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# Parasympathetic neural activity and the reciprocal regulation of innate antiviral and inflammatory genes in the human immune system

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ABSTRACT

The vagus nerve mediates parasympathetic nervous system control of peripheral physiological processes including cardiovascular activity and immune response. In mice, tonic vagal activation down-regulates inflammation via nicotinic acetylcholine receptor-mediated inhibition of the pro-inflammatory transcription factor NF-κB in monocyte/macrophages. Because Type I interferon and pro-inflammatory genes are regulated reciprocally at the level of transcription factor activation and cell differentiation, we hypothesized that vagal activity would up-regulate Type I interferon response genes concurrently with inflammatory downregulation in human immune cells. We mapped empirical individual differences in the circulating leukocyte transcriptome and vagal activity indexed by high frequency (0.15–0.40 Hz) heart rate variability (HF-HRV) in 380 participants in the Midlife in the US study. Here we show that promoter-based bioinformatics analyses linked greater HF-HRV to reduced NF-κB activity and increased activity of IRF transcription factors involved in Type I interferon response (independent of β-antagonists, BMI, smoking, heavy alcohol consumption, and demographic factors). Transcript origin analyses implicated myeloid lineage immune cells as targets, representing per-cell alterations in gene transcription as HF-HRV was not associated with differential prevalence of leukocyte subsets. These findings support the concept of parasympathetic inhibition of pro-inflammatory gene expression in humans and up-regulation of Type I interferons that could augment host defense against viral infections.

## 1. Introduction

The role of the vagus nerve in down-regulating inflammation has been established in model systems, but its broader impact on human immunology remains largely unclear. In LPS-stimulated human macrophage cultures, administration of acetylcholine, the primary vagal neurotransmitter, attenuated the release of the pro-inflammatory cytokines TNFα, IL-1ß, and IL-6 but not the anti-inflammatory IL-10 (Borovikova et al., 2000). In vagotomized rats, administration of LPS elicited the release of TNFα in serum, an effect that was significantly decreased by electrical stimulation of the distal vagus (Borovikova et al., 2000). Vagal activity inhibits the migration of leukocytes to sites of inflammation in model systems (Saeed et al., 2005), in part by its action on the reticuloendothelial system of the liver and spleen where cytokines are produced. These vagal anti-inflammatory effects are dependent on an α7 subunit of the nicotinic acetylcholine receptor (α7nAChR) (Wang et al., 2003) - NFκB pathway (De Waal Malefyt, 1991; Nishiki, 2004; Zhang et al., 2017b) in macrophages. In mice with a knockout of the α7 subunit, LPS causes unrestrained TNFα, IL1-ß, and IL-6 responses in serum, liver, and spleen (Borovikova et al., 2000; Wang et al., 2003). Administration of α7nAChR agonists decrease this cytokine release (Wang et al., 2004). These model system data support the existence of a cholinergic anti-inflammatory reflex (Tracey, 2009).

Analysis of oscillations in heart rate (HR) may provide a window on human vagal physiology in vivo and therefore a method to probe the cholinergic anti-inflammatory reflex in humans. HR typically is not steady but rather, it varies cyclically and at different frequencies. Beat-to-beat variation in HR reflects modulation of autonomic outflow to the heart (Akselrod et al., 1981; Saul, 1990). Oscillations in the high frequency (HF) range (0.15–0.40 Hz) range are widely recognized to reflect

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cardiac vagal regulation (Saul et al., 1991). Slower oscillations, in the low frequency (LF) range (0.04–0.15 Hz), are the product of both cardiac parasympathetic and sympathetic modulation (Pomeranz et al., 1985). Because HF heart rate variability (HF-HRV) reflects cardiac vagal modulation, it should relate to vagally regulated inflammatory processes and some data support this connection. Using data from 757 participants in the CARDIA study, we reported that HF-HRV during a seated rest was inversely related to serum IL-6 and CRP (Sloan et al., 2007). Similarly, we reported inverse relationships between HF HRV and serum levels of fibrinogen and CRP in 1153 participants from the MIDUS (Midlife in US) study (Cooper et al., 2015). In 661 healthy employees from an airplane manufacturing plant in Germany, HRV measured as the root mean squared successive difference (rMSSD) was inversely related to both white blood cell count and CRP (Thayer and Fischer, 2009). In 1601 participants in the Young Finns Study, rMSSD and HF-HRV were significantly and marginally inversely related to CRP (Haarala et al., 2011). In 643 older adults free of heart disease, SDNN, a global measure of HRV, was inversely related to serum CRP (Sajadieh et al., 2004). These associations are consistent with mechanistic data from animal models showing that experimental induction of inflammation can suppress HRV (Masson et al., 2015) and that vagus nerve stimulation can increase HRV in parallel with reductions in TNFα (Samniang et al., 2016) or NF-κB p65 nuclear translocation (Sun et al., 2013). However, the signaling pathways that mediate relationships between vagal activity in inflammatory protein expression in humans have not yet been defined.

Beyond inflammation, little is known about the effects of vagal activity on other aspects of human immune response such as innate antiviral responses or adaptive immune responses by B and T lymphocytes. However, it is likely that broader effects exist because the parasympathetic division of the autonomic nervous system is reciprocally cross-regulated by the sympathetic division of the autonomic nervous system, and the sympathetic nervous system is known to exert pervasive effects on the innate immune system, including pro-inflammatory effects reciprocal to those associated with parasympathetic activity as well as inhibition of innate antiviral responses mediated by the Type I interferon system (Collado-Hidalgo et al., 2006; Irwin and Cole, 2011). As such, it is conceivable that HF-HRV might be associated with a reciprocal increase in activity of the Type I interferon system in addition to decreased activity of the pro-inflammatory system. To address this hypothesis, and to clarify the gene regulatory pathways through which parasympathetic activity regulates innate immune response in humans, we mapped the empirical differences in leukocyte gene expression associated with individual differences in HF-HRV and quantified activity of two major gene regulatory axes that characterize cellular differentiation and anti-microbial effector responses in the myeloid lineage immune cells that mediate innate immune responses (i.e., monocytes, dendritic cells, and neutrophil granulocytes) – inflammatory effector genes regulated by the pro-inflammatory transcription factor, NF-κB, and Type I interferon effector genes involved in innate antiviral responses mediated by the Interferon Response Factor (IRF) family of transcription factors (Amit et al., 2009; Siegal, 1999).

## 2. Materials and methods

### 2.1. Participants and study protocol

Data were collected from participants in the Midlife in the US (MIDUS) Refresher (MR) cohort (Weinstein et al., 2019), part of the larger MIDUS study of the behavioral, psychological and social factors accounting for age-related variation in health and well-being in a national sample of middle-aged and older Americans (Brim et al., 2004). MR consisted of five projects, including a Biomarker Project (P4). Biomarker data were collected during 1.5-day visits to a clinical research center (CRC) at the University of Wisconsin–Madison, the University of California, Los Angeles, or Georgetown University.

### 2.2. HRV assessment

After an overnight stay at the CRC, a fasting blood sample was drawn. Participants then were provided with a light breakfast, but no caffeine consumption was permitted. Following breakfast, they began the HRV data collection protocol. ECG electrodes were placed on the left and right shoulders, as well as in the left lower quadrant. ECG was recorded in Lead II. Respiration bands were placed around the chest and abdomen and the finger cuff of a Finometer beat-to-beat blood pressure monitor was placed around the middle finger of the nondominant hand. Respiration was calibrated using an 800 cc spirobag (Ambulatory Monitoring Systems, Ardsley, NY). Data were recorded during an 11 min seated baseline as part of a more extensive psychophysiology protocol with exposure to challenging stimuli and recovery periods. Here we report HRV data from this resting baseline. Analog ECG signals were digitized at 500 Hz by a 16-bit A/D conversion board (National instruments, Austin, TX) and passed to a microcomputer.

The ECG waveform was submitted to an R-wave detection routine implemented by custom-written software, resulting in an RR interval series. Errors in marking R waves were corrected by visual inspection. Ectopic beats or noisy segments were corrected by interpolation. Files in which>20% of the RRIs were interpolated were excluded from analysis. High frequency (0.15– 0.40 Hz) HRV was computed based on 300-s epochs, using an interval method for computing Fourier transforms similar to that described by DeBoer, Karemaker, and Strackee (DeBoer et al., 1984). The mean value of HF-HRV from the two baseline 300-s epochs was computed, with the last 60 s excluded from analysis.

### 2.3. RNA profiling

Genome-wide transcriptional profiling was conducted on approximately $10^7$ peripheral blood mononuclear cells (PBMCs) collected from MIDUS participants by antecubital venipuncture followed by standard Ficoll density gradient centrifugation, as previously described (Love et al., 2010). Total RNA was extracted from PBMC (Qiagen RNeasy Mini QiaCube), checked for suitable mass ($\geq$10 ng by NanoDrop ND1000 spectrophotometry; achieved mean = 7457 ng, range 163–27104) and integrity (RNA Integrity Number $\geq$ 3 by Agilent TapeStation electrophoresis; achieved mean = 5.1 $\pm$ SD 1.5), and assayed by RNA sequencing in the UCLA Neuroscience Genomics Core Laboratory using Lexogen QuantSeq 3′ FWD cDNA library synthesis and multiplex DNA sequencing on an Illumina HiSeq 4000 instrument with single-strand 65-nt sequence reads (all following the manufacturers' standard protocol). Samples yielded an average of 11.0 million sequence reads (SD 1.6 million), each of which was mapped to the GRCh38 human transcriptome sequence using the STAR aligner (Dobin et al., 2013) to generate transcript counts per million mapped transcripts (TPM). An average 96.0% (SD 1.0%) of reads mapped successfully and endpoint sample validity metrics showed an average inter-sample profile consistency mean r = 0.86 (SD = 0.08). Among 670 samples meeting input RNA quality criteria (RNA Integrity Number $\geq$ 3), 538 (80%) met the assay endpoint validity criterion of profile consistency r $\geq$ 0.85. TPM values were floored at 1 and log2-transformed for analysis as described below. (No additional RNAseq read-level QC or sample normalization was required because STAR accounts for variations in read quality during mapping and transcript quantification, and TPM normalization controls for variations in library size/sequencing depth.)

To map the empirical transcriptomic correlates of HF-HRV while controlling for potential confounders, primary analyses used standard linear statistical models to estimate the magnitude of difference in average transcript abundance over a 4-SD range of variation in HF-HRV (i.e., ranging from a lower limit of 2 SD below the mean HF-HRV to 2 SD above) while controlling for age, sex, race (White vs Non), body mass index (kg/m$^2$), educational attainment, history of regular smoking, history of alcoholism, and average drinks of alcohol per week over the last month. Ancillary analyses additionally controlled for the prevalence

of major leukocyte subsets within the circulating leukocyte pool by including as covariates the abundance of 8 mRNAs encoding differentiation markers for T lymphocytes (*CD3D*, *CD3E*), CD4 + and CD8 + T lymphocyte subsets (*CD4*, *CD8A*), B lymphocytes (*CD19*), natural killer cells (CD16/*FCGR3A*, CD56/*NCAM1*), and monocytes (*CD14*). Leukocyte subset marker abundance was quantified as the RNA sequencing-derived TPM value for each marker transcript (i.e., italicized gene symbols in the previous sentence). Among the 543 valid transcriptome profiles, 163 (30%) were missing data on HF-HRV or covariates, leaving a final analyzed sample size of 380. Genes showing minimal level or variation in expression (mean $< 0.5$ log2 units or SD $= 0$) were excluded from analysis, leaving 11,680 analyzable transcripts. Among those, linear model analyses identified 1,178 transcripts that showed a point estimate of $\geq 1.25$-fold differential expression / 4-SD HF-HRV and these genes served as input into higher-order bioinformatics analyses of transcription factor activity and cellular origin. (Primary analyses took point estimates of differential expression as input into higher-order bioinformatics analyses because previous research has found point estimate-based screening to provide more reliable results than screening based on p-values (Cole et al., 2003; Norris and Kahn, 2006; Shi et al., 2008; Witten and Tibshirani, 2007).

Analyses of transcription factor activity utilized the TELiS promoter sequence database (Cole et al., 2005) as in previous research (Cole et al., 2020; Miller et al., 2014) to test three a priori-specified hypotheses regarding activity of transcription control pathways involved in 1) down-regulated activity of the pro-inflammatory transcription factor, NF-κB (indicated by over-representation of NF-κB binding sites in promoters of down-regulated genes relative to up-regulated genes, quantified by the TRANSFAC position-specific weight matrix V$NFKB_Q6), and 2) Type I interferon innate antiviral responses (IRFs; indicated by under-representation of V$IRF1_Q6 in up- vs. down-regulated genes). TELIS bioinformatics analyses assess changes in the expression of putative transcription factor target genes (identified by the presence of consensus transcription factor-binding motifs in the gene's core promoter sequence) and does not involve assessment of mRNAs that encode the transcription factor or its signaling pathway (because transcription factors are generally activated through post-translational modification of their protein structure, rather than by transcriptional changes in protein abundance). Inference of transcription factor activity from statistical analysis of TELiS-annotated target genes has been well validated against other measures such as DNA-binding assays (e.g., electrophoretic mobility shift, chromatin immunoprecipitation) or experimental induction of transcription factor activity (e.g., by ligand-induced activation, genetic over-expression) (Cole et al., 2005). TELiS analyses were conducted using 9 different parametric combinations of core promoter DNA sequence length (−300, −600, and − 1000 to + 200 nucleotides surrounding the RefSeq-designated transcription start site from the GRCh38 human genome sequence) and transcription factor-binding motif (TFBM) detection stringency (TRANSFAC mat_sim values of 0.80, 0.90, and 0.95) (Cole et al., 2005). Log2-transformed TFBM ratios (comparing prevalence in promoters of up- vs. down-regulated genes) were averaged across the 9 parametric combinations and tested for statistical significance using standard errors derived from bootstrap resampling of linear model residual vectors (controlling for potential correlation across genes) to quantify sampling variability in the set of differentially expressed genes.

To assess the role of myeloid lineage immune cells (classical and non-classical monocytes, dendritic cells, and neutrophils) in mediating HF-HRV-associated transcriptome differences, the same set of up- and down-regulated genes served as input into Transcript Origin Analysis as previously described (Cole et al., 2011) and previously applied (Cole et al., 2020; Miller et al., 2014), with cell type-specific gene expression reference profiles derived from publicly available data on isolated leukocyte subsets (Gene Expression Omnibus GSE1133 and GSE101489). Transcript Origin Analysis uses an external reference database of differential gene expression in isolated leukocyte subsets (i.e., GSE1133 and GSE101489) to quantify the contribution of specific cell types to an arbitrary set of differentially expressed genes (e.g., the set of genes up-regulated or down-regulated in association with HF-HRV). The reference data define the extent to which each gene transcript is preferentially expressed by each subtype of leukocyte (using a quantitative "cell type diagnosticity score" as defined in (Cole et al., 2011)), allowing the arbitrary gene set to be tested for significant difference in the mean diagnosticity score across cell types. Results were again tested for statistical significance using standard errors derived from bootstrap resampling of linear model residual vectors (controlling for potential correlation across genes) to quantify sampling variability in the set of differentially expressed genes that served as analytic input.

We tested the hypothesis that greater vagal activity, indexed by HF-HRV, would be associated with reduced expression of pro-inflammatory genes in circulating blood, and perhaps also with increased activity of the other major dimension of innate immune transcriptional response involving the Type I interferon system (Amit et al., 2009; Siegal, 1999; Su et al., 2004).

## 3. Results

### 3.1. Participants

863 participants were enrolled in the MR Biomarker Project. Of these, 195 were excluded from HRV analysis because of noisy data or ectopic beats in excess of 20% of the total number of RRIs. 670 participants provided RNA assay consent and blood samples sufficient for RNA sequencing; 538 of those (80%) yielded high-quality RNA profiles suitable for substantive analysis. This analysis included data from all 380 participants who had valid measures of both HRV and gene expression (see Supplementary Fig. 1). Mean age ± SD was 50.35 ± 13.0 years. 192 were women and 188 were men. Participants' BMI was 30.67 ± 7.81 kg/$m^2$. 109 were non-white and 271 were white. Mean level of education was completion of 4 years of college.

### 3.2. Transcriptional analysis

Genome-wide transcriptional profiling of peripheral blood mononuclear cell samples identified 1,178 gene transcripts showing $> 1.25$-fold difference in average expression across the span of HF-HRV activity scores ranging from 2 SD below the sample mean to 2 SD above (i.e., the general range of variation in a normal distribution) – 522 transcripts up-regulated $> 1.25$-fold and 656 transcripts down-regulated (SI Data File 1). These genes served as input into this study's primary analysis testing the role of the pro-inflammatory transcription factor NF-κB as a potential mediator of HF-HRV-related differences in gene expression. These analyses were conducted using the TELiS promoter-based bioinformatics analysis to quantify the relative prevalence of transcription factor-binding motifs for NF-κB and IRF transcription factors in the promoters of the up- vs. down-regulated gene sets (i.e., the relative prevalence of NF-κB or IRF target genes among genes regulated in association with HF-HRV). As shown in Fig. 1, results linked greater levels of HF-HRV to reductions in bioinformatically inferred NF-κB activity (0.54-fold relative prevalence of NF-κB TFBMs in the promoters of up- vs. down-regulated genes, average log2 TFBM ratio -0.890 ± standard error 0.221, $p = .0001$, controlling for age, sex, race, body mass index, educational attainment, smoking history, and alcohol use). Parallel analyses of the IRF transcription factor linked HF-HRV to greater levels of bioinformatically inferred Type I interferon signaling (1.28-fold relative prevalence, log2 TFBM ratio 0.357 ± 0.146, $p = .016$). Similar results emerged in analyses that additionally controlled for the relative prevalence of 10 mRNAs marking major leukocyte subsets (NF-κB: 0.38-fold, −1.41 ± 0.01, $p < .0001$; IRF: 1.23-fold, 0.295 ± 0.01, $p < .0001$), indicating the effects did not stem from any correlated variations in leukocyte subset prevalence, as well as in unadjusted analyses that did not include any covariates (NF-κB: 0.72-fold, -0.478 ± 0.207, $p = .022$;

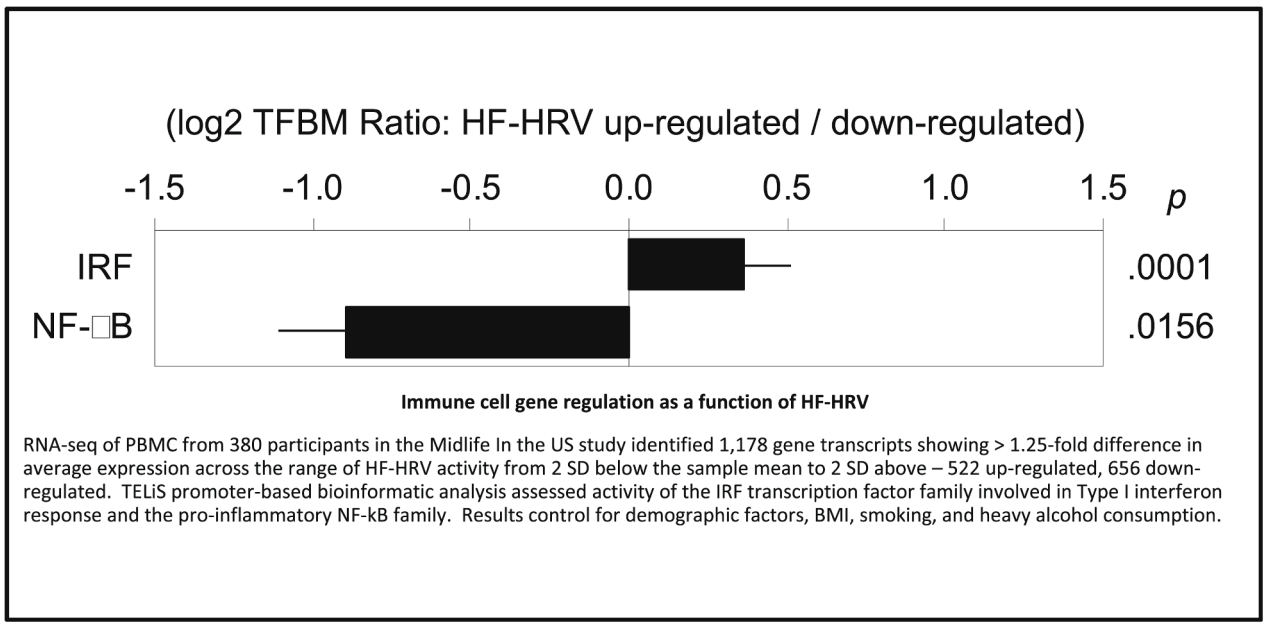


**Fig 1.** Transcription factor activity (log2 TFBM Ratio: HF- HRV up-regulated/ down-regulated).

IRF: 1.25-fold, 0.318 ± 0.064, p < .0001). Ancillary analyses also controlled for the use of β -adrenergic antagonist medications that can affect both HF-HRV (0.35 SD elevation in this sample, p = .025) and immune cell gene regulation (as reflected by reduced NF-κB activity and increased IRF activity in this sample; NF-κB: 0.52-fold, -0.936 ± 0.387, p = .016; IRF: 1.44-fold, 0.529 ± 0.255, p = .039) and continued to find a significant differences in bioinformatic indications of gene regulation as a function of HF-HRV (NF-κB: 0.56-fold, -0.845 ± 0.216, p < .0001; IRF: 1.31-fold, 0.385 ± 0.143, p = .008).

In circulating blood, pro-inflammatory and Type I interferon transcriptional programs are mediated predominately by myeloid lineage immune cells including monocytes, dendritic cells, and neutrophil granulocytes (Amit et al., 2009; Murphy, 2011; Siegal, 1999; Su et al., 2004). To determine the role of these myeloid cell types in mediating the transcriptomic correlates of HF-HRV, we conducted Transcript Origin Analyses (Cole et al., 2011) to quantify the extent to which HF-HRV-related gene transcripts derived disproportionately from monocytes (including both classical and non-classical subtypes), dendritic cells (including myeloid and plasmacytoid subtypes), and neutrophils. This analysis uses an external database of differential gene expression in isolated leukocyte subsets to quantify the contribution of specific cell types to HF-HRV-associated differences in genome-wide transcriptional profiles (genes listed in SI File 1). As shown in Fig. 2 (left panel), gene transcripts down-regulated in association with HF-HRV derived preferentially from monocytes, including both classical and non-classical monocyte subsets (classical: 0.64 ± 0.20, p = 0.0006; non-classical: 0.58 ± 0.15, p = 0.0001). Down-regulated genes also derived preferentially from two other myeloid lineage cell populations: neutrophil granulocytes (0.60 ± 0.20, p = 0.0014) and BDCA1+/CD1c + myeloid dendritic cells (0.31 ± 0.11, p = 0.0020). Results showed no significant contribution to the HF-HRV down-regulated transcriptome from the other two circulating dendritic cell populations involving BDCA2 + plasmacytoid dendritic cells or BDCA3 + dendritic cells (both p > .50). By contrast, genes up-regulated in association with HF-HRV (Fig. 2, right panel) derived preferentially from BDCA2 + plasmacytoid dendritic cells (0.22 ± 0.09, p = 0.0088) and from lymphoid lineage CD4 + T cells (0.60 ± 0.16, p = 0.0001), CD8 + T cells (0.31 ± 0.12, p = 0.0061), and B cells (0.34 ± 0.16, p = 0.0186). These cellular contributions to HF-HRV-related gene expression appear to stem from per-cell differences in gene regulation as Transcriptome Representation Analyses (Powell et al., 2013) testing for differential prevalence of leukocyte subpopulations found no significant differences in association with HF-HRV for any cell type examined (all p > 0.20).

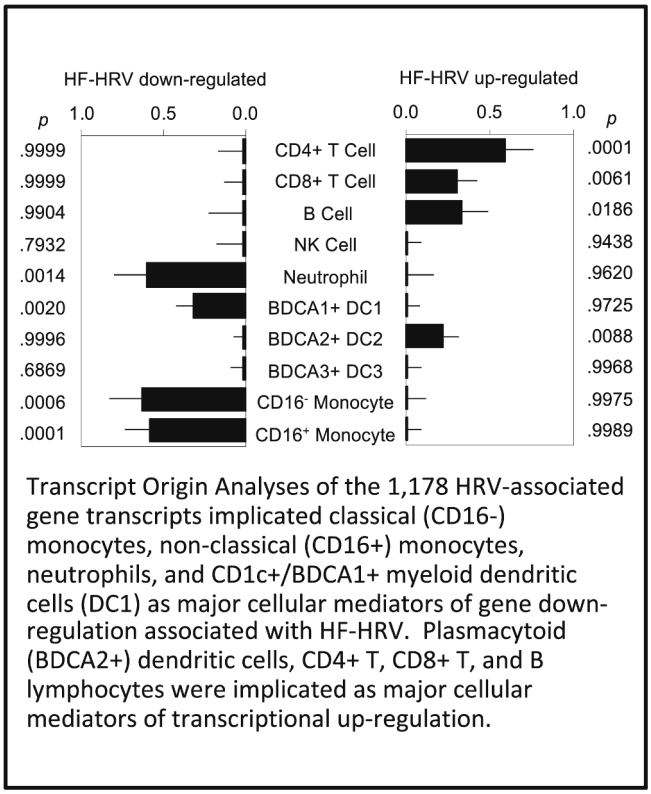


**Fig 2.** Cellular origin.

## 4. Discussion

In genome-wide transcriptional profiles from circulating leukocyte samples, vagally-mediated HF-HRV is associated with reduced activity of the pro-inflammatory NF-κB transcription control pathway and

increased activity of Type I interferon-related antiviral response genes mediated by IRF transcription factors. This reciprocal shift in the relative equilibrium of pro-inflammatory and antiviral gene expression emerged despite control for demographic factors (age, sex, race), health behavioral risk factors (body mass index, smoking history, alcohol use), the use of β -adrenergic antagonist medications, and the relative prevalence of major leukocyte subsets within the assayed cell samples. The reciprocal effects for NF-κB and IRF target genes are consistent with previous data documenting distinct inflammatory and antiviral transcriptional networks in innate immune cell (Amit et al., 2009; Murphy, 2011; Siegal, 1999; Su et al., 2004). The relation of HF-HRV to the overall equilibrium of these two gene systems is consistent with previous experimental data showing direct anti-inflammatory effects of cholinergic signaling through the α7nAChR on NF-κB activity (Huston and Tracey, 2011; Wang et al., 2003). However, these effects may also be mediated in part by parasympathetic inhibition of sympathetic nervous system activity, the latter of which can induce a complementary "conserved transcriptional response to adversity" (CTRA) transcriptional program that is mediated by β -adrenergic signaling (Heidt et al., 2014; McKim et al., 2018; Powell et al., 2013) and characterized by up-regulated expression of NF-κB target genes and down-regulated expression of IRF target genes (i.e., a profile reciprocal to that linked to HF-HRV in this study) (Cole, 2019). The present data suggest both mechanisms may be operative, as HF-HRV and β -adrenergic antagonists were each associated with reduced NF-κB and increased IRF activity net of the other's influence. However, future research experimentally dissociating sympathetic and parasympathetic signaling will be required to clarify the relative contributions of these two autonomic pathways to the shifting equilibrium of pro-inflammatory and antiviral signaling observed here.

Analyses of the cellular origins of differentially expressed transcripts identified myeloid lineage immune cells (monocytes, dendritic cells, and neutrophils) as major mediators of the transcriptomic variation associated with HF-HRV. Genes down-regulated in association with HF-HRV derived preferentially from monocytes in general, and more specifically from both classical and non-classical monocyte subsets. These results are consistent with the indications of decreased NF-κB activity as monocytes are the chief source of pro-inflammatory gene expression in the circulating immune cell pool (Amit et al., 2009; Murphy, 2011; Su et al., 2004). Likewise, the preferential derivation of HF-HRV-up-regulated genes from plasmacytoid (BDCA2+) dendritic cells is consistent with the observed up-regulation of IRF activity as this cell type is known to be the primary source of Type I interferon in circulating blood (Siegal, 1999). Transcriptome representation analyses of cell subset abundance failed to indicate any significant association of HF-HRV with the relative prevalence of these cell types (or any others) within the PBMC pool. Such results suggest that the transcriptomic variations observed here likely derive predominately from per-cell changes in gene expression (e.g., due to variations in sympathetic and parasympathetic signal transduction and downstream gene regulation). Analyses of cell-type selective gene expression thus converge with the primary analyses of transcription factor activity in linking the regulatory equilibrium of pro-inflammatory and antiviral gene expression with parasympathetic activity as indexed by HF-HRV.

Although this study has several strengths in terms of representativeness, sample size, rigorous HRV assessment, and comprehensive transcriptome profiling, the present results are limited in several respects. Although these findings are consistent with previous experimental data from animal models (Borovikova et al., 2000; De Waal Malefyt, 1991; Nishiki, 2004; Saeed et al., 2005; Tracey, 2009; Wang et al., 2004; Wang et al., 2003; Zhang et al., 2017a), they come from a correlational analysis and a causal effect of parasympathetic activity on gene expression cannot be established from this study. The transcriptomic analyses tested pre-specified hypotheses regarding transcriptional control pathways and cellular mechanisms, but additional transcriptional correlates of HF-HRV likely exist and may be identified in future unbiased discovery analyses. This study did not involve any measures of disease, so the health significance of the observed differences in gene expression remains to be determined in future research.

## 5. Conclusions

Despite these limitations, this study clarifies the gene-regulatory mechanisms linking vagally-mediated HRV to reduced inflammatory biology in humans and it also identifies a reciprocal increase in type I interferon activity that may enhance host resistance to viral infections. As predicted from previous analyses in model systems (Collado-Hidalgo et al., 2006; Tracey, 2002; Tracey, 2009), human HF-HRV was associated with down-regulated NF-κB activity and up-regulated IRF activity under physiological conditions. These findings suggest that interventions to enhance vagal activity could potentially have a favorable impact on inflammation-related chronic diseases such as atherosclerosis, neurodegenerative diseases, and cancer, as well as host resistance to viral infections.

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## Author contributions

RPS designed and oversaw the HRV data collection protocol and was responsible for computation of HRV. SWC developed and implemented the transcript origin analyses. Both authors were responsible for writing the manuscript.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.08.217.

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