GENETICS

In Vitro Genotoxic and Cytotoxic Effects of Protein Somatic Products from Helminths on Donor Blood Lymphocytes

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Protein somatic products of adult helminths *Hymenolepis nana* and *Toxocara canis* and secretory-excretory somatic product of *Trichinella spiralis* larvae *in vitro* produced genotoxic and cytotoxic effects on donor blood lymphocytes, which manifested in accumulation of single-chain breaks, alkaline-labile sites in nuclear DNA, and apoptotic cells. This effect depended on the concentration of parasitic protein products during coculturing.

Key Words: *helminths; alkaline single cell gel electrophoresis; donor lymphocytes; genotoxicity; cytotoxicity*

The alkaline single cell electrophoretic (DNA comet) assay is a highly sensitive fluorescence microscopic method of identification of primary damage to DNA molecules in individual cells [14]. This method is used to study the genotoxic and cytotoxic effect of environmental factors on cells [7].

Protein products of dwarf tapeworms (*Hymenolepis nana*), *Trichocephalus trichiuris*, acaridae, and trichina larvae cocultured with peripheral blood lymphocytes from donors *in vitro* produce a clastogenic effect [4]. Little is known about the ability of protein somatic products (SP) from *Hymenolepis nana*, *Toxocara canis*, and *Trichinells spiralis* to produce a genotoxic effect and cause apoptosis in donor peripheral blood lymphocytes.

Here we studied the genotoxic and cytotoxic effect of protein products from *H. nana*, *T. canis*, and *T. spiralis* on donor blood lymphocytes during *in vitro* coculturing.

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MATERIALS AND METHODS

The blood was taken from 10 healthy donors (5 men and 5 women, 25-30 years). Protein SP were obtained from *H. nana* and *T. canis* as described elsewhere [1]. The secretory-excretory SP (SESP) of *T. spiralis* larvae was obtained by the method of O.-Ya. L. Bekish [2]. Total protein concentration was measured by the biuret method [3].

The cells from each donor were cultured in 10 flasks (1 control and 9 experimental flasks). The culture mixture contained 0.5 ml lymphocyte suspension, 2. 5 ml RPMI 1640 medium, and 350 μg gentamicin. SP of *H. nana* and *T. canis* and SESP of *T. spiralis* were used in final concentrations of 100, 200, and 400 μg per 1 ml culture medium. The volume of sterile 0.9% NaCl added to control flasks was similar to that of parasitic SP. Culturing was performed at 37°C for 24 h. The lymphocyte suspension was washed 2 times with 3 ml RPMI 1640 medium at 1100 rpm and 8°C. The supernatant was removed and the pellet was diluted with RPMI 1640 medium to a final concentration of $1\text{-}5\times10^6$ cells/ml. RPMI 1640 medium (200 μl) was added to the cell suspension from control flasks (100 μl). Incubation was performed with 100 μM H_2O_2 at 37°C for 5 min (positive control). The remaining lym-

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phocytes from control suspensions served as negative control.

Alkaline single cell gel electrophoresis was performed as described elsewhere [13] with modifications [8]. This method allowed us to estimate the number of single-chain breaks and alkaline-labile sites in nuclear DNA after incubation with genotoxic factors. Micropreparations were stained with ethidium bromide and examined under a Mikmed-3 luminescence microscope (LOMO, ×600). Comet images in micropreparations were photographed with a Nikon Coolpix-4500 digital camera. Damage to the DNA molecule was assayed in digital images (CASP v. 1.2.2) [9]. We examined 100 cells. The length of the comet tail (pixels) and percentage of tail DNA were estimated (Fig. 1, *a*, *b*). The tail moment is calculated by multiplication of the tail length on tail DNA percentage and serves as a general international criterion of the genotoxic effect of environmental factors [9]. The percentage of apoptotic cells in 100 randomly selected cells was calculated to evaluate the cytotoxic effect of parasitic products on donor lymphocytes (Fig. 1, *c*). The results were analyzed using Statistica 6.0 software. The data were expressed as arithmetic mean± standard deviation (*M±SD*). The significance of differences was estimated by Student's *t* test.

RESULTS

SP of *H. nana* and *T. canis* and SESP of *T. spiralis* produced a genotoxic effect on cultured lymphocytes. They *in vitro* increased the number of single-chain breaks and alkaline-labile sites in nuclear DNA of cells migrating during single cell electrophoresis (Table 1). The severity of damage to nuclear DNA increased with increasing the concentration of helminthic protein SC. Changes in the tail moment were characterized by clear-cut dose dependence. The tail moment increased by 2.6 times with increasing in the dose of *H. nana* SP from 200 to 400 μg/ml. Increasing the concentration of *T. canis* SP and *T. spiralis* SESP from 100 to 200 and 400 μg/ml was accompanied by the increase in the tail moment by 1.7-2.4 times.

Our results are consistent with published data that coculturing of human T lymphocytes Jurkat with adult *Necator americanus* and secretory-excretory protein products is accompanied by an increase in the degree of cell DNA fragmentation [6]. The degree of changes linearly increased with an increase in the concentration of helminthic protein products [6]. The number of gene mutations at the *hprt* locus increased in peripheral blood lymphocytes from patients with neurocysticercosis [11].

Helminthic protein SP exhibited not only genotoxic, but also cytotoxic activity. It was manifested in a sharp increase in the number of apoptotic cells in the culture of donor lymphocytes. The effect was specific and depended on the type of parasitic protein products added to cultured blood lymphocytes. *H. nana* SP in a dose of 400 μg/ml increased the count of apoptotic cells by 5.5 times. The count of apoptotic cells observed after the addition of *T. canis* SP and *T. spiralis* SESP in doses of 200 and 400 μg/ml surpassed the negative control.

The cytotoxic effect of *T. canis* SP did not depend on its concentration. The parasitic product in different concentrations increased the count of apoptotic cells by 3.7 times, which surpassed the negative control. The count of apoptotic cells increased by 1.7 times after increasing the concentration of *T. spiralis* SESP from 200 to 400 μg/ml.

TABLE 1. Alkaline Single Cell Electrophoretic Assay of Donor Peripheral Blood Lymphocytes Cultured with Protein SP and SESP of Helminths (*M±SD*)

Group		Length of comet tail, pixels	Comet tail DNA, %	Tail moment	Apoptotic cells, %
Negative control		3.77±1.74	1.90±0.55	0.13±0.04	0.40±0.52
Positive control (100 μM H_2O_2)		24.90±5.97*	14.63±6.23*	3.89±2.42*	5.20±0.79*
H. nana SP, μg/ml	100	6.61±5.10	2.92±1.56	0.24±0.27	0.70±0.67
	200	20.11±6.58*	10.02±2.81*	2.02±0.80*	0.89±0.60
	400	32.14±14.92*°	13.76±6.75*°	5.28±4.15*°	2.20±0.79*
T. canis SP, μg/ml	100	14.60±6.77*	7.91±4.40*	1.35±1.38*	1.40±0.52*
	200	22.67±6.12*+	10.69±4.17*	2.63±1.53*+	1.44±0.53*
	400	32.43±9.17*°	15.86±4.43*°	5.03±1.76*°	1.60±0.70*
T. spiralis SESP, μg/ml	100	17.30±9.15*	14.35±11.69*	3.31±3.64*	1.00±1.25
	200	33.00±9.75*+	15.96±5.86*	5.50±3.32*+	3.56±0.88*
	400	44.14±12.07*	26.42±9.07*°	12.31±5.87*°	6.10±1.37*°

Note. $p<0.01$-0.05: *compared to the negative control; +compared to 100 μg/ml SP and SESP; °compared to 200 μg/ml SP and SESP.

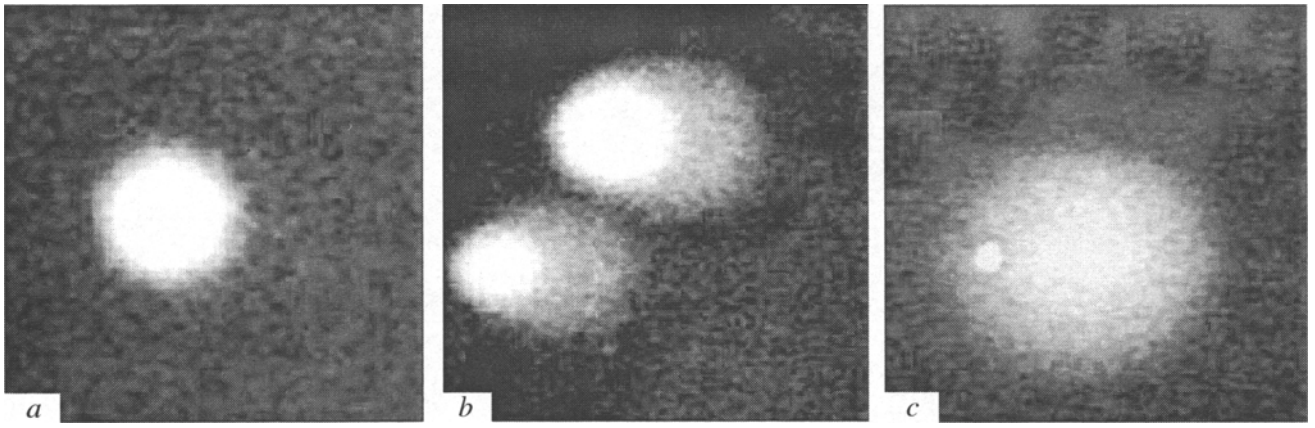

Fig. 1. Comets of donor blood lymphocytes during alkaline single cell electrophoresis. Ethidium bromide staining, ×600. Normal cell (*a*), cell with 10-12% DNA damage in the comet tail (*b*), and apoptotic cell (*c*).

Our findings are confirmed by the results of experiments on mice with acute and chronic schistosomosis and observation of patients with asymptomatic Mansoni schistosomosis [5,10]. The intensity of apoptosis in splenic and granulomatous T lymphocytes increased in patients with schistosomal invasion. Culturing of human T lymphocytes (Jurkat) with *Taenia crassiceps* cysticerci, adult *N. americanus*, and their excretory-secretory protein products was accompanied by an increase in the count of apoptotic cells, which depended on the amount of parasites and concentration of protein products [6,12].

Our results show that protein SP of *H. nana* and *T. canis* and SESP of *T. spiralis in vitro* produced a genotoxic effect on donor blood lymphocytes. It was manifested in an increase in the number of single-chain breaks and alkaline-labile sites in nuclear DNA. This effect depended on the concentration of protein SP and SESP. When the concentration of parasitic products was doubled, the degree of changes increased by 1.7-2.7 times. Protein SP of *H. nana* and *T. canis* and SESP of *T. spiralis* also possessed cytotoxic activity and increased the number of apoptotic cells. The effect was specific and observed under the influence of *H. nana* SP (400 μg/ml), *T. canis* SP (100, 200, and 400 μg/ml), and *T. spiralis* SESP (200 and 400 μg/ml). Toxocarosis protein SP in low and high doses produced a similar cytotoxic effect. However, the count of apoptotic cells increased by 1.7 times only after a 2-fold increase in the concentration of *T. spiralis* SESP.

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