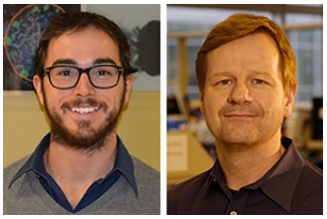
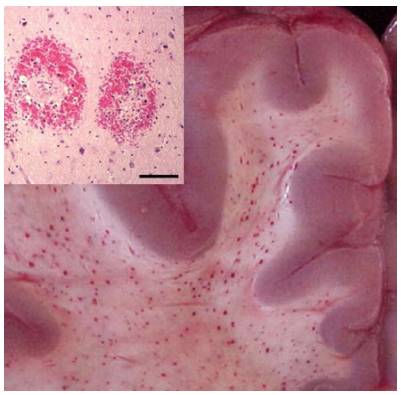
A novel immune regulator links malaria and inflammatory bowel disease



Insight from Brandon Sack (left) and Stefan Kappe (right)

Malaria is caused by *Plasmodium* parasites and is commonly thought of as a disease resulting from the cyclic infection and destruction of red blood cells. However, a subset of infected individuals will develop "cerebral malaria"—a pathogenic neuroinflammation, presumably caused by parasitized red blood cells sequestering in the brain. Those affected, usually children under the age of five, can die or suffer permanent cognitive impairment long after successful treatment of infection. The exact causes of cerebral malaria are poorly understood, but mouse models of experimental cerebral malaria have identified $CD8^+$ T cells as key players in the development of this debilitating condition.

In this issue, Kennedy et al. used an unbiased genome-wide screen of ENU-mutagenized mice to identify a novel gene, *Ccdc88b,* that influences T cell activation and function and that is associated with resistance to cerebral malaria following infection with the mouse malaria parasite *Plasmodium berghei*. They found *Ccdc88b* to be expressed in T cells and myeloid cells. T cells from *Ccdc88b*$^{-/-}$ knockout mice were impaired in their ability to become activated and to secrete effector cytokines in response to stimulation via their T cell receptor. Importantly, unlike T cells from wild-type mice, adoptive transfer of T cells from knockout mice did not confer cerebral malaria to recipient mice.



DOROVINI-ZIS, K., ET AL. 2011. *AM. J. PATHOL.* 178:2146–2158; IMAGE © ELSEVIER

Postmortem histology showing hemorrhages in the brain of a cerebral malaria victim.

With over 300 million malaria infections each year, cerebral malaria causes substantial mortality. Understanding the molecular and cellular etiology of cerebral malaria will lead to better identification and treatment of those most at risk. Furthermore, $CD8^+$ T cells have a duplicitous role in malaria infection—both helping to clear the parasite from the liver and blood and in orchestrating the damaging neuroinflammation seen in cerebral malaria. Understanding the events leading to a pathogenic T cell response could also help inform the design of malaria vaccines that eliminate, but do not exacerbate, disease.

The authors' work also extends beyond models of malaria, as a region in the human genome that contains *Ccdc88b* has been implicated in autoimmune disorders such as inflammatory bowel disease, multiple sclerosis, and Crohn's disease. This further supports the idea that *Ccdc88b* is a novel and important regulator of immune function. It will be fascinating to confirm the role of *Ccdc88b* in other immune disease models and to determine whether this pathway could be manipulated to quell overzealous T cells in malaria pathogenesis and beyond.

Kennedy, J.M., et al. 2014. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20140455.

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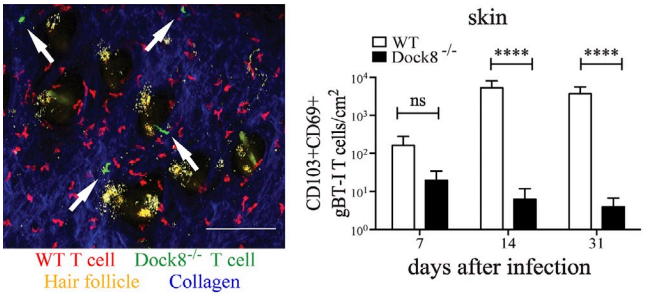
T cells require DOCK8 for flexibility and function

Insight from Stuart Tangye

In this issue, Zhang et al. report a novel function for DOCK8 (dedicator of cytokinesis 8) in controlling the structural integrity of lymphocytes. DOCK8 deficiency severely compromised survival of T cells during trafficking through dense tissue networks, impairing skin-specific protective antiviral immune responses.

Identifying the genetic lesions underlying primary immunodeficiencies has an immediate impact on disease diagnosis, therapy, and patient management. It can also provide mechanistic explanations for disease pathogenesis, such as the absence of T and NK cells in X-SCID due to the requirement for γc in IL-7R and IL-15R signaling, and the lack of switched Ig isotypes in hyperimmunoglobulin M syndrome due to the requirement for CD40/CD40L interactions in this process. However, there are other examples where identifying the mutant gene in a particular disease opens up a new field of study, for the simple reasons that little was known about the function of this gene in immune cells. The discovery that biallelic loss-of-function mutations in *DOCK8* causes autosomal recessive hyper IgE syndrome falls into this second category. DOCK8 is a guanine nucleotide exchange factor that activates small GTPases such as Cdc42. Previous studies revealed that cytoskeletal defects in DOCK8-deficient B cells, T cells, and NK cells impairs their effector function. However, it is not clear why individuals with *DOCK8* mutations specifically develop severe viral infections of the skin. Zhang et al. theorized that since DOCK8-deficient lymphocytes exhibited normal chemotaxis and initial migration into human tissue, these cells may be impaired

in their ability to traffic through the dense tissue networks of the skin. By analyzing humans, mice, and cell lines, DOCK8-deficient T and NK cells were found to have aberrant morphologies under conditions that replicated skin infiltration and penetration, and this ultimately contributed to an unusual form of cell death termed "cytothripsis" (cell shattering). This morphological effect was replicated by abolishing expression of Cdc42 or p21-activated kinase (PAK), but not RAC1/2 or the WAS protein, thereby establishing that DOCK8 operates in this setting by activating these regulators of actin polymerization. Overall, the stress experienced by DOCK8-deficient cells moving through dense tissue networks abrogated the generation and maintenance of tissue-resident memory $CD8^+$ T cells that are important for protective immunity at such sites.



In adoptive transfer experiments, Dock8-deficient T cells were undetectable in skin one month after HSV infection compared with normal numbers of control T cells. Left panel: two photon imaging of the skin 17 days after infection with HSV; right panel: numbers of HSV-specific T cells in skin at various times after infection with HSV.

This selective inability of effector T cells lacking DOCK8 to efficiently localize to tissues high in collagen content and provide potent antiviral immunity at these sites may explain why DOCK8-deficient patients have heightened susceptibility to skin-trophic viral infections, yet systemic viral infections are more effectively controlled. These findings reveal the morphological flexibility of immune cells that is required for them to execute effector function in nonlymphoid tissues and the critical function of DOCK8/Cdc42/PAK in this process. It will be important to elucidate the mechanism by which DOCK8 integrates into TCR signaling, as well as to assess virus-specific skin-resident memory cells in DOCK8-deficient humans to establish a paucity of these cells at these sites. Identification of the components that regulate lymphocyte integrity, motility, and survival under conditions of migratory stress may provide an opportunity to enhance tissue-specific immunity and memory in patients with germline mutations in this pathway. Eventually, shedding light on the pathology arising in the skin may result in improved outcomes for patients with this often-fatal immunodeficiency.

Zhang, Q., et al. 2014. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141307.

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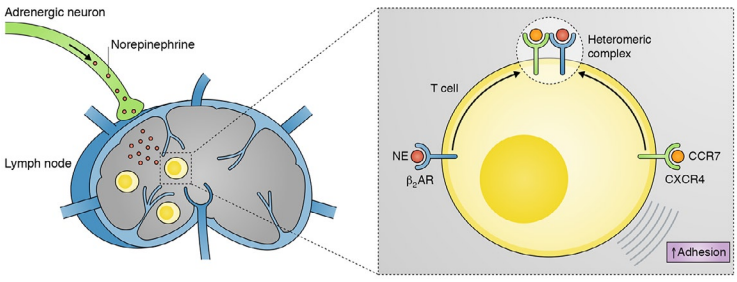
Lymphocyte called home: β_2-adreneric neurotransmission confines T cells to lymph nodes to suppress inflammation

Insight from Kevin Tracey

The immune and nervous systems sense changes in the environment, mobilize host responses, and establish memories of threatening events. In this issue, an important study by Nakai et al. using experimental models of T cell inflammation shows that neurotransmission through β_2-adrenergic signals in lymph nodes restricts T cell egress, thereby limiting potentially damaging tissue inflammation. This reveals a previously unrecognized neural mechanism for suppressing T cell mediated tissue damage.

Like neurons, lymphocytes and other hematopoietic cells express neurotransmitter receptors. Ligand binding mediates intracellular signal transduction that modulates the expression of genes involved in immunological responses including cytokine production, cell proliferation and migration. Historically, immunology studies of neurotransmitter receptor signaling were descriptions of the biological effects of neurotransmitter receptor signaling on immune responses, regardless of the neurotransmitter's cell of origin. Recent convergence of neurophysiology and immunology, however, has delineated the maps of specific neural circuits that regulate immunity. Elucidation of these genetic and molecular mechanisms has significant implications for understanding innate and adaptive immunity.



Adrenergic signals via β_2-adrenergic receptor (β_2AR) in T cells lead to the formation of heteromeric protein complexes of β_2AR with CCR7 and CXCR4. This culminates in increased adhesion to lymph node stroma and T cell retention.

It has been known for decades that adrenergic neurons innervate lymph nodes. Missing was the answer to how such neurons might regulate T lymphocytes. In the present study, ablation of adrenergic

signaling to lymph nodes, or deletion of β_2-adrenergic receptors from lymphocytes resulted in lymphopenia in lymph nodes. β_2-adrenergic receptors physically associated with CCR7 and CXCR4, which control lymphocyte retention in lymph nodes, resulting in prolonged RAC1 signaling. CCR7 and CXCR4 signals enhanced lymph node retention of B and T cells, respectively. Moreover, activation of β-adrenergic signals inhibited lymph node transit of antigen-primed T cells, significantly curtailing the development of paralysis in EAE and attenuating inflammation in DTH. This indicates that β_2-adrenergic neural signaling can override T cell damage to tissues.

Immunity cannot be fully understood without understanding the neural circuits that reflexively modulate it. The growing list of reflexive neural circuits that specifically influence immunity provides an understanding of distinct molecular and neurophysiological mechanisms that inhibit and stimulate immunity. These circuits operate reflexively, meaning that sensory input stimulates the outgoing, efferent arc of the circuit to regulate immunity. The incoming signals can be activated by cytokines, products of tissue injury, and even by bacterial products directly. The innate immune system is thus not alone on the front line against infection and injury; this role is shared with sensory neurons. Closed feedback loops through these neural circuits can exert significant influence on the development of innate and adaptive immunity.

So, if the question being studied is "what is the basis of immunity and how is it controlled?", then it is time to face up to the answers provided by studies of neural circuits that reflexively control immune responses. Nearly every cell of the body is within signaling range of sensory neurons capable of monitoring their biochemical, metabolic, and physical states. And all of the major organs of the immune system are targeted by neurons that can in turn transmit reflexive neural signals.

The challenge for immunologists today is to delineate specific neural circuit mechanisms regulating specific aspects of immunity. In the 20th century, Charles Sherrington taught that the nervous system is built upon single units of reflex action; the time has arrived to apply these principles to immunology. This represents a major advance over studies of generalized neurotransmitter responses to hematopoietic cells. Mapping specific input and output neural circuits has opened new insights into understanding immunity and holds significant opportunity for learning how to therapeutically modulate it.

Nakai, A., et al. 2014. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141132.

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Sleeping our Alzheimer's risk away

Insight from Ryan Watts

Are we getting enough sleep? Clinical investigation has revealed an inverse correlation between the accumulation of amyloid plaques in the brain (a hallmark of Alzheimer's disease pathology) and the amount of sleep. Of course, these correlations do not prove causation, which requires a detailed analysis of the relationship between sleep and plaque formation. Now, a new study using mouse models of Alzheimer's disease (AD) provides compelling genetic evidence linking sleep with AD pathology and concludes that getting more sleep will reduce amyloid-β (Aβ) plaque formation, at least in mice.

The primary constituent of amyloid plaques in the brains of patients with AD is the amyloidogenic peptide, Aβ. Aβ production is regulated by synaptic activity, with increased activity resulting in more Aβ production. Animal models that express the human amyloid precursor protein (APP), often with mutations to enhance production of Aβ, produce robust Aβ plaque pathology. Studies in these mice have shown that amyloid pathology disrupts sleep patterns and that reducing pathology can reverse these deficits. Furthermore, pharmacological inhibition of the wakefulness-modulating pathway, which is mediated by orexin signaling, reduced plaque formation. But genetic validation of the role of orexin signaling and the impact of modulating this pathway on sleep and amyloid plaque pathology remained to be explored.

In this issue, Roh et al. report data from two different mouse models of AD combined with genetic deletion of orexin. Loss of orexin resulted in decreased wakefulness (increased sleep) and a subsequent reduction in amyloid pathology and Aβ concentrations in brain. Two possible explanations for these results were tested: orexin may act directly on the neurons that overproduce Aβ, or, orexin may impact pathology as a result of modulating the sleep–wake cycle. The authors found that rescue of orexin in neurons that modulate sleep,

but not directly in neurons in the region of plaque formation, increased wakefulness and thus increased amyloid pathology, supporting the second explanation. Sleep deprivation in AD mice that lacked orexin resulted in increased plaque formation, demonstrating a causal link between sleep and amyloid pathology.

The implications of these findings on human behavior and potential pharmacological approaches to prevent AD are far-reaching. The totality of data linking sleep and the risk of developing AD suggests that lack of sleep increases amyloid pathology and that increased amyloid pathology negatively impacts sleep. As a result, the risk of AD and the relationship to sleep are self-perpetuating. Furthermore, sleep modulation may be a viable consideration for drug development in AD, however the current evidence points towards prevention, rather than treatment of existing disease. These data also suggest that consideration should be given to lifestyle choices that place sleep as a priority. Increasing our quality and quantity of sleep may reduce the risk of developing amyloid pathology, ultimately protecting us from developing AD.

Roh, J.H., et al. 2014. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141788.

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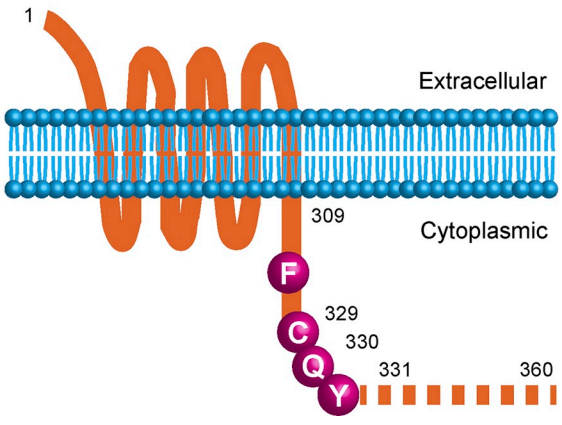
CCR4 drives ATLL jail break

Insight from Kevin Shannon

Adult T cell leukemia/lymphoma (ATLL), caused by human T cell lymphotropic virus 1 (HTLV-1), is an aggressive cancer that is refractory to current therapies. The long latency and low overall penetrance of ATLL in HTLV-1–infected individuals infers the need for cooperating events, which include somatic *JAK3, NOTCH1*, and *FAS* mutations. While overexpression of CCR4 is a hallmark of ATLL, it is not clear whether dysregulation of CCR4 function contributes to disease pathogenesis. In this issue, Nakagawa et al. report recurrent somatic mutations in the CCR4 chemokine receptor in ~25% of ATLL cases, implicating these mutations in ATLL pathogenesis.

The authors performed RNA transcriptome analysis of two ATLL cases and targeted sequencing of additional ATLL patient samples and cell lines. Remarkably, the *CCR4* mutations found in primary ATLL specimens were heterozygous and introduced missense or truncating mutations in a conserved carboxy-terminal domain of CCR4 involved in negative regulation. Together, these findings suggested a dominant gain-of-function mechansim of action. Indeed, elegant functional studies revealed defective internalization of these mutant CCR4 proteins as well as enhanced migration and chemotaxis in response to chemokines. The authors also demonstrated hyperactive PI3 kinase/Akt signaling in ATLL cells expressing CCR4 mutant proteins.

So, how do *CCR4* mutations promote malignant growth? The authors provide two logical and nonexclusive explanations. First, these mutations might enable ATLL cells to migrate to and colonize niches in tissues such as skin and lymph nodes that are favorable for cancer cell survival and proliferation. If this idea is correct, it is possible that patients with and without *CCR4* mutations will exhibit specific patterns of tissue involvement and disease evolution. Second, dysregulated PI3K signaling downstream of mutant CCR4 might be the key biochemical driver contributing to clonal selection of ATLL cells. Given this underlying biology, it is reasonable to speculate that "seed" and "soil" both contribute to the aberrant growth of ATLL tumors with somatic *CCR4* mutations. For example, because *CCR4* mutations render PI3K signaling hypersensitive to chemokine stimulation, specific tissue microenvironments likely favor ATLL growth through paracrine mechanisms.



Schematic of CCR4 mutant isoforms in ATLL

An exciting aspect of these new mechanistic insights is their potential for clinical translation. A "first generation" anti-CCR4 antibody called KW-0761 is showing promise in early phase clinical trials. It will be interesting to determine whether mutant *CCR4* is a predictive biomarker of sensitivity to this and other anti-CCR4 agents. Deep genomic analysis of tumors with *CCR4* mutations that relapse after an initial response will provide additional insights. PI3 kinase inhibitors—both alone and in combination with other agents—are another potential therapeutic strategy for improving outcomes in this relentless cancer.

Nakagawa, M., et al. 2014. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20140987.

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