National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Overview of animal models in vaccine testing

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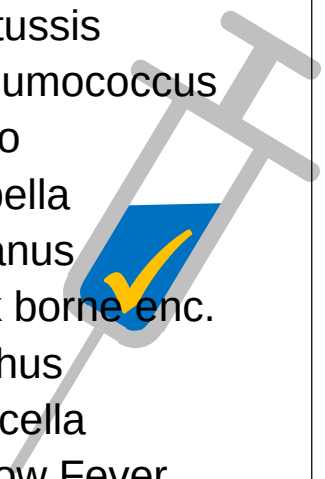
List of Effective Vaccines

Marketed vaccines

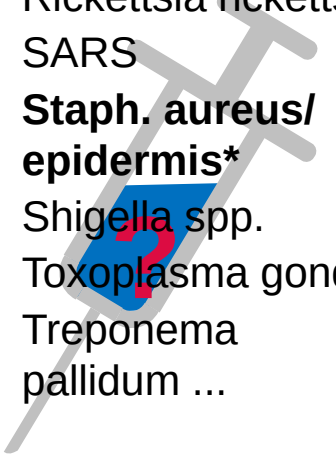
- Cholera
- Diphtheria
- H. influenza B
- Hepatitis A/B
- Influenza
- Measles
- Men AWYC
- Mumps
- Pertussis
- Pneumococcus
- Polio
- Rubella
- Tetanus
- Tick borne enc.
- Typhus
- Varicella
- Yellow Fever



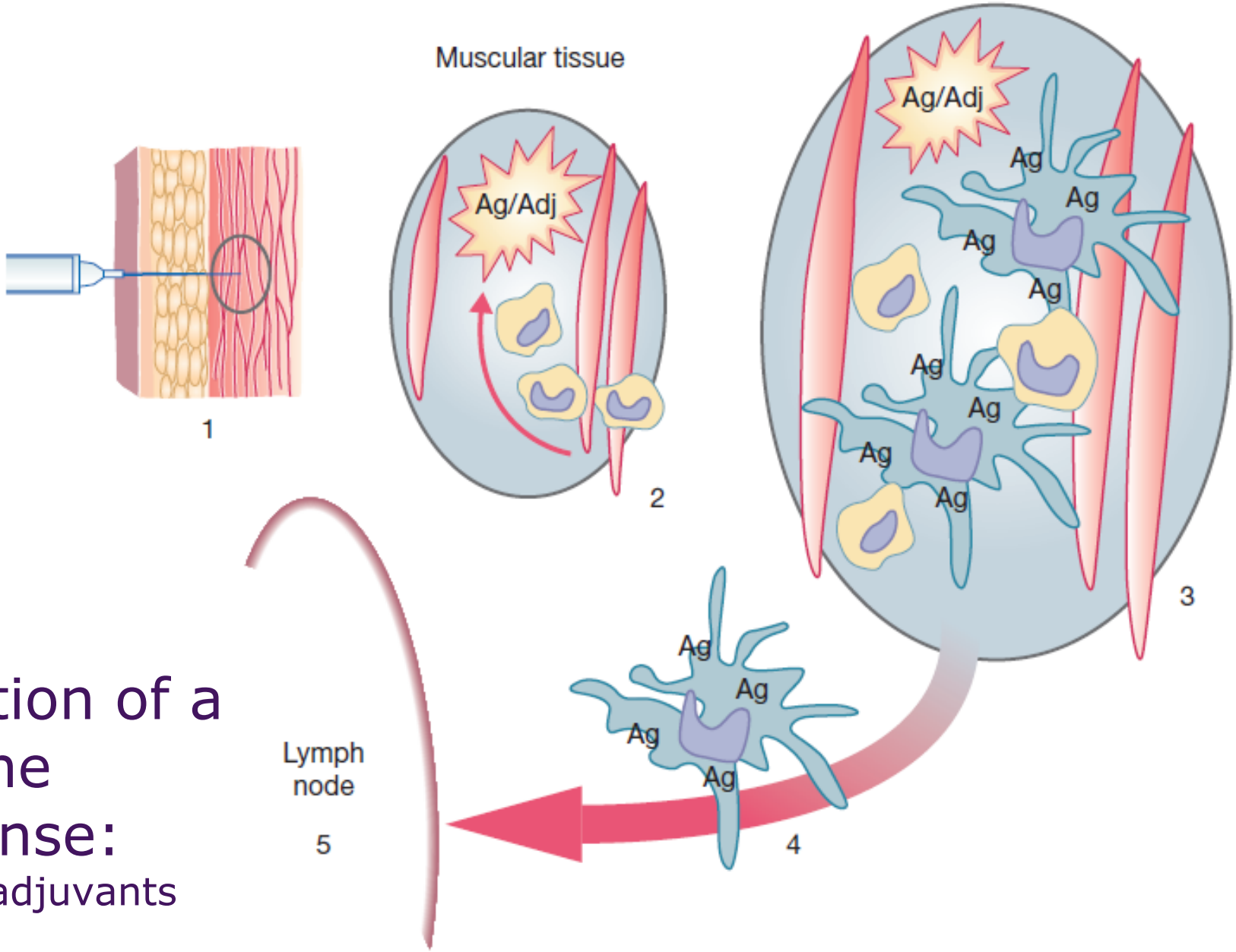
No satisfactory vaccines against

- Borrelia burgdorferi
- Chlamydia spp.
- Cytomegalovirus
- Enterococcus
- **Group A strep.***
- **Group B strep.***
- Hepatitis C*
- HIV
- **Jap Enc V***
- Legionella pneumophila
- Mycoplasma pneumoniae
- **TB (in adults)***
- Leishmaniasis

» Malaria
» Neisseria gonorrhoeae
» Nontypeable Haemophilus inf
» Plasmodium vivax
» Pseudomonas aeruginosa
» Rickettsia rickettsii
» SARS
» **Staph. aureus/ epidermis***
» Shigella spp.
» Toxoplasma gondi
» Treponema pallidum ...



Initiation of a vaccine response:
Role of adjuvants



From Siegrist, 2011

Regulatory Guidelines

EU CPMP/SWP/465/95 issued 1997

Preclinical Pharmacological and Toxicological testing of vaccines

WHO issued 2005

Guidelines on preclinical testing of vaccines

FDA issued 2007 (Final)

Considerations for Reproductive Toxicity studies for Preventive Vaccines for Infectious Disease Indications

EU issued 2006

Guideline on Adjuvants in Vaccines

Purposes in using animal models

- Proof-of-concept testing
  - Immunogenicity
  - Challenge studies
- Safety testing
  - Toxicity testing (inactivated vaccines)
  - Viral safety testing (live-attenuated vaccines)
  - Batch-release testing (?)
- Quality testing (not discussed)
  - Adventitious agents

Limitations of Animal Experiments

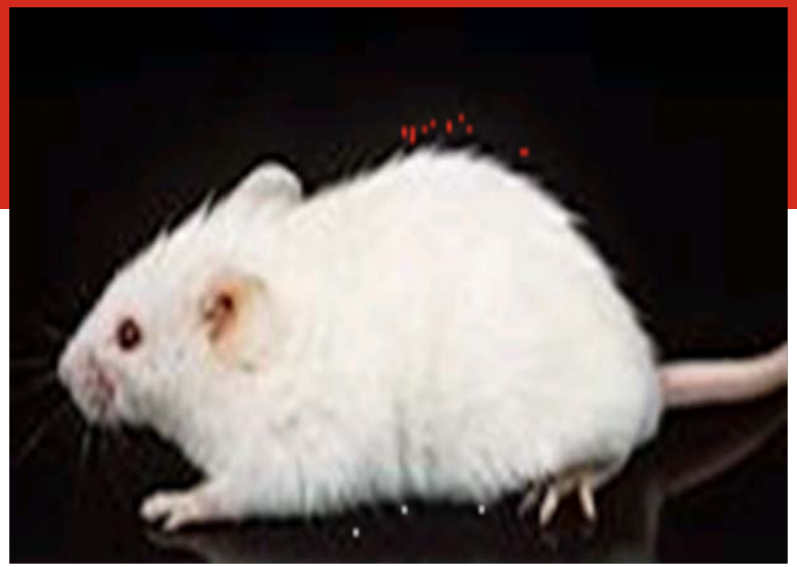
- Pathogenesis and immune responses are frequently species-specific
  - E.g. risks of autoimmunity difficult to predict
- Potential safety concerns may not necessarily indicate a problem in humans

Proof-of-concept testing

- Immunogenicity
  - Level of Antibody production
  - Class and subclass characterization of antibodies
  - Cell-mediated Immunitiy
  - Duration of the immune response
- Challenge by pathogenic organisms (disease models) (preferred)
  - Characterization of immune response only insufficient

EU Note for Guidance on Preclinical pharmacological and Toxicological Testing of Vaccines CPMP/SWP/465/95

- Special situation not covered by the Guideline
  - Passive immune transfer
  - Serological testing, bactericidal assays



Proof-of-concept testing

Immunogenicity models

Most often used:

- Mouse
  - Background: animal of the immunologists. Most of immunological reagents are available for mice
  - Outcome might be strain-dependent
    - E.g. Influenza testing with Baxter's Celvapan
  - Outcome dependent on formulation
    - Adjuvant testing: Mice lie with some TLR4 agonists and LPS-analogues
  - Serological testing, bactericidal assays
    - N.Meningitidis vaccine

Proof-of-concept testing INFLUENZA

- Challenge models, i.e. disease models
  Various examples
  - Influenza vaccine classical models
    - Ferret disease model
    - Mouse model
  - More recent models
    - Guinea pig model
    - Pig model
    - Nonhuman Primate model

Influenza ferret model

1933 Initial successful experiment: 2 ferrets (Smith et al)

- Two-day incubation period
- Temperature rises abruptly 48 and 96 hours after infection
- Coincidently the ferret looks ill, is quiet and lethargic
- Refused food
- Eyes watery and watery discharge from the nose

Unique feature of ferrets:

Viral shedding

Other important aspects

- Cross-protectivity
  - Inherent protection in case of antigenic drift
  - Protection between clades
    - By whole virion vaccines (e.g. Baxter)
    - By adding adjuvants

Other animal models

Pigs (minipig ??)

- Disease symptoms
  - Nasal discharge
  - Dry cough
  - Labored breathing
  - Fever
  - Weight loss
  - Replication of virus

Morbidity high, mortality low (except with bacterial infections)

Advantage compared with Ferrets:

For pigs a high number of reagents is available

Göttingen-Ellegaard Minipig

Proof-of-concept testing Human Papilloma Virus

- Challenge models, i.e. disease models
  - Cottontail rabbit papillomavirus (CRPV) induce tumours
    - cutaneous rather than mucosal.
  - Canine oral papillomavirus (COPV)
    - infects and induces lesions at a mucosal site (oral mucosa).
- Various examples
  - Studies with species-specific papillomaviruses have demonstrated the possibility to vaccinate against infection and development of tumour lesions using virus-like particles formed by recombinant viral capsid proteins
    - Breitburd et al 1995
    - Jansen et al 1995
    - Suzich et al 1995

Proof-of-concept testing Human Papilloma Virus

- Passive immune transfer
  - African green monkeys were immunised with HPV-11 VLPs.
  - Sera from immunised monkeys neutralised HPV-11 in an ex vivo model for HPV infection (human foreskin tissue was infected with HPV and then implanted into athymic mice).
  - Significant levels of HPV-11-neutralising antibodies were observed in cervicovaginal secretions (Lowe et al 1997).

Proof-of-concept testing Smallpox

- Challenge models, i.e. disease models
  - Variola: human smallpox, no real animal models
  - Vaccinia: cowpox, "general" pox antigen
  - Monkeypox: specific for monkeys e.g. macaques

- Various examples

  Small pox vaccine (Vaccinia)
  - › Monkeypox challenge after Vaccinia vaccination Zaucha et al, 2001 Laboratory Investigation
  - › BALB/c Mouse with cowpox virus infection Martinez et al, 2000 Arch. Path. Lab. Medicine

Proof-of-concept testing Smallpox

FDA

– Animal rule (two species) to prove efficacy in case clinical studies are not feasible (smallpox guideline)

- If the efficacy of a product (e.g. a preventive vaccine) cannot be proven clinically, proof-of-concept studies (challenge studies) in two animal species might replace this request.

EMA

– Animal rule (mouse and monkey, before clinical trials)

– Use of comparator in development

– Check of pock formation in humans

Proof-of-concept testing with Dengue vaccines

- Challenge models, i.e. disease models, are lacking
  - antibodies directed against the Dengue virus E protein neutralize the virus and have been shown to protect animals when actively induced by experimental vaccines or when passively administered, prior to challenge (i.e. passive immunization)
  - However, a correlation between the titre of neutralizing antibodies in serum, as determined in an in vitro neutralizing antibody assay (PRNT50), and protection has not been established for any of the four serotypes of virus.
  - NHP-models usually low level of viremia and no disease symptoms. Used to study pathogenesis.

Proof-of-concept testing with Dengue vaccines

- Transgenic models
  - Dengue vaccine in AG129 transgenic mice (interferon α/β/γ-receptor-deficient mice) (Johnson and Röhrig, 1999)
  - Innate immunity is deficient in these mice
  - Enable to a certain extent the development of vaccines
    - Viral virulence (affinity for glysocaminoglycans)
    - Model used to adapt the vaccine
    - Sensitive to all 4 DENV serotypes
  - Passive transfer of antiserum from DENV-1 immune mice reduced viral burden.
  - However, additional mouse models are needed

Animal models in safety testing

- Safety testing
  - Toxicity of inactivated vaccines
  - Toxicity of adjuvants alone and in combination with antigens
  - Viral safety of live-attenuated viral vaccines
    - Neurotropism, viscerotropism

Safety testing for inactivated vaccines (1)

- Repeated dose toxicity testing
  - Single dose testing generally not needed

- Relevant animal species/strain
  - Rat
  - Rabbit (because of im administration?)
  - Immune response sufficient as criterium, or should the species be sensitive to the disease?

- Dosing schedule
  - What is the origin of N+1?
  - Is this useful after 3-4 injections?

Safety testing for inactivated vaccines (2)

- Choice of dose
  - Human dose in presumed final formulation (effects might be dependent on formulation)

- Route of administration
  - Using method of vaccine administration (specific device?)
  - Skin vaccination (pigs, minipigs)
  - Intramuscular route (with human dose large muscels are required)

- Timing of evaluation of endpoints.
  - Is length of immunization in animals predictable for humans?

Safety testing for adjuvanted vaccines

EU Guideline on Adjuvants in Vaccines (2006)

The intended action is to induce long-lasting changes in the immune system by influencing the sensitivity to **defined** antigens.

Default position:

- Testing in two species (including non-rodent) unless justified
- Some adjuvants might exert high level of species specificity (e.g. some cytokines): one animal species is sufficient
- Ideally the selected species should be same in which the proof-of-concept has been studied

Safety issues with adjuvants

Association with Autoimmunity

adjuvants may increase immune response to non-target antigens

Effects during pregnancy

adjuvants may disturb the delicate balance in early pregnancy

Live-attenuated vaccines

- Neurotropism
  - Viral infectivity in neuronal cells
    Non-human primates
    Protocol in WHO Yellow fever Guideline
    Classical test, difficult to be replaced. Is it relevant?

- Viscerotropism
  - Due to virulence in visceral organs
    e.g. liver and spleen with yellow fever.

Conclusions on Animal Models

- Proof-of-concept testing in animal disease models
  - Immunogenicity combined with serological assays
  - Challenge in disease models
  - Passive immune transfer

- Safety
  - Toxicity testing (inactivated vaccines)
    - Repeated dose toxicity
    - Developmental toxicity
  - Toxicity testing of adjuvanted vaccines
    - Association with autoimmunity(?)
  - Viral safety (live-attenuated vaccines)
    - Neurotropism
    - Viscerotropism