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Mass-Spectrometric Analysis of Proteome of Microvesicles Produced by NK-92 Natural Killer Cells

A. V. Korenevskii, Yu. P. Milyutina, A. A. Zhdanova, K. M. Pyatygina, D. I. Sokolov, and S. A. Sel'kov

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Membrane extracellular microvesicles serve as carriers of a wide range of molecules, the most important among these are proteins, lipids, and nucleic acids. Cytotoxic proteins of natural killer cells play a key role in the realization of their cytolytic functions. An important stage in understanding of the distant communication of cells and mechanisms of its regulation is analysis of the proteome composition of microvesicles. We studied the proteomic composition of microvesicles produced by NK-92 natural killer cells. Granzyme A, a specific protein of cytotoxic cells, has been identified in the microvesicles by QTOF-mass spectrometry. It was shown that heat shock proteins, components of the ubiquitin—proteasome system, enzymes of protein biosynthesis and energy metabolism, nuclear and serum proteins, as well as cytoskeleton proteins are associated with the microvesicles.

Key Words: *mass spectrometry; proteomic analysis; natural killer cells; microvesicles; granzymes*

Among post-genomic approaches to the study of vital processes in the body, an important place is occupied by proteomic analysis of proteins and peptides, the factors of the final stage of cell signaling [1]. Achievements in proteomics and peptidomics help to solve issues related to structural characteristics of proteins, intracellular signaling, post-translational modifications, and cell communication. Of particular importance is application of proteomic techniques in medical molecular diagnostics allowing the search for potential protein markers. It is now beyond doubt that proteomic studies can contribute to solving the problems of various branches of medicine, *e.g.* in the field of medical immunology [29].

Extracellular membrane microvesicles (MV) produced by cells as a result of local changes in the membrane structure, are currently considered as effective mediators of physiological and pathological processes. MV, among which ectosomes and exosomes are distinguished, serve as carriers of a wide range of molecules; proteins, lipids, and nucleic acids carried by MV have the paramount functional importance [10]. Natural killer (NK) cells, a subpopulation of lymphocytes destroying virus-infected and tumor cells through contact cytolysis and participating in mechanisms of immunological tolerance in the mother—fetus system are of particular interest as the source of MV [23]. There are indirect data that NK cells can produce MV: $CD56^+$ MV and leukocyte-derived MV with different phenotypes were found in peripheral blood plasma [22].

Isolation and characterization of various extracellular MV produced by NK cells were described in some recent reports [9,15,21,30]. Cytotoxic activity of exosomes of activated NK cells of the peripheral blood against various tumor cell lines related to the expression of FasL on their surface [15,30] and the presence of NK cell effector molecules perforin [9,15,30], granzyme A, granzyme B, and granulysin [9] was revealed. The authors of these studies consider promising the use of various NK cell-derived extracellular MV preparations in the therapy of various forms of cancer.

D. O. Ott Research Institute of Obstetrics, Gynecology, and Reproductology, St. Petersburg, Russia. ***Address for correspondence:*** a.korenevsky@yandex.ru. A. V. Korenevskii

In the above studies, mainly activated peripheral blood NK cells were used [9,21]; the use of MV derived from these cells for immunotherapy is associated with significant complications (the search for suitable donors, production of sufficient amounts of MV, *etc*.) [15]. The use of MV obtained from NK cells of NK-92 cell line seems more promising for this purpose, because of easy procedure of their isolation in sufficiently large amounts and pronounced cytotoxic effect [15]. In the context of clinical application of MV obtained from NK cells of NK-92 cell line for the therapy of tumors, a pressing task is analysis of functional potencies, phenotype, and content of these MV allowing evaluation of both positive and possible negative side effects of this therapy.

Cytotoxic proteins of NK cells (perforin, granzymes, FasL, granulyzin, and polypeptide LL-37) play the key role in their cytolytic functions [2,13,20,26]. An important step in understanding of distant communication of cells and mechanisms of its regulation is analysis of the proteomic composition of MV that can contribute to the cytotoxic effect of NK cells against target cells. The data on MV production by NK cells and their proteomic spectrum would provide information about previously unknown mechanisms of NK cell interaction with target cells under normal conditions and during inflammation. To solve this problem, isolation of MV followed by mass spectrometric analysis of their proteome in a model experiment with NK cells of the NK-92 line can be used.

MATERIALS AND METHODS

Cell culturing. NK-92 cells (ATCC) derived from large granular peripheral blood lymphocytes of a 50-year-old man with rapidly progressive non-Hodgkin's lymphoma reproduce the main phenotypical and functional characteristics of activated NK cells [8]. NK-92 cells is maintained in a suspension culture that requires subculturing every 48 h. The cells were cultured in complete culture medium αMEM (BioloT) supplemented with 12.5% fetal calf serum, 12.5% inactivated equine serum depleted of MV, 0.2 mM myoinositol, 0.02 mM folic acid, 2 mM L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin, 10 mM HEPES, 0.1 mM 2-mercaptoethanol (Sigma-Aldrich), and 500 U/ml recombinant IL-2 (Roncoleukin; Biotech). The cells were cultured in a humid atmosphere at 37°C and 5% CO_2. Cell viability evaluated by trypan blue exclusion was ≥96%.

Microvesicle isolation. Due to the absence of common standards for isolation and characterization of MV, various currently used methodological approaches allow isolation of MV fractions with different degree of purity and concentration [14]. MV separated from NK-92 cells were isolated by the modified method of differential centrifugation [22] in $Ca^{2+}Mg^{2+}$-free Hanks solution (Sigma-Aldrich); to this end, supernatants were sequentially centrifuged at 200*g* (room temperature, 10 min) and 9900*g* (4°C, 10 min). The pellet was washed twice with cold PBS (Sigma-Aldrich) and re-centrifuged at 19,800*g* (4°C, 20 min). The supernatant was discarded and the pellet was washed several times with cold PBS at 19,800*g* (4°C, 20 min). The purified pellet was resuspended in deionized water of the MilliQ standard in the presence of protease inhibitor mixture (cOmplete, EDTA-free; Roche Diagnostics GmbH) in a concentration specified by the manufacturer and stored at -80°C until analysis. This protocol enables isolation of MV with a diameter of 100-200 nm with sufficient purity and minimal losss of the biomaterial; during this procedure, MV are separated successively from coarse cell debris particles and large apoptotic bodies, as well as from exosomes [12].

Laser correlation analysis. The granulometric analysis of MV produced by NK-92 cells was performed by dynamic light scattering on a Zetasizer NanoZS laser correlation spectrometer (Malvern Instruments) with particle measurement limits from 0.3 nm to 10 μ. MV for this assay were isolated as described above. MV diameter was calculated using Zetasizer Software 7.11 (Malvern Instruments).

Preparation of cell extracts and MV. Frozen cells (3×10^6) and MV were thawed, subjected to 3 freeze/thaw cycles, and then intensively homogenized in a glass homogenizer for 5 min. The resulting material was concentrated by evaporation on the CentriVap vacuum concentrator (Labronco) over 2-3 h until complete removal of fluid. Then, 160 μl ReadyPrep 2-D Starter Kit Rehydration/Sample Buffer containing 8 M urea, 2% CHAPS, 50 mM DL-dithiothreitol, 0.2% Bio-Lyte 3/10 ampholyte, and 0.001% bromophenol blue (Bio-Rad Laboratories) was added to the residue. The mixture was incubated for 30 min (to a state close to homogeneous), after which the cell debris was removed by centrifugation at 16,000*g* (4°C, 10 min) and the supernatant was sampled for further analysis.

Measurement of total protein concentration. Protein content in cells and MV was measured by using Bradford protein assay on a NanoDrop One spectrophotometer and NanoDrop One Viewer software (Thermo Fisher Scientific).

2D electrophoresis. Cell and MV proteins were separated by 2D gel electrophoresis. Isoelectrofocusing was performed on Mini-Protean 7 cm IPG strips with immobilized pH 3-10 gradient in the Protean i12 IEF Cell chamber (Bio-Rad Laboratories) under conditions of active rehydration at 50 V (20°C, 12 h) followed by separation at 14,000 V (20°C, 5 h). The

cell and MV extracts were applied to the strips in such a way that protein content in strip was approximately the same and sufficient to obtain valid data. To insulate the strips in the isoelectrofocusing chamber from the environment, they were coated with mineral oil.

After isoelectrofocusing of the proteins, the strips were incubated in Equilibration Buffer I (375 mM Tris HCl, 6 M urea, 2% SDS, and 3% DL-dithiothreitol; pH 8.8) and thereafter under the same conditions in Equilibration Buffer II (375 mM Tris HCl, 6 M urea, 2% SDS, and 3.7% iodoacetamide; pH 8.8) (Bio-Rad Laboratories).

Separation of the proteins by their molecular weights was conducted in 10% PAAG using commercial Mini-Protean TGX Stain-Free PrecastGel (Bio-Rad Laboratories) in a Mini-Protean Tetra System vertical electrophoresis chamber (Bio-Rad Laboratories) in alkaline TGS buffer solution containing 25 mM Tris, 192 mM glycine, 0.1% SDS (Sigma-Aldrich) at 200 V.

Preparation of samples for mass spectrometric analysis. After electrophoresis, the gels were visualized on a ChemiDoc Touch Gel Imaging System (Bio-Rad Laboratories) and stained with Coomassie Brilliant Blue R-250 Solution (Bio-Rad Laboratories). Then, gel spots containing the target proteins were cut out with a scalpel and minced. To remove the dye and SDS, the gel fragments were washed three times with 50% acetonitrile solution in 30 mM Tris buffer (pH 8.2; Sigma-Aldrich) for 15 min at room temperature. After removal of the solution, the gel fragments were dehydrated by 10-min incubation in acetonitrile and after removal of acetonitrile, the samples were drired for 40 min at 4°C. A solution of modified bovine trypsin (20 ng/ml; Promega) was added to dried samples and incubated on ice for 1 h and then at 37°C for 4 h until complete rehydration of the gel. Trypsin excess was removed and the samples were incubated with 50 µl 30 mM Tris buffer (pH 8.2) for 16-18 h at 37°C. The tryptic peptide mixtures were extracted three times from the gel with 50% water solution of acetonitrile containing 0.1% formic acid (Sigma-Aldrich) in an ultrasonic bath for 20 min. The peptide in the resultant solution were dried at 4°C and dissolved in a mobile phase A (5% acetonitrile in 0.1% formic acid) for further separation by HPLC.

Liquid chromatography mass spectrometry of peptide mixtures. The peptides were separated on a Zorbax 300SB-C18 analytical column (3.5 µ, 150 mm×100 µ; Agilent Technologies) in mobile phase B gradient (90% acetonitrile in 0.1% formic acid) at a flow rate of 20 µl/min.

Mass spectra of tryptic peptides were recorded on a ESI-Q-TOF 6538 UHD quadrupole-time-of-flight mass spectrometer (Agilent Technologies) coupled with Agilent 1260 high-performance liquid chromatograph (Agilent Technologies). The ion analysis was performed at a frequency of 3 spectra per second in the automatic tandem MS/MS analysis mode with 2+, 3+, *n*+mother ions.

Analysis of mass spectrometric data was carried out using Spectrum Mill MS Proteomic Workbench software (Rev B.04.00.127, Agilent Technologies). The mgf-files were used for database searches with the MASCOT search engine (Matrix Science, London, UK); the search was conducted against the UniProt (SwissProt) database with a taxonomic restriction for the species *Homo Sapiens* and tolerance for peptide ions 20 ppm, for fragmentary ions 0.1 Da. Parallel search was performed using the database of inverted and random amino acid sequences (decoy). Data validation was carried out using the autovalidation procedure with a cut-off value of false positives (FDR) of 1%. For identified proteins, their correspondence to actual positions on PAAG was verified.

RESULTS

Granulometric analysis showed that the size of MV produced by NK-92 cells was 190-460 nm, which corresponded to the diameter of ectosomes (100-1000 nm), and the peak of MV distribution (40.8%, *n*=3) corresponded to 295 nm (Fig. 1). These findings are in line with the data of other researchers on the size of MV formed by different cells [3,28]. Analysis of particles in the supernatant obtained after MV isolation by differential centrifugation showed that their sizes was 16-190 nm, the peak of particle distribution (<30%) corresponded to 28 nm.

The total protein content in NK-92 cells and their MV was 60.2±6.1 and 2.5±0.3 µg/10^6 source cells, respectively. These data allowed calculating protein load of strips for isoelectrofocusing and its alignment relative to cells and their MV.

During electrophoretic separation of proteins in two directions (isoelectric point and molecular weight), electrophoregrams were obtained corresponding to protein profile of cells and their MV (Fig. 2).

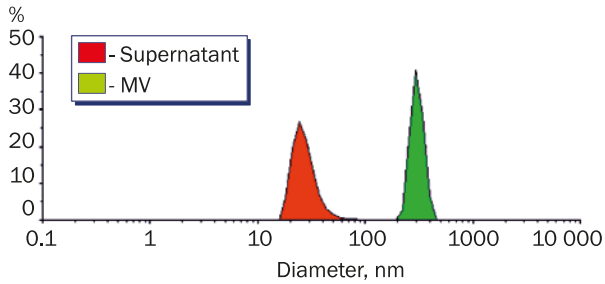

Fig. 1. Granulometric analysis of microvesicles produced by NK cells of the NK-92 cell line and of the supernatant obtained after MV isolation by differential centrifugation.

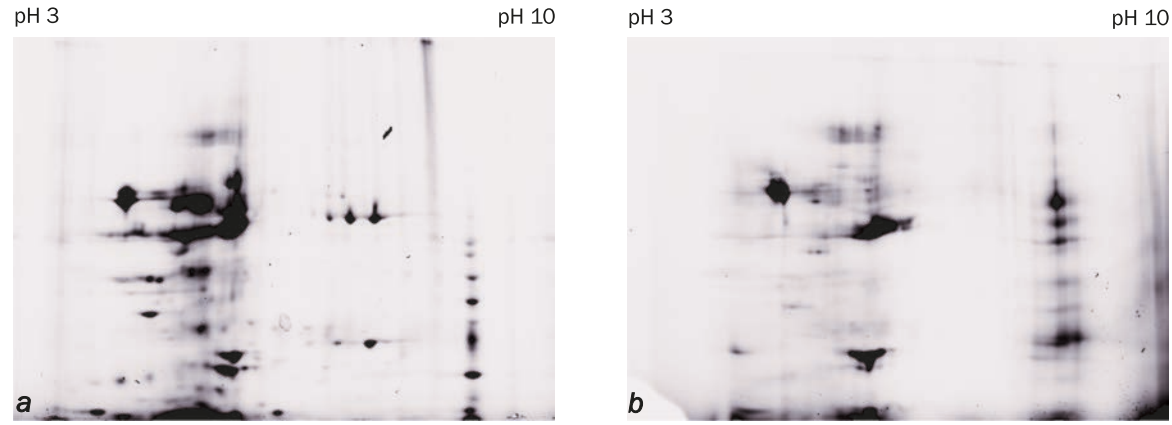

Fig. 2. 2D electrophoregrams of proteins in NK-92 cells (*a*) and MV produced by these cells (*b*).

The results suggest that the major and minor components of cell and MV proteome differ significantly. However, the absence of proteins identified in MV in source cells cannot unambiguously indicate their exclusively secretory nature. To elucidate the causes for differences in cell and MV proteome, an attempt was undertaken to analyze partial proteome of MV and compare it with the proteome of cells producing these MV and with the data reported previously.

The results of liquid chromatography-mass spectrometric analysis of protein fractions of NK-92 cell and MV lysates are presented in Tables including all proteins identified in spots on PAAG after 2D gel electrophoresis. A total of 52 proteins that perform various functions (Table 1) were identified in cells and MV, which is only a small part of the known NK cell proteome [16,19]. The individual proteomic composition of NK-92 cells and their MV is presented in Table 2.

The NK-92 cell line used in our study allows reproducing *in vitro* the basic biochemical processes taking place in NK cells *in vivo*, including the processes leading to spontaneous secretion of MV into the extracellular environment. The data of 2D gel electrophoresis suggest that NK-92 cells and MV formed by these cells carry a wide spectrum of proteins with molecular weight varying from 10 to 125 kDa and isoelectric point from 3.0 to 10.0. Using mass spectrometric analysis, we identified proteins with various functions and properties enabling intricate regulation of cell metabolism.

In light of MV biogenesis, their proteome consists of protein molecules of cellular origin. Proteomes of MV derived from platelets, plasma microparticles, mature lymphocytes, endotheliocytes, mast cells, and some other cells are now relatively well studied, and relevant data have been presented by many authors [7,17]. These studies showed that MV produced by blood cells and endotheliocytes contain both nonspecific proteins characteristic of all cell types and specific proteins involved in functioning of a particular cell. Proteins specific for a certain cell type are most often presented by T cell receptors, proteins of the immunoglobulin superfamily (CD54 of B cells), selectin family (P-selectin of platelets), and some other proteins involved in the immune response [4]. Proteins present in MV irrespective of their origin are most often involved in vesicle formation process. These proteins naturally include tetraspanins (CD9, CD63, CD81, and CD82), heat shock proteins (HSP70, HSP90), cytoskeleton proteins, enzymes of various metabolic pathways, adhesion and recognition molecules, and major histocompatibility complex (MHC) proteins [25]. In our study, proteins that belong to some of the listed classes, in particular chaperones, actin, vimentin, and other cytoskeleton components, as well as proteins participating in the metabolism were also found in the

TABLE 1. Total Protein Composition of NK-92 Cells and Their MV Determined Using Liquid Chromatography Mass Spectrometry

Group of proteins	Total number of proteins	% of total amount of proteins
Cytoskeleton proteins	19	36.5
Chaperones	5	9.6
Cytotoxic proteins	1	1.9
Proteasome degradation proteins	4	7.7
Protein biosynthesis enzymes	6	11.5
Energy metabolism enzymes	10	19.2
Histones	3	5.8
Heterogeneous nuclear ribonucleoproteins	1	1.9
Serum proteins	2	3.8
Transmembrane transporters	1	1.9

TABLE 2. Individual Proteome Composition of Natural Killer NK-92 Cells and Their Microvesicles Determined by Liquid Chromatography Mass Spectrometry

Protein	AN UniProt (SwissProt)	MW, kDa	pI	Number of peptides in cells	Number of peptides in MV
Cytoskeleton proteins					
Actin, cytoplasmic 1 (β-actin)	P60709	41.7	5.2	7 (19.7)	—
Actin, cytoplasmic 2 (γ-actin)	P63261	42.1	5.3	6 (21.0)	12 (56.8)
Actin, aortic smooth muscle	P62736	42.4	5.2	3 (10.0)	3 (8.7)
Vimentin	P08670	53.7	5.1	17 (36.6)	6 (10.1)
Keratin, type I cytoskeletal 9	P35527	62.3	5.1	2 (4.1)	2 (4.1)
Keratin, type I cytoskeletal 10	P13645	59.0	5.1	2 (3.9)	
Keratin, type II cytoskeletal 1	P04264	66.2	8.3	3 (5.5)	4 (7.3)
Keratin, type II cytoskeletal 8	P05787	53.7	5.5	N/A	2 (4.1)
Keratin, type II cytoskeletal 79	Q5XKE5	57.8	8.3	6 (9.3)	
Keratin, type II cytoskeletal 80	Q6KB66	50.5	8.3	7 (12.2)	
POTE ankyrin domain family member F	A5A3E0	123.1	5.8	7 (19.7)	N/A
Tropomyosin α-3 chain	P06753	33.0	4.7	N/A	17 (51.1)
Tropomyosin α-4 chain	P67936	28.6	4.7	N/A	
Tropomyosin β chain	P07951	32.9	5.1	N/A	
Tubulin α-1B chain	P68363	50.1	4.9	N/A	3,5 (15.9)
Tubulin β chain	P07437	49.7	4.8	9 (22.7)	
Tubulin β-4B chain	P68371	49.8	4.8	7 (16.6)	
Chaperones					
10 kDa heat shock protein, mitochondrial	P61604	10.9	8.9	N/A	9 (23.5)
60 kDa heat shock protein, mitochondrial	P10809	61.1	5.7	18 (29.8)	
71 kDa heat shock cognate protein	P11142	70.9	5.4	2 (4.3)	
105 kDa heat shock protein	Q92598	96.7	5.3	N/A	
Calreticulin	P27797	48.3	4.3	2 (6.9)	20 (52.0)
Cytotoxic proteins					
Granzyme A	P12544	29.5	9.7	N/A	2 (8.3)
Proteasome degradation proteins					
14-3-3 Protein ζ/δ	P63104	27.7	4.7	N/A	5 (22.4)
Proteasome subunit α type-5	P28066	26.4	4.7	N/A	2 (15.8)
Proteasome subunit α type-7	O14818	27.9	8.8	N/A	
Proteasome activator complex subunit 2	Q9UL46	27.4	5.5	N/A	
Protein biosynthesis enzymes					
78 kDa glucose-regulated protein	P11021	72.3	5.1	11 (24.3)	N/A
Stress-70 protein, mitochondrial	P38646	73.7	5.9	5 (9.4)	N/A
40S ribosomal protein SA	P08865	33.0	4.8	N/A	3 (12.8)
Eukaryotic initiation factor 4A-II	Q14240	46.6	5.3	N/A	2 (5.6)
Protein disulfide-isomerase A3	P30101	56.8	6.0	4 (11.0)	8 (18.0)
Elongation factor 1-α1	P68104	50.1	9.4	N/A	2 (4.9)
Energy metabolism enzymes					
ATP synthase subunit β, mitochondrial	P06576	56.6	5.3	8 (21.3)	N/A

TABLE 2.

Protein	AN UniProt (SwissProt)	MW, kDa	pI	Number of peptides in cells	Number of peptides in MV
Glyceraldehyde-3-phosphate dehydrogenase	P04406	36.2	8.8	2 (11.6)	N/A
α-Enolase	P06733	47.5	7.0	15 (41.8)	23 (57.6)
β-Enolase	P13929	47.3	7.8	4 (14.0)	N/A
γ-Enolase	P09104	47.6	4.9	2 (9.2)	N/A
L-lactate dehydrogenase B chain	P07195	36.6	5.7	N/A	2 (7.1)
L-lactate dehydrogenase A-like 6B	Q9BYZ2	41.9	8.8	N/A	3 (10.2)
Phosphoglycerate kinase 1	P00558	45.0	8.7	N/A	6 (19.6)
Fructose-bisphosphate aldolase A	P04075	39.9	8.7	7 (8.7)	N/A
Fructose-bisphosphate aldolase C	P09972	39.5	8.2	2 (6.4)	N/A
Nucleoproteins					
Histone H2A type 1-B/E	P04908	14.1	11.1	N/A	2 (21.5)
Histone H2B type 1-M	Q99879	14.0	10.3	N/A	2 (19.0)
Histone H4	P62805	11.4	11.4	N/A	3 (29.1)
Heterogeneous nuclear ribonucleoprotein F	P52597	46.0	5.4	N/A	2 (7.9)
Serum proteins					
Apolipoprotein A-I	P02647	30.8	5.6	N/A	2 (4.1)
Serum albumin	P02768	71.4	5.9	N/A	3 (7.2)
Transmembrane transporters					
Chloride intracellular channel protein 1	O00299	26.9	5.1	N/A	2 (11.2)

Note. AAC, amino acid coverage; MW, molecular weight; pI, isoelectric point; N/A in the graph indicates the non-detection or unreliable detection of the protein in the experimental series.

studied MV. The cytoskeleton proteins together with enzymes participating in various metabolic processes naturally constituted the major part of the identified MV proteome. We also identified some specific proteins, in particular granzyme A, a representative of serine protease family expressed in cytotoxic T cells and NK cells and surprisingly some nuclear proteins.

Molecular mechanism of MV formation implies the presence of components of the submembrane actin network and other elements of the cytoskeleton [6]. The data of the mass-spectrometric analysis suggest that isolated MV may have ectosomal nature. For instance, we identified different isoforms of cytoskeleton proteins, as well as nuclear proteins (histones and heterogeneous nuclear ribonucleoproteins), which, along with mitochondrial proteins (β-subunit of mitochondrial ATPase) and endoplasmic reticulum (calreticulin) or Golgi comples detected by us are not identified in exosomes according to available published reports [5]. This assumption is confirmed by the presence of chloride intracellular ion channel protein 1 in the isolated MV. However, it is known that cytotoxic protein granzyme A identified by us in the MV is present in lytic granules of NK cells; being released in the cytoplasm, this protein is inactivated via formation of a complex with serpin [11]. The presence of granzymes in exosomes was reported previously [15], while larger ectosomes, or extracellular MV, mainly contain cytoplasmic protein isoforms. At the same time, there are data that large microparticles can include other compartments and cell organelles [27]. Thus, detection of granzymes in MV differentiated by size does not allow one to unequivocally determine the origin of these particles. In clarifying this issue, it might be helpful to use, along with the approach presented in the paper, additional methods, including analysis of specific exosomal proteins, *e.g.* Alix, or, conversely, cytoplasmic membrane proteins [24].

The presence of histones in MV fraction revealed by us most likely suggests that they contain admixture of apoptotic bodies rather than nuclear proteins, because the size of apoptotic bodies (200-5000 nm) partially overlap with that of MV (100-1000 nm) in the range of particle diameters in the isolated fraction

(190-460 nm) [10]. It should be noted that we could not achieve reproducibility of results on histones in cells, which is probably due to the fact that the isoelectric point of most histones exceeds 10, while the isoelectrofocusing of proteins was carried out in the pH range of 3-10.

The detected various isoforms of cytoskeletal keratin can indicate contamination of samples with cytokeratin. Keratin, type II cytoskeletal 1 is often present in samples as a contaminant and is detected by mass spectrometric analysis in fractions that do not correspond to its isoelectric point and molecular weight. This protein was detected by other researchers in similar analysis of different biological samples [18].

Various proteomic research strategies based on a combination of different techniques have been described by now. Protein identification is a complex multistage process that does not always lead to reproducible results. The proposed scheme for studying protein composition of MV can produce good results in qualitative description of the protein profile. However, the search for target protein by using this approach is associated with considerable difficulties.

The electrophoregrams obtained for the proteome of the cells and their MV differ by protein profile, which makes it difficult to search for a particular protein by isoelectrofocusing followed by electrophoretic separation of the peptides by the molecular weight. In addition, analysis produces unreliable data for proteins with isoelectric point lying outside the pH gradient used in the study.

We also obtained data on the size of MV produced by NK-92 cells as well as reliable data on the content of individual proteins in MV. However, for a more detailed analysis of the MV proteome by liquid chromatography-mass spectrometry, it would be advantageous to include additional steps during their isolation such as ultracentrifugation, immunoprecipitation, and probably the use of protein fractionation methods that would prevent the loss of biological material during washing out the tryptic peptides from the gel.

Based on our findings we can conclude that the protein profiles of NK-92 cells and MV produced by these cells differ, which is confirmed by mass-spectrometric identification; apart from universal ubiquitous proteins, MV contain granzyme A, which is a specific protein of cytotoxic cells. Therefore, MV containing one of the granzymes and probably other cytotoxic proteins can contribute to the cytotoxic effect of NK cells. Our preliminary data on the proteome of the MV produced by NK-92 cells can expand existing concepts of distant cell communication and give some evidence about new mechanisms of NK cell interaction with target cells. The presented mass spectrometric analysis will be useful for further proteomic studies of MV produced by cells involved in the formation of immunological tolerance, both under physiological conditions and under conditions of inflammatory reaction accompanying various pathological processes.

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