Auto-antibodies and Autoimmune Disease during Treatment of Children with Chronic Hepatitis C

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Abstract

Objectives—Auto-antibodies were studied in a well-characterized cohort of children with chronic hepatitis C (CHC) during treatment with PEG-IFN and ribavirin to assess the relationship to treatment and development of autoimmune disease.

Methods—114 children (5–17 years), previously screened for the presence of high titer autoantibodies, were randomized to Peg-IFN with or without ribavirin. Anti-nuclear (ANA), anti-liver-kidney-microsomal (LKM), anti-thyroglobulin (TG), anti-thyroid peroxidase (TPO), insulin (IA2), anti-glutamic acid decarboxylase (GAD) antibodies were measured after trial completion using frozen sera.

Results—At baseline,19% had auto-antibodies: ANA (8%), LKM (4%), and GAD (4%). At 24 and 72 weeks (24 weeks after treatment completion), 23% and 26% had auto-antibodies (p=0.50, 0.48 compared to baseline). One child developed diabetes and two hypothyroidism during treatment; none developed autoimmune hepatitis. At 24 weeks, the incidence of flu-like symptoms, gastrointestinal symptoms, and headaches were 42%, 8% and 19% in those with auto-antibodies vs. 52%, 17%, and 26% in those without (p=0.18, 0.36, and 0.20, respectively). In children with negative HCV PCR at 24 weeks, there was no difference in the rate of early virologic response /sustained virologic response respectively in those with auto-antibodies 76%/ 69%, vs 58%/65% in those without (p=0.48).

Conclusions—Despite screening, we found autoantibodies commonly at baseline, during treatment for CHC and after. The presence of antibodies did not correlate with viral response, side effects, or autoimmune hepatitis. Neither screening nor archived samples assayed for thyroid and diabetes-related antibodies identified the 3 subjects who developed overt autoimmune disease, diabetes (1) and hypothyroidism (2).

Keywords

INTRODUCTION

The literature is replete with reports of *de novo* manifestations of autoimmune diseases occurring during the treatment of Chronic Hepatitis C (CHC) with interferon (IFN) based therapies. The most common diseases reported are thyroid disease[1], type 1 insulin dependent diabetes[2], and autoimmune hepatitis[3]. Rarer manifestations have included celiac disease[4], autoimmune thrombocytopenia[5], and autoimmune hemolytic anemia[6]. These complications are serious and, in some cases, permanent after cessation of therapy. Thyroid disease can occur in patients with CHC prior to treatment [7] and in as many as 15% of adults during IFN treatment [1, 7]. A nearly 5% incidence has been reported for diabetes [2]. These findings have implications for the risk and benefit of treating CHC with IFN-based therapies. To limit potential risk, some clinicians have suggested utilizing antibodies to screen and monitor the onset of disease. However, it is unclear if the presence of antibodies prior to treatment correlates with subsequent development of autoimmune disease [8].

Although combination therapy with PEG- IFN and ribavirin has become an established and effective therapy for CHC in children [9] little is known about the development of auto-antibodies and their predictive utility in children. The PEDS-C trial provided a unique opportunity to study a well characterized cohort of North American children regarding the impact of IFN on the development of auto-antibodies, the predictive value of antibody presence on the development of symptomatic complications, and the impact of baseline antibody on response to treatment [10]. In this study, we applied a standardized approach to screening our population to minimize risk to our treatment population. All children entering

the treatment portion of the clinical trial were excluded if they had increased titers of ANA, ASMA, LKM or TPO. In the 114 remaining children, ages 5 to 17 years, a population typical of those a clinician would treat in practice, we assessed auto-antibodies, using stored frozen sera obtained before, during, and after treatment in the PEDS-C trial of PEG-IFN alfa 2a with and without ribavirin. We evaluated the impact of auto-antibodies on response to therapy, tolerance of therapy, and development of *de novo* autoimmune disease.

MATERIALS AND METHODS

Subjects

Eligible children included treatment-naïve children from 5 to 17 years old with CHC infection documented by HCV RNA in serum on 2 occasions at least 6 months apart and a liver biopsy consistent with CHC. Details of the trial design and therapeutic outcomes were previously published[10–11] and the study was registered at www.ClinicalTrials.gov, NCT00100659. Subjects were enrolled by investigators at 11 U.S. medical centers from December 2004 until May 2006. The present study was approved by institutional review boards at each site and was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice Guidelines and local regulatory requirements. Children were excluded if there was evidence of decompensated liver disease or serological tests suggesting hepatitis A, hepatitis B, human immunodeficiency virus infection, or autoimmune hepatitis (ANA>1:160, ASMA >1:80, LKM >60 IU/ml, Covance labs)[10–11]. A total of 9 children were excluded from the study at screening: 5 with ANA, 1 with SMA, and 3 with LKM.

Treatment

Subjects were randomly assigned 1:1 to receive either PEG-IFN alfa-2a and RV or PEG-IFN alfa-2a and placebo[10]. PEG-IFN 2a was administered at a dose of 180 μg/1.73m^2 body surface area (maximum 180 μg) subcutaneously once weekly. Ribavirin was administered at a dose of 15 mg/kg/day orally in 2 doses (maximum 1200 mg/day if   75 kg and 1000 mg if < 75 kg). Placebo was supplied in the same dosing regimen as RV. Patients without detectable HCV RNA at 24 weeks were continued on treatment for another 24 weeks, whereas those who had detectable HCV RNA at 24 weeks were considered treatment failures. Treatment was stopped in those subjects who had been given PEG-IFN alfa-2a with RV and who did not achieve viral clearance at 24 weeks. Patients who failed treatment with PEG-IFN alfa-2a plus placebo were offered "open-label" therapy with PEG-IFN alfa-2a plus RV for another 48 weeks (stopping after 24 weeks if HCV RNA remained positive). Further details of the study design and outcome results of the trial have been previously reported [10–11]. Drug dose reductions were instituted for toxicities according to protocol.

Measurement

Auto-antibodies: All samples from weeks 0, 24 and 72 in the PEDS-C trial were stored at −80 degrees Celsius until analysis and were tested in a single lab simultaneously at the completion of the clinical trial. Figure 1 illustrates the times at which sera were obtained. Baseline and week 24 sera were tested from all subjects. Only those who were HCV negative at 24 weeks and continued on medication to complete a 48 week course of therapy were tested at the 72 week time point (24 weeks off therapy).

Auto-antibodies were measured by FDA-approved ELISA in a CLIA approved lab and reported in WHO international units. All tests were performed in the fully accredited Immune Disorders Laboratory at Johns Hopkins University (Clinical Laboratories Improvement Act CLIA #21D0709511). Positive cut-off values were all based on WHO standards established for each test. Thyroid-associated auto-antibodies included antibodies to TG (QUANTA Lite® ELISA) and TPO (QUANTA Lite® TPO ELISA) and were

measured by commercially manufactured ELISA kits (Inova Diagnostics, San Diego, CA). Positive values were defined as levels exceeding the normal reference range of 100 units. Auto-antibodies LKM and ANA were measured utilizing Inova Diagnostics QUANTA Lite® ELISA assays. Positive results for LKM-1 auto-antibodies included values exceeding 25 units. A moderate positive result for ANA auto-antibodies fell within the range of 20–60 units; however, for the purpose of this study, only results exceeding 60 units (strong positive) were considered to be positive. Detection of F-actin antibodies was done via commercial QUANTA Lite® Actin IgG ELISA (Inova Diagnostics, San Diego, CA). Values exceeding 30 units ("moderate to strong positive") were considered positive. GAD and IA-2 auto-antibodies associated with the presence of Type 1 diabetes, were measured using a GAD/IA-2 Auto-antibody Screen ELISA Kit (KRONUS, Inc., Star, ID). GAD positive values were defined as values exceeding 5 IU/ml. IA-2 positive values were defined as values exceeding 20 units.

Routine laboratory assessments were performed at Covance and included free T4, thyroid stimulating hormone, complete blood count and ALT among other tests that are fully described in Schwarz et al[10–11].

Statistics

The generalized estimating equation (GEE) was utilized to analyze the study outcome by determining the correlation between autoantibody presence and treatment outcome. The presence of positive auto-antibodies at 24 and 72 weeks, treatment type, and visit time point functioned as covariates. All category variables were summarized using frequency and percentage. The comparison of adverse events between patients with and without auto-antibodies was performed using Fisher's exact test. All continuous factors were reported as mean and standard deviation at each group. A two sided T-test or a Wilcoxon Rank-sum test was used to compare the significance of autoantibody presence and absence in relation to continuous variables (e.g., Knodell score). A P-value equal to or less than 0.05 was considered significant.

RESULTS

Antibody presence at baseline, 24 weeks and 72 weeks

114 treatment-naïve children with HCV entered the randomized trial. Mean age was 10.7 years, 55% were male, 75% Caucasian and the majority of children acquired their infection in the perinatal period[10]. Genotype 1 accounted for 81%. Auto-antibodies common to autoimmune hepatitis (ANA, LKM, anti-F-Actin), thyroid disease (TPO, TG) and diabetes (IA-2, GAD) were assessed (Table 1). At baseline 19 % of children had auto-antibodies. The most common auto-antibody was ANA (8%) followed by LKM (4%) and GAD (4%). Children with hepatitis-associated antibodies at baseline were younger, 7.8 vs 11.1 years (p=0.0007) and had slightly lower BMI Z-scores, −0.11 vs 0.70 (p=0.01) but did not differ from those without antibodies in terms of sex, genotype, viral load, Knodell inflammation or fibrosis scores (p= 1.00, 0.46, 0.13, 0.84 and 0.41 respectively).

After 24 weeks of PEG-IFN therapy, the overall prevalence of antibodies was 23% with ANA remaining the most common. At the 72 week time point, 26% of subjects had auto-antibodies. The antibody titers were not significantly different between time points before, during or 24 weeks after completion of therapy (P-value =0.58 for TPO, 0.53 for TG, 0.86 for F-Actin, 0.22 for ANA, 0.68 for IA-2). Mean antibody titers did not increase at 24 or at 72 weeks, whether assessed in all subjects or only in those with initial positive titers. The rate of antibodies becoming positive in those who were negative at baseline was up to 3% for liver-specific antibodies; among those who were positive for hepatitis-related

autoantibodies at baseline, antibody subsequently became negative in 1 of 9 children with ANA, 2 of 5 children with LKM, and in the 1 child with F actin.

Relationship between antibody presence and EVR/ SVR

Of the 114 subjects in the trial, 61% achieved viral suppression at week 24. Baseline hepatitis-associated antibody positivity did not significantly affect subsequent EVR and SVR. No significant differences in response at 24 weeks were found when comparing those with any auto-antibodies and those without (Table 2). EVR in those with antibodies at week 24 was 76% (19/25), and in those without antibodies at week 24 was 58% (48/83). SVR among this group of children who had undetectable HCV-RNA at 24 weeks was 69% (11/16) in those with antibodies and 65% (30/ 46) in those without. (p=0.46) Additionally there was no difference in the rate of antibody positivity at week 72 in those who achieved SVR vs those who did not (27% vs 24%, p=0.50). The children who relapsed at week 72 were compared to those who achieved SVR. Again, there was no statistically significant association of auto-antibodies with SVR or relapse based on GEE analysis. When comparing the hepatitis-associated antibody positive and negative groups who had achieved SVR, there was no significant difference in the frequency of non-genotype I or initial combo therapy, parameters that might have influenced SVR.

Auto-antibodies and end-organ disease

Three children developed significant autoimmune disease (Table 3). A 6 year old girl developed insulin dependent diabetes mellitus (IDDM) after 32 weeks of study drug. She developed severe hyperglycemia at 32 weeks of therapy. Study drug was discontinued at week 32 and she continued to require insulin. Study testing on stored sera revealed negative IA-2 antibody at each time point and negative GAD at baseline and week 24; GAD was positive at week 72. Two girls, ages 11 and 13, developed clinical hypothyroidism requiring treatment, neither child had positive TG or TPO at baseline. In the 11 year old at 24 weeks, thyroid stimulating hormone (TSH) was elevated to 45.33 IU/ml at week 24 and her TG increased from 19 units at baseline to 264 units at week 24. Subsequent TSH normalized on replacement therapy and she continued thyroid medication after study completion. The 13 year old girl developed elevated TSH 30 IU/ml and low free T4 0.6ng/dl at 36 weeks of study treatment; thyroid-specific auto-antibodies were not detected. She remained on thyroid hormone therapy with normal thyroid function tests after achieving SVR. Four children had elevated TPO and none of these children had abnormal T4 or TSH. Of the 7 with positive TG, only the one described above developed thyroid disease.

Symptoms, ALT and auto-antibodies

ALT elevation was examined in those children with ANA, LKM or F-Actin. At baseline, week 24 and week 72, children with and without these antibodies had similar ALT values. Of those with autoimmune hepatitis-related antibodies the highest ALT was 126 U/L (Table 4). In contrast, the highest ALT values, those exceeding 5 times the upper limit of normal (ULN,) all occurred among those children without antibodies to ANA, LKM or F-Actin. The most common side effects of treatment were flu-like symptoms (67%), GI symptoms (33%) and headaches (24%). Comparing 24 week data for flu-like symptoms, GI symptoms and headaches, side effects were not more frequent in those with or without antibodies.

DISCUSSION

This is the first study to prospectively and longitudinally assess the presence and development of auto-antibodies in children with CHC. Despite use of conventional screening techniques that excluded 9 children who had antibodies to ANA, LKM and SMA, we found that almost 20% of the enrolled subjects had baseline antibodies using an ELISA

technique. We found thyroid-, diabetes-, and hepatitis-related antibodies to be common in these children. The frequency of auto-antibodies, however, did not increase significantly with treatment in this cohort, nor did it change significantly six months after completion of treatment. The presence of auto-antibodies did not impact response to therapy. Furthermore, despite screening, three children (3%), none of whom had organ-specific auto-antibodies at baseline, developed *de novo* clinical autoimmune disease, 2 with hypothyroidism and 1 with IDDM.

Previous studies have presented conflicting results about the importance of antibodies before and during treatment with interferon based therapies. This study has methodologic advantages. All samples were analyzed simultaneously on well-standardized FDA-approved and CLIA-certified (except for IA2) immunoassays carried out by ELISA. This technology allows large numbers of samples to be simultaneously and objectively measured in WHO units; the assays are quantitative and represent a continuous function, as opposed to immunofluorescence assays which are subjective and discontinuous measures not amenable to statistical analysis in an epidemiologic study. Since these tests are performed under the proficiency program of the College of American Pathologists, the results can be compared nationally from one clinical laboratory to another.

Auto-antibodies have been reported in both adults and children with CHC. Auto-antibodies are seen in 70% of adults with CHC; 41% are ANA positive, 9% are SMA positive [12]. Gregorio et al screened for auto-antibodies in 51 Italian children with CHC and found 65% to be positive (90% of treated patients, 65% of untreated, p=0.12); SMA was the most common antibody (67%), followed by ANA (10%) and LKM (8%)[13]. Bortolotti reported positive SMA in 17.5%, LKM in 10% and ANA in 7.5% of 40 children with CHC [14–15]. Jara found ANA in 13%, SMA in 3%, and LKM in 10% of 30 children, and 7 children developed ANA during treatment[16]. In our prospectively studied North American cohort, the frequency is less and at least in part explained by our exclusion of children with high titer AIH related antibodies. In this study, the frequency of F-actin antibody (the cytoskeletal target of SMA) was less than 2%, LKM less than 5%, and ANA at most 13%. Other potential contributors to the difference in antibody frequency between this study population and that reported by Bortolotti, include differences in the populations studied, differences in the laboratory techniques for measuring auto-antibodies, as well as, our use of a stringent standard of strong positivity as defined by the manufacturer for each assay, rather than designating moderate or weak results as positive. These methodologic and population differences may account for variability in frequencies reported among these different studies.

It is notable that no child in this study, with or without antibodies, developed evidence of autoimmune hepatitis. Although we excluded 9 children with AIH related antibodies, we still identified children who had moderate and strong positive antibody by ELISA in the archived samples. No child had an ALT flare during treatment that limited therapy, similar to the experience of Jara's group, who did not exclude children with autoantibodies from treatment as long as they did not have features of autoimmune hepatitis[16]. In contrast Bertolotti et al. reported in a study of only 40 subjects, that 5 of 7 LKM-positive children, had a hepatitis flare during treatment[14]. Gregorio reported the need to discontinue treatment in several auto-antibody positive children who developed worsening hepatitis while on interferon[13]. New LKM antibodies during treatment and association with a hepatitis flare has been reported in one patient [17].

Like most adult studies, we did not detect a relationship between auto-antibody appearance and treatment outcome [8, 18–20]. In our study, there was no significant difference in 72 week treatment outcomes (only those who were virus negative at 24 weeks were analyzed)

between the auto-antibody positive and negative groups, with SVR of 70% and 65%, respectively.

Of particular note is that neither our screening antibodies nor our archived samples assayed for thyroid and islet cell antibodies identified the subjects who developed the most serious complications. As previously discussed, one child treated in this study developed IDDM at 32 weeks of treatment. She was negative for GAD and IA-2 antibodies at the start of treatment and at 24 weeks. GAD antibody was positive in the study week 72 sample tested. Diabetes has been reported to occur rarely in patients with CHC who are treated with IFN [2]; among 11,000 adults with viral hepatitis treated with interferon in a retrospective European report, 10 (0.08%) developed diabetes. Several adult CHC patients with positive diabetes-antibodies who did not develop diabetes with IFN treatment have been reported [21, 22]. Wirth reported one girl who developed diabetes mellitus while being treated with peg IFN and ribavirin for hepatitis C[23]. In the present study, up to 9.3% of subjects had positive diabetes related antibodies at some point, with the notable development of clinical diabetes in only one child, who was antibody negative at baseline and 24 weeks. For comparison, diabetes antibody positivity in the general pediatric population range from 0.5–2.9% % [24]. Auto-antibodies were thus not helpful in identifying the child who would develop diabetes in this study, although the literature suggests that these autoantibodies are strong predictors of subsequent development of diabetes in non-hepatitis C patients [25].

The development of thyroid disease is common in adults with CHC, both with and without treatment. Most studies report a rate of 10% or below[26,27] Auto-antibody positivity prior to treatment has been associated with an increased risk[27] of clinical autoimmune disease and new auto-antibodies develop during treatment in 5–45% [28]. In the pediatric literature, about 20% of children treated with interferon and ribavirin developed thyroid autoantibodies, and about 10% developed transient or permanent hypothyroidism requiring treatment [9, 16, 23, 29]. In the present report, the prevalence of thyroid auto-antibodies was lower than in most series at around 3%, with the frequency of anti-TG trending up from 0% at baseline to 6.5% at week 72. It thus appears that thyroid auto-antibodies as well as clinical thyroid disease occur uncommonly in children treated with IFN and at risk children cannot be identified prospectively.

We found that auto-antibodies are commonly detected in children with CHC infection and *de novo* autoimmune disease still occurs despite screening at baseline. Even sensitive antibody measures did not predict subjects at risk. Three children developed clinical disease, two with hypothyroidism and one with IDDM. In all 3 cases, screening antibodies as well as baseline and on-treatment antibodies did not identify risk until the disease developed. These data affirm that a variety of autoantibodies are common in children with CHC before, during and after treatment with Peg-IFN based therapy, Our screening at baseline may have accounted for our lower frequency of complications compared to historic reports. Even with screening and subsequent autoantibody assays, we were not able to identify children who would develop autoimmune disease. We provide fresh insight into the limited clinical role for following these antibodies to predict complications when treating children with CHC.

Acknowledgments

Abbrevations

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ALT	Alanine Aminotransferase
ANA	Anti-Nuclear Antibody
ASMA	Anti-Smooth Muscle Antibody
CHC	Chronic Hepatitis C
EVR/SVR	Early Viral Response/ Sustained Viral Response
GAD	Anti-Glutamic Acid Decarboxylase
GEE	Generalized Estimating Equation
HCV	Hepatitis C Virus
HCV RNA	Hepatitis C Virus Ribonucleic Acid
IA2	Insulinoma antigen
IDDM	Insulin Dependent Diabetes Mellitus
IFN	Interferon
LKM	Anti-Liver-Kidney Microsomal
LKM-1	Anti-Liver-Kidney Microsomal Type 1
RV	Ribavirin
SMA	Smooth Muscle Antibody
TG	Anti-Thyroglobulin
TPO	Anti-Thyroid Peroxidase
TSH	Thyroid Stimulating Hormone
VR	Viral Response

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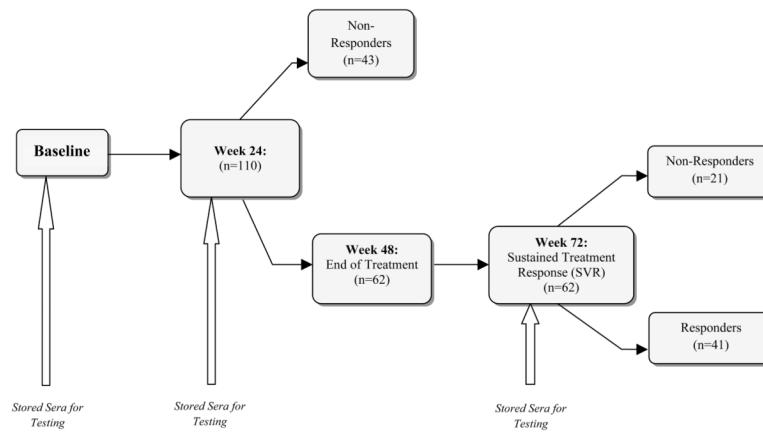
Figure 1.
The consort diagram shows treatment and decision points during the Peds-C Trial. At baseline, week 24 and week 72 serum was stored and frozen. The stored serum was used for analysis of serum auto-antibodies after completion of the clinical trial.

Table 1

Results of antibody testing on stored sera from baseline, week 24 and week 72. Last row provides the number of subjects who had any antibody positive.

Autoantibody	Baseline (n=114)			Week 24 (n=108)			Week 72 (n=62)		
	# Pos (%)	Mean	Range	# Pos (%)	Mean	Range	# Pos (%)	Mean	Range
TPO (>100 units)	3 (2.6)	489	422–589	3 (2.8)	444	353–497	2 (3.2)	196.5	112–281
TG (>100 units)	0 (0)	0	0	3 (2.8)	257.7	222–287	4 (6.5)	292	123–431
LKM-1 (> 25 units)	5 (4.4)	37.1	25.8–44.5	4 (3.7)	46.9	31.9–89.9	2 (3.2)	26.1	26–26.1
ANA (> 60 units)	9 (7.9)	104.5	60.1–157.7	9 (8.3)	97.3	60.3–160.2	8 (12.9)	94.2	60.6–189.2
F-ACTIN (>30 units)	1 (0.9)	44.9	44.9	1 (0.9)	33.8	33.8	1 (1.6)	39.6	39.6
GAD (>5IU/ml)	5 (4.4)	19.6	6.1–35.6	10 (9.3)	23.7	8–45.2	1 (1.6)	27.9	27.9
AI-2 (>20 units)	3 (2.6)	46.7	22.6–85.2	2 (1.9)	27.8	20.4–35.2	3 (4.8)	44.3	22–64.7
ANY ANTIBODY									
# Pos (%)	22 (19)			25 (23)			16 (25)		

Table 2

Comparison of responders to non-responders at weeks 24 and 72. Of those achieving EVR, 28% had antibodies and of those achieving SVR, 27% had antibodies. EVR in those with antibodies was 19 out of 25 (76%), whereas EVR in those without antibodies was 48 out of 83 subjects (58%). SVR in those with anti-bodies was 11 of 16 (69%) and in those without 30 of 46 (65%).

Autoantibody	Week 24 EVR				Week 72 SVR		
	EVR+ (n=67)	EVR − (n=43)	Total (n=108)	SVR + (n=41)	SVR − (n=21)	Total (n=62)	
	# Pos (%)	# Pos (%)	# Pos (%)	# Pos (%)	# Pos (%)	# Pos (%)	
TPO (>100 units)	0 (0)	3 (6.9)	3 (2.8)	2 (4.9)	0 (0)	2 (3.2)	
TG (>100 units)	1 (1.4)	2 (4.6)	3 (2.8)	1 (2.4)	3 (14.3)	4 (6.5)	
LKM-1 (> 25 units)	3 (4.4)	1 (2.3)	4 (3.7)	2 (4.9)	0 (0)	2 (3.2)	
ANA (> 60 units)	5 (7.5)	4 (9.3)	9 (8.3)	6 (14.6)	2 (9.5)	8 (12.9)	
F-ACTIN (>30 units)	1 (1.4)	0 (0)	1 (0.9)	0 (0)	1 (4.7)	1 (1.6)	
GAD (>5IU/ml)	8 (12)	2 (4.7)	10 (9.3)	1 (2.4)	0 (0)	1 (1.6)	
AI-2 (>20 units)	2 (2.9)	0 (0)	2 (1.9)	3 (7.3)	0 (0)	3 (4.8)	
Any	19 (28)	6 (14)	25 (23)	11 (27)	5 (24)	16 (26)	

Table 3

Genotype, antibody pattern and therapeutic response for the children who developed either insulin dependent diabetes or hypothryoidism

Antibody (nl range)	11 year old girl with hypothyroidism			13 year old girl with hypothyroidism			6 year old girl with IDDM		
	BL	24	72	BL	24	72	BL	24	72
TPO (0–100)	0	0	0	0	0	0	0	0	0
TG (0–100)	19	264	94	13	54	17	11	9	16
LKM-1 (0–25)	1.7	1.3	1.5	1.6	1.2	1.2	1.1	1.8	1.2
ANA (0–20nl:20–60moderate:>60 high)	10.4	14.9	10.6	23.6	25.1	26.9	8.2	7.1	11.2
F-ACTIN (0–30)	9.6	9.1	7.3	14.8	7.5	6.5	9.6	3.0	12.5
GAD (0–5)	1.9	0	1.3	0.3	0	0.8	1.0	2.7	27.9
AI-2 (0–20)	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0
Treatment Group	Peg-IFN + Placebo			Peg-IFN + Placebo			Peg-IFN + RBV		
SVR (yes/no)	Yes			Yes			Yes		
Genotype	1a			1a			1a		

Table 4

Association between ALT and presence of antibodies associated with autoimmune hepatitis. The top panel provides data for those subjects with positive antibodies to LKM-1, ANA or F-Actin. The bottom panel provides data for those subjects with no detectable antibodies. Among those with liver associated auto-antibodies, there was no increase risk of hepatic flare or hepatitis as assessed by ALT.

Liver Specific Autoantibodies	Baseline (n=114)			Week 24 (n=108)			Week 72 (n=62)		
	#Pos (%)	ALT (Mean)	ALT (Range)	#Pos (%)	ALT (Mean)	ALT (Range)	#Pos (%)	ALT (Mean)	ALT (Range)
LKM-1	5 (4.4)	64.4	26–117	4 (3.7)	43.75	14–95	2 (3.2)	17	14–20
ANA	9 (7.9)	44.1	25–69	9 (8.3)	49.89	14–126	8 (12.9)	26.6	10–106
F-Actin	1 (0.9)	54	54	1 (0.9)	129	129	1 (1.6)	106	106

Liver Specific Autoantibodies	Baseline (n=114)			Week 24 (n=108)			Week 72 (n=60)*		
	# Without (%)	ALT (Mean)	ALT (Range)	# Without (%)	ALT (Mean)	ALT (Range)	# Without (%)	ALT (Mean)	ALT (Range)
LKM-1	109 (95.6)	53.77	10–254	104 (96.3)	50.52	7–316	58 (96.7)	23.8	7–109
ANA	105 (92.1)	55.1	10–254	99 (91.67)	50.31	7–316	52 (86.7)	23.3	7–109
F-Actin	113 (99.1)	54.2	10–254	107 (99.1)	49.7	7–316	59 (98.3)	22.2	7–109

*
For week 72, two subjects were missing ALT levels. Therefore, the n=60.