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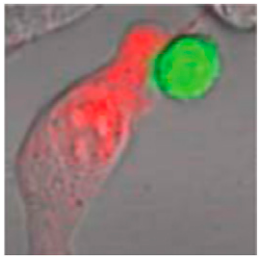
A View to a Kill



Target cell killing by CTLs and NK cells begins with exocytosis of lytic granules across the immunological synapse followed by perforin pore formation in the target cell membrane. Due to the dynamic nature of these cytotoxic synapses, it has been difficult to accurately characterize their spatiotemporal properties. Lopez et al. (p. 2328) addressed this issue by using a time-lapse live cell microscopy technique to measure real-time NK cell and CTL engagement and killing of target cells. In human NK cells and CTLs, a single Ca^{2+} flux occurred rapidly after target cell engagement and was quickly followed by a perforin-mediated breach of the target cell membrane. Similar results were observed in mouse NK cells, although the delivery of the lethal hit was delayed. In contrast, mouse CTLs demonstrated multiple fluctuations in intracellular Ca^{2+} prior to perforin pore formation, which occurred significantly more slowly than with NK cells. A subset of both human and mouse NK cells degranulated multiple times while contacting a single target cell. Interestingly, perforin formed pores only on the target cell and not on the killer cell, although CTL membranes were not intrinsically resistant to perforin. Further use of this technique will aid in teasing apart the kinetics of NK cell and CTL Ca^{2+} flux and degranulation, as well as the unidirectional nature of perforin pore delivery.

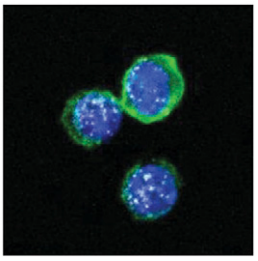
Universal Flu Vaccines?

Influenza A virus (IAV) infection, known for causing significant death even in nonpandemic years, can be ameliorated with vaccines that generate potent neutralizing Abs. However, selective pressure on the Ab targets, IAV hemagglutinin (HA) and neuraminidase, driven by previous vaccination or IAV infection, can quickly render potential vaccines obsolete. By contrast, anti-IAV $CD8^+$ T cell responses are generated against more conserved epitopes and thus provide protection against a wider variety of IAV subtypes. In vitro studies have shown that influenza virus–like particles (VLPs) stimulate influenza-specific $CD8^+$ T cell responses. To test this in vivo, Hemann et al. (p. 2486) assessed $CD8^+$ T cell responses after intranasal administration of VLPs containing HA and matrix protein 1 of the A/PR/8/34 influenza virus. After vaccination, there was a significant increase in pulmonary HA-specific $CD8^+$ T cells that mediated protection against homotypic IAV challenge. $CD8^+$ T cell depletion supported the conclusion that these cells were responsible for viral protection. Importantly, VLP vaccination provided heterosubtypic protection, working against IAV strains that could evade neutralizing Abs induced by previous vaccines. Thus, VLPs containing $CD8^+$ T cell Ags can generate protective responses in vivo, and using this method to induce cross-protective $CD8^+$ T cell responses may lead to the development of a universal IAV vaccine.

Deep Diving into the *Igh*

Ab diversity is maintained through several gene rearrangements, including those of the V, D, and J segments mapping within the *Igh* locus. There are over 100 functional V_H gene segments and their usage is nonrandom, indicating that preferential selection occurs. To determine the mechanism of this selection, Choi et al. (p. 2393) used deep sequencing of pro-B cell *Igh* 5′RACE-products. After unbiased amplification, chromatin immunoprecipitation sequencing (ChIP-seq) was performed for the transcriptional repressor CCCTC-binding factor (CTCF), the cohesin subunit Rad21, RNA polymerase II, and several histone modifications, and RNA-Seq was performed on sense and antisense noncoding germline transcripts. Comparison with non-rearranging *Igh* genes from $RAG^{-/-}$ pro-B cells by computational analysis allowed assessment of accessibility elements (CTCF, Rad21, etc.) and led the authors to organize the *Igh* locus into four distinct domains. Proximal V genes were characterized by a CTCF site near the recombination signal sequence (RSS) and a lack of noncoding RNA and histone marks. Distal V genes were found to have higher levels of histone marks and noncoding RNA, perhaps to compensate for the effects of distance from CTCF sites and a less optimal RSS. The authors have found a complex system that regulates selection of gene segments within the four domains of the *Igh* locus for recombination and subsequent expression.

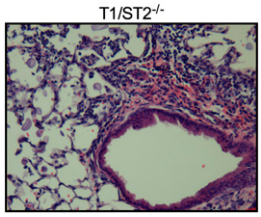
Cooperating To Get to Th9



Differentiation of IL-9–producing Th9 cells can be induced by TGF-β in vitro, but the role of this cytokine in Th9 development in vivo remains unclear. In this issue, Tamiya et al. (p. 2360) analyzed Th9 differentiation in mice with T cell–specific deletion of the TGF-β–activated transcription factors Smad2 and Smad3 in a model of OVA-induced asthma. Relative to controls, mice with Smad2/3 deficiency had lower IL-9 production and attenuated asthma. In vitro analysis revealed that Smad2 and Smad3 were redundantly required for TGF-β–mediated IL-9 production via direct activation of *Il9* transcription. Smad2/3 were found to bind to and cooperate with the transcription factor IFN regulatory factor 4 (IRF4) to induce Th9 differentiation. In the absence of IRF4, Smad2/3 could neither be recruited to nor bind to the *Il9* promoter. In the absence of Smad2/3, IRF4 could bind to the *Il9*

promoter, but failed to activate *Il9* transcription. This study reveals a molecular mechanism for TGF-β–mediated induction of Th9 differentiation, an important step toward understanding the regulation of allergic immune responses.

IL-33 Influences Infection



Infection with the opportunistic fungal pathogen *Cryptococcus neoformans* can be cleared by a Th1 response or become progressive in the presence of a Th2 response. Flaczyk et al. (p. 2503) hypothesized that the epithelial-derived cytokine IL-33, which targets cell types including Th2 cells, could regulate susceptibility to progressive *C. neoformans* infection. IL-33 expression in the lungs of BALB/c mice increased following infection with the virulent *C. neoformans* strain H99, which induces a Th2 response, but not with the less virulent 52D strain, which induces a Th1 response. Compared with wild-type mice, infection of mice lacking the IL-33 receptor subunit T1/ST2 resulted in significantly reduced growth and dissemination of the H99 strain. T1/ST2 was found to be key to the lung Th2 bias caused by this virulent infection. Following H99 infection, T1/ST2$^{-/-}$ mice had increased inflammatory lung infiltrates consisting of neutrophils and classically activated macrophages, along with decreases in eosinophil recruitment and mucus hypersecretion. Infection of wild-type mice resulted in expansion of alternatively activated macrophages and of type-2 pulmonary innate lymphoid cells (ILC2) that were associated with IL-5 and IL-13 expression, and these cells were reduced in the absence of T1/ST2. Taken together, these data reveal that IL-33 modulates both innate and adaptive immunity to promote susceptibility to virulent strains of *C. neoformans*.

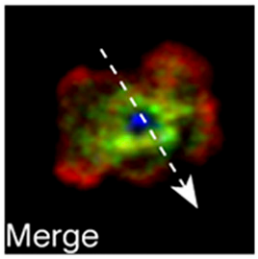
Dulling Divisions

Innate and adaptive T cell subsets undergo distinct patterns of proliferation following thymic selection. To clarify these differing proliferative dynamics and address whether regulatory T cells (Tregs) proliferate after positive selection, Föhse et al. (p. 2384) developed a genetic system of T cell pulse labeling. Transgenic mice were generated with a fluorescent reporter gene inserted into the 3′ untranslated region of the *Tcrd* C region. This reporter was transcribed in all double-negative thymocytes and then maintained in γδ T cells, but excised during Vα-Jα recombination in αβ T cells; thus, αβ T cell division was indicated by dilution of the fluorescent label. After positive selection, CD4$^+$ and CD8$^+$ thymocytes underwent one to two divisions and then, following thymic exit, demonstrated very little proliferation under steady-state or inflammatory conditions. In contrast, iNKT cells underwent intrathymic cell division and completely lost the fluorescent label during thymic development. CD4$^+$Foxp3$^+$ Tregs and CD4$^+$ conventional T cells proliferated to similar degrees upon thymic exit. However, Tregs in the secondary lymphoid organs had lower levels of fluorescence than conventional T cells, suggesting Treg activation and proliferation in the periphery. Thus, this mouse model can clarify T cell proliferative dynamics by reporting T cell expansion in vivo.

Teaching Tolerance to DCs

Tolerogenic DCs (tDCs), particularly those generated ex vivo, have beneficial effects when administered in animal models of autoimmunity and graft rejection. tDCs display an immature phenotype and are efficient at Ag uptake but express low levels of costimulatory molecules. These factors led Matsumoto et al. (p. 2247) to try treating human DCs with protein kinase C inhibitors (PKCI) to create stable tDCs. PKCI-treated DCs were semimature in phenotype and secreted IL-10. In addition, they induced naive CD4$^+$ T cells to produce IL-10 and differentiate into Foxp3$^+$ regulatory cells. PKCI-treated DCs also expressed CCR7 and migrated toward CCR7 ligands. Taken together, the data indicated that PKCI-treated DCs were capable of eliciting a strong immunosuppressive response. Neither the administration of proinflammatory cytokines nor LPS changed the phenotype of PKCI-treated DCs, perhaps because PKCI blocked the canonical and noncanonical NF-κB activation pathways that lead to DC maturation. The high level of IL-10 produced by these unique DCs was induced by a combination of intracellular cAMP accumulation and NF-κB inhibition. Finally, the ability of mouse PKCI-treated DCs to prevent graft-versus-host disease in an acute animal model led the authors to conclude that PKCI-treated DCs are good candidates for tolerance-inducing therapy, and are appropriate as clinical-grade tDCs.

Synaptic Stability



Formation of an immunological synapse (IS) between a B cell and an APC requires BCR recognition of Ag followed by assembly of an LFA-1 integrin cluster. To better understand the regulation of B cell motility and adhesion during IS formation, Saez de Guinoa et al. (p. 2742) investigated the involvement of the scaffold protein vinculin, which links integrins at the plasma membrane to the actin cytoskeleton. Vinculin was recruited to the actin-rich peripheral supramolecular activation cluster (pSMAC) of the B cell IS after BCR binding to Ag and LFA-1 binding to ICAM-1. This localization coincided with a local increase in PIP_2 at the pSMAC and required the activity of Syk, which is known to be important for integrin activation following BCR stimulation. Phosphorylated Syk localized to the central SMAC (cSMAC). In the absence of vinculin recruitment to the IS, B cells failed to firmly adhere to the APC and instead migrated away in response to CXCL13, carrying Ag clusters with them. Vinculin-mediated firm adhesion and F-actin assembly at the IS required the activity of nonmuscle myosin-II. By identifying vinculin as a key mediator of B cell arrest and adhesion in IS formation, this study dissects some of the molecular requirements for B cell activation.