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Common Genetic Variants Modulate Pathogen-Sensing Responses in Human Dendritic Cells

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Supplementary Materials: Materials and Methods

Supplementary Text

Figs. S1 to S5

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Abstract

Little is known about how human genetic variation affects the responses to environmental stimuli in the context of complex diseases. Experimental and computational approaches were applied to determine the effects of genetic variation on the induction of pathogen-responsive genes in human dendritic cells. We identified 121 common genetic variants associated in *cis* with variation in expression responses to *E. coli* lipopolysaccharide, influenza or interferon- β (IFN β). We localized and validated causal variants to binding sites of pathogen-activated STAT and IRF transcription factors. We also identified a common variant in *IRF7* that is associated in *trans* with type I interferon induction in response to influenza infection. Our results reveal common alleles that explain inter-individual variation in pathogen sensing and provide functional annotation for genetic variants that alter susceptibility to inflammatory diseases.

Introduction

Susceptibility to complex diseases depends on both genetic predisposition and exposure to environmental factors, with interactions between the two (GxE interactions) likely contributing substantially to disease risk (1, 2). However, the extent and mechanisms by which common human genetic variants interact with the environment remain poorly explored and have been difficult to detect in clinical studies (1, 3). Genetic analysis of molecular traits – such as gene expression profiles – offers a promising way to dissect the molecular mechanisms underlying GxE interactions. Expression quantitative trait locus (eQTL) studies have been used to map genetic variants contributing to variation in gene expression, but have largely focused on steady state expression in humans (4, 5), thus excluding GxE interactions. In model organisms, differences in growth conditions or treatment with various stimuli have revealed the existence of response eQTLs (reQTLs) (6-9), defined as QTLs associated with the change in expression after stimulation. Here, we sought to identify reQTLs in humans, to explain the mechanism by which the environment interacts with these variants, and to determine whether these variants are associated with immune-mediated diseases.

We used dendritic cells (DCs) of the innate immune system as a model system for reQTL studies, with physiological and clinical relevance. DCs play a direct role in the host recognition of pathogens using specialized sensors that engage well-characterized signaling and transcriptional networks. For example, bacterial lipopolysaccharide (LPS) activates two distinct arms of the Toll-like receptor 4 (TLR4) pathway, whereas influenza infection primarily activates the RNA-sensing Toll-like receptors (e.g., TLR3) and the RIG-I-like receptors (e.g., RIG-I) (10). These, in turn, lead to the translocation of transcription factors from the cytoplasm into the nucleus to induce the expression of immune genes, including IFN β secretion that engages the type I interferon response pathway to induce the expression

of hundreds of anti-viral effectors. Genetic studies have associated common variants near many genes in these pathways with risk of different inflammatory diseases (11, 12). DCs also play a critical role in the pathologic immune responses underlying inflammatory diseases (11-13), also reflected in recent genome-wide association studies (GWAS) of several diseases (14-17), especially inflammatory bowel disease (14).

Results

Assessing the impact of genetic variation on pathogen-sensing in primary human DCs

We developed an integrated experimental and computational pipeline (Fig. 1A) to identify variability in human DC responses and associate this variability with common genetic variants. First, we optimized a high-throughput protocol to isolate primary CD14⁺CD16^{lo} monocytes from human blood samples (figs. S1A–S1F), differentiate them into monocyte-derived dendritic cells (MoDCs), and stimulate them with three immunostimulatory agents (Fig. 1B): *E. coli* lipopolysaccharide (LPS), influenza virus, or IFN β (a cytokine induced by LPS and influenza). Second, we collected genome-wide transcript profiles from resting and stimulated DCs from 30 healthy individuals and computationally identified a ‘signature’ of 415 genes that would be informative of variation in the response in a larger cohort. Third, we generated 1,598 transcriptional profiles (using an amplification-free platform suitable for small cell numbers (18)) from DCs isolated from 534 healthy individuals (295 Caucasians, 122 African-Americans, 117 East Asians; table S1) in four states: resting, LPS-stimulated, influenza-infected and IFN β -stimulated. Finally, we associated expression variation with genetic variation to identify *cis*- and *trans*-eQTLs and reQTLs, and investigated candidate reQTLs for their mechanism of action.

Variability of pathogen-sensing responses between individuals

To assess the inter-individual variability of DC responses, we first profiled genome-wide expression using microarrays in resting, LPS-stimulated and influenza-infected MoDCs isolated from each of 30 healthy individuals (18 Caucasians, 6 Asians, 6 African-Americans). We found 1,413 genes that were regulated in LPS- or influenza-treated cells [$\log_2(\text{fold change}) > 0.75$ or < -1.5 ; FDR < 0.01 ; fig. S2A, table S2A; see table S2B–I for enriched biological processes] at 5 hrs and 10 hrs, respectively (time points selected to have maximal expression of induced clusters; figs. S1B and S1C).

We quantified reproducibility in these responses by recalling 12 of the 30 donors 2-9 months after the first collection for MoDC isolations and profiling (table S1); we identified genes whose inter-individual expression variance is significantly higher than their intra-individual variance based on the serial replicates (taking into account known covariates including gender, age and population and unknown ones using Surrogate Variable Analysis (SVA) (19)). 222 of the 1,413 regulated genes (16%) showed significantly higher (FDR < 0.1) inter- than intra-individual variability, either in their absolute expression or in the differential expression (stimulated/baseline level) to at least one stimulus (Fig. 2A and table S3). These results suggest that there is consistent variation in these traits that may have a genetic basis.

Expression profiling of a pathogen response signature

Mapping the genetic basis of inter-individual variation in pathogen responses requires profiling of DC gene expression in a larger cohort; this poses a substantial technical challenge given the limited numbers of primary cells and multiple stimuli. Furthermore, the responses of DCs to virus, bacterial ligands and interferon are limited in scope, and do not encompass the entire genome. We therefore defined a 415 gene signature set (Fig. 2B and table S3) that could be monitored in small numbers of cells using a sensitive multiplex RNA detection system (18), allowing us to scale up our study. The signature consisted of: (1) all 222 of 1,413 regulated genes with greater inter- than intra-individual variability in the microarray study; (2) all 24 regulated genes exhibiting greater inter- than intra-population variability (FDR < 0.1; table S3) in the microarray study; (3) 76 genes comprising the known components of the TLR4, TLR3, RIG-I and IFNAR pathways (Fig. 1B) (10, 20-22); (4) 61 regulated genes that play key roles in the DC response (e.g. *IFNB1* and *IFITM3*) (22-24); (5) 28 regulated genes residing in loci previously associated with autoimmune or infectious diseases (25); and (6) 35 control genes including those that had among the lowest inter-individual variance in the microarray profiles. This representative signature allowed us to monitor genes with high inter-individual variability, as well as key components and responses of the pathogen-sensing pathway.

We then generated 1,598 transcriptional profiles, using the 415-gene signature, from MoDCs isolated from 534 healthy individuals (and 37 serially-collected replicates) in up to four conditions: resting (528 individuals), LPS-stimulated (356), influenza-infected (342) and IFN β -stimulated (284) (Figs. 2C, S1E, S1F, table S1). The IFN β stimulus allowed us to partition genes that were induced by both LPS and influenza (cluster III) into those induced secondary to type I IFN signaling (cluster IIIa) or by other mechanisms shared between these bacterial and viral sensing pathways (cluster IIIb) such as NF- κ B or AP-1 activation (Figs. 2C and 1B). The signature expression profiles clustered similarly to those from the microarray analysis (Fig. 2C and fig. S2C), and captured most of the variance in the genome-wide profile (cross validation, >0.99 gtPCC; fig. S2D (26)), demonstrating the utility of the signature to capture the genome-wide response.

reQTLs modulate cellular responses to pathogens

We mapped *cis*-eQTLs and *cis*-reQTLs by testing for association between common SNPs (minor allele frequency >5%; genotyped with Illumina Human OmniExpress BeadChip) and variation in either absolute transcript abundance (eQTL) or stimulation-induced change in transcript abundance (reQTL) in a nearby gene (in which the transcriptional start site or stop codon is within 1Mb of the SNP). We pooled individuals from the three human populations together and included covariates and principal components to increase statistical power while avoiding systematic confounders such as population structure and expression heterogeneity. We identified 264 genes with *cis*-eQTLs in at least one condition (permutation FDR < 0.05; Fig. 3A, figs. S3A–C, table S4A–D) (27). Notably, 22/264 genes also associated with additional independent *cis*-eQTLs after conditioning on the top five most significant SNPs in each *cis*-eQTL region (table S6 and fig. S3D) (27), reflecting the complexity of the regulatory landscape around each gene.

We detected 121 *cis*-reQTLs (Fig. 3B and table S4E–G; permutation FDR < 0.05; 91% internal reproducibility in at least 2 human populations, table S5), the subset of genetic variants that affect the induction of gene expression by LPS, influenza or IFN β . Of the 121 genes with *cis*-reQTLs, 7 associations were found only in the influenza condition (e.g., *IFNA21*, Fig. 3C and fig. S3B; meta-analysis, $P < 1 \times 10^{-5}$, permutation FDR 0.02–0.03), 15 in both the LPS and influenza conditions (e.g., *TEC*; Figs. 3C and S3B), and 57 in all three stimulation conditions (e.g. *ARL5B*, *SLFN5*, *CLEC4F*; Figs. 3C–3E and S3B), likely reflecting activation of the shared IFN β pathway. We hypothesized that causal variants driving *cis*-reQTLs alter the sequence of genomic elements that respond to transcription factors downstream of the pathogen-sensing receptors (Fig. 3F), and represent gene-by-environment interactions.

To validate *cis*-reQTLs, we quantified allele-specific expression for pan-stimulation-specific associations in heterozygote individuals (Figs. 3D and 3E). This was feasible for a gene with an exonic SNP (*CLEC4F* rs2075221) that was the most significant reQTL SNP, and for a gene with an exonic SNP (*SLFN5* rs11651240) in linkage disequilibrium (LD) with the most significant reQTL SNP (rs11867191, $R^2 = 0.501$ CEU). As predicted, transcripts derived from the major and minor alleles differed in expression after stimulation with LPS (*SLFN5*, $P < 0.001$, t-test; *CLEC4F*, $P < 0.01$), influenza (*SLFN5*, $P < 0.001$; *CLEC4F*, $P < 0.01$) or IFN β (*SLFN5*, $P < 0.001$; *CLEC4F*, $P < 0.001$) but not at baseline (Figs. 3D and 3E).

***cis*-reQTLs that alter the sequence of TF binding sites**

We hypothesized that the causal variants underlying *cis*-reQTLs functionally alter the chromosomal binding sites of transcription factors activated by one or more stimuli. To fine map reQTLs, we performed a *trans*-ethnic meta-analysis (28) of imputed variants in each population (~10M). We then examined whether the most highly associated meta-reQTLs overlap with transcription factor (TF) binding sites identified in high-throughput human ChIP-Seq datasets (e.g., ENCODE) or computationally-predicted conserved regulatory elements (29–31). We found substantial enrichment (table S7) of known binding sites for TFs from the STAT family (TFs that are known to be activated downstream of Type I IFN signaling). The most significant enrichment over background was found for STAT2 (116-fold, binomial $P < 2.55 \times 10^{-21}$) and STAT1 (126-fold, binomial $P < 2.98 \times 10^{-13}$) binding sites (derived from ChIP-seq in IFN α -stimulated K562 cells) within *cis*-eQTLs after IFN β stimulation.

Among the 57 genes (e.g., *SLFN5*, *CLEC4F*, *ARL5B*; Fig. 3F) with *cis*-reQTLs in all 3 stimuli, we observed that the most significant *cis*-reQTL in the: (1) *SLFN5* locus (rs11080327) lies in an ENCODE ChIP-Seq signal (29) for STAT1 (Figs. 4A–C); (2) *CLEC4F* locus (rs35856355) alters a commonly occurring cytosine in a canonical ISRE (interferon-stimulated response element) that is a target of IFN-activated transcription factors (Figs. 4B and 4C) (22, 30–32); and (3) *ARL5B* locus (rs2130531) changes a guanine in the canonical ISRE motif (Figs. 4B and 4C). Additional *cis*-reQTLs that alter putative STAT binding sites based on ChIP-Seq data or predicted ISRE motifs include: rs10086852 (*PTK2B*), rs1981760 (*NOD2*), rs73023464 (*C19ORF12*), rs1331717 (*IFI44*) and rs12064196 (*IFI44*) (Fig. 4A). Because STAT transcription factors are activated downstream of the type

IFN receptor (IFNAR) and bind to ISRE motifs (Fig. 4A) (22), we hypothesized that the SNPs in these sites are likely causal SNPs within their respective *cis*-reQTL regions.

***cis*-reQTLs affect differential binding of stimulus-activated transcription factors**

To experimentally validate our predicted regulatory mechanisms, we determined whether IFN β -activated transcription factors bind differentially at these SNPs. Using radiolabeled 24-26 bpdsDNA probes in electrophoretic mobility shift assays (EMSA), we found that probes encompassing the major alleles (rs35856355^C and rs2130531^G, which are associated with increased expression in *CLEC4F* and *ARL5B*), but not the minor alleles, shifted after incubation with IFN β -stimulated MoDC nuclear lysates (Fig. 4D). Consistently, non-radiolabeled major but not minor allele probes competed for binding to the IFN β -stimulated nuclear factors (fig. S4A). By incubating with antibodies to transcription factors known to bind the ISRE element, we found that IRF1 super-shifted the low MW band and STAT2 and IRF9 super-shifted the higher MW band, consistent with the known complex of these latter two proteins (32) (Fig. 4D). These results suggested that the SNPs in *CLEC4F* and *ARL5B* alter the binding of IFN-activated IRF1, STAT2 and IRF9 transcription factors.

To directly test the functional effects of the SNPs, we cloned 150-200 bp genomic regions surrounding rs11080327 (*SLFN5*), rs35856355 (*CLEC4F*) and rs2130531 (*ARL5B*) upstream of a luciferase reporter driven by a minimal promoter (Fig. 4E). For each region, we created two constructs that differ only at the respective SNPs. Since almost all cell types respond to IFN β stimulation, we transfected the reporter constructs into HEK-293 cells and stimulated the cells with IFN β . Consistent with our hypothesis, the constructs containing the major alleles of rs11080327 (*SLFN5*), rs35856355 (*CLEC4F*) and rs2130531 (*ARL5B*) induced respectively 11.6-fold, 2.1-fold and 1.5-fold more luciferase production than the cognate constructs containing the minor alleles (t-test, $P < 0.001$, $P < 0.001$ and $P < 0.01$, respectively; Fig. 4E). Furthermore, mutation of motif-conserving nucleotides across the *CLEC4F* ISRE motif reduced induction by IFN β while mutation of non-conserved nucleotides did not (fig. S4B), consistent with the ISRE consensus motif.

Finally, to further test (33) the functionality of rs11080327 – the *SLFN5* variant with the strongest functional effect – in its native chromosome, we directly edited the genome using the CRISPR system (34, 35), converting the heterozygous rs11080327^{A/G} in HEK-293 cells to a homozygous rs11080327^{G/G} (Fig. 4F and fig. S4C). We then stimulated both native and CRISPR-converted cells with IFN β . The fold induction of *SLFN5* decreased from 3.64 in heterozygous cells to 1.03 in the converted rs11080327 (G) homozygotes, while the induction of other genes was unaffected (Fig. 4F and fig. S4C).

The enrichment, EMSA, reporter assays and directed mutagenesis results suggest that we have identified causal variants in some of these *cis*-reQTL regions and demonstrate that the reQTLs occur due to differential binding of stimulus-activated transcription factors.

A *cis*-eQTL associating with *IRF7* is a *trans*-reQTL

We next attempted to detect cases where variation in gene expression is explained by distant genetic variants acting in *trans*. We noted that *cis*-eQTLs for regulator genes tend to have

smaller effect sizes and less significant *P*-values than those for regulated genes (fig. S3E). To decrease the multiple testing burden of detecting *trans* associations, we restricted testing SNPs local to genes on our gene signature set. A number of these are *cis*-eQTLs associated with the expression of genes encoding regulators (*e.g.*, transcription factors) in pathways that we stimulated (Fig. 5A). Of these, rs12805435 was the most significant, associating with the expression of the master anti-viral transcription factor *IRF7* in *cis* only after influenza, LPS or IFN β stimulation (table S4A–D). This SNP associated with both the expression and induction of 7 additional genes in *trans* (*i.e.* the transcriptional start site or stop codon is located > 1 Mb from the SNP) only after influenza infection: *NMI*, *IFNA4*, *IFNA10*, *IFNA13*, *IFNA21*, *IFNA17* and *IFNA5* ($P < 6.26 \times 10^{-5}$ in expression, $P < 1.68 \times 10^{-5}$ in differential expression; Fig. 5B and table S8–10).

To test whether the *trans*-associated genes are indeed targets of *IRF7*, we infected MoDCs that overexpressed *IRF7* or a control gene with influenza virus, and found that *IRF7* overexpression induced the expression of *IFNA2*, *IFNA13* and *IFNA14* in influenza-infected cells (Fig. 5C). In addition, in HEK-293 cells, which normally express very low levels of *IRF7*, we overexpressed *IRF7* and observed induction of *NMI*, *IFNA4*, *IFNA10*, *IFNA13*, *IFNA14* and *IFNA21* (Fig. 5D), suggesting that *IRF7* is sufficient to drive downstream expression. We have thus identified a stimulus-specific *cis*-eQTL associated with *IRF7* expression, which is also a *trans*-reQTL that underlies the variability of IFN induction in response to influenza infection in humans.

reQTLs in DCs overlap with autoimmune and infectious disease SNPs from GWAS

We examined the subset of loci associated in genome-wide association studies (GWAS) with inflammatory disorders. We first extended an analysis of Crohn's disease in which loci are enriched for genes specifically expressed in DCs (12, 14) to multiple sclerosis, celiac disease, psoriasis and leprosy (fig. S5A). We found that genes nearest to the susceptibility loci for these diseases were not only enriched in DC-specific genes but also in genes induced by LPS and/or influenza (fig. S5B), suggesting that some of the GWAS loci modulate the expression of the corresponding genes in activated DCs.

Supporting this hypothesis, 15 *cis*-reQTLs and 23 *cis*-eQTLs that were not significant at baseline but only became significant after stimulation are the same SNPs previously identified in GWAS of autoimmune and infectious diseases, including Crohn's disease, multiple sclerosis, celiac disease, psoriasis and leprosy (25) (Figs. 6A, 6B and table S11). These include: *NOD2* with leprosy (rs9302752), *IRF7* with systemic lupus erythematosus (rs4963128), *TRAF1* with rheumatoid arthritis (rs881375), and *CREM* with Crohn's disease (rs12242110) and ulcerative colitis (rs4246905). For example, rs9302752 – a SNP previously associated with susceptibility to leprosy (17) – was associated in our study with expression of *NOD2* and its response under IFN β stimulation ($P = 3.49 \times 10^{-25}$) but not at baseline (Fig. 6A and table S11); *NOD2* plays a known role in pathogen sensing and possibly mycobacterial immunity (36). Similarly, rs4963128 – a variant associated with SLE (37) – was associated in our study with the expression of *IRF7* after IFN β stimulation ($P = 1.10 \times 10^{-16}$) but not at baseline (Fig. 6A), in line with the importance of type I IFN responses in SLE pathogenesis (38). We note that rs4963128 is on the same haplotype ($R^2 =$

0.69, $D' = 0.94$) as the *IRF7* SNP rs12805435 that is associated with the *trans*-reQTL effect described above. These results suggest a role for innate immune pathogen-sensing pathways in the pathogenesis of these inflammatory disorders.

Discussion

While genetic association studies have identified alleles that confer disease risk, little is known about how these genetic variants contribute to disease through their effects on specific biological processes and their interaction with environmental stimuli. We addressed this question by quantifying the dendritic cell response to pathogens in a set of genotyped individuals, and then leveraged our understanding of these pathways to explain the mechanisms underlying the observed genotype-environment-phenotype interactions.

Many of the QTL associations we identified are only detectable in the presence of specific stimuli, underscoring the need to activate cells to capture additional genotype-phenotype relationships (39-41). Furthermore, few eQTL studies have definitively identified the causal variants underlying the associations. By measuring variability in hundreds of individuals, applying stimuli that partially overlap in their downstream pathways, and leveraging genomic datasets such as the 1000 Genomes Project and ENCODE (29, 42), we (i) pinpointed causal variants in reQTL regions and (ii) identified the stimulus-activated transcription factors that bind differentially at these SNPs, explaining some of the GxE interactions. Our dataset can thus be used to explore mechanisms of GxE interactions (43) and are consistent with DNaseI-sensitivity QTL (44) and ChIP-Seq QTL (45) studies that showed that differential transcription factor binding between individuals is pervasive in resting cells.

The reQTLs we identified provide genetic explanations for inter-individual variation in innate immune responses. This is best exemplified by the *trans*-reQTL in the *IRF7* locus that regulates the type I IFN anti-viral response. Our study reveals the effects of this *trans*-reQTL on target genes (anti-viral IFN module) in the context of a particular cell type (DCs) and in response to specific ligands (influenza). The changes in this immune response are, in turn, likely to impact organismal phenotypes that are driven by the IFN module, including susceptibility to viral infections and autoimmune diseases like SLE.

Overall, our high-throughput experimental pipeline and integrative analysis of primary human dendritic cells reveals abundant gene-by-environment interactions, points to the effects of disease variants on pathogen detection, and motivates extending our approach to a wide variety of immune cell types and stimulatory conditions to better explain the impact of human genetic variation on the immune response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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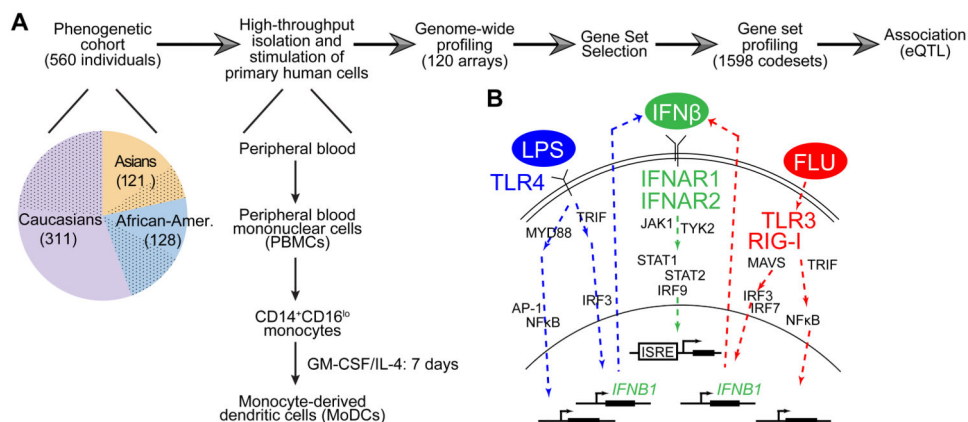


Fig. 1. A strategy to identify gene-by-environment interactions in the innate immune responses of primary human dendritic cells

(A) Strategy used to identify baseline and response expression quantitative trait loci (eQTLs and reQTLs), consisting of five steps: (i) high-throughput isolation and stimulation of primary human MoDCs from 560 healthy individuals (dotted slices, male; solid-colored slices, female) collected as part of the PhenoGenetic cohort; (ii) whole-genome gene expression measurements in a subset of the cohort; (iii) selection of signature gene set, consisting of regulators and regulated genes; (iv) digital multiplex gene expression measurements of signature genes in the entire cohort; and (v) mapping of genetic variation to expression variation. GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-4, interleukin-4. (B) Model of innate immune pathways activated by three stimuli demonstrating their downstream relationships. Lipopolysaccharide (“LPS”) from *E. coli* engages the TLR4 receptor; interferon-beta (“IFN β ”) engages the heterodimeric IFNAR receptor; influenza A/PR8 (NS1) (“FLU”) engages the cytoplasmic TLR3 and RIG-I receptors. Receptor engagement activates signal transduction cascades that regulate expression of inflammatory genes, IFNs and IFN-stimulated genes. IFNAR activation also occurs during LPS and FLU stimulations because LPS and FLU both induce IFN production, leading to activation of ISREs. JAK1, Janus kinase 1.

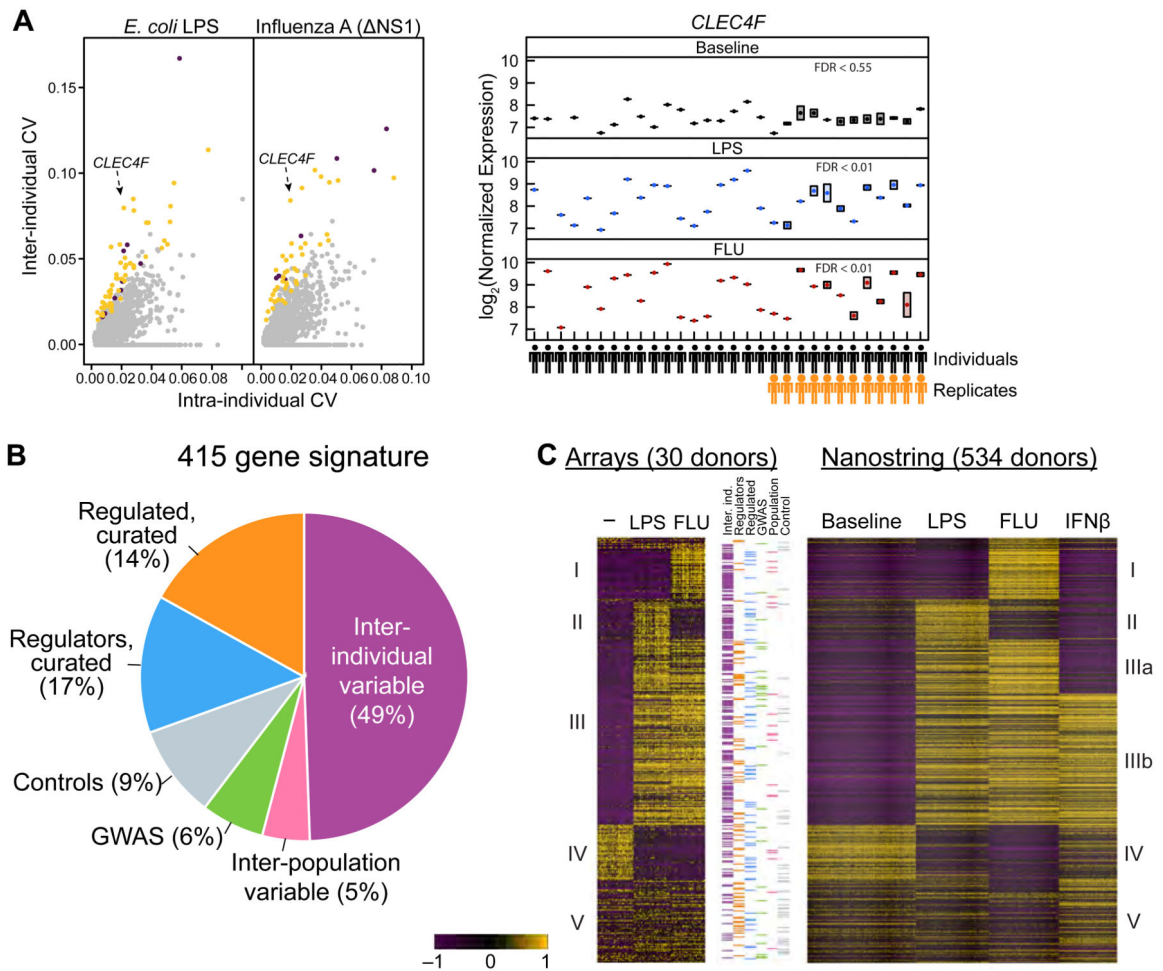


Fig. 2. Genome-wide expression profiles in MoDCs reveal response phenotypes

(A) Coefficient of variation (CV) of gene expression between 30 different donors (“Inter-individual CV”) plotted against CV of expression within 12 serial replicate samples (“Intra-individual CV”) for each differentially regulated (fold change >0.75 or <-1.5) gene following LPS or FLU stimulation. Yellow (up-regulated) and purple (down-regulated) circles represent genes with significant (moderated *t* test, FDR < 0.1) inter- vs. intra-individual variation. Right, log₂(expression, microarray data) of *CLECF* in baseline, LPS-stimulated and FLU-infected MoDCs from 30 donors and 12 replicates, demonstrating example of a gene that shows significant (FDR < 0.01) inter- vs. intra-individual variation following LPS and FLU stimulations but not at baseline (fig. S2B). Standard error of replicate samples (*n* = 12) is shown for each sample. (B) Pie chart of 415 signature genes selected for Nanostring codeset: 222 (49%) are regulated genes that showed significant (mixed model variance components test, permutation FDR < 0.1) inter- vs. intra-individual variability; 61 (14%) are curated, regulated genes with a known function in the innate immune response; 76 (17%) are curated regulators in the TLR4, TLR3, RIG-I and IFNAR pathways; 41 (9%) are control genes including low-variance genes, sex-specific genes and non-expressed genes; 28 (6%) are regulated genes that were reported in the regions of autoimmune and infectious disease GWAS; and 21 (5%) are regulated genes that showed

significant inter- versus intra-population variability. (C) Gene expression heatmap of the 415-gene signature in MoDCs from the microarray study (30 individuals) and the Nanostring study (534 individuals). Each row represents a gene; each column represents a donor sample at baseline, stimulated with LPS, infected with FLU or stimulated with IFN β . Rows were clustered by *k*-means clustering of Nanostring dataset with major clusters (I, II, IIIa, IIIb and IV) labeled. Between the two heat maps, each row was labeled with colored dashes corresponding to one of the 6 categories described in (B).

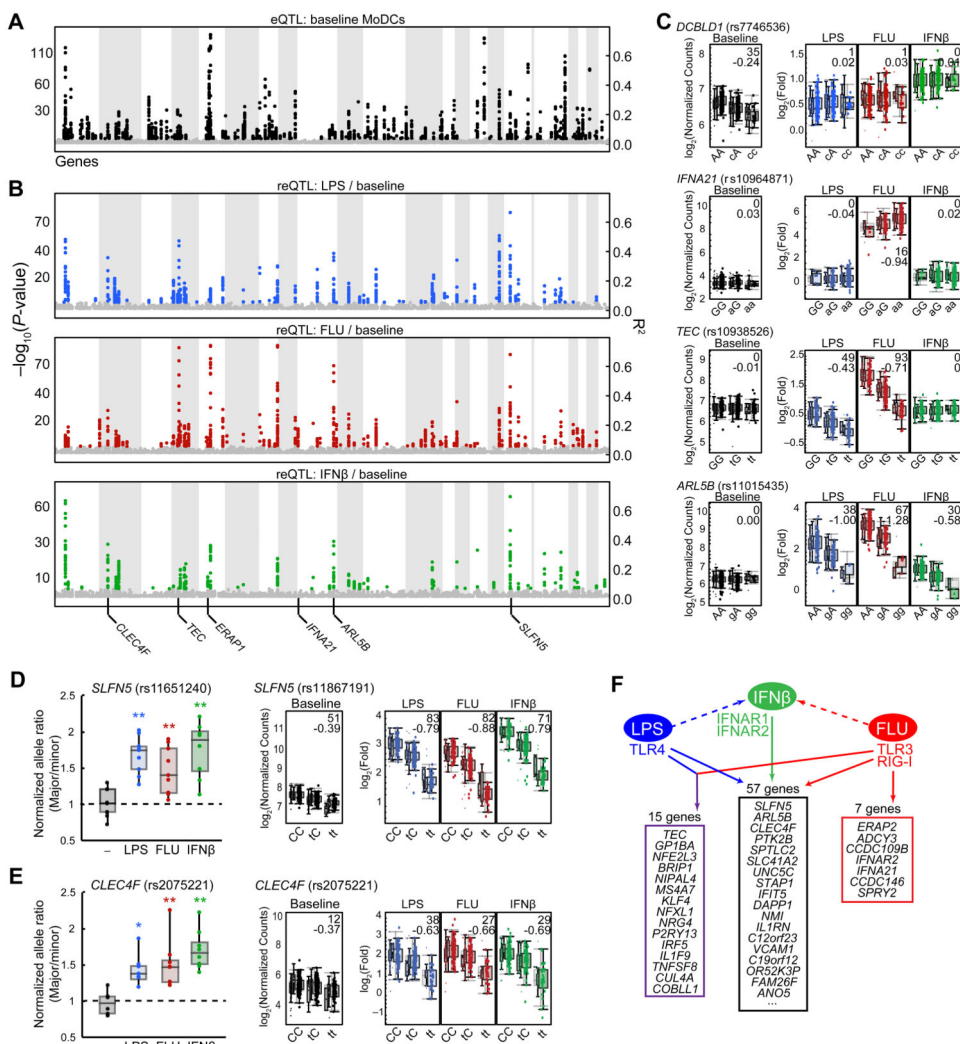


Fig. 3. Association analysis reveals cis-eQTLs and cis-reQTLs (A and B) Manhattan plot of cis-eQTLs (A, baseline expression) and cis-reQTLs (B, LPS-, FLU- and IFN β -stimulated fold changes relative to baseline) showing $-\log_{10}(P)$ values (left y axis) and R^2 values (right y-axis) for all cis-SNPs, which are displayed on the x-axis with associated genes ordered by their chromosomal location. (C) Box-whisker plots showing gene expression (left; $\log_2(\text{nCounts})$, y-axis) or fold change (right; $\log_2(\text{fold})$, y-axis) of *DCBLD1*, *IFNA21*, *TEC* and *ARL5B* in resting, LPS-stimulated, FLU-infected and IFN β -stimulated MoDCs as a function of genotype of the respective cis-SNPs (x-axis: rs27434, rs10964871, rs10938526 and rs11015435). African Americans, Asians and Europeans in this order are displayed as separate box-whisker plots adjacent to each other in each condition. $-\log_{10}(P)$ values) and β statistics are displayed in top right corners. (D and E) Allelic imbalance analysis of *SLFN5* (D) and *CLEC4F* (E) in resting, LPS-stimulated, FLU-infected and IFN β -stimulated MoDCs, showing the ratio of gene expression between the major and minor alleles in heterozygote (rs11651240 for *SLFN5*, rs2075221 for *CLEC4F*) cDNA samples ($n = 8$ in (D); $n = 9$ in (E)) normalized to the ratio in the corresponding genomic DNA samples; significant deviation from 1.0 (dashed line) is consistent with allelic

imbalance. Data are from one experiment representative of three (mean and standard deviation shown). * $P < 0.01$, ** $P < 0.001$, compared to unstimulated cells (Student's t -test). On the right panels, box-whisker plots showing expression (left; $\log_2(\text{nCounts})$, y-axis) or fold change (right; $\log_2(\text{fold})$, y-axis) of *SLFN5* (D) and *CLEC4F* (E) in resting, LPS-stimulated, FLU-infected and IFN β -stimulated MoDCs as a function of genotype of the respective cis-SNPs: rs11867191 and rs2075221. (F) Schematic showing the different combinations of stimuli leading to significant cis-reQTLs, with the most significant examples listed. Specificity to conditions was defined with M -value > 0.9 taken as the inclusion criteria and M -value < 0.1 taken as the exclusion criteria for each condition.

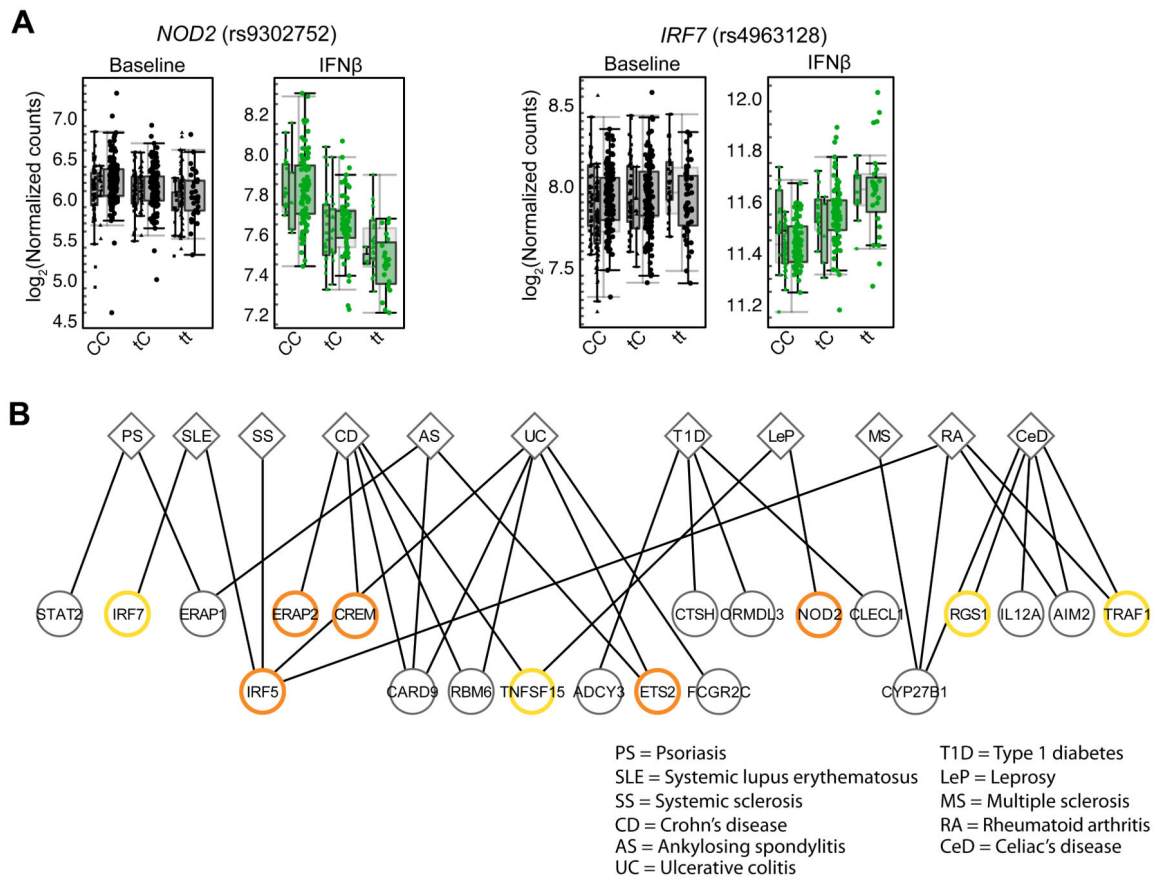


Fig. 6. Autoimmune and infectious disease-associated SNPs are cis-eQTLs and cis-reQTLs
(A) Expression ($\log_2(\text{nCounts})$) of *NOD2* in resting and IFN β -stimulated MoDCs from 184 Caucasians as a function of genotype of the leprosy GWAS SNP, rs9302752 (left). Right, expression ($\log_2(\text{nCounts})$) of *IRF7* in resting and IFN β -stimulated MoDCs from 184 Caucasians as a function of genotype of the SLE GWAS SNP, rs4963128. **(B)** Plot showing overlap of genome-wide significant ($P < 5 \times 10^{-8}$) GWAS SNPs with cis-eQTLs and reQTLs in MoDCs, with clinical phenotypes connected to corresponding gene expression phenotypes by lines. Orange circles represent cis-reQTLs ($P < 10^{-7}$); yellow circles represent stimulus-specific cis-eQTLs ($P < 10^{-7}$).