MICROBIOLOGY AND IMMUNOLOGY

Phagocyte Activation with Anti-ICAM-3 Monoclonal Antibodies

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Monoclonal antibodies ICO-60 clustered as CDw50 (anti-ICAM-3) drastically boosted the chemiluminescence of human neutrophils, depending on the dose of antibodies, initial chemiluminescence, and individual human reactivity. The contact-adhesion interactions of neutrophils treated with ICO-60 and of intact neutrophils were associated with intensification of chemiluminescence. ICO-60 sharply increased the adhesion and aggregation of human neuroblasts and suppressed these phenomena induced by phorbol ester. The expression of ICAM-3 on neuroblasts is proposed.

Key Words: *neutrophils; neuroblasts; oxygen metabolism; anti-ICAM-3 antibodies (ICO-60)*

The functional activity of monoclonal antibodies (MAb) ICO-60 obtained by A. Yu. Baryshnikov, clustered as CDw50 (anti-ICAM-3), is analyzed. The ICAM-3 contraceptor for integrin molecule LFA-1 was recently discovered and is being intensively studied at present [3,4].

MATERIALS AND METHODS

Neutrophils were isolated from venous blood of healthy donors and patients with the asthmatic triad in a Ficoll-Verografffin density gradient (C=1.114), and their spontaneous or induced (by 50 ng/ml phorbol ester (PMA) or zymosan) luminol-dependent chemiluminescence (CL) was studied using an LKB L1251 luminometer [1]. In addition, an original method was used: neutrophils from one subject were divided into two portions, 2×10^6 cells from one of them were used untreated or pretreated with PMA or MAb ICO-60, after which 10 mM iodacetamide and then 100,000 intact neutrophils from the other portion were added, and CL was studied. In some experiments we studied CL of neutrophils treated with MAb ICO-60 in different dilutions. A neuroblast suspension was isolated by pipetting and washing from the brain of 8-9-week fetuses obtained during induced abortions. Adhesion of these neuroblasts to polystyrene and aggregation under the effect of PMA or MAb ICO-60, which were incubated for 30 min with the cells at 37°C, were studied. In some experiments the cells were pretreated for 15 min with ICO-60, then washed on a centrifuge, and incubated for 30 min more.

RESULTS

ICO-60 antibodies caused a potent activation of spontaneous CL of neutrophils, whereas MAb CD11b detecting the expression of Mac-1 integrin did not affect or even suppressed it (Fig. 1). It is noteworthy

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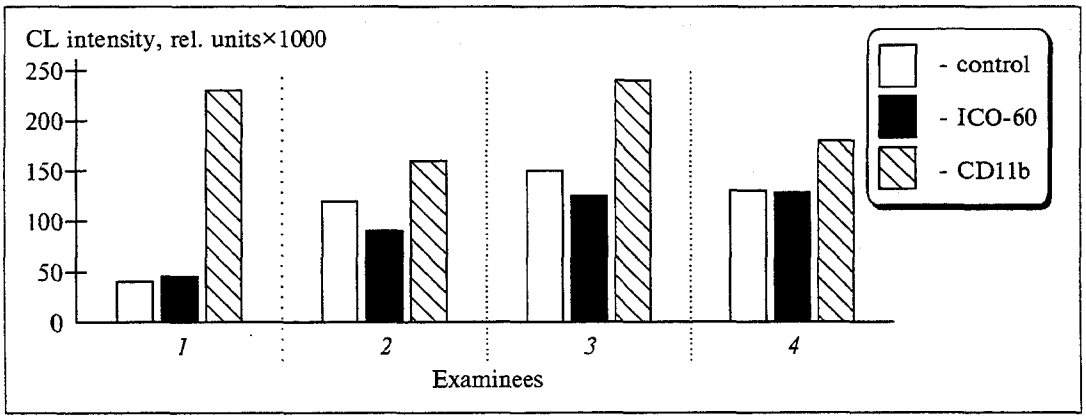

Fig. 1. Effect of ICO−60 monoclonal antibodies on spontaneous chemiluminescence (CL) of neutrophils.

that ICO-60 in different dilutions variously activated the neutrophils of different subjects. Either the predominant activity of high concentrations of MAb, or equal activities of all concentrations used, or no activity at all were observed (Fig. 2). Evidently, this depended not only on individual features of the examinees, but also on the initial level of CL: at very high levels the effects of MAb did not always manifest themselves. The latter hypothesis was confirmed during analysis of the effect of ICO-60 on CL induced by zymosan phagocytosis in neutrophils: it was weak or absent in the majority of cases.

We observed interesting results during induction of neutrophil CL by contact-adhesion interactions with autologous cells treated with ICO-60 and inhibited by iodacetamide. Such cells caused a potent burst of oxygen metabolism in them, the intensity of this burst surpassing the analogous activity of autologous neutrophils inhibited or pretreated with PMA and inhibited with iodacetamide (Fig. 1). It is worthy of note that neutrophil activation with antibodies was not inferior to the similar effect of PMA on these cells or was even higher.

ICO-60 caused extremely well-expressed adhesion and aggregation of human neuroblasts, which were most pronounced when the cells were washed free of antibodies. Addition of MAb to PMA reduced the activating potential of phorbol ester. The efficacy of MAb depended on their concentration (Table 2).

Hence, MAb ICO-60 appreciably activated oxygen metabolism of neutrophils, and this property depended to a certain measure on the dose of anti-

TABLE 1. Effect of ICO-60 Monoclonal Antibodies on the Cell-to-Cell Contact-Adhesion Interactions of Neutrophils

Series	CL	Number of neutrophils, 10^6	CL, rel. units		
			1	2	3
I	Spontaneous	0.5	0.479	0.349	1.196
	Induced by:				
	PMA	-"-	0.604	0.330	0.768
	zymosan	-"-	44.19	66.22	49.27
	PMA+5 mM EDTA	-"-	0.643	0.183	0.394
	ICO-60	-"-	0.709	2.621	0.803
II	Spontaneous	-"-			0.691
	Induced by ICO-60	-"-			1.083
III	Spontaneous	0.1	0.200	0.167	
	Induced by ICO-60	0.1	0.220		
	Neutrophils (treated with iodacetamide)	2.0			
	+intact neutrophils	0.1	0.204	0.246	0.45
	Neutrophils (treated with ICO-60, then iodacetamide)	2.0			
	+intact neutrophils	0.1	0.339	0.816	1.387

Note. In series I neutrophils were kept at 4°C for 3-4 h; in series II neutrophils were pretreated with PMA and then iodacetamide, after which intact neutrophils were added. *Patient with the asthmatic triad during remission; 1 and 2 are donors.

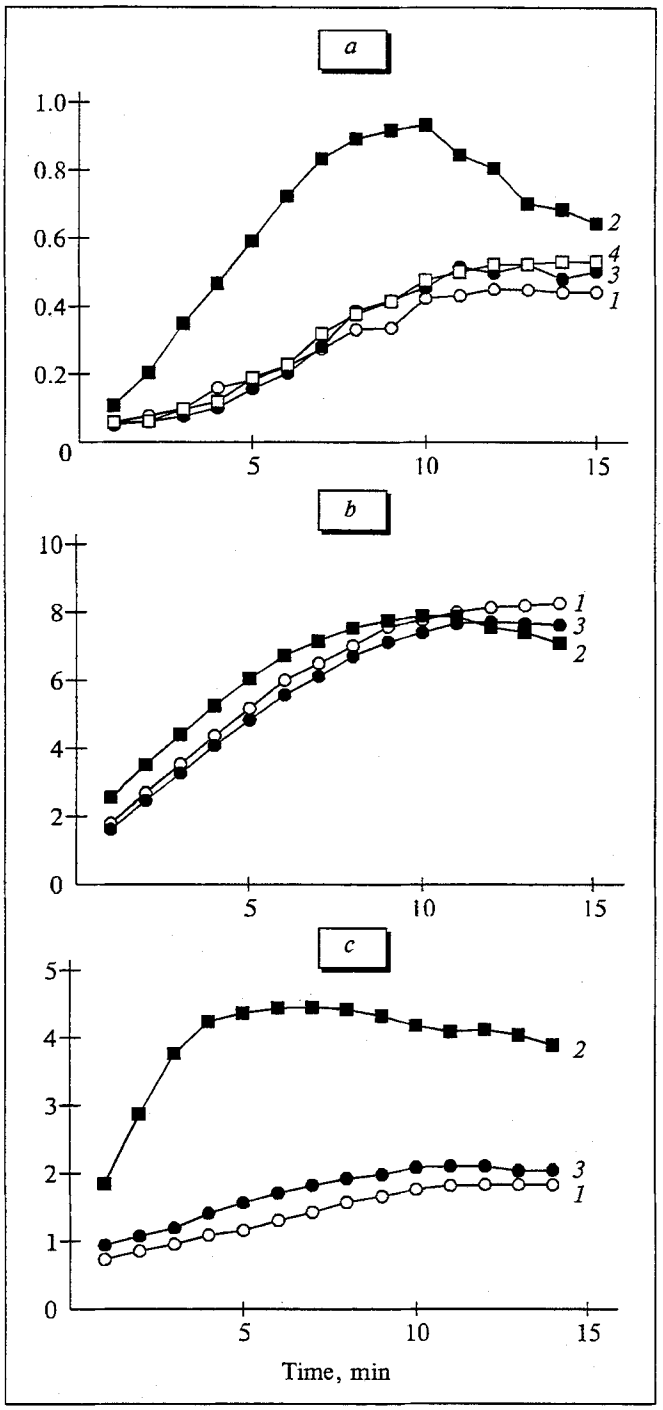

Fig. 2. Effect of ICO–60 monoclonal antibodies (MAb) on spontaneous chemiluminescence (CL) of neutrophils in donors (*a*) and patients with the asthmatic triad (*b*, *c*). Ordinates: CL intensity, rel. units. *1*) control; *2*) MAb 1:20; *3*) MAb 1:100; *4*) MAb 1:500.

TABLE 2. Effect of ICO-60 Monoclonal Antibodies (MAb) on Neuroblast Adhesion and Aggregation

Cell treatment	Number of adhesive cells/aggregates		
	1	2	3
Control	150/15	130/14	183/15
PMA	820/34	1976/165	1001/40
PMA+ICO-60	1002/19	1076/17	-
ICO-60[1]	2324/196	2457/138	2564/140[3]
ICO-60[2]	881/104	844/79	1310/65[4]

Note. [1]Cells were treated with MAb, then washed. [2]MAb were not washed. [3]Cells were treated with MAb 1:1000. [4]Cells were treated with MAb 1:100 (not washed). Dash: not assessed. In experiment 1 neuroblasts of the brain stem were used, in experiments 2 and 3 cerebrocortical neuroblasts.

bodies, the initial level of oxygen metabolism, and the physiological reactivity of cells from different individuals. Neutrophils treated with ICO-60 and metabolically inactivated induced a more potent oxygen burst in a mixed suspension of intact neutrophils than did intact cells. Direct activation of neutrophils with ICO-60 indicates the involvement of integrin contraceptor ICAM-3 interacting with the adhesion molecule LFA-1 in the functional activity of neutrophils, and hence we might expect that blocking of donor cell ICAM-3 with specific MAb should suppress the activation of the recipient cell through contact-adhesion interaction with LFA-1. But our results indicate the opposite and suggest that the ICAM-3—LFA-1 relationship is not involved here, but rather that ICO-60 enhances the expression of some other receptors and their ligands, maybe ICAM-1, ICAM-2, or integrins. Moreover, the results permit us to propose the presence of ICAM-3 contraceptor, heretofore detected only on leukocytes [2], on neuroblasts as well.

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