A *Yersinia pestis* vaccine development to ensure the safety of humankind

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BACKGROUND

Although it is difficult to ascertain, it is estimated that throughout history have died over **200 million** people because of plague. That is because without an effective treatment, 50–60% of cases of bubonic plague are fatal, while untreated septicaemic and pneumonic plague are **invariably fatal**. [1]

Plague has been used as a weapon many times throughout history, like in 1347 when the Mongols catapulted bodies of infested people into Caffa or during the Second World War when Japan sent rice carrying *Yersinia pestis.*
Yet the most disturbing precedent is the antibiotic-resistant *Yersinia pestis* that the Russians tried to develop. [2]

Nowadays plague can be treated with **streptomycin** or **gentamicin** reducing mortality to only 1-5% of those infected. However, an efficient vaccine that develops protective immune response is lacked. [1] [3]

That raises the question of what would happen if these antibiotics didn't work?

Figure 1. Nicotiana benthamiana infiltrated with the cultures of *Agrobacterium*

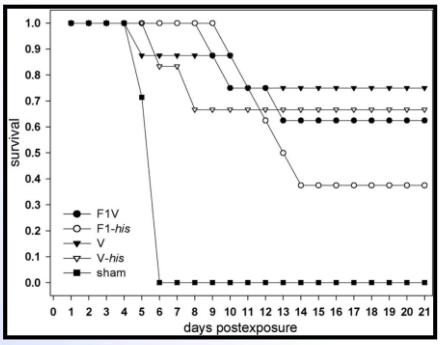

Figure 4. Survival of vaccinated Hartley guinea pigs to aerosolized *Y. pestis* [4].

OBJECTIVES

- Create a subcutaneous vaccine capable of inducing not only humoral but also cellular response.
- Try some different adjuvants and establish which one is the fittest for human vaccination.
- Use viral vectors for expression of recombinant F1 and V proteins in leaves of *N. benthamiana*, to prove that is a rapid, cheap and efficient expression system.
- Develop a vaccine that can protect humankind in case of a terrorist attack with an antibiotic-resistant *Yersinia pestis.*

WORKING PLAN

It has been decided to use a recently developed transfection technology that relies on *Agrobacterium* as an infective systemic agent that delivers deconstructed tobacco mosaic virus (TMV) viral replicons. This improved process is being used to simultaneously start transient gene amplification and high-level expression in all mature leaves of a plant. This technology, combines advantages of three biological systems: vector efficiency and efficient systemic DNA delivery of *Agrobacterium*, speed and expression of a plant RNA virus, as well as low production costs of a plant. [4]

Subclone the genes into the viral vectors

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Expression of viral vectors in *Agrobacterium*

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Infect the plant (*fig. 1*)

→ Confirm infiltration (*fig. 2*)

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Ten days of viral expression

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Purification of the proteins

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Construction of different adjuvant vaccines

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Start the trials on guinea pigs (*fig. 3*)

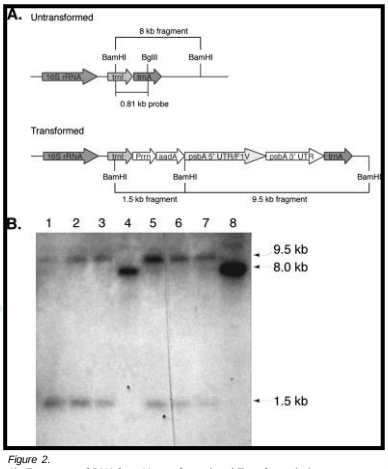

Figure 2.
A) Fragments of DNA from Untransformed and Transformed plants
B) Southern blot analysis of transgenic lines. Transformed with the integration (9,5kb). And without the integration (8kb) [5].

Adjuvant	B.S.*1	1st Shot	B.S.	2nd Shot	B.S	3rd Shot	B.S	Trial*2
Aluminum Hydroxide	Day 0 Before first dose	Day 0	Day 15	Day 30	Day 45	Day 60	Day 75	Day 90
MF59								
QS-21								
IL-12								
Gram-negative flagellin								
No adjuvant								

Figure 3. Vaccines with the corresponding adjuvant will be administrated to 8 guinea pigs each.
*1: Blood sample
*2: Trial with lethal dose 50

EXPECTED RESULTS AND BENEFITS

- It can be expected that the control group will have fewer antibodies in serum and therefore it is also expected a higher rate of deaths.
- It is expected a lower response from Aluminium hydroxide.
- A high level of cellular response with IL-12, QS-21 and MF59 adjuvants.
- Better results than in other studies (fig. 4).

- Determine and optimize the best suited adjuvants for the vaccine.
- Identify flaws in the current system of vaccination
- Provide safety to population in case of a bioterrorist attack.

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