the reverse trancriptase inhibitor efavirenz and by AMD3100, a drug that blocks HIV-1 entry via the HIV-1 coreceptor CXCR4. This suggests that the inflammatory response to abortive HIV-1 infection is triggered by premature termination of viral DNA elongation, which signals caspase-1 and inflammasome activation and the maturation and release of bioactive IL-1β in these $CD4^+$ T cells. Capsase-1 and inflammasome activation are required for IL-1β production in this system. Therefore, caspase-1-dependent cell death, known as pyroptosis, is a plausible mechanism of CD4 depletion.

DNA damage cascades also play a role in death of activated $CD4^+$ T cells that are infected with HIV-1. Cooper et al. (2013) demonstrated that virus-induced $CD4^+$ T cell killing is triggered by integration. Cell death in this system was associated with productive rather than abortive infection. The mechanism of killing following viral integration involved the activation of DNA-dependent protein kinase (DNA-PK), a central integrator of the DNA damage response, which causes phosphorylation of p53 and histone H2AX.

Taken together, these studies highlight the emerging complexity of innate sensing of HIV-1, with multiple pathways in different cell types leading to a variety of outcomes. Lahaye and colleagues speculate that enhancing innate sensing might represent a strategy for enhancing HIV-1 control and producing more effective vaccines. However, manipulation of the innate sensing systems might also result in increased pathogenesis. Therefore, additional studies in this area would be of significant value, particularly in systems that allow interaction between $CD4^+$ T cells and other immune system cells so that the total effects of such manipulations on all cell types and the overall immune response can be determined.

REFERENCES

Baldauf, H.M., Pan, X., Erikson, E., Schmidt, S., Daddacha, W., Burggraf, M., Schenkova, K., Ambiel, I., Wabnitz, G., Gramberg, T., et al. (2012). Nat. Med. *18*, 1682–1687.

Cooper, A., García, M., Petrovas, C., Yamamoto, T., Koup, R.A., and Nabel, G.J. (2013). Nature *498*, 376–379.

Deeks, S.G., Tracy, R., and Douek, D.C. (2013). Immunity *39*, 633–645.

Doitsh, G., Cavrois, M., Lassen, K.G., Zepeda, O., Yang, Z., Santiago, M.L., Hebbeler, A.M., and Greene, W.C. (2010). Cell *143*, 789–801.

Gao, D., Wu, J., Wu, Y.T., Du, F., Aroh, C., Yan, N., Sun, L., and Chen, Z.J. (2013). Science *341*, 903–906.

Goldstone, D.C., Ennis-Adeniran, V., Hedden, J.J., Groom, H.C.T., Rice, G.I., Christodoulou, E., Walker, P.A., Kelly, G., Haire, L.F., and Yap, M.W. (2011). Nature *480*, 379–382.

Laguette, N., Sobhian, B., Casartelli, N., Ringeard, M., Chable-Bessia, C., Ségéral, E., Yatim, A., Emiliani, S., Schwartz, O., and Benkirane, M. (2011). Nature *474*, 654–657.

Lahaye, X., Satoh, T., Gentili, M., Cerboni, S., Conrad, C., Hurbain, I., Marjou, A., Lacabaratz, C., Leleievre, J.-D., and Manel, N. (2013). Immunity *39*, this issue, 1132–1142.

Manel, N., Hogstad, B., Wang, Y., Levy, D.E., Unutmaz, D., and Littman, D.R. (2010). Nature *467*, 214–217.

Sun, L., Wu, J., Du, F., Chen, X., and Chen, Z.J. (2013). Science *339*, 786–791.

New Twist on an Ancient Innate Immune Pathway

Stephane Lajoie[1] and Marsha Wills-Karp[1,*]
[1]Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA
*Correspondence: mwkarp@jhsph.edu
http://dx.doi.org/10.1016/j.immuni.2013.11.015

Activation of the complement system has long been known to be regulated by a series of steps involving fluid-phase convertases. In this issue of *Immunity*, Liszewski et al. (2013) report the discovery of an intracellular cathepsin-L-dependent C3 activation pathway.

The complement system can be activated by "hard-wired" pattern-recognition receptors (PRRs) that have evolved to recognize pattern-associated molecular patterns (PAMPs). PRRs in the complement system recognize exogenous and endogenous "danger" motifs. Recognition receptors in the complement system (i.e., specific antibody, mannan-binding lectin [MBL], C1q, and natural immunoglobulin M [IgM]) activate three separate complement pathways referred to as the classical, lectin, and alternative. Although each of these pathways is activated by distinct PRRs, they all culminate in activation of the complement factor 3 (C3), the central step in complement activation. The C3 convertase (C3bBb) converts the inactive yet abundant C3 component into the biologically active effector fragments referred to as anaphylatoxins (C3a and C3b). C3a in turn binds its G-protein-coupled receptor, C3aR, on the surface of cells, whereas C3b can either bind its receptor, CD46, or bind to more of the C3 convertase, creating the C5 convertase [C3(H2O)BbP3b], which leads to the generation of C5a and C5b. C5b initiates the terminal enzymatic cascade of the lytic membrane attack complex, which mediates lysis of pathogens and unprotected host cells.

Although liver-generated circulating anaphylatoxins undoubtedly play a role in pathogen control systemically, emerging evidence suggests that anaphylatoxins are also produced by immune cells, including T cells. Once produced, they bind their receptors on the T cell

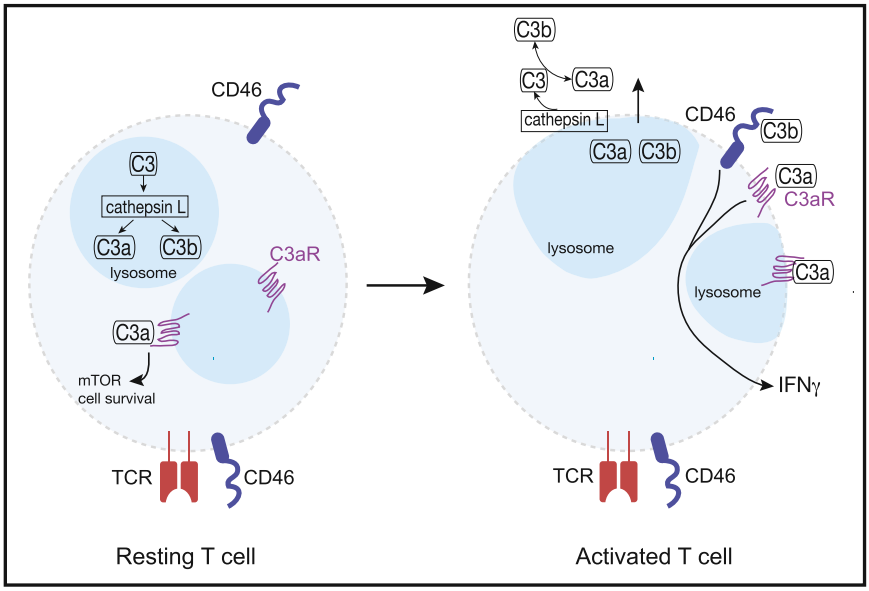

Figure 1. Cathepsin L-dependent Intracellular and Extracellular Complement Activation Pathways

In resting T cells, the complement factor C3 is contained intracellularly in performed stores and is proteolytically cleaved to C3a and C3b, by cathepsin L within lysosomes. Intracellularly generated C3a signals via engagement of intracellular C3aRs promote cell survival via mTOR activation. Unstimulated T cells do not express surface C3, C3a, C3b, the C3aR, or cathepsin L. However, upon engagement of the TCR alone or the TCR plus CD46, T cells release C3, C3a, and C3b, as well as cathepsin L and express surface C3aR. On the cell surface, cathepsin L cleaves C3 to generate C3a and C3b. Subsequent binding of C3a to C3ar and C3b to CD46 induces T cell secretion of cytokines such as IFN-γ and IL-17A.

surface and regulate adaptive T cell immunity (Heeger and Kemper, 2012; Cardone et al., 2010). However, to date, the exact mechanism(s) governing anaphylatoxin production in T cells are not well understood. In pursuit of the mechanism by which C3a is released from human T cells, Liszewski et al. (2013) speculated that T cell-intrinsic mechanisms might be regulating the rapid release of the C3-cleavage product C3a. Based on their observation that a series of endosomal or lysosomal proteases were expressed in T cells, they first explored whether these cathepsins (B, G, L), might play a role in cleavage of C3 into C3a and C3b in human T cells. They found that cathepsin L (CTSL), but no other cathepsin, cleaved C3 into its active fragments (C3a, C3b). Interestingly, the other cathepsins degraded C3 without generation of biologically active C3a and C3b—suggesting specificity of CTSL for this reaction. Similarly, CTSL-dependent cleavage appears to be specific to C3 and C4, because CTSL does not cleave C5 into its activation fragments. In support of an interaction between CTSL and C3 and C3a in intact cells, they demonstrated that CTSL and C3 colocalized to lysosomal compartments and that inhibition of CTSL activity with a cell-permeable CTSL inhibitor reduced intracellular levels of C3a. These results suggested that CTSL generates "tonic" C3a from intracellular pools of C3 in resting T cells. Because neither the C3aR nor the C3b receptor CD46 were expressed on resting T cells, the functional consequence of producing C3a intracellularly was unclear. However, the authors gained an important clue to the potential function when they noted that the C3aR was colocalized with C3 in lysosomes. Based on the proximity between the C3a and its receptor C3aR intracellularly, they proposed a scenario in which CTSL cleaves C3 into C3a and C3b and C3a in turn binds its receptor intracellularly to regulate basal T cell function (Figure 1). Because C3aR engagement on $CD4^+$ T cells activates the kinase mTOR, which is required for T cell survival in vivo (Strainic et al., 2008), they tested the hypothesis that intracellular C3a-C3aR engagement mediated mTOR and resting T cell survival. Indeed, CTSL inhibition and small interfering RNA (siRNA) knockdown of C3aR in resting T cells resulted in reduced mTOR phosphorylation and reduced T cell viability. Although the exact logistics of C3a-C3aR engagement were not elucidated, these results suggest the intriguing possibility that C3a engages its G protein coupled receptor, C3aR, on the surface of intracellular lysosomes, not on the plasma membrane. These fascinating findings suggest a new pathway of enzymatic cleavage of C3, challenging the traditionally held belief that complement activation only occurs through a series of serum convertases.

To explore whether C3 mediates similar processes in activated T cells, the authors examined the levels and cellular localization of C3, C3a, and C3aR upon TCR activation. They show that TCR activation induces shuttling of the intracellular stores of C3aR to the cell surface and amplifies intracellular CTSL-mediated cleavage of C3 into C3a and C3b and induces extracellular, cell surface CTSL-mediated C3 activation. Subsequent extracellular C3aR and CD46 engagement by C3a and C3b, respectively, leads to induction of the T helper 1 (Th1) cell cytokine, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) (Figure 1). CTSL inhibition of TCR-activated T cells results in a reduction in the secretion of the Th1 cell cytokines, IFN-γ and TNF-α, and interleukin-17A (IL-17A), whereas it had no effect on Th2 cell cytokine production. This sequence of events is consistent with the lack of robust Th1 cell responses in CD46- and C3-deficient patients (Le Friec et al., 2012). Interestingly, this phenomenon was not observed in CTSL-deficient mice in which CTSL was maintained only in thymic epithelium, suggesting that mouse and human cells might differ in regards to the role of CTSL regulation of C3 cleavage. This species difference in CTSL regulation of C3a-C3aR might explain some of the conflicting reports of C3aR expression on mouse T cells. The differential expression of the C3aR between resting and activated T cells appears to represent a fail-safe mechanism designed to prevent unnecessary complement activation in the absence of pathogens, but at the same time allows maintenance of a supply of resting T cells that can be called into action if necessary. This elaborate rheostat mimics the protective mechanisms utilized to protect host cells against serum complement activation products.

Because T cell hyperactivation and aberrant complement activation are prominent features of several autoimmune disorders, the authors sought to determine whether modulation of cathepsin L pathways normalized T cell cytokine production in T cells from patients with autoimmune arthritis. Strikingly, they found that intracellular C3a levels, mTOR activity, and IFN-γ levels were higher in blood T cells from patients with autoimmune arthritis as compared to those obtained from healthy individuals. Importantly, pharmacological targeting of CTSL reversed the heightened IFN-γ production observed in the patient's T cells. The normalization of their IFN-γ productive capacity was accompanied with a reduction in intracellular C3a levels and mTOR activity. Although these findings will need to be confirmed in a larger study, the insights afforded by this study raise the possibility that aberrant regulation of the steps involved in intracellular C3 activation might underlie susceptibility to autoimmune arthritis. More broadly, these findings have implications for a wide spectrum of human disorders associated with complement dysregulation, including other autoimmune diseases, sepsis, age-related macular degeneration, graft rejection, and asthma, to name a few.

The current study highlights a role for CTSL-dependent C3a-driven production of the Th1 cell cytokines. Although these studies suggest that CTSL-mediated C3a generation is specifically associated with enhanced Th1 cells and IFN-γ production, other studies have shown that C3a regulates the production of the signature cytokines of other $CD4^+$ T cell subsets such as Th17 (Lajoie et al., 2010) and Th2 cells (Zhang and Köhl, 2010). Studies have also shown that T regulatory (Treg) cells express C3aR and C5aR and that signaling through these receptors inhibits $Foxp3^+$ expression and Treg cell function (Strainic et al., 2013). Moreover, blockade of these complement pathways in both mouse and human $CD4^+$ T cells favored their transformation to $Foxp3^+$ Treg cells and as a consequence limits the clinical expression of graft-versus-host disease (van der Touw et al., 2013). The known ability of Treg cells to suppress the expansion and cytokine production of other $CD4^+$ T cell subsets suggests that the effect of C3a blockade on IFN-γ levels in autoimmune arthritis patients might be secondary to C3aR-mediated suppression of Treg cell cytokine production and function.

Evidence supporting the conceptual model that intracellular T cell production of C3 and C3a occurs independently of that generated in the liver was provided by the observation that T cells derived from C3-deficient patients, which do not have measurable serum C3 or C3a levels, contain both C3 messenger RNA (mRNA) and C3a protein. Although the T cells from all the patients examined contained C3a proteins, the levels were variable between patients and appeared to be dependent upon specific genetic polymorphisms in the respective C3 genes. These results highlight the possibility that previous assumptions made about the role of C3 in immunoregulation, which were based on serum complement component deficiencies in humans, might need to be reevaluated.

Although pursuit of CTSL and C3 pathways as therapeutic targets for the treatment of T cell-mediated disorders is a tempting option, much remains to be learned about the role of intracellular activation of C3 in T cell-mediated disorders. For example, it remains to be shown whether enhanced T cell survival and cytokine production of T cells from autoimmune patients and/or other complement-associated diseases is due to enhanced basal T cell expression of C3 or to enhanced CTSL cleavage of C3 into C3a. It also remains to be determined whether specific SNPs in C3 preferentially render it susceptible or resistant to CTSL-mediated cleavage. These results will undoubtedly fuel further investigation into the role of dysregulated CTSL-mediated intracellular C3 activation in health and disease.

REFERENCES

Cardone, J., Le Friec, G., Vantourout, P., Roberts, A., Fuchs, A., Jackson, I., Suddason, T., Lord, G., Atkinson, J.P., Cope, A., et al. (2010). Nat. Immunol. *11*, 862–871.

Heeger, P.S., and Kemper, C. (2012). Immunobiology *217*, 216–224.

Lajoie, S., Lewkowich, I.P., Suzuki, Y., Clark, J.R., Sproles, A.A., Dienger, K., Budelsky, A.L., and Wills-Karp, M. (2010). Nat. Immunol. *11*, 928–935.

Le Friec, G., Sheppard, D., Whiteman, P., Karsten, C.M., Shamoun, S.A.-T., Laing, A., Bugeon, L., Dallman, M.J., Melchionna, T., Chillakuri, C., et al. (2012). Nat. Immunol. *13*, 1213–1221.

Liszewski, M.K., Koley, M., Le Friec, G., Leung, M., Bertram, P.G., Fara, A.F., Subias, M., Pickering, M.C., Drouet, C., Meri, S., et al. (2013). Immunity *39*, this issue, 1143–1157.

Strainic, M.G., Liu, J., Huang, D., An, F., Lalli, P.N., Muqim, N., Shapiro, V.S., Dubyak, G.R., Heeger, P.S., and Medof, M.E. (2008). Immunity *28*, 425–435.

Strainic, M.G., Shevach, E.M., An, F., Lin, F., and Medof, M.E. (2013). Nat. Immunol. *14*, 162–171.

van der Touw, W., Cravedi, P., Kwan, W.H., Paz-Artal, E., Merad, M., and Heeger, P.S. (2013). J. Immunol. *190*, 5921–5925.

Zhang, X., and Köhl, J. (2010). Expert Rev. Clin. Immunol. *6*, 269–277.