Affinity Mass Spectrometry Measurement of Targeted Cytokines under Physiological Conditions

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Certain cytokine levels in serum can serve as sentinels of inflammatory function in animals. We are developing a method for large-scale screening of cytokine levels in serum samples from mutant mouse populations as a possible means to detect genetic conditions that lead to chronic inflammatory diseases such as Crohn's disease or arthritis. The goal of this work is to detect mutant animals which have relatively subtle abnormalities leading to chronic inflammatory diseases. To this end, we have developed a method for concentration and purification of tumor necrosis factor alpha (TNF-α), a 17 kDa proinflammatory cytokine that has a relatively high serum concentration in acute reactions and seems to be an early effector in inflammatory cytokine cascades. The method is based on affinity capture of cytokines from serum using an antibody-derivatized micron-sized bead support, followed by detection and semi-quantitation by MALDI-MS. The capture was optimized and characterized by using sensitive ^{125}I labeling experiments.[1] The selectivity of affinity separations is complemented by the m/z measurement capability of mass spectrometry.[2]

Capture molecules were covalently linked to ca. 1 μm diameter aminopolystyrene or fragmented Sephacryl beads using glutaraldehyde linkages[3] or CNBr activation,[4] at 7-15 μg IgG per mg beads. TNF-α-specific capture molecules included purified rat monoclonal antibody to mouse TNF-α (RatxTNF-α), purified goat polyclonal antibody to mouse TNF-α (GxTNF-α), and a recombinant soluble TNF-α receptor protein (

We are currently working to improve sensitivity by eluting the TNF-α from the capture beads into smaller volumes, and to improve the precision of MALDI quantitation using more homogeneous sample preparation techniques and internal standards, with the goal of rapid throughput sample analysis at physiologically relevant concentrations.

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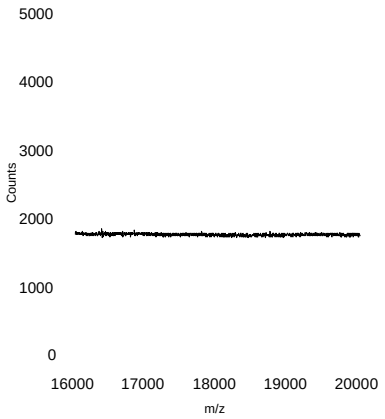
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