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Sialoside-Binding Macrophage Lectins in Phagocytosis of Apoptotic Bodies

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Abstract—Elimination of apoptotic bodies is one of the important functions of macrophages. The aim of this work was to study the role of macrophage lectins in this process. Macrophage lectins were probed with neoglycoconjugates Glyc-PAA-fluo where carbohydrate is linked to fluorescein-labeled polyacrylamide (MW 30 kD). It was shown that neoglycoconjugates containing a Neu5Acα2-3Gal fragment bound to macrophages isolated from blood of healthy donors. Besides, carbohydrate chains containing the same fragment were revealed on apoptotic bodies. Phagocytosis of apoptotic bodies by macrophages was inhibited with sialooligosaccharide ligands of siglec-5 and MAbs to siglec-5. Thus, siglec-5 expressed on macrophages could participate in phagocytosis of apoptotic bodies. In addition, the role of siglecs in engulfment of apoptotic bodies by tumor-associated macrophages was studied. The phagocytic potency of macrophages isolated from blood of breast cancer patients was lower than engulfment ability of macrophages obtained from healthy donors and depended on tumor degree. Staining of macrophages obtained from blood of tumor patients with sialylated Glyc-PAA-fluo probes was more intense than that of macrophages from healthy donors; phagocytosis of apoptotic bodies by tumor-associated macrophages was inhibited by carbohydrates that are known to be ligands for siglecs.

Key words: apoptosis, breast cancer, glycoconjugates, phagocytosis, siglecs

Apoptosis, or programmed cell death, is the mechanism used by the organism for elimination of foreign cells, genetically imperfect cells, and immune cells which have completed their functions [1]. Apoptotic bodies are rapidly eliminated from the organism by phagocytes: macrophages, neutrophils, dendritic cells, and natural killer cells [2, 3]. Elimination of apoptotic bodies is an important stage in the organism's life activity. Disorders in this process result in intoxication and finally in the death of the organism.

Tumor cells in early stages of transformation also die through apoptosis. The surviving tumor cells realize some changes increasing their antioxidant activity, and this makes them resistant to macrophages and natural killers [2, 3]. Impaired recognition by phagocytes of tumor cells increases the tumorigenicity and metastasizing activity of the transformed cells.

Studies on the mechanism of phagocytosis, in particular, of receptors responsible for the primary recognition seems to be a key for approaches to directed stimulation of phagocytosis during early stages of oncogenesis.

Lectins, along with the vitronectin receptor, CD36, phosphatidylserine receptor, and scavenger receptor, are involved in phagocytosis via binding to complementary carbohydrates of target cells (lectin-mediated phagocytosis) [3]. During inflammation, neutrophils are phagocytized by fibroblasts by means of a fucose/mannose-specific lectin [4]. Macrophages and immature dendritic cells

Abbreviations: FITC) fluorescein isothiocyanate; Glyc-PAA) polyacrylamide glycoconjugates; GM-CSF) granulocyte and macrophage colony-stimulating factor; fluo) fluorescein residue; PBS) phosphate-buffered saline containing 0.01 M Na_2HPO_4, 0.01 M NaH_2PO_4, 0.15 M NaCl (pH 7.3); PBA) PBS supplemented with 0.2% BSA; $SiaLe^x$) Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc; 3′SiaLac) Neu5Acα2-3Galβ1-4Glc; 6′SiaLac) Neu5Acα2-6Galβ1-4Glc; 3′SiaTF) Neu5Acα2-3Galβ1-3GalNAcα; $3'SiaLe^c$) Neu5Acα2-3Galβ1-3GlcNAc; $SiaT_n$) Neu5Acα2-6GalNAcα; $6^{(Gal)}OSuSiaLe^x$) Neu5Acα2-3(6-HSO_3)Galβ1-4(Fucα1-3)GlcNAc; $6^{(GlcNAc)}OSuSiaLe^x$) Neu5Acα2-3Galβ1-4(Fucα1-3)(6-HSO_3)GlcNAc; Sia_2) Neu5Acα2-8Neu5Acα.

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can engulf tumor cells in early stages of breast, lungs, intestine, ovaries, and pancreas cancers with involvement of a galactose-binding lectin, which interacts with the mucin O-chain carbohydrates carrying Galβ1-3GalNAc/Thr/Ser epitope [5]. The same lectin interacts with biantennal galactose-containing chains of desialylated low density lipoprotein and, thus, is involved in the engulfment and accumulation of the lipoprotein by blood macrophages of patients with coronary heart failure and atherosclerosis [6]. Galectin-3 is reported to be involved in the lectin-mediated phagocytosis. Indeed, elimination of myelin in diseases of the peripheral nervous system is associated with the recognition by galectin (MAC-2) of carbohydrate chains of myelin [7]. Moreover, in galectin-3 knockout mice phagocytosis of apoptotic bodies by macrophages is reduced [8].

There are scarce data on the involvement of siglecs in lectin-mediated phagocytosis. Sialoside-binding lectins, such as siglec-1, siglec-5, and siglec-11, are expressed on macrophages. Siglecs recognize sialylated carbohydrate chains of glycoproteins and glycolipids of the cell membrane [9-11]. At present, 11 siglecs are known. Except siglec-4 (a myelin-associated protein), which is identified only on cells of the central and peripheral nervous systems, siglecs are exposed on immune cells and peripheral blood cells. By amino acid sequence, siglecs are divided into two groups. The first group includes sialoadhesin (siglec-1), CD22 (siglec-2), and siglec-4, which are 25-30% homologous. The other group (CD33-like proteins) includes siglecs with the 50-80% homology to siglec-3 (CD33) [10, 11]. The structure and carbohydrate specificity of siglecs are intensely studied, but their biological role is poorly understood. Sialylated chains of MUC1 and CD43 are reported to be ligands for siglec-1 [12, 13]. Binding of siglec-1 to CD43 lead to contact stimulation between T-cells and macrophages. Besides, siglec interaction with mucin carbohydrate chains provides increase of cell adhesion to tumor-associated macrophages in breast cancer. Siglec-1 and siglec-5 were recently shown to be involved in phagocytosis of the bacterium *Neisseria meningitides* through binding to the fragment Neu5Acα2-3Gal of lipopolysaccharide [14].

The purpose of the present work was to study the role of siglecs in phagocytosis of apoptotic bodies by macrophages, including tumor-associated ones.

MATERIALS AND METHODS

The following reagents were used: bovine serum albumin (BSA) (Serva, Germany); Ficoll-Paque (Amersham, UK); the culture medium RPMI-1640, fetal calf serum, glutamine, an antibiotic-antimycotic (Invitrogen, Great Britain); GM-CSF (Lumax, France); poly(2-hydroxyethyl methacrylate) and fluorescein isothiocyanate (FITC) (Sigma, USA); digoxigenin-labeled lectins from *Maakia amurensis* (MAA) and *Sambucus nigra* (SNA), FITC-labeled IgG antibodies to digoxigenin (Roche, Germany); Glyc-PAA-fluo and Glyc-PAA glycoconjugates (Lectinity, Russia); phycoerythrin-labeled antibodies to the human antigen CD45 (anti-CD45-RPE), FITC-labeled antibodies to macrophage-associated antigens (Coltag, USA); FITC-labeled streptavidin and FITC-labeled antibodies to mouse IgG (Sigma). Other reagents were from Reakhim (Russia). Monoclonal antibodies to siglec-5 and siglec-7 were presented by P. R. Crocker (Great Britain).

Mononuclear cells were isolated from blood of healthy donors and of patients with breast cancer during surgical operations in the Russian Research Center for Roentgen Radiology, Russian Ministry of Public Health. The stage of the patient's disease was characterized by the formula $T_xN_yM_z$, where T is the tumor, N are metastases located near the tumor along the lymphoid ducts, M are distant metastases; $x = 1$ corresponds to tumor size no more than 2 cm, $x = 2$ corresponds to tumor size of 2-5 cm; y and z were the numbers of metastases. Blood samples were taken from six patients: $T_4N_2M_0$ (one patient), $T_2N_1M_0$ (two patients), $T_2N_0M_0$ (two patients), and $T_1N_0M_0$ (one patient).

Isolation of mononuclear leukocytes. Mononuclear cells were isolated in the gradient of Ficoll-Paques. Briefly, 4 ml of Ficoll-Paques was added to the blood sample (10 ml) of a healthy donor or patients diluted twofold in PBS. Cell suspension was centrifuged at 2800 rpm for 30 min. The fraction containing lymphocytes and monocytes was washed twice in PBS by centrifugation at 800 rpm for 10 min at 4°C and cultured in 5 ml of RPMI-1640 containing 10% fetal calf serum and 2 mM glutamine. To obtain macrophages, cells were cultured in medium supplemented with GM-CSF (100 μg/ml) for 4 days at 37°C in the presence of 5% CO_2. The macrophages were phenotyped with interaction of antibodies directed to macrophage antigens (CD11b, CD14, and CD36), B-lymphocytes (CD40), and dendritic cells (CD86, CD83).

Labeling of apoptotic bodies with fluorescein isothiocyanate. Cell lines of human breast carcinoma MCF-7 (ATCC No. CRL-2351) and of human melanoma M44 presented by J. LePendu (France) were used. The cells were cultured in the RPMI-1640 medium supplemented with 10% fetal calf serum and 2 mM glutamine. Apoptosis of the cells was induced by UV-radiation for 2 min. The induction of apoptosis resulted in flotation of the produced apoptotic bodies. After 48 h, supernatant containing the apoptotic bodies was collected. The induction of apoptosis was confirmed by electrophoresis of DNA in 1% agarose gel [15]. The electrophoregram showed fragmentation of DNA that suggested the apoptosis (data not presented). The cells were washed thrice in RPMI-1640 by centrifugation at 1200 rpm, resuspended in 1 ml of the

same medium, and supplemented with FITC solution in the same medium (15 μmol per 10^6 cells). The cells were incubated at 37°C for 40 min. After completion of the reaction, the cells were washed thrice in PBA (PBS supplemented with 0.2% BSA).

Determination of expression of carbohydrate structures on M44 and MCF-7 cells after the induction of apoptosis. Adhesive (a control) cells were removed from the plastic with Versene solution. The suspension of apoptotic cells was washed thrice in PBA with centrifugation at 1200 rpm. Fifty microliters of lectin solution digoxigenin labeled was added to the U-well plate (Nunc, Denmark) containing $2 \cdot 10^5$ cells per 100 μl. Cells were incubated for 30 min at 4°C, with a subsequent washing in PBA and incubation with FITC-labeled antibodies to digoxigenin (diluted 1 : 10 in PBA) for 30 min. On completion of the reaction, the cells were washed in PBA by centrifugation at 1200 rpm and resuspended in 2 ml of PBS. The cells were analyzed using a DACO Galaxy laser flow cytometer (Denmark) at 488 nm. To 100 μl of the cell suspension 2 ml of PBS was added and carefully mixed. The cell fluorescence was determined at room temperature. Data were processed with the Flowmax program (DACO Galaxy). In each sample, at least 5000 cells were analyzed.

Identification of lectins of the macrophage surface. The cells were washed thrice by centrifugation in PBA. Cells ($2 \cdot 10^5$ per 100 μl) were incubated in U-well plate with 50 μl of 100 μM Glyc-PAA-fluo for 40 min at 4°C. On completion of the reaction, the cells were washed by triple centrifugation in PBA at 1200 rpm. The stained cells were analyzed as described above.

Phagocytosis of apoptotic bodies by macrophages. Apoptotic bodies were washed thrice by centrifugation in RPMI-1640 at 1200 rpm for 3 min. A 0.5-ml cell suspension sample containing macrophages (10^6 cells/ml) was cultured in a 24-well plate (Nunc) pretreated with 2% poly(2-hydroxyethyl methacrylate) in ethanol to prevent the cell adhesion. To the macrophages 0.5 ml of FITC-labeled apoptotic bodies (10^6 cells/ml) were added. The plate was incubated overnight at 37°C in the atmosphere with 5% CO_2. To evaluate inhibition of phagocytosis, the macrophages were washed thrice in RPMI-1640 and incubated for 40 min at 37°C with inhibitors (100 μM Glyc-PAA or 10 mM Neu5Acα-OBn), or lectins (SNA or MAA, 1 μg/ml), or monoclonal antibodies to siglecs (10 μg/ml). After completion of the reaction, the cells were washed, resuspended in 0.5 ml of RPMI-1640, and cultured in a 24-well plate as described above. Forty-five microliters of the cell suspension and 5 μl of anti-CD45-RPE (10 μg/ml) were incubated in PBA in a U-well plate for 1 h and washed twice in PBA. The cell fluorescence was determined by flow cytometry as described above.

Statistical analysis on the data of phagocytosis by macrophages of breast cancer patients was performed using the Wilcoxon–Mann–Whitney U test [16].

RESULTS

Interaction of macrophages with Glyc-PAA-fluo. Phenotype of macrophages isolated from peripheral blood of healthy donors was confirmed by flow cytometry. The fluorescent signal was displaced positively in the case of cell interaction with antibodies to macrophage antigens CD11c, CD14, CD36, and CD40 (Fig. 1). However, the cells did not bind with antibodies to dendritic cell antigens CD83 and CD86 and with antibodies to the monocyte antigen CD15. These findings suggested that the monocytes differentiated into macrophages. The interaction of macrophages with fluorescein-labeled glycoconjugates containing Neu5Ac was also studied (table). The macrophages bound to oligosaccharides containing the disaccharide fragment Neu5Acα2-3Gal, i.e., to 3′SiaLac, 3′SiaTF, and 3′SiaLe^c. The macrophages did not bind to SiaLe^x, although this molecule also contained the Neu5Acα2-3Gal fragment. The interaction of macrophages with a derivative containing Neu5Acα2-6Gal, i.e., with 6′SiaLac, was weaker than with the 2-3 isomer (3′SiaLac). Glycoconjugates containing neuraminic acid as monosaccharide, or 2-8 disialosides (Sia_2), or Neu5Acα2-6GalNAcα ($SiaT_n$) did not interact with macrophages, but pretreatment of the cells with neuraminidase provided the binding to all sialylated glycoconjugates (table). Because Neu5Acα2-3Gal is the most typical ligand for siglec-1 and siglec-5, these results suggested that siglec-1 or siglec-5 should be exposed on the macrophage surface. This is also supported by data on the interaction with antibodies to siglec-5 (data not presented; similar results are published in [17]).

Binding of macrophages isolated from peripheral blood of a healthy donor to sialylated fluorescence labeled glycoconjugates (results are presented for one of three independent experiments)

Glyc-PAA-fluo	Interaction of macrophages	
	before treatment with neuraminidase	after treatment with neuraminidase
Neu5Acα*	–	+
3'SiaLac	+	+
6'SiaLac	+/–	+
3'SiaTF	+	+
3'SiaLe^c	+	+
$SiaT_n$	–	+
Sia_2	–	+
SiaLe^x	–	n.a.**

* Similar results were observed with neoglycoconjugates Neu5Acα which had either aminopropyl- or *para*-glycylamidobenzyl spacer.
** n.a., not analyzed.

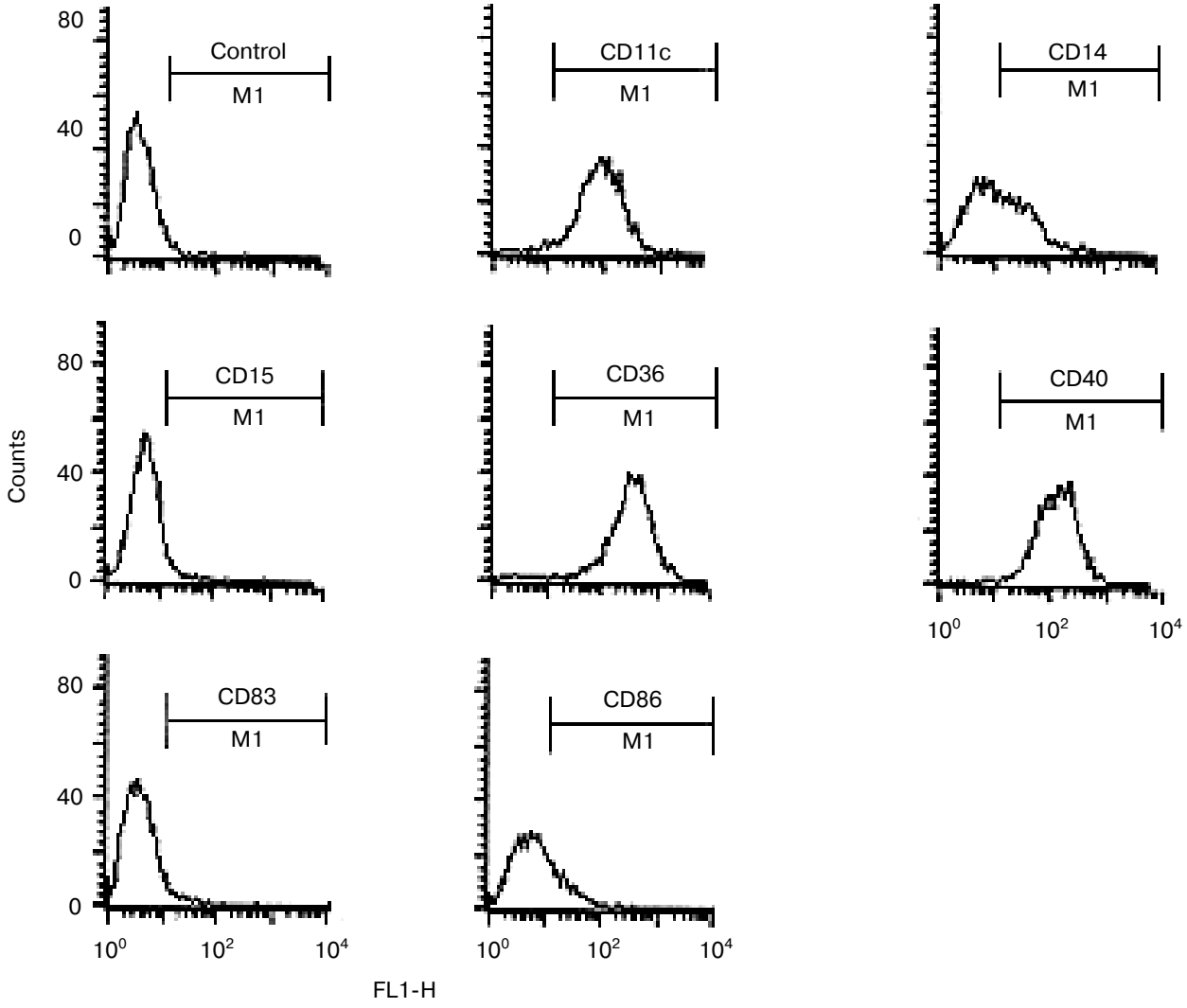

Fig. 1. Phenotype of macrophages isolated from the peripheral blood of healthy donor. The logs of fluorescence intensity (Y-axis) are plotted against cell number. FITC-labeled antibodies against mouse IgG were used as a negative control. Results of one of three experiments are presented.

Interaction of apoptotic bodies with sialic acid specific lectins SNA and MAA. Apoptosis of the M44 cells was induced by UV radiation and then interaction of apoptotic bodies with plant lectins SNA and MAA recognizing Neu5Acα2-6Gal and Neu5Acα2-3Gal, respectively, was studied (Fig. 2). The two lectins bound with the apoptotic bodies, but their interaction was weaker than with the initial cells. These results suggested that ligands (and, consequently, also the binding sites) for siglecs should remain on the apoptotic bodies.

Phagocytosis of apoptotic bodies. Engulfment of apoptotic bodies by macrophages was studied by flow cytometry. The apoptotic bodies were labeled with FITC. Because the leukocyte-associated antigen CD45 was expressed on the macrophages, we evaluated only phagocytosis by the CD45-positive macrophages (Fig. 3b). Addition of EDTA inhibited the engulfing activity of the macrophages (Fig. 3c), and this is in agreement with the literature data on involvement of Ca^{2+} in phagocytosis [3, 18]. To reveal the specificity of lectin involved in elimination of the apoptotic bodies, the macrophages were preincubated with sialylated saccharides or glycoconjugates Glyc-PAA. $SiaLe^x$-PAA had no effect on the engulfment by macrophages of apoptotic bodies (Fig. 3d), while benzyl glycoside Neu5AcαOBn (Fig. 3e) or its polyacrylamide conjugate (data not presented) decreased by 50% the phagocytosis. The engulfment by macrophages of the apoptotic bodies was also decreased in the presence of antibodies to siglec-5 (Fig. 3f) but not to siglec-7 (data not presented).

Then involvement of sialoside-binding lectins was studied in phagocytosis of apoptotic bodies by macro-

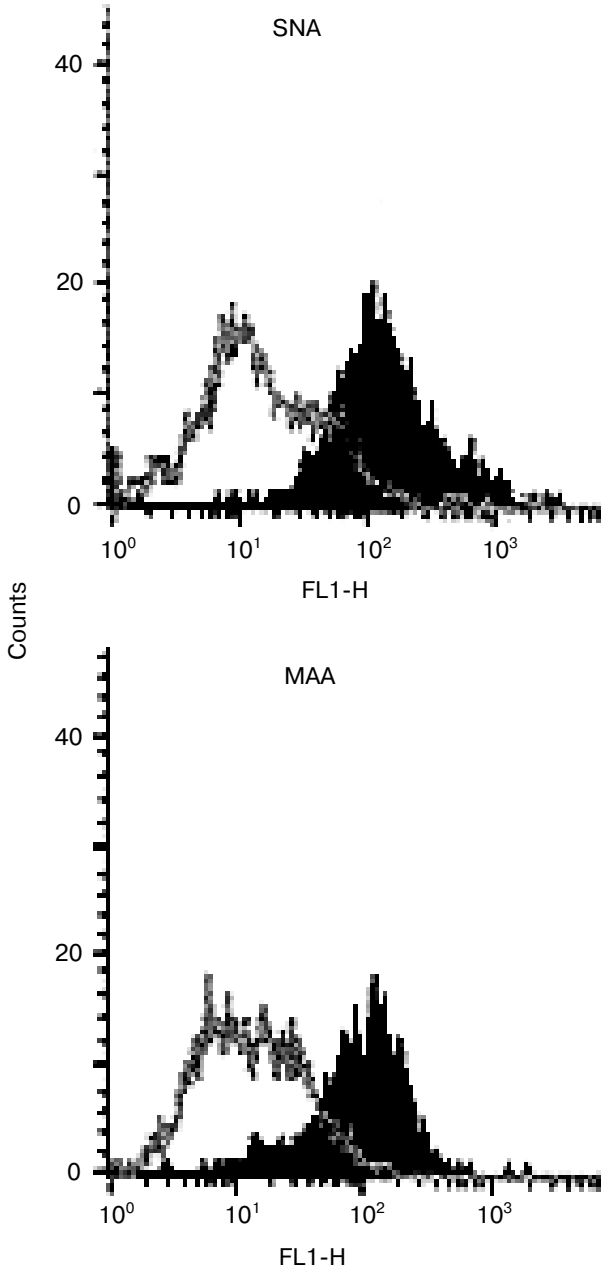

Fig. 2. Flow cytometry analysis of interaction between the M44 cells (the darkened area) and the apoptotic bodies obtained from these cells (the light area) with lectins SNA and MAA.

phages of breast cancer patients. The apoptotic bodies were obtained from the breast carcinoma MCF-7 cells, and the macrophages were isolated from peripheral blood of the patients. Phagocytosis was assessed by number of the CD45-positive macrophages, which had engulfed the FITC-labeled apoptotic bodies. Flow cytometry analysis of the blood cells showed that macrophages and neutrophils could engulf apoptotic bodies whereas monocytes are inactive (Fig. 4). The phagocytosis by macrophages from the patients' blood was 6-10% and phagocytosis by macrophages of healthy donors was up to 41% (Fig. 3b). In three patients with metastases, the phagocytosis was 6-10%, and in two patients without metastases it was 17%. In all cases, the engulfment by neutrophils was no more than 8% and that of monocytes was lower than 2% (Fig. 4).

Interaction of Glyc-PAA-fluo with macrophages from blood of patients with breast cancer. The Glyc-PAA-fluo interactions with macrophages from blood of a healthy donor and with those of a patient with breast cancer are shown in Fig. 5. The cells poorly interacted with the L-selectin ligand $6^{(GlcNAc)}$OSuSiaLe^x, and this indicated the cell differentiation into macrophages. However, the patient's macrophages bound with sialylated Glyc-PAA-fluo even without the pretreatment with neuraminidase. The interaction of the patient's macrophages with 3′SiaLac and 6′SiaLac was fourfold and with Sia_2 tenfold higher than the interaction of the macrophages isolated from blood of a healthy donor.

Phagocytosis of apoptotic bodies by macrophages from blood of a patient with breast cancer. Analysis of MCF-7 cells by means of lectins SNA and MAA showed that after apoptosis induction as in the case of M44 cells, Neu5Acα-containing glycans were detected on apoptotic bodies. Quantities of sialylated glycans on the apoptotic bodies obtained from the M44 and MCF-7 cells were approximately the same, as shown by flow cytometry using MAA and SNA lectins (data not presented).

To identify the lectin-binding carbohydrate chains of apoptotic bodies during their elimination by tumor-associated macrophages, phagocytosis was studied in the presence of two types of inhibitors: carbohydrates capable of binding to siglecs and plant lectins capable of binding to the corresponding carbohydrate chains on apoptotic bodies. Preincubation of apoptotic bodies with MAA recognizing Neu5Acα2-3Gal inhibited the engulfment of apoptotic bodies, whereas SNA, which bound Neu5Acα2-6Gal, did not affect the phagocytosis (Fig. 6a). The engulfment of apoptotic bodies was also prevented by incubation of macrophages with 3′SiaLac-PAA or Neu5AcαOBn, while other derivatives of N-acetylneuraminic acid, such as 6′SiaLac-PAA and Sia_2-PAA, had no effect (Fig. 6b).

DISCUSSION

Elimination of apoptotic bodies is an important function of macrophages. As a tumor grows, it suppresses the phagocytic and cytotoxic functions of macrophages. First, this is associated with secretion by tumor cells of cytokines, which inhibit the functions of macrophages [2, 19-21]. To make tumor-associated macrophages act against a tumor is an important problem of antitumor therapy. To activate interaction of macrophage receptors with the appropriate ligands on apoptotic bodies seems to

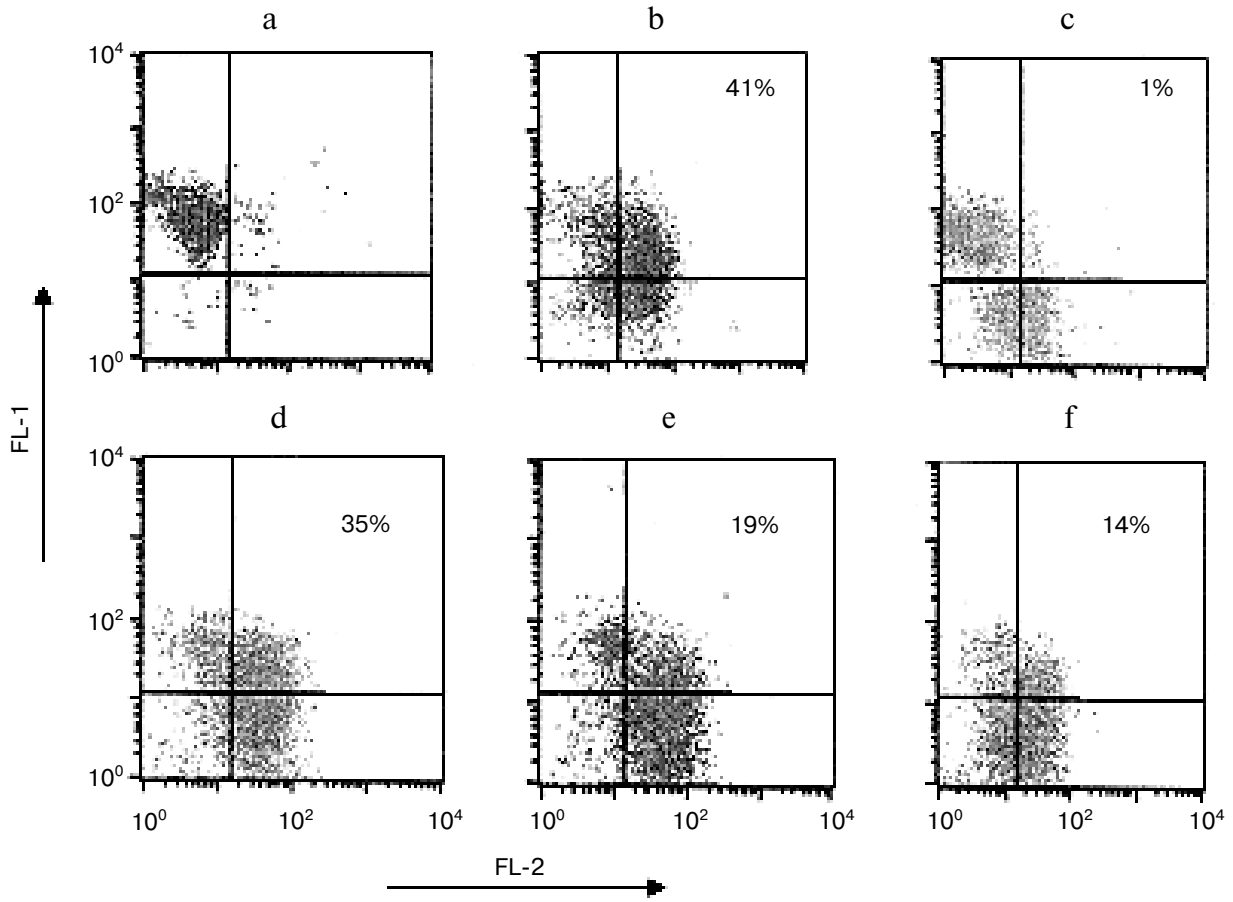

Fig. 3. Phagocytosis by macrophages of apoptotic bodies obtained from M44 cells. Two-parametric histogram displays light scatter of logarithmic FITC (X-axis) and RPE (Y-axis) fluorescence intensities. Phagocytosis was assessed by the number of the CD45-positive macrophages, which had engulfed FITC-labeled apoptotic bodies. a) The population of macrophages bound to anti-CD45-RPE. b-f) The population of CD45-positive macrophages which had engulfed apoptotic bodies: b) phagocytosis of apoptotic bodies; c) the same in the presence of 2 mM EDTA; d, e, f) phagocytosis in the presence of 100 μM $SiaLe^x$-PAA, 10 mM Neu5Acα-OBn, and antibodies to siglec-5 (10 μg/ml), respectively. Presented here are results of one of three experiments with macrophages isolated from the peripheral blood of healthy donors.

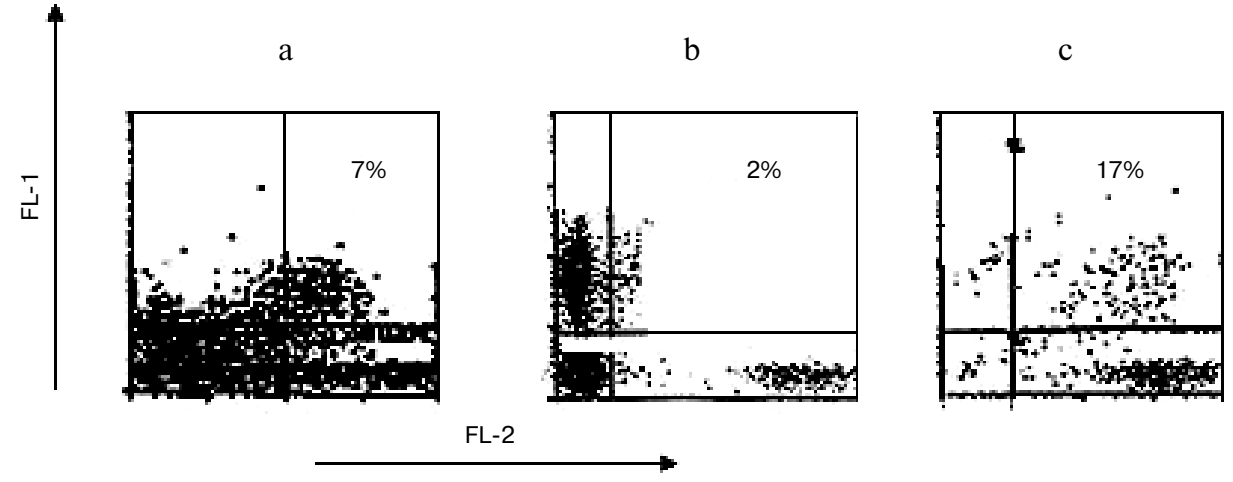

Fig. 4. Phagocytosis of apoptotic bodies by neutrophils (a), monocytes (b), and macrophages (c) from peripheral blood of a patient with breast cancer. Results of one of three experiments are presented.

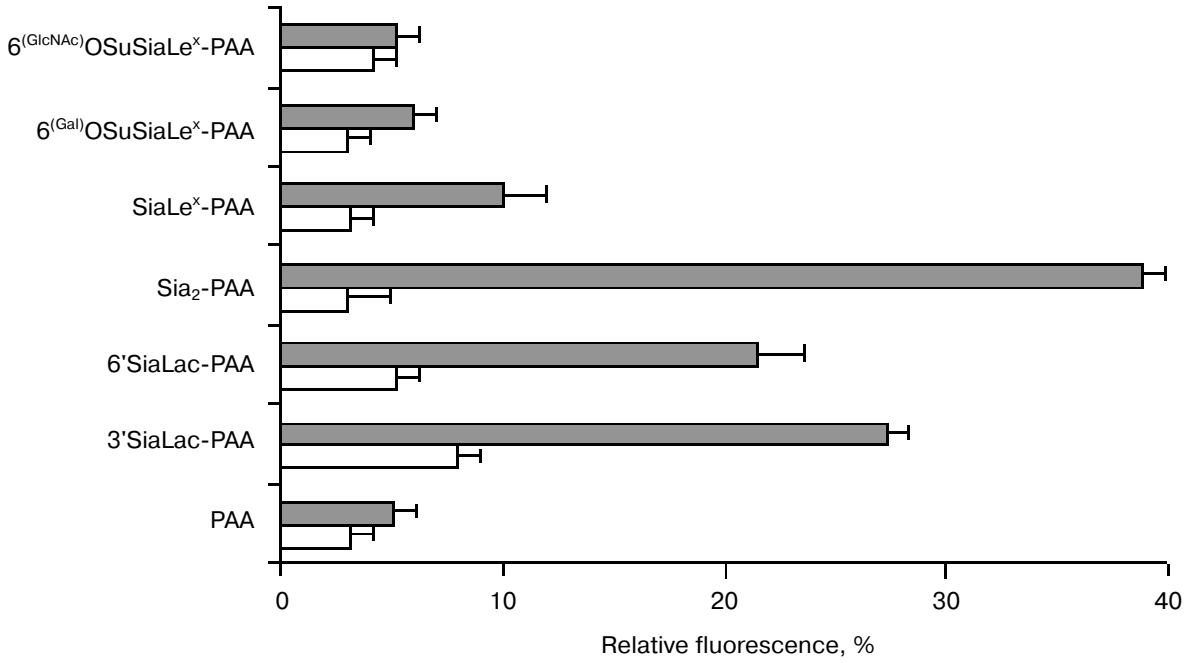

Fig. 5. Flow cytometry analysis of interaction of macrophages from peripheral blood of a healthy donor (light bars) and a breast cancer patient (dark bars) with Glyc-PAA-fluo (100 μM). Along the abscissa axis, the increase in fluorescence, which was calculated by the formula $[(I_i/I_0)\cdot 100] - 100\%$, where I_i was the fluorescence intensity after incubation of the macrophages with Glyc-PAA-fluo, I_0 was the fluorescence intensity after incubation of the macrophages with PAA-fluo (control). Results of one of three experiments are presented.

be an approach for recovery of the engulfment ability of macrophages with subsequent events. Therefore, it is interesting to reveal functional receptor–ligand pairs during phagocytosis.

In the present work, we studied the involvement of siglecs in phagocytosis of apoptotic bodies by macrophages from peripheral blood of healthy donors and also by macrophages from blood of patients with breast cancer.

Siglecs were detected on the macrophages by means of polyacrylamide neoglycoconjugates and phagocytosis was studied afterwards.

Macrophages preferentially bound to Neu5Acα2-3Gal-containing neoglycoconjugates. However, the macrophages pretreated with neuraminidase also recognized Neu5Acα2-6Gal and Neu5Acα2-8Neu5Acα. Such an increased interaction was explained by the decreased masking *cis*-interaction of siglecs with the neighboring sialylated carbohydrate chains [11, 12, 22]. We interpret the findings as follows: the cells contain a Neu5Acα2-3Gal-specific siglec, which is only partially masked by Neu5Acα2-3Gal-containing glycans, whereas a Neu5Acα2-6Gal-specific siglec (or siglecs) is completely masked by Neu5Acα2-6Gal chains. We have earlier found that siglec-1 and siglec-5 display the highest affinity for Neu5AcαOBn (unpublished). Considering our data on the interaction with Neu5AcαOBn, the literature data on the expression of siglec-1 and siglec-5 on macrophages, and the interaction of macrophages with monoclonal antibodies to siglec-5, it was suggested that siglec-5 should be involved in recognizing Neu5Acα-containing carbohydrate chains of apoptotic bodies during their phagocytosis by macrophages, but involvement of siglec-1 is also possible.

Phagocytosis was studied on the human melanoma cell line M44 characterized by a high content of sialylated glycans, which could be potential partners for siglecs. To identify targets of macrophage siglecs on apoptotic bodies, the carbohydrate composition of the M44 cells was studied after the induction of apoptosis. The exposition of both Neu5Acα2-3Gal- and Neu5Acα2-6Gal-containing glycans was found to be decreased, which was in agreement with the earlier observations on human colon carcinoma HT-29 cells [15].

Then we studied phagocytosis as it is, i.e., involvement of macrophage siglecs in this process. The engulfment of apoptotic bodies by macrophages was inhibited in the presence of a carbohydrate ligand for siglec-5 and also of antibodies to siglec-5, whereas antibodies to siglec-7 did not affect phagocytosis. These findings support the involvement of the macrophage siglec-5 in the lectin-mediated phagocytosis.

We also studied whether macrophages isolated from blood of patients with breast cancer retained the ability for siglec-mediated phagocytosis. Macrophages and neutrophils of the patients engulfed apoptotic bodies, while

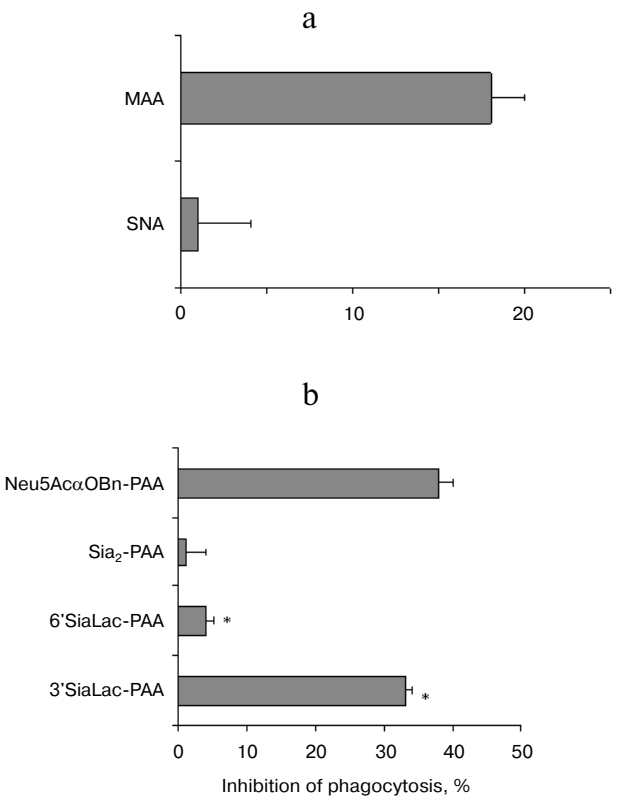

Fig. 6. Inhibition by lectins (a) and Glyc-PAA (b) of phagocytosis of apoptotic bodies obtained from the MCF-7 cells by macrophages from peripheral blood of a patient with breast cancer. The inhibition was calculated as $100 - [(F_i/F_0)\cdot 100]$, where F_i was number of the CD45-positive macrophages which engulfed FITC-labeled apoptotic bodies in the presence of an inhibitor, F_0 was the same in the absence of inhibitors. Results of one of three experiments are presented. * $p_U < 0.05$, the significance of differences in the presence or absence of Glyc-PAA.

monocytes failed to do this. The phagocytic activity of macrophages of patients with tumors, including those with metastases, was several times *lower* than the activity of macrophages of healthy donors. But the binding of macrophages of the patients with sialyl-glycoconjugates was *higher* than that of macrophages of healthy donors. The increased expression of siglecs could be due to secretion by the tumor cells of cytokines, some of which, e.g., IL-4, can stimulate the synthesis of siglecs [22]. The increased secretion of siglecs on macrophages was favorable for the tumor, because the recognition by siglecs of sialylated receptors prevented the recognition of the tumor cells as "foreign". Phagocytosis of apoptotic bodies resulting from the MCF-7 cells was inhibited by Neu5Acα-OBn-PAA and 3′SiaLac-PAA, but not by Sia_2-PAA and 6′SiaLac-PAA. Note that macrophages interacted with 6′SiaLac-PAA. Both siglec-5 and siglec-1 displayed a certain affinity for disialoside, but it was significantly less pronounced than the affinity for Neu5Acα2-3Gal and Neu5AcαOBn [23, 24]. This explained the absence of disialoside effect on phagocytosis in our experiments. In addition to siglec-1 and siglec-5, siglec-3 was expressed on macrophages, and 6′SiaLac was the preferential ligand for this siglec. It seems that some sialoside-binding lectins, first of all siglec-5, promoted phagocytosis, and other siglecs were not involved in elimination of apoptotic bodies.

Sialylation of the cell surface influenced the lectin-mediated adaptive cell immunity and was manifested, in particular, by masking ligands for galectins of these cells. Indeed, masking of polylactosamine chains of T-lymphocytes by 2-6 sialylation inhibited apoptosis of lymphocytes induced by galectin-1 [25] and sialylation of glycolipids prevented their interaction with galectin-3 [26, 27]. We described earlier the galectin-dependent phagocytosis of apoptotic bodies by THP-1 cells of macrophage origin [28]. In the present study, we also observed the galectin-dependent phagocytosis of apoptotic bodies, but this was the phagocytosis by macrophages only of patients with tumors and not by macrophages of healthy donors. It seems that both siglecs and galectin-1 can be involved in phagocytosis of apoptotic bodies by macrophages. It remains to be elucidated whether the involvement of siglecs and galectins in apoptosis is concerted, independent, or antagonistic.

From the standpoint of oncotherapy, the findings suggest that directional changes in the carbohydrate composition of apoptotic bodies (in particular, by creating additional binding sites for siglecs or galectins) is promising for activation of their phagocytosis and, as a result, for the subsequent exposition of tumor antigens to cells of the immune system.

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REFERENCES

1. Samuilov, V. D., Oleskin, A. V., and Lagunova, E. M. (2000) *Biochemistry (Moscow)*, **65**, 873-887.
2. Deichman, G. I. (2000) *Biochemistry (Moscow)*, **65**, 78-94.
3. Hart, S. P., Haslett, C., and Dransfield, I. (1996) *Experientia*, **52**, 950-956.
4. Hall, S., Savill, J. S., Henson, P. M., and Haslett, C. (1994) *J. Immunol.*, **153**, 3218-3227.
5. Sakamaki, T., Imai, Y., and Irimura, T. (1995) *J. Leukocyte Biol.*, **57**, 407-414.

6. Barlett, A., Grewal, T., De Angelis, E., Myers, S., and Stanley, K. K. (2000) *Atherosclerosis*, **153**, 219-230.
7. Reichert, F., Saada, A., and Rotshenker, S. (1994) *J. Neurosci.*, **14**, 3231-3245.
8. Sano, H., Hsu, D. K., Apgar, J. R., Yu, L., Sharma, B. B., Kuwabara, I., Izui, Sh., and Liu, F.-T. (2003) *J. Clin. Invest.*, **112**, 389-397.
9. Angata, T., and Brinkman-van der Linden, E. C. M. (2002) *Biochim. Biophys. Acta*, **1572**, 294-316.
10. Crocker, P. R., and Varki, A. (2001) *Immunology*, **103**, 137-145.
11. Crocker, P. R., and Varki, A. (2001) *Trends Immunol.*, **22**, 337-342.
12. Van den Berg, T. K., Nath, D., Ziltener, H. J., Vestweber, D., Fukuda, M., van Die, I., and Crocker, P. R. (2001) *J. Immunol.*, **166**, 3637-3640.
13. Nath, D., Hartnell, A., Happerfield, L., Miles, D. W., Burchel, J., Taylor-Papadimitriou, J., and Crocker, P. R. (1999) *Immunology*, **98**, 213-219.
14. Jones, C., Virji, M., and Crocker, P. R. (2003) *Mol. Microbiol.*, **49**, 1213-1225.
15. Rapoport, E., and LePendu, J. (1999) *Glycobiology*, **9**, 1337-1343.
16. Gubler, E. V., and Genkin, A. A. (1973) in *Nonparametric Statistical Tests in Biomedical Researches* [in Russian], Meditsina, Leningrad, pp. 72-75.
17. Cornish, A. L., Freeman, S., Forbes, G., Ni, J., Zhang, M., Cepeda, M., Gentz, R., Augustus, M., Carter, K. C., and Crocker, P. R. (1998) *Blood*, **92**, 2123-2132.
18. Rubartelli, A., Poggi, A., and Zocchi, M. R. (1997) *Eur. J. Immunol.*, **27**, 1893-1900.
19. Lesimple, T., Moisan, A., and Toujias, L. (1998) *Res. Immunol.*, **149**, 663-671.
20. Jackson, P., and Evans, S. R. T. (2000) *J. Surg. Oncol.*, **75**, 146-154.
21. Elgert, K., Alleva, D. G., and Mullins, D. W. (1998) *J. Leukoc. Biol.*, **64**, 275-290.
22. Crocker, P., Hartnell, A., Munday, J., and Nath, D. (1997) *Glycoconjugate J.*, **14**, 601-609.
23. Blixt, O., Collins, B. E., van den Nieuwenhof, I. M., Crocker, P. R., and Paulson, J. C. (2003) *J. Biol. Chem.*, **278**, 31007-31019.
24. Brinkman-van der Linden, E. C. M., and Varki, A. (2000) *J. Biol. Chem.*, **275**, 8625-8632.
25. Amano, M., Galvan, M., He, J., and Baum, L. (2003) *J. Biol. Chem.*, **278**, 7469-7475.
26. Feizi, T., Solomon, J. C., Yuen, Ch.-T., Ching, G. K., Frigeri, L. G., Hsu, D. K., and Liu, F.-T. (1994) *Biochemistry*, **33**, 6342-6349.
27. Holicova, Z., Hrdlickova-Cela, E., Plzak, J., Smatana, K., Jr., Betka, J., Dvorankova, B., Esner, M., Wasano, K., Andre, S., Kaltner, H., Motlik, J., Hercogova, J., Kodet, R., and Gabius, H. J. (2002) *APMIS*, **110**, 845-856.
28. Rapoport, E., Khaidukov, S., Baidina, O., Bojenko, V., Moiseeva, E., Pasynina, G., Karsten, U., Nifant'ev, N., LePendu, J., and Bovin, N. (2003) *Eur. J. Cell. Biol.*, **82**, 295-302.