Published in final edited form as:

Am J Obstet Gynecol. 2006 August ; 195(2): 394.e1–394.24.

Human spontaneous labor without histologic chorioamnionitis is characterized by an acute inflammation gene expression signature

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Abstract

OBJECTIVE—The purpose of this study was to identify which biological processes may be involved in normal labor.

STUDY DESIGN—Transcriptional profiles for chorioamniotic membranes (n=24) and blood (n=20) were generated from patients at term with no labor (TNL) and in labor (TIL).

RESULTS—Expression of 197 transcripts ($P \leq 0.02$) differentiated TIL and TNL chorioamniotic membrane samples. Gene Ontology analysis indicated that TIL samples had increased expression of multiple chemokines and transcripts associated with neutrophil and monocyte recruitment. Microarray results were verified using quantitative real-time RT-PCR with independent samples. Transcriptional profiles from blood RNA revealed no Gene Ontology category enrichment of discriminant probe sets.

CONCLUSION—Labor induces gene expression changes consistent with localized inflammation, despite the absence of histologically detectable inflammation.

Keywords

chorioamniotic membrane; blood, transcriptional profile; microarray; acute inflammation; parturition; chorioamnionitis; chemokines; cytokines

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Condensation

Genome-wide transcriptome analysis of chorioamniotic membranes at term after spontaneous labor reveals a localized acute inflammation gene expression signature in the absence of histologic inflammation.

This research was supported by the Intramural Research Program of the National Institute of Child Health and Human Development, NIH, DHHS.

Introduction

Human parturition involves “a common pathway” manifested clinically by uterine contractions, cervical ripening and chorioamniotic membrane/decidual activation, culminating in membrane rupture.¹ The chorioamniotic membranes undergo complex anatomical and biochemical events that lead to membrane rupture.^{2,3} Morphological, biochemical and biophysical studies suggest that rupture of membranes results from the application of acute or chronic stress on localized areas of the membranes that are weaker.⁴

Cervical ripening has been likened to an inflammatory response.⁵ Indeed, analyses of inflammatory mediators in gestational tissues have demonstrated that the expression of these mediators is increased during normal term labor.^{5,6} Although these studies of individual cytokines have hinted at parturition as an inflammatory process, the full extent of the involvement of inflammation has not yet been established.

Global expression analyses using genomic approaches including oligonucleotide and cDNA microarrays are capable of exploring the full extent of gene expression changes associated with parturition. Multiple studies have used genomic approaches to study gene expression changes in chorioamniotic membranes associated with parturition.^{6–10} The gene expression studies performed with chorioamniotic membranes at term, however, have not been unbiased. Specifically, gene expression analyses that use arrays with small numbers of genes selected for their involvement in the immune response are biased due to the number of genes and their selection.^{9,11} In addition, other genomic analyses of fetal membranes at term have focused on gene expression profiles after first culturing tissues in organ culture.¹⁰ It is not clear, however, to what degree the culture conditions influence gene expression profiles.

We undertook this study to identify the biological processes involved during normal spontaneous labor using an unbiased genome-wide approach and analyzed the transcriptome in the chorioamniotic membranes and maternal blood.

Materials and Methods

Study design

A prospective cohort study was designed to examine differential gene expression of the chorioamniotic membranes and blood between patients not in labor (TNL) and those in spontaneous labor (TIL) at term. The inclusion criteria were: 1) gestational age consistent with term gestation (38 – 41.5 weeks); 2) no medical or obstetrical complications of pregnancy; and 3) normal pregnancy outcome including an infant who was of appropriate weight for gestational age (between the 10th and 90th percentile) without congenital anomalies and APGAR scores above 7.

The TNL patients were scheduled to have elective Caesarean sections for obstetrical indications, namely, a history of a previous Caesarean section. The TIL patients had a normal spontaneous vaginal delivery (Table I). All placentas were subjected to histological examination by a pediatric pathologist (YMK) and a criterion for inclusion was the absence of histological chorioamnionitis.¹²

The interval after rupture of membranes represents the time between rupture of membranes and delivery. TIL patients at admission were in active labor, as determined by their cervical dilatation state (median 4.5 cm, interquartile range 3.7 – 5.0 cm). The duration of labor was calculated by the difference between the time of admission and the time of delivery.

All women provided written informed consent prior to the collection of tissues. The collection of samples was approved by the Institutional Review Boards of both Wayne State University School of Medicine and the National Institute of Child Health and Human Development.

Isolation of total RNA

The protocols for procurement and RNA isolation from chorioamniotic membranes have been described previously.⁸ For the microarray experiments, 12 RNA samples from each clinical group were used. Real-time quantitative RT-PCR was carried out with 18 of the original 24 samples since some samples were exhausted in the array experiments. In addition, 25 new samples were analyzed including 13 new TIL, and 12 new TNL samples. Blood was drawn into collection tubes, which are part of the PAXgeneTM Blood RNA System (PreAnalytiX GmbH, Hombrechtikon, Switzerland) and processed according to manufacturer's instructions (Qiagen Inc., Valencia, California, USA). For the microarray experiments, 12 TIL and 8 TNL blood samples were used.

Microarrays

Microarray expression data from the chorioamniotic membranes were collected at Expression Analysis Inc. (www.expressionanalysis.com; Durham, North Carolina, USA). A total of 10 µg of total RNA extracted from chorioamniotic membranes was used for labeling and labeled RNA was hybridized to Affymetrix HG-U133A and HG-U133B microarrays using standard conditions as described (Affymetrix Technical Manual, www.affymetrix.com).

Microarray data generated from RNA isolated from blood were collected at the Applied Genomics Technology Center of Wayne State University (www.agtc.wayne.edu; Detroit, MI, USA). Microarray probe synthesis using 1 µg of total RNA was subjected to two rounds of *in vitro* transcription amplification as described in the GeneChip Eukaryotic Small Sample Target Labeling Technical Note (Version I, 2001; www.affymetrix.com) with the following modifications. After the initial conversion of mRNA into cDNA and the subsequent *in vitro* transcription reaction, 2 µg of *in vitro* transcribed complementary RNA (cRNA) was used for the second round of amplification. In addition, all of the cDNA from the second amplification step was used as the starting material to generate biotin-labeled cRNA as described by the manufacturer's protocol for the BioArray HighYield RNA Transcript Labeling Kit (Enzo, Farmingdale, New York, USA). Finally, 20 µg of labeled cRNA was serially hybridized to both the HG-U133A and HG-U133B arrays (Affymetrix, Santa Clara, California, USA).

Data Analysis

Oligonucleotide microarrays were analyzed with the R Statistical package¹³ using some of the packages provided by the Bioconductor Project (www.bioconductor.org). In particular, extensive use was made of the following libraries: "affy",¹⁴ "annaffy" (written by C. Smith; www.bioconductor.org) and "Biobase".¹⁵ Array data were pre-processed by first applying quantile normalization followed by a sequence-specific expression model as implemented in the R library "gcrma", which is a perfect-match method.¹⁶ Normalized microarray data underwent discriminant analysis using a permutation-based t-test to rank genes whose expression was significantly different between TNL and TIL samples. Probe sets were considered significantly different if the *P* value generated from the permuted t-test was ≤ 0.02 and there was a minimum average expression difference of 1.4-fold between the TNL and TIL groups. These thresholds were chosen to maintain the false discovery rate (FDR)¹⁷ at 10% or less for the chorioamniotic membrane arrays; a rate considered acceptable for exploratory analyses. Specifically, the FDR for the probe sets from the HG-U133A arrays was 6.6%, and 10% from the HG-U133B arrays. Given the FDR values and number of discriminant genes for each set of arrays, these results indicate that no more than 16 probe sets were false positives. The FDR associated with the probe sets from the HG-U133A and HG-U133B arrays was 12.8%

and 21.1%, respectively for the blood arrays. The point P value was calculated based on all permutations using the library “multtest”¹⁸. Some of the computations were performed on a linux cluster running LAM MPI (www.lam-mpi.org) using the R library “Rmpi”¹⁹ and the library “snow” (written by L. Tierney, A. J. Rossini and N. Li; cran.r-project.org).

Hierarchical clustering and clustered image maps were generated using Cluster 3.0²⁰ and Java TreeView (written by A.J. Saldanha; <http://jtreeview.sourceforge.net>). Hierarchical cluster analysis of samples was undertaken to explore sample classes within clinical groupings. Gene Ontology (GO) annotations were applied (www.geneontology.org) and interpreted based on results produced by the software GO Tree Machine.²¹ GO annotation provides hierarchically structured classifications for genes under the broad categories “Molecular Function,” “Biological Process” and “Cellular Component.”²² GO Tree Machine uses Fisher's exact test to calculate the significance of representation of GO Biological Process categories relative to the frequency expected to occur by chance given the GO annotation for the U133 set.²¹ Since identical GO categories may appear at multiple hierarchical levels, the number of distinct statistical tests for a specific category was determined and used to correct the point-wise P value using the Sidak correction.²³ The analysis was restricted to GO Biological Process categories at level 4 of the hierarchy to provide a coherent interpretation of the data.

A Kruskal-Wallis test was used to determine the significance of an association of TIL sub-classes with respect to both the interval after rupture of membranes and the duration of labor. In addition, an empirical Bayesian generalized linear model was used for the analysis of regression coefficients to determine the significance of association of the “acute inflammation gene expression signature” with both the interval after rupture of membranes and the duration of labor.²⁴ A similar statistical model was used to determine the significance of TIL and TNL sub-classes with respect to the “acute inflammation gene expression signature” using logistic regression.²⁴

Real-time quantitative RT-PCR assays

TaqMan (Applied Biosystems) 5'-nuclease qRT-PCR assays were used to measure mRNA levels independently for eight genes listed below. The GenBank accession number or the Reference Sequence database accession, followed by the Gene IDs, respectively, are listed in parenthesis after each gene symbol: IL8 (C013615; 3576), TLR2 (F502291; 7097), CXCL1 (NM_001511; 2919), CXCL2 (NM_002089; 2920), CXCL3 (NM_002090; 2921), CCL20 (NM_004591; 6364), IL6 (NM_000600; 3569), and PBEF (NM_005746; 10135). Full length gene names can be found at www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=gene. Gene expression determinations were as described.⁸ Differential expression between clinical groups was calculated using log-transformed data and a one-sided Mann-Whitney U test.

Results

Identification of discriminant genes

Transcriptional profiling of chorioamniotic membrane tissue was carried out to identify molecular mechanisms involved in human parturition. Tissue samples were obtained from 12 patients who had normal spontaneous labor at term (term in labor; TIL), and from 12 patients who were not in labor at term (term no labor; TNL) and delivered by elective Cesarean section (Table I). Using both Affymetrix HG-U133A and HG-U133B arrays allowed an unbiased analysis of virtually all known and predicted transcripts (<http://www.affymetrix.com/products/arrays/specific/hgu133.affx>). Discriminant analysis demonstrated that there were 185 and 39 probe sets from the HG-U133A and HG-U133B arrays, respectively, which differed in expression between TIL samples and their TNL

counterparts. These discriminant probe sets correspond to 197 unique transcripts in total, as defined by NCBI Gene. The complete list of the 224 probe sets is presented in Table II.

The discriminant probe sets were subjected to hierarchical cluster analysis to investigate the consistency of gene expression patterns across similar clinical samples (Figure 1). As reflected in the dendrogram in the upper part of Figure 1, variation in gene expression within clinical groupings was evident suggesting that sub-classes exist within clinical samples. Gene expression patterns within sub-classes of clinical samples were subsequently investigated (Figure 1; refer to subsequent results and discussion).

The sub-cluster of genes representing probe sets with the lowest discriminant P values is shown in Figure 2. There were two different probe sets measuring the expression of each of IL8, SOD2, PBEF1, and PHLDA1 (Figure 2). Each of the probe sets in the pairs clustered with one another demonstrating the reproducibility of the data, as well as the consistency of gene expression across similar samples (Figure 2). This sub-cluster depicted genes that were previously implicated in parturition including IL8⁹ and SOD2.²⁵

Gene expression changes in chorioamniotic membranes undergoing spontaneous labor are associated with the inflammatory response

The sub-cluster of genes with the smallest P values included many genes known to be involved in the inflammatory response (Figure 2). Specifically, the first 12 probe sets represent genes that have been definitively associated with the inflammatory response (Figure 2). To systematically characterize biologically meaningful patterns of gene expression changes, including the observation that many genes involved in acute inflammation were increased in TIL samples, Gene Ontology (GO) annotation was applied to all discriminant genes. The number of genes for each significantly enriched Biological Process category was plotted next to the number of genes expected to appear by chance and is presented in Figure 3A.

Among the down-regulated genes, there was no enrichment for GO categories. Among the genes with increased expression, six GO Biological Process categories were enriched to a statistically significant level and belonged to the following six GO Biological Processes: response to wounding ($P < 3.5 \times 10^{-7}$), taxis ($P < 3.5 \times 10^{-7}$), response to pest/pathogen/parasite ($P < 5.9 \times 10^{-5}$), response to biotic stimulus ($P < 0.0049$), response to abiotic stimulus ($P < 0.019$), and viral genome replication ($P < 0.012$) (Figure 3A). These significantly enriched GO Biological Process categories included 30 genes (represented by 33 unique probe sets) from the discriminant gene list. In addition to these genes, there were a number of other genes in the discriminant gene list that were relevant to the inflammatory response. Specifically, we included in subsequent interpretation six genes (represented by 9 unique probe sets) from the GO-defined Molecular Function categories of: chemokine activity, cytokine activity or cytokine binding. Collectively, these results confirmed the initial observation that a localized inflammatory response characterizes many of the gene expression differences observed between the TIL and TNL chorioamniotic membranes.

Hierarchical clustering of genes in significant GO categories reveals coordinated involvement of genes associated with acute inflammation

The probe sets from the GO categories indicated above were subjected to hierarchical cluster analysis to further investigate their expression patterns (Table III, Figure 3B). The analysis revealed sub-classes in both TNL and TIL samples. The dendrogram of the patient samples shows three major clusters that are colored for clarity (Figure 3B).

The TNL chorioamniotic membrane samples show a cluster of four samples that have decreased expression of most genes (referred to as “low expressors”, fuchsia leaves on the top

dendrogram, Figure 3B). Adjacent is a cluster with five samples that show decreased expression of genes close to the center of the heat map and generally little change in the remaining genes (“average expressors”, black leaves, Figure 3B). We refer to the specific sub-cluster of genes in the center of the heat map as the central gene cluster and its dendrogram leaves have been colored red for clarity (Figure 3B). Finally, there is a three-sample cluster that shows some genes over-expressed (“high expressors”, red leaves, Figure 3B). It is interesting that this last group of three TNL samples cluster most closely with a set of three TIL samples (Figure 3B). The significance of these TNL sub-classes was verified by analysis of regression coefficients for all pair-wise comparisons as follows: high vs. average ($P \leq 0.022$), high vs. low ($P \leq 0.0031$), and average vs. low ($P \leq 0.0018$).

Similarly, the TIL samples appear to have a unimodal distribution that can be divided into three groups. There are five TIL samples that over-expressed nearly every discriminant gene to a high degree and form a tight sub-cluster which we refer to as “high expressors” (orange leaves, Figure 3B). Moreover, four TIL samples exhibit high over-expression of the central gene cluster and generally show modest or no over-expression of the remaining genes (“average expressors”: blue leaves, Figure 3B). Finally, three TIL samples generally display very little change in gene expression of any genes (“low expressors”: green leaves, Figure 3B). These TIL sub-classes exhibit significant differences in gene expression between all pair-wise comparisons as follows: high vs. average ($P \leq 0.017$), high vs. low ($P \leq 0.0084$), and average vs. low ($P \leq 0.0073$). Thus, hierarchical clustering of the probe sets belonging to statistically enriched and inflammation-related GO Biological Process categories reveals unique features in gene expression patterns.

Acute inflammation is a physiological process with distinct phases which can be initiated by the activation of toll-like receptors (TLR2 and TLR4) which recognize among other patterns, epitopes found on pathogenic bacteria as well as epitopes derived from host products²⁶. TLR2 or TLR4 activation leads to the release of neutrophil-specific chemokines and subsequent neutrophil infiltration into the site of inflammation.²⁷ This neutrophil infiltration phase is followed by a monocyte infiltration phase triggered by the release of monocyte-specific chemokines.^{27,28} Monocytes differentiate into macrophages and dendritic cells.²⁹

To investigate the gene expression differences within TIL samples and determine the relationship between the different GO Biological Processes, a Venn diagram was constructed and is presented in Figure 3C. With the exception of the GO category of “viral genome replication,” central to all GO Biological Processes are six genes (whose expression increased during labor) that are involved in the specific recruitment of neutrophils (bold-faced, dark blue genes, Figure 3C).³⁰ Moreover, these six genes form the central gene cluster which demonstrates that they are coordinately expressed (Figure 3B). This list of genes included IL8 (CXCL8) and CXCL6 (GCP-2), which function by signaling through the CXCR1 receptor (Table III).^{31,32} CXCL1 (Gro α), CXCL2 (Gro β), CXCL3 (Gro γ) and CXCL5 (ENA78) serve to recruit neutrophils by signaling through the CXCR2 receptor (Table III).^{31,32} Indeed, four of these six genes were among probe sets with the smallest P values that discriminated the TNL and TIL chorioamniotic membrane samples (Table II). Furthermore, with the exception of one IL8 probe set, these six genes changed in expression in a relatively concerted manner such that each gene exhibited an average increase in expression of between 5.0 and 6.5-fold in the TIL samples relative to their TNL counterparts (Table III).

The transition of acute inflammation from a primarily neutrophil-specific infiltration to a predominately monocyte-specific infiltration is thought to be controlled by expression of IL6 and SOCS3.^{28,29,33,34} Both IL6 and SOCS3 appear coordinately over-expressed in “high” and “average expressor” TIL samples (fourth and fifth gene from the bottom, Figure 3B). These two genes are indicated on the Venn diagram (boldfaced, green symbols, Figure 3C).

Multiple genes involved in monocyte recruitment are also over-expressed in most TIL “high” and “average over-expressors”; they appear near the bottom of the gene-specific dendrogram and are also central to nearly all GO Biological Process categories shown (bold-faced, red genes, Figure 3C). Thus, the monocyte-specific chemokines CCL3 (MIP1 α), CCL4 (MIP1 β), and CCL20 (MIP3 α) exhibit over-expression in a pattern remarkably similar to the neutrophil-specific gene cluster (Figure 3B). The CCR1 receptor, which is specific to CCL3 and CCL4,³² exhibits decreased expression in TIL samples (third gene from the top, Figure 3B). Down regulation of CCR1 has been observed during the differentiation of monocytes into activated and mature dendritic cells in response to CCL3 and CCL4.³⁵ Other receptors involved in monocyte chemotaxis are over-expressed in most TIL samples including the gene for formyl peptide receptor 1 (FPR1) which is found on monocytes and serves as the receptor for chemotactic peptides released by activated neutrophils including cathepsin G³⁶ (Figure 3B, 12th gene from the bottom).

Since acute inflammation can be initiated by TLR2 or TLR4 activation, it is also noteworthy that TLR2 is over-expressed predominantly in the “high expressor” TIL samples (sixth row from the top, Figure 3B). Neutrophil infiltration into sites of inflammation requires that blood-borne neutrophils bind to ICAM1,²⁸ which is also over-expressed 1.6-fold on average in TIL samples (gene 25, Table II). IL1A, which is over-expressed in TIL samples and is central to nearly all GO Biological Process categories presented in Figure 3C, is a generalized activator of inflammation (seventh gene from the top, Figure 3B). Thus, “high” and “average TIL expressors” exhibit over-expression of numerous genes that are known to be involved in several phases of the acute inflammatory process from the initiation phase through the neutrophil- and monocyte-specific infiltration phases.^{27,28} We refer to these genes as the “acute inflammation gene expression signature” (Figures 3B, 3C).

The “acute inflammation gene expression signature” was not associated with either the interval after membrane rupture or the duration of labor

To determine whether the interval after rupture of membranes or the duration of labor was driving expression of genes in the “acute inflammation gene expression signature” in “high” and “average TIL expressors”, two statistical analyses were carried out. First, a Kruskal-Wallis test demonstrated that there is no association of “expressor” sub-class with either the interval after rupture of membranes ($P \geq 0.36$), or the duration of labor ($P \geq 0.67$). Second, the analysis of regression coefficients of the “acute inflammation gene expression signature” demonstrated that there was no association with either the interval after rupture of membranes ($P \geq 0.72$), or the duration of labor ($P \geq 0.70$).

While genes in the “acute inflammation gene expression signature” are frequently over-expressed in the TIL membranes compared to TNL membranes, it is noteworthy that a subset of these genes are already highly expressed in TNL samples. In order to investigate this finding more closely, the discriminant probe-sets were rank ordered by mean TNL expression, and each value was divided by the number of discriminant probe sets (224) and expressed as a percentile. The probe-sets annotated by GO are shown with this percentile, along with the average expression value for the TIL and TNL samples (Table III). Given that the arrays were quantile normalized, we considered that an expression level above the 50th percentile to be high. Using this criterion, there is a high average expression in TNL samples of 3 of 6 neutrophil-specific chemokines (CXCL1, CXCL2 and IL8), 1 of 2 transition genes (SOCS), and 2 of 4 monocyte-specific chemokines (CCL3 and CCL4) (Table III). Thus, while virtually all of the genes involved in neutrophil- and monocyte-specific chemotaxis are over-expressed in the TIL samples relative to their TNL counterparts, half of these genes were already highly expressed in the TNL samples.

Genes associated with acute inflammation had increased expression after spontaneous labor in a new set of chorioamniotic membrane samples

In order to verify and extend the analysis of discriminant gene expression changes occurring during labor, qRT-PCR was used to measure the mRNA levels of eight selected genes in the “acute inflammation gene expression signature” (Table IV). Sufficient RNA was available from the original samples to perform qRT-PCR with 9/12 TIL samples and 9/12 TNL samples. We also validated the results with 25 new chorioamniotic membranes consisting of 12 TNL and 13 TIL samples. Specifically, four representative categories of mRNA were measured based on their respective involvement during specific phases of acute inflammation: 1) TLR2 as a potential initiator of inflammation; 2) Neutrophil-specific chemokines IL8, CXCL1, CXCL2 and CXCL3, because neutrophil recruitment is required during the early phase of acute inflammation;^{37,38} 3) IL6 as a regulator of the transition from a primarily neutrophil-specific infiltration to a predominantly monocyte-specific infiltration;^{33,39} and 4) CCL20 since the ensuing monocyte-specific infiltration phase is driven by monocyte-specific chemokines.⁴⁰ In addition PBEF transcript levels were measured to further assess its expression in chorioamniotic membranes during labor^{9, 10}.

Gene expression values measured by qRT-PCR were remarkably consistent with the GeneChip-based expression measurements (Table IV). Indeed, all qRT-PCR-based gene expression measurements were differentially expressed between the TNL and TIL samples using the same statistical threshold applied to the microarray data ($P \leq 0.02$) and all had high Pearson correlation coefficients that ranged from a low of 0.68 (TLR2, $P \leq 0.0001$; Table IV) to a high of 0.93 (CXCL1, $P \leq 0.0001$; Table IV). Furthermore, qRT-PCR-derived fold changes for CXCL1, CXCL2 and CXCL3 varied by less than 15% of the fold change determined by microarray while the remaining genes (IL6, CCL20 and PBEF) yielded noticeably increased fold change using qRT-PCR measurements compared with the microarray measurements.

Next, qRT-PCR was used to assay gene expression with a new replication set of samples consisting of 13 TIL and 12 TNL samples. Gene expression changes measured by qRT-PCR in the replication set continued to exhibit significant differential expression between TIL and TNL samples (Table IV). The expression level of PBEF appeared to follow those of the other genes measured and were consistent with its involvement and expression in parturition^{9, 10, 41} and the suggestion that this gene may be involved in acute inflammation.⁴²

Taken together, qRT-PCR measurements of gene expression in both the original and the replication set indicated that transcripts involved in multiple, discrete phases of acute inflammation increased in expression in TIL chorioamniotic membrane samples relative to their TNL counterparts.

Gene expression changes in blood of patients in spontaneous labor do not exhibit an inflammatory response

Since genes associated with the inflammatory response show considerable increases in expression in TIL chorioamniotic membranes, we explored whether this response was localized. To this end, transcriptional profiles of blood samples from 12 new TIL patients were compared with 8 new TNL patients. The TIL samples were from patients in active labor (median cervical dilatation 4.0 cm, interquartile range: 3.9 – 4.5 cm). There were 220 and 23 probe sets (from the HG-U133A and HG-U133B arrays, respectively), that were differentially expressed between the groups when using the same statistical criteria applied to the chorioamniotic membranes (data not shown). These 243 discriminant probe sets correspond to 220 unique transcripts in total, as defined by the Gene database.

These discriminant genes were then analyzed for enriched representation of Biological Processes defined by GO annotation. After correcting P values for multiple tests, no GO categories were significantly enriched ($P \leq 0.02$). Furthermore, only six of the discriminant genes from the blood analysis were common with the discriminant genes found in the chorioamniotic membrane analysis (USP9X, ASF1A, CXCL1, EDD, PP784, SMARCA2). Of these six genes, only CXCL1 (GRO1) is known to be involved in acute inflammation. Thus, although the TIL chorioamniotic membranes exhibited the “acute inflammation gene expression signature” at the end of labor, no such pattern was observed in the TIL blood samples from similar patients in active labor.

Comment

Examination of a global transcription profile that reflects an acute inflammatory response extend prior gene-by-gene observations of inflammatory proteins.⁶ Although other studies have shown that inflammatory genes increase in expression in fetal membranes during labor, no study has yet presented sufficient data to clearly demonstrate an orchestrated gene expression signature.⁹ The analysis presented here demonstrates that multiple transcripts controlling each of the defined steps of acute inflammation increase during labor. Specifically, we observed increases in multiple cytokines and chemokines that are known to orchestrate acute inflammation. It is noteworthy that the “acute inflammation gene expression signature” appears to be coordinately expressed and is not associated with either the interval after rupture, or the duration of labor.

Consistent with previous analyses of chorioamniotic membranes measuring one, or several genes at a time, IL8,^{9, 25, 43, 44} IL6,⁴⁵ PBEF,^{9, 10} TLR2⁴⁶ and SOD2²⁵ were over-expressed in TIL samples compared to TNL samples (Table II). Indeed, the expression levels of these mRNAs, or their corresponding proteins, have previously been found to be elevated in samples such as chorioamniotic membranes, myometrium, cervix, or amniotic fluid from TIL patients.

Although numerous stimuli can initiate an acute inflammatory response, one possible sequence may begin with the activation of pattern recognition receptors including TLR2 and TLR4, which are found on the surface of epithelial cells and resident macrophages. TLR2 activation can occur by binding to products from yeast, mycoplasmas and gram-positive bacteria.⁴⁷ TLR4 recognizes bacterial lipopolysaccharide and can also bind host-derived products that are degraded during acute inflammation.²⁶ Activated TLR2 and TLR4 cause a release of chemokines that result in neutrophil recruitment and activation.²⁷ Neutrophils recruited by this mechanism bind to ICAM1 receptors and migrate to the site of chemokine release.²⁸ It is, therefore, striking that the mRNA levels for both TLR2 and ICAM1 increased 1.6-fold and 2.1-fold, respectively, in the TIL chorioamniotic membrane samples relative to their TNL counterparts (Table II· Table III). The subsequent phase of the acute inflammatory response involves the recruitment of monocytes that will go on to differentiate into macrophages and dendritic cells at the site of inflammation.²⁹ This phase of the inflammatory response is preceded by the production of chemokines responsible for monocyte-specific recruitment and differentiation.^{27, 28} It is, therefore, noteworthy that the mRNA levels of monocyte-specific chemokines increased in the TIL samples (discussed below). The transition to the monocyte-specific phase appears to be controlled by IL6 and SOCS3,^{28, 39, 48, 49} both of which were increased in the TIL samples relative to TNL samples (Tables II and III).

Multiple lines of evidence are consistent with the “acute inflammation gene expression signature” eliciting the recruitment and activation of neutrophils in the context of an acute inflammatory response. First, genes specifically implicated in neutrophil recruitment and activation had statistically the most significant changes in their expression (Tables II and III). It is remarkable that IL8, CXCL1, CXCL2, and CXCL3 were among the 10 most discriminant

probe sets (Table III) and are coordinately expressed (Figure 3B). Furthermore, the mRNA levels of all of these genes increased in the TIL samples in a similar manner from 4-fold to 6.5-fold relative to the TNL samples (Tables II and III). Finally, hierarchical clustering indicated that the same four genes exhibited similar expression patterns across multiple samples (Figure 2, top five genes). Interestingly, CXCL5, another neutrophil-specific chemokine was also in the same sub-cluster, even though its *P* value was not as significant (Figure 2, eighth gene from the top, Table III).⁵⁰ Taken together, the “acute inflammation gene expression signature” is consistent with neutrophil recruitment and activation seen during a classical inflammatory response.

Changes in the “acute inflammation gene expression signature” were also consistent with monocyte recruitment and differentiation. Specifically, a set of genes that was central to all GO Biological Process categories depicted in Figure 3C (with the exception of “viral genome replication”) were four genes whose expression increased during spontaneous labor and which are known to be involved in the chemotaxis and differentiation of monocytes. These genes are FPR1, CCL3 (MIP1 α), CCL4 (MIP1 β) and CCL20 (MIP3 α) (Figure 3C, Table III).³⁸ Both CCL3 and CCL4 signal through the CCR1 receptor³² that had a decreased expression in TIL samples (Table III). Decreased expression of CCR1 occurs when monocytes differentiate into activated and mature dendritic cells.³⁵

In contrast to the gene expression patterns in TIL chorioamniotic membranes at the end of labor, blood samples of TIL patients in active labor did not exhibit an increase in the “acute inflammation gene expression signature”. Taken together, our results indicate that differentially increased expression of the inflammatory response signature is independent of the duration of labor, as well as the interval after rupture, and does not manifest systemically during the early stages of the active phase of labor.

The fact that the “acute inflammation gene expression signature” is not associated with either the interval after rupture of membranes or the duration of labor, suggests that this gene expression signature is not a simple consequence of these two events. The results presented here can be interpreted in the context of two, non-exclusive models^{51–53}. The first has been previously proposed as the hypo- or hyper-immune responder model. An alternative model proposes that genes associated with acute inflammation may function in tissue homeostasis.

The principle line of evidence consistent with the hypo- or hyper-immune responder model derives from hierarchical clustering of the 42 probe sets belonging to enriched GO categories. This hierarchical clustering suggests the existence of TIL and TNL sub-classes. While the distribution of gene expression appeared to be unimodal, it was simple to discern TIL samples that were either “high”, “average”, or “low expressors”. We suggest that the TIL “low expressor” and “high expressor” samples may correspond to genetically predetermined hypo- and hyper-immune responders, respectively.^{51–53} Previous studies have measured the expression of the cytokines IL1B, IL6, and IL8 in the cervical fluid and discovered that patients with low concentrations of 2 of 3 of these cytokines were more likely to subsequently develop clinical chorioamnionitis.⁵³ We consider that the five TIL samples showing high expression are more likely to correspond to hyper-immune responders (orange leaves of top dendrogram, Figure 3B).^{51, 52} Conversely, the three TIL low expressors are more likely to correspond to hypo-immune responders (green leaves, Figure 3B).^{52, 53} Since three TNL samples clustered with the three TIL low expressors, it is also possible that these three TNL samples correspond to immune hyper-responders that have not yet been subject to the stimulus of labor (red leaves, Figure 3B). Our results demonstrate clearly that labor increases expression of genes involved in acute inflammation. Given that the “acute inflammation gene expression signature” is over-expressed in the absence of detectable inflammation, the fetal membranes may be primed for a massive inflammation reaction, in the case of a triggering event.⁵² Although a previous study

analyzed cervical fluid and its effects on chorioamnionitis⁵³, our study focuses exclusively on normal chorioamniotic membranes that have undergone labor or not. Nevertheless, our results demonstrate that sub-classes of TIL samples are evident and can be differentiated on the level of expression of genes associated with acute inflammation.

The second model is predicated on the expression levels of inflammatory genes in TNL samples. Although TIL patient samples demonstrated over-expression of the “acute inflammation gene expression signature”, the TNL samples did show high levels of a considerable fraction of these inflammatory genes. Thus, about half of the genes associated with each of the three principle phases of acute inflammation exhibited mean expression levels in the TNL samples that were at least at the 50th percentile or higher in the discriminant probe sets. Specifically, 3 of 6 neutrophil-specific chemokines, 1 of 2 transition genes and 2 of 4 monocyte-specific chemokines were expressed at high levels in TNL samples. Given that all of the chorioamniotic membrane samples were selected based on their lack of inflammation (as defined by histological examination of the extra-placental membranes) this result implies that genes involved in acute inflammation may serve functions not traditionally associated with acute inflammation. In this context, TLR2 signaling has been shown to be necessary for normal epithelial homeostasis in the mouse intestine.⁵⁴ It is, therefore, conceivable that TLR2 and other genes associated with acute inflammation may be involved in maintaining tissue homeostasis of the chorioamniotic membranes.

This study has investigated global transcriptional changes during labor in chorioamniotic membranes. Nevertheless, multiple lines of evidence are consistent with the translation of transcripts from the inflammatory response signature into protein products. First, we observed gene expression changes that have been reported previously to be temporally linked, including the down-regulation of CCR1 mRNA expression in response to the binding of CCR1 receptor protein by its ligands CCL3 and CCL4.³⁵ Second, studies measuring protein in chorioamniotic membranes are consistent with the results presented here and have demonstrated over-expression of IL8,^{9,25,43,44} IL6,⁴⁵ TLR2⁴⁶ and SOD2²⁵ protein in TIL samples compared to their TNL counterparts. Finally, virtually all previous studies measuring amniotic fluid protein levels of genes in the inflammatory response signature are consistent with our results including the protein levels of IL1A, IL1B, IL6, IL8 and CCL3 (MIP1 α) (See Table V for references and details). Since protein in amniotic fluid is most likely derived from the fetus and the fetal membranes, the level probably reflects synthesis and secretion by the membranes and fetal neutrophils.

The previous protein studies were performed for one or at most a few gene products at a time. Although they hinted at the role of inflammation during parturition, the transcriptional profile established here provides, for the first time, the full extent of gene expression associated with acute inflammation during parturition in the chorioamniotic membrane. Thus, while transcriptional activity does not necessarily reflect translational activity, for many of the genes we have investigated in the “acute inflammation gene expression signature”, we find that increased transcription is consistent with multiple studies demonstrating increased protein levels.

In conclusion, the results presented here suggest that labor induces gene expression changes in chorioamniotic membranes consistent with a localized acute inflammatory response, despite the absence of histological evidence of inflammation. Further research is required to determine which of the two models we propose explains the clinical and biochemical findings.

Acknowledgements

We thank Ms. Magdalena Skunca, Ms. Irene Broner, Ms. Carrie Couyoumjian, Ms. Tamara Briazova and Mr. Guy M. Lenk for expert technical assistance in RNA isolations and Dr. Susan Land and Mr. Daniel Lott at the Applied

Genomics Technology Center of Wayne State University for performing the microarray hybridizations and scanning with the RNA samples isolated from blood. We gratefully acknowledge the expert secretarial assistance of Ms. Krista Mitchell. We thank Dr. David Womble and Mr. Daniel Liu at the Bioinformatics Facility of the Center for Molecular Medicine and Genetics for configuring the appropriate software and giving us access to the "BlueGene" Linux cluster. We thank Drs. Kyle Furge and Wendell Jones for critical comments on the manuscript.

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Figure 1. Hierarchical clustering of probe sets that discriminate the chorioamniotic membrane samples of TIL patients from their TNL counterparts

A permutation based t-test was used to find genes with altered expression between TIL and TNL samples. The top 224 probe sets ($P \leq 0.02$) with a minimum average expression difference of 1.4-fold are shown.

Figure 2. Subcluster of discriminant probe sets with the lowest *P* values

A sub-cluster of probe sets taken from the hierarchical clustering shows genes previously implicated in parturition and genes involved in the acute inflammatory response. Row labels correspond to the permuted t-test *P* value followed by the HUGO Gene Nomenclature Committee (HGNC) official gene symbol and include the most commonly used alternative gene symbol. The color scale and column labels follow the same conventions as Figure 1.

Figure 3. Analysis of microarray data from chorioamniotic membranes using Gene Ontology categories

A, Graph of Gene Ontology (GO) Biological Process categories that are over-represented in the 224 discriminant probe sets. Significantly enriched GO Biological Processes from hierarchical level 4 are indicated below each set of bars. Black bars represent the number of observed, and grey bars represent the number of expected genes for that category. Symbols: ***: $P < 0.00059$; **: $P < 0.0049$; and *: $P < 0.019$. B, Hierarchical clustering of discriminant probe sets from selected GO categories. Discriminant probe sets either belonged to significantly enriched GO Biological Processes categories (shown in Figure 3A), or GO Molecular Activities corresponding to chemokine, cytokine, or cytokine binding (Table III). The color scale, row and column labels follow the same conventions as Figure 1. Sub-clusters of the array samples on the top dendrogram have been colored for clarity. The central gene cluster has been colored red on the left dendrogram and is discussed in the text. C, Venn diagram illustrating the relatedness of all significantly enriched GO Biological Process categories. The square box represents probe sets that were chosen by their Molecular Function. Colored gene symbols: dark blue, neutrophil chemotaxis and activation; red, monocyte chemotaxis and activation; and green, transition of acute inflammation from a predominantly neutrophilic to monocytic infiltration.

Table I
Clinical and demographic characteristics of the study samples for chorioamniotic membrane microarrays

Characteristic	Term In Labor (n = 12)	Term No Labor (n = 12)
Maternal age (y)	21.5(21 – 24) ^A	29(22 – 33) ^A
Ethnicity		
African American	11	7
Caucasian	1	2
Asian	0	1
Hispanic	0	1
Others	0	1
Gestational Age at Delivery (wk)	40.2(39.2 – 40.7) ^A	39.0(38.7 – 39.2) ^A
Birthweight (g)	3540(3145 – 3685) ^A	3590(3360 – 3910) ^A
Dilatation at Admission (cm)	4.5 (3.7 – 5.0) ^A	0
Duration of Labor (h)	5.7 (4.8 – 8.4) ^A	0
Interval after rupture (h)	5.8 (4.2 – 8.5) ^A	0

^AValues are median (interquartile range).

Table II
Complete list of 224 probe sets showing differential expression between TIL and TNL samples.

Rank	Probe Set	Gene ID	Symbol ^A	Alias ^B	Gene Name ^C	Fold Change ^D	P-value ^E
1	209774_x_at	2920	CXCL2	GRO2, GROb, MIP2, MIP2A, SCYB2, MGSA, MIP, CINC	chemokine (C-X-C motif) ligand 2	6.5	0.000004
2	211506_s_at	3576	IL8	K60, NAF, GCP1, IL, LECT, LUCT, NAPI, 3, CXCL8, GCP, LYNA, MDNCF, MONAP, NAP, SCYB8, TSG, AMCF, b	interleukin 8	18.4	0.000012
3	205289_at	650	BMP2	GRO3, GROg, MIP2B, SCYB3, MIP, CINC	bone morphogenetic protein 2	4.6	0.000021
4	207850_at	2921	CXCL3	BMP2A	chemokine (C-X-C motif) ligand 3	6.5	0.000029
5	205290_s_at	650	BMP2	CCa2, CRYB2, CRYB2A, D22S665	bone morphogenetic protein 2	3.7	0.000046
6	206777_s_at	1415	CRYBB2	K60, NAF, GCP1, IL, LECT, LUCT, NAPI, 3, CXCL8, GCP, LYNA, MDNCF, MONAP, NAP, SCYB8, TSG, AMCF, b	crystallin, beta B2	2.5	0.000064
7	202859_x_at	3576	IL8		interleukin 8	4.0	0.000072
8	212548_s_at	23045	KIAA0826		KIAA0826 protein	-1.5	0.000077
9	204470_at	2919	CXCL1	GRO1, GROa, MGSA, NAP, SCYB1, MGSA	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	5.7	0.000092
10	205476_at	6364	CCL20	CKb4, LARC, ST38, MIP3A, MIP, SCYA20	chemokine (C-C motif) ligand 20	4.9	0.000097
11	201631_s_at	8870	IER3	DIF2, IEX1, PRG1, DIF, GLY96, IEX, IEX	immediate early response 3	4.3	0.0001
12	205207_at	3569	IL6	HGF, HSF, BSF2, IL, IFNB2	interleukin 6 (interferon, beta 2)	5.3	0.00015
13	225955_at	284207	LOC284207		hypothetical protein LOC284207	1.7	0.00018
14	209289_at	4781	NFIB	NFIB2, NFIB3, NF1	nuclear factor I/B	-1.9	0.00024
15	227697_at	9021	SOC3	CIS3, Cish3, SSI, SOCS, MGC71791	suppressor of cytokine signaling 3	2.1	0.00029
16	202259_s_at	10443	CG005	UAP, UBAP, NAG20, MGC8710	hypothetical protein from BCRA2 region	-1.6	0.00029
17	46270_at	51271	UBAP1		ubiquitin associated protein 1	1.5	0.0003
18	202122_s_at	10226	TIP47	PP17, TIP47, MGC2012, MGC11117	cargo selection protein (mannose 6 phosphate receptor binding protein)	1.5	0.00033
19	207316_at	3036	HAS1	HAS	hyaluronan synthase 1	1.6	0.00035
20	224809_x_at	26277	TINF2	TIN2	TERF1 (TRF1)-interacting nuclear factor 2	1.5	0.00037
21	217738_at	10135	PBEF	PBEF	pre-B-cell colony-enhancing factor	2.0	0.0004
22	214446_at	22936	ELL2	U	elongation factor, RNA polymerase II, 2	1.4	0.00042
23	36711_at	23764	MAFF		v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	2.1	0.00045
24	224739_at	64840	PPN	PPN, por, MG61, PORC, MGC29687	likely ortholog of mouse porcupine homolog (Drosophila)	1.6	0.00061
25	202637_s_at	3383	ICAM1	BB2, CD54	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	1.6	0.00066
26	200666_s_at	3337	DNAIB1	HSPF1	DnaI (Hsp40) homolog, subfamily B, member 1	2.1	0.00067
27	212185_x_at	4502	MT2A	MT2	metallothionein 2A	-1.0	0.00072
28	235549_at	255488	IBRDC2		IBR domain containing 2	1.9	0.00074
29	212327_at	22998	KIAA1102		KIAA1102 protein	-2.1	0.00076
30	202643_s_at	7128	TNFAIP3	A20, TNFAIP2	tumor necrosis factor, alpha-induced protein 3	2.6	0.00078
31	215223_s_at	6648	SOD2	IPO, MNSOD	superoxide dismutase 2, mitochondrial	2.6	0.00086
32	205193_at	23764	MAFF	U	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	1.5	0.00093

Rank	Probe Set	Gene ID	Symbol ^A	Alias ^B	Gene Name ^C	Fold Change ^D	Pvalue ^E
33	216607_s_at	83530	CYP51P2		cytochrome P450, subfamily 51 pseudogene 2	2.3	0.00097
34	207196_s_at	10318	TNIP1	VAN, NAF1, ABIN, KIAA0113	TNFAIP3 interacting protein 1	1.7	0.001
35	209706_at	4824	NKX3-1	NKX3A, NKX3	NK3 transcription factor related, locus 1 (Drosophila)	1.5	0.0011
36	203665_at	3162	HMOX1	HO, HK286B10	heme oxygenase (decycling) 1	2.5	0.0011
37	212328_at	22998	KIAA1102	PBEF	KIAA1102 protein	-2.5	0.0012
38	217739_s_at	10135	PBEF		pre-B-cell colony-enhancing factor	2.1	0.0013
39	201502_s_at	4792	NFKBIA	IKBA,MAD,NFKBI	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	2.0	0.0014
40	204924_at	7097	TLR2	TIL4	toll-like receptor 2	2.1	0.0014
41	242239_at	221078	FLJ23743		hypothetical protein FLJ23743	-1.4	0.0014
42	212199_at	114932	MGC9651		hypothetical protein MGC9651	-1.4	0.0014
43	213524_s_at	50486	G0S2		putative lymphocyte G0/G1 switch gene	3.0	0.0015
44	205239_at	374	AREG			2.6	0.0016
45	209037_s_at	10938	EHD1		EH-domain containing 1	1.6	0.0016
46	203045_at	4814	NIN1	NIN1, NINJURIN	ninjurin 1	1.5	0.0016
47	219955_at	54596	FLJ10884		hypothetical protein FLJ10884	-2.1	0.0016
48	214974_x_at	6374	CXCL5	SCYB5, ENA	chemokine (C-X-C motif) ligand 5	5.3	0.0017
49	229218_at	1278	COL1A2	O14	collagen, type I, alpha 2	-1.5	0.0017
50	207442_at	1440	CSF3	ER	colony stimulating factor 3 (granulocyte)	3.0	0.0018
51	205767_at	2069	EREG	GCSF, G, MGC45931	epiregulin	2.5	0.002
52	227698_s_at	57799	RAB40C	RARL, RASL8C	RAB40C, member RAS oncogene family	1.6	0.0022
53	201325_s_at	2012	EMP1	TMP, CL	epithelial membrane protein 1	1.9	0.0022
54	213528_at	92342	MGC9084		hypothetical protein MGC9084	-1.4	0.0022
55	206157_at	5806	PTX3	TSG	pentaxin-related gene, rapidly induced by IL-1 beta	3.0	0.0023
56	204956_at	4507	MTAP	MSAP, c86fus	methylthioadenosine phosphorylase	-1.5	0.0023
57	203699_s_at	1734	DIO2	SeY, TXDI2	deiodinase, iodothyronine, type II	-2.1	0.0023
58	216840_s_at	3908	LAMA2	LAMM	laminin, alpha 2 (merosin, congenital muscular dystrophy)	-1.6	0.0024
59	242444_at	114904	CIQTNF6	CTRP6, ZACRP6	Clq and tumor necrosis factor related protein 6	1.9	0.0025
60	226907_at	81706	PPP1R14C	KEP1, NY, CPI17	protein phosphatase 1, regulatory (inhibitor) subunit 14C	2.1	0.0026
61	217996_at	22822	PHLDA1	TDAG51, DTIP1B11	pleckstrin homology-like domain, family A, member 1	3.0	0.0028
62	205087_at	25950	RWDD3	DKEZP566K023	RWD domain containing 3	-1.5	0.0028
63	228485_s_at	23446	CDW92	CTL1,CHTL1	CDw92 antigen	2.0	0.0029
64	212048_s_at	57648	KIAA1522		KIAA1522 protein	1.6	0.0031
65	213243_at	157680	COH1	CHS1, VPS13B, KIAA0532, DKEZp31310811	Cohen syndrome 1	-1.5	0.0031
66	202011_at	7082	TJPI	Zo	tight junction protein 1 (zona occludens 1)	-1.5	0.0031
67	206359_at	9021	SOC3		suppressor of cytokine signaling 3	2.1	0.0032
68	224927_at	170954	KIAA1949		KIAA1949 protein	1.6	0.0034
69	205664_at	22944	KIN	BTCD, KIN17	KIN, antigenic determinant of recA protein homolog (mouse)	-1.6	0.0034
70	205100_at	9945	GFPT2	GFAT2	glutamine-fructose-6-phosphate transaminase 2	2.6	0.0036
71	203815_at	2952	GSTT1		glutathione S-transferase theta 1	-2.0	0.0036
72	213796_at	NA	NA		NA	2.3	0.0037
73	220044_x_at	51747	LUC7A		cisplatin resistance-associated overexpressed protein	-1.5	0.0037
74	218810_at	80149	FLJ23231		hypothetical protein FLJ23231	2.0	0.0038
75	211456_x_at	350817	NA		NA	-1.0	0.0039

Rank	Probe Set	Gene ID	Symbol ^A	Alias ^B	Gene Name ^C	Fold Change ^D	Pvalue ^E
76	215385_at	NA	NA		NA	-1.4	0.0039
77	216080_s_at	2237	FEN1	MF1, RAD2, FEN	flap structure-specific endonuclease 1	1.9	0.0042
78	214751_at	90333	LOC90333		hypothetical protein LOC90333	-1.6	0.0042
79	208137_x_at	81856	MGC5384	MGC5384	hypothetical protein MGC5384	-1.6	0.0043
80	225185_at	22808	MRAS	M, RRAS3, R	muscle RAS oncogene homolog	1.9	0.0044
81	209704_at	22823	M96		likely ortholog of mouse metal response element binding transcription factor 2	-1.6	0.0044
82	217997_at	22822	PHLDA1	TDAG51, DTIP1B11	pleckstrin homology-like domain, family A, member 1	3.0	0.0045
83	228846_at	4084	MAD	MXD1	MAX dimerization protein 1	2.3	0.0045
84	243134_at	NA	NA		NA	-1.5	0.0045
85	214715_x_at	90338	KR18	F11, HZF5, KR18, HKr18, FLJ00032, KIAA1611	KRAB zinc finger protein KR18	-1.6	0.0045
86	212774_at	10472	ZNF238	RP58, TAZ, ZBTB18, C2H2	zinc finger protein 238	-1.9	0.0046
87	216841_s_at	6648	SOD2	IPO, MNSOD	superoxide dismutase 2, mitochondrial	2.0	0.0047
88	222853_at	23767	FLRT3		fibronectin leucine rich transmembrane protein 3	2.0	0.0047
89	202685_s_at	558	AXL	UFO		1.4	0.0049
90	212207_at	23389	KIAA1025	KIAA1025, TRAP240L, PROSIT240	KIAA1025 protein	-1.6	0.0049
91	219232_s_at	112399	EGLN3	PHD3, HIPPH3, FLJ21620	egl nine homolog 3 (C. elegans)	-1.2	0.0049
92	221599_at	28971	PTD015	FLJ21035	PTD015 protein	-1.7	0.005
93	202133_at	25937	TAZ		transcriptional co-activator with PDZ-binding motif (TAZ)	-1.4	0.0051
94	200664_s_at	3337	DNAJB1	HSPF1	DnaJ (Hsp40) homolog, subfamily B, member 1	1.7	0.0052
95	224368_s_at	57446	NDRG3	FLJ13556	NDRG family member 3	1.7	0.0053
96	205114_s_at	6348	CCL3	MIP1A, SCYA3, LD78ALPHA, MIP	chemokine (C-C motif) ligand 3	2.3	0.0054
97	205119_s_at	2357	FPR1	FPR, FMLP	formyl peptide receptor 1	2.0	0.0057
98	222549_at	9076	CLDN1	CLD1, SEMP1	claudin 1	1.7	0.0057
99	204894_s_at	8639	AOC3	HPAO, VAPI, VAP		-1.4	0.0058
100	212761_at	6934	TCF7L2	TCF4, TCF	transcription factor 7-like 2 (T-cell specific, HMG-box)	-2.0	0.0058
101	235629_at	NA	NA		NA	-2.6	0.006
102	203083_at	7058	THBS2	TSP2	thrombospondin 2	-1.9	0.006
103	230660_at	56256	D1667H12.2	D1667H12	hypothetical protein D1667H12.2	-1.4	0.006
104	209377_s_at	9324	HMGH3	TRIP7, PNAS	high mobility group nucleosomal binding domain 3	-2.0	0.006
105	209703_x_at	25840	DKFZP586A0522		DKFZP586A0522 protein	-1.4	0.0061
106	212921_at	56950	SMYD2	HSKM, ZMYND14	SET and MYND domain containing 2	-1.5	0.0063
107	218303_x_at	51315	LOC51315		hypothetical protein LOC51315	-1.4	0.0064
108	204836_at	2731	GLDC	GCE, NKH, GCSP, HYGN1	glycine dehydrogenase (decarboxylating; glycine decarboxylase, glycine cleavage system protein P)	-2.3	0.0064
109	206765_at	3759	KCNJ2	IRK1, LQT7, HHIRK1, KIR2, HHIRK1	potassium inwardly-rectifying channel, subfamily J, member 2	-1.9	0.0065
110	219250_s_at	23767	FLRT3		fibronectin leucine rich transmembrane protein 3	2.3	0.0066
111	221009_s_at	51129	ANGPTL4	ARP4, FIAP, PGAR, HFARP, PPARG, pp1158, ANGPTL2		2.8	0.0068
112	205595_at	1830	DSG3	PVA, CDHF6	desmoglein 3 (pemphigus vulgaris antigen)	1.4	0.0068
113	234066_at	9173	IL1RL1	T1, ST2, DER4, ST2L, ST2V, FIT, MGC32623	interleukin 1 receptor-like 1	2.3	0.0069
114	219564_at	3773	KCNJ16	KIR5, MGC33717	potassium inwardly-rectifying channel, subfamily J, member 16	-2.1	0.0069

Rank	Probe Set	Gene ID	Symbol ^A	Alias ^B	Gene Name ^C	Fold Change ^D	Pvalue ^E
115	212538_at	23348	zizimin1	ZIZ1, KIAA1058, zizimin1	zizimin1	-2.5	0.007
116	211464_x_at	839	CASP6	MCH2	caspase 6, apoptosis-related cysteine protease	-1.5	0.007
117	225910_at	284019	LOC284019		hypothetical protein LOC284019	-1.7	0.007
118	242051_at	NA	NA		NA	-1.7	0.0071
119	218691_s_at	8572	RIL		LJM domain protein	1.6	0.0074
120	216243_s_at	3557	IL1RN	IRAP, IL1F3, IL1RA, ICIL, MGC10430	interleukin 1 receptor antagonist	1.9	0.0075
121	204984_at	2239	GPC4		glypican 4	-1.9	0.0075
122	201471_s_at	8878	SQSTM1	p60, p62, PDB3, ZIP3	sequestosome 1	1.5	0.0076
123	219802_at	79912	FLJ22028		hypothetical protein FLJ22028	-1.4	0.0076
124	225018_at	56907	Spir-1	Spir, KIAA1135	Spir-1 protein	1.7	0.0078
125	206461_x_at	4496	MT1H	MT1	metallothionein 1H	2.0	0.0079
126	219313_at	54762	DKFZp434C0328		hypothetical protein DKFZp434C0328	-2.0	0.0079
127	243683_at	9643	MORF4L2	MRGX, MORFL2, KIAA0026	mortality factor 4 like 2	-2.1	0.008
128	213546_at	222161	DKFZp586I1420		hypothetical protein DKFZp586I1420	-1.5	0.008
129	217185_s_at	10729	ZNF259P	354J5	zinc finger protein 259, pseudogene	1.4	0.0082
130	208694_at	5591	PRKDC	XRCC7	protein kinase, DNA-activated, catalytic polypeptide	-1.7	0.0082
131	201099_at	8239	USPX	DFRX	ubiquitin specific protease 9, X-linked (fat facets-like, Drosophila)	-1.4	0.0083
132	218506_x_at	84656	Interim: N-PAC;	HIBDL	Interim: cytokine-like nuclear factor n-pac	1.4	0.0085
133	224169_at	10886	GPR74	NPF2, NPGPR	G protein-coupled receptor 74	2.5	0.0086
134	202708_s_at	8349	HIST2H2BE	H2B, GL105, H2B, H2B, H2BFQ	histone 2, H2be	1.5	0.0086
135	214224_s_at	5303	PIN4	EPVH, PAR14	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting, 4 (parvulin)	-1.4	0.0086
136	205681_at	597	BCL2A1	GRS, BFL1, HBP1, BCL2L5		2.3	0.0088
137	204742_s_at	23047	APRIN	AS3, CG08, FLJ23236, KIAA0979		-1.5	0.0088
138	207606_s_at	94134	ARHGAP12	FLJ10971, FLJ20737, FLJ21785		-1.5	0.0088
139	201960_s_at	23077	PAM	PAM, FLJ10106, KIAA0916	protein associated with Myc	-1.9	0.0089
140	201219_at	54764	ZRANB1	TRABID	zinc finger, RAN-binding domain containing 1	-1.5	0.0089
141	205568_at	366	AQP9	SSC1, HsT17287		1.7	0.0092
142	212987_at	22858	ICK	MRK, LCK2, KIAA0936, MGC46090	intestinal cell (MAK-like) kinase	-1.6	0.0093
143	209239_at	4790	NFKB1	KBF1, EBP, MGC54151, NFKB, NFKB, NF	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)	1.4	0.0096
144	212266_s_at	6430	SFRS5	HRS, SRP40	splicing factor, arginine/serine-rich 5	-1.3	0.0096
145	210873_x_at	200315	APOBEC3A	ARP3, PHRBN		1.5	0.0097
146	203380_x_at	6430	SFRS5	HRS, SRP40	splicing factor, arginine/serine-rich 5	-1.6	0.0097
147	241681_at	NA	NA		NA	-1.7	0.0097
148	203427_at	25842	ASF1A	CIA, DKFZP547E2110		-1.4	0.0098
149	208882_s_at	51366	DD5	HYD, KIAA0896	progesterin induced protein	-1.5	0.0099
150	226031_at	55610	FLJ20097	KIAA1861	hypothetical protein FLJ20097	-1.5	0.0099
151	218490_s_at	55900	ZNF302	ZNF327, ZNF135L, ZNF140L	zinc finger protein 302	-1.6	0.0099
152	202284_s_at	1026	CDKN1A	P21, CIP1, SDIL, WAF1, CAP20, CDKN1, MDA	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.7	0.01
153	200790_at	4953	ODC1		ornithine decarboxylase 1	1.5	0.01
154	208018_s_at	3055	HCK	JTK9	hemopoietic cell kinase	1.4	0.01
155	209290_s_at	4781	NFIB	NFIB2, NFIB3, NF1	nuclear factor I/B	-1.5	0.01
156	203132_at	5925	RB1	RB, OSRC	retinoblastoma 1 (including osteosarcoma)	-1.5	0.01
157	205099_s_at	1230	CCR1	CKR, HMI45, CMKBR1, MIP1aR, SCYAR1	chemokine (C-C motif) receptor 1	-1.6	0.01
158	210675_s_at	5801	PTPRR	EC, PCPTP1, PTP, PTPBR7	protein tyrosine phosphatase, receptor type, R	-2.0	0.01
159	209242_at	5178	PEG3	PW1, KIAA0287	paternally expressed 3	-1.7	0.01

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160	202833_s_at	5265	SERPINA1	PI, A1A, AAT, PI1, AIAT, MGC9222, MGC23330	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	2.0	0.011
161	209182_s_at	11067	DEPP	FIG, DEPP	decidual protein induced by progesterone	2.0	0.011
162	242809_at	9173	ILIRL1	TI, ST2, DER4, ST2L, ST2V, FIT, MGC32623	interleukin 1 receptor-like 1	2.0	0.011
163	224840_at	2289	FKBP5	P54, FKBP51, FKBP54, PPIase, Ptg	FK506 binding protein 5	1.7	0.011
164	206336_at	6372	CXCL6	GCP2, CKA, GCP, SCYB6	chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	2.3	0.011
165	210118_s_at	3552	IL1A	IL1, IL, IL1F1, IL1	interleukin 1, alpha	2.1	0.011
166	218919_at	79752	FLJ14007		hypothetical protein FLJ14007	-1.6	0.011
167	215978_x_at	152719	LOC152719		hypothetical protein LOC152719	-1.6	0.011
168	225667_s_at	151354	NSE1		NSE1	-1.4	0.011
169	216187_x_at	NA	NA		NA	-1.6	0.011
170	220079_s_at	84196	USP48	USP31, FLJ11328, FLJ20103, FLJ23054, FLJ23277, MGC14879	ubiquitin specific protease 48	-1.4	0.011
171	202644_s_at	7128	TNFAIP3	A20, TNFAIP2	tumor necrosis factor, alpha-induced protein 3	2.3	0.012
172	218136_s_at	51312	MSCP	HT015, PRO1278, PRO1584	mitochondrial solute carrier protein	1.7	0.012
173	203234_at	7378	UPP1	UP, UPP, UPASE, UDRPASE	uridine phosphorylase 1	1.6	0.012
174	226710_at	349185	NA		NA	1.5	0.012
175	201169_s_at	8553	BHLHB2	DEC1, STRA13, Stra14		3.0	0.012
176	212616_at	80205	FLJ12178	AD013	hypothetical protein FLJ12178	-1.5	0.012
177	218128_at	4801	NFYB	HAP3, CBF, CBF,	nuclear transcription factor Y, beta	-1.9	0.012
178	206084_at	5801	PTPRR	EC, PCPTP1, PTP, PTPBR7	protein tyrosine phosphatase, receptor type, R	-1.7	0.012
179	221185_s_at	84223	DKFZp434B27	FLJ23571	hypothetical protein DKFZp434B27	2.3	0.013
180	209305_s_at	4616	GADD45B	MYD118, GADD45BETA, DKFZP566B133	growth arrest and DNA-damage- inducible, beta	1.7	0.013
181	201192_s_at	5306	PITPN	VIB1A, PITPNA	phosphatidylinositol transfer protein	1.4	0.013
182	205547_s_at	6876	TAGLN	SM22, SMCC, WS3	transgelin	-1.7	0.013
183	233893_s_at	57654	KIAA1530		KIAA1530 protein	-1.4	0.013
184	213519_s_at	3908	LAMA2	LAMM	laminin, alpha 2 (merosin, congenital muscular dystrophy)	-1.4	0.013
185	205029_s_at	2173	FABP7	MRG, FABPB, B	fatty acid binding protein 7, brain	-3.0	0.013
186	201579_at	2195	FAT	ME5, CDHF7	FAT tumor suppressor homolog 1 (Drosophila)	-1.6	0.013
187	201695_s_at	4860	NP	PNP	nucleoside phosphorylase	1.7	0.014
188	208581_x_at	4501	MT1X	MT1, MT	metallothionein 1X	2.0	0.014
189	212144_at	25777	UNC84B	SUN2, KIAA0668	unc-84 homolog B (C. elegans)	1.4	0.014
190	210992_x_at	2213	FCGR2B	CD32, FCG2, FCGR2, IGFR2	Fc fragment of IgG, low affinity IIb, receptor for (CD32)	1.4	0.014
191	208246_x_at	54782	FLJ20006	NBL, CAG3A, CTG3a, NUMBR, NUMB, TNRC23	hypothetical protein FLJ20006	-1.6	0.014
192	242195_x_at	9253	NUMBL	CDLS, IDN3, IDN3, FLJ11203, FLJ12597, FLJ13354, FLJ13648, DKFZp434L1319	numb homolog (Drosophila)-like	-1.5	0.014
193	212483_at	25836	IDN3	RMP, URI, NNX3, FLJ10575	IDN3 protein	-1.4	0.014
194	222266_at	8725	C19orf2	KIAA1226	chromosome 19 open reading frame 2	-1.5	0.014
195	234762_x_at	57486	NLN	CSP1, DSCI, RCN1, MCPI1, ADAPT78	neurolysin (metallopeptidase M3 family)	-1.5	0.014
196	208370_s_at	1827	DSCR1	ADRP, MGC10598	Down syndrome critical region gene 1	1.7	0.015
197	209122_at	123	ADFP			2.0	0.015
198	212657_s_at	3557	ILIRN	IRAP, IL1F3, IL1RA, ICIL, MGC10430	interleukin 1 receptor antagonist	3.2	0.015
199	216268_s_at	182	JAG1	AGS, AHD, AWS, HJ1, JAGL1	jagged 1 (Alagille syndrome)	-1.7	0.015
200	212637_s_at	11059	WWP1	AIP5, Tti11, hSDRP1, DKFZp434D2111	WW domain-containing protein 1	-1.4	0.015

Rank	Probe Set	Gene ID	Symbol ^A	Alias ^B	Gene Name ^C	Fold Change ^D	P-value ^E
201	204103_at	6351	CCL4	ACT2, G, LAG1, Act, MIP1B, SCYA4, AT744, MIP	chemokine (C-C motif) ligand 4	2.0	0.016
202	201737_s_at	10299	TEB4				
203	203566_s_at	178	AGL	GDE	similar to S. cerevisiae SSM4	-1.5	0.016
204	205047_s_at	440	ASNS	TS11		-1.9	0.016
205	211924_s_at	5329	PLAUR	CD87, UPAR, URKR	plasminogen activator, urokinase receptor	1.7	0.017
206	228132_at	84448	ABLM2	KIAA1808		1.7	0.017
207	224896_s_at	NA	NA		NA	1.5	0.017
208	228221_at	126969	MGC45474		hypothetical protein MGC45474	1.4	0.017
209	209717_at	7813	EV15	NB4S	ecotropic viral integration site 5	1.7	0.017
210	204779_s_at	3217	HOXB7	HOX2, HOX2C, HHO, Hox	homeo box B7	-1.4	0.017
211	214594_x_at	5205	ATP8B1	BRIC, FIC1, PFIC, ATPIC, PFIC1		-1.4	0.017
212	218701_at	51110	CGI-83	CGI		-1.6	0.017
213	205030_at	2173	FABP7	MRG, FABPB, B	lactamase, beta 2	-1.7	0.017
214	209012_at	7204	TRIO		fatty acid binding protein 7, brain	-2.3	0.017
215	212201_at	23141	KIAA0692		triple functional domain (PTPRF interacting)	-1.6	0.017
216	236534_at	149428	BNIP-S	PP753, BNIP, BNIP1, BNIP2, BNIP3, BNIP4	KIAA0692 protein	1.4	0.018
217	203255_at	80204	FBXO11	VIT1, FBX11, FLJ12673, MGC44383, UG063H01	Bcl2/adenovirus E1B interacting protein like	2.0	0.018
218	201204_s_at	6238	RRBP1	ES130, ES	F-box protein 11	-1.4	0.018
219	218705_s_at	28966	SNX24	SBB131	ribosome binding protein 1 homolog 180kDa	-1.5	0.018
220	217707_x_at	NA	NA		sorting nexin 24	-1.4	0.018
221	218309_at	55450	CaMKIIN1 ^a	MGC22256	NA	-2.0	0.018
222	209324_s_at	6004	RGS16	RGS, A28, A28	kinase II		
223	209285_s_at	23272	RAP140	KIAA1105	regulator of G-protein signalling 16	1.7	0.019
224	209710_at	2624	GATA2	NFE1B, MGC2306	retinoblastoma-associated protein 140	-1.6	0.019
					GATA binding protein 2	-1.4	0.02

^A Symbol taken from the LocusLink Database and corresponds to official HUGO Gene Nomenclature Committee (HGNC) symbols.

^B Alias refers to alternative symbols.

^C Gene Name corresponds to the official HGNC name.

^D Fold change refers to expression change in TIL relative to TNL samples.

^E P values were calculated using a permuted Student's t-test comparing TIL samples with their TNL counterparts.

Table III

List of 42 discriminant probe sets from selected GO categories^A.

Probe Set	Gene ID	TNL Percentile ^B	Symbol	Gene Name	Fold Change ^C	Pvalue ^D	Mean ^E	
							TNL	TIL
204894_s_at	8639	12	AOC3	amine oxidase, copper containing 3 (vascular adhesion protein 1)	-1.4	0.0058	2.5	2
205568_at	366	15	AQP9	aquaporin 9	1.7	0.0092	2.9	3.7
205239_at	374	50	AREG	amphiregulin (schwannoma-derived growth factor)	2.6	0.0016	5.8	7.2
205290_s_at	650	18	BMP2	bone morphogenetic protein 2	4.5	0.000021	3.1	5.3
205289_at	650	19	BMP2	bone morphogenetic protein 2	3.7	0.000046	3.1	5
205476_at	6364	5	CCL20	chemokine (C-C motif) ligand 20	4.9	0.000097	2.1	4.4
205114_s_at	6348	84	CCL3	chemokine (C-C motif) ligand 3	2.2	0.0054	8.8	10
204103_at	6351	79	CCL4	chemokine (C-C motif) ligand 4	2	0.016	8.2	9.2
205099_s_at	1230	62	CCR1	chemokine (C-C motif) receptor 1	-1.6	0.01	6.7	6
206777_s_at	1415	6	CRYBB2	crystallin, beta B2	2.4	0.000064	2.2	3.5
207442_at	1440	2	CSF3	colony stimulating factor 3 (granulocyte)	3.0	0.0018	1.8	3.4
204470_at	2919	66	CXCL1	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	5.6	0.000092	7	9.5
209774_x_at	2920	53	CXCL2	chemokine (C-X-C motif) ligand 2	6.4	4.4E-06	6.1	8.8
207850_at	2921	8	CXCL3	chemokine (C-X-C motif) ligand 3	6.4	0.000029	2.3	5
214974_x_at	6374	26	CXCL5	chemokine (C-X-C motif) ligand 5	5.2	0.0017	3.8	6.2
206336_at	6372	8	CXCL6	chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	2.2	0.011	2.2	3.4
205767_at	2069	19	EREG	epiregulin	2.5	0.002	3.1	4.4
210992_x_at	2213	32	FCGR2B	Fc fragment of IgG, low affinity IIb, receptor for (CD32)	1.4	0.014	4.2	4.7
205119_s_at	2357	43	FPR1	formyl peptide receptor 1	2.0	0.0057	5.1	6.1
210118_s_at	3552	35	IL1A	interleukin 1, alpha	2.1	0.011	4.5	5.6
234066_at	9173	91	IL1RL1	interleukin 1 receptor-like 1	2.3	0.0069	9.8	11
242809_at	9173	96	IL1RL1	interleukin 1 receptor-like 1	2.0	0.011	11	12
216243_s_at	3557	20	IL1RN	interleukin 1 receptor antagonist	1.9	0.0075	3.1	4
212657_s_at	3557	38	IL1RN	interleukin 1 receptor antagonist	3.2	0.015	4.7	6.4
205207_at	3569	44	IL6	interleukin 6 (interferon, beta 2)	5.3	0.00015	5.2	7.6
211506_s_at	3576	39	IL8	interleukin 8	18.4	0.000012	4.9	9.1
202859_x_at	3576	93	IL8	interleukin 8	4.0	0.000072	10	12
208581_x_at	4501	99	MT1X	metallothionein 1X	2.0	0.014	12	13
209239_at	4790	44	NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)	1.4	0.0096	5.2	5.7
201502_s_at	4792	95	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	2.0	0.0014	11	12
201192_s_at	5306	58	PITPN	phosphatidylinositol transfer protein	1.4	0.013	6.4	6.9
211924_s_at	5329	49	PLAUR	plasminogen activator, urokinase receptor	1.7	0.017	5.7	6.5
s206157_at	5806	38	PTX3	pentaxin-related gene, rapidly induced by IL-1 beta	3.0	0.0023	4.8	6.4
209324_s_at	6004	26	RGS16	regulator of G-protein signalling 16	1.7	0.019	3.7	4.5
202833_s_at	5265	15	SERPINA1	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	2.0	0.011	2.9	3.9
227697_at	9021	20	SOCS3	suppressor of cytokine signaling 3	2.1	0.0032	3.1	4.2
206359_at	9021	83	SOCS3	suppressor of cytokine signaling 3	2.1	0.00029	8.6	9.7
216841_s_at	6648	59	SOD2	superoxide dismutase 2, mitochondrial	2.0	0.0047	6.6	7.6
215223_s_at	6648	74	SOD2	superoxide dismutase 2, mitochondrial	2.6	0.00086	7.6	9
204924_at	7097	16	TLR2	toll-like receptor 2	2.1	0.0014	3	4.1
207196_s_at	10318	63	TNIP1	TNFAIP3 interacting protein 1	1.7	0.001	6.8	7.6
212637_s_at	11059	12	WWP1	WW domain-containing protein 1	-1.4	0.015	2.5	2

^A Probe sets either belonged to significantly enriched GO Biological Processes or GO Molecular Activities corresponding to chemokine, cytokine or cytokine binding.

^B TNL percentile refers to the rank order of the mean TNL expression divided by the number of discriminant probe sets (224) and was expressed as a percentage.

^C Fold change refers to expression change in TIL relative to TNL.

^D *P* values were calculated using a permuted Student's *t*-test comparing TIL samples with their TNL counterparts.

^E Mean values are expressed as log₂-transformed arbitrary units.

Table IV

Comparison of GeneChip array data to qRT-PCR results of selected genes in original study samples and replication set

Gene symbol	Gene name	Original sample set				Replication set	
		Array Fold change [*]	qRT-PCR Fold change [†]	P value [‡]	Correlation [§]	qRT-PCR Fold change ^{//}	P value [‡]
TLR2	Toll like receptor 2	2.1	3.1	0.0558	0.68	3.1	0.0194
IL8	Interleukin 8	18.4	10.1	0.0011	0.92	N/A	N/A
		4.9			0.87		
CXCL1	Chemokine (C-X-C motif) ligand 1	5.7	6.6	0.0002	0.93	2.8	0.0140
CXCL2	Chemokine (C-X-C motif) ligand 2	6.5	5.9	0.0028	0.88	5.3	0.0009
CXCL3	Chemokine (C-X-C motif) ligand 3	6.5	7.1	0.0003	0.87	4.0	0.0033
IL6	Interleukin 6	4.9	10.0	0.0017	0.92	4.6	0.0072
CCL20	Chemokine (C-X-C motif) ligand 20	5.3	23.4	0.0003	0.91	10.1	0.0009
PBEF	Pre-B-cell colony-enhancing factor	2.1	3.6	0.0062	0.80	2.3	0.0265
		1.9			0.78		

* Fold change represents the average increase in 13 new TIL samples relative to 12 new TNL samples.

[†] Fold change refers to expression change in TIL relative to TNL. There are 2 probe sets for IL8 and PBEF on the GeneChip.

[‡] The P values were calculated using a Wilcoxon rank sum test comparing TIL samples with their TNL counterparts.

[§] Correlation indicates the Pearson correlation coefficient between 9 TIL and 9 TNL samples for which both array and qRT-PCR measurements exist (P < 0.0001). Two correlation coefficients appear for IL8 and PBEF because each of these genes is represented by 2 probe sets on the array.

^{//} Comparison was between 9 TIL and 9 TNL samples.

Table V

Summary of protein determinations of cytokines in amniotic fluid samples.

Study	Protein	Gene Symbol	Number of TIL Subjects	Centrality ^A TIL (ng/mL)	Number of TNL Subjects	Centrality ^A TNL (ng/mL)	PValue ^{B,C}
Keelan, J.A. 2004 ⁵⁰	ENA78	CXCL5	16	1.4	39	1.7	0.0010 ^D
Cohen, J. 1996 ⁵⁵	GRO α	CXCL1	20	2.1	20	1.9	N.S.
Opsjln, S.L. 1993 ⁵⁶	IL1	IL1	25	0.680	24	0.188	0.0001
Romero, R. 1989 ⁵⁷	IL1 α	IL1A	21	Not Determined	16	Not Determined	0.0010
Romero, R. 1990 ⁵⁸	IL1 β	IL1B	41	Not Determined	39	Not Determined	0.002
Romero, R. 1992 ⁵⁹	IL1 β	IL1B	30	0.0515	19	0	0.0017
Cox, S.M. 1997 ⁶⁰	IL6	IL6	175	11.5*	120	0.446*	0.0010
Fukuda, H. 2002 ⁶¹	IL6	IL6	7	1.97	11	0.119	0.0010
Hebisch, G. 2001 ⁶²	IL6	IL6	7	12.561	36	0.269	0.001
Olah, K.S. 1996 ⁶³	IL6	IL6	10	1.5	19	0.01	0.0080
Opsjln, S.L. 1993 ⁵⁶	IL6	IL6	25	4.8	24	0.399	0.0001
Romero, R., 1990 ⁵⁸	IL6	IL6	40	19.5	31	13.0	0.0500
Santhanam, U. 1991 ⁶⁴	IL6	IL6	40	74	33	15	0.05
Hebisch, G. 2001 ⁶²	IL8	IL8	7	9.553	33	1.556	0.001
Laham, N. 1993 ⁶⁵	IL8	IL8	6	3.896*	12	0.969*	0.0030
Olah, K.S. 1996 ⁶³	IL8	IL8	10	Not Indicated	19	Not Indicated	N.S.
Romero, R. 1991 ⁶⁶	IL8	IL8	49	2.68	38	0.000	0.0500
Dudley, D.J. 1996 ⁶⁷	MIP1 α	CCL3	36	Not Indicated	29	Not Indicated	0.0010
Romero, R. 1994 ⁶⁸	MIP1 α	CCL3	20	0.017	19	0.000	0.0010

^A Centrality describes median except for * which are mean.^B All but one study (Cox, et. al.) used non-parametric statistical tests.^C N.S. indicates expression change was reported to be not significant.^D Direction of expression change was opposite to that reported in this paper.