The Journal of Immunology

IN BRIEF | DECEMBER 15 2008

IN THIS ISSUE

Online Issn: 1550-6606
Print Issn: 0022-1767

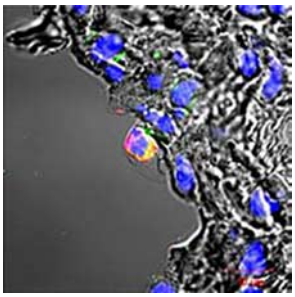
2008

J Immunol (2008) 181 (12): 8185–8186.
https://doi.org/10.4049/jimmunol.181.12.8185

THE JOURNAL OF IMMUNOLOGY

In This Issue

Antimicrobial Epithelial Cells



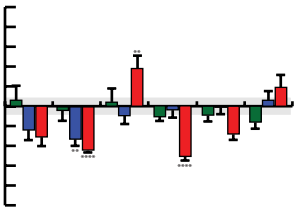
Mycobacterium tuberculosis can infect lung epithelial cells in addition to alveolar macrophages, but the potential role of epithelial cells in antimycobacterial host defense is unclear. Lipocalin 2 (Lcn2) mediates host defense against *Escherichia coli* infection through iron sequestration, leading Saiga et al. (p. 8521) to address the possible role of Lcn2 in mycobacterial infection. In *Mycobacterium bovis* bacillus Calmette-Guérin-infected mice, Lcn2 was secreted from alveolar macrophages and type II alveolar epithelial cells. Lcn2 was demonstrated to inhibit mycobacterial growth in vitro via iron sequestration, and $Lcn2^{-/-}$ mice were much more susceptible to *M. tuberculosis* infection than were wild-type mice. During the early phase of mycobacterial infection, the absence of Lcn2 did not appear to affect the infection of alveolar macrophages but instead resulted in greatly increased infection of alveolar epithelial cells in these mice compared with Lcn2-sufficient animals. Further analysis of $Lcn2^{-/-}$ cells and the use of endocytosis inhibitors indicated that Lcn2 was endocytosed by alveolar epithelial cells, where it limited the intracellular growth of mycobacteria. Alveolar epithelial cells therefore play an active role in protection from mycobacterial infection through the actions of Lcn2.

Regulatory Contamination

Regulatory T cells (Treg) have potent immunosuppressive activity that can control autoimmunity and allograft rejection. Using a standard protocol for ex vivo isolation and expansion of Treg, Vogtenhuber et al (p. 8767) observed the outgrowth of a novel suppressive cell population. $CD4^+CD25^+$ cells were isolated and then expanded using anti-CD3 mAb and IL-2 stimulation, and the resulting cells were highly enriched with a $CD4^{low/-}CD25^+$ T cell population. During the culture period, this population acquired more potent in vitro suppressive activity than $CD4^+CD25^+$ Treg, but they were neither derived from nor dependent on the presence of these "normal" Treg. The $CD4^{low/-}CD25^+$ T cells contained subpopulations including γδ T, $CD8^+$ T, and NKT cells and demonstrated an activated phenotype. These cells did not express FoxP3 yet mediated cell contact-dependent suppression that was independent of TGF-β and IL-10. As Treg generally comprise a small fraction of a host's total T cells, in vitro expansion is necessary for immunotherapeutic use. However, these data demonstrate that such expansion does not solely expand conventional Treg. This identification of a novel population of $CD4^{low/-}CD25^+$ regulatory T cells will therefore be useful both for understanding immunosuppression and for potential development of Treg-based therapy.

The Power of D_H

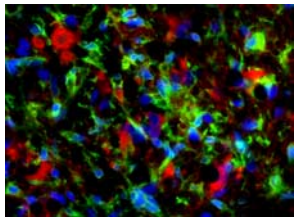
The CDR3 region of the Ig H chain (CDR-H3) provides much of the variability of the Ig Ag-binding site but incorporates D_H gene segments in only one of six possible reading frames, RF1. Within the CDR-H3, tyrosine and glycine residues are heavily favored, and many of these residues are provided by the use of RF1. Two articles in this issue address the immune rationale and potential mechanism of this reading frame selectivity. First, Zemlin et al. (p. 8416) assessed the role of D_H sequence in the regulation of reading frame usage by creating transgenic mice bearing a single frame-shifted *DFL16.1* D_H region (Δ*D-DμFS*). These mice showed a skewed preference for reading frame RF2 usage and were used to test a number of hypotheses for RF1 selectivity. Use of RF2 led to a CDR-H3 repertoire enriched for valine rather than the normally preferred tyrosine and glycine, and there were no compensatory changes observed in V_H or J_H usage that might temper this increased hydrophobicity. Somatic selection for tyrosine residues was observed only in the minority of mature B cells that developed using RF1 for D_H translation, but was not seen during B cell development. Instead, Dμ-mediated suppression via an allelic exclusion-like mechanism had a dominant effect on the CDR-H3 repertoire. These data indicate that germline D_H sequence plays a major role in determining the CDR-H3 repertoire, but a complex interplay of factors ultimately determines the composition of this Ag-binding region.



In the second article, Schelonka et al. (p. 8409) took the analysis of the Δ*D-DμFS* mouse a step further and addressed the effects of RF2 preference on B cell development and function. B cells from Δ*D-DμFS* mice were compared with those of mice limited to either a single normal D_H gene segment (Δ*D-DFL*) or a single inverted D_H segment that resulted in a preference for charged amino acids in the CDR-H3 (Δ*D-iD*). Although all three of these mouse strains had fewer immature B cells than wild type, Δ*D-DFL* mice had normal numbers of mature B cells, whereas Δ*D-DμFS* and Δ*D-iD* animals had reduced mature B cells in the spleen and bone marrow. Unlike the mice bearing an inverted D_H, Δ*D-DμFS* mice had normal numbers of marginal zone B cells and peritoneal cavity B-1a cells. Analysis of Ab responses in Δ*D-DμFS* mice revealed decreased Ab titers to T-independent Ags and variable effects on Ab responses to T-dependent Ags. These data indicate that changes in the amino acid composition of CDR-H3 regions by

alterations in D_H reading frame usage strongly influence B cell development and Ab production. Taken together, these studies help us understand why RF1 is so heavily favored in the D_H regions of all jawed vertebrates and indicate the importance for a balance between the diversity afforded by CDR-H3 regions and structural constraints important for Ag recognition.

Complementing Neurodegeneration



Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by motor neuron loss. The classical complement pathway has been implicated in the pathogenesis of ALS, but the involvement of specific complement factors has not been addressed. Woodruff et al. (p. 8727) therefore analyzed complement involvement in superoxide dismutase $(SOD1)^{G93A}$ rats, a rodent model of ALS. Complement activation, indicated by C3/C3b deposition, was observed in areas of motor neuron degeneration in end-stage $SOD1^{G93A}$ animals. Analysis of the receptor for complement factor C5a (C5aR) demonstrated expression on motor neurons of both wild-type and $SOD1^{G93A}$ rats and up-regulation on astrocytes, but not microglia, in end-stage disease. The alternate receptor for C5a, C5L2, was also expressed on motor neurons but was not expressed on microglia or astrocytes in the spinal cord. Treatment of $SOD1^{G93A}$ rats with the C5aR antagonist PMX205 significantly delayed disease onset, extended survival time, and reduced motor deficits compared with untreated animals. This antagonist also reduced astrocyte, but not microglial, proliferation in the lumbar spinal cord. These data suggest that C5a inhibitors may have therapeutic potential in such devastating neurodegenerative diseases as ALS, for which there is currently no effective treatment.

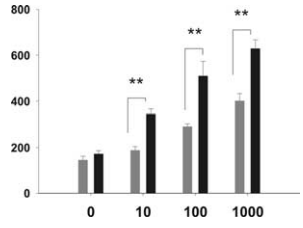
Killing TB

Control of *Mycobacterium tuberculosis* (Mtb) infection requires both $CD4^+$ and $CD8^+$ T cells, but the mechanism by which $CD8^+$ T cells protect against Mtb is not fully defined. Woodworth et al. (p. 8595) hypothesized that $CD8^+$ T cell cytolytic activity is required for this protection and sought to identify the mechanism(s) by which $CD8^+$ T cells might kill Mtb. In vivo cytotoxicity assays showed that Mtb-specific $CD8^+$ T cells used a hierarchy of cytolytic mechanisms dominated by perforin-mediated killing that could partially compensate for one another. Residual cytolytic activity in perforin-deficient mice was mediated by CD95/CD95L and TNFRp55 pathways and was particularly dependent upon CD95L in the lung. However, adoptive transfer of perforin-deficient $CD8^+$ T cells into Mtb-infected mice demonstrated that $CD8^+$ effector T cells absolutely required perforin to protect recipients against infection despite the availability of other cytotoxic pathways. Mtb-specific $CD4^+$ T cells were also observed to have anti-Mtb cytolytic activity; however, in contrast to the results with $CD8^+$ CTL, $CD4^+$ CTL-mediated killing was not dependent on perforin and CD95. This systematic analysis of the cytolytic mechanisms at play in Mtb infection has implications for future vaccine strategies to combat this widespread pathogen.

Rethinking Immunity to Malaria

The *Plasmodium* parasites, which cause malaria, go through a complex life cycle in their insect and mammalian hosts. Sporozoites injected into host skin by an infected mosquito invade the liver and become liver-stage parasites (the pre-erythrocytic stage), which are then released into the circulation to infect erythrocytes (the blood or erythrocytic stage). Acquired immunity to these parasites has been thought to be stage specific, particularly based on studies showing that successful immunization to the pre-erythrocytic stage does not induce protection to blood-stage parasites. Belnoue et al. (p. 8552) tested this theory by assessing whether immunity to blood-stage parasites could induce cross-stage immunity to the pre-erythrocytic stage. The authors immunized mice with RBCs infected with *Plasmodium yoelii* and then challenged them with homologous sporozoites after the original erythrocytic infection was cleared by treatment with chloroquine. Interestingly, this immunization protocol was able to greatly reduce the burden of pre-erythrocytic infection. Protection to liver-stage challenge was mediated by T cells through a process requiring NO, while protection to blood-stage infection was mediated by Abs. These data encourage a reassessment of malaria pathogenesis and have important implications for malaria vaccine development.

Suppressing Macrophages



The serine/threonine kinase AMP-activated protein kinase (AMPK) regulates energy homeostasis and has been proposed to suppress inflammatory responses. As recent data have questioned this anti-inflammatory role, Sag et al. (p. 8633) examined the potential of AMPK to regulate macrophage activity. The authors first determined that the anti-inflammatory stimuli IL-10 and TGF-β rapidly activated AMPK in macrophages, whereas the proinflammatory stimulus LPS inactivated AMPK. Inhibition of AMPK expression via siRNA led to an increase in the proinflammatory mediators TNF-α, IL-6, and COX-2. Use of a dominant-negative AMPK mutant also increased TNF-α and IL-6 expression and correspondingly decreased IL-10 expression, whereas a constitutively active AMPK mutant decreased these proinflammatory mediators and increased IL-10. These mutant forms of AMPK were also used to address the possible macrophage signaling mechanisms involved in this observed anti-inflammatory activity of AMPK. AMPK was found to negatively regulate NF-κB activity but to activate Akt, leading to inactivation of glycogen synthase kinase 3-β (GSK3-β) and activation of cyclic AMP-responsive element binding protein (CREB). These data suggest that AMPK may be a master regulator of macrophage differentiation to an anti-inflammatory phenotype.

Summaries written by Jennifer Hartt Meyers, Ph.D.