

Immune mechanisms of responsiveness to the combined treatment with IFNa2b and Isoprinosine in chronic hepatitis C

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Introduction

Hepatitis C is a major health problem, considering that there are 150 million chronic carriers throughout the world. Prevention of HCV infection with efficient vaccination is not yet available, and treatment is the only option for eliminating the chronic infection. Currently, IFNa is used as the most wide-spread therapy of CHC, but the sustained response rate constitutes 8-15%. A successful combination of IFNa with ribovirin enhances the responsiveness rate up to 30-40%, but this kind of treatment has numerous side-effects and not all patients are eligible due to that factor. Thus, the issue of treatment is still being discussed.

The immune system's function is clearance of virus and infected cells. Viral antigens are presented on macrophages and B-lymphocytes and recognized by CD4+helper cells producing TH1 cytokines (IFNg, IL-2), which activate CD8+ cells (CTL) for killing of the infected cells. CD8+ cells recognize the modification of HLA Class1 with viral antigen on the infected cells and in the presence of TH1 cytokines activate their cytolytical function and clear the virus. They also produce TH1 cytokines (IFNg, TNFa) that are able to inhibit the replication of virus without killing the cells. Excessive activity of TH1 cytokines is antagonized and controlled by TH2 cytokines, IL-4 and IL-10 that up-regulate humoral immunity. B-lymphocytes produce neutralizing antibodies that bind the extracellular viral pattern. When all these protective immune mechanisms work together, the virus is effectively eliminated and there is an acute, cyclic form of HCV infection.

Unfortunately, up to 80% of patients have chronic hepatitis C with a high outcome rate of liver cirrhosis. The predominant chronic character of liver inflammation in HCV infection demonstrates the inability of the immune system to stop the pathological process. In chronically infected CHC patients, the dichotomy of TH1/TH2 cytokine balance in liver and peripheral blood develops: TH1 cytokines predominate over TH2 in liver, while in periphery TH2 cytokines predominate over TH1.

Thus, the therapy strategies are to limit the replication of HCV (to minimize "the harmful" blocking effects of HCV antigens) and to activate the protective immune response. Following this pathogenically approved therapeutic approach for the treatment of CHC, we used rIFNa (rialdiron, Lithuania) in a combination with immunomodulator Isoprinosine (Newport Synthesis LTD, Ireland), a drug with inosine-related activity.

This pilot investigation was conducted in Latvia and Lithuania and was organized as a double-blind, placebo-controlled study.

Materials and methods

CHC patients selected on the basis of virological, biochemical, morphological criteria were randomized into 2 groups undergoing the following treatment courses:

- **ACTIVE GROUP** (20 patients): IFNa, 3 MU x 3W, 24 weeks; Isoprinosine, p.o. 3g/day, divided, for 5 days a week/ 2 weeks, followed by one week off and repetition of the scheme, 24 weeks.
- **PLACEBO GROUP** (21patients): IFNa, 3 MU x 3W + placebo (the same scheme as for Isoprinosine), 24 weeks.

Treatment efficacy was determined by:

- Anti-HCV (ELISA, Abbot), HCV-RNA (PCR, Amplicor, Hoffman la Roch).
- Serum ALT elevation.
- Liver biopsy showing the features of chronic active or persistent/ lobular hepatitis.

Cytokine balance (TH1cytokines: IFNg, IL-12 and TH2 cytokines: IL-4, IL-10) was detected in whole peripheral blood (WPB). **The levels of cytokines were tested by ELISA** (PharMingen):

- Spontaneous cytokine production.
- Mitogens-induced (PHA, LPS) production.
- Antigen-induced (core peptides 1-17, 73-91 and NS4 peptide 1921-1941) production.

4 periods of sampling were used for cytokine analysis:

- P1, before the treatment.
- P2, 1-3 months treatment.
- P3, 4-6 months treatment.
- P4, follow up (6 months after the treatment).

Results

The baseline characteristics of both the groups – active and placebo – were quite similar.

	ACTIVE	PLACEBO
• Sex, %M	12/20 (60%)	12/21(57%)
• Age, medium	39.2	36.1

HCV serotype:

• Type 1	9/20 (45%)	12/21 (57%)
• Type 2+3	9/20 (45%)	6/21 (29%)
• Others	2/20 (10%)	3/17 (14%)

Response:

• Complete (N ALT and HCV RNA neg after 6 months of treatment)	9/20 (45%)	7/21 (33%)
• Sustained (N ALT and HCV RNA neg after 6 months of follow up)	5/20 (25%)	3/21 (14%)

Figure 1. Ag-induced IL-10 production in WPB of responders, active vs placebo

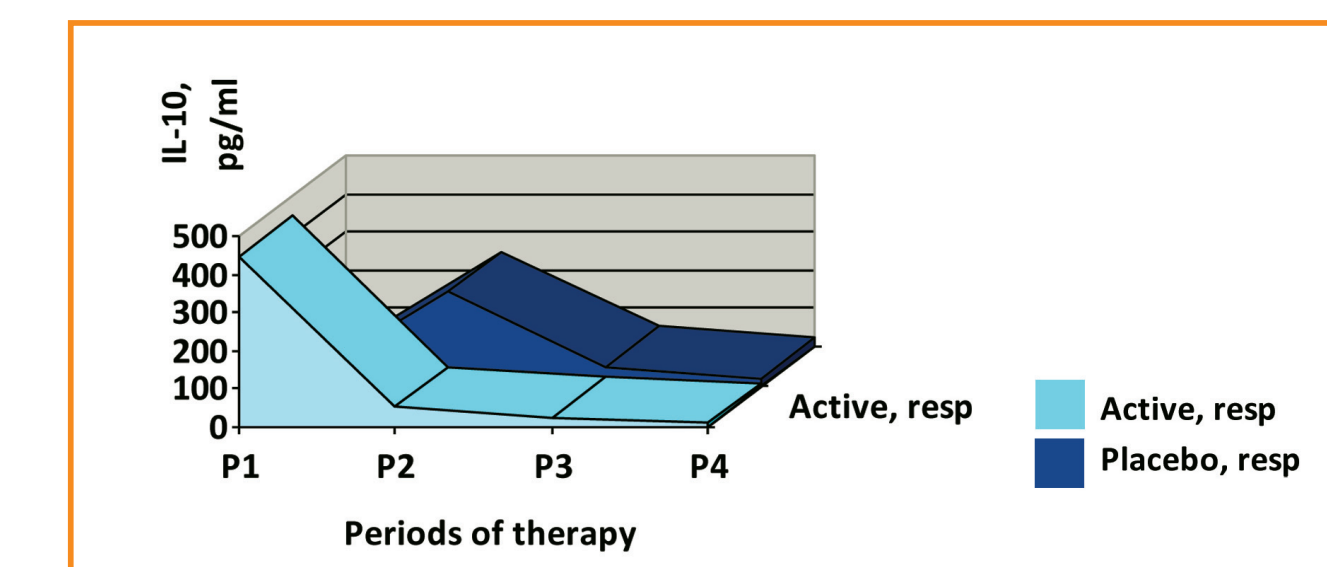


Figure 2. Spontaneous production of IFNg in WPB of responders, active vs placebo

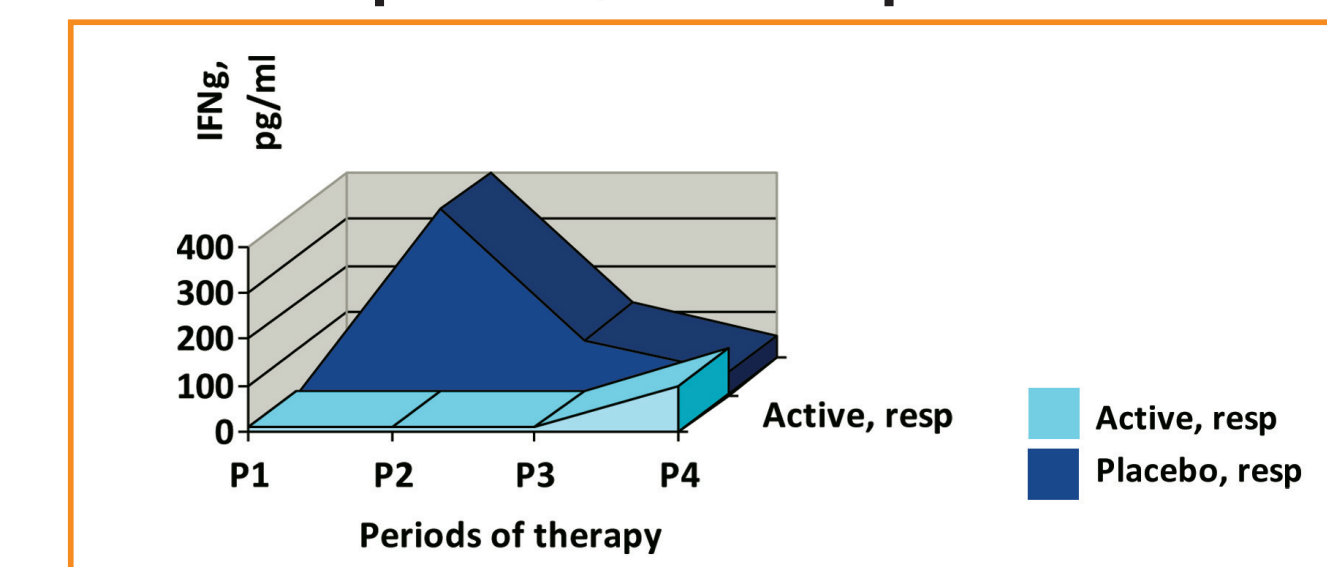


Figure 3. Ag-induced IL-10 production in WPB of active group, responders vs non-responders

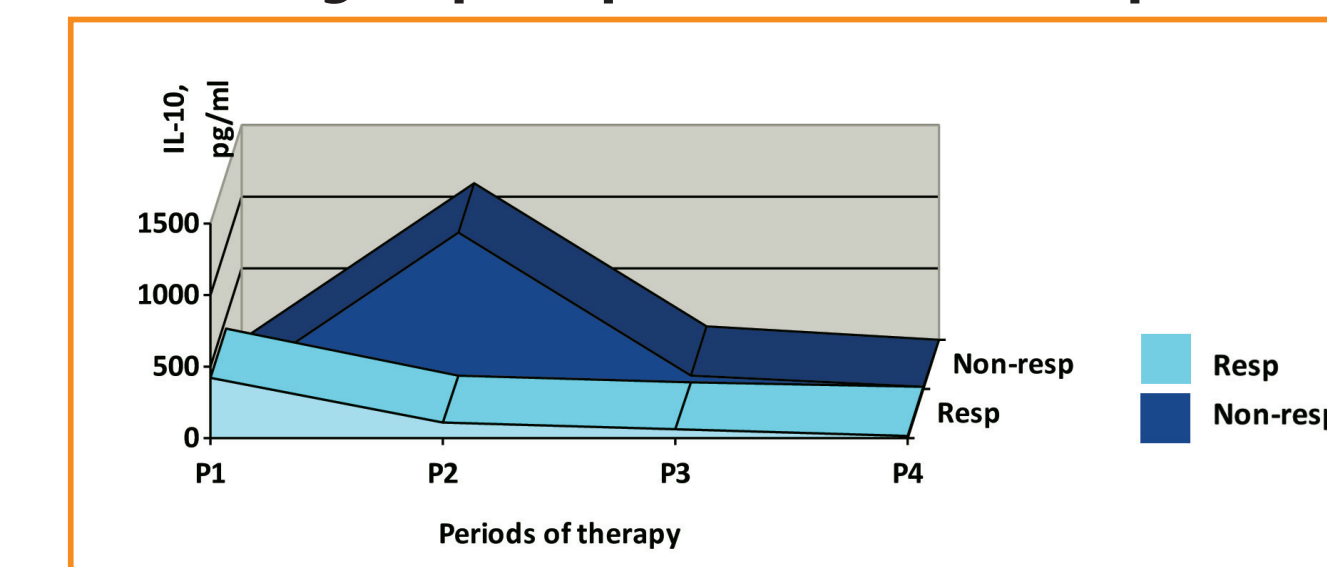
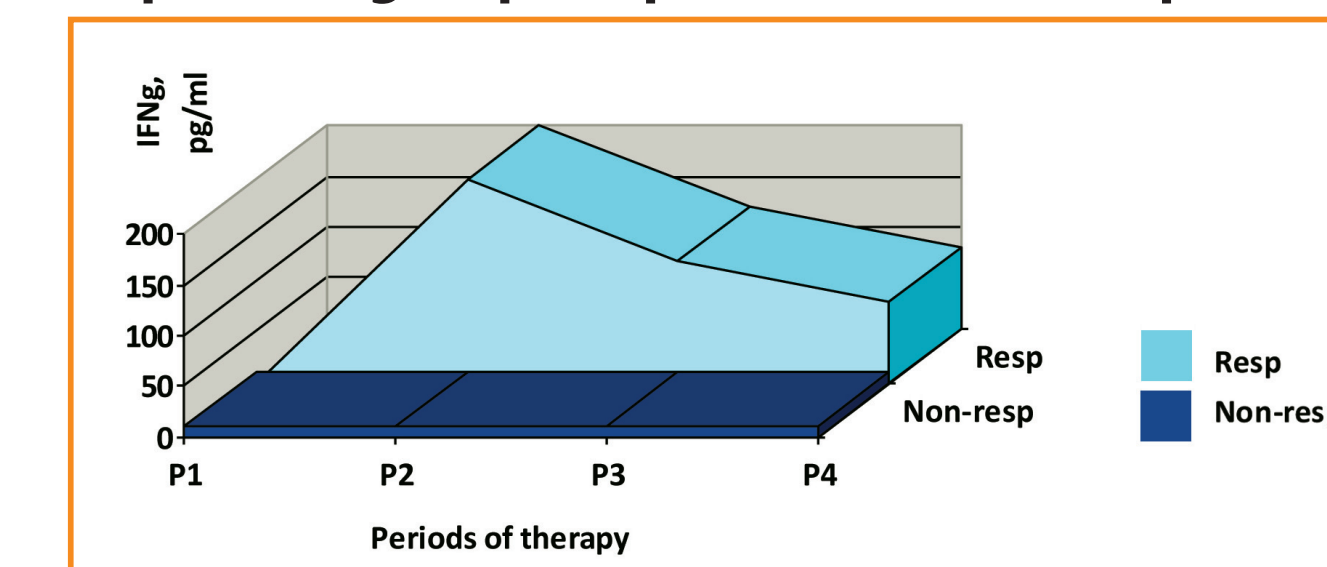


Figure 4. PHA-induced IFNg production in WPB of placebo group, responders vs non-responders



• We analyzed the dynamics of TH1/TH2 cytokine production in WPB in periods 1-4 of both the groups. There was no difference in the levels of TH1/TH2 cytokines in the non-responders to therapy in all periods of sampling. However, the secretion of antigen-specific IL-10 production was higher in the active responders vs the placebo responders, p2 (P<0.05, Fig.1). Spontaneous production of IFNg was higher in the placebo responders vs the active responders, p3 (P<0.05, Fig.2).

• To assess the immune mechanisms of responsiveness, we compared the levels of TH1/TH2 cytokines in the responders vs the non-responders in period 1-4. In the active group, the levels of antigen-specific IL-10 was lower in the responders vs the non-responders, p2 (P<0.05, Fig.3). In the placebo group, the levels of PHA-induced IFNg (24h) was higher in the responders vs the non-responders, (P<0.05, Fig.4).

Summary of results

- Patients for the active and the placebo group were selected on baseline parameters (sex, age, HCV serotypes).
- There is a difference in sustained response in the active group vs the placebo group (25% and 14% respectively).
- The dynamics of TH1/TH2 cytokine production is similar in the active and the placebo group in non-responders, but differs in responders to therapy.
- IFNa induces elevation of IL-10 production in both the active and the placebo group right at the beginning of the treatment.
- Suppression of Ag-specific IL-10 takes place only in the active group and seems to be mediated by Isoprinosine.
- Down-regulation of HCV-specific IL-10 production is beneficial for the responsiveness to the treatment in the active group.
- In the placebo group, the response is linked to late activation of IFNg production, but the rate of response is lower vs the active group.

Conclusions

- There are beneficial effects of IFNa + Isoprinosine vs IFNa monotherapy in terms of sustained response.
- Poor response to IFNa monotherapy in CHC is due to IFNa-induced activation of IL-10 production.
- Isoprinosine reverses the effect of IFNa on IL-10 production. Down-regulation of the levels of HCV-specific IL-10 leads to an enhancement of responsiveness to the treatment.

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