REFERENCES

Baehrecke, E.H. (2002). Nat. Rev. Mol. Cell Biol. *3*, 779–787.

Berry, D.L., and Baehrecke, E.H. (2007). Cell, this issue.

Blommaart, E.F., Krause, U., Schellens, J.P., Vreeling-Sindelarova, H., and Meijer, A.J. (1997). Eur. J. Biochem. *243*, 240–246.

Gorski, S.M., Chittaranjan, S., Pleasance, E.D., Freeman, J.D., Anderson, C.L., Varhol, R.J., Coughlin, S.M., Zuyderduyn, S.D., Jones, S.J., and Marra, M.A. (2003). Curr. Biol. *13*, 358–363.

Kuranaga, E., and Miura, M. (2007). Trends Cell Biol. *17*, 135–144.

Levine, B. (2007). Nature *446*, 745–747.

Maiuri, M.C., Zalckvar, E., Kimchi, A., and Kroemer, G. (2007). Nat. Rev. Mol. Cell Biol. *8*, 741–752.

Schweichel, J.U., and Merker, H.J. (1973). Teratology *7*, 253–266.

Scott, R.C., Juhasz, G., and Neufeld, T.P. (2007). Curr. Biol. *17*, 1–11.

Xie, Z., and Klionsky, D.J. (2007). Nat. Cell Biol. *9*, 1102–1109.

Synapse Remodeling, Compliments of the Complement System

Lawrence Fourgeaud[1] and Lisa M. Boulanger[1,2,*]

[1]Division of Biological Sciences, Section of Neurobiology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA

[2]Silvio Varon Chair in Neuroregeneration

*Correspondence: lboulanger@ucsd.edu

DOI 10.1016/j.cell.2007.11.031

A growing body of evidence indicates that some proteins known for their immune functions also have distinct nonimmune functions in the normal uninjured central nervous system. In this issue, Stevens et al. (2007) demonstrate an unexpected requirement for molecules of the complement cascade in the remodeling of synaptic connections in the developing visual system.

Traditionally, immune molecules have been associated with neurons only in the context of pathological conditions such as brain injury, neuroinflammation, and autoimmune disorders. However, our definition of neuroimmunology is expanding, based on mounting evidence that certain proteins that were originally identified in the immune system also have nonimmune functions in the central nervous system (e.g., Boulanger et al., 2001; Goddard et al., 2007; Huh et al., 2000; Loconto et al., 2003; Oliveira et al., 2004; Syken et al., 2006). The work now presented by Stevens et al. (2007) reinforces this emerging concept and introduces a new set of players—members of the complement cascade.

The complement cascade is an arm of the innate immune system and is composed of over 30 small proteins and protein fragments that are normally found in inactive forms in the blood. The complement cascade can be triggered via three basic mechanisms: the classical, lectin, and alternative pathways. The classical pathway is initiated by binding of the complement protein C1q. All three pathways converge on complement C3, which triggers a sequence of proteolytic events that amplify the signal and can lead to formation of the cell-killing membrane attack complex. In these cascades, both C1q and C3 selectively bind to pathogens and potentially toxic cellular debris and mark them for destruction and clearance by phagocytosis.

In the current study, Stevens et al. found using a gene chip screen that mRNA encoding C1q is upregulated by purified neurons from the developing mouse eye (retinal ganglion cells) in vitro in response to astrocytes. Punctate C1q immunoreactivity was detected at postnatal day 5 (P5) in the developing retina and in the dorsal lateral geniculate nucleus (dLGN), where retinal ganglion cell axons from both eyes initially send exuberant, overlapping projections. These retinal projections undergo activity-dependent remodeling during the first two postnatal weeks, such that selective strengthening of some connections and weakening of others results in the establishment of the adult pattern of distinct, nonoverlapping eye-specific layers. Imaging of the dLGN during this remodeling (at P5) showed that some C1q protein was colocalized with either the postsynaptic marker PSD95 or the presynaptic marker SV2, whereas less C1q was detected at sites of close apposition between the two markers. Because such close apposition is a hallmark of mature stable synapses, this pattern is consistent with the presence of C1q at nascent or retracting synaptic connections. Importantly, the timing of C1q expression coincided closely with the

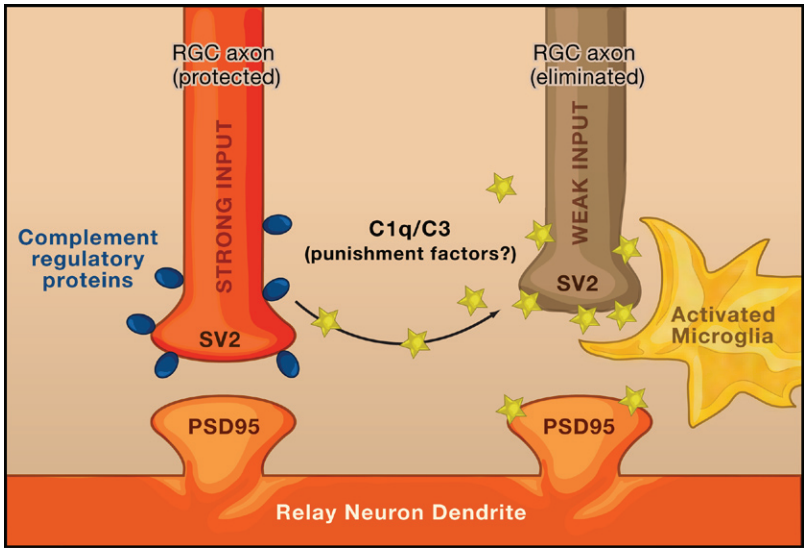


Figure 1. Complement Protein Function in Developmental Synapse Remodeling
As proposed by Stevens et al. (2007), the complement proteins C1q and C3 may be released by strong synapses made by retinal ganglion cells (RGCs) in the developing dorsal lateral geniculate nucleus (dLGN). These molecules may bind to neighboring weaker synapses, marking them for destruction perhaps through phagocytosis by activated microglia. Strong synapses also may express complement regulatory proteins that could protect them from complement-mediated destruction.

period of active remodeling in the visual system, with the highest levels at early postnatal ages (P5) when remodeling is ongoing and declining expression after the second postnatal week (P15) when remodeling is largely complete.

To characterize the function of C1q in the development of the mouse visual system, Stevens et al. first looked at the structure of retinogeniculate projections in C1q-deficient mice and found that retinal ganglion cell axons from the two eyes retained a higher degree of anatomical overlap than in wild-type mice. This was apparent both late in the remodeling process (P10) and after it is normally complete (P30). The authors then performed elegant electrophysiological recordings on relay neurons in the P30 dLGN. At this age, the majority of relay cells in wild-type animals receive active inputs from only one or two retinal axons. In contrast, the majority of these relay cells in mice lacking C1q were innervated by four or more functional retinal inputs, indicating that the persistent anatomical overlap of inputs from the two eyes correlates with aberrant retention of functional connections. Furthermore, a remarkably similar phenotype was observed in mice deficient for C3, supporting the possibility that C1q may affect synapse remodeling through activation of either all or part of the classical complement cascade. The authors further showed that although many more retinal ganglion cell inputs were retained in mice deficient in C1q, the majority of the synapses were weak, with each LGN neuron receiving only one or two strong inputs. This polarization of synaptic strength is thought to be an early step in remodeling and may normally lead to anatomical retraction of the functionally weaker inputs. This key result suggests that the normal remodeling process has begun in neurons lacking C1q but then is either interrupted or delayed.

Stevens et al. also examined C1q expression in the DBA/2J mouse model of glaucoma, a disorder characterized by degeneration of retinal ganglion cells. They found that C1q immunoreactivity was strikingly elevated in the retinas of adult mice with either early or moderate glaucoma, mimicking the labeling seen at earlier ages in wild-type mice. This raises the tantalizing possibility that the pathogenesis of glaucoma, and possibly other neurodegenerative disorders, involves reactivation of a developmental remodeling pathway involving complement.

How might complement proteins contribute to synapse remodeling? The authors hypothesize that C1q and C3 act as tags to mark synapses for elimination (Figure 1), perhaps analogous to the way they mark pathogens and debris for clearance in their immune capacity. In this model, astrocytes stimulate retinal ganglion cells that are electrically active in the appropriate levels and patterns to release soluble C1q and C3. These molecules then act as spreading "punishment signals" that bind to neighboring weaker synapses resulting in their physical removal, possibly through phagocytosis by activated microglia (Figure 1).

The current study demonstrates that complement cascade proteins are required for remodeling of the developing retinogeniculate projection, but details of the proposed model remain to be tested. For example, are the levels or function of C1q regulated by electrical activity in the developing visual system, and are neurons the primary source of complement proteins in the dLGN? If so, do strong synapses also selectively upregulate protective factors, such as known membrane-bound complement regulatory proteins, in order to escape "punishment" by their own secreted complement proteins (Figure 1)? It will also be important to determine when these molecules act. For example, is it early in the remodeling process, when they might label imprecise connections and initiate their removal, or later, when they might recruit cellular effectors to clean up debris in the wake of selective axon weakening? Importantly, the current studies clearly demonstrate that loss of C1q causes retention of inappropriate connections; it is unknown if the converse, an increase in C1q, is sufficient to cause removal of synapses, or if C1q is simply permissive for normal remodeling processes. Also, as C1q and C3 are both secreted soluble proteins, their presence in neurons implies the existence of as yet unknown neuronal complement receptors.

The complement cascade can recruit the adaptive immune response, in part by regulating expression of members of the major histocompatibility complex (MHC) class I gene family. Notably, the increased overlap in retinal projections

in the LGN of mice lacking C1q or C3 is remarkably similar to that seen in mice deficient for cell surface MHC class I (A. Datwani and C.J. Shatz, personal communication). Thus, it is possible that C1q and C3 act by inducing expression of MHC class I in the developing visual system. Future work might resolve this question by determining whether MHC class I levels are reduced in mice lacking C1q or C3 (or vice versa) and assessing whether the effects of MHC class I deficiency and C1q deficiency on visual system development occlude one another, which would suggest that they act in functionally convergent pathways.

Activated microglia are the only cells of the brain known to express C3 receptors (Gasque et al., 1998), indicating that they may participate in synaptic remodeling mediated by C3 (Figure 1). If microglia contribute to this process, one prediction is that higher numbers of microglia should be present in the developing brain specifically during the period of active remodeling. This is in fact the case in some brain regions (e.g., Maslinska et al., 1998). High numbers of activated microglia have also recently been reported in postmortem brain samples from patients with autism (Vargas et al., 2005), raising the possibility that microglia might contribute to pathological changes in connectivity in this neurodevelopmental disorder.

The results reported by Stevens et al. add to growing evidence that the immune system and nervous system make different use of some of the same molecular machinery. This molecular overlap could act as a point of either beneficial or harmful crosstalk between the two systems in injury and disease states and hints at new therapeutic directions for a wide variety of neurological disorders. Much additional work is needed to elucidate the precise molecular mechanisms by which proteins of the innate and adaptive immune system also participate in normal brain development and plasticity. Fortunately, this work will be greatly facilitated by the knowledge and experimental tools established when these proteins were first characterized in the immune system.

REFERENCES

Boulanger, L.M., Huh, G.S., and Shatz, C.J. (2001). Curr. Opin. Neurobiol. *11*, 568–578.

Gasque, P., Singhrao, S.K., Neal, J.W., Wang, P., Sayah, S., Fontaine, M., and Morgan, B.P. (1998). J. Immunol. *160*, 3543–3554.

Goddard, C.A., Butts, D.A., and Shatz, C.J. (2007). Proc. Natl. Acad. Sci. USA *104*, 6828–6833.

Huh, G.S., Boulanger, L.M., Du, H., Riquelme, P.A., Brotz, T.M., and Shatz, C.J. (2000). Science *290*, 2155–2159.

Loconto, J., Papes, F., Chang, E., Stowers, L., Jones, E.P., Takada, T., Kumanovics, A., Fischer Lindahl, K., and Dulac, C. (2003). Cell *112*, 607–618.

Maslinska, D., Laure-Kamionowska, M., and Kaliszek, A. (1998). Folia Neuropathol. *36*, 145–151.

Oliveira, A.L., Thams, S., Lidman, O., Piehl, F., Hokfelt, T., Karre, K., Linda, H., and Cullheim, S. (2004). Proc. Natl. Acad. Sci. USA *101*, 17843–17848.

Stevens, B., Allen, N.J., Vazquez, L.E., Howell, G.R., Christopherson, K.S., Nouri, N., Micheva, K.D., Mehalow, A.K., Huberman, A.D., Stafford, B., et al. (2007). Cell, this issue.

Syken, J., Grandpre, T., Kanold, P.O., and Shatz, C.J. (2006). Science *313*, 1795–1800.

Vargas, D.L., Nascimbene, C., Krishnan, C., Zimmerman, A.W., and Pardo, C.A. (2005). Ann. Neurol. *57*, 67–81.

The Exoneme Helps Malaria Parasites to Break out of Blood Cells

Chris J. Janse[1] and Andrew P. Waters[1,*]

[1]Department of Parasitology, Leiden University Medical Centre, Albinusdreef 2, 2333ZA Leiden, The Netherlands

*Correspondence: waters@lumc.nl

DOI 10.1016/j.cell.2007.11.026

Malaria parasites must invade the erythrocytes of its host, to be able to grow and multiply. Having depleted the host cell of its nutrients, the parasites break out to invade new erythrocytes. In this issue of *Cell*, Yeoh et al. (2007) discover a new organelle, the exoneme, that contains a protease SUB1, which helps the parasite to escape from old erythrocytes and invade new ones.

The malaria parasite (*Plasmodium*) is a unicellular, obligate intracellular protozoan that must invade, colonize, replicate within, and emerge from various cells types of the mammalian host or mosquito vector in order to complete its life cycle. Although the basic structure of *Plasmodium* is comparable to a standard eukaryotic cell, it is capable of producing distinct invasive forms and specialized organelles that are evolved to recognize and invade the correct cell type. For growth and multiplication in the bloodstream of its vertebrate host,