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Thesis for doctoral degree (Ph.D.) 2010

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Stockholm 2010

Published by Karolinska Institutet.

ISBN 978-91-7409-544-9

Printed by
REPROPRINT AB
Stockholm 2010
www.reproprint.se
Gårdsvägen 4, 169 70 Solna

“Less is more.”

ABSTRACT

Nanotechnology is a fast developing area, which refers to research and technology development at the "nanometer" scale, ranging from 0.1-100 nm. The properties of nanomaterials offer the ability to interact with complex biological functions, implying enormous opportunities for novel applications within medicine. However, there is little information available concerning the potential toxicity of nanoparticles and what influence such particles have on the immune system, e.g. on dendritic cells (DCs). DCs are the most efficient antigen presenting cells, having a capacity to initiate and direct immune responses against foreign material. The aim of this thesis was to study effects of differently sized and shaped nanomaterials in the interaction with primary human monocyte derived DCs (MDDCs), thereby obtaining an insight on what impact these materials have on the immune system and their potential use in medical applications. In addition, we wanted to determine if endogenous nanoparticles (exosomes), produced by various cells, are natural targeting vehicles.

We show that conventionally produced gold nanoparticles had a maturing effect on human MDDCs, but this was found to be a result of lipopolysaccharide (LPS) contamination. By modification of the production process, clean particles were obtained, which had practically no effect on phenotype or cytokine production of MDDCs. These findings emphasize the importance of retaining high purity during the production of nanoparticles, since possible contaminants may interfere with the assessment of nanoparticles' biological effects and result in hazardous particles.

To investigate whether various shapes of gold nanoparticles affect MDDCs differently, a novel method was developed for the preparation of gold nanorods with high aspect ratios (ARs) based on a self-seeded surfactant-mediated protocol. The biocompatibility of these high AR gold nanorods, with potential use in thermal therapy, was compared with spherical gold nanoparticles. Both materials had no or minor effects on MDDCs' viability and phenotype, thus shape did not seem to affect the biocompatibility of gold.

To determine whether the size of the particle is important for its biocompatibility, the impact of mesoporous silica nano- (270 nm) and microparticles (2.5 µm) was compared. Size- and concentration-dependent effects were seen where the smaller particles and lower concentrations affected MDDCs to a minor degree compared to the larger particles and higher concentrations, both in terms of viability, uptake, and immune regulatory markers. Thus, the larger particles have promising features to serve as an immune-stimulant, with possible T cell modulatory properties, while the smaller particles are more suitable for neutral drug delivery systems.

Finally, we evaluated whether exosomes of different origins are selectively targeting different immune cells. Results revealed that exosomes derived from human MDDCs and breast milk preferably associated with monocytes, whereas exosomes from an Epstein-Barr virus (EBV) transformed B cell line (LCL1) selectively targeted B cells. The interaction between LCL1-derived exosomes and peripheral blood B cells was dependent on CD21 on B cells and the exosome associated EBV glycoprotein gp350. This finding suggests that exosome-based vaccines can be engineered for specific B-cell targeting by inducing gp350 expression.

To summarize, the work included in this thesis has contributed to the understanding of how particles of various materials, shapes and sizes affect and interact with human MDDCs. The knowledge gained is of importance for the further development of the studied materials within various medical applications. This thesis also highlights the potential use of endogenous nanoparticles, exosomes, as targeting delivery vehicles in medical applications.

LIST OF PUBLICATIONS

I. Helen Vallhov, Jian Qin, Sara M. Johansson, Niklas Ahlborg, Mamoun A. Muhammed, Annika Scheynius, and Susanne Gabrielsson. The importance of an endotoxin-free environment during the production of nanoparticles used in medical applications.
Nano Lett. 2006, 6(8):1682-6.
Highlighted in Nanowerk Spotlight http://www.nanowerk.com/ August 14th, 2006 and in the "Currents" section of Analytical Chemistry's October 1st, 2006, issue.

II. Fei Ye, Helen Vallhov, Jian Qin, Evangelia Daskalaki, Abhilash Sugunan, Muhammet S. Toprak, Andrea Fornara, Susanne Gabrielsson, Annika Scheynius, and Mamoun Muhammed. Synthesis of high aspect ratio gold nanorods and their effects on human antigen presenting cells.
Submitted.

III. Helen Vallhov, Susanne Gabrielsson, Maria Strømme, Annika Scheynius, and Alfonso E. Garcia-Bennett. Mesoporous silica particles induce size dependent effects on human dendritic cells.
Nano Lett. 2007, 7(12):3576-82.

IV. Helen Vallhov*, Sara M. Johansson*, Cindy Gutzeit, Qin Li, Sherree Friend, Thaddeus C. George, Annika Scheynius, and Susanne Gabrielsson. Exosomes released by Epstein-Barr virus transformed B cells selectively target B cells through CD21-gp350 interactions.
*Shared first authorship.
Submitted.

Publication not included in the thesis:

Nagarjun Konduru, Yulia Y. Tyurina, Wei Feng, Liana V. Basova, Natalia A. Belikova NA, Huelya Bayir, Katherine Clark, Marc Rubin, Donna Stolz, Helen Vallhov, Annika Scheynius, Erika Witasp, Bengt Fadeel, Padmakar D. Kichambare, Alexander Star, Elena R. Kisin, Ashley R. Murray, Anna A. Shvedova, and Valerian E. Kagan. Phosphatidylserine targets single-walled carbon nanotubes to professional phagocytes *in vitro* and *in vivo*.
PLoS One. 2009;4(2):e4398.

CONTENTS

LIST OF ABBREVIATIONS

AAS	Atomic absorbtion spectroscopy
ACD	Allergic contact dermatitis
APC	Antigen presenting cell
AR	Aspect ratio
Ab	Antibody
BCR	B cell receptor
BET	Bruner, Emmett and Teller (developers of the method)
cAMP	Cyclic adenosine monophosphate
CLSM	Confocal laser scanning microscopy
CTAB	Cetyltrimethylammonium bromide
DC	Dendritic cell
DDC	Dermal dendritic cell
EBNA	EBV-specified nuclear antigen
EBV	Epstein-Barr virus
ELISA	Enzyme-linked immunosorbent assay
ELISpot	Enzyme linked immunospot assay
EPR	Enhanced permeability and retention
FITC	Fluorescein isothiocyanate
FOXP3	Fork-head box protein 3
GM-CSF	Granulocyte-macrophage colony stimulating factor
gp	glycoprotein
HRTEM	High resolution transmission electron microscopy
IDC	Interstitial dendritic cell
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
LAL	Limulus amebocyte lysate
LFA	Leukocyte function-associated antigen
LC	Langerhans cell
LCL	Lymphoblastoid cell line
LMP	Latent membrane protein
LOC	Lab-on-chip
LPS	Lipopolysaccharide
LSPR	Localized surface plasmon resonance
MARCO	Macrophage receptor with collagenous structure
mDC	Myeloid dendritic cell
MDDC	Monocyte-derived dendritic cell
MHC	Major histocompatibility complex
MLR	Mixed lymphocyte reaction
MRI	Magnetic resonance imaging
MVB	Multivesicular body
NIR	Near infrared
NLDFT	Nonlocal Density Functional Theory
PBMC	Peripheral blood mononuclear cells

pDC	Plasmacytoid dendritic cell
PE	Phycoerythrin
PEG	Polyethylene glycol
PLG	Poly(lactide-co-glycolide)
ppm	Parts per million
PRR	Pattern recognition receptor
SEMi	Scanning electron microscopy
SERS	Surface-enhanced Raman scattering
SPR	Surface plasmon resonance
TCR	T cell receptor
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
TGF	Tumor growth factor
T_H	T helper cell
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	T regulatory cell
XRD	X-ray diffraction

1 INTRODUCTION

1.1 THE WORLD OF NANOTECHNOLOGY

Nanotechnology refers to research and technology development at the atomic and molecular scale, leading to the controlled manipulation and study of structures in the "nanometer" size, normally ranging from 0.1-100 nm - the same basic size as small biological entities (1). The area of nanomaterials has been increasingly expanding during the last decade, ranging from optical systems, electronic, chemical industries, to environmental engineering and medicine (1), which up to now has resulted in approximately 800 nanoparticle-containing consumer products (2). This new class of advanced materials possesses special properties including a large surface area to volume ratio, whereby the materials take on novel properties compared to those seen in the bulk scale, such as dramatic changes of their magnetic, physiochemical, and electronic properties (3). Additionally, the ability to modify the outer layer of nanomaterials gives further rise to new possible applications.

1.1.1 Nanomedicine

The properties of nanomaterials offer the ability to interact with complex biological functions at the scale of biomolecules, implying enormous opportunities for novel applications in medicine, including diagnostics, medical imaging, targeted drug delivery and immune therapy (1, 4).

It is now possible to produce nanofluidic systems for lab-on-chip (LOC) diagnostics, which enables the detection of biological disease markers in the process of routine screening (1). The small dimension of the LOC reduces processing times and the amount of reagents needed, thereby making routine diagnostics more costs-efficient. In addition, it can be fabricated with many channels, allowing for massively parallel chemical analyses and more sensitive detection (5).

In magnetic resonance imaging (MRI), nanosized contrast agents have greater magnetic susceptibility than traditional contrast agents like gadolinium, and depending on size and surface coating they can be enriched at specific sites in the body (4, 6). As an example, superparamagnetic iron oxide nanoparticles in the size of 5-10 nm are able to reach lymph nodes and bone marrow. Hereby, the lymph node status of patients with breast carcinoma can be investigated (4).

By using nanoparticles in drug delivery, the solubility and stability of drug agents can be enhanced and drug release can be controlled. By targeting the particles directly to e.g. the tumour site, a systemic release is avoided, thereby minimizing drug-induced side effects (7). The targeted delivery can either be actively or passively achieved. Active targeting requires that the particle is coated with a ligand which is specifically directed to a tissue or cell-specific receptor, while passive targeting can occur due to e.g. the enhanced permeability and retention (EPR) effect at tumour sites (8). This effect is based on increased hypervascularity and enhanced permeability around tumour tissues, which implies that nanoparticles tend to accumulate in tumours much more

than they do in normal tissues (9). The physiochemical characteristics of the nanocarrier and the nature of the target cell are decisive for which internalization pathway is used, as well as the intracellular fate of the nanoparticle. The release of the drug into the enzymatic environment of lysosomes or directly into the cell cytoplasm, has an important impact on the activity of the drug (10). There are various kinds of nanoparticles, such as liposomes, polymers, micelles, nanoshells and carbon nanotubes, which all have the potential to be used in drug delivery or are already in use (4). Doxil is one type of liposome-drug, encapsulating doxorubicin within a liposome, which is already in clinical use against ovarian cancer (11).

The engineering of materials that can modulate the immune system is a new emerging field. For therapeutic use, such as immune therapy, materials are now being engineered to target antigen presenting cells (APC), especially dendritic cells (DCs), which have a central role in the immune system, as described later. It has also become clear that materials can be modulated to be delivered through specific intracellular pathways, which allows a better control of how antigens (foreign substances) are presented to T lymphocytes (12). Moreover, materials are being designed to combine several features, e.g. by both being an efficient delivery system and by being an adjuvant. Adjuvants are substances, which are added in vaccines to mimic danger signals, thereby augmenting an immune response towards antigens. Today, aluminium-based mineral salt (alum) is the most used adjuvant. However, studies indicate that it is a poor adjuvant for antibody induction and induces T_H2 rather than T_H1 cytokine responses (13). Therefore, the development of new vaccine-delivery systems is pointing towards the use of nano- or microparticles - having similar dimensions as pathogens, which the immune system is used to interact with, and which may also, depending on physiochemical properties, have some adjuvant effects. Hereby, it is ensured that both the antigen and the adjuvant are co-delivered into the same population of APCs, which often results in more efficient immune responses (11, 13). Extensive studies have been done on poly(lactide-co-glycolide) (PLG) polymer particles, for example for their use as adjuvants. It has been demonstrated that particles below 10 µm in size were more immunogenic than larger ones, which is probably a consequence of impaired phagocytic uptake by APCs when particles become too large (14). The use of polymeric nanoparticles is also under investigation, due to their higher surface area, which enables a higher antigen-polymer ratio (15).

1.1.2 Safety in nanotechnology

During the last years this fast developing area of nanotechnology has become aware of the importance to study possible toxicological and immunological effects caused by nanomaterials, which can differ compared to similar material in bulk-scale. Here it is not just important to study the toxicity of nanoparticles with intended use for *in vivo* administration, but also all particles which are produced in high amounts, thereby causing a risk of unintentional exposure to the environment. A challenge in evaluating risk associated with the use, and also the production of nanomaterials, is the many different routes of entry into the body and possible sites of interaction with biological systems, and the diversity and complexity of the types of materials available. The shape alone can differ from spheres, nano-fibers, nano-tubes to two dimensional structures such as thin films. In addition, the surface characteristics of the particles influence the way nanoparticles interact with different blood constituents. Nanoparticles whose

surfaces are not modified to prevent absorption of opsonins (proteins that give an eat-me-signal to cells) are readily removed from the blood stream by e.g. phagocytosing macrophages. If this is not requested, surface hydrophobicity or charge can be changed, e.g. by coating the particles with polyethylene glycol (PEG). However, this is not true for all kinds of particles, which demonstrates the complexicity of understanding particle structure-activity relationships (11, 16).

Since nanomedicine is a relatively new field, there are until now no formal standards for assessing the immune toxicity of nanoparticles. However, as more nanoscale drugs are formulated to target the immune system and since the immune system is our primary defence against foreign invasion, it is increasingly important to investigate the immune responses caused by these nanoscale formulations. Phagocytic cells, such as macrophages and DCs, are immune cells which are specialized in responding to foreign material and will therefore most probably come in close contact with nanoparticles. Therefore, the effects on these cells are important to study. The understanding of how nanoparticles, in various materials, shapes and sizes interact with immune cells is not only important for predicting potential adverse effects, but will also enable the optimization of nanoparticle design for future biomedical applications.

To evaluate the potential toxic and immunogenic nanoeffects, a close collaboration between material and biological scientists is required. The nanomaterial needs to be thoroughly characterized before being applied to cells, thereby enabling a better understanding of how different properties of nanoparticles can affect the biological response (17). However, it also important to be aware that the size of the produced nanoparticles may be increased when added to cell media, since they can be covered with plasma proteins, forming a protein corona (18).

Cell lines are commonly used to study the effects of nanomaterials, since they are relatively easy to handle. However, they are usually cancer-cell-lines, such as Jurkat leukemia T cells or HeLa cells derived from cervical cancer cells, which have different characteristics compared to healthy cells, and are thus mainly useful for performing preliminary screenings of potential toxic or immunogenic effects. To approach a more *in vivo* like situation and to see a true response variability between different individuals, one may rather use human primary cells. However, thorough *in vitro* studies need also to be compared with *in vivo* studies involving animal models. It is also important to note that traditionally analytical *in vitro* assays cannot always be combined with nanoparticles. The optical and catalytic properties of particles sometimes interfere with the reagents and detection methods used in these assays and can thus produce unreliable results. Among others, the Limulus Amebocyte Lysate (LAL) assay used to assess contamination of the bacterial product lipopolysccharide (LPS) and the viability assay based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) have been reported to be affected by various nanoparticles (19-21). Due to these limitations, it is required to carefully validate the applied methods and sometimes run at least two or more independent test systems in parallel, which then can be verified and compared with each other (2, 19).

1.2 ENGINEERED NANOPARTICLES

The amount of novel nanoparticles developed is constantly increasing, involving various modifications of liposomes, polymers, micelles and inorganic nanoparticles. Some of the most promising inorganic materials are gold nanoparticles and mesoporous silica particles, which are currently under investigation for several applications.

1.2.1 Gold nanoparticles

1.2.1.1 The applications of gold in biomedicine

Gold is seen as one of the most stable and inert materials (22). It exists at a metallic ground state (0), but it has also two additional oxidation states (I and III). The pharmacological and toxicological potentials of these three states differ profoundly. Metallic gold was already used as a medicinal agent as early as 2500 BC, and in 1890 Robert Koch demonstrated the cytotoxic effect of gold (I) salts on the tubercle bacillus *in vitro*, and it was therefore used as a treatment for tuberculosis until the late 1930s. Similar therapy was extended to the treatment of rheumatoid arthritis (RA), which is based on the immune suppressive effect of gold salts, although their mechanism of action is not fully understood (22, 23). Additionally, due to its anti-corrosive characteristics, gold (0) has been used as a prosthetic in dentistry and as the predominant component of dental alloys (22).

The most recent application is to employ gold in nanotechnology in the area of biomedicine, starting from biosensing to drug and gene delivery systems. Nano-sized gold nanoparticles can be readily functionalized with a wide range of biomolecules such as DNA (24, 25) and proteins (26), which make them suitable for various applications. Moreover, gold nanoparticles hold great promises due to its photothermal and optical properties (27-29). These properties of gold nanoparticles arise from the resonant oscillation of their free electrons in the presence of light, also known as localized surface plasmon resonance (LSPR). Hereby, photons are released, defined as scattering, which is a process which finds great utility in optical and imaging fields. Other photons will be directly absorbed again and converted to heat, which mechanism finds applications in thermal therapy. The interplay between scattering and absorption is known as the surface plasmon resonance (SPR) spectrum (30, 31). The absorption of gold nanoparticles is highly dependent on shape and size (31). Anisotropic nanoparticles, e.g. nanorods, are interesting, since they show tuneable longitudinal plasmon absorption bands depending on their aspect ratios (ARs), that is, length-to-width ratio (32). Therefore, gold nanorods are excellent candidates for diagnostic tools using surface-enhanced Raman scattering (SERS) (33). For *in vivo* applications, gold nanoparticles exhibit great potential for use in thermal therapy, where soft tissues and blood vessels supplying e.g. cancer cells is affected through high temperatures generated by particles' local absorption of laser energy. This effect is evaluated for its application in drug release and cancer therapy (34-36). Among others, there is currently a pilot study conducted in patients with refractory head and neck cancer (www.nanospectra.com). Gold nanorods with high ARs are especially useful within this area, owing to their strong absorption and scattering of electromagnetic radiation in the near-infrared (NIR) region, where the optical absorption in tissue is minimal and penetration optimal (34, 37).

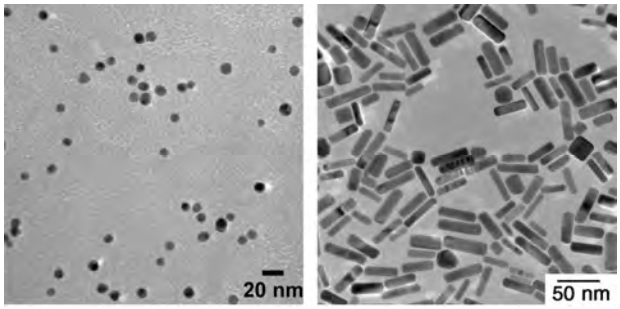

Figure 1. Images of spherical and rod shaped gold nanoparticles taken by transmission electron microscopy (TEM). Modified from Paper I and II.

1.2.1.2 Methods to produce gold nanoparticles

There exist various relatively simple methods for the production of spherical gold nanoparticles. One of the most widely used involves the reduction of chloroauric acid ($HAuCl_4$) to neutral gold (Au^0) by agents such as sodium citrate (38) or sodium borohydrate (39). However, the synthesis of gold nanorods, especially those with high ARs, is not as straightforward as for spherical particles and therefore hard to scale up. They often involve multi-step protocols, based on pre-made spherical nanoparticles, which then act as seeds (40). To enhance the yield of produced nanorods, different additives have been evaluated, such as nitric acid, which has also been proven to be valuable for receiving high AR nanorods (41). The mechanism behind the increase of AR with nitric acid is unknown. The size and shape of gold nanoparticles can be assessed by transmission electron microscopy (TEM), while the concentration is measured with atomic absorption spectroscopy (AAS).

1.2.1.3 Toxic and immunogenic effects of gold nanoparticles

Gold is clearly a good candidate for biological purposes since it is the least corrosive and most biologically inert of all metals. When gold salts have been used in rheumatic patients, there have been some minor side effects encountered in about half of all patients (22). The side effects include hypersensitivity reactions of skin and mucous membranes, and much less frequently more serious toxicities like blood dyscrasias, pulmonitis, nephrotoxicity, nephritic syndrome and allergic contact dermatitis (ACD). Gold (0) used as a prosthesis, rarely triggers any toxicity or immune responses at all. However, owing to the small size of gold nanoparticles, spherical (42-44) and rod shaped (45) particles are often engulfed by cells. This close interaction of nanoparticles with cells and in addition, due to the fact that the properties of gold may be altered in the nanorange, underlines the importance to study their potential toxic and immunogenic effects.

Some of the first toxicological studies were mainly performed on cell lines. Hereby, size-dependent toxic effects were reported, where larger spherical gold particles in the range of 4 to 18 nm had no impact, while particles in the size of 1.4 nm implicated a strong toxicity towards human cancer and healthy cell lines, due to their ability to bind to DNA (46-48). In another study no toxicity could be detected, even at higher

concentrations of gold nanoparticles, but instead it was demonstrated that the gold-salt precursor solution itself was toxic (48). These results highlight the importance of testing pure nanoparticle preparations, and could also explain why sometimes similar studies have different conclusions. To note, some conflicting results may also arise from different synthesis protocols and experimental set-ups.

Gold nanorods are typically prepared in the presence of a high concentration of micelle-forming detergents such as cetyltrimethylammonium bromide (CTAB), which have raised some concerns regarding their toxicity. However, recent studies have shown that if the surfactant concentration can be reduced or replaced with alternative capping agents, then the gold nanorods themselves only induce low cytotoxicity (48-50).

The knowledge of the immune modulatory properties of spherical gold nanoparticles is poor. One study on a macrophage cell line (RAW264.7) was undertaken, where gold nanoparticles in the size of 3.5 nm were shown to be non-toxic and have no impact on the secretion of the proinflammatory cytokines tumor necrosis factor (TNF)-α and IL1-β (51). Concerning gold nanorods, there are no studies reporting on the immunogenic effects, thus efforts are needed to bring some light into this area to understand their suitability in medical applications.

1.2.2 Mesoporous silica particles

1.2.2.1 The applications of mesoporous silica particles in biomedicine

Nanoporous materials such as mesoporous silicates are comprised of a honeycomb-like porous structure with empty channels of nanosized dimensions (52). They are being utilized in a variety of bio-related applications as a result of their internal pore volumes, tailorable surfaces, and high chemical and thermal stabilities. Moreover, mesoporous materials offer extremely high surfaces areas, usually around 1000 m^2/g, and are typically composed of monodispersed particles with amorphous silica walls (52). First discovered in 1992 (53), the material properties have many advantages in several important applications including their use as catalyst, adsorbents and in separation and purification technologies (54, 55). In medicine, mesoporous materials may offer advantages over current particles in tissue engineering, labelling and bioseparation material technologies, and transfection devices. In particular, they hold great promises as drug delivery systems, since they have a high drug-loading capacity, thereby reducing the quantity of matrix materials needed for administration.

Of the many nanoporous materials available, covering a pore size spectrum ranging from 0.5 to 500 nm, those in the mesoscale (2-50 nm) are of particular interest in biotechnologies since a large proportion of biomolecules are within this size range. Hence, the adsorption of active pharmaceutical molecules into stabile, non-erosive mesoporous materials has been explored offering the potential to control (delay) drug release, enhance drug dissolution, promote drug permeation across the intestinal cell wall and improve drug stability under the extreme environment of the gastro-intestinal tract when administered orally (56). Different types of drug molecules (including macromolecules) can be adsorbed into mesoporous micro- and nanoparticles by modifying the pore architecture and surface chemistry of the carrier material (57).

Recently Zhao *et al*., reported on a mesoporous silica-based double drug delivery system for glucose-responsive controlled release of insulin and cyclic adenosine monophosphate (cAMP) (58). In this work, insulin was immobilized on the exterior surface of the particles and also served as caps to encapsulate cAMP molecules inside of the mesopores. The release of both insulin and cAMP from the particles could be triggered by the introduction of saccharides, such as glucose. Mesoporous silica particles have also, among others, been suggested as a delivery system for hydrophobic anticancer drugs, thereby underlining the possibility to use these materials to solve the problem of insolubility of many other cancer drugs (59).

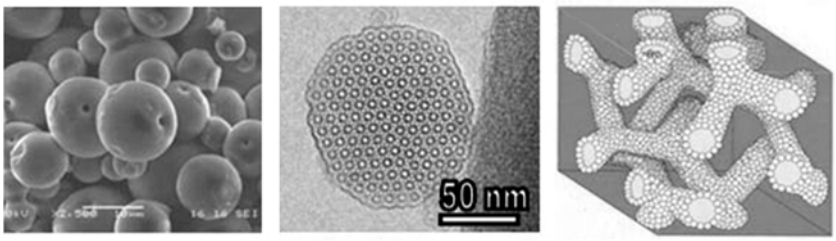

Figure 2. Images of mesoporous silica particles taken by scanning electron microscopy (SEMi, left) and by transmission electron microscopy (middle), where the latter shows a cross section of a cubic particle, demonstrating the pores. The schematic 3D image to the right shows a possible pore connectivity within a particle. (Images are taken by Dr. Alfonso Garcia-Bennett, The Ångström Laboratory, Uppsala University)

1.2.2.2 Methods to produce mesoporous silica particles.

These types of materials are produced through a sol-gel route, which means that a solution contains all the necessary inorganic precursors and that the growth of the particles is controlled through temperature or pH. The production of mesoporous silica particles relies on the use of an organic template, typically an amphillic surfactant, and hydrolysing and condensing silica species. The result is an organic micellar template supported by an amorphous silica wall. Through calcinations or solvent extraction of the template the pores are revealed, which size can be assessed with nitrogen adsorption-desorption isotherm measurements and the NLDFT method (60). To measure the size of mesoporous silica particles scanning electron microscopy (SEMi) can be applied.

1.2.2.3 Toxic and immunogenic effects of mesoporous silica particles

Some initial work has been conducted to identify the biocompatibility of silica materials. However, these studies are focusing on the effects of crystalline zeolites or on bioactive glass (61, 62). There were also no reports found on the effects of mesoporous silica particles on cells in the immune system when we initiated our studies, but which are needed to fully understand the potential of mesoporous silica particles.

1.3 OUR BODY'S DEFENSE AGAINST FOREIGN INVADERS

Our immune defense is a complex system spread throughout the body, involving specialized lymphoid tissues and billions of cells of various types, which interact with each other and form an army to defend our body against foreign invaders, such as microorganisms. The immune system is divided into two main parts – the innate and the adaptive immune system (63).

Before an immune response is induced, a pathogen must by-pass the skin and epithelial surfaces, which provides a physical, chemical and microbiological barrier. If a microorganism is able to circumvent these barriers, the innate immune system is induced and acts immediately. It provides a first line defense against infections, which depends on the recognition and destruction of common pathogens by phagocytic cells called macrophages and neutrophils. Several different receptors are involved in the recognition of the pathogen, such as pattern recognition receptors (PRRs) or Toll-like receptors (TLRs). NOD-like receptors (NLRs) form large complexes called inflammasomes, which are sensors, specialized in recognizing intracellular microbes within the cytosol (64). With the recognition of the invaders, cytokines and inflammatory mediators are released that restrict the spread of infection by tissue swelling and enables the recruitment of additional cells, such as monocytes which after arrival mature into macrophages. However, the innate immune system can be overcome by many pathogens, and it does not lead to any known immunological memory. Therefore, a more versatile means of defense, which in addition provides increased protection against future reinfection with the same pathogen, has evolved, called the adaptive immune system (63).

The induction of the adaptive immune response begins when a pathogen is ingested and processed into peptides by an APC, such as an immature dendritic cell (DC) in the infected tissue. Thereby, the dendritic cell starts to mature and migrate through the lymph to the regional lymph nodes, where they interact with recirculating lymphocytes, by expressing co-stimulatory molecules, cytokines and peptides on major histocompatibility complex (MHC) molecules. Naive antigen-specific T lymphocytes are thereby activated and start to proliferate and differentiate into effector cells than can eliminate the infectious agent by directly killing infected cells or by activating other cells in the immune system, such as antigen-specific B lymphocytes. In contrast to the fast innate immune response, the adaptive immune response needs several days to develop. However, a subset of the proliferating lymphocytes differentiates into memory cells, which are ready to be quickly reactivated in a secondary immune response when encountering the same pathogen again (63).

1.3.1 Dendritic cells

1.3.1.1 DC subsets

At least four functional distinct subsets of human DCs have been identified: plasmacytoid DCs (pDCs) – also known as natural IFN-producing cells, blood monocyte-derived DCs (MDDCs), dermal or interstitial DCs (DDCs, IDCs) and

Langerhans' cells (LCs). The three last named are also referred to as conventionally or myeloid DCs (mDCs). All subsets originate from the bone marrow, however, it is unclear whether they have a common progenitor or if they originate from different lineages early in the development (65).

1.3.1.2 Dendritic cells' role in an immune response

When DCs are immature and spread all over the body, concentrated in tissues, they are efficient in detecting and engulfing foreign agents, thus called "the sentinels of the immune system" (66). The uptake is mediated by endocytosis, which encompasses several mechanisms including receptor-mediated endocytosis, phagocytosis and macropinocytosis. The size of the foreign agent to be taken up is decisive for which mechanism is involved. Receptor mediated endocytosis allows the uptake of macromolecules, involving e.g. c-type lectins, Fc- and scavenger receptors. Particulate and soluble antigens are efficiently internalized by phagocytosis and macropinocytosis, respectively, both being actin dependent resulting in the formation of large intracellular vacuoles. Phagocytosis is also receptor mediated, while macropinocytosis is a non-specific process, allowing DCs to sample large amounts of surrounding fluid (67).

During the sampling DCs may encounter a danger signal, which they receive e.g. by a microbial structure binding to a DC associated TLR or PRR (68). Hereby, DCs start to mature, as seen by increased surface expression of CD83, and migrate from its peripheral site to a regional lymph node. Here they develop into the most efficient type of APC, having an exceptional capacity to interact with T cells and B cells. By expressing different levels of co-stimulatory molecules such as CD40, CD80 and CD86, and antigens loaded onto MHC class I and II, they can induce antigen-specific immune responses, or immunological tolerance. Depending on the type of stimulus and DC subset, they also express various cytokines, such as interferon (IFN)-α, inteleukin (IL)-10 and IL-12, affecting the outcome of the immune response. This dual capacity, both being immune activating and tolerogenic, gives DCs the ability to dictate immune responses and has evoked hopes that they can be manipulated *in vitro* or *in vivo* for use in immune therapy (63, 69).

1.3.1.3 DCs in immune therapy

Dendritic cell vaccines can be made by harvesting patient's monocytes, which are then cultured with GM-CSF and IL-4, generating monocyte derived DCs (MDDCs). Thereafter, these are activated with co-stimulatory agents and loaded with tumor specific antigens. A large amount of clinical trials have established the safety of these cellular based vaccines and have shown to activate the immune system. However, they are still under an investigational phase and their efficacy needs to be improved (70).

1.3.2 T lymphocytes

1.3.2.1 T cells' role in an immune response

T cells originate from the bone marrow and develop in the thymus into a population of mature naive T cells, each having an antigen specific T cell receptor (TCR). Thereafter, they start to circulate through the peripheral circulation. Once in the secondary lymphoid tissues they can be activated by their specific antigen presented by APCs, such as DCs. For full activation, also a second signal is required, which is delivered by co-stimulatory molecules. Depending on the signals received the T cells develop into

an effector T cell. To be able to respond efficiently to different kinds of infections, there exist several different subsets of effector T cells, such as $CD4^+$ T helper cells (T_H1, T_H2), cytotoxic $CD8^+$ T cells and regulatory T (Treg) cells (63).

1.3.2.2 T helper cells

T helper cells carry the CD4 co-receptor, which is associated with the TCR; together they bind to the MHC class II:peptide complex on APCs. The function of CD4 T cells is principally to help other cells to achieve their effector function, thus called T helper cells. T_H1 cells stimulate eradication of intracellular pathogens by activating macrophages through IFN-γ. They also stimulate inflammation, and the production of opsonizing antibodies (Abs) that enhance the phagocytosis of pathogens. T_H2, which evolved to enhance elimination of parasitic infections, are characterized by the production of IL-4, IL-5 and IL-13, which are potent activators of Ab producing B cells and have a central role in IgE-mediated allergic diseases (63). More subsets are constantly suggested, such as T_H0, producing both IL-4 and IFN-γ (71), and T_H17, which is characterized by the release of IL-17 and IL-22. T_H17 is involved in several autoimmune diseases and in immune responses that are essential for protection against extracellular bacteria and fungi (72).

1.3.2.3 Cytotoxic T cells

Cytotoxic T cells carry the CD8 co-receptor complexed with their TCR. After activation by APCs through an MHC class I:peptide complex they migrate to the periphery to resolve the infection. They kill infected cells that display peptide fragments of cytosolic pathogens, especially viruses, bound to MHC class I molecules (63).

1.3.2.4 Regulatory T cells

Regulatory T cells are needed to regulate potentially dangerous immune responses caused by hazardous self-reactive T cells in the periphery. By expressing IL-10 and tumor growth factor (TGF)-β they prevent autoimmune diseases by maintaining self-tolerance, suppress allergy and enable feto-maternal and oral tolerance. The transcription factor fork-head box protein 3 (FOXP3) is currently considered the most reliable marker of Treg cells (73, 74).

1.3.3 B lymphocytes

1.3.3.1 B cells' role in an immune response

B cells are generated and develop in the bone marrow to immature B cells. Thereafter, they leave and start to circulate through the periphery, ending up in the secondary lymphoid organs. Here, B cells become activated, if a specific antigen binds to their B cell receptor (BCR). Usually they also require additional signals as through the B-cell co-receptor (CD21:CD19:CD81) and by T cell help through CD40-CD40L interactions and cytokines. Activation leads to proliferation and differentiation into plasma B cells, which secrete a diverse repertoire of immunoglobulins (Ig), but with a single antigen specificity. This enables the B-cell response to be highly specific. The produced Abs cover the surface of pathogens to either neutralize a pathogen, or they can coat a pathogen to promote the uptake by phagocytosing cells (63).

1.3.3.2 Epstein-Barr virus infection of B cells

The B-cell receptor, CD21, is also used by the Epstein-Barr virus (EBV) (75), which is a member of the herpesvirus family, consisting of double stranded DNA, an icosahedral capsid and an envelope containing viral glycoproteins (76). EBV specifically binds to CD21 on B cells through the interaction of the virus-encoded glycoprotein (gp)350, which dominates the external viral envelope. Thereafter, EBV becomes endocytosed into vesicles, involving the gp42:gp85:gp25 complex and gp110, and is subsequently released into the cytoplasm (77). More than 90 % of the human population is infected by EBV, which is mainly transmitted by saliva. After primary infection, EBV establishes a latent infection in B cells that persists for life, which is believed to be controlled by humoral immunity and cytotoxic T cells (78). EBV has three transcriptionally different forms of latency: latency I, II and latency III. During latency III all latent genes are expressed, comprising of EBV-specified nuclear antigens (EBNAs) and latent membrane proteins (LMPs), which are important to maintain latency and play possible roles in the immortalization of infected cells (79). However, EBV can be activated, thereby entering the lytic phase, where it can cause a wide spectrum of malignancies, such as Burkitt's lymphoma, follicular dendritic cell tumor and nasopharyngeal carcinoma (80). In this phase, EBV expresses over 80 viral replication associated genes, and interferes with many cellular functions of its host, but these mechanisms are still not fully understood (81).

1.4 ENDOGENOUS NANOPARTICLES – EXOSOMES

Exosomes are nanosized (30-100 nm in size), endogenous nanovesicles, which were first described in 1987 as waste products of maturing reticulocytes (82). Since then they have been found to be released from a wide variety of cell types like DCs (83), B cells (84), T-cells (85), neurons (86) and epithelial cells (87). In addition, they have been isolated from various biological fluids including plasma, bronchoalveolar lavage and human breast milk (88, 89).

1.4.1 Exosome formation and composition

Exosomes originate from the intraluminal vesicles of late endosomal compartments called multivesicular bodies (MVBs), in which the exosomes are formed by inward budding of the limiting endosomal membrane (90, 91). When the MVBs fuse with the outer cell membrane, the exosomes are released extracellularly (84, 92). Proteins and lipids are also sorted at the limiting membrane of endosomes during the formation of the intraluminal vesicles and therefore the released exosomes contain molecules, reflecting their origin from late endosomes (93). The composition of exosomes varies also depending on their cell type from which they are secreted and the activation state of the cell (94). Exosomes from APCs express MHC class I and II molecules, which are loaded with antigen peptides, and co-stimulatory molecules like CD80 and CD86 (90), as well as integrins and sphingolipids (95). In contrast, exosomes from T cells carry CD3 (85) and from B cells CD19 and surface Ig (96). However, they do also contain common components. They are for example enriched in tetraspanins, such as CD9, CD63 and CD81, which are involved in cell adhesion and T-cell co-stimulation, and can be used for the identification of exosomes. Moreover, they express CD55 and CD59, which make them resistant to complement lysis, thereby making them stable *in vivo* (97).

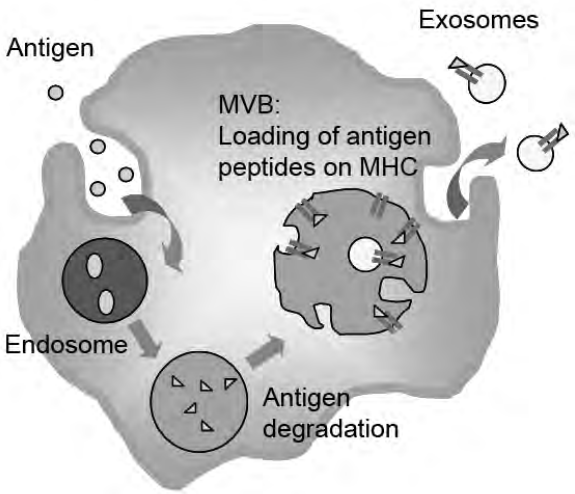

Figure 3. Schematic picture of how exosomes are formed and loaded with MHC class II:antigen complexes in antigen presenting cells. Antigens are taken up by the cell into endosomes, where the antigens are degraded into peptides. The peptides are loaded onto MHC class II in multivesicular bodies (MVB), while also vesicles are formed by inward budding of the limiting membrane, which are released to the cell exterior as exosomes (modified from (98)).

Exosomes are commonly isolated and washed by a series of ultracentrifugation steps. To distinguish them from other small vesicular structures or large protein aggregates, it is sometimes required to do a complete separation by a sucrose gradient. TEM and immune electron microscopy are some of the methods, which can be used to identify and characterize exosomes, but they can also be analyzed by flow cytometry if they are pre-bound to beads. There is no known method to quantify the numbers of exosomes, why one has to rely on protein concentration measurements of isolated exosome fractions.

1.4.2 Exosome function and involvement with infectious agents

From being considered as a waste product, exosomes have been implicated in several potential functions in the immune system. They are considered key players in cell-cell communication since they can transfer antigen/MHC complexes (99-101) and mRNA as well as microRNA (102) between cells. Milk exosomes have been suggested to be involved in tolerance induction (89), which has also been observed for exosomes derived from intestinal epithelial cells, where they are called tolerosomes (103). In contrast, B cell- (104, 105) and DC-derived exosomes (106) have mainly been implicated in T-cell stimulation in an antigen-specific manner.

It has also been shown that exosomes are involved in cell-to-cell spread of infectious agents including prions and mycoplasma (107). Moreover, HIV exploits the exosome pathway in macrophages for the release of virions, leading to HIV particles displaying markers found on exosomes, such as tetraspanins and MHC molecules (108). The EBV virus latent membrane protein 1 (LMP1), which inhibits proliferation of PBMC, has

also been found on exosomes derived from a lymphoblastoid cell line and from malignant epithelial cells (109, 110).

1.4.3 Exosomes in immune therapy

The therapeutic potential of exosomes was discovered in 1998, when Zitvogel *et al.* showed that the injection of tumor peptide loaded DC-derived exosomes could reverse tumor growth in mice (83). Since then two clinical phase I trials have been conducted and one phase II trial is ongoing involving DC-derived exosomes and patients with non-small cell lung cancer or with metastatic melanoma. Both phase I trials suggested that exosome administration is safe and showed some promising results on tumors (111, 112). There are also different types of exosomes that are being investigated in vaccine strategies against various pathogens (107). However, since the mechanism of how exosomes interact with the immune system is unclear, further investigations are needed within this area, which hopefully will enable the optimization of future exosome-based therapies.

2 AIMS OF THE THESIS

The overall aims of this thesis were to study the effects of different sized and shaped nanomaterials in the interaction with primary human dendritic cells, thereby obtaining an insight of what impact these materials have on the immune system and of their potential use in medical applications. In addition, we wanted to determine if endogenous nanoparticles (exosomes) can be used as targeting vehicles. The more specific aims were to:

I: Investigate the potential immunomodulatory effects of spherical gold nanoparticles (7 nm) on MDDCs.

II. To find a new facile method for the preparation of gold nanorods with high ARs suitable for thermal therapy and to evaluate whether various shapes of gold nanoparticles affect MDDC viability and phenotype differently.

III. Compare the potential toxic and immunomodulatory effects of mesoporous silica nano- (270 nm) and microparticles (2.5 µm) on MDDCs.

IV. Determine if exosomes with different origin target specific cell populations, thereby clarifying the mechanism of how exosomes interact with the immune system, which hopefully will enable the optimization of future immune therapies.

3 METHODOLOGY

Methods used for paper I-IV are described in detail in the respective "Materials and methods" sections. Below follows an overview of each method with references to the papers in which they are used:

Atomic absorption spectroscopy (AAS) [I, II]	Determines the concentration of a particular metal element in a sample.
BET method [III]	Calculates the surface areas of solids by physical adsorption of gas molecules.
Confocal laser scanning microscopy (CLSM) [III, IV]	Optical imaging technique enabling scanning through cells.
Enzyme-linked immunosorbent assay (ELISA) [III]	Quantitative technique to measure specific substances such as cytokines in a solution.
Enzyme linked immunospot assay (ELISpot) [I]	Antibody and enzyme based detection of cytokine release on a per cell basis.
Exosome preparation [IV]	Isolation of exosomes by ultracentrifugation.
Flow cytometry (FACSCalibur and FACSAria) [I-IV]	Laser based analysis of cells in flow using fluorochrome conjugated antibodies.
Generation of MDDCs [I-IV]	Peripheral blood monocytes, which in the presence of IL-4 and GM-CSF differentiate into MDDCs.
High resolution transmission electron microscopy (HRTEM) [II]	Allows the imaging of the crystallographic structure of a sample at an atomic scale.
ImageStream® [IV]	Technique that combines flow cytometry with fluorescence microscopy.
Limulus Amebocyte Lysate (LAL) endochrome assay [I-III]	Enzyme based analysis to detect LPS in a solution.
MACS cell separation [I-IV]	Cell separation based on magnetic cell specific microbeads.
Mixed lymphocyte reaction (MLR) [I]	Proliferation assay of T cells using ^{3}[H]-thymidine incorporation and scintillation measurement.

NanoSight® [IV]	Optical microscope detecting nanoparticles.
Nitrogen adsorption-desorption isotherm measurements [III]	Method to obtain pore size distribution curves.
NLDFT method [III]	Measures the pore size distribution of a solid material.
PI and Annexin-V assay [II, III]	Viability test measuring early apoptosis and late apoptosis/necrosis.
Preparation of spherical gold nanoparticles [I, II]	Produced with sodium borohydride as a reducing agent.
Refractometer [IV]	Measures the density of e.g. a liquid.
Scanning electron microscopy (SEMi) [III]	Images a sample surface by scanning it with a high-energy beam of electrons.
Standard gradient centrifugation with Ficoll Paque [I-IV]	Density separation of mononuclear cells from blood.
Statistical analyses [III, IV]	Wilcoxon's signed-rank test for dependent samples and Student's t-test.
Sucrose gradient fractionation [IV]	Continuous density gradient separation by ultracentrifugation of exosomes.
Synthesis of low AR gold nanorods [II]	Produced with sodium borohydride as a reducing agent and silver nitrate.
Synthesis of mesoporous silica particles [III]	Produced with N-lauroyl-amino acid anionic surfactants and alkoxysilanes.
Thermogravimetric analysis (TGA) [III]	Determine changes in weight in relation to change in temperature.
Transmission electron microscopy (TEM) [I-IV]	Microscopy technique using a beam of electrons instead of light.
Trypan blue exclusion [I-IV]	Dye exclusion test to verify cell viability.
Western blot analysis [IV]	Gel electrophoretic separation of proteins and transfer to membranes for antibody detection.
X-ray diffraction (XRD) [II, III]	Tool to investigate a structure on the atomic scale.

4 RESULTS AND DISCUSSION

4.1 THE IMPORTANCE OF AN ENDOTOXIN-FREE ENVIRONMENT DURING THE PRODUCTION OF NANOPARTICLES USED IN MEDICAL APPLICATIONS (PAPER I)

Gold nanoparticles comprise several interesting properties, which make them attractive for various bio-related applications, including biosensing, drug delivery and thermal therapy (113). In this paper, we decided to explore if gold nanoparticles are as bio-compatible as gold in bulk scale and thus suitable as inert carriers for therapeutic applications, or if they have possible immune modulating effects, thereby affecting DC maturation.

Conventionally laboratory (Royal Institute of Technology) produced spherical gold nanoparticles (7 nm Ø), stabilized with human serum albumin (HSA), were added to human immature MDDCs, derived from healthy blood donors, at a concentration of 0.5, 5 and 50 µg Au/ml. Flow cytometric analysis of MDDCs co-cultured with these nanoparticles for 24 h resulted in a dose-dependent increased expression of CD80, CD83, as well as CD86 and MHC class II. When adding gold nanoparticles to PBMC it was also found that the nanoparticles induced the expression of IL-12, known to be produced by mDCs, but not of INF-α, which is produced by pDCs.

However, the strong maturation of MDDCs led us to suspect that the gold nanoparticles were contaminated with endotoxins, which are found in the outer membrane of gram-negative bacteria, and are members of a class of phospholipids called lipopolysaccharides (LPS) (114). It is also known that LPS induces IL-12 production by mDCs, but not by pDCs since the latter cells lack the Toll-like receptor 4 (TLR4) to which LPS binds (115). Therefore, LPS analyses were performed with the LAL endochrome assay. The results revealed concentrations of 12-51 ng LPS/ml in the nanoparticle batches, corresponding to final concentrations of 0.5 -11 ng LPS/ml in the cell cultures, which are sufficient to stimulate MDDCs (116, 117). To confirm that the up-regulation of maturation-related molecules was only induced by the LPS and not by the nanoparticles as well, we performed experiments where we added the LPS-blocking agent polymyxin B. Herby, the dose-dependent maturation of MDDCs was abolished, suggesting that LPS was responsible for the maturation of the DCs, rather than the nanopaticles themselves.

Efficient removal of LPS is difficult to achieve due to its extreme heterogeneity, both in terms of composition and structure, and due to its high stability (114). Thus, the safest approach is to keep the entire production process contaminant-free from the beginning. Therefore, we optimized the synthesis procedures by synthesizing nanoparticles in fume hoods, replacing distilled water with ultra-pure water (<0.25 ng LPS/ml) and the consistent use of gloves. In addition, both the surroundings and the glassware were cleaned with PyroCLEAN™ before being used in the synthesis experiments. In this way, LPS concentrations were lowered 16 - 425 times to 0.12 - 0.75 ng/ml, resulting in a final endotoxin concentration of 3 - 48 pg/ml for cells treated with 50 μg gold nanoparticles/ml. These levels are within the critical limits mentioned before (116, 117), and are also low enough to be administered into humans according to the Food and Drug Administration (FDA) guidelines (www.fda.gov). MDDCs that were exposed to gold nanoparticles with this low endotoxin content showed only a minor up-

regulation of surface molecules. Corresponding results were seen for the expression of IL-12 and IFN-α, where a few or no cytokine producing cells could be detected with the ELISpot assay used.

Since a major function of DCs is to deliver signals for T-cell stimulation, the functionality of MDDCs co-cultured with gold nanoparticles was assessed by investigating the impact of nanoparticles on lymphocyte activation by MDDCs. In an autologue setting, MDDCs pre-incubated with low-LPS nanoparticles did not induce a proliferative response in CD14-depleted PBMCs, while high-LPS nanoparticles did, suggesting that high-LPS nanoparticles can trigger an unwanted immune response. Furthermore, in an allogeneic setting, high-LPS nanoparticles potentiated the allogeneic reaction, whereas low-LPS nanoparticles did not. These results suggest that low-LPS nanoparticles are not interfering with MDDC-T cell interactions.

To conclude, this paper, which was highlighted in *Nanowerk Spotlight* and in *Analytical Chemistry*, emphasizes the importance of maintaining high purity during the production process of nanoparticles to be able to assess their true biological affects; which also underlines how important it is to combine the knowledge within materials science with that of biomedicine when generating new advanced materials. Our data also suggest that the exposure of contaminated nanoparticles during the production and handling might be hazardous, and hence precautions need to be taken to avoid inhalation of particles. Moreover, we show that gold nanoparticles produced in a clean environment have a low impact on maturation and functionality of DCs, thus having a potential to be used as inert carriers for biomedical applications.

4.2 SYNTHESIS OF HIGH ASPECT RATIO GOLD NANORODS AND THEIR EFFECTS ON HUMAN ANTIGEN PRESENTING DENDRITIC CELLS (PAPER II)

The previous study (Paper I) showed that spherical gold nanoparticles had no major impact on MDDCs. Next we wanted to explore whether the shape of gold nanomaterials has any importance concerning biocompatibility and immune modulating characteristics. Therefore, we set out to compare the effects of spherical gold nanoparticles with high AR gold nanorods on MDDCs.

To receive high AR gold nanorods, we here developed a new facile method without the use of pre-made gold nanoparticles seeds, thereby avoiding labor and time intensive multi-step protocols. The method is based on that a gold precursor is reduced into metallic gold NRs in two stages, where Au(III) is reduced into Au(I) by ascorbic acid and then to Au(0) by sodium borohydride, in the presence of CTAB. The effect of different acids, such as HNO_3, HCl, H_2SO_4 and H_3PO_4, on the formation of gold nanorods was examined. The use of all acids with the concentration of 1 mM resulted in the formation of gold nanorods, but those synthesized with the addition of HNO_3 were found to have the highest AR with an average of ~21. These results suggest that nitrate ions play a key role in the growth of long nanorods with high ARs.

Furthermore, we also studied the effect of different concentrations of HNO_3 (0.2-72 mM) on the morphology of gold nanorods. The length and AR of gold nanorods were found to be greatly influenced by the HNO_3 concentration, where the average length

varied from ~21 to 447 nm and the AR changed from ~1 to 26. The highest AR was achieved using 3 mM HNO_3, while a further increase of HNO_3 concentration resulted in the formation of shorter nanorods. High resolution transmission electron microscopy (HRTEM) revealed that at a low concentration of nitric acid intermediate formed twinned nuclei are formed, which are responsible for the growth of nanorods, whereas at a high concentration of nitric acid intermediate formed single crystal nuclei are formed resulting in the isotropic growth of gold nanoparticles. We propose that the mechanism of growth of high AR gold nanorods, in this method, can be both attributed to the effect on elongation of surfactant micelles by nitrate ions and the process of Ostwald ripening (118).

To evaluate the biocompatibility of high AR gold nanorods (AR ~21), the viability and immune modulatory effects of these rods were tested on human primary MDDCs and compared with the effects caused by gold nanorods with low AR produced according to Jana *et al*. (AR ~4.5) (119) and with 7 nm spherical gold NPs from paper I. After co-culture of MDDCs with particles (0.5, 5, or 50 µg/mL) for 24 h cell death by necrosis (uncontrolled cell death) and apoptosis (programmed cell death) was measured by propidium iodide (PI) and Annexin V-fluorescein staining, respectively. Both nanorods with a high AR and spherical gold nanoparticles did not seem to have an impact on cell viability. In contrast, nanorods with a low AR at the highest concentration induced a considerable decrease in viability from 84 ± 6.6 % (n = 3; medium control) to 57 ± 18 % (n = 3), involving mainly late apoptosis and necrosis. It was seen that the different effects caused by the two rod types were not due to different morphologies, but could be attributed to that toxic CTAB could not be as efficiently washed away from the surface of low AR nanorods as from high AR rods.

The biocompatibility of the high AR gold nanorods were further evaluated in terms of immune modulatory effects on MDDCs. Results showed that they, similar to spherical gold nanoparticles, did not have any major impact on the expression of CD40, CD80, CD83 or CD86 on MDDCs. Both types of particles were also efficiently internalized by cells, ending up in vesicular structures, probably endolysosomes, as detected by TEM.

In summary, we here developed a new simple method for the preparation of gold nanorods with high ARs. Neither these high AR nanorods nor spherical gold nanoparticles had any major impact on viability or on immune regulatory markers on MDDCs, thus different morphologies of gold nanoparticles did not seem to cause any different cellular responses. Low AR gold NRs induced considerable cell death, which was rather due to surface bound CTAB. These results support the further development of high AR gold nanorods for medical applications such as thermal therapy.

Our results in Paper I and Paper II have now been further strengthened by Brandenberger *et al*., who recently demonstrated by using an epithelial-airway model, consisting of alveolar epithelial-like cells, human monocyte derived macrophages and DCs, that spherical gold nanoparticles in the size of 15 nm deposited at the air-liquid interface had no impact on the triple cell co-culture. Here, they were both looking at the mRNA induction of pro-inflammatory and oxidative stress markers, as well as protein induction of pro- and anti-inflammatory cytokines (120). However, another study highlights that smaller spherical gold particles (1.4 nm) trigger necrosis of carcinoma cells (HeLa), which they explain by induced oxidative stress and mitochondrial damage (121). The size dependent effect is also reported by Yen *et al*., where 2-4 nm sized

spherical gold nanoparticles induced apoptosis and the up-regulation of pro-inflammatory genes, while larger particles showed no toxic effects on a murine macrophage line (122). Similar results have been achieved when gold nanoparticles have been intravenously injected into mice, where smaller particles dispersed more quickly and were more toxic compared to larger ones (123, 124). Thus, gold nanoparticles above the size of 4 nm seem to be the most promising candidates for medical applications.

4.3 MESOPOROUS SILICA PARTICLES INDUCE SIZE DEPENDENT EFFECTS ON HUMAN DENDRITIC CELLS (PAPER III)

Mesoporous silica particles hold great promise for drug delivery applications, due to their internal pore structures and high surface areas. Our primary goal here was to determine the effect of mesoporous particles of different sizes and structures on human MDDCs.

We chose to compare the effects of mesoporous silica nano- (272 nm) and microparticles (2.5 µm), shown to posses 3-dimensional (3D) cubic cylindrical and cubic cage type pore geometries, respectively (125). Pore sizes were 3.9 nm for AMS-6 and 2.8 nm for AMS-8, and both materials had surface areas above 500 m^2/g.

AMS-6 and AMS-8 (0.5, 5 or 50 µg/mL) were co-cultured with immature MDDCs for either 24 or 48 h. A significant decrease in viability, measured by PI and Annexin-V staining, from 89 ± 1.1 % (n = 4) in the medium control to 81 ± 0.8 % (n = 4) and to 69 ± 1.3 % (n = 4) was detected for the MDDCs treated with the highest concentration of AMS-6 and AMS-8 for 24 h, respectively. A significant difference in viability was also observed between the MDDC samples co-cultured with either AMS-6 or AMS-8, 50 µg/mL. After 48 h only the highest concentration of AMS-8 induced a significant decrease in viability. To note, the silica particles themselves did also absorb PI, which needed to be taken into consideration while analyzing the toxicity results. Therefore, we also employed trypan blue exclusion as a complimentary method to study cell viability, which results supported our previous findings.

AMS-8 also had a higher immune modulatory impact on MDDCs, compared to AMS-6. At 5 and 50 µg per mL, AMS-8 induced a significant increase of $CD86^+$ cells and a corresponding significant decrease of $CD80^+$ and $CD40^+$ cells compared to MDDCs cultured in medium alone. For AMS-6, a significant effect was only seen for CD40, which expression was reduced at the highest concentration of nanoparticles. Both types of particles induced a slight decrease of MHC class I, but only at the highest concentration used. There was no significant effect on the DC maturation marker CD83, nor on MHC class II. Looking at the cytokine expression, both kinds of mesoporous silica materials induced the production of IL-12p70 by MDDCs, which was most pronounced for AMS-8 at 50 µg/mL. However, they did not cause any IL-10 production.

By using FITC-conjugated silica particles, it was seen that both types of mesoporous particles were internalized by MDDCs within 10-60 min at 37ºC. This was not observed at 4ºC, suggesting that an active mechanism, such as endocytosis, is involved in the cellular uptake seen at 37 ºC. The uptake of particles was linked to the increased percentage of CD86 seen before. In addition, more $CD86^+$ cells containing

FITC-labeled silica particles were observed for AMS-8 than for AMS-6. TEM images supported our data, where an uptake was seen for both unconjugated AMS-6 and AMS-8. They also showed that AMS-6 was encapsulated into vesicular compartments, whilst around the larger spheres no membrane like structures could be detected, suggesting a cytosolic localization.

In conclusion, these data show that both size and concentration of mesoporous silica particles correlate with effect, where the smaller silica particles and lower concentrations in general affected MDDCs to a minor degree compared to the larger particles and higher concentrations, both in terms of viability, uptake and immune regulatory markers. Although the cells remain in a relative immature state, an induction of IL-12p70 and CD86 and a decreased expression of CD80 and CD40 was detected. We also conclude that the size of particles has an impact on cellular localization, where the larger particles seemed to be able to escape from the endolysomal entrapment, which has also been previously reported for cytochrome c coated mesoporous silica particles (126). Our data suggest different applications for the tested mesoporous silica material due to the size, where AMS-8 has promising features to serve as an adjuvant/regulant, with possible T cell modulatory properties, whereas AMS-6 is rather a potential candidate for a more neutral drug delivery system.

Following our study the number of toxicological papers involving mesoporous silica particles has increased rapidly. One study by Huang *et al*. demonstrates, by comparing different ARs, that the shape of mesoporous silica particles also has an impact on the cellular functions, seen on human melanoma (A375) cells (127). There are also recent studies, which suggest, as Paper III, size dependent effects of silica particles, but where nanosized materials are more readily taken up by cancer cells (HeLa cells) (128), and do also cause higher cytotoxicity in endothelial cells (EAHY926) compared to larger particles which induce a very low toxic response (129). The latter results seem to partly contradict our findings, but can be explained by the use of different cells, type and sizes of mesoporous silica particles, thereby demonstrating how demanding this research field is, due to the immense variations within this class of materials.

The detected toxic response seen for some of the mesoporus silica particles can be prevented by ammonium functionalization, which was demonstrated by Tao *et al*. (130). Furthermore, it has now been shown that porous silica particles are degraded into renally cleared components in mice with no evidence of toxicity (131), which are promising results for the future use of these particles. However, an *in vivo* study highlight that the toxicity is also dependent on the administration route, where subcutaneous injection in mice caused no toxicity over a three month period, whereas intra-peritoneal and intra-venous injections resulted in euthanasia or even death (132).

There are still few studies on the effects of mesoporous silica particles on cells in the immune system. Thakur *et al*. has demonstrated that particles in the size of 1 µm have no toxic effect on primary alveolar macrophages and are thought to bind to the scavenger receptor MARCO (macrophage receptor with collagenous structure) (133). Another study done on primary human monocyte derived macrophages shows that mesoporous silica particles (350 nm) do not have an impact on cell viability or function of macrophages, and are internalized through an active process of endocytosis (134).

To date, several studies prove that mesoporous particles have a potential to be used as drug delivery systems, although there are still too few and some contradicting studies, as mentioned above, to be able to fully understand their biocompatibility. However, the number of papers is involving mesoporous silica particles are rapidly increasing, thus; hopefully in a near future we will have a full picture of the potential use of these particles in medicine.

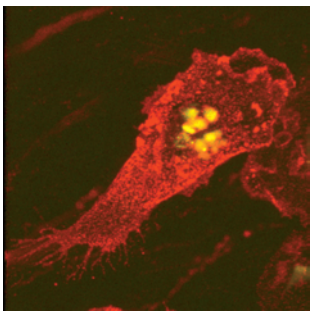

Figure 4. Confocal image of an HLA-DR/Alexa Fluor 546 labelled MDDC with engulfed FITC-conjugated microsized mesoporous silica particles (AMS-8), shown at a maximum projection displayed in 2D (400 × magnification) (Paper III).

4.4 EXOSOMES RELEASED BY EPSTEIN-BARR VIRUS TRANSFORMED B CELLS SELECTIVELY TARGET B CELLS THROUGH CD21-GP350 INTERACTIONS (PAPER IV)

The therapeutic potential of naturally occurring endogenous nanoparticles, exosomes, is currently being evaluated in clinical studies for use in cancer immunotherapy (135) and in vaccine strategies against various pathogens (107). However, there are still fundamental mechanistic questions that remain to be elucidated to understand the role of exosomes *in vivo* and the full potential of exosomes in clinical treatment. Here, we explored the nature of exosome-cell communication by investigating whether exosomes of various human cellular origins target specific immune cells in PBMC.

We chose to study exosomes isolated from human MDDCs, an Epstein-Barr virus (EBV) transformed lymphoblastoid B cell line (LCL1), and from human breast milk, which were stained with a green fluorescent membrane dye, PKH67. The different exosomes were then co-incubated with PBMC for 1 or 4 h and analyzed for association with different cell populations.

Flow cytometry showed that after 1 h ~46 % of the monocytes (HLA-DR$^+$CD14$^+$) were associated with DC exosomes, whereas only ~17 % of B cells (HLA-DR$^+$CD14$^-$ CD19$^+$) were positive for DC exosomes. Similar tendencies were seen for milk exosomes. In contrast, LCL1 exosomes showed a reverse pattern of association with a strong preference for B cells. 63 % of the B cells were positive for LCL1 exosomes whereas on average only 17 % of the monocytes had associated with these exosomes at 1 h. Even after 4 h, less than 8 % of either CD4$^+$ or CD8$^+$ T cells showed associations with any of the exosomes, which could be explained by that we used *ex vivo* PBMC, likely to contain mainly non-stimulated T cells with a low lymphocyte function-associated antigen (LFA)-1 expression (136). Confocal laser scanning microscopy

(CLSM) confirmed the association of exosomes with cells and showed that they mainly localized on the surface of B cells, whereas they were often internalized by monocytes. The association and localization of exosomes was further verified by a novel quantitative technique which combines flow cytometry with simultaneous imaging by fluorescence microscopy (ImageStream).

The association of most exosomes with cells decreased when incubation was performed at 4ºC instead of 37ºC, indicative of an active, probably phagocytic, uptake by cells. However, this was not seen for the interaction between LCL1 exosomes and B cells, suggesting the involvement of adhesion molecules or surface receptors, such as the human complement receptor 2 (CD21) or integrins. We found out that CD21 was involved, by using anti-CD21 Abs, which efficiently blocked the interaction between LCL1 exosomes and peripheral blood B cells. By comparing the LCL1 exosomes with exosomes from an EBV negative Burkitt's lymphoma B cell line, BJAB (137), we found out that BJAB exosomes bound to a 10 fold lower extent to B cells compared to LCL1 exosomes, suggesting the involvement of EBV-derived or -induced proteins in the binding to CD21. Known ligands to CD21 include among others the low affinity receptor for IgE (CD23) and the EBV envelope glycoprotein gp350, which is a lytic protein critical for viral attachment to B cells (75). These ligands were blocked by either anti-gp350 (138) or anti-CD23 Abs (139). The binding of LCL1 exosomes to B cells was substantially reduced when blocking gp350, but no reduction in exosome binding was seen when CD23 was blocked. This observation suggests that gp350 mediates the exosome binding to CD21 on B cells. The presence of gp350 in LCL1 cells was confirmed by flow cytometry analysis, and for exosomes in sucrose gradient by immunoblotting, where gp350 co-localized with HLA-DR and CD81. These markers also partly co-localized with the EBV-encoded latent membrane protein 1 (LMP1), which has been previously found on exosomes (110).

Since we found gp350 on both LCL1 cells and exosomes, some of the LCL1 cells could be in the lytic stage of the EBV life cycle, thus producing free virus particles into the EBV transformed B-cell cultures. To exclude the possibility that virions contaminated our exosome preparations and thereby give a false positive signal for exosomes, we analyzed the exosome preparations from LCL1 cells alone or in combination with primary B cells by TEM and immune EM. No particles in the size of EBV (200 nm), or any naked high density virus capsids were detected in three different exosome preparations or on the B-cell surface. Only vesicles of approximately 100 nm were seen, which were positive for CD63 and HLA-DR, indicative of exosomes. These results are also in line with our findings with Western blot, where gp350 co-localized with HLA-DR and CD81. The size distribution was further investigated by an optical microscope (NanoSight), which quantitatively confirmed the lower size range within the LCL1- and BJAB-exosome preparations, compared to EBV particles. Thus, it is likely that the LCL1 cells do not support the morphogenesis of complete EBV particles and their subsequent release, or that only very few virions are produced and thus hard to detect but also unlikely to affect our results. Hence, we conclude that exosomes from LCL1 cells contain gp350 and are able to target B cells.

In this paper we show that exosomes preferably associate with monocytes. However, the cell preference of exosomes may be changed towards B cells through transformation, which we demonstrated here by comparing exosomes from an EBV negative with an EBV positive B-cell line (LCL1). Exosomes derived from EBV transformed B cells might have a role in long-term immune protection against EBV infection, by preventing the spread of virions by exosomal blocking of the EBV entry

receptor on B cells (CD21). Alternatively, the induction of gp350-exosome production could be used by the virus to increase its immune modulatory potential. Our findings do also suggest how exosomes can be engineered in the laboratory, e.g. by inducing the expression of gp350, to redirect their cellular targeting to B cells. This finding may potentiate the therapeutic usefulness of exosomes in the treatment of cancer and inflammatory diseases, since it was recently demonstrated that exosomes require the support of activated B cells for generating antigen specific T-cell responses *in vivo* (140).

The hypothesis that the induction of gp350-exosome production could help EBV to increase its immune modulatory potential is in line with a recent study, which demonstrates the existence of galectin-9 carrying exosomes in plasma from patients with EBV-associated nasopharyngeal carcinoma (141). Galectin-9 is known to induce apoptosis in mature Th1 lymphocytes and could thereby allow tumor cells to evade the immune system. Our suggestion to further explore the applications of exosomes and gp350 in immune therapy could among others be realized by using nanotechnology. Hereby, it has recently been possible to develop artificial exosomes by coating liposomes with MHC class I/peptide complexes and a range of ligands for adhesion, activation and survival T-cell receptors. These have been shown to be able to both activate and expand functional antigen specific T cells (142). Thus, translational research, combining nanotechnology and the knowledge of the role of exosomes in different immune responses, has the potential to generate new tools for vaccination and immune therapy.

5 CONCLUSIONS

Paper I. In this study we demonstrated that conventionally produced gold nanoparticles had a maturing effect on human MDDCs, which we found to be a result of LPS contamination. By modification of the production process, clean particles were obtained, which had practically no effect on phenotype or cytokine production of MDDCs. These findings emphasize the importance of retaining high purity during the production of nanoparticles, since possible contaminants may interfere with the assessment of nanoparticles' biological effects and result in harmful particles, which may also be hazardous to individuals producing and handling these materials.

Paper II. To be able to investigate whether various shapes of gold nanoparticles affect MDDC viability and phenotype differently, we here developed a novel method for preparing gold nanorods with high AR based on a self-seeded surfactant-mediated protocol. The biocompatibility of these high AR gold nanorods was compared with spherical gold nanoparticles used in Paper I on MDDCs. Both types of particles had no or minor effects on MDDC's viability and phenotype, thus shape did not seem to affect the biocompatibility of gold. These findings support the potential use of high AR gold nanorods in medical applications, such as thermal therapy.

Paper III. In this study we evaluated whether the size of the particle is important for its biocompatibility, by comparing the effects of mesoporous silica nano- (270 nm) and microparticles (2.5 µm) on MDDCs. Size- and concentration-dependent effects were seen where the smaller particles and lower concentrations affected MDDCs to a minor degree compared to the larger particles and higher concentrations, both in terms of viability, uptake, and immune regulatory markers. These results demonstrate the importance of size in the interaction with MDDCs and support the further development of mesoporous silica particles as drug- and vaccine-delivery systems.

Paper IV. Here we investigated whether exosomes of different origins can selectively target different immune cells. Results revealed that exosomes derived from human MDDCs and breast milk preferably associated with monocytes, whereas exosomes from an Epstein-Barr virus (EBV) transformed B cell line (LCL1) selectively targeted B cells. The interaction between LCL1-derived exosomes and peripheral blood B cells was dependent on CD21 on B cells and the EBV glycoprotein gp350. The targeting of EBV-B cell exosomes might have a role in controlling further viral spread during acute infection by blocking CD21. Our novel findings also suggest that exosome-based vaccines can be engineered for specific cellular targeting.

In summary, the work included in this thesis has contributed to the understanding of the importance to use clean nanoparticles in the evaluation of their biocompatibility. By studying gold and mesoporous nanomaterials, we have demonstrated how particles of various materials, shapes and sizes affect and interact with human MDDCs differently. The gained information also supports the further development of theses materials, both within the area of thermal therapy and in the field of drug and vaccine delivery. Moreover, this thesis suggests the use of endogenous nanoparticles, exosomes, derived form EBV transformed B cells, as a targeting delivery system within immune therapy.

6 FUTURE PERSPECTIVES

This thesis gives an insight into what to consider while testing nanomaterials for future medical applications with focus on how various nanoparticles affect immune cells, especially DCs. Still, many questions remain and new ones have arisen during this work.

The finding that conventionally produced nanoparticles contained LPS (Paper I) highlight the importance to always carefully characterize the produced material before starting biocompatibility experiments. The results also emphasize the importance of close collaboration between scientists within the field of materials and biomedicine when generating new advanced materials, to prevent mistakes which with the right knowledge can easily be avoided.

We have learnt that both gold and mesoporous particles hold great promises for future medical applications (Paper I-III). However, a general problem in nanomedicine is that most studies looking at the impact of nanoparticles, are performed with different methods, cells and with various concentrations. There exists also a huge variability of how similar particles are produced. Hereby, it becomes sometimes hard to compare our results with others. One way to solve this problem would be to use a reference material as a golden standard and agree on if the concentration should be measured as µg/mL, surface area/mL and/or as parts per million (ppm).

Moreover, the effects studied by us and mostly by others are on one single cell type, which in addition are very often cell lines. The latter are very helpful in large screening studies to project potential toxic or immunogenic effects. However, the increased use of primary cells is important, since they show true individual variations from donor to donor, as also seen in all our experiments (Paper I-IV). To approach an even more *in vivo* like situation and to understand what effect on one cell type means for other surrounding cells, the development of multi cellular systems is needed. If these systems become sufficient sophisticated they may also in the future replace some of the *in vivo* experiments in animal models, which are today often needed to verify *in vitro* experiments (143). Therefore, we have now developed a novel system to address the question whether the mesoporous silica induced up-regulation of CD86 on MDDCs has any importance (Paper III). Is it significant enough to e.g. stimulate naive T cells into effector T cells? This method is based on a DC/naive T-cell co-culture system, where MDDCs are pre-cultured with mesoporous silica particles and thereafter added to naive T cells. Preliminary results show that the naive T cells are affected by the MDDCs, especially by those which have been pre-treated with AMS-8, as seen by the increased number of IFN-α, IL-4 and IL-13 producing cells, using ELISpot as a read out system (Figure 5). Hence, even if AMS-8 only induces a minor up-regulation of CD86 (Paper III), it is still enough to activate T cells. However, how these effects are achieved, e.g. through specific receptors or cellular up-take routes (67), still needs to be elucidated, as well as why there is a difference in cellular localization within MDDCs depending on size. The understanding of these mechanisms will also enable the optimization of nanoparticle design for future biomedical applications.

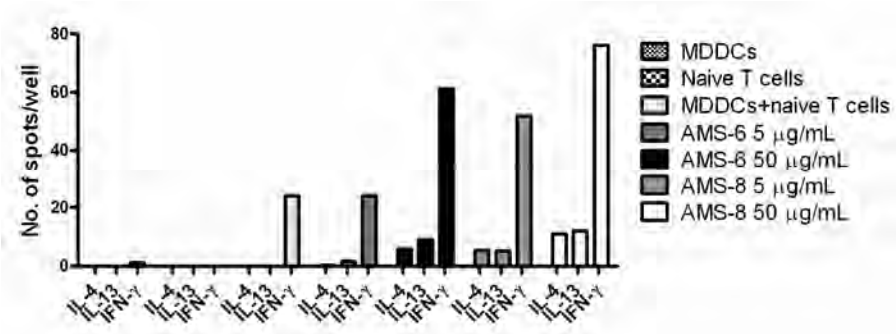

Figure 5. ELISpot analysis of the expression of IL-4, IL-13 and IFN-γ by naive T cells after co-culture with AMS-6/AMS-8 treated MDDCs in an autologous setting. MDDCs and naive T cells, as separate and in combination, are used as negative controls. Results shown are one representative experiment out of two using cells from two different healthy blood donors.

The minor immunogenic effect seen with silica particles demonstrates the importance to be careful while producing and handling these kinds of materials, but they also further underline the potential use of mesoporous silica microparticles as an adjuvant, which we suggested in Paper IV. Thus, we have now produced another type of mesoporous silica particle, SBA-15, which has similar size, but larger pores, and seem to have immunogenic effects on MDDCs comparable to AMS-8. Thereby, we have been able to load these with the model-antigen ovaalbumin (OVA). We have now also functionalized SBA-15 with n-propyl cyanide, which, as seen in preliminary results, seem to improve the biocompatibility of these materials. In a continuation we would now like to explore the use of SBA-15 in vaccine delivery systems, starting with *ex vivo* and thereafter proceed with *in vivo* animal studies.

The application of gold nanoparticles, especially of the high AR gold nanorods, in thermal therapy still needs to be confirmed by NIR radiation of cells containing nanorods, followed by *in vivo* studies. Here it may also be necessary to cover the gold rods with PEG to ensure a long enough circulation time in blood.

For both gold nanorods and mesoporous silica particles there are still questions about their fate in the body. Thus, biodegradability studies and long-term *in vivo* studies in animal models are needed to see if the materials are easily secreted from the body, or if they accumulate in different organs and tissues. Noninvasive *in vivo* imaging, where whole living animals are studied, is a method which could be applicable for this purpose (144).

The usefulness of endogenous nanoparticles, exosomes, in therapy still needs further investigations. Our results show that exosomes from EBV transformed B cells selectively target B cells (Paper IV). If confirmed *in vivo*, these findings could have great implications for cancer vaccines. However, this approach could have limitations in a clinical setting, due to the presence of LMP1. LMP1 is known to inhibit T-cell proliferation (110) and has been claimed to have oncogenic properties (145). Still, several studies have shown the potential of exosomes, derived from EBV transformed B cells, to activate T cells (84, 104). In addition, the relative expression of LMP1 and

gp350 on exosomes may differ during the latent and lytic phase of the EBV life cycle, and therefore the exosome cultures may be in the future fine-tuned for optimal clinical applications. An alternative strategy would be to modify DC exosomes, which already have been implicated in clinical studies (135) to express gp350.

Our novel finding of targeted B-cell inter-communication via exosomes might also reflect the situation *in vivo* in EBV infected individuals. Currently, we are investigating whether this is a mechanism for long-term immune protection against EBV infection, by preventing the spread of virions by exosomal blocking of the EBV entry receptor on B cells (CD21). Alternatively, the induction of exosome production could be used by the virus to increase its immune modulatory potential. To understand which role these exosomes have in EBV infection and spreading, as well as in regulation of other immune responses, further investigations are needed.

Within the Seventh-Framework Programme of the European Commission (EC-FP-7 - NANOMMUNE, www.nanommune.com), investigations have been initiated to further understand how nanoparticles affect the immune system, thereby hopefully answering some of the questions mentioned above. This study has also a unique focus on exosomes, where one wants to understand whether exosomes, both in terms of production and phenotype, are affected by engineered nanoparticles.

7 POPULÄRVETENSKAPLIG SAMMANFATTNING

Nanoteknik är ett snabbt växande fält, vars betydelse kommer att vara stor inom många områden i framtiden. Några exempel på dess betydelse är utvecklingen av starkare material, effektivare elektronik och mer träffsäkra läkemedel som kan skilja t.ex. cancerceller från friska celler. Upp till 800 nanoteknikprodukter har redan nått marknaden, såsom fläckfria kläder, slitstarka tennisbollar och solkrämer som inte syns på huden. I naturen har däremot nanotekniken funnits mycket länge. Bl.a. producerar våra celler i kroppen små blåsor (30-100 nm), s.k. exosomer, som tros vara viktiga för kommunikationen mellan olika celler. En nanometer är en miljondels millimeter. Själva ordet nano kommer från grekiskans ord för dvärg och hit hör partiklar och strukturer som är mellan 1 och 100 nanometer (nm) stora. Det som gör nanotekniken intressant är att material beter sig annorlunda vid så små storlekar, eftersom deras magnetiska, kemiska och elektroniska egenskaper helt kan förändras. Däremot vet man inte vad dessa nya egenskaper har för betydelse för miljön och människans hälsa. Därför satsas nu allt mer resurser på detta forskningsområde.

Ett sätt att undersöka hur nanopartiklar påverkar oss är att studera hur celler från människans blod beter sig när de kommer i kontakt med nanopartiklar. Det handlar t.ex. om vita blodkroppar som monocyter, B-celler, T-celler och dendritiska celler (DC). DC har en central roll när det gäller att sätta igång en immunreaktion när kroppen attackeras av främmande organismer eller substanser. De är på ständig jakt efter inkräktare och om de hittar något främmande kan de bli aktiverade, vilket innebär att de producerar signalmolekyler på sin yta och utsöndrar signaler i form av lösliga ämnen. Dessa signalmolekyler hjälper DC att kommunicera med T-celler och B-celler som i sin tur för ett immunsvar vidare mot det främmande ämnet.

Målet med den här avhandlingen har varit att undersöka hur nanopartiklar i olika material, storlekar och former påverkar DC, genom att bl.a. mäta hur mycket signalmolekyler de producerar och titta på cellernas överlevnad. Därmed får man en indikation på hur dessa nanopartiklar påverkar vårt immunförsvar, men man får också en idé om vilka material som är lovande för användning inom medicin. Vi har dessutom velat undersöka om exosomer, producerade av olika celltyper, styrs till specifika celler i kroppen och i så fall om de skulle kunna användas inom medicinen som biologiska målinriktade transportörer av läkemedel.

I en första studie tittade vi på hur sfäriska guldpartiklar (7 nm i diameter) påverkar DC. Guldnanopartiklar är ett spännande material p.g.a. deras stabilitet och möjlighet att kunna fästa andra molekyler på sin yta. De kan också lätt bli upphettade av infrarött ljus, som gör dem intressanta för användning inom bl.a. cancerbehandling (värmeterapi). Vi fann först att guldnanopartiklarna aktiverade DC, vilket dock visade sig vara p.g.a. att partiklarna var förorenade med bakteriella produkter. Genom att förändra produktionsprocessen av partiklarna lyckades vi få renare partiklar, som inte hade någon påverkan på DC:s signalmolekyler. Att DC förblev opåverkade innebär att guldnanopartiklarna verkar vara lovande för framtida medicinska tillämpningar. Dessa upptäckter visar också hur viktigt det är att hålla en hög renhet vid produktionen av

nanopartiklar och att noggrant fastställa deras egenskaper innan de ingår i cell- eller djurstudier, för att därigenom undvika missvisande resultat.

I nästa studie ville vi undersöka om en annan form på guldnanopartiklarna har en större inverkan på DC jämfört med sfäriska guldpartiklar. Därför utvecklade vi en enkel metod för att producera stavformade guldpartiklar som hade ett högt längd/bredd-förhållande med en längd på 410 nm och en bredd på 18 nm. Att de har ett högt längd/bredd-förhållande gör dem fördelaktiga för användning inom värmeterapi eftersom de är lättare att hetta upp med infrarött ljus jämfört med sfäriska guldpartiklar. Stavarna produceras bl.a. med hjälp av salpetersyra, vars koncentration visade sig vara avgörande för att få ett högt längd/bredd-förhållande. Cellstudier visade att dessa stavformade guldpartiklar liksom sfäriska guldpartiklar praktiskt taget inte hade någon påverkan på DC, vare sig man tittar på DC:s överlevnad eller signalmolekyler, trots att man såg att de togs upp effektivt av cellerna. Dessa resultat visar att formen i detta fall inte har någon betydelse för huruvida DC påverkas och att de nyutvecklade stavformade guldpartiklarna verkare lovande för användning inom värmeterapi.

I vår tredje studie valde vi att jämföra porösa kiselpartiklar i olika storlekar (270 nm respektive 2.5 µm). Dessa kiselpartiklar är fyllda med porer, på samma sätt som en bikupa. Porerna skulle man kunna fylla med läkemedel för transport till önskad plats inom kroppen. Studier på DC visade att de mindre kiselpartiklarna och lägre koncentrationer av partiklar hade mindre påverkan på DC:s överlevnad och signalmolekyler jämfört med de större partiklarna, som framför allt hade en påverkan på en specifik signalmolekyl som heter CD86. Ju fler partiklar cellerna tog upp, desto mer CD86 fick de på sin yta, som kan innebära att DC har möjlighet att börja kommunicera med T-celler. Dessa resultat visar att de små porösa kiselpartiklarna passar bättre för att användas som neutrala läkemedelstransportörer, medan de större skulle kunna användas inom områden där man önskar få ett immunsvar, t.ex. vid vaccineringar.

Sista studien fokuserade på exosomer, kroppsegna nanoblåsor. Exosomer har redan visat lovande resultat för användning inom cancerterapi, där deras uppdrag är att visa upp delar av cancercellen för T-celler och B-celler för att därigenom starta ett immunsvar mot tumören. I denna studie tittade vi på om exosomer producerade av DC och B-celler, och sådana exosomer som har hittats i bröstmjölk, dras till specifika vita blodkroppar. Resultaten visade att de flesta exosomerna attraheras till monocyter. Om B-cellerna däremot har blivit infekterade med Epstein-Barr virus (EBV) förändras deras exosomer så att de istället dras till ytan på B-celler. Förändringen beror på att dessa exosomer bär ett EBV-protein (gp350) på sin yta som specifikt binder till B-cellens yta. Dessa resultat väcker tankar om vilken funktion dessa exosomer har hos EBV-infekterade personer, men visar också att de skulle kunna användas inom t.ex. cancerbehandlingar där B-cellen spelar en viktig roll.

Den här avhandlingen har med dessa studier ökat kunskapen om hur olika nanopartiklar påverkar DC och hur exosomer kan omdirigeras för att binda till specifika cellpopulationer, vilket förhoppningsvis efter vidareutveckling kan leda till framtida läkemedel.

8 ACKNOWLEDGEMENTS

My time as a PhD student has been a special period of my life to remember, thanks to many people in science as well as others around me who have inspired and encouraged me to reach my goal. I especially would like to thank:

Annika Scheynius, my main supervisor. Thank you for believing in me, sharing your enormous knowledge within research, and guiding me through my adventures in the nanoworld! You have been very encouraging, enthusiastic and supporting in all situations. Thank you also for always keeping your door open!

My co-supervisor, **Susanne Gabrielsson**. Thank you for your fantastic support! You finally turned me into an exosome person as well, which I am very happy for, although at times I almost felt like a virus person… Your talent to see opportunities, where others see failures has been very inspiring, as has your ability to combine research with a glass of champagne!

My co-authors: **Jian Qin, Sara Johansson, Niklas Ahlborg, Mamoun Muhammed, Fei Ye, Evangelia (Eva) Daskalaki, Abhilash Sugunan, Muhammet Toprak, Andrea Fornara, Maria Strømme, Alfonso Garcia-Bennett, Bengt Fadeel, Valerian Kagan, Anna Shvedova, Sherree Friend, Thaddeus George, Cindy Gutzeit and Qin Li.** Thank you all for great collaborations!

Our very helpful and "always-finding-a-solution" previous and present administrators, **Anne Rasikari, Gerd Franzon Lundquist and Catharina Johansson**. A special thanks to Catharina for also being so helpful in the lab.

All previous and present members of the exosome group**: Sara Johansson**, for always having a strong and happy spirit and lots of style! Thank you also for all support in the lab when I was new and all our great times together! **Lotta**, I have never seen a person working so efficiently in a lab as you! Thank you for your great laughter! **Mukti,** for always being so caring and helpful, it's so nice to have you back! And also thank you for your nice Bangladesh evenings! **Ulf**, thank you for all your positive energy you spread throughout the lab, always being open for new challenges! **Britta**, finally I am not the only nanoperson – I was so glad when you joined! Thank you for your never-ending fighting spirit and for our great days in Lisbon! **Patricia**, for bringing some Spanish spice into our group, and for always being so positive! **Cindy**, how would I have managed without you? Thank you for your support, for keeping me company in front of the FACS in late evenings and for our great time in Berlin with tasty "Orangenbier"! **Tanja**, **Karen** and **Zekiye,** for all the uplifting words during the writing of my thesis! I am looking forward to getting to know you better when I have found my way out of my writing room!

All the members of the NanoNet and NANOMMUNE group, for fruitful discussions and sharing all your knowledge with me. A special thanks to: **Erika Witasp,** with whom I could share all my "nano" problems and who was incredible in turning endless

hours in front of the FACS to an enjoyable event. **Andrea Kunzmann** and **Tina Thurnherr** for all our nice dinners and great times, even when we were almost blown away!

All current and previous members of the Scheynius clinical allergy research unit and **everyone** at L2:04! Thank you all for creating such a nice working atmosphere, sharing all your knowledge, and for all nice parties, lunches and fikas! A special thanks to:

Josefine, for being someone to share Janeway frustrations with, for keeping me company in my "thesis tunnel", and for your great sense of humour! By the way, you have fantastic taste! **Erika**, for always being so supportive, keeping me company at the gym, and for all nice walks to Fridhemsplan! **Maiike**, thanks for "forcing" me to the gym! Also for letting me experience peculiar Dutch music during late nights, while making never ending movies… **Emilie**, thank you for sensing when I was in desperate need for chocolate and coffee – I could never have had a better desk neighbour! Thanks also for organizing all these nice parties and film clubs! **Jeanette**, for always having something to say and for keeping me company during weekends! My sister apologizes again for your fainting and I am sorry for Victor's tough treatment!

All my roommates, previous and present: **Maria, Jeanette, Sara L., Ola, Emilie, Christine, Erika, Lotta, Theresa, Mukti, Maaike, Monica, Sarah T, Justus**, for really good company, sharing great laughs and thoughts about everything and nothing!

Christine R., thank you for always being there, sharing ups and downs, and being so easy to talk to. Thank you for all our fun times, from University, London to Croatia! You are a true friend for life!

Stort tack till alla mina vänner och min familj:

Mina fantastiska vänner från Forskarskolan, **Anna**, med din underbara stil och framåtanda, **Johanna**, med dina påhittigheter från maskerad till hästteater, **Vilija**, med din oändliga energi och litauiska kokkonst, **Mantas**, med din entusiasm och ditt medryckande dragspelande, och **Kasper** & **Daniel** som också tillhör gänget, tack för alla upptåg och fantastiska cocktailkvällar! Jag vill också tacka mina trofasta vänner, **Annette** och **Sonja**, för att ni alltid har funnits där, även om jag önskade att jag hade haft er på närmare håll! Detsamma gäller för **Maria H**, som jag har upplevt många yror med och som jag har både det ena och andra att tacka för, men framför allt min Claes! **Hannele**, tack för att du är en sådan härlig optimist och vän som alltid ställer upp i vått som torrt, och **Maria G.** som har följt mig så länge – tack för alla härliga upplevelser, alltifrån "Geschmackprobe", till vilda hästar och "björnjakt" i djupa finska skogar!

Stort tack till min härliga, ständigt växande familj, som betyder så mycket för mig! **Mamma Nilla** och **Pappa Per** som alltid har funnits där, stöttat och trott på mig, även om jag nog alltid kommer att förbli er minsting! Mina syskon **Jonas**, **Karin** och **Annika** som jag har haft så roligt med under uppväxten, och även kommer att ha i framtiden! Alla syskonbarnen – **Rasmus**, **Lukas** och **Filip** – som sprider så mycket glädje och ger livet en extra dimension! **Michaela**, **Victor** och **Thomas** för att ni är så härliga att umgås med och ställer upp på en del familjeknasigheter! Mina svärföräldrar,

Margareta och **Bosse**, för att jag känner mig så välkommen hos er och att ni gör allt för att förstå ”nano”. Tack också till Irma och Selma för ert moraliska stöd, och till min ständiga supporter L.K.!

Min **Claes**, som är det allra finaste jag har.
Tack för all din fantastiska support, tilltro och kärlek!
Jag älskar dig!♥

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