Arch. Immunol. Ther. Exp. (2012) 60:61–68
DOI 10.1007/s00005-011-0153-y

ORIGINAL ARTICLE

Neutrophil Function and Apoptosis in Patients with Chronic Hepatitis C Treated with Pegylated Interferon α and Ribavirin

Elżbieta Jabłonowska · Kamila Wójcik · Barbara Kur · Przemysław Lewkowicz · Marek Nocuń

Received: 30 January 2011 / Accepted: 1 July 2011 / Published online: 8 December 2011

Abstract The role of neutrophils in the pathogenesis of chronic hepatitis C as well as the effect of pegylated interferon α (PEG-IFN-α) and ribavirin treatment on neutrophil function is not precisely known. The study included 32 patients with CCH aged between 19 and 58 years (mean 33.5 years). Before and after 12 weeks of treatment with Peg-IFN-α and ribavirin, intracellular reactive oxygen species (ROS) level, expression of adhesion molecules CD11b/MAC-1, CD16, CD18 and CD62L on neutrophils, as well as apoptosis and necrosis of these cells were analyzed with the use of flow cytometry. During antiviral therapy, a statistically significant decrease of mean fluorescence intensity for CD16 high and CD62 and increase for CD11b/MAC-1 along with the increased apoptosis and decreased necrosis of neutrophils were observed. After 12 weeks of treatment, intracellular ROS production by unstimulated neutrophils did not change, but after stimulation with phorbol 12-myristate 13-acetate, statistically significant increase of ROS level was observed. During PEG-IFN-α and ribavirin treatment, activation of neutrophil function and increased ROS production were reported, which possibly resulted in accelerated apoptosis of these cells.

Keywords HCV · IFN · Neutrophils · Apoptosis

Abbreviations

CHC	Chronic hepatitis C
Fmlp	*N*-formyl-methionyl-leucyl-phenylalanine
HCV	Hepatitis C virus
MFI	Mean fluorescence intensity
PEG-IFN-α	Pegylated interferon α
PMA	Phorbol 12-myristate 13-acetate
PMNs	Polymorphonuclear neutrophils
ROS	Reactive oxygen species
SD	Standard deviation
TNF-α	Tumor necrosis factor α
TRIAL/Apo-2	Ligand TNF-related apoptosis-inducing ligand

Introduction

The World Health Organization (2000) estimates that 3% of the world population is infected with the hepatitis C virus (HCV). Persistent infection can lead to chronic hepatitis C (CHC), complicated by cirrhosis and carcinoma. It is estimated that one out of five infected persons develops cirrhosis after 20 years from the infection. The antiviral treatment with pegylated interferon α (PEG-IFN-α) and ribavirin does not eliminate HCV in approximately half of the treated patients. It is well known that interferon α (IFN-α) has antiviral, immunoregulatory and antiproliferative activity. The IFN-α stimulates transcriptional activation of hundreds of genes with antiviral efficacies. Under the

E. Jabłonowska (✉) · K. Wójcik
Department of Infectious Diseases and Hepatology, Medical University of Łódź, Kniaziewicza 1/5, 91-347 Lodz, Poland
e-mail: elajablonowska@gmail.com

B. Kur
Department of Occupational Diseases and Toxicology, Nofer Institute of Occupational Medicine, Lodz, Poland

P. Lewkowicz
"Gameta" Infertility Center, Lodz, Poland

M. Nocuń
Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine, Lodz, Poland

influence of IFN-α, the secretion of other cytokines, e.g., tumor necrosis factor α (TNF-α), increases (Larrea et al. 1998) which stimulates target cells and results in the synthesis of pro-inflammatory cytokines. IFN-α leads to augmented production of reactive oxygen species (ROS), also increases expression of adhesive molecules and participates in the regulation of apoptosis (Bazhanova 2005; Cascao et al. 2009; Melley et al. 2005). Increased ROS production in patients with CHC can inhibit HCV replication (Choi et al. 2004) and contribute to elimination of HCV. On the other hand, uncontrolled production of ROS can result in hepatocytes injury. Several authors suggest the role of ROS in liver cell damage. In numerous studies, increased lipid, protein and nucleic acid peroxidation in the blood and liver biopsy specimens from patients with CHC has been demonstrated (De Maria et al. 1996; Farinati et al. 1995; Mutlu-Turkoglu et al. 1997; Shimoda et al. 1994). Decreased levels of reduced glutathione in red blood cells and peripheral blood mononuclear cells, as well as increased glutathione turnover have also been reported (Farinati et al. 1995; Mutlu-Turkoglu et al. 1997; Swietek and Juszczyk 1997). Some authors report that in patients with CHC, treatment with IFN-α lowers the level of thiobarbituric-acid-reacting compounds and down-regulates the activity of glutathione peroxidase, with a simultaneous increase in sulfur-hydrogen groups (Mutlu-Turkoglu et al. 1997). Polymorphonuclear neutrophils (PMNs) belong to the group of main cells producing ROS. Their role in pathogenesis of CHC has not been thoroughly examined. In a previous study, an increased production of in vitro ROS by both stimulated and unstimulated peripheral blood PMNs in patients with CHC was proved. We decided to analyze the effect of Peg-IFN-α and ribavirin treatment on PMNs oxygen metabolism, expression of adhesion molecules, CD11b, CD16, CD18 and CD62L, and apoptosis of neutrophils in patients with CHC.

Materials and Methods

The study population consisted of 32 patients who did not have any other systemic or inflammatory diseases. Patients with liver cirrhosis, other causes of liver disease, previous immunosuppressive or antiviral treatment, and pregnant women were excluded from this study.

The diagnosis of chronic hepatitis was based on the results of liver biopsy specimen examination. HCV infection was established based on the presence of viral genetic material detected by the RT-PCR method. Genotype of HCV and HCV viral load was determined in all patients. Combined therapy using PEG-IFN-α 2a (Pegasys; Roche, Switzerland) and ribavirin (Copegus) was applied. Pegasys was administered subcutaneously once a week at a dose of 180 μg. Ribavirin was administered per os daily as dose dependent on patient's weight (less than 60 kg: 1,000 mg; above 60 kg: 1,200 mg). The decision whether the therapy would be continued was made 12 weeks after its introduction. Patients with undetectable HCV viremia continued the therapy until 24 or 48 weeks (genotype 3: 24 weeks, genotype 1 or 4: 48 weeks). In patients with a less than 2 log decrease in viral load, the therapy was stopped. In patients with a more than 2 log decrease in viral load, the therapy was continued—for genotype 3 until 24 week, for genotypes 1 and 4 until 48 weeks if viral load in the 24th week was undetectable. The patients were examined at the beginning of the therapy and after 2, 4, 8, 12, 16, 20, 26, 32, 38, and 48 weeks of treatment. During each control visit, physical examination and basic laboratory tests were performed as follows: peripheral blood image with differential white cells count, platelet cells number, and ALT activity (to complete the clinical picture only) were determined. During 7 days prior to the examination, none of the patients received any anti-inflammatory treatment or any other medications which could affect neutrophil activity. During 4 weeks prior to the examination, none of the patients showed (besides the typical general symptoms associated with PEG-IFN-α treatment) any acute symptoms of infection. The experimental protocol was approved by the Ethics Committee of Medical University of Łódź, and informed consent was obtained from all participants of the study.

Flow Cytometry

All analyses were performed on heparinized whole blood (Li-Heparin, Monovette Blood Collection System, Sarsted, Nümbrecht, Germany) with the use of FACS Canto II Flow Cytometer (Becton–Dickinson, San Jose, CA, USA). Granulocytes were identified by forward and right scatter (FSC/SSC) dot plot. Ten thousand events within the granulocyte gate were counted per sample. The data were analyzed using FACS Diva software (Becton–Dickinson, San Jose, CA, USA). Prior to each analysis, the flow cytometer was calibrated by BD Cytometer Setup and Tracking Beads (BD Biosciences, San Jose, CA, USA). Percent of positive labeled cells and mean fluorescence intensity (MFI) were used to quantify the responses.

CD11b/MAC-1, CD16, CD18 and CD62L Expression

Fifty microliters of heparinized blood was incubated with monoclonal mouse anti-human antibody against specific antigens: CD11b-PE (clone D12.11), CD62L-PE (SK11), CD16-FITC (NKP15i) and CD18-FITC (L130) (all provided by BD Pharmingen, San Jose, CA, USA) for 30 min at room temperature in the dark. After incubation, the erythrocytes were lysed with BD FASC Lysing Solution

(BD Biosciences) for 10 min. After lysis, the cells were washed once in BD CellWASH solution and resuspended in BD CellFIX solution (BD Biosciences).

Measurement of ROS

Intracellular ROS level was assessed by the use of commercially available assay kit (Phagoburst Orpegen Pharma, Heidelberg, Germany) according to the manufacturer's instructions. Briefly, a portion of 100 µl of blood was transferred into 5-ml polypropylene FACS tubes and preincubated for 10 min on ice cold water bath. The blood was then mixed with plain buffer (negative control) or one of the following stimulants: the chemotactic peptide *N*-formyl-methionyl-leucyl-phenylalanine (fMLP, 0.83 µM), phorbol 12-myristate 13-acetate (PMA; 1.35 µM), or suspension of opsonized *Escherichia coli* ($0.17–0.33 \times 10^9$ bacteria per ml). The samples were incubated for 10 min at 37°C in a water bath. After that, cells were incubated with dihydrorhodamine 123 for 10 min at 37°C. After lysing of erythrocytes and washing, propidium iodide solution was added to exclude aggregation artefacts of bacteria or cells during the flow cytometric analysis.

Apoptosis and Necrosis

Apoptosis and necrosis was measured using the FITC Annexin V Apoptosis Detection Kit I (BD Biosciences Pharmingen, San Jose, CA, USA). According to the manufacturer's protocol, blood erythrocytes were lysed by incubation of 200 µl of blood with BD Pharm Lyse Lysing Buffer for 10 min at room temperature. After washing, leukocytes were resuspended in Annexin V Binding Buffer at a concentration of 1×10^6 cells/ml. Hundred microliters of cell solution (1×10^5 cells/ml) was transferred into FACS tubes and incubated for 15 min at room temperature with FITC-conjugated Annexin-V and propidium iodide. After adding 400 µl of Annexin V Binding Buffer, samples were measured on a flow cytometer within 30 min. Apoptotic cells were defined as those demonstrating positive staining for Annexin-V-FITC (with negative or positive PI staining, respectively, for early and late stage of apoptosis), while necrotic cells were PI-positive and Annexin-V-FITC-negative. Viable cells were defined as double negative for both stains.

Statistical Analysis

The results are presented as arithmetic mean ± standard deviation (SD) of mean for the normally distributed parameters or as a median and interquartile range (from lower quartile, Q1 to upper quartile, Q3) for data showing departures from normality (the assumption of normality was assessed with the Shapiro–Wilk test). The significance of differences between the groups in flow cytometry parameters was determined with Wilcoxon signed-rank test.

Table 1 Characterization of the patients with CHC

Parameter	Mean ± SD	Min–max
Age	33.5 ± 12.5	19.0–58.0
ALT (U/l)	82.6 ± 70.2	24.0–310.0
GPT (U/l)	61.0 ± 35.0	11.0–136.0
HCV viral load ($\times 10^3$)	2,855.3 ± 2,394.1	107.0–7,810.0
Histopathology staging (S)[a]	2.1 ± 0.9	0–4
Histopathology grading (G)[a]	1.3 ± 1.2	0–4

[a] Estimation according to Scheuer

Table 2 Expression of CD11b, CD16, CD18, and CD62L on peripheral blood granulocytes

	Before treatment	3 months of treatment	Significance
CD11b			
%	99.6 (99.3–99.8)	98.8 (98.2–99.4)	$p = 0.002$
MFI	6,839 (5,275–10,079)	9,545 (7,927–10,994)	$p = 0.05$
CD16low			
%	3.2 (1.9–4.7)	3.2 (2.1–4.7)	NS
MFI	1,369 (1,150–2,246)	1,295 (1,172–1,913)	NS
CD16high			
%	93.5 (91.8–95.3)	91.6 (89.5–93.9)	$p = 0.0376$
MFI	22,278 (19,037–26,380)	19,957 (15,703–22,178)	$p = 0.0011$
CD18			
%	99.5 (98.9–99.7)	98.7 (97.4–99.4)	$p < 0.0001$
MFI	2,589 (2,342–3,178)	2,985 (2,611–3,504)	NS
CD62L			
%	99.1 (98.5–99.4)	96.4 (94.0–98.7)	$p < 0.0001$
MFI	5,956 (5,032–8,058)	3,009 (2,222–4,242)	$p < 0.0001$

Data are presented as median and interquartile range (Q1–Q3)

MFI mean fluorescence intensity, *NS* nonsignificant

Results

The study group consisted of 32 subjects: 20 men and 12 women. The mean age of patients was 33.5 ± 12 years (19–58). A total of 26 patients were infected with HCV genotype 1, 4 patients with HCV genotype 3, and 2 with HCV genotype 4. The characterization of the study group is shown in Table 1.

In the 12th week of treatment, HCV-RNA was not detected in the blood of 21 patients (complete early virological response). In eight patients, more than 2 logs decrease in viral load was reported (partial early virological response), whereas in three patients lack of virological response was observed.

Expression of CD11b, CD16, CD18 and CD62L on peripheral blood granulocytes are presented in Table 2.

During treatment, we observed decreasing MFI for CD16 high and CD62L and increasing for CD11b (Fig. 1). Apoptosis of granulocytes increased but necrosis decreased during antiviral therapy (Table 3; Fig. 2). After 3 months of treatment with PEG-IFN-α and ribavirin, intracellular ROS production by unstimulated neutrophils did not change, but after stimulation with PMA, statistically significant increase of ROS level was observed (Fig. 3). The level of ROS production by neutrophils after stimulation by *E. coli* and fMLP is presented in Table 4. Laboratory data

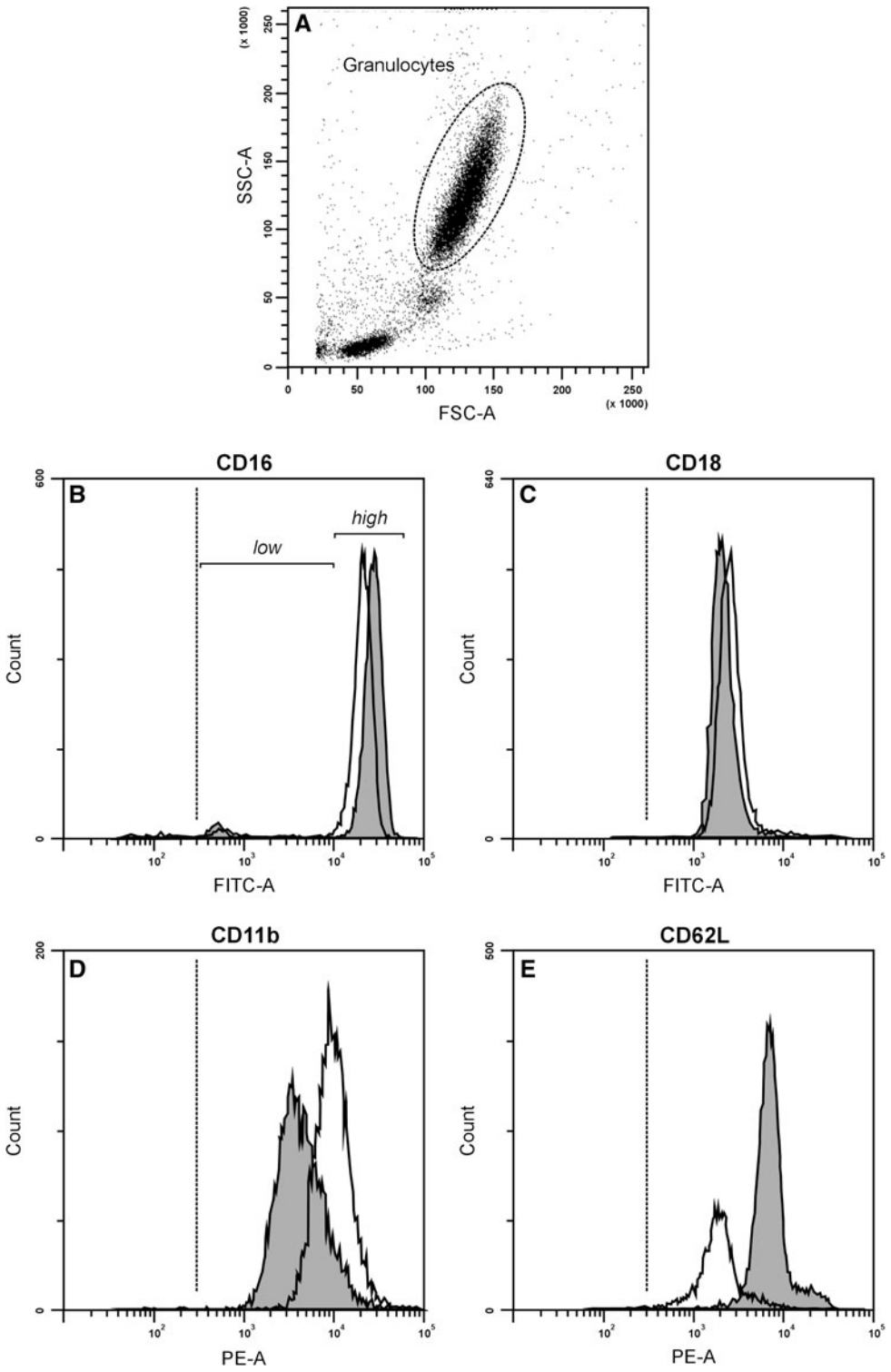

Fig. 1 Flow cytometric analysis of expression of specific surface markers on whole blood granulocytes. A representative experiment is shown. Human peripheral whole blood granulocytes were identified and gated based on forward and side scatter (FSC/SSC) characteristics (**a**). Histograms present expression of CD16 (**b**, both gated subpopulations with low and high fluorescence intensities), CD18 (**c**), CD11b (**d**), and CD62L (**e**) on granulocytes before (*solid*) and after (*unfilled*) 3-month combined therapy with PEG-IFN-α and ribavirin. Gates were set up according to isotype control data (*dashed line*)

Table 3 Apoptosis and necrosis of peripheral blood granulocytes

	Before treatment	3 months of treatment	Significance
Apoptotic cells (%)			
Early ($AnexV^+PI^-$)	14.7 (10.3–18.3)	20.2 (13.7–31.3)	$p < 0.0001$
Late ($AnexV^+PI^+$)	1.6 (0.9–2.1)	2.3 (1.7–3.4)	$p = 0.017$
Total ($AnexV^+$)	16.3 (11.6–20.3)	21.7 (15.8–33.2)	$p < 0.0001$
Necrotic cells (%)			
($AnexV\text{-}PI^+$)	3.0 (1.7–5.3)	1.2 (0.4–1.9)	$p < 0.0001$

Data are presented as median and interquartile range (Q1–Q3)

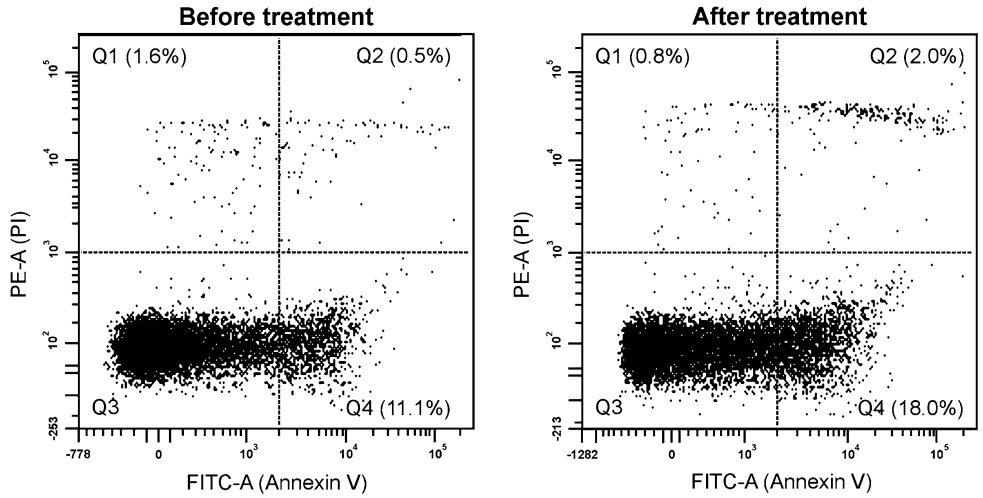

Fig. 2 Flow cytometric analysis of apoptosis/necrosis of whole blood granulocytes. A representative experiment is shown. *Dot plots* show percentage of apoptotic/necrotic peripheral whole blood granulocytes (gated by forward and side scatter (FSC/SSC) characteristics—see Fig. 1) before and after 3-month combined therapy with PEG-IFN-α and ribavirin. Gated granulocytes were subdivided into subpopulations based on Annexin V-FITC and propidium iodide (PI) staining characteristics. Subpopulations are defined as Annexin V(−)PI(+) (*Q1* necrosis), Annexin V(+)PI(+) (*Q2* late apoptosis), Annexin V(−)PI(−) (*Q3* viable cells) and Annexin V(+)PI(−) (*Q4* early apoptosis)

during treatment such as blood morphology, ALT activity, and levels of GPT, showed results typical for CHC treatment with IFN-α (data not shown).

Discussion

Neutrophils are cells with a short life span. They migrate at the infection site, eliminate pathogen and undergo spontaneous apoptosis. Neutrophils eliminate pathogens using an aerobic system and/or oxygen-independent killing systems (defensins) (Zeman 1993). ROS promote the synthesis of hypochlorous acid, which reacts with primary amines to form relatively stable chloramines. The chloramines promote long-lasting oxidation, which results in nuclear factor-κB inhibitor inactivation and increased release of pro-inflammatory cytokines, which is accompanied by increased cytotoxic activity (Al Nedawi et al. 2004; Jiang et al. 2003; Marcinkiewicz 1997). The stimulation of neutrophils by IFN-α or TNF-α induces the receptor and integrin expressions, which augment the cell response after agonist stimulation (Brar et al. 1993; Little et al. 1994). After completing its task, PMNs must be removed to prevent them from causing damage to healthy tissues.

Increased ROS production was demonstrated in patients with CHC. De Maria et al. (1996) observed processes which involved lipid and protein oxidation. In another study, increased levels of lipid peroxidation products and increased activity of superoxide dismutase were found in mononuclear peripheral blood cells from patients with CHC. Simultaneously, glutathione level was decreased and the level of oxidized glutathione was increased in 35% of the patients with CHC (Mutlu-Turkoglu et al. 1997). Those unfavorable processes were the consequence of increased ROS formation and insufficient total antioxidant status.

The effect of PEG-IFN-α and ribavirin treatment on neutrophils function is not precisely known.

Crucial to therapeutic standards is that IFN-α was found to cause an increase in ROS formation in vitro. Stimulation of ROS release could account for the inhibition of HCV replication (Choi et al. 2004; Little et al. 1994). Theoretically, applying IFN-α could intensify the unfavorable

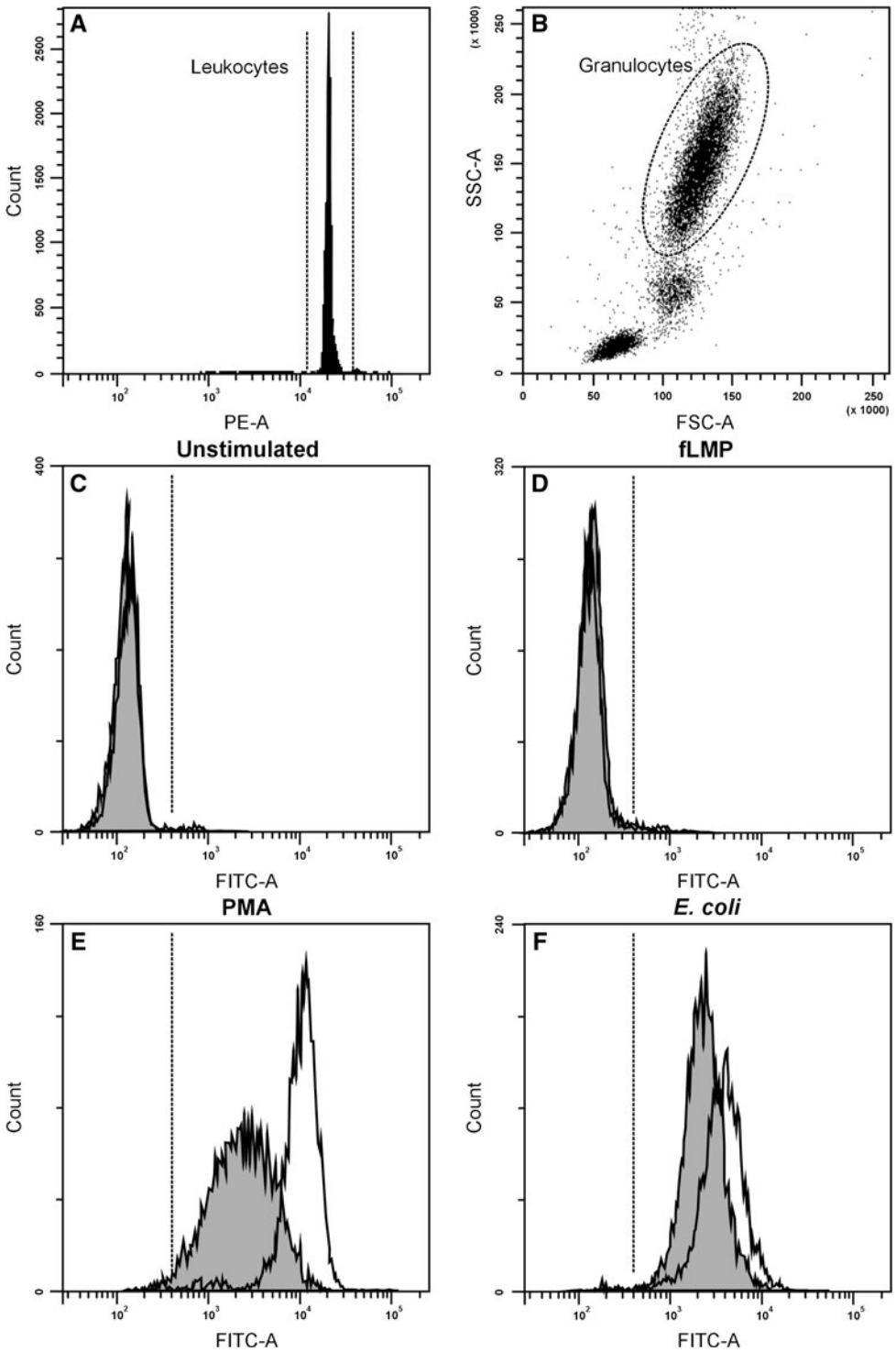

Fig. 3 Flow cytometric analysis of ROS production by whole blood granulocytes. A representative experiment is shown. Human peripheral whole blood granulocytes were identified and gated based on propidium iodide staining (**a**) and forward and side scatter (FSC/SSC) characteristics (**b**). Histograms present fluorescence intensity of dihydrorhodamine 123 staining of unstimulated cells (**c**) or cells stimulated with one of the following stimulants: *N*-formyl-methionyl-leucyl-phenylalanine (fMLP; **d**) phorbol 12-myristate 13-acetate (PMA; **e**) or suspension of opsonized *Escherichia coli* (**f**), before (*solid*) and after (*unfilled*) a 3-month combined therapy with PEG-IFN-α and ribavirin. Gates were set up according to unstimulated cells (*dashed line*)

phenomenon of antioxidative stress. However, in several studies, therapy with IFN-α resulted in lowering the increased levels of the compounds which react with thio-barbituric-acid and in diminishing glutathione peroxidase activity with an increase in the total amount of sulfur-hydrogen groups (Mutlu-Turkoglu et al. 1997), which would suggest an inhibitory effect on oxidative stress. In our study, PEG-IFN-α and rivabirin treatment contributed to the up-regulation of CD11b and CD18 expression on the PMNs surface and increase in ROS production upon stimulation with PMA, which indicated the activation of PMNs. On the other hand, the lower expression of CD16 high and CD62L molecules and acceleration of PMNs apoptosis were observed during antiviral treatment.

Table 4 ROS production by peripheral blood granulocytes

	Before treatment	3 months of treatment	Significance
Unstimulated			
%	0.4 (0.2–0.7)	0.5 (0.1–0.9)	NS
MFI	1,223 (1,073–1,477)	1,293 (1,050–1,754)	NS
fMLP			
%	0.8 (0.5–1.2)	1.3 (0.6–2.6)	$p = 0.04$
MFI	1,520 (1,372–1,675)	1,485 (1,313–1,872)	NS
PMA			
%	98.1 (97.1–99.5)	98.8 (97.6–99.8)	$p = 0.02$
MFI	4,148 (3,484–6,338)	9,572 (7,523–12,985)	$p < 0.0001$
E. coli			
%	94.4 (88.6–99.3)	92.3 (83.1–97.9)	$p = 0.03$
MFI	2,941 (2,245–3,563)	3,154 (2,289–4,373)	NS

Data are presented as median and interquartile range (Q1–Q3)

MFI mean fluorescence intensity, *NS* nonsignificant

Piazzolla et al. (1996) found a decrease in ROS formation in patients who were effectively treated with IFN-α. However, ROS production was increased under the influence of adhesion molecules in their study. In our previous study an increase in ROS production during treatment with IFN-α and TFX was observed (Jablonowska et al. 2005). In another study conducted by Giorgini et al. (2009), similarly to our investigation, during treatment with PEG-IFN-α and ribavirin, an increase of PMA-induced oxidative burst was observed. Giorgini et al. (2009) observed an increase not only in ROS production, but also in PMNs chemotaxis during treatment. The authors speculated that the conjunction of decreased number of neutrophils (caused by myelotoxic effect of IFN) and their activation could suggest the possibility of a drug-induced cellular selection, in which the more resistant and functionally active cells survived the myelotoxic effect of drugs (Giorgini et al. 2009).

Spontaneous neutrophil apoptosis can be delayed or accelerated by some cytokines. For instance, granulocyte colony-stimulating factor can extend the neutrophils' life span, and IFN-α in vitro is known to reduce neutrophil apoptosis. Tecchio et al. (2004) demonstrated that upon incubation with therapeutic concentrations of IFN-α, human neutrophils are induced to accumulate TRIAL/Apo-2 ligand (TNF-related apoptosis-inducing ligand) mRNA and release a soluble form of TRIAL that fully retains proapoptotic activities toward TRIAL-sensitive leukemic cell lines. The authors concluded that IFNs act as specific stimuli for sTRAIL production by neutrophils. Release of endogenous sTRIAL by activated neutrophils did not correlate with an increased apoptosis of the same cells, as IFN-α resulted in significantly prolonged neutrophil survival (Tecchio et al. 2004). On the contrary, Renshaw et al. (2003) demonstrated acceleration of human neutrophil apoptosis by a soluble version of TRAIL. It was previously shown that phagocytosis initiated a molecular cascade of events that accelerated apoptosis in human PMNs. During the induction of phagocytosis-induced apoptosis, 32 genes encoding key surface molecules including receptors for IL-8β, IL-10α, IL-13α1, IL-15α, IL-17, IL-18, C1q, and formyl peptide, Toll-like receptor 6, P-selectin (CD62), were down-regulated (Kobayashi et al. 2002). Moreover, ROS production not only has implications related to microbial cell killing and collateral oxidative damage, but also has potential functions in modulation of the inflammatory response, including apoptosis (Melley et al. 2005). Rates of PMNs apoptosis may be increased by ROS (Watson 2002). The induction of apoptosis by ROS may be of fundamental importance to neutrophil removal from a side of inflammation.

In this study, neutrophil activation observed during IFN-α and ribavirin treatment coexisted with increased apoptosis of these cells. In some studies conducted in in vitro conditions, apoptosis of PMNs was delayed by IFN (Sakamoto et al. 2005; Tecchio et al. 2004; Wang et al. 2003). This analysis does not correspond with our results, but experiments conducted in vitro cannot be directly compared to studies performed in vivo. Another significant difference is that unlike the above-mentioned study, our patients developed CHC and received PEG-IFN-α with ribavarin. During PEG-IFN-α and ribavirin treatment, increasing percentage of apoptotic neutrophiles coinciding with decreased expression of CD16 high molecules were observed. Moreover, during antiviral treatment we also observed decreased expression of CD62L. The decrease of those molecules on PMNs is known to be characteristic of neutrophils that have undergone apoptosis (Hart et al. 2000).

Co-occurence of the increased ROS production and the decrease in HCV viremia in the 12th week of treatment with IFN and ribavirin can indicate that neutrophils play an important role in elimination of HCV infection. It is well known that excessive ROS production can cause oxidative damage of cells. However, ROS have also been proved to act as key mediators in signaling cascades and subtoxic levels of ROS can regulate inflammatory response. ROS production has potential autocrine and paracrine functions in the modulation of the inflammatory response, which would explain the observed increase in apoptosis of these cells.

It is worth noting that lymphocytes and neutrophils also become infected in the course of HCV, which can alter their function. However, in the study by Toro et al. (1998) no differences were found in the production of ROS between HCV-infected neutrophils and neutrophils with no HCV-RNA sequences.

We conclude that during IFN treatment, activation of neutrophil function and increased ROS production were reported, which possibly resulted in accelerated apoptosis of these cells.

Acknowledgments The study was supported by an internal grant from the Medical University of Łódź (502-11-724) and Nofer Institute of Occupational Medicine (IMP 11.3). The skillful technical assistance of Ms Jolanta Wojno-Kulinska is gratefully acknowledged.

References

Al Nedawi KN, Czyz M, Bednarek R et al (2004) Thymosin beta 4 induces the synthesis of plasminogen activator inhibitor 1 in cultured endothelial cells and increases its extracellular expression. Blood 103:1319–1324

Bazhanova ED (2005) Interferon alpha involvement in apoptosis regulation. Zh Evol Biokhim Fiziol 41:101–106

Brar DW, Borden EC, Proctor RA (1993) Recombinant interferon-gamma preserves human granulocyte bactericidal and chemoluminescence activities. J Infect Dis 168:128–134

Cascao R, Rosario HS, Fonseca JE (2009) Neutrophils: warriors and commanders in immune mediated inflammatory diseases. Acta Reumatol Port 34:313–326

Choi J, Lee KJ, Zheng Y et al (2004) Reactive oxygen species suppress hepatitis C virus RNA replication in human hepatoma cells. Hepatology 39:81–89

De Maria N, Colantoni A, Fagiuoli S et al (1996) Association between reactive oxygen species and disease activity in chronic hepatitis C. Free Radic Biol Med 21:291–295

Farinati F, Cardin R, De Maria N et al (1995) Iron storage, lipid peroxidation and glutathione turnover in chronic anti-HCV positive hepatitis. J Hepatol 22:449–456

Giorgini A, Capsoni F, Podda M et al (2009) Treatment with PEG-interferon and ribavirin for chronic hepatitis C increases neutrophil and monocyte chemotaxis. Ann NY Acad Sci 1173:847–857

Hart SP, Ross JA, Ross K et al (2000) Molecular characterization of the surface of apoptotic neutrophils: implications for functional downregulation and recognition by phagocytes. Cell Death Differ 7:493–503

Jablonowska E, Tchorzewski H, Lewkowicz P et al (2005) Reactive oxygen intermediates and serum antioxidative system in patients with chronic C hepatitis treated with IFN-alpha and thymus factor X. Arch Immunol Ther Exp 53:529–533

Jiang Z, Seo JY, Ha H et al (2003) Reactive oxygen species mediate TGF-beta1-induced plasminogen activator inhibitor-1 upregulation in mesangial cells. Biochem Biophys Res Commun 309:961–966

Kobayashi SD, Voyich JM, Buhl CL et al (2002) Global changes in gene expression by human polymorphonuclear leukocytes during receptor-mediated phagocytosis: cell fate is regulated at the level of gene expression. Proc Natl Acad Sci USA 99:6901–6906

Larrea E, Beloqui O, Munoz-Navas MA et al (1998) Superoxide dismutase in patients with chronic hepatitis C virus infection. Free Radic Biol Med 24:1235–1241

Little R, White MR, Hartshorn KL (1994) Interferon-alpha enhances neutrophil respiratory burst responses to stimulation with influenza A virus and FMLP. J Infect Dis 170:802–810

Marcinkiewicz J (1997) Neutrophil chloramines: missing links between innate and acquired immunity. Immunol Today 18:577–580

Melley DD, Evans TW, Quinlan GJ (2005) Redox regulation of neutrophil apoptosis and the systemic inflammatory response syndrome. Clin Sci 108:413–424

Mutlu-Turkoglu U, Ademoglu E, Turkoglu S et al (1997) The effects of interferon-alpha on serum lipid peroxidation and total thiol content in patients with chronic active hepatitis-C. Res Commun Mol Pathol Pharmacol 96:357–361

Piazzolla G, Giannelli G, Antonelli G et al (1996) Effects of interferon-alpha treatment on neutrophil oxidative metabolism, lymphocyte proliferation and monocyte HLA class I antigen expression in patients with chronic hepatitis C. Immunopharmacol Immunotoxicol 18:529–548

Renshaw SA, Parmar JS, Singleton V et al (2003) Acceleration of human neutrophil apoptosis by TRAIL. J Immunol 170:1027–1033

Sakamoto E, Hato F, Kato T et al (2005) Type I and type II interferons delay human neutrophil apoptosis via activation of STAT3 and up-regulation of cellular inhibitor of apoptosis 2. J Leukoc Biol 78:301–309

Shimoda R, Nagashima M, Sakamoto M et al (1994) Increased formation of oxidative DNA damage, 8-hydroxydeoxyguanosine, in human livers with chronic hepatitis. Cancer Res 54:3171–3172

Swietek K, Juszczyk J (1997) Reduced glutathione concentration in erythrocytes of patients with acute and chronic viral hepatitis. J Viral Hepat 4:139–141

Tecchio C, Huber V, Scapini P et al (2004) IFNalpha-stimulated neutrophils and monocytes release a soluble form of TNF-related apoptosis-inducing ligand (TRAIL/Apo-2 ligand) displaying apoptotic activity on leukemic cells. Blood 103:3837–3844

Toro F, Conesa A, Garcia A et al (1998) Increased peroxide production by polymorphonuclear cells of chronic hepatitis C virus-infected patients. Clin Immunol Immunopathol 88:169–175

Wang K, Scheel-Toellner D, Wong SH et al (2003) Inhibition of neutrophil apoptosis by type 1 IFN depends on cross-talk between phosphoinositol 3-kinase, protein kinase C-delta, and NF-kappa B signaling pathways. J Immunol 171:1035–1041

Watson RW (2002) Redox regulation of neutrophil apoptosis. Antioxid Redox Signal 4:97–104

WHO (2000) Hepatitis C—global surveillance (update). Wkly Epidemiol Rec 75:18–19

Zeman K (1993) Current views of the role of neutrophils in inflammatory processes. Pol J Immunol 18:3–44