MICROBIOLOGY AND IMMUNOLOGY

Effect of Nicotinamide on the Development of Allergic Encephalomyelitis

Yu. L. Zhitnukhin, I. V. Litvinenko, and R. P. Ogurtsov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 2, pp. 180-182, February, 1998
Original article submitted February 22, 1997

Daily injections of nicotinamide to guinea pigs immunized with an encephalitogenic preparation from day 1 through 16 postimmunization suppress the development of clinical manifestations of experimental allergic encephalomyelitis and reduce mortality of experimental animals. In nicotinamide-treated animals, weakened delayed-type hypersensitivity skin reactions to myelin and adhesive activity of peripheral blood leukocytes as well as enhanced cytochrome P-450-dependent monooxygenase activity are noted.

Key Words: *experimental allergic encephalomyelitis; nicotinamide; delayed-type hypersensitivity; leukocyte adhesion; monooxygenase activity*

Experimental allergic encephalomyelitis (EAE) is an inflammatory demyelinating disease of the central nervous system, which allows investigators to study the mechanisms of induction and regulation of autoimmune reactions and effects of various drugs in neurological and systemic immune-mediated disorders [15]. EAE is characterized by delayed-type hypersensitivity (DTH) reactions, monocyte/macrophage being predominant component of cell infiltrate [10]. It has been shown that tumor necrosis factor-α, interleukin-1, and interferon-γ enhance the expression of cell adhesion molecules-1 [14] and major histocompatibility complex class II antigens. Enhanced production of these cytokines is associated with pathogenesis of autoimmune disorders and inflammatory tissue lesions in multiple sclerosis and EAE [11]. It has been shown that nicotinamide (NA) inhibits interferon-γ-induced expression of cell adhesion molecules and major histocompatibility complex antigens in cultured vascular endotheliocytes [9,12] and activates microsomal enzymes (monooxygenases) in human liver [4]. The latter is of particular interest in light of inhibition of cytochrome P-450-dependent monooxygenases in autoimmune disorder [1]. The aim of the present study was to test the effect of NA on the development of EAE, DTH reactions, adhesive properties of leukocytes, and activity of microsomal enzymes in animals immunized with an encephalitogenic preparation.

Department of Immunology, Institute of Experimental Medicine, Russian Academy of Medical Sciences; Department of Nervous Diseases, Military Medical Academy, St. Petersburg

MATERIALS AND METHODS

Experimental allergic encephalitis was modeled in guinea pigs (350-400 g) by single subcutaneous inoculation (in fore footpads) of 10 mg (wet weight) myelin in 0.1 ml Freund's complete adjuvant. Myelin was isolated from rabbit spinal cord by differential centrifugation in sucrose density gradient [3]. The disease onset, duration of neurological disorders, and mortality of immunized animals were assessed. Some animals were intraperitoneally injected with 5% NA (two injections per day, 50 mg/animal). On day 10 of the latent period of EAE, all guinea pigs were injected with 1 mg myelin in 0.1 ml physiological saline, and skin DTH reactions were evaluated after 24 h by measuring infiltration and erythema zones.

0007-4888/98/0002-0156$20.00

TABLE 1. Induction of EAE after Administration of NA(*M±m*)

Series	Period of NA administration	Number of animals	Cases of EAE		Latency, days	Died	
			number	%		number	%
Control	—	20	15	75.0	14.6±0.38	10	50.0
Experiment	1-16+	19	6	31.6*	15.5±0.88	3	15.8

Note. +After inoculation of encephalitogenic preparation; *$p<0.01$, **$p<0.02$ compared with corresponding parameters in the control group.

Adhesive activity of leukocytes was studied on the 12th day of latent period by fluorimetrically measuring cell adhesion to Falkon Plastic-3034 microtitration plates [5]. Induction of the cytochrome P-450 system was assessed by half-elimination period for antipyrine. To this end, antipyrine was injected intramuscularly (15 mg/kg, single injection), and its serum concentration was measured 1 and 3 h postinjection [6]. The data were processed statistically using the Student *t* test.

RESULTS

Neurological symptoms of EAE such as myasthenia, ataxia, and motor paresis and paralysis were noted in some animals at different times after immunization, the minimum latency being 12 days. Table 1 shows the data on the development and outcome of EAE in NA-treated and untreated animals. Daily NA injections for 16 days after inoculation of encephalitogenic preparation reduced morbidity and mortality in experimental animals, and prolonged latency of EAE in 31.6% guinea pigs, in which injection of NA did not prevent the development of EAE.

In view of the fact that NA inhibited the development of EAE, it was interesting to test the ability of NA to affect some immunological and biochemical parameters of the pathological process. Data on *in vivo* DTH reactions, adhesive properties of blood leukocytes, and antipyrine elimination rate are summarized in Table 2. Skin reactions to myelin were less frequent and pronounced in NA-treated group. Similar changes were noted for adhesive properties of leukocytes: NA reduced the number of adherent cells more than 6-fold. On day 8 postimmunization, half-elimination period for antipyrine was shorter in NA-treated animals, while in controls (intraperitoneal injection of physiological saline) this parameter was the same before and after immunization.

Our findings indicate that NA reduces morbidity and mortality of experimental animals in EAE. This was accompanied by a reduction in the frequency and degree of DTH reaction to myelin and activation of cytochrome P-450-dependent monooxygenase system. Our data prove the existence of a direct correlation between induction of EAE and DTH, confirm the assumption that the immunomodulatig effect of cytochrome P-450 inductors is due to functional interrelationship between the enzyme and immune systems [2], and are in conformity with previous data on reduced activity of the monooxygenase system during the development of DTH reaction and on restriction of DTH reaction caused by activation of the monooxygenase system [7]. Experimental studies revealed increased adhesiveness of peripheral blood leukocytes at the end of latent period and its pronounced decrease caused by NA. It should be noted that leukocyte migration through vascular endothelium is a key event in the development of multiple sclerosis and EAE. This process depends on cooperative effect of adhesion molecules which are expressed on endothelial cells and leukocytes and mediate their interaction rendering autoreactive T cells capable of crossing the blood-brain barrier [8]. Thus, suppressive effect of NA with respect to EAE can be explained by its various activities: activation of liver microsomal

TABLE 2. Effects of NA on DTH Reaction, Leukocyte Adhesiveness, and Antipyrine Metabolism in EEA (*M±m*)

Series	Number of animals	DTH reaction to myelin			Leukocyte adhesiveness[1]	Antipyrine test $T_{1/2}$, h	
		abs.	%	Diameter, mm		-1[2]	+8[2]
Control	20	19	95.0	15.8±0.7	148.7±19.1	1.17±0.11	1.13±0.12
Experiment	19	10	52.6*	8.2±0.6*	22.9±2.3*	1.38±0.09	1.03±0.12**

Note. [1]Number of adherent cells; [2]days before or after inculation; *$p<0.001$ compared with corresponding parameters of the control group, **$p<0.02$ compared with the same group.

enzymes, inhibition of DTH reaction to encephalitogenic agent and suppression of leukocyte adhesiveness, as is was demonstrated in the present study. Tumor necrosis factor-α-regulated expression of cell adhesion molecules-I on vascular endothelial cells and interferon-γ induction of major histocompatibility complex antigens play an important role in the development of EAE [11]. Taking into account the ability of *in vitro* inhibition of this expression by NA [9,12], one can assume the existence of such an effect *in vivo* during the development of EAE. This mechanism probably prevents the disturbances of the blood-brain barrier, a key step in the mechanism of autoimmune demyelinating diseases.

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