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The role of *Ifng* in alterations in liver gene expression in a mouse model of fulminant autoimmune hepatitis

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Abstract

Background/Aims—BALB/c mice with a homozygous deficiency in the *Tgfb1* gene are a model of fulminant autoimmune hepatitis (AIH), spontaneously and rapidly developing Th1-mediated IFN- γ -dependent necroinflammatory liver disease. We sought to understand the molecular basis for fulminant Th1 liver disease and the specific role of the *Ifng* gene.

Methods—Global gene expression in livers from BALB/c *Tgfb1*^{-/-} mice with and without an intact *Ifng* gene was assessed by microarray analysis. Expression patterns were confirmed by quantitative reverse transcriptase-polymerase chain reaction. Gene ontology clustering analysis was performed to identify altered pathways. The contributions of *Ifng* to altered expression pathways were quantified.

Results—Over 100 genes were strongly (> 10-fold) upregulated, most encoding proteins involved in immune function/response. Chemokines were the most prominently upregulated group, with eight chemokine genes upregulated > 10-fold. *Ifng* was necessary for the upregulation of CXC chemokines gene, but not of CC chemokine genes. By quantitative analysis, *Ifng*'s role in liver gene upregulation varied greatly among overexpressed genes.

Conclusions—Gene expression changes indicate a particularly important and heretofore unappreciated role for chemokines in fulminant AIH. *Ifng* has an important role in expression of some but not all genes. *Ifng* is dichotomous in the regulation of distinct chemokine subfamilies: specifically, *Ifng* is critical for overexpression of specific CXCL genes but dispensable for overexpression of specific CCL genes. These results provide a clearer understanding of the role of *Ifng* in the molecular basis of necroinflammatory liver disease.

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Keywords

autoimmune hepatitis; chemokine; gene expression; interferon- γ – Th1

Introduction

The disease autoimmune hepatitis (AIH) is the clinical manifestation of break-through of normal hepatic tolerance mechanisms, with persistent immune-mediated destruction of hepatocytes (1). Disease progression is typically controlled through chronic use of immunosuppressive medications. However, as many as 10–20% of patients fail medical therapy (2,3) and may require orthotopic liver transplantation. Prominent features of AIH include a strong genetic influence (4,5), a failure of normal tolerogenic mechanisms in the liver (6–8) and a probable Th1 pathogenesis involving the accumulation of CD4⁺ T cells producing IFN- γ . Liver infiltrating CD4⁺ T cells from biopsies of AIH patients are found in portal tracts on biopsy and produce copious IFN- γ *ex vivo* (9,10). Understanding the mechanisms responsible for the pathogenic activity of Th1 CD4⁺ T cells in the AIH liver is important to devise new rational therapies for this difficult disease.

The cytokine transforming growth factor- β 1 (TGF- β 1) is a central player in the development and maintenance of immune tolerance (11). We have demonstrated that TGF- β 1 plays a key role in the maintenance of immune tolerance in the liver. Mice homozygous for a targeted deletion of the *Tgfb1* gene rapidly accumulate CD4⁺ T cells in the liver; furthermore, BALB/c-background *Tgfb1*^{null/null} homozygotes (*Tgfb1*^{−/−} mice) develop a severe fulminant necroinflammatory liver disease with widespread hepatocellular damage (12). The CD4⁺ T-cell subset is intimately involved in pathogenesis, as its monoclonal antibody-mediated depletion in presymptomatic mice prevents the development of necroinflammatory liver disease (13). CD4⁺ T cells isolated from *Tgfb1*^{−/−} livers are highly skewed to the Th1 phenotype, producing copious IFN- γ (12). *Ifng*^{−/−}*Tgfb1*^{−/−} double knockout mice are largely protected from liver disease (12), establishing the Th1 cytokine IFN- γ as a key factor in the development of hepatocellular damage. However, the specific role of IFN- γ in the development of liver pathology in this mouse model has not been determined.

To further investigate the pathology involved in the development of fulminant necroinflammatory liver disease, we profiled liver gene expression in *Tgfb1*^{−/−} mice using microarray. To advance our understanding of the pathological role of IFN- γ , we similarly examined livers from mice doubly deficient in both *Ifng* and *Tgfb1*.

Methods

Mice

Mice were bred at the Dartmouth Medical School in an American Association Accreditation of Laboratory Animal Care-accredited animal care facility and were treated humanely according to National Institutes of Health guidelines. The derivations of *Tgfb1*^{−/−} mice and *Ifng*^{−/−}*Tgfb1*^{−/−} mice are published (12). Litters were screened by PCR of DNA from tail snips for *Tgfb1* genotypes as described (14).

RNA purification and application of Affymetrix GeneChip® microarrays

Livers were dissected from 11-day-old BALB/c mice of the following genotypes (three mice per genotype): *Ifng*^{+/+}*Tgfb1*^{+/+}, *Ifng*^{+/+}*Tgfb1*^{−/−}, *Ifng*^{−/−}*Tgfb1*^{+/+} and *Ifng*^{−/−}*Tgfb1*^{−/−}. Livers were homogenized in TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and liver

RNA prepared. Gene expression analysis used a total of 12 GeneChip® Mouse Genome 430 2.0 microarrays (Affymetrix, Santa Clara, CA, USA), which were processed with standard labelling, one-cycle amplification, hybridization, washes and scanning, completed at the Dartmouth Genomics and Microarray Laboratory.

Microarray data analysis

Resulting data were analysed using GeneTraffic™ version 3.2 (Stratagene, La Jolla, CA, USA). Expression values in CHP format were imported into the GeneTraffic suite and subjected to robust multi-array average (RMA) normalization using established algorithms (15). For multitest, data in CEL format were uploaded into the Bioconductor Microarray Analysis server (Bioinformatics Shared Resource, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA) and similarly standardized using RMA. Significance was determined using a standard Wilcoxon's rank sum test with Benjamini and Hochberg false discovery rate correction for multitest (16). Resulting data were annotated and visualized using the exploratory visual analysis (EVA) program (17) by the Computational Genetics facility at Dartmouth-Hitchcock Medical Center (Lebanon, NH, USA). Reported statistical significance of cluster enrichment was determined using EVA. Cluster analysis was performed using GeneTraffic. Genes were clustered by hierarchical analysis using the Pearson correlation distance metric. Results were visualized using the imaging tools provided by GeneTraffic. Additionally, the lists containing genes that Bioconductor analysis identified as differentially expressed were uploaded to the Database for Annotation, Visualization and Integrated Discovery [DAVID; NIAID, NIH, <http://david.abcc.ncifcrf.gov/>, (18)]. Verification of clustering and pathway analysis was performed on the DAVID online suite.

Determination of the relative contribution of *Ifng* to aberrant gene expression in *Tgfb1*^{-/-} mice

For each gene, the following formula was used to calculate the contribution of *Ifng* to *Tgfb1*^{-/-}-specific expression, and expressed as a ratio (*R*):

$$R = \frac{\left[\left(E_{Ifng(-/-)Tgfb1(-/-)} \right) / \left(E_{Ifng(-/-)Tgfb1(+/-)} \right) \right]}{\left[\left(E_{Ifng(+/-)Tgfb1(-/-)} \right) / \left(E_{Ifng(+/-)Tgfb1(+/-)} \right) \right]}$$

where *E* is the mean expression level (in arbitrary units) for three genotypically matched mice. For each gene, this formula incorporates the fold-change in expression when on an *Ifng*^{-/-} background, calculating the ratio of expression changes on *Ifng*^{-/-} vs. *Ifng*^{+/+} backgrounds. A ratio of ~1 indicates that upregulation of the gene in *Tgfb1*^{-/-} liver is intact in the absence of functional IFN-γ and is therefore IFN-γ independent. A ratio of ~0 indicates that upregulation of the gene in *Tgfb1*^{-/-} liver is eliminated in the absence of IFN-γ and is therefore IFN-γ dependent. A ratio between 0 and 1 indicates that upregulation of the gene is attenuated but not eliminated in the absence of IFN-γ and is therefore partially IFN-γ dependent.

Real-time quantitative reverse transcriptase-polymerase chain reaction

Reverse transcription was performed using the Taqman® RT Kit (Applied Biosystems, Foster City, CA) according to the manufacturer and used oligo-dT primers. PCR amplicons using specific primers [supporting information Table S1; (19–22)] were detected by SYBR Green. Each reaction was carried out in triplicate, and the mean was used in calculations. All values were measured in relation to the level of β-actin mRNA in the same sample. Gene

expression was quantified relative to *Tgfb1*^{+/+} controls using the 2⁻ (experimental CT - β -actin CT) method (23).

Results

Large changes in gene expression accompany fulminant hepatitis in *Tgfb1*^{-/-} mice

We used microarray to identify genes whose expression level was altered in total liver RNA from *Tgfb1*^{-/-} mice, all of whom exhibited fulminant liver necroinflammation. Of 28 287 genes queried, 111 (0.39%) were upregulated > 10-fold, and 43 and 11 were upregulated > 20- and 40-fold respectively (Table 1). Using equivalent cut-offs, 11, 4 and 0 unique genes were downregulated more than 10-, 20- and 40-fold respectively. Thus, fulminant necroinflammatory disease in *Tgfb1*^{-/-} livers is more associated with gene upregulation than with gene downregulation.

The 111 genes upregulated at least 10-fold were grouped using EVA according to their gene ontology (GO) function. Genes falling into the cytokine, receptor, GTPase and peptidase groups were the most highly enriched ($P < 0.05$; Fig. 1). From these four GO function groups, specific genes upregulated more than 10-fold are listed in Table 2. In the cytokine gene group, eight of nine genes thus identified are members of the chemokine class of signalling proteins, encoding four CXC chemokines, four CC chemokines, as well as IL-1 β . The receptor activity group comprises a diverse list that includes Fc receptors, colony stimulating factor receptors, chemokine receptors and membrane-spanning 4A receptors. The GTPase activity group includes three guanylate nucleotide-binding proteins and a T-cell-specific GTPase. The peptidase activity group includes several granzymes, matrix metalloproteinases and cathepsins.

The 57 genes downregulated at least fivefold are distributed widely among various GO groups, with more prominent representation among the oxidoreductase activity, lyase activity and transferase activity groups (data not shown). For the 11 genes downregulated > 10-fold, five are in the oxidoreductase activity group, including two cytochrome P450 family members (supporting information Table S2). Functional groups associated with upregulated genes were largely nonoverlapping with those associated with downregulated genes ($P < 0.05$; data not shown). Groups associated with upregulated gene expression are generally populated by immune genes and genes encoding proteases, whereas groups associated with downregulated gene expression are generally populated by genes encoding proteins involved in microsomal function and oxidative metabolism. This distinction was independently confirmed by annotation and analysis using the DAVID database (18) (data not shown).

Ifng is important for the upregulation of some but not all genes in inflamed livers from *Tgfb1*^{-/-} mice

To more precisely define at the molecular level which changes in gene expression are dependent upon IFN- γ , we carried out microarray analyses of livers from *Tgfb1*^{-/-} mice on an *Ifng*^{-/-} background, along with livers from *Tgfb1*^{+/+}(*Ifng*^{-/-}) littermate control mice. Genes previously found to be upregulated > 10-fold in *Tgfb1*^{-/-} (*Ifng*^{+/+}) livers were then clustered using GeneTraffic according to expression in *Ifng*^{-/-}*Tgfb1*^{-/-} livers (data not shown). Eighteen percent of genes upregulated in livers from *Tgfb1*^{-/-} mice were not upregulated in livers from *Tgfb1*^{-/-} mice on an *Ifng*^{-/-} background, whereas 13% of genes upregulated in livers from *Tgfb1*^{-/-} mice were upregulated equivalently whether on an *Ifng*^{-/-} background or a *Ifng*^{+/+} background. The remaining 69% were partially upregulated in livers from *Tgfb1*^{-/-} mice on an *Ifng*^{-/-} background. The magnitude of the specific contribution of *Ifng* is depicted visually in Figure 2A and can be seen to vary greatly for 30

individual genes upregulated > 20-fold in *Tgfb1*^{-/-} liver (Fig. 2A); a similar pattern is seen for genes upregulated > 10-fold. A similar analysis of downregulated genes shows that most genes are suppressed equivalently in livers from *Tgfb1*^{-/-} mice, whether they are on an *Ifng*^{+/+} background or on an *Ifng*^{-/-} background. Thus, in general, *Ifng* has a much greater influence on gene upregulation than gene downregulation in this model of fulminant hepatitis.

Confirmation of expression patterns by quantitative reverse transcriptase-polymerase chain reaction

To confirm expression patterns revealed by microarray, we examined the expression of selected genes by quantitative reverse transcriptase-polymerase chain reaction (QRT-PCR) using liver RNA isolated from an additional four to six mice per genotype group. By microarray analyses, the genes H2Ea and CCL3 are both strongly upregulated in *Tgfb1*^{-/-} liver. However, overexpression of H2Ea is largely dependent upon *Ifng*, whereas the overexpression of CCL3 is independent of *Ifng*. Similar patterns of expression were observed when these genes were specifically measured by QRT-PCR (supporting information Fig. 1A). Moreover, we examined three genes downregulated on microarray in *Tgfb1*^{-/-} liver (Keg, SerpinA6 and Ces); all yielded similar patterns of expression when analysed using QRT-PCR (supporting information Fig. 1B). As another demonstration of quality, the measured expression of the housekeeping gene β -actin was similar among microarray samples (supporting information Fig. 1C).

Specific chemokine genes are strongly overexpressed in hepatic *Tgfb1*^{-/-} livers

Because chemokines represented eight of nine genes in the cytokine GO function group, we more thoroughly examined the expression of chemokines and their receptors. Chemokines are a diverse family of small secreted proteins further categorized based on the relative positions of N-terminal cysteine residues. Among the CXC sub-family, four (CXCL2/Gro- β , CXCL9/Mig, CXCL10/IP-10 and CXCL11/ITAC) were upregulated in *Tgfb1*^{-/-} livers > 10-fold, and expression was strongly dependent upon *Ifng* (Table 3, Fig. 3A). Other CXCL chemokines were either not detected or not elevated in inflamed livers (Table 3). Expression of genes encoding receptors for CXC chemokines (CXCR1–5) was similar between *Tgfb1*^{-/-} livers and control livers (data not shown). Among the other chemokine families, seven CCL chemokines (CCL2, 3, 4, 5, 6, 8, 24) were upregulated (Fig. 4A). Unlike the four strongly upregulated CXCL chemokines, upregulation of each CCL chemokine was not dependent on *Ifng* (Fig. 3B). Among known or probable receptors for these chemokines, three (CCR1, 2, 5) were upregulated; expression was independent of *Ifng* (Fig. 4B). The CCR3 receptor, which binds CCL8 and CCL24, was not upregulated in *Tgfb1*^{-/-} liver. No change in expression was observed for ligands in the C or CX₃C subfamilies (data not shown).

Discussion

The strong consistent changes in gene expression combined with reproducibility at QRT-PCR using additional biological replicates indicate that the profiling data are high quality, and allow us to draw conclusions with confidence. The aggressive phenotype observed in BALB/c *Tgfb1*^{-/-} mice, in which fulminant liver disease is 100% penetrant by 11 days of age (13), readily translates into robust gene expression differences. Most upregulated genes are involved in immune function or response, encoding chemokines, cytokines, their receptors, acute phase proteins, tetraspanins, major histocompatibility complex class II proteins, proteins important for antigen presentation and a variety of proteases and granzymes. These observations are consistent with the important role of the immune system in fulminant hepatitis and with previous histological and flow cytometric findings that T

cells, macrophages and neutrophils are abundant in the liver in this model system (13,24). By contrast, the downregulated genes are not involved in immune function *per se* but encode proteins involved in a variety of biological functions. Except for a few genes involved in oxidoreductase activity, functional relationships among downregulated genes are not readily apparent.

The Th1 cytokine IFN- γ is important for liver damage in *Tgfb1*^{-/-} mice. Recent studies from our laboratory indicate that CD4⁺ T cells – but not CD8⁺ T cells, NK cells or other cell types – serve as the relevant source of IFN- γ for liver pathology in *Tgfb1*^{-/-} mice (25). Notably, studies in AIH patient samples also implicate CD4⁺ T-cell-derived IFN- γ in disease pathogenesis (9,10). However, whereas liver disease in *Tgfb1*^{-/-} mice is significantly ameliorated when IFN- γ is absent, *Ifng*^{-/-}*Tgfb1*^{-/-} livers are not completely devoid of lesions and still exhibit T-cell accumulation in liver (25), some periportal inflammation (12) and a modest transaminitis developing after about 3 weeks of age (12). Thus, whereas IFN- γ is required for the full-blown necroinflammatory liver disease phenotype, there must be IFN- γ -independent pathways leading to dysregulation of the liver immune system and some liver pathology in *Tgfb1*^{-/-} mice.

The microarray results provide molecular support for this contention; the requirement for *Ifng* varies greatly among overexpressed genes. For some genes [e.g. encoding the macrophage scavenger receptor Marco or the GM-CSF-receptor subunit 2 (Csf2rb2)], upregulation is *Ifng* independent. For others [e.g. encoding the class II protein H2-Ea or the polymeric IgA receptor (Pigr)], upregulation is *Ifng* dependent. For others [e.g. encoding serum amyloid A1 (Saa1) or IL-1 receptor antagonist (IL1rn)], upregulation is partially *Ifng* dependent. Indeed, the role of *Ifng* in regulating gene expression in this system is remarkably complex. The curve in Figure 2A is continuous, with no obvious break to distinguish *Ifng*-dependent vs. *Ifng*-independent genes. Interestingly, downregulated genes behave differently, in that they are generally more refractory to the absence of *Ifng* and it is easier to define a ‘break point’ (asterisk in the inset to Fig. 2B) that distinguishes genes that do not require *Ifng* from those that do.

Strong chemokine upregulation is a prominent gene expression feature associated with fulminant liver disease in this mouse model. Chemokines are related members of a large family of small protein inflammatory mediators that potently elicit the chemotaxis of immune cells participating in inflammation. Chemokines are classified into four subfamilies based on the relative positions of N-terminal cysteine residues (CXC, CC, C and CX₃C). Th1 cells preferentially express the chemokine receptors CXCR3 and CCR5 (26) and the ligands that interact with these receptors are chemoattractive for Th1 cells. CXCR3 is the exclusive receptor for three potent Th1 attracting ligands, CXCL-9, -10 and -11, all of which are strongly upregulated at the mRNA level in *Tgfb1*^{-/-} liver in an *Ifng*-dependent fashion. CCR5 is an important receptor for the ligands CCL3, 4, 5, and 8, all of which are strongly upregulated, but in an *Ifng*-independent fashion. This dichotomy suggests that there are redundant pathways for the chemotaxis and accumulation of Th1 cells in *Tgfb1*^{-/-} liver, one (via CXCR3) that is *Ifng* dependent, and one (via CCR5) that is *Ifng* independent.

Chemokine response pathways represent attractive therapeutic targets for autoimmune disease (27), so elucidating their roles may open up new treatment avenues. Chemokine signalling pathways have been implicated in the pathogenesis of a variety of autoimmune diseases including rheumatoid arthritis (28), systemic lupus erythematosus (29) and autoimmune thyroiditis (30), as well as in inflammation due to graft-versus-host disease (31,32). In autoimmune demyelinating disease of the central nervous system, engagement of certain chemokine receptors is important for the homing of autoreactive T-lymphocytes to sites of demyelination and neuronal injury (33); however, other chemokines have been

associated with neuroprotection (34), indicating complex roles for chemokines in the pathogenesis of autoimmune inflammatory disorders. Clinical trials using chemokine receptor antagonists in multiple sclerosis patients have met with limited success (35), although new approaches to the development of chemokine receptor antagonists may help to realize their therapeutic potential (36).

Only a small number of studies have specifically examined chemokines in autoimmune liver diseases. Plasma levels of CXCL9 and CXCL10 are significantly increased in primary biliary cirrhosis (PBC) patients and rise with disease progression. Portal areas of PBC livers stain for both chemokines. In addition, CXCR3-positive cells are more numerous in peripheral blood and found in portal areas of diseased livers (37). CXCL10 is highly upregulated in sera of AIH patients, and hepatocytes around portal tracts in AIH liver strongly express CXCL10 mRNA. Successful treatment of AIH with prednisolone correlates with a sharp decrease in serum CXCL10 levels (38). Fulminant hepatic failure is associated with early and strong upregulation of CCL2, 3, 4 and 5 (39). Together, these data indicate that several chemokines are overexpressed in patients with autoimmune liver disease in serum and (where investigated) in liver. The results presented here provide additional impetus to understand the role of chemokines in the pathogenesis of autoimmune inflammatory liver diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Enrichment of gene ontology (GO) function groups with genes upregulated in inflamed livers. Genes significantly upregulated (10-fold or greater, $P < 0.05$ as determined by Wilcoxon's rank-sum test) in *Tgfb1*^{-/-} liver RNA were grouped by GO function using the exploratory visual analysis program. Bars show the $-\log_{10}$ (significance) for the enrichment of each group.

Fig. 2.

Requirement of *Ifng* for gene overexpression in liver inflammation varies among upregulated genes. (A) For 30 genes upregulated > 20-fold in *TGF-β1*^{-/-} livers, the contribution of *Ifng* to upregulation of each gene was calculated as in 'Methods' and expressed as a ratio. A high ratio (~1) indicates that the gene is upregulated equivalently in livers in *Tgfb1*^{-/-} mice on either *Ifng*^{+/+} or *Ifng*^{-/-} backgrounds; i.e. gene upregulation is IFN-γ independent. A low ratio (approaching 0) indicates that the gene is upregulated in livers in *Tgfb1*^{-/-} mice on an *Ifng*^{+/+} background but not on an *Ifng*^{-/-} background; i.e. gene upregulation is *Ifng* dependent. Intermediate ratios indicate partial *Ifng* dependence. A ratio > 1 indicates that *Ifng* actually suppresses gene upregulation, such that gene expression is greater when both *Tgfb1* and *Ifng* are interrupted. Inset: The same analysis applied to over 100 genes upregulated > 10-fold. Gene identifiers are not shown, because of image resolution limitations. The data show that genes upregulated in *Tgfb1*^{-/-} liver cannot be readily classified into *Ifng*-dependent and *Ifng*-independent groups. Rather, the sigmoidal shape of the 'curve' shows that *Ifng*'s involvement is different for each gene, and that a continuum best describes *Ifng*'s role in regulation. (B) A similar analysis was applied to 11 genes downregulated > 10-fold, and (inset) over 50 genes downregulated > five-fold (gene identifiers not shown). By comparison with upregulated genes, most downregulated genes are fairly refractory to the presence or absence of an intact *Ifng* gene. The asterisk indicates a natural 'breakpoint' in the curve. The few genes below this breakpoint more strongly depend upon *Ifng* for their downregulation.

Fig. 3.

Ifng is differentially required for the overexpression of distinct chemokines. Selected genes were reanalysed by quantitative real-time reverse transcriptase-polymerase chain reaction (QRT-PCR) using an additional four to six 11-day-old mice per genotype. (A) Four upregulated CXCL genes (CXCL2, 9, 10 and 11) and (B) three upregulated CCL genes (CCL4, CCL5, CCL8) were measured by QRT-PCR. QRT-PCR data are normalized to a β -actin control QRT-PCR. Results from microarray and QRT-PCR are shown side-by-side. Units are arbitrary. The y-axes are in log₁₀ format.

Fig. 4. Strong upregulation of specific CC chemokines and their receptors in *Tgfb1*^{-/-} livers. Microarray results for (A) seven CCL genes and (B) four CCR receptor genes are shown. Units are arbitrary. The y-axes are in log₁₀ format. Dotted lines indicate known or putative ligand-receptor interactions, underscoring the substantial redundancy in this system.

Table 1

Numbers of genes upregulated or downregulated in inflamed livers

Fold change*	Genes	
	<i>n</i> [†]	% [‡]
40	11	0.039
20	43	0.15
10	111	0.39
5	ND	ND
1/5	57	0.20
1/10	11	0.039
1/20	4	0.014
1/40	0	0.0

* Fold change indicates the threshold used to classify genes based on expression in *Tgfb1*^{-/-} livers vs. *Tgfb1*^{+/+} livers.

[†] The number of genes on the microarray significantly ($P < 0.05$ by Wilcoxon's rank sum test) upregulated or downregulated at or above each fold change threshold was determined.

[‡] The percentage was calculated by dividing the number of genes indicated at each fold change by the total number of mouse genes (28 287; http://nar.oxfordjournals.org/cgi/reprint/33/suppl_1/D471.pdf).

ND, not determined.

Table 2

Gene ontology grouping of genes induced > 10-fold

Cluster	Symbol	Gene or protein name *	Fold induction
Cytokine activity, <i>n</i> = 9	Cxcl11	Chemokine (C-X-C motif) ligand 11	51.6
	Cxcl9	Chemokine (C-X-C motif) ligand 9	29.5
	Cxcl2	Chemokine (C-X-C motif) ligand 2	25.9
	Ccl6	Chemokine (C-C motif) ligand 6	22.4
	Ccl5	Chemokine (C-C motif) ligand 5	17.9
	Il1b	Interleukin 1 β	16.8
	Cxcl10	Chemokine (C-X-C motif) ligand 10	11.1
	Ccl3	Chemokine (C-C motif) ligand 3	10.9
	Ccl24	Chemokine (C-C motif) ligand 24	10.5
Receptor activity, <i>n</i> = 22	Ms4a6d	Membrane-spanning 4-domains, subfamily A, member 6D	39.6
	Fcgr3a	Fc fragment of IgG, low affinity IIIa, receptor	38.1
	Marco	Macrophage receptor with collagenous structure	26.8
	Csf2rb1	Colony stimulating factor 2 receptor, β 1, low-affinity (g-m)	26.3
	Il1rn	Interleukin 1 receptor antagonist	24.7
	Cd274	CD274 antigen	24.0
	Fpr-rs2	Formyl peptide receptor, related sequence 2	22.1
	Ccr1	Chemokine (C-C motif) receptor 1	21.5
	Csf2rb2	Colony stimulating factor 2 receptor, β 2, low-affinity (g-m)	20.8
	Cd300lf	CD300 antigen like family member F	17.7
	Pigr	Polymeric immunoglobulin receptor	15.8
	Ccr5	Chemokine (C-C motif) receptor 5	13.4
	Il2rg	Interleukin 2 receptor, γ -chain	13.4
	Fpr1	Formyl peptide receptor 1	13.0
	Fcgr3	Fc receptor, IgG, low affinity III	12.7
	Ms4a6b	Membrane-spanning 4-domains, subfamily A, member 6B	12.3
	Gpr109a	G protein-coupled receptor 109A	12.0
	Clec4d	C-type lectin domain family 4, member d	11.9
	Procr	Protein C receptor, endothelial	11.8
	Fcgr1	Fc receptor, IgG, high affinity I	11.7
	Ms4a4b	Membrane-spanning 4-domains, subfamily A, member 4B	11.4
	Ms4a8a	Membrane-spanning 4-domains, subfamily A, member 8A	10.7
GTPase activity, <i>n</i> = 5	5830443L24Rik	RIKEN cDNA 5830443L24 gene	27.7
	Gbp1	Guanylate nucleotide-binding protein 1	23.6
	Gbp2	Guanylate nucleotide binding protein 2	19.2
	Tgtp	T-cell-specific GTPase	13.2
	Gbp3	Guanylate nucleotide-binding protein 3	12.6
Peptidase activity, <i>n</i> = 9	Gzmb	Granzyme B	44.3
	Mmp13	Matrix metalloproteinase 13	39.3
	Mmp12	Matrix metalloproteinase 12	34.4

Cluster	Symbol	Gene or protein name [*]	Fold induction
	Ctsc	Cathepsin C	18.0
	Adam8	A disintegrin and metallopeptidase domain 8	12.4
	Gzma	Granzyme A	10.7
	Serpinb6b	Serine (or cysteine) peptidase inhibitor, clade B, member 6b	10.5
	Ctss	Cathepsin S	10.4
	Casp4	Caspase 4, apoptosis-related cysteine peptidase	10.2

^{*} Genes induced > 10-fold and populating the four most highly enriched clusters (Fig. 1) are listed.

Table 3

CXCL chemokine expression in inflamed livers

Receptor	Cognate ligands	Alternate names	Change in expression in <i>Tgfb1</i> ^{-/-} liver	
			<i>Ifng</i> ^{+/+}	<i>Ifng</i> ^{-/-}
CXCR1	CXCL5*	ENA-78		
CXCR2	CXCL1	KC/Gro- α	++	+
	CXCL2	Gro- β	++	
	CXCL5*	ENA-78		
	CXCL7	NAP-2		
CXCR3	CXCL9	Mig	++	
	CXCL10	IP-10	++	
	CXCL11	I-TAC	++	
CXCR3-B [†]	CXCL4	PF4		+
CXCR4	CXCL12	SDF-1 α	-	-
CXCR5	CXCL13	BCA-1		++
CXCR6	CXCL16	-	+	+
Unknown	CXCL14	BRAK		
Unknown	CXCL15	Lungkine		

* CXCL5 binds to both CXCR1 and CXCR2.

[†] Alternative splice form of CXCR3.

+, increase of two- to 10-fold; ++, increase of > 10-fold; -, decrease of two- to 10-fold.