Effects of Immune Complexes with Different Molecular Weights on Functional Activity and Intracellular pH of Neutrophils Exposed or Not Exposed to UV Light

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Loading of neutrophils with immune complexes changed their functional activity and intracellular pH. These changes depended on the size of immune complexes. UV irradiation of neutrophils loaded with immune complexes led to a molecular weight-dependent decrease in intracellular pH. After irradiation the chemiluminescence response to latex increased only in neutrophils loaded with non-phagocytized immune complexes with low (less than 10 units IgG) and high molecular weights (more than 25 units IgG).

Key Words: *immune complexes; intracellular pH; chemiluminescence; UV irradiation; neutrophils*

Recent studies showed that many diseases are associated with the presence of immune complexes (IC). IC with various physicochemical properties were found in the blood from patients with systemic lupus erythematous, psoriasis, glomerulonephritis, viral hepatitis, autoimmune thyroiditis, and other diseases. These IC determine the course, clinical signs, and outcome of pathological processes. Phagocytizing cells, including neutrophils, eliminate IC from the body. The interaction between neutrophils and IC depends on the size, composition, and concentration of these complexes. IC modify receptors on cells and change their functional activity. However, the role of messenger systems (*e.g.*, intracellular pH, pH_i) in the regulation of functional and metabolic activity of cells remains unclear.

Here we studied the effects of model IC with various molecular weights on functional activity and pH_i of mouse peritoneal neutrophils irradiated or nonirradiated with UV light.

MATERIALS AND METHODS

Peripheral blood neutrophils were routinely isolated in a Ficoll-Hypaque density gradient (Pharmacia) [6].

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Complexes of aggregated immunoglobulins (IgG) isolated from the plasma of healthy donors by ion-exchange chromatography on DEAE-Sephadex (Pharmacia) balanced with 0.02 M phosphate buffer (pH 7.5) were used as model IC [4]. Human IgG in various concentrations (1-6 mg/ml) were aggregated by 20-min incubation at 63°C to obtain model IC with various molecular weights [2]. Aggregated IC had the mean molecular weight of 7-28 units IgG.

Neutrophils were incubated for 20 min in media containing aggregated IC with various molecular weights in a concentration of 6 mg/ml. Unbound IC were removed by centrifugation at 460*g*. The ratio of proteins unbound to neutrophils was estimated on a SF-26 spectrophotometer.

Luminol-dependent chemiluminescence (CL) was measured routinely on a PKhL-1 chemiluminometer [1]. Polystyrene latex particles with a diameter of 0.95 μ (activating agent) were added to the cell suspension in a 40:1 ratio. CL of cells was calculated in percents of the control. Nonirradiated and IC-non-loaded mouse peritoneal neutrophils served as the control.

pH_i of individual cells was measured microfluorometrically. Fluorescein diacetate (FDA) was used as a fluorescence probe. FDA possessing high hydro-phobicity easily passes the plasma membrane and enters

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the cytoplasm. In the cytoplasm FDA is rapidly hydrolyzed by intracellular nonspecific esterases. The conformational state of FDA determining changes in fluorescence depends on pH_i, which allows estimating this parameter. Neutrophils were loaded with the probe by incubation with 5 μg/ml FDA (Serva) for 15 min. Nonhydrolyzed FDA was washed out with Hank's solution (pH 7.4). The fluorescence intensity was measured on a LYuMAM-I3 luminescent microscope equipped with a FMEL-IA photometric tip at 520 and 568 nm. pH_i was estimated by calibration curves. To construct these calibration curves the equilibrium between incubation medium pH and pH_i was established by nigericin.

UV irradiation was performed using a LOS-2 laboratory spectral irradiator equipped with an interference light filter with light transmission maximum at 334 nm and spectral interval of not more than 20 nm, respectively. The power density was 10 mW/cm^2. The dose of irradiation was set by varying the time of exposure. Irradiated and control cells were kept in dark at 20°C.

RESULTS

The intensity of latex-induced CL decreased only in neutrophils loaded with small and intermediate model IC (Table 1). By contrast, the CL response of neutrophils loaded with high-molecular-weight IC markedly increased compared to the control. Loading of neutrophils with small and intermediate IC (7-20 units IgG) was followed by an increase in spontaneous CL ($p<0.01$). Long-term incubation (more than 1 h) of cells with high-molecular-weight IC did not modulate their spontaneous CL.

pH_i in control samples changed insignificantly over 1.5 h (7.35). Twenty-minute incubation of neutrophils with IC having various molecular weights led to alkalization of the cytoplasm, which depended on the size of complexes. Small and intermediate IC (7-20 units IgG) increased pH_i, while IC with molecular weights of 25 and 28 units IgG decreased pH_i more than by 0.1.

UV irradiation of neutrophils changed parameters of CL. The intensity of spontaneous CL decreased independently on the size of IC incubated with neutrophils. However, the intensity of latex-induced CL in neutrophils depended on the molecular weight of IC. The CL response of neutrophils loaded with intermediate IC did not change after irradiation. At the same time, UV irradiation increased the CL response of neutrophils loaded with small and, particularly, large IC (by several times).

Changes in CL of irradiated neutrophils were accompanied by a decrease in pH_i. The degree of acidification of the intracellular content depended on the size of IC. pH_i in neutrophils loaded with small and intermediate IC did not differ from that in irradiated cells non-loaded with IC. However, pH_i in neutrophils incubated with large IC was much lower than in irradiated control cells. pH_i of irradiated neutrophils loaded with various IC underwent more pronounced changes with a decrease in the size of complexes (Table 2).

Pronounced CL response of neutrophils loaded with large IC is related to sensitization of cells with these complexes. High-molecular-weight IC are weakly phagocytized, but modulate redistribution of receptors on plasma membranes. This is probably associated with acidification of the intracellular content within physiological limits, since the decrease in pH_i intensifies expression of CD18 molecules necessary for adhesion and phagocytosis on the cell surface.

As differentiated from large IC, intermediate IC are intensively phagocytized by neutrophils, which leads to exhaustion of cell reserves and decrease in the intensity of CL. The cells exhausted after phagocytosis require intensification of synthetic activity, which is mediated by activation of glycolysis. Previous studied showed that the intensity of glycolysis sharply increases with acidification of the medium. Low-molecular-weight IC are not phagocytized by neutrophils, but cause their degranulation. This is accompanied by activation of glycolysis and acidification of the cytoplasm.

UV irradiation causes acidification of the intracellular content, which primarily depends on the initial pH_i. The higher is the initial pH_i, the more pronounced changes in pH_i after irradiation. Acidification of the neutrophil cytoplasm within physiological limits pro-

TABLE 1. Effects of IC with Various Molecular Weights on CL and pH_i of Mouse Peritoneal Macrophages ($M \pm m$)

Parameter		Average molecular weight of IC, units IgG					
		7	10	15	20	25	28
CL, % of control	spontaneous	128.4±10.6*	132.1±16.3*	137.1±11.6*	140.4±18.7*	112.1±15.4	117.4±16.3
	latex-induced	67.4±16.8*	71.7±12.2*	70.9±19.5*	62.2±15.6*	138.6±18.8*	143.7±12.1*
pH_i		7.54±0.02*	7.51±0.02*	7.43±0.03*	7.48±0.02*	7.22±0.02*	7.19±0.03*

Note. *$p<0.01$ compared to the control. Here and in Table 2: pH_i of nonirradiated control cells is 7.35±0.01.

TABLE 2. Effects of UV Irradiation on CL and pH_i of Mouse Peritoneal Macrophages Loaded with IC (*M*±*m*)

Parameter	Irradiated control cells	Average molecular weight of IC, units IgG					
		7	10	15	20	25	28
CL, % of nonirradiated control cells							
spontaneous	77.9±4.6	75.4±5.1*	70.9±7.9*	79.8±10.2*	73.8±7.4*	72.2±7.3*	65.0±10.3*
latex-induced	346.9±48.3	285.6±34.8*	192.5±41.3*	87.1±16.6*	96.7±27.6*	338.6±41.5	343.2±49.8
pH_i	7.15±0.03	7.14±0.02	7.18±0.03	7.15±0.02	7.19±0.02	7.01±0.03*	7.09±0.02*
ΔpH_i after irradiation	—	-0.40±0.03*	-0.33±0.03*	-0.28±0.03*	-0.27±0.03*	-0.20±0.04*	-0.10±0.03*

Note. *$p<0.01$ compared to irradiated control cells.

motes the increase in functional activity of cells. However, it does not necessarily happen. In our experiments UV irradiation did not increase the intensity of latex-induced CL in cells loaded with intermediate IC, although pH_i dropped to a level typical of irradiated control cells (Table 2). The CL response of neutrophils loaded with small IC markedly increased after irradiation, although their pH_i did not differ from the initial level.

Our results indicate that loading of neutrophils with small and intermediate IC causes acidification of the intracellular content followed by a decrease in the intensity of their CL response. The interaction of large IC with neutrophils is accompanied by insignificant acidification of the cell cytoplasm and intensification of latex-induced CL. UV irradiation decreases pH_i of neutrophils, which depends on the size of IC bound to cells. After irradiation spontaneous CL of neutrophils decreases independently on the size of IC bound to cells. However, the CL response to latex increases only in irradiated neutrophils loaded with small and large IC.

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