CHANGES IN EXPRESSION OF CLASS II ANTIGENS OF THE MAJOR HISTOCOMPATIBILITY COMPLEX INDUCED BY INTERFERON PREPARATIONS STUDIED BY ELISA

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Interferons (IFN) are natural regulators of the immune response. This property of IFN largely depends on their ability to modify expression of class II antigens of the major histocompatibility complex (MHC) on immunocompetent cells. The most effective regulator of expression of class II antigens of MHC is IFN-γ [8, 12, 13].

Preparations of IFN-γ enhance expression of class II MHC antigens dose-dependently on human and mouse cell membrane. Enhancement of antigen expression is linked with synthesis of proteins and mRNA and is blocked by cAMP stimulators [5, 14, 16].

Competitive analysis has shown the presence of different receptors for two types of IFN: IFN-α and IFN-β (type II) [4, 10]. In certain immune interactions the two types of IFN exhibit opposite properties. During the study of the effect of IFN-α/β and IFN-γ on the phagocytic function of mouse peritoneal macrophages it was shown that preliminary incubation of the cells with IFN-α/β enhances phagocytosis whereas treatment with IFN-γ, on the contrary, depresses phagocytic activity of macrophages [11]. On treatment with IFN-α and IFN-β, expression predominantly of class I MHC antigens is observed [7, 15], whereas under the influence of IFN-γ, both classes I and II of MHC are expressed. The study of the combined effect of IFN of types I and II on expression of class II MHC antigens revealed a decrease in the expression of these antigens under the influence of IFN-α and IFN-β, when induced beforehand by IFN-γ [9].

The study of the effect of different IFN preparations on expression of class II antigens is important not only to explain the mechanism of their action, but also for their practical use. We have developed a highly sensitive version of ELISA to determine expression of MHC class II antigens on cells [2, 3].

The aim of this investigation was to study by ELISA the action of preparations of human IFN-α and IFN-γ on expression of MHC class II antigens on mouse macrophages, using monoclonal antibodies to products of subregions I-A and I-E of the H-2 complex.

EXPERIMENTAL METHOD

CBA and C57BL/6 mice (from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR) were used. Spleen cells, obtained by homogenization of the tissue and removal of contaminating erythrocytes by osmotic lysis, were divided into two fractions: macrophages, adherent to plastic, and lymphocytes (nonadherent). This fractionation was done by culturing splenocytes in medium 199 with the addition of 10% embryonic calf serum (ECS) for 1.5 h at 37°C, in an atmosphere containing 5% CO_2.

The IFN preparations consisted of "leukinferon" (II, for injection), a preparation of IFN-α with antiviral activity of 8×10^3 IU/ml, and immune (IFN-γ) – an experimental batch of preparations with antiviral activity of 12.8×10^3 IU/ml, prepared at the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. The antiviral activity of the IFN preparations was determined by a micromethod based on delay

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of the cytopathic action of vescicular stomatitis virus in a diploid culture of human embryonic fibroblasts. Activity was expressed in international units (IU) of activity of the B69/19 standard, calculated by comparison with the national reference preparation R_9, prepared by the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. Expression of MHC class II antigens on macrophages was determined before treatment of the cells with IFN, and again after incubation for 24 and 48 h in the presence of different doses of the same preparation or a mixture of the preparations. In the control experiments macrophages were incubated under similar conditions in culture medium without the addition of IFN. The medium for culture of the cells was RPMI-1640, containing 10% ECS, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 5 mM HEPES-blocker, 100 U/ml of penicillin, and 100 μg/ml of streptomycin, pH 7.2-7.4.

Expression of MHC class II antigens on splenic macrophages of mice was assessed with the aid of monoclonal antibodies of hybridomas 10, 2-16 and 3/14, specific for antigens $I\text{-}A^k$ and $I\text{-}E^k$ respectively. For this purpose we used supernatants of hybridoma cultures, ascites fluid from a hybridoma tumor, and also antibodies isolated by affinity chromatography on Sepharose-protein A.

Affinity-isolated rabbit antibodies to mouse immunoglobulin G, in a working dilution of 1:500-1:1000, labeled with horseradish peroxidase by the periodate method ("Sigma"), were used as the conjugate.

ELISA was carried out on flat-bottomed planchets (USSR), treated beforehand with a 0.001% solution of poly-L-lysine ("Sigma") in phosphate-buffered saline (PBS); pH 7.3. The cells for study were added in a concentration of 4×10^6 ml, in a volume of 50 μl per well. After centrifugation the cells were fixed with 50 μl of a 0.5% solution of glutaraldehyde ("Reanal") in PBS for 15 min. The planchets were washed twice with PBS and then treated with a 0.1 M solution of glycine ("Reanal"), after which a 0.1% solution of bovine serum albumin (BSA) in PBS was added to the wells, and the planchets were incubated overnight at 4°C. After washing with 0.1% solution of Tween-20 ("Merck") in PBS, the conjugate was applied in a volume of 100 μl per well, which was followed by further incubation, washing, and addition of substrate solution, namely 0.004% H_2O_2 in 0.1 M citrate-phosphate buffer, pH 4.5. After incubation for 30 min the reaction was stopped with 50% sulfuric acid and estimated colorimetrically, by determining levels of absorption at a wavelength of 492 nm (E^0_{492}) on a "Titertek Multiscan" instrument ("Flow Laboratories"). The results were expressed in optical density units, and each cell population was tested in four repetitions. Splenic macrophages from C57BL/6 mice, with a haplotype giving no cross reactions with monoclonal antibodies of hybridomas 10, 2-16 and 3/14, and also systems without monoclonal antibodies or without monoclonal antibodies and conjugate, by means of which the background of nonspecific adsorption of the conjugate on the cells and the natural peroxidase activity of the cells could be detected respectively, were used as the controls.

The experimental results were subjected to statistical analysis with determination of the mean values, mean errors, and confidence intervals. The differences between the mean values was assessed by Student's t test.

EXPERIMENTAL RESULTS

The investigations show that the optical density, measured at 492 nm on splenic macrophages of CBA mice before culture was: 0.553 ± 0.050 for the product of the I-A subregion; 0.587 ± 0.038 for the product of the I-E subregion. In control investigations for specificity of interaction of the monoclonal antibodies, optical density, measured in the same way on splenic macrophages of C57BL/6 mice for products of subregions I-A and I-E varied between limits of 0.389 ± 0.009 and 0.373 ± 0.036. In the study of nonspecific adsorption of the conjugate and the natural peroxidase activity of the cells, the optical density values were: 0.273 ± 0.020-0.303 ± 0.025 in the first case, and 0.133 ± 0.013-0.162 ± 0.008 in the second case, irrespective of the strain of mice. The somewhat higher level of optical density obtained in the control tests on macrophages of C57BL/6 mice compared with the background of natural peroxidase activity of the cells and nonspecific sorption of the conjugate can probably be explained by interaction of the monoclonal antibodies with Fc-receptors on the macrophage membrane [6]. Thus the absolute values of optical density include extinction values obtained as a result of the natural peroxidase activity of the cells, nonspecific sorption of the conjugates, and interaction of monoclonal antibodies wih macrophagal membrane Fc-receptors. These control groups were set up for all types of cells tested, and the

TABLE 1. Stimulation of Expression of MHC Class II Antigens on Splenic Macrophages of CBA Mice under the Influence of Interferon Preparations

Preparation	Dose, IU/ml	I-A		I-E	
		24h	48 h	24 h	48 h
IFN-α (leukinferon)	0,5	0,639±0,023*	1,081±0,030*	0,750±0,021*	1,076±0,030*
	100	0,622±0,020	1,169±0,038	0,749±0,035*	1,083±0,021*
IFN-γ	0,5	0,567±0,020	0,867±0,032*	0,693±0,016*	0,963±0,023*
	5	0,683±0,026*	1,064±0,015*	0,754±0,016*	1,004±0,015*
	25	0,732±0,027*	1,115±0,032*	0,788±0,019*	1,147±0,024*
	100	0,431±0,014*	0,412±0,025*	0,591±0,028*	0,631±0,015*
Control	—	0,567±0,041	0,752±0,072	0,664±0,037	0,879±0.058*

Legend. *) Values differing statistically significantly ($p \leq 0.05$) from control values.

TABLE 2. Combined Effect of IFN-α and IFN-γ Preparation on Expression of MHC Class II Antigens on Splenic Macrophages of CBA Mice

Time in culture, h	Dose of IFN-α and IFN-γ, IU/ml	I-A			I-E		
		—	0,5	100	—	0,5	100
24	0,5	0,567±0,020	0,550±0,023	0,640±0,033	0,693±0,016	0,761±0,016	0,815±0,021
	5	0,683±0,026	0,616±0,018	0,587±0,030*	0,754±0,016	0,768±0,016	0,680±0,028*
	25	0,732±0,027	0,630±0,017*	0,602±0,021*	0,788±0,019	0,799±0,016	0,716±0,010
48	0,5	0,867±0,032	1,054±0,022	1,093±0,020	0,963±0,023	1,036±0,014	1,082±0,019
	5	1,064±0,015	1,112±0,036	0,978±0,007*	1,004±0,015	1,150±0,025	0,914±0,009*
	25	1,115±0,032	0,997±0,036*	1,081±0,016*	1,147±0,024	0,998±0,023*	1,093±0,012*

Legend. *) Values differing statistically significantly ($P \leq 0.05$) from values of expression of class II antigens under the influence of the IFN-γ preparation alone.

optical density values obtained varied within the limits of the control values of extinction of the natural peroxidase activity of the cells and nonspecific sorption of the conjugate.

Incubation of monocytes in the culture medium for 24 and 48 h without IFN preparations led to a spontaneous increase in expression of MHC class II antigens. After culture for 48 h the optical density values for products of the I-A and I-E subregions were: 0.752 ± 0.072 and 0.879 ± 0.058, and they differed statistically significantly from levels of expression of the class II antigens on the original cell populations.

On treatment of macrophages with the IFN preparations, enhanced expression of products of the I-A and I-E MHC subregions was discovered. On incubation of the cells with different doses of IFN expression of MHC class II antigens increased at different rates depending on the type of IFN preparation and the dose added before culture. The increase in the optical density values under the influence of IFN preparations, discovered by ELISA, may be evidence of an increased density of the class II antigens tested in the cell population.

Table 1 gives values of optical density characterizing the level of expression of products of the I-A and I-E subregions of the mouse H-2 complex under the influence of two IFN preparations after culture for 24 and 48 h.

Determination of the optical density values showed that the greatest expression of the two types of class II antigens was observed under the influence of the IFN-γ preparation in a dose of 25 IU/ml after 48 h in culture. IFN-α II for injections also increased expression of the MHC class II antigens. A dose of the IFN-γ and IFN-α preparations of 0.5 IU/ml did not lead to any significant increase in expression of products of the I-A and I-E subunits of the H-2 complex on the macrophagal membrane in the course of culture. The dose of IFN-γ of 100 IU/ml caused inhibition of expression of MHC class II antigens. Optical density values in cell populations treated with this dose of IFN-γ were stastistically significantly lower than optical density values for control cells, cultured without IFN preparations. This phenomenon was observed for products both of the I-A subregion and of the I-E subregion of the H-2 complex.

The dynamics and the level of expression of class II antigens on unstimulated mouse cells and the same cells stimulated by IFN were similar in type for products of I-A and I-E of MHC.

The study of the combined effect of the IFN-α and IFN-γ preparations on expression of MHC class II antigens on splenic macrophages (Table 2) show that expression of class II antigens to optimal and suboptimal doses of IFN-γ decreased under the influence of IFN-α, and differed statistically significantly from the optical density values in the control and extinction characterizing expression of MHC class II antigens under the influence of the IFN-γ preparation alone. Thus in the case of simultaneous exposure of splenic macrophages to preparations of IFN-α and IFN-γ, a mutually weakening effect was exhibited on expression of I-A and I-E antigens of the mouse H-2 complex.

IFN preparations thus not only prevent the natural fall of expression of class II antigens on macrophages during their long-terms culture, but also enhance this expression, mainly on account of an increase in density of the class II antigen molecules in the cell population [1].

The spontaneous increase in expression of MHC class II antigens in the control can probably be explained by the insufficient purity of the cell population. The presence of even slight contamination with T-cells can induce immune interactions and can lead to the production of an endogenous IFN-γ, which induces expression of MHC class II antigens.

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