NEURODEGENERATION

A human microglia-like cellular model for assessing the effects of neurodegenerative disease gene variants

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Microglia are emerging as a key cell type in neurodegenerative diseases, yet human microglia are challenging to study in vitro. We developed an in vitro cell model system composed of human monocyte-derived microglia-like (MDMi) cells that recapitulate key aspects ofmicroglia phenotype and function. We then used this model system to perform an expression quantitative trait locus (eQTL) study examining 94 genes from loci associated with Alzheimer’s disease, Parkinson’s disease, and multiple sclerosis. We found six loci (CD33, PILRB, NUP160, LRRK2, RGS1, and METTL21B) in which the risk haplotype drives the association with both disease susceptibility and altered expression of a nearby gene (cis-eQTL). In the PILRB and LRRK2 loci, the cis-eQTL was found in the MDMi cells but not in human peripheral blood monocytes, suggesting that differentiation of monocytes into microglia-like cells led to the acquisition of a cellular state that could reveal the functional consequences of certain genetic variants. We further validated the effect of risk haplotypes at the protein level for PILRB and CD33, and we confirmed that the CD33 risk haplotype altered phagocytosis by the MDMi cells. We propose that increased LRRK2 gene expression by MDMi cells could be a functional outcome of rs76904798, a single-nucleotide polymorphism in the LRRK2 locus that is associated with Parkinson’s disease.

INTRODUCTION

Genome-wide association studies (GWAS) and sequencing studies have implicated the innate immune system in neurodegenerative diseases, specifically Alzheimer’s disease (AD), Parkinson’s disease (PD), and multiple sclerosis (MS). An increasing number of disease-specific genetic loci that contain innate immune-specific genes are being identified. For example, CD33 is known to play a role in AD as shown by our group and others (1–3), and TREM2 has been implicated in frontotemporal dementia, PD, and amyotrophic lateral sclerosis in addition to AD (4–9). To leverage GWAS findings for therapeutic targeting, the GWAS associations must first be translated into functional outcomes, as has been done for CD33. We have demonstrated that the CD33 risk allele leads to increased CD33 expression on the surface of monocytes and to diminished internalization of amyloid-β 42 peptide, supporting a genotype-to-phenotype association for this AD single-nucleotide polymorphism (SNP) (1). Mapping gene expression as a quantitative trait [expression quantitative trait locus (eQTL) analysis] can identify which genes in a locus modulate their expression in response to a SNP; then, one can relate the direction of the association with expression to the direction of association with other traits, such as disease susceptibility. Because the number of these eQTL studies has increased, it has become clear that many eQTLs are dependent on cell type, activation state, or environment. This observation reflects the fact that epigenomic features that are specific to a differentiation state, for example, influence access to the sequence variant and hence influence the transcriptional output of the locus (10–12).

We have previously reported a number of monocyte-specific eQTL associations for MS, AD, and PD susceptibility variants (13). Given that MS, AD, and PD are all diseases of the central nervous system (CNS), we wanted to further examine these susceptibility loci in innate immune cells in the context of the CNS environment. Microglia are the resident innate immune cells of the CNS, and they are critical regulators of CNS homeostasis in health and of inflammatory responses in disease (14–16). There is also strong evidence that in diseases such as stroke, brain trauma (17), AD (18, 19), PD (20, 21), and MS (22), peripheral leukocytes, including monocytes, infiltrate the brain and differentiate into effector cells such as macrophages that are found at the site of pathology. Therefore, for genetic studies, we would ideally isolate these resident microglia and infiltrating macrophages from a large number of subjects. However, because of the difficulty in acquiring these cells from the human brain, alternative methods of obtaining these cells are needed. In one approach, patient-derived cells can be reprogrammed into human induced pluripotent stem cells (hiPSCs) and then differentiated into neurons or certain glial cells (23–25). The differentiation of hiPSCs into microglia, however, remains technically challenging, takes several months (26), and is currently not performed on the scale needed for genetic studies.

The investigation of genetically-driven changes in gene expression in microglia and infiltrating macrophages from patients with neurodegenerative diseases is limited by lack of access to these cells.
from the number of subjects required to perform well-powered genomic and functional analyses. However, a number of protocols do exist to differentiate human monocytes toward a microglia-like phenotype (27–32). These protocols have leveraged the plasticity of immune cells to direct human primary bone marrow–derived monocytes toward a microglia-like phenotype through coculture with astrocytes or astrocyte-conditioned medium, or via stimulation with recombination human cytokines. These differentiated cells have been shown to have a ramified morphology, a phagocytic ability including engulfing synapses, and a CX3CR1(CCR2−) phenotype (27, 29, 32). They also express the proteins P2RY12 and TMEM119 (32). Because these cells do not share the embryonic origin of the vast majority of resident microglia in the nondiseased brain (33), it is possible that they are more similar to infiltrating macrophages that have taken up residence in the CNS. There is currently no robust marker capable of distinguishing these two cell types in humans. A study by Etemad et al. (27) generated these cells by culturing ex vivo human monocytes in the presence of macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), nerve growth factor–β (NGF–β), and chemokine ligand 2 (CCL2), all of which are important for microglia development and survival (33–37). In addition, Ogihidani et al. (29) developed ramified microglia-like cells from human monocytes using a combination of GM-CSF and interleukin-34 (IL-34). IL-34 shares a receptor (CSF-1R) with M-CSF and is known to be important for directing the differentiation of microglia (38). These studies have shown that inducible microglia/macrophages display features observed in CNS resident microglia and that they are optimal for high-throughput studies to enable genetic analyses. Because the protocols use recombiant cytokines and serum-free media, potential batch-to-batch variation, which could arise from astrocytes or astrocyte conditioned media, is reduced.

Here, we used an in vitro model system composed of human monocytes differentiated using CNS cytokines that are known to be critical for the microglia microenvironment in vivo. We refer to these cells as human monocyte-derived microglia-like (MDMi) cells. We leveraged the capacity of this system to be deployed on a moderate scale to perform a genetic study of genes associated with susceptibility to several neurodegenerative diseases. To validate this in vitro system, we compared our human MDMi to human embryonic and induced pluripotent stem cell–derived microglia (ESC/iPSC) (26), ex vivo–derived human microglia (39), and murine microglia (40). Protocols that in vitro polarize microglia and macrophages toward proinflammatory “M1” and anti-inflammatory “M2” phenotypes allowed us to study molecular mechanisms that may help to distinguish microglia from other myeloid cells. It has been shown that the M1 phenotype of human microglia is distinct from the M1 phenotype of monocyte-derived macrophages (MDMs) (41), which has allowed us to characterize our inducible microglia/macrophages. With the generation of a detailed transcriptomic reference and an understanding of the characteristics of our model system in hand, we have evaluated the effect of common genetic variation on the expression of genes found in susceptibility loci for MS, PD, and AD.

**RESULTS**

**Differentiation of monocytes into MDMi cells**

We first identified the genes that are more highly expressed in our MDMi model system when compared to a tissue-level profile from human dorsolateral prefrontal cortex (DLPFC). Specifically, human peripheral monocytes from five young healthy individuals were stimulated with GM-CSF, M-CSF, CCL2, NGF–β, and IL-34 for 10 days and analyzed for gene expression using RNA-sequencing (RNA-seq). We then compared RNA-seq data from the MDMi model system to the RNA-seq data from the DLPFC of 238 individuals without a pathological diagnosis of AD. We then assembled a list of 368 genes with a threefold difference in expression in this analysis (fold-change genes) (table S1). These fold-change genes were then compared to published lists of microglia-enriched genes in ESC/iPSC-derived microglia (pMGL) (26), in ex vivo murine microglia (P60MG) (40), and in ex vivo human microglia isolated from resected brain tumor tissue or epileptic foci after surgery (HuMG) (39). There was more overlap in these gene lists among the three human models compared to the gene list for mouse microglia. We found that MDMi and pMGL shared 203 genes (55%) of the MDMi differentially expressed genes (Fig. 1A). Further, MDMi cells had 118 genes (32%) of the fold-change genes, in common with enriched genes in microglia from surgically resected human brain tissue (HuMG), whereas HuMG shared 100 genes (29%) of the pMGL-enriched genes. In comparison, MDMi cells only shared 24 genes (6.5%) with the enriched genes in murine p60 microglia (40). Similarly, the pMGL cells shared 19 genes (5.5%) and the brain tissue–derived microglia shared 34 genes (5.4%) with the enriched genes in murine p60 microglia.

The comparison of the data sets clearly demonstrated intermammalian differences in microglia gene expression; however, we also examined specific genes that have been reported to be key for microglial function in the murine system. For example, we found up-regulation of genes such as TGFBR1 (P < 0.0001), PROS1 (P = 0.0005), P2RX7 (P = 0.0058), and C1QB (P = 0.0007) (Fig. 1B) in our MDMi cell model system relative to ex vivo human peripheral blood monocytes and MDMs. Notably, P2RY12 and TMEM119, other murine microglia signature genes, were not up-regulated compared to ex vivo monocytes, although P2RY12 mRNA was up-regulated in MDMi cells compared to MDMs (fig. S1A). Therefore, we examined the proteins encoded by these genes and determined that both TMEM119 and P2RY12 were highly expressed in MDMi cells compared to monocytes from the same individuals (Fig. 1C and fig. S1, B to D). Microglia are thought to be long-lived cells, whereas monocytes and MDMs are thought to be more transient. To explore this, we kept the MDMi cells and the MDM cells in culture for 30 days and found that the apoptotic activator gene BAX was highly expressed in ex vivo monocytes and MDM cells cultured for 30 days, but not in MDMi cells (fig. S2).

We also evaluated the function of MDMi cells and demonstrated that, like primary human microglia, MDMi cells differed from peripheral monocytes with respect to their response to environmental stimuli (41). Under conditions driving an M1 but not an M2 phenotype, IL10 expression was significantly higher (P = 0.0018) in MDMi cells compared to macrophages from the same individuals (Fig. 1D), which is concordant with a published report (41).

**Functional consequences of genetic variants in human peripheral blood monocytes and MDMi cells**

Using a Fluidigm high-throughput quantitative polymerase chain reaction (qPCR) chip, we measured 94 genes found in loci associated with one of three neurodegenerative diseases (AD, MS, or PD) in MDMi cells from 95 young, healthy subjects of European ancestry with genome-wide genotype data. From this mRNA data set, we
performed an eQTL study to identify genotype-driven effects on the expression of nearby genes in MDMi cells [cis-eQTL analysis (cis-eQTL)] (Fig. 2A). Specifically, for each gene measured, we evaluated whether SNPs found within 1 Mb of the transcription start site of the measured gene were associated with the gene’s expression. At a false discovery rate (FDR) < 0.05, we identified cis-eQTL associations for 35 genes in MDMi cells (table S2). After linkage disequilibrium (LD) pruning of the list of SNPs with an MDMi eQTL (eliminating those SNPs with R² > 0.2, with the top SNP in a locus), 141 SNPs with a cis-eQTL remained. We compared our MDMi eQTLs to our previously published human peripheral blood monocyte eQTL data derived from 211 young, healthy subjects of European ancestry (13). We found that 82 (58.1%) of these SNPs were also cis-eQTLs in human monocytes (global FDR < 0.05) (table S3). Of those eQTLs that were shared, 100% (82 of 82) shared the same direction of effect in MDMi cells and monocytes, showing that differentiation did not affect the function of these variants. Correlations of the effect size for those SNPs with R² versus monocytes (R² versus monocytes) illustrates the fact that certain haplotypes influenced gene expression very differently in monocytes and MDMi cells, with clear peaks of associations in MDMi cells and no effect in our larger sample of subjects with monocytes that underwent transcriptional profiling.

**Association of disease-specific SNPs with gene expression in MDMi cells**

To provide mechanistic insights into the effect of GWAS-derived SNPs on disease susceptibility, we merged our cis-eQTL results with a list of disease-associated SNPs influencing AD (42, 43), PD (44), and MS (45). Of the 70 disease-associated SNPs within 1 Mb of our genes of interest, we found seven eQTL associations at a secondary, targeted FDR of 0.05 (Table 1). The SNP/gene expression associations for rs10838725/NUP160, rs701006/METTL21B, and rs1323292/RGS1 were also found in human peripheral blood monocytes (13); the associations for rs1476679/PILRB and rs76904798/LRRK2 appeared to be unique to MDMi cells (Table 1). Figure 3A (top) shows the cis-eQTL shared between monocytes and MDMi cells, where the association for RGS1 and the MS SNP rs1323292 was similar in both...
cell types. Figure 3A (bottom) shows an example of a differentiation state–specific cis-eQTL in which PTK2B and its respective disease-associated SNP rs28834970 was observed in the monocyte data set but not in the MDMi Fluidigm data set. On the other hand, significant associations were found in MDMi cells for the PILRB gene (thought to be an activating immune receptor) and the AD SNP rs1476679...
that were not seen in the human peripheral blood monocyte data set. Specifically, the rs1476679\(^T\) risk allele was associated with increased PILRB expression in MDMi cells (\(P = 0.00084\)) but not in monocytes (\(P = 0.19\)) (Fig. 3B, top). These results were replicated via PCR in a smaller study examining an independent set of individuals from whom we assessed both monocytes and MDMi cells (Fig. 3B, middle). PILRB protein expression mirrored the mRNA expression data in both monocytes and MDMi cells (Fig. 3B, bottom). In MDMi cells, a one-way analysis of variance (ANOVA) revealed a significant effect of genotype on PILRB mRNA \([F(2, 34) = 6.78, P = 0.0034]\) and protein \([F(2, 31) = 5.15, P = 0.0112]\) expression that was not present in monocytes \(\text{[mRNA]}: F(2, 34) = 1.67, P = 0.2037; \text{protein}: F(2, 31) = 2.14, P = 0.1346\].

Of particular interest, we examined the GWAS SNP for PD at the LRRK2 locus (44). The role of LRRK2 in PD has been of great interest because dominant mutations in the gene have been associated with familial PD (46). The PD GWAS rs76904798\(^T\) risk allele was associated with increased LRRK2 expression in MDMi cells \(\text{(}P = 8.92 \times 10^{-7}\text{)}\), whereas there was a nonsignificant trend \((P = 0.0177)\) in monocytes \((\text{Fig. } 4A)\). Thus, the differentiation of the monocytes to MDMi cells appeared to enhance the magnitude of the correlation between the PD SNP rs76904798 and LRRK2; the effect size of the SNP was significantly different between the two cell types \((P < 0.05)\). Critically, using the colocalization method (which assesses whether two association signals are consistent with a shared causal variant) (47), we found that the PD association and the eQTL SNP colocalized in MDMi cells \(\text{(}P = 0.0177)\), but not in monocytes \((\text{Fig. } 4B)\). This suggested that the disease SNP regulated gene expression in the in vitro MDMi cell model only and not in human monocytes.

### Differential expression of CD33 isoforms in MDMi cells

CD33 is a cell surface protein expressed by myeloid cells, and higher CD33 expression in the brain has been associated with more advanced cognitive decline and AD (2, 48). We recently demonstrated that individuals with the rs3865444\(^C\) risk genotype have increased CD33 on the surface of their monocytes compared to those with the rs3865444\(^A\) protective genotype (1). Furthermore, alternative splicing of CD33 generates two isoforms of the protein: full-length CD33\(^m\) and truncated CD33\(^m\), which lacks the immunoglobulin V-set domain encoded by exon 2 (49). The AD SNP rs3865444 or a SNP in high LD leads to alternative splicing of exon 2, which is the primary mechanism of the genetically driven differential expression of CD33 (3, 13).

Here, we found a significant effect of genotype on CD33 expression. There was an increase in full-length CD33\(^m\) mRNA expression \([F(2, 75) = 7.74, P = 0.0009]\) and a decrease in CD33\(^m\) mRNA expression \([F(2, 75) = 42, P < 0.0001]\) in MDMi cells from subjects with the rs3865444\(^C\) risk genotype; the changes in gene expression were dose-dependent (Table 1) (Fig. 5A). Similar findings were observed for the CD33 protein \((\text{Fig. } 5B)\). Densitometry analysis of the Western blot revealed a significant effect of genotype on CD33\(^m\) protein expression in monocytes \((P = 0.034)\) and MDMi cells \((P = 0.009)\) from the same individuals. The effect of genotype on CD33\(^m\) protein expression was only observed in MDMi cells \((P = 0.016)\) and not in monocytes \((P = 0.124)\) [as we had previously reported (13)]. The genotype-dependent difference in CD33 surface expression was further confirmed in MDMi cells using high content imaging \((P = 0.001; \text{Fig. } 5C)\). Myeloid cells, such as infiltrating macrophages or microglia, are thought to be involved in AD through phagocytosis of amyloid-\(\beta\) that accumulates in neuritic amyloid plaques, a neuropathological feature of AD (50). Thus, we tested whether MDMi cells from subjects with the rs3865444\(^C\) CD33 risk allele showed reduced activation of microglia and, therefore, a reduced phagocytic ability compared to MDMi cells from subjects with the protective allele. We found reduced phagocytic uptake of fluorescently labeled dextran in MDMi cells bearing the rs3865444\(^C\) risk allele \((P = 0.047; \text{Fig. } 5D)\), similar to what we had previously reported for monocytes (1).

### DISCUSSION

The recent identification of microglia-specific gene signatures has enabled us to examine the phenotype of MDMi cells through gene expression changes rather than subtle shifts in mean fluorescence intensity representing the expression of specific surface markers or changes in morphology. Here, we show that monocytes cultured for 10 days in the presence of specific cytokines up-regulate expression of microglia-specific genes, including the key microglial gene TGFBR1, compared to monocytes not treated with cytokines (51). Other genes highly expressed by MDMi cells include C1QB, a critical mediator of synaptic refinement and plasticity (52), and PROS1, which directs microglia to phagocytose apoptotic cells (53). A role for P2RX7, another highly expressed MDMi gene, has been described by Monif et al. (54), who found that P2RX7 drives microglia activation and proliferation. Thus, MDMi cells express many genes that are important for microglia function.

There is no single functional assay that defines microglia. Several groups have used the ability to phagocytose material as a functional...
readout; however, other myeloid cells, including monocytes and macrophages, share this ability. Synaptic pruning is another characteristic function, but this is difficult to model in vitro. We leveraged a recent study (41) that characterized the functional properties of human microglia in vitro by assessing cytokine production when they were driven toward an M1 or M2 inflammatory phenotype. Consistent with this study, we demonstrate here that, under M1 conditions, MDMi cells produced more IL-10 compared to MDM cells; no difference was seen between MDMi cells and MDM cells under M2 conditions. We also found that the MDMi cells survived for up to 30 days in culture without up-regulating the proapoptotic gene BAX in contrast to MDM cells, which did up-regulate BAX and showed increased cell death. These findings highlight the functional differences between MDMi cells and MDM cells.

It is difficult to differentiate human microglia and CNS-infiltrating blood-derived macrophages; the role of infiltrating blood-derived macrophages in the brain remains poorly understood but may be related to disease. In AD, inflammation and microglial activation have
been extensively studied, but the contribution of blood-derived immune cells remains unclear. Recent data suggest that blood-derived monocytes may play a role in amyloid-β clearance. For example, more perivascular amyloid-β was observed in CCR2-deficient Tg2576 AD mice, which show reduced early recruitment of monocytes (35), compared to Tg2576 mice expressing CCR2. In support of this protective role, increasing monocyte recruitment has been reported to delay the progression of AD (55, 56). On the other hand, infiltrating monocytes may enhance tissue damage in MS. For example, MDMs were found to initiate demyelination in the experimental autoimmune encephalomyelitis (EAE) rodent model of MS (57). Furthermore, blocking monocyte recruitment to the CNS blocked EAE progression, suggesting that these infiltrating cells may be required for disease progression in MS (58). An argument could be made that our MDMi cell model system more likely represents blood-derived macrophages that infiltrate the CNS rather than embryonically derived microglia, but we need more knowledge about these two cell types before we can decide which cell type our model more faithfully recapitulates.

GWAS studies have identified new loci associated with disease susceptibility. In determining the mechanistic outcomes of these genetic variants, it has been demonstrated that eQTLs are context-dependent, existing only in certain cell types and activation states (12, 13). To extend our understanding of the modulation of gene expression by genetic variants in our in vitro model of microglia, we associated MDMi RNA expression with SNPs. We examined the expression of 94 genes in loci associated with increased risk of developing AD, MS, or PD in MDMi cells from 95 young, healthy individuals and compared their expression to microarray data from peripheral blood monocytes from 211 individuals generated in an earlier study (13). The expression of many of these genes was affected by genetic variation in the MDMi cells. For a number of genes, genetic regulation of gene expression was determined by completely different SNPs in the MDMi cells compared to the monocytes (Fig. 2). This demonstrates that the genotype-driven gene expression differences between monocytes and MDMi cells can be context-specific and highlights the importance of examining...
genotype-induced gene expression differences in CNS-relevant cell types.

A striking example of a cell type–specific cis-eQTL was found in the PILRB gene. We found an association in MDMi cells between PILRB gene expression and the AD SNP rs1476679 that was not seen in the monocyte eQTL data set. On the basis of a Bayesian colocalization analysis (47) that sought to determine whether our MDMi eQTL and the International Genomics of Alzheimer’s Project stage 1 (42) AD susceptibility GWAS effects were driven by a shared causal variant, it appeared more likely that the eQTL and AD GWAS effects were not driven by a shared variant (posterior probability eQTL and AD associations driven by a shared causal variant = 0.04). This result suggested that whereas the AD SNP displayed some association with PILRB expression, the effect of the rs1476679 SNP was probably mediated by another mechanism, and the association may be the result of partial LD between the AD and eQTL causal variants.

Activation of monocytes has been shown to highlight different eQTL associations (12). We found that the differentiation of MDMi cells identified additional associations (for example, with the PILRB gene) and enhanced some associations that were weakly seen in monocytes (for example, as observed for LRRK2). Pathogenic mutations in LRRK2 are the most common genetic cause of familial PD (59, 60). High LRRK2 expression has been discovered recently in myeloid cells, but not in T cells, suggesting a functional role for LRRK2 in the innate immune system (61). In addition, LRRK2 is expressed in microglia where it modulates the proinflammatory response in these cells (62). In our data, the monocyte eQTL SNP rs10784428 (56) was associated with LRRK2 expression in both monocytes and MDMi cells, whereas the PD-associated SNP rs76904798 was associated with LRRK2 expression only in MDMi cells (Fig. 4). This suggested that LRRK2 may have a context-specific role that may also vary with disease state. These results highlight the potential use of MDMi cells for studying the functional role of the LRRK2 variant in microglia.

On the basis of the expression of CD33 in human microglia (1) and our recent characterization of the AD risk gene CD33 in monocytes where we found increased CD33 surface protein in individuals carrying the CD33 rs3865444C/rs6714679C risk genotype, we explored whether this was also the situation in MDMi cells. We found that MDMi cells recapitulated the previously described expression and functional effects of the risk allele, but we also observed differential protein expression of the truncated CD33 in MDMi cells that was not seen in monocytes (63). Notably, whereas the CD33 locus has been associated with AD at a threshold of genome-wide significance (64), it fell below that threshold in a recent analysis (42). Further evaluation of these data and additional replication data supporting the role of CD33 in non-European populations (65–67) and AD-related endophenotypes (1) support its association with AD. Functional studies also link CD33 to TREM2 (68), complementing these genetic findings. Regardless of the association with AD, the CD33 variant has an important functional effect on myeloid cells. The differential effect of the CD33 variant in MDMi cells compared to monocytes suggests that the disease-associated effect of rs3865444 may differ subtly between microglia and monocytes. To confirm this, future work should investigate whether the isoform-specific pattern we observed is also seen in postmortem primary human microglia from genotyped individuals.

Our study presents the detailed characterization of an in vitro cellular model system that can be used to investigate the effects of genetic variants associated with neurodegenerative diseases on human innate immune cells. The characterization of eQTL effects using MDMi cells illustrates the utility of the model system for moderate-throughput experiments in which 95 different subjects were examined in parallel. MDMi cells may offer an important tool for translational studies that could complement data from biopsy-derived or autopsy-derived microglia and macrophages.

However, there are some limitations to our study that should be considered. First, MDMi cells were derived from peripheral blood monocytes and may be different from microglia derived from the yolk sac, especially in terms of epigenetic changes that occur over a lifetime. Despite this, our data suggest that MDMi cells may enable the study of cells that share many features with microglia, for example, brain macrophages that differentiate from infiltrating monocytes exposed to the CNS cytokine milieu. As we continue to use this cellular system to model human microglia and macrophages, we will identify additional strengths and limitations of this model system, as has been the case for monocyte-derived dendritic cells, which are routinely used to model in vivo dendritic cells (69).

A second limitation is that monolayers of MDMi cells do not resemble the three-dimensional (3D) spatial arrangement of microglia in the CNS, and stiff plastic culture dishes do not resemble the physical environment of the brain. Therefore, a recently described 3D culture system using a collagen construct may enable the study of these MDMi cells in a more relevant tissue-like microenvironment (70). In addition, because of unknown reasons, we found that in about 10% of our subjects, their monocytes did not survive the differentiation process. This may have implications in studies where the sample size is small. To control for this, we excluded subjects from analysis who had poorly differentiated MDMi cells based on visual observations of morphology.

Notably, the MDMi cells in the present study were derived from frozen peripheral blood mononuclear cells (PBMCs). It should be possible to collect many PBMC samples and bank them for use in large-scale studies, such as the eQTL analyses presented here, that are performed in a single batch, thus minimizing the effect of experimental variation. In addition, the use of a well-defined set of recombinant cytokines to generate MDMi cells will further reduce variability compared to approaches involving astrocyte-conditioned medium, for example.

In terms of understanding the functional outcomes of GWAS SNPs, it is now clear that they must be examined in the correct cell type and context. Because of the limitations of acquiring human microglia in sufficient quantity from large numbers of human subjects (as is needed for genetic association studies) and a lack of a cell line that adequately mimics human microglia, we suggest that the MDMi cell model system could overcome this challenge. In addition to genetic studies, MDMi cells may be useful in drug screening where biochemical or functional changes in response to drugs could be monitored when microglia are thought to be the target cell type. Additional potential applications of MDMi cells may include the exploration of various biological behaviors of human microglia in neurodegenerative disorders. Coculturing MDMi cells with human neurons and astrocytes would enable the modeling of innate immune dysfunction in a more complex system where the target cell is interacting with other relevant cell types. Finally, given that the differentiation protocol takes just 10 days, one could envisage that in the future, MDMi cells derived from a patient’s monocytes could be rapidly characterized to help with drug selection and disease management (71). In conclusion, we propose that our in vitro translational
tool for generating microglia-like cells quickly and easily from adult blood could be useful for exploring microglia function and dysfunction.

**MATERIALS AND METHODS**

**Study design**

The overall aim of the study was to characterize and refine a translational research tool in which microglia-like cells were generated from human peripheral blood monocytes in the presence of specific cytokines. We refer to these cells as MDMi. The objective of the first portion of the study was to determine whether MDMi cells expressed known microglia genes and whether they had a functional phenotype that was similar to microglia. To do this, we derived MDMi cells from the peripheral venous blood of healthy control volunteers from the PhenoGenetic Project. This is a living tissue bank that consists of healthy subjects who are recontactable and can therefore be recalled based on their genotype. A total of 1753 healthy subjects >18 years of age have been recruited from the general population of Boston. Subjects were free of chronic inflammatory, infectious, and metabolic diseases and are of diverse ethnicities (29% are non-Caucasian), and 62.7% are women. The median age was 24 years. The objective of the second portion of the study was to extend our understanding of the genetic modulation of gene expression in our in vitro MDMi cell model. To do this, we associated MDMi RNA expression with SNPs. We used this model system to perform an eQTL study examining the expression of 94 genes in loci associated with increased risk of developing AD, MS, or PD in MDMi cells from 95 young, healthy individuals from the PhenoGenetic Project. We compared the expression of these genes in MDMi cells to microarray data from monocytes from 211 individuals (also from the PhenoGenetic Project) generated in an earlier study (13). For the eQTL analysis in monocytes (n = 211), which included only Caucasians, the median age was 25 years and 56.9% were women. For the eQTL analysis in MDMi cells (n = 95), also from Caucasian subjects only, the median age was 29 years and 67.4% were women. Informed consent was obtained from all human subjects. All blood draws and data analyses were done in compliance with protocols approved by the institutional review boards of each institution.

**Induction of MDMi and MDM**

PBMCs were separated by Lymphoprep gradient centrifugation (StemCell Technologies). PBMCs were frozen at a concentration of \(1 \times 10^7\) to \(3 \times 10^7\) cells ml\(^{-1}\) in 10% dimethyl sulfoxide (DMSO; Sigma-Aldrich) or 90% (v/v) fetal bovine serum (FBS; Corning). Before each study, aliquots of frozen PBMCs from the PhenoGenetic cohort were thawed and washed in 10 ml of phosphate-buffered saline. Monocytes were positively selected from whole PBMCs using anti-CD14 microbeads (Miltenyi Biotec) and plated at the following densities per well: 1 \(\times 10^5\) cells (96-well plate), 3 \(\times 10^5\) cells (24-well plate), and 1 \(\times 10^5\) cells (6-well plate). To induce the differentiation of MDMi, we incubated monocytes under serum-free conditions using RPMI-1640 Glutamax (Life Technologies) with 1% penicillin/streptomycin (Lonza) and Fungizone (2.5 µg/ml; Life Technologies) and a mixture of the following human recombinant cytokines: M-CSF (10 ng/ml; BioLegend, 574806), GM-CSF (10 ng/ml; R&D Systems, 215-GM-010/CF), NGFβ (10 ng/ml; R&D Systems, 256-GF-100), CCL2 (100 ng/ml; BioLegend, 571404), and IL-34 (100 ng/ml; R&D Systems, 5265-IL-010/CF) under standard humidified culture conditions (37°C, 5% CO₂) for up to 15 days. MDMs were generated by incubating monocytes with M-CSF (20 ng/ml) in RPMI plus 10% FBS. The cells were used for experiments and characterization at different time points as indicated. For the 30-day culture, both MDM and MDMi cells were moved on day 15 to unconditioned RPMI media until day 30.

**Statistical analyses**

Statistical analyses for Figs. 1 (A and D) and 5 (B to D) were performed using Prism 5 (GraphPad Software). Comparisons of protein and mRNA expression data across groups were analyzed by Student’s t test or one-way ANOVA as appropriate, and significant differences were deconvoluted using Tukey’s multiple comparison test. Probability values of <0.05 were considered to represent statistically significant differences.

**SUPPLEMENTARY MATERIALS**

www.sciencetranslationalmedicine.org/cgi/content/full/9/421/eaai7635/DC1

Materials and Methods

Fig. S1. Higher expression of P2RY12 and TREM119 in MDMi cells compared to monocytes.

Fig. S2. The proapoptotic gene BAX is increased in monocytes and MDMi cells compared to MDMi.

Fig. S3. Many SNPs have differential eQTLs in MDMi cells compared to monocytes.

Table S1. Differentially expressed genes in MDMi cells compared to bulk brain tissue.

Table S2. Cis-eQTLs in MDMi cells.

Table S3. LD-pruned list of cis-eQTLs in MDMi cells.

References (72–77)


Ryan et al., Sci. Transl. Med. 9, eaai7635 (2017) 20 December 2017
A human microglia-like cellular model for assessing the effects of neurodegenerative disease gene variants

Katie J. Ryan, Charles C. White, Kruti Patel, Jishu Xu, Marta Olah, Joseph M. Replogle, Michael Frangieh, Maria Cimpean, Phoebe Winn, Allison McHenry, Belinda J. Kaskow, Gail Chan, Nicole Cuerdon, David A. Bennett, Justin D. Boyd, Jaime Imitola, Wassim Elyaman, Philip L. De Jager and Elizabeth M. Bradshaw

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The secret life of microglia

In a new study, Ryan et al. characterized a cellular model of monocytes differentiated into a microglial-like state. They then demonstrated the effectiveness of this human in vitro cell model system for identifying functional consequences of genetic variation associated with neurodegenerative disease risk by performing a cis-expression quantitative trait locus (cis-eQTL) study. The authors identified six neurodegenerative disease–associated loci with genotype-driven gene expression differences in their cell model system. Two of these cis-eQTLs were not seen in ex vivo human monocytes, emphasizing the importance of examining the cell type of interest when investigating functional consequences of genetic variants.