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# INSIDE LAB INVEST

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## Building a better assay: SARS detection by MRT-PCR gene chip

Emergence of the severe acute respiratory syndrome (SARS) is a sobering reminder of the devastating local and global effects that a new pathogen can inflict. This highly contagious disease spreads rapidly within communities, internationally via air travel, and carries about a 10% mortality rate. On the other hand, the rapid isolation and characterization of a novel SARS coronavirus (SARS-CoV) as the etiologic agent of this disease resulted from international cooperation among public health officials, collaborating laboratories of the World Health Organization, and the Centers for Disease Control. Ongoing worldwide surveillance requires sensitive and specific assays for early detection. Two currently used serologic tests; enzyme-linked immunosorbent assay (ELISA) and an indirect immunofluorescence assay (IFA) are most accurate for SARS diagnosis during the convalescent phase when viral titers are high. However, the low viral load during the incubation period, which can range from 2 to 16 days, and during the first week of symptoms hampers early detection. Increased sensitivity is provided by real-time reverse transcriptase-polymerase chain reaction (RT-PCR), but the current assay only identifies one region of the SARS-CoV sequence. The WHO and CDC recommend documentation of more than one genome sequence for definitive confirmation. Inside this issue, **Juang *et al***[1] (p. 1085) introduce an improved method, which combines multiplex RT-PCR (MRT-PCR) with a diagnostic gene chip assay. The technique was highly sensitive (detecting $<10$ copies of SARS-CoV) and specific (using multiple sequence-specific probes that recognize more than one genome region) when applied to 53 clinical samples. It allowed for accurate detection, confirmation and even semiquantitative estimation of viral load. Expansion of the gene chip could even allow for the simultaneous assay of other coronaviruses or related respiratory pathogens. The virulence of this pathogen appears to have been matched by aggressive, international, multidisciplinary public health countermeasures; this new assay should help!

### Reference

1 Juang J-L, Chen T-C, Jiang SS, *et al*. Coupling multiplex RT-PCR to a gene chip assay for sensitive and semi-quantitative detection of severe acute respiratory syndrome-coronavirus. Lab Invest 2004;84:1085–1091.

## The quiet clean-up of apoptosis: monocytes, no swelling

Extravasation of plasma and plasma proteins from the vasculature is a key feature of the inflammatory response, giving rise to characteristic swelling of soft tissue. Mobilization of neutrophils from the circulation into the interstitial space is a well-established inducer of plasma extravasation. Neutrophils mobilize in response to the presence of proinflammatory bacterial products, the release of chemotactic factors such as Complement 5a (C5a), or the presence of necrotic cellular debris. In contrast, cellular apoptosis does not incite an inflammatory reaction. Rather, a monocytic pattern of leukocyte emigration ensues, unaccompanied by neutrophil emigration or tissue swelling. The premise that monocyte-predominant tissue infiltration does not induce plasma extravasation has not been directly tested. In this issue, **Miyamoto *et al***[1] (p. 1126) used guinea-pigs to induce monocyte-predominant subcutaneous infiltrates by intradermal injection of the cross-linked homodimer of S19 ribosomal protein (RP S19 dimer), a protein released from apoptotic cells. Plasma extravasation did not occur. It did not occur in animals with elevated peripheral blood monocyte levels. It did not occur despite coinjection of prostaglandin $E_2$, a vasodilatory agent. While RP S19 dimer is an agonist of the chemotactic C5a receptor on monocytes, it is an antagonist of the neutrophil chemotactic C5a receptor. These authors showed that plasma extravasation did not occur when C5a was coinjected with RP S19 dimer; nor did neutrophil extravasation occur. Hence, one must conclude that plasma extravasation is specific for a neutrophilic inflammatory response, and is not the result of physical leakage of plasma during monocyte emmigration. This limited response to apoptosis is highly advantageous, given the routine occurrence of apoptosis and a 'quiet' pattern of monocytic infiltration during tissue development and remodeling.

### Reference

1 Miyamoto K, Kitamoto Y, Tokunaga H, *et al*. Protective effect of vascular endothelial growth factor/vascular permeability factor 165 and 121 on glomerular endothelial cell injury in the rat. Lab Invest 2004;84:1126–1136.

## Sailing against ischemia–reperfusion injury: lower the mast (cells)

Ischemia–reperfusion (IR) injury causes significant morbidity associated with trauma and organ trans-

plantation. In addition, the US Navy (and other navies) have been interested for quite a while in the pathobiology of the postischemic reperfusion injury that occurs during treatment of decompression sickness. Regardless of the manner of injury, reintroduction of blood flow following ischemia causes deleterious and in some cases irreversible effects on tissue function. Such effects have been attributed to toxic metabolites and oxygen radicals related to neutrophils. More recently, nitric oxide (NO) levels regulated by NO synthetase II (NOS II) emerged as a possible mediator. NOS II is localized almost exclusively in mast cells. In this issue, **Bortolotto *et al***[1] (p. 1103) present definitive evidence that mast cells of skeletal muscle play a regulatory role in the pathology of IR injury in this tissue. In prior publications, this group and others reported that mast cell-deficient mice were less susceptible to the development of necrosis after IR injury than wild-type mice of the same strain. The current study uses mast cell-depleted mice, which were engrafted with bone marrow-derived mast cells of normal littermates. When subjected to IR injury, these 'reconstituted' animals showed significantly decreased skeletal muscle viability compared to nonengrafted mast cell-deficient controls. This work confirms that mast cells can contribute to skeletal muscle injury during postischemic reperfusion. This study has wide-ranging implications for understanding the mechanisms of IR injury and for treating the conditions associated with this pathology. Up anchor, lower the mast (cells), and all ahead full!

### Reference

1 Bortolotto SK, Morrison WA, Han XL, *et al.* Mast cells play a pivotal role in ischemia reperfusion injury to skeletal muscles. Lab Invest 2004;84: 1103–1111.

## Integration of Epstein–Barr virus in critical sites of the host genome: a potential oncogenic event

Epstein–Barr virus (EBV) is a ubiquitous member of the herpesvirus family that establishes a life-long persistent infection in more than 90% of the human population worldwide. Following primary infection, the virus persists in B cells, usually as a harmless passenger. However, EBV can transform B cells, which may result in the development of lymphoproliferative diseases. Approximately 20% of Burkitt's lymphomas (BL) in the United States contain EBV DNA and the virus has been etiologically linked to the genesis of these tumors. The link between EBV and BL is further supported by evidence that EBV infection precedes the development of malignancy in some tumors, although the precise pathogenetic mechanisms that may permit EBV to induce BL remain unclear.

The EBV genome is present in BL cells as multiple nuclear episomes. There is also evidence for chromosomal integration of viral genome in some BL lines. Integration may be an important mechanism for interaction of EBV with cellular genes, particularly those involved in cell-growth regulation and tumorigenesis, but analysis of integrated EBV DNA is complicated by the presence of the episomal form of EBV in the nucleoplasm. In this issue, **Luo *et al***[1] (p. 1193) examine a human BL cell line (NAB) that contains one copy of EBV DNA integrated in the 2p13 chromosomal region of the host genome, a site that may be amplified in B-cell and Hodgkin's lymphomas. The authors cloned the breakpoint at the site of EBV integration into the host chromosomal DNA and found that the integration site is near genes such as *REL* and *BCL11A*; genes known to be involved in tumorigenesis or RNA processing. *REL* encodes a NF-kappaB transcription factor and *BCL11A* encodes a zinc-finger transcription factor that is overexpressed in some B-cell and Hodgkin's lymphomas. Furthermore, the expression levels of *REL* were increased, perhaps due to dysregulation caused by the integration event. In addition, the authors found that transcription of the viral gene LMP2 may be disrupted by the event.

Understanding the oncogenic potential of EBV remains a very challenging task. Although episomal expression of EBV proteins may play an important role in tumorigenesis, this study shows that integration of EBV DNA into the host genome could represent another oncogenic mechanism if the integration sites overlap with genes encoding proteins biologically critical in tumorigenesis.

### Reference

1 Luo W-J, Takakuwa T, Ham MF, *et al.* Epstein–Barr virus is integrated between REL and BCL-11A in American Burkitt lymphoma cell line (NAB-2). Lab Invest 2004;84:1193–1199.