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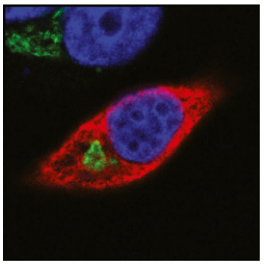
Teaching an Old Peptidase New Tricks



The signal peptide peptidase (SPP), not to be confused with the signal peptidase (SP), is an aspartyl protease responsible for intramembrane cleavage and release of leader peptides left in the endoplasmic reticulum (ER) membrane. While examining TAP-independent peptide loading pathways, Oliveira et al. (p. 4020) discovered that SPP mediated ER release of C-terminal peptides for presentation on MHC I. Specifically, they found SPP to be responsible for generating a ceramide synthase C-terminal 9-mer peptide epitope (Trh4) that was expressed at the cell surface as a peptide/MHC I complex. MHC I presentation of Trh4 was increased in the absence of TAP activity, and inhibitors of aspartyl proteases inhibited peptide release from the ER. Through the use of mutagenesis, the authors demonstrated that both the C-terminal location of the epitope and transmembrane region "helix-breaking" residues upstream of the peptide were necessary for cleavage and release of Trh4. The use of siRNA confirmed that SPP was responsible for the release and presentation of this epitope, independent of SP. This study demonstrates that SPP has a novel function in the generation of C-terminal epitopes for presentation by cleaving them through transmembrane proteolysis in the ER.

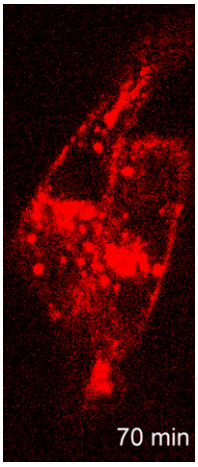
The Price of Internalizing Inhibition

The inhibitory Fc receptor, FcγRIIB (CD32b) plays an important role in mAb immunotherapy as genetic deficiency or deletion of FcγRIIB can enhance the effects of certain mAbs that bind to Fcγ receptors. However, the data are far from clear, and specific in vivo manipulation of FcγRIIB has not been possible, leading Williams et al. (p. 4130) to create and analyze a panel of mouse mAbs specific to FcγRIIB that do not cross-react with other FcγRs. Promisingly, these mAbs were able to enhance phagocytosis and stimulate programmed cell death of target cells. However, in the context of several mouse tumor models, these FcγRIIB mAbs had limited therapeutic activity. The authors demonstrated that the anti-FcγRIIB mAbs were rapidly removed from in vivo circulation; further experiments showed that both FcγRIIB and mAbs were internalized from the surfaces of both tumor and host cells. This occurred within 24 h following administration, explaining the low therapeutic potential of these mAbs. In vitro, there was almost no mAb internalization found with human target cells, raising the tantalizing possibility that these mAbs may be more effective in human disease. Thus, the authors determined that mAb-based immunotherapy can cause internalization of either reagent or target receptor. In addition, further work will determine whether the unique effects of FcγRIIB immunotherapy can be harnessed in human disease.

Breaking the Epithelial Barrier

Genital epithelial cells (GECs) provide a physical barrier, respond to incoming pathogens, and are involved in early innate immune responses in the female reproductive tract (FRT). During sexual transmission in the genital mucosa, HIV-1 gp120 initiates inflammation and barrier disruption. However, the early events following HIV-1 exposure in the FRT are poorly understood. To better delineate these events, Nazli et al. (p. 4246) stimulated GECs with gp120 and determined that the resulting proinflammatory cytokine upregulation and disruption in barrier integrity were dependent on TLR2- and TLR4-mediated induction of NF-κB. Treatment of GECs to neutralize canonical HIV-1 receptors showed that heparin sulfate (HS), but not CD4, CXCR4, or CCR5, was an essential cofactor in gp120-induced TLR2 and TLR4 signaling and that removal of HS blocked gp120 activation of NF-κB. Additional bead-binding assays demonstrated that gp120 could bind directly to TLR2, TLR4, and HS on host epithelial cells. As with seminal plasma (SP) from HIV-1–infected men, stimulation of GECs with uninfected SP spiked with gp120 induced TNF-α and disrupted the epithelial barrier. Furthermore, HIV-1–infected SP combined with gp120 had an additive effect on TNF-α production. In this study, the authors demonstrate a mechanism by which HIV-1 directly activates the innate immune response in the FRT during sexual transmission.

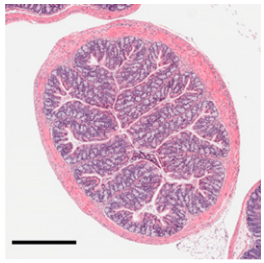
mAbs To Manage Metastasis



Antibodies that target and inhibit the catalytic ability of CD73, a cell-surface ecto-5′-nucleotidase, have been shown to reduce tumor growth and increase T cell cytotoxicity. Terp et al. (p. 4165) used the monoclonal anti-human CD73 Ab (AD2) to determine if blocking this cell surface molecule would have an effect on tumor metastasis that was independent of the ectonucleotidase activity and primary tumor growth inhibition. They found that AD2 did inhibit metastasis through a mechanism involving the clustering and internalization of CD73. This effect was independent of CD73 cross-linking, as both whole IgG and Fab fragments of AD2 were effective. AD2 was capable of blocking tumor metastasis but not primary tumor growth in a murine metastatic tumor model. Ex vivo treatment of tumor cells

with AD2 prior to transfer into mice resulted in inhibition of tumor extravasation, colonization, and metastasis. Further experiments demonstrated that AD2 had little effect on the ectonucleotidase activity of CD73. siRNA-mediated reduction of CD73 surface expression on tumor cells had similar effects. The authors demonstrated that these effects were epitope-specific and restricted to AD2. Thus, the authors show that Ab-mediated internalization of CD73 was effective in inhibition of metastasis in animal models of human tumors and conclude that Ab therapies against CD73 should target its surface expression, not solely its enzymatic activity.

Tcf7 Regulates ILC Subsets



Innate lymphoid cells (ILCs) are critical mediators of mucosal immunity and are comprised of several discrete subsets defined by distinct transcription factors and patterns of cytokine production. Mielke et al. (p. 4383) characterized the molecular pathways leading to the development of these subsets in mice in the presence or absence of *Tcf7*, which encodes T cell factor 1 (TCF-1), a transcription factor important in NK and T cell differentiation. *Tcf7* was highly expressed in ILC subsets within the small intestine lamina propria. Using chimeric mice reconstituted with a mixture of $Tcf7^{-/-}$ and $Tcf7^{+/+}$ fetal liver cells, the authors confirmed that *Tcf7* was essential for the development of both ILC2 and $NKp46^{+}$ ILC3 subsets. $Tcf7^{-/-}$ mice had a reduction in the frequency and number of the ILC2 subset, leading to a decrease in the production of IL-15 and IL-13 and a subsequent reduction in eosinophil infiltration in a lung inflammation model. TCF-1 was also required for the development of the $ROR\gamma t^{+}NKp46^{+}$ ILC3 subset, but not the NCR^{-} ILC3 subset, through a pathway involving T-bet and Notch. An enhanced susceptibility to intestinal *C. rodentium* infection in $Tcf7^{-/-}$ mice was observed, due to a reduction in IL-22 production by $NKp46^{+}$ ILC3 that led to increased mucosal hyperplasia and crypt damage. Taken together, the data demonstrate that *Tcf7* is an essential regulator of ILC2 and $NKp46^{+}$ ILC3 development that contributes to the immune response to infection.

Complementing Infection

The complement component C1q, part of the C1 complex, is essential for activation of the classical complement pathway. This pathway functions as a first line of defense against invading pathogens, such as *Streptococcus pneumoniae* (pneumococcus), which employs multiple evasion strategies to counteract host defenses. In this issue, Agarwal et al. (p. 4235) demonstrate a novel mechanism by which pneumococci use host C1q for adhesion and invasion. Incubation of pneumococci with normal human serum demonstrated that C1q, both purified and as part of the C1 complex, could bind directly to pneumococci in an Ab- and serotype-independent manner. Interestingly, the authors found that the pneumococcal capsular polysaccharide limited binding to C1q by masking surface-exposed proteins that function as major ligands for C1q. Infection of several endothelial and epithelial cell types with C1q-pretreated pneumococci increased both the adherence and invasion of pneumococci. These effects were mediated by the C1q C-terminal globular heads and the N-terminal collagen stalks, respectively. Although C1q showed significant binding to other gram-positive and gram-negative pathogens, C1q did not enhance the number of host cell–associated bacteria in cell infection assays, suggesting that the function of C1q in pneumococcal infection is specific. The authors demonstrate here a unique role for C1q as a "molecular bridge" between host cells and pneumococci that functions to increase bacterial adhesion and invasion.