The Journal of Immunology

In This Issue

J Immunol 2017; 198:4551-4552; ;
doi: 10.4049/jimmunol.1790008
http://www.jimmunol.org/content/198/12/4551

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

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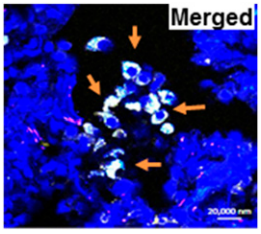
Vγ2Vδ2 T Cells Manage Macaque Mycobacteria

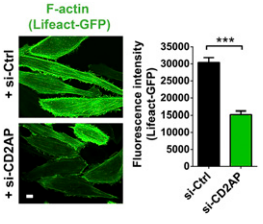


Vγ2Vδ2 T cells, which are present only in primates, make up a majority of the γδT cells in humans and recognize phosphoantigens, including those produced by *Mycobacterium tuberculosis*. Although studies have suggested that Vγ2Vδ2 T cells could be protective against tuberculosis, additional data are necessary to prove this possibility. Qaqish et al. (p. 4753) undertook adoptive transfer studies in rhesus macaques to determine whether these cells could indeed protect against this deadly disease. Macaque Vγ2Vδ2 T cells expanded in vitro from PBMCs had tissue-trafficking effector memory or central memory phenotypes, and upon stimulation produced IFN-γ, TNF-α, and granzyme A and could inhibit the growth of intracellular bacteria. Following adoptive transfer into uninfected macaques, these in vitro–expanded cells rapidly trafficked into the airways, where they remained for at least 7 d. In macaques that received Vγ2Vδ2 T cells 3 d after high-dose *M. tuberculosis* infection, Vγ2Vδ2 T cells were detected in the bronchoalveolar lavage fluid during the early phase of infection at a significantly higher frequency and expressed higher levels of the proliferative cell marker Ki67 than in infected animals that received IL-2–stimulated autologous PBLs or saline. Compared with infected controls, infected macaques that received Vγ2Vδ2 T cells maintained their body weight, had lower levels of *M. tuberculosis* bacteria in the lung, and showed little or no dissemination of bacteria to extrapulmonary sites. Tuberculosis lesions were mainly restricted to the right caudal lung lobe (the infection site) in animals that received Vγ2Vδ2 T cells, whereas control animals showed lesions throughout the lungs, as well as in the spleen, liver, and kidneys. This proof-of-concept study indicates that Vγ2Vδ2 T cells can protect against *M. tuberculosis* infection in nonhuman primates, supporting the integration of strategies addressing the activities of these cells into the development of vaccines or treatments for human tuberculosis.

EZH2 Eases Checkpoint Passage

To ensure that mature B and T cells express functional IgH or TCRβ-chains, respectively, developing lymphocytes must pass through a pre–Ag receptor checkpoint, in which pre-BCR or pre-TCR signaling inhibits p53-mediated apoptosis. Transcription of the *Cdkn2a* gene in pre-B cells stabilizes p53, whereas pre-BCR signaling induces p53 degradation and allows pre-B cells to pass through the pre–Ag receptor checkpoint. A histone methyltransferase known to play a role in lymphocyte development, EZH2, catalyzes methylation of H3K27 and is hypothesized to affect the pre–Ag receptor checkpoint, although the mechanism by which this might occur remains unclear. To better understand the involvement of EZH2 in lymphoid development, Jacobsen et al. (p. 4682) generated mice with lymphoid-specific deletion of EZH2 ($Ezh2^{\Delta/\Delta}$ mice). In these mice, development of B and T cells was arrested at or prior to the pre–Ag receptor checkpoint, but development of innate lymphoid (NK and ILC2P) cells was not impaired. Consistent with these observations, $Ezh2^{\Delta/\Delta}$ pro-B cells and DN3 thymocytes had markedly reduced H3K27me3 modifications and upregulated a large number of genes, relative to controls, whereas these differences were not seen in NK or ILC2P cells. One of the genes that was upregulated in these B and T cell precursors was *Cdkn2a*, and its upregulation was associated with stabilization of p53 and increased apoptosis in $Ezh2^{\Delta/\Delta}$ adaptive lymphoid cells. EZH2 inhibitors could also induce apoptosis and cell cycle alterations in wild-type pro-B cells, supporting the importance of EZH2 in the survival and proliferation of these cells. In $Ezh2^{\Delta/\Delta}$ mice that also lacked expression of *Cdkn2a*, B and T cell maturation was at least partially restored and levels of p53 protein were reduced, relative to $Ezh2^{\Delta/\Delta}$ mice. Taken together, these data suggest that EZH2 delays expression of *Cdkn2a* during B and T cell development and that this delay allows B and T lymphocyte precursors time to assemble functional pre–Ag receptors and thus pass the pre–Ag receptor checkpoint.

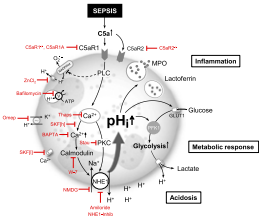
CD2AP Keeps ICAM-1 in Check



Leukocyte diapedesis, an important step in inflammation, is mediated by interaction of β2 integrins on leukocytes with clustered ICAM-1 on endothelial cells lining blood vessels. Very little is known about how ICAM-1 clustering is regulated to prevent excessive diapedesis and inflammation. In this issue, Schaefer et al. (p. 4823) identified CD2-associated protein (CD2AP) as a negative regulator of ICAM-1. The C-terminal portion of CD2AP was found to bind directly to the intracellular domain of clustered ICAM-1 in TNF-α–stimulated primary human endothelial cells. Depletion of CD2AP in these cells using small interfering RNAs significantly increased the speed and frequency of neutrophil transmigration and shifted the prevailing type of migration from the paracellular (through endothelial cell junctions) to the transcellular (through the endothelial cell body) pathway. This increased diapedesis was linked to increases in neutrophil adhesion and ICAM-1 clustering in endothelial cells in which CD2AP was knocked down. CD2AP depletion altered the organization of the F-actin cytoskeleton and reduced recruitment of F-actin to clustered ICAM-1,

suggesting that CD2AP links ICAM-1 to the actin cytoskeleton. Recruitment of cortactin to ICAM-1 and activation of the RhoGTPase Rac1 that is normally induced by binding to ICAM-1 were also impaired following CD2AP knockdown. Mechanical force has previously been shown to promote ICAM-1–induced signaling and neutrophil transmigration, and the authors found that application of such force reduced CD2AP binding to clustered ICAM-1, suggesting the existence of a negative feedback loop. Mechanical force applied to clustered ICAM-1 induced PI3K activation, and this activation was inhibited by CD2AP depletion, suggesting that CD2AP is required for mechanosensitive signaling through ICAM-1. This study indicates that the actin-binding adaptor protein CD2AP controls ICAM-1–mediated signaling and leukocyte diapedesis and could serve as a therapeutic target for the reduction of excessive inflammation.

Pumping Up Neutrophil pH_i with C5a



The complement activation product C5a functions as a central molecule in the development of inflammation in the setting of sepsis. To date, studies have demonstrated that high levels of C5a result in overactivation of important neutrophil functions such as phagocytosis, cytokine release, and induction of respiratory burst. Given the dependence of neutrophil function on modulation of intracellular pH (pH_i), in this issue, Denk et al. (p. 4846) sought to define a mechanistic link between complement activation and neutrophil pH_i. Incubation of human neutrophils with recombinant C5a at physiologically relevant levels induced a rapid elevation of pH_i that was dependent on C5a receptor-1 (C5aR1) and the proton transporter sodium/hydrogen exchanger 1 (NHE1). Treatment of neutrophils with signaling inhibitors prior to C5a stimulation revealed the dependence of C5a-induced activation of NHE1 on protein kinase C, intracellular calcium, and calmodulin. C5a also regulated neutrophil release of myeloperoxidase and lactoferrin, both of which were significantly inhibited after NHE1 inhibition. Furthermore, glycolytic flux in neutrophils appeared to be pH dependent, as glucose uptake and glycolysis were greatly increased following C5a stimulation and were significantly reduced following the addition of an NHE1 inhibitor. Neutrophils from septic mice and humans exhibited elevated pH_i, and the pH_i shift observed in murine neutrophils during sepsis was similar to the shift in pH_i seen after in vitro exposure of neutrophils to C5a. Importantly, administration of a C5aR1 antagonist immediately after induction of sepsis in mice normalized pH_i, suggesting that hyperlactatemia and extracellular acidification during sepsis is caused by complement-activated immune cells. Taken together, these data demonstrate a mechanistic link between the complement system and neutrophil metabolic activation through modulation of neutrophil pH_i, and indicate that complement inhibition during sepsis might balance the physiological mechanisms required for neutrophil metabolism and pH homeostasis.