Absorption and Digestion of Phagocytized Objects by Mononuclear Phagocytes during Rheumatoid Arthritis

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Radioisotope study of mononuclear phagocytes from patients with rheumatoid arthritis showed impaired ingestion of bacteria in the presence of pronounced digestive activity. Excessive accumulation methylumbelliferyl phosphate β-glucuronide (product of hydrolysis catalyzed by glucuronidase released from cells) into the incubation medium was observed. This was probably related to the predominance of extracellular digestion.

Key Words: *rheumatoid arthritis; mononuclear phagocytes; lysosomal enzymes; phagocytosis; exocytosis*

Macrophage lysosomal enzymes cause injury to the articular tissue in rheumatoid arthritis (RA) [3]. Activity of these enzymes in the blood and joint tissue increases in this pathology [4]. The damaging effect of lysosomal enzymes is associated with specific features of absorption and digestion by mononuclear phagocytes (MP) during RA [1].

MATERIALS AND METHODS

We examined blood MP from 24 RA patients and 24 healthy donors without hereditary autoimmune diseases. The cells were isolated in a Ficoll-Verografin density gradient. MP were separated by adhesion [2].

The object of phagocytosis *Staphylococcus aureus* strain Wood was grown on media with C^{14}-labeled amino acids and opsonized with pooled native sera (blood group IV). The microbial suspension was added to MP suspension (10^3 microbial bodies per cell) and incubated at 37°C for 30 min. Under these conditions the count of MP in samples practically did not decrease during incubation. Cell viability determined in the trypan blue exclusion test was 95-98%. The cells were pelleted by centrifugation. The total cell-bound radioactivity, intracellular radioactivity (after removal of surface membrane proteins with trypsin), and radioactivity of supernatants (label bound to high-molecular-weight and low-molecular weight products of bacterial peptide degradation) were measured on a BETA analyzer (PO MA) [5]. Samples from donors and patients were analyzed simultaneously.

The release of lysosomal glucuronidase from MP was assayed by measuring fluorescence of the hydrolysis product methylumbelliferyl phosphate β-glucuronide (MUP-β-glucuronide (Sigma), fluorescent substrate analogue) during the interaction of cells with opsonized zymosan for 30 min. The measurements were performed on a MPF 44 B spectrophotometer (Perkin Elmer) at an excitation wavelength of 325 nm. The contents of the substrate and product were measured at 448 and 375 nm, respectively. The release of glucuronidase was determined by the product/substrate fluorescence ratio measured before and 0.5, 15, and 30 min after addition of zymosan.

The results were analyzed by Student's test and Mann—Whitney test.

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TABLE 1. Distribution of Radioactive Label Bound to High-Molecular-Weight (HMWP) and Low-Molecular-Weight Peptides (LMWP) in Supernatants, Intracellular Bacterial Peptides, and Bacterial Peptides Bound to Surface Membrane of MP from RA Patients and Healthy Donors after 30-min Incubation with Phagocytized Objects (cpm)

Characteristic		Healthy donors	RA patients
Radioactivity of bacterial peptides, cpm			
supernatants	HMWP	1650.3±101.9	1910.6±99.9*
	LMWP	215.79±10.10	202.43±9.80
MP-bound	on cell membranes	1293.0±98.9	1299.3±32.5
	in cells	348.09±35.30	79.28±19.50*
	% of intracellular label	21.2	5.75
Radioactivity of phagocytized objects added to the mixture of MP		3493.7±45.9	

Note. *$p<0.05$ compared to healthy donors.

RESULTS

Considerable differences were revealed in the absorption of phagocytized objects by MP from RA patients and healthy donors (Table 1). Cells from healthy donors intensively ingested bacteria. By the end of incubation 21.2% cell-bound labeled peptides were found in MP. In RA patients this index was 5.75%. Digestive activity of MP was estimated by the content of high-molecular-weight and low-molecular-weight peptides in supernatants. Despite poor absorption of objects by MP from RA patients, the amount of labeled products formed after degradation of bacterial peptides did not decrease. Moreover, the content of high-molecular-weight peptides in patients surpassed that in healthy donors (Table 1).

We hypothesized that during this period MP from RA patients extracellularly digest opsonized bacteria with the involvement of released lysosomal enzymes. The intensity of this process in RA patients is so high that the amount of labeled products formed after degradation of bacterial peptides in supernatants does not differ from that in healthy donors. It should be emphasized that MP from healthy donors intracellularly digest foreign material. To test this hypothesis we studied the release of lysosomal glucuronidase during the interaction of MP with opsonized zymosan.

Control experiments showed that the substrate and hydrolysis product were present in the incubation medium, but were not found in MP after removal of the medium. Products of MUP-β-glucuronide hydrolysis were detected in the suspension of MP from RA patients and healthy donors (Fig. 1). It can be hypothesized that extracellular digestion of phagocytized objects is not only a pathological process. The suspension of MP from RA patients more significantly accumulated the hydrolytic product in various periods after addition of zymosan.

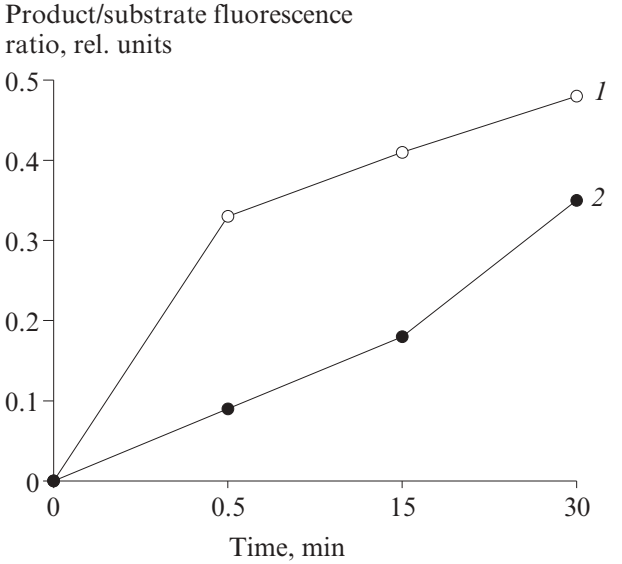

Fig. 1. Fluorescence of glucuronidase product MUP-β-glucuronide in the suspension of mononuclear phagocytes from patients with rheumatoid arthritis (*1*) and healthy donors (*2*).

The excessive release of glucuronidase from MP in RA patients is consistent with decelerated internationalization of phagocytized objects. High-intensity extracellular digestion probably contributes to massive tissue damage and plays a role in the pathogenesis of RA.

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REFERENCES

1. M. I. Arleevskaya and A. P. Tsibulkin, *Immunologiya*, No. 6, 62-70 (1997).
2. S. D. Duglas and P. G. Kui, *Study of Phagocytosis in Clinical Practice* [in Russian], Moscow (1993).
3. V. A. Nasonova and N. V. Bunchuk, *Rheumatoid Diseases* [in Russian], Moscow (1997).
4. A. S. Poltyrev, *Ter. Arkhiv*, No. 9, 147-151 (1990).
5. M. I. Arleevskaya, D. G. Khaliullina, and A. P. Tsibulkin, *Russ. J. Immunol.*, **7**, No. 1, 69-72 (2002).