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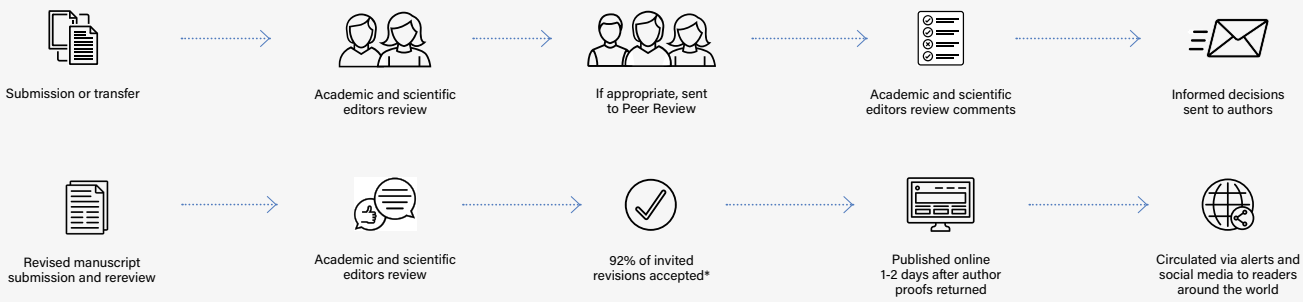

JEM Journal of Experimental Medicine

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On the cover: A human tonsil section analyzed by imaging mass cytometry, using DNA intercalator staining and merged signals from membrane markers to detect individual cells. Image ©2019 Durand et al. Related source: Durand, M., T. Walter, T. Pirnay, T. Naessens, P. Gueguen, C. Goudot, S. Lameiras, Q. Chang, N. Talaei, O. Ornatsky, T. Vassilevskaia, S. Baulande, S. Amigorena, E. Segura. 2019. Human lymphoid organ cDC2 and macrophages play complementary roles in T follicular helper responses. *J. Exp. Med.* 216: 1561–1581. https://doi.org/10.1084/jem.20181994

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HUMAN *IL2RB* MUTATIONS CAUSE AUTOIMMUNITY AND IMMUNODEFICIENCY

Two groups of researchers describe patients with a loss of immunity and peripheral immune tolerance arising from autosomal recessive mutations in the *IL2RB* gene

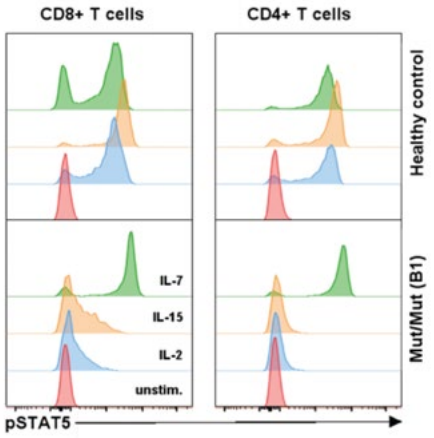
The cytokine interleukin-2 (IL-2) plays a key role in helping the immune system fight infections while avoiding attacks on the body's own, healthy tissues. Primarily produced by $CD4^+$ helper T cells upon exposure to foreign antigens, IL-2 stimulates the proliferation of both T cells and natural killer (NK) cells to fight invading microbes. But IL-2 also supports peripheral tolerance by promoting the development of regulatory T cells and the clonal deletion of peripheral, self-reactive T cells.

The high-affinity IL-2 receptor (IL-2R) is a trimeric protein composed of α, β, and γ chains. The β and γ subunits additionally participate in IL-15 signaling, which also regulates CD8 T and NK cell development, differentiation, and proliferation. Moreover, the γ subunit is a component of the receptors for multiple other cytokines. IL-2/IL-15 binding induces tyrosine phosphorylation of the IL-2R β and γ subunits, as well as the associated kinases JAK1 and JAK3. This leads to the phosphorylation and activation of the transcription factor STAT5 and the induction of genes promoting cell proliferation and survival.

Mutations in many of these signaling components are known to cause severe disease in humans. Mutations in *IL2RA*, encoding the IL-2R α chain, for example, cause an early-onset autoimmune syndrome, whereas mutations in *IL2RG*, encoding the γ subunit, cause a severe combined immunodeficiency associated with the loss of both T and NK cells. Hypomorphic mutations in *IL2RG* have also been reported to cause autoimmunity and immunodeficiency. But no defects had ever been reported in the gene encoding IL-2Rβ until two groups of researchers independently identified several kindreds with autosomal recessive mutations in *IL2RB*.

A team led by Elena Hsieh, Cullen Dutmer, and Ross Kedl at the University of Colorado School of Medicine identified two affected siblings in Tajikistan. And a team led by Michael Lenardo, of the National Institute of Allergy and Infectious Diseases, Kenneth Smith, of the University of Cambridge, Caroline Rooryck, of the University of Bordeaux, and Sophie Hambleton, from Newcastle University, discovered four affected families of South Asian, Middle Eastern, and Eastern European origin.

Altogether, the two teams identified seven pediatric patients that survived past birth and three that died perinatally. "All the patients that survived the neonatal period had recurrent infections, as well as autoimmune disease, leading to early death in most cases," says Lenardo. Common symptoms included enteropathy, dermatitis, autoimmune hemolytic anemia, and elevated immunoglobulin levels in the blood, as



Compared with cells from a healthy control, $CD8^+$ and $CD4^+$ T cells from a patient with a hypomorphic *IL2RB* mutation show reduced STAT5 phosphorylation in response to IL-2 or IL-15 stimulation.
Credit: Zhang et al., 2019

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ORIGINAL PAPER

Zhang, Z., F. Gothe, P. Pennamen, J.R. James, D. McDonald, C.P. Mata, Y. Modis, A.M. Alazami, M. Acres, W. Haller, C. Bowen, R. Döffinger, J. Sinclair, S. Brothers, Y. Zhang, H.F. Matthews, S. Naudion, F. Pelluard, H. Alajlan, Y. Yamazaki, L.D. Notarangelo, J.E. Thaventhiran, K.R. Engelhardt, H. Al-Mousa, S. Hambleton, C. Rooryck, K.G.C. Smith, and M.J. Lenardo. 2019. Human interleukin-2 receptor β mutations associated with defects in immunity and peripheral tolerance. *J. Exp. Med.* 216:1311–1327.
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well as increased susceptibility to infection by CMV and other herpesviruses.

Hsieh and colleagues' two patients carried a homozygous, nine-nucleotide in-frame deletion in *IL2RB* predicted to disrupt a conserved WSXWS motif in the extracellular domain of the receptor. "This motif is common to type I cytokine receptors and influences receptor folding, trafficking, binding, and signaling," Hsieh says. Accordingly, this deletion appears to be a hypomorphic mutation that reduces the surface levels of IL-2Rβ and results in dysregulated IL-2/15-dependent STAT5 phosphorylation.

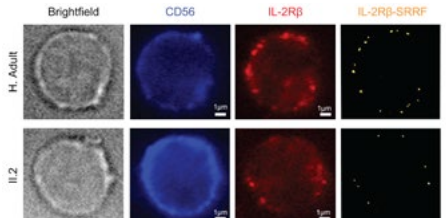
Among Lenardo and colleagues' patients, two families carried a missense mutation that also acts hypomorphically by causing IL-2Rβ to be sequestered in the endoplasmic reticulum instead of being transported to the cell surface. A third family carried a missense mutation that perturbs IL-2 binding to the receptor, and the fourth carried a nonsense mutation that truncates IL-2Rβ before its transmembrane domain. This latter mutation had the most severe effect, causing three children in the family to die perinatally with signs of in utero autoimmunity.

The patients with hypomorphic *IL2RB* alleles had fewer regulatory T cells, likely contributing to their various early-onset autoimmune symptoms. Other T cell subsets remained but were largely unresponsive to IL-2 or IL-15 stimulation. The patients' NK cells, on the other hand, still responded to both cytokines and were present in higher numbers compared with healthy controls. This contrasts with IL-2Rβ-knockout mice, which have diminished numbers of NK cells. The difference may reflect the hypomorphic nature of the human mutations, and the fact that NK cells express particularly high levels of IL-2R, enabling enough signaling activity to persist and drive NK cell proliferation.

However, further analyses revealed that NK cell development was perturbed in hypomorphic *IL2RB* patients. Most of the NK cells present in these children were immature cells, whereas terminally differentiated NK cells, which are crucial to fighting herpesvirus infections, were largely absent. "This suggests a developmental functional defect in the *IL2RB* hypomorphic patients, which may contribute to their CMV susceptibility," says Hsieh. "Together, our findings highlight the critical role of IL-2/15 signaling in T and NK cell development and antiviral immunity and provide insight into the mechanisms that regulate the intricate interface between self-tolerance and host immunity."

"The presence of both immunodeficiency and autoimmune disease as defining features of IL-2Rβ deficiency is consistent with the multifaceted role of IL-2 signaling biology in the immune system," says Lenardo.

Though most of the children studied ultimately died of their ailments, two of the patients were successfully treated by hematopoietic stem cell transplantation. In the future, an alternative therapeutic approach could involve hyperstimulating IL-2 signaling in hypomorphic *IL2RB* patients. The researchers also note that their findings should encourage prenatal screening for *IL2RB* mutations and genetic counseling for at-risk families.



Both immunofluorescence (red) and stream real-time superresolution microscopy (orange) show that IL-2Rβ levels are reduced on the surface of NK cells expressing CD56 (blue) from a hypomorphic *IL2RB* patient (bottom) compared with a healthy control (top).
Credit: Fernandez et al., 2019

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Fernandez, I.Z., R.M. Baxter, J.E. Garcia-Perez, E. Vendrame, T. Ranganath, D.S. Kong, K. Lundquist, T. Nguyen, S. Ogolla, J. Black, C. Galambos, J.C. Gumbart, N. Dawany, J.R. Kelsen, E.F. de Zoeten, R. Quinones, H. Eissa, M.R. Verneris, K.E. Sullivan, R. Rochford, C.A. Blish, R.M. Kedl, C.M. Dutmer, and E.W.Y. Hsieh. 2019. A novel human *IL2RB* mutation results in T and NK cell–driven immune dysregulation. *J. Exp. Med.* 216:1255–1267.
https://doi.org/10.1084/jem.20182015

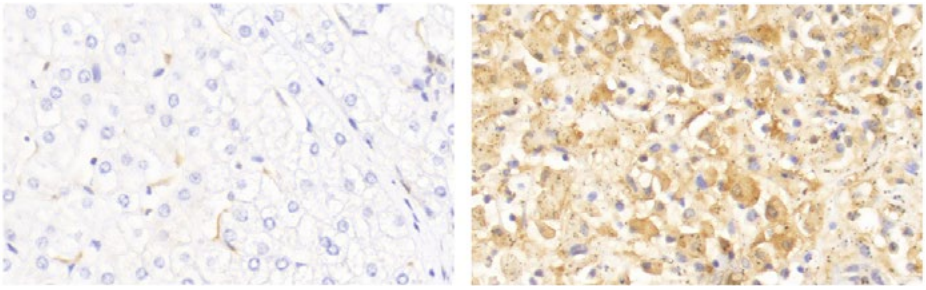
IL-18BP DEFICIENCY CAUSES FULMINANT VIRAL HEPATITIS

Excessive IL-18 signaling causes the body's immune system to attack and kill healthy liver cells, leading to catastrophic liver failure in response to hepatitis A infection

The hepatitis A virus (HAV) infects the liver and usually causes a relatively mild illness that clears up in a matter of weeks or months. But as many as 1 in 200 HAV patients suffer a much more severe response known as fulminant viral hepatitis (FVH) that is characterized by a rapid loss of liver tissue and catastrophic liver failure, resulting in the release of toxins that damage the brain. The condition is usually fatal unless the patient receives a liver transplant.

Other hepatitis viruses can also cause FVH, but the reason why some patients suffer such a severe response to infection is unclear. It typically occurs in children and young adults who are otherwise healthy and have no prior history of liver disease or immunodeficiencies.

A team of researchers led by Jean-Laurent Casanova at The Rockefeller University in New York identified an 11-year-old girl in France who died of FVH after becoming infected with HAV. Casanova and colleagues, including first author Serkan Belkaya, sequenced the girl's DNA and found that she carried identical 40 nucleotide deletions in both copies of the gene encoding interleukin-18 binding protein (IL-18BP). This loss-of-function mutation prevents IL-18BP from binding and neutralizing the proinflammatory cytokine IL-18.



IL-18 levels (brown) are low in the liver of a healthy individual (left) but are drastically elevated in the liver of a young girl with FVH resulting from a mutation in the *IL18BP* gene (right).
Credit: Belkaya et al., 2019

Casanova and colleagues discovered that both IL-18 and IL-18BP are secreted by hepatocytes and macrophages in the liver and that IL-18 levels are elevated in FVH patients. The researchers determined that, in the absence of IL-18BP, IL-18 enhances NK cells' ability to target and kill liver cells, whether they are infected with HAV or not. Addition of IL-18BP blocked this IL-18–induced toxicity, suggesting that IL-18BP usually prevents an excessive reaction to HAV infection but that patients carrying mutations in this gene are susceptible to FVH.

"Our findings provide a proof of principle that FVH can be caused by inborn errors in single genes," Casanova says. "Human IL-18BP injections have been approved for clinical use for indications unrelated to liver conditions and has been proposed as a treatment for preventing acetaminophen-induced liver damage. Neutralizing IL-18 with IL-18BP might be beneficial to patients with FVH caused by HAV and possibly other viruses as well."

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ORIGINAL PAPER

Belkaya, S., E. Michailidis, C.B. Korol, M. Kabbani, A. Cobat, P. Bastard, Y.S. Lee, N. Hernandez, S. Drutman, Y.P. de Jong, E. Vivier, J. Bruneau, V. Béziat, B. Boisson, L. Lorenzo-Diaz, S. Boucherit, M. Sebagh, E. Jacquemin, J.-F. Emile, L. Abel, C.M. Rice, E. Jouanguy, and J.-L. Casanova. 2019. Inherited IL-18BP deficiency in human fulminant viral hepatitis. *J. Exp. Med.* 216:1777–1790.
https://doi.org/10.1084/jem.20190669

A VIRULENCE FACTOR IN CIS AND TRANS

The *Toxoplasma gondii* protein ROP16 suppresses the host immune response by altering the behavior of both infected and uninfected macrophages

The intracellular parasite *Toxoplasma gondii* produces numerous virulence factors that alter the host's immune response and allow the parasite to survive and replicate. Many of these factors are released from a specialized secretory organelle called the rhoptry.

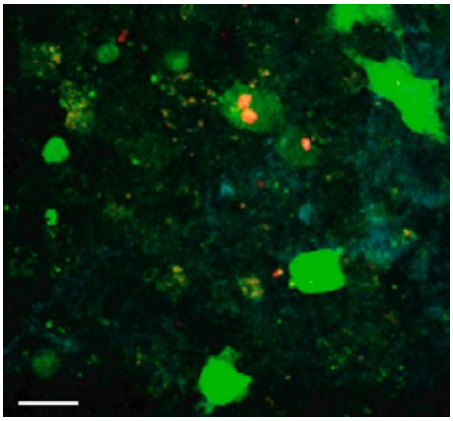
"The effects of these virulence factors have been studied primarily in cells infected with *T. gondii*," says Christopher Hunter, from the University of Pennsylvania School of Veterinary Medicine. "But we now appreciate that rhoptry proteins can also be injected into host cells without parasite invasion and their impact on uninfected cells is not well understood."

The team, including co-first authors Longfei Chen and David Christian, and collaborators from the University of Arizona Anita Koshy and Josh Kochanowsky, were particularly interested in the virulence factor ROP16, a protein kinase present in *T. gondii* rhoptries that can directly phosphorylate the host cell transcription factors STAT3 and STAT6. This causes macrophages to adopt an anti-inflammatory, M2 phenotype, but how this impacts the parasite's survival and replication, and whether ROP16 can modify the behavior of uninfected, as well as infected, macrophages was unclear.

These questions became addressable when Koshy generated parasites that express Cre recombinase in the rhoptries. Using these parasites to infect reporter mice allowed the researchers to distinguish infected macrophages from macrophages that had been injected with rhoptry proteins. "We had previously recognized that some cells receive this injection but are not actively infected with parasites," Koshy says. "So, we thought if we can look at cells that only received the injection we could ask what the consequences of that injection are."

The researchers discovered that injection of rhoptry proteins was sufficient to induce M2 polarization in a STAT6 and STAT3 dependent manner. Transcriptional profiling revealed that injection altered the expression of ~600 macrophage genes, many of them related to M2 cell function. However, full infection altered the expression of a further ~1,200 genes, and was required to suppress many genes involved in antimicrobial activity and protective immunity.

To investigate the role of ROP16 in these changes, Hunter and colleagues infected mice with a *T. gondii* strain lacking the protein kinase. *rop16*-deficient parasites were unable to induce the M2 phenotype by either infection or injection of macrophages. Instead, infected mice displayed higher numbers of pro-inflammatory, M1-type macrophages. This correlated with increased production of the cytokines IL-12 and IFN-γ, higher numbers of parasite-specific T cells, and reduced parasite burden.



The omentum of a *T. gondii*–infected mouse shows that a green fluorescent reporter indicating exposure to rhoptry proteins is expressed in uninfected cells as well as in cells containing the parasite (red).
Credit: Chen et al., 2019

ROP16 is therefore a key virulence factor that acts in cis (within infected cells) and in trans (when injected into uninfected cells) to induce the development of M2 macrophages and prevent an effective T cell–mediated response to infection. "There are a number of potential mechanisms whereby M2 macrophages can antagonize the development of cell-mediated immunity, and we are currently trying to understand the basis for the ROP16-mediated suppression of T cell responses," Hunter says.

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https://doi.org/10.1084/jem.20181757

RIPK1 CAN KILL WITHOUT ITS KINASE ACTIVITY

Simultaneous innate immune priming and TAK1 inactivation by microbial pathogens causes RIPK1 to induce inflammatory cell death and sepsis independently of its kinase activity

In response to various stimuli, the kinase activity of RIPK1 can induce three different modes of cell death: necroptosis, pyroptosis, and apoptosis. For example, RIPK1 kinase activity can drive pyroptosis by activating the NLRP3 inflammasome complex, leading to the proteolytic activation of proinflammatory cytokines and the pore-forming membrane protein gasdermin D.

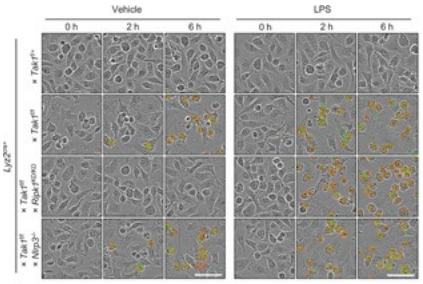
In 2018, Thirumala-Devi Kanneganti and colleagues at St. Jude Children's Research Hospital found that, to prevent unnecessary inflammation and cell death, RIPK1 activity is normally limited by another kinase, TAK1. Inflammasome assembly usually requires a priming signal emitted by invading pathogens or damaged tissue, but macrophages lacking TAK1 undergo spontaneous inflammasome activation and cell death in a RIPK1 kinase activity–dependent manner. The resulting inflammation causes myeloid proliferation and neutrophilia in mice.

TAK1 itself is a key component of proinflammatory signaling, however, and many microbial pathogens have evolved to inhibit TAK1 signaling. "We therefore wanted to investigate how microbial priming affects the role of RIPK1 in inflammasome activation and cell death under conditions of TAK1 inactivation," Kanneganti says.

Kanneganti and colleagues, including co-first authors R.K. Subbarao Malireddi and Prajwal Gurung, mimicked the potential effects of microbial infection by treating *Tak1*-deficient macrophages with bacterial LPS and other molecules that activate Toll-like receptors and prime inflammasome assembly. These microbial priming signals induced macrophage cell death independently of RIPK1 kinase activity.

Under these conditions, the researchers discovered, RIPK1 can instead act as a scaffold to promote the assembly of a protein complex that also contains the protease caspase-8 and the inflammasome proteins NLRP3 and ASC. This complex drives apoptosis as well as inflammasome activation and pyroptosis. Moreover, under the same conditions, RIPK1 can also stimulate RIPK3-mediated necroptosis in a kinase activity–independent manner. Thus, a single macromolecular complex can drive three different cell death pathways, which the authors collectively refer to as PANoptosis (Pyroptosis, Apoptosis, and Necroptosis).

In vivo, the high levels of inflammatory cell death induced by simultaneous microbial priming and TAK1 inactivation might make animals prone to sepsis. When given in combination with a TAK1 inhibitor, even a low (10-µg) dose of bacterial LPS killed 60% of wild-type mice. The mice were only partially protected when RIPK1's kinase activity was inhibited, but were fully protected when both RIPK3 and caspase-8 were inactivated to block PANoptotic cell death. RIPK3 and caspase-8 inactivation also prevented the neutrophilia associated with TAK1 deficiency.



Treatment of TAK1-null macrophages with LPS results in the death of both macrophages expressing wild-type RIPK1 (second row) and those expressing kinase-dead RIPK1 (third row), indicating that the cell death is occurring independently of RIPK1 kinase activity.
Credit: Malireddi et al., 2020

The work suggests that microbes have evolved to modulate the host immune response by inactivating central nodes of inflammatory signaling such as TAK1. "Our data support the concept that cells have coevolved to sense TAK1 inactivation as a danger signal to drive RIPK1 kinase activity–independent inflammatory cell death, PANoptosis, and bypass the pathogen-mediated immune evasion strategy," Kanneganti says. "Our findings also highlight the complexity in regulating inflammatory cell death and identify potential components of the PANoptotic cell death complex that could be targeted to treat myeloid proliferation and sepsis."

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ORIGINAL PAPER

Malireddi, R.K.S., P. Gurung, S. Kesavardhana, P. Samir, A. Burton, H. Mummareddy, P. Vogel, S. Pelletier, S. Burgula, and T.-D. Kanneganti. 2020. Innate immune priming in the absence of TAK1 drives RIPK1 kinase activity–independent pyroptosis, apoptosis, necroptosis, and inflammatory disease. *J. Exp. Med.* 217:e20191644.
https://doi.org/10.1084/jem.20191644

HOW SKIN γδ T CELLS STAY POSITIONED

Sphingosine-1-phosphate receptor 2 and CD69 cooperate to keep γδ T cells from leaving the dermis

Skin is a crucial barrier that must stand up to physical insults and contact with microbes, and a population of dermal γδ T cells in mice and humans serves in defense. These cells are very mobile, constantly patrolling beneath the skin, yet they stay in the dermis. Brian J. Laidlaw, Elizabeth E. Gray, Jason G. Cyster, and colleagues at the University of California, San Francisco, discovered what keeps those cells in the skin.

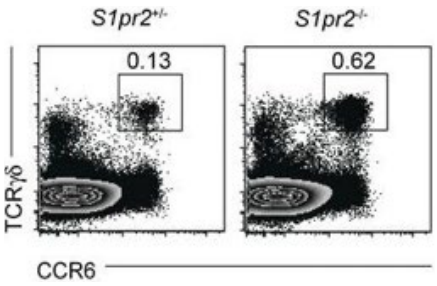
Effector and memory αβ T cells exit the skin via skin-draining lymph nodes, a process that is mediated by the cells' S1P receptor 1 (S1PR1) that binds to the lipid sphingosine-1-phosphate (S1P) produced by skin lymphatics. To see whether S1P plays a role in γδ T cell positioning, the researchers generated mice deficient in sphingosine kinase 1 and 2 *(Sphk1/2)*, which results in reduced S1P levels. Unexpectedly, *Sphk1/2* knockout mice showed an increase in the frequency and number of γδ T cells in skin-draining lymph nodes, indicating that S1P has a role in keeping γδ T cells from accumulating there.

S1PR2 is a G protein–coupled receptor that inhibits migration in some cell types. Because S1P prevents γδ T cells from accumulating in skin draining lymph nodes, the team asked whether S1PR2 might help retain γδ T cells in the skin. *S1pr2* knockout mice showed an increase in γδ T cells in skin-draining lymph nodes, indicating that S1PR2 normally keeps dermal γδ T cells from leaving the skin.

In contrast, the researchers found that S1PR1 promotes the exit of γδ T cells from the skin, and *S1pr1* mRNA levels were higher in γδ T cells from the skin-draining lymph node.

The cell surface protein CD69 is known to repress S1PR1 activity, and the researchers found that γδ T cells in the dermis expressed increased levels of *Cd69* mRNA and protein. *Cd69* knockout mice showed more γδ T cells in skin draining lymph nodes, indicating that CD69 keeps γδ T cells in the dermis by regulating S1PR1.

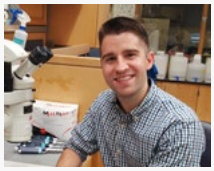
To explore whether CD69 cooperates with S1PR2 to prevent γδ T cells from exiting the skin, the team generated mice with both *Cd69* and *S1pr2* knocked out. They observed a decrease in the frequency and number of γδ T cells in the dermis of these mice compared to mice lacking either *Cd69* or *S1pr2*, suggesting that S1PR2 and CD69 cooperate to keep γδ T cells in the skin.



S1pr2 knockout mice display an increase in the frequency and number of CCR6⁺ γδ T cells present in the skin-draining lymph node.
Credit: Laidlaw et al., 2019

"Inducing γδ T cell exit from the skin by local administration of an S1PR2 antagonist may be an effective treatment for diseases such as psoriasis that involve an overactive tissue-resident lymphocyte response," Laidlaw says.

RESEARCHER DETAILS



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Laidlaw, B.J., E.E. Gray, Y. Zhang, F. Ramírez-Valle, and J.G. Cyster. Sphingosine-1-phosphate receptor 2 restrains egress of γδ T cells from the skin. 2019. *J. Exp Med.* 216:1487–1496.
https://doi.org/10.1084/jem.20190114

BCOR ENHANCES T HELPER 17 CELL FORMATION

BCL6 corepressor works together with KDM2B to generate T cell subset that helps promote clearance of extracellular bacteria

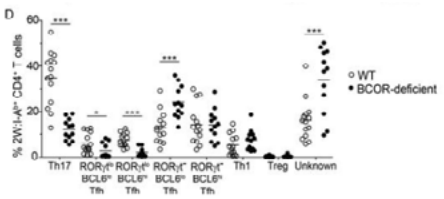
T helper (Th) cells protect their hosts from pathogens at mucosal surfaces. When a naive Th cell recognizes a microbial peptide via its T cell receptor (TCR), it differentiates into a specialized helper to optimally attack the pathogen. Jessica A. Kotov, Micah D. Gearhart, Marc K. Jenkins, and colleagues at the University of Minnesota Medical School studied the signals responsible for generating differentiated Th17 cells and found a crucial role for BCL6 corepressor (BCOR).

Th17 cells secrete cytokines that act on other immune cells, including neutrophils, to promote clearance of extracellular bacteria, such as *Streptococcus pyogenes* (*Sp*), *Klebsiella pneumoniae*, and *Bordetella pertussis*. But in order to become a Th17 cell, a naive Th cell must have key genes activated and repressed. The team previously found that the transcription factor BCL6 is involved in determining the fate of Th cells that differentiate into follicular helper cells by recruiting BCOR, a component of a variant Polycomb repressive complex 1.1 (PRC1.1). They wanted to see whether BCOR is also involved in deciding Th17 fate.

To generate a robust Th17 response, the team compared *Sp* infections in wild-type mice and mice with T cells deficient in BCOR. Naive Th cells clonally expanded in both groups of mice, but T cell BCOR-deficient mice had significantly smaller fractions of Th17 and other Th cell subsets. BCOR deficiency also led to reduced IL-17A production by Th17 cells. Mice with a *Bcl6* mutation in T cells did not have different numbers of Th17 compared to wild type, however, indicating that BCOR does not cooperate with BCL6 to promote Th17 differentiation.

To confirm the role of the PRC1.1 complex in Th17 differentiation, the team looked at the histone demethylase KDM2B, a second unique component of PRC1.1. Using CRISPR/Cas9, they deleted *Kdm2b* or *Bcor* in naive Th cells, introduced these cells into mice, and inoculated the mice with heat-killed *Sp. Kdm2b* or *Bcor* deletion did not affect T cell proliferation but the absence of either gene reduced the population of Th17 cells, confirming the importance of the PRC1.1 complex in Th17 differentiation. "These results indicate that BCOR and KDM2B drive uncommitted cells to become Th17 cells," Kotov says.

Genome-wide expression and BCOR chromatin immunoprecipitation studies revealed that BCOR directly represses



T cell BCOR-deficient mice had significantly smaller fractions of Th17 and other Th cell subsets compared to wild-type mice.
Credit: Kotov et al., 2019

Lef1, *Runx2*, and *Dusp4* genes. CRISPR/Cas9-targeted disruption of *Lef1*, *Runx2*, and *Dusp4* in naive Th cells enhanced Th17 differentiation, indicating that these genes normally act to suppress Th17 production. The team's research shows that "PRC1.1 components BCOR and KDM2B work together to enhance Th17 cell formation by repressing Th17 fate suppressors," says Kotov.

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ORIGINAL PAPER

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IL-2 SCALES THE SIZE OF THE T REG POPULATION

Self-reactive thymocytes modulate the size of the regulatory T cell population generated in the thymus through production of IL-2

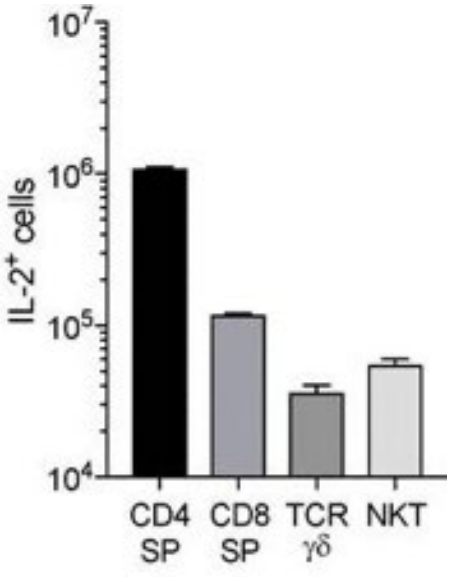
Regulatory T (T reg) cells protect against life-threatening autoimmunity. The size of the T reg cell population needs to be tightly-controlled, to suppress autoimmunity and inflammation, while allowing for protection against pathogens. Saskia Hemmers, Alexander Y. Rudensky, and colleagues at the Sloan Kettering Institute found that the size of the T reg pool is adjusted by the amount of the cytokine interleukin-2 (IL-2).

T reg cells are a specialized subset of $CD4^+$ T cells defined by expression of the transcription factor Foxp3. Expression of Foxp3, and T reg differentiation in the thymus, is dependent upon T cell receptor (TCR) and interleukin-2 (IL-2) signaling. However, the cellular sources of IL-2 that promote T reg cell differentiation in the thymus and maintenance outside the thymus have been debated.

To identify the specific cellular sources of IL-2, the team generated mice in which cells with a "life history" of IL-2 expression were permanently marked or "fate mapped" by a fluorescent reporter protein. They found that the majority of IL-2–producing cells in the thymus are CD4 single-positive (CD4SP) thymocytes, which are precursors of specialized T cells, including T regs. They also noticed that T reg cells emerged from the thymus on the same day that IL-2-producing cells were first detected, suggesting production of IL-2 is a limiting factor for T reg cell generation.

When the researchers treated mice with a bacterial super-antigen that stimulates TCRs, it led to an overall expansion of T reg cells and IL-2 "fate-mapped" cells in the thymus. The majority of the increase in IL-2 was contributed by CD4SP thymocytes receiving strong TCR stimulation, but there was also "history" of IL-2 production observed in a subset of thymic T reg cells. In mice with IL-2 deleted from T cells, T reg expansion was blunted after super-antigen exposure. These results suggest that T reg precursor cells, upon receiving a strong TCR signal, can produce IL-2, which in turn promotes their own differentiation into T reg cells.

To learn about the features of thymocytes producing IL-2, the team performed single-cell RNA sequencing analysis of CD4 thymocyte subsets. Their data revealed that IL-2 was expressed in self-reactive CD4SP thymocytes, some of which will go on to become T reg cells. Additionally, "IL-2–expressing thymocytes exhibited a gene expression pattern characteristic of TCR engagement, NF-κB signaling, and tonic type I IFN signaling and served as a key source of IL-2 for developing thymic T reg cells," says Hemmers.



CD4 single-positive thymocytes represent the major source of IL-2 in the thymus.
Credit: Hemmers et al., 2019

Because of the crucial role T regs play in protecting against autoimmunity, IL-2 scaling of the T reg population by CD4SP thymocytes that are self-reactive could play an important role in preventing autoimmunity.

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ORIGINAL PAPER

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NEW METHOD TO DETECT "HIDDEN" HIV-1

A combination of quadruplex qPCR and next-generation sequencing can detect latent reservoirs of HIV-1

Currently, HIV-1 patients have to take antiretroviral drugs for life because some immune cells remain infected with the virus, though the virus is not replicating. Numerous scientists are looking for a way to permanently shut down this "latent reservoir" of HIV-1, which requires sensitive measurement of the reservoir. Christian Gaebler, Michel C. Nussenzweig, and colleagues at Rockefeller University reveal a new high-throughput method for detecting the HIV-1 genome in tiny samples.

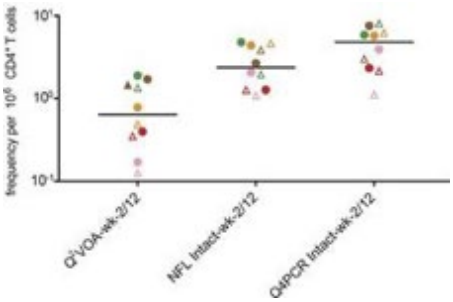
"HIV-1 integrates into the host genome, where it is transcribed to produce infectious virions," Gaebler explains. "In a small number of CD4⁺ T cells, the integrated virus is silenced and becomes latent. Combination antiretroviral therapy is highly effective in suppressing HIV-1 infection and preventing disease progression; however, this therapy does not eliminate the virus due to the existence of the latent reservoir."

In order to evaluate true cures for HIV-1, which don't require long-term treatment, researchers need a specific and precise tool to identify the latent HIV-1 reservoir. Viral outgrowth assays were the standard for assessing latent HIV-1, however this method fails to detect ultralow levels of latent virus in patients initially thought to be cured. Near full-length (NFL) sequencing can detect ultralow virus levels but is labor intensive.

To maximize HIV-1 detection, the team designed quantitative PCR (qPCR) primers/probes that cover four conserved regions of the viral genome, including the packaging signal (PS), group-specific antigen (*gag*), polymerase (*pol*), and envelope (*env*). Though 99% of 578 genomes from the Los Alamos HIV genome database were positive for at least one of the combinations of two primer/probe sets, selecting just two probes was not sensitive enough to accomodate the genome variations.

To accommodate HIV-1 sequence diversity, the team developed a multiplex qPCR strategy for simultaneous detection of all four probes in a 384-well format to allow for high-throughput screening. They call the method Quadruplex qPCR or Q4PCR. Using CD4⁺ T cell samples from HIV-1 patients enrolled in a clinical trial, the team compared Q4PCR with NFL sequencing and viral outgrowth assays performed on the same samples.

The team found that NFL and viral outgrowth assays showed fewer intact and inducible proviruses per million CD4⁺ T cells than Q4PCR. However, the requirement for hybridization with a third or fourth probe decreases Q4PCR's sensitivity, so the team screened samples with all four primer/probe sets and subsequently sequenced viruses positive for any two Q4PCR primer/probe sets. "The combination of four-probe qPCR and next-generation sequencing is a highly sensitive and specific method for measuring intact proviruses in the HIV-1 latent reservoir," Gaebler says.



Viral outgrowth assays (Q2VOA) and near full-length (NFL) sequencing showed fewer intact and inducible proviruses per million CD4⁺ T cells than quadruplex qPCR (Q4PCR). Credit: Gaebler et al., 2019

RESEARCHER DETAILS

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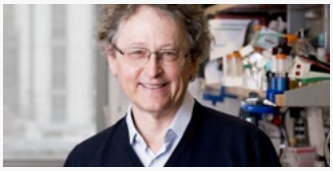

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https://doi.org/10.1084/jem.20190896

"ADAPTIVE" NATURAL KILLER CELLS CORRELATE WITH PROTECTION FROM MALARIA

In people exposed to *Plasmodium falciparum*, "adaptive" natural killer cells appear to protect against malaria

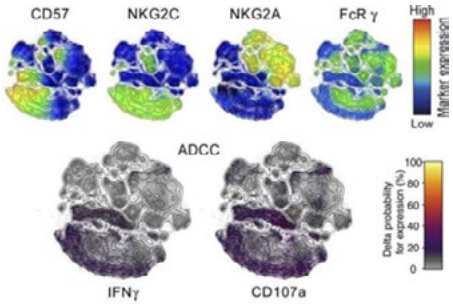
Plasmodium falciparum is a mosquito-transmitted parasite that causes half of all malaria cases, amounting to over 400,000 deaths in 2018, making it the deadliest parasite in humans. Geoffrey T. Hart, Eric O. Long, and colleagues at the University of Minnesota and the National Institute of Allergy and Infectious Diseases wanted to know how antibodies acquired during *P. falciparum* infection provide immunity to malaria, and found a crucial role for natural killer (NK) cells.

Complete immunity to *P. falciparum* is rare, even after years of exposure, though "clinical immunity," which reduces the severity of the disease, is common. Antibodies against malaria antigens can trigger a range of helpful immune responses, including antibody-dependent cellular cytotoxicity (ADCC) by NK cells in vitro. However, the precise role of NK-mediated ADCC in protection against malaria in vivo wasn't known.

Recently, "adaptive" NK cells with enhanced ADCC activity were found to develop in response to cytomegalovirus (CMV) infection. These NK cells increase expression of NKG2C and lose expression of the transcription factor promyelocytic leukemia zinc finger (PLZF) and of the signaling Fc receptor γ-chain (FcRγ). The researchers found a large frequency of "adaptive" NK cells in blood samples from Malian individuals naturally exposed to *P. falciparum*.

The researchers observed that individuals that had more FcRγ-negative NK cells before malaria transmission season had resistance to malaria symptoms and delayed onset of disease during transmission season, whereas acute malaria was associated with fewer PLZF-negative and FcRγ-negative NK cells. "Among all phenotypic markers tested, FcRγ-negative was the only one strongly associated with every measure of resistance: reduced parasite load, increased probability of remaining disease free, and delayed disease onset during the transmission season," Hart says.

Through in vitro functional assays on patient NK cells, the researchers found that ADCC responses were mostly limited to the FcRγ-negative NK cell subset. When the researchers added plasma (containing antibodies) from malaria-resistant Mali adults to *P. falciparum*–infected red blood cells in vitro, it triggered robust degranulation by NK cells obtained from malaria-exposed individuals. Within that pool of degranulating NK cells, they observed an enrichment in FcRγ-negative cells, suggesting a critical role for these "adaptive" NK cells in combating malaria.



t-SNE analysis of NK cell subsets shows that FcRγ-negative cells (blue areas in the top right plot), known as "adaptive" NK cells, show the highest probability of IFN-γ production and degranulation (CD107a) in antibody-dependent cellular cytotoxicity assays (purple areas in the bottom plots).
Credit: Hart et al., 2019

"Our results suggest that NK cells play a critical and underappreciated role in reducing malaria morbidity through ADCC-mediated clearance of parasites from the blood. Consideration of antibody-dependent NK cell responses to *P. falciparum* antigens is therefore warranted in the design of malaria vaccines," Hart says.

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