COMMENTARY

To eat, or not to eat, that is the question: Neural stem cells escape phagocytosis in autism with macrocephaly

Simon T. Schafer[a,1] and Fred H. Gage[a,1]

Autism spectrum disorder (ASD) is a highly heritable neurodevelopmental disorder that is thought to emerge very early in development. Cerebral overgrowth, which is clinically referred to as macrocephaly, is frequently observed in children with ASD, and brain imaging studies have reported increases in both gray- and white-matter volume (1–6). Children with these early signs of brain enlargement have been shown to be part of an ASD group with high probability of receiving a diagnosis (3, 5–7). The presence of additional numbers of neurons and increased cortical thickness in the prefrontal cortex of the postmortem brain from ASD patients complement these clinical observations (8). Copy number variation (CNV) in the 16p region of chromosome 16 has been linked to ASD and can manifest in opposing head sizes. Deletion of 16p11.2, which is probably one of the most well-known CNVs linked to ASD, generally leads to macrocephaly, whereas duplications in this region have been associated with smaller head sizes (9–12). The cellular mechanisms that underlie these opposing phenotypes remain unknown.

Considering that live human brain tissues are inaccessible to study the cellular mechanisms that are contributing to these phenomena, the advent of induced pluripotent stem cell (iPSC) technologies has propelled research on this front as it allows researchers to generate any type of cell from human skin or blood. In PNAS, Li et al. take advantage of such an approach to explore the cellular mechanisms that could be involved in gray- and white-matter enlargement related to ASD (13). Here, iPSCs from a cohort of 16p11.2 deletion and duplication carriers were used to derive specific cellular models of the developing brain, in particular neural stem cells (NPCs), the precursors that will continue to form the cells of the gray matter, and oligodendrocyte precursors (OPCs), which contribute to establishing the white matter.

In the developing brain, a specialized population of tissue-resident macrophages contributes to maintaining a fine balance between the generation and elimination of neural stem cells through a process known as phagocytosis. Phagocytosis is a complex process that is involved in removal of opsonized and nonopsonized targets, such as pathogens, cellular debris, and apoptotic cells. Mounting evidence suggests that this process may also be important throughout early neural development and homeostasis as well as during brain repair. Under normal conditions, classical "eat me" and "don't eat me" signals associated with phagocytosis have been shown to maintain cellular homeostasis in different tissue types (14, 15). Various types of cancer have been shown to overexpress CD47 and can directly bind with SIRPα, which is mainly located on macrophages. The binding of CD47 with SIRPα transmits a "don't eat me" signal through which certain cancers evade immune clearance mechanisms (16, 17). Targeting these macrophage immune checkpoints has been shown to be promising for cancer treatment (16, 18).

Neural Stem Cells Escape Phagocystosis through Overexpression of CD47 "Don't Eat Me" Signals

In PNAS, Li et al. expand on this concept and identify that CD47, a cellular "don't eat me" signal, is overexpressed in NPCs derived from subjects with 16p11.2 deletion syndrome as measured by gene expression and the presence of the CD47 protein on the cell surface (13). At the same time, calreticulin (CRT), a dominant proapoptotic "eat me" signal, appeared highly up-regulated in 16p11.2 deletion carriers within the population of CD47-positive cells. In contrast to the subjects with 16p11.2 deletion syndrome, NPCs derived from duplication carriers with normal head circumferences retained levels of CD47 and CRT similar to unaffected controls. Interestingly, similar to what has been observed in cancer, the cells seemed to escape recognition and subsequent CRT-mediated

[a]Laboratory of Genetics, Salk Institute for Biological Studies, La Jolla, CA 92037

Author contributions: S.T.S. and F.H.G. wrote the paper.

The authors declare no competing interest.

See companion article, "Overexpression of CD47 is associated with brain overgrowth and 16p11.2 deletion syndrome," 10.1073/pnas.2005483118.

[1]To whom correspondence may be addressed. Email: sschafer@salk.edu or gage@salk.edu.

Published April 21, 2021.

phagocytosis, as shown by an elegant coculture assay with human blood-derived macrophages from unaffected subjects. These observations suggest that NPCs from 16p11.2 deletion carriers bypass an essential cellular elimination process due to high levels of CD47, which protects them from CRT-mediated phagocytosis during brain development.

Besides increases in gray matter, several studies have also reported changes in white matter volume that are present in 16p11.2 deletion carriers with macrocephaly (9–12). OPCs are important subtypes of glia cells that can differentiate into oligodendrocytes to provide support and insulation for axons in the central nervous system. Interestingly, 16p11.2 deletion carriers showed no changes in CD47 levels at early stages of OPC differentiation, whereas marked differences emerged at later stages of OPC development. Similar to what has been observed in NPCs, the OPCs from macrocephalic 16p11.2 deletion carriers appeared to be protected from CRT-mediated phagocytosis. Notably, the "protective" effect was confined to NPCs and more mature OPCs, as the levels of phagocytosis and CD47 remained unaffected at early stages of OPC differentiation as well as at the preceding pluripotent stem cell stage.

Blocking "Don't Eat Me" Signals as a Potential Therapeutic Path Forward?

Treatment with CD47-blocking antibodies can restore phagocytosis of CD47-overexpressing cancer cells without affecting the normal cells, because blocking such "don't eat me" signals leads to phagocytosis only if potent "prophagocytic" signals such as CRT are present; these signals are, however, absent on normal cells (16–18). In the present study, the authors showed that antibody-mediated blockade of CD47 in 16p11.2 deletion NPCs was sufficient to restore the rate of phagocytosis to control levels. Given the promising observations from the in vitro system, the authors next explored to what degree the findings were relevant in an in vivo setting. NPCs or OPCs derived from control, 16p11.2 deletion and duplication carriers were intracerebrally injected into NOD-scid IL2gammanull mouse pups. The NPC or OPC target populations were then assessed with regard to microglia-mediated phagocytosis in vivo. Similar to what had been observed in the in vitro experiments, NPCs as well as OPCs from macrocephalic 16p11.2 deletion carriers appeared to evade CRT-mediated phagocytosis. Strikingly, pretreatment as well as intraperitoneal injections with the CD47 blocking antibody were sufficient to increase phagocytosis of NPCs and OPCs from 16p11.2 deletion carriers, whereas the duplication carriers and controls remained unaffected (13). These results highlight the possibility that CD47 could serve as a promising target for future treatment of 16p11.2 with macrocephaly, allowing for clearing of overabundant progenitor cells in the brain. In fact, the Hu5F9-G4 antibody blocking CD47 has been used in human clinical trials and has proven to be a beneficial macrophage immune checkpoint inhibitor for treating non-Hodgkin's lymphoma (19). While these studies are still in their infancy, they harbor potential for future treatment strategies.

The study by Li et al. (13) shows that contrasting clinically well-defined groups of patients allows underlying cellular mechanisms to be revealed that are relevant for endophenotypes related to ASD. As this study mainly focused on neural cells, future studies are needed to explore if possible deficiencies in brain-resident immune cells of patients may further enhance such phenotypes. It would also be interesting to see how these findings relate to the enhanced progenitor cell proliferations that are observed in other nonsyndromic forms of ASD. Given that CD47 may be important for other cellular processes during brain development (e.g., synapse elimination), future studies may need to explore if other stages of brain development are affected in these individuals. Nevertheless, it is tempting to speculate that NPCs that circumvent developmental clearance mechanisms may have selective expansion advantages to clonally outcompete the "normal" progenitor pools.

The study by Li et al. shows that contrasting clinically well defined groups of patients allows underlying cellular mechanisms to be revealed that are relevant for endophenotypes related to ASD.

How an overproduction or overabundance of progenitor cells during early brain development can lead to structural and functional alterations that are associated with ASD at later stages is currently not known. Thus, future studies may need to establish how the observed neurodevelopmental alterations unfold as the brain matures. Forthcoming technological advancements and clinically well-defined stem cell–based disease models may allow us to disentangle the cellular mechanisms underlying specific endophenotypes related to ASD at a finer level of granularity. As therapeutic interventions are still far from being specific, the findings of this current study point to a new and promising direction for targeting amenable clinical endophenotypes in ASD, thereby providing a window of opportunity for intervention or mitigation of symptoms. Future studies that explore the specificity of these mechanisms for ASD and in the context of macrocephaly will undoubtedly further advance the field.

Acknowledgments

We thank members of the F.H.G. laboratory for discussions, M. Pena and M. L. Gage for editorial suggestions, and B. Coyne for administrative assistance. Research reported in this publication was supported by grants from the NIH under Awards MH095741 and MH090258. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. This work was supported by the JPB Foundation, Lynn and Edward Streim, Annette C. Merle-Smith, and AHA-Allen Initiative in Brain Health and Cognitive Impairment award made jointly through the American Heart Association and The Paul G. Allen Frontiers Group (19PABH134610000). S.T.S. was supported by a National Alliance for Research on Schizophrenia & Depression Young Investigator Grant from the Brain & Behavior Research Foundation (Leichtung Family Investigator).

1 J. K. Lee *et al.*, Longitudinal evaluation of cerebral growth across childhood in boys and girls with autism spectrum disorder. *Biol. Psychiatry*, 10.1016/j.biopsych.2020.10.014 (2020).
2 D. S. Andrews *et al.*, A longitudinal study of white matter development in relation to changes in autism severity across early childhood. *Biol. Psychiatry* **89**, 424–432 (2021).
3 E. Courchesne *et al.*, Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study. *Neurology* **57**, 245–254 (2001).
4 E. Courchesne, R. Carper, N. Akshoomoff, Evidence of brain overgrowth in the first year of life in autism. *JAMA* **290**, 337–344 (2003).
5 H. C. Hazlett *et al.*; IBIS Network; Clinical Sites; Data Coordinating Center; Image Processing Core; Statistical Analysis, Early brain development in infants at high risk for autism spectrum disorder. *Nature* **542**, 348–351 (2017).

6 R. W. Emerson *et al.*; IBIS Network, Functional neuroimaging of high-risk 6-month-old infants predicts a diagnosis of autism at 24 months of age. *Sci. Transl. Med.* **9**, eaag2882 (2017).
7 E. Courchesne *et al.*, Mapping early brain development in autism. *Neuron* **56**, 399–413 (2007).
8 A. P. A. Donovan, M. A. Basson, The neuroanatomy of autism - A developmental perspective. *J. Anat.* **230**, 4–15 (2017).
9 A. M. Maillard *et al.*; 16p11.2 European Consortium, The 16p11.2 locus modulates brain structures common to autism, schizophrenia and obesity. *Mol. Psychiatry* **20**, 140–147 (2015).
10 A. Y. Qureshi *et al.*; Simons VIP Consortium, Opposing brain differences in 16p11.2 deletion and duplication carriers. *J. Neurosci.* **34**, 11199–11211 (2014).
11 J. P. Owen *et al.*; Simons VIP Consortium, Aberrant white matter microstructure in children with 16p11.2 deletions. *J. Neurosci.* **34**, 6214–6223 (2014).
12 Y. S. Chang *et al.*, Reciprocal white matter alterations due to 16p11.2 chromosomal deletions versus duplications. *Hum. Brain Mapp.* **37**, 2833–2848 (2016).
13 J. Li *et al.*, Overexpression of CD47 is associated with brain overgrowth and 16p11.2 deletion syndrome. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2005483118 (2021).
14 P.-A. Oldenborg *et al.*, Role of CD47 as a marker of self on red blood cells. *Science* **288**, 2051–2054 (2000).
15 P.-A. Oldenborg, H. D. Gresham, F. P. Lindberg, CD47-signal regulatory protein α (SIRPalpha) regulates Fcgamma and complement receptor-mediated phagocytosis. *J. Exp. Med.* **193**, 855–862 (2001).
16 M. P. Chao *et al.*, Anti-CD47 antibody synergizes with rituximab to promote phagocytosis and eradicate non-Hodgkin lymphoma. *Cell* **142**, 699–713 (2010).
17 M. P. Chao *et al.*, Calreticulin is the dominant pro-phagocytic signal on multiple human cancers and is counterbalanced by CD47. *Sci. Transl. Med.* **2**, 63ra94 (2010).
18 D. Kim *et al.*, Anti-CD47 antibodies promote phagocytosis and inhibit the growth of human myeloma cells. *Leukemia* **26**, 2538–2545 (2012).
19 R. Advani *et al.*, CD47 blockade by Hu5F9-G4 and rituximab in non-Hodgkin's lymphoma. *N. Engl. J. Med.* **379**, 1711–1721 (2018).