A pathway analysis of poly(I:C)-induced global gene expression change in human peripheral blood mononuclear cells


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Huang, C. Chris, Karen E. Duffy, Lani R. San Mateo, Bernard Y. Amegadzie, Robert T. Sarisky, and M. Lamine Mbow. A pathway analysis of poly(I:C)-induced global gene expression change in human peripheral blood mononuclear cells. Physiol Genomics 26: 125–133, 2006.—To gain global pathway perspective of ex vivo viral infection models using human peripheral blood mononuclear cells (PBMCs), we conducted expression analysis on PBMCs of healthy donors. RNA samples were collected at 3 and 24 h after PBMCs were challenged with the Toll-like receptor-3 (TLR3) agonist polyinosinic acid-polycytidylic acid [poly(I:C)] and analyzed by internally developed cDNA microarrays and TaqMan PCR. Our results demonstrate that poly(I:C) challenge can elicit certain gene expression changes, similar to acute viral infection. Hierarchical clustering revealed distinct immediate early, early-to-late, and late gene regulation patterns. The early responses were innate immune responses that involve TLR3, the NF-κB-dependent pathway, and the IFN-stimulated pathway, whereas the late responses were mostly cell-mediated immune response that involve activation of cell adhesion, cell mobility, and phagocytosis. Overall, our results expanded the utilities of this ex vivo model, which could be used to screen molecules that can modulate viral stress-induced inflammation, in particular those mediated via TLRs.

Polyinosinic acid-polycytidylic acid; microarray; Toll-like receptor; viral infection; ex vivo

MANY VIRUSES PRODUCE double-stranded RNA (dsRNA) during their replication cycle. It is either the genetic material (for some RNA viruses) or an essential intermediate or byproduct for viral RNA synthesis. It is known that dsRNA induces the synthesis of interferon (IFN), a family of cytokines that can be produced as a result of Toll-like receptor (TLR) signaling cascade (1, 21, 29). In turn, IFNs may bind to the cell surface IFN receptors and activate the transcription of IFN-stimulated genes (ISG) whose products inhibit various stages of virus replication (28).

dsRNA is a potent and global modulator of mammalian gene expression. In addition to IFN-induced genes, other mammalian genes can be induced by dsRNA directly without the involvement of IFN (28). These genes are collectively referred to as dsRNA-stimulated genes (DSG) (12). In many in vitro assays, a synthetic dsRNA analog polyinosinic acid-polycytidylic acid, or Poly(I:C), has been demonstrated to have certain stimulatory effects similar to viral dsRNA, most notably strong induction of IFN-α/β (1, 8, 11), although it is unlikely that poly(I:C) possesses all the properties of various viruses.

TLRs are type I transmembrane proteins characterized by an extracellular leucine-rich portion that exhibits considerable structural divergence and is necessary for the recognition of different ligands. TLRs recognize conserved patterns derived from microbial pathogens identified as pathogen-associated molecular patterns (PAMPs). Interaction of TLR with a PAMP triggers several signaling cascades that lead to cytokine secretion (2). There are 10 human TLRs; different TLR ligands can include different cytokine secretion profiles. In addition, TLRs are able to expand their repertoire of ligands by forming homo- or heterodimers as well as binding different adapter proteins (24). TLRs also contain a highly conserved cytoplasmic Toll-IL-1 receptor (TIR) domain that, through different adapter molecules such as MyD88, TIRAP, TRIF, or TRAM, connects the receptors to different intracellular signaling pathways (31). dsRNA is a ligand for TLR3, which is highly expressed in immature dendritic cells (DC) as well as epithelial and endothelial cells (1).

Although poly(I:C) has been used in many experimental studies, there have been few attempts to identify the gene expression profiles it generates. Geiss et al. (12) reported a microarray analysis that identified genes regulated by poly(I:C) in human glioma-derived GRE cells that are devoid of the type I IFN loci. Because human peripheral blood mononuclear cells (PBMCs) or their components such as T cells, master cells, dendritic cells, and macrophages have been used in many expression profiling studies stimulated by viruses (7, 14, 15, 17, 19), it is invaluable to obtain a global gene expression profile of poly(I:C)-stimulated human PBMCs to gain a systematic view of the utilities.

MATERIALS AND METHODS

Sample collection and ex vivo treatment. Informed consent forms were obtained from all participating subjects. Whole blood was collected from three human donors into heparin-coated syringes. PBMCs were isolated via a FicollPaque PLUS gradient (Amer- sham/GE Healthcare). After one wash with Hanks’ balanced salt solution (HBSS), the PBMCs were resuspended in Red Blood Cell Lysis solution (Sigma-Aldrich, St. Louis, MO) for 10 min. After three washes with HBSS, the cells were resuspended in RPMI 1640 media (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 0.1 mM nonessential amino acids (Invitrogen), 1 mM sodium pyruvate (Invitrogen), and 10 μg/ml gentamicin (Sigma-Aldrich). The cells were plated in 48-well plates at a concentration of 3 × 10^6 cells/well (0.5 ml/well), incubated ~30 min at 37°C, and then treated with 5 μg/ml poly(I:C) (Amersham/GE Healthcare). Poly(I:C) was reconstituted to 2 mg/ml in PBS and heated at 50°C to solubilize.
RNA isolation. To harvest RNA, samples were lysed using Nucleic Acid Purification Lysis solution (Applied Biosystems, Foster City, CA). RNA was prepared using ABI PRISM 6100 Nucleic Acid PrepStation (DNase step included). RNA quality was verified with the 2100 BioAnalyzer (Agilent Technologies, Palo Alto, CA). Samples that demonstrated high quality (i.e., the ratio of 28S rRNA to 18S rRNA was >1.7) and had a minimum of 1 μg of RNA were submitted for microarray analysis.

Microarray process. In this study, PBMCs were collected from three healthy donors. Samples were treated with poly(I:C) or media for 3 or 24 h (total of 4 different treatment groups). All 12 samples were run in duplicate for a total of 24 arrays. Each clone is printed as duplicate spots on a given chip; thus four technical replicates were generated for each clone. A single intensity value for each clone was generated after averaging the quadruplet after smoothing spline normalization. The microarray contains 8,132 unique human cDNA clones representing 6,198 unique genes collected from Research Genetics [Integrated Molecular Analysis of Genomes and their Expression (IMAGE) Consortium] and Incyte Genomics (Santa Clara, CA). Some clones are represented by more than one clone on the array. All clones have been verified by DNA sequencing.

To make the probe from the sample RNA, one round of T7 polymerase-based linear RNA amplification was performed by RT of RNA with a T7 promoter oligo(dT) primer, and Cy3-dCTP-labeled fluorescent cDNA probes were synthesized from the amplified RNA as described (27). The probes were heated to 95°C for 2 min, cooled, and applied to the slides. The slides were covered with glass coverslips, sealed, and hybridized at 42°C overnight. Microarrays were scanned with an Agilent G2565AA Microarray Scanner (Agilent Technologies, Palo Alto, CA). Fluorescence intensity for each feature of the array was obtained using Imagene version 4.2 software (BioDiscovery, Los Angeles, CA).

Data analysis. With the use of GeneSpring (Redwood City, CA) version 7.2, the averaged intensity for each clone was further normalized across all samples. Chip-to-chip normalization was performed by dividing the averaged intensity of each clone by the median intensity of a chip. The intensity of each clone was then normalized to the median intensity of that clone in the untreated group at corresponding time points. The intensity data were then log2 transformed to approximate normal distribution. Multifactorial ANOVA was conducted, using Partek Pro (St. Charles, MO) version 6.0, with treatment, time, donor, and chip batch as independent factors. Multiple testing correction was applied through Benjamini-Hochberg false discovery rate (FDR) (3), with the P value cutoff set at 0.05. In addition, post hoc analysis by Fishers least significant difference (LSD) was conducted. Genes showing significant changes due to treatment (P < 0.05 after FDR adjustment) were identified and imported back into GeneSpring for fold-change filtering, clustering analysis, and graphic representation. Fold change is calculated based on the mean intensity value from the three donors.

Gene Ontology (GO) analysis was performed at the Database for Annotation, Visualization and Integrated Discovery (DAVID 2.0; http://apps1.niaid.nih.gov/david/) (9). GoCharts were obtained by inputting the gene list of significant interest and selecting GO from a list of functional annotations. Pathway analysis was performed in Ingenuity 3.0 (Ingenuity Systems, Mountain View, CA) and PathwayAssist 3.0 (Ariadne Genomics, Rockville, MD), according to instructions provided by the vendors.

Real-time quantitative PCR. RNA samples from the 3-h time point (from 2 donors) and the 24-h time point (from 1 donor) were transcribed into cDNA using the iScript Synthesis Kit (Bio-Rad Laboratories, Hercules, CA), which uses both random hexamers and oligo(dT) as the primers for synthesis. Real-time PCR was performed on the ABI PRISM 7900HT Sequence Detection System using a TaqMan Low Density Array, which included duplicate wells of 20 target genes on a 384-well card (Applied Biosystems). RNA-to-cDNA (150 ng) in a 100-μl volume containing TaqMan Universal PCR Master Mix (Applied Biosystems) and water was used in each sample port for real-time PCR. The endogenous control 18S rRNA was used to normalize the samples using the ΔΔCT method of relative quantitation (where Ct is threshold cycle), with SDS software version 2.1 (Applied Biosystems). The endogenous control β-actin was included to confirm accurate normalization of the samples.

Cytokine analysis. Cell supernatants were collected at 3 and 24 h post-poly(I:C) stimulation and frozen at −20°C until analysis. Cytokine and concentrations in the supernatants were measured using Luminex (Austin, TX) technology. A Luminex Kit (Biosource International, Camarillo, CA) is used to measure the following cytokines/chemokines: IL-6, IL-12, tumor necrosis factor TNFa, and IFNy. Sample acquisition and analysis were performed using the Luminex 100 IS (Luminex) with STarStation software (Applied Cytometry Systems, Sacramento, CA). The results of two measurements were averaged to determine a final concentration.

RESULTS

Analysis of differentially expressed genes and gene clusters. To identify differentially expressed genes, we applied multifactorial ANOVA, taking treatment, time, donor, and chip batch as independent factors. As shown in Table 1, a majority of the difference in gene expression is due to factors other than the treatment. However, differential gene expression of a group of 165 clones is identified as specific for the treatment, after multiple comparison adjustment by FDR (3). This list of clones was further reduced to 145 clones by a post hoc test (P < 0.05) and to 111 clones by fold-change filtering, at least 1.5-fold compared with medium alone treatment at either 3 or 24 h. A complete list of the raw and normalized intensity value of the entire microarray study can be found in Supplemental Table S1 (available at the Physiological Genomics web site).

After hierarchical clustering analysis for the 111 clones was performed, a clear expression regulation pattern emerged. Figure 1 shows the gene tree structure horizontally and individual biological replicates vertically. Three clusters of upregulated genes and one cluster of downregulated genes can be identified which match the temporal cascade of gene expression by poly(I:C) stimulation: immediate early responding, early-to-late responding, and late responding genes.

Immediate early responding genes. The first cluster represents genes that were upregulated by poly(I:C) stimulation at 3 h, with expression declining at 24 h. There are four genes in this cluster: IL-6, IFNB1, interferon-induced protein with tet-

Table 1. ANOVA factors and nos. of significant changes due to these factors

<table>
<thead>
<tr>
<th>Variable Name</th>
<th>Cutoff P Value</th>
<th>No. of clones with significant P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (3 h, 24 h)</td>
<td>0.0338416</td>
<td>5.504</td>
</tr>
<tr>
<td>Subject (n = 3)</td>
<td>0.0378136</td>
<td>6.150</td>
</tr>
<tr>
<td>Batch (2)</td>
<td>0.00342474</td>
<td>557</td>
</tr>
<tr>
<td>Treatment [media, poly(I:C)]</td>
<td>0.00101451</td>
<td>165</td>
</tr>
<tr>
<td>Poly(I:C) vs. media†</td>
<td>0.00089154</td>
<td>145</td>
</tr>
</tbody>
</table>

Poly(I:C), polyinosinic acid-polycytidylic acid. *False detection rate (FDR) adjusted; 5% identified may be false positive. †Result of a post hoc test.
ratricopeptide repeat IFIT1, and KCNK 17 (Table 2). Another gene, protein kinase R (PKR), which was significantly changed by ANOVA but did not meet the 1.5-fold change cutoff, was also shown by TaqMan analysis as upregulated in the same fashion as the rest of the genes in this cluster and is also listed in Table 2.

We also profiled TLR3, which is a receptor for poly(I:C), and two additional cytokines, TNFα and IL-12p35, that were not upregulated by poly(I:C) stimulation.

Table 2. Immediate early response genes to poly(I:C) stimulation in PBMCs

<table>
<thead>
<tr>
<th>GenBank ID</th>
<th>Common Name</th>
<th>Description</th>
<th>Fold Change at 3 h</th>
<th>Fold Change at 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_031460</td>
<td>KCNK17; TALK2; TASK4</td>
<td>potassium channel, subfamily K, member 17</td>
<td>3.33</td>
<td>1.55</td>
</tr>
<tr>
<td>NM_001548</td>
<td>IFIT1; G10P1; IFI56; IFNA11;</td>
<td>interferon-induced protein with tetratricopeptide repeats 1</td>
<td>3.10 (680)</td>
<td>1.82 (80.6)</td>
</tr>
<tr>
<td>NM_000600</td>
<td>IL6; HGF; HSF; BSF2; IL-6; IFNB2</td>
<td>interleukin 6 (interferon, beta 2)</td>
<td><strong>1.60</strong> (28.6)</td>
<td><strong>1.06 (+)</strong></td>
</tr>
<tr>
<td>NM_002176</td>
<td>IFNB1; IFB; IFF</td>
<td>interferon, beta 1, fibroblast</td>
<td>1.55 (20.6)</td>
<td>1.01 (1.4)</td>
</tr>
<tr>
<td>NM_002759</td>
<td>EIF2AK1; PKR; PRKR</td>
<td>eukaryotic translation initiation factor 2-alpha kinase 2</td>
<td>1.22 (11.7)</td>
<td>1.14 (5.8)</td>
</tr>
<tr>
<td>NM_003265*</td>
<td>TLR3</td>
<td>toll-like receptor 3</td>
<td>(14.7)</td>
<td>(1.7)</td>
</tr>
<tr>
<td>NM_174355*</td>
<td>IL12A; IL-12p35</td>
<td>interleukin 12A</td>
<td>(13.7)</td>
<td>(6.7)</td>
</tr>
<tr>
<td>NM_000594*</td>
<td>TNF; DIF; TNFA; TNFSF2;</td>
<td>tumor necrosis factor (TNF superfamily, member 2)</td>
<td>(5.1)</td>
<td>(2.5)</td>
</tr>
</tbody>
</table>

Fold change is calculated based on the mean intensity value from 3 donors. Boldface indicates average value of 2 clones for the same gene on the microarray. *Indicates gene that did not pass statistical test in the microarray analysis. Fold change by TaqMan validation (in parentheses) is based on the mean of 2 of the 3 donors at 3 h and 1 of the 3 donors at 24 h. + Indicates that the gene expression level can be reliably detected in poly(I:C)-treated samples but not in media alone-treated samples.
known to be associated with viral infection-induced inflammation (7) by TaqMan PCR analysis. Indeed, all of them displayed the pattern of an immediate early responding gene. These genes did not show significant changes in our microarray analysis for several reasons. IL-12p35 is not on our microarray. TLR3 is normally expressed at a very low level (CT >35 in a TaqMan assay) that cannot be reliably detected by our microarray. Expression of TNFα is primarily regulated at the level of mRNA stability (4, 18), rendering it difficult to be captured by our microarray.

A complete list of the TaqMan data of the 19 genes we profiled can be found in Supplemental Table S2.

**Early-to-late responding genes.** Figure 2 lists the second cluster of genes that were upregulated by poly(I:C) stimulation. Genes in this cluster were upregulated at 3 h poststimulation; some stayed relatively constant, and others had even higher expression at 24 h. There are 43 clones in this cluster that can be mapped to 37 genes in the RefSeq database. We have conducted TaqMan PCR validation for 12 genes on that list, and the results are summarized Table 3. In all cases, the microarray result was confirmed. We also discovered, by TaqMan analysis, IFNγ (IFNG) to be highly upregulated by poly(I:C) stimulation. It failed to be detected in our microarray because of a nonperforming probe.

**Late responding genes and downregulated genes.** Figure 3 lists a third cluster of genes that were upregulated by poly(I:C) stimulation only at 24 h poststimulation. There are 37 clones in this cluster that can be mapped to 37 genes in the RefSeq database.

**Cytokine analysis.** We measured cytokine level in the supernatants at 3 and 24 h post-poly(I:C) stimulation. While there was not much detectable protein at 3 h, significant protein levels of IL-6, IL-12, and TNFα were detected at 24 h (Fig. 5), indicating a delay of protein production of these early genes. We also observed robust protein production of IFNγ at 24 h; IFNG was found to be an early-to-late responding gene.
Table 3. List of early-to-late response genes to poly(I:C) stimulation in PBMCs that were validated by TaqMan PCR

<table>
<thead>
<tr>
<th>GenBank</th>
<th>Common</th>
<th>Description</th>
<th>Fold Change at 3 h</th>
<th>Fold Change at 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_007315</td>
<td>STAT1; ISGF-3; STAT91</td>
<td>signal transducer and activator of transcription</td>
<td>5.38 (16.5)</td>
<td>3.74 (5.7)</td>
</tr>
<tr>
<td>NM_006074</td>
<td>TRIM22; RNF94; STAF50;</td>
<td>tripartite motif-containing 22</td>
<td>2.76 (6.8)</td>
<td>3.43 (5.4)</td>
</tr>
<tr>
<td>NM_173842</td>
<td>IL1RN; IRA; IL1RA;</td>
<td>interleukin-1 receptor antagonist</td>
<td>2.24 (5.8)</td>
<td>4.29 (50.5)</td>
</tr>
<tr>
<td>NM_055531</td>
<td>IFI16; IFNGIP1</td>
<td>interferon, gamma-inducible protein 16</td>
<td>2.02 (6.6)</td>
<td>1.70 (7.8)</td>
</tr>
<tr>
<td>NM_002468</td>
<td>MYD88</td>
<td>myeloid differentiation primary response gene</td>
<td>1.79 (4.7)</td>
<td>1.76 (2.3)</td>
</tr>
<tr>
<td>NM_005409</td>
<td>CXCL11; I-TAC; SCYB111</td>
<td>chemokine (C-X-C motif) ligand 11</td>
<td>6.18 (3.155)</td>
<td>25.04 (123.5)</td>
</tr>
<tr>
<td>NM_006274</td>
<td>CCL19; MCP-3; SCYA19;</td>
<td>chemokine (C-C motif) ligand 19</td>
<td>1 (++)</td>
<td>2.36 (625)</td>
</tr>
<tr>
<td>NM_005623</td>
<td>CCL8; MCP-2; SCYA8; SCYA10</td>
<td>chemokine (C-C motif) ligand 8</td>
<td>6.35 (1,077)</td>
<td>17.28 (122)</td>
</tr>
<tr>
<td>NM_000043</td>
<td>TNFRSF6; FAS; APT1; CD95; FASTM</td>
<td>tumor necrosis factor receptor superfamily, member 6</td>
<td>1.85 (5.5)</td>
<td>3.34 (11.5)</td>
</tr>
<tr>
<td>NM_00639</td>
<td>TNFSF6; FASL; CD178; CD95L;</td>
<td>tumor necrosis factor (ligand) superfamily, member 6</td>
<td>1.35 (3.2)</td>
<td>1.70 (6.9)</td>
</tr>
<tr>
<td>NM_00593</td>
<td>TAP1; APT1; PSF1;</td>
<td>transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)</td>
<td>1.91 (5.1)</td>
<td>3.00 (6.0)</td>
</tr>
<tr>
<td>NM_00619*</td>
<td>IFNG; IFG; IFI</td>
<td>interferon, gamma</td>
<td>(252)</td>
<td>(588.2)</td>
</tr>
</tbody>
</table>

Fold change is calculated based on the mean intensity value from 3 donors. Bold face indicates average value of 2 clones for the same gene on the microarray. *Indicates gene that did not pass statistical test in the microarray analysis. Fold change by TaqMan validation (in parentheses) is based on the mean of 2 of the 3 donors at 3 h and 1 of the 3 donors at 24 h. + Indicates that the gene expression level can be reliably detected in poly(I:C)-treated samples but not in media alone-treated samples.

Fig. 3. Upregulated late responding genes to poly(I:C) stimulation in PBMCs. A cluster of 43 upregulated late responding clones. Color legend is at left.
Fig. 4. Downregulated genes to poly(I:C) stimulation in PBMCs. A cluster of 20 downregulated clones. Color legend is at left.

Fig. 5. Cytokine analysis in the cell supernatants 24 h post-poly(I:C) stimulation. Each solid square represents an average of 2 measurements before poly(I:C) stimulation, and each solid circle represents an average of 2 measurements 24 h posttreatment. Data from all 3 donors are shown along with the average. TNF, tumor necrosis factor; IFN, interferon.

viruses. As result, there is a wealth of knowledge on viral-
data confirmed that this is a valid system: that poly(I:C) is a
used in many virology and immunology studies. Overall, our

two Ingenuity networks that are involved in viral function,
addition, 16 of 32 secondary response genes can be mapped to
constructed pathway based on the ResNet database (25, 26) and
Ingenuity network involved in immune response. A recon-
27 of 30 primary response genes could be mapped to an
response genes, both up- and downregulated (Figs. 3 and 4), are
3 h only (such as RANK), are combined into a primary
genes (Table 2 and Fig. 2), including those downregulated at
pathway analysis, the immediate early genes and early-to-late
expression that is inducible primary immune responses at the transcript level [see
review by Jenner and Young (16)]. Overall, our result matches
well with previously reported genes that include TLRs and
their adaptors, proinflammatory cytokines, chemokines and
their receptors, cell adhesion molecules, antigen presenting
molecules, and transcription factors and apoptosis regulators
(16). These genes are generally mapped along the TLR3-,
NF-κB-, and IFN-stimulated signaling pathways (Fig. 5).

The most noticeable gene is TLR3, which is a receptor for
dsRNA that plays a key role in bridging innate and adaptive
immunities. Human TLR3 is highly expressed in immature
dendritic cells and macrophages in PBMCs. Our gene expres-
expression profiling analysis showed that TLR3 gene expression was
upregulated at 3 h post-poly(I:C) stimulation. Previously,
TLR3 has been shown to be upregulated in mast cells (19) as
well as human endothelial and epithelial cells (30) after
poly(I:C) stimulation.

Other genes along the TLR3 signaling pathway were also
upregulated. For example, gene expression of MyD88, which
is arguably the best characterized TLR adaptor (2), is upregu-
lated by poly(I:C) stimulation. The death domain of MyD88
recruits members of the IL-1 receptor-associated kinases
IRAK-1 and IRAK-4. These kinases are autophosphorylated,
leading to association with TRAF6, which then mediates the
activation of MAPKs as well as the IkB kinases IKKα and
IKKβ. The result is the activation of activator protein (AP)-1
and NF-κB transcription factors and expression of a wide
variety of proinflammatory cytokines, such as TNFα, IL-6,
IL-12, and IFNα, -β, and -γ; all have been observed in our
study.

The key cytokine that regulates innate immune responses
against viruses is IFN-α/β (5). The major pathway of intracel-
lar signaling used by IFN-α/β and their receptors accesses
the tyrosine kinases Jak 1 and Tyk 2, activating signal trans-
ducer and activator of transcription (STAT1) and STAT2 to
form a STAT1/STAT2 heterodimer. In our study, we observed
an increase of STAT1 message by poly(I:C) stimulation. More-
over, several genes involved in antigen presentation that are
regulated by STAT1, such as TAP1 (20, 23), PSMB8, and

DISCUSSION

This study, being the first global expression profiling on
poly(I:C)-stimulated human PBMCs, has generated compre-
hsensive information on the experimental system that can be
used in many virology and immunology studies. Overall, our
data confirmed that this is a valid system: that poly(I:C) is a
TLR ligand that mimics certain aspects of viral infection by
triggering key molecular events such as a rapid innate immune
response followed by cellular and humoral immune responses.

Many previously published studies focused on the immedi-
ate early genes that were turned on hours poststimulation by
viruses. As result, there is a wealth of knowledge on viral-

Table 4. Biological process GO terms for top hits (minimum of 4 genes) from the primary and secondary response genes

<table>
<thead>
<tr>
<th>Biological Process GO Term (4th level)</th>
<th>Primary Response Genes (44)</th>
<th>Secondary Response Genes (47)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count</td>
<td>Percentage</td>
</tr>
<tr>
<td>Respond to biotic stimulus</td>
<td>27</td>
<td>61.4%</td>
</tr>
<tr>
<td>Respond to pest/pathogen/parasite</td>
<td>17</td>
<td>36.4%</td>
</tr>
<tr>
<td>Protein metabolism</td>
<td>16</td>
<td>36.4%</td>
</tr>
<tr>
<td>Innate immune response</td>
<td>12</td>
<td>27.3%</td>
</tr>
<tr>
<td>Response to wounding</td>
<td>12</td>
<td>27.3%</td>
</tr>
<tr>
<td>Cell surface receptor-linked signal</td>
<td>11</td>
<td>25%</td>
</tr>
<tr>
<td>Programmed cell death</td>
<td>9</td>
<td>20.5%</td>
</tr>
<tr>
<td>Response to abiotic stimulus</td>
<td>8</td>
<td>18.2%</td>
</tr>
<tr>
<td>Intracellular signalling cascade</td>
<td>8</td>
<td>18.2%</td>
</tr>
<tr>
<td>Taxis</td>
<td>7</td>
<td>15.9%</td>
</tr>
<tr>
<td>Regulation of apoptosis</td>
<td>5</td>
<td>11.4%</td>
</tr>
<tr>
<td>Regulation of I-kappaB kinase/NF-kappaB cascade</td>
<td>4</td>
<td>9.1%</td>
</tr>
<tr>
<td>Positive regulation of signal transduction</td>
<td>4</td>
<td>9.1%</td>
</tr>
<tr>
<td>Negative regulation of programmed cell death</td>
<td>4</td>
<td>9.1%</td>
</tr>
</tbody>
</table>

*Term/gene cooccurrence probability, a method that identifies functional categories overrepresented in a gene list relative to the representation within the proteome of a given species (http://apps1.niaid.nih.gov/david/). GO, Gene Ontology.

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G1P2, were also upregulated. For example, induction of G1P2 causes natural killer (NK) cell proliferation and an augmentation of non-major histocompatibility complex (MHC)-restricted cytotoxicity (22).

We also identified a set of chemokines, CCL8, CXCL9, and CXCL11, that are known as IFNγ-induced chemokines by dendritic cells and macrophages in response to viral stimulation (15). Interestingly, although chemokine CCL2 was upregulated at 3 and 24 h post-poly(I:C) stimulation, its receptor, CCR2, was downregulation at 24 h. It has been hypothesized that cytokines that rapidly induce chemokine expression often downregulate chemokine receptor expression in a delayed manner, thereby limiting the chemokine response(6).

Another interesting finding is the upregulation of both Fas [TNF receptor superfamily (TNFRSF) member 6] and Fas ligand genes [TNF super family (TNFSF) member 6]. The Fas/FasL system is responsible for infection-induced cell death but also plays an important role in lymphocyte-mediated cytotoxicity. FasL may be upregulated in directly infected cells to enhance killing of responding immune cells and facilitate immune evasion. Immune cells that target directly infected cells can induce Fas-mediated apoptosis (10).

Previous studies showed that poly(I:C) could also signal through a TLR3-independent pathway via PKR, which is a cytoplasmic dsRNA binding protein that can mediate dsRNA signaling through its dimerization and the recruitment of TRAFs, which can then link both IKK and MAPK activation pathways (13). In our study, we observed an upregulation of the PKR gene, supporting the role PKR plays in poly(I:C)-induced pathways.

It is important to treat the primary and secondary responses separately. The primary response genes consist of many cytokines and chemokines that are transiently expressed and must be tightly controlled. The secondary response genes, on the other hand, are mostly effectors of the primary response genes and need to stay up- or downregulated for a period of time to achieved cellular and humoral response to infection. Our gene ontology and pathway analysis showed that, although these two groups of genes share common functions such as response to biotic stimuli, there are major differences (Table 4). For example, the secondary response genes encode more receptors and adhesion molecules that are essential for phagocytosis.

Because of the ease of access to human PBMCs and their clinical utility for noninvasive diagnostics, ex vivo stimulation,
such as with poly(I:C), is a useful system for a variety of applications such as the screening of compounds that antagonize or modify molecular pathways of virus-induced inflammation, apoptosis, and cellular responses. Understanding the advantages of this type of ex vivo system is important, and our results demonstrated the value of a comprehensive analysis.

NOTE ADDED IN PROOF

This study was conducted on samples donated by employees at the author’s laboratory. Informed consent was obtained, and steps were taken to protect the privacy of the donors, including the storage of delinked samples. While this privately sponsored noninterventional study on donated samples does not require Institutional Review Board (IRB) review and approval under applicable federal regulations, the authors regret that the IRB review was not obtained, given that this is inconsistent with the Journal’s policies. The authors are taking steps to ensure that all such future research at their company is reviewed by an IRB.

From the Editor: As is stated in our ethical policies, all human or animal studies must have IRB or Institutional Animal Care and Use Committee approval or their equivalent.

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DISCLOSURES

The opinions or assertions contained herein are not to be construed as official or as reflecting the views of Centocor Research & Development, Incorporated.

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