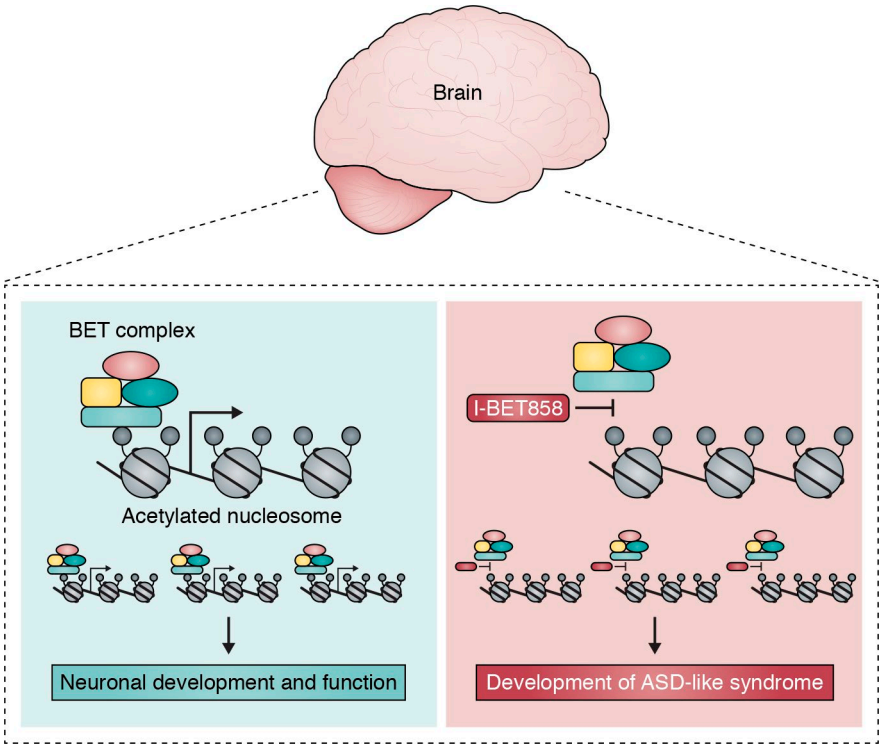
# Regulating BETs at central station

Insight from Mark Dawson

The establishment of complex behaviors, memory, and language has long fascinated humanity. It is, after all, what makes us human. Understanding this complexity presents one of the most significant challenges in biomedical research. Autism spectrum disorders (ASDs) are a group of neurodevelopmental disorders often characterized by repetitive and stereotyped behavior, impaired social development, and diminished language skills. ASDs are estimated to affect up to 1:100 children, many of whom require lifelong social support. The advent of the genomic era has provided unprecedented insight into the molecular architecture of ASDs, and genes controlling transcription and chromatin remodeling appear central to pathogenesis of ASDs.

In this issue, Sullivan et al.demonstrate that pharmacological suppression of the critical BET bromodomain transcriptional regulators induces an ASD-like syndrome in mice. The BET proteins contain tandem bromodomains that mediate their interaction with chromatin by binding acetylated lysines on histone tails. They function as part of multisubunit chromatin complexes to nuance and facilitate transcription. BET inhibitors disrupt this protein–protein interaction by displacing the BET proteins from chromatin, leading to transcriptional repression. These drugs have shown promise in a range of human pathologies, including inflammation and cancer, and clinical trials in a variety of malignancies are underway.

Sullivan et al. report on their development of a novel BET bromodomain inhibitor (I-BET858) with excellent central nervous system (CNS) penetration. Although the BET proteins are not known to be mutated in ASDs, treating mice with I-BET858 resulted in the selective down-regulation of genes associated with synaptic transmission and neuronal development, several of which have previously been implicated in pathogenesis of ASDs. BET inhibition did not affect the expression of housekeeping genes or the immediate early response genes that are activated by brain-derived neutrophic factor (BDNF); instead, I-BET858 preferentially reduced the transcriptional output from longer genes (>100 kb), many of which mediate the secondary response to BDNF stimulation. The functional consequence of these transcriptional changes was the manifestation of an ASD-like syndrome. Interestingly, the behavioral changes induced by BET inhibition appeared to be independent of effects on memory formation, and, crucially, these features were only partially reversed after cessation of the drug.




**BET bromodomain transcriptional complexes interact with chromatin to regulate transcription of genes associated with normal neuronal development and function. Pharmacological suppression of BET complexes with a novel inhibitor, I-BET858, induces an autism spectrum disorder–like syndrome in mice.**

This study offers some intriguing insights into the functional interplay between transcription regulation and complex behaviors. It also provides the impetus to understand at the molecular level why certain extra-long genes, associated with ASDs, appear to be preferentially regulated by BET proteins. Importantly, the clinical consequences of these findings need further exploration. On the one hand, the novel CNS-permeable inhibitor offers a therapeutic opportunity to patients with primary or metastatic CNS tumors. But it also emphasizes the importance of vigilant monitoring of patients currently on these therapies for subtle behavioral and cognitive deficits.

Sullivan, J.M., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20151271

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# A phospholipase linkAGE to SARS susceptibility

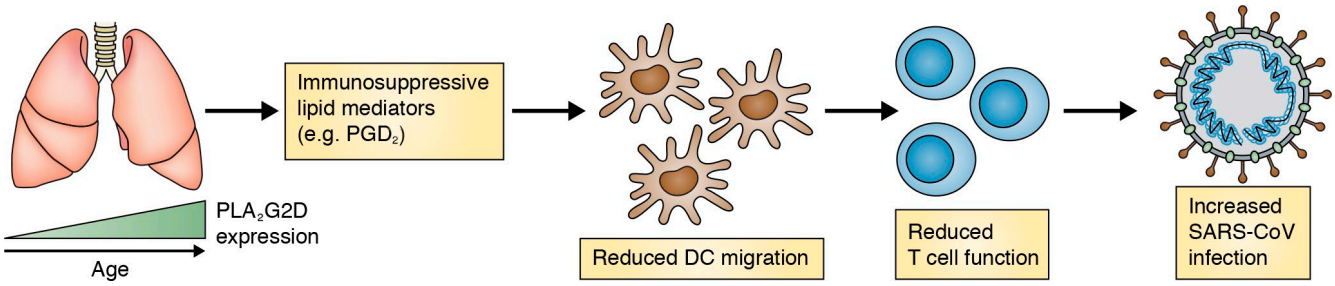
**Insight from John Schoggins**

During the aging process, immune functions decline, rendering the host more vulnerable to certain viruses. The mechanisms underlying this age-dependent susceptibility to infection are an active area of research. In this issue, Vijay et al. show that secreted phospholipase $A_2$ ($PLA_2$) group IID ($PLA_2G2D$) is critical in determining the impact of age on host susceptibility to severe acute respiratory syndrome coronavirus (SARS-CoV).

$PLA_2$ hydrolyzes membrane phospholipids to release arachdonic acid, which is metabolized by multiple enzymes (e.g., cyclooxygenases and lipoxygenases) to generate functionally diverse lipid mediators including prostaglandins and leukotrienes. This group previously linked prostaglandin $D_2$ ($PGD_2$) expression in mouse lungs to defective immune responses and age-related susceptibility to SARS-CoV. Blocking $PGD_2$ function restored immune responses and increased survival in older, infected mice. How and why $PGD_2$ expression is elevated with age is the focus of the current study.

The authors first compared the transcriptomes of $CD11c^+$ cells from the lungs of young or middle-aged mice. Of all genes in the arachidonic acid cascade, only *Pla2g2d* was elevated in the older mice. Lipid profiling of lung tissue revealed that older mice also had increases in multiple lipid mediators including $PGD_2$. Using mice lacking *Pla2g2d*, the authors showed that $PLA_2G2D$ was uniquely responsible for age-dependent increases in lipid mediator expression. Moreover, in the absence of *Plas2g2d*, dendritic cell migration and T cell responses were enhanced in older mice. Notably, $Pla2g2d^{-/-}$ mice challenged with SARS-CoV had a striking survival advantage when compared with $Plas2g2d^{+/+}$ mice. Mechanistically, the authors were able to link *Pla2g2d* expression with markers of oxidative stress.

This study provides strong genetic evidence that lipid mediators produced downstream of $PLA_2G2D$ contribute to age-dependent susceptibility to viral infection. These findings once again highlight lipid mediators as double-edged swords in immunology. Whereas some prostaglandins, including $PGD_2$, have beneficial immunosuppressive effects on age-related oxidative stress, they can also adversely affect immune cell function, thereby dampening desirable antiviral responses. The observation that enhanced $PLA_2G2D$ signaling is detrimental in a virus-infected host suggests that therapies targeting this pathway may benefit older patients infected with SARS-CoV. However, the treatment may need to be tailored to specific lipid mediators to avoid dampening natural immunosuppressive responses and exacerbating comorbidities such as asthma, allergies, or autoimmune disorders.




**Aging lungs have higher $PLA_2G2D$ expression and elevated levels of lipid mediators, some of which naturally combat age-related oxidative stress by exerting immunosuppressive functions. The trade-off is an increased susceptibility to certain viral infections, including SARS-CoV and influenza A virus.**

Vijay, R., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20150632

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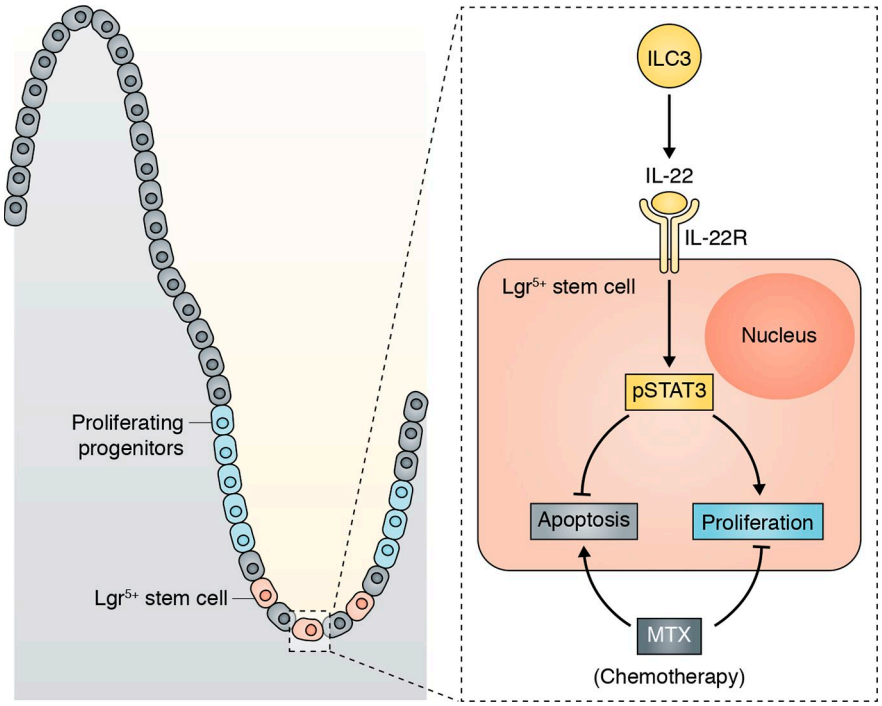
# ILC3s protect intestinal stem cells from chemotherapy

Insight from Gérard Eberl

Type 3 innate lymphoid cells (ILC3s) play a critical role in intestinal immunity by providing an array of cytokines that reinforce the epithelial barrier and keep the enormous symbiotic microbiota at a safe distance. One of these cytokines, interleukin (IL)-22, induces the production of antimicrobial peptides by epithelial cells and inhibits their apoptosis. In this issue, Aparicio-Domingo et al. report that ILC3s and IL-22 protect epithelial stem cells from death induced by methotrexate (MTX), an antifolate drug widely used in treatments against cancer and autoimmunity. ILC3s thereby limit the intestinal pathology associated with such treatments.

Mice treated with MTX rapidly develop intestinal pathology characterized by a loss in Lgr5$^+$ epithelial stem cells and, as a consequence, suffer a decrease in epithelial proliferation and intestinal architecture. However, the mice fully recover seven days after cessation of treatment, via a process involving phosphorylation of the transcription factor Stat3 in epithelial cells and the recovery of full proliferative capacity. In the absence of ILC3s, posttreatment reinduction of Stat3 phosphorylation and epithelial proliferation fails, and the intestine does not heal. Several cytokines are produced by ILC3s in this context, such as GM-CSF, lymphotoxin, IFNγ, and IL-22, but IL-22 was the only one found to activate Stat3 and is required to protect Lgr5$^+$ cells from the deleterious effects of MTX.




**ILC3s protect intestinal Lgr5$^+$ stem cells and proliferating progenitors from damage induced by the chemotherapeutic agent MTX. ILC3s produce IL-22, which activates the transcription factor Stat3, an inhibitor of apoptosis and promoter of proliferation in epithelial cells.**

These data extend the roles of ILC3s in intestinal immunity: not only do they induce the production of effectors that target microbes, but they also increase epithelial resistance to injury. Together, these effects reinforce the intestinal barrier to allow for a good living for both the host and its symbiotic microbiota. It appears now that ILC3s also protect us from the side effects of chemotherapy, which remains our best shot at fighting tumors. Such side effects may be further attenuated by promoting the activity of ILC3s, for example by delivering agonists of RORγt, a transcription factor and nuclear hormone receptor required for the generation of ILC3s. Conversely, ILC3s and their counterparts in adaptive immunity, the Th17 cells, have been implicated in autoimmunity and IBD, and antagonists of ILC3s and Th17 cells are being developed to combat these types of pathologies. However, it is becoming clear that great care must be taken when considering inhibition of ILC3s, as this inhibition would also be expected to weaken the intestinal barrier.

Aparicio-Doming, P., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20150318

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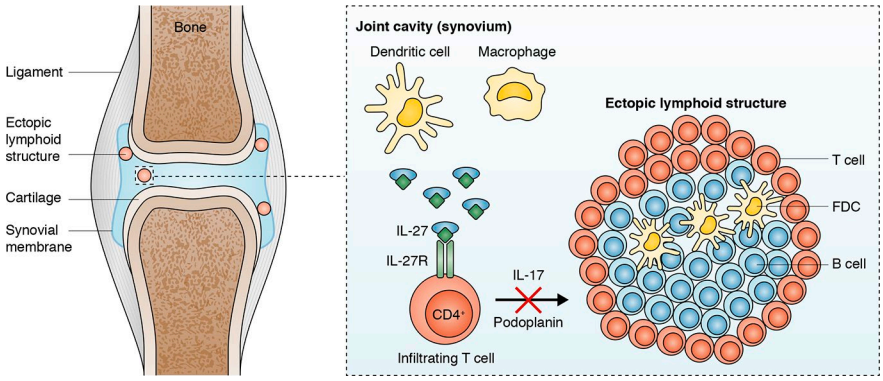
# IL-27 shakes up the establishment of ectopic lymphoid structures

**Insight from (left to right) Alejandro Villarino, John O'Shea, and Christopher Hunter**

The artist Keith Haring once wrote, "Good and evil are not complete opposites… in fact, [they] are often one and the same." Such dualism is commonplace in the field of cytokine biology, and among the most salient examples of this is interleukin (IL)-27, which was initially classified as proinflammatory but later found to have critical antiinflammatory properties. In this issue, Jones et al. uncover a novel regulatory function of IL-27 that is relevant for the pathogenesis of rheumatoid arthritis (RA).

Using a mouse model of RA, the authors discover that IL-27 is a potent inhibitor of ectopic lymphoid-like structures (ELSs), which tend to occur at sites of chronic inflammation, like arthritic joints, and are associated with severe forms of rheumatic disease. They first demonstrate that both ELS formation and synovial pathology are exaggerated in mice lacking the IL-27 receptor (IL-27R) and then link these phenomena to the well-documented ability of IL-27 to regulate IL-17, a cytokine known to promote ELSs in chronic disease settings. Thus, a model emerges whereby IL-27 limits ELS formation and, in turn, slows RA progression by restricting the ability of infiltrating T cells to produce IL-17, as well as other pro-ELS agents like podoplanin, which was found to be coexpressed with IL-17 and subject to IL-27–mediated inhibition.

The present work also yields potentially valuable insights into the pathogenesis and treatment of RA. Although synovial ELSs are known to correlate with lymphoid infiltrates and disease severity, the cellular factors that control ELS formation have only recently begun to be appreciated. The authors implicate IL-27 by demonstrating an inverse relationship between cytokine production and the appearance of synovial ELS, which, notably, are highly enriched for IL-27R–expressing T cells. Based on loss-of-function studies in mice, they propose a causal link and go one step further to endorse IL-27 as a predictive biomarker for stratifying RA patients into ELS-negative and -positive subtypes. The ability to make this distinction at early stages of disease could provide both a rationale for selective deployment of ELS-targeting drugs and an impetus for development of IL-27–based biologics. Differences between ELS-negative and -positive forms of disease may also explain why IL-27 appears to limit pathology in some, but not all, models of RA. This host-protective quality may become apparent in models with overt ELS involvement, as with the antigen-driven protocol used here, whereas its more damaging, proinflammatory qualities may dominate in settings where ELSs are not prevalent, such as during the onset of disease or in models with more diffuse infiltrates.




**IL-27 limits development of ELSs in arthritic joints. CD4⁺ T cells drive the pathogenesis of RA, in part by promoting the formation of ELSs within the inflamed synovium. IL-27, produced by a variety of cell types including DCs and macrophages, limits this process by acting directly on infiltrating T cells to restrict their ability to produce IL-17 and other pro-ELS factors, like podoplanin.**

The work also raises new questions about other cytokines and cell types that regulate ELS formation and maintenance, whether this pathway is operative in other disease settings that feature ELSs (such as MS), and whether the results form this (or any) mouse model translate to the human disease. But fear not, as Haring said, "[if we] don't understand anything. That is, I think, the key to understanding everything."

Jones, G.W., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20132307

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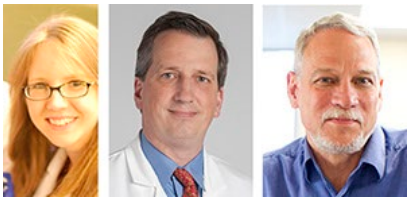
# Peripheral macrophages not ADept at amyloid clearance

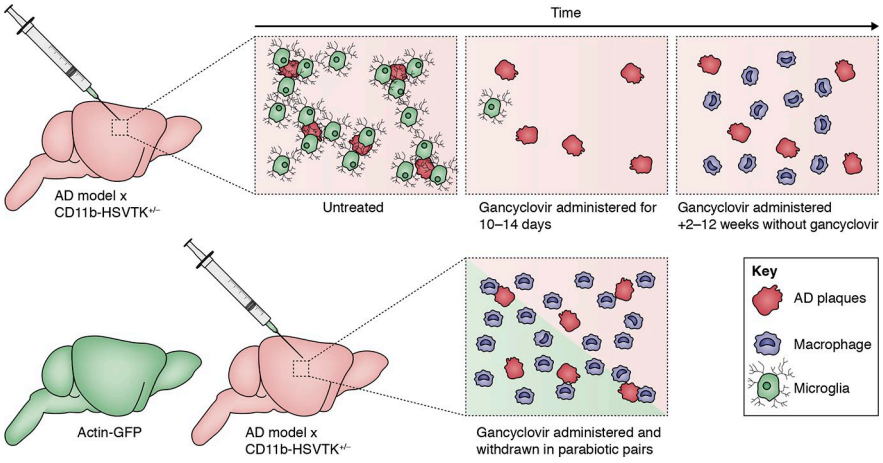


Insight from (left to right) Taylor Jay, Bruce Lamb, and Gary Landreth

In this issue, Prokop et al. and Varvel et al. demonstrate that replacing resident microglia with peripherally derived myeloid cells, remarkably, does not impact pathology in Alzheimer's disease (AD) mouse models.

Increasing evidence suggests a link between neuroinflammation and amyloid pathology in AD. Inflammatory myeloid cells accumulate around amyloid plaques in the human AD brain and in AD mouse models. However, these cells fail to mount a productive phagocytic response to effectively clear amyloid from the brain. It has been proposed that microglia become dysfunctional because of age or disease state and that perhaps replacing these cells with a more competent, blood-derived myeloid cell population would result in amyloid clearance and ameliorate pathology. Indeed, a variety of studies have suggested that infiltration and engraftment of peripheral myeloid cells in the CNS could alter AD-like pathologies.

In the current issue, both investigators use mouse models in which herpes simplex virus thymidine kinase (HSVTK) is expressed under the control of the CD11b promoter. HSVTK can modify ganciclovir into a toxic product. So, upon intracerebroventricular administration of ganciclovir, CD11b-expressing resident microglia were eliminated from their AD mouse models. Prokop et al. performed parabiosis experiments to demonstrate that 28 days after the withdrawal of ganciclovir administration, peripherally derived myeloid cells infiltrate and repopulate the CNS. Surprisingly, these peripherally derived cells failed to home to amyloid plaques, and the authors found no resultant change in amyloid pathology. Varvel et al. reported similar results two weeks after repopulation by peripheral myeloid cells, but after longer-term engraftment in the CNS, the peripherally derived cells do eventually cluster around amyloid deposits. Remarkably, despite the near total replacement of resident microglia by peripherally derived myeloid cells, the authors found no significant change in amyloid deposition.




**The authors cross multiple AD mouse models to their CD11b-HSVTK model of microglial depletion. In untreated mice (top panels) myeloid cells cluster around plaques. After 10–14 days of intraventricular gancyclovir administration, microglia are largely eliminated from the brain. After 2–12 weeks of recovery after gancyclovir administration, myeloid cells repopulate the CNS, but these cells do not associate with amyloid deposits. There is no change in amyloid deposition at any stage compared with nondepleted mice. To determine the origin of repopulating myeloid cells, the authors use labeled parabiotic pairs (botton panels) and demonstrate that repopulation after gancyclovir administration occurs via infiltration of peripherally derived myeloid cells.**

Together, these studies suggest that replacement of microglia by peripherally derived myeloid cells is not sufficient to enhance amyloid clearance and ameliorate AD pathology. Rather, it appears that the tissue microenvironment plays a key role in regulating myeloid cell function as it relates to amyloid clearance regardless of cell ontogeny. Despite these findings, it is still possible that recruitment of specific myeloid cell subsets in AD could contribute to plaque clearance, as the global elimination of microglia that drove repopulation in these studies may not attract the same cells that enter the CNS in the context of AD. Nevertheless, the findings in these studies suggest that largescale replacement of microglia with peripheral myeloid cells in itself is unlikely to be an effective therapeutic strategy to promote amyloid clearance in AD patients.

Prokop, S., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20150479
Varvel, N.H., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20150478

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