JOURNAL OF VIROLOGY, Apr. 2007, p. 3391–3401 Vol. 81, No. 7
0022-538X/07/$08.00+0 doi:10.1128/JVI.02640-06
Copyright © 2007, American Society for Microbiology. All Rights Reserved.

Changes in Gene Expression during Pegylated Interferon and Ribavirin Therapy of Chronic Hepatitis C Virus Distinguish Responders from Nonresponders to Antiviral Therapy▽†

Milton W. Taylor,[1]* Takuma Tsukahara,[1] Leonid Brodsky,[3] Joel Schaley,[1] Corneliu Sanda,[1] Matthew J. Stephens,[2] Jeanette N. McClintick,[2] Howard J. Edenberg,[2] Lang Li,[2] John E. Tavis,[4] Charles Howell,[5] and Steven H. Belle[6] for the Virahep-C Study Group

Department of Biology, Indiana University, Bloomington, Indiana 47401[1]; Department of Biochemistry and Molecular Biology and Center for Medical Genomics, Indiana University School of Medicine, Indianapolis, Indiana[2]; Institute of Evolution, University of Haifa, Haifa, Israel[3]; Department of Molecular Microbiology and Immunology, Saint Louis University School of Medicine, St. Louis, Missouri[4]; Department of Medicine, University of Maryland, Baltimore, Maryland[5]; and Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania[6]

Received 29 November 2006/Accepted 19 January 2007

Treating chronic hepatitis C virus (HCV) infection using pegylated alpha interferon and ribavirin leads to sustained clearance of virus and clinical improvement in approximately 50% of patients. Response rates are lower among patients with genotype 1 than with genotypes 2 and 3 and among African-American (AA) patients compared to Caucasian (CA) patients. Using DNA microarrays, gene expression was assessed for a group of 33 African-American and 36 Caucasian American patients with chronic HCV genotype 1 infection during the first 28 days of treatment. Results were examined with respect to treatment responses and to race. Patients showed a response to treatment at the gene expression level in RNA isolated from peripheral blood mononuclear cells irrespective of degree of decrease in HCV RNA levels. However, gene expression responses were relatively blunted in patients with poor viral response (<1.5 $\log_{10}$-IU/ml decrease at 28 days) compared to those in patients with a marked (>3.5 $\log_{10}$-IU/ml decrease) or intermediate (1.5 to 3.5 $\log_{10}$-IU/ml decrease) response. The number of genes that were up- or down-regulated by pegylated interferon and ribavirin treatment was fewer in patients with a poor response than in those with an intermediate or marked viral response. However AA patients had a stronger interferon response than CA patients in general. The induced levels of known interferon-stimulated genes such as the 2′5′-oligoadenylate synthetase, MX1, IRF-7, and toll-like receptor TLR-7 genes was lower in poor-response patients than in marked- or intermediate-response patients. Thus, the relative lack of viral response to interferon therapy of hepatitis C virus infection is associated with blunted interferon cell signaling. No specific regulatory gene could be identified as responsible for this global blunting or the racial differences.

Chronic hepatitis C virus (HCV) infection is a major cause of chronic liver disease and liver cancer (1, 2). An estimated 170 million persons worldwide, including 3 million in the United States, are actively infected with HCV. The prevalence of HCV infection varies by race and ethnicity, HCV infection being two times more common in African-Americans (AA) than Caucasian Americans (CA). The incidence of hepatocellular carcinoma is also greater in AA (9). The current recommended treatment for chronic HCV infection is the combination of pegylated alpha interferon (peginterferon) and the oral antiviral drug ribavirin given for 24 or 48 weeks (11, 15, 23, 31). This regimen is effective in eradicating virus in 70 to 80% of patients with genotype 2 or 3 HCV infection, but in only 40 to 50% of individuals infected with genotype 1, the most common genotype in the United States. For unknown reasons AA are less likely to respond to interferon-based therapy of HCV infection than CA (18, 20, 25, 27).

Alpha interferon mediates its antiviral and pharmacological effects by binding to type I interferon receptors on the cell surface membrane, which leads to transcription of up to 1,000 interferon-stimulated genes, presumably via the Janus-activated kinase 1 (JAK1)-STAT (signaling transducers of activation and transcription) signaling pathway (28, 30). A potential explanation for a lack of response to interferon therapy of HCV infection is an underlying deficient cellular response to interferon with a blunted response to interferon signaling, this being more common among AA patients than CA patients. To test this hypothesis, global gene expression in peripheral blood mononuclear cells (PBMC) before and during the first 28 days of therapy with peginterferon and ribavirin was analyzed in a cohort of AA and CA patients with genotype 1 HCV infection. These patients were undergoing therapy in the Study of Viral Resistance to Antiviral Therapy for Hepatitis C (Virahep-C), a large, prospective, multicenter study designed to define the differences in response rates among AA and CA patients and to determine clinical, immunological, host genetic, viral genetic, and interferon cell signaling factors that were associated with lack of response to treatment (5). The current analysis summarizes results of global gene expression in PBMC during the first 28 days of therapy, comparing patients with a marked

* Corresponding author. Mailing address: Department of Biology, Indiana University, Bloomington, IN 47401. Phone: (812) 855-3340. Fax: (812) 855-6705. E-mail: taylor@indiana.edu.

† Supplemental material for this article may be found at http://jvi.asm.org/.

▽ Published ahead of print on 31 January 2007.

TABLE 1. Baseline participant characteristics by response group

Feature	Value for patients with:			*P*
	Marked response	Intermediate response	Poor response	
No.				
Total	27	17	25	
AA	13 (48%)	9 (53%)	11 (44%)	0.85[a]
Male	17 (63%)	14 (82%)	19 (76%)	0.33[a]
Body wt (kg)				
Mean (SD)	84.7 (22.5)	92.1 (23.4)	93.1 (14.6)	0.28[b]
Median (25th, 75th)	79.8 (70.8, 97.1)	94.3 (81.2, 99.8)	93.9 (79.8, 104.3)	
HCV RNA level ($\log_{10}$ IU/ml)				
Mean (SD)	6.0 (0.8)	6.5 (0.6)	6.4 (0.5)	
Median (25th, 75th)	5.7 (5.3, 6.8)	6.7 (6.2, 6.9)	6.4 (6.2, 6.6)	0.11[c]
ALT (mg/dl)				
Mean (SD)	101.7 (94.1)	69.5 (28.7)	129.8 (106.3)	
Median (25th, 75th)	68.0 (42.0, 123.0)	65 (52.0, 81.0)	83.0 (58.0, 164.0)	0.13[c]
Liver histology				
Ishak necroinflammatory score (0–18)				
Mean (SD)	8.2 (2.6)	7.2 (2.4)	7.8 (3.3)	
Median (25th, 75th)	8 (6, 9)	8 (6, 9)	7 (5, 9)	0.48[c]
Ishak fibrosis score (0–6)				
Mean (SD)	2.1 (1.0)	1.7 (1.3)	2.4 (1.4)	
Median (25th, 75th)	2 (1, 3)	1 (1, 2)	2 (2, 3)	0.08[c]

[a] Chi-square test.
[b] Analysis of variance.
[c] Kruskal-Wallis test.

(decrease of more than 3.5 $\log_{10}$ at 28 days following treatment initiation), intermediate (1.4 $\log_{10}$ to 3.5 $\log_{10}$ decrease), or poor (<1.4 $\log_{10}$ decrease) viral response.

MATERIALS AND METHODS

Patient treatment and samples. The Virahep-C study enrolled a cohort of 196 AA and 205 CA participants from eight U.S. clinical centers, who were recruited between July 2002 and December 2003. The institutional review boards of participating centers approved the protocol, and all patients gave informed, written consent for both the therapy and investigations of viral, immunological, and host cell-signaling responses. Eligible patients were naive to interferon and ribavirin treatment and had detectable HCV RNA in serum, and nearly all had a liver biopsy performed within the previous 18 months showing chronic hepatitis. Only patients who were born in the United States and designated themselves as "African-American/black" or "Caucasian/white" were eligible. The clinical protocol called for participants to be treated for up to 48 weeks with peginterferon-2a (Pegasys; Roche Pharmaceuticals, Nutley, NJ) in a dose of 180 μg weekly by self-administered subcutaneous injection and ribavirin (Copegus; Roche) orally in a dose of 1,000 or 1,200 mg daily based on body weight of less than 75 kg or equal to or greater than 75 kg. Serum samples were tested for HCV RNA levels using a quantitative PCR-based assay (Cobas Amplicor HCV monitor test, version 2.0; Roche) on days 0 (pretreatment), 1, 2, 7, 14, and 28 and at weeks 12, 24, 48, and 72. PBMC were collected from patients before treatment (day 0) and on days 1 (after the initial supervised injection of peginterferon), 2, 7, 14, and 28. Treatment was discontinued at week 24 in participants whose serum was still HCV RNA positive by a sensitive qualitative PCR-based assay (Roche Cobas Amplicor HCV test, v2.0) that had a lower limit of sensitivity of 50 IU/ml. The primary end point of the study was a sustained virologic response, defined as lack of detectable serum HCV RNA in serum drawn 24 weeks after completing treatment. The overall results of this study have been published and demonstrated that the sustained virological response rate was higher among CA patients (52%) than AA patients (28%) and that the racial difference was not explained by clinical features such as age, gender, weight, severity of the underlying hepatitis, pretreatment viral levels, or amount of drug taken (6).

From the Virahep-C cohort, 72 patients who did not have dose reductions of either peginterferon or ribavirin in the first 28 days of treatment were selected such that 12 patients of each race (CA and AA) were included by virological-response category. The three categories of response were marked, defined as a decrease in HCV RNA levels of more than 3.5 $\log_{10}$ IU/ml or to an undetectable level on day 28; intermediate, defined as a decrease of 1.4 to 3.5 $\log_{10}$ IU/ml on day 28; and poor, defined as less than a 1.4 $\log_{10}$ IU/ml decline on day 28 relative to baseline. These definitions were made a priori in an attempt to analyze the biological basis for virological responses. Of these, RNA adequate to provide gene expression information was not obtained from three patients.

PBMC preparation. PBMC were collected in sodium-heparin cell preparation tubes at day 0 (before treatment) and days 1, 2, 7, 14, and 28 after initiation of treatment. Whole blood was diluted with an equal volume (8 ml) of phosphate-buffered saline (PBS), carefully layered over a 10-ml Ficoll-Hypaque gradient (Amersham/Pharmacia, Piscataway, NJ), and centrifuged at 800 rpm for 20 min at room temperature. The buffy coat layer was transferred to a 15-ml RNase-free tube, diluted with PBS, and centrifuged at 100 × *g* for 15 min at room temperature. The supernatants were discarded, and the PBMC were retained.

RNA extraction. Samples were shipped overnight by express courier at 4°C to a central repository, where RNA was isolated on arrival. The PBMC were lysed in 1 ml of TRI reagent (Molecular Research Center Inc., Cincinnati, OH). The PBMC lysate was mixed with 1-bromo-3-chloropropane phase separation agent for 1 min and incubated at room temperature for 15 min. After centrifugation for 15 min at 12,000 rpm and 4°C, RNA was precipitated from the supernatant overnight at −20°C with an equal volume of isopropanol and 1/10 volume of 7.5 M ammonium acetate. The precipitate was washed twice with 75% ethanol and then with 95% ethanol. RNA was briefly air dried and then further purified using RNeasy columns (QIAGEN, Valencia, CA). The amount and quality of RNA were determined by spectrophotometry and by electrophoresis through 1% agarose with ethidium bromide, and RNA quality was analyzed by the Agilent Bioanalyzer according to the manufacturer's instructions. Samples that did not show two clear bands of rRNA were discarded.

RNA labeling and hybridization. Preparation of cDNA and cRNA and labeling were carried out according to the protocols recommended by Affymetrix (Santa Clara, CA) in the GeneChip expression analysis technical manual, as previously described (34).

Array analysis and data processing. The microarrays were scanned using a dedicated model 3000 scanner controlled by GCOS software. The average intensity on each array was normalized by global scaling to a target intensity of 1,000. Data were extracted using the Affymetrix Microarray Suite 5 (MAS5) algorithm and exported into a custom-designed database (MicroArray Data Portal) in the Center for Medical Genomics (Indiana University-Purdue University Indianapolis, Indianapolis). All DNA microarray chips were analyzed for unequal distribution or artifacts as described previously (4). Any chip shown to be defective was corrected or dropped from the analysis.

The MicroArray Data Portal, in addition to its role as a database and analytical tool, is an informatics platform with active links from each sequence to

several public databases. Sequence information for each gene on the HG-U133A GeneChip was obtained by parsing the HG-U133 target file obtained from the Affymetrix informatics website, http://www.affymetrix.com/analysis/download_center.affx). A GenBank accession number and a Unigene cluster were used to match sequences to their corresponding LocusLink number, gene symbol, and map position and to link with Gene Ontology (GO) terms and *Enzyme Nomenclature* (19) EC numbers. EC numbers were then used in conjunction with the Ligand database to link genes to KEGG pathways (www.genome.ad.jp/kegg/).

Statistical analysis. The MAS5 data were filtered to eliminate any gene that was not called present in at least 50% of the samples in any one group (fraction present ≥ 0.5) (24). Changes (n-fold) for each gene were calculated using the ratio of the MAS5 signals of the baseline and the posttreatment time. If the signal for the posttreatment time point was greater than the baseline the change was calculated as $+\text{average}_{\text{posttreatment}}/\text{average}_{\text{baseline}}$; otherwise, the change was calculated as $-\text{average}_{\text{baseline}}/\text{average}_{\text{posttreatment}}$. The asymptotic standard errors (ASE) were estimated using the delta method, and 95% confidence intervals were calculated by multiplying the ASE by 1.96, with the product added to and subtracted from the change.

Welch's t test using the MAS5 signals was used to test for differences in gene expression between CA and AA, and one-way analysis of variance was used to test for differences among the three response groups.

For each gene, the expression levels of posttreatment time points were compared to the baseline (pretreatment) expression levels using a paired t test of the MAS5 signals. Genes whose P value ≤ 0.001 and for which the absolute value of the change was at least 1.5-fold were selected as significant. Because of the filtering and differences in power, the numbers of genes considered to be significant in mutually exclusive and exhaustive subgroups will not necessarily add up to the number of genes considered to be significant in the entire sample. Genes that are significant in both racial groups contribute twice to the sum of genes but only once to the number of significant genes in the entire sample. On the other hand, gene expression differences that meet the change criterion may not meet the criterion of a P value <0.001 in either racial group but, due to the increased number of observations for the entire sample compared to each racial group, do have a P of <0.001 for the entire sample; such genes do not contribute to the sum of significant genes across racial groups but do contribute to the number of significant genes in the entire sample.

All analyses were performed using the R statistical language and environment.

Microarray data accession number. Microarray data presented in this paper have been deposited with NCBI/GEO under accession no. GSE7123.

RESULTS

The baseline features of the three response groups are shown in Table 1. The participants who were included in this analysis of gene expression were not representative of the total Virahep-C cohort since they were selected for nearly equal representation in the various race and response groups early in the study and the total cohort did not divide equally into these categories at the end of the study. Baseline features were similar across response groups, though marked responders had somewhat lower HCV RNA levels than the other response groups. However, this difference was not statistically significant ($P = 0.11$). Ultimately, sustained virological responses occurred in 81% of the marked responders, 35% of the intermediate responders, and 8% of the poor responders. Characteristics of AA and CA were also similar (Table 2), although AA tended to have lower alanine aminotransferase (ALT) values than did CA ($P = 0.01$), a difference that was also found in the total Virahep-C cohort (6). Racial differences in sustained virologic response were not apparent (39% for versus 47% for CA; $P = 0.51$), though they were in the total cohort, due to the selection criteria within each race whereby the three early-response groups were nearly equally represented in each race.

Oligonucleotide microarray analysis of gene expression in PBMC demonstrated less difference among poor, intermediate, and marked viral responders before therapy than would be expected by chance. For example, of the 10,910 genes whose expression was detected at baseline in at least one patient in each response category (and whose fraction present was at least 0.5 in any one group) only 73 differed across response groups at a P of ≤ 0.01 and 2 at a P of ≤ 0.001, whereas 109 and 11, respectively, would be expected by chance at these significance levels.

TABLE 2. Baseline patient characteristics by race

Feature	Value for:		P
	AA	CA	
No.			
Total	33	36	
Male	24 (73%)	26 (72%)	0.96[a]
Body wt (kg)			
Mean (SD)	91.7 (23.3)	87.6 (17.2)	0.41[b]
Median (25th, 75th)	84.4 (78.9, 104.3)	91.2 (75.8, 100.2)	
HCV RNA level ($\log_{10}$ IU/ml)			
Mean (SD)	6.2 (0.7)	6.3 (0.7)	
Median (25th, 75th)	6.4 (5.5, 6.8)	6.5 (5.7, 6.7)	0.54[c]
ALT (mg/dl)			
Mean (SD)	79.2 (63.2)	126.6 (104.9)	
Median (25th, 75th)	63.0 (47.0, 83.0)	91.5 (56.0, 169.0)	0.01[c]
Liver histology			
Ishak necroinflammatory score (0–18)			
Mean (SD)	7.8 (2.3)	7.8 (3.2)	
Median (25th, 75th)	7.0 (6.0, 9.0)	7.5 (5.5, 9.0)	0.60[c]
Ishak fibrosis score (0–6)			
Mean (SD)	1.9 (1.3)	2.3 (1.1)	
Median (25th, 75th)	2.0 (1.0, 2.0)	2.0 (1.5, 3.0)	0.16[c]

[a] Chi-square test.
[b] Analysis of variance.
[c] Wilcoxon rank sum test.

Global gene expression response is greater in marked responders than in poor responders. Gene expression in PBMC changed substantially during peginterferon and ribavirin therapy, with major changes being evident by days 1 and 2 after the initial injection of peginterferon and administration of ribavirin. The numbers of genes that were significantly modified (absolute value of change greater than 1.5-fold and $P \leq 0.001$) at each time point for each response group and for each racial group within the response group are shown in Table 3. Many genes were altered in expression at the early time points in patients in all three response groups. The number of differentially expressed genes dropped between day 2 and day 7 and increased again slightly between days 7 and 28. Postbaseline PBMC samples were generally taken before administration of interferon. For only one subject at two time points, samples were taken 4 h after administration of interferon; this did not appear to affect the results for this patient.

In another study, similar experiments were run with RNA samples taken directly from patient PBMC and cultured in vitro for 6 or 24 h with interferon/ribavirin, and similar patterns of gene induction or down regulation were obtained (34). Likewise, samples processed immediately (without shipping) in an ongoing study show very little difference from the data in these experiments.

Within marked responders, there were more genes changed in AA than in CA at every time point (Table 3). Among poor responders, the same relationship held except at day 28. The relationship was more mixed in intermediate responders,

TABLE 3. Number of genes (proportion present > 0.5) modified (at least 1.5-fold change; $P \leq 0.001$) during peginterferon and ribavirin treatment

Day (*n*)	No. of genes modified for:								
	Marked responders			Intermediate responders			Poor responders		
	AA	CA	All	AA	CA	All	AA	CA	All
1 (69)	1,373	553	1,250	233	198	760	228	195	418
2 (68)	586	336	669	177	110	384	206	87	272
7 (68)	190	94	293	40	59	180	9	4	58
14 (63)	181	151	240	50	74	183	50	9	72
28 (61)	229	117	307	21	45	133	40	56	149

where more genes changed expression in AA than CA in the first 2 days after treatment but more genes changed expression in CA than in AA after that.

The number of genes that changed in expression was greater in the marked responders than in the intermediate or poor responders at all time points. There was a smaller difference in the numbers of genes that changed in expression between the intermediate and poor responders at most points. Figures 1 and 2 show the numbers of genes whose expressions increased or decreased, respectively, using the change filter of 1.5-fold and a P value of ≤ 0.001. The numbers of genes that were increased in gene expression were far higher in the marked responders than in either the intermediate or poor responders, and the intermediate responders had numbers intermediate between the other two groups. Slightly more genes were up- than down-regulated. Although the differences among the three response categories of patients held for both up- and down-regulation of genes, the decline over time in numbers of down-regulated genes was much sharper than the decline in up-regulated genes.

Compared to the baseline expression, there was an interferon response in all three categories of patients at each time point. The increases in four well-defined interferon-stimulated genes (2′5′-oligoadenylate synthetase 1 [OAS1] and OAS2, MX1, and MX2 genes) are shown in Fig. 3. At all time points, there was a significant difference in increases of these four genes between the marked and poor virological responders ($P < 0.04$ at each time point). There was less of a difference between marked and intermediate responders, and this difference did not reach statistical significance at every time point.

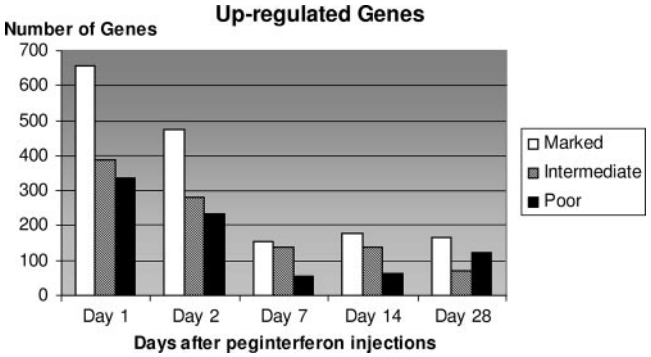

FIG. 1. Number of genes up-regulated ($P < 0.001$; ≥1.5-fold change) at each time point compared to baseline in each response category of patient.

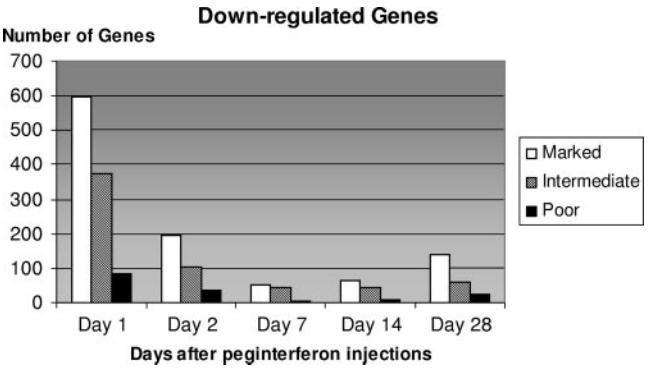

FIG. 2. Number of genes down-regulated ($P < 0.001$; ≥1.5-fold change) at each time point compared to baseline in each category of patients.

Table 4 presents a selected group of genes that were up-regulated in marked and poor responders by function. These genes were chosen from the total list (See Table S1 in the supplemental material) because of previous association with interferon activity or because they form a functional group. The change for genes in poor responders was consistently lower than that found in marked responders. Supplemental Table S1 presents a list of individual induced genes, for patients of all three response groups at different time points after treatment initiation. Supplemental Table 2 presents a list of those genes that were down-regulated on different days. The majority of genes that were down-regulated encode products which are involved in translational regulation, such as eukaryotic elongation or translation factors and ribosomal proteins. In general, these were down-regulated substantially in marked-response patients but marginally in poor- and intermediate-response patients. As in the case of up-regulated genes in patients, the magnitude of the decrease in gene expression in poor responders was considerably less than in marked or intermediate responders. Table 5 compares the response between AA patients and CA patients on day 1. The responses in terms of change (*n*-fold) were very similar between the two groups.

Most of the genes that were up-regulated during therapy and that are presented in Table 4 are well-known interferon-induced genes that have been described as being increased in expression in human PBMC in vitro with interferon stimulation (75% concordance) (34). Many of the genes that were induced at days 1 and 2 were no longer increased in expression in PBMC collected at days 7, 14, and 28 (see Tables S1 and S2 in the supplemental material). Among the major genes with early transient expression were those encoding chemokines CXCL10 (IP10), CXCL11, CCL8, and TNFSF10 (TRAIL). Several of these genes are known to be strongly induced by gamma interferon (5, 14). Other gamma interferon-induced genes such as the WARS, INDO, and caspase genes were also induced transiently at a low level during the first 24 h after the initial injection of peginterferon (see Table S1 in the supplemental material). However, gamma interferon mRNA was not detectable, nor was gamma interferon detectable by enzyme-linked immunosorbent assay (data not shown). Other genes

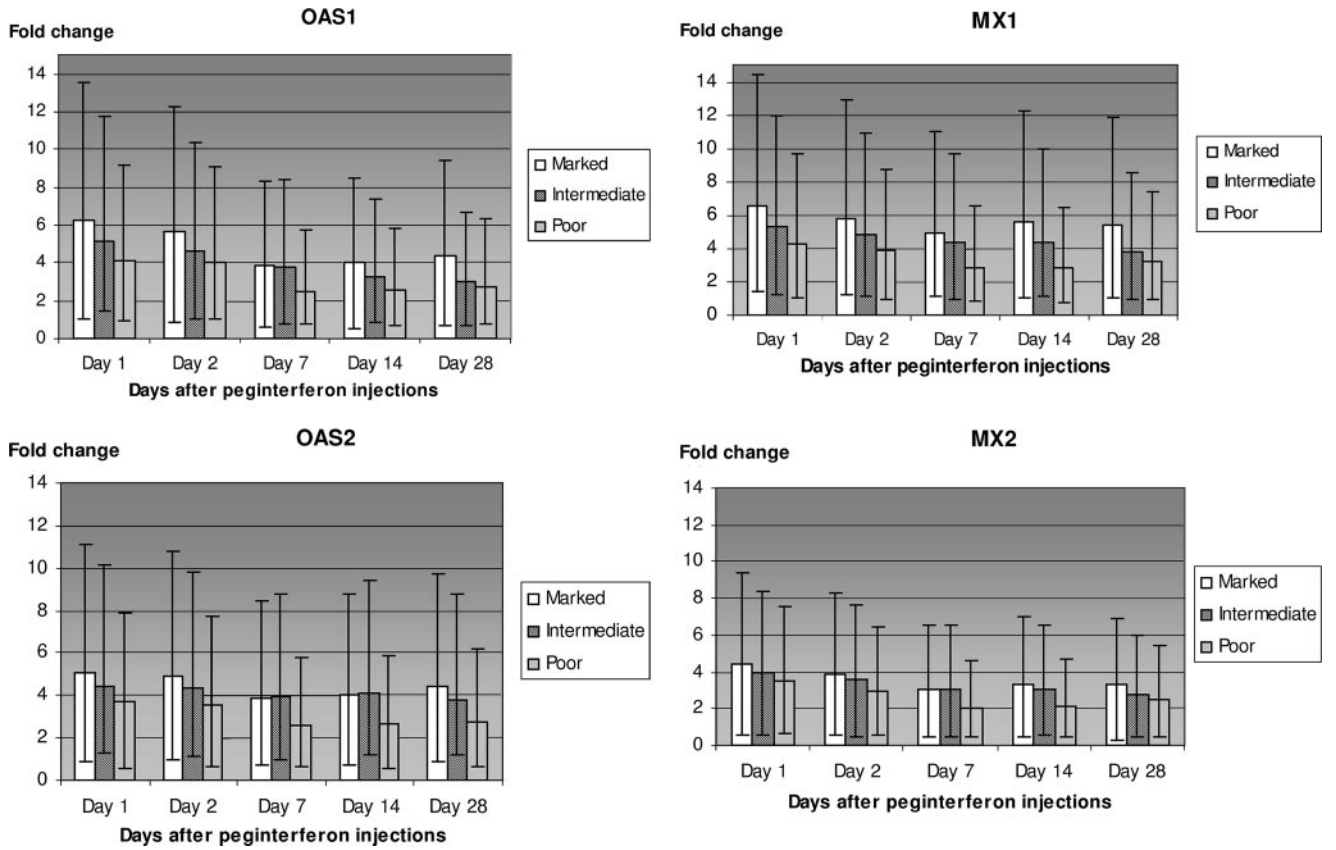

FIG. 3. Increase in mRNA as detected by microarrays for oligo(A) synthetase 1 and 2 and MX1 and MX2 in all three response categories of patients at days 1, 2, 7, 14, and 28 after initiation of treatment.

transiently induced include the interleukin-1Ra (IL-1Ra) gene, previously shown to be induced only during the first 48 h of treatment (7). A subgroup of genes showed no change with time in any of the patient categories. These included those encoding IFI44, IFI44L, IFIH1, MX1, OAS2, SP100, SP110, IFIT1, IFITM1, and HERC6, genes known to be important in the antiviral response. Other genes such as the IFI27 gene increased with time in all three classes of patients. The function of this gene is unknown, but it was induced to very high levels (approximately 100-fold) by day 28. Other genes such as the carbonic anhydrase 1 gene were induced late (days 14 and 28) after treatment initiation.

DISCUSSION

The major finding of this study was that patients who exhibited a vigorous early virological response to treatment with peginterferon and ribavirin had concurrent vigorous alterations in PBMC gene mRNA levels, including genes whose levels were induced and repressed. Recognizing that several of the genes examined have expressions that may not meet the assumptions of the *t* tests, the analyses were repeated using a nonparametric rank sum test. Though this affected the results for some genes, the main conclusion that the number of genes whose levels were induced or repressed was greater among marked responders than poor responders remained true. Included in these genes whose expression was strongly altered were the classic interferon-induced genes. Thus, among marked responders (whose HCV RNA levels decreased by 3.5 logs or more 1 month after starting therapy) 655 genes were increased and 595 genes decreased in expression within 24 h of the first injection of peginterferon. For comparison, among poor responders (whose HCV RNA levels decreased by less than 1.4 logs after 1 month of therapy) the number of up-regulated genes was only 336 and only 82 were down-regulated within 24 h of starting therapy (Fig. 1 and 2). This difference was not due to lack of compliance because the initial doses of peginterferon and ribavirin were administered under observation. These findings suggest that poor or nonresponse to interferon-based therapy in chronic HCV infection may be due to a blunted induction of interferon-responsive genes. The finding that large number of genes are "down-regulated" in marked responders and not in poor responders may be indicative of a lack of sufficient oligo(A) synthetase, resulting in lowered activation of RNase L (26, 28, 29). However, a core of genes did appear to be actively down-regulated, since genes involved in translation regulation such as translation elongation factor and ribosomal protein genes appeared to be down-regulated in both marked and poor responders. Such genes have previously been reported to be down-regulated by alpha interferon in PBMC in culture (34).

In the Virahep-C study, AA had lower rates of sustained response and higher levels of serum HCV RNA than CA at

TABLE 4. Change in gene expression[a]

Function/description	Response	Symbol	Fold change in gene expression on day:				
			1	2	7	14	28
Antiviral response							
Interferon-stimulated gene 20 kDa	Marked	ISG20	3.21	2.49	2.21	2.07	2.16
Interferon-stimulated gene 20 kDa	Poor	ISG20	2.75	2.11	1.65	1.50	1.57
2′5′-Oligoadenylate synthetase 1 (40/46 kDa)	Marked	OAS1	7.03	6.78	4.18	3.80	4.29
2′5′-Oligoadenylate synthetase 1 (40/46 kDa)	Poor	OAS1	4.94	4.86	2.82	2.98	3.01
2′5′-Oligoadenylate synthetase 2 (69/71 kDa)	Marked	OAS2	5.10	4.91	3.88	4.04	4.44
2′5′-Oligoadenylate synthetase 2 (69/71 kDa)	Poor	OAS2	3.67	3.55	2.57	2.63	2.77
2′5′-Oligoadenylate synthetase-like	Marked	OASL	7.83	6.72	3.57	4.09	4.16
2′5′-Oligoadenylate synthetase-like	Poor	OASL	5.56	4.94	2.64	2.63	2.99
Viperin, cig5	Marked	RSAD2	18.64	17.94	10.3	14.28	15.58
Viperin, cig 5	Poor	RSAD2	12.11	12.1	6.70	7.13	7.66
Apoptosis							
XIAP associated factor 1	Marked	HSXIAPAF1	3.48	4.00	3.42	3.74	3.52
XIAP associated factor 1	Poor	HSXIAPAF1	2.87	2.99	2.36	2.41	2.56
TRAIL	Marked	TNFSF10	5.4	4.33	2.31	2.16	2.42
TRAIL	Poor	TNFSF10	3.28	2.99	**1.56**	**1.59**	**1.67**
Cell proliferation							
Lysosomal-associated membrane protein 3	Marked	LAMP3	11.52	6.61	5.5	5.13	6.19
Lysosomal-associated membrane protein 3	Poor	LAMP3	7.18	4.57	2.78	2.69	3.45
Chemokines							
Chemokine (C-C motif) ligand 2	Marked	CCL2	58	41.07	16.24	23.59	15.65
Chemokine (C-C motif) ligand 2	Poor	CCL2	**52.43**	**27.33**	**7.07**	**75.62**	**10.38**
Chemokine (C-C motif) receptor 1	Marked	CCR1	4.22	3.88	2.21	2.86	2.82
Chemokine (C-C motif) receptor 1	Poor	CCR1	2.84	**2.86**	**1.78**	2.08	**1.84**
Chemokine (C-C motif) ligand 8	Marked	CCL8	11.18	5.28	2.01	2.47	2.85
Chemokine (C-C motif) ligand 8	Poor	CCL8	**7.04**	2.70	**1.40**	**1.57**	**1.56**
Chemokine (C-X-C motif) ligand 11	Marked	CXCL11	5.09	2.22	**−1.01**	**1.00**	**−1.07**
Chemokine (C-X-C motif) ligand 11	Poor	CXCL11	4.83	**2.66**	**3.66**	**1.33**	**1.22**
Chemokine (C-X-C motif) ligand 10	Marked	CXCL10	24.42	11.88	1.79	1.93	2.00
Chemokine (C-X-C motif) ligand 10	Poor	CXCL10	19.95	8.94	1.51	**1.47**	1.56
Complement pathway							
Complement component 3a receptor 1	Marked	C3AR1	3.62	3.28	1.76	2.22	1.92
Complement component 3a receptor 1	Poor	C3AR1	2.38	2.32	**1.35**	**1.49**	**1.33**
Serine (or cysteine) proteinase inhibitor	Marked	SERPING1	11.93	7.09	2.19	2.57	**2.43**
Serine (or cysteine) proteinase inhibitor	Poor	SERPING1	8.88	5.65	1.94	2.29	2.24
Endonuclease							
Liver RNase (neurotoxin)	Marked	RNAse2	2.85	2.70	2.10	2.71	2.24
Liver RNase (neurotoxin)	Poor	RNAse2	2.19	2.13	**1.74**	**1.64**	1.82
Helicases							
Hypothetical protein FLJ20035	Marked	FLJ20035	4.56	4.38	4.10	4.06	4.27
Hypothetical protein FLJ20035	Poor	FLJ20035	3.36	2.85	2.42	2.16	2.66
Interferon induced with helicase C domain 1	Marked	IFIH1	5.59	4.99	3.52	3.70	4.21
Interferon induced with helicase C domain 1	Poor	IFIH1	3.59	3.25	2.24	2.20	2.48
Immune response							
Alpha interferon-inducible protein (clone IFI-15K)	Marked	G1P2	10.51	9.76	6.53	7.72	8.35
Alpha interferon-inducible protein (clone IFI-15K)	Poor	G1P2	7.43	6.93	3.89	4.24	4.75
Alpha interferon-inducible protein (clone IFI-6-16)	Marked	G1P3	4.11	4.09	3.02	3.22	3.07
Alpha interferon-inducible protein (clone IFI-6-16)	Poor	G1P3	3.39	2.67	**1.73**	1.99	2.29
Alpha interferon-inducible protein 27	Marked	IFI27	27.98	41.54	61.26	100.47	121.84
Alpha interferon-inducible protein 27	Poor	IFI27	21.45	35.28	52.67	69.51	89.4
Interferon-induced protein 35	Marked	IFI35	4.75	4.18	2.77	2.58	2.71
Interferon-induced protein 35	Poor	IFI35	3.56	3.00	**1.82**	**1.87**	1.95
Interferon-induced protein 44	Marked	IFI44	6.62	6.31	6.03	6.59	6.77
Interferon-induced protein 44	Poor	IFI44	4.29	4.12	3.41	3.36	4.09
Interferon-induced protein 44	Marked	IFI44L	9.09	9.36	9.13	10.68	10.67
Interferon-induced protein 44	Poor	IFI44L	5.91	5.93	5.02	4.95	6.06
Interferon-induced protein with tetratricopeptide repeats 1	Marked	IFIT1	17.57	17.1	10.72	12.27	13.55
Interferon-induced protein with tetratricopeptide repeats 1	Poor	IFIT1	9.23	9.23	5.79	5.27	6.31
Interferon-induced protein with tetratricopeptide repeats 3	Marked	IFIT3	11.95	10.3	6.38	7.53	8.47

Continued on facing page

TABLE 4—*Continued*

Function/description	Response	Symbol	Fold change in gene expression on day:				
			1	2	7	14	28
Interferon-induced protein with tetratricopeptide repeats 3	Poor	IFIT3	7.42	6.73	3.56	3.74	4.23
Interferon-induced protein with tetratricopeptide repeats 5	Marked	IFIT5	3.32	3.19	2.63	2.72	2.94
Interferon-induced protein with tetratricopeptide repeats 5	Poor	IFIT5	2.49	2.30	2.05	2.02	2.00
Interferon-induced transmembrane protein 1 (9-27)	Marked	IFITM1	1.86	1.76	1.67	1.71	1.95
Interferon induced transmembrane protein 1 (9-27)	Poor	IFITM1	1.71	1.63	**1.42**	**1.41**	1.62
Interferon-induced transmembrane protein 3 (1-8U)	Marked	IFITM3	2.71	2.45	1.89	2.00	1.91
Interferon-induced transmembrane protein 3 (1-8U)	Poor	IFITM3	2.28	2.20	1.60	1.65	1.82
IL-1 receptor antagonist	Marked	IL1RN	5.83	4.12	**1.43**	1.99	**1.98**
IL-1 receptor antagonist	Poor	IL1RN	4.21	2.93	**1.44**	2.05	1.75
Myxovirus (influenza virus) resistance 1	Marked	MX1	6.51	5.80	4.95	5.57	5.39
Myxovirus (influenza virus) resistance 1	Poor	MX1	4.29	3.90	2.83	2.87	3.26
Myxovirus (influenza virus) resistance 2	Marked	MX2	4.39	3.89	3.07	3.28	3.30
Myxovirus (influenza virus) resistance 2	Poor	MX2	3.46	2.94	2.04	2.12	2.49
Inflammatory response							
Heparanase	Marked	HPSE	4.16	2.45	1.73	2.05	1.80
Heparanase	Poor	HPSE	2.97	1.92	**1.39**	1.50	1.59
Sialoadhesin	Marked	SN	44.54	39.65	23.14	33.58	26.4
Sialoadhesin	Poor	SN	18.17	16.45	9.37	11.92	12.41
JAK-STAT cascade							
N-myc (and STAT) interactor	Marked	NMI	3.07	2.68	1.91	1.93	2.08
N-myc (and STAT) interactor	Poor	NMI	2.19	2.01	**1.50**	**1.46**	1.59
Signal transducer and activator of transcription 1 (91 kDa)	Marked	STAT1	2.38	2.05	1.88	1.85	2.09
Signal transducer and activator of transcription 1 (91 kDa)	Poor	STAT1	1.98	1.77	**1.46**	**1.37**	1.61
Phospholipid scrambling							
Phospholipid scramblase 1	Marked	PLSCR1	4.61	4.22	2.92	3.32	3.50
Phospholipid scramblase 1	Poor	PLSCR1	3.24	2.97	**2.17**	2.01	2.50
RNA editing							
Apolipoprotein B mRNA editing enzyme	Marked	APOBEC3A	6.03	4.66	2.63	2.93	2.94
Apolipoprotein B mRNA editing enzyme	Poor	APOBEC3A	4.29	3.53	2.08	2.15	2.22
Signal transduction							
Membrane-spanning 4 domains, subfamily A, member 4	Marked	MS4A4A	7.00	7.36	2.35	2.89	2.48
Membrane-spanning 4 domains, subfamily A, member 4	Poor	MS4A4A	3.68	4.39	**1.61**	**1.71**	1.89
Toll pathway							
Toll-like receptor 7	Marked	TLR7	7.17	5.12	2.90	3.19	2.79
Toll-like receptor 7	Poor	TLR7	3.93	3.07	**1.70**	**1.66**	**1.80**
Transcription factor/repressor							
CD38 antigen (p45)	Marked	CD38	2.61	2.15	1.89	1.86	1.90
CD38 antigen (p45)	Poor	CD38	2.43	2.03	1.64	**1.46**	1.56
Homeobox (expressed in ES cells) 1	Marked	HESX1	23.01	17.4	9.44	10.46	9.53
Homeobox (expressed in ES cells) 1	Poor	HESX1	17.56	12.78	**9.01**	5.74	6.23
Gamma interferon-inducible protein 16	Marked	IFI16	2.28	2.05	1.94	1.80	1.80
Gamma interferon-inducible protein 16	Poor	IFI16	1.84	1.87	1.55	**1.45**	1.51
Interferon regulatory factor 7	Marked	IRF7	4.56	4.25	3.12	3.20	3.37
Interferon regulatory factor 7	Poor	IRF7	3.34	3.11	2.04	2.19	2.33
Lectin, galactoside-binding, soluble, 9 (galectin 9)	Marked	LGALS9	3.04	2.86	1.86	1.81	2.07
Lectin, galactoside-binding, soluble, 9 (galectin 9)	Poor	LGALS9	2.30	2.07	**1.32**	**1.61**	1.65
Lymphocyte antigen 6 complex, locus E	Marked	LY6E	4.59	4.93	3.68	3.31	3.85
Lymphocyte antigen 6 complex, locus E	Poor	LY6E	3.71	3.71	**2.25**	**2.95**	**2.86**
Nuclear antigen Sp100	Marked	SP100	2.04	1.90	1.89	1.82	1.75
Nuclear antigen Sp100	Poor	SP100	1.78	1.78	1.51	**1.37**	**1.43**
SP110 nuclear body protein	Marked	SP110	1.98	2.03	1.76	1.80	1.85
SP110 nuclear body protein	Poor	SP110	1.64	1.64	**1.46**	**1.46**	1.53
T-box 3 (ulnar mammary syndrome)	Marked	TBX3	7.36	6.13	4.26	4.42	4.42
T-box 3 (ulnar mammary syndrome)	Poor	TBX3	4.91	4.02	2.47	2.35	2.6
Ubiquitin pathway							
Hect domain and RLD 5	Marked	HERC5	8.64	7.50	4.64	5.06	5.46
Hect domain and RLD 5	Poor	HERC5	5.92	5.09	2.88	2.85	3.18
Hect domain and RLD 6	Marked	HERC6	5.61	5.46	5.84	5.54	5.61

Continued on following page

TABLE 4—*Continued*

Function/description	Response	Symbol	Fold change in gene expression on day:				
			1	2	7	14	28
Hect domain and RLD 6	Poor	HERC6	4.19	4.07	3.59	3.35	3.76
Promyelocytic leukemia	Marked	PML	3.68	2.89	1.80	1.93	1.91
Promyelocytic leukemia	Poor	PML	3.07	2.26	**1.49**	1.60	1.60
Ubiquitin-conjugating enzyme E2L 6	Marked	UBE2L6	2.97	2.52	1.76	1.81	1.82
Ubiquitin-conjugating enzyme E2L 6	Poor	UBE2L6	2.54	2.19	**1.42**	**1.45**	1.53
Ubiquitin-specific protease 18	Marked	USP18	12.53	10.06	7.10	8.66	9.96
Ubiquitin-specific protease 18	Poor	USP18	6.89	5.80	4.00	3.95	4.89
Unknown function							
28-kDa interferon-responsive protein	Marked	IFRG28	4.68	4.87	3.38	3.52	3.76
28-kDa interferon-responsive protein	Poor	IFRG28	3.43	3.09	**2.16**	2.10	**2.11**

[a] Changes in gene expression for marked responders and poor responders at days 1, 2, 7, 14, and 28 after initiation of treatment. Data were derived from Table S1 in the supplemental material. Numbers in boldface represent values below the cutoff of a 1.5-fold change or a P value of >0.001.

almost all time points (6). The current analyses show that differences in the number of genes whose expression changed at least 1.5-fold between marked and poor responders in both AA and CA patients occurred within 24 h of starting treatment (Tables 4 and 5; Fig. 1 and 2). The level of gene expression and number of genes induced or down-regulated were considerably higher among marked virological responders than among poor responders (see Tables S1 and S2 in the supplemental material). However, it is puzzling that AA had higher numbers of genes induced and slightly higher levels of gene expression at day 1 than others.

Alpha interferon is known to act through induction of a large number of genes, the exact number and pattern of which have only been partially identified (33, 34). In this study, 801 genes were found to be increased during peginterferon and ribavirin therapy; many, but not all, of these were known interferon-induced genes (see Tables S1 and S2 in the supplemental material). While global interferon-induced gene expression was less among poor responders than marked responders, no specific gene could be linked to the differences in responses or to racial differences. Thus, poor or nonresponse appeared to be a global blunting of interferon cell signaling, rather than the lack of induction or function of a specific antiviral gene product. These data are in agreement with recent findings in which the gene expression of nonresponders was lower than that of responders when PBMC were cultured from such patients (16). Despite differences in the microarray systems used and assessment of in vitro versus in vivo responses, the levels of induction (*n*-fold) for many genes were remarkably similar. However, He et al. (16) found that the levels of gene induction in white patients was higher than in black patients. However, we could not find any difference between the racial groups in this study in levels of gene expression. The present study demonstrates that, controlling for virological response, gene expression changes were actually more common, within 2 weeks of treatment initiation, in AA than CA patients who had a marked or poor response. The reason for this difference is not known.

Previous studies using cell culture systems have suggested that HCV replication or presence of HCV antigens may interfere with specific interferon-induced gene products, such as the well characterized antiviral enzymes OAS, protein kinase R, and adenosine deaminase (8, 10, 12). The present analysis, in contrast, suggests that lack of response to administered interferon was due to an ongoing physiological defect that causes blunted regulation of interferon responsiveness. The blunted response might be due to a prior inflammatory response, interferon receptor deficiency or dysfunction, or lack of afferent cell signaling through the JAK-STAT pathway. In this regard, several recent studies in vitro and in vivo have suggested that a deficiency in STAT1 activation or DNA binding occurs in patients with chronic HCV infection (22). Such findings are compatible with the findings in this study. In fact genes such as the IRF-7 gene (Tables 4 and 5), a key gene in induction of interferon, was induced compared to baseline at lower levels in poor responders than in marked-response patients, as was the cig 5 (viperin) gene, previously identified as being important in the interferon response to hepatitis C virus (17). Toll-like receptor 7 (TLR7) has been shown to be important in the recognition of single-stranded viral RNA and subsequent signaling of the interferon, IκB kinase α/β/γ, and mitogen-activated protein kinase cascades leading to NF-κB and AP-1 activation and to IRF-7 and interferon production (13). Expression of the IRF-7 gene was increased from baseline to levels almost twice as high in marked-response than in poor-response patients and was thus strongly induced by peginterferon/ribavirin combination therapy.

Several limitations of the present findings deserve mention. First and foremost, the analysis of gene expression was conducted on PBMC and not on hepatocytes that harbor replicating HCV. Analysis of hepatocytes, however, requires liver biopsy, an invasive procedure which cannot be done repeatedly in humans during interferon therapy. Furthermore, analyses on liver tissue include PBMC and other nonparenchymal cells, and changes in expression in liver tissue may not reflect effects on hepatocytes only. Responses in PBMC are more likely to reflect a global response and not be under the local control of replicating virus or disease activity, which may modulate interferon responses. The chimpanzee model of HCV infection offers a potential approach to analyzing intrahepatic gene expression during interferon therapy (3, 21, 32). However, chimpanzees respond minimally to human alpha interferon therapy, and interpretation of results has to take into consideration interspecies differences.

TABLE 5. Changes on day 1 for CA and AA and the combined group[a]

Function/description	Symbol	Fold change for:			*P*
		All	CA	AA	
Antiviral response					
Interferon-stimulated gene 20 kDa	ISG20	2.94	2.71	3.19	0.15
2′5′-Oligoadenylate synthetase 1, 40/46 kDa	OAS1	5.77	5.29	6.26	0.32
2′5′-Oligoadenylate synthetase 2, 69/71kDa	OAS2	4.37	4.16	4.61	0.38
2′5′-Oligoadenylate synthetase-like	OASL	6.68	6.19	7.15	0.28
Radical *S*-adenosyl methionine domain containing 2	RSAD2	15.66	14.77	16.56	0.60
Apoptosis					
XIAP associated factor-1	HSXIAPAF1	3.40	3.17	3.61	0.50
TRAIL	TNFSF10	4.45	4.05	4.88	0.25
Cell proliferation					
Lysosomal-associated membrane protein 3	LAMP3	9.17	8.91	9.42	0.77
Chemokines					
Chemokine (C-C motif) ligand 2	CCL2	46.88	39.68	53.27	0.36
Chemokine (C-C motif) receptor 1	CCR1	3.58	3.46	3.69	0.68
Chemokine (C-C motif) ligand 8	CCL8	9.58	7.85	10.91	0.29
Chemokine (C-X-C motif) ligand 11	CXCL11	5.62	5.17	6.19	0.48
Chemokine (C-X-C motif) ligand 10	CXCL10	21.81	19.51	24.34	0.25
Complement pathway					
Complement component 3a receptor 1	C3AR1	3.02	2.80	3.31	0.21
Serine (or cysteine) proteinase inhibitor	SERPING1	10.64	9.51	11.94	0.28
Endonuclease					
Liver RNase (neurotoxin)	RNAse2	2.54	2.49	2.60	0.73
Helicase					
Hypothetical protein FLJ20035	FLJ20035	3.92	4.10	3.73	0.42
Interferon induced with helicase C domain 1	IFIH1	3.91	3.69	4.09	0.64
Immune response					
Alpha interferon-inducible protein (clone IFI-15K)	G1P2	8.78	7.80	9.89	0.22
Alpha interferon-inducible protein (clone IFI-6-16)	G1P3	3.75	3.42	4.11	0.31
Alpha interferon-inducible protein 27	IFI27	25.98	21.91	29.71	0.39
Interferon-induced protein 35	IFI35	3.96	3.63	4.34	0.31
Interferon-induced protein 44	IFI44	5.32	5.62	5.02	0.66
Interferon-induced protein 44	IFI44L	7.38	7.25	7.52	0.49
Interferon-induced protein with tetratricopeptide repeats 1	IFIT1	12.68	13.10	12.30	0.88
Interferon-induced protein with tetratricopeptide repeats 3	IFIT3	9.40	9.62	9.19	0.78
Interferon-induced protein with tetratricopeptide repeats 5	IFIT5	2.98	2.89	3.08	0.84
Interferon induced transmembrane protein 1 (9-27)	IFITM1	1.82	1.77	1.86	0.55
Interferon induced transmembrane protein 3 (1-8U)	IFITM3	2.49	2.35	2.63	0.49
IL-1 receptor antagonist	IL1RN	4.76	4.19	5.47	0.31
Myxovirus (influenza virus) resistance 1	MX1	5.29	5.47	5.12	0.11
Myxovirus (influenza virus) resistance 2	MX2	3.92	3.67	4.19	0.65
Inflammatory response					
Heparanase	HPSE	3.65	3.22	4.19	0.03
Sialoadhesin	SN	28.31	28.66	28.02	0.96
JAK-STAT cascade					
N-myc (and STAT) interactor	NMI	2.60	2.47	2.76	0.35
Signal transducer and activator of transcription 1 (91 kDa)	STAT1	2.17	2.11	2.24	0.54
Phospholipid scrambling					
Phospholipid scramblase 1	PLSCR1	4.00	3.75	4.29	0.31
RNA editing					
Apolipoprotein B mRNA editing enzyme	APOBEC3A	4.88	4.26	5.70	0.06
Signal transduction					
Membrane-spanning 4 domains, subfamily A, member 4	MS4A4A	5.51	4.68	6.40	0.07

Continued on following page

TABLE 5—*Continued*

Function/description	Symbol	Fold change for:			*P*
		All	CA	AA	
Toll pathway					
Toll-like receptor 7	TLR7	5.66	5.37	5.98	0.51
Transcription factor/repressor					
CD38 antigen (p45)	CD38	2.46	2.29	2.64	0.16
Homeobox (expressed in embryonic stem cells) 1	HESX1	22.41	19.63	25.53	0.24
Gamma interferon-inducible protein 16	IFI16	2.07	2.04	2.10	0.67
Interferon regulatory factor 7	IRF7	3.84	3.49	4.22	0.13
Lectin, galactoside-binding, soluble, 9 (galectin 9)	LGALS9	2.56	2.49	2.63	0.74
Lymphocyte antigen 6 complex, locus E	LY6E	3.95	3.79	4.13	0.73
Nuclear antigen Sp100	SP100	1.92	1.96	1.88	0.69
SP110 nuclear body protein	SP110	1.71	1.75	1.67	0.65
T-box 3 (ulnar mammary syndrome)	TBX3	5.93	5.90	5.96	0.94
Ubiquitin pathway					
Hect domain and RLD 5	HERC5	7.30	7.13	7.47	0.75
Hect domain and RLD 6	HERC6	5.01	4.82	5.24	0.43
Promyelocytic leukemia	PML	3.31	3.18	3.44	0.48
Ubiquitin-conjugating enzyme E2L 6	UBE2L6	2.69	2.51	2.90	0.07
Ubiquitin-specific protease 18	USP18	9.62	8.37	11.10	0.12
Unknown function					
28-kDa interferon-responsive protein	IFRG28	4.31	3.69	5.01	0.01

[a] Changes (*n*-fold) were defined as $+\text{average}_{\text{day 1}}/\text{average}_{\text{day 0}}$, if $\text{average}_{\text{day 1}} > \text{average}_{\text{day 0}}$, or as $-\text{average}_{\text{day 0}}/\text{average}_{\text{day 1}}$, if $\text{average}_{\text{day 1}} < \text{average}_{\text{day 0}}$. The standard error of a change (n = fold) was estimated by the delta method. A two-sample Z test was constructed to compare changes (n = fold) between CA and AA according to the equation $Z = FC_{CA} - FC_{AA}/(\text{std.}FC_{CA}^2 + \text{std.}FC_{AA}^2)^{1/2}$, where Z is approximately standard normal, FC_{CA} and FC_{AA} are the changes (n-fold) for CA and AA, respectively, and $\text{std.}FC_{CA}$ and $\text{std.}FC_{AA}$ are corresponding standard errors.

A final limitation to this study was that it was based upon viral kinetic analyses done during the first 28 days of therapy and was not based on results of sustained virological responses. This design was purposeful, in that early virological responses are highly predictive of ultimate responses and are not affected by nonbiologic factors, such as dose modification, compliance, and dropout. Only patients who took the full prescribed dose of peginterferon were selected. Furthermore, the differences between responders and nonresponders in the strength of gene induction were found even at day 1, which occurred after an observed administration of peginterferon and ribavirin at the initiation of treatment. Thus, by using early viral responses, purely biological factors associated with response and nonresponse could be assessed.

In this study, a poor virological response to peginterferon and ribavirin therapy of HCV infection was found to be associated with global, blunted changes in interferon-responsive gene expression. These results indicate that the blunted response is not specific to the liver or to virally infected cells. This hyporesponsiveness may be determined by host genetics, or it may be due to an environmentally induced lesser sensitivity to interferon. It is also possible that PBMC are exposed to viral proteins in circulation or to hepatocyte-associated HCV antigens which might alter the immune response of such cells to interferon treatment.

ACKNOWLEDGMENTS

This study was funded as a cooperative agreement by the National Institute of Diabetes and Digestive and Kidney Diseases with cosupport from the Intramural Research Program of the National Cancer Institute and with further support under a Cooperative Research and Development Agreement with Roche Laboratories, Inc. Grant numbers are as follows: U01 DK60329, U01 DK60340, U01 DK60324, U01 DK60344, U01 DK60327, U01 DK60335, U01 DK60352, U01 DK60342, U01 DK60345, U01 DK60309, U01 DK60346, U01 DK60349, and U01 DK60341. Other support was received from the National Center for Research Resources General Clinical Research Centers Program, grants M01 RR00645 (New York Presbyterian), M02 RR000079 (University of California, San Francisco), M01 RR16500 (University of Maryland), M01 RR000042 (University of Michigan), and M01 RR00046 (University of North Carolina). The Center for Medical Genomics is supported in part by grants from the Indiana 21st Century Research and Technology Fund and the Indiana Genomics Initiative (supported in part by the Lilly Endowment, Inc.).

Members of Virahep-C contributing to the study include, from the Beth Israel Deaconess Medical Center, Boston, MA, Nezam Afdhal (principal investigator) and Tiffany Geahigan (research coordinator); from the New York-Presbyterian Medical Center, New York, NY, Robert S. Brown, Jr. (principal investigator), Lorna Dove (coinvestigator), Shana Stovel (study coordinator), and Maria Martin (study coordinator); from the University of California, San Francisco, San Francisco, Norah Terrault, (principal investigator), Stephanie Straley, Eliana Agudelo, Melissa Hinds (clinical research coordinator), and Jake Heberlein (clinical research coordinator); from Rush University, Chicago, IL, Thelma E. Wiley (principal investigator) and Monique Williams (study coordinator); from the University of Maryland, Baltimore, Charles D. Howell (principal investigator), Kelly Gibson (project coordinator), Karen Callison (study coordinator), and Jane Lewis (study coordinator); from the University of Miami, Miami, FL, Lennox J. Jeffers (principal investigator), Shvawn McPherson Baker (coinvestigator), Maria DeMedina (project manager), and Carol Hermitt (project coordinator); from the University of Michigan, Ann Arbor, Hari S. Conjeevaram (principal investigator), Robert J. Fontana (coinvestigator), and Donna Harsh (study coordinator); from the University of North Carolina, Chapel Hill, Michael W. Fried (principal investigator [K24 DK066144]), Scott R. Smith (coinvestigator), Dickens Theodore (coinvestigator), Steven Zacks (coinvestigator), Roshan Shrestha (coinvestigator), Karen Dougherty (coinvestigator), Paris Davis (study coordinator), and Shirley Brown (study coordinator); from St. Louis University, St. Louis, MO, John E. Tavis (principal investigator),

Adrian Di Bisceglie (coinvestigator), Ermei Yao (coinvestigator), Maureen Donlin (coinvestigator), Nathan Cannon (graduate student), and Ping Wang (lab technician); from Cedars-Sinai Medical Center, Los Angeles, CA, Huiying Yang (principal investigator), George Tang (project scientist), and Dai Wang (project scientist); from the University of Colorado Health Sciences Center, Denver, Hugo R. Rosen (principal investigator), James R. Burton (coinvestigator), and Jared Klarquist (lab technician); from Veteran's Administration, Portland, OR, Scott Weston (lab technician); from Indiana University, Bloomington, Milton W. Taylor (principal investigator), Corneliu Sanda (postdoctoral associate), Takuma Tsukahara (statistician), and Mary Ferris (lab assistant); from the Data Coordinating Center, Graduate School of Public Health at the University of Pittsburgh, Pittsburgh, PA, Steven H. Belle (principal investigator), Richard A. Bilonick (statistician), Geoffrey Block (coinvestigator), Jennifer Cline (data manager), Marika Haritos (statistician), KyungAh Im (statistician), Stephanie Kelley (data manager), Sherry Kelsey (coinvestigator), Laurie Koozer (project coordinator), Sharon Lawlor (data coordinator), Stephen B. Thomas (coinvestigator), Abdus Wahed (statistician), Yuling Wei (project coordinator), Leland J. Yee (consultant), and Song Zhang (statistician); from the National Institute of Diabetes and Digestive and Kidney Diseases, Patricia Robuck (project scientist), James Everhart (scientific advisor), Jay H. Hoofnagle (scientific advisor), Edward Doo (scientific advisor), T. Jake Liang (scientific advisor), and Leonard B. Seeff (scientific advisor); and from the National Cancer Institute, David E. Kleiner (central pathologist).

We thank Mary Ferris for the excellent record keeping and entering of data into the portal at the Center for Medical Genetics. We thank Ron Jerome and Chunxiao Zhu for expert assistance with the microarray studies, which were carried out using the facilities of the Center for Medical Genomics at Indiana University School of Medicine. We also thank Song Zhang and Jia Li from the data coordinating center, Pittsburgh, for statistical support and Jay H. Hoofnagle for help in editing the manuscript.

REFERENCES

1. **Armstrong, G. L., A. Wasley, E. P. Simard, G. M. McQuillan, W. L. Kuhnert, and M. J. Alter.** 2005. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. Ann. Intern. Med. **144:**705–714.
2. **Benvegnu, L., M. Gios, S. Boccato, and A. Alberti.** 2004. Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. Gut **53:**744–749.
3. **Bigger, C. B., B. Guerra, K. M. Brasky, G. Hubbard, M. R. Beard, B. A. Luxon, S. M. Lemon, and R. E. Lanford.** 2004. Intrahepatic gene expression during chronic hepatitis C virus infection in chimpanzees. J. Virol. **78:**13779–13792.
4. **Brodsky, L., A. Leontovich, M. Shtutman, and E. Feinstein.** 2004. Identification and handling of artifactual gene expression profiles emerging in microarray hybridization experiments. Nucleic Acids Res. **32:**e46.
5. **Cassatella, M. A., S. Gasperini, F. Calzetti, A. Bertagnin, A. D. Luster, and P. P. McDonald.** 1997. Regulated production of the interferon-gamma-inducible protein-10 (IP-10) chemokine by human neutrophils. Eur. J. Immunol. **27:**111–115.
6. **Conjeevaram, H. S., M. W. Fried, L. J. Jeffers, N. A. Terrault, T. E. Wiley-Lucas, N. Afdhal, R. S. Brown, S. H. Belle, J. H. Hoofnagle, D. E. Kleiner, and C. D. Howell.** 2006. Peginterferon and ribavirin treatment in African Americans and Caucasian American patients with chronic hepatitis C genotype I. J. Gastroenterology **131:**470–477.
7. **Cotler, S. J., T. Craft, M. Ferris, M. Morrisey, J. McCone, K. R. Reddy, A. Conrad, D. M. Jensen, J. Albrecht, and M. W. Taylor.** 2002. Induction of IL-1Ra in resistant and responsive hepatitis C patients following treatment with IFN-con1. J. Interferon Cytokine Res. **22:**549–554.
8. **Duong, F. H., V. Christen, J. M. Berke, S. H. Penna, D. Moradpour, and M. H. Heim.** 2005. Upregulation of protein phosphatase 2Ac by hepatitis C virus modulates NS3 helicase activity through inhibition of protein arginine methyltransferase 1. J. Virol. **79:**15342–15350.
9. **El-Serag, H. B.** 2004. Hepatocellular carcinoma: recent trends in the United States. Gastroenterology **127:**S27–S34.
10. **Foy, E., K. Li, R. Sumpter, Jr., Y. M. Loo, C. L. Johnson, C. Wang, P. M. Fish, M. Yoneyama, T. Fujita, S. M. Lemon, and M. Gale, Jr.** 2005. Control of antiviral defenses through hepatitis C virus disruption of retinoic acid-inducible gene-I signaling. Proc. Natl. Acad. Sci. USA **102:**2986–2991.
11. **Fried, M. W., M. L. Shiffman, K. R. Reddy, C. Smith, G. Marinos, F. L. Goncales, Jr., D. Haussinger, M. Diago, G. Carosi, D. Dhumeaux, A. Craxi, A. Lin, J. Hoffman, and J. Yu.** 2002. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N. Engl. J. Med. **347:**975–982.
12. **Gale, M., Jr., and E. M. Foy.** 2005. Evasion of intracellular host defence by hepatitis C virus. Nature **436:**939–945.
13. **Garcia-Sastre, A., and C. A. Biron.** 2006. Type 1 interferons and the virus-host relationship: a lesson in detente. Science **312:**879–882.
14. **Gasperini, S., M. Marchi, F. Calzetti, C. Laudanna, L. Vicentini, H. Olsen, M. Murphy, F. Liao, J. Farber, and M. A. Cassatella.** 1999. Gene expression and production of the monokine induced by IFN-gamma (MIG), IFN-inducible T cell alpha chemoattractant (I-TAC), and IFN-gamma-inducible protein-10 (IP-10) chemokines by human neutrophils. J. Immunol. **162:**4928–4937.
15. **Hadziyannis, S. J., H. Sette, Jr., T. R. Morgan, V. Balan, M. Diago, P. Marcellin, G. Ramadori, H. Bodenheimer, Jr., D. Bernstein, M. Rizzetto, S. Zeuzem, P. J. Pockros, A. Lin, and A. M. Ackrill.** 2004. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann. Intern. Med. **140:**346–355.
16. **He, X. S., X. Ji, M. B. Hale, R. Cheung, A. Ahmed, Y. Guo, G. P. Nolan, L. M. Pfeffer, T. L. Wright, N. Risch, R. Tibshirani, and H. B. Greenberg.** 2006. Global transcriptional response to interferon is a determinant of HCV treatment outcome and is modified by race. Hepatology **44:**352–359.
17. **Helbig, K. J., D. T. Lau, L. Semendric, H. A. Harley, and M. R. Beard.** 2005. Analysis of ISG expression in chronic hepatitis C identifies viperin as a potential antiviral effector. Hepatology **42:**702–710.
18. **Howell, C., L. Jeffers, and J. H. Hoofnagle.** 2000. Hepatitis C in African Americans: summary of a workshop. Gastroenterology **119:**1385–1396.
19. **International Union of Biochemistry and Molecular Biology.** 1992. Enzyme nomenclature. Academic Press, San Diego, CA.
20. **Jeffers, L. J., W. Cassidy, C. D. Howell, S. Hu, and K. R. Reddy.** 2004. Peginterferon alfa-2a (40 kd) and ribavirin for black American patients with chronic HCV genotype 1. Hepatology **39:**1702–1708.
21. **Lanford, R. E., B. Guerra, H. Lee, D. Chavez, K. M. Brasky, and C. B. Bigger.** 2006. Genomic response to interferon-alpha in chimpanzees: implications of rapid downregulation for hepatitis C kinetics. Hepatology **43:**961–972.
22. **Lin, W., W. H. Choe, Y. Hiasa, Y. Kamegaya, J. T. Blackard, E. V. Schmidt, and R. T. Chung.** 2005. Hepatitis C virus expression suppresses interferon signaling by degrading STAT1. Gastroenterology **128:**1034–1041.
23. **Manns, M. P., J. G. McHutchison, S. C. Gordon, V. K. Rustgi, M. Shiffman, R. Reindollar, Z. D. Goodman, K. Koury, M. Ling, and J. K. Albrecht.** 2001. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet **358:**958–965.
24. **McClintick, J. N., and H. J. Edenberg.** 2006. Effect of filtering by Present call on analysis of microarray experiments. BMC Bioinformatics **7:**49.
25. **Muir, A. J., J. D. Bornstein, and P. G. Killenberg.** 2004. Peginterferon alfa-2b and ribavirin for the treatment of chronic hepatitis C in blacks and non-Hispanic whites. N. Engl. J. Med. **350:**2265–2271.
26. **Nijs, J., and K. De Meirleir.** 2005. Impairments of the 2-5A synthetase/RNase L pathway in chronic fatigue syndrome. In Vivo **19:**1013–1021.
27. **Reddy, K. R., J. H. Hoofnagle, M. J. Tong, W. M. Lee, P. Pockros, E. J. Heathcote, D. Albert, T. Joh, et al.** 1999. Racial differences in responses to therapy with interferon in chronic hepatitis C. Hepatology **30:**787–793.
28. **Samuel, C. E.** 2001. Antiviral actions of interferons. Clin. Microbiol. Rev. **14:**778–809.
29. **Silverman, R. H.** 2003. Implications for RNase L in prostate cancer biology. Biochemistry **42:**1805–1812.
30. **Stark, G. R., I. M. Kerr, B. R. Williams, R. H. Silverman, and R. D. Schreiber.** 1998. How cells respond to interferons. Annu. Rev. Biochem. **67:**227–264.
31. **Strader, D. B., T. Wright, D. L. Thomas, and L. B. Seeff.** 2004. Diagnosis, management, and treatment of hepatitis C. Hepatology **39:**1147–1171.
32. **Su, A. I., J. P. Pezacki, L. Wodicka, A. D. Brideau, L. Supekova, R. Thimme, S. Wieland, J. Bukh, R. H. Purcell, P. G. Schultz, and F. V. Chisari.** 2002. Genomic analysis of the host response to hepatitis C virus infection. Proc. Natl. Acad. Sci. USA **99:**15669–15674.
33. **Tan, H., J. Derrick, J. Hong, C. Sanda, W. M. Grosse, H. J. Edenberg, M. Taylor, S. Seiwert, and L. M. Blatt.** 2005. Global transcriptional profiling combination of type I and type II demonstrates the interferon enhances antiviral and immune responses at clinically relevant doses. J. Interferon Cytokine Res. **25:**632–649.
34. **Taylor, M. W., W. M. Grosse, J. E. Schaley, C. Sanda, X. Wu, S. C. Chien, F. Smith, T. G. Wu, M. Stephens, M. W. Ferris, J. N. McClintick, R. E. Jerome, and H. J. Edenberg.** 2004. Global effect of PEG-IFN-alpha and ribavirin on gene expression in PBMC in vitro. J. Interferon Cytokine Res. **24:**107–118.