

# Characterization of Liver Leukocyte Infiltrates and Features of Cytokine Profile under Viral Hepatitis-Induced Immunosuppression

Olga V. Lebedinskaya, Irina N. Kabanovskaya, Anna S. Lasareva, Nelly K. Akhmatova,  
Anatoliy P. Godovalov, Andrey V. Horinko, and Mikhail V. Kiselevsky

**Abstract**—The nature, prevalence, cellular composition of leukocyte infiltrates and immunohistochemical characteristics of their constituent cells in the liver of patients with chronic viral hepatitis B and C were investigated. It was found that the area of distribution and cellular composition of infiltrates depended on the virus type and process activity. The expediency of immunohistochemical study using leukocyte infiltrates from liver biopsies of patients with viral hepatitis aimed at clarifying diagnosis, making prognosis, and choice of optimal treatment with elements of immune correction is emphasized.

**Keywords**—Viral hepatitis, leukocyte infiltration, immunohistochemical characteristics, immunosuppression.

## I. INTRODUCTION

LOCAL reactions of the immune system under viral hepatitis infection are manifested as lymphoid tissue hyperplasia in liver parenchyma. Inflammatory process in liver is followed by dystrophic and degenerative changes, hepatocyte necrosis, and thereafter the stroma collagenization at the sites of parenchymal destruction occurs [2]. This pathological state is accompanied by composition alteration in immunocompetent cells and their functional disturbance that probably result in the formation of the immunodeficient state [1]. The development of immunosuppressive and immunopathological reactions leads to difficulty in viral elimination and process chronization [3]. To work out novel methods of diagnosis and therapy for liver viral damage there seems topical to study pathological reactions that provoke the formation of chronic disease.

O.V. Lebedinskaya is with the Acad. E.A. Wagner Perm State Medical Academy, 61-74, P.Osipenko str., Perm, Russia (e-mail: lebedinska@mail.ru).

I.N. Kabanovskaya is with the Acad. E.A. Wagner Perm State Medical Academy, 26, Petropavlovskaya str., Perm, Russia (e-mail: lebedinska@mail.ru).

Anna S. Lasareva is with the State Institution N.N. Blokhin Russian Research Cancer Centre, RAMS, 24, Kashirskoe str., Moscow, Russia.

N.K. Akhmatova is with the I.I. Mechnikov Scientific-Research Institute of Vaccine and Sera, RAMS, 5a, Maliy Casenniy, Moscow, Russia (phone: +7(495) 916-07-74; e-mail: anelly@mail.ru).

A.P. Godovalov is with the Acad. E.A. Wagner Perm State Medical Academy, 26, Petropavlovskaya str., Perm, Russia (e-mail: AGodovalov@gmail.com).

A.V. Horinko is with the Acad. E.A. Wagner Perm State Medical Academy, 26, Petropavlovskaya str., Perm, Russia.

M.V. Kiselevsky is with the State Institution N.N. Blokhin Russian Research Cancer Centre, RAMS, 24, Kashirskoe str., Moscow, Russia.

There is evidence accumulated on disparity between the level of proinflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$  and IL-6) in blood serum and morphological changes in the liver of patients with chronic viral hepatitis.

Aim of investigation – reveal the morphological peculiarities of leukocyte infiltrates in virus hepatitis B- or C-damaged liver depending on the activity of pathological process and to study the level of proinflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , and IL-6) and IFN- $\alpha$  in patients with chronic hepatitis B and C that demonstrate different degrees of histological activity.

## II. MATERIALS AND METHODS

### A. Patients

Liver biopsies from 37 patients with chronic viral hepatitis were analyzed, of which 13 were with chronic hepatitis B (CHB) and 24 – with chronic hepatitis C (CHC). Diagnosis «chronic viral hepatitis» was made based on syndromes of hepato- and splenomegaly, cholestasis and cytolysis. Anti-viral therapy was not realized prior to patients' examination. Etiological verification of the diagnosis was supported by detection of serological markers (HBsAg, anti-HBcor, IgM, anti-Hbcor, total, anti-HCV IgG) with immuno-enzyme assay (IEA).

13 patients with chronic viral hepatitis C and 10 patients with chronic viral hepatitis B under the age of 22 to 61 yrs were examined. Each group was subdivided in subgroups depending on morphological changes in liver being detected in biopsy: patients with histological activity index (HAI) up to 9 points (7 individuals with CHC and 8 ones with CHB) and group with HAI over 9 points (6 individuals with CHC and 2 ones with CHB).

### B. Histological Methods

Disease activity was determined using conventional method of semi-quantitative evaluation of histologic activity of inflammatory process and fibrosis according to R. Knodel (adapted by K. Ishak) relatively to hepatitis B and C. Paraffin sections of liver biopsies from patients with different disease activities (minimal, low, moderate, and high) were stained with hematoxylin-eosin and Van-Hison method.

### C. Immunohistochemical Examination

Immunohistochemical examination was carried out using cryosections with the use of labeled monoclonal antibodies to lymphocyte surface markers (CD3, CD16, CD56, CD4, CD25, CD8, CD20), dendritic cells (CD1a) and macrophages (CD14). Double staining with antibodies labeled by fluorochromes such as phycoerythrin (PE) and fluorescein isothiocyanate (FITC) was applied.

### D. Cytokine Profile

To resolve the task the cytokine profile (TNF- $\alpha$ , IFN- $\alpha$ , IL-1 $\beta$ , and IL-6) was determined with IEA method using test-systems (ProCon, Russia), where the cytokine control level amounted up to 50 pg/ml.

## III. RESULTS

In case of minimal activity of the chronic process the connective tissue of portal tracts demonstrates lymphocyte infiltration being minor with CHB and more pronounced with blast forms presence with CHC. Certain leukocytes could be found within the sinusoid lumen. Walls of some sinusoids demonstrate enlarged Kupffer cells (Fig. 1).

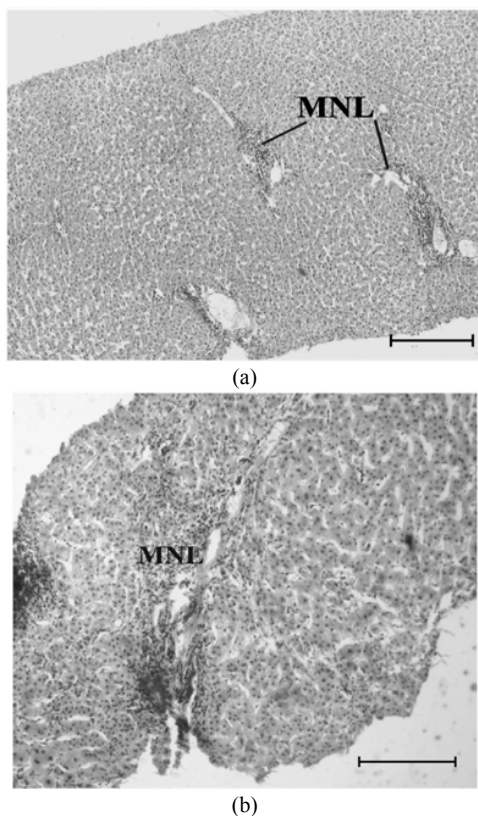


Fig. 1 Microphotographs of histologic slides of liver samples of patients with hepatitis B and C (1a - leukocyte infiltrates in liver of a patient with CHB, stained by Van Gison, scale bar: 100  $\mu$ m; 1b - leukocyte infiltrates in liver of a patient with CHC, stained by hematoxylin-eosin, scale bar 100 $\mu$ m; MNL – mononuclear leucocytes)

Low activity pathological process in liver damaged with hepatitis B or C virus is also found to result in extensive leukocyte infiltration of proliferating connective tissue around triads, when this tissue is mainly composed of lymphocytes and minor amount of granulocytes. Inside lobules one could observe enlarged Kupffer cells that are detached from sinusoid walls. Chronic hepatitis C is manifested in minor lymphocyte clusters within lobules, and in huge number of blast forms in periportal infiltrates. Hepatic morphological changes under moderate activity of pathological process are exhibited in extensive periportal leukocyte infiltration that is represented by lymphoid cells, and with CHC the multiple blast forms are present as well (Fig. 2).

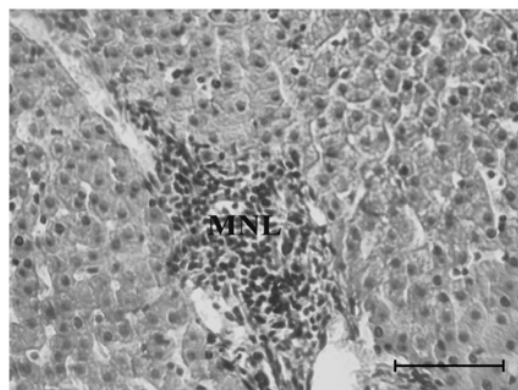


Fig. 2 Microphotograph of histologic slid of liver sample of patients with hepatitis C (leukocyte infiltrates in liver of a patient with hepatitis C; stained by hematoxylin-eosin; scale bar: 30  $\mu$ m)

Chronic hepatitis B is characterized by that inside liver lobules some leukocytes could be found that predominantly are of lymphocyte origin. Liver lobules of patients infected with hepatitis C are defragmented where lymphocyte-infiltrated proliferated connective tissue could be found. Dilated intra-lobular sinusoids are filled with lymphocytes, macrophages and activated Kupffer cells both with hepatitis B and C. Patients with highly active chronic process in hepatitis virus-affected liver demonstrate lymphoid infiltration both in proliferated connective tissue in portal tracts (Fig. 3) and inside altered liver lobules (Fig. 4).

Infiltrates demonstrate primarily lymphocytes, blast forms and macrophages. Lymphocyte clusters are highly compact and possess more clear boundaries than with low and moderate activity of the disease.

Immunohistochemical investigation demonstrated that liver infiltrates of patients with chronic viral hepatitis B or C comprise predominantly the following lymphocyte subsets: T-lymphocytes (CD3+), NKT-cells (CD3+/CD16+/CD56+; fig. 5, 6), NK-cells (CD16+/CD56+), T-regulatory lymphocytes (CD4+/CD25+), cytotoxic lymphocytes (CD8+).

Cytokine profile in blood serum of all patients analyzed showed that the TNF- $\alpha$ , IFN- $\alpha$ , IL-1 $\beta$ , and IL-6 content did not exceed the control values (up to 50 pg/ml).

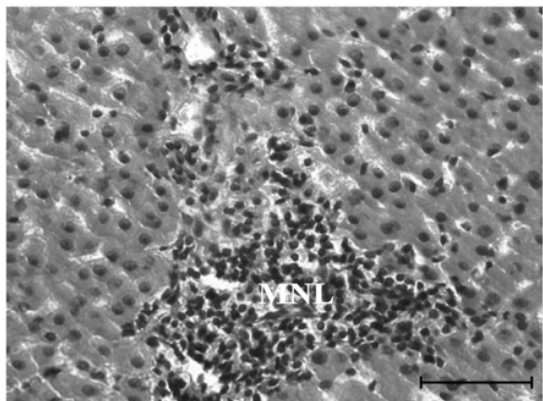


Fig. 3 Microphotograph of histologic slide of liver samples of patients with hepatitis B (leukocyte infiltrates in liver of a patient with hepatitis B; stained by hematoxylin-eosin; scale bar: 70  $\mu$ m)

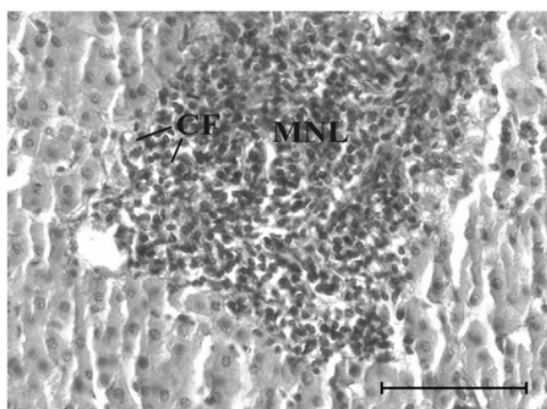


Fig. 4. Microphotograph of histologic slide of liver samples of patients with hepatitis C (leukocyte infiltrates in liver of a patient with hepatitis B; stained by Van Gison; scale bar: 70  $\mu$ m)

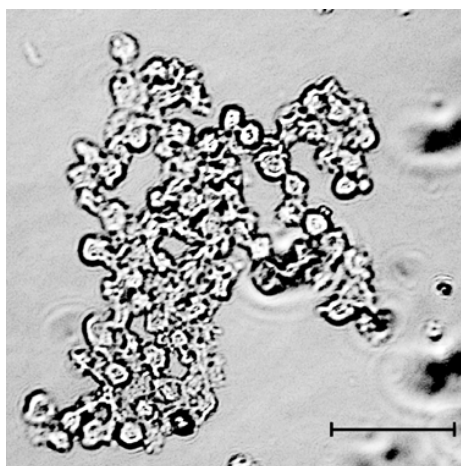


Fig. 5 Mononuclear leukocytes (MNL) of leukocyte infiltrates in liver of a patient with viral hepatitis B. Microphotographs of MNLs stained by fluorescent monoclonal antibodies to cell-surface markers CD3/CD16/CD56 in cytopspins in bright field. Scale bar: 50  $\mu$ m

However, the group with CHC and HAI over 9 points demonstrated reliable decrease of TNF- $\alpha$  ( $6.3 \pm 3.2$ ) and IL-1 $\beta$  ( $12.8 \pm 1.1$ ) as compared with the group with CHC and HAI below 9 points, where TNF- $\alpha$  had  $12.3 \pm 11.0$  and IL-1 $\beta$  –  $44.6 \pm 8.7$  values. IFN- $\alpha$  ( $24.8 \pm 9.3$ ) and IL-6 ( $13.3 \pm 3.4$ ) in patients with CHC and HAI over 9 points, and IFN- $\alpha$  ( $34.8 \pm 9.9$ ) and IL-6 ( $14.3 \pm 2.2$ ) in subjects with CHC and HAI below 9 points did not reliably differ.

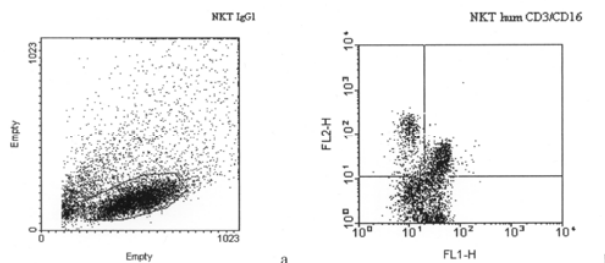


Fig. 6 DotPlot flow cytometry analysis of cell-surface markers CD3 (FITC), NK (PE) of mononuclear leukocytes (MNL) isolated from liver samples of patients with hepatitis B: a – hepatic MNL distribution; b – hepatic MNL distribution with double staining (CD3/CD16)

Spontaneous production of IFN- $\alpha$  varied within physiological values among those being examined. The level of TNF- $\alpha$  a spontaneous production in the group of patients with CHC and HAI below 9 points varied from 0.7 pg/ml to 1017 pg/ml; similar variations were observed in patients with CHC and HAI over 9 points (from 0.3 to 990 pg/ml). Individuals with CHB demonstrated the variation in the level of spontaneous production within the control values, but in the group with HAI over 9 points this level was by 2-fold lower ( $7 \text{ pg/ml} \pm 0.5$ ) than in the group with HAI below 9 points ( $16.6 \text{ pg/ml} \pm 6.2$ ). The level of IL-1 $\beta$  spontaneous production varied from 0.4 pg/ml to 1803 pg/ml in the group of patients with CHC and HAI <9 points (1803 pg/ml), similar variations were revealed with the group of subjects with CHC and HAI >9 points (from 11 to 1320 pg/ml). In the group with CHB and HAI <9 points the IL-1 $\beta$  level varied within the control values, whereas patients with CHB and HAI >9 points demonstrated 2-fold exceeding of the control values (102 pg/ml and 171 pg/ml).

IL-6 spontaneous production approached highest values in patients with CHC and HAI >9 points, where values varied from 16 to 2095 pg/ml, and in subjects with CHC and HAI <9 points the level was within the control ones. Among the patients with HCB and HAI <9 and HAI >9 points the IL-6 level complied with the control values.

#### IV. DISCUSSION

Thus, liver leukocyte infiltrates of CHB or CHC patients are dominated by cells of lymphoid origin; vast number of blast forms and macrophages occur among leukocytes following the disease activation. Population of lymphoid cells in leukocyte infiltrates is mainly represented by T-

lymphocytes, NK- and NKT-cells. Area of distribution and cell content in leukocyte infiltrates of patient liver are found to depend on virus type and activity of chronic process. Patients with hepatitis C have more extensive leukocyte infiltration with blast form predominance than hepatitis B patients. Liver infection with hepatitis C virus results not only in periportal area-located infiltrates but to the intra-lobular occurrence of large numbers of leukocytes even under minimal activity of pathological process. While conducting the morphological assay of liver biopsy from CHB and CHC patients aimed at making diagnosis, prognosis for a disease and selection of optimal therapy, including immunotherapy it seems appropriate to focus not only on the fibrosis activity, but also on the features of leukocyte infiltrates in affected organ such as the degree of their distribution, nature, cell composition, immunohistochemical peculiarities of their cells.

Results obtained evidence for different roles of proinflammatory cytokines in realization of pathomorphological changes in liver following the infection with viral hepatitis B and C. CHB and CHC patients demonstrated that the liver leukocyte infiltrates (LI) are predominantly represented by T-lymphocytes (CD3+), NKT-cells (CD3+CD16+CD56+), NK-cells (CD16+CD56+), T-regulatory lymphocytes (CD4+CD25+), and cytotoxic T-lymphocytes (CD8+). Cytotoxic lymphocytes (CD8+) and NK-cells (CD16+CD56+) being revealed in liver LI from CVH individuals were found to be similar in composition with cells from liver tissue infiltrates under autoimmune hepatitis. For the first time we have observed the T-regulatory cell (CD4+CD25+) complex in liver parenchyma of those patients that provoked the suppression of cell immune response following the virus and damaged tissue elimination and provided the progression of persisting viral infection with autoimmune component.

#### V. CONCLUSION

Complex of T-regulatory (CD4+CD25+) cells being revealed by us in leukocyte infiltrates evidences for the presence of morphofunctional basis for the formation of autoimmune manifestations against a background of persisting viral infection.

#### ACKNOWLEDGMENT

The work was supported by RFBR grant 11-04-96037r\_ural\_a and Perm Region Administrative Body.

#### REFERENCES

- [1] K.H. Meyer zum Buschenfelde "Immunopathology of chronic liver diseases", *Verh. Dtsch. Ges. Pathol.*, vol. 79, 1995, pp. 186–197.
- [2] M. Muller, H.E. Blum, F. Er, R. Thimme, N. Semmo "Cellular immune response in hepatitis C infection: Role of CD154 expression and clinical relevance", *Dtsch. Med. Wochenschr.*, vol. 136(20), 2011, pp. 1073–1077.
- [3] A. Weber, Y.T. Boege, F. Reisinger, M. Heikenwälder "Chronic liver inflammation and hepatocellular carcinoma: persistence matters", *Swiss. Med. Wkly.*, vol. 141, 2011, pp. 1–9.