Articolo originale

Original article

Circulating Apo 2L levels decreased in genotype II hepatitis C with pegylated interferon-2 alpha treatment

Riduzione dei livelli circolanti di Apo 2L in corso di epatite C genotipo II in seguito a trattamento con interferone alfa-2

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INTRODUCTION

Chronic hepatitis C (HCV) infects approximately 170 million people and causes more than 350 000 deaths every year [1]. Information regarding pathogenetic mechanism of acute hepatitis C infection is limited. Following innate immune activation, cellular immunity, including natural killer (NK) cell activation and antigen-specific CD8 cell proliferation occurs [2]. CD8+ T lymphocytes directly kill infected cells via direct cell-cell contact, and release antiviral cytokines (e.g. IFN, TNF) [3].
Besides IFNs produced *in vivo*, IFN-α and IFN-β are currently employed therapeutics. The well known example of the treatment of chronic HCV infection is pegylated IFN-α (Peg IFN-α) [4]. IFN binds to its receptor on the infected hepatocyte surface. Although hepatitis C virus (HCV) replicates in hepatocytes, interferon (IFN) induction and immediate cell death block the virus within the cell.
Another pathway of viral clearance occurs via the ligands and receptors of the TNF-α family: TNFα/TNF-receptor 1, CD95/CD95 Ligand and TRAIL (TNF-related apoptosis-inducing ligand)/Trail receptor-1 and -2, respectively. It was shown that lymphocytes that recognize the viral antigen on hepatocytes became activated and expressed cytolytic Fas ligand, while hepatocytes in the vicinity of lymphocytes exhibited enhanced Fas expression and become susceptible to FasL-mediated death [5]. Ligand binding induces the formation of signal complex, resulting in the activation of caspase-8, which culminates apoptosis of hepatocyte [3].
It has been previously reported that sFas might play a role in allergic rhinitis [6]. In addition, an unexpected difference in serum sFas levels was observed between allergic rhinitis patients and bronchial asthma patients, although no difference exists in sFas-L between adult asthma patients and healthy controls [7]. Reflective of a common mechanism in allergic conditions, we observed that soluble Apo 2L levels of severe persistent allergic asthma patients decreased after anti-IgE treatment using omalizumab [8]. The increase in the eosinophil levels in allergic asthma has been previously reported, and it is thought to reflect an increase in peripheral blood eosinophil survival promoted by Apo 2L

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Table 1 - Laboratory characteristics of the patients (group IA, IB, IC).

Parameter	*PRE-interferon (IA) (n=11)*	*POST-6.hafta (IB) (n=11)*	*POST-12.hafta (IC) (n=11)*
TRAIL (Apo 2L) (pg/mL)	250.24±12.9	223.23±8.41	183.39±8.12
HCV RNA (IU/mL)	1583350.8±395745.6	4705.08±1638.8	711.8±518.7
HGB (g/dL)	12.9±0.5	11.9±0.9	11.1±0.7
WBC (mm3)	7783.4±731.3	4008.9±118.6	3216.67±282.80
Platelet (mm3)	175889.9±13361.7	142496.8±12010.39	135996.8±12565.9
GGT (U/L)	59.3±1.7	37±2.1	35±0.1
MPV (um3)	11.8±0.1	11.23±0.43	11.27±0.32
ALT (U/L)	82.5±5.2	43.8±6.5	27.6±3.5
AST (U/L)	57.33±17.00	30.33±6.42	27.42±2.89
AFP (ng/mL)	4.65±0.69	3.67±0.39	2.93±0.40

[9]. Mechanisms are involved in the increased susceptibility of human mast cell to Apo 2L-induced apoptosis after IgE-dependent activation. We speculated that the physiological functions of sApo 2L in allergic conditions, and the elucidation of the molecular mechanisms by which sTRAIL:TRAIL^{receptor} signals cells, would be of significant interest to the scientific allergy community in the coming years [6-8, 10, 11]. Previously the expression level of sTRAIL was found to be much higher in the HBV-infected group than that of the healthy controls [12]. In this study, we aimed to investigate the relationship between IFN treatment response, HCV viral load and sApo 2L levels.

PATIENTS AND METHODS

Eleven HCV-treatment naïve HCV-infected patients were treated with weight-based ribavirin daily in addition to either weekly pegIFN alpha-2b at 1.5 ug/kg, weekly pegIFN alpha 2a, or alpha-2b IFN at 900 mcg every two weeks.
All patients gave written informed consent approved by the Institutional Review Board prior to enrollment in the studies. Intensive serum monitoring was completed at study visits on day 0 (pretreatment), weeks 4, 6 and 12.

RESULTS

Mean baseline HCV-RNA level was 1583350.8 ± 395745.6 IU/mL; TRAIL level was 250.24 ± 12.9; ALT level was 82,5 ± 5.2; AST level was 57.33 ± 17.00 and AFP level was 4.65±0.69 (Table 1).
HCV-RNA levels decreased gradually with PegIF-α 2 treatment and the differences were significant between day 0 and week 4 (p=0.001, p<0.005); between day 0 and week 12 (p=0.001, p<0.005); between weeks 6 and 12 (p=0.01, p<0.05). sApo 2L levels decreased with PegIF-α 2 treatment and the differences were significant between day 0 and week 4 (p=0.01, p<0.005); between day 0 and week 12 (p=0.001, p<0.000); between week 4 and week 12 (p=0.005, p<0.001); between weeks 6 and 12 (p=0.01, p<0.05). ALT, AST and GGT levels also decreased significantly between day 0 and week 12 (p=0.003, p<0.005, p=0.02, p<0.05 and p=0.003, p<0.005 respectively).
We also observed significant decrease in AFP level and the differences were significant be-

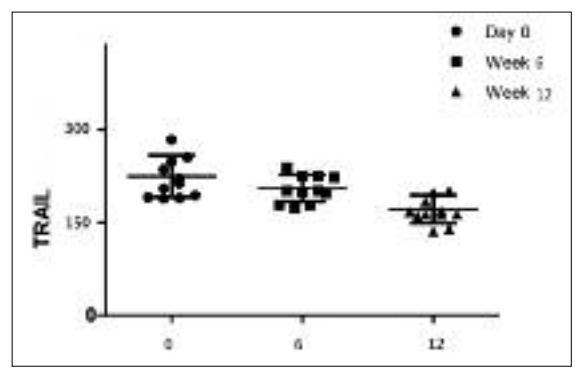

Figure 1 - STRAIL levels. Group IA (mean+SEM): 250.24±12.9; Group IB (mean+SEM): 223.23±8.41; Group IC (mean+SEM): 183.39±8.12.

tween day 0 and week 12 (p=0.006, p<0.01); between weeks 6 and 12 (p=0.023, p<0.05).

DISCUSSION

Apoptosis (programmed cell death) is an active physiological process that leads to the ordered destruction of cells without the release of intracellular contents into the extracellular environment (which would otherwise cause an inflammatory reaction and tissue damage) [13]. It is a process fundamental to maturation and homeostasis of the immune system. Apoptosis can be induced passively, through lack of essential survival signals, or actively, through ligand induced trimerization of specific death receptors of the tumor necrosis factor (TNF) receptor family, such as Fas (or CD95), the TNF receptor, or the TNF related apoptosis inducing ligand (TRAIL: Apo 2L) receptor [14]. Engagement of the Fas/FasL system is now acknowledged to contribute to increased apoptosis. Recent interest had focused on Apo 2L, with several studies indicating that sApo 2L is involved in the pathophysiology of different disease states including cancer, viral infections, autoimmune diseases and inflammation. Defective apoptosis following interaction of Apo 2L with its ligand may contribute to these effects [15-19].
The role of sApo 2L and another apoptotic marker FAS/FASL systems in colon cancer patients treated with chemotherapeutic agents was discussed in some reported studies [20-22]. Serum sApo 2L levels can be used to assess prognosis of metastatic colon cancer patients especially in the early evaluation phase following the bevacizumab treatment. Certain anti-cancer agents also upregulate Apo 2L and Apo 2L receptor expression on tumor cells, thus sensitizing cells to apoptosis [23, 24]. In this study we also showed that circulating Apo 2L level decreased in hepatitis C with the pegilated interferon-2 alpha treatment. We previously reported that circulating Apo 2L level decreased after omalizumab treatment in severe asthma patients. We suggested that this is possibly the evidence of pro-inflammatory effect suppressed within vascular bed [7, 8, 10, 25]. Recently, our study group also showed that sApo 2L level was lower than for healthy individuals with high levels in blister fluid in a patient with toxic epidermal necrolysis [26]. Recently, TRAIL-receptor-2, 4 and caspase-8 were found to be up-regulated in HCV patients, whereas TRAIL-receptor-3 to be down-regulated and sustained virologic response correlated with high expression of TRAIL and pro-apoptotic TRAIL-R2 on HCV-infected hepatocytes [27]. Althoug we didn't include genotyping of HCV, which influences the responce to PegIFN, we suggest that, decreased level of circulating Apo 2L may reflect its increased binding to its ligand expressed on hepatocytes or lymphocytes under the influence of PegIFN treatment [28]. Taken together, our results and those of others suggest that characterization of the specific receptor systems activated, and the pro-inflammatory factors regulated, by TRAIL *in vivo* may lead to the development of novel therapeutic strategies for diseases as diverse as infection, autoimmunity and allergy.

Conflict of interest
All authors declare that they have no conflict of interest.

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Keywords: sTRAIL, hepatitis C, pegylated interferon- 2 alpha.

SUMMARY

Pro-inflammatory factors regulated by TRAIL *in vivo* may lead to the development of novel therapeutic strategies for diseases as diverse as infection, autoimmunity and allergy. In this study we aimed to investigate the relationship between IFN treatment response, HCV viral load and sApo 2L levels. Eleven HCV-treatment naïve HCV-infected patients were treated with pegIFN alfa-2a. Intensive serum circulating Apo 2L levels were monitored at study visits on day 0 (pretreatment), and in weeks 4, 6 and 12. HCV-RNA and sApo 2L levels decreased gradually with PegIF-α 2 treatment and the differences were significant between day 0 and week 4 (p=0.001, p<0.005 and p=0.01, p<0.005 respectively); between day 0 and week 12 (p=0.001, p<0.005 and p=0.001, p<0.000 respectively); between weeks 6 and 12 (p=0.01, p<0.05 and p=0.01, p<0.05 respectively). We suggest that decreased levels of circulating Apo 2L may reflect its increased binding to its ligand expressed on hepatocytes or lymphocytes under the influence of PegIFN treatment.

RIASSUNTO

I fattori pro-infiammatori regolati in vivo da TRAIL possono condurre allo sviluppo di strategie terapeutiche innovative per diverse patologie quali infezioni, malattie autoimmuni e allergie. Questo studio è stato condotto nell'intento di indagare la relazione tra la risposta al trattamento con IFN, carica virale di HCV e livelli di sApo 2L. Undici pazienti con infezione da HCV, e naïve *per il trattamento anti-HCV, sono stati trattati con pegIFN alfa-2a. I livelli sierici circolanti di Apo 2L sono stati monitorati nel corso dello studio al giorno 0 (pretrattamento) e alle settimane 4, 6 e 12. I livelli di HCV-RNA e sApo 2L si sono ridotti progressivamente in seguito al trattamento con PegIF-α 2 e le differenze sono risultate significative tra il giorno 0 e la settimana 4 (p=0,001, p<0,005 e p=0,01, p<0,005 rispettivamente); tra il giorno 0 e la settimana 12 (p=0,001, p<0,005 e p=0,001, p<0,000 rispettivamente); tra la settimana 6 e la settimana 12 (p=0,01, p<0,05 e p=0,01, p<0,05 rispettivamente). Noi suggeriamo che la riduzione dei livelli circolanti di Apo 2L possa rifletterne un maggiore legame con il suo ligando espresso su epatociti o linfociti, sotto l'influenza del trattamento con PegIFN.*

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