This item is the archived peer-reviewed author-version of:

Monoclonal antibody binding to the macrophage-specific receptor sialoadhesin alters the phagocytic properties of human and mouse macrophages

Reference:

De Schryver Marjorie, Cappoen Davie, Elewaut Dirk, Nauwynck Hans J., Maes Louis, Caljon Guy, Cos Paul, Delputte Peter.- Monoclonal antibody binding to the macrophage-specific receptor sialoadhesin alters the phagocytic properties of human and mouse macrophages

Cellular immunology - ISSN 0008-8749 - 312(2017), p. 51-60

Full text (Publisher's DOI): <http://dx.doi.org/doi:10.1016/J.CELLIMM.2016.11.009>

To cite this reference: <http://hdl.handle.net/10067/1382850151162165141>

Monoclonal antibody binding to the macrophage-specific receptor sialoadhesin alters the phagocytic properties of human and mouse macrophages

Marjorie De Schryver¹, Davie Cappoen¹, Dirk Elewaut², Hans J. Nauwynck³, Louis Maes¹, Guy Caljon¹, Paul Cos¹ and Peter L. Delputte^{1,*}

¹ Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp (UA), Antwerp 2610, Belgium

² Unit for Molecular Immunology and Inflammation, VIB Inflammation Research Center, Ghent 9052, Belgium Department of Rheumatology, Ghent University, Ghent University Hospital, Ghent 9000, Belgium

³ Laboratory of Virology, Ghent University, Merelbeke 9820, Belgium

* Corresponding author: peter.delputte@uantwerpen.be

E-mail addresses: marjorie.deschryver@uantwerpen.be (M. De Schryver); davie.cappoen@uantwerpen.be (D. Cappoen); dirk.elewaut@ugent.be (D. Elewaut); hans.nauwynck@ugent.be (H.J. Nauwynck); louis.maes@uantwerpen.be (L. Maes); guy.caljon@uantwerpen.be (G. Caljon); paul.cos@uantwerpen.be (P. Cos) and peter.delputte@uantwerpen.be (P.L. Delputte)

Highlights

- Human & mouse macrophages were triggered with a sialoadhesin(Sn)-specific antibody
- Sn triggering almost completely blocked phagocytosis of fluorescent beads
- No effect was seen for antibody-coated beads taken up via Fc-mediated phagocytosis
- Phagocytosis of bacteria was higher in mouse macrophages after Sn triggering

Abstract

Sialoadhesin (Sn) is a surface receptor expressed on macrophages in steady state conditions, but during inflammation, Sn can be upregulated both on macrophages and on circulating monocytes. It was shown for different species that Sn becomes internalized after binding with monoclonal antibodies. These features suggest that Sn is a potential target for immunotherapies. In this study, human and mouse macrophages were treated with anti-Sn monoclonal antibodies or F(ab')₂ fragments and the effect of their binding to Sn on phagocytosis was analyzed. Binding of antibodies to Sn resulted in delayed and reduced phagocytosis of fluorescent beads. No effect was observed on Fc-mediated phagocytosis or phagocytosis of bacteria by human macrophages. In contrast, an enhanced phagocytosis of bacteria by mouse macrophages was detected. These results showed that stimulation of Sn could have different effects on macrophage phagocytosis, depending both on the type of phagocytosis and cellular background.

Keywords ¹

Sialoadhesin / CD169 / Siglec-1, phagocytosis, macrophages, inflammation, immunotherapy

1 Introduction

Macrophages are a type of phagocytes that play a role in both the innate and adaptive immune system. Along with neutrophils, macrophages are the first defensive cells to meet intruding microorganisms. Upon recognition through a set of cell specific receptors, phagocytic cells are able to engulf the microorganisms to prevent further spread in a process called phagocytosis. Phagocytosis is often associated with the release of cytokines and chemokines and unlike neutrophils, the more specialized macrophages can present antigens to T cells, activating the adaptive immune system [1, 2]. In some cases, pathogens like *Legionella* and *Mycobacterium*, abuse phagocytosis by macrophages to evade the immune system while preventing phagosome maturation by inhibiting phagosome-lysosome fusion [2]. To that respect, modulation of phagocytosis could be interesting in some diseases, for instance in chronic obstructive pulmonary disease and hemophagocytic syndromes. In chronic obstructive pulmonary disease, a reduced bacterial phagocytosis was observed in macrophages [3, 4]. In contrast, upregulated phagocytosis of erythrocytes and platelets was observed in hemophagocytic syndromes [5]. Modulation of phagocytosis could aid in these conditions to obtain an elevated or reduced phagocytosis rate in the macrophages. Indeed, modulation of phagocytosis was shown by targeting transmembrane proteins with monoclonal antibodies (mAbs) leading to reduced phagocytosis of apoptotic cells [6, 7]. It was shown that treating porcine sialoadhesin (Sn; Siglec-1, CD169), a macrophage-specific receptor, with anti-Sn mAbs also reduced phagocytosis of fluorescent beads in porcine primary macrophages [8].

Sn is expressed on a subset of tissue resident macrophages in steady state conditions, but during inflammation Sn is upregulated on macrophages, monocytes and possibly also on dendritic cells [9]. For instance, during arthritis, the expression of Sn was shown to increase with the severity of the disease [10]. Sn is part of the Siglec family (sialic acid-binding Ig-like lectin). Other Siglecs, CD22 and CD33, are able to internalize after mAb binding, moreover, antibodies are being used for the development of immunotoxins for anti-cancer immunotherapy [11, 12]. Studies with the porcine *Arterivirus* and HIV-1, show that Sn can mediate uptake of virus into early endosomes and in the case of HIV-1 can also mediate trans-infection [13-18]. Further research then showed that in different species, anti-Sn mAbs are able to induce internalization of Sn [18-21]. Interestingly, unlike most other Siglecs, no known signalization motif has yet been identified for Sn, even more, the short cytoplasmic tail of Sn is not conserved between species [22]. However recently, the interaction of mouse Sn (mSn) has been observed with DNAX-activating protein of 12 kDa (DAP12), suggesting Sn could contain a still unknown signalization motif [23]. Sn, described as a non-phagocytic receptor, is like other Siglecs able to bind with sialic acids, which are terminal nine-carbon sugar molecules found on vertebrates and pathogens [24, 25]. It was shown that pathogens containing sialic acids are able to bind with Sn, as observed for *Campylobacter jejuni* and *Neisseria meningitidis*. Moreover, these bacteria were more easily phagocytosed after binding with Sn in a sialic acid-dependent manner [26, 27]. Group B *Streptococcus* could also bind to Sn in a sialic acid-dependent manner, moreover, it was

¹ Abbreviations: BHI, Brain Heart Infusion; DAP12, DNAX-activating protein of 12 kDa; DAPI, 4',6-diamidino-2-phenylindole; IFN- α , interferon- α ; mAb, monoclonal antibody; MDMs, monocyte-derived macrophages; Siglec, sialic acid-binding Ig-like lectin; Sn, Sialoadhesin; TLR, Toll-like receptor

shown that in primary mouse macrophages, Sn contributes to the bactericidal activity of macrophages against group B *Streptococcus* [28].

The interesting expression pattern of Sn during inflammation, the interaction of Sn with sialic acids and the capacity of anti-Sn mAbs to induce internalization of Sn, makes Sn a potential target for immunotherapy. Some possibilities for targeting Sn have already been explored for porcine Sn (pSn). PSn was targeted with immunotoxins, which resulted in killing of primary porcine macrophages [16] or with fusion proteins containing virus epitopes, which resulted in the induction of virus-specific antibodies [29]. MSn expressed on mouse splenic macrophages was targeted using anti-Sn mAbs for antigen targeting, activating both cellular and humoral immune responses [30]. Not only anti-Sn mAbs were used, mSn and hSn have also been targeted using liposomal nanoparticles coated with glycan ligands [20, 31, 32]. Nevertheless, using mAbs in immunotherapy could induce undesirable side effects on the host cell [33].

Previous research showed that targeting Sn with specific mAbs also blocks phagocytosis, which can be considered as an unwanted side effect [8]. However, this was only shown in porcine macrophages, and due to the non-conserved amino acid sequence and the lack of a known signalization motif in the cytoplasmic tail of Sn amongst different species [22], the outcome could be different in mouse and human macrophages. Moreover, for porcine macrophages the effect on phagocytosis was only investigated with polystyrene beads and not with more relevant assays such as Fc-mediated phagocytosis or phagocytosis of bacteria. Therefore, in this study, the effect of stimulation with anti-hSn mAbs and anti-mSn mAbs on different types of phagocytosis was analyzed on human and mouse macrophages. Our results show that the effect of anti-Sn mAbs on phagocytosis drastically differs between the types of phagocytosis. While a substantial reduction is seen in the phagocytosis of fluorescent beads, the Fc-mediated phagocytosis and phagocytosis of bacteria are unaffected and sometimes even slightly increased.

2 Material and methods

All products were purchased from Thermo Scientific, unless otherwise stated.

2.1 Ethical statement

The Ethical Committee for Animals of the University of Antwerp authorized experiments regarding BALB/c mice; permit number 2014-17. The Ethical Committee of the Antwerp University Hospital and the University of Antwerp authorized the use of human monocytes; permit number 16108.

2.2 Primary macrophages

Human monocytes were obtained from buffy coats of healthy volunteers from the Belgian Red Cross-Flanders. The mononuclear cells were isolated from the buffy coats using Ficoll (Sigma-Aldrich). CD14⁺ monocytes were isolated using MACS separation following manufacturer's instructions (Miltenyi Biotec). The monocytes were seeded in RPMI with 10% inactivated human serum, 1% non-essential amino acids, 1% sodium pyruvate and 1% glutamine. After two days in culture, human IFN- α was added to the cells during three days to obtain hSn-positive monocyte-derived macrophages (MDMs).

BALB/c mice were sacrificed and their primary bone marrow cells were collected in RPMI medium. Red blood cells were removed using an ammonium-chloride-potassium lysis buffer (VWR and Janssen Chimica). Macrophages were seeded on coverslips in a 24-well plate in RPMI with 10% inactive fetal

bovine serum, 1% non-essential amino acids, 1% sodium pyruvate, 1% glutamine and with addition of L929 supernatant. L929 supernatant was retrieved from L929 cells, kindly provided by dr. C. Uyttenhove (Ludwig Institute for Cancer Research, Brussels, Belgium). After three days in culture, mouse interferon-alpha (IFN- α) was added to the cells, to increase the number of Sn-positive macrophages.

2.3 Production of antibodies and F(ab')₂ fragments

Anti-hSn mAb 12A4 and anti-mSn mAb SySy94 were produced and purified as previously published [21]. F(ab')₂ fragments were produced as previously described [34]. Briefly, PNGase F (New England Biolabs) was added to purified mAbs to allow proteolytic cleavage following manufacturer's instructions. Pepsin agarose beads were added to cleave the mAbs after the deglycosylation step. The F(ab')₂ fragments were purified using a Zeba spin column with a 40 kDa cut off. Control antibodies were used for hSn and mSn using respectively 40-1a and normal rat IgG control (R&D). The hybridoma 40-1a developed by Joshua Sanes was obtained from the Developmental Studies Hybridoma Bank, created by the NICHD of the NIH and maintained at The University of Iowa, Department of Biology, Iowa City, IA 52242.

2.4 Phagocytosis assay

Macrophages were treated with anti-Sn mAbs or F(ab')₂ fragments 12A4 or SySy94 during different times, from 1 to 24 hours. Control antibodies were added as control for the binding of the Fc domain of the mAbs with Fc receptors. Antibodies were added at a concentration of 2,5 μ g/ml. Carboxylate-modified polystyrene yellow-green fluorescent beads (Sigma-Aldrich) were added to the macrophages and cells were kept at 37°C to induce phagocytosis during 1 or 6 hours. As a negative control for the phagocytosis, macrophages were kept at 4°C during the experiment. Finally, macrophages were washed to remove unphagocytosed beads, fixed with 4% paraformaldehyde (Merck) and permeabilized using 0,5% Triton X-100 (Sigma-Aldrich). Macrophages were further stained with Texas Red®-X phalloidin to stain F-actin, located just beneath the plasma membrane and cell nuclei were visualized with 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich). Each experiment was repeated independently three times, phagocytosed particles of 50 different macrophages were count.

2.5 IgG-coated fluorescent beads

Human and mouse IgG (Sigma-Aldrich) were dissolved in 50 mM MES buffer (Sigma-Aldrich) to a final concentration of 2 mg/ml. Carboxylate-modified polystyrene yellow-green fluorescent beads were added to the suspension and were incubated for 15 min at room temperature. 1-Ethyl-3-(3-Dimethylaminopropyl)carbodiimide (Sigma-Aldrich) was added to the solution before adjusting the pH to 6,5. Glycine (VWR) was added to a final concentration of 100 mM and the suspension was incubated for 30 min at room temperature. Beads were centrifuged and washed with phosphate buffered saline.

2.6 Bacteria

Gram-negative *Escherichia coli* (ATCC 8739) and Gram-positive *Staphylococcus aureus* (ATCC 25923) were grown in LB broth overnight at 37°C. Both bacteria are encapsulated. Gram-positive and encapsulated *Streptococcus pneumoniae* (ATCC 6301), was grown in Brain Heart Infusion (BHI) broth during 6 hours at 37°C. Bacteria were heat-inactivated for 30 min at 65°C before being used in phagocytosis assays.

2.7 Microscopy

Images were acquired using an Axio Observer inverted microscope and a Compact Light Source HXP 120C with Filter set 49, 20 and 10 for blue, red and green fluorophores respectively (Zeiss). Images were analyzed and background was reduced using the ImageJ software.

3 Results

3.1 Effect of anti-sialoadhesin mAbs on the phagocytosis capacity of sialoadhesin-positive macrophages using carboxylate-modified beads

Previous research showed that phagocytosis of porcine macrophages was downregulated after treatment with anti-pSn mAbs [8]. Due to the lack of known signaling sequences in the cytoplasmic tail of Sn of different species and because the cytoplasmic tail of Sn of different species is not conserved [22], we investigated the effect of anti-Sn mAbs on phagocytic processes in human and mouse macrophages.

Primary Sn-positive human and mouse macrophages were treated with anti-Sn mAbs or F(ab')₂ fragments. In order to exclude effects of the Fc domain of the antibody, control mAbs were included as controls. Macrophages without antibodies were used as reference for phagocytosis in normal conditions. Previous research for pSn, showed that at a concentration of 2,5 µg/ml, a clear effect was observed on the phagocytosis of beads [8]. Therefore, this concentration was also used in these experiments. Antibodies were added 24 hours prior to performing the phagocytosis assay. Microscopic images showed a difference in phagocytic uptake of beads (Figure 1). When no mAbs or control mAbs were added to the macrophages, macrophages showed a clear phagocytosis of the beads. When anti-Sn mAbs or F(ab')₂ fragments were added to the macrophages during 24 hours, hardly any phagocytosis was observed, both in human and mouse primary macrophages. Quantification of the number of phagocytosed beads was performed to analyze the effect of the stimulation of anti-Sn mAbs or F(ab')₂ fragments on primary human and mouse macrophages. As a negative control, macrophages were kept at 4°C instead of 37°C. The control showed indeed no uptake of beads (Figure 2). When the macrophages were placed at 37°C and phagocytosis was allowed, a high amount of intracellular beads was seen in human (mean of 5 beads per cell) and mouse (mean of 18 beads per cell) macrophages, both when no antibodies were added and when control antibodies were added. When anti-Sn mAbs or F(ab')₂ fragments were added to human and mouse primary macrophages, a reduction was observed in the amount of phagocytosed beads (Figure 2).

Figure 1: Microscopic overview of phagocytosis of human and mouse primary macrophages after stimulation with anti-Sn mAbs or F(ab')₂ fragments. Human and mouse primary Sn-positive macrophages were treated with no mAbs, control mAbs, anti-Sn mAbs or F(ab')₂ fragments during 24 hours. After inducing phagocytosis by adding yellow-green fluorescent beads for 1 hour, macrophages were fixed and permeabilized. Texas Red®-X phalloidin (red) was added to stain F-actin. Cell nuclei were stained with DAPI (blue). Scale bar indicates 10 μm.

Figure 2: Phagocytosis assay of Sn-positive macrophages after 24 hours stimulation with anti-Sn mAbs or F(ab')₂ fragments. Anti-Sn mAbs or F(ab')₂ fragments were added to human MDMs and mouse bone marrow-derived macrophages during 24 hours. No mAbs or control mAbs were used as reference or control respectively. Fluorescent beads were added to macrophages and were placed at 37°C to allow phagocytosis, as a control, macrophages were kept at 4°C. After 1 hour, macrophages were fixed and permeabilized. For each condition, the number of phagocytosed beads in fifty macrophages was counted. Data represent mean and standard deviation of three independent repeats.

3.2 Time-dependent effect of sialoadhesin stimulation on phagocytosis using beads

Our results illustrate that a 24 hour stimulation with anti-Sn mAbs or F(ab')₂ fragments strongly reduced phagocytosis. To analyze if this effect would also occur upon shorter incubation times, macrophages were triggered with anti-Sn mAbs or F(ab')₂ fragments for a shorter period, ranging from 1 to 10 hours. Again, a high amount of phagocytosed beads was seen in untreated macrophages as well as in macrophages that were treated with control antibodies (Figure 3). When anti-Sn mAbs or F(ab')₂ fragments were added to the macrophages, a decrease in the amount of phagocytosed beads was observed for all incubation times tested (Figure 3).

Figure 3: Effect of phagocytosis of different stimulation durations with anti-Sn mAbs or F(ab')₂ fragments on primary macrophages. Primary human and mouse Sn-positive macrophages were treated with antibodies for different times prior to the addition of fluorescent beads during 1 hour at 37°C. Macrophages were fixed, permeabilized and further analyzed using fluorescence microscopy. For each condition, fifty macrophages were analyzed for amount of phagocytosed beads. Data represent mean and standard deviation of three independent repeats.

3.3 Effect on phagocytosis after pulse stimulation of Sn

To analyze how long the effect of Sn antibody stimulation on phagocytosis lasts, the effect of a pulse stimulation of 1 hour was analyzed. Anti-Sn mAbs and F(ab')₂ fragments were added to the macrophages during 1 hour. After the pulse incubation of antibodies on macrophages, macrophages were washed and kept in culture until the phagocytosis assay 24, 48 and 72 hours later. Again, a clear reduction in phagocytosis by macrophages was observed at all times tested, showing that even a short, one hour pulse stimulation has a clear effect on phagocytosis of beads up to three days later (Figure 4).

Figure 4: Effect on phagocytosis with a short pulse stimulation of Sn. A stimulation of 1 hour was performed on human and mouse Sn-positive macrophages. Macrophages were washed and after 24, 48 or 72 hours incubation, fluorescent beads were added to the macrophages. Macrophages were fixed, permeabilized, and further analyzed using microscopy. The amount of phagocytosed beads was analyzed for fifty macrophages in each condition. Data represent mean and standard deviation of three independent repeats.

3.4 Effect of mAb stimulation of Sn on long duration phagocytosis

Our findings show that stimulation of macrophages with Sn-specific antibodies almost completely blocks phagocytosis of beads. However, only 1 hour of phagocytosis was allowed and macrophages are known to be able to phagocytose for longer times. Therefore, we allowed phagocytosis for 6 hours after the macrophages were treated with Sn-specific antibodies for 24 hours. These results showed still a reduction, but not an almost complete block of phagocytosis as observed before (Figure 5).

Figure 5: Effect of mAbs triggering of Sn on long duration phagocytosis. Human and mouse primary macrophages were triggered with antibodies for 24 hours prior phagocytosis. Fluorescent beads were added during 6 hours to the macrophages. Macrophages were fixed, permeabilized and further stained with Texas-Red®-X phalloidin. For each condition, fifty macrophages were analyzed for phagocytosed beads with fluorescence microscopy. Results show mean and standard deviation of three independent repeats.

3.5 Effect of anti-Sn mAbs on Fc-mediated phagocytosis

Fc-mediated phagocytosis is an important functionality of macrophages. Antibodies produced by the host can bind to pathogens and macrophages recognize the Fc domain of the antibody with Fc receptors inducing Fc-mediated phagocytosis [2]. The effect of stimulation of macrophages with anti-Sn mAbs or F(ab')₂ fragments was analyzed for Fc-mediated phagocytosis. Human or mouse IgG were covalently bound to fluorescent beads and added to the macrophages to induce Fc-mediated phagocytosis after macrophages were pre-treated during 24 hours with anti-Sn mAbs or F(ab')₂ fragments. IgG-coated beads were phagocytosed both in untreated macrophages and in macrophages treated with control mAbs (Figure 6). Approximately the same amount of phagocytosed IgG-coated beads was observed compared to phagocytosis with uncoated beads (Figure 2). Surprisingly, macrophages treated with anti-Sn mAbs or F(ab')₂ fragments also showed clear phagocytosis of IgG-coated beads and no differences in phagocytosis was observed with untreated or control-mAb treated macrophages (Figure 6).

Figure 6: Effect of Sn triggering on Fc-mediated phagocytosis. Fluorescent beads covalently bound with IgG were added to primary human and mouse macrophages treated for 24 hours with anti-Sn mAbs or F(ab')₂ fragments. Macrophages were fixed after 1 hour of phagocytosis and permeabilized. Macrophages were further stained with Texas-Red®-X phalloidin. For each condition, phagocytosed beads of fifty macrophages were counted. Data represents mean and standard deviation of three independent repeats.

3.6 Effect of adding Sn antibodies to macrophages on the phagocytosis of bacteria

A well-known and crucial characteristic of macrophages is the phagocytosis of pathogens [35]. The effect of treating primary Sn-positive macrophages with anti-Sn mAbs or F(ab')₂ fragments on phagocytosis of bacteria was analyzed. The phagocytosis assay was performed using heat-inactivated bacteria; Gram-negative *E. coli* and Gram-positive *S. pneumoniae* and *S. aureus*, where *S. pneumoniae* and *S. aureus* are encapsulated. Anti-Sn mAbs or F(ab')₂ fragments were added to primary human or mouse macrophages during 24 hours as stimulation, before the phagocytosis assay. Control mAbs or no antibodies were added as control and reference respectively. Stimulation of human MDMs with anti-Sn mAbs or F(ab')₂ fragments did not result in major differences of phagocytosis of bacteria compared to the reference and control macrophages (Figure 7). Unexpectedly, for primary mouse macrophages, an increase in the amount of phagocytosed bacteria was observed in cells treated with anti-Sn mAbs or F(ab')₂ fragments compared to the controls. These results were observed for all bacteria tested (Figure 7).

Figure 7: Analysis of phagocytosis of bacteria in primary human and mouse macrophages after stimulation with Sn-specific antibodies. Anti-Sn mAbs or F(ab')₂ fragments were added to primary human and mouse Sn-positive macrophages during 24 hours. Heat-inactivated bacteria were added to the macrophages and phagocytosis was allowed during 1 hour. Macrophages were fixed, permeabilized and further stained with Texas-Red®-X phalloidin. DAPI was used to stain all cellular and bacterial nuclei. Fifty macrophages were counted for amount of phagocytosed bacteria. Data represent mean and standard deviation of three independent repeats.

3.7 Comparing the phagocytosis of different cell types after stimulation with anti-Sn mAbs

Previous experiments showed that treatment with anti-Sn mAbs or F(ab')₂ fragments has an effect on the phagocytosis by macrophages. Since macrophages obtained from different anatomical sites can display different functionalities, the effect on the phagocytosis was compared in different cell types of the same species, mouse alveolar macrophages and mouse bone marrow-derived macrophages. Cells were stimulated during 24 hours prior phagocytosis. Treatment with Sn mAbs or F(ab')₂ fragments had no influence on Fc-mediated phagocytosis in both cell types and the phagocytosis of pathogen *S. pneumoniae* was augmented in both alveolar and bone marrow-derived macrophages, as shown in Figure 8. Interestingly, the phagocytosis of fluorescent beads was not similar for mouse alveolar and bone marrow-derived macrophages. Alveolar macrophages did not show a drastic reduction in phagocytosis (Figure 8). A more detailed analysis of phagocytosis of beads (Table 1) reveals different patterns of phagocytosis. Some macrophages show an almost complete block in phagocytosis, while other macrophages show enhanced phagocytosis upon stimulation with anti-Sn mAbs or F(ab')₂ fragments.

Figure 8: Effect of Sn stimulation with anti-Sn mAbs or F(ab')₂ fragments on phagocytosis in different types of mouse macrophages. Fluorescent beads, IgG-coated beads or *S. pneumoniae* were added to mouse alveolar macrophages or mouse bone marrow-derived macrophages after being treated during 24 hours with antibodies. Phagocytosis was allowed for 1 hour at 37°C. For each condition, the amount of phagocytosed beads in fifty macrophages was quantified. Data represent mean and standard deviation of three independent repeats.

Table 1: Phagocytosis of fluorescent beads by mouse alveolar macrophages and bone marrow-derived macrophages. Fluorescent beads were added to alveolar macrophages or bone marrow-derived macrophages after being treated during 24 hours with antibodies. Phagocytosis was allowed for 1 hour. The mean $\pm$ standard deviation of three independent repeats are shown: the amount of phagocytosed beads per cell (first row), the percentage of cells performing phagocytosis (second row) and the mean of the number of phagocytosed beads in the cells performing phagocytosis (third row).

Mouse alveolar macrophages			
	Mean of phagocytosed beads	Percentage of cells performing phagocytosis	Mean of phagocytosed beads of only phagocytosing cells
No mAb	10,86 $\pm$ 3,19	62,66 $\pm$ 6,42	16,78 $\pm$ 3,18
Control mAb	11 $\pm$ 2,25	56,66 $\pm$ 9,86	19,51 $\pm$ 5,37
SySy94 mAb	13,42 $\pm$ 4,44	36 $\pm$ 6	36,16 $\pm$ 8,88
SySy94 F(ab') ₂	13,55 $\pm$ 2,61	44 $\pm$ 2	30,25 $\pm$ 4,80

Mouse bone marrow-derived macrophages			
	Mean of phagocytosed beads	Percentage of cells performing phagocytosis	Mean of phagocytosed beads of only phagocytosing cells
No mAb	17,29 $\pm$ 7,36	78 $\pm$ 10,58	21,38 $\pm$ 7,83
Control mAb	19,54 $\pm$ 7,07	85,34 $\pm$ 10,06	22,16 $\pm$ 5,26
SySy94 mAb	0,41 $\pm$ 0,14	3,34 $\pm$ 3,06	2,78 $\pm$ 2,41
SySy94 F(ab') ₂	1,96 $\pm$ 1,82	19,34 $\pm$ 17	6,47 $\pm$ 2,86

4 Discussion

When microorganisms are able to breach the first line of defense comprised of the skin and mucosal barriers specialized phagocytes are essential to clear invading pathogens [36]. These phagocytes prevent further spread of the microbes within the host by engulfing and subsequent enzymatic destruction of the germs. Of these phagocytes, macrophages play a crucial role, not only in the phagocytosis but also in signaling for the recruitment and activation of other immune cells. Recognition of pathogens within the host is mediated through a set of receptor molecules on the cellular surface of the phagocyte, which upon binding can alter the cell's behavior and can trigger phagocytosis [1, 36, 37]. Besides the phagocytosis of pathogens, macrophages can also phagocytose and degrade apoptotic cells by recognizing them through surface receptors [2]. Phagocytosis has been described as the uptake of particles larger than 0,5 μ m, and was shown to be actin-dependent [38]. In some cases, phagocytosis is compromised, such as during chronic obstructive pulmonary disease, where phagocytosis of bacteria by macrophages is reduced [3, 4]. In contrast, during hemophagocytic syndromes, an uncontrolled and unwanted phagocytosis of erythrocytes and platelets is observed [5]. For such diseases, therapeutic modulation of phagocytosis could be interesting. Modulation of the phagocytosis of apoptotic cells has previously been shown upon targeting cell surface receptors with mAbs [6, 7]. Furthermore, it was shown that sialoadhesin (Sn), a macrophage-specific receptor could also be a potential target for modulation of phagocytosis, since triggering porcine macrophages with an anti-pSn mAb, reduced phagocytosis of beads [8]. Sn is the prototypic member of the Siglec family (sialic-acid binding IgG-like lectin). Unlike other Siglecs, Sn does not contain any known signalization motif and furthermore the amino acid sequence of the short cytoplasmic tail is not conserved between species [22]. Despite this lack of conservation between species, our results showed that the phagocytosis of carboxylate-modified beads is reduced when hSn and mSn are triggered with antibodies, similar to what was previously observed with porcine macrophages [8].

It has been suggested that Sn could be used as target for immunotherapy. Indeed, during inflammation and infection, an upregulation of Sn is observed on macrophages, monocytes and potentially dendritic cells. This was observed for rheumatoid arthritis on both resident and infiltrating macrophages in synovial tissue and upon HIV-1 infection on blood monocytes [39, 40]. During cancer, an increased number of Sn-positive macrophages was observed in liver and spleen [9, 41]. Therapeutic antibodies can be used to modulate cell functioning or to eliminate cells using immunotoxins [42]. In both cases, potential unwanted side effects of the mAb binding must be identified before it can be used in immunotherapy. For Sn, a receptor for which the function is not known and which does not contain any known signalization motifs [22], it is difficult to predict what the side effects might be of mAb binding. Our results confirm previous results obtained with porcine macrophages and show that the effect of mAb binding to Sn on phagocytosis has already an effect after 1 hour of stimulation. Moreover, this effect lasted for a long time, since a pulse stimulation for 1 hour still reduced phagocytosis 72 hours later. Interesting, since the effect of stimulation of Sn has a long-term effect, less stimulation of Sn is needed in potential targeting of Sn when modulating the phagocytosis through Sn stimulation. Therefore, we analyzed a longer duration of phagocytosis of beads after Sn stimulation. This showed that the impact on the amount of phagocytosed beads was diminished. These results further suggest that treating hSn and mSn with anti-Sn mAbs or F(ab')₂ fragments delays the phagocytic capacity of macrophages, but does not completely shut down the phagocytosis mechanism of macrophages. Amine-modified beads were also used to induce phagocytosis instead of carboxylate-modified beads (data not shown). The surface charge of the beads is different, carboxylate-modified beads are negatively charged while amine-modified beads are more neutral [43]. Phagocytosis with amine-modified beads showed that hardly any beads were phagocytosed and Sn stimulation had no effect on this phagocytosis, presumably because almost no phagocytosis was observed in non-treated controls. Clearly, the charge of particles influences the phagocytosis capacity of macrophages. Similarly, the surface charge of macrophages may influence phagocytosis. In our study, we used anti-Sn mAbs that interfere with the binding of sialic acids to Sn [21]. Previous studies have shown that Siglecs on cell surface can interact with negatively charged sialic acids also present on the same cell surface in *cis* interactions [44]. Binding of the mAb to Sn could interfere with these *cis* interactions which results in an increased number of unbound sialic acids on the cell surface and a possible increase in negative charge of the cell surface. Therefore, the zeta-potential, which gives an indication of the surface charge of particles [45], was measured for the macrophages after the different stimulations [46, 47]. These results showed that all cells were negatively charged and that no major differences were observed upon stimulation, suggesting that the observed effect is not due to changes in surface charge (data not shown).

Macrophages can phagocytose pathogens which are matured in the phagosome and later phagolysosome where the pathogen gets killed by the cell [48]. However, macrophages are sometimes also targeted by several pathogens to evade the immune system, such as *Mycobacterium tuberculosis* [49]. The pathogen phagocytosis by macrophages is an important feature for maintaining proper clearance, therefore, the effect of Sn stimulation on phagocytosis of bacteria was analyzed. Heat-inactivated Gram-negative *E. coli* and Gram-positive *S. aureus* and *S. pneumoniae*, all encapsulated, were used instead of the beads for analyzing the effect of Sn stimulation on phagocytosis of bacteria. In primary human macrophages, no differences were observed after treating the macrophages with anti-Sn mAbs or F(ab')₂ fragments. The macrophages were still able to induce phagocytosis. However, primary mouse macrophages showed an increase in phagocytosis of

bacteria when treated with anti-Sn mAbs or F(ab')₂ fragments. These results are in contrast with the effect of Sn stimulation on phagocytosis of beads. The major difference between both phagocytosis mechanisms is the ability of the macrophage to bind with receptors phagocytosing bacteria instead of fluorescent beads. For instance, lipopolysaccharide on the membrane of *E. coli* and lipopeptides on *S. aureus* are able to bind with surface receptors on macrophages, respectively Toll-like receptor 4 (TLR) and TLR2 [50, 51]. Besides using different macrophage receptors for phagocytosis, the phagocytosis was increased for all different bacteria when mSn was treated. It could be that in mouse macrophages and not human macrophages, the stimulation of Sn results in an upregulation of receptors recognizing bacteria, resulting in a better phagocytosis. Interestingly, Sn as well as other members of the Siglec family, is able to recognize sialic acids, found on terminal glycans in vertebrates or pathogens [24]. Some bacteria misuse this feature and bind with Sn in a sialic acid-dependent manner. This was shown for *Campylobacter jejuni* and *Neisseria meningitides*, where the presence of Sn increased phagocytosis by macrophages [26, 27, 52]. However, this effect is not because Sn acts as a phagocytic receptor but rather because it enhances binding to macrophages and facilitates interaction with other receptors that are involved in the phagocytosis of the bacteria. It was shown that for bacteria containing sialic acids and interacting with Sn, the interaction can be blocked or diminished by adding anti-Sn mAbs [27, 53]. The antibodies used in our experiments also block sialic acid binding. However, this had no effect on the phagocytosis of the bacteria tested in human macrophages and phagocytosis was even augmented in mouse macrophages.

Several receptors on phagocytes recognize opsonins, which are host proteins such as IgG antibodies. The host antibodies can recognize the pathogen or apoptotic cell with their F(ab')₂ fragments and the Fc domain remains available for binding with Fc receptors on macrophages leading to Fc-mediated phagocytosis [54]. The effect of Sn stimulation on Fc-mediated phagocytosis was analyzed. These results showed no differences in phagocytosis after anti-Sn mAb or F(ab')₂ fragment stimulation compared to untreated macrophages or macrophages treated with control antibodies. Similar to pathogen phagocytosis, where receptors are being triggered, Fc-mediated phagocytosis was not affected after Sn stimulation. This may indicate that stimulation of Sn with antibodies does not affect receptor-mediated phagocytosis.

The effect of Sn-specific mAbs on phagocytosis was compared between mouse alveolar and bone marrow-derived macrophages, since macrophages obtained from different anatomical sites may display different functionalities. No differences between different macrophages were observed for Fc-mediated phagocytosis and phagocytosis of the pathogen *S. pneumoniae*. However, a drastic difference was observed between the two types of macrophages for the phagocytosis of fluorescent beads. An almost complete block in the mean number of phagocytosed beads was seen in bone marrow-derived macrophages while there was no effect in alveolar macrophages. The cellular background could explain the differences between the alveolar macrophages and the bone marrow-derived macrophages. Alveolar macrophages are important cells for a first defense and are therefore considered to be more actively phagocytosing cells. Interestingly, in porcine alveolar macrophages phagocytosis of beads is drastically reduced upon treatment with Sn-specific mAbs, which is in contrast with the results observed in our experiments [8]. Intriguingly, although mean values of the number of phagocytosed beads were identical between controls and anti-Sn mAbs treated cells, we could identify two different patterns of phagocytosis in mouse alveolar macrophages. Some macrophages showed almost no phagocytosis, while others had enhanced phagocytosis upon stimulation with anti-Sn mAbs or F(ab')₂ fragments. This seems to imply the existence of two

functionally different alveolar macrophage populations. Indeed, previously it was shown that alveolar macrophages can be divided into two functional subtypes [55, 56], which could explain our results.

To confirm that the effects we observed are directly related to mAbs binding to Sn, we also analyzed phagocytosis in macrophages from Sn-deficient mice compared to wild type mice (supplementary data Figure S1). The effects on phagocytosis were observed only in wild type and not in macrophages from Sn-deficient mice, confirming that the effect we observe is directly related to Sn.

Sn has the potential to be used as immunotherapeutic target in certain diseases where Sn is upregulated, by modulating macrophage functionality or eliminating these cells using immunotoxins. Our findings show that there are no serious side effects that could interfere with the use of Sn-specific mAbs as immunotherapy. Indeed, phagocytosis of pathogens and Fc-mediated phagocytosis is still functional.

Our results show that stimulation of Sn with anti-Sn mAbs can modulate phagocytosis. An augmented phagocytosis is observed in hemophagocytic syndromes and modulating phagocytic cells using anti-Sn mAbs to counter this property could reduce phagocytosis. However, our results showed only a reduced phagocytosis of beads after Sn stimulation, while phagocytosis is observed for particles that interact with phagocytic receptors. In the case of hemophagocytic syndromes, there is an elevated expression of the phagocyte receptor CD36 on leukocytes, suggesting the phagocytosis is receptor-dependent and that Sn targeting will have no effect [57]. In contrast, targeting Sn for modulation phagocytosis in diseases with reduced phagocytosis could be interesting. In chronic obstructive pulmonary disease, a reduced bacterial phagocytosis was observed. The phagocytosis might augment after stimulating Sn with anti-Sn mAbs. Our results showed that there was no effect on bacterial phagocytosis in human macrophages, but in contrast an increased phagocytosis of bacteria in mouse macrophages. Further research is needed to analyze if stimulation of Sn can increase phagocytosis in specific diseases.

5 Acknowledgements

We would like to acknowledge Pim-Bart Feijens and Rik Hendrickx for their help with experiments. We also would like to thank P.R. Crocker for the use of the macrophages derived from sialoadhesin-deficient mice. This project was supported by the Agency for Innovation by Science and Technology in Flanders (IWT, Belgium), Marjorie De Schryver is a Ph.D. fellow of the IWT–Flanders. The Research Foundation-Flanders (FWO-Vlaanderen; 12N5915N) funds Davie Cappoen. Guy Caljon is supported by a UA research fund (TT-ZAPBOF 33049).

This article is based upon work from the COST Action BM1404 Mye-EUNITER (www.mye-euniter.eu), supported by COST (European Cooperation in Science and Technology). COST is part of the EU Framework Program Horizon 2020.

6 References

- [1] C.A. Janeway Jr, P. Travers, M. Walport, M.J. Shlomchik, Principles of innate and adaptive immunity, (2001).
- [2] A. Aderem, D.M. Underhill, Mechanisms of phagocytosis in macrophages, *Annu Rev Immunol*, 17 (1999) 593-623.
- [3] A.E. Taylor, T.K. Finney-Hayward, J.K. Quint, C.M. Thomas, S.J. Tudhope, J.A. Wedzicha, P.J. Barnes, L.E. Donnelly, Defective macrophage phagocytosis of bacteria in COPD, *The European respiratory journal*, 35 (2010) 1039-1047.

- [4] S. Hodge, G. Hodge, R. Scicchitano, P.N. Reynolds, M. Holmes, Alveolar macrophages from subjects with chronic obstructive pulmonary disease are deficient in their ability to phagocytose apoptotic airway epithelial cells, *Immunology and cell biology*, 81 (2003) 289-296.
- [5] D.N. Fisman, Hemophagocytic syndromes and infection, *Emerging infectious diseases*, 6 (2000) 601-608.
- [6] L.P. Kane, T cell Ig and mucin domain proteins and immunity, *Journal of immunology*, 184 (2010) 2743-2749.
- [7] A.C. Foks, D. Engelbertsen, F. Kuperwaser, N. Alberts-Grill, A. Gonen, J.L. Witztum, J. Lederer, P. Jarolim, R.H. DeKruyff, G.J. Freeman, A.H. Lichtman, Blockade of Tim-1 and Tim-4 Enhances Atherosclerosis in Low-Density Lipoprotein Receptor-Deficient Mice, *Arteriosclerosis, thrombosis, and vascular biology*, 36 (2016) 456-465.
- [8] M.I. De Baere, H. Van Gorp, H.J. Nauwynck, P.L. Delputte, Antibody binding to porcine sialoadhesin reduces phagocytic capacity without affecting other macrophage effector functions, *Cellular immunology*, 271 (2011) 462-473.
- [9] A.S.G. O'Neill, T.K. van den Berg, G.E.D. Mullen, Sialoadhesin - a macrophage-restricted marker of immunoregulation and inflammation, *Immunology*, 138 (2013) 198-207.
- [10] Y.S. Xiong, Y. Cheng, Q.S. Lin, A.L. Wu, J. Yu, C. Li, Y. Sun, R.Q. Zhong, L.J. Wu, Increased expression of Siglec-1 on peripheral blood monocytes and its role in mononuclear cell reactivity to autoantigen in rheumatoid arthritis, *Rheumatology*, 53 (2014) 250-259.
- [11] G.G. Bornstein, Antibody Drug Conjugates: Preclinical Considerations, *The AAPS Journal*, 17 (2015) 525-534.
- [12] J.P. Leonard, D.M. Goldenberg, Preclinical and clinical evaluation of epratuzumab (anti-CD22 IgG) in B-cell malignancies, *Oncogene*, 26 (2007) 3704-3713.
- [13] M. Pino, I. Erkizia, S. Benet, E. Erikson, M.T. Fernandez-Figueras, D. Guerrero, J. Dalmau, D. Ouchi, A. Rausell, A. Ciuffi, O.T. Keppler, A. Telenti, H.G. Krausslich, J. Martinez-Picado, N. Izquierdo-Useros, HIV-1 immune activation induces Siglec-1 expression and enhances viral trans-infection in blood and tissue myeloid cells, *Retrovirology*, 12 (2015) 37.
- [14] P.L. Delputte, W. Van Breedam, I. Delrue, C. Oetke, P.R. Crocker, H.J. Nauwynck, Porcine arterivirus attachment to the macrophage-specific receptor sialoadhesin is dependent on the sialic acid-binding activity of the N-terminal immunoglobulin domain of sialoadhesin, *Journal of virology*, 81 (2007) 9546-9550.
- [15] H. Van Gorp, W. Van Breedam, P.L. Delputte, H.J. Nauwynck, The porcine reproductive and respiratory syndrome virus requires trafficking through CD163-positive early endosomes, but not late endosomes, for productive infection, *Archives of virology*, 154 (2009) 1939-1943.
- [16] P.L. Delputte, H. Van Gorp, H.W. Favoreel, I. Hoebeke, I. Delrue, H. Dewerchin, F. Verdonck, B. Verhasselt, E. Cox, H.J. Nauwynck, Porcine sialoadhesin (CD169/Siglec-1) is an endocytic receptor that allows targeted delivery of toxins and antigens to macrophages, *PloS one*, 6 (2011) e16827.
- [17] I. Delrue, H. Van Gorp, J. Van Doorselaere, P.L. Delputte, H.J. Nauwynck, Susceptible cell lines for the production of porcine reproductive and respiratory syndrome virus by stable transfection of sialoadhesin and CD163, *BMC biotechnology*, 10 (2010) 48.
- [18] N. Izquierdo-Useros, M. Lorizate, M.C. Puertas, M.T. Rodriguez-Plata, N. Zangger, E. Erikson, M. Pino, I. Erkizia, B. Glass, B. Clotet, O.T. Keppler, A. Telenti, H.G. Krausslich, J. Martinez-Picado, Siglec-1 is a novel dendritic cell receptor that mediates HIV-1 trans-infection through recognition of viral membrane gangliosides, *PLoS biology*, 10 (2012) e1001448.
- [19] N. Vanderheijden, P.L. Delputte, H.W. Favoreel, J. Vandekerckhove, J. Van Damme, P.A. van Woensel, H.J. Nauwynck, Involvement of Sialoadhesin in Entry of Porcine Reproductive and Respiratory Syndrome Virus into Porcine Alveolar Macrophages, *Journal of virology*, 77 (2003) 8207-8215.
- [20] W.C. Chen, N. Kawasaki, C.M. Nycholat, S. Han, J. Pilotte, P.R. Crocker, J.C. Paulson, Antigen delivery to macrophages using liposomal nanoparticles targeting sialoadhesin/CD169, *PloS one*, 7 (2012) e39039.

520 [21] M. De Schryver, H. Van Gorp, I. Hoebeke, B. De Maeyer, K. Ooms, I. Pintelon, L. Maes, P. Cos,
521 H. Nauwynck, P. Delputte, Development and Characterization of New Species Cross-Reactive
522 Anti-Sialoadhesin Monoclonal Antibodies, *Antibodies*, 5 (2016) 7.

523 [22] M. Klaas, P.R. Crocker, Sialoadhesin in recognition of self and non-self, *Seminars in*
524 *immunopathology*, 34 (2012) 353-364.

525 [23] Q. Zheng, J. Hou, Y. Zhou, Y. Yang, B. Xie, X. Cao, Siglec1 suppresses antiviral innate immune
526 response by inducing TBK1 degradation via the ubiquitin ligase TRIM27, *Cell research*, 25
527 (2015) 1121-1136.

528 [24] S. Pillai, I.A. Netravali, A. Cariappa, H. Mattoo, Siglecs and Immune Regulation, *Annual*
529 *Review of Immunology*, 30 (2012) 357-392.

530 [25] P.R. Crocker, S. Gordon, Properties and distribution of a lectin-like hemagglutinin
531 differentially expressed by murine stromal tissue macrophages, *The Journal of experimental*
532 *medicine*, 164 (1986) 1862-1875.

533 [26] A.P. Heikema, M.P. Bergman, H. Richards, P.R. Crocker, M. Gilbert, J.N. Samsom, W.J. van
534 Wamel, H.P. Endtz, A. van Belkum, Characterization of the specific interaction between
535 sialoadhesin and sialylated *Campylobacter jejuni* lipooligosaccharides, *Infect Immun*, 78 (2010)
536 3237-3246.

537 [27] C. Jones, M. Virji, P.R. Crocker, Recognition of sialylated meningococcal lipopolysaccharide
538 by siglecs expressed on myeloid cells leads to enhanced bacterial uptake, *Molecular*
539 *Microbiology*, 49 (2003) 1213-1225.

540 [28] Y.C. Chang, J. Olson, A. Louie, P.R. Crocker, A. Varki, V. Nizet, Role of macrophage
541 sialoadhesin in host defense against the sialylated pathogen group B *Streptococcus*, *Journal of*
542 *molecular medicine*, 92 (2014) 951-959.

543 [29] K. Ooms, H. Van Gorp, S. Botti, T. Van Gaever, P.L. Delputte, H.J. Nauwynck, Evaluation of
544 viral peptide targeting to porcine sialoadhesin using a porcine reproductive and respiratory
545 syndrome virus vaccination-challenge model, *Virus research*, 177 (2013) 147-155.

546 [30] H. Veninga, E.G.F. Borg, K. Vreeman, P.R. Taylor, H. Kalay, Y. van Kooyk, G. Kraal, L. Martinez-
547 Pomares, J.M.M. den Haan, Antigen targeting reveals splenic CD169+macrophages as promoters
548 of germinal center B-cell responses, *European Journal of Immunology*, 45 (2015) 747-757.

549 [31] N. Kawasaki, J.L. Vela, C.M. Nycholat, C. Rademacher, A. Khurana, N. van Rooijen, P.R.
550 Crocker, M. Kronenberg, J.C. Paulson, Targeted delivery of lipid antigen to macrophages via the
551 CD169/sialoadhesin endocytic pathway induces robust invariant natural killer T cell activation,
552 *Proceedings of the National Academy of Sciences of the United States of America*, 110 (2013)
553 7826-7831.

554 [32] C.M. Nycholat, C. Rademacher, N. Kawasaki, J.C. Paulson, In Silico-Aided Design of a Glycan
555 Ligand of Sialoadhesin for in Vivo Targeting of Macrophages, *Journal of the American Chemical*
556 *Society*, 134 (2012) 15696-15699.

557 [33] T.T. Hansel, H. Kropshofer, T. Singer, J.A. Mitchell, A.J. George, The safety and side effects of
558 monoclonal antibodies, *Nature reviews. Drug discovery*, 9 (2010) 325-338.

559 [34] D.S. Wilson, J. Wu, P. Peluso, S. Nock, Improved method for pepsinolysis of mouse IgG(1)
560 molecules to F(ab')(2) fragments, *Journal of immunological methods*, 260 (2002) 29-36.

561 [35] T.H. Mogensen, Pathogen recognition and inflammatory signaling in innate immune
562 defenses, *Clinical microbiology reviews*, 22 (2009) 240-273, Table of Contents.

563 [36] C.A. Janeway, Jr., R. Medzhitov, Innate immune recognition, *Annu Rev Immunol*, 20 (2002)
564 197-216.

565 [37] E. Kong, M.A. Jabra-Rizk, The great escape: pathogen versus host, *PLoS pathogens*, 11
566 (2015) e1004661.

567 [38] R.C. May, L.M. Machesky, Phagocytosis and the actin cytoskeleton, *Journal of cell science*,
568 114 (2001) 1061-1077.

569 [39] A.C. van der Kuyl, R. van den Burg, F. Zorgdrager, F. Groot, B. Berkhout, M. Cornelissen,
570 Sialoadhesin (CD169) expression in CD14+ cells is upregulated early after HIV-1 infection and
571 increases during disease progression, *PloS one*, 2 (2007) e257.

- [40] A. Hartnell, J. Steel, H. Turley, M. Jones, D.G. Jackson, P.R. Crocker, Characterization of human sialoadhesin, a sialic acid binding receptor expressed by resident and inflammatory macrophage populations, *Blood*, 97 (2001) 288-296.
- [41] S. Muerkoster, O. Wachowski, H. Zerban, V. Schirmacher, V. Umansky, M. Rocha, Graft-versus-leukemia reactivity involves cluster formation between superantigen-reactive donor T lymphocytes and host macrophages, *Clinical Cancer Research*, 4 (1998) 3095-3106.
- [42] A.M. Scott, J.D. Wolchok, L.J. Old, Antibody therapy of cancer, *Nature reviews. Cancer*, 12 (2012) 278-287.
- [43] M. Dawson, D. Wirtz, J. Hanes, Enhanced viscoelasticity of human cystic fibrotic sputum correlates with increasing microheterogeneity in particle transport, *The Journal of biological chemistry*, 278 (2003) 50393-50401.
- [44] P.R. Crocker, Mammalian carbohydrate recognition systems, Springer Science & Business Media 2012.
- [45] D.A. Haydon, The surface charge of cells and some other small particles as indicated by electrophoresis. II. The interpretation of the electrophoretic charge, *Biochimica et biophysica acta*, 50 (1961) 457-462.
- [46] Y. Zhang, M. Yang, N.G. Portney, D. Cui, G. Budak, E. Ozbay, M. Ozkan, C.S. Ozkan, Zeta potential: a surface electrical characteristic to probe the interaction of nanoparticles with normal and cancer human breast epithelial cells, *Biomedical microdevices*, 10 (2008) 321-328.
- [47] O.V. Bondar, D.V. Saifullina, Shakhmaeva, II, Mavlyutova, II, T.I. Abdullin, Monitoring of the Zeta Potential of Human Cells upon Reduction in Their Viability and Interaction with Polymers, *Acta naturae*, 4 (2012) 78-81.
- [48] A. Aderem, Phagocytosis and the inflammatory response, *The Journal of infectious diseases*, 187 Suppl 2 (2003) S340-345.
- [49] G. Weiss, U.E. Schaible, Macrophage defense mechanisms against intracellular bacteria, *Immunological reviews*, 264 (2015) 182-203.
- [50] L.M. Stuart, J. Deng, J.M. Silver, K. Takahashi, A.A. Tseng, E.J. Hennessy, R.A. Ezekowitz, K.J. Moore, Response to *Staphylococcus aureus* requires CD36-mediated phagocytosis triggered by the COOH-terminal cytoplasmic domain, *The Journal of cell biology*, 170 (2005) 477-485.
- [51] D.M. Underhill, H.S. Goodridge, Information processing during phagocytosis, *Nature reviews. Immunology*, 12 (2012) 492-502.
- [52] R. Huizinga, A.S. Easton, A.M. Donachie, J. Guthrie, W. van Rijs, A. Heikema, L. Boon, J.N. Samsom, B.C. Jacobs, H.J. Willison, C.S. Goodyear, Sialylation of *Campylobacter jejuni* lipooligosaccharides: impact on phagocytosis and cytokine production in mice, *PloS one*, 7 (2012) e34416.
- [53] A.P. Heikema, R.I. Koning, S. Duarte dos Santos Rico, H. Rempel, B.C. Jacobs, H.P. Endtz, W.J. van Wamel, J.N. Samsom, Enhanced, sialoadhesin-dependent uptake of Guillain-Barre syndrome-associated *Campylobacter jejuni* strains by human macrophages, *Infect Immun*, 81 (2013) 2095-2103.
- [54] K. Kwiatkowska, A. Sobota, Signaling pathways in phagocytosis, *BioEssays : news and reviews in molecular, cellular and developmental biology*, 21 (1999) 422-431.
- [55] H. Garn, A. Siese, S. Stumpf, A. Wensing, H. Renz, D. Gerns, Phenotypical and functional characterization of alveolar macrophage subpopulations in the lungs of NO₂-exposed rats, *Respiratory research*, 7 (2006) 4.
- [56] L.K. Johnston, C.R. Rims, S.E. Gill, J.K. McGuire, A.M. Manicone, Pulmonary macrophage subpopulations in the induction and resolution of acute lung injury, *American journal of respiratory cell and molecular biology*, 47 (2012) 417-426.
- [57] G. Janka, S. Imashuku, G. Elinder, M. Schneider, J.I. Henter, Infection- and malignancy-associated hemophagocytic syndromes. Secondary hemophagocytic lymphohistiocytosis, *Hematology/oncology clinics of North America*, 12 (1998) 435-444.