TIME DEPENDENCE OF CLASS I AND II HLA-ANTIGEN SHEDDING. CORRELATION BETWEEN FLUCTUATIONS OF HLA-ANTIGEN SHEDDING AND OF ^{51}Cr-LABELED MACROMOLECULES

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UDC 612.118.221.2:612.112.94

KEY WORDS: lymphocytes; membrane; HLA-A, B- and DR-antigens; shedding; ultradian rhythm.

The basic mechanisms regulating synthesis and expression of antigens of the major histocompatibility system have been studied for membrane-associated H-2- and HLA-molecules, i.e., at the cellular level. A definite concentration of MHCS products also has been found in biological fluids [10, 11], but the principles governing their secretion into the extracellular medium have not been adequately studied. One of the mechanisms of secretion of these MHCS molecules is shedding: a temperature- and energy-dependent process, effected by the cytoskeleton [5]. It has been postulated that shedding is, on the one hand, a compensatory mechanism for removal of an excess of plasmalemma fragments of lymphocytes during circulation of the membrane, and on the other hand, an important component for the release of secretion by lymphoid cells. With the exception of plasma cells, lymphoid cells are characterized mainly by an intermittent type of secretion [3].

The aim of this investigation was to study the temporal characteristics of shedding of HLA-antigens of the I and II classes, and also of material bound with ^{51}Cr, for we know that spontaneous release of the label from cells is due to trivalent ^{51}Cr ions bound with macromolecules and, in particular, with proteins [8, 9].

EXPERIMENTAL METHOD

Mononuclear cells (MC) were isolated from heparinized venous blood [4] and incubated in complete culture medium (RPMI-1640 with 10% fetal calf serum, 2 mM L-glutamine, and 10 mM

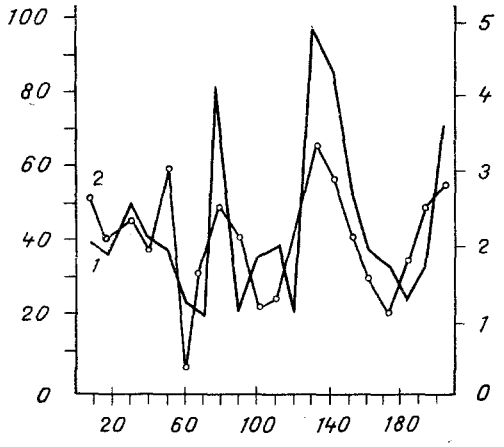

Fig. 1. Shedding of DR-antigens and ^{51}Cr-macromolecules at 22°C. Abscissa, time (in min); ordinate: on the left — units of reaction velocity (in relative units of the content of DR-antigens), on right — ratio of radioactivity (in cpm) of cell residue to that of supernatant. 1) HLA-DR-proteins, 2) ^{51}Cr-macromolecules.

Research Institute of Clinical Immunology, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 9, pp. 327-329, September, 1987. Original article submitted July 15, 1986.

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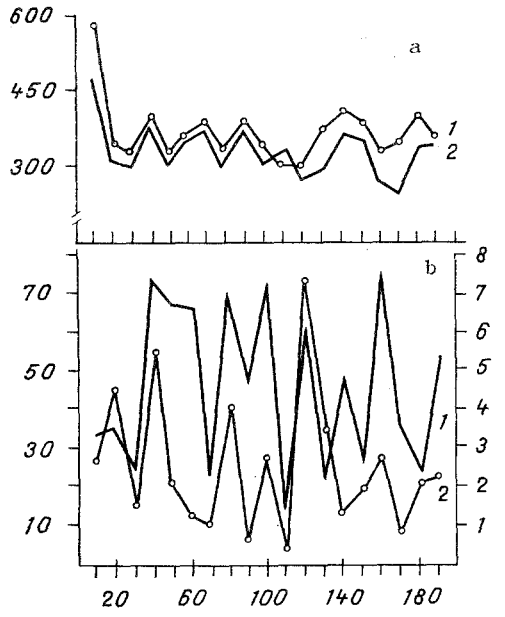

Fig. 2

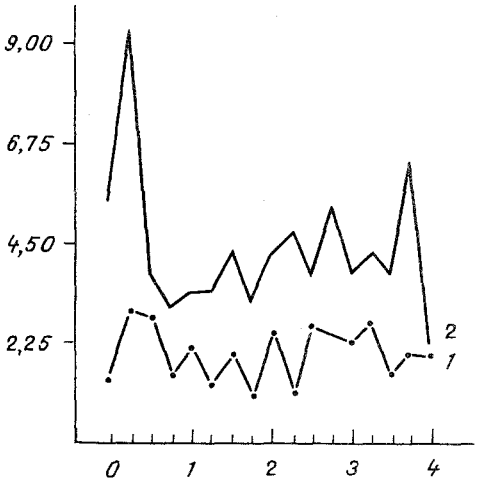

Fig. 3

Fig. 2. Shedding of HLA-A9, -Bw40-, and -DR-antigens and of ^{51}Cr-macromolecules at 37°C. a: 1) HLA-A9, 2) HLA-Bw40; b: 1) HLA-DR, 2) ^{51}Cr-macromolecules. Abscissa, time (in min); ordinate: a, b (on left) — units of reaction velocity, b (on right) — ratio of radioactivity of cell residue to that of supernatant.

Fig. 3. Reduction of secretion—reabsorption of ^{51}Cr-labeled macromolecules by mononuclear cells after pretreatment with colchicine (10^{-7} M) and vinblastine (10^{-8} M). 1) Experiment, 2) control. Abscissa, time (in h). Ordinate, ratio of radioactivity of cell residue to radioactivity of supernatant.

HEPES-buffer, in siliconized glass tubes in a concentration of 2.5×10^6 cells/ml. In a parallel series some cells were labeled with ^{51}Cr (in the form of sodium chromate) in a dose of 0.37 MBq/ml for 30 min, and resuspended after three washings in complete medium in a concentration of 0.5×10^6 cells/ml. Unlabeled cells were subjected to the same manipulations, i.e., they were incubated and washed parallel with the labeled cells. In separate experiments MC from five healthy blood donors were treated before labeling with ^{51}Cr (and with subsequent washing) with a mixture of colchicine (10^{-7} M) and vinblastine (10^{-8} M) for 90 min, with mitomycin C (50 μg/ml, 30 min), or irradiated in a dose of 2000 rads. Untreated cells from the same donors served as the control. To maintain a constant concentration of MC in unit volume the cell suspensions were inclined at 75° on a shaker (120 cycles/min in the horizontal plane), and triplet 1-ml samples were taken during incubation for 3 h. After centrifugation the supernatants of unlabeled cells were used to determine soluble HLA-antigens, whereas for the labeled cells the ratio of radioactivity of the cell sediment and of the supernatant was estimated. An LKB-Wallak RIA Gamma 1271 γ-counter was used. MHCS antigens in the supernatant were assayed by the use of monospecific anti-A9 and anti-Bw40 typing sera (Behringwerke, West Germany) and IKO-1 monoclonal antibodies (Gor'kii Research Institute of Epidemiology and Microbiology) against monomorphic HLA-DR specificities. This determination was done nephelometrically on a Beckman ICS II immunochemical system (USA). A healthy man of the A9, Bw40 HLA-phenotype served as the donor. Nonparametric tests were used for statistical analysis of the data: the serial r test [1], Spearman's coefficient of correlation, and Wilcoxon's W test. The numerical data are presented in the $M \pm \sigma$ form.

EXPERIMENTAL RESULTS

The titer of histocompatibility antibodies of the I and II classes determined in the supernatant of MC was found to change periodically (Figs. 1 and 2). Since the rises and falls of their level relative to the median depended statistically significantly on time ($p < 0.05$ by the r test) and the period was 20-30 min, these processes were classed as circumhoralian

TABLE 1. Phase Relationships of Shedding of HLA-A9-, HLA-Bw40-, and HLA-DR- Antigens and of ^{51}Cr-Labeled Macromolecules (^{51}Cr-M) According to Results of Correlation Analysis

Series of Experiments	Experimental conditions	Spearman's coefficient of correlation (ρ) and level of its significance
I (22 °C)	HLA-DR ^{51}Cr-M	1—2 ρ =0,64 ($p<0,05$)
II (37 °C)	HLA-DR ^{51}Cr-M	1—2 ρ =0,63 ($p<0,05$)
	HLA-A9	3—4 ρ =0,61 ($p<0,05$)
	HLBw40	1—3 ρ =—0,57 ($p<0,05$)
III (37 °C)	HLA-Bw40 HLA-A9	1—2 ρ =—0,62 ($p<0,05$)

(ultradian) cell rhythms [2, 6]. When the experiment was conducted at room temperature (22°C) the mean period was 30 min (Fig. 1), at 37°C it was 20 min (Fig. 2). The change of period was possibly due to an increase in flowability of the membrane and acceleration of metabolic processes. Similar changes of period also were observed for fluctuations of ^{51}Cr-labeled macromolecules (Figs. 1 and 2). The periodic rises and falls of the level of secreted products are evidence of continuous secretion—reabsorption processes.

Oscillations of shedding of MHCS antigens of classes I and II were opposite in phase (Table 1). This rule still applied on redetermination 1 week later. The course of secretion—reabsorption of antigens A9 and Bw40 was almost completely in phase, and quantitatively speaking the oscillations were very similar. Shedding of ^{51}Cr-labeled macromolecules was in phase with shedding of DR-antigens. This rule was observed at 22°C (Fig. 1) and was still observed 1 week later at 37°C (Fig. 2). The close correlation between this process and secretion—reabsorption of MHCS molecules was used as a model of this process in experiments to study cytoskeletal regulation of shedding. All agents which disturbed cytoskeletal function considerably reduced the amplitude of the oscillations. Compared with the control, taken as 100%, after exposure to colchicine and vinblastine the mean amplitude was 49.0 ± 12.1% ($p < 0.01$), after irradiation it was 56.9 ± 19.3% ($p < 0.05$), and after treatment with mitomycin C it was 58.7 ± 17.9% ($p < 0.05$). The result of one experiment is given in Fig. 3.

The basis for the secretion—reabsorption processes observed in these experiments is evidently provided by components of the membrane recycling process such as endocytosis and exocytosis [3, 7].

LITERATURE CITED

1. S. A. Aivazyan, I. S. Enyukov, and L. D. Meshalkin, Applied Statistics [in Russian], Moscow (1983).
2. V. Ya. Brodskii, Tsitologiya, 18, 397 (1976).
3. S. N. Bykovskaya and E. V. Gruntenko, T-Lymphocytes in Antitumor Immunity [in Russian], Novosibirsk (1982).
4. A. Böyum, Scand. J. Clin. Lab. Invest., Suppl. 97, 51 (1968).
5. S. G. Emerson, J. Pretell, and R. E. Cone, Exp. Clin. Immunogenet., 1, 9 (1984).
6. F. Halberg and G. S. Katinas, Int. J. Chronobiol., 1, 31 (1973).
7. M. D. Houslay and K. K. Stanley, Dynamics of Biological Membranes, Chichester (1983).
8. R. Kurth and G. Medley, Immunology, 29, 803 (1975).
9. E. Martz, Cell. Immunol., 26, 313 (1976).
10. Y. Miyakawa, N. Tanigaki, V. P. Kreiter, et al., Transplantation, 15, 312 (1973).
11. D. O. Schmid and S. Cwik, Tissue Antigens, 2, 255 (1972).