université PARIS-SACLAY

FACULTÉ DE PHARMACIE

Sécurité d'emploi des vaccins et effets secondaires

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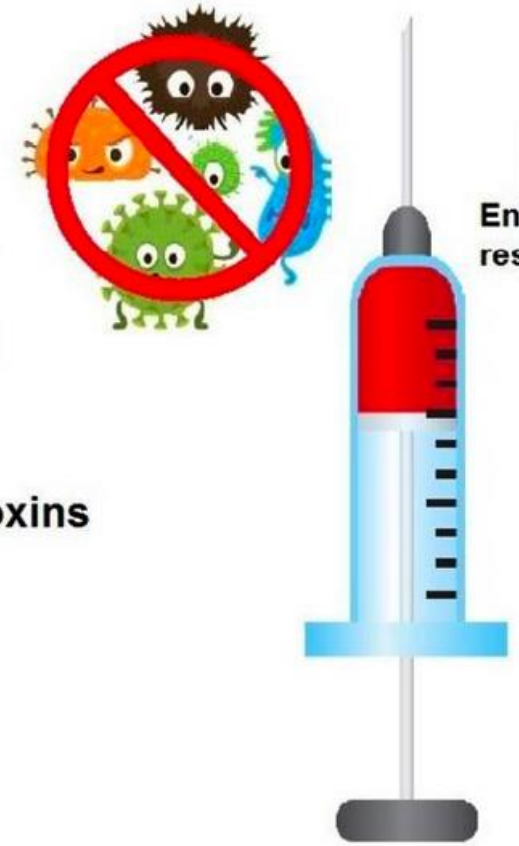
VACCINE COMPONENTS

ACTIVE COMPONENT

- **Antigen**
  - Weakened microbes
  - Inactivated microbes
  - Parts of microbes
  - Modified microbial toxins



EXCIPIENTS

- **Adjuvants**
  - Enhance body's immune response towards antigens
- **Stabilizers**
  - Protect vaccine from harsh processing conditions
- **Preservatives**
  - Prevent contamination during use of multiple-dose preparations
- **Antibiotics**
  - Prevent microbial contamination during manufacture
  - Used to inactivate microbes in toxoid vaccines
- **Trace Components**
  - Minute quantities of residual components used during manufacture
  - (ie: inactivating agents, antibiotics, egg proteins etc)

https://covid19.trackvaccines.org/vaccine-types/

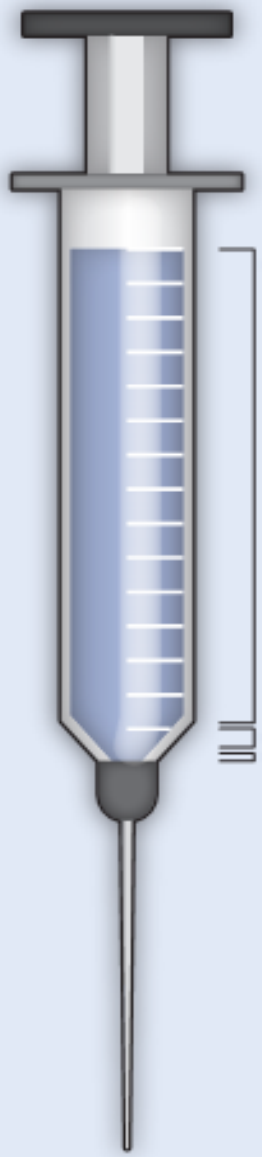
Vaccine components

- **The antigen:**
  - Live attenuated vaccines (smallpox)
  - Inactivated vaccines (rabbies)
  - Bacterial polysaccharides
  - Modified viral or bacterial vectors encoding antigens
  - Peptides, recombinant proteins
  - DNA, RNA (Synthetic vaccines)
  - Encapsulated DNA (Sars CoV 2)
- **Adjuvant:**
  - Enhance the immune response
- **Excipients:**
  - Needed for product formulation
- **Contaminants, impurities**

Quels sont les risques ?

CALCULATING RISKS

Some vaccines have risks that are common but mild. A few have more serious risks, but these are very rare.



1 COMMON: MORE THAN 1 IN 100 DOSES
Redness, swelling or soreness at the site of an injection are common for many vaccines, as are mild fevers. Nausea, vomiting and diarrhoea have been reported for a few.

2 LESS COMMON: 1 IN 100 TO 1 IN 100,000
High fevers can occur in this range, as can fever-induced convulsions from vaccines such as that for measles, mumps and rubella (1 in 3,000 doses).

3 RARE: 1 IN 100,000 TO 1 IN 1 MILLION
Preliminary data suggest that current rotavirus vaccines are associated with intussusception, an infolding of the bowel, in about 1 in 100,000 first doses, but the overall risk is unclear. Severe allergic reactions to some vaccines are generally less common than this, in the order of 1 in 1 million.

4 INCONCLUSIVE: NOT ENOUGH DATA
Guillain-Barré syndrome, a paralytic disorder, has been associated with some seasonal influenza vaccines, but a causal link has not been firmly established. Serious disorders have been reported after other vaccinations, but many are so rare that determining causality is difficult.

Source: US Centers for Disease Control and Prevention. For more information, see go.nature.com/s7rfio

Possible origins of vaccine toxicity

- For small molecules safety evaluation is mainly based on regulatory and experimental toxicological studies
- For therapeutic proteins/gene or cell therapy, toxicity is mainly anticipated based on the mode of action and the target (antibodies, recombinant proteins…)

- For vaccines it is both: components need to be tested (adjuvants, preservatives, excipients…) for direct toxicity and mode of action can be an issue
  - Exagerated immune reaction augmented by adjuvants : reactogenicity
  - Autoimmunity due to mode of action: antigen…
  - Direct toxicity link to a chemical components = organ toxicity

Immune response to the vaccine antigen

- Cross-reactivity between self antigen and vaccine-produced antibodies
- Anaphylactoid reactions
- Hypersensitivity reactions

Adjuvant

- Reactogenicity
  - Cytokine release
  - Vasoactive amines…
- Target organ toxicity
- Immunogenicity ?
- Auto-immunity ?

Hypersensitivity, Autoimmunity

Non-specific activation of the immune system, Inflammation

Target organ toxicity

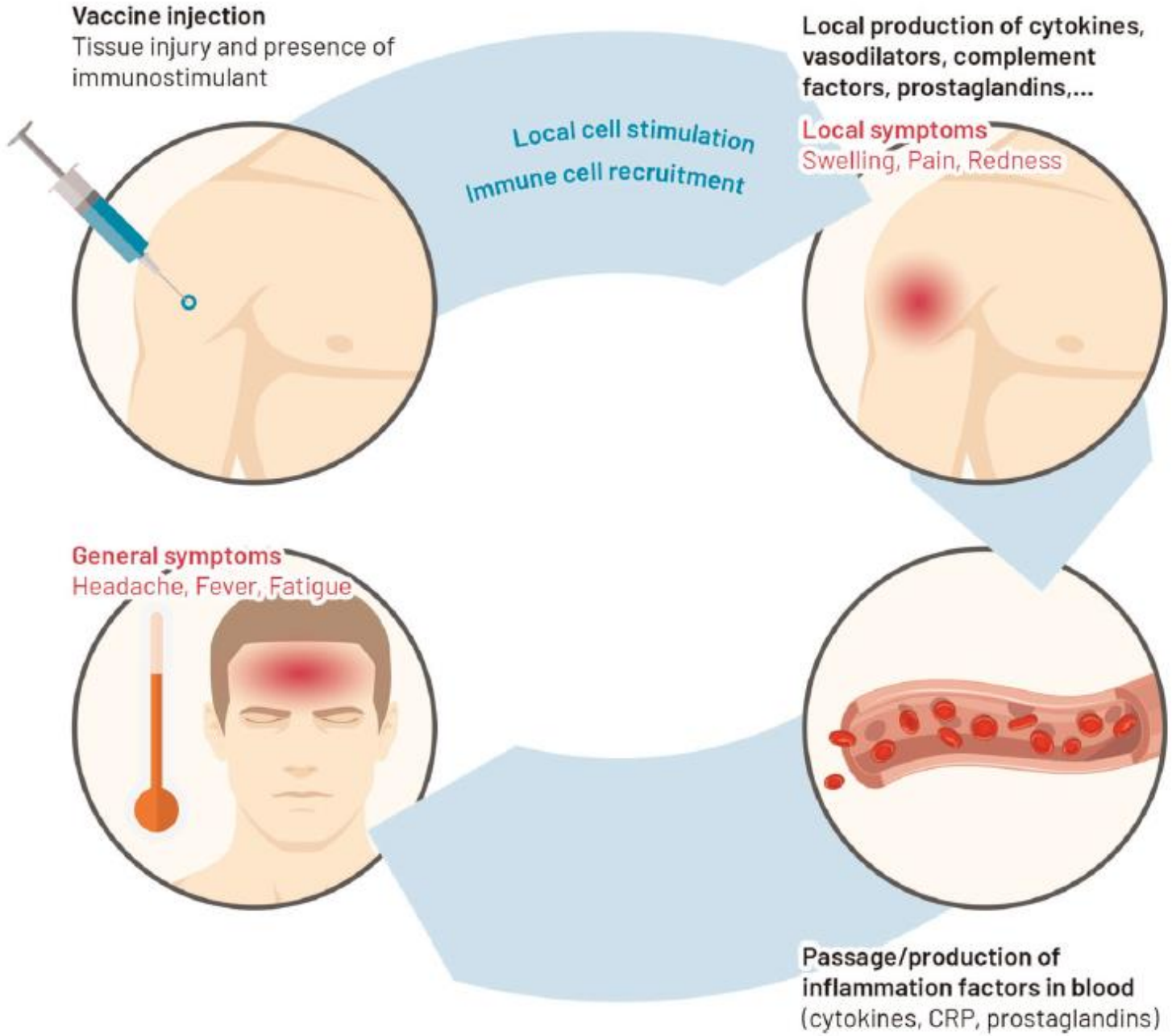
Contaminants, impurities

Immunogenicity (gelatin, ovalbumin…), direct toxicity

Reactogenicity

Reactogenicity

- Redness, swelling or pain at the site of injection are common for many vaccines under the term of reactogenicity.
- Occurs soon after vaccine injection (within 24 hrs).
- Represents the physical manifestation of the inflammatory response to vaccination, and can include:
  - Injection-site pain, redness, swelling or induration at the injection site
  - Systemic symptoms, such as fever, myalgia, or headache.
  - High fever can be present at a rate of 1/100 to 1/100 000
    - fever-induced convulsions can be present from vaccines such as measles, mumps or rubella.
  - But it is usually mild and self-limiting and rarely have serious medical consequences
- Mainly due to the adjuvant component of the vaccine but not only (tissue injury, inactivated or live-attenuated pathogens…).
- Can lead to needle fear, long-term negative attitudes and non-compliant behaviors impacting vaccination success.



Hervé C et al, npg vaccines (2019)

- All vaccines can activate pattern recognition receptors (PRRs) and provoke mediators production
  - PRRs are expressed by immune cells (monocytes, macrophages, mast cells, dendritic cells, keratinocytes, skeletal muscle cells)
  - Role: recognition of what is harmful by the immune system, create inflammatory conditions
- Macrophages and mast cells, are key target cells
- Within minutes after injection:
  - Proinflammatory cytokines and chemokines production
  - Complement factors (C3a and C5a) activation
  - Vasoactive amines and bradykinin : vasodilatation
  - Cell recruitment from blood, redness and swelling
- **Adjuvants can participate and enhance reactogenicity: mode of action = vaccine specific immune response**

Type of adjuvant

- **Two major classes:**
- ***Vehicles***
  - Components that present vaccine antigens to the immune system in an optimal manner
    - controlled release and depot delivery systems to increase the specific immune response to the antigen
  - Mineral salts, emulsions, liposomes, virosomes (nanoparticles made of viral proteins like influenza hemagglutinin and phospholipids), biodegradable polymer microspheres, QuilA, QS21/saponins, immune stimulating complexes (i.e. ISCOM, ISCOMATRIX)
- ***Immunostimulants***
  - Components that directly engage the immune system to increase responses to antigens (e.g. TLR/NLR/RLR ligands, cytokines, bacterial exotoxins)
  - Activation of the inflammasome

Adjuvants in approved vaccines

- **Aluminium salts (phosphate, hydroxide, alum)**
  - Depot formation: antigen release
  - Particulate structure formation: DC uptake
  - Augmentation of MHC class expression and antigen presentation
  - Inflammasome activation: chemokines, cytokines
- **MF59**
  - Oil (squalene, shark oil) in water nano-emulsion
  - Depot effect: increase antigen uptake
  - Stimulation of cytokine and chemokine production
- **AS03** (squalene, tocopherol-based oil-in-water emulsion)
- **MPL** (monophosphoryl lipid A)
  - Glycolipid from *salmonella* cell membranes
  - TLR-4 ligand
  - AS04 = MPL + alum
- **Other adjuvants**
  - Saponins (Quil A, ISCOM, QS-2)
  - AS01 (MPL liposomal formulation)
  - AS02 (oil in water emulsion containing QS-21)
  - TLR-7 (imiquimod) and TLR-8 (resiquimod) agonists in cancer vaccines
  - CpG 1018
  - “Adjuvant systems” (proprietary) ex: Matrix-M™ (Nuvaxovid™)

In resume

- It is unknown whether the specific molecular pathways that cause symptoms are independent from pathways involved in the generation of antigen-specific response
- This knowledge is required for the design of less reactogenic vaccines or targeted strategies aimed at reducing the severity of symptoms
- Reactogenicity is highly variable among the human population
  - Factors influencing reactogenicity
    - Host factors (age, gender, body mass index, stress…)
    - Vaccine factors (composition, injection route, adjuvants…)

Auto-immunity

« Observed » cases of vaccine-induced « autoimmunity »

- « Swine flu-H1N1 » vaccine (1976): Guillain-Barré syndrome (GBS)
  - ~45 million persons were vaccinated over a 10-week period against an H1N1 influenza virus of swine
  - GBS is a rare, acute, often post-infectious, immune-mediated disorder of the peripheral nervous system with rapidly evolving, bilateral, ascending motor neuron paralysis
    - The mortality rate is low with intensive care support
  - Restrospective analysis found a range of 4,9 to 11,7 cases of GBS per 1 million adult vaccinees in the 6 weeks after immunization
    - Incidence of GBS in the general population estimated to be 0.6–4.0 cases per 100,000 per year
  - GBS may be expected to occur at a background rate of 0.07–0.46 cases per 100,000 vaccinees within 6 weeks of any vaccination
  - Putative mechanism
    - Molecular mimicry with antigens on infecting pathogens (antibodies to gangliosides ie. anti-GM1)
    - Hypothesis: formation of sialic acid–Hemagglutinin complexes that mimic GM1 ganglioside in susceptible hosts due to low levels of viral neuraminidase in the vaccine preparation (formulation problem)

H1N1 vaccination and narcolepsia (2009-2010)

- August 2010: possible association between exposure to AS03 adjuvanted pandemic H1N1 vaccine and occurrence of narcolepsy-cataplexy in children and adolescents in Sweden and later in Finland
  - Recommended discontinuation of this vaccine in these countries
  - At the same time, France reported 6 cases, 5 following the AS03 adjuvanted vaccine and 1 following an inactivated split pandemic H1N1 vaccine
- **Simakajornboon N et al, Sleep (2022)** "Report from the pediatric working group of the sleep research network"
  - Significant increase in pediatric narcolepsy incidence after the 2009 H1N1 pandemic in the United States.
  - Magnitude of increase is lower than reported in European countries and in China.
  - Temporal correlation between monthly H1N1 infection and monthly narcolepsy incidence, suggests that H1N1 infection may be a contributing factor to the increased pediatric narcolepsy incidence after the 2009 H1N1 pandemics.
- **Juvet LK et al, Front Immunol (2021)** "Norwegian Institute of Public Health"
  - Main systematic review on narcolepsy found a 5 to 14-fold increased risk in children, and a 2 to 7- fold increased risk in adults after vaccination with Pandemrix™
  - The attributable risk of narcolepsy one year after vaccination was 1 case per 18 400 vaccine doses in children/adolescents, and 1 case per 181 000 vaccine doses in adults

Type 1 Narcolepsy (T1N) = auto-immune disease ?

- Type 1 Narcolepsy (T1N) is a disabling neurological disorder that affects 1/2000 individuals
  - Excessive daytime sleepiness, disturbed nocturnal sleep, sleep paralysis, hypnagogic hallucinations and sudden episodes of bi-lateral losses of muscle tone, triggered by emotions (cataplexy)
- Caused by the loss of (> 90%) orexin/hypocretin (HCRT)-producing neurons
- Highly (98%) associated with HLA class II haplotype DQA1*01:02/DQB1*06:02 (DQ0602)
- Also strongly associated with T cell receptor (TCR) alpha polymorphism and other immune response genes (cathepsin H, tumor necrosis factor (ligand) superfamily member 4)
- All Pandemrix™-associated narcolepsy cases were positive for HLA class II DQB1*06:02
- All these evidences support that T1N is an autoimmune disorder.

Other reported putative cases of auto-immunity due to vaccine

- Hepatitis B vaccine: multiple sclerosis
  - « Risks of HBV vaccination are only theoritical » (2003)
  - **No association found**
- Measles, mumps and rubella vaccines: aseptic meningitis
  - Related to the mumps virus component (Urabe strain vs Jeryl Lynn strain)
  - Infection by the live attenuated vaccine and not autoimmune disease ?
  - Still not resolved
- Varicella vaccines: neuropathy
  - 400 « possible » immune-mediated adverse events out of 9,7 millions doses of Varivax®

Mechanisms of autoimmunity

- **Creation of new antigens : neo-epitopes**
  - Specific lymphocytes for these « new antigens » will not be deleted by thymic selection
  - «Altered self» (pi-concept and drugs)
  - Formation of neo-epitopes (CYP450 and drugs…)
- **Revelation of cryptic antigen**
  - After extensive cytolysis/necrosis
  - « non-tolerized » antigens
- **Cross-reactivity**
  - Cross-reactivity (anti-procainamide antibodies and histones…)
- **Molecular mimicry**
  - Molecular mimicry to self antigens (infections…)
- **Dysruption of self tolerance**
  - Alteration of T-reg lymphocytes function

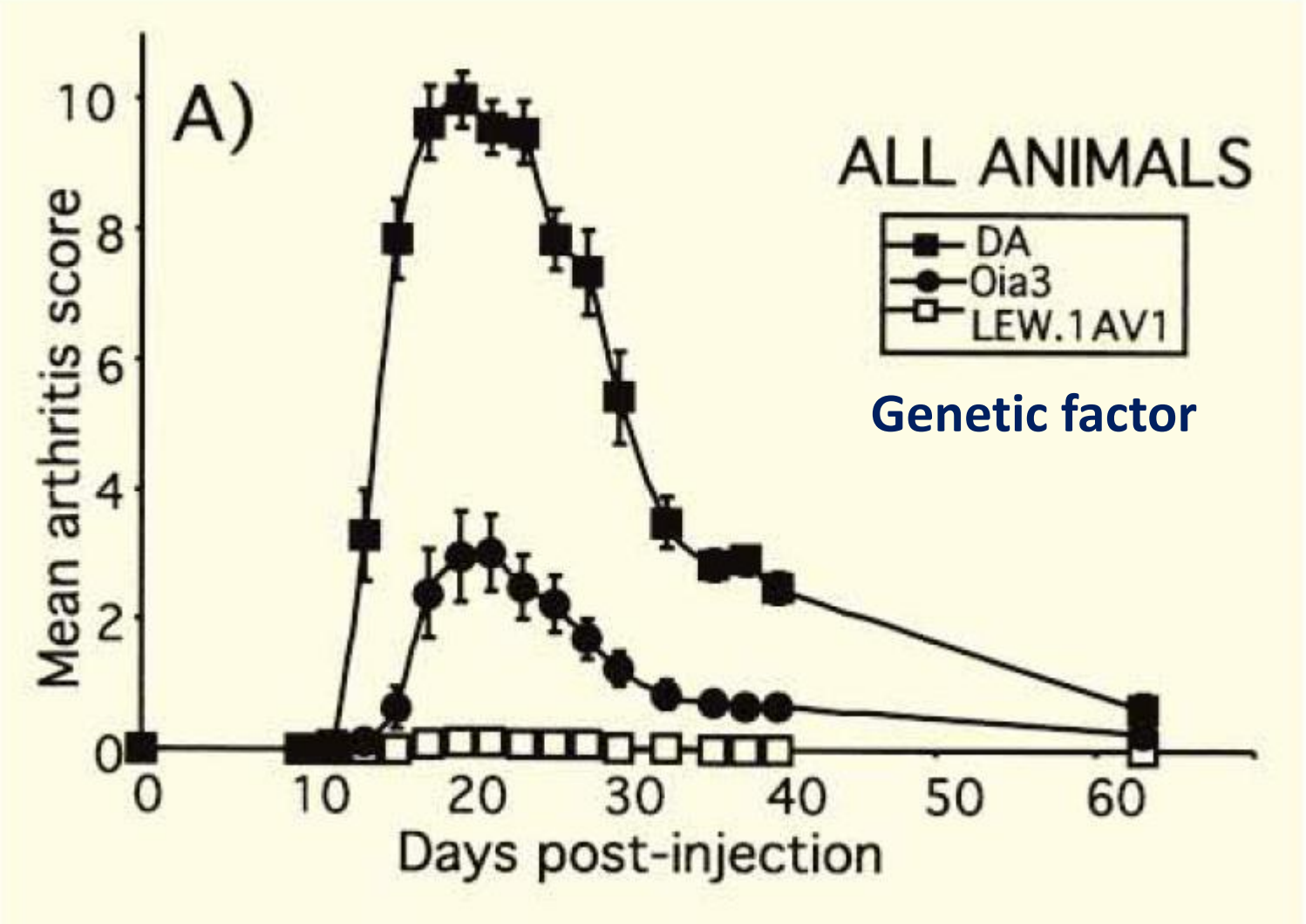
Vaccine and autoimmunity

- To establish a role for vaccines in autoimmunity you need several conditions
  - Infections should be linked to autoimmunity
    - Transition from viral infection to autoimmunity may occur in selected cases
  - Mechanisms whereby exposure to infectious antigens leads to autoimmunity must be established
  - Evidence must be present that vaccinated patients developed an autoimmune disease. Association doesn’t mean causality

Could adjuvants increase the risk of autoimmunity?

- **Some animal data have suggested a link between vaccine/adjuvants and autoimmunity**
  - Complete Freund's adjuvants (mineral oil, Mycobacterium) induces Experimental Allergic Encephalitis
  - Squalene (adjuvant component of AS03, AF03) can induce arthritis in rats and lupus in mice
  - Adjuvant can induce lupus auto-antibodies in mice
- **Human clinical observations**
  - Gulf war syndrome ????
  - Macrophagic myofaciitis and fibromyalgia ????

Autoimmune arthritis: Squalene



Rikard Holmdahl. Immunological Reviews (2001)

Allergy to vaccine components

- Measles, mumps, and rubella vaccines and influenza, yellow-fever, and tick-borne encephalitis vaccines
  - may contain low amounts of **ovalbumin** and be responsible for anaphylactic reactions in egg-allergic patients
- Anaphylactic reactions have been reported in patients without ovalbumin allergy
  - In patients immunized with ovalbumin- and **gelatin**-containing vaccines, such as measles, mumps, and rubella vaccines
  - In patients immunized with other gelatin-containing vaccines such as Japanese encephalitis virus (JEV) and varicella vaccines

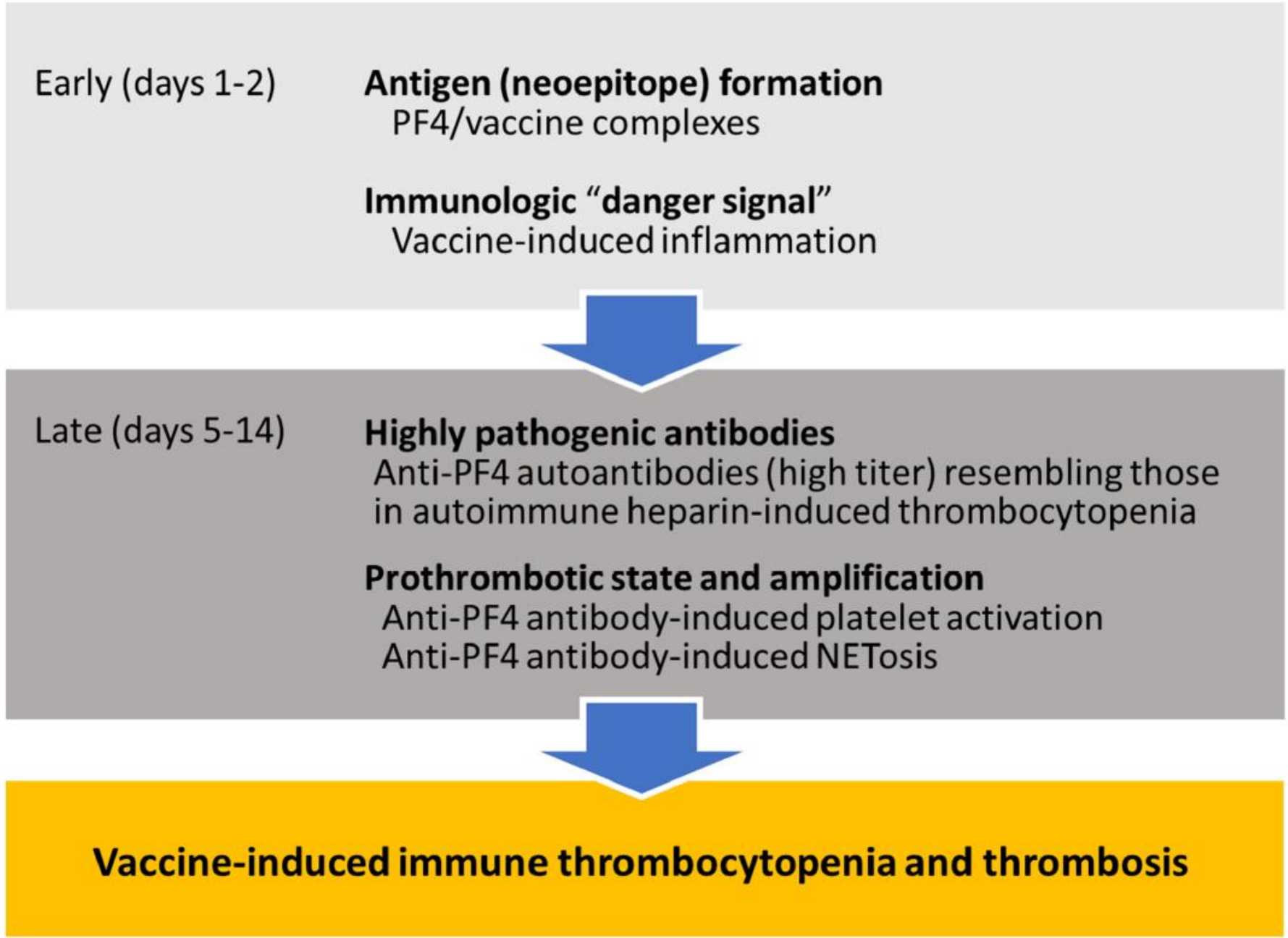
- Severe reactions have been reported in neonates receiving a first injection of BCG and in patients after a booster injection of the BCG vaccine
  - Diagnosis was based on the presence of **dextran** HS anti-dextran antibody (IgM/IgG) in mother's serum and cord blood and in the serum of patients
  - Anti-dextran antibodies in patients may result from previous immunization with BCG or from occult sensitization by saccharides expressed on the outer membrane of bacterial microorganisms
- *Mechanisms of dextran reactions*
  - Circulating immune complex (CIC) formation between preexisting anti-dextran IgG antibodies and dextran injected with the BCG vaccine
  - Complement system activation by CIC, and mast cell and basophil activation by complement derived factors
- Those sensitizations may be responsible for low levels of anti-dextran-reacting antibodies (IgM/IgG) in serum of up to 70 % of healthy subjects

Key questions Hypersensitivity

- True anaphylactic reactions are rare event
- How to prevent anaphylactoid reactions ?
- Immunogenicity of adjuvant: could it play a role in vaccine hypersensitivity ?
- Is there a link with the type of immune response (TH2 vs TH1) ?
- Vaccine composition and pre-immunisation of patients to natural components ?

Vaccine-Induced Prothrombotic Immune Thrombocytopenia (VIPIT) Following AstraZeneca COVID-19 Vaccination

- Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a rare but severe immunological reaction to the non-replicable adenoviral vector-based COVID-19 vaccines.
- Extreme activation of platelets and the coagulation system leads to a high risk of death from venous or arterial thrombosis or secondary haemorrhage.
- Platelet count and D-dimer are the most reliable markers.
- **Mechanisms**
  - Visualized vaccine components are forming antigenic complexes with platelet factor 4 (PF4) on platelet surfaces.
  - The anti-PF4 antibodies obtained from VITT patients bound on these complexes.
  - PF4/vaccine complex formation and the vaccine-stimulated proinflammatory milieu trigger a pronounced B cell response that results in the formation of high-avidity anti-PF4 antibodies in VITT patients.
  - High-titer anti-PF4 antibodies potently activated platelets in the presence of PF4 or DNA and polyphosphate polyanions.
  - Anti-PF4 VITT patient antibodies also stimulated neutrophils to release NETs in a platelet PF4-dependent manner.
- Which patients are immunized ? Role for T-cells and adaptive immunity ?

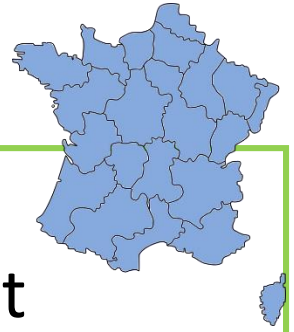

Early (days 1-2)
Antigen (neoepitope) formation
PF4/vaccine complexes
Immunologic "danger signal"
Vaccine-induced inflammation
Late (days 5-14)
Highly pathogenic antibodies
Anti-PF4 autoantibodies (high titer) resembling those in autoimmune heparin-induced thrombocytopenia
Prothrombotic state and amplification
Anti-PF4 antibody-induced platelet activation
Anti-PF4 antibody-induced NETosis
Vaccine-induced immune thrombocytopenia and thrombosis

Chiffres de la vaccination contre la COVID-19

MONDE

4 101 203 911 personnes ayant au moins reçu une dose

- 24 vaccins développés contre la Covid-19



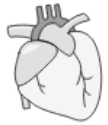
FRANCE

53 769 307 de personnes complétement vaccinées (au 15/09/2023)

- 18,9% de non vaccinés
- 156 788 000 injections tous vaccins confondus
- **193 934 cas déclarés** suite à la vaccination tous *vaccins confondus.
  - ✓ *6 vaccins autorisés en France : Comirnaty-Spikevax-*Vaxzevria-Janssen*-Nuvaxovid-Vidpertyn

Effets Indésirables d’intérêts, imputables aux vaccins COVID 19

Réactogènicité



Myocardite/Péricardite

Thrombocytopénie Thrombotique Induite par le Vaccin (TTIV)

Troubles menstruels : **Saignements menstruels abondants/importants**

Reported issues with vaccines: real and fake/not proven

- Rotavirus vaccines are suspected to be associated with intussusception in about 1/100 000 first doses, mechanism unknown
- Guillain-Barré syndrome: neuromuscular paralysis
  - rare reports, unknown association and incidence
    - 5.8 cases per million first doses of adenovirus vectored COVID-19 vaccines, otherwise not distinguishable from incident naturally occurring cases
  - 1000 cases (mean age, 47 years) of GBS reported after vaccination in the United States between 1990 and 2005 (hep B and influenza vaccines)
  - Not possible to rule out an effect in susceptible individuals
- Myofasciitis macrophages and aluminium salts
  - Well-described; persistent histological lesion with macrophage infiltration
- Narcolepsia associated with H1N1 vaccination
- Auto-immunity
  - SARS-CoV-2 vaccine ChAdOx1 nCov-19 and severe thromboembolic complication
- Pericarditis and mRNA vaccines
  - mRNA covid-19 vaccines are associated with a rare but heightened risk of acute myocarditis and pericarditis
  - More frequent in young adults after the second injection
  - Primary hypercatecholaminergic state ? 16 mechanisms proposed.
- *Hepatitis B vaccine and multiple sclerosis: not proven, no recorded association*
- *Aluminium salts and chronic fatigue syndrome or fibromyalgia: not proven, no recorded association*

But…

- Black S et al. The Lancet (2009)

- “if a cohort of 10 million individuals was vaccinated in the UK, 21,5 cases of Guillain-Barré syndrome and 5,75 cases of sudden death would be expected to occur within 6 weeks of vaccination as coincident background cases. In female vaccinees in the USA, 86·3 cases of optic neuritis per 10 million population would be expected within 6 weeks of vaccination. 397 per 1 million vaccinated pregnant women would be predicted to have a spontaneous abortion within 1 day of vaccination”.

Regulatory requirements for non-clinical safety evaluation of vaccines

Guidelines for non-clinical evaluation of vaccines

WHO

- WHO Guidelines on nonclinical evaluation of vaccines. WHO Technical Report Series, No. 927, 2005 Annex 1
- WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines. WHO Technical Report Series No. 987, 2014; WHO Expert Committee on Biological Standardization Sixty-fourth report
- WHO Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines. WHO Technical Report Series No 941, 2007 Annex 1

EMA

https://www.ema.europa.eu/en/human-regulatory/research-development/scientific-guidelines/multidisciplinary/multidisciplinary-vaccines

- EMA. Guideline on dossier structure and content for pandemic influenza. Vaccine marketing authorization application. 2009
- EMA. Guideline on influenza vaccines. Non-clinical and clinical module. 2016
- EMA. Guideline on Adjuvants in Vaccines for Human Use. 2005
- EMA. Guideline-non-clinical-studies-required-first-clinical-use-gene-therapy-medicinal-products. 2008
- EMA. Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products. 2018

FDA

- FDA. Guidance for Industry : Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications FDA/CBER. 2006.
- FDA. Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Disease Indications FDA/CBER. 2007
- FDA. Quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines. 2011

Animal models, selection

- **Safety studies**
  - Important to document the pharmacological activity/immune response of the vaccine in the presence and absence of adjuvant in species used for safety studies
  - Observe and immune response enhanced by the adjuvant through a similar mechanism as expected in humans
  - In compliance with GLPs
  - Not necessary to conduct the nonclinical safety study in the same animal species used for proof-of-concept or non-clinical pharmacology studies
  - Nonhuman primates should be used only if no other relevant animal species is available.
  - ***Use of a single species is generally acceptable***
- **Pharmacology studies**
  - The nature and extent of an immune response (humoral and cellular) determines the efficacy of a vaccine.
  - Might be different in animals and in man
  - A proof-of-concept needs to be provided from non-clinical investigations before clinical trials can be started.
  - Proof-of-concept studies be undertaken using an animal species in which:
    - An immune response to the vaccine antigen is developed/Documented immune response to the vaccine (humoral and/or cell mediated immune response) is needed
    - The immune response to the antigen is enhanced by the adjuvant through a mechanism similar to that expected in humans
    - Ideally, the animal immune response should mimics the human one

Other issues for safety studies

- Dose = highest intended human dose
  - Commonly, only one dose tested
- Evaluation of the adjuvant alone can be important for novel adjuvants that have not been studied previously or will be used in multiple different vaccine formulations
  - Advisable to include additional (lower and higher) doses of the adjuvant component(s) in order to identify a safe dose that could be used in a first-in-human clinical trial, as well as safety signals that should be monitored in the proposed clinical trial.
- Use the intended route for clinical administration
  - Feasibility related to route of administration
    - Intranasal route in rodents (possible delivery to the lungs)
  - Rabbit is sufficiently large that administration of the human dose can generally be achieved
    - Preferred species for IM injection, for other aspects is the rabbit really useful ? Is the immune response always characterized in Rabbits ? Adjuvant mechanism has been evaluated in Rabbits ?
- Availability of historical data
  - Ex: Recent historical data for ferrets or marmoset are scarce
- Availability of reagents for immune analysis
  - Ex **Rodents** vs rabbit or dogs

In conclusion

- **Safety issues have been documented with vaccines but concern most of the time very few susceptible individuals**
- **Reactogenicity is common and can be severe in some individuals**
- **Importance to build an extensive knowledge on the mechanism of action of adjuvants and its possible extrapolation to man**
  - ex mouse TLR vs Human TLR…
  - Early in the development ?
  - Will help to define and predict potential side effects
- **Important to understand the local effects of adjuvants and their consequences for safety (fever…)**
- **Important to understand the distribution of adjuvants and its consequence to systemic effects**
- **Autoimmunity still an issue and a frequent asked question**
  - Animal models are of no help to date
  - The value of administration of very high dose of adjuvant using non conventional route of administration are highly debatable for human safety
  - Working on signal detection and pharmaco-epidemiological studies in humans should be developed
- **Distinguish novel vaccines from new vaccines**
  - Novel: new adjuvant, need extensive evaluation
  - New: contains known products, perform regulatory non-clinical approaches

Néanmoins…

- Le vaccin reste un médicament qui est évalué sur un rapport bénéfice/risque
- Le risque (maladies infectieuses) n'est plus perceptible par la société des « pays développés » car présent à des incidences très faibles grâce à la vaccination
  - **Le bénéfice n'est donc plus évaluable par la population**
  - **Tout événement « toxique » « attribué » à un vaccin devient donc insupportable car provoqué chez un individu sain**
- Les acteurs de la santé publique se doivent d'informer en toute connaissance la population sur le risque de ne pas être vaccinée
  - Sur une base scientifique
  - En évitant la polémique
- Nos acteurs de santé connaissent-ils suffisamment les vaccins, la vaccination et les adjuvants ?