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Innate Immune Responses to TREM-1 Activation: Overlap, Divergence, and Positive and Negative Cross-Talk with Bacterial Lipopolysaccharide

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TREM-1 (triggering receptor expressed on myeloid cells-1) is an orphan immunoreceptor expressed on monocytes, macrophages, and neutrophils. TREM-1 associates with and signals via the adapter protein DAP12/TYROBP, which contains an ITAM. TREM-1 activation by receptor cross-linking has been shown to be proinflammatory and to amplify some cellular responses to TLR ligands such as bacterial LPS. To investigate the cellular consequences of TREM-1 activation, we have characterized global gene expression changes in human monocytes in response to TREM-1 cross-linking in comparison to and combined with LPS. Both TREM-1 activation and LPS up-regulate chemokines, cytokines, matrix metalloproteases, and PTGS/COX2, consistent with a core inflammatory response. However, other immunomodulatory factors are selectively induced, including SPP1 and CSF1 (i.e., M-CSF) by TREM-1 activation and IL-23 and CSF3 (i.e., G-CSF) by LPS. Additionally, cross-talk between TREM-1 activation and LPS occurs on multiple levels. Although synergy in GM-CSF protein production is reflected in commensurate mRNA abundance, comparable synergy in IL-1β protein production is not. TREM-1 activation also attenuates the induction of some LPS target genes, including those that encode IL-12 cytokine family subunits. Where tested, positive TREM-1 outputs are greatly reduced by the PI3K inhibitor wortmannin, whereas this attenuation is largely PI3K independent. These experiments provide a detailed analysis of the cellular consequences of TREM-1 activation and highlight the complexity in signal integration between ITAM- and TLR-mediated signaling. ***The Journal of Immunology,*** **2008, 180: 3520–3534.**

The initial host response to infection consists of the activation of myeloid lineage cells of the innate immune system. Myeloid cells sense pathogens through the cell surface and intracellular pattern recognition receptors (PRRs)[2] that bind to conserved microbial molecular structures. An example of PRRs are the Toll-like receptors or TLRs (1, 2). TLR4, for example, recognizes LPS, a cell-wall component of Gram-negative bacteria. Activation in response to such stimuli triggers a number of antimicrobial defenses, including phagocytosis, bactericidal agent release, and proinflammatory cytokine release. The latter modulates local tissue responses and stimulates additional innate and adaptive immune responses to assist in pathogen clearance. In addition to TLRs, myeloid cells express triggering receptors expressed on myeloid cells (TREMs), a family of receptors of the Ig superfamily (3). Although reports of putative TREM ligands exist (4–8), biologically active ligands for any of the TREM receptors have yet to be identified. The first characterized of the TREMs was TREM-1, which is expressed on $CD14^+$ monocytes, macrophages, and neutrophils (9–11). Activation of TREM-1 using a cross-linking Ab triggers degranulation, respiratory burst, Ca^{2+} mobilization, phagocytosis, and cytokine release, consistent with cellular activation (9, 12–14). Additionally, TREM-1 activation synergizes with LPS and other PRR ligands in the production of some proinflammatory cytokines (9, 12–14). These and other findings indicate that cells of the innate immune system can integrate signals from multiple stimuli to deliver an appropriately tailored response (15).

TREM-1, TREM-2, and, in mice, TREM-3, contain an extracellular Ig variable region but lack a cytoplasmic signaling domain. These receptors associate with the general immunoreceptor adapter protein DAP12/TYROBP via a transmembrane charge interaction (9, 16, 17). DAP12 contains an ITAM, and current models for TREM signaling are largely based on our understanding of DAP12 signaling in the relevant cell types (3, 18, 19). According to these models, ligand binding triggers tyrosine phosphorylation of the DAP12 ITAM by Src family kinases, resulting in the recruitment and activation of the nonreceptor tyrosine kinase Syk. Syk, in turn, activates multiple effector pathways and molecules, including the PI3K/Akt pathway, phospholipase C-γ, and the Ras/ERK MAPK pathway. Consistent with this model, phosphorylation of phospholipase C-γ, Akt, and ERK have been shown experimentally downstream of TREM-1 activation (9, 20, 21). TREM-1 activation also results in phosphorylation of the transmembrane linker protein NTAL (non-T cell activation linker), which modulates TREM-1 signaling (21). Additional Syk substrates and downstream effector molecules are presumably also activated in response to TREM-1 activation (19, 22), but this has yet to be shown experimentally.

The mechanism(s) by which TREM-1 activation together with PRR ligands amplifies inflammatory responses are not yet fully understood. Ligand-driven TLR4 oligomerization activates IRAK (IL-1R-associated kinase) kinases and TBK1 (TNFR-associated NF-κB kinase-binding kinase-1), which ultimately lead to the activation of MAPK/IκB kinase (IKK) and IRF3 (IFN-regulatory factor-3), respectively (2). Signaling pathways downstream of

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[2] Abbreviations used in this paper: PRR, pattern recognition receptor; TREM, triggering receptor expressed on myeloid cells; IKK, IκB kinase; GIMAP, GTPase of immunity-associated proteins.

TREM-1 activation and LPS therefore share some downstream features but are considerably divergent, particularly in more receptor-proximal events. A contributing factor to positive cross-talk with prolonged treatment may be that PRR ligands can up-regulate TREM-1 expression (9, 11, 12, 23, 24). Additionally, either LPS or TREM-1 activation can induce both TLR4 and TREM-1 to colocalize to lipid rafts, indicating more immediate receptor cross-talk (21). Furthermore, recent experiments indicate that TREM-1 expression may be required to maintain steady-state levels of key TLR4 signaling molecules (25). Consistent with these observations, TREM-1 blockade reduces serum TNF and IL-1β levels and is protective in mouse models of LPS-induced shock and microbial sepsis (24). TREM-1 therefore plays a critical role in amplifying the uncontrolled inflammatory responses elicited in these models, and anti-TREM-1 therapies are candidates for the treatment of sepsis. Moreover, endogenous ligands exist for TLRs, and innate immune responses are more generally implicated in sterile inflammation and tissue homeostasis (26–28). As such, they are potential targets for autoimmune disease therapy, which is highlighted most clearly by the success of anti-TNF therapies for treating rheumatoid arthritis and other inflammatory disorders (29). Interestingly, we have recently observed that TREM-1 is disproportionately up-regulated and functionally present in disease tissue from rheumatoid arthritis patients, indicating a potential modulatory role for TREM-1 in autoimmune disease (our unpublished observations).

Additional TREM family members contain an ITIM or other tyrosine-based signaling motif (3). Cellular responses to TREM ligands may therefore be determined by a balance of activating and inhibitory signals, although this remains speculative. Recent work, however, indicates that simple categorization of these and other receptors as either activating or inhibitory may be an oversimplification (30, 31). Indeed, in microglial cells TREM-2 promotes phagocytosis while suppressing proinflammatory cytokine production (32), and TREM-2 expression dampens inflammation in mouse models of multiple sclerosis (8, 33). In addition, macrophages deficient in DAP12, TREM-2, or Syk are hyperresponsive to TLR ligands (34–36). Furthermore, while DAP12 knockout mice are protected in high-dose LPS-induced shock and microbial sepsis models (37), they have increased mortality in a D-galactosamine-sensitized, LPS-induced shock model (35). The in vivo results are difficult to deconvolute given the different experimental protocols and number of DAP12-paired immunoreceptors (18); yet, taken together these data indicate that DAP12 and/or DAP12-paired receptors can potentially have activating and/or inhibitory roles in innate immune responses (19, 38).

Although TREM-1 activation and PRR ligands are both proinflammatory and can act in concert to amplify inflammation, the extent of overlap in the responses they elicit and any divergence have not been investigated in detail. To address this, we have analyzed global gene expression changes in human monocytes after either TREM-1 activation or LPS treatment. We analyzed a relatively short time point of 2 h to bias the results toward direct effects of these activating stimuli. Large changes in gene expression are observed in response to both treatments, including overlap in a commonly regulated set of inflammatory genes. We also identify immunomodulatory factors that are preferentially induced by one treatment over the other. To investigate the cross-talk between these stimuli, we included for analysis cells receiving a combined treatment of TREM-1 activation plus LPS. By leveraging this data, we find that positive cross-talk between these pathways can occur by multiple mechanisms. Moreover, we show that TREM-1 activation can suppress the induction of key immunomodulatory factors by LPS. We further find that, where tested, this positive cross-talk and negative cross-talk differ in their sensitivity to the PI3K inhibitor wortmannin.

Materials and Methods

Monocyte isolation and cell culture

Human monocytes were purified from anonymous healthy donor buffy coats obtained from Massachusetts General Hospital (Boston, MA). Buffy coats were stored at 4°C overnight for cell isolation the following day. Monocytes were isolated by negative selection using RosetteSep (Stem Cell Technologies, catalog no. 15068) according to the manufacturer's protocol by density centrifugation over Histopaque (Sigma-Aldrich, catalog no. H8889). Monocytes were maintained in RPMI 1640 (Mediatech, catalog no. 15-040-CV) supplemented with 10% FBS (Sigma-Aldrich, catalog no. F-9423) that had been heat inactivated. All incubations were at 37°C in a tissue culture incubator maintained at 5% CO_2.

Cell culture treatments

For TREM-1 activation, tissue culture-treated plates were preincubated with an appropriate volume of 5 μg/ml anti-TREM-1 Ab (R&D Systems, catalog no. MAB1278) in PBS overnight in a tissue culture incubator. Wells were washed twice with PBS immediately before cell addition. As a control, wells received the same treatment with an isotype-matched murine IgG1 Ab (anti-*Eimeria tenella*; Wyeth). For LPS treatment, gel filtration chromatography-purified LPS from *Salmonella enterica* (Sigma-Aldrich, catalog no. L2262) was added to a final concentration of 1 ng/ml. In the wortmannin studies, cells in RPMI 1640/10% FBS were preincubated with wortmannin (Sigma-Aldrich, catalog no. W1628) at a final concentration of 100 nM for 30 min in polypropylene tubes before seeding. The final DMSO concentration in DMSO- and wortmannin-treated cultures was 0.1%.

Microarray

Monocytes (5×10^6) received the following six treatments in 12-well tissue culture-treated plates: untreated, isotype control, anti-TREM-1, LPS, isotype control plus LPS, and anti-TREM-1 plus LPS. Total RNA was isolated after 2 h using QIAshredders and RNeasy Miniprep kits according to the manufacturer's protocol (Qiagen, catalog nos. 79654 and 74104, respectively). Total RNA yields ranged from 1 to 6 μg. Residual genomic DNA was removed by DNase treatment and phenol/chloroform extraction followed by ethanol precipitation using standard techniques. Microarray on Affymetrix Human Genome U133 plus 2.0 arrays was performed according to established protocols. For each array, all probe sets were normalized to a mean intensity value of 100. The default GeneChip operating software (GCOS) statistical values were used for all analyses. Monocytes from a total of 11 healthy donors were analyzed.

Microarray data analysis

Only qualifiers present at >50 signal units and called "present" in >66% of treatment groups were considered for analysis. Normalized signal values were transformed to $\log_2$ values before ANOVA analysis. Qualifiers with $p < 0.01$ and fold change >2.0 between any treatment groups were used to generate the heat map in Fig. 1 and for subsequent analyses. Fold changes were calculated from the following comparisons: anti-TREM-1 to isotype control Ab (TREM), LPS to untreated (LPS), and anti-TREM-1 plus LPS to isotype control Ab (combined or dual). Fold reductions are reported as negative fold changes. For genes represented by multiple qualifiers, we selected the qualifier with the highest average intensity in the untreated sample for analysis.

Real-time RT-PCR analysis

Total RNA from 5×10^6 monocytes was isolated after 2 h of treatment using QIAshredders and RNeasy Plus Miniprep kits (Qiagen, catalog no. 74134). Three hundred nanograms of total RNA was analyzed by real-time RT-PCR in a 20-μl reaction volume using an RT-PCR master mix (Applied Biosystems, catalog no. 4309169) and an Applied Biosystems 7900HT fast real-time PCR machine. The following inventoried real-time RT-PCR primer/probe sets from Applied Biosystems were used: assay identifiers Hs99999905_m1 (*GAPDH*), Hs00174097_m1 (*IL1B*), Hs00171266_m1 (*CSF2*), Hs00233688_m1 (*IL12B*), and Hs00372324_m1 (*IL23A*). Fold inductions relative to GAPDH were normalized to isotype control treated samples, which were set to a value of 1.0. All real-time RT-PCR were performed in duplicate.

ELISA measurements

Cell culture media were analyzed using the following kits: R&D Systems catalog no. DY338 (INHBA), R&D Systems catalog no. DOST00 (SPP1),

R&D Systems catalog no. DMC00 (M-CSF), R&D Systems catalog no. DCS50 (G-CSF), and eBioscience catalog no. 88-7239 (IL-23). IL-1β, GM-CSF, and IL-12p40 levels in cell culture media were determined using custom-coated multispot plates and a Sector Imager 6000 according to the manufacturer's protocol (Meso Scale Discovery). All ELISA measurements were performed on duplicate treatments.

Results

Global gene expression analysis of purified human monocytes

Initially, we evaluated our experimental system by testing the effect of activating TREM-1 on human monocytes using an anti-TREM-1 Ab. This treatment reproducibly resulted in the secretion of multiple proinflammatory cytokines into cell culture media (see below and data not shown). The response was observed only when the Ab was prebound to plates, consistent with TREM-1 activation via receptor cross-linking. Additionally, an isotype-matched control Ab and other Abs tested had no effect (see below and data not shown). Our system therefore faithfully reproduces some of the events described by other groups using similar methods to activate TREM-1 (9, 12–14).

To investigate the cellular consequences of TREM-1 activation, we examined global gene expression changes following treatment with the anti-TREM-1 Ab. For comparison, we analyzed cells receiving LPS treatment and also cells receiving a combined treatment of anti-TREM-1 plus LPS. Briefly, purified human monocytes from 11 anonymous healthy donors were treated with anti-TREM-1 Ab, 1 ng/ml LPS, or both and subjected to microarray analysis. We analyzed a relatively short time point of 2 h to minimize the contribution of secondary and/or differentiation effects while generating gene expression changes suitable for high-confidence analysis. As controls, cells received either no treatment or treatment with an isotype-matched control Ab (isotype). Fold changes in mRNA expression were determined using the following comparisons: anti-TREM-1 to isotype (TREM), LPS to untreated (LPS), and α-TREM-1 plus LPS to isotype (combined or dual). Additional details on statistical analyses are provided in *Materials and Methods*, and the complete microarray results have been deposited in the Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo/) of the National Center for Biotechnology Information and are accessible through GEO Series accession number GSE9988.

Genes significantly regulated ($p < 0.01$) with a fold change of >2.0 between any treatment groups were selected for our initial analysis. A hierarchical clustering algorithm was used to group qualifiers with similar patterns of expression. A heat map of the relative expression of these qualifiers is shown in Fig. 1, both for individual donor (Fig. 1*A*) and as average expression (Fig. 1*B*). The isotype control Ab was essentially inert, as there was little difference between isotype and untreated and between isotype plus LPS and LPS alone. As seen in Fig. 1, there were significant and consistent changes in gene expression across donors with either TREM-1 activation or LPS. The combined treatment of TREM-1 activation plus LPS was predominantly a union of these individual treatments. The exception was a subset of LPS-induced genes antagonized by TREM-1 activation, denoted by an asterisk (Fig. 1*B*) and discussed below.

Given the large number of genes with changes in expression, we restricted most of our subsequent analyses to genes that passed our initial filtering criteria and had fold changes of >4 with either TREM-1 activation or LPS. Unassigned qualifiers were eliminated for the following analyses. By these criteria, 232 genes were up-regulated >4-fold with either TREM-1 activation or LPS. Of these, 69 genes were up-regulated >4-fold in both treatments or >4-fold in only one treatment but within 2-fold in a direct pairwise comparison between the two treatments. We have categorized these genes as commonly up-regulated. The remainder of genes up-regulated >4-fold with either TREM-1 activation or LPS have been categorized as treatment specific or treatment biased. These consist of 62 genes for TREM-1 activation and 101 genes for LPS. Hypothetical and predicted genes that met the above criteria are not listed in the tables but are provided in the figure legends, as are *p* value ranges for the relevant data sets. A brief description of regulated genes accompanies the tables, with implications of select findings reserved for *Discussion*.

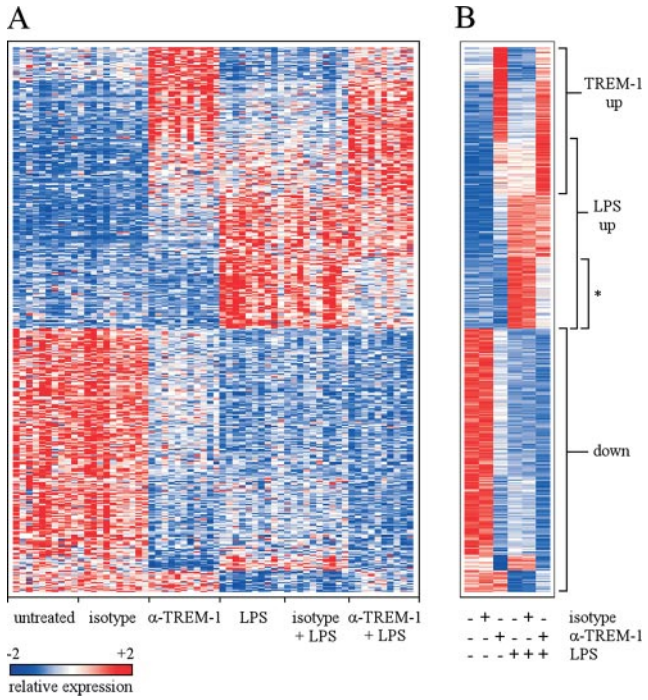

FIGURE 1. Heat map clustering of microarray data. Purified human monocytes from 11 anonymous healthy donors received the indicated treatments for 2 h before RNA isolation and microarray analysis. Treatments included no treatment (untreated), isotype control Ab (isotype), anti-TREM-1 cross-linking Ab (column labeled "α-TREM-1"), 1 ng/ml LPS (column labeled "LPS"), isotype plus 1 ng/ml LPS (column labeled "isotype + LPS"), and anti-TREM-1 plus 1 ng/ml LPS (column labeled "α-TREM-1 + LPS"). Shown are the relative expression in individual donors (*A*) and average intensity (*B*) for those qualifiers with fold change of >2.0 ($p < 0.01$) between any treatment groups. Columns in *A* represent individual donors and rows in both *A* and *B* represent individual qualifiers. Bracketed regions in *B* indicate heat map regions corresponding to qualifiers up-regulated by TREM-1 activation (TREM-1 up) or LPS (LPS up) and genes down-regulated with either treatment. The asterisk (*) designates a cluster of LPS-regulated qualifiers whose induction is antagonized when LPS is combined with TREM-1 activation.

Genes up-regulated by both TREM-1 activation and LPS, and down-regulated genes

The genes we categorized as commonly up-regulated are listed in Table I. Provided are fold changes with TREM-1 activation (Table I, column labeled "TREM"), LPS (column labeled "LPS"), and combined TREM-1 activation plus LPS (column labeled "Dual"), ranked by fold induction with TREM-1 activation. Represented on this list are TNF superfamily members and modulators (in order of appearance: *TNFSF15*, *BRE*, *TNF*), chemokines (*CXCL3*, *CXCL2*, *CCL20*, *CXCL5*, *CCL3*), other cytokines and mitogenic factors (*CSF2*, *IL6*, *AREG*), matrix metalloproteinases (*MMP1*, *MMP10*), and *PTGS2*/*COX2*. These results are consistent with both TREM-1

Table I. *Commonly up-regulated genes*[a]

Gene	Description	Fold Induction		
		TREM	LPS	Dual
INHBA	TGF-β family (inhibin βA)	96.7	97.0	219.7
TNFSF15	TNF ligand superfamily, 15 (VEGI/TL1A)	30.2	4.4	39.9
MMP1	Matrix metalloproteinase 1 (interstitial collagenase)	29.1	7.3	64.9
GPCR5A	GPCR (orphan)	17.8	6.0	18.6
F3	Coagulation factor III	13.3	32.5	77.7
DTNBP1	Dystrobrevin binding protein 1	13.0	9.7	14.5
MAD1L1	MAD1 mitotic arrest deficient-like 1 (yeast)	12.7	4.9	22.6
EDN1	Endothelin 1	11.5	7.9	31.5
CXCL3	Chemokine (GRO-γ)	11.2	31.7	30.9
GEM	Ras superfamily GTPase	10.9	5.3	17.0
TFPI2	Tissue factor pathway inhibitor 2	10.3	15.5	42.0
HES4	BHLH transcription factor	10.0	5.2	13.4
CSF2	CSF 2 (GM-CSF)	9.6	18.9	192.4
MMP10	Matrix metalloproteinase 10 (stromelysin 2)	9.6	16.0	56.8
CXCL2	Chemokine (GRO-β/MIP-2α)	7.8	16.0	16.4
EGR1	Early growth response 1; C2H2 Zn^{2+} finger protein	7.5	5.3	15.4
NR4A2	Nuclear receptor subfamily 4, A2	6.6	2.4	8.1
ARRDC4	Arrestin domain containing 4	6.5	3.3	6.1
PLD1	Phospholipase D1, PC-specific	6.4	12.2	28.3
ASNS	Asparagine synthetase	6.3	5.6	7.9
HOMER1	Homer homolog 1 (*Drosophila*); Ca^{2+} channel modulator	6.0	2.8	5.6
PLEKHC1	Pleckstrin homology domain containing, C1	5.8	5.1	9.0
FOSL1	FOS-like Ag 1 (FOS family)	5.8	3.5	8.4
E2F7	E2F transcription factor 7	5.5	18.1	31.9
EGR3	Early growth response 3 (C2H2 Zn^{2+} finger protein)	5.5	2.9	6.0
ELOVL7	ELOVL family, elongation of long chain fatty acids, 7	5.5	2.5	4.8
SLAMF7	Signaling lymphocytic activation molecule family, 7	5.3	2.7	4.7
ABL2	v-Abl Abelson MLV oncogene homolog 2	5.3	4.6	6.8
CCL20	Chemokine (MIP-3α)	5.1	11.6	10.3
CXCL5	Chemokine (ENA-78)	5.1	5.1	8.9
CCL3	Chemokine (MIP-1α)	5.0	6.0	7.8
PALM2-AKAP2	PALM2-AKAP2 co-transcribed product	5.0	5.7	8.0
SYNJ2	Synaptojanin 2; PI phosphatase and Rac1 interactor	4.9	11.5	8.4
PTGS2	Prostaglandin-endoperoxide synthase 2 (COX-2)	4.7	19.2	19.4
IL6	Interleukin 6	4.6	125.5	74.7
ADORA2B	Adenosine A2β GPCR	4.6	3.5	5.2
PHLDA1	Pleckstrin homology-like domain, A1	4.6	2.7	4.6
ATP1B1	ATPase, Na^+/K^+ transporting, β1	4.5	4.1	4.1
NEDD4L	NEDD-4-like (ubiquitin ligase)	4.5	3.5	8.7
RGS1	Regulator of G-protein signaling 1	4.3	2.1	4.5
MAFF	v-Maf homolog F; bZIP protein	4.3	5.4	7.7
NME7	Nucleoside diphosphate kinase	4.3	3.3	3.7
GLA	Galactosidase, α	4.3	2.1	5.0
BRE	TNFR p55 modulator	4.3	16.7	10.6
OKL38	pregnancy-induced growth inhibitor (OSGIN1)	4.1	1.9	4.2
TNF	TNF-α	4.1	16.2	19.8
AREG	EGF family (amphiregulin)	4.1	7.2	6.5
ETS2	v-Ets AEV E26 oncogene homolog 2 (avian)	3.7	5.9	8.5
ANKRD15	Ankyrin repeat domain 15	3.6	7.1	7.2
ADRB2	β-2-adrenergic GPCR, surface	3.3	6.2	9.0
MCF2L2	MCF.2 cell line derived sequence; Rho family GEF	3.3	4.0	4.6
SFMBT2	Scm-like with four mbt domains 2	3.3	5.9	5.8
HES1	Hairy and enhancer of split 1 homolog (bHLH)	3.2	4.7	4.2
SERPINB2	Serine (or cysteine) proteinase inhibitor, B2 (PAI-2)	3.2	4.8	5.0
CCRL2	Chemokine (C-C motif) receptor-like 2	2.9	4.7	5.5
FAD158	Factor for adipocyte differentiation 158	2.9	4.1	4.5
CCL18	Chemokine (MIP-4)	2.8	4.3	6.2
STARD4	START domain containing 4; cholesterol homeostasis	2.7	4.6	4.0
PHLDA2	Pleckstrin homology-like domain, A2	2.7	4.3	4.9
HECW2	HECT, C2, WW domain containing ubiquitin ligase 2	2.6	4.0	4.1
GADD45B	Growth arrest and DNA-damage-inducible, β	2.6	4.6	4.5
YRDC	Ischemia/reperfusion inducible protein	2.1	4.5	3.7

[a] Genes up-regulated >4-fold with TREM-1 activation and >4-fold with LPS or >4 fold in one treatment and within 2-fold in a direct pairwise comparison between treatments. Shown are fold inductions with TREM-1 activation (column labeled "TREM"), LPS (column labeled "LPS"), and TREM-1 activation plus LPS (column labeled "Dual"), ranked by fold induction with TREM-1 activation. The *p* values for genes up-regulated >4-fold with TREM-1 activation in this table ranged from 1.7×10^{-3} to 1.5×10^{-10}, and those for LPS ranged from 4.1×10^{-3} to 2.0×10^{-14}. Genes that met the filtering criteria but are not listed are *C20orf139*, *KIAA1718*, *LOC348938*, *LOC401151*, *LOC401588*, *LOC92162*, and *MGC4504*.

activation and LPS eliciting a proinflammatory response. Also present in Table I are INHBA, coagulation and vascularization factors (*F3*, *EDN1*, *TFPI2*, *SERPINB2*), transcription and DNA binding factors (*HES4*, *EGR1*, *FOSL1*, *E2F7*, *EGR3*, *MAFF*, *ETS2*, *HES1*), and factors involved in lipid metabolism and/or signaling (*PLD1*, *ELOVL7*, *SYNJ2*, *GLA*, *STARD4*). In the combined (dual)

Table II. *Down-regulated genes*[a]

Gene	Description	Fold Induction		
		TREM	LPS	Dual
CCR2	Chemokine (C-C motif) receptor 2	−8.4	−7.2	−11.2
DHX57	DEAH-box RNA/DNA helicase	−6.5	−3.0	−9.6
GIMAP6	GTPase, immunity-associated protein 6	−6.3	−5.5	−13.9
GIMAP7	GTPase, immunity-associated protein 7	−5.8	−9.7	−8.1
FUCA1	Fucosidase, α-L-1, tissue	−5.6	−5.6	−8.9
ADORA3	Adenosine A3 GPCR	−5.6	−4.9	−5.6
CX3CR1	Chemokine (C-X3-C motif) receptor 1	−5.5	−9.9	−16.1
TSCOT	Thymic stromal cotransporter (SLC64A2)	−5.0	−8.1	−6.2
CST3	Cystatin C; cysteine protease inhibitor	−4.9	−4.4	−4.9
BMF	Bcl2 modifying factor	−4.6	−4.0	−5.5
CCL8	Chemokine (MCP-2)	−4.6	−1.5	−6.1
FPRL2	Formyl peptide receptor-like 2; DC maturation modulator	−4.4	−3.1	−8.8
CARD12	Caspase recruitment domain family, 12 (NLRC4)	−4.3	−5.4	−11.9
LILRB1	Leukocyte Ig-like receptor, B1; ITIM-containing	−4.2	−2.1	−7.1
TLR1	TLR 1	−4.1	−8.1	−11.4
FAM26B	Family with sequence similarity 26, B	−4.1	−6.5	−7.7
GIMAP8	GTPase, immunity-associated protein 8	−3.8	−4.0	−5.8
SAP30	Sin3-associated polypeptide; HDAC complex component	−3.6	−4.2	−4.1
GIMAP1	GTPase, Iimmunity-associated protein 1	−3.4	−5.1	−9.1
ARRDC2	Arrestin domain containing 2	−3.2	−4.1	−4.7
RGS19	Regulator of G-protein signaling 19	−3.0	−4.2	−4.6
UBADC1	Ubiquitin-associated domain containing 1 (GBDR1)	−2.6	−4.5	−6.0
NALP12	NACHT, LRR and PYD containing 12 (NLRP12/Monarch1)	−2.4	−4.0	−3.8
PFKFB4	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	−1.9	−4.7	−3.4
CECR6	Cat eye syndrome chromosome region, candidate 6	−1.3	−5.9	−5.1
OLIG1	Oligodendrocyte bHLH transcription factor 1	−1.2	−4.5	−2.5
ZNF555	Zn^{2+} finger protein 555	−1.1	−4.9	−2.3
OLIG2	Oligodendrocyte bHLH transcription factor 2	3.1	−5.6	1.4

[a] Genes down-regulated >4-fold (fold induction less than −4 fold) with either TREM-1 activation or LPS. Shown are fold inductions with TREM-1 activation (column labeled "TREM"), LPS (column labeled "LPS"), and TREM-1 activation plus LPS (column labeled "Dual"), ranked by fold reduction with TREM-1 activation. *p* values for genes down-regulated >4-fold with TREM-1 activation in this table ranged from 5.6×10^{-3} to 5.7×10^{-12}, and those for LPS ranged from 2.4×10^{-3} to 1.1×10^{-14}. Genes that met the filtering criteria but are not listed are *C9orf59*, *FLJ12442*, *FLJ33641*, *LOC90120*, *MGC2941*, and *MGC17791*.

treatment, the expression change for the majority of the genes in Table I was within 2-fold of the sum of those in individual treatments. One notable exception was *CSF2* (i.e., GM-CSF), whose mRNA induction was significantly increased in combined treatment with respect to individual treatments (9.6-, 18.9-, and 192.4-fold with TREM-1 activation, LPS, and combined treatment, respectively).

As seen in Fig. 1, a comparable number of genes were down-regulated in our analysis as were up-regulated, although there was less treatment specificity among these genes. Table II lists genes with fold changes of less than −4 (i.e., down-regulated >4-fold) with either TREM-1 activation or LPS. Among these genes are chemokine receptors (*CCR2*, *CX3CR1*), transcription factors (*OLIG1*, *ZNF555*, *OLIG2*), GTPases of immunity-associated proteins (*GIMAP6*, *GIMAP7*, *GIMAP8*, *GIMAP1*), and CCL8. Additionally, TLR1 and NOD-like receptors (*CARD12*, *NALP12*) are also on this list. An example of a gene that was not commonly down-regulated is the oligodendrocyte transcription factor OLIG2, which was up-regulated 3.1-fold by TREM-1 activation and down-regulated 5.6-fold by LPS (Table II). By and large, however, relatively few genes were down-regulated in one treatment but not the other. The dynamic range in down-regulation was lower than that for up-regulation, as expected given the limiting kinetic contribution of mRNA half-lives to the analysis.

Genes preferentially up-regulated by either TREM-1 activation or LPS

Tables III and IV list those genes that are preferentially up-regulated by either TREM-1 activation or LPS, respectively. The lists are ranked by how biased the genes were, i.e., the ratio in a direct pairwise comparison of TREM to LPS (Table III) or LPS to TREM (Table IV). Genes preferentially induced by TREM-1 activation include *SPRY2*, cytokines and related molecules (*TNFSF14*, *CSF1*, *SPP1*, *CCL7*, *IL1F5*, *LIF*), metallothioneins (*MT1K*, *MT1E*, *MT1F*), phosphatases (*DUSP14*, *DUSP4*), transcription factors (*EGR2*, *ATF3*), factors involved in lipid metabolism and/or signaling (*EDG3*, *PPARG*, *LPL*, *PPAP2B*, *PLCXD1*, *NPC1*, *FABP3*, *ACSL3*), and MMP19. Genes preferentially induced by LPS include interleukins (*IL23A*, *IL12B*, *EBI3*, *IL1F9*, *IL10*, *IL1A*, *IL18*), IL receptors (*IL15RA*, *IL2RA*, *IL7R*), cytokines and related molecules (*CSF3*, *CCL23*, *CXCL1*, *TSLP*, *CCL5*, *CLC*, *EREG*, *TNFSF9*), factors involved in lipid metabolism and/or signaling (*SGPP2*, *PLA1A*, *MGLL*), kinases (*MAP3K8*, *RIPK2*, *MAP3K4*, *TBK1*, *PIM3*), regulators of NF-κB signaling (*TNIP3*, *NFKBIZ*), *CCR7*, and *CIAS1*. In general, genes preferentially induced by TREM-1 activation were largely unaffected with concomitant LPS treatment. However, as seen in Fig. 1 and Table IV, some genes preferentially induced by LPS were clearly antagonized by concomitant TREM-1 activation. This repressive effect is addressed in more experimental detail below.

Confirmation of selected microarray data and of factors preferentially induced by TREM-1 activation or LPS

The microarray results pointed to a number of immunomodulatory factors with similar or divergent regulation by TREM-1 activation and LPS. For follow-on studies, we focused on selected genes with potentially important functional and/or mechanistic implications. These included (with fold changes for TREM and LPS, respectively, in parentheses): INHBA, a TGF-β family member (96.7 and 97.0); SPP1, a cytokine-like extracellular matrix protein also known as OPN or osteopontin (28.0 and 3.7); IL-23, a heterodimeric p40/p19 cytokine important for Th17 cell maintenance

Table III. *Genes preferentially up-regulated by TREM-1 activation*[a]

Gene	Description	Fold Induction			Ratio[b]
		TREM	LPS	Dual	
SPRY2	Sprouty 2; RTK and MAPK modulator	78.4	1.1	51.0	73.0
MT1K	Metallothionein 1K	20.7	1.7	11.6	18.7
TNFSF14	TNF ligand superfamily, 14 (LIGHT)	8.9	−1.6	7.8	17.1
CRTAM	Class-I MHC-restricted T cell associated molecule	9.5	−1.5	4.4	15.4
LRFN4	LRR and fibronectin type III domain containing, 4	4.7	−2.3	2.3	11.6
EDG3	Sphingolipid GPCR, 3 (S1P3)	7.2	−1.9	4.6	10.8
CSF1	CSF 1 (M-CSF)	22.0	1.8	26.2	10.4
HTRA4	HtrA serine protease 4	5.7	−2.5	3.4	10.1
LY9	Lymphocyte Ag 9 (CD229, SLAMF3)	7.7	−1.2	3.0	10.1
SPP1	Secreted phosphoprotein 1 (OPN)	28.0	3.7	27.2	8.3
HSD3B7	OH-δ-5-steroid dehydrogenase, 3 β- and δ-isomerase 7	5.3	−1.1	2.2	7.6
PPARG	Peroxisome proliferative activated receptor, γ	7.8	1.6	5.8	6.7
SGNE1	Secretory granule, neuroendocrine protein 1 (SCG5)	13.2	1.2	8.0	6.6
GIPR	Gastric inhibitory polypeptide receptor	6.4	−1.1	4.4	6.5
MT1E	Metallothionein 1E	4.5	−1.2	3.3	6.5
RNUT1	U snRNA transporter (SNUPN)	5.6	−1.4	4.3	6.3
LPL	Lipoprotein lipase	10.9	1.9	7.5	6.2
DUSP14	Dual specificity phosphatase 14	4.9	−1.1	2.3	6.0
MMP19	Matrix metalloproteinase 19	10.1	2.1	9.7	5.4
SPINK1	Serine protease inhibitor, Kazal type 1	12.5	2.1	12.6	5.4
MOAP1	Modulator of apoptosis 1	4.5	−1.1	4.3	5.1
MT1F	Metallothionein 1F	4.3	1.1	3.3	5.0
EGR2	Early growth response 2; C2H2 Zn^{2+} finger protein	13.2	1.9	10.6	4.9
GREM1	Gremlin 1 (BMP antagonist)	9.2	1.9	5.8	4.8
RAMP	RA-regulated nuclear matrix-associated protein (DTL)	7.1	−1.1	6.4	4.7
TM7SF4	DC-specific transmembrane protein (DCSTAMP)	8.7	1.4	7.9	4.5
ASPH	Aspartate β -hydroxylase; Ca^{2+} homeostasis	8.7	3.4	7.2	4.4
WBP5	WW domain binding protein 5	4.5	−1.0	3.6	4.4
RIS1	Ras-induced senescence 1	5.8	1.4	2.7	4.4
PPAP2B	Phosphatidic acid phosphatase, 2B	5.0	1.2	3.5	4.4
PLCXD1	PI-specific PLC, X domain containing 1	5.3	1.7	3.7	3.8
NPC1	Putative membrane protein (cholesterol trafficking)	4.8	1.7	4.4	3.5
FABP3	Fatty acid binding protein 3	5.1	1.8	5.3	3.2
ACSL3	Acyl-CoA synthetase long-chain, 3	4.2	1.4	3.2	3.1
SLCO4A1	Solute carrier organic anion transporter family, 4A1	5.9	1.6	7.1	3.1
ATF3	Activating transcription factor 3	4.9	1.2	3.9	3.1
RGS13	Regulator of G-protein signaling 13	4.5	1.5	3.9	3.1
FNDC3	Fibronectin type III domain containing 3	5.1	1.2	4.0	3.0
NRIP3	Nuclear receptor interacting protein 3	4.0	1.6	3.5	3.0
THBD	Thrombomodulin	4.6	1.4	3.3	2.9
CCL7	Chemokine (MCP-3)	5.1	2.3	3.3	2.7
TBC1D7	TBC1 domain family, member 7	4.2	1.9	3.9	2.6
DUSP4	Dual specificity phosphatase 4	14.0	3.6	8.5	2.6
SNAPC1	Small nuclear RNA activating complex, polypeptide 1, 43 kDa	5.5	2.1	5.8	2.6
RHOBTB3	Rho-related BTB domain containing 3	5.6	1.8	7.3	2.6
IL1F5	IL 1 family, 5 (δ)	6.4	2.2	9.4	2.4
EIF2AK3	Eukaryotic translation initiation factor 2-α kinase 3	4.1	1.9	4.1	2.4
DSCR1	Down syndrome critical region, 1; calcineurin inhibitor	5.4	2.2	7.4	2.3
HBEGF	Heparin-binding EGF-like growth factor	6.7	2.1	7.6	2.2
CKS2	CDC28 protein kinase regulatory subunit 2	5.4	2.7	6.1	2.2
NT5E	5′-Nucleotidase, ecto (CD73)	5.1	2.4	4.0	2.2
LIF	Leukemia inhibitory factor; cytokine	7.1	1.7	23.9	2.1
RRAD	Ras-related associated with diabetes	7.2	3.5	10.5	2.1
GCLM	Glutamate-cysteine ligase, modifier subunit	6.3	3.4	6.9	2.1
RGS16	Regulator of G-protein signaling 16	4.5	1.8	5.8	2.0

[a] Genes up-regulated >4-fold with TREM-1 activation that are not commonly up-regulated. Shown are fold inductions with TREM-1 activation (column labeled "TREM"), LPS (column labeled "LPS"), TREM-1 activation plus LPS treatment (column labeled "Dual"), and the ratio of average expression in a direct pairwise comparison of TREM-1 activation to LPS (ratio). Genes are ranked by ratio, i.e., how biased their induction was toward TREM-1 activation when compared to LPS. *p* values for genes up-regulated >4-fold with TREM-1 activation in this table ranged from 7.7×10^{-4} to 2.6×10^{-12}. Genes that met the filtering criteria but are not listed are *C6orf114*, *C6orf128*, *C9orf47*, *KIAA1199*, *KIAA1393*, *LOC440995*, and *MGC33212*.

[b] TREM/LPS ratio from direct pairwise comparison.

(−1.1 and 31.8 for *IL12B*/p40, and 1.1 and 54.4 for *IL23A*/p19); and the colony stimulating factors CSF1 (22.0 and 1.8), CSF2 (9.6 and 18.9), and CSF3 (1.3 and 45.2). The CSFs are mitogenic factors for either monocyte lineage cells (CSF1, i.e., M-CSF), neutrophils (CSF3, i.e., G-CSF), or both (CSF2, i.e., GM-CSF). The levels of these proteins secreted into cell culture media following either TREM-1 activation or LPS were measured over an 8-h time course (Fig. 2). Here and throughout, comparable results were obtained using multiple monocyte preparations with representative data provided. Monocytes produced INHBA and GM-CSF in response to both TREM-1 activation and LPS (Fig. 2*A*). However, SPP1 and M-CSF were much more strongly produced in response to TREM-1 activation when compared with LPS (Fig. 2*B*). In contrast, IL-23 and G-CSF were detectably produced only in response

Table IV. *Genes preferentially up-regulated by LPS*[a]

Gene	Description	Fold Induction			
		TREM	LPS	Dual	Ratio[b]
IL23A	IL 23, α subunit (IL-23p19)	1.1	54.4	18.4	76.4
TNIP3	TNFAIP3 interacting protein 3 (ABIN-3)	1.2	124.2	14.3	64.7
CSF3	Colony stimulating factor 3 (G-CSF)	1.3	45.2	36.4	54.4
IL12B	IL 12, β subunit (IL-12p40)	−1.1	31.8	4.0	31.1
EBI3	EBV induced gene 3 (IL-27, β subunit)	−1.9	10.5	2.8	20.9
CCL23	Chemokine (MIP-3)	−2.0	5.6	1.5	16.8
PTTG1IP	Pituitary tumor-transforming 1 interacting protein	−1.6	8.0	2.9	15.2
TIFA	TRAF-interacting with a Forkhead-associated domain	−2.3	6.7	2.7	14.8
IL1F9	IL 1 family, 9 (ϵ)	2.7	34.3	23.5	13.9
KCNJ2	K^+ inwardly-rectifying channel, J2	−2.3	5.7	1.6	13.0
BF	B-factor; complement factor B (CFB)	1.2	13.0	4.7	12.6
GBP3	Guanylate binding protein 3	−2.2	4.9	2.3	10.7
CIAS1	Cold autoinflammatory syndrome 1 (NLRP3/cryopyrin)	−1.2	7.6	5.1	9.8
CDC2	Cell division cycle 2, G1 to S and G2 to M	1.6	12.8	6.5	9.4
TPRT	*Trans*-Prenyltransferase (PDSS1)	−1.0	9.5	5.7	9.2
CXCL1	Chemokine (GRO-α)	2.6	21.7	9.2	9.1
TSLP	Thymic stromal lymphopoietin (cytokine)	3.6	30.8	37.6	9.1
NFKBIZ	NF-κB gene enhancer in B-cells inhibitor, ζ	−1.8	4.4	3.4	8.2
PDCD1LG1	Programmed cell death 1 ligand 1 (B7-H1)	2.0	17.6	6.6	7.7
ZBTB10	Zn^{2+} finger and BTB domain containing 10	−1.1	6.4	4.1	7.6
LAMP3	Lysosomal-associated membrane protein 3 (DC-LAMP)	1.1	10.5	3.8	7.2
MCOLN2	Mucolipin 2; cation channel	−1.3	5.1	2.0	7.2
IL15RA	IL 15 receptor, α	−1.4	4.2	2.2	6.6
ICAM4	Intercellular adhesion molecule 4	−1.7	5.1	2.4	6.5
HAS1	Hyaluronan synthase 1	2.1	9.6	4.1	6.5
CCL5	Chemokine (RANTES)	−1.0	6.9	2.9	6.4
GPR43	GPCR 43 (FFAR2)	−1.3	4.8	1.7	6.1
PMAIP1	PMA-induced protein 1; Bcl-2 family (NOXA)	2.9	16.1	11.3	6.0
SGPP2	Sphingosine-1-phosphate phosphatase 2	1.3	9.0	4.2	5.9
CCRN4L	CCR4 carbon catabolite repression 4-like (*Saccharomyces cerevisiae*)	1.4	7.3	4.4	5.9
TPD52	Tumor protein D52	3.0	8.7	14.6	5.9
PLEKHF2	Pleckstrin homology domain containing, F2	−1.3	4.8	2.0	5.6
SYNPO2	Synaptopodin 2	−1.1	6.7	3.1	5.4
CASP5	Caspase 5; apoptosis-related cysteine protease	−1.3	4.8	1.8	5.4
MAP3K8	MEK kinase 8 (TPL2)	1.3	8.0	3.4	5.4
HS3ST3B1	Heparan sulfate (glucosamine) 3-*O*-sulfotransferase 3B1	−1.3	4.3	2.0	5.3
IL10	IL 10	1.5	12.1	7.2	5.3
EZH2	Enhancer of zeste (polycomb group) homolog	−1.7	4.2	1.6	5.2
MAP3K4	MEK kinase 4	1.5	8.9	6.1	5.2
IL1A	Interleukin 1, α	2.1	10.3	9.6	5.1
GJB2	Gap junction protein, β2, 26kDa (connexin 26)	2.9	14.0	12.7	5.1
BIRC3	Baculoviral IAP repeat-containing 3 (CIAP2)	−1.3	4.2	2.2	5.1
TRGV9[c]	TCR γ alternate reading frame protein	−1.0	6.2	1.8	4.8
SLAMF1	Signaling lymphocytic activation molecule family, 1	1.2	5.7	6.3	4.7
PLA1A	Phospholipase A1, A	1.6	8.5	4.7	4.7
GADD45A	Growth arrest and DNA-damage-inducible, α	2.0	8.8	8.1	4.7
IL2RA	IL 2 receptor, α	1.3	5.9	3.1	4.3
IL18	IL 18	1.9	7.6	4.9	4.2
RIPK2	Receptor-interacting serine-threonine kinase 2	1.1	4.9	3.2	4.0
ADAMDEC1	ADAM-like, decysin 1 (disintegrin metalloproteinase)	1.2	5.4	3.2	4.0
TBK1	TANK-binding kinase 1	1.2	4.9	4.1	4.0
RHOH	Ras homolog gene family, H	1.1	4.5	2.2	3.9
IL7R	Interleukin 7 receptor	1.2	5.2	2.7	3.9
PTX3	Pentaxin-related gene, induced by IL-1β (TNFAIP5)	1.8	7.0	5.4	3.8
CLC	Cardiotrophin-like cytokine (lysophospholipase)	1.1	4.2	4.2	3.8
RAPGEF2	Rap guanine nucleotide exchange factor (GEF) 2	1.4	5.5	4.2	3.8
ARL8	ADP-ribosylation factor-like 8 (Ras superfamily)	2.4	8.2	8.0	3.7
GBP1	Guanylate binding protein 1, interferon-inducible, 67 kDa	1.5	5.0	3.3	3.6
NDP	Norrie disease (pseudoglioma)	1.5	9.2	2.7	3.6
TRAF1	TNF receptor-associated factor 1	1.1	4.1	3.2	3.6
SAV1	Salvador homolog (WW domain containing)	1.5	6.4	4.7	3.5
XTP2	HbxAg transactivated protein 2 (BAT2D1)	1.2	4.3	4.2	3.4
SOCS3	Suppressor of cytokine signaling 3	1.3	4.3	2.8	3.4
G0S2	Putative lymphocyte G0/G1 switch gene	2.1	6.1	5.4	3.4
TWISTNB	TWIST neighbor	1.4	4.9	2.8	3.3
SPG3A	Spastic paraplegia 3A GTPase; vesicle trafficking	2.5	11.0	8.1	3.3
CFLAR	CASP8 and FADD-like apoptosis regulator	1.4	4.7	4.0	3.3
DUSP2	Dual specificity phosphatase 2	2.7	5.7	5.7	3.1
TEX14	Testis expressed sequence 14	3.2	13.4	15.2	3.1
SIAH2	Seven in absentia homolog; ubiquitin ligase	2.1	7.0	4.2	3.0

(*Table continues*)

Table IV. (*Continued*)

Gene	Description	Fold Induction			Ratio[b]
		TREM	LPS	Dual	
CCR7	Chemokine (C-C motif) receptor 7	2.5	7.1	7.3	2.9
UPB1	Ureidopropionase, β; pyrimidine degradation	1.3	4.9	3.1	2.8
FJX1	Four jointed box 1 (*Drosophila*)	1.1	4.1	1.8	2.7
MBNL1	Muscleblind-like (*Drosophila*)	1.5	4.0	2.7	2.7
EREG	EGF family (epiregulin)	2.0	5.0	3.7	2.7
ID2	Inhibitor of DNA binding 2; HLH containing	1.7	4.8	3.7	2.5
MGLL	Monoglyceride lipase	1.6	5.4	4.1	2.5
DNAJB4	DnaJ (Hsp40) homolog, subfamily B, member 4	2.9	6.2	6.1	2.4
FLT1	Fms-related tyrosine kinase 1; VEGF/VEGFA receptor	3.4	9.4	7.1	2.3
HEY1	Hairy/enhancer-of-split related; bHLH containing	2.4	5.4	5.0	2.3
PIM3	Pim-3 oncogene; serine/threonine kinase	2.1	4.5	4.7	2.2
TNFSF9	TNF ligand superfamily, member 9	1.7	4.9	2.5	2.1

[a] Genes up-regulated >4-fold with LPS that are not commonly up-regulated. Shown are fold inductions with TREM-1 activation (column labeled "TREM"), LPS (column labeled "LPS"), TREM-1 activation plus LPS treatment (column labeled "Dual"), and the ratio of average expression in a direct pairwise comparison of LPS to TREM-1 activation (ratio). Genes are ranked by ratio, i.e., how biased their induction was towards LPS when compared to TREM-1 activation. *p* values for genes up-regulated >4-fold with LPS in this table ranged from 1.2×10^{-3} to 3.6×10^{-14}. Genes that met the filtering criteria but are not listed are *C10orf78*, *C21orf71*, *FLJ14490*, *FLJ23231*, *FLJ25590*, *FLJ32499*, *KIAA0286*, *KIAA0376*, *LOC90167*, *LOC123872*, *LOC285628*, *LOC338758*, *LOC341720*, *LOCLOC374443*, *LOC387763*, *LOC400581*, *LOC441366*, *MGC10744*, and *MGC11082*.

[b] LPS/TREM ratio from direct pairwise comparison.

[c] TRGV9/TARP.

to LPS (Fig. 2*C*). These results are consistent with the microarray data and identify immunomodulatory factors that are preferentially induced by either TREM-1 activation or LPS. To our knowledge, this is the first report of cytokines or related factors induced by TREM-1 activation that are not also induced by LPS.

Cross-talk between TREM-1 activation and LPS: synergy

Consistent with published reports (9, 12–14), we routinely observed increased production of some cytokines when TREM-1 activation was combined with LPS. This effect was most striking with the cytokines IL-1β and GM-CSF. However, the microarray data indicated that these observations had different mechanistic explanations, because TREM-1 activation and LPS synergized in the induction of GM-CSF mRNA (Table I and see above) but not IL-1β mRNA. By microarray, *IL1B* was induced 1.8-, 1.9-, and 1.8-fold by TREM-1 activation, LPS, and combined treatment, respectively. We pursued these observations by comparing IL-1β and GM-CSF mRNA levels to protein production in individual

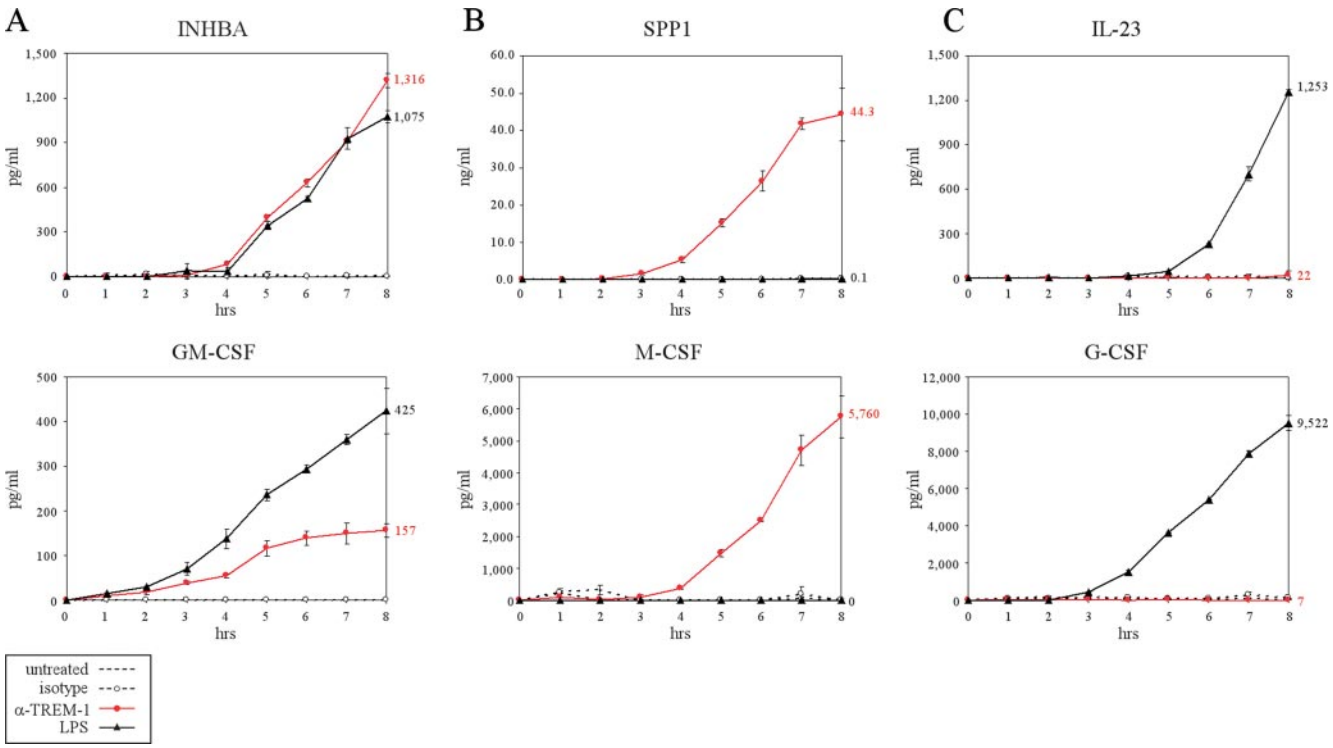

FIGURE 2. Confirmation of selected microarray data and identification of factors preferentially induced by either TREM-1 activation or LPS. ELISA measurements of protein levels in cell culture media over an 8-h time course are shown. *A*, INHBA and GM-CSF are induced by both TREM-1 activation (red solid line) and LPS (black solid line). *B*, SPP1 and M-CSF are preferentially induced by TREM-1 activation. *C*, IL-23 and G-CSF are preferentially induced by LPS treatment. The picogram or nanogram (SPPI) per milliliter values of these factors after 8 h for TREM-1 activation (red) and LPS (black) are provided. α-TREM-1, anti-TREM-1 cross-linking Ab.

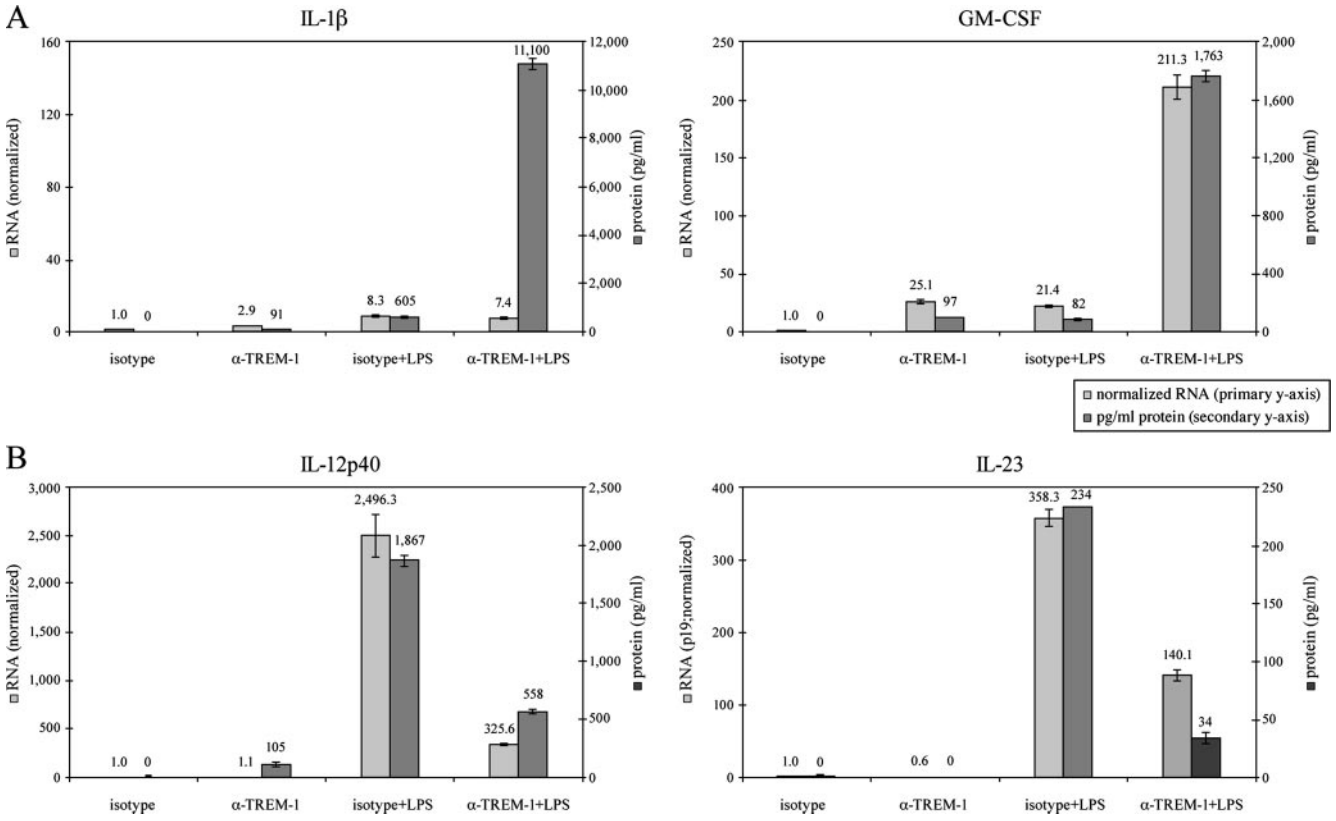

FIGURE 3. mRNA and protein level determination for factors where TREM-1 activation synergizes with LPS in protein production (IL-1β and GM-CSF) or antagonizes LPS-induced protein production (IL-12p40 and IL-23). *A*, Two-hour mRNA (light gray) and 8-h protein (dark gray) for IL-1β and GM-CSF after the indicated treatments. Treatments included isotype control Ab ("isotype") and anti-TREM-1 cross-linking Ab ("α-TREM-1") with or without 1 ng/ml LPS. mRNA inductions were determined by real time RT-PCR and normalized to GAPDH mRNA (primary *x*-axis). Protein levels in cell culture media were determined by ELISA and are shown as picograms per milliliter (secondary *y*-axis). *B*, Two-hour IL-12p40 and IL-23p19 mRNA and 8 h IL-12p40 and IL-23 protein, determined as in *A*.

monocyte preparations (Fig. 3*A*). mRNA levels were determined by real-time RT-PCR at 2 h posttreatment. Protein levels in cell culture media were assayed after 8 h of treatment, as this yielded suitable levels for analysis of these secreted factors.

Both TREM-1 activation and LPS induce IL-1β protein production (91 and 605 pg/ml, respectively), with LPS routinely a more potent stimulus (Fig. 3*A*). In all experiments, isotype plus LPS was indistinguishable from LPS alone and, for clarity, is reported as representative of the LPS response. Cells receiving a combined treatment of TREM-1 activation plus LPS produced 11,100 pg/ml IL-1β protein. However, this amplification was not reflected in the levels of IL-1β mRNA. TREM-1 activation, LPS, and combined treatment induced IL-1β mRNA 2.9-, 8.3-, and 7.4-fold, respectively. Similarly, both TREM-1 activation and LPS induce GM-CSF protein production (97 and 82 pg/ml, respectively), and comparable synergy is seen as with IL-1β (1,783 pg/ml GM-CSF with combined treatment). By real-time RT-PCR, GM-CSF mRNA was induced 25.1-, 21.4-, and 211.3-fold with TREM-1 activation, LPS, and combined treatment, respectively. Therefore, while the synergy between TREM-1 activation and LPS in IL-1β production appears to occur posttranscriptionally (i.e., in protein translation, processing, or secretion), that in GM-CSF production is likely a direct result of increased mRNA abundance.

Cross-talk between TREM-1 activation and LPS: repression

As seen in Fig. 1 and Table IV, TREM-1 activation noticeably attenuated the mRNA accumulation of some LPS target genes. Table V lists those genes up-regulated >2-fold by LPS that were antagonized >2-fold when LPS was combined with TREM-1 activation, as determined in a direct pairwise comparison of combined treatment to LPS. Some of these genes are present in Table IV, but the relaxed fold induction criteria (>2-fold vs >4-fold in Table IV) returned additional genes of potential interest. Genes in Table V are ranked by fold antagonization by TREM-1 activation. Among the most highly antagonized genes were *IL12B* (p40 of IL-12 and IL-23), *IL23A* (p19 of IL-23), *CCL23*, *TNIP3*, and *EBI3* (a p40-like subunit of IL-27). Additionally, genes implicated in and/or related to type I IFN responses (*IRF1*, *IFIT2*) were also induced by LPS and antagonized by concomitant TREM-1 activation.

We investigated these findings in more detail by analyzing 2-h IL-12p40 and IL-23p19 mRNA levels by real-time RT-PCR and 8-h IL-12p40 and IL-23 protein levels in cell culture media, as above (Fig. 3*B*). Consistent with the microarray results, the TREM-1 activation effect (denoted by an arrow, →) was to attenuate LPS-induced IL-12p40 and IL-23p19 mRNAs 7.6-fold (2,496.3→325.6 fold induction) and 2.6-fold (358.3→140.1 fold induction), respectively. These transcripts are extremely rare in the absence of LPS, which may influence the absolute but not relative fold induction values. Consistent with this mRNA attenuation, TREM-1 activation reduced the levels of IL-12p40 and IL-23 proteins produced in response to LPS 3.4-fold (1,267→558 pg/ml) and 6.9-fold (234→34 pg/ml), respectively. IL-12, a heterodimer of p40 and IL12A-encoded p35, was not detectable over the time course of these experiments, nor was the *IL12A* transcript detectably induced by LPS in the microarray experiments (data not

Table V. *Genes up-regulated by LPS that are antagonized by TREM-1 activation*[a]

Gene	Description	Fold Induction			
		TREM	LPS	Dual	Ratio[b]
IL12B	IL 12, β subunit (IL-12p40)	−1.1	31.8	4.0	−7.2
CCL23	Chemokine (MIP-3)	−2.0	5.6	1.5	−5.9
TNIP3	TNFAIP3 interacting protein 3	1.2	124.2	14.3	−5.3
IL23A	IL 23, β subunit (IL-23p19)	1.1	54.4	18.4	−4.6
EBI3	EBV induced gene 3 (IL-27, β subunit)	−1.9	10.5	2.8	−3.8
CMKOR1	CXCR7 (orphan)	−1.5	3.7	1.3	−3.6
KCNJ2	K^+ inwardly-rectifying channel, J2	−2.3	5.7	1.6	−3.4
WNT5A	Wingless-type MMTV integration site family, 5A	−2.4	2.6	−1.6	−3.4
HAS1	Hyaluronan synthase 1	2.1	9.6	4.1	−3.4
PTTG1IP	Pituitary tumor-transforming 1 interacting protein	−1.6	8.0	2.9	−3.3
BF	B factor (properdin)	1.2	13.0	4.7	−3.1
THBS1	Thrombospondin 1	−1.9	2.8	−1.2	−3.0
TMPO	Thymopoietin (LAP2)	−1.3	3.4	1.1	−2.9
OASL	2′-5′-Oligoadenylate synthetase-like	−1.6	3.0	1.3	−2.8
MCOLN2	Mucolipin 2; cation channel	−1.3	5.1	2.0	−2.8
GPR43	G protein-coupled receptor 43	−1.3	4.8	1.7	−2.7
PLAC8	Placenta-specific 8	−1.6	2.6	−1.1	−2.7
CXCL1	Chemokine (GRO-α)	2.6	21.7	9.2	−2.6
TRGV9[c]	TCR γ alternate reading frame protein	−1.0	6.2	1.8	−2.6
G1P2	Interferon, α-inducible protein (ISG15)	−1.3	2.9	1.5	−2.6
IFIT2	Interferon-inducible with tetratricopeptide repeats 2	−2.6	2.6	1.4	−2.5
PDCD1LG1	Programmed cell death 1 ligand 1	2.0	17.6	6.6	−2.4
TIFA	TRAF-interacting with a Forkhead-associated domain	−2.3	6.7	2.7	−2.4
CDC2	Cell division cycle 2, G1 to S and G2 to M	1.6	12.8	6.5	−2.3
IRF1	Interferon regulatory factor 1	−1.4	2.1	−1.1	−2.3
OAZ2	Ornithine decarboxylase antizyme 2 (polyamine synthesis)	−2.1	2.4	−1.1	−2.3
MLF1IP	MLF1 interacting protein	−1.7	3.5	1.4	−2.3
SLC1A3	Solute carrier family 1, 3 (glutamate transporter)	−1.4	3.9	1.7	−2.3
CASP5	Caspase 5, apoptosis-related cysteine protease	−1.3	4.8	1.8	−2.2
INDO	Indoleamine-pyrrole 2,3 dioxygenase (CD107B)	−1.3	2.9	1.3	−2.2
CDKN2B	Cyclin-dependent kinase inhibitor 2B (inhibits CDK4)	1.1	2.7	1.5	−2.2
IL15RA	IL 15 receptor, α	−1.4	4.2	2.2	−2.2
RHOU	Ras homolog gene family, member U	−1.2	3.5	1.4	−2.2
CCL5	Chemokine (RANTES)	−1.0	6.9	2.9	−2.1
LAMP3	Lysosomal-associated membrane protein 3	1.1	10.5	3.8	−2.1
PLEKHF2	Pleckstrin homology domain containing, F2	−1.3	4.8	2.0	−2.1
RIN2	Ras and Rab interactor 2	−1.9	2.3	1.1	−2.0
HS3ST3B1	Heparan sulfate (glucosamine) 3-*O*-sulfotransferase 3B1	−1.3	4.3	2.0	−2.0
PIM2	Pim-2 oncogene (serine/threonine kinase)	1.0	3.7	1.7	−2.0
EPM2AIP1	EPM2A (laforin) interacting protein 1	−1.7	2.5	1.2	−2.0
MAP3K8	MEK kinase 8 (TPL2)	1.3	8.0	3.4	−2.0

[a] Genes up-regulated >2-fold with LPS treatment that were antagonized >2-fold by TREM-1 activation as determined by a direct pairwise comparison of TREM-1 activation plus LPS to LPS. Shown are fold inductions with TREM-1 activation (column labeled "TREM"), LPS (column labeled "LPS"), TREM-1 activation plus LPS treatment (column labeled "Dual"), and the ratio of average expression in a direct pairwise comparison of dual to LPS treatment (ratio). Genes are ranked by ratio, i.e., the fold antagonization with TREM-1 activation. *p* values for genes up-regulated >2-fold with LPS in this table ranged from 6.4×10^{-3} to 9.6×10^{-12}, and those for ratio ranged from 6.7×10^{-3} to 6.8×10^{-7}. Genes that met the filtering criteria but are not listed are *C7orf16*, *C13orf18*, *KIAA0984*, *KIAA1533*, *LOC341720*, and *MGC20235*.

[b] Dual/LPS ratio from direct pairwise comparison.

[c] TRGV9/TARP.

shown). This is consistent with a requirement of IFN-γ for IL-12 production by monocytes in response to LPS (39).

Synergy is wortmannin sensitive, whereas significant repression persists in the presence of wortmannin

The class I PI3Ks are heterodimeric enzymes consisting of a p110 catalytic subunit (p110α, β, δ or γ) bound to a p85 regulatory subunit (p85α or β, p55α or γ, or p50α; collectively referred to as p85; (40). Association of the regulatory subunit with activated receptor complexes at the plasma membrane allows the catalytic subunit to convert phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol 3,4,5-trisphosphate, a lipid second messenger molecule. Inhibitors of PI3K are known to potentiate TLR signaling (41). The precise underlying mechanisms are not known, but accordingly PI3K inhibitors such as wortmannin increase the activity of several transcription factors in response to LPS (42–44), and decrease survival in mouse models of microbial sepsis (45, 46). In contrast, wortmannin blocks some signaling events downstream of TREM-1 activation (21).

We therefore tested the effect of wortmannin on the synergy in IL-1β and GM-CSF production and the attenuation in IL-12p40 and IL-23 production that we observe when TREM-1 activation is combined with LPS (Fig. 4). An opposite effect of wortmannin is seen, for example, in IL-1β production where wortmannin reduced the amount of IL-1β generated by TREM-1 activation (from 658 to 31 pg/ml) but increased the amount generated by LPS (from 1,173 to 9,662 pg/ml). This trend was observed with a number of cytokines, including TNF and IL-8 (data not shown). Consistent with an important role of PI3K in TREM-1-induced cytokine production, the contribution of TREM-1 activation to synergistic IL-1β and GM-CSF production was eliminated or greatly reduced in the presence of wortmannin. The TREM-1 activation effect (denoted by →) on LPS-induced IL-1β was 1,173→33,756 pg/ml in DMSO-treated cells and 9,662→5,038 pg/ml in wortmannin-treated cells. For GM-CSF, the TREM-1 activation effect was 81→899 pg/ml in DMSO-treated cells and 105→184 pg/ml in wortmannin-treated cells. With regard to repression, TREM-1

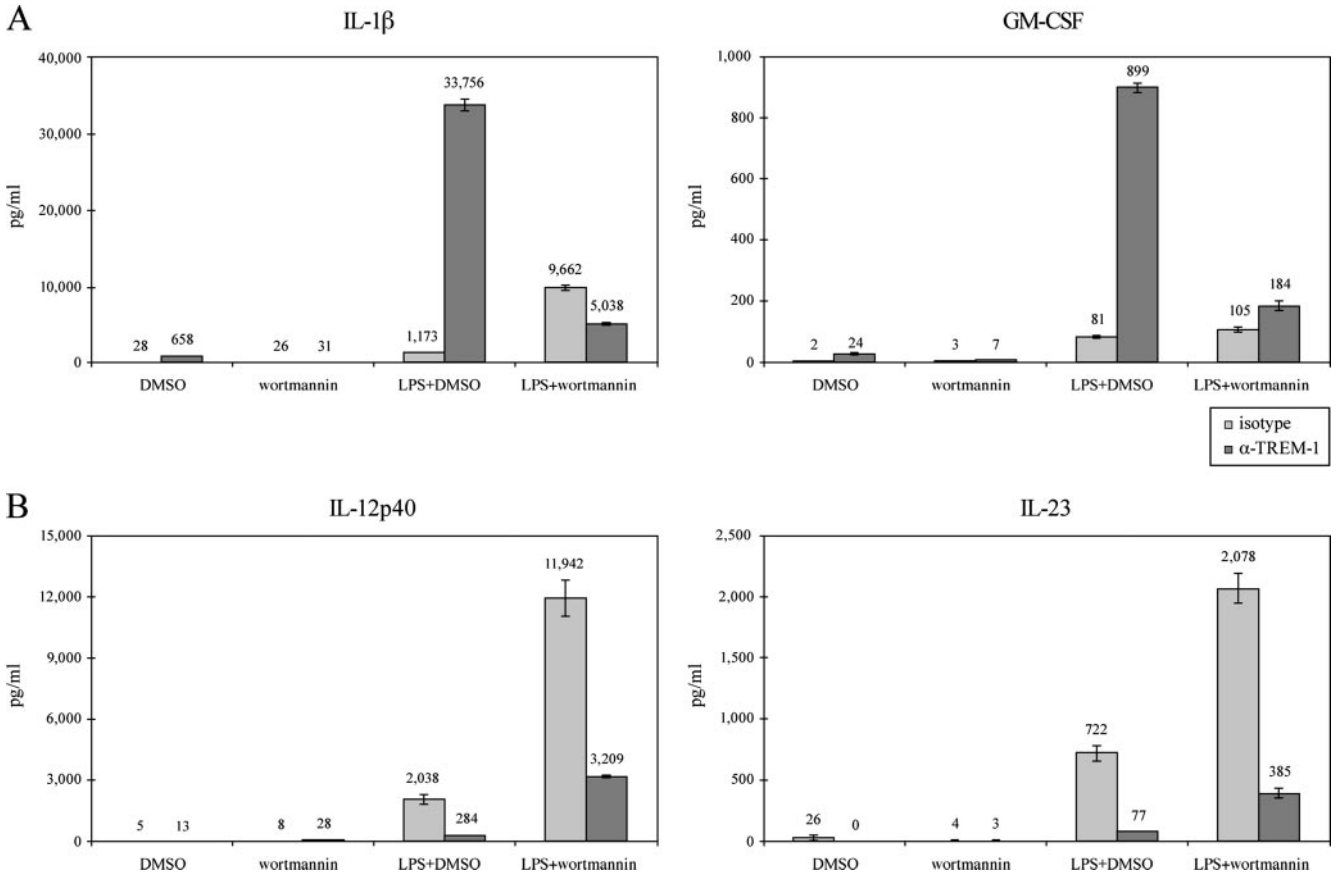

FIGURE 4. Effects of the PI3K inhibitor wortmannin on TREM-1 activation-mediated synergy (*A*) and repression (*B*). IL-1β, GM-CSF, IL-12p40, and IL-23 protein levels were determined in cell culture media after 8 h with the indicated treatments. Treatments included DMSO, 100 nM wortmannin, 1 ng/ml LPS plus DMSO, and 1 ng/ml LPS plus 100 nM wortmannin. Within each treatment group the cells also received treatment with either isotype control Ab (light gray) or anti-TREM-1 (α-TREM-1) cross-linking Ab (dark gray).

activation attenuated LPS-induced IL-12p40 and IL-23 protein levels 7.2-fold (2,038→294 pg/ml) and 9.4-fold (722→77 pg/ml), respectively, in DMSO-treated cells. In wortmannin-treated cells, the attenuation was 3.7-fold (11,942→3,209 pg/ml) and 5.4-fold (2,078→385 pg/ml), respectively. The levels of LPS-induced IL-12p40 and IL-23 were elevated significantly by wortmannin, yet attenuation by TREM-1 activation, although reduced, is still evident. Therefore, while the synergy between TREM-1 activation and LPS in IL-1β and GM-CSF largely requires PI3K signaling, some PI3K-independent aspect of TREM-1 signaling contributes to the attenuation of LPS-induced IL-12p40 and IL-23. The different mechanisms of synergy, and the difference in sensitivity to wortmannin of synergy and repression, highlight the complexity in cross-talk between TREM-1 activation and LPS. These results are summarized in Fig. 5.

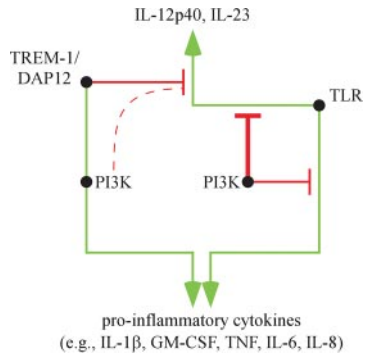

FIGURE 5. Circuit diagram for the cross-talk between TREM-1 activation and TLR signaling. Both TREM-1 activation and LPS elicit the production of multiple proinflammatory cytokines, including IL-1β, GM-CSF, TNF, IL-8, and IL-6. TREM-1-mediated cytokine production requires PI3K signaling and is blocked by the PI3K inhibitor wortmannin. TLR activation (here via LPS treatment) additionally induces the production of IL-12p40 and IL-23. In contrast to TREM-1 activation, LPS-mediated cytokine production is broadly inhibited by PI3K, with IL-12p40 and IL-23 production more strongly amplified by wortmannin (indicated by the thicker line). In addition, TREM-1 activation attenuates LPS-induced IL-12p40 and IL-23 production. The mechanism remains to be determined and, while there appears to be some PI3K contribution (indicated by the dashed line), this repression contains a PI3K-independent component.

Discussion

In this study we report global gene expression changes in human monocytes in response to TREM-1 activation, LPS, or combined TREM-1 activation plus LPS treatment. Our objectives were to: 1) characterize in detail the cellular consequences of TREM-1 activation; 2) identify overlap and divergence between TREM-1 activation and LPS; and 3) identify and investigate points of signaling cross-talk between TREM-1 activation and LPS. Both TREM-1 activation and LPS elicited robust gene expression changes at 2 h posttreatment. In the following section we have limited the mention of regulated genes to those we feel have potentially important functional implications.

TREM-1 activation and LPS overlap in their up-regulation of a number of immunomodulatory factors, including proinflammatory cytokines and chemokines (TNF, IL-6, GM-CSF, CXCL2, CXCL3, CXCL5, CCL3, CCL20), degradative proteases (MMP1,

MMP10), and PTGS2/COX2 (Table I). These genes are among those comprising a common response to TLR ligands or pathogens (47), and our data are consistent with TREM-1 activation triggering a core inflammatory response. Additional proinflammatory genes were induced by both treatments but did not meet the fold induction criteria we used to generate the preceding Tables. IL-8 mRNA, for example, was up-regulated 2.3- and 3.3-fold by TREM-1 activation and LPS, respectively, and IL-8 protein is readily detectable in cell culture media in response to either treatment (data not shown). Among genes induced by TREM-1 activation and LPS in Table I are INHBA, a TGF-β family member that is up-regulated in inflammatory disorders and arthropathies (48–50), and the TNF superfamily ligand TNFSF15 (TL1A), a Th1 polarizing cytokine that is up-regulated in autoimmune disease (51). A number of genes were also down-regulated by TREM-1 activation and LPS (Fig. 1 and Table II). These included the chemokine receptors CCR2 (consistent with previous reports for LPS; Refs. 52, 53) and CX3CR1, indicating that both treatments induce a nonmigratory phenotype with respect to the potent monocyte chemoattractants CCL2/MCP-1 and CX_3CL1/fractaline, the ligands for CCR2 and CX_3CR1, respectively (54–56). Notably, GTPases of immunity-associated proteins (GIMAPs) are highly represented among down-regulated genes (Table II; GIMAPs 1, 6, 7, and 8). Although the precise function of GIMAPs is not known, in mice they may regulate T cell survival by modulating Bcl-2 family member activity (57–59). GIMAP expression is lymphoid restricted in mice but is broader in humans (59), and their down-regulation by TREM-1 activation and LPS may point to an important role in myeloid cell survival.

The comparative aspect of our experiments allowed us to identify factors that are preferentially induced by TREM-1 activation or LPS. Although both treatments induced GM-CSF, M-CSF was preferentially induced by TREM-1 activation, and G-CSF was induced by LPS but not by TREM-1 activation. IL-23 was also specifically induced by LPS (see below). These microarray findings were confirmed by measurement of protein levels in cell culture media (Fig. 2). GM-CSF is mitogenic for both macrophages and neutrophils, whereas M-CSF and G-CSF are lineage-specific cytokines that support the proliferation of macrophages and neutrophils, respectively. Additionally, when combined with RANKL (receptor activator of NF-κB ligand), M-CSF promotes macrophage differentiation into bone-resorbing osteoclasts (60). The functional implication of M-CSF production by TREM-1 activation may also extend to SPP1/OPN, which was strongly and preferentially induced by TREM-1 activation (Fig. 2). SPP1 is a secreted adhesive glycophosphoprotein with cytokine/chemokine-like properties that interacts with CD44 and various integrins (61). Among other roles (62), SPP1 may, as an immobilized component of the extracellular matrix, anchor osteoclasts to sites of bone resorption (63). TREM-1 activation, therefore, through the production of M-CSF and SPP1 could potentially contribute to osteoclast maintenance and function (64). Interestingly, DAP12 knockout mice have severe osteopetrosis (65–67) and although currently this is attributed to defective TREM-2 function in osteoclasts (68, 69), our results raise the possibility that defective TREM-1 function may also contribute to this phenotype. Other genes preferentially induced by TREM-1 activation were the metallothionein family members MT1K, MT1E, and MT1F, which are cysteine-rich heavy metal-binding proteins implicated in binding, thereby reducing intracellular levels of available zinc (70). This may be of functional importance, because high intracellular zinc can suppress TNF and IL-1β production by monocytes (71) and inhibit LPS-induced expression of MHC class II and costimulatory molecules (72). All of these possibilities are currently speculative, yet they point to potentially significant cellular events that would be difficult to uncover in the absence of global gene expression profiling.

These data illustrate the overlap and previously uncharacterized divergence in monocyte responses to TREM-1 activation and LPS. Furthermore, while TREM-1 activation can amplify some cellular responses to TLR ligands such as LPS, the extent of this cross-talk and any underlying mechanisms have not been investigated in detail. Our analysis of cells receiving a combined treatment of TREM-1 activation plus LPS uncovered several points of cross-talk between these pathways. TREM-1 activation and LPS synergize in IL-1β and GM-CSF protein production, yet synergy in mRNA accumulation was evident for GM-CSF but not for IL-1β. TREM-1 activation therefore appears to impact a posttranscriptional step in the production of IL-1β, which is regulated at multiple points before its externalization in mature form. These include inflammasome-mediated processing of pro-IL-1β (73) and noncanonical secretory mechanisms such as lysosomal exocytosis (74) or microvesicle shedding (75). However, we were unable to make any further mechanistic conclusions, as both activated caspase-1 (which cleaves pro-IL-1β to mature IL-1β) and pro-IL-lβ were difficult to detect using freshly isolated monocytes, the latter even in the presence of caspase-1 inhibitors (data not shown). Nonetheless, our findings point to mechanistically distinct interplay between TREM-1 activation and LPS in the synergistic production of some cytokines and to a potential role for TREM-1 activation in inflammasome activity and/or secretory pathways. Despite the different underlying mechanisms for synergistic IL-1β and GM-CSF protein production, both require PI3K because the contribution of TREM-1 activation to synergy was essentially eliminated in the presence of wortmannin (Fig. 4).

TREM-1 activation has been reported to attenuate the induction of the immunosuppressive cytokine IL-10 in response to LPS, in support of a proinflammatory role for TREM-1 (12). By microarray, IL-10 mRNA was induced 1.5-, 12.1-, and 7.2-fold by TREM-1 activation, LPS, and combined treatment, respectively (Table IV). Consistent with this, we generally observed reduced LPS-induced IL-10 protein levels with concomitant TREM-1 activation, although in some monocyte preparations the levels were essentially unchanged (data not shown). We find, however, that a number of genes specifically induced by LPS are antagonized by concomitant TREM-1 activation. Strikingly, three of the top five most highly antagonized genes encode IL-12 cytokine family subunits (*IL12B*, *IL23A*, and *EBI3*; Table V). *IL12B* encodes a p40 subunit that heterodimerizes with either *IL12A*-encoded p35 (to form IL-12) or *IL23A*-encoded p19 (to form IL-23). *EBI3* encodes a recently characterized p40-related subunit of IL-27 (76). Functionally, IL-12 is a central regulator of the polarization of naive $CD4^+$ T cells toward a Th1 phenotype; IL-23 is important for the maintenance of Th17 cells, which have been implicated in autoimmune disease progression; and IL-27 has roles in naive $CD4^+$ T cell proliferation and in early Th1 responses (77). To confirm the microarray data, we analyzed the TREM-1 activation effect on LPS-induced IL12-p40 and IL23-p19 mRNA levels and IL-12p40 and IL-23 protein production. mRNA accumulation in response to LPS was reduced with concomitant TREM-1 activation, consistent with strong transcriptional regulation of the promoters for these genes, and the protein levels in cell culture media were accordingly reduced (Fig. 3). Repression of LPS-induced IL-12 family cytokines unexpectedly implicates TREM-1 activity in the potential dampening of Th1 and Th17 responses. Interestingly, a recent report demonstrates that DAP12 deficiency leads to amplified Th1 responses to mycobacterial and viral infection in vivo (78).

In contrast to the synergy we observe in IL-1β and GM-CSF production, significant (albeit less) repression of LPS-induced IL-12p40 and IL-23 by TREM-1 activation persisted in the presence of wortmannin (Fig. 4). This was an intriguing result because PI3K inhibitors are known to potentiate IL-12 production in response to LPS (41, 42, 79, 80), pointing to an inhibitory role for PI3K signaling. A simple explanation for the repression we observe, therefore, would invoke PI3K activation downstream of TREM-1. However, our data indicate that some PI3K-independent aspect of TREM-1 signaling contributes to the repressive effect. Similar to TREM-1, the activation of a number of receptors, including some that signal via ITAM motifs, can dampen TLR-induced IL-12 production (81–88), and the precise underlying mechanisms are likely to be complex.

Accumulating evidence suggests that ITAM signaling can have both activating and inhibitory effects on immune responses. For example, while sustained aggregation of FcαRI (which signals via the ITAM-containing FcRγ) by IgA-immune complexes promotes cell activation, low level receptor stimulation via serum IgA or anti-FcαRI Fab activates the protein phosphatase SHP-1, which induces a state refractory to activation (89). In addition, weak activation of the TCR-coupled CD3ζ, which also contains an ITAM, results in SHP-1 activation (79). Therefore, current hypotheses to explain ITAM-mediated inhibitory signaling hinge largely on high- vs low-avidity ligands (19, 38). Our experiments, which use plate-bound anti-TREM-1 Ab, may not reveal some of this complexity. Nonetheless, TREM-1 activation clearly repressed the induction of IL-12 family member genes in response to LPS, potentially indicating mechanistic commonality in the negative regulation of this important cytokine family.

Among other LPS-induced genes that were antagonized by TREM-1 activation were *CCL23*, *TNIP3*, and *IRF1* (Table V). CCL23 is one of the ligands for the chemokine receptor CCR1, which is expressed on NK cells, monocytes, and immature dendritic cells. The attenuation of TNIP3, an A20-binding inhibitor of NF-κB (ABIN), was confirmed by real-time RT-PCR in multiple monocyte donors (data not shown). TNIP3 has been shown previously to be induced by LPS and to negatively regulate IKK signaling (90). Reduced TNIP3 levels when LPS is combined with TREM-1 activation could potentially sustain IKK activation. In contrast, attenuation of IRF1 mRNA induction indicates that TREM-1 activation could interfere with the late transcriptional induction of type I IFN genes in response to LPS. An additional inhibitory role may result from TREM-1 activation-induced *PPARG* (Table III), which encodes a nuclear hormone receptor that inhibits a subset of LPS-induced genes (91, 92). All of these effects would be late points of potential cross-talk between TREM-1 activation and LPS (i.e., postsynthesis of induced factors) that most likely would not impact our 2-h microarray analysis.

Recently, it has become clear that monocyte differentiation in response to different activating stimuli can yield phenotypically distinct macrophage populations (93). Treatment with IFN-γ together with TLR ligands results in classically activated macrophages (ca-Mϕ or M1) that have a high Th1 polarizing function. Nonclassical treatments give rise to alternatively activated macrophages (aa-Mϕ or M2), a further subdivided population that includes macrophages derived from IL-4 or IL-13 treatment, FcγR ligation, glucocorticoids, and other treatments and that appear to have roles in Th2 biasing and/or tissue homeostasis. Notably, we observe gene expression changes that correlate well, although not perfectly, with different macrophage activation states. For example, *CCR7*, *IL2RA*, *IL15RA*, and *IL7R* are candidate membrane receptor markers for M1 macrophages (94) and all are LPS-biased genes in our study (Table IV). In contrast, *TNFSF14* (LIGHT) is a candidate marker for M2 macrophages (95) and *PPARG* is required for M2 maturation (96). Both *TNFSF14* and *PPARG* are TREM-1 biased genes in our study (Table III). These are early indications of potential direction or propensity toward classical M1 or alternative M2 activation states with LPS and TREM-1 activation, respectively.

In summary, our data confirm the proinflammatory effect of TREM-1 activation and uncover several novel findings. First and foremost, we have identified shared and divergent gene expression changes between TREM-1 activation and LPS in key immunomodulatory factors. These findings have potentially important functional implications for the activation of these respective pathways. For example, TREM-1 activation, by inducing M-CSF and SPP1, may promote osteoclast function and, by dampening LPS-induced IL-12 family member cytokines, may impact T cell responses in vivo. Additionally, TREM-1 activation appears to bias monocyte differentiation toward an alternatively activated phenotype. It will therefore be of particular interest to characterize the effect of TREM-1 deficiency or antagonization with respect to these findings, as they provide clues into the in vivo role of TREM-1 activation in innate and adaptive immune responses. Through the analysis of selected microarray results, we further uncover some of the mechanistic complexity in signal integration of TREM-1 activation and LPS, with PI3K as an important contributory node in this network. Because many immunoreceptors signal via DAP12 and/or ITAM motifs, these findings may be applicable to the cross-talk between ITAM- and TLR-mediated signaling more generally.

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Disclosures

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