



ES2A EEP-02

Vaccine strategies

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Abstract

In a context of a pandemic and major doubts towards vaccination among populations, this paper deals with vaccine strategies, especially how mRNA vaccines are revolutionizing them. It is clear that immunology represents a complex field of science, and nowadays the breakthroughs in biotechnologies are offering new possibilities to accelerate vaccine development, efficiency and safety as well as a better understanding our immune system. This paper aims at summarizing the different vaccine techniques with a special attention to mRNA vaccines¹, in order to have an overview of the current vaccine strategies which are going to evolve.

Acknowledgements

I cannot express enough thanks to my teacher Véronique Stoven for her interesting lessons about molecular and synthetic biology, which inspired me this topic. I absolutely loved doing research and writing this report.

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¹Messenger RiboNucleic Acid

1 Introduction

Vaccines represent an amazing invention which saved millions of lives in the world since 2000 [7]. Even if vaccination is not new, the recent pandemic of COVID-19 brought to the forefront this field, generating great advances but also important debates about safety, ethics and politics. It is necessary to take into account all these changes and to adapt to be avoid a backward step and the rejection of these opportunities.

Therefore it would be interesting to better understand the recent evolutions in vaccine strategies and technologies and the consequences it may have on society.

Firstly, I will briefly recall the history of vaccination, detailing the different types of vaccines which have been developed. Secondly, I will present the technology of mRNA (messenger ribonucleic acid) vaccines which are currently the main source of progress. Thirdly, I will observe how society is integrating these developments and what issues remain to be solved.

This theme is closely linked to the mechanics of the immune system, in medicine, which will not be detailed in this article due to the needed knowledge in medicine. In any case, I would like to thank Ms. Stoven for her molecular biology course and her help in the writing of this article.

2 A brief history of vaccines

2.1 Discovery of the principle

The principle of vaccination could come from the idea of mithridatism, that is to say the ability to gain protection against a poison by taking several benign amounts.

Variolization, which corresponds to injecting smallpox pustules to gain immunity against smallpox, began in China around the 10th century [3]. Before the scientist Jenner, several persons realized variolization in England. However, in 1798, this is Jenner who made a link between cowpox and smallpox: cowpox could an attenuated version of smallpox.

The idea of vaccination is to create an individual, long-term and efficient protection against a pathogen (like a virus or a bacteria), without causing serious symptoms. This is made possible thanks to the memory of human's immune system.

The word "vaccination" comes from the Latin word "vacca", which means "cow".

The immune system at a glance

In the medical field, the immune system is mainly composed of two parts:

- The innate immune system. After the infection, it is the first system to react. It is mainly represented by Natural Killers (NK) cells, phagocytes and dendritic cells which play an important role to initiate the adaptive response².
- The adaptive (or acquired) immune system. This one is subdivided in two categories:
 - The cellular response, thanks to lymphocytes T (thymus) CD8+ (cluster of designation). They are cytotoxic and they can kill the infected cells. To be efficient, in most cases, they need the major histocompatibility complex.
 - The humoral response, thanks to lymphocytes B (Bursa of Fabricius). They produce antibodies (immunoglobulines, abbreviated Ig), which are proteins able to neutralize microbial effectors, from bacteria and viruses. Antibodies need antigens of the pathogens, which are proteins expressed by the pathogen, to recognize them.

Lymphocytes T CD4+ (helper) regulate both responses. The specificity of the adaptive immune system is its memory thanks to specific T and B memory cells, and it can be easily observed using the concentration of immunoglobulines in the blood.

The graph 1 shows that the response after the first infection is less important and slower after the second one. It also compares the concentration of IgM, an immunoglobuline which are non-specific,

²The details for each different type of cells will not be covered in this report.

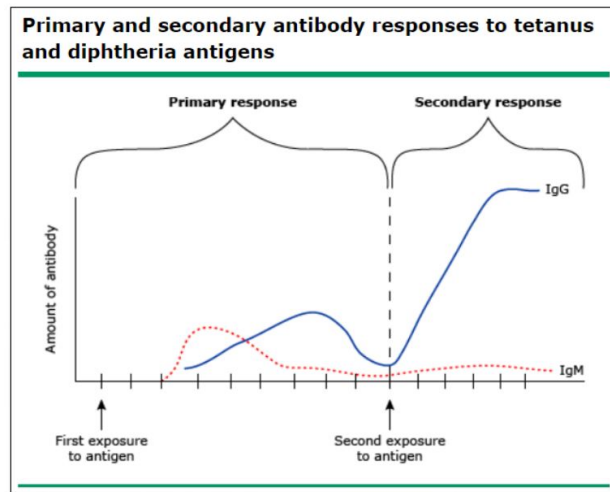


Figure 1: Primary and secondary responses to specific antigens [11]

versus IgG, which are more specific microbial effectors such as toxins or proteins: it proves that the adaptive immune system becomes efficient after the innate system, and that there is a memory effect on the second infection.

The cellular response is more complicated to quantify.

As regards pathogens, the incubation period can be short (pneumococcus) or long (hepatitis B virus).

It is important to notice that the immune system is very different according to the age of the person: the main phenomenon when ageing is immunosenescence, which makes the immune system less reactive and dynamic.

2.2 First generation of vaccines

It is only between 1870 and 1885 with Pasteur's works (helped by Emile Roux and Emile Duclaux, and based on Robert Koch's findings), that the first vaccines were developed [12]. The first official vaccines that he conceived were against chicken cholera, anthrax, swine erysipelas and then rabies.

The first generation of vaccines consists in pathogens which are live, weakened or killed. Live weakened vaccines are the most immunogenic, but they should be prepared carefully. Despite being efficient, there are still high risks using them if virus are not attenuated enough: one can develop the disease and transmit it to an immunocompromised person. Otherwise, vaccines can be killed for example with heat/chemical treatment, and they are less dangerous, however there is a need for adjuvants (from the Latin word "adjuvare", which means "to help") to make them immunogenic, and the response is mainly humoral and less cellular.

Adjuvants can be very diverse and numerous as well as their mechanism of action: killed mycobacteria, oils, aluminium salts, microparticles, squalanes, ligands of PRRs... They aim at amplifying the reaction for a whole population and each individual (especially old people whose immune system is less dynamic) and at reducing the quantity of active substance needed (dose sparing). The dosage should be very cautious not to hide the active substance.

To prepare those vaccines, cell culture is often needed. To weaken viruses, other environments than human cells are used such as chicken cells so that the virus is no more adapted to human cells...

To prepare a live weakened vaccine, the virus or the bacteria is replicated in adverse conditions several times. Main vaccines of this type are BCG (Bacillus Calmette-Guérin vaccine, against tuberculosis), the Mumps vaccine. It is important to note that different vaccines can be combined together, like with the MMRV vaccine (Mumps, Measles, Rubella and Varicella: a tetravalent vaccine). It is not dangerous for health and avoid too many injections for children, who must develop an immunity for many diseases in the first years.

Thanks to the development of immunology and microbiology, in particular the ability to isolate pathogens, vaccines were better understood and it led to the development of a new type of vaccines.

The method of production of these vaccines also evolved over time. With genetic engineering and reverse genetics, that is to say, contrary to forward genetic, the ability to know which phenotypes can be controlled by different genetic sequences, it allows a better treatment of viruses to select inactivated vaccines.

2.3 Second generation of vaccines

This generation demands more development and technological advances, to use subunits of viruses such as protein antigens or inactivating toxins produced by the pathogen. There is also the need for adjuvant, which are substances which trigger a powerful immune response ; otherwise the vaccine would not be effective. The response is again mainly humoral: sometimes it cannot activate Toll-Like Receptors on dendritic cells needed for a complete response.

Subunits vaccines (also called acellular vaccines) are stable and safe, with no risk to induce the disease but the right part of the virus should be chosen. They can be protein-based, with polysaccharides, which is a sugar capsule produced by some pathogens (however it has less effect than proteins), or both (it is then called conjugate). Inactivated toxins are called anatoxin is a toxin which has lost his toxic power thanks to heat and formaldehyde, but not totally its immunogenic ability.

To produce these units, the pathogen is cultivated at large scale before using chemicals to destroy and separate the parts of the pathogen.

Lots of recent vaccines use this technique: influenza, hepatitis A, HPV (Human papillomavirus infection), pertussis, tetanus (anatoxin). The first approved subunit was against hepatitis B in 1981 in the United States. At that time, it was a breakthrough because it represented the first vaccine to protect against liver cancer and against a sexually transmitted disease.

2.4 Towards a third generation

The third generation of vaccines can be described as genetic vaccines, since it is using genetic sequences to support information of the pathogen. It was made possible thanks to the latest discoveries in biotechnologies and genetics such as gene sequencing [4].

There are three main categories:

- Viral vector vaccines: a specific gene of the pathogen is put into an innocuous virus for humans. This harmless virus can deliver the selected genetic material of the pathogen into the human cells. Then the cells produce proteins from the pathogen which can be used to train the immune system as a classic vaccine. For instance, the vaccines from the firm AstraZeneca and the Oxford University or Janssen against Covid-19 rely on this technique. A major drawback of viral vector vaccines is that it also makes us immune against the vector virus, which in the future will have difficulties to deliver the genetic material.
- mRNA vaccines: this will be discussed in the next section.
- pDNA (Plasmid DesoxyriboNucleic Acid) vaccines: the gene from the pathogen is encoded with DNA instead of RNA and it is injected in our cell thanks to the same technique as mRNA vaccines. Nevertheless it must be carefully studied because of the possible effects on human DNA.

3 mRNA vaccines: a promising technology

3.1 Principle

It is important to understand the differences between *in vitro*, *in vivo* and *in situ*.

The two first incentive were quicker development (with a financial and medical interest) and a wider and safer immune response. However, it can lead to other uses and it creates new perspectives as regards therapies.

cf [8]

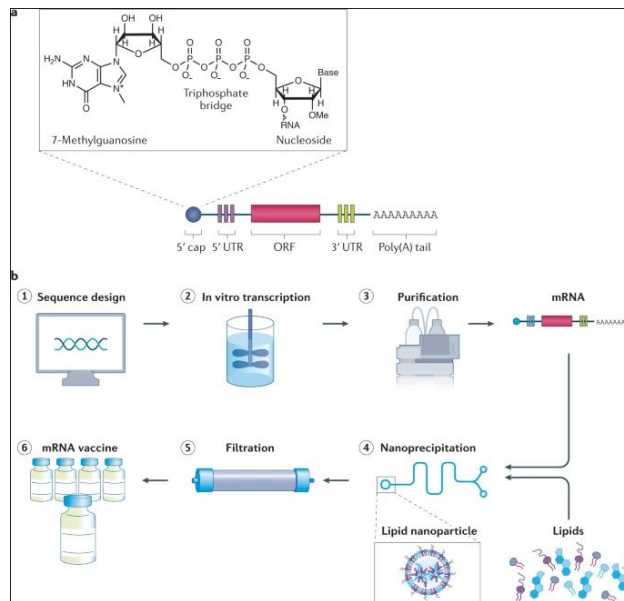


Figure 2: Synthesis of [10]

3.2 Development

The first tests were realized in the 1990s by Wolff. [17] But much progress has been needed in the 2000s as regards the synthesis of mRNA.

The main steps to develop a mRNA vaccine are:

- First, scientists have to find an antigen that can characterize the pathogen thanks to reverse genetics.
- Second, the gene encoding this antigen is sequenced. The sequence can be shared on the Internet all the world, allowing global research and speeding up development.
- Then, the gene can be synthesized thanks to recent biotechnologies like artificial gene synthesis, often called DNA printing.
- This DNA constitutes a DNA template plasmid, and translated *in vitro* into mRNA. All this process is realized thanks to enzymes, especially the RNA polymerase. The DNase then degrades the template DNA. It is also an enzyme which adds the required 5' cap, stabilizing the molecule.
- A step of purification is then critical to obtain an efficient mRNA vaccine. Enzymes, remaining DNA and incorrect transcripts are removed. There are also double-stranded RNA (dsRNA) which can alter the immune activation if they are still present. Finally

Nowadays, there are two major types of mRNA vaccines:

- Conventional mRNA which only corresponds to the studied gene
- Self-amplifying mRNA which also encodes the replication machinery to produce many antigens.

3.3 Advantages and drawbacks

Among the technologies of third generation, it is the most promising compared to viral vectors and pDNA vaccines in terms of efficiency.

The main advantages are:

- It embodies the idea of "vaccine on demand". It would allow people to face epidemics such as for Zika and Ebola as well as pandemics.

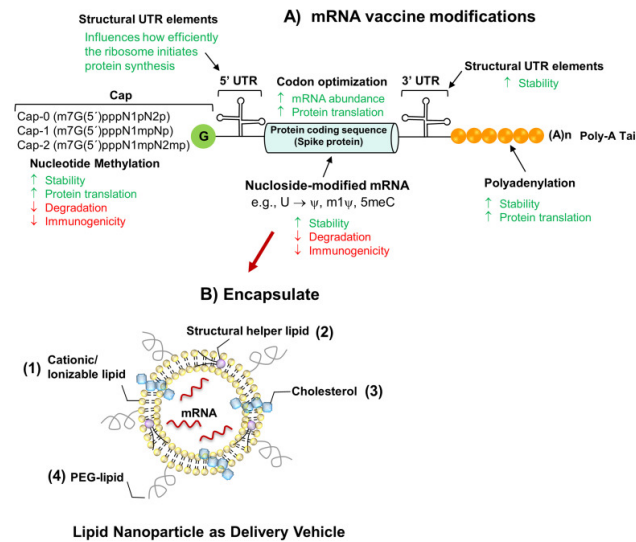


Figure 3: Synthesis of [6]

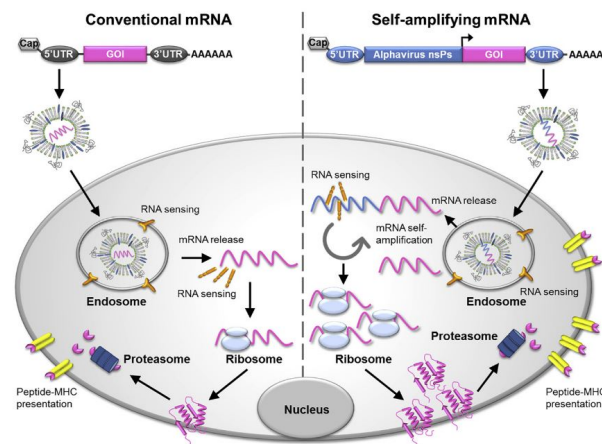


Figure 4: Synthesis of [9]

- It creates a complete immune response (humoral and cellular).

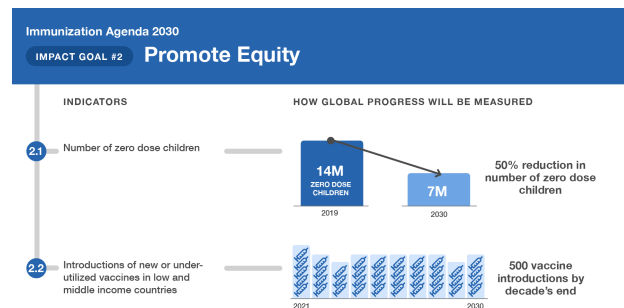
The main drawbacks are:

- It is very difficult to preserve the vaccine because mRNA is not stable.

Disease	Maximum number of cases (year)	Number of cases in 2014
Diphtheria	206,939 (1921)	0
Measles	894,134 (1941)	72
Mumps	152,209 (1968)	40
Pertussis	265,269 (1934)	311
Polio (paralytic)	21,269 (1952)	0
Rubella	57,686 (1969)	0
Tetanus	1,560 (1923)	0
<i>Hemophilus influenzae</i> type B infection	~20,000 (1984)	134
Hepatitis B	26,611 (1985)	58

4 New stakes for vaccines in the 21st century

4.1 World inequalities

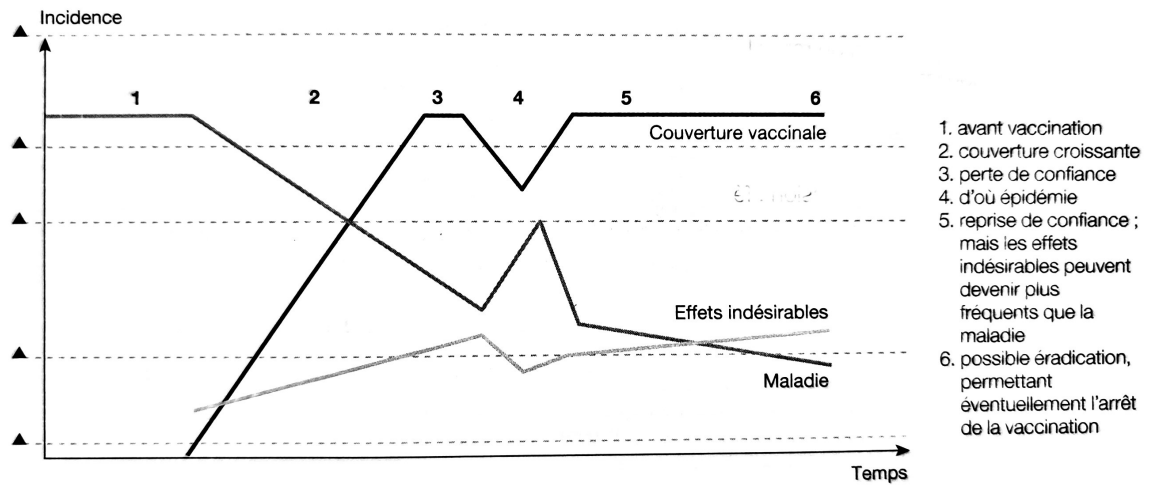


4.2 Public policies

Good manufacturing practice (abbreviated GMP) represents a minimum standard for medicine manufacturers, for instance useful to produce mRNA at an industrial level. In the US, the FDA is determining it, and in Europe it is the European Medicines Agency.



F-146-1 : Phases induites par la vaccination sur l'épidémiologie d'une maladie, la perception de celle-ci, et la perception du risque lié à la vaccination



Firstly it is an individual protection.

Secondly it protects the whole population thanks to herd immunity: the pathogen is less likely to be transmitted ; otherwise it would be exponential, as people observed for the Covid-19 pandemic.

4.3 New uses and technical challenges

Currently, there is no real therapeutic vaccines.

5 Conclusion

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A Source code

The source code of this document is available at <https://github.com/florian6973/biology-report>. It was written using Visual Studio Code and compiled with TeX Live (Windows).

In this repository a beamer is also available, to present this report.