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Proteomic and bioinformatics profile of paired human alveolar macrophages and peripheral blood monocytes

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Abstract

Little is known about proteomic differences between pluripotent human peripheral blood monocytes (MN) and their terminally-differentiated pulmonary counterparts, alveolar macrophages (AM). To better characterize these cell populations, we performed a label-free shotgun proteomics assessment of matched AM and MN preparations from eight healthy volunteers. With an FDR of less than 0.45%, we identified 1754 proteins within AM and 1445 from MN. Comparison of the two proteomes revealed that 1239 of the proteins found in AM were shared with MN, whereas 206 proteins were uniquely identified in MN and 515 were unique to AM. Molecular and cellular functions, protein classes, development associations, and membership in physiological systems and canonical pathways were identified among the detected proteins. Analysis of biologic processes represented by these proteomes indicated that MN were most prominently enriched for proteins involved in cellular movement and immune cell trafficking. In contrast, AM were enriched for proteins involved in protein trafficking, molecular transport, and cellular assembly and organization. These findings provide a baseline proteomic resource for further studies aimed at better understanding of the functional differences between MN and AM in both health and disease.

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Keywords

Alveolar macrophage; Cell biology; Human immune response; Lung; Peripheral blood monocytes; Shotgun proteomics

1 Introduction

The mononuclear phagocyte system (MPS) consists of committed myeloid progenitor cells of the bone marrow, blood monocytes of the peripheral circulation, and differentiated tissue macrophages. The majority of cell division within the MPS occurs in monoblast and promonocyte populations within the bone marrow. As these cells mature into monocytes (MN), they enter the peripheral blood, in which they circulate through all compartments of the body. MN remain in the bloodstream for less than 48 h before either entering specific tissue compartments or dying. Once they cross into tissue sites, MN undergo their final stage of differentiation in response to various inflammatory and immune stimuli present in the new environment, and become terminally-differentiated tissue macrophages that display a more extended life expectancy of 2–5 weeks [1, 2].

As circulating members of the MPS, MN play an important role in the immune response and host defense against pathogens. They are also involved in removal of apoptotic cells and in scavenging toxic compounds to maintain homeostasis. However, their main function is as a systemic reservoir of myeloid precursors for renewal of tissue macrophages [3–5]. Because of their accessibility, MN are commonly studied as surrogates for various macrophage populations, but they do not possess a terminally-differentiated phenotype and are therefore unlikely to express responses identical to those of specific tissue macrophages. Alveolar macrophages (AM) represent a distinct macrophage population that reside in the distal compartments of the lung, i.e. the alveolar space, where they function as a first line of defense against inhaled insults such as particulate matter, environmental toxins and microorganisms [6–8]. AM display phagocytic and microbicidal capabilities, and serve as antigen-presenting cells. In addition, they perform a complex immunoregulatory role as producers of a wide variety of pro- and anti-inflammatory agents that modulate lymphocyte, epithelial, and endothelial function and promote fibroblast migration and replication [9,10]. Disordered AM responses contribute to the pathogenesis of many pulmonary diseases, such as chronic obstructive pulmonary disease, asthma, idiopathic pulmonary fibrosis, and pulmonary sarcoidosis [11–14]. Further, among defined tissue macrophage populations, AM of healthy individuals are uniquely accessible for detailed study via the procedure of fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) [15].

Global proteomics studies can provide crucial information on the functional makeup of a cell relevant to its ability to respond to distress or insult. We hypothesized that MN and AM possess substantially distinct proteomes that are relevant to their discrete functional roles. Currently, little is known about the comparative proteomes of these two cell types. Prior studies relied upon application of 2D electrophoresis prior to LC-MS/MS analysis. This approach was based on visual assessment of differences in the densities of whole proteins within the samples and resulted in characterization of 27 proteins that were present to

differing degrees between MN and AM [16]. In contrast, we employed a shotgun proteomics approach to study matched MN and AM samples from healthy non-smoking adult volunteers. This methodology does not involve a labeling step and, by analyzing all detectable peptides within a sample, avoids limitations to detection based on protein mass and membrane-association. Our findings therefore reflect a comprehensive overview of the proteome profile of each cell type with limited subcellular location bias.

2 Materials and methods

2.1 Human subjects

Healthy non-smokers aged 18–50 were recruited for participation in research blood drawing and bronchoscopy procedures. Exclusion criteria included a history of chronic lung disease or respiratory symptoms, as well as use of any inhaled or systemic immunosuppressive agents in the six months prior to study participation. All volunteers consented to research participation according to protocols approved by the Institutional Review Boards of the University Hospitals Case Medical Center and the Louis Stokes Cleveland Department of Veterans Affairs Medical Center.

2.2 Isolation of blood monocytes

Peripheral blood was obtained by venipuncture. Mononuclear cells were isolated by density sedimentation, as previously described [17]. To remove platelets, cells were washed two more times at $120 \times g$ for 10 min. MN were then isolated by adherence to polystyrene plates, followed by additional removal of non-adherent lymphocytes via light vortexing of the plates. Residual adherent MN were covered with cold PBS, cooled to 4 C for 15 min, and dislodged with sterile plastic scrapers. Following washing, MN were resuspended in Iscove's Modified Dulbecco's Medium (IMDM, BioWhittaker, Walkersville, MD) with 1% penicillin G (P-3032, Sigma, St. Louis, MO) and assessed for purity using flow cytometry (see below).

2.3 Fiberoptic bronchoscopy and bronchoalveolar lavage

All bronchoscopies were performed in the Dahms Clinical Research Unit of University Hospitals Case Medical Center using previously described protocols [18]. BAL fluid was aliquoted into 50 mL polypropylene tubes and immediately centrifuged at $300 \times g$ for 10 min. Supernatants were removed, and BAL cells were resuspended in IMDM with 1% penicillin G.

2.4 Sample purity assessment

AM cell differentials were determined by light microscopy counting of 300 cells on Wright-Giemsa-stained cytospin preparations (LeukoStat; Fisher Diagnostics, Pittsburgh, PA). Assessment of MN purity was performed using flow cytometry after staining with anti-CD3 and anti-CD4 antibodies, (BD Bioscience, San Jose, CA), with MN being defined as the $CD4^+/CD3^-$ population. Samples were included in the proteomics analysis only when purity of >90% of viable cells was achieved for both AM and MN samples of a given donor.

2.5 Preparation of protein samples

After isolation, cells were re-suspended in IMDM with 2% fetal bovine serum and 1% penicillin G, aliquoted into 1.5 mL microfuge tubes, and incubated at 37 C overnight. The next morning, samples were centrifuged at $300 \times g$ for 10 min. Supernatant was then removed and cell pellets snap frozen by immersion in liquid nitrogen for 5 min. prior to storage at -80°C . When sample collection was complete, approximately $1-2 \times 10^6$ cells for MN and matched AM from all eight volunteers were thawed and processed simultaneously for proteomic analysis. 100 μL of 4% SDS with 1X protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) was added to cells. Each sample underwent three 5s pulse sonications with a probe sonicator at 15% amplitude and 5s rest intervals between repetitions. Lysates were then incubated on ice for 45 min. and pulse-sonicated again before an additional 5.5 h incubation on ice.

Following cell lysis, samples were processed using a filter-aided sample preparation (FASP) cleanup protocol with Amicon Ultra MWCO 3K filters (Millipore, Billerica, MA) as previously described [19]. Samples were reduced and alkylated on the filters with 10 mM DTT (Acros, Fair Lawn, NJ) and 25 mM iodacetamide (Acros, Fair Lawn, NJ), respectively, after which they were concentrated to a final volume of 40 μL in 8 M urea. Protein concentration was performed using the Bradford Method as adapted to the manufacturer's instructions (Bio-Rad, Hercules, CA).

Following reduction and alkylation, 5 μg of total protein was aliquoted for enzymatic digestion. Urea concentration was adjusted to 4 M using 50 mM Tris pH 8 and proteins were digested with mass spectrometry grade lysylendopeptidase (Wako Chemicals, Richmond, VA) in an enzyme/substrate ratio of 1:20 for 2 h at 37 C. Urea concentration was further adjusted to 2 M using 50 mM Tris pH 8 and lysyl peptides were additionally digested with sequencing grade trypsin (Promega, Madison, WI) in an enzyme/substrate ratio of 1:20 at 37 C overnight. Finally, samples were diluted in 0.1% formic acid (Thermo Scientific, Rockford, IL) prior to LC-MS/MS analysis. 400 fmol of Pierce[®] Retention Time Calibration Mixture (Thermo Scientific, Rockford, IL) was spiked into samples to track retention times and mass drift across all samples. MN samples showed a maximum retention time drift of 2.07 min across nine tracked peptides for all eight samples, whereas AM samples showed a 3.49 min maximum retention time drift across the same nine tracked peptides for all eight samples. Additionally, the mass drift was accounted for using the same nine tracked peptides in both cell types; MN showed a maximum 4.15 ppm error while AM had a 3.35 ppm maximum mass error.

2.6 Reverse phase LC-MS/MS analysis

Four hundred nanograms of 8 AM and 8 MN peptide digests were randomized with blanks between and loaded on a column in a 14 μL injection for a total of 16 LC/MS/MS runs. Resulting data was acquired on an OrbitrapVelos Elite mass spectrometer (Thermo Electron, San Jose, CA) equipped with a Waters nanoACQUITY LC system (Waters, Taunton, MA). Peptides were desalted in a trap column (180 $\mu\text{m} \times 20$ mm, packed with C18 Symmetry, 5 μm , 100A, Waters, Taunton, MA) and subsequently resolved in a reversed phase column (75 $\mu\text{m} \times 250$ mm nano column, packed with C18 BEH130, 1.7 μm , 130A (Waters, Taunton,

MA). LC was carried out at ambient temperature at a flow rate of 300 nL/min using a gradient mixture of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The gradient employed ranged from 4 to 44% solvent B over 210 min. Peptides eluting from the capillary tip were introduced into the nanospray mode with a capillary voltage of 2.4 kV. A full scan was obtained for eluted peptides in the range of 380–1800 amu followed by 25 data dependent MS/MS scans. MS/MS spectra were generated by collision-induced dissociation of the peptide ions at normalized collision energy of 35% to generate a series of b- and y-ions as major fragments. In addition a one-hour wash was included between each sample.

2.7 Protein identification and quantification

Individual raw LC-MS/MS data files were analyzed for protein identification through Mascot Daemon version 2.3.2 (Matrix Sciences, London, UK). Key search parameters were Trypsin for enzyme; a maximum of 1 missed cleavage; peptide charge states of +2 to +3; peptide tolerance of 10 ppm; and MS/MS tolerance of 0.8 Da. Oxidation of methionine was a variable modification. Identifications were merged, the spectra summed and then quantified using spectral counting (selecting normalized total spectra) in Scaffold version 4.4.0 (Proteome Software, Inc. Portland, Oregon). The protein probability was determined using a protein threshold of 99%, two peptides and a peptide threshold of 95% [20, 21]. A false discovery rate (FDR) was calculated using ProteinProphet algorithm for the 213 073 spectra examined [21]. Both MASCOT and Scaffold strategies utilized the IPI-human database from June 2010 (86 392 sequences). Over 1700 unfiltered proteins were identified in AM samples with a 0.35% FDR, whereas an FDR of 0.41% was observed for over 1400 unfiltered proteins observed in MN samples.

2.8 Functional proteome classification using IPA and PANTHER

Classification and analysis of identified proteins was performed using PANTHER (Protein ANalysisTHrough Evolutionary Relationships) (www.pantherdb.org) and Ingenuity Pathway Analysis Tool (IPA, Ingenuity® Systems, www.ingenuity.com) software. Initial characterization (protein localization and molecular categorization) was based on division of all observed proteins as uniquely identified within AM, uniquely identified within MN, or identified in both cell types. To allow a more comprehensive assessment of the differences between the proteomes of AM and MN, further analysis (protein class, molecular and cellular function, disease and physiology system association, and canonical pathway analysis) utilized expanded data sets that included proteins enriched within one cell type or the other in addition to those that were unique for that cell type. In this assessment, “enrichment” was based on ratio of the abundance of each detected protein within AM compared to MN samples, with cut-off criteria as fold-change of 10 (i.e., proteins enriched within AM) or 0.1 (i.e., proteins enriched within MN). This approach produced two data sets for analysis, proteins unique or enriched within AM (set 1), and proteins unique or enriched within MN (set 2). The lists of protein identifiers for the two datasets were generated by Scaffold based on protein accession number. PANTHER was used to annotate proteins with regard to their protein class. Calculation of *p*-values in this analysis is based on application of binomial statistics. Evaluation of identified proteins in terms of subcellular location, molecule type, molecular and cellular function, disease association, physiological

system development and function, and canonical pathways was performing using IPA, in which *p*-values are determined via a right-tailed Fisher's exact test.

3 Results

3.1 Protein identification and proteome annotation

Paired samples of purified MN and AM from eight healthy volunteer (age 23–36) that had a purity of >90% were prepared and analyzed concurrently in a proteomics and bioinformatics pipeline. Mean cell numbers were 1.79×10^6 for AM samples (range 1.18 – 2.00×10^6) with a purity of 94.08% ($\pm 1.83\%$), whereas MN samples had a mean cell number of 1.58×10^6 (range 1.00 – 2.00×10^6) with a purity of 94.05% ($\pm 2.97\%$).

For both cell types, 400 ng of peptide digest was analyzed across eight paired volunteer samples for a total of 16 LC/MS/MS runs using the settings previously described in the methods. All raw data files for each cell type were merged together for analysis. General findings of the proteins identified in this shotgun proteomics experiment are displayed in Fig. 1. We obtained similar proteome coverage across both cell types as illustrated in the Venn diagram. Mapping of identified peptides revealed more than 1700 non-redundant proteins within AM samples and over 1400 proteins within MN samples. 1239 proteins shared by both proteomes represented approximately 85.7% of the proteins identified within the MN samples and 70.6% of those detected within AM samples. Accordingly, 206 proteins were identified within MN samples only, whereas 515 were identified within AM alone. (Listing of all proteins identified and their presence within samples from AM, MN or both cell types is presented in Supporting Information Table 1; identified peptides and their spectral counts are provided in Supporting Information Table 2.) Initial categorization utilized IPA to identify subcellular locations of the identified proteins. In comparison to proteins uniquely identified within AM, proteins found within MN samples alone displayed modest enrichment of extracellular, nuclear, and plasma membrane proteins, as well as noteworthy depletion of cytoplasmic proteins (Fig. 1). The molecular category of each protein detected in MN and/or AM samples was also assessed using IPA (Fig. 2). In the category of enzymes, proteins detected within MN samples only were underrepresented compared to those found uniquely within AM (Fig. 2, inset). Proteins unique to MN were relatively depleted of kinases, peptidases, translation regulators, and transporters, but showed enrichment for G-coupled receptors, ion channels, transcription regulators, and transmembrane receptors (Fig. 2, main figure).

To obtain a more comprehensive view of the proteomic differences between AM and MN, our further analysis focused on proteins that were detected in samples of one cell type to a 10-fold greater extent than the other, in addition to the proteins that were uniquely identified within samples of one cell type alone (i.e., sets 1 and 2 as defined in the Methods section, and detailed in Supporting Information Table 3). Set 1 (identified as unique or enriched within AM) consisted of 541 proteins, whereas set 2 (unique or enriched within MN) consisted of 231 proteins. Annotation of identified proteins within these sets for categorization of protein class was performed using PANTHER. As illustrated in Fig. 3, proteins classified as oxidoreductases were found to be the most commonly observed class present to a similar extent within both AM and MN. The proteins classes that were more

prominent in AM samples than MN included chaperones, cytoskeletal proteins, defense/immunity proteins, hydrolases, isomerases, kinases, ligases, lyases, phosphatases, proteases, structural proteins, and transferases. In contrast, MN samples displayed greater proportions of proteins classified as calcium-binding proteins, cell adhesion molecules, cell junction proteins, enzyme modulators, extracellular matrix proteins, membrane traffic proteins, nucleic acid binding proteins, receptors, signaling molecules, and transcription factors.

3.2 Analysis of molecular and cellular proteome functions

IPA was also used to evaluate the molecular and cellular functions of proteins unique or enriched within MN or AM. As shown in Fig. 4, proteins unique or enriched within AM were most significantly associated with the functions of protein trafficking, molecular transport, cellular assembly and organization, protein synthesis, small molecule biochemistry, amino acid metabolism, metabolic disease, hereditary disorder, and developmental disorder. In contrast, proteins unique or enriched within MN were significantly associated with cellular movement, immune cell trafficking, cancer, organismal injury and abnormalities, cell-to-cell signaling and interaction, hematological system development and function, inflammatory response, and organismal functions. Overlap in functions of proteins differentially identified in AM and MN was found for the functions of infectious diseases, dermatological diseases and conditions, and cell death and survival, indicating that different sets of proteins associated with these categories were identified within each cell type.

3.3 Disease and physiologic system association of identified proteins

Evaluation of disease association of identified proteins was performed using IPA as well. Proteins unique or enriched within MN were found to be most significantly associated with cancer (p -value $2.14\text{e-}13$) and organismal injury and abnormalities (p -value $2.14\text{e-}13$), followed by inflammatory response (p -value $2.91\text{e-}13$), infectious disease (p -value $4.77\text{e-}12$), and cardiovascular disease (p -value $6.31\text{e-}10$). In contrast, proteins identified as unique or enriched within AM were most significantly associated with infectious diseases (p -value $2.81\text{e-}09$), followed by developmental disorders (p -value $1.47\text{e-}09$), hereditary disorder (p -value $1.47\text{e-}09$), metabolic disease (p -value $1.47\text{e-}09$), and dermatological diseases and conditions (p -value $4.88\text{e-}06$). Analysis of the proteins unique to AM also revealed 11 proteins associated with lysosomal storage disease, a category of rare inherited metabolic disorder that results from defects in lysosomal function [21] (p -value $1.47\text{e-}07$, data not shown).

The top two most significant physiological functions in MN enriched proteins were hematological system development and function (p -value $2.91\text{e-}13$), and immune cell trafficking (p -value $9.09\text{e-}16$). Functions that were significantly associated with proteins unique or enriched within AM included endocrine system development and function (p -value $5.86\text{e-}09$), cardiovascular system development and function (p -value $6.07\text{e-}04$), immune cell trafficking (p -value $1.71\text{e-}04$), and connective tissue development and function (p -value $7.62\text{e-}04$).

3.4 Canonical pathway analysis

IPA was also used to identify canonical pathways associated with the proteins unique or enriched within AM or MN samples (Fig. 5, detailed in Supporting Information Table 4). Of the top 12 pathways associated with each cell type, only one, liver X receptor (LXR)/retinoid X receptor (RXR) activation, was common to both protein sets. Other canonical pathways associated with proteins unique or enriched within AM included clathrin-mediated endocytosis signaling, NAD salvage pathway II, L-cysteine degradation III, superpathway of methionine degradation, role of pattern recognition receptors in recognition of bacteria and viruses, NRF2-mediated oxidative stress response, glutathione biosynthesis, phagosome maturation, NAD phosphorylation and dephosphorylation, phenylalanine degradation and methylmalonyl pathway. In contrast, canonical pathways most strongly associated with proteins unique or enriched in MN were oxidative phosphorylation, extrinsic prothrombin activation pathway, intrinsic prothrombin activation pathway, mitochondrial dysfunction, TREM1 signaling, coagulation system, paxillin signaling, caveolar-mediated endocytosis signaling, tumoricidal function of hepatic natural killer cells, purine nucleotides de novo biosynthesis II, and ceramide biosynthesis.

4 Discussion

This study provides the first assessment of the human MPS involving a comprehensive comparison of the baseline proteome of MN with that of AM. We utilized a solution-based proteomic study of volunteer-matched cell populations to demonstrate that, despite substantial overlap, there are significant differences between the proteomes of terminally-differentiated AM and their circulating MN precursors. In many cases, these differences appear to correspond to the differing lifespans, localization, and primary functions of these MPS populations.

MN are most notable for being short-lived pluripotent cells that serve primarily to mobilize mononuclear phagocytes from the bone marrow and through peripheral blood into specific tissues where differentiation can occur [22, 23]. The differentiation of MN is mediated by multiple transcription factors, such as PU.1, interferon regulatory factor 8 (IRF8), and Kruppel-like factor 4 (KLF-4). Proteins associated with transcriptionally-mediated differentiation processes are typically located within the nucleus, but require extra-nuclear exportation. Accordingly, the state of readiness for differentiation could account for the enrichment for nuclear, plasma membrane, and extracellular proteins observed within MN [24], and is also consistent with the association of the MN proteome with the function of hematological system development. Also at the level of molecular and cellular function, the prominent association of proteins unique and enriched within MN with cellular movement, immune cell trafficking, as well as cell-to-cell signaling and interaction is consistent with the critical function of these circulating cells in replenishing specific differentiated phagocyte populations by delivering themselves from the bloodstream into specialized organ sites. Canonical pathway analysis of proteins unique to or enriched within MN also identified multiple pathways associated with thrombosis (extrinsic and intrinsic prothrombin activation pathways and coagulation system). MN have been implicated as contributing to inflammation and plaque instability within atherosclerotic lesions [25], however they also

serve a physiologic role in clotting as a main source for coagulation factor III (aka tissue factor or thromboplastin) [26]. In contrast, AM presumably would not require the capacity to contribute to hemostasis as residents of the typically blood-free environment of the alveoli.

Our analysis of the AM proteome likewise provides some correlation to known features of these cells. For example, the association of these proteins with lysosomal storage disease most likely relates to the central role played by lysosomes in the host defense function of AM; phagocytosis of pathogens results in their delivery to lysosomes where they are enzymatically degraded and thereby inactivated [27, 28]; the importance of this process to AM function is similarly reflected in the identification of phagosome maturation as a prominent pathway associated with the AM proteome. Likewise, the observation that proteins unique or enriched within AM are associated with endocrine system development and function may reflect the known prevalence of a large number of neuroendocrine cells within the lung [29]. Although interactions between these cells and AM are incompletely understood, recent studies demonstrate that AM express functional receptors for locally-produced neurotransmitters [30, 31]. More pointedly, analysis of associations with proteins unique or enriched within AM identified several canonical pathways (clathrin-mediated endocytosis signaling, role of pattern recognition receptors in recognition of bacteria and viruses, phagosome maturation) that are consistent with known functions of these cells as the first line of respiratory host defense. AM function in the initial immune recognition of a wide range of pathogens respond through their expression of multiple families of pattern recognition receptors (PRRs) including Toll-like receptors (TLRs), NOD-like receptors (NLRs), retinoic acid inducible gene I-like receptors (RLRs), and C-type lectin receptors (CLRs) [32]. Mannose receptor (also designated as CD206) is a specific CLR that is a hallmark of differentiated tissue phagocytes including AM, and which mediates recognition and uptake of a variety of organisms via binding to mannose and other bacterial cell wall components [33]. Endocytosis of mannose receptors and their subsequent recycling to the plasma membrane is itself a clathrin-dependent process [34], further indicating the interaction of these pathways with known AM functions.

Our findings provide a baseline proteomic resource for further studies aimed at better understanding functional differences between MN and AM in both health and disease. Although the findings noted above are consistent with some of the known functional distinctions between these cell types, our analysis has also identified other differences in the proteomes of MN and AM that are not as readily amenable to intuitive interpretation. Further investigation of these novel and, at times, unexpected findings may provide a means to further understand the processes by which tissue-specific differentiation of cells of the MPS occurs, and how these serve to facilitate the role of AM in protective and pathologic responses within the human lung.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AM	alveolar macrophage
BAL	bronchoalveolar lavage
BEH	bridged ethylsilicone/silica hybrid
Cdc42	cell division control protein 42 homolog
CLR	C-type lectin receptor
FASP	filter-aided sample preparation
IMDM	Iscoe's modified Dulbecco's medium
IPA	Ingenuity Pathway Analysis
IRF8	interferon regulatory factor 8
KLF-4	Kruppel-like factor 4
LXR	liver X receptor
MN	peripheral blood monocyte
MPS	mononuclear phagocyte system
NLR	NOD-like receptors
PANTHER	Protein ANalysis THrough Evolutionary Relationships
PRR	pattern recognition receptors
RLR	retinoic acid inducible gene I-like receptors
RXR	retinoid X receptor
TLR	Toll-like receptors
TREM1	triggering receptor expressed on myeloid cells 1

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Figure 1. General differences in the proteins identified within AM and MN using a shotgun proteomics approach (detailed in Supporting Information Table 1). Venn diagram (inset) indicates the numbers of proteins detected within matched human AM (black), and MN (light gray) alone, as well as those which were detected in both samples (dark gray). Subcellular distribution of the identified proteins within each category is illustrated in the bar graph (main figure).

Figure 2.

Differing molecular function profiles of proteins in MN and AM. Figures illustrate proteins unique to AM (black), unique to MN (light gray), and common to both cell types (dark gray). Proteins unique to MN were less frequently identified as enzymes than those of AM (inset). Proteins found in MN alone were relatively enriched for G-couple protein receptors, ion channels, transcription regulators, and transmembrane receptors, whereas proteins unique to AM were enriched for kinases, peptidases, translation regulators, and transporters (main figure).

Figure 3.

Functional categorization of proteins enriched within MN and AM, based on being identified to a 10-fold greater extent, or uniquely identified within that cell type (as detailed in Supporting Information Table 3). Bar charts illustrate the molecular and cellular classes of proteins enriched or uniquely identified within AM (black) or MN (gray), as accessed via PANTHER and detailed within the text. Results are displayed as a percentage of the total proteins represented by each protein class.

Figure 4.

Distinct patterns of molecular and cellular function were observed for proteins enriched or uniquely identified within AM (black) or MN (gray). As illustrated, several molecular and cellular functions were significantly associated with the proteins that were unique or enriched in AM or MN samples. Attribution of certain functions (e.g. cell death and survival, infectious diseases) to more than one cell population indicates that distinct sets of proteins associated with those functions were identified within AM and MN. Significance is represented as $-\log$ of p -values; the vertical line corresponds 1.30 (i.e. $-\log$ of $p = 0.05$).

Figure 5.

Canonical pathways of proteins enriched or uniquely identified within AM (black) and MN (gray). Figures display the 12 canonical pathways most significantly associated with each sample as determined by IPA analysis, as detailed in the text (and in Supporting Information Table 3). The bimodal distribution of pathways associated with the AM and MN samples are consistent with the distinct functional profiles of these populations. Significance is again illustrated as $-\log$ of p -values, with the vertical line corresponding to $p = 0.05$.