as a secondary or primary process although **higher tumor burden may be driving B-cell response**

Expansion of γδ T-cells

- Expansion of **γδ** T-cells (also called fetal-type lymphocyte) is of unclear significance as these are *primarily expressed in the first three years of life*
- As these cells are **inherently autoreactive**, they do not require antigen presentation from APC and can recognize antigen directly in tissue
- TUMOR v NON-TUMOR: no differences…

Pranzatelli et al., *Neurology,* 2004

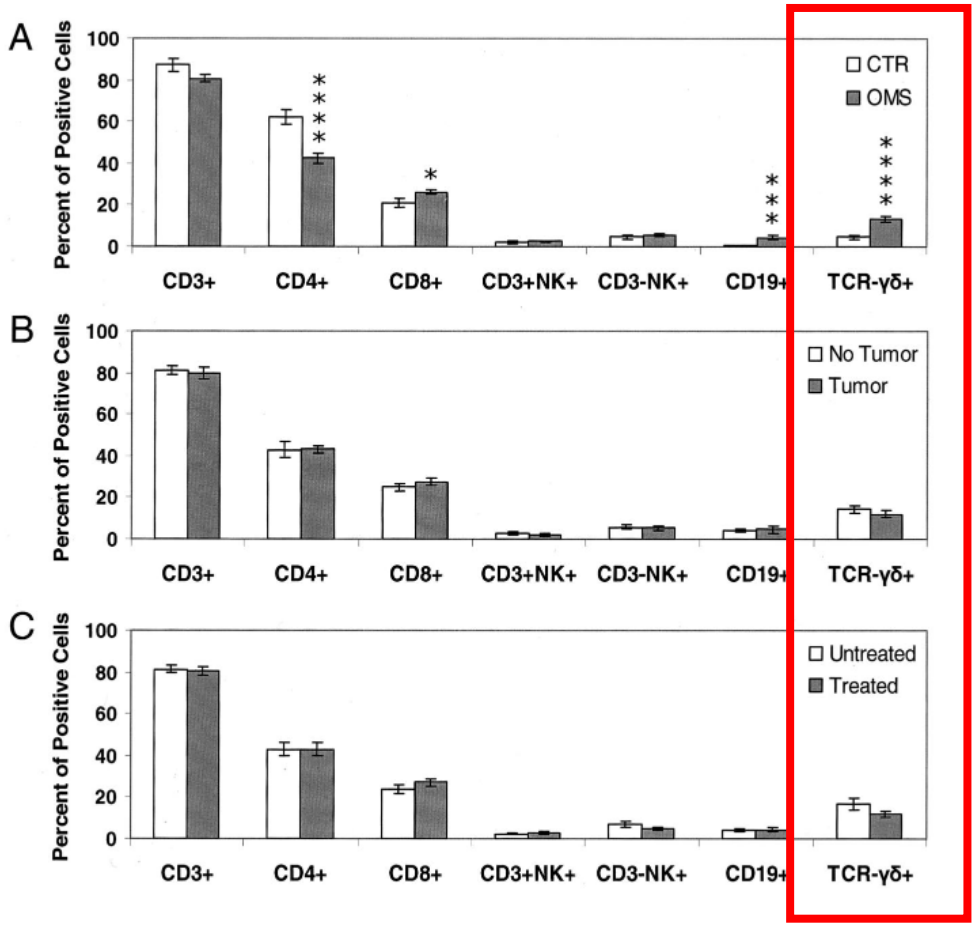


*Figure 1. Distribution of CSF lymphocyte population. (A) In opsoclonus–myoclonus syndrome (OMS), the percentage of $CD3^+$ cells and $CD4^+$ T-cells was reduced compared with controls, but increases were found for $CD19^+$ B-cells, γδ T-cells, and $CD8^+$ T-cells. There were no statistically significant differences between tumor and no-tumor groups (B) or untreated and treated groups (C). Data are means ± SEM. The B-cell percentage for controls was 0.7 ± 0.2%. Asterisks signify statistical significance by t-tests: *0.01 ≤ p < 0.05, ***0.0001 ≤ p < 0.001, **** p < 0.0001.*

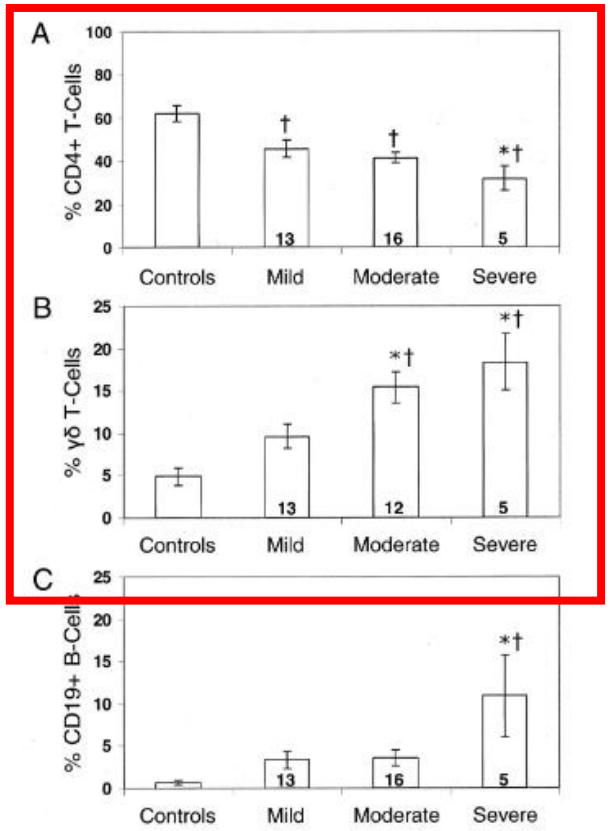
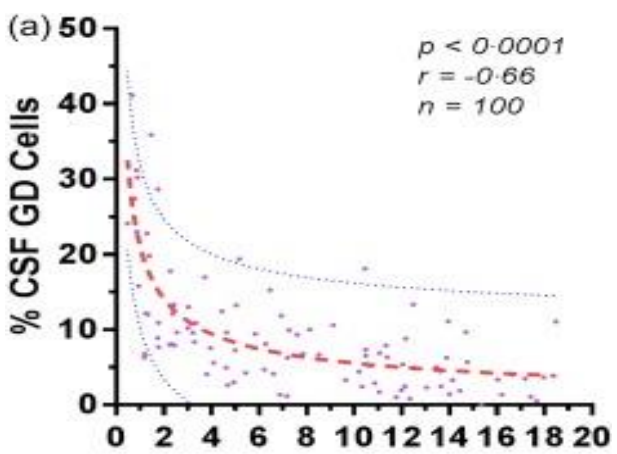


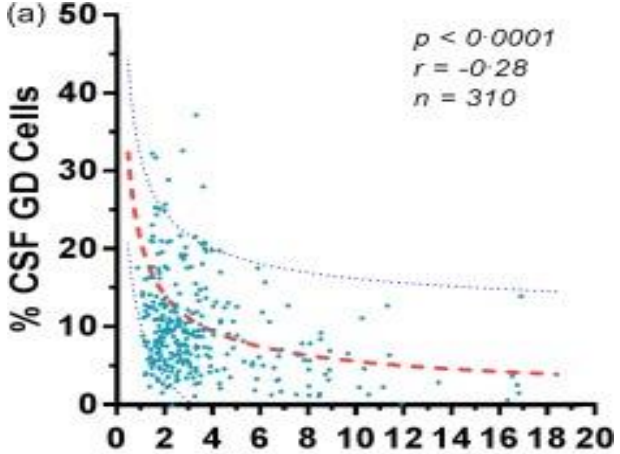
Figure 3. Relation between neurologic severity (total score) in opsoclonus–myoclonus syndrome (OMS) and percentage of CSF $CD4^+$ T-cells (A), γδ T-cells (B), and $CD19^+$ B-cells (C). Sample size is shown at the base of each column. Data are means ± SEM. Asterisks indicate statistically significant differences between OMS severity categories on Duncan test, p < 0.05. Dagger indicates significant differences between OMS and control subjects. Analysis of variance with linear trend analysis revealed that the more severely affected children (higher total score) had a lower percentage of CSF $CD4^+$ T-cells ($F = 20.6$, $p \leq 0.0001$). In contrast, the percentage of CSF γδ T-cells increased with severity ($F = 25.6$, $p \leq 0.0001$), being nearly double in the severe category, and the percentage of CSF $CD19^+$ B-cells was also higher ($F = 21.2$, $p \leq 0.0001$).

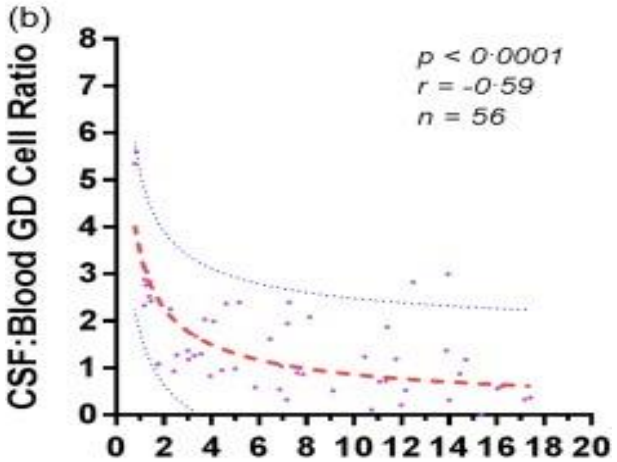
Pranzatelli et al., *Neurology,* 2004

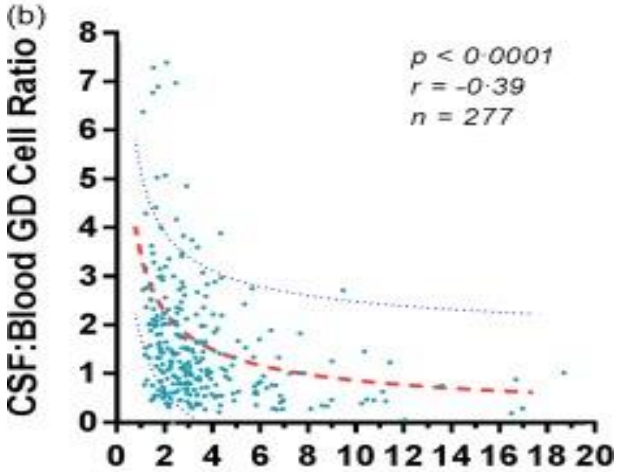
But…

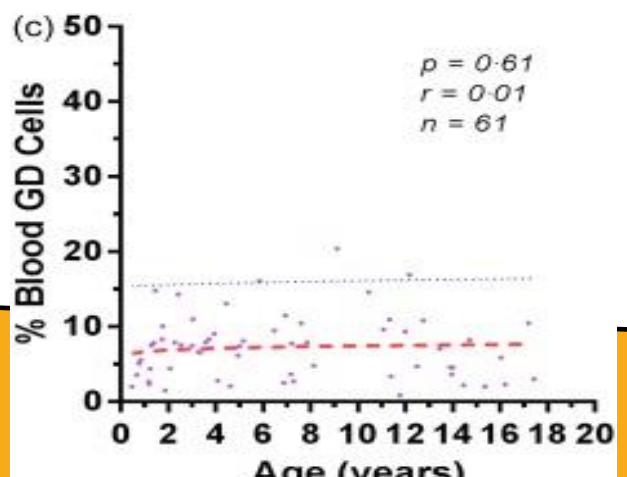
γδ T-cells decrease in the CSF and serum with age

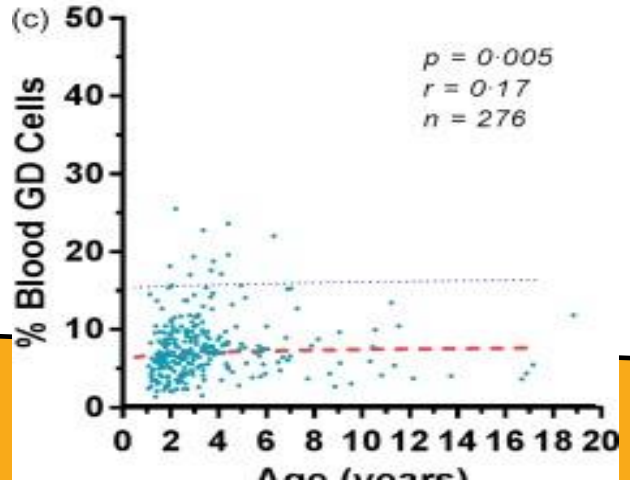












Pranzatelli et al., *Clin Exp Immunol,* 2018

T-CELL REDUCTION IS GOOD?

Administration of Steroid Sparing Therapies Decrease CD4+ in CSF

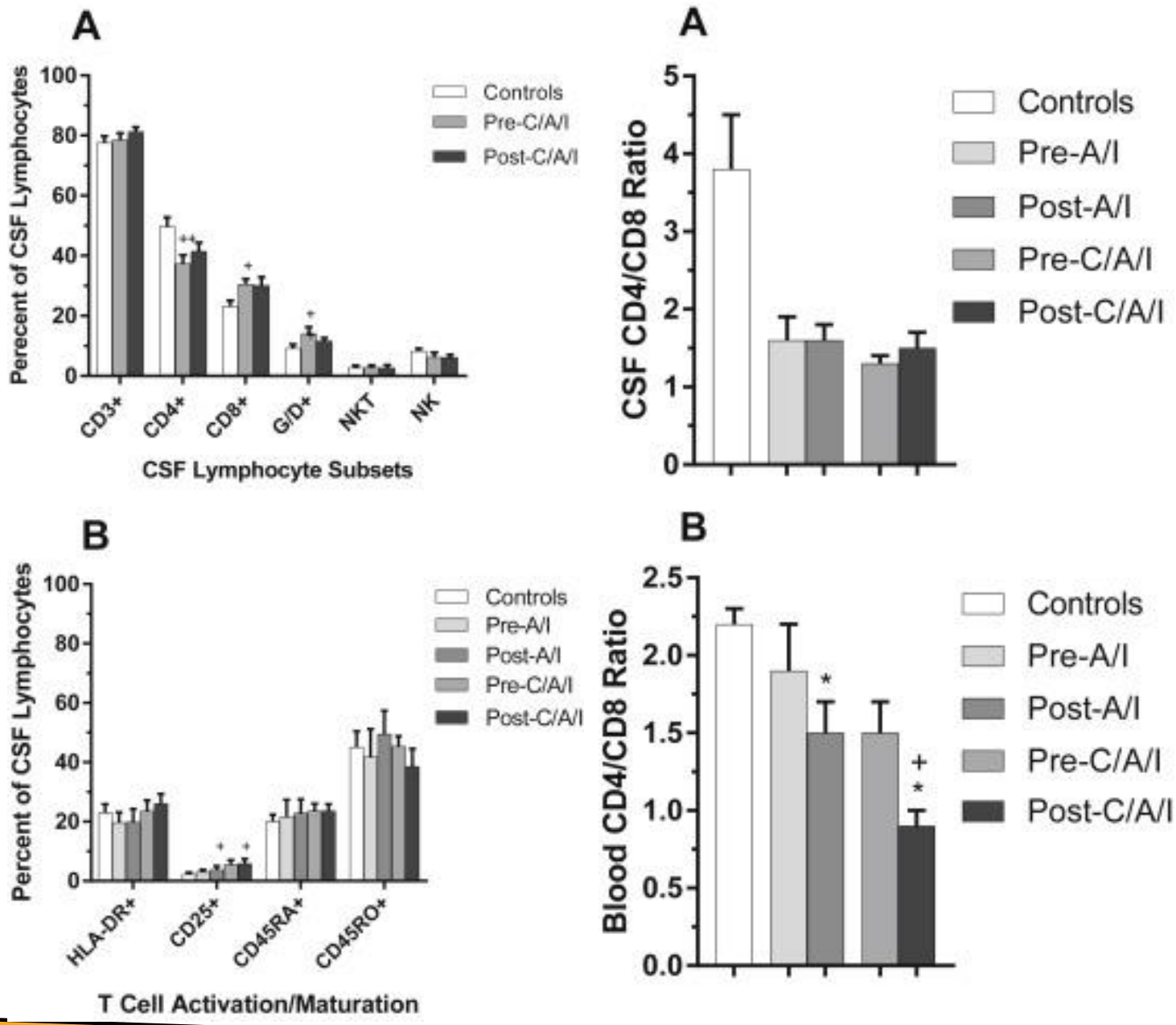
- Utilization of 6MP decreases CD4+ cell populations in the CSF by 21% with further reductions in NK cells (32%)
- Subsequent increases in Th2 (helper cell) responses which correlate closely with clinical improvement

Tumors May Drive T-Cell Response

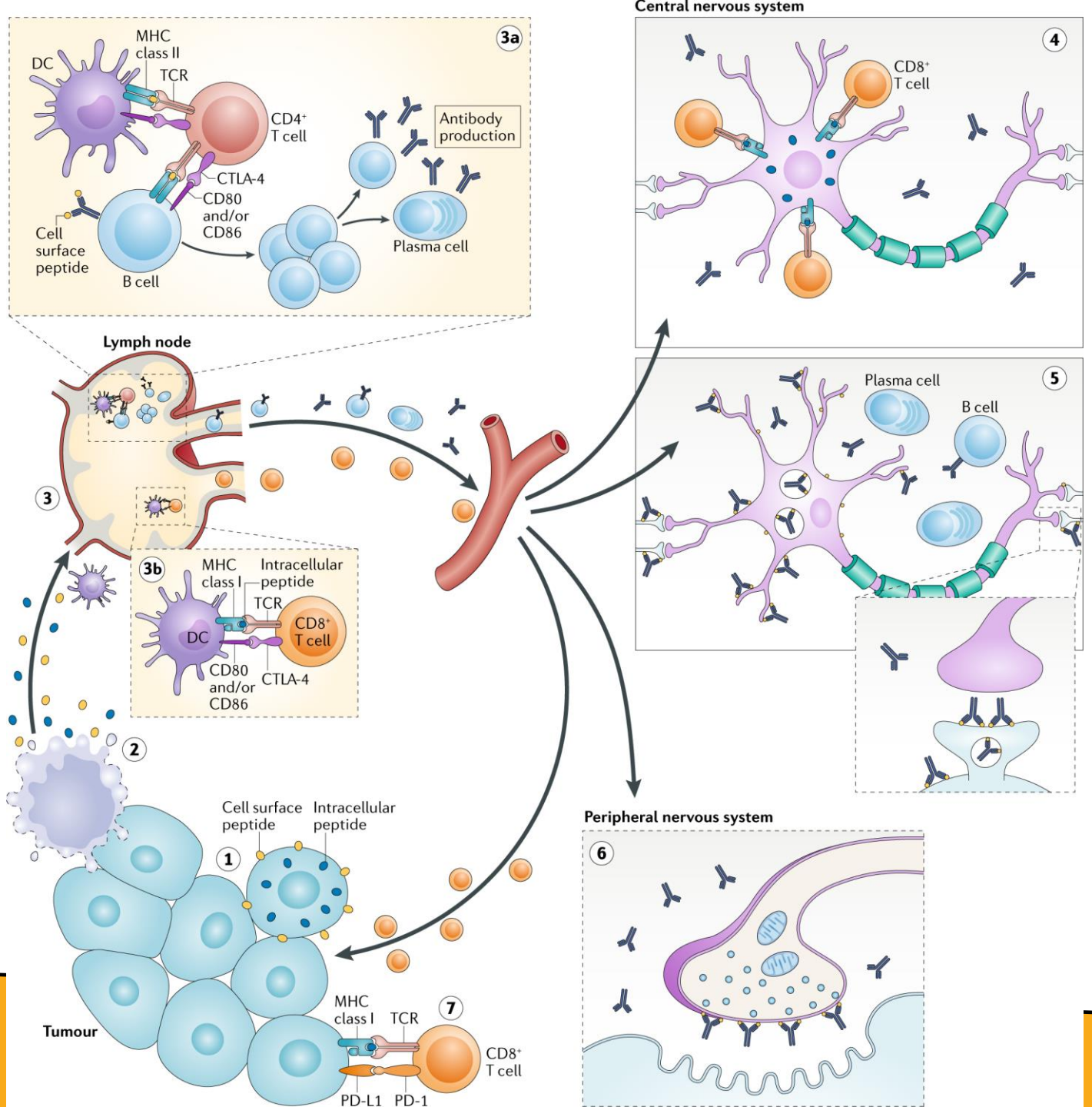
- NB in OMS is associated with more immunogenic profile as previously shown
- Bao et al (2021) reported that T-cells are of higher prevalence in higher risk (>18 mo) patients and those with OMS
- High CD3+ cells are correlated with favorable oncologic prognosis regardless of CD4/CD8 ratio

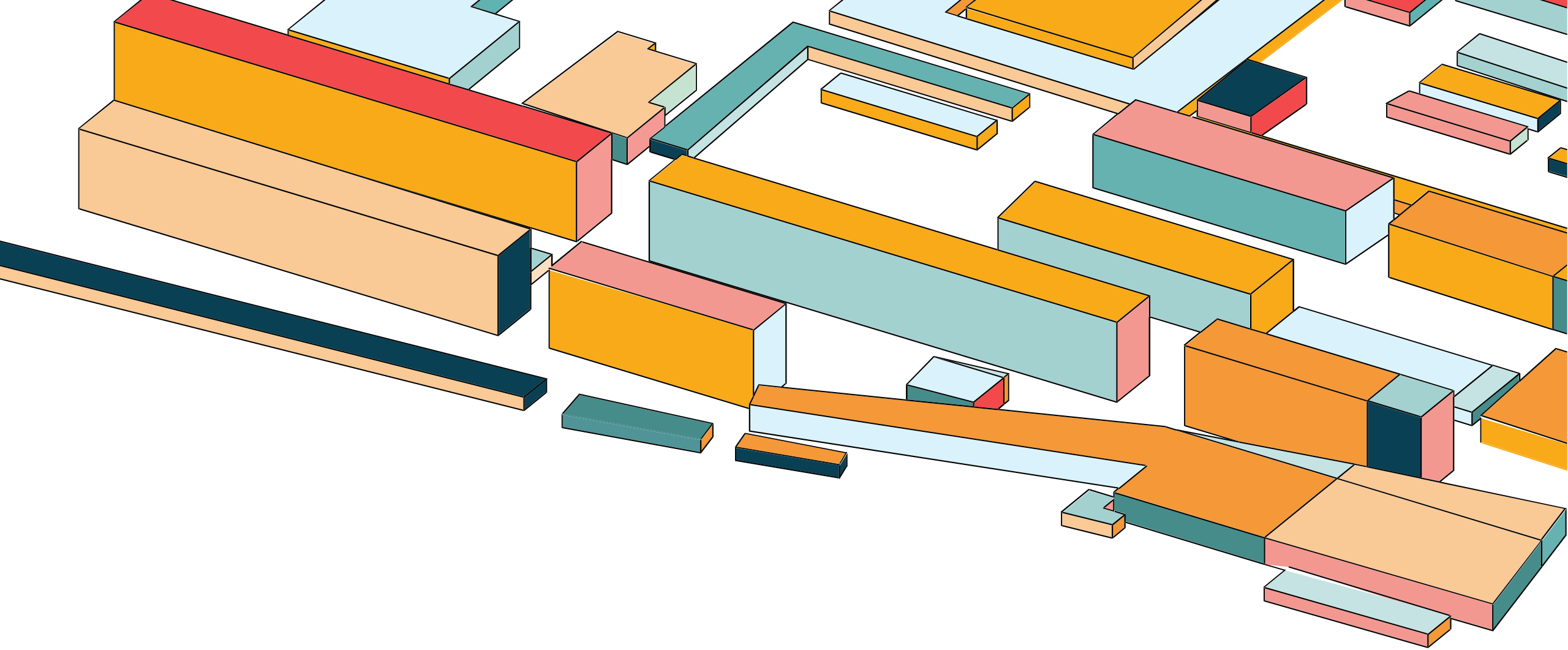
Pranzatelli et al., *J Transl Immunol,* 2017; Bao et al., *J Immunother Cancer,* 2021; Mina et al., *Oncoimmunology,* 2015

"The main neuroimmunological observation was that low-dose cyclophosphamide combination immunotherapy exerted demonstrable anti-B cell effects in CSF rather than restorative effects on CSF lymphocyte phenotypic abnormalities involving T cells."



Pranzatelli et al., *Eur J Paediatr Neurol,* 2018


3a
MHC class II
DC
TCR
CD4+ T cell
Antibody production
CTLA-4
CD80 and/or CD86
Cell surface peptide
B cell
Plasma cell
Central nervous system
4
CD8+ T cell
Lymph node
3
Plasma cell
B cell
5
3b
MHC class I
Intracellular peptide
TCR
DC
CD8+ T cell
CD80 and/or CD86
CTLA-4
2
Cell surface peptide
Intracellular peptide
1
Peripheral nervous system
6
Tumour
MHC class I
TCR
7
CD8+ T cell
PD-L1
PD-1

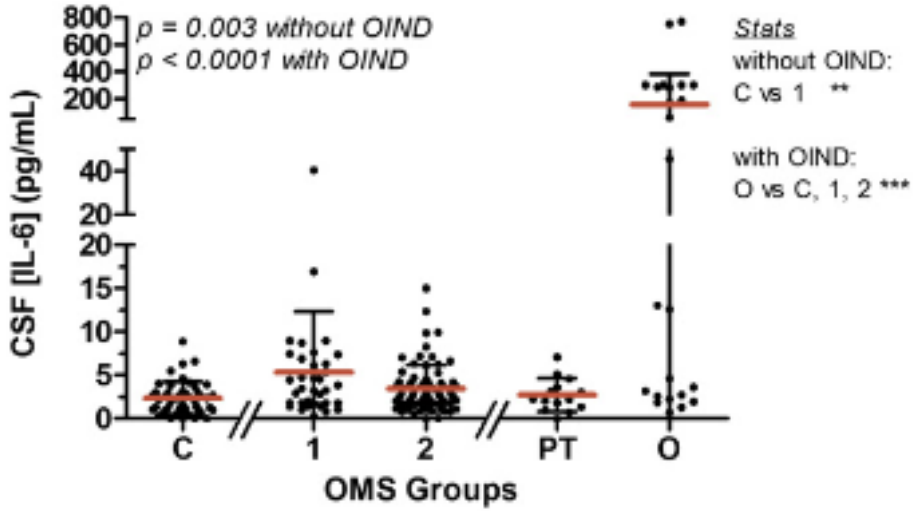


KEEP CALM AND CHEMOKINE ON

SERUM/CSF CYTOKINES

Serum and CSF Cytokines also Have Been Identified in Active OMS

- CSF Cytokines are highly variable and poorly predictive of untreated v. treated OMS status
- Similarly weak signal detected in serum cytokine analysis in IL-Ra (high), IL-4 (high), IL-13 (high), IL-6 (high), CCL4 (high), IL-16 (high), CCL3 (low), and CCL11 (high)
- Although aberrations were detected, and can be linked to a variety of other neuroinflammatory disorders with similar patterns, nothing emerged as specific for OMS



C. Controls *(47)*
1. Untreated OMS *(35)*
2. Treated OMS *(77)*
PT. Previously-treated OMS *(14)*
O. OIND *(24)*

Fig 1. CSF IL-6 concentration as measured by ELISA. In the legend, the n value for each group is given within parentheses. Horizontal lines indicate the mean ± 1 SD. Post-hoc tests of means (*) are shown to the right of each graph under "Stats." The level of statistical significance in Tukey post-hoc tests is indicated by the number of symbols: *$0.01 \leq p < 0.05$, **$0.001 \leq p < 0.01$, ***$0.0001 \leq p < 0.001$. The previously-treated group is shown for comparison but was not part of the statistical analysis.

Pranzatelli et al., *J Neurol Sci,* 2013

CHEMOKINES AND CYTOKINES

- Pranzatelli et al. (2013) reported on elevations in **CCL17**, **CCL21**, and **CCL22** in untreated OMS and was **predictive of both disease severity and likelihood of response** to corticosteroids and ACTH.

- This was in comparison to CCL19 which was expressed in the CSF but was not altered by therapy of any type.

- Interestingly, CCL21 is a ligand for CCL7

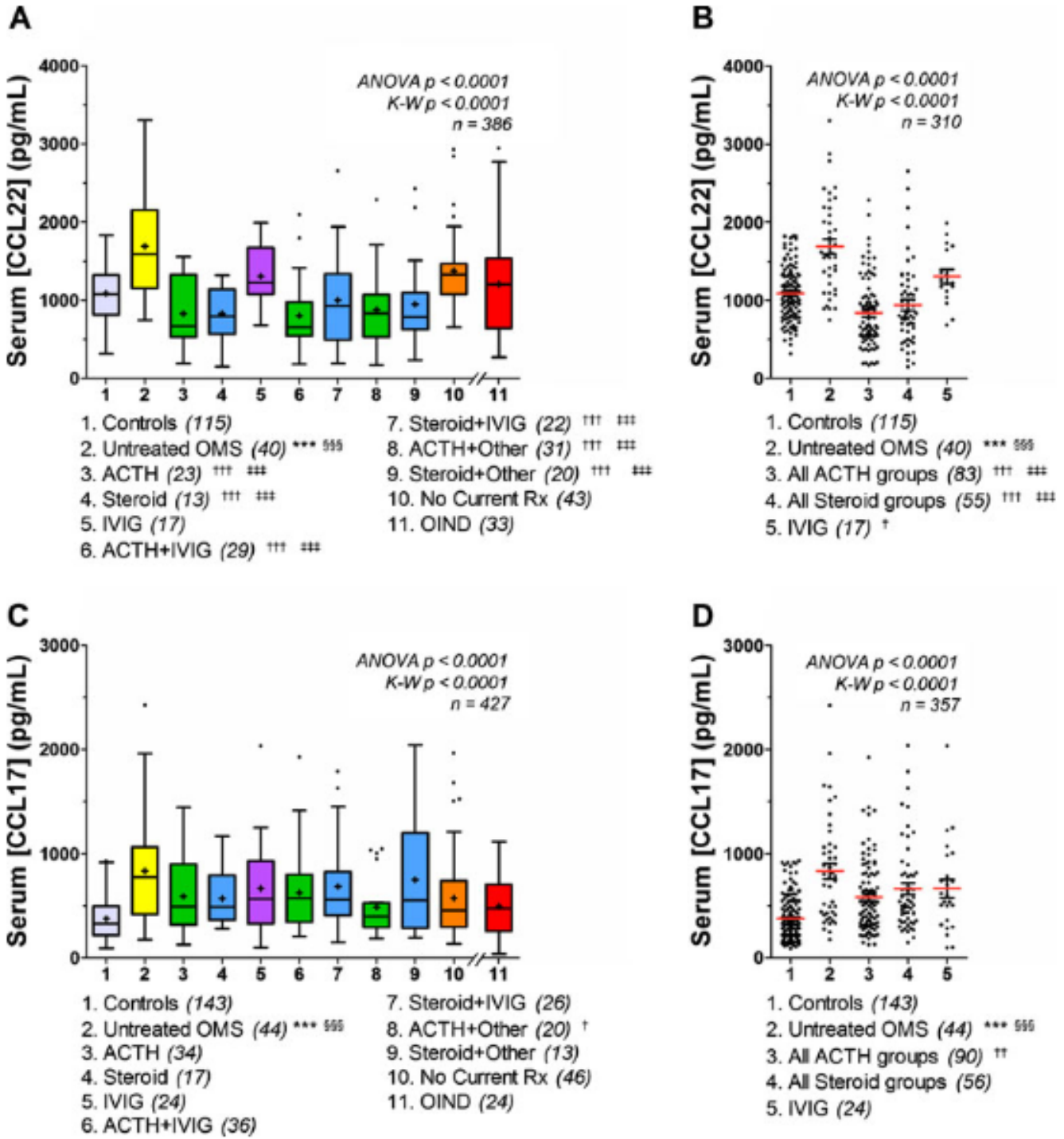
- *CCL7 is associated with receptor balance of tolerance and regulation of immune responses.*

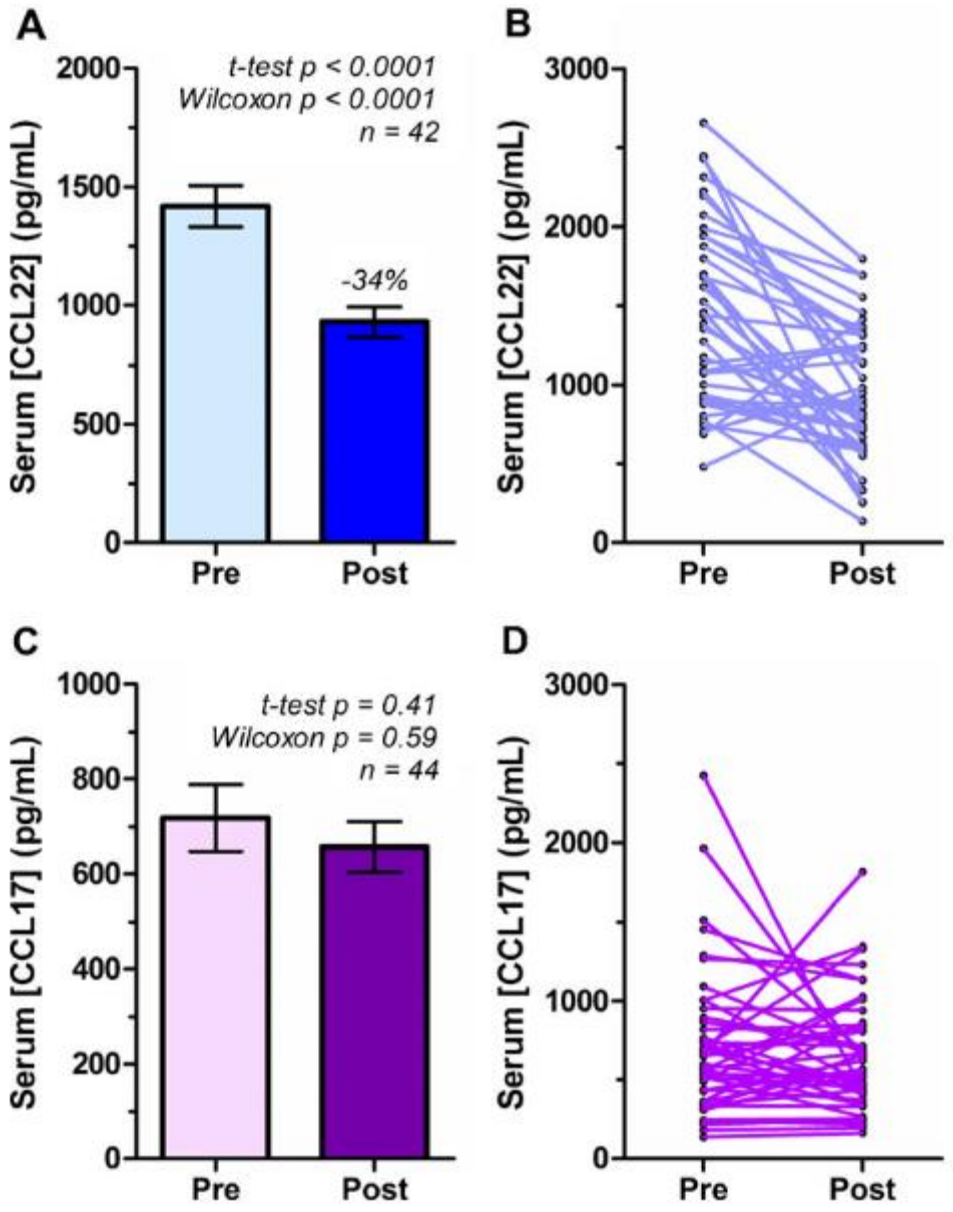
- CCL22 and CCL17 are ligands for CCL4.

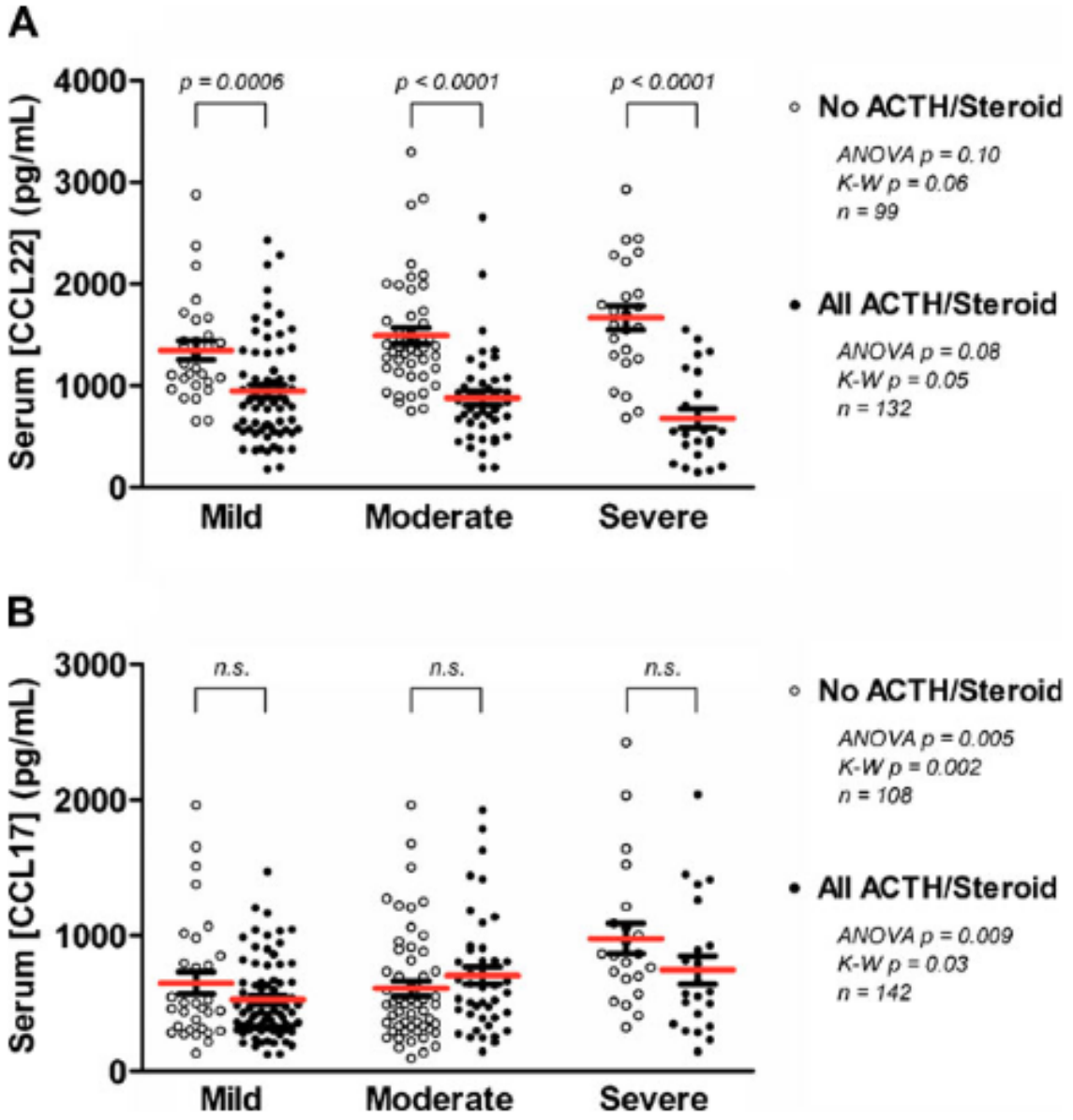
- *CCL4 is a chemoattractant for natural killer cells*, monocytes and various other immune cells in the site of inflamed or damaged tissue

Pranzatelli et al., *J Clin Immunol,* 2013; Pranzatelli et al., *Cytokine,* 2013

Fig. 1 **a** Cross-sectional comparison of serum CCL22 concentration in NIND controls, untreated OMS, various OMS active treatment groups, previously-treated OMS, and OIND. Post-hoc statistical comparisons of means were between untreated OMS and NIND controls (*) and between OMS treatment groups (†). Medians were also compared (§ and ‡, respectively) by the K-W test. The number of symbols denotes the level of significance: * $0.01 \leq p < 0.05$; ** $0.001 \leq p < 0.01$; *** $0.0001 \leq p < 0.001$. Means are indicated by + in the box, medians by the line in the box, the 75th/25th IQR by the upper and lower sides of the box, and the range by the bars. Dots indicate statistical outliers. **b** Collapse of the 3 ACTH groups into 'All ACTH groups' and the 3 steroid groups into 'All Steroid groups' for CCL22 analysis. The line indicates the mean. **c** Cross-sectional comparison of CCL17 concentration. **d** Combined ACTH groups and combined steroid groups for CCL17 analysis







Pranzatelli et al., *J Clin Immunol,* 2013

ARE WE BARKING UP THE WRONG TREE?

CCL4 Seems Like A Reasonable Therapeutic Target

- Overexpression of CCL17 and CCL22 ligands in OMS and a variety of other neuroimmune and autoimmune conditions is present
- In vitro experiments have indicated that these neutraligands can inhibit CCL17 and/or CCL22-induced intracellular calcium responses, CCR4 endocytosis, and human T-cell migration.

There is always a but…

- Blockade of CCL4, CCL17 and CCL22 have not been able to augment disease in EAE models of MS
- Anti-CCL4 antibodies (Mogamulizumab) have been used in a verity of infectious diseases (HTLV) and neoplastic disorders (T-cell lymphoma) with reasonable safety although dermatologic side effects seem common (including SJS!)

Sato et al., *NEJM,* 2018; Ishi et al., *Clin Cancer Res,* 2010; Scheu et al., *Int J Mol Sci,* 2017

OTHER BIOMARKERS?

Neurofillament Light Chain Also Elevated in OMS

- Pranzatelli et al (2014) reported that CSF NfL is elevated in children with OMS and that more severe cases were correlated with higher levels of NfL.
- Correlation between NfL and other CSF biomarkers of B-cell mediated disease (next slide)
- Use of immunotherapy, as expected, reduced NfL concentrations

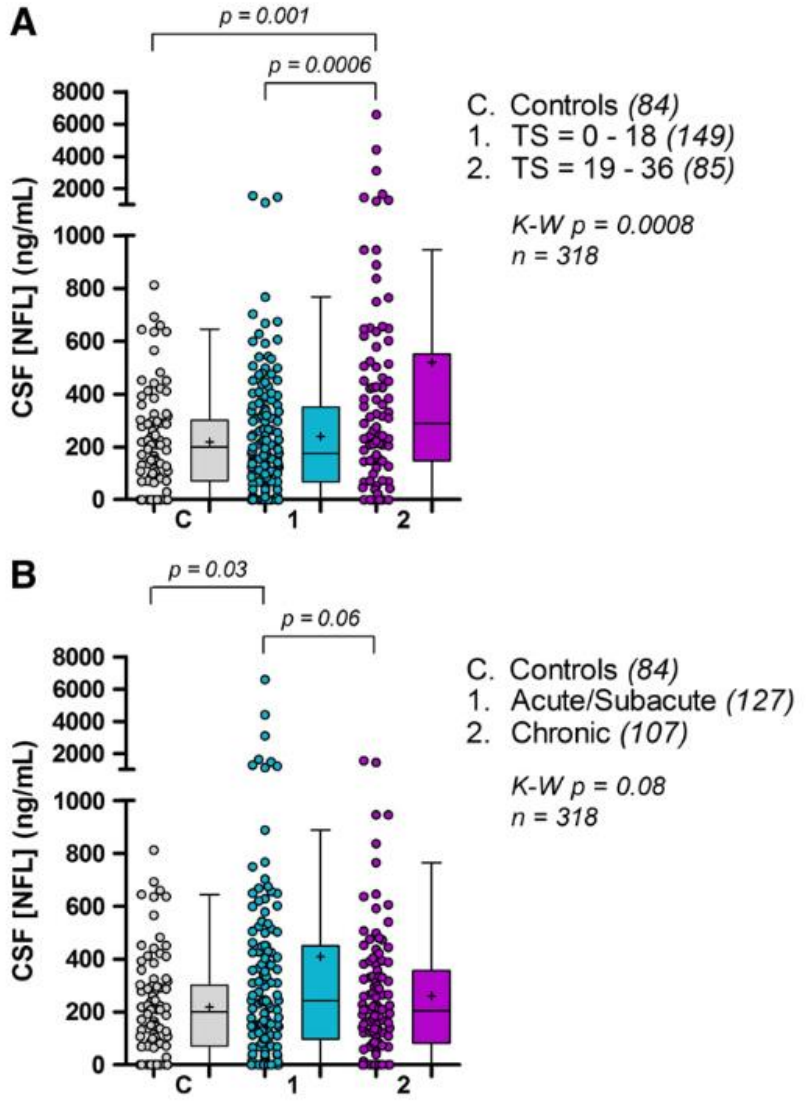


Fig. 2. Relation of CSF NFL to OMS severity and OMS duration. (A) Both raw data and box graphs are shown. Patients were grouped by a median split of Total Score (TS ≤ 18 vs ≥19) to compare higher vs lower severity OMS on CSF NFL. *p*-Values above brackets are Wilcoxin test results. (B) Patients were grouped as having OMS < 1 year (acute/subacute) or ≥1 year (chronic).

Pranzatelli et al., *J Neuroimmunol,* 2014

Table 2
Relation of brain-related proteins and inflammatory proteins and cells in OMS.

	Controls	OMS		3-Group	OMS
		'High' CSF NFL	'Normal' CSF NFL	*p*-Value[a]	*p*-Value[b]
Untreated OMS					
n	84	23	28		
CSF CXCL13 (pg/mL)	0 (0–1.9)	16 (6–43)	6 (0.1–14)	<0.0001	0.01†
CSF NSE (pg/mL)	3227 (2340–6807)	5825 (5081–7218)	3937 (2999–4916)	0.03	0.003†
CSF pNFH (pg/mL)[c]	0 (0–128)	22 (0–104)	0 (0–7.4)	0.06	n.s.
All OMS[d]					
n	84	57	177		
CSF leukocytes (cu mm)	1 (0.5–2)	3 (2–6)	1 (1–3)	0.0003	0.03
Total CSF B cells (%)	1.0 (0.3–1.5)	3.7 (1.6–7.1)	1.9 (0.5–3.7)	<0.0001	<0.0001†
CSF BAFF (pg/mL)	125 (97–155)	138 (89–212)	109 (72–161)	0.01	0.008†
CSF CXCL13 (pg/mL)	0 (0–1.9)	6.6 (0.1–15.8)	2.9 (0–6.8)	<0.0001	0.002†
Serum CCL21 (pg/mL)	459 (412–542)	572 (465–700)	462 (332–577)	0.01	0.004†

Data are medians with IQR. n.s., not significant.
CSF NSE and pNFH were not measured in treated OMS.
[a] Statistically significant uncorrected *p* values from K–W tests.
[b] Significant results from M–W tests after Bonferroni corrections are indicated by a cross.
[c] Excluding an extreme outlier in controls (1860 pg/mL) and the 'high' OMS group (2742 pg/mL).
[d] In 'All OMS', 40% of cases were untreated in the 'high' NFL group; 16% in the 'normal' NFL group.

Pranzatelli et al., *J Neuroimmunol,* 2014

EMERGING DATA

COVID19

Post-Infectious Syndrome and Provocation of Relapses
(everything is related to COVID)

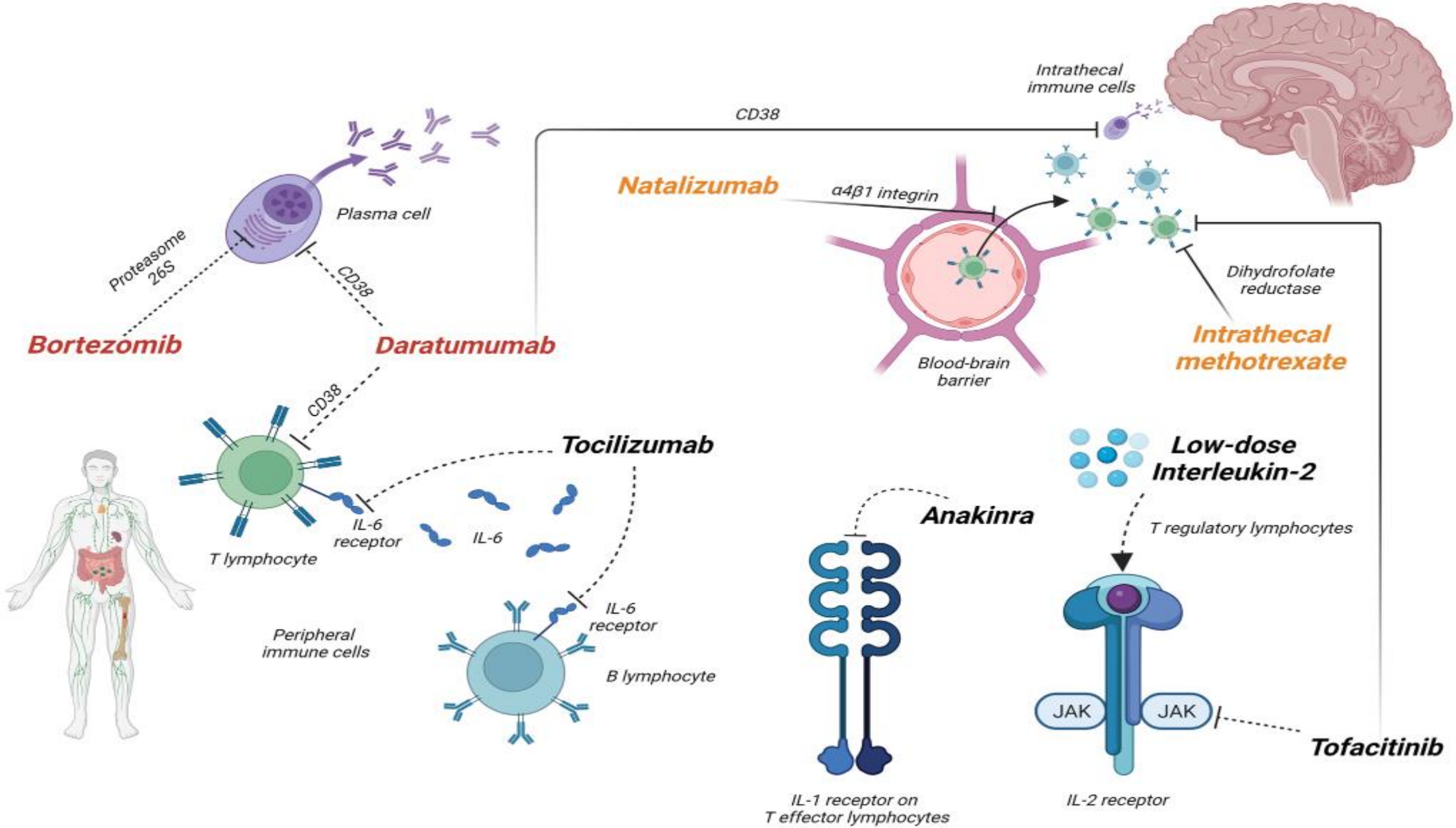
GluD2 in CSF

Case Report of Clonal GluD2 Ab in Serum and CSF
(been there, done that)

Increased Autoimmunity

Personal and Familial Autoimmunity Increased in Paraneoplastic and Non-Paraneoplastic Cases

Ishaq et al., *Front Neurol,* 2021; Noguchi et al. *Ped Blood Cancer,* 2022, ; Santoro et al., *Neurol Neuroimmunol Neuroinflamm,* 2021


Intrathecal
immune cells
CD38
Natalizumab
α4β1 integrin
Plasma cell
Proteasome
26S
CD38
Bortezomib
Daratumumab
Blood-brain
barrier
Dihydrofolate
reductase
Intrathecal
methotrexate
CD38
Tocilizumab
Low-dose
Interleukin-2
Anakinra
T regulatory lymphocytes
IL-6
receptor
IL-6
T lymphocyte
Peripheral
immune cells
IL-6
receptor
B lymphocyte
JAK
JAK
Tofacitinib
IL-1 receptor on
T effector lymphocytes
IL-2 receptor

REMAINING QUESTIONS

B-Cell/Antibodies

Is there a specific target?
Is this process primarily B-cell/antibody driven?

Genetics

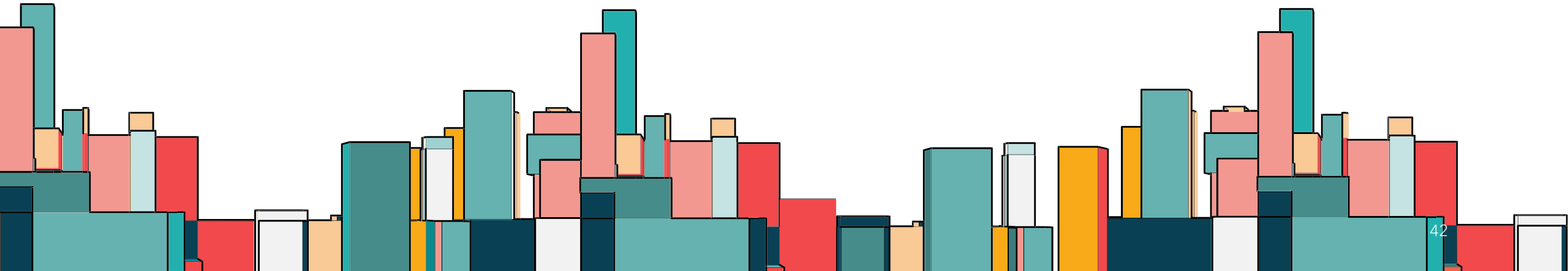
Does predilection for autoimmunity make the difference for who develops OMS and who does not?

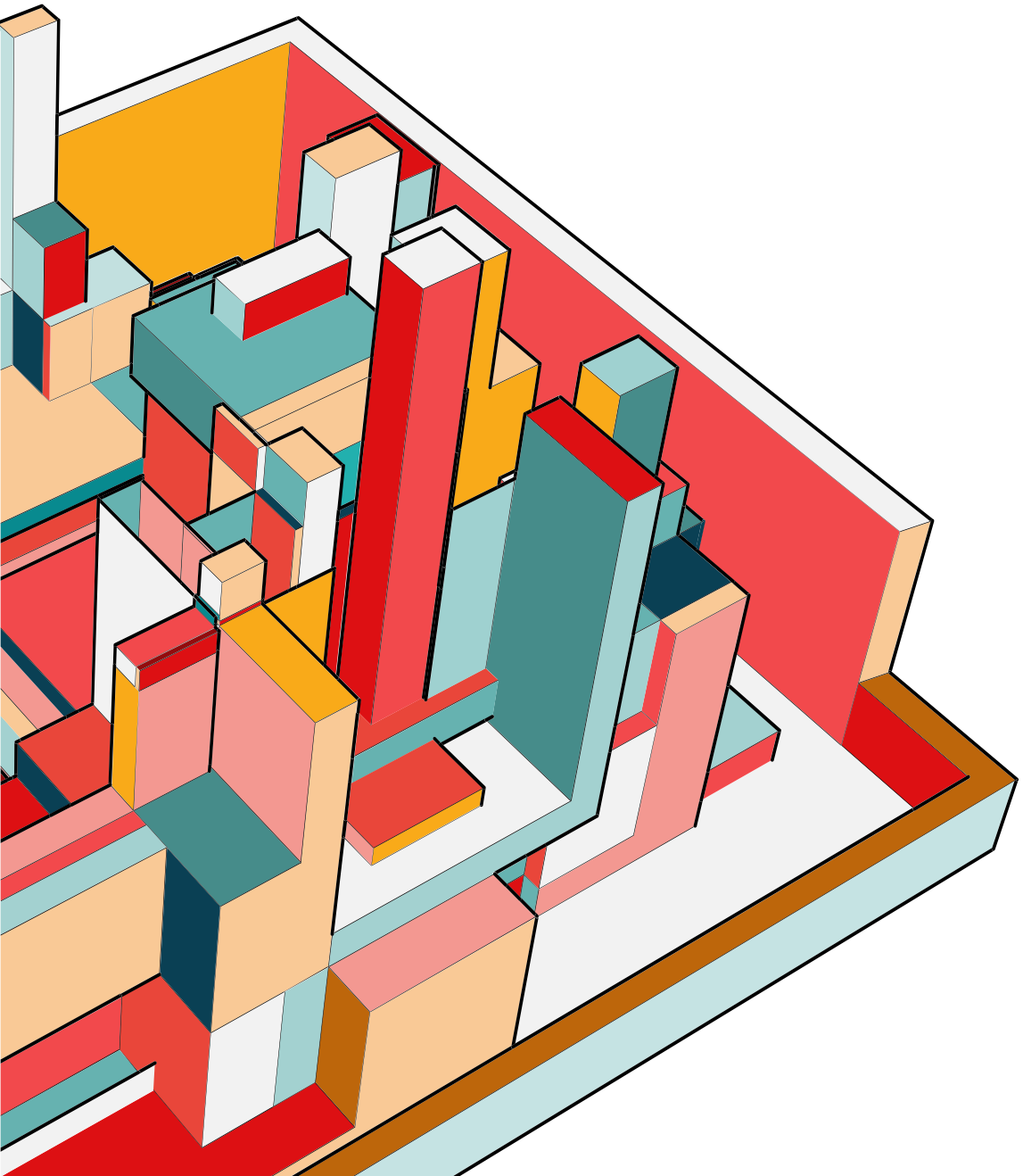
Role of T-Cells

What role does T-cell expansion play in the acute and chronic forms of the disease?
Do T-cell intervention change outcomes?

Chemokines/Cytokines

Are these smoke to the B/T-cell fire?





SUMMARY

OMS appears to be primarily driven through B-cell dysregulation although no definitive autoantibody has been identified. Promising target in GluK2. The role of T-cell activation of B-cells remains unclear.

Cytokine and chemokine signaling, via B and T cell mediated mechanisms may be the activators of the potent immunogenic response in individuals who develop OMS versus those that don't. Potential uses as biomarkers of disease activity/severity.

Genetic/inheritable modifiers may have a role in the development of abnormal immunologic signaling and cellular expansion.

THANK YOU

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