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Aptamer-linked immobilized sorbent assay: the next ELISA?

For the past 30 years, enzyme-linked immunosorbent assay (ELISA) has been the primary means for detecting antigens in biological samples. The first applications of ELISA in the early 1970s were in identification of pathologically relevant immunoglobulins, especially towards infectious agents such as salmonella, rubella, and syphilis. The invention of ELISA was dependent upon the ability to produce specific antibodies, a process greatly assisted by the development in 1975 of hybridomas to generate monoclonal antibodies. Now, ELISA is versatile technique for the detection of virtually any protein or peptide, with sensitivities of detection down to the nanomolar or picomolar range. As antibodies can be immobilized on solid substrates, high throughput microarray assay systems can be deployed. Concentrations of antigens can be quantified, as can binding affinity to the antibody employed in the assay. Hence, ELISA is one of the major assays now available for biological research and clinical diagnostics.

Against this background is the current explosion of high-throughput and high-sensitivity assays for nucleotides. Generally taking the form of solid substrate-based microarray assays, there are numerous high-sensitivity detection techniques currently in use. Hence, nucleotide sequences across the full extent of the genome or transcriptome can be analyzed in single sample runs.

Of recent interest are aptamer-based nucleotide assays. Aptamers are single-stranded oligonucleotides, with lengths measured in the tens of nucleotides; they may be either DNA or RNA molecules. These single-stranded nucleic acid molecules have highly defined tertiary structures, which allow them to form stable and specific complexes with a variety of molecular targets: small molecules such as amino acids; proteins as well as nucleotide sequences; and even whole viruses. Hence, aptamers can act as surrogates for antibodies for the detection of proteins and peptides in biological samples. Distinction can be made between closely related protein or nucleic acid targets on the basis of binding affinities, which exhibit dissociation constants in the nanomolar to picomolar range. Notably, the degree of molecular distinction between protein targets by aptamer-based assays may exceed that of antibodies. Key advantages of aptamers over antibodies are that, being shorter, aptamers can be reproducibly synthesized on solid substrates, they are more stable in harsh environments (especially DNA aptamers), and can be easily labeled with fluorescent or other reporters during their synthesis. The first reports of aptamer-based assays were in 1990,[1,2] but their use is not as widespread as ELISA.

In the current issue, **Vivekananda and Kiel**[3] (p. 610) report a DNA-aptamer assay for identification of *Francisella tularensis* subspecies *japonica* antigen. *F. tularensis* is an intracellular, nonmotile, nonsporulating, Gram-negative bacterial pathogen that causes tularemia in man and animals. The most common subspecies causing tularemia in man are the main subspecies, *tularensis* and *holarctica*. These organisms are highly infectious; fewer than 25 organisms are capable of causing disease when spread by aerosol. Although natural transmission of this organism to the human population is through insect bites, contaminated water sources, or handling of infected animals, interest in *F. tularensis* has increased recently because of its potential use as an agent of biological warfare. Tularemia bacteremia is one of the high-ranking pathogens categorized by the Centers for Disease Control as a category A agent.

From a library of 10^{14}–10^{16} different sequences, these authors successfully identified a set of 25 unique DNA sequences of 99 nucleotides, that exhibited high binding affinity to tularemia bacterial antigen from *F. tularensis* subspecies *japonica.* This is the only antigen commercially available for tularemia assay detection. When these sequences were placed in a sandwich Aptamer-linked immobilized sorbent assay (ALISA), the aptamer cocktail exhibited specificity in its ability to bind to tularemia bacterial antigen from subspecies *japonica, holarctica* and *tularensis*, but not to closely related *Bartonella henselae.* The ALISA consistently outperformed an ELISA for detection of tularemia bacterial antigen, both for the *japonica* and *holarctica* subspecies. Hence, these authors have developed a DNA-based ALISA which may be well suited for the detection of bio-warfare agents, in which robust, nonperishable and inexpensive reagents capable of operating in severe and extreme environmental conditions are needed. On a broader front, the aptamer approach (ALISA) may have performance superior to ELISA for detection of biological molecules in both research and clinical applications, potentially replacing antibodies as the preferred detection methodology.

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Blocking stem cell factor attenuates airway hypersensitivity and fibrosis in a model of chronic asthma

Chronic asthma pathogenesis results from complex interactions between inflammation, airway obstruction, airway hypersensitivity and responsiveness, and airway fibrosis/remodelling. Leukocyte infiltration and peribronchial accumulation play a major role in these processes, and among these leukocytes, eosinophils have been identified as the major perpetrators of chronic bronchial injury. Previous studies have indicated that stem cell factor (SCF), mostly known as a major mast cell activation factor, participates in acute asthma pathophysiology, and this may involve a direct effect on eosinophils. In this issue, **Berlin *et al***[1] (p. 557) used a chronic cockroach-allergen-induced asthma mouse model to demonstrate that blocking SCF has beneficial effects not only in attenuating the severity of the airway inflammation and hyperresponsiveness, but also in decreasing the amount of bronchial fibrosis, therefore limiting long-term bronchial injury. In this clinically relevant model, multiple applications of cockroach antigen using sequential intranasal followed by intratracheal challenges, resulted in exacerbated airway hyperreactivity, increased eosinophil infiltration and pronounced peribronchial fibrosis, a feature that is less obvious in the more acute model elicited with a single-antigen challenge. Local inhibition of c-kit/SCF interactions by intratracheal administration of an anti-SCF antibody, or local treatment with imatinib, an inhibitor that blocks SCF/c-kit-associated RTK, during the last two intratracheal challenges, reduced the levels of the CCL6 and CCL17 inflammatory chemokines in the lungs and significantly decreased lung collagen deposition, as assessed by both measurements of lung hydroxyproline levels and by histological assessment of peribronchial fibrosis. As in the acute model, SCF blocking or inhibition resulted in a significant decrease in eosinophil accumulation, reinforcing the link between these leukocytes and asthma pathogenesis. Interestingly, however, blocking SCF in established disease had no impact on the number of mast cells in the lungs. It was already known that local SCF production within the airway affects immediate mast cell activation, alters cytokine and chemokine production, and is associated with mucus overexpression. What this new study shows is that therapeutic local inhibition of SCF significantly improves clinical outcome and attenuates bronchial injury without apparent effect on the mast cells. While the mechanism by which it occurs has still to be elucidated, especially regarding eosinophil activation, the SCF pathway presents a promising target for intervention in chronic asthma.

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Glomerular cell apoptosis in diabetes

Long-standing hyperglycemia is the initiator of the glomerular changes associated with type 1 and type 2 diabetes mellitus (DM). These consist of extensive glomerular remodelling involving both extracellular and cellular compartments; the resulting nephropathy is the most common cause of end-stage renal disease in western countries. In diabetic glomerulopathy, extracellular matrix (ECM) expansion, which is due to an imbalance between synthesis and degradation, is the eye-catching event. It is no wonder that until recently ECM expansion has attracted more attention than changes in the cellular compartment, which is reduced in number on account of enhanced cell death through apoptosis.[1]

Although numerous studies in experimental DM models and in humans suggest that glomerular cell loss plays a role in glomerular injury, regulation of the cellular apoptosis induced by high glucose is still poorly understood. The few studies carried out on human and murine mesangial cells indicate that high glucose can induce apoptosis by an oxidant-dependent mechanism involving the redox-sensitive transcription factor nuclear factor-κB (NF-κB), and upregulation of the Bax/Bcl-2 ratio, with the consequent release of cytochrome *c* from mitochondria and caspase 9 activation.[2,3]

In this issue, **Khera *et al***[4] (p. 566) identify a novel pathway that links high glucose to mesangial cell apoptosis and involves both NF-κB and the TGF-β system. These authors demonstrate the role of TGF-β1 as an effector of the proapoptotic effect of high glucose. Not only is TGF-β1 overexpressed in mesangial cells upon exposure to high glucose, but also mesangial cells exhibit enhanced sensitivity to high glucose-induced TGF-β1 signalling. Further studies are needed to evaluate precisely the amount that the proapoptotic effect of high glucose that depends on enhanced sensitivity to TGF-β signalling. The data of Khera *et al*[4] are in keeping with the role of TGF-β1 in the regulation of several functional changes seen in mesangial cells cultivated at under

high glucose conditions, such as enhanced expression of ECM proteins and enhanced glucose uptake through the induction of GLUT-1 (insulin-independent glucose transporter) expression. TGF-β1 can increase intracellular glucose in mesangial cells and overproduction of ECM proteins even at normal extracellular glucose concentrations.[5]

The injurious effect of high-glucose exposure has been attributed to various biochemical alterations of intracellular metabolism, including (a) increased glucose availability for the polyol and hexosamine pathways, (b) protein kinase-C activation, (c) nonenzymatic glycation, and (d) oxidative stress. Recent experimental evidence suggests that enhanced glucose flux through glycolysis results in increased superoxide production in the mitochondria. This event can trigger redox-sensitive signalling pathways that activate the above pathways of HG injury.[6] p66Shc, an adaptor protein encoded by the mammalian proto-oncogene SHC locus, controls oxidative stress response and life span. This protein has been recently shown to participate in mitochondrial ROS production by serving as a redox enzyme oxydizing cytochrome *c*, with the effect of generating proapoptotic H_2O_2 in response to specific stress signals. Redox-defective mutants of p66Shc cannot elicit mitochondrial ROS generation or swelling *in vitro*, nor can they mediate mitochondrial apoptosis *in vivo*.[7]

Recent data from our laboratories suggest that p66Shc-deficient mice are also resistant to DM glomerulopathy. p66Shc-deficient mice, when made diabetic, exhibit a low degree of ECM deposition. Furthermore, their glomerular cell death rate does not increase in comparison with diabetic wild-type mice. Likewise, mesangial cells isolated from p66Shc-deficient mice show little or no glucose-induced apoptosis or increased deposition of ECM in comparison with mesangial cells isolated from wild-type mice. Finally, high glucose does not increase intracellular ROS levels in the mesangial cells of diabetic p66Shc-deficient mice.[8]

Most recently, glomerular cell apoptosis has been recognized as an early event in the nephropathy of murine type-1 and 2 DM models *in vivo*, with increasing cell death levels parallelling the inception and progression of urinary albumin excretion.[9] Another recent acquisition is that podocytes have been identified as the main glomerular cell type that undergoes apoptosis[8,9] in response to glucose-induced ROS production.[9]

In summary, further studies should be undertaken to highlight the pathways of the proapoptotic effect of high glucose on glomerular cells. The role of the different cell types involved, in particular podocytes and mesangial cells, should be differentiated. A better knowledge of apoptosis pathways, cell targets, and signalling modulation could open the way to new, exciting clinical intervention measures for the prevention and reduction of DM glomerulopathy.

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