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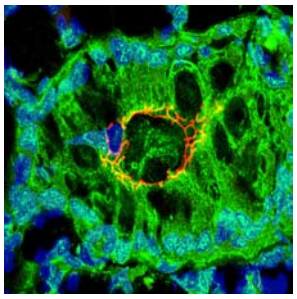
THE JOURNAL OF IMMUNOLOGY

In This Issue

PAR-ting the Epithelial Barrier



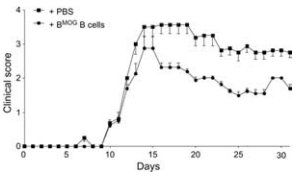
During gut inflammation, large numbers of polymorphonuclear neutrophils (PMNs) migrate across the intestinal epithelium and disrupt the epithelial barrier. Protease-activated receptors (PARs) have been shown to regulate epithelial barrier function, leading Chin et al. (p. 5702) to analyze whether PMN proteases and PARs might interact to disrupt the intestinal epithelial barrier during inflammation. In vitro experiments suggested that, in the presence of a chemoattractant gradient, PMN serine proteases increased epithelial permeability without cleaving apical junction proteins and enhanced PMN transepithelial migration. The PMN contact-induced increase in epithelial permeability depended on the activation of PAR-1 and PAR-2, which were localized to the basolateral surfaces of epithelial cells. A downstream mechanism for this process was suggested by the observation that activation of PAR-1 and PAR-2 led to phosphorylation of myosin light chain kinase and myosin light chain. Finally, treatment of epithelial monolayers with PAR-1 and PAR-2 small interfering RNA inhibited PMN transepithelial migration, further connecting PMN activities to PAR activation. The identification of this mechanism by which PMNs and PARs interact to disrupt the epithelial barrier and induce PMN transepithelial migration may have important implications for the understanding of mucosal inflammatory disease.

Redundancy of Fyn

The Src family kinase Fyn participates in both the propagation and inhibition of TCR signaling as well as in signaling promoting Th2 differentiation. Although Fyn is clearly important for signaling induced by CD3 crosslinking, its role in the more physiological situation of MHC:peptide stimulation of T cells is not clear. To address this issue, Mamchak et al. (p. 5374) generated DO11.10 TCR-transgenic, Fyn-deficient mice on the BALB/c background. $CD4^+$ T cells in these mice, including $Foxp3^+$ regulatory T cells, developed normally and splenic $CD4^+$ T cells were not different in number or phenotype from those of wild-type mice. DO11.10 wild-type and DO11.10 Fyn-deficient $CD4^+$ T cells proliferated identically upon Ag stimulation and did not differ in their requirements for costimulation. Fyn deficiency did not affect the formation or stability of T cell:APC conjugates, nor did it impair the induction of a Th2 response to *Nippostrongylus brasiliensis* infection. However, the Fyn-deficient cells consistently produced more IL-4 and less IFN-γ than their wild-type counterparts in vitro. Under Th1-promoting conditions in vivo, Fyn-deficient DO11.10 T cells produced more IFN-γ than did wild-type DO11.10 T cells. Although Fyn may suppress cytokine responses under some conditions, these data suggest that its role in TCR signaling in vivo may be largely redundant.

PD-1 and Active Tolerance

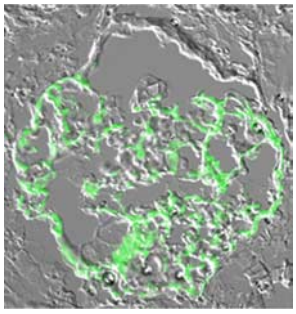
Costimulatory and coinhibitory pathways are important for the induction and regulation of T cell responses. Programmed death 1 (PD-1) is an inhibitory molecule that has recently been shown to be involved in T cell exhaustion and tolerance to pathogens. Two articles in this issue, using very different models, demonstrated that PD-1 is actively involved in peripheral tolerance to autoimmunity and transplantation. First, Koehn et al. (p. 5313) examined the role of PD-1 in tolerance to allogeneic skin grafts following costimulatory blockade. Skin grafts expressing OVA were transplanted into bone marrow chimeric mice possessing a population of detectable graft-specific (OT-1) T cells, and recipients were treated with Abs to block the CD28- and CD40-mediated costimulatory pathways. Grafts were accepted by mice that had low frequencies of OT-1 T cells but not by those with higher frequencies. Mice with surviving grafts showed deletion of many OT-1 T cells; however, a small population of OT-1 T cells persisted, and these cells up-regulated PD-1. Blockade of PD-1 led to graft rejection and allowed the tolerized OT-1 T cells to regain the ability to proliferate and secrete IFN-γ. These data suggest that PD-1 actively maintains transplantation tolerance by preventing donor-specific T cells from attacking the graft.



In the second article, Frommer et al. (p. 5748) assessed the role of B cells in the induction of tolerance to experimental autoimmune encephalomyelitis (EAE). To this end, they generated transgenic mice with B cell-specific presentation of the encephalitogenic epitope of myelin oligodendrocyte glycoprotein (MOG) on MHC II. These B^{MOG} mice were resistant to EAE induction through a mechanism independent of $CD4^+CD25^+$ regulatory T cells and IL-10. Resting or activated MOG-presenting B cells from B^{MOG} mice transferred into wild-type recipients could also induce tolerance and thereby inhibit EAE development. MOG-specific T cells interacting with MOG-presenting B cells failed to proliferate to any significant extent but did up-regulate the inhibitory costimulatory molecules CTLA-4, PD-1, BTLA, and CD5. Of these molecules, CTLA-4 and PD-1 were found to be important for the induction of peripheral $CD4^+$ T cell tolerance by MOG-expressing B cells. Further adoptive transfer experiments to clarify the mechanism of B cell-induced tolerance determined that MOG-specific $CD4^+$ T cells that had encountered Ag on B cells underwent activation-induced

cell death upon re-exposure to Ag on activated APCs. These data suggest that constitutive MHC II-mediated Ag presentation by B cells induces peripheral $CD4^+$ T cell tolerance by sensitizing self-reactive cells to deletion. Together, these articles indicate that PD-1 plays an active role in tolerance induction that could be exploited to enhance graft acceptance or to prevent or treat autoimmune disease.

Foxn1 and Thymic Fate



Differentiation of the thymic epithelial anlage is vital to successful thymopoiesis and requires the activity of the Foxn1 transcription factor. During development, bone morphogenetic protein (BMP) is expressed in the future thymic anlage, whereas the BMP inhibitor Noggin is expressed in the future parathyroid. To determine how *Foxn1* expression is initiated and maintained during thymopoiesis, Soza-Ried et al. (p. 5272) developed an elegant lineage tracing system. Interference with BMP signaling by a *Noggin* transgene under control of the *Foxn1* promoter caused the loss of *Foxn1* expression in thymic epithelial cells and a consequent lack of thymopoiesis. These epithelial cells did not assume a parathyroid fate but instead reverted to an uncommitted epithelial state. To further analyze the relationship between BMP and *Foxn1* and assess its evolutionary relevance, the authors utilized a zebrafish model. In zebrafish larvae, inhibition of BMP signaling completely extinguished *foxn1* expression and resulted in a severe decrease in thymopoiesis and diversity of TCR-β rearrangements. This evidence of evolutionary conservation further indicates the importance of extrinsic signals in the maintenance of *Foxn1* expression. The authors also suggest that Foxn1 induction of thymic epithelial specification may be a default pathway in the developing thymus that must be inhibited to allow development of other cell types.

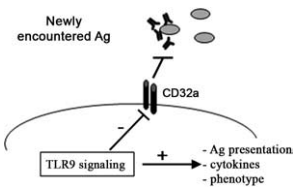
Flexibility in B Cell Memory

Human mature B cells can be divided into naive and memory populations by the expression of CD27. The roles of $IgM^+IgD^+CD27^+$ memory B cells and $IgM^-IgD^-CD27^+$ switched memory B cells in T cell-independent (TI) vs T cell-dependent (TD) Ab responses are controversial and difficult to study in the human system. IgM^+ memory B cells have been proposed to participate solely in TI immune responses, but this is debated. Moens et al. (p. 5306) hoped to resolve this controversy by studying the responses of human B cell subsets to pneumococcal Ags in a SCID mouse system. B cell-depleted PBMCs were transferred into SCID recipients along with naive mature B cells, IgM^+ memory B cells, or switched memory B cells, and mice were then immunized with heat-inactivated *Streptococcus pneumoniae* or pneumococcal Ags. Switched memory B cells produced IgG Abs to both TI and TD Ags. Surprisingly, IgM^+ memory B cells produced specific IgM or IgG responses to both pneumococcal TI polysaccharide Ags and TD protein Ags. A patient with hyper-IgM syndrome also developed IgG responses to *S. pneumoniae* serotype 3 polysaccharide Ags following administration of a pneumococcal vaccine, supporting the relevance of the SCID mouse results to human immunology. These data clarify the relative roles of human memory B cell subsets in the immune response to pneumococcal Ags and indicate that IgM^+ memory B cells can class switch and respond to TD Ags.

Surviving Complement Attack

To survive in human hosts, microbial pathogens must develop mechanisms to evade complement. Many bacteria therefore interact with complement inhibitors to escape opsonization and phagocytosis. The periodontal pathogen *Porphyromonas gingivalis*, which mediates much of its proteolytic activity via gingipain cysteine proteases, is strongly resistant to complement-mediated destruction. Potempa et al. (p. 5537) therefore analyzed whether *P. gingivalis* could bind the complement inhibitor C4b-binding protein (C4BP). Indeed, C4BP bound to all 13 clinical and laboratory strains of *P. gingivalis* tested. Biochemical analysis determined that two sites on the C4BP α-chains, the complement control protein (CCP) domains CCP1 and CCP6–7, bound to the hemagglutinin/adhesin domain of the *P. gingivalis* gingipain HRgpA. Capture of C4BP by *P. gingivalis* increased as the bacteria aged and formed aggregates, and the presence of C4BP was able to inhibit complement deposition on the bacterial surface. *P. gingivalis* may therefore protect itself from complement attack not only via degradation of complement components by gingipains, but also by acquisition of complement inhibitor C4BP.

Controlling Ag Uptake



Plasmacytoid dendritic cells (pDCs) are a major subset of dendritic cells in human peripheral blood that, upon stimulation by TLR ligands, produce large amounts of type I IFNs and mature into competent APCs. Different classes of TLR9 ligands affect pDCs in distinct ways. CpG-A oligonucleotides (ODN) localize to early endosomes and induce IFN production, CpG-B ODN localize to late endosomes and induce pDC maturation, and CpG-C ODN can mediate both effects in both locations. Benitez-Ribas et al. (p. 5219) assessed whether these TLR ligands could also affect the ability of pDCs to present exogenous Ags to T cells. Indeed, treatment of pDCs with CpG-C inhibited pDC uptake and presentation of immune complexes via CD32a without altering CD32a cell surface expression. Prevention of endosomal acidification restored CD32a-mediated immune complex internalization, suggesting that TLR9 stimulation was required for the observed effects of CpG-C. Analysis of different classes of CpG ligands indicated that CpG-B or CpG-C in the late endosomes inhibited CD32a-mediated Ag uptake and presentation, whereas CpG-A in the early endosomes had no effect. Specific TLR9 ligands can therefore directly modulate pDC Ag presentation, and the authors speculate that down-modulation of Ag-presenting capacity upon pDC maturation might prevent responses to self-Ags.

Summaries written by Jennifer Hartt Meyers, Ph.D.