ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LIX, N 1, 37 SECTIO D 2004

Chair and Department of Infectious Diseases, Skubiszewski Medical University of Lublin

HANNA FOTA-MARKOWSKA, ROMA MODRZEWSKA,
ROMANA ROLLA-SZCZEPAŃSKA, DARIUSZ BIELEC,
JOANNA KRZOWSKA-FIRYCH

Dynamics of proinflammatory cytokines serum levels during viral hepatitis type A

The aim of the work was to assess the dynamics concerning IL-2, sIL-2R and IL-4 serum levels in the course of an acute viral hepatitis A infection.

Hepatitis A virus (HAV) is classified under the genus *Hepatovirus* of the *Picornaviridae* family (5). HAV antigen was detected in the liver, spleen, kidney, abdominal lymph nodes, duodenum and tonsils of experimentally infected primates (10, 11). Its presence was confirmed in the cytoplasm of hepatocytes and Kupffer's cells (10, 11). Liver biopsy specimens taken from patients with acute classic viral hepatitis A showed the following morphologic changes: focal necrosis, acidophilic or ballooning degeneration, increase in binucleation and mitotic activity, hypertrophy of Kupffer's cells whose cytoplasm is packed with lipofuscin, and inflammatory infiltrates, mainly with lymphocytes, in portal areas (9).

The pathogenic mechanism leading to liver tissue injury in hepatitis A is unclear. It is thought that the cause of hepatocytes injury is not the cytopathic effect of HAV. There are some HAV strains which cause culture cells destruction, but it was showed that HAV infection of human fibroblasts culture or hepatoma cell line leads to an apparent, persistent infection (4, 7, 14). At the beginning of HAV infection in humans large quantities of infectious virus are produced in the liver and excreted in stool before the recognizable onset of hepatic disease (5).

The recent studies have established that the cause of liver tissue injury in hepatitis A course is immune response to HAV infection. B a b a et al., showed that natural killer cells and lymphokine activated killer cells from the blood of patients with hepatitis A caused lysis of HAV infected fibroblasts *in vitro* (1). F l e i s c h e r et al. found in liver biopsies from patients with acute phase of hepatitis A that $CD8^+$ T lymphocytes dominated over $CD4^+$ lymphocytes. Approximately 50% of all $CD8^+$ clones showed specific cytotoxicity against autologous fibroblasts infected with HAV and produced interferon γ (6).

Cytokines play an important role both in hepatic inflammation and hepatic regeneration (13). There are some papers concerning significance of these mediators in hepatitis A. In this study we describe serum concentration of interleukin 2 (IL-2), soluble interleukin 2 receptor (sIL-2R) and interleukin 4 (IL-4) in patients with hepatitis A.

MATERIAL AND METHODS

We examined 48 patients, 24 of them at the age of 19 to 35 years (13 men and 11 women), who were hospitalised at the Department of Infectious Diseases, Medical University of Lublin for the

acute virus hepatitis A infection. The serum concentrations of IL-2, sIL-2R and IL-4 were assessed with the use of ELISA methods: IL-2 levels with Intertest-2 Human Interleukin-2 ELISA Kit (Genzyme), sIL-2R levels with the Predicta Interleukin-2 Receptor Kit (Genzyme) and IL-4 levels with the use of Intertest-4 Human Interleukin-4 ELISA Kit (Genzyme). In patients the assessments were done twice – in the first and in the third week of the hospitalisation (assessments first and second). In the control group which consisted of 24 healthy persons at the age of 18 to 37 years (12 men and 12 women), the assessments were done once. The obtained data were analysed with the use of c-Cochran and Cox statistical test.

Table 1. Il-2, sIL-2, IL-4 serum level during acute viral hepatitis type A

Parameter	Group	Assay	X	SD	Comparison		
					I z K	II z K	I z II
IL-2 [pg/ml]	Ct	K	147.5	27.3			
	P	I	98.7	32.2	$p < 0.01$ **	$p > 0.05$	$p > 0.05$
		II	123.3	34.0			
sIL-2R [pg/ml]	Ct	K	816.5	233.5			
	P	I	2380.5	885.3	$p < 0.01$ **	$p < 0.01$ **	$p > 0.05$
		II	1939.5	845.9			
IL-4 [ng/ml]	Ct	K	68.17	34.23			
	P	I	35.5	20.5	$p < 0.05$ *	$p > 0.05$	$p > 0.05$
		II	49.8	26.4			

Ct – control group
P – patients with viral hepatitis type A

RESULTS

We observed that IL-2 serum level was independent of sex in the control group. As the internal norm we assumed the values between 147.5 ± 27.3 pg/ml. The level of IL-2 in the first assessment in patients was 98.7 ± 32.2 pg/ml, in the second assessment – 123.3 ± 34.0 pg/ml. We observed a statistically important decrease in levels of IL-2 in the first assessment in patients compared to the assessment in the control group ($p < 0.001$), whereas the differences observed in the second assessment in patients and the assessment in the control group were of a random type ($p < 0.05$). There was no statistical difference observed in IL-2 levels in the first and the second assessment ($p > 0.05$).

In the control group the level of sIL-2R was independent of sex, its mean value was 816.5 ± 233.5 pg/ml. The sIL-2R level in the first and second assessments in patients amounted to 2380 ± 885.3 pg/ml and 1939 ± 845.9 pg/ml, accordingly. We observed a statistically important increase of the sIL-2R level in the first and second assessment compared to the control group ($p < 0.01$). However, we did not observe a statistical difference in sIL-2R levels between both assessments in patients.

The IL-4 mean level in the control group was 68.17 ± 34.23 pg/ml. In patients, the mean sIL-4 levels were 35.5 ± 20.5 ng/ml (first assessment) and 49.0 ± 26.4 ng/ml (second assessment). We observed a statistically important decrease in IL-4 levels in the first assessment in comparison to the control group ($p < 0.05$). There was no statistical difference, either in IL-4 levels between the second assessment in patients and the assessment in the control group, or between both assessments in patients ($p > 0.05$).

DISCUSSION

In recent years, there have been only a few reports about dynamics of interleukin 2 (IL-2) and interleukin 4 (IL-4) serum level changes during acute liver inflammatory diseases, especially those caused by primary hepatotropic viruses, however, the crucial role of these cytokines in pathophysiology of different liver injuries is confirmed (13, 15).

IL-2, similarly to IL-4 and GM-CSF, is an pleiotropic, biologic active protein, which is most important regulator of growth and differentiation of T, NK and LAK cells, controls B cells proliferation and stimulates immunoglobulin secretion (13, 15).

Our research showed that the serum IL-2 level in control group was 147.5 ± 27.3 pg/ml. We have observed a significant decrease in the serum level of this interleukin during the acute stage of viral hepatitis type A in comparison to control.

It is known that immune reaction stimulated by IL-2 is mediated by binding to specific receptor sIL-2R. This receptor complexes of three polypeptide chains: α ,β, γ and the concentration of each of them generating the affinity to interleukin 2. Inteleukin 2 receptors are expressed mainly on activated lymphocytes T, B and also on stimulated monocytes. Released from targets cells serum IL-2R (sIL-2R) fraction represents a small soluble fraction (13).

Our research showed that the serum sIL-2R concentration in control group was estimated 816.5 ± 233.5 pg/ml. We observed a significant increase in the level of this parameter during acute stage of viral hepatitis type A in comparison to control group.

It must be underlined that the sIL-2R expression is induced not only by antigens but by interleukin 2 itself too, and that the T, B cells, stimulated with antigens, express receptors with high affinity to IL-2. Therefore, it is not possible to exclude that increase in the serum IL-2R level is stimulated rather by HAV antigens than its own IL-2 property. In that way, it seems to be possible that equivocal high sIL-2R concentration and decreased IL-2 level observed in our research during the acute viral hepatitis type A is a consequence of effective receptor binding. It was observed during acute stage of viral hepatitis type B also (8, 10,11).

Our research showed that the serum IL-4 concentration in control group was estimated at 68.17 ± 34.23 ng/ml. We observed a significant decrease in its level during acute viral hepatitis type A in comparison to control.

The experimental data attempt to identify a specific trait, distinct from known cytokines genes, encoded T cell membrane protein, which regulates and controls production of IL-4 by T cell. T cell protein, coded by human homologue of this trait, play a receptor role for hepatitis virus type A, so we cannot exclude that this is a possible way of decrease in the serum IL-4 level (12).

It will be valuable to extend the observation to estimate the correlation of some subpopulations of T cells in such a clinically defined group, especially that immune mechanisms of HAV infection are unclear so far.

CONCLUSIONS

1. We observed a statistically important decrease in IL-2 and IL-4 serum levels in patients with acute viral hepatitis A infection in the first week of the hospitalisation compared to values observed in the control group. We also observed an increase in sIL-2R levels in the course of the disease. The dynamics of IL-2, sIL-2R and IL-4 serum levels in patients with acute hepatitis A infection was of a random type.

2. The assessment of IL-2, sIL-2R and IL-4 serum levels can be helpful in examining the cellular response in viral hepatitis A infection.

REFERENCES

1. Baba M. et al.: Cytolytic activity of natural killer cells and lymphokine activated killer cells against hepatitis A virus infected fibroblasts. J. Clin. Lab. Immunol., 40, 47, 1993.
2. Cohen J. I. et al.: Hepatitis A virus infection in a chimpanzee: duration of viremia and detection of virus in saliva and throat swabs. J. Infect. Dis., 160, 887, 1989.
3. Coulepis A. G. et al.: Detection of hepatitis A virus in the faces of patients with naturally acquired infection. J. Infect. Dis., 141, 151, 1980.
4. Cromeans T. et al.: Replication kinetics and cytopathic effect of hepatitis A virus. J. Gen. Virol., 70, 2051, 1989.
5. Cuthbert J. A.: Hepatitis A: Old and new. Clin. Microbiol. Rev., 14, 38, 2001.
6. Fleischer B. et al.: Clonal analysis of infiltrating T lymphocytes in liver tissue in viral hepatitis A. Immunol., 69, 14, 1990.
7. Frosner G. G. et al.: Propagation of human hepatitis A virus in a hepatoma cell line. Infection, 7, 303, 1979.
8. Gauss-Muller V., Deinhardt F.: Effect of hepatitis A virus infection on cell metabolism *in vitro*. Proc. Soc. Exp. Biol. Med., 175, 10, 1984.
9. Ishak K.G.: Light microscopic morphology of viral hepatitis. Am. J. Clin. Pathol., 65, 787, 1976.
10. Karayiannis P. et al.: Hepatitis A virus replication in tamarins and host immune response in relation to pathogenesis of liver cell damage. J. Med. Virol., 18, 261, 1986.
11. Mathiesen L.R. et al.: Localization of hepatitis A antigen in marmoset organs during acute infection with hepatitis A virus. J. Infect. Dis., 138, 369, 1978.
12. McIntire J. J. et al.: Identification of Tapr (a airway hyperreactivity regulatory locus) and the linked Tim gene family. Nat. Immunol., 2 (12), 1109, 2001.
13. Simpson K. J. et al.: Cytokines and the liver. J. Hepatol., 27, 1120, 1997.
14. Vallbracht A. et al.: Persistent infection of human fibroblasts by hepatitis A virus. J. Gen. Virol., 65, 609, 1984.
15. Witor A. et al.: Stężenie wybranych cytokin w surowicy chorych na ostre wirusowe zapalenie wątroby typu A. Hepatologia Polska, 7, 2, 83, 2000.

SUMMARY

In this study we assessed the dynamics concern ıg IL-2, sIL-2R and IL-4 serum levels in the course of an acute viral hepatitis A infection. The st ıdy group consisted of 48 persons. The serum concentrations of IL-2, sIL-2R and IL-4 were assessed with the use of ELISA methods. In patients the assessments were done twice – in the first and third week of the hospitalisation. In the control group, which consisted of 24 healthy persons the assessments were done once. The obtained data were analysed with the use of c-Cochran and Cox statistical test. We observed a statistically important decrease in IL-2 and IL-4 serum levels in patients with acute viral hepatitis A infection in the first week of the hospitalisation compared to values observed in the control group. We also observed an increase in sIL-2R levels in the course of the disease. The dynamics of IL-2, sIL-2R and IL-4 serum levels in patients with acute hepatitis A infection was of a random type. The assessment of IL-2, sIL-2R and IL-4 serum levels can be helpful in examining the cellular response in viral hepatitis A infection.

Dynamika poziomu wybranych cytokin prozapalnych w surowicy krwi chorych w przebiegu ostrego wirusowego zapalenia wątroby (wzw A)

W przedstawionej pracy przeanalizowano dynamikę stężenia IL-2, sIL-2R oraz IL-4 w surowicy krwi chorych w przebiegu ostrego wirusowego zapalenia wątroby typu A. Badaniami objęto grupę 48 osób. Stężenie IL-2, sIL-2R oraz IL-4 oznaczano w surowicy krwi metodą ELISA. W grupie chorych badania wykonywano dwukrotnie: w pierwszym i w trzecim tygodniu hospitalizacji. W grupie kontrolnej, obejmującej 24 osoby zdrowe – jednorazowo. Uzyskane dane liczbowe poddano analizie statystycznej za pomocą testu c-Cochrana i Coxa. Stwierdzono istotny statystycznie spadek stężenia IL-2 oraz IL-4 w surowicy krwi chorych na ostre wzw typu A w pierwszym tygodniu hospitalizacji w porównaniu z grupą osób zdrowych. W przebiegu choroby obserwowano wzrost poziomu sIL-2R w porównaniu z kontrolą. Dynamika zmian poziomu IL-2, sIL-2R oraz IL-4 w surowicy krwi chorych w przebiegu ostrego wzw typu A miała charakter losowy. Oznaczanie poziomu IL-2, sIL-2R oraz IL-4 w surowicy krwi może być pomocne w ocenie odpowiedzi komórkowej w przebiegu ostrego wirusowego zapalenia wątroby typu A.