Differences in the immune response and hormonal status thus revealed were thus due mainly to a considerable decrease in the values of the parameters with age in C57BL mice.

We are not inclined to regard weakening of reactivity of the HHACS as a factor responsible for the lower level of immunologic reactivity. For example, the possiblity cannot be ruled out that a higher immune response may be observed in C57BL mice to other antigens differing from SRBC. However, the results revealed correlation between the intensity of the immune response to T-dependent antigen and the level of HHACS function, and this correlation was particularly strong in animals with a particular genotype during agine.

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STATE OF ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY AND *IN VITRO* EFFECT OF INTERFERON ON K CELL FUNCTION IN CHILDREN WITH CHRONIC VIRUS HEPATITIS B

B. S. Kaganov, Yu. I. Zimin, V. F. Uchaikin, M. Z. Saidov, and A. M. Sorokin

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Recent investigations have demonstrated the role of cell-mediated cytotoxic reactions in the pathogenesis of virus hepatitus B (VHB). It has been shown that expression of antigens on the surface of liver cells, arising during intracellular replication of hepatitis B virus, cytolytic immune processes aimed at eliminating infected hepatocytes are activated. The possibility of involvement of T killer cells, natural killer cells, and K cells in the mechanism of destruction in acute and chronic VHB also have been demonstrated [1, 5, 9]. K cells mediate antibody-dependent cellular cytotoxicity (ADCC). With the aid of a receptor for the Fc fragment of IgG, found on their surface, K cells bind with antibodies, reacting with antigens of target cells, and exert a cytolytic reaction [12]. In chronic hepatitis B (CHB) all the conditions are found for induction of ADCC. The presence of antibodies of the IgG class, directed against the expressed virus antigen (HB_cAg) [14] in the serum and on the surface of the hepatocytes, and the discovery of antibodies against hepatocyte membrane antigen (LSP) [10] are evidence that the conditions are met for cytolysis by circulating K cells.

Department of Infectious Diseases of Children, N. I. Pirogov Second Moscow Medical Institute. Fourth Main Board, Ministry of Health of the USSR, Moscow.

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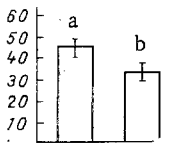

Fig. 1

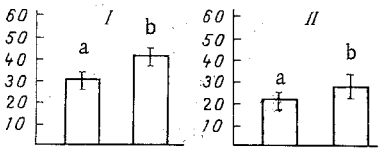

Fig. 2

Fig. 1. K cell function in healthy children (a) and in children with CHB (b). Here and in Fig. 2: ordinate, % cytotoxicity (mean results with standard error shown).

Fig. 2. Effect of IF on K cell function in healthy blood donors (I) and patients with CHB (II). a) Initial K cell activity, b) activity of K cells incubated with IF.

One factor influencing killer cell activity is interferon (IF) [7, 8, 11, 15]. The immunomodulating and antiviral effects of IF are the basis for its use in the treatment of virus infections and cancer [4].

The aim of this investigation was to study ADCC and to determine the *in vitro* effect of IF on K cell activity in patients with CHB and healthy blood donors (control group).

EXPERIMENTAL METHOD

To assess K cell activity a spectrophotometric method based on determination of the relative quantity of hemoglobin, released from lysed target cells (sheep's red blood cells — SRBC) into the incubation medium was used [2]. Mononuclear peripheral blood cells isolated in a system of one-step Ficoll—Verografin density gradient [6] were incubated in medium 199 containing 20 mM HEPES, 2 mM L-glutamine, 100 U/ml of penicillin, and 100 μg/ml of streptomycin, together with SRBC sensitized with rabbit antibodies (experiment) or intact (control), for 18 h at 37°C in a humid atmosphere with 5% CO_2, in round-bottomed microplates. The effector:target ratio was 5:1 (2×10^5 mononuclear cells and 4×10^4 SRBC per sample). All tests were repeated 3-4 times. At the end of incubation the plates were centrifuged for 10 min at 400g. The optical density (A_{610}) of the supernatants of the experimental and control samples was measured at a wavelength of 610 nm with the aid of benzidine reagent on a Model 25 spectrophotometer ("Beckman Instruments"). The degree of cytolysis was determined by the equation:

$$\% \text{ cytotoxicity} = \left(\frac{A_{610} \text{ in experiment} - A_{610} \text{ in control}}{A_{610} \text{ during maximal lysis of SRBC}}\right) \times 100\%.$$

Human leukocytic IF (N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) with specific activity of 3×10^6 IU/ml was used. To determine the effect of IF on K cell activity, IF was added to the incubation medium in a final concentration of 250 IU/ml. The optimal IF concentration was chosen in preliminary experiments. Mononuclear cells and target cells were incubated for 18 h simultaneously in medium containing IF and in medium without IF. Incubation with IF did not affect the viability of the effector cells.

ADCC was determined in 49 childern with CHB aged from 2 to 14 years. On the basis of the results of punch biopsy of the liver chronic active hepatitis (CAH) was diagnosed in 26 patients and chronic persistent hepatitis (CPH) in 23.

The effect of IF on ADCC *in vitro* was evaluated in 15 patients (in nine children with CAH and in six with CPH), forming part of the total group of patients with CHB, and also in 14 healthy adult blood donors, To determine normal values during childhood, K cell activity was estimated in 20 children, aged from 4 to 13 years, free from infectious diseases, and undergoing investigation and treatment for squint.

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

On incubation of human blood mononuclear cells with SRBC, sensitized with hyperimmune rabbit antiserum, lysis of the SRBC took place and was measured spectrophotometrically as the quantity of hemoglobin released into the incubation medium. Lymphocytes with the phenotype FcR^+ are responsible for this cytopathogenic effect against SRBC [2].

As the investigations showed, in normal clinically healthy children, with an effector: target ratio of 5:1, 44.8 ± 2.6% of target cells died during 18 h of incubation. In children with acute VHB, K cell activity exceeded normal values in the first and second weeks of the disease: The percentage cytotoxicity was 58.9 ± 5.5 ($P < 0.05$) and 74.6 ± 7.0 ($P < 0.01$) respectively. Later K cell activity declined and returned to normal values during the period of convalescence (6th-9th weeks of the disease). On the basis of these data it was postulated that in acute VHB, in the course of K cell killing, cytolysis of hepatocytes carrying virus antigens on their membrane took place, and in that way target cells were eliminated, thus completing the infectious process [3].

Unlike acute VHB, in children with CHB ADCC was below normal and the percentage cytotoxicity was 33.5 ± 2.0 ($P < 0.001$, Fig. 1). It can be tentatively suggested that the depressed K cell activity was the cause of disturbance of effective elimination of infected hepatocytes and of persistence of hepatitis B virus.

After incubation of peripheral blood mononuclear cells from patients with CHB with IF, no significant increase in cytotoxicity was found in the ADCC reaction: The initial percentage cytotoxicity was 22.2 ± 3.3, and after incubation with IF it rose to 28.7 ± 4.2 (difference not significant, $P > 0.05$; Fig. 2, II).

Meanwhile incubation of peripheral blood mononuclear cells from healthy blood donors in the presence of IF significantly increased the intensity of ADCC. Under normal conditions, in blood donors with an effector:target ratio of 5:1, the percentage cytotoxicity during 18 h of incubation was 30.5 ± 3.0, whereas after incubation of the effector cells with IF it was 41.7 ± 3.0 ($P < 0.02$; Fig. 2, I).

The activating effect of IF on ADCC of the blood donors can probably be explained by an increase in the cytotoxic potential of individual K cells and (or) its stimulating effect on differentiation of K precursor cells. Similar conclusions were drawn from a study of the stimulating effect of IF on natural killer (NK) cells [7, 13]. Since, in the modern view, subpopulations of K and NK cells are similar or even identical, they may share the same mechanisms of regulation of their activity [7, 11, 15].

If *in vitro* thus has different effects on K cell activity of patients with CHB and healthy blood donors. The weak stimulating effect of IF on ADCC in CHB may be associated with a reduction in the number of K cells or pre-K cells in the peripheral blood and (or) their low sensitivity to IF.

It can be postulated that the reduction in K cell activity in CHB is the result of quantitative or qualitative disturbances at the level of the effector cell population or their precursors. The appearance of resistance to the stimulating action of IF at the level of the K-cell and (or) the pre-K-cell population in CHB must be taken into account when combined measures of immunomodulating therapy are planned.

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