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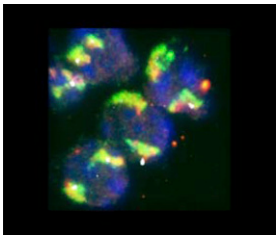
THE JOURNAL OF IMMUNOLOGY

In This Issue

CD8 Relocates for Expression



During interphase, each chromosome exists in an uncondensed form in a defined space within the nucleus called its chromosomal territory (CT). Because *cis*-regulatory elements are often located large distances from their target genes, and/or the transcriptional machinery is restricted to defined regions within the cell nucleus, a gene may have to "loop out" from its sub-CT (sCT) to achieve the necessary physical interactions required for transcription. To determine if the CD8 gene locus must undergo positional changes to be expressed, Ktistaki et al. (p. 5686) used three-dimensional techniques to asses its relative position to its sCT and regulatory elements while silenced or actively transcribed. During thymocyte development, the CD8α gene locus localized within its sCT boundary in $CD4^-$ $CD8^-$ double-negative and CD4 single-positive (SP) thymocytes, whereas the CD8α locus had moved out of its sCT in $CD4^+$ $CD8^+$ double-positive and CD8 SP populations. Likewise, in mature lymphocyte populations, the CD8α gene was found within its sCT for nonexpressing B cells and CD4 SP cells but had discernibly repositioned itself outside of the sCT within CD8 SP cells. The greater CD8α/β gene locus was also found to be clustered spatially with *cis*-regulatory elements in CD8-expressing cells. This work reveals a correlation between the spatial organization of the CD8 gene locus relative to its sCT and its expression status.

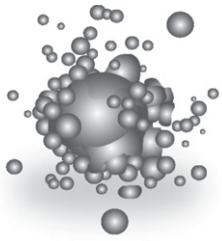
Cell-Specific TNF-α Production

Both TLR3 and TLR4 stimulation induce dendritic cells and macrophages to release the proinflammatory cytokine TNF-α. Normal levels of TNF-α production require signaling through the adaptor protein TRIF. In this issue, Gais et al. (p. 5842) investigated the mechanism(s) by which TRIF controls TNF-α biosynthesis. In response to TLR4 stimulation, TRIF signaling was required for the efficient translation of TNF-α mRNA but dispensable for TNF-α mRNA transcription and mRNA stability in both bone marrow (BM)-derived dendritic cells and BM-derived macrophages. By contrast, TRIF signaling was necessary for efficient TNF-α mRNA induction when peritoneal macrophages were stimulated with TLR4. Moreover, in response to TLR3 stimulation, TNF-α mRNA production by BM-derived macrophages also required TRIF. Despite these differences, TRIF-mediated control of TNF-α protein expression was linked to the prolonged activation of p38 MAPK and the p38 effector kinase MK2 in all cell types evaluated. These data reveal cell-type specific roles for TRIF in regulating TNF-α biosynthesis.

ISGlylating Virulence

Type 1 IFNs play an important role in cellular resistance to influenza A virus (IAV) infection by inducing the expression of endogenous factors with antiviral activities. One IFN-stimulated gene (ISG) is the ubiquitin homolog ISG15, which directly regulates the activity of target proteins through its conjugation (ISGylation) to specific lysine residues. In this issue, Tang et al. (p. 5777) demonstrate that ISG15 attenuates IAV activity via ISGylation of the IAV virulence factor nonstructural protein 1 (NS1). Initial experiments revealed that NS1 interacted with the type 1 IFN-inducible ISG15 E3 ligase Herc5. Both IFN-β treatment and virus infection induced Herc5-mediated ISGlyation of IAV-NS1, which disrupted its interactions with protein targets. ISGlyation also inhibited IAV-NS1 homodimerization, thereby impairing its ability to interact with RNA targets, and was correlated with decreased viral gene induction. Herc5-mediated ISGlyation of IAV-NS1 derived from the H1N1 strain A/PR8/34 generated two forms of ISGylated IAV-NS1 via the modification of two sets of lysine residues. Markedly, more virulent H5N1 IAV strains displayed only one ISGlyated form of NS1. These data support previous reports that mice deficient in ISG15 exhibit increased susceptibility to IAV infection.

Lymphocyte Longevity



Apoptosis

In healthy individuals, the expression of CD57 on $CD8^+$ T cells correlates with a shorter lifespan and lower numbers compared with $CD8^+CD57^-$ T cells. Previous studies have suggested that the lowered life span for $CD8^+CD57^-$ T cells results from an increased rate of apoptosis. In this issue, Wood et al. (p. 5582) investigated a possible correlation between the heat shock protein (Hsp) 27, previously shown to exhibit antiapoptotic activity, and the disparate life spans of these T cell subsets. When baseline expression levels of Hsp27 in $CD57^+$ and $CD57^-$ $CD8^+$ T cells of normal human donors were compared, Hsp27 levels were consistently lower in $CD8^+CD57^+$ T cells. After 48 h in culture, ~50% of $CD8^+CD57^+$ T cells were undergoing apoptosis compared with only ~10% of $CD8^+CD57^-$ T cells. For both subsets, Hsp27 levels were highest in freshly harvested cells and decreased significantly as the cells underwent apoptosis. This suggested that the initially lower levels of Hsp27 expression in $CD8^+CD57^+$ cells enhanced their susceptibility to apoptosis. Correspondingly, overexpression of Hsp27 in $CD8^+CD57^+$ cells reduced apoptosis, whereas Hsp27 depletion from

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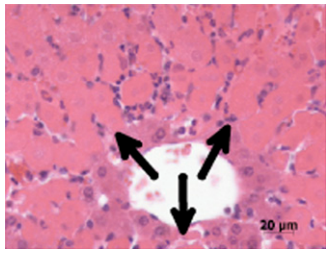
$CD8^+CD57^-$ cells increased their rate of apoptosis. Finally, the antiapoptotic effect of Hsp27 was linked to its inhibition of caspase-3 activity. These data define a link between Hsp27 expression levels and the survival rate of $CD8^+CD57^+$ T cells.

Rehabilitating Kupffer Cells



Kupffer cells (KCs) are liver-specific macrophages that are important players in normal liver function but have also been negatively implicated in destructive inflammatory responses, such as hepatic ischemia/reperfusion (I/R) injury. Ellet et al. (p. 5849) report in this issue that when I/R is combined with bowel congestion, KCs are actually protective. To investigate the role of KC in hepatic injury, murine livers were depleted of KC (>98%) by injection of liposomal clodronate (LC) 48 h prior to ischemia induction. Following I/R, LC-treated mice had decreased survival (~55%) compared with diluent-treated control mice (~95%). In the absence of I/R, serum alanine aminotransferase levels were unaffected by LC treatment, but they substantially increased in LC-pretreated animals following I/R compared with controls. Furthermore, confluent zones of necrosis around pericentral regions in the liver were found in LC-treated mice subjected to I/R. Liver sinusoidal endothelial cell activation was found to be substantially increased in LC-treated mice. This was due to the loss of KC-derived IL-10, as the pretreatment of LC-treated mice with IL-10 resulted in decreased endothelial cell activation and increased survival rates post I/R treatment. Thus, by releasing IL-10, KCs promote survival after hepatic I/R with bowel congestion. These findings have implications for the treatment of I/R-induced hepatic injuries.

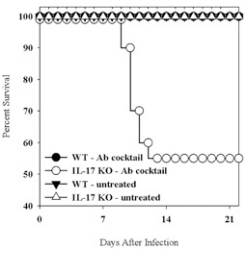
NKT Cells Prime with IFN-γ

CD1d-restricted NKT cells are important players in the inflammatory immune response. To investigate NKT cell function during graft rejection, Mattarollo et al. (p. 5663) used the K5mOVA transgenic mouse, in which epithelial keratinocytes express OVA, as a skin graft model. When K5mOVA skin was grafted onto NKT cell-deficient recipients initial graft rejection was significantly delayed compared with wild-type (wt) recipients. The transfer of in vitro-activated OVA-specific $CD8^+$ T cells into NKT cell-deficient graft recipients overcame the delayed effect. Decreased numbers of OVA-specific, IFN-γ–producing $CD8^+$ T cells were found in NKT cell-deficient graft recipients compared with wt mice. However, a second graft evoked similar kinetics in both NKT-deficient and wt mice, indicating that the memory $CD8^+$ T cell response was NKT independent. Grafted wt mice exhibited an increased ratio of IFN-γ to IL-17 production compared with NKT-deficient mice in draining lymph nodes. When NKT-deficient graft recipients were reconstituted with $IFN\text{-}\gamma^{+/+}$ NKT cells, they exhibited faster graft rejection than those reconstituted with $IFN\text{-}\gamma^{KO}$ NKT cells. In contrast, graft rejection was not enhanced by increased IL-17 levels. These studies reveal that NKT cell-derived IFN-γ promotes the rejection of skin grafts by enhancing the primary response of skin-derived Ag-specific $CD8^+$ T cells.

Culled by Cathepsin B

The pattern recognition receptor TLR9 recognizes unmethylated DNA containing CpG motifs and is expressed on various cell types that include early hematopoietic cells, B cells, and APCs. Stimulation of TLR9 on common lymphoid progenitors is known to inhibit their differentiation along the B cell lineage while promoting their differentiation into dendritic cells. To elucidate the effects of TLR9 signaling on B cell-committed lymphopoiesis, Lalanne et al. (p. 5678) studied the effects of CpG DNA treatment on purified adult mouse pro- and pre-B cells. In culture, the addition of CpG DNA strongly inhibited IL-7–induced proliferation of pro-B cells but not pre-B cells. Inhibition was due to increased cell death directly caused by TLR9-mediated signaling rather than TLR9-induced soluble factors, such as type I IFN. These results were confirmed in vivo by exploring the effects of CpG/TLR9 signaling in a mouse model that exhibited defective B cell lymphopoeisis. Finally, TLR9-induced pro-B cell death was caspase independent and induced by the release of the lysosomal cysteine protease cathepsin B into the cytosol. These data define a negative regulatory role for CpG/TLR9-mediated signaling during B cell development at the pro-B cell stage that is mediated by the activity of cathepsin B.

Combat-Ready Double Negatives



For many types of respiratory pathogens, such as the intracellular bacteria *Francisella tularensis,* respiratory vaccination invokes a superior protection against infection than that provided by traditional parenteral vaccination. To gain insights into the biological basis of these differences, Cowley et al. (p. 5791) investigated the differential responses of mice to intranasal (i.n.) and intradermal (i.d.) vaccinations with the *F. tularensis* live vaccine strain (LVS). Interestingly, double-negative (DN) αβ T cells were found to effectively respond to sublethal i.d. *F. tularensis* infection, but they constituted only a minor population of splenic and lung T cells. In contrast, similar numbers of DN T cells and $CD8^+$ T cells were recruited to the lungs in response to i.n. LVS infection. During the acute phase of the infection, pulmonary DN T cells produced copious amounts of IFN-γ and significantly higher levels of IL-17A than either $CD4^+$ or $CD8^+$ T cells on day 7 postinfection. Furthermore, IL-17A–deficient mice exhibited greater susceptiblity to i.n. than i.d. LVS infection. These data show an important role for DN T cell-derived IL-17A in the pulmonary response to LVS infection, thereby establishing that i.n. and i.d. vaccinations evoke disparate immune responses. These observations could lead to improved vaccine efficacy for respiratory pathogens.

Summaries written by Meredith G. Safford, Ph.D.