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Influence of IFN-γ on gene expression in normal human bronchial epithelial cells: modulation of IFN-γ effects by dexamethasone

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Pawliczak, Rafal, Carolea Logun, Patricia Madara, Jennifer Barb, Anthony F. Suffredini, Peter J. Munson, Robert L. Danner, and James H. Shelhamer. Influence of IFN-γ on gene expression in normal human bronchial epithelial cells: modulation of IFN-γ effects by dexamethasone. Physiol Genomics 23: 28–45, 2005. First published June 28, 2005; 10.1152/physiolgenomics.00011.2005.—Interferon gamma (IFN-γ) plays a role in a variety of lung inflammatory responses, and corticosteroids are frequently employed as a treatment in these conditions. Therefore, the effect of IFN-γ, of the corticosteroid dexamethasone (Dex), or of both on gene expression was studied in normal human bronchial epithelial (NHBE) cells. NHBE cells were exposed to medium alone, IFN-γ (300 U/ml), Dex (10−7 M), or both IFN-γ and Dex for 8 or 24 h. Gene expression was examined using oligonucleotide microarrays. A principal components analysis demonstrated that the IFN-γ treatment effect was the primary source of differences in the data. With a 5% false discovery rate, of the 66 genes upregulated by IFN-γ by twofold or greater at 8 h and 287 genes upregulated at 24 h, coinubcation with Dex inhibited the expression of 2 genes at 8 h and 45 genes at 24 h. Prominent among these were cytokines and secreted proteins. Dex cotreatment increased expression of 65 of the 376 genes that were inhibited by IFN-γ by 50% at 24 h. The majority of these genes encode cell cycle or nuclear proteins. Dex alone increased the expression of only 22 genes and inhibited the expression of 7 genes compared with controls at 24 h. The effect of Dex on IFN-γ-induced changes suggests a specific, targeted effect on IFN-γ responses that is substantially greater than the effect of Dex alone. Dex had little effect on the immediate early response to IFN-γ but a significant effect on the late responses.

interferon; inflammation, cytokines, lung cells, corticosteroids, transcriptional regulation

INTERFERON GAMMA (IFN-γ) is a pleuripotent cytokine produced by T lymphocytes and natural killer cells. It has been well documented that IFN-γ plays a role in lung inflammation, including antiviral, antitumor, and other immune responses (7, 21). It stimulates antigen presenting cells and major histocompatibility complex (MHC) class II molecule expression, driving specific immune responses through antigen recognition and antibody production. In the lung, IFN-γ signaling has been implicated in viral infections, sarcoidosis, mycobacterial infections, cystic fibrosis, and hypersensitivity pneumonia (8, 23–25, 39). IFN-γ may play a role as a modifier of cytokine/chemokine production and may influence cellular recruitment into inflammatory lesions. The binding of IFN-γ to its surface receptor activates the receptor-associated tyrosine kinases JAK1 and JAK2. JAKs tyrosine phosphorylate and activate the latent cytosolic transcription factor STAT1, which then dimerizes, translocates to the nucleus, and binds to the γ-activated sequence (GAS) elements of IFN-γ-responsive genes, resulting in gene activation (3, 13, 18). Although IFN-γ has been reported to increase cyclooxygenase-2 (2) and ICAM-1 (10) expression in epithelial cells, its effect on global pattern of gene expression has not been well studied.

Dexamethasone (Dex), like many glucocorticosteroids, exerts its anti-inflammatory action through binding to a cytoplasmic glucocorticoid receptor that, after transport to the nucleus, interacts with glucocorticoid receptor response elements (GRE), influencing transcriptional activity of a variety of genes. It might also result in MAPK inhibition and repression of binding of nuclear factors such as NF-κB and activator protein-1 to their respective response elements or inhibition of transcription in response to DNA binding by these factors (4, 27). Glucocorticoids are effective in the treatment of immune and inflammatory disorders affecting the lung and other organs.

Therefore, it was of interest to study the effect of IFN-γ on gene expression in normal human bronchial epithelial (NHBE) cells and, further, to investigate the role of glucocorticosteroids in modulating IFN-γ-induced effects.

EXPERIMENTAL PROCEDURES

Materials. NHBE cells were obtained from Cambrex BioSciences (Walkersville, MD) and were cultured according to the company’s protocol. Cell passage 3 was used in all experiments. Cells were grown in uncoated T-75 flasks (Becton Dickinson, Bedford, MA) in BEGM medium (Cambrex BioSciences) with growth factors. Medium was changed 48 h before starting an experiment to medium without hydrocortisone. All experiments were conducted in medium without hydrocortisone. IFN-γ was obtained from Roche Molecular Biochemicals (Indianapolis, IN), and Dex was from Calbiochem (San Diego, CA).

Experimental design. Four treatments were applied [control, IFN-γ (300 U/ml), Dex (10−7 M), and a combination of IFN-γ and Dex (IFN-Dex) for 8 h (n = 4) or for 24 h (n = 5)]. For each time point, the complete treatment design was applied to separate sets of flasks of cells. The design was carefully balanced to avoid any potential batch or replicate effects.

RNA isolation, reverse transcription, and in vitro transcription. After incubation with the indicated treatments, medium was removed and cells were washed three times with ice-cold 1× HBSS without Ca2+ and Mg2+ (BioSource International, Rockville, MD). Total RNA was isolated from the cells using RNeasy Mini Kit and Qiasheader column, both from Qiagen (Valencia, CA). The quality of total RNA was assessed by visualization of intact 18S and 28S
ribosomal RNA bands using a 1.2% formaldehyde agarose gel (Ambion, Austin, TX) stained with Syber Green II (Molecular Probes, Eugene, OR). Ten micrograms of total RNA were reverse transcribed using the SuperScript II Custom Kit (Invitrogen, Carlsbad, CA), employing Th(dT)1-primer: 5’-GGC CAG TGA ATT GTA ATA CGA-3’ (Genset, La Jolla, CA). First-strand synthesis occurred at 42°C followed by second-strand synthesis at 16°C. The cDNA was treated with phenol-chloroform-isoamyl alcohol (25:24:1; Sigma-Aldrich, St. Louis, MO) followed by ethanol precipitation. The equivalent of 1 µg of cDNA was used as a template for the in vitro transcription and biotin labeling reaction, which was performed using a BioArray High Yield RNA Transcription Labeling Kit (Enzo Life Sciences, Farmingdale, NY). The RNasy Mini Kit (Qiagen) was used to clean up the labeled cRNA. Twenty micrograms of adjusted, labeled cRNA were fragmented by heating at 95°C for 35 min in 1X fragmentation buffer (40 mM Tris-acetate, pH 8.1, 100 mM magnesium acetate, and 100 mM potassium acetate). cRNA and fragmented cRNA were visualized using a 1.2% formaldehyde agarose gel (Ambion) stained with Syber Green II (Molecular Probes). The Spectromax Plus spectrophotometer (Molecular Devices, Sunnyvale, CA), using the SoftMax Plus software, was used to quantify total RNA and cRNA. Fifteen micrograms of fragmented biotinylated cRNA were used to make the hybridization cocktail, which also contained herring sperm DNA (0.1 mg/ml; Promega), BSA (0.5 mg/ml; Invitrogen), 50 µM control oligonucleotide B2, and eukaryotic hybridization controls (bioB, bioC, bioD, and Cre at 1, 5, 25, and 100 µM, respectively) (Affymetrix). The hybridization cocktail was heated at 99°C for 5 min followed by 45°C for 5 min. Two hundred microliters of hybridization cocktail, containing 10 µg of fragmented biotinylated cRNA, were hybridized to an Affymetrix Human Genome HG-U95Av2 microarray for 16 h at 45°C. The microarrays were washed and stained by the Affymetrix Fluidics Station using the standard format as describe by Affymetrix. The probe array was stained with streptavidin-phycocerythrin (Molecular Probes) solution and enhanced by use of an antibody solution containing 0.5 mg/ml biotinylated anti-streptavidin (Vector Laboratories, Burlingame, CA). An Affymetrix Scanner was used to scan the probe arrays. Each array was scanned twice. Gene expression was quantitated using Microarray Suite Version 4.0 (MAS4.0; Affymetrix).

Data analysis. Results from the MAS4.0 analysis were transferred to a laboratory information management system (LIMS; Affymetrix). These files were utilized to analyze and transform the data.

Data normalization and transformation. Average difference values from the MAS4.0 software were further normalized and transformed with symmetric adaptive transform (26). This approach incorporates a quantile normalization with a variance-stabilizing transform. The result is a data set in which the underlying variance is stable regardless of the average expression level.

Quality control. The RNA extraction, preparation, hybridization, and scanning were performed in a core laboratory by a single biologist. The quality of the results was assessed by comparison of parameters such as percent present calls and scaling factors to historical values for this laboratory. Furthermore, to detect possible outliers or other artifacts among the 20 samples, a principal components analysis (PCA) was performed on the transformed 24-h results. PCA is a statistical technique for representing high-dimensional data in a few dimensions. The principal components represent the samples as orthogonal linear combinations of the 12,625 probe set values, chosen so that the first component explains the largest fraction of the total variance, the second, the next largest, and so on. After inspection of the first five principal components, no apparent outliers among the samples were observed. The batch effect could be seen in the second principal component. However, because of the balanced experimental design, comparisons between treatment groups are not confounded by batch.

Selection of differentially expressed genes. Multiple criteria were used to conservatively select differentially expressed genes. Two statistical tests were utilized: the consistency test (26) and the paired t-test. The consistency test measures whether a gene is behaving consistently across replicates. The paired t-test compares the average difference between treatments to its standard error. The P values for
each test were converted to the corresponding false discovery rate (FDR) (6). Three comparisons were made to measure the interferon effect (IFN-γ vs. control), the Dex effect (Dex vs. control), and the Dex reversal of IFN-γ effect (IFN-Dex vs. IFN-γ).

Fold change for each comparison was computed after increasing negative average difference values to zero and adding 10 to all values to dampen the large variance resulting from denominator values near zero. Furthermore, to avoid selection of genes at or near the detection

Table 2. IFN-upregulated genes at 8 h

| GenBank Unigene | Name | Symbol | Fold Change |
|-----------------|------|--------|-------------|
| **Cytokines and secreted proteins (11)** |
| X72755*         | chemokine (C-X-C motif) ligand 9 | CXCL9 | 184.26 |
| X02530          | chemokine (C-X-C motif) ligand 10 | CXCL10 | 165.47 |
| AF030514        | chemokine (C-X-C motif) ligand 11 | CXCL11 | 161.35 |
| M26683*         | chemokine (C-C motif) ligand 2, MCP1 | CCL2 | 18.58 |
| X54486          | serine (or cysteine) proteinase inhibitor, clade G, member 1 | SERPING1 | 10.18 |
| AF031167*       | interleukin 15 | IL15 | 8.16 |
| M13755          | interferon, alpha-inducible protein (clone IFN-15K) | GIP2 | 7.52 |
| J04080          | complement component 1, s subcomponent | C1S | 4.31 |
| **Cell surface and membrane proteins (11)** |
| U77643          | secreted and transmembrane 1 | SECTM1 | 48.74 |
| M24283*         | intercellular adhesion molecule 1 (CD54) | ICAM1 | 48.31 |
| X57522          | transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) | TAP1 | 12.69 |
| D11151          | endothelin receptor type A | EDNRA | 7.65 |
| AF035279*       | interleukin 15 receptor, alpha | IL15RA | 6.95 |
| M13560*         | CD74 antigen | CD74 | 6.22 |
| D28137*         | bone marrow stromal cell antigen 2 | BST2 | 5.79 |
| **Signal transduction (7)** |
| L78833*         | interferon-induced protein 35 | IFI35 | 25.70 |
| M14660*         | interferon-induced protein with tetratricopeptide repeats 2 | IFIT2 | 23.46 |
| M33882*         | myxovirus (influenza virus) resistance 1 | MX1 | 23.32 |
| M55543*         | guanylate binding protein 1, interferon-inducible, 67kDa | GBP1 | 20.54 |
| AB000373*       | suppressor of cytokine signaling 1 | SOCS1 | 17.56 |
| M55543*         | guanylate binding protein 2, interferon-inducible | GBP2 | 10.30 |
| L08488          | inositol polyphosphate-1-phosphatase | INPP1 | 7.90 |
| **Transcriptional regulation (10)** |
| L05072*         | interferon regulatory factor 1 | IRF1 | 35.30 |
| M97935*         | signal transducer and activator of transcription 1, 91kDa | STAT1 | 11.69 |
| M060228*        | retinoic acid receptor responder (tazarotene induced) 3 | RARRES3 | 6.50 |
| X2200           | tripartite motif-containing 22 | TRIM22 | 6.42 |
| U53831*         | interferon regulatory factor 7 | IRF7 | 5.73 |
| U32849*         | N-myc (and STAT) interactor | NMI | 5.01 |
| M87503*         | interferon-stimulated transcription factor 3, gamma 48kDa | ISGF3G | 4.64 |
| **Metabolism (15)** |
| M34455*         | indoleamine-pyrrole 2,3 dioxygenase | IDO | 23.57 |
| X59892*         | tryptophanyl-tRNA synthetase | WARS | 16.67 |
| AA808961*       | proteasome (prosome, macropain) subunit, beta type, 9 | PSMB9 | 9.88 |
| U08021          | nicotinamide N-methyltransferase | NNMT | 9.72 |
| M11810          | retinoic acid receptor responder (tazarotene induced) 3 | RARRES3 | 6.50 |
| AB006746*       | phospholipid scramblase 1 | PLSCR1 | 6.51 |
| AA883502        | ubiquitin-conjugating enzyme E2L 6 | UBE2L6 | 6.23 |
| AB003151        | carbonyl reductase 3 | CBRR3 | 5.65 |
| M87434*         | 2’-5’-oligoadenylate synthetase 2, 69/71kDa | OAS2 | 5.14 |
| AJ225089        | 2’-5’-oligoadenylate synthetase-like | OASL | 4.95 |
| J04164*         | 6-pyruvoyltetrahydropterin synthase | PTS | 4.31 |
| **Cell cycle (3)** |
| AFO26939*       | interferon-induced protein with tetratricopeptide repeats 4 | IFIT4 | 94.15 |
| **Nuclear proteins (4)** |
| L22342*         | interferon-stimulated transcription factor 3, gamma 48kDa | ISGF3G | 4.64 |
| M62800*         | interferon-stimulated transcription factor 3, gamma 48kDa | ISGF3G | 4.64 |
| U88964*         | interferon-stimulated transcription factor 3, gamma 48kDa | ISGF3G | 4.64 |
| **Other (5)** |
| AB000115        | Chromosome 1 orf 29 | C1orf29 | 4.87 |
| D28915          | Interferon-induced protein 44 | IFI44 | 4.03 |

Total = 66. *Listed in the database of interferon (IFN)-responsive genes cited in Ref. 14. No. in parentheses is total no. in that category. Complete list of genes is presented in Supplemental Table S2.
### A. Dex-upregulated genes at 8 h

| GenBank | Unigene   | Name                                           | Fold Change |
|---------|-----------|------------------------------------------------|-------------|
| J03764  | Hs.82085  | plasminogen activator inhibitor type 1          | 4.82        |
| U76702  | Hs.433827 | follistatin-like 3 (secreted glycoprotein)      | 2.86        |
| AF056087| Hs.7306   | secreted frizzled-related protein 1             | 2.42        |
| U64197  | Hs.75498  | chemokine (C-C motif) ligand 20                 | 2.28        |
| J05070  | Hs.151738 | matrix metalloproteinase 9                     | 1.64        |
| J02973  | Hs.2030   | Thrombomodulin                                 | 1.61        |

#### Cytokines and secreted proteins (6)

#### Cell surface and membrane proteins (3)

#### Signal transduction (3)

#### Transcriptional regulation (7)

#### Cell cycle (1)

### B. Dex-downregulated genes at 8 h

| GenBank | Unigene   | Name                                           | Fold Change |
|---------|-----------|------------------------------------------------|-------------|
| M17017  | Hs.624    | interleukin 8                                  | 0.36        |
| AB000220| Hs.171921 | semaphorin                                     | 0.48        |
| X02419  | Hs.77274  | plasminogen activator, urokinase               | 0.52        |

#### Cytokines and secreted proteins (3)

#### Cell surface and membrane proteins (2)

#### Signal transduction (2)

#### Transcriptional regulation (2)

#### Cell cycle and nuclear proteins (3)

#### Metabolism (5)

#### Other (3)

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**A:** dexamethasone (Dex)-upregulated genes. Total = 24. **B:** Dex-downregulated genes. Total = 18.
limit of the assay, the mean average difference (AD) value was required to be >10 and the transformed AD value >0.5, and at least 60% of the replicates must be called “present” in at least one treatment group. The subset of genes in which Dex appeared to reverse the IFN-γ effect was further filtered by analysis of a second experiment in which the effect of IFN-Dex was compared with the effect of IFN-γ plus the vehicle for Dex, DMSO (IFNDMSO; 1:250,000 dilution) \( n = 3 \). The filter for genes upregulated by IFN-γ (2-fold or greater) and reversed by cotreatment with Dex was IFNDMSO/IFNDex >1.5. The filter for genes downregulated by IFN-γ (50% or greater) and reversed by cotreatment with Dex was IFNDMSO/IFNDex <0.75.

Cluster analysis. Those genes that changed in response to IFN-γ or Dex treatment were subjected to hierarchical cluster analysis. The clustering was performed in JMP 5.1 software (SAS, Cary, NC) using the Ward method. Characterization of several observed gene clusters was performed using GO-SCAN, a software tool that selects Gene Ontology (GO) terms (GO consortium) that are significantly enriched in the cluster compared with the entire chip (developed by and available on request from J. Barb and P. J. Munson). GO-SCAN computes the Fisher exact test for each GO category and selects those which are overrepresented in each gene list.

Gene expression measurements using real-time PCR. Real-time PCR mRNA quantification using a TaqMan system [Applied Biosystems (ABI), Rockville, MD] was employed to confirm data obtained from microarray experiments. cDNA obtained from extracted mRNA using random hexamers was used to perform, in triplicate, real-time PCR. Probe and primers sets were obtained from ABI or designed with the use of the software Primer Express (ABI). The sequences of the primers and probes are presented in Table 1. Real-time PCR was conducted using a high-capacity cDNA archival kit (ABI) and run on a 7900HT Sequence Detection System (ABI) according to the manufacturer’s directions. RNase P1 gene was used as a standard. Cells incubated without IFN-γ and Dex were used as a control. Relative gene expression is presented as a fold induction compared with control ± SE. Correlation of logarithm of fold change of microarray data with real-time PCR data was calculated using JMP 5.1.

Secreted protein detection. Monocyte chemoattractant protein-1 (MCP-1), RANTES (regulated on activation, normal T expressed and secreted), and interleukin-8 (IL-8) were detected in cell culture supernatants obtained from cells exposed to IFN-γ, Dex, or both using ELISA methods (R&D Systems, Minneapolis, MN), performed according to the manufacturer’s instructions. Data are expressed in picograms per milliliter ± SE. Statistical comparisons were by Student’s t-test.

RESULTS

NHBE cells were studied after exposure to IFN-γ, 300 U/ml, for 8 or 24 h. Exposure of NHBE cells to IFN-γ resulted in substantial changes in gene expression at 8 h and with more remarkable changes at 24 h. A PCA of changes in gene expression at 24 h (Fig. 1) demonstrates that the principal

Fig. 2. A cluster diagram of 684 genes altered by IFN-γ or by Dex at 24 h. For columns comparing IFN or Dex with control cells, red signifies increased expression and green signifies decreased expression relative to control cells. For the column IFNDex-IFN, in which cells treated with IFN and Dex were compared with cells treated with IFN alone, red indicates increased expression of cells treated with IFN and Dex compared with cells treated with IFN alone and green indicates decreased expression. IFN-C indicates comparison of array results from cells treated with IFN-γ vs. control cells; IFN-Dex-IFN indicates comparison of array results from cells treated with Dex and IFN-γ vs. IFN-γ alone. Dex-C indicates comparison of array results of cells treated with Dex vs. control cells. \( n = 5 \) for each group. See RESULTS for details.
Table 4. IFN-upregulated genes at 24 h: cytokines and secreted proteins and cell surface and membrane-bound proteins

| GenBank Unigene Name | Fold Change |
|----------------------|-------------|
| Chemokine (C-X-C motif) ligand 9 | 437.84 |
| Chemokine (C-X-C motif) ligand 10 | 352.07 |
| Chemokine (C-X-C motif) ligand 11 | 271.67 |
| Endothelial cell growth factor 1 | 91.62 |
| Clade C G1 inhibitor member 1 | 44.98 |
| Secreted and transmembrane protein 1 | 39.93 |
| Endothelial cell growth factor 1 platelet-derived | 31.64 |
| Complement component 1 r subcomponent | 27.04 |
| Small inducible cytokine A5 RANTES/chemokine (C-C motif) ligand 5 | 26.54 |
| Small inducible cytokine A2, MCP1/chemokine (C-C motif) ligand 2 | 26.50 |
| Complement component 1 s subcomponent | 23.53 |
| Interferon-stimulated protein 15 kDa | 20.38 |
| Interleukin 8 | 12.88 |
| Matrix metalloproteinase 10 stromelysin 2 | 11.80 |
| Clusterin complement lysis inhibitor SP-40 | 11.68 |
| Plasminogen activator inhibitor 2, clade B, member 2 | 8.41 |
| GR02 oncogene/chemokine (C-X-C motif) ligand 2 | 7.70 |
| Serine or cysteine proteinase inhibitor clade B member 1 | 7.42 |
| Serum amyloid A1 | 6.25 |
| Complement component 1 s subcomponent | 5.83 |
| Interleukin 15 | 5.37 |
| Vascular endothelial growth factor C | 5.23 |
| Bone morphogenetic protein 2 | 5.05 |
| Cathepsin C | 4.56 |
| Matrix metalloproteinase 1 | 4.33 |
| Inhibin beta A | 4.12 |

*Listed in the database of IFN-responsive genes cited in Ref. 14. Complete list of genes is presented in Supplemental Table S4.
determinant of differences in the data is IFN-γ treatment effect. Whereas treatment with IFN-γ resulted in a substantial change in global gene expression (principal component 1), treatment of cells with Dex alone had only a minor effect (principal component 3). Dex treatment had a more pronounced effect on cells concomitantly exposed to IFN-γ.

**IFN-γ**- and Dex-induced changes in gene expression at 8 h. With an FDR of 5% and a threshold of twofold or greater change, IFN-γ treatment of NHBE cells for 8 h increased expression of 66 genes, 61 of which have a known function or cellular localization and thus could be annotated. These genes are listed in Table 2 and include cytokines, cell surface and adhesion molecules, transcription factors, genes involved in cellular metabolism, and cell cycle and nuclear proteins. Thirty-four of these 66 genes were previously listed as interferon-responsive genes from studies of leukocytes (Ref. 14) and are marked with an asterisk (*) next to the GenBank number in this and other tables. With the same 5% FDR and a threshold of 50% reduction in expression, no genes were identified for which IFN-γ at 8 h reduced expression. Dex treatment for 8 h increased expression of only 24 genes even with a relaxed fold-change threshold of 1.6 (Table 3A). Dex treatment for 8 h decreased expression of 18 genes even with a relaxed fold-change threshold of 40% reduction in expression compared with control (Table 3B). Dex cotreatment with IFN-γ for 8 h resulted in the inhibition of only two genes upregulated by IFN-γ alone. These genes are TNFSF10 (Hs.387871), reduced 31% from IFN-γ stimulation alone, and indoleamine-pyrrolo dioxygenase (Hs.840), reduced 38% from IFN-γ stimulation alone. Therefore, Dex-induced changes in gene expression at 8 h were relatively minor, and Dex inhibition of IFN-γ-induced changes in gene expression at 8 h were minimal.

**IFN-γ**-induced changes in gene expression at 24 h. Using an FDR of 5% and a change threshold of twofold or greater, IFN-γ treatment of NHBE cells for 24 h increased gene expression of 287 genes, 252 of which could be annotated. IFN-γ treatment for 24 h also decreased (FDR 5%, 50% or greater change) expression of 376 genes, of which 312 could be annotated. Dex treatment alone increased expression of 22 genes (FDR 5%, 1.6-fold) and inhibited expression of 7 genes (FDR 5%, 40% or greater change). In experiments in which Dex was used in coinubcation with IFN-γ, Dex inhibited expression of 45 (of 287) genes induced by IFN-γ. We observed also that Dex increased expression of 65 of a total of 376 genes inhibited by IFN-γ alone. A cluster diagram of these genes is presented in Fig. 2. Genes prominently induced by IFN-γ treatment for 24 h are represented in red in the column IFN-C. These include a cluster designated A. GO-SCAN anal-

### Table 5. IFN-upregulated genes at 24 h: signal transduction and transcriptional regulation

| GenBank | UniGene | Name                                                                 | Fold Change |
|---------|---------|----------------------------------------------------------------------|-------------|
| AA203213 | Hs.432233 | interferon-stimulated protein 15 kDa                              | 125.30      |
| M33882* | Hs.76391 | myxovirus influenza resistance protein 1                           | 63.99       |
| L78833* | Hs.50842 | interferon-induced protein 35                                       | 49.35       |
| AF026939* | Hs.181874 | interferon-induced protein with tetratricopeptide repeats 4         | 44.93       |
| M55542* | Hs.62661 | guanylate binding protein 1 interferon-inducible 67kDa             | 35.53       |
| X67325* | Hs.278613 | interferon alpha-inducible protein 27                             | 28.35       |
| M55543* | Hs.171862 | guanylate binding protein 2 interferon-inducible                    | 19.18       |
| AB005043 | Hs.50640 | JAK binding protein                                                  | 14.84       |
| U29970* | Hs.265827 | interferon alpha-inducible protein clone IFI-6-16                   | 14.46       |
| L22342* | Hs.241510 | interferon-induced protein 41 30kDa                                | 14.39       |
| Y11999 | Hs.21453 | inositol triphosphate 3-kinase C                                    | 13.24       |
| U72882* | Hs.50842 | interferon-induced protein 35                                       | 11.17       |
| U19261 | Hs.2134 | TNF receptor-associated factor 1                                    | 9.20        |
| J03909* | Hs.14623 | interferon gamma-inducible protein 30                               | 8.65        |
| L24564 | Hs.1027 | Ras-related associated with diabetes                               | 7.85        |
| M92357 | Hs.101382 | tumor necrosis factor alpha-induced protein 2                      | 7.59        |
| M59465 | Hs.211600 | tumor necrosis factor alpha-induced protein 3                      | 7.51        |
| U04636* | Hs.196384 | cyclooxygenase 2                                                   | 7.03        |
| X68277 | Hs.171695 | dual specificity phosphatase 1                                    | 5.69        |
| U19523 | Hs.86724 | GTP cyclohydrolase 1 dopa-responsive dystonia                     | 5.58        |
| L08488 | Hs.32309 | inositol polyphosphate-1-phosphatase                               | 5.01        |
| AF039555 | Hs.2288 | visinin-like 1                                                    | 4.25        |
| L05072* | Hs.80645 | interferon regulatory factor 1                                     | 28.62       |
| AF060228 | Hs.17466 | retinoic acid receptor responder tazarotene induced 3              | 18.17       |
| M97936* | Hs.21486 | signal transducer and activator of transcription 1 91kDa            | 15.73       |
| U88964* | Hs.183487 | interferon stimulated gene 20kDa                                   | 12.87       |
| X82200 | Hs.318501 | stimulated trans-acting factor 50 kDa                              | 7.00        |
| L19871 | Hs.460 | activating transcription factor 3                                   | 5.73        |
| M29893 | Hs.6906 | v-ral oncogene homolog                                             | 5.61        |
| S76638 | Hs.73090 | NFKB2                                                              | 5.25        |
| U18671 | Hs.72988 | signal transducer and activator of transcription 2 113kDa          | 4.98        |
| U32849* | Hs.54483 | N-myc and STAT interactor                                           | 4.92        |
| U53831* | Hs.166120 | Interferon regulatory factor 7                                     | 4.90        |
| M60618 | Hs.77617 | nuclear antigen Sp100                                             | 4.13        |

*Listed in the database of IFN-responsive genes cited in Ref. 14. Complete list of genes is presented in Supplemental Table S5.

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ysis of group A reveals that this group is significantly enriched in genes coding for signal transduction proteins, MHC class II proteins, G protein-coupled receptors, chemokine receptors, chemokines, and cytokines. The complete GO-SCAN analysis of this cluster is presented in Table A of the Supplemental Materials (available at the Physiological Genomics web site).\(^1\) A subset of group A that is prominently reversed by the cotreatment of Dex with IFN-\(\gamma\) is identified as group D. Analysis of this cluster reveals significant enrichment of G protein receptor-binding proteins, chemokine receptors, chemokines, and cytokines (Table D of Supplemental Materials). Two other clusters of IFN-\(\gamma\)-upregulated genes are designated B and C. GO-SCAN analysis of cluster B is enriched in genes coding for proteins involved in cysteine peptidase activity, caspase activity, antiviral responses, and proteosome activity (Table B of Supplemental Materials). As may be seen in the second column, this cluster is little altered by cotreatment with Dex. A fourth cluster, C, includes genes induced by IFN-\(\gamma\) and altered by Dex cotreatment. GO-SCAN analysis of this group reveals enrichment with genes associated with G protein-coupled receptors, chemokine receptors, chemokines, cytokines, and antiviral response genes. Finally, genes downregulated by IFN-\(\gamma\) treatment are presented in green in the column IFN-C. A cluster of these genes for which the IFN-\(\gamma\)-effect is altered by Dex cotreatment is presented as cluster E. GO-SCAN analysis of this cluster reveals that it is enriched in genes involved in nucleotide binding, DNA and RNA metabolism, chromatin, and cell proliferation (Table E of Supplemental Materials). The principal GO terms overrepresented in each of the clusters are presented in the Supplemental Materials ("GO Summary Table").

\*Listed in the database of IFN-responsive genes cited in Ref. 14. Complete list of genes is presented in Supplemental Table S6.
regulation. IFN-γ influenced expression of 39 genes playing roles in cellular metabolism. These include genes involved in the ubiquitin-proteasome pathways and 2',5'-oligoadenylate synthase genes. Twenty-two genes involved in cell cycle and differentiation were induced by exposure to IFN-γ, including neuregulin-1 and caspases-1, -4, and -10. A list of 40 genes of unclassified function, primarily expressed sequence tags (ESTs) and KIAA gene products, is included in Supplemental Materials (Supplemental Table S11).

Genes downregulated by IFN-γ at 24 h in NHBE cells. Expression of 16 genes encoding secreted proteins was decreased by IFN-γ by 50–90% compared with control cells. This group included plasminogen activator inhibitor type-1, metallothionein-1L, activated platelet protein-1, cathepsin L2, and others (Table 7 and Supplemental Materials). Forty-three genes encoding cell surface and membrane-associated proteins were downregulated by IFN-γ. IFN-γ downregulated expression of adenosine-A2β receptor, integrin-ß6, and transforming growth factor (TGF)-ß receptor type-2. Exposure to IFN-γ inhibited expression of 29 genes involved in signal transduction including RAB4, TYRO3, protein tyrosine kinase, regulator of G protein signaling-3 (RGS3), and protein tyrosine kinase-7 (PTK7). A full list of signal transduction genes inhibited by IFN-γ is presented in Table 8 (and Supplemental Materials). IFN-γ also inhibited expression of 37 genes involved in transcriptional regulation. This group includes hepatocyte nuclear factor-3ß, transcription factor nuclear receptor subfamily 2, group F (NR2F2), and the TGF-ß-stimulated protein TSC-22. Six genes involved in translation regulation were inhibited by exposure to IFN-γ. Table 9 (and Supplemental Materials) presents 85 genes involved in cellular metabolism that were inhibited by IFN-γ. Eight genes encoding cytoskeleton proteins were inhibited by IFN-γ. IFN-γ is known to have an inhibitory effect on cell proliferation. Consistent with that effect, the expression of 42 cell cycle proteins was inhibited by exposure to IFN-γ (Table 10 and Supplemental Materials). These include cyclins A2, B1, B2, and F as well as cyclin-dependent kinase-4. Expression of 47 genes encoding nuclear proteins was also inhibited by IFN-γ, including proliferating cell nuclear antigen and histone family members. Sixty-seven other genes inhibited by IFN-γ, including genes not annotated, are listed in the Supplemental Materials (Supplemental Table S12).

Influence of Dex on gene expression in NHBE cells. The effect of Dex on gene expression in NHBE cells was studied. Even with the alteration of the filter criteria to include genes upregulated by 60% or more, only 22 genes were significantly induced by Dex compared with control cells (Table 11). The gene most dramatically induced was serum amyloid A1. Similarly, the effect of Dex alone in downregulation of genes in NHBE cells was modest. Even with the filter criteria relaxed to include genes altered by >40%, the list only included seven genes (Table 11).

Influence of Dex on IFN-γ-induced gene expression. The effect of Dex on IFN-γ-induced changes in gene expression was also studied. Using the list of IFN-γ-upregulated genes, we
IFN-\(\gamma\), DEX, AND GENE EXPRESSION IN HUMAN LUNG CELLS

Table 8. IFN-downregulated genes at 24 h: signal transduction, transcriptional regulation, and translation regulation

| GenBank | Unigene | Name | Fold Change |
|---------|---------|------|-------------|
| AF035959 | Hs.24879 | phosphatidic acid phosphatase type 2C | 0.11 |
| AB016811 | Hs.111554 | ADP-ribosylation factor-like 7 | 0.13 |
| M28211 | Hs.119007 | RAB4 member RAS oncogene family | 0.17 |
| S62539 | Hs.96063 | insulin receptor substrate 1 | 0.27 |
| U27655 | Hs.82294 | regulator of G-protein signalling 3 | 0.32 |
| D17517 | Hs.301 | TYRO3 protein tyrosine kinase | 0.33 |
| D31762 | Hs.153954 | TRAM-like protein | 0.34 |
| Y11312 | Hs.132463 | phosphoinositide-3-kinase class 2 beta polypeptide | 0.34 |
| M68941 | Hs.73826 | protein tyrosine phosphatase non-receptor type 4 | 0.37 |
| Y12735 | Hs.127355 | dual-specificity tyrosine- \(\gamma\) phosphorylation regulated kinase | 0.38 |
| X16302 | Hs.99816 | beta-catenin-interacting protein ICAT | 0.39 |
| U33635 | Hs.90572 | PTK7 protein tyrosine kinase 7 | 0.40 |

**Signal transduction (29)**

- **Transcriptional regulation (37)**
- **Translation regulation (6)**

Complete list of genes is presented in Supplemental Table S8.

evaluated genes for which Dex reduced the IFN-\(\gamma\) effect by 30% or more. In contrast to the effect of Dex alone on NHBE cells, the effect of Dex on IFN-\(\gamma\)-stimulated cells was more pronounced. Of the 287 genes upregulated by IFN-\(\gamma\), 45 were inhibited by Dex treatment with IFN-\(\gamma\) (Table 12). A substantial portion of the Dex effect was on genes coding for cytokines and secreted proteins and cell surface and membrane proteins. While 40 of the 66 genes induced by IFN-\(\gamma\) at the 8-h time point were also induced at the 24-h time point, only 1 of the 45 genes induced by IFN-\(\gamma\) and inhibited by Dex at 24 h was induced at the earlier 8-h time point, MCP-1. Forty-four of the 45 genes induced by IFN-\(\gamma\) and inhibited by Dex at 24 h were only induced at the latter (24 h) time point and were not genes that were early responders to IFN-\(\gamma\) treatment. Similarly, we studied the effect of Dex coinubation on genes downregulated by IFN-\(\gamma\). Of the list of 376 genes downregulated by IFN-\(\gamma\), Dex cotreatment increased expression of 65 genes by at least twofold compared with IFN-\(\gamma\) treatment alone (Table 13). These genes downregulated by IFN-\(\gamma\) and reversed by Dex were predominantly cell cycle and nuclear proteins. Therefore, the genes upregulated by IFN-\(\gamma\) and reversed by Dex are functionally distinct from those downregulated by IFN-\(\gamma\) and reversed by Dex.

**Confirmatory experiments.** To verify and validate data obtained from microarray studies, two additional techniques were employed: real-time PCR mRNA quantification and ELISA quantitation of secreted proteins. Real-time PCR mRNA quantification was performed for eight genes found to be upregulated, downregulated, or unchanged in expression in response to one or more of the treatments. The following gene products were selected: Cox-2, IL-7 receptor, MAPK kinase kinase (MAPKKK), matrix metalloproteinase-10 (MMP-10), plasminogen activator inhibitor-2 (PAI-2), serum amyloid A, superoxide dismutase (SOD), and vascular endothelial growth factor (VEGF) (see Table 1). Comparison between data obtained from microarrays and real-time PCR expression estimation for all eight genes selected is presented in Table 14. The comparison of the logarithm of fold change for all treatments resulted in a correlation coefficient of 0.97 (\(P < 0.0001\)). The logarithm of fold change in real-time PCR data was linearly proportional to the microarray data, with a proportionality
constant slightly >1. Of interest, serum amyloid A1 mRNA was induced by treatment with IFN-γ and by treatment with Dex (Tables 4 and 11). Treatment of cells with both IFN-γ and Dex resulted in a dramatically increased gene expression for serum amyloid A. To obtain further confirmation that changes in mRNA levels correspond with enhanced protein production, three secreted proteins were detected using ELISA, RANTES, IL-8, and MCP-1 (Fig. 3). RANTES and MCP-1 were not detectable in control supernatants. Exposure to IFN-γ increased RANTES levels to 630 ± 63 pg/ml and MCP-1 to 2,274 ± 82 pg/ml at 24 h. Similar exposure increased IL-8 production from 822 ± 45 pg/ml (control cell supernatants) to 1,696 ± 71 pg/ml. The effect of IFN-γ on secretion of these three cytokines was substantially inhibited by Dex. Therefore, changes in mRNA expression by real-time PCR and changes in protein production appear to be consistent with the detected changes in mRNA expression by microarray.

**DISCUSSION**

IFN-γ is a pleiotropic Th1-type cytokine generated by activated T lymphocytes and natural killer cells. IFN-γ has antiproliferative, antiviral, and immune-modulating properties. IFN-γ might modulate protein expression at the transcriptional, posttranscriptional, and posttranslational levels. IFN-γ binds to its cellular membrane receptors and induces STAT1 recruitment and phosphorylation, leading to activation transcription of several genes. STAT1 activation involves at least JAK1 and

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**Table 9. IFN-downregulated genes at 24 h: metabolism and cytoskeleton proteins**

| GenBank | Unigene  | Name                                      | Fold Change |
|---------|----------|-------------------------------------------|-------------|
| M74542  | Hs.575   | aldehyde dehydrogenase 3                  | 0.08        |
| M57951  | Hs.278892| UDP glycosyltransferase 1 family polypeptide A4 | 0.10        |
| U035861 | Hs.431175| aldo-keto reductase family 1 member C1    | 0.13        |
| U46689  | Hs.159608| aldehyde dehydrogenase 10 fatty aldehyde dehydrogenase | 0.17        |
| M90656  | Hs.151393| glutamate-cysteine ligase catalytic subunit | 0.20        |
| AF02887 | Hs.169907| glutathione S-transferase A4              | 0.20        |
| U37100  | Hs.116724| aldo-keto reductase family 1 member B1    | 0.20        |
| AF029893| Hs.8526  | beta-1 3-N-acetylcysteaminyltransferase    | 0.22        |
| D17793  | Hs.78183 | aldo-keto reductase family 1 member C3    | 0.24        |
| M81600  | Hs.406515| NADPH quinone oxidoreductase              | 0.24        |
| AB02967 | Hs.323462| DEAD/H box 30, DDX30                     | 0.25        |
| AF030249| Hs.196176| dienoyl-CoA isomerase                     | 0.25        |
| AT797997| Hs.155020| methyltransferase of Williams Beuren syndrome | 0.26        |
| AF009767| Hs.132898| fatty acid desaturase 1                   | 0.27        |
| AF037335| Hs.5338  | carbonic anhydrase XII                    | 0.27        |
| AL035079| Hs.76359 | catalase                                  | 0.28        |
| X83467  | Hs.76781 | ATP-binding cassette sub-family D ALD member 3 | 0.28        |
| AF014398| Hs.5753  | inositol myo 1 or 4 monophosphatase 2     | 0.28        |
| U97105  | Hs.173381| dihydropyrimidinase-like 2                | 0.29        |
| X53463  | Hs.2704  | glutathione peroxidase 2 gastrointestinal  | 0.29        |
| M95263  | Hs.82609 | hydroxymethylbilane synthase              | 0.32        |
| U00238  | Hs.311   | Phosphoribosyl pyrophosphate amidotransferase | 0.32        |
| X15334  | Hs.173724| creatine kinase brain                     | 0.33        |
| Y17448  | Hs.46634 | kynurenine aminotransferase               | 0.33        |
| X78669  | Hs.79088 | reticulocalbin 2 EF-hand calcium binding domain | 0.34        |
| X92106  | Hs.78943 | blemogenic hydrolase                      | 0.34        |
| U24183  | Hs.75160 | phosphofructokinase muscle                | 0.34        |
| D00723  | Hs.77631 | glycine cleavage system protein H aminomethyl carrier | 0.35        |
| X96752  | Hs.8110  | L-3-hydroxyacyl-Coenzyme A dehydrogenase short chain | 0.36        |
| W26480  | Hs.132898| fatty acid desaturase 1                   | 0.36        |
| X16832  | Hs.288181| cathepsin H                              | 0.36        |
| L38298  | Hs.118131| 5-10-methenyltetrahydrofolate synthetase  | 0.37        |
| Y09008  | Hs.78853 | uracil-DNA glycosylase                    | 0.37        |
| AB020316| Hs.134015| uronyl 2-sulfotransferase                 | 0.38        |
| AL049699| Hs.42336 | malic enzyme 1 NADP dependent cytosolic   | 0.38        |
| U57650  | Hs.155939| inositol polyphosphatase-5-phosphatase 145kDa | 0.38        |
| U28042  | Hs.41706 | DEAD/Asp-Glu-Ala-Ala/His box polypeptide 10 RNA helicase | 0.38        |
| AB000359| Hs.306173| phosphatidlyinositol glycan class C pseudogene 1 | 0.39        |
| U34804  | Hs.272462| sulfotransferase family cytosolic 1A phenol-prefering member 2 | 0.39        |
| AB003151| Hs.88778 | carbonyl reductase 1                      | 0.40        |
| U76421  | Hs.85302 | adenosine deaminase RNA-specific B1 homolog of rat RED1 | 0.40        |
| D00860  | Hs.56    | phosphoribosyl pyrophosphate synthetase 1 | 0.40        |
| L12711  | Hs.89643 | transketolase Wernicke-Korsakoff syndrome | 0.40        |
| X71129  | Hs.74047 | electron-transfer-flavoprotein beta polypeptide | 0.40        |

| Cytoskeleton proteins (8) |

| GenBank | Unigene  | Name                                      |
|---------|----------|-------------------------------------------|
| M19267  | Hs.77899 | tropomyosin 1 alpha                        | 0.38        |
| AL008583| Hs.107374| chromobox homolog 6                        | 0.38        |

Complete list of genes is presented in Supplemental Table S9.
JAK2 proteins. IFN-γ may activate additional signal transduction pathways such as Pyk2 and ERK1/2 (35), the Src-family kinase Fyn (36), and the adaptor proteins c-Cbl, CrkL, and CrkII pathway (17, 28). It is also involved in signaling through G protein-linked signaling molecules such as Rap-1 (1). IFN-γ signaling might also utilize the protein tyrosine phosphatases SHP-1 and SHP-2. Finally, a secondary link to NF-κB activation is possible by the induction of NOD1 and NOD2 gene and protein expression by IFN-γ (19, 30, 32) or by induction of TNF-α expression. IFN-γ-signaling pathways were reviewed in detail by Ramana et al. (29). It is possible that both STAT-dependent and -independent pathways might be overlapping.

Complete list of genes is presented in Supplemental Table S10.

Table 10. IFN-downregulated genes at 24 h: cell cycle, nuclear proteins, and other

| GenBank | Unigene | Name | Fold Change |
|---------|---------|------|-------------|
| U63743  | Hs.69360| kinesin-like 6 mitotic centromere-associated kinesin | 0.13 |
| AL080146| Hs.194698| cyclin B2 | 0.22 |
| Z29066  | Hs.155704| NIMA never in mitosis gene a related kinase 2 | 0.29 |
| S78187  | Hs.155752| cell division cycle 25B | 0.29 |
| AF017790| Hs.58169| highly expressed in cancer rich in leucine heptad repeats | 0.29 |
| X62048  | Hs.75188| wee 1 S. pombe homolog | 0.30 |
| U37426  | Hs.8878 | kinesin family member 11 | 0.30 |
| U01038  | Hs.77597| polo Drosophila like kinase | 0.30 |
| U37022  | Hs.95577| cyclin-dependent kinase 4 | 0.31 |
| L25876  | Hs.8443 | cyclin-dependent kinase inhibitor 3 CDK2-associated phosphatase | 0.34 |
| M37712  | Hs.183418| cell division cycle 2-like 1 PITSRE proteins | 0.34 |
| U21090  | Hs.74598| polymerase DNA directed delta 2 regulatory subunit 50kDa | 0.35 |
| A3175913| Hs.156346| topoisomerase DNA II alpha 170kDa | 0.35 |
| Z24459  | Hs.3548 | mature T-cell proliferation 1 | 0.35 |
| U03911  | Hs.78934| mus81 E. coli homolog 2 colon cancer nonpolyposis type 1 | 0.35 |
| AF086868| Hs.159651| death receptor 6 | 0.36 |
| U05540  | Hs.82906| CDC20 cell division cycle 20 | 0.37 |
| X67155  | Hs.270845| kinesin-like 5 mitotic kinase-like protein 1 | 0.39 |
| M87339  | Hs.35120| replication factor C activator 1 4 37kDa | 0.39 |
| M65488  | Hs.84318| replication protein A1 70kDa | 0.39 |
| AL096744| Hs.115521| REV3 yeast homolog like catalytic subunit of DNA polymerase zeta | 0.39 |
| D88357  | Hs.184572| cell division cycle 2 G1 to S and G2 to M | 0.40 |

**Cell cycle (42)**

| GenBank | Unigene | Name | Fold Change |
|---------|---------|------|-------------|
| AF016371| Hs.9880 | peptidyl prolyl isomerase H. cyclophilin H | 0.12 |
| X2209   | Hs.268515| meningioma disrupted in balanced translocation 1 | 0.12 |
| X74794  | Hs.154443| Minichromosome maintenance 4 | 0.14 |
| AF052432| Hs.275675| katanin p80 WD40-containing subunit B 1 | 0.20 |
| M97287  | Hs.74592 | special AT-rich sequence binding protein 1 binds to nuclear matrix | 0.21 |
| M15796  | Hs.78996 | proliferating cell nuclear antigen | 0.22 |
| D8587   | Hs.155462| minichromosome maintenance 6 | 0.22 |
| AA255502| Hs.46423 | H4 histone family member G | 0.22 |
| D38073  | Hs.179565| minichromosome maintenance 3 | 0.22 |
| Y12478  | Hs.198308| tryptophan rich basic protein | 0.22 |
| AB017019| Hs.170311| heterogeneous nuclear ribonucleoprotein D-like | 0.23 |
| D21063  | Hs.57101 | minichromosome maintenance 2, mitotin | 0.27 |
| U66618  | Hs.250581| SWI/SNF actin dependent regulator of chromatin subfamily d member 2 | 0.27 |
| U23803  | Hs.77492 | heterogenous nuclear ribonucleoprotein A0 | 0.27 |
| X72889  | Hs.198296| SWI/SNF actin dependent regulator of chromatin subfamily a member 2 | 0.28 |
| U28946  | Hs.3248 | mus81 E. coli homolog 6 | 0.29 |
| D63880  | Hs.5719 | chromosome condensation-related SMC-associated protein 1 | 0.30 |
| U30872  | Hs.77204 | centromere protein F 350/400kD mitosin | 0.31 |
| X62534  | Hs.80684 | high-mobility group nonhistone chromosomal protein 2 | 0.32 |
| M97856  | Hs.243886| autosomal histone-binding nuclear protein | 0.32 |
| D55167  | Hs.77152 | minichromosome maintenance 7 | 0.33 |
| U18300  | Hs.77602| damage-specific DNA binding protein 2 48kDa | 0.33 |
| X14850  | Hs.147097| H2A histone family member X | 0.33 |
| Z83738  | Hs.182432| H2B histone family member E | 0.34 |
| D21089  | Hs.320 | xeroderma pigmentosum complementation group C | 0.35 |
| AC004770| Hs.4756 | flap structure-specific endonuclease 1 | 0.33 |
| L37747  | Hs.89497 | Lamin B1 | 0.35 |
| HG4074-HT4344| Hs.50758| SMC4 structural maintenance of chromosomes 4 yeast like 1 | 0.38 |
| AB019987| Hs.108112| histone fold protein CHRAC17 DNA polymerase epsilon p17 subunit | 0.39 |
| AF070640| Hs.184572| heterogenous nuclear ribonucleoprotein H3 2H9 | 0.39 |
| U35451  | Hs.77254| chromobox homolog 1 Drosophila HP1 beta | 0.40 |

**Nuclear proteins (47)**

| GenBank | Unigene | Name | Fold Change |
|---------|---------|------|-------------|
| Complete list of genes is presented in Supplemental Table S10. |

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human peripheral blood leukocytes (14). We identified a similar number of genes activated by IFN-γ in human bronchial epithelial cells. In this paper, we have concentrated on chemokines and secreted proteins, membrane proteins, cell cycle proteins, signal transduction proteins, and others. In NHBE cells, IFN-γ increased expression of a number of C-X-C and C-C chemokines. We observed a multifold increase in RANTES, MCP-1, and IL-8 expression. Similar data were obtained by others (15, 34). In contrast, Casola et al. (9) reported a lack of IFN-γ effect on RANTES transcription in A549 cells. Expression of all three chemokines not only was induced by IFN-γ, but this effect was also inhibited by Dex (33, 37). Other secreted proteins were induced, including several genes potentially important in airway remodeling: MMP-1 and -10, cathepsin C, a disintegrin and metalloproteinase-8, and TGF-β.

Exposure to interferons induces expression of several transcription factors known as interferon-induced proteins (11, 31). Similarly, in our experiments, IFN-γ induces expression of

| GenBank   | Unigene        | Name                                                                 | Fold Change |
|-----------|----------------|----------------------------------------------------------------------|-------------|
| A.        |                | **Secreted proteins (3)**                                            |             |
|           | AA829286       | serum amyloid A1                                                      | 5.05        |
|           | AF056087       | secreted frizzled-related protein 1                                  | 3.21        |
|           | J03764         | plasminogen activator inhibitor type 1                               | 1.71        |
|           | **Cell surface proteins (1)**                          |             |
|           | X76180         | amiloride sensitive sodium channel, SCNN1A                           | 2.32        |
|           | **Signal transduction (3)**                             |             |
|           | L13463         | regulator of G-protein signalling 2, 24kDa                           | 4.76        |
|           | X68277         | dual specificity phosphatase 1                                       | 2.81        |
|           | X89416         | protein phosphatase 5 catalytic subunit                               | 2.49        |
|           | **Transcriptional regulation (1)**                      |             |
|           | D50920         | Thyroid hormone receptor associated protein 100                      | 1.97        |
|           | **Cell cycle (2)**                                     |             |
|           | M93311         | metallothionein 3 growth inhibitory factor                           | 1.62        |
|           | D38073         | minichromosome maintenance 3                                         | 1.61        |
|           | **Translation regulation (1)**                          |             |
|           | H68340         | RNA helicase-related protein                                          | 1.71        |
|           | **Metabolism (9)**                                     |             |
|           | U42031         | FK506-binding protein 5                                               | 3.96        |
|           | X59834         | glutamate-ammonia ligase (glutamine synthase)                         | 2.72        |
|           | AF017060       | aldehyde oxidase 1                                                    | 1.94        |
|           | AA587372       | ubiquitin-conjugating enzyme E2M                                      | 1.86        |
|           | R92331         | metallothionein 1E                                                    | 1.66        |
|           | AA420624       | monoamine oxidase A                                                   | 1.63        |
|           | R93527         | metallothionein 1H                                                    | 1.61        |
|           | M13485         | metallothionein 1B                                                    | 1.60        |
|           | M10943         | metallothionein 1F                                                    | 1.60        |
|           | **Other (2)**                                          |             |
|           | W29045         | retinal cDNA                                                          | 2.41        |
| B.        | AA522537       | putative prostate cancer susceptibility protein                        | 1.68        |
|           |                | **Secreted proteins (1)**                                            |             |
|           | X07820         | matrix metalloproteinase 10 stromelysin 2                            | 0.22        |
|           | **Cell membrane protein (1)**                           |             |
|           | N74607         | aquaporin 3                                                           | 0.50        |
|           | **Signal transduction (1)**                             |             |
|           | U04636         | prostaglandin-endoperoxide synthase 2 (COX-2)                         | 0.55        |
|           | **Transcriptional regulation (3)**                      |             |
|           | L05072         | interferon regulatory factor 1                                        | 0.28        |
|           | U53476         | MMTV integration site family member 7A                               | 0.53        |
|           | M11433         | retinol-binding protein 1 cellular                                    | 0.54        |
|           | **Metabolism (1)**                                     |             |
|           | AF038451       | anterior gradient 2 Xenopus laevis homolog                            | 0.51        |

A: Dex-upregulated genes. B: Dex-downregulated genes.
several IFN-induced proteins (protein 15 kDa and 35 kDa and interferon-induced protein with tetratricopeptide repeats). Other signal transduction/transcription pathway genes were also induced by IFN-γ treatment. These include Toll-like receptor-2 and MYD88, eight TNF-related signaling genes, and genes in the NF-κB pathway. In this study, we have evaluated the effect of Dex on gene expression in resting NHBE cells and the effect of Dex as an inhibitor of interferon-induced changes in gene expression. Functional genomic studies of the effect of Dex on myeloma cell gene expression and

| GenBank | Unigene | Name | IFNC | IFN/Dex/IFN | IFN/Dex/C | Dex/C |
|---------|---------|------|------|-------------|-----------|-------|
| M21121 | Hs.241392 | chemokine (C-C motif) ligand 5, RANTES | 26.54 | 0.23 | 6.11 | 1.00 |
| M26683* | Hs.303649 | chemokine (C-C motif) ligand 2, MCP1 | 26.50 | 0.35 | 9.28 | 1.14 |
| M28130* | Hs.624 | interleukin 8 | 12.88 | 0.35 | 4.45 | 0.50 |
| X07820 | Hs.2258 | matrix metalloproteinase 10 (stromelysin 2) | 11.80 | 0.04 | 0.52 | 0.22 |
| M22489 | Hs.73853 | bone morphogenetic protein 2 | 5.05 | 0.52 | 2.62 | 1.06 |
| M13509 | Hs.83169 | matrix metalloproteinase 1 | 4.33 | 0.20 | 0.87 | 0.42 |
| J03634* | Hs.727 | inhibin, beta A | 4.12 | 0.44 | 1.81 | 1.10 |
| X04430* | Hs.93913 | interleukin 6 (interferon, beta 2) | 3.36 | 0.45 | 1.50 | 1.07 |
| X70340 | Hs.170009 | transforming growth factor, alpha | 3.18 | 0.48 | 1.53 | 0.94 |

**Cell surface and membrane proteins (10/67)**

| GenBank | Unigene | Name | IFNC | IFN/Dex/IFN | IFN/Dex/C | Dex/C |
|---------|---------|------|------|-------------|-----------|-------|
| U91512 | Hs.11342 | ninjurin 1 | 24.60 | 0.64 | 15.83 | 1.47 |
| X59770 | Hs.25333 | interleukin 1 receptor, type II | 18.30 | 0.20 | 3.67 | 0.82 |
| D10923 | Hs.137555 | chemokine receptor; GTP-binding protein | 9.30 | 0.40 | 3.70 | 0.78 |
| X74039 | Hs.179657 | plasminogen activator, urokinase receptor | 7.99 | 0.64 | 5.11 | 0.78 |
| S71326 | Hs.50964 | CEA-related cell adhesion molecule 1 | 7.30 | 0.55 | 4.03 | 0.88 |
| M29696 | Hs.362807 | interleukin 7 receptor | 5.14 | 0.48 | 2.48 | 1.09 |
| U25997 | Hs.25590 | stanniocalcin 1 | 3.02 | 0.41 | 1.24 | 0.87 |
| M21419 | Hs.24147 | tumor necrosis factor-alpha | 2.80 | 0.41 | 1.14 | 0.77 |
| U04636* | Hs.19638 | cyclooxygenase 2 | 7.03 | 0.52 | 3.68 | 0.55 |
| U19523 | Hs.86724 | GTP cyclohydrolase 1 | 5.58 | 0.67 | 3.75 | 0.70 |
| M57730 | Hs.399713 | ephrin-A1 | 2.35 | 0.44 | 1.02 | 0.59 |

**Signal transduction (6/44)**

| GenBank | Unigene | Name | IFNC | IFN/Dex/IFN | IFN/Dex/C | Dex/C |
|---------|---------|------|------|-------------|-----------|-------|
| U19261 | Hs.2134 | TNF receptor-associated factor 1 | 9.20 | 0.29 | 2.68 | 0.95 |
| M92357 | Hs.1011382 | tumor necrosis factor alpha-induced protein 2 | 7.59 | 0.43 | 3.29 | 0.86 |
| U04636* | Hs.196384 | cyclooxygenase 2 | 7.03 | 0.52 | 3.68 | 0.55 |
| U19523 | Hs.86724 | GTP cyclohydrolase 1 | 5.58 | 0.67 | 3.75 | 0.70 |
| U91616 | Hs.182885 | NFκB inhibitor, epsilon | 2.53 | 0.68 | 1.71 | 0.81 |

**Transcriptional regulation (3/34)**

| GenBank | Unigene | Name | IFNC | IFN/Dex/IFN | IFN/Dex/C | Dex/C |
|---------|---------|------|------|-------------|-----------|-------|
| S76638 | Hs.73090 | NFκB 2 (p49/p100) | 5.25 | 0.54 | 2.85 | 0.78 |
| M16038 | Hs.80887 | v-yes-1 oncogene homolog | 3.34 | 0.56 | 1.87 | 0.89 |
| U57094 | Hs.50477 | RAB27A | 3.28 | 0.40 | 1.31 | 0.92 |

**Metabolism (6/39)**

| GenBank | Unigene | Name | IFNC | IFN/Dex/IFN | IFN/Dex/C | Dex/C |
|---------|---------|------|------|-------------|-----------|-------|
| AL031983 | Hs.44532 | ubiquitin D | 295.17 | 0.49 | 145.63 | 1.00 |
| AF026941 | Hs.17518 | vepirin | 39.43 | 0.43 | 16.83 | 1.00 |
| L13972 | Hs.301698 | sialyltransferase 4A | 10.41 | 0.24 | 2.54 | 0.92 |
| M76665 | Hs.275215 | hydroxysteroid (11-beta) dehydrogenase 1 | 7.93 | 0.45 | 3.55 | 0.96 |
| AB005038 | Hs.199270 | cytochrome P450, subfamily XXVIIIB | 3.57 | 0.62 | 2.21 | 1.15 |
| U92315 | Hs.94581 | sulfotransferase family 2B, member 1 | 3.34 | 0.43 | 1.42 | 0.94 |

**Cell cycle (2/23)**

| GenBank | Unigene | Name | IFNC | IFN/Dex/IFN | IFN/Dex/C | Dex/C |
|---------|---------|------|------|-------------|-----------|-------|
| U27467 | Hs.227817 | BCL2-related protein A1 | 3.75 | 0.62 | 2.31 | 1.19 |
| AF035444 | Hs.154036 | tumor suppressing candidate 3 | 2.47 | 0.48 | 1.18 | 0.71 |

**Other (3/36)**

| GenBank | Unigene | Name | IFNC | IFN/Dex/IFN | IFN/Dex/C | Dex/C |
|---------|---------|------|------|-------------|-----------|-------|
| AF016045 | Hs.97905 | ovo-like 1 (Drosophila) | 5.42 | 0.51 | 2.77 | 1.25 |
| AB023155 | Hs.174188 | neuron navigator 3 | 4.53 | 0.43 | 1.95 | 1.36 |
| M21302 | Hs.355542 | small proline-rich protein 2A | 2.35 | 0.47 | 1.11 | 0.86 |

Total = 45. Nos. in parentheses indicate IFN-stimulated genes altered by Dex over IFN-stimulated genes nnotated in Tables 4 – 6. *Listed in the database of IFN-responsive genes cited in Ref. 14. †M26683 is the only gene listed that was upregulated by IFN-γ at 8 and 24 h.
Table 13. Dex reversal of IFN-downregulated genes at 24 h

| GenBank | Unigene | Name | Fold Change |
|---------|---------|-----------------|-------------|
|         |         | **Cytokines and secreted proteins (2/16)** |         |
| AA224832 | Hs.380778 | metallothionein 1L | 0.22 2.39 0.52 1.52 |
| M14083 | Hs.82085 | plasminogen activator inhibitor type 1 | 0.39 2.47 0.97 1.44 |
|         |         | **Cell surface proteins (2/43)** |         |
| X68487 | Hs.45743 | adenosine A2b receptor | 0.21 2.76 0.59 1.14 |
| AF032862 | Hs.72550 | hyaluronan-mediated motility receptor RHAMM | 0.44 2.28 1.00 1.11 |
|         |         | **Signal transduction (3/31)** |         |
| U73960 | Hs.10706 | ADP-ribosylation factor-like 4 | 0.41 3.56 1.47 1.30 |
| AF011468 | Hs.250822 | serine/threonine kinase 6 | 0.42 3.24 1.37 1.14 |
| M86699 | Hs.169840 | TTK protein kinase | 0.44 2.05 0.91 0.99 |
|         |         | **Transcriptional regulation (2/38)** |         |
| U94319 | Hs.82110 | PC4 and SFRS1 interacting protein 2 | 0.19 2.58 0.49 1.05 |
| AL096880 | Hs.27801 | zinc finger protein 278 | 0.38 2.84 1.08 1.37 |
|         |         | **Metabolism (11/84)** |         |
| A1797997 | Hs.155020 | putative methyltransferase | 0.26 4.29 1.11 1.43 |
| U00238 | Hs.311 | phosphoribosyl pyrophosphate amidotransferase | 0.32 2.50 0.81 1.34 |
| M95623 | Hs.82609 | hydroxymethylbilane synthase | 0.32 3.33 1.06 1.15 |
| U24183 | Hs.75160 | phosphoribosyl pyrophosphate synthetase 1 | 0.34 2.08 0.83 1.33 |
| AF038195 | Hs.150922 | BCS1 yeast homolog | 0.42 2.39 1.07 1.21 |
| U28042 | Hs.41706 | DEAD/H box polypeptide 10 | 0.45 2.48 1.13 1.24 |
|         |         | **Cell cycle (17/43)** |         |
| U63743 | Hs.69360 | kinesin-like 6 centromere-associated kinesin | 0.13 5.99 0.77 1.11 |
| AL080146 | Hs.194968 | cyclin B2 | 0.22 3.06 0.66 1.02 |
| AF017790 | Hs.58169 | expressed in cancer/rich in leucine repeats | 0.29 3.57 1.05 1.41 |
| U37426 | Hs.8878 | kinesin family member 11 | 0.30 2.18 0.65 1.36 |
| U01038 | Hs.77597 | polo Drosophila like kinase | 0.30 3.97 1.19 1.45 |
| U37022 | Hs.95577 | cyclin-dependent kinase 4 | 0.34 2.39 0.75 1.15 |
| L25876 | Hs.84113 | cyclin-dependent kinase inhibitor 3 | 0.35 2.30 0.78 0.92 |
| AI375913 | Hs.156346 | DNA topoisomerase II alpha 170kDa | 0.35 2.39 0.84 1.14 |
| U05340 | Hs.82096 | CDC20 cell division cycle 20 homolog | 0.37 2.84 1.06 1.27 |
| U67155 | Hs.270845 | kinesin-like 5 mitotic kinesin-like protein 1 | 0.39 2.70 1.05 0.98 |
| D88357 | Hs.184572 | cell division cycle 2 G1 to S and G2 to M | 0.40 3.22 1.30 1.17 |
| U65410 | Hs.79078 | MAD2 mitotic arrest deficient yeast homolog 1 | 0.41 2.24 0.92 1.18 |
| D26361 | Hs.3104 | Kinesin Family Member 14 | 0.42 2.03 0.86 1.01 |
| X51688 | Hs.85137 | cyclin A2 | 0.42 2.72 1.14 1.38 |
| U30872 | Hs.77024 | protein arginine N-methyltransferase 3 | 0.45 2.48 1.13 1.24 |
| J04031 | Hs.172665 | methylenetetrahydrofolate dehydrogenase | 0.46 2.19 1.01 1.26 |
| U73379 | Hs.93002 | ubiquitin carrier protein E2-C | 0.50 2.15 1.07 1.08 |
|         |         | **Nuclear proteins (21/47)** |         |
| AF016371 | Hs.9880 | peptidyl prolyl isomerase H (cyclophilin H) | 0.12 4.93 0.61 1.05 |
| X74794 | Hs.154443 | minichromosome maintenance 4 | 0.20 3.53 0.70 1.45 |
| AA255502 | Hs.54623 | histone family member G | 0.22 3.54 0.78 1.24 |
| D84557 | Hs.155462 | minichromosome maintenance 6 | 0.22 2.63 0.57 1.44 |
| M15796 | Hs.78996 | proliferating cell nuclear antigen | 0.22 2.44 0.54 1.32 |
| D21063 | Hs.57101 | minichromosome maintenance 2, mitotin | 0.27 2.54 0.68 1.58 |
| U66618 | Hs.250581 | SWI/SNF related regulator of chromatin | 0.27 2.44 0.67 1.24 |
| D63880 | Hs.5719 | chromosome condensation-related protein 1 | 0.30 2.16 0.64 1.03 |
| U30872 | Hs.77024 | centromere protein F 350/400kDa mitosin | 0.31 2.12 0.66 1.05 |
| X62534 | Hs.80964 | high-mobility group nonhistone protein 2 | 0.32 2.94 0.96 1.19 |
| M97856 | Hs.243886 | autoantigenic histone-binding protein | 0.32 2.39 0.76 1.26 |
| AC004770 | Hs.4756 | flap structure-specific endonuclease 1 | 0.33 2.53 0.84 1.55 |
| D55716 | Hs.77152 | minichromosome maintenance 7 | 0.33 2.70 0.99 1.33 |
| X14850 | Hs.147097 | H2A histone family member X | 0.34 2.43 0.83 1.53 |
| L37747 | Hs.89097 | Lamin B1 | 0.36 2.27 0.81 1.34 |
| AB019987 | Hs.50758 | SMC4 structural maintenance of chromosomes 4 | 0.38 2.02 0.76 0.97 |
| AF070640 | Hs.108112 | histone fold protein CHRAC17 | 0.39 2.17 0.85 1.2 |
| AJ131180 | Hs.173980 | nuclear matrix protein NMP200 | 0.43 2.48 1.08 1.45 |
on ocular cell gene expression have been reported (12, 20, 22). The effect of Dex on resting cells in our study was relatively minor at 8 or 24 h. While the effect of Dex on IFN-γ treated cells after coinoculation for 8 h was minimal, the effect on IFN-γ-treated cells at 24 h was more remarkable. Furthermore, the effect of Dex on IFN-γ-induced changes in gene expression appeared to be selective. To some extent, our hypothesis that glucocorticosteroids can at least in part reverse the proinflammatory action of IFN-γ has been confirmed. For example, IFN-γ exposure increases cyclooxygenase-2 expression up to sevenfold, and this effect was partially inhibited by Dex. The effects of Dex and IFN-γ on cyclooxygenase-2 expression have been reported earlier (5, 16). In our experimental model, IFN-γ induced TNF-α and TGF-β expression. In both cases, the IFN-γ effect was reversed by Dex. In addition, IL-8, RANTES, and MCP-1 expression was increased by IFN-γ treatment and reversed by Dex coinoculation. Similar data regarding IL-6 expression were also reported by others (38).

Of the 45 genes induced by IFN-γ at 24 h and modulated by coinoculation with Dex, only 1 gene was induced by IFN-γ at 8 h, MCP-1. The remaining 44 genes were only induced at 24 h. It seems possible that these genes are a part of a secondary amplification dependent on earlier IFN-γ-induced changes in gene expression. If this is the case, then the Dex inhibition at 24 h might be related to an inhibition of factors in this secondary round of gene expression. For example, at least 12 genes in the TNF-α and NF-κB-signaling pathways were induced by IFN-γ at 24 h. These include TNF-α, TNF receptor-associated factor, TNF-α-induced proteins-2, -3, and -6, TNFRSF1A-associated death domain, TNF ligand superfamily member 10 (TRAIL), receptor TNFRSF-interacting serine-threonine kinase, and TNF-induced protein GG2-1. In addition, four genes in the NF-κB pathway were induced. These include NF-κB inhibitor, epsilon, NF-κBIA, NF-κB2, and Rel(p65). Dex coinoculation with IFN inhibited the induction of four of the genes listed above: TNF receptor-associated factor-1, RNFA-induced protein-2, NF-κB inhibitor, epsilon, and NF-κB2 (p49/100) (Table 12). It further raises the possibility that some of the Dex inhibition of IFN-γ-induced changes at 24 h may be inhibition of secondary changes in gene expression resulting from Dex inhibition of activation of transcription factors such as NF-κB (4, 27).

In this paper, the role of IFN-γ in modulation of gene expression was not only measured at the mRNA level, but data obtained from microarray experiments were subsequently confirmed in two ways. First, changes in mRNA expression of 8 genes studied using a microarray approach were confirmed utilizing a real-time PCR expression detection system (as shown in Table 14). Second, we measured expression of selected proteins as well. IL-8, RANTES, and MCP-1 production was increased with IFN-γ treatment, suggesting that for these genes, mRNA levels and protein production are closely related.

To summarize, we present information regarding groups of genes regulated by IFN-γ in NHBE cells. These data include a number of genes that are up- and downregulated by this proinflammatory cytokine. We suggest that several groups of genes might be upregulated by IFN-γ, and this effect might be at least in part altered by glucocorticosteroids. Further studies involving detailed analysis of pro-

Table 14. Comparison of RT-PCR and microarray results

| Gene  | GenBank No. | RT-PCR Fold Change | Array | IFN/C Fold Change | Dex/C Fold Change | IFN + Dex/C Fold Change |
|-------|-------------|--------------------|-------|-------------------|------------------|------------------------|
| COX2  | U04636      | 18.8 ± 0.5         | 7.0   | 0.6 ± 0.06        | 0.6              | 6.7 ± 0.9              |
| IL7-R | M28064      | 24.9 ± 1.6         | 5.1   | 0.7 ± 0.09        | 1.1              | 9.6 ± 0.6              |
| MAPKK | AP002715    | 0.7 ± 0.05         | 0.5   | 1.1 ± 0.06        | 0.9              | 0.7 ± 0.2              |
| MMP10 | X07830      | 16.1 ± 1.4         | 11.8  | 0.1 ± 0.01        | 0.2              | 11.0 ± 0.06            |
| PAI2  | Y00630      | 19.6 ± 0.9         | 8.4   | 1.1 ± 0.05        | 0.9              | 11.0 ± 0.06            |
| SAA   | AA829286    | 12.4 ± 1.8         | 6.3   | 13.9 ± 1.1        | 6.4              | 117.4 ± 12.3           |
| SOD   | X07834      | 36.5 ± 1.9         | 23.1  | 1.1 ± 0.02        | 0.8              | 19.3 ± 0.7             |
| VEGF  | U43142      | 8.6 ± 0.5          | 5.2   | 1.1 ± 0.06        | 0.8              | 6.1 ± 0.4              |

*n = 3.
RESULTS for details. Cells were treated with or without IFN-γ into medium from normal human bronchial epithelial (NHBE) cells. Culture medium was harvested and assayed for MCP-1, RANTES, or IL-8.

Fig. 3. Release of monocyte chemoattractant protein (MCP)-1, RANTES (regulated on activation, normal T expressed and secreted), and interleukin (IL)-8 into medium from normal human bronchial epithelial (NHBE) cells. Cells were treated with or without IFN-γ, Dex, or IFN-γ and Dex for 24 h. Culture medium was harvested and assayed for MCP-1, RANTES, or IL-8. Results are expressed as pg/ml culture medium. For each point, n = 5. *P < 0.001, IFN-γ vs. control. **P < 0.001, IFN-γ vs. IFN/Dex treatments. See Results for details.

mot regions of IFN-γ-regulated genes may help to determine signal transduction pathways involved in these processes.

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