Anti-Methylene Diphenyl Diisocyanate (MDI) Murine Monoclonal (IgG) Antibodies

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Relevance: As immune proteins that affinity mature in vivo to attain high binding specificity for antigenic/allergenic epitopes, antibodies are useful reagents for numerous clinical and research applications. Monoclonal antibodies (mAbs) against common high molecular weight allergens (e.g. dust mite, cat allergens) have proven useful for standardizing diagnostic preparations, and quantitative measurements of allergen.

Research Purpose: We sought to develop murine hybridomas that secrete MDI-specific IgG.

Methods: Hybridomas were developed by fusing spleen cells of MDI immunized mice, with the SP2/O murine myeloma cell line. High secreting subclones were isolated by limiting dilution cloning, allowing ready purification of milligram quantities of mAb from minimal volumes (<500 ml) of culture supernatant.

Analysis: Hybridomas secreting MDI-specific IgG were identified by ELISA analysis of culture supernatant for immunoglobulin with differential binding to MDI-albumin vs. unconjugated albumin, using a secondary reagent with specificity for the Fc-region of the gamma constant region.

Results: Six different clones were isolated that secrete IgG1/kappa antibodies, which specifically recognize MDI conjugated to human albumin (and a panel of other carrier proteins), but do not bind hexamethylene diisocyanate, toluene diisocyanate, or isothiocyanate conjugated proteins. The purified mAbs recognize MDI-albumin in plate (ELISA) and membrane (Western blot) bound assay formats. Interestingly, two additional anti-albumin mAbs were serendipitously isolated, with specificity for human, rabbit, and dog albumin (which possess MDI susceptible di-lysine motif at positions 413/414), but not mouse or bovine albumin (which lack the 413/414 di-lysine).

Conclusions: A panel of IgG mouse mAbs with specificity for MDI-conjugated proteins has been successfully developed and demonstrates the exquisite MDI specificity the immune system is capable of developing.

Implications: The new MDI-specific IgG mAbs will be especially useful for tracking MDI in future studies, and may form the basis of assays that quantitate MDI (biomarkers) in clinical samples.

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Enrichment and Measurement of a Signature MDI Human Serum Albumin Peptide Adduct

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Purpose: The purpose of this study was to develop and validate a signature peptide biomarker LC/MS/MS method to enable the monitoring of 4,4′-methylene diphenyl diisocyanate (4,4′-MDI) adducted to human serum albumin (HSA) in plasma collected from workers occupationally exposed to MDI.

Relevance: 4,4′-MDI is used to produce polyurethane. Exposure to isocyanates like MDI may exacerbate airway disorders such as occupational asthma. Therefore it is essential to have a reliable and specific method for biomonitoring the occupational exposure of workers to MDI.

Methods: A murine anti-4,4′-MDI monoclonal IgM antibody was bound to magnetic beads and utilized for enrichment of the MDI adducted HSA. Following enrichment, trypsin digestion was performed to generate the predictable MDI adducted HSA signature peptide biomarkers that were quantified by liquid chromatography tandem mass spectrometry (LC/MS/MS).

Analysis: An Agilent 6530 LC/QTOF system was utilized for intact adducted protein analysis and an Agilent 6490 LC/MS/MS system operated in multiple reaction monitoring (MRM) mode was utilized for quantification of the adducted peptide biomarker.

Results: In vitro results showed that exposure of 1mM 4,4′-MDI to HSA produces major MDI adducts of both HSA isomers. LC/MS/MS quantitation of the 1 mM 4,4′-MDI adducted HSA trypsin digest was performed. The longer MDI adducted K(MDA)VPQVSTPTLVEVSR peptide was quantifiable and it's concentration was 276.63 ng/mL which accounted for 10.31% of the total MDI adducted albumin when 0.1 mg/mL of HSA was digested. No quantifiable amounts resulted for MDI adducted K(MDA) and YTK(MDA) amino acid or short peptide, respectively. These results demonstrated that the primary site of adduction appears to be K(MDA)VPQVSTPTLVEVSR. The MDI adducted HSA remained stable over 8 days when stored under both -80°C and room temperature conditions. IgM magnetic bead capture of the adducted 4,4′-MDI HSA protein showed that enrichment is favorable to that without bead capture as evident by the ability to pull down and quantify adducted albumin with higher concentrations of plasma present.

Conclusions: The MDI adducted HSA is stable at both -80°C and room temperature over 8 days. Of the possible 413/414 HSA signature on the peptide adducts, the 414 lysine on K(MDA)VPQVSTPTLVEVSR appears to be the primary site of adduction. Sensitivity of the signature peptide method in matrix standards is comparable to Sabbioni's (2010) lysine amino acid methodology with the added benefit of more selectivity. Additional work is planned with human exposure samples.

Implications: Biological monitoring is advantageous as sampling is less time-consuming and HSA protein adduct biomarkers have long half-lives and may be used to monitor long-term exposure. In addition HSA protein adducted biomarkers reflect the absorbed dose which integrates such factors as lung ventilation, alternative routes of exposure and acquired metabolic characteristics. Thus future work may correlate albumin related biomarker levels, rather than air levels, to potential sensitization, resulting from MDI occupational exposure.

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