We Invest in Quality.

Advanced Vaccines in Modern Medicine

Phospholipid-Based Delivery Systems
Phospholipids are essential components of advanced vaccines
Phospholipids enable the delivery of immunostimulants, antigens and RNA to the target site and trigger the desired immune response
Cationic lipids are the most advanced complexing agents for RNA vaccines
Vaccine Adjuvants

The routine use of vaccines is one of the most outstanding accomplishments of modern medicine. The first major milestone was the eradication of smallpox. Nowadays, emerging pathogens like Sars-CoV-2 require innovative vaccination approaches [1]. Besides the prevention of infectious diseases, vaccination is also an emerging field to prevent and treat cancer.

The first generations of vaccines were made by use of live attenuated organisms or inactivated organisms, followed by specific antigens and most recently by antigen encoding mRNA. Antigens often induce only a low immune response. In such cases, adjuvants are needed to boost and/or modulate the immune response. Adjuvants can be classified into carrier systems and immunostimulants [2-4].

Immunostimulants

In 1925, Ramon demonstrated for the first time in horses that artificial enhancement of diphtheria and tetanus antitoxin levels by the addition of immunostimulants like agar, metallic salts and saponins is possible. In the 1940s, first trials were performed with water-in-oil emulsions as adjuvants. These so-called Freund adjuvants comprised mineral oil emulsions. Freund adjuvants are no longer used in marketed vaccines as they are poorly tolerated due to the non-degradable mineral oils present. Although the use of aluminium salts is well established, immunostimulants like saponins, Monophosphoryl Lipid A (MPL) or cationic lipids are used more frequently [3-4].

Carrier Systems

Carrier systems include e.g. oil-based emulsions, Immune Stimulating Complexes (ISCOMs), liposomes, lipoplexes and lipid nanoparticles [7-8].

Natural and synthetic phospholipids, mainly phosphatidylcholine, often called “lecithin” in American literature, and other phospholipids relevant for the adjuvant use are often explored and attract more and more attention (Fig. 1). Such systems are used as carriers for immunostimulants, antigens or mRNA along with cationic lipids like DOTAP for complexation (Fig. 2). In addition, certain phospholipids of the carrier systems like DOPC and DOPE play a major role in intracellular processing.

Phospholipid-based carrier systems exhibit advantages over other nanoparticles:

- Wide range of particle sizes and compositions possible
- High efficiency of antigen entrapment-compatibility with hydrophilic, hydrophobic-, and amphiphilic antigens
- Biodegradable & safe after any route of administration

**Fig. 1:** Increasing interest in vaccines & phospholipids: Number of peer-reviewed publications from 1995 to 2019 (Source: Lipoid internal database)
Commercialized Vaccines with Phospholipids

The most prominent marketed vaccine with liposomal adjuvants technology is GSK’s Shingrix® for vaccination against Varicella zoster virus infections (shingles). Beyond liposomes, ISCOMs, open cage-like lipid particles made of Quillaja saponins, cholesterol and phosphatidylcholine, are used. They can be found in commercial products, e.g. in animal vaccines against equine influenza (Tab. 1).

### Table 1: Marketed vaccines with phospholipids

<table>
<thead>
<tr>
<th>Trade Product</th>
<th>Indication</th>
<th>Formulation</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equilis® Frequenza Te</td>
<td>Equine Influenza/Tetanus</td>
<td>ISCOM</td>
<td>MSD/Intervet International BV, Netherlands</td>
</tr>
<tr>
<td>Equilis West Nile suspension for injection</td>
<td>West Nile virus infection</td>
<td>ISCOM</td>
<td>MSD/Intervet International BV, Netherlands</td>
</tr>
<tr>
<td>Equip® FT</td>
<td>Influenza/Tetanus</td>
<td>ISCOM</td>
<td>Zoetis Inc., USA</td>
</tr>
<tr>
<td>Shingrix®</td>
<td>Shingles</td>
<td>liposomes</td>
<td>GSK plc, UK</td>
</tr>
</tbody>
</table>

### Table 2: Vaccines in clinical research with phospholipids

<table>
<thead>
<tr>
<th>Project/Product</th>
<th>Indication</th>
<th>Status 03/2020</th>
<th>Formulation</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACI-24 and ACI-35 vaccine</td>
<td>Alzheimer</td>
<td>Phase VII</td>
<td>liposomes</td>
<td>AC Immune SA, Switzerland</td>
</tr>
<tr>
<td>DepoVax™/VaccMax</td>
<td>Cancer, infectious diseases</td>
<td>Phase VII</td>
<td>emulsion</td>
<td>Immuno Vaccine Technologies Inc., Canada</td>
</tr>
<tr>
<td>IvAC Mutanom</td>
<td>Cancer</td>
<td>Phase II</td>
<td>Lipoplex Liposome</td>
<td>BioNTech SE, Germany</td>
</tr>
<tr>
<td>Rabies Vaccine</td>
<td>Rabies, RSV</td>
<td>Phase I</td>
<td>Lipid nanoparticles</td>
<td>CureVac AG, Germany</td>
</tr>
<tr>
<td>mRNA-1647</td>
<td>CMV mononucleosis</td>
<td>Phase VII</td>
<td>Lipid nanoparticles</td>
<td>Moderna Inc., USA</td>
</tr>
<tr>
<td>Versamune™</td>
<td>HPV associated cancers</td>
<td>Phase II</td>
<td>Lipoplex</td>
<td>POS Biotechnology Corp., USA</td>
</tr>
<tr>
<td>GSK3277511A</td>
<td>COPD</td>
<td>Phase II</td>
<td>liposomes</td>
<td>GSK plc, UK</td>
</tr>
<tr>
<td>GSK3844766A</td>
<td>RSU</td>
<td>Phase II</td>
<td>liposomes</td>
<td>GSK plc, UK</td>
</tr>
<tr>
<td>Mosquirix™/M72/AS01E</td>
<td>Malaria/Tuberculosis</td>
<td>Phase VII</td>
<td>liposomes</td>
<td>GSK plc, UK</td>
</tr>
</tbody>
</table>

Abbreviations:
AS01: Adjuvant system 01, DOPC: 1,2-Dioleoyl-sn-glycero-3-phosphocholine, DOPE: 1,2-Dioleoyl-sn-glycero-3-phospho-ethanolamine, DOTAP: N-[1-(2,3-Dioleoyloxypropyl]-N,N,N-trimethylammoniummethyl-sulfate, DOTMA: 1,2-di-O-octadecenyl-3-trimethylammonium propane (chloride salt), GSK: GlaxoSmithKline plc., HIV: Human Immunodeficiency Virus, ISCOMs: Immunestimulating complexes, MPL: Monophosphoryl Lipid A, MSD: Merck Sharp and Dohme, PC: Phosphatidylcholine, PEG: Polyethylene glycol, QS21: Highly purified saponin; derivative from the Quillaja saponaria Molina tree, mRNA: Messenger Ribonucleic acid, RSV: Respiratorisches Syncytial-Virus
Concluding Remarks

The extremely successful introduction of the Shingrix® vaccine product from GSK for prophylaxis of herpes zoster [2019: £ 1.8 billion] \[9\] and the market presence of veterinary vaccines show the enormous potential of phospholipids as components of adjuvants. The use of phospholipids like DOPC and DOPE in adjuvants may therefore become more relevant.

Cationic materials are needed as complexing agents for mRNA vaccines, and cationic lipids, especially DOTAP, are most advanced.

Vaccines in Clinical Research with Phospholipids

Promising clinical research with vaccines is underway, for instance the studies being performed by GSK with the AS01 adjuvant in various diseases (Tab. 2). The adjuvant is comprised of liposomes with DOPC, cholesterol, MPL and QS21 (Quillaja saponaria Molina). In addition, more and more mRNA-based vaccines are studied clinically, e.g. by BioNTech, CureVac and Moderna. mRNA is i.e. encapsulated in lipid nanoparticles (Fig. 2), which use e.g. phospholipids like DOPE and DOPC and cationic lipids like DOTAP and DOTMA as complexing agents.

Fig. 2: Schematic illustration of carrier systems for immunostimulants, antigens and mRNA.
References

[9] GSK, Press Release. GSK delivers 2019 sales of £ 33.8 billion + 10 % AER, + 8 % CER (Pro-forma +4 % CER*), Feb. 5 [2020].

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