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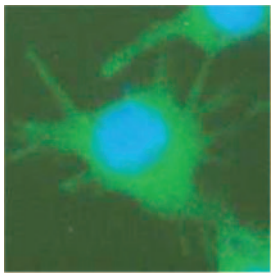
IN THIS ISSUE

Hsp72 Protects against Inflammation



Previous work by Xiao and collaborators showed that heat shock of macrophages reduces LPS-induced release of the nuclear protein high mobility group box 1 (HMGB1), which is proinflammatory in its excreted form. Continuing those studies, Tang et al. (p. 1236) in the Xiao laboratory showed that LPS- or TNF-α-induced HMGB1 translocation from nucleus to cytoplasm was inhibited in murine macrophages overexpressing transfected heat shock protein 72 (Hsp72). Hsp72 overexpression also prevented cytoplasmic translocation of the nuclear importin CRM1 (chromosome region maintenance 1), which mediates the movement of HMGB1 from nucleus to cytoplasm in stimulated cells. Normal CRM1/HMGB1 interaction in the stimulated cells was attenuated by Hsp72 overexpression, whereas Hsp72/HMGB1 interaction was increased. Heat shocked macrophages overexpressing Hsp72 had reduced levels of released TNF-α and IL-1β compared with controls. Prior heat shock or overexpression of Hsp72 in macrophages inhibited HMGB1-induced phosphorylation of MAPKs, IκBα degradation, NF-κB p65 nuclear translocation, NF-κB DNA binding, and expression of TNF-α and IL-1β. The experiments indicate that the protective role of heat shock in lethal sepsis and infection in mice occurs by Hsp72 inhibition of CRM1-mediated translocation of HMGB1 in macrophages and by Hsp72 prevention of the release of proinflammatory HMGB1.

SHPS-1 Required for EAE/MS

The Src homology 2 domain-containing protein tyrosine phosphatase substrate-1 (SHPS-1) protein found on dendritic cells (DCs), macrophages, and neutrophils interacts with CD47 on T cells and RBCs. This interaction has positive and negative effects in the immune system and may impact autoimmune diseases. Tomizawa et al. (p. 869) found that their mutant mice, with SHPS-1 deleted in most of the cytoplasmic region, were resistant to induced experimental autoimmune encephalomyelitis (EAE), the mouse model for multiple sclerosis (MS). Mutant splenic T cells, primed with a myelin oligodendrocyte glycoprotein (MOG) peptide, produced less IFN-γ, IL-2, and IL-17 than wild-type T cells; fewer CD11c$^+$ DCs and CD4$^+$ and CD8$^+$ T cells were seen in the spleens of mutant mice. In vitro proliferation and IFN-γ, IL-2, and IL-10 production were reduced in MLRs of mutant DCs with wild-type CD4$^+$ or CD8$^+$ T cells, whereas a normal proliferative response was noted in a MLR with wild-type DCs and mutant T cells. OVA-specific CD4$^+$ T cells proliferated poorly in culture with OVA-pulsed mutant DCs, and the production of TNF-α and IL-6 was impaired in LPS- or CpG-treated mutant bone marrow-derived DCs. Similarly, IL-12-induced production of IFN-γ was reduced in splenic DCs from SHPS-1 mutant mice. Adoptively transferred MOG-primed wild-type T cells had reduced ability to induce EAE in SHPS-1 mutant mice. These results indicate that SHPS-1 on DCs is required for priming CD4$^+$ T cells in MOG peptide-induced EAE. The authors suggest that SHPS-1 might serve as a target in the treatment of MS.

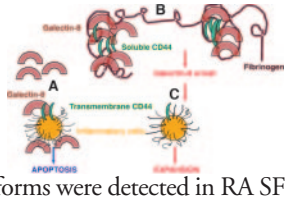
A Triggering Ag in SLE

Putterman and coworkers showed cross-reactivity of anti-dsDNA Abs and kidney-eluted Abs from nephritic lupus mice with α-actinin. To look at α-actinin Ab cross-reactivity with nuclear Ags, Deocharan et al. (p. 1313) in the Putterman laboratory immunized non-autoimmune BALB/c mice with α-actinin. They found high serum IgG1 Ab reactivity against chromatin by ELISA compared with controls immunized with adjuvant only. Immunized mice also had higher glomerular Ig deposition. Chromatin proteins that reacted with immune sera were identified by immunoblotting followed by matrix-assisted laser desorption ionization mass mapping and proteomic analysis on reactive bands. The identities of high mobility group box proteins (HMGB) 1 and 3 and heat shock protein 70 (HSP70) were confirmed by anti-HMGB1 and anti-HSP70 Ab binding to chromatin immunoblots, anti-α-actinin Ab binding to immunoblots of chromatin immunoprecipitated by anti-HMGB1 and anti-HSP70 Abs, and by immune serum binding to immunoblots of purified HMGB1 and HSP70 proteins. α-Actinin, chromatin, HMGB1, and HSP70 were bound by a panel of nephrophilic, but not non-nephrophilic, Abs in ELISA. A linear amino acid sequence common to α-actinin, HMGB1, HMGB3, and HSP70 was discovered using alignment programs and shown by a prediction program to lie within B cell epitope regions. The demonstration that α-actinin immunization of non-autoimmune mice generates Abs cross-reactive with nuclear Ags suggests that α-actinin might be a triggering Ag in systemic lupus erythematosus in humans.

Regulating Apoptosis of RA SF

Golan and collaborators showed that the inflammatory modulator CD44vRA, a splice variant of CD44, is expressed only on synovial fluid (SF) cells from joints of rheumatoid arthritis (RA) patients. In a continuation of their work, Eshkar Sebban et al. (p. 1225) found that soluble CD44vRA bound the glycosylated mammalian lectin galectin-8 (gal-8) with high affinity in plasmon surface resonance. Gal-8 blocked the binding of anti-CD44vRA to CD44-negative human B cells transfected with a vector expressing CD44vRA or

wild-type CD44 utilizing exon 6. Anti-gal-8 mAb binding to RA SF cells was increased by preincubation of the cells with gal-8, whereas the preincubation of gal-8 with soluble CD44vRA reduced gal-8 binding to SF cells. Several gal-8 isoforms were detected in RA SF cells by RT-PCR, and one isoform was detected in the SF of RA patients, but not in controls, by immunoblotting. A triple complex of CD44 proteins, gal-8, and fragments of proinflammatory fibrinogen identified by mass spectrometry were coimmunoprecipitated from RA SF by anti-CD44 and anti-fibrinogen mAbs; all three proteins were detected on immunoblots by mAbs against them. Soluble CD44vRA reduced the gal-8-induced apoptosis of RA SF cells in vitro. Gal-8-induced JNK phosphorylation was highest in transfected cells expressing CD44vRA or a form of CD44 lacking exon 6 compared with transfected cells expressing wild-type CD44. The authors propose that the sequestration of soluble gal-8 in a triple complex with soluble CD44vRA and fibrinogen reduces the proapoptotic, anti-inflammatory activity of gal-8 in the joints of RA patients.



Differences between Organizers

Specific hemopoietic cells called inducers are required for the organogenesis of lymph nodes (LNs) and Peyer's patches (PPs) in the peripheral lymphoid tissues (PLTs) of mice. The inducers interact with surrounding mesenchymal cells called organizers to drive PLT organogenesis. To define differences in the organizer populations of PP and LN, Okuda et al. (p. 804) confirmed the presence of two equal cell populations, double-positive $(DP)^{high}$ (ICAM-1^{high} VCAM-1^{high}) and DP^{med} (ICAM-1^{med} VCAM-1^{med}), in mesenteric LNs (MLNs), but a predominant DP^{high} cell population in PPs. Only the DP^{high} organizer cells in MLN expressed a receptor activator of the NF-κB ligand. Gene expression profiles of DP^{high} cells from the whole mesentery (MLN cells) or whole intestine (PP cells) of day 17.5 embryos, obtained by microarray and confirmed by quantitative PCR analysis, indicated further differences between the two organizers. A variety of cytokines, chemokines, and molecules (including transcription factors) induced during the differentiation of several mesenchymal cell lineages were expressed at higher levels in the MLN cells. The expression of several homeostatic chemokines and other cytokines was higher in PP cells. With the exception of the receptor activator of the NF-κB ligand and IL-6, which remained higher in MLN DP^{high} cells, these differences were not apparent in a comparison of MLN and PP populations from 4-day-old mice. These results suggest that within PLTs the MLN organizer is more active than the PP organizer, possibly reflecting the earlier development of MLNs in the embryo.

Fighting Tuberculosis with IL-1R

Although the control of infection by *Mycobacterium tuberculosis* requires several TLR signals mediated by the intracellular adaptor protein MyD88, it is not known whether the MyD88-controlled IL-1R or IL-18R pathways are also involved. On p. 1178, Fremond et al. found that bone marrow-derived macrophages from *IL-1R1*$^{-/-}$ and *MyD88*$^{-/-}$, but not *IL-18R*$^{-/-}$, mice had reduced IL-12 p40 levels but up-regulated expression of the costimulatory molecules CD40 and CD86 after in vitro stimulation by *M. tuberculosis*. A similar reduction of IL-12p40 was not seen in *IL-1R1*$^{-/-}$ dendritic cells exposed to bacteria. Infection of mice with a virulent strain of *M. tuberculosis* resulted in early death of *IL-1R1*$^{-/-}$ and *MyD88*$^{-/-}$, but not *IL-18R*$^{-/-}$ or wild-type, mice. Lungs from infected *IL-1R1*$^{-/-}$ and *MyD88*$^{-/-}$ mice lacked granulomas and had severe inflammation, mononuclear and neutrophil infiltration, and high bacteria counts compared with control animals. Expression of NO and related nitrogen intermediates was similar in all the strains. Local concentrations of cytokines and chemokines, as measured in lung homogenates, indicated elevated levels of several inflammatory cytokines in infected *IL-1R1*$^{-/-}$ mice compared with infected wild-type mice. A defect in the internalization of mycobacteria by *MyD88*$^{-/-}$ lung cells was not present in infected *IL-1R1*$^{-/-}$ animals. In contrast to the defects seen in the *IL-1R1*$^{-/-}$ and *MyD88*$^{-/-}$ animals, mice lacking the common adaptor for the TLR2 and TLR4 pathways had only a partially impaired response to infection. The data suggest that upstream IL-1R signaling is essential to MyD88-mediated control of the early immune response to *M. tuberculosis* infection in mice.

Endogenous Retrovirus in MS

Transporters of alanine, serine, cysteine, and threonine (ASCTs) on brain cells are needed for neuronal functioning. However, ASCTs are receptors for the human endogenous retrovirus HERV-W, and high transcript levels of Syncytin-1, the HERV-W envelope glycoprotein, are found in the brains of multiple sclerosis (MS) patients. In their study of the role of Syncytin-1 in MS, Antony et al. (p. 1210) measured increased Syncytin-1, but decreased ASCT1, expression in astrocytes of MS lesions compared with non-MS control brains. In vitro, TNF-α induced *Syncytin-1* mRNA in normal astrocytes, and Syncytin-1-pseudotyped virions infected only astrocytes and monocyte-derived macrophages. The expression of ASCT1 was down-regulated while that of several endoplasmic reticulum (ER) chaperone genes was up-regulated in astrocytes in MS brains and in astrocytes transfected with a vector overexpressing Syncytin-1. Transfection of one of the ER chaperones in astrocytes increased inducible NO synthase (iNOS) and down-regulated ASCT1; supernatants from the transfected cells decreased oligodendrocyte viability in culture. A NOS inhibitor reversed the transcriptional repression of ASCT1 in astrocytes overexpressing Syncytin-1. Stereotaxic injection of TNF-α into brains of mice carrying the Syncytin-1 transgene induced Syncytin-1, proinflammatory cytokine, and ER chaperone expression in astrocytes and reduced expression of oligodendrocyte/myelination markers. The injected transgenic brains also had an increased influx of $CD3^+$ T cells and iNOS immunoreactivity but decreased ASCT1 levels. TNF-α-injected transgenic mice exhibited neurobehavioral abnormalities. The authors show that human endogenous retrovirus-W (HERV-W) Syncytin-1 induces changes in astrocytes and in TNF-α-injected Syncytin-1 transgenic mice that mimic findings in MS patients.

Summaries written by Dorothy L. Buchhagen, Ph.D.