Nanomedicine for infectious diseases

Harald Unterweger¹, Stefan Lyer¹, Christina Janko¹, Ralf P Friedrich¹, Iwona Cicha¹ ,
Rainer Tietze¹ & Christoph Alexiou^{*1}

¹Department of Otorhinolaryngology, Head & Neck Surgery, Section of Experimental Oncology & Nanomedicine (SEON), Else Kröner-Fresenius-Stiftung-Professorship, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Glueckstr. 10a, 91054, Erlangen, Germany

*Author for correspondence: c.alexiou@web.de

First draft submitted: 5 March 2020; Accepted for publication: 12 March 2020; Published online: 12 May 2020

Keywords: drug-resistant bacteria • drug-delivery nanosystems • tuberculosis • vaccines • viral infections

Multilamellar particle-based vaccines for efficient protection against infectious pathogens

Evaluation of: Fan Y, Stronsky SM, Xu Y *et al.* Multilamellar vaccine particles elicits potent immune activation with protein antigens and protects mice against Ebola virus infection. *ACS Nano* 13(10), 11087–11096 (2019).

Repeated outbreaks of infectious pathogens, such as Ebola virus or Zika virus, have a large impact on global health [1,2]. Viral vector-based vaccines achieve promising protection rates against these diseases, but there are still remaining concerns associated with these traditional vaccine approaches, such as pre-existing immunity or safety issues [3]. Protein antigens are an alternative to these live vector vaccines, but they also have their disadvantages.

In their work, Fan *et al.* addressed these limitations by developing multilamellar vaccine particles (MVPs) based on lipid-hyaluronic acid particles with incorporated protein antigens. Depending on the incorporated antigen, these MVPs varied in their physical properties. While the pristine unilamellar liposomes possessed a mean hydrodynamic size of 130 nm, a polydispersity index (PDI) of 0.27 and a ζ potential of +19.5 mV, the MVPs incorporated with ovalbumin (OVA) and monophosphoryl lipid A (MPLA) had a size of 280 nm, a PDI of 0.18 and a ζ potential of 18.0 mV. Incorporation of MPLA and EBOV GP led to a larger diameter (350 nm) and PDI (0.26) and a slightly less negative ζ potential. The encapsulation efficiency for OVA was 18%, corresponding to 9 μ g per mg lipid, and 36% for EBOV GP, corresponding to 15 μ g per mg lipid.

In vitro studies using bone marrow-derived dendritic cells demonstrated an enhanced antigen delivery and an improved antigen processing by dendritic cells induced by this MVP delivery concept. Furthermore, robust antigen-specific CD8⁺ and CD4⁺ T-cell response, as well as humoral responses were triggered in naive C57BL/6 mice, which could be observed even 4 months after vaccination with MVP-OVA.

Based on these results, MVP-EBOV GP were tested as a vaccine against Ebola virus exposure. For this purpose, mice received a single dose of the particles, with a 3 μ g EBOV GP load. In comparison to soluble MPLA + EBOV GP vaccination, in other words without particle carrier, this approach led to on average 128-fold higher GP-specific IgG serum titers. Moreover, the protection against the lethal Ebola virus exposure significantly increased from 10% to 80%.

In conclusion, Fan *et al.* provided extensive proof-of-concept studies to show the remarkable performance of MVP as vaccine delivery system for recombinant protein antigens.

– Written by H Unterweger.

Arming macrophages with antibacterial peptides to combat multi-resistant sepsis pathogens

Evaluation of: Hou X, Zhang X, Zhao W *et al.* Vitamin lipid nanoparticles enable adoptive macrophage transfer for the treatment of multidrug-resistant bacterial sepsis. *Nat. Nanotechnol.* 15(1), 41–46 (2020).

Largely unknown to the general public, 30 million people worldwide suffer from sepsis every year and it is still the most common cause of death in hospitals [4]. Clinical data increasingly indicate that macrophages and monocytes, which are important components of the innate immune system, are damaged in the course of sepsis. This immensely

worsens the prognosis in patients suffering from multi-resistant pathogens [5]. Additionally, some bacteria, such as *Staphylococcus aureus* or *Escherichia coli* developed mechanisms to escape their clearance by these immune cells [6].

The aim of Hou *et al.*'s work was to enhance the bactericidal ability of immune cells by transfecting them with an mRNA coding for a construct of an antimicrobial peptide (IB367), combined to cathepsin B (Cat-B) and a Cat-B-sensitive linker. After transcription of the gene construct, Cat-B should shuttle into the lysosomes, where the peptide can carry out its antimicrobial effect.

Among nanoparticles synthesized from five different vitamin-derived lipids, the vitamin C-based formulation (V_CL) revealed the highest transfection efficiency of a luciferase-mRNA into RAW264.7 cells, outperforming Lipofectamine 3000 and electroporation 10- and 50-fold, respectively. Upon optimization of the nanoparticles, the authors demonstrated that a mass ratio of V_CL/2-dioleoyl-*sn*-glycero-3-phosphoethanolamine/cholesterol of 30/30/50, and a mass ratio of V_CL/mRNA being 15/1, further enhanced the transfection efficiency sevenfold. Subsequent studies in RAW cells showed that the V_CL-nanoparticles (V_CLN) are internalized via caveolae-mediated endocytosis and that the construct is effectively shuttled into the lysosomes. Further, the V_CLN showed a 33–87% antibacterial efficiency, which could be impaired by inhibiting the Cat-B-shuttle-function.

In an immunosuppressed mouse model, a single combined intraperitoneal (ip.) and intravenous (iv.) administration of RAW cells or bone marrow-derived macrophages transfected with full-construct nanoparticles, resulted in a significantly higher animal survival rate after ip. infection with methicillin-resistant *S. aureus* (MRSA) alone or ip. coinfection with MRSA and multi-drug resistant *E. coli*. A second treatment effectively eradicated the remaining colony forming bacteria in those animals that did not show full response after the first treatment.

Being aware that waiting for approximately 1 week for autologous blood mononuclear cells to be prepared may be too long for many of the sepsis patients, the authors propose to use pluripotent stem cells or allogeneic 'universal' macrophages to overcome this drawback.

Taken together, Hou *et al.* report an interesting novel approach to one of the most important threats for human health worldwide. Even if other therapies are developed for patients in acute diseases settings, using autologous mononuclear cells could be helpful for patients suffering from long-lasting persistent sepsis induced by biofilms that arise for example at the surfaces of artificial heart valves or other implants.

– Written by S Lyer and C Alexiou.

Increasing the immune response at the infection site by loading neutrophils with nanoparticles for light-induced suicide

Evaluation of: Zhang P, Zhao Q, Shi M *et al.* Fe₃O₄@TiO₂-laden neutrophils activate innate immunity via photosensitive reactive oxygen species release. *Nano Lett.* 20(1), 261–271 (2020).

Bacterial infections remain a challenge in modern medicine. Due to the rise of drug-resistant pathogens, systemic infections can become life threatening. Phagocytic cells such as neutrophils and macrophages form the first line of defense and, ideally, remove invaders rapidly and efficiently by phagocytosis and other mechanisms. However, virulence factors released by bacteria can prevent adequate immune activation [7]. To overcome this problem, Zhang *et al.* developed photocatalytic nanoparticles to be incorporated into neutrophils. When activated by light, these nanoparticles release reactive oxygen species, triggering neutrophil apoptosis with cytokine release, which induces the recruitment of macrophages to the target area and the termination of immune suppression. The synthesized nanoparticles had an iron oxide (Fe₃O₄) core and TiO₂ shell (termed 'FTO' nanoparticles), enabling both photoactivation and magnetic isolation of the target cells. To load neutrophils *in vivo* with the FTO nanoparticles, the authors injected fetal bovine serum containing lipopolysaccharide into the abdominal cavity of mice to induce the recruitment of neutrophils. After 6 h, nanoparticle injection was carried out, resulting in their complete uptake by neutrophils (Neu-FTO). After a further 2 h, the neutrophils were extracted from the abdominal cavity, followed by a magnetic separation to remove unloaded cells. Transmission electron microscopy (TEM) and inductively coupled plasma-atomic emission spectroscopy (ICP-AES) confirmed an efficient nanoparticle uptake into the neutrophils (1.75×10^{-5} µg of Fe per cell). Due to the lipopolysaccharide stimulation, neutrophils showed an activated phenotype with enhanced surface levels of CD14 and TLR4. Neu-FTO were still able to express TNF-α, IL-6, and IFN-γ and were active against bacteria. With near-ultraviolet light illumination, reactive oxygen species was produced by FTO within the neutrophils inducing cell death accompanied by release of the macrophage chemokines MCP-1 and MIP-1β. In a mouse model of necrotizing fasciitis with a local infection with MRSA characterized by immune suppression, immunohistochemistry of the infected skin confirmed *in vivo* that Neu-FTO induced neutrophil apoptosis accompanied by macrophage recruitment, resolving immune

suppression. Investigation of the blood, 3 days postinfection showed that macrophage infiltration after Neu-FTO treatment cleared pathogens and controlled infection. The results were confirmed in a high dose intraperitoneal infection model to study the salvage effect of Neu-FTO on fatal infections. Here, a large number of bacteria (1×10^{10} CFU/ml) were injected into the abdominal cavity once per day for three consecutive days. In the untreated control group, blood smears revealed a large number of bacteria and all mice died by day 3. Neu-FTO treatment, however, prevented invasion of bacteria into the blood and more than 50% of the mice were alive after 3 days.

Summarizing, the authors showed that with nanoparticle-loaded neutrophils the body's own immune responses can be activated to eliminate bacterial infection. This system is especially promising to fight against drug-resistant bacterial strains.

– Written by C Janko.

Double-layered M2e-NA protein nanoparticles as a universal influenza vaccine

Evaluation of: Wang Y, Deng L, Gonzalez GX *et al.* Double-layered M2e-NA protein nanoparticle immunization induces broad cross-protection against different influenza viruses in mice. *Adv. Healthcare Mater.* 9(2), e1901176 (2020).

Influenza is mainly transmitted by droplets and aerosols originating from respiratory secretions of infected individuals. The virus is responsible for annual epidemics and recurring global pandemics, representing a major cause of morbidity and mortality worldwide [8,9]. Influenza vaccination is recommended for vulnerable risk groups such as pregnant women, children, elderly persons, individuals with specific chronic medical conditions and healthcare workers [10]. However, due to the genetic instability, the resulting antigenic drift or shift, and low immunogenicity of conserved epitopes, no universal influenza vaccine is yet available that could provide a long-lasting protection, although promising candidates are currently being evaluated in clinical trials [11]. A nanoparticle-based approach for the development of a broad-scope and durable vaccine were recently described by Wang *et al.* [12]. They produced structure-stabilized fusion proteins of the small tetrameric influenza M2 ectodomain (M2e) and the influenza neuroaminidase (NA) subtypes N1 and N2 (M2e-N1 and M2e-N2). The fusion proteins were then crosslinked by 3'-dithiol-bis-(sulfosuccinimidylpropionate) (DTSSP) to the surface of M2e nanoparticles to generate self-disassembling double-layered protein nanoparticles. The roughly spherical M2e-N1 had size of 211.0 nm and a negative ζ potential of -27.77 mV. M2e-N2 nanoparticles were 190.4 nm in size, with slightly less negative ζ potential (-21.03 mV). The loading ratios of the fusion protein to the total protein content were 62.1% for M2e-N1 and 59.3% for M2e-N2.

In mice, immunization with the double-layered nanoparticles significantly increased NA- and M2e-specific IgG antibody levels compared with immunization with the soluble protein mixture (sM2e+NA). Strong neutralization against both homologous and heterologous NA influenza viruses was observed in sera, and the infected animals completely survived high doses of virus. The nanoparticle immunization groups lost less bodyweight, had a reduced viral titer, a lower degree of immune cell infiltration and inflammation, and a significantly increased number of IFN- γ secreting CD4 and CD8 T cells in the lungs compared with mice receiving the soluble protein mixture. Moreover, in nanoparticle-immunized mice a more durable immune protection and stronger cross-protection against heterosubtypic influenza viruses was achieved compared with the mice groups receiving the soluble proteins.

In summary, Wang *et al.* presented a new recombinant protein-based, highly effective influenza vaccine which, unlike the egg-based production of traditional seasonal vaccines, can be produced very quickly and in high quantities. The slow disassembly and protein release contributed to a longer antigen retention, processing and presentation, consequently leading to the increased immune response. Moreover, the novel particles triggered a broadly cross-protective immune response against both M2e and various NA subtypes, indicating their potential as a universal influenza vaccine, either alone or in more complex vaccine formulations.

– Written by RP Friedrich.

Mannose-selenium-isoniazid nanoparticles: a targeted weapon against tuberculosis

Evaluation of: Pi J, Shen L, Yang E *et al.* Macrophage-targeted isoniazid-selenium nanoparticles promote antimicrobial immunity and synergize bactericidal destruction of tuberculosis bacilli. *Angew. Chem. Int. Ed. Engl.* 59(8), 3226–3234 (2020).

TB remains the number one cause of death among infectious diseases, being responsible for about 1.6 million deaths yearly [13]. According to the current estimates, about 25% of the world population is infected with *Mycobacterium*

tuberculosis (MTB), mainly due to the very high transmission rate and a poor efficacy of the current therapies [14]. MTB is a non-motile slow-dividing bacillus, which upon transmission invades and replicates within alveolar macrophages. A thick capsule-like cell wall, rich in lipids and mycolic acid [15,16], constitutes a potent protective barrier that prevents both the phagolysosomal destruction of MTB inside macrophages and the entry of exogenous drugs into the bacteria.

In their paper, Pi *et al.* addressed the problem of MTB escape from phagolysosomal destruction and the poor efficacy of therapeutic approaches to TB. Based on the known antimicrobial effects of selenium, the authors designed selenium-chitosan nanoparticles. To improve the targeting efficacy, the particle shell was functionalized with mannose (Man-Se NPs), which is avidly internalized by macrophages. Furthermore, isoniazid, an antibiotic used to treat TB, was incorporated in the particles via hydrogen bonding between amino groups of chitosan and drug molecules (Ison-Man-Se NPs).

The produced nanoparticles were characterized in terms of physico-chemical properties and the presence of elemental selenium and selenides. Man-Se NPs had a hydrodynamic size of approximately 40 nm and the Ison-Man-Se NPs were slightly larger (45 nm). The *in vitro* cytotoxicity of the particles was considerable at 10 µg/ml in the tested cell lines (THP-1 monocytic cells and human lung microvascular endothelial cells), but the doses up to 1 µg/ml were well tolerated. The uptake of NPs by macrophages was greatly enhanced upon functionalization with mannose and occurred mainly via clathrin-mediated endocytosis, leading to lysosomal accumulation. This type of intracellular distribution has proven beneficial for drug release, with nearly doubled rate of isoniazid release at pH 5.3 compared with pH 7.4. The authors subsequently investigated the bactericidal efficacy of the produced nanoparticles in the THP-1-derived macrophages infected with bacillus Calmette-Guérin (BCG) or H37Rv (MTB strain H37Rv). Whereas Man-Se NPs and free isoniazid showed dose-dependent inhibition of BCD and H37Rv in macrophages, Ison-Man-Se NPs exhibited very potent synergistic bactericidal effects. The mechanisms of Ison-Man-Se NPs actions were therefore investigated, showing that these particles promoted fusion of phagosome-localized MTB into the lysosomes, thus enhancing both the lysosomal and the drug-induced destruction of bacilli. Furthermore, in contrast to free isoniazid, Ison-Man-Se NPs induced autophagy and apoptosis in BCG-infected macrophages and prevented the bacteria-induced polarization to M2 phenotype, thus contributing to antibacterial immunity.

These data indicate that, provided sufficient preclinical safety, Ison-Man-Se NPs can represent a highly potent weapon against active and latent TB.

– Written by I Cicha.

Rapid monitoring of the progress of infectious diseases

Evaluation of: Belushkin A, Yesilkoy F, González-López JJ *et al.* Rapid and digital detection of inflammatory biomarkers enabled by a novel portable nanoplasmonic imager. *Small* 16(3), 1906108 (2020).

In a world full of smartphones, tablets and other practical devices, more portable digital assistants are also finding their way into medicine. That this is more than just a gadget shows the urgent need for fast and accurate diagnostic tools, especially for life-threatening diseases, which require immediate treatment [17]. Rapid, reliable and cost-efficient diagnostic tools can drastically improve the care of life-threatening infections at different clinical settings, especially in facilities that are far away from high-end laboratory diagnostic centers. Belushkin *et al.* have made a promising contribution in this direction by developing a plasmonic imager, which is able to detect very sensitively procalcitonin (PCT) and C-reactive protein (CRP), two severe bacterial infection-related inflammation markers [18]. The determination of these biomarkers is not new, but the previous methods are based on complex, time-consuming and expensive turbidometric or chemiluminescent immunoassays. None of these systems are really transportable.

The reported approach utilizes gold nanoparticles (Gold-NPs), corresponding to a complementary gold nanoarray, and allows the quantification of the biomarkers in complex media. The detection mechanism is based on a large-area surface plasmon resonance on gold nano-hole arrays. Those arrays exhibit optical transmission at its resonance wavelength when they are illuminated in a collinear optical path. If even a single Gold-NP binds to the arrays, the plasmonic resonance is distorted and suppresses the optical transmission very sensitively. As a result, the detection of single Gold-NP-labelled molecules is possible. For the quantification of PCT and CRP, the respective antibody-conjugated Gold-NPs bind to the gold nano-hole array surface that exposes the complementary antibody, if the respective biomarker is present in the sample. The system was miniaturized to the extent that it can be read out on a portable device. The respective arrays for multiplexed biosensing are bioprinted on the sensor surface. This manufacturing process yields to highly uniformly structured chips with negligible variation in optical properties.

The analytical performance regarding the specified sepsis biomarkers is 21 pg ml⁻¹ for PCT and 36 pg ml⁻¹ for CRP, with a dynamic range over three orders of magnitude, from the two-digit picogram to the sub-microgram range. Thus, the performance of the method is exactly in the clinically relevant range where tens of pg PCT ml⁻¹ for healthy volunteers and several hundred pg ml⁻¹ for patients with systemic inflammatory response syndrome are registered. As clinically relevant CRP concentrations range from a few hundred µg ml⁻¹ to several hundred µg ml⁻¹, corresponding dilutions are required for this marker.

In summary, this device provides a powerful tool for point-of-care diagnostics of inflammatory processes with special focus on monitoring the progress of infectious disease.

– Written by R Tietze.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

1. Aruna A, Mbala P, Minikulu L *et al.* Ebola virus disease outbreak – Democratic Republic of the Congo, August 2018–November 2019. *MMWR Morb. Mortal. Wkly Rep.* 68(50), 1162–1165 (2019).
2. Correa-Morales F, Gonzalez-Acosta C, Mejia-Zuniga D *et al.* Surveillance for Zika in Mexico: naturally infected mosquitoes in urban and semi-urban areas. *Pathog. Glob. Health* 113(7), 309–314 (2020).
3. Ura T, Okuda K, Shimada M. Developments in viral vector-based vaccines. *Vaccines (Basel)* 2(3), 624–641 (2014).
4. Reinhart K, Daniels R, Kissoon N, Machado FR, Schachter RD, Finfer S. Recognizing sepsis as a global health priority – a WHO resolution. *New Engl. J. Med.* 377(5), 414–417 (2017).
5. Hotchkiss RS, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. *Lancet Infect. Dis.* 13(3), 260–268 (2013).
6. Garzoni C, Kelley WL. *Staphylococcus aureus*: new evidence for intracellular persistence. *Trends Microbiol.* 17(2), 59–65 (2009).
7. Kobayashi SD, Malachowa N, Deleo FR. Neutrophils and bacterial immune evasion. *J. Innate Immun.* 10(5–6), 432–441 (2018).
8. Bresee JS, Fry A, Sambhara S, Cox NJ. Inactivated influenza vaccines. In: *Plotkin's Vaccines* (7th Edition). Plotkin SA, Orenstein WA, Offit PA, Edwards KM (Eds.), Elsevier, Amsterdam, The Netherlands (2018).
9. Cunha BA. Influenza: historical aspects of epidemics and pandemics. *Infect. Dis. Clin. North Am.* 18(1), 141–155 (2004).
10. Vaccines against influenza WHO position paper – November 2012. *Wkly. Epidemiol. Rec.* 87(47), 461–476 (2012). www.who.int/wer/2012/wer8747.pdf
11. Phillipson JE, Babecoff R, Ben-Yedidia T. Is a universal influenza vaccine feasible? *Ther. Adv. Vaccines Immunother.* 7, 1–6 (2019).
12. Wang Y, Deng L, Gonzalez GX *et al.* Double-layered M2e-NA protein nanoparticle immunization induces broad cross-protection against different influenza viruses in mice. *Adv. Healthc. Mater.* 9(2), e1901176 (2019).
13. Floyd K, Glaziou P, Zumla A, Raviglione M. The global tuberculosis epidemic and progress in care, prevention, and research: an overview in year 3 of the end TB era. *Lancet Resp. Med.* 6(4), 299–314 (2018).
14. Bloom BR, Atun R, Cohen T *et al.* Tuberculosis. In: *Major Infectious Diseases* (3rd Edition). Holmes KK, Bertozzi S, Jha P (Eds.). The World Bank, DC, USA (2017).
15. Queiroz A, Riley LW. Bacterial immunostat: mycobacterium tuberculosis lipids and their role in the host immune response. *Rev. Soc. Bras. Med. Trop.* 50(1), 9–18 (2017).
16. Angala SK, Belardinelli JM, Huc-Claustre E, Wheat WH, Jackson M. The cell envelope glycoconjugates of *Mycobacterium tuberculosis*. *Crit. Rev. Biochem. Mol. Biol.* 49(5), 361–399 (2014).
17. Sinha M, Jupe J, Mack H, Coleman TP, Lawrence SM, Fraley SI. Emerging technologies for molecular diagnosis of sepsis. *Clin. Microbiol. Rev.* 31(2), e00089-17 (2018).
18. Li Q, Gong X. Clinical significance of the detection of procalcitonin and C-reactive protein in the intensive care unit. *Exp. Ther. Med.* 15(5), 1–6 (2018).