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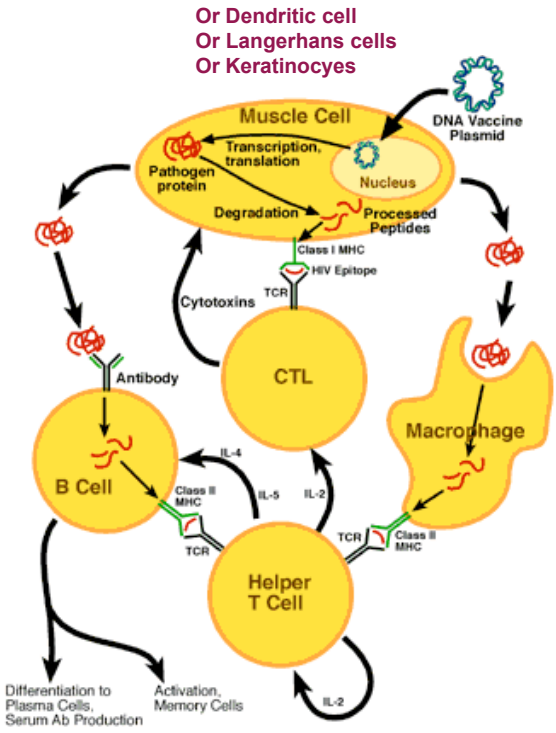
Vaccins ADN

- Principe:
  - ADN nu plasmidique
  - Expression in vivo après injection directe
  - Présentation antigénique par:
    - Cellules transfectées
    - Directe si Ag sécrété
    - Cross-présentation
  - Réponse immunitaire complète

- Avantages:
  - Pas de problème de restriction HLA pour les peptides immunodominants
  - Immunité cellulaire +++
  - Immunité humorale
  - Stockage, coût
  - Possibilité de coupler à des adjuvants génétiques
    - CpG, ADN cytokines

- Mais…
  - Pas ou peu d'anticorps neutralisants: liée à un défaut de conformation de la protéine
  - Risques d'intégration (?)



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Stage of development

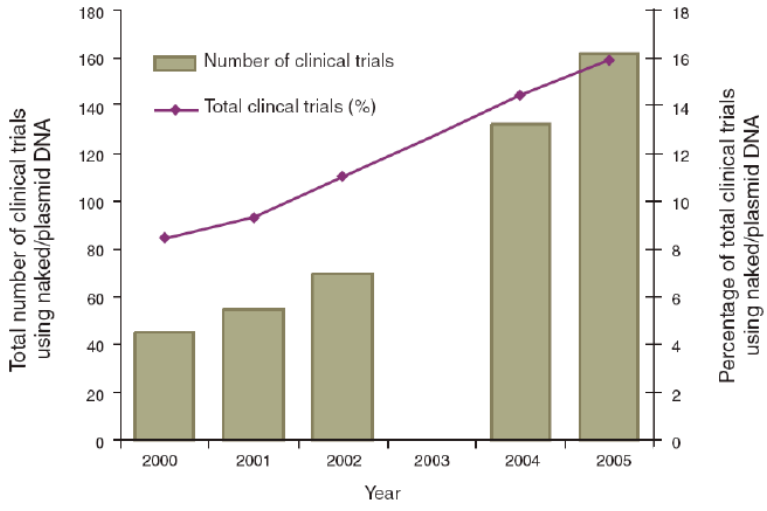

Figure 2 Plot showing increasing usage of naked/plasmid DNA in gene therapy trials throughout the world[29]. Both the number of trials and the percentage of total trials using naked or plasmid DNA has been increasing since 2000. No data were available later than 2003.

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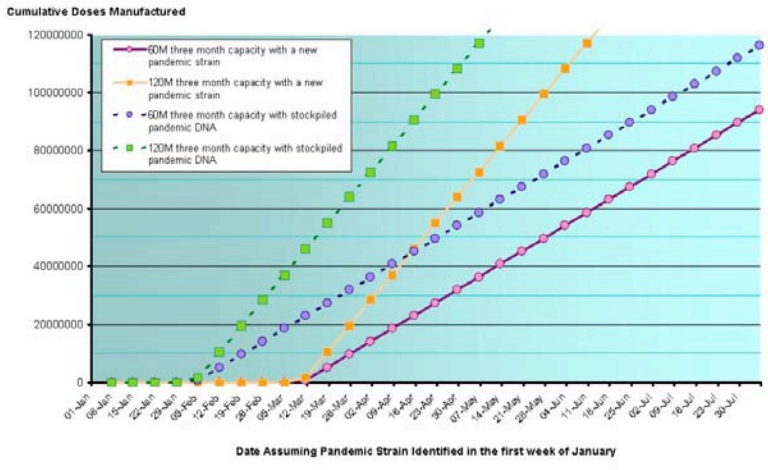
DNA vaccine production

PowderMed's estimation

The very small amounts of DNA required to create an immune response means that **the entire UK population could receive two doses of a pandemic vaccine obtained from under 250g of DNA.** This modest scale and the straightforward production of a DNA vaccine using a well-characterized *E. coli*-based fermentation process means that the **manufacturing scale-up risk is much lower** for PowderMed's DNA vaccine than for a cell-based vaccine. The DNA technology also means that **vaccine can be produced with certainty, irrespective of virus strain, which is not the case for egg or cell based approaches.**

A PowderMed DNA vaccine will also provide a number of **unique advantages** in the face of a pandemic, these include: **no requirement for cold chain storage or distribution, easy needle free administration,** which does not require medically trained personnel and **the ability to stockpile** for prolonged periods. These factors provide significant post manufacture logistical flexibility and will further contribute directly to saving more lives by distributing and administering vaccine faster



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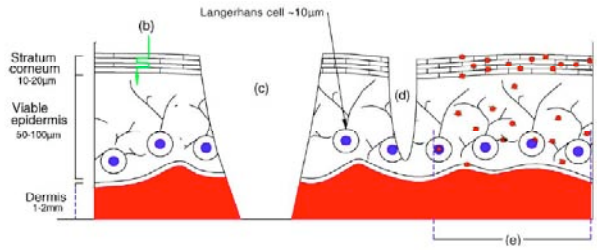
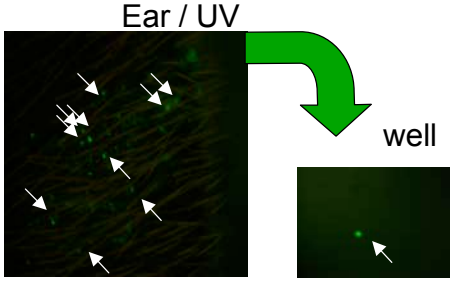
Modalités d'administration

- Techniques:
  - Injection IM / SC (c)
  - Electroporation
  - Jet (c): Biojector, Dermojet, …
  - Biobalistique (e): Gene Gun, PowderJect, …
  - Dermographe
  - Patch (b)

	Cd	Ep	Dj	Gg
	Carditoxin	Electropo	Dermojet	Gene gun
DNA (µg)	100	10	10	0.5
injection	IM	IM	ID	ID



- Importance du choix d'administration de l'ADN:
  - Muscle : longévité cellulaire assure une production antigénique prolongée
  - Peau : riche en APC



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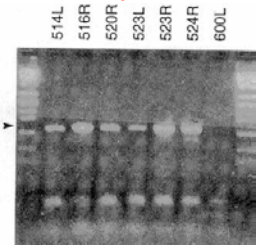
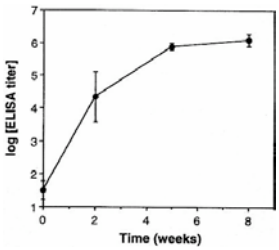
Protection with DNA vaccine : IM injection

NP expression

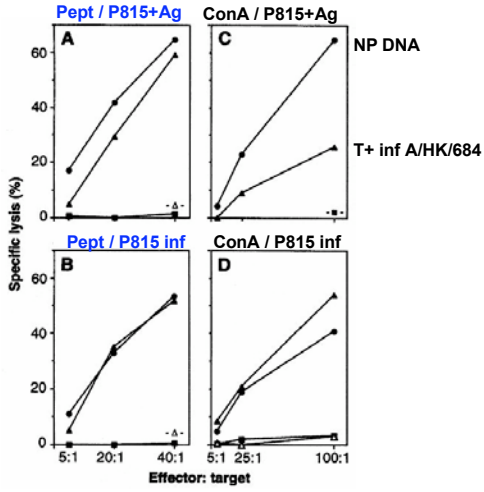


NP =conserved viral protein
NP A/PRT/8/34

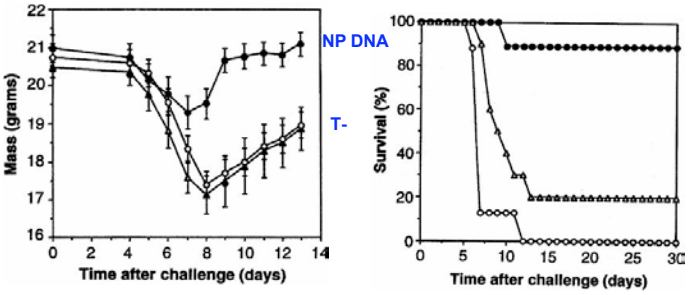
Antibody



CTL



A/HK/68 challenge



Heterologous Protection Against Influenza by Injection of DNA Encoding a Viral Protein
Ulmer, Jeffrey B.; Science 1993

IM injection 400µg

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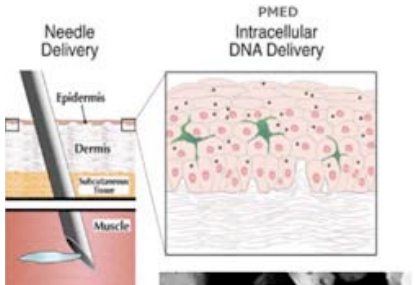
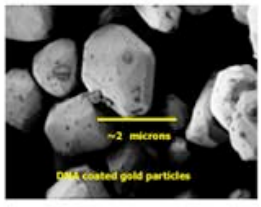
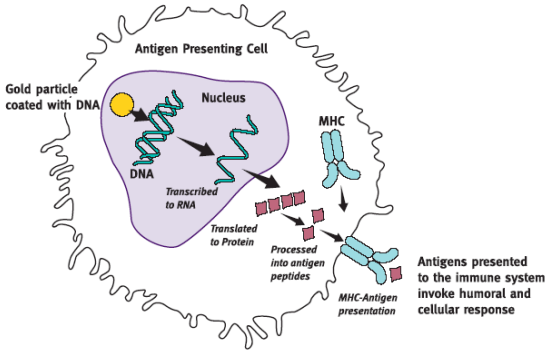
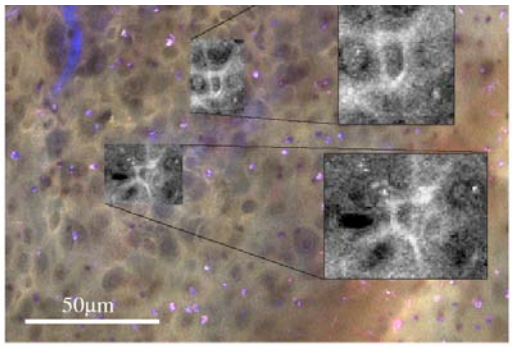
Biobalistic delivery









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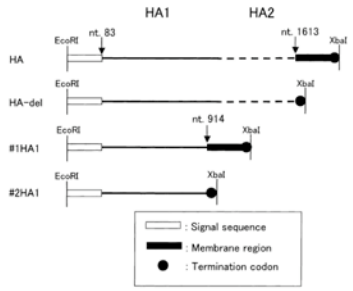
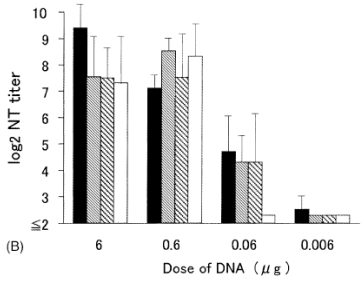
Protection with DNA vaccine : Gene Gun

DNA vaccines



HA neutralization



CTL

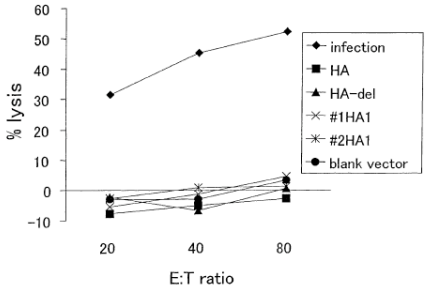


Fig. 5. CTL responses of immunized mice. Two weeks after the third immunization, spleen cells from the immunized mice were cocultured for 6 days with irradiated syngenic spleen cells infected with A/WSN/33. CTL activity was assayed by ^{51}Cr-release against ^{51}Cr-loaded P815 target cells infected with A/WSN/33. Data are mean values of two mice and one infected mouse as a positive control.

Challenge

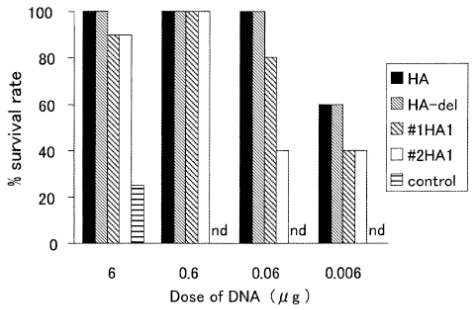


Fig. 4. Protection from death after virus challenge. Two weeks after the third immunization, the mice (BALB/c) were challenged with 20 LD_{50} of A/WSN/33. The vertical line shows mouse survival. At a dose of 6 μg: $n = 8$–10 except for the HA-del group ($n = 5$). At doses of 0.006–0.6 μg: $n = 5$. nd: not done.

ELSEVIER
Vaccine 21 (2003) 3118–3125
www.elsevier.com/locate/vaccine

Analysis of epitope recognition of antibodies induced by DNA immunization against hemagglutinin protein of influenza A virus

Ken Tonegawa [a,b], Eri Nobusawa [b], Katsuhisa Nakajima [b,*], Takashi Kato [a], Takeo Kutsuna [a], Kazumichi Kuroda [c], Toshikatsu Shibata [c,d], Yuichi Harada [d], Atsushi Nakamura [a], Makoto Itoh [a]

Immunization with Gene gun

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•PowderMed : DNA vaccine

Oxford, UK, 1st August 2005

PowderMed has announced that it has progressed its H5N1 Avian Influenza Vaccine programme into the final stages of preclinical development.

DNA influenza vaccine consists of the **pPJV1671** plasmid containing the **HA gene from A/Panama/2007/99**.

Phase I : 1, 2 or 4µg of DNA

•The delivery device is a fully developed and patented system, called **Particle Mediated Epidermal Delivery** (PMED™), whereby gold particles coated in the vaccine DNA are propelled into the skin using high-pressure helium.

PowderJect® Particle Mediated Epidermal Delivery (PMED™) technology

ELSEVIER
Vaccine xxx (2005) xxx–xxx
www.elsevier.com/locate/vaccine

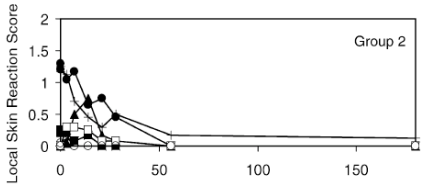
Epidermal DNA vaccine for influenza is immunogenic in humans

Robert J. Drape, Michael D. Macklin, Lori J. Barr, Suzanne Jones [1], Joel R. Haynes, Hansi J. Dean *

Abstract

A phase I clinical trial was conducted to evaluate a monovalent influenza DNA vaccine containing the HA gene from A/Panama/2007/99 delivered by particle-mediated epidermal delivery (PMED). Three groups of 12 healthy adult subjects received a single dose on day 0 of either 1, 2 or 4 μg of DNA vaccine, delivered as 1, 2 or 4 PMED administrations. The PMED influenza DNA vaccine elicited serum hemagglutination-inhibition (HAI) antibody responses at all three dose levels, with the highest and most consistent responses in subjects vaccinated with the highest dose level. Antibody responses were greatest at the last time point tested, day 56. Treatment-related reactions were mild to moderate, and included skin reactions at the vaccine site. These results provide a preliminary indication of the safety and immunogenicity of a prototype epidermal DNA vaccine for influenza.

Group 1 : 1 x 1µg on day 0
Group 2 : 2 x 1µg on day 0
Group 3 : 4 x 1µg on day 0



Serum antibody responses, seroconversion and seroprotection rate

Group	Day	GMT (range)	Seroconversion[a] (%)	Seroprotection[b] (%)	Mean GMT increase (fold)
1	0	16 (5–40)	–	17 (2/12)	–
	14	23 (5–160)	8 (1/12)	42 (5/12)	1.4
	21	28 (10–240)	17 (2/12)	33 (4/12)	1.7
	56	44 (10–320)	33 (4/12)	58 (7/12)	**2.8**
2	0	17 (5–40)	–	33 (4/12)	–
	14	29 (10–60)	17 (2/12)	50 (6/12)	1.7
	21	36 (20–80)	8 (1/12)	58 (7/12)	2.1
	56	65 (20–320)	**67 (8/12)**	**92 (11/12)**	**3.9**
3	0	12 (5–40)	–	8 (1/12)	–
	14	21 (5–80)	17 (2/12)	25 (3/12)	1.8
	21	40 (10–160)	33 (4/12)	67 (8/12)	3.4
	56	97 (40–640)	**64 (7/11)**	**100 (11/11)**	**8.1**

Values meeting CPMP criteria are bold.
[a] Seroconversion is defined as either a negative pre-vaccination titer (≤10) to a post-vaccination titer ≥40, or a significant increase in antibody titer, i.e. at least a 4-fold increase between pre- and post-vaccination titers where the pre-vaccination titer is ≥10.
[b] Seroprotection rate is defined as the proportion of subjects achieving a titer ≥40.

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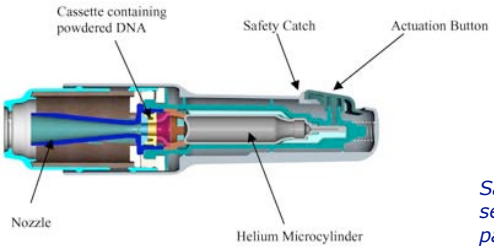
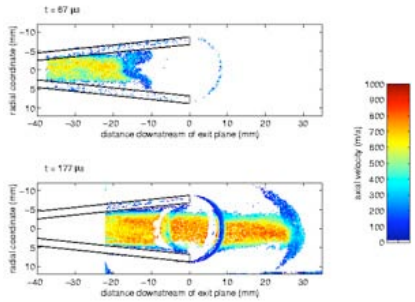
Particle-Mediated Epidermal Delivery (PMED)



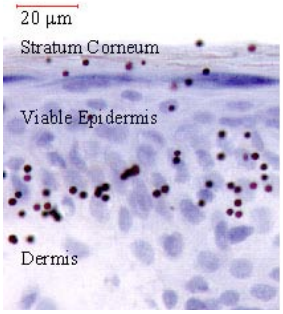


Sample particle velocity maps of earlier prototypes obtained with Doppler Global Velocimetry (DGV), Quinlan (1999), Quinlan et al. (2001).

Advantages of PMED™ over Needle and Syringe Vaccine Delivery

Needle and Syringe (NS) Injection	PMED Advantage
NS injection of vaccines bypasses the immune rich network of the epidermis	PMED delivers antigenic compounds to the epidermis, which is rich with immune cells, therefore eliciting a stronger cell mediated and humoral response
NS vaccines require large amounts of material to be delivered which can be expensive	Because the epidermis is rich in immune sentry cells, PMED requires less material (for example 1,000-fold less DNA than NS) to elicit an immune response, decreasing both risk and cost and ease of production scale up
Most NS vaccine formulations require refrigeration for stability, making transport and storage more costly	PMED formulations are stable at room temperature, making transport and storage simple and inexpensive
Any vaccine that involves needles requires training for administration which increases the cost	PMED is simple and easy to use
Vaccines that involve needles can be painful and risk accidental needle stick injury for the health care provider	PMED is needle-free and poses no needle stick risk to the healthcare provider

Sample histology section of gold particles delivered to the skin. Kendall, Mitchell and Bellhouse (2001).



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Adjuvants génétiques

- ODN CpG
  - Activation TLR-9 par CpG non-méthylés
- Plasmides codant pour
  - Cytokines:
    IL-2, GM-CSF, IL-12, IL-15,..
  - Chimiokines
    IFNg-inducible protein-10 [IP-10], MΦ inhibitory protein-1 [MIP-1], RANTES, …
  - Molécules de co-stimulation

ELSEVIER
Vaccine xxx (2003) xxx–xxx
www.elsevier.com/locate/vaccine

The use of Th1 cytokines, IL-12 and IL-23, to modulate the immune response raised to a DNA vaccine delivered by gene gun

Jonathan Williman, Euan Lockhart, Lynn Slobbe, Glenn Buchan, Margaret Baird*
Department of Microbiology and Immunology, University of Otago, PO Box 56, Dunedin, New Zealand

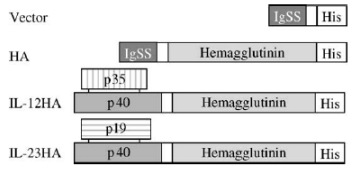

Fig. 1. Schematic representation of proteins produced by each vaccine construct. Both the vector and HA contain a IgSS for secretion of the protein out of the transfected cells. All constructs finish with a V5 epitope and His tag.

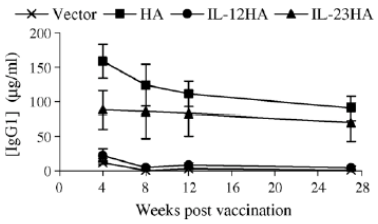

Fig. 4. Mice vaccinated with HA or IL-23HA produced high levels of long-lived IgG1. Fusion of IL-12 to the HA gene severely suppressed all antibody production. Serum from individual mice was assayed on ELISA plates coated with heat inactivated influenza virus. Data shows mean and standard error from eight mice per group and is representative of two individual experiments. No specific IgG2a was detected in the serum from any mice.

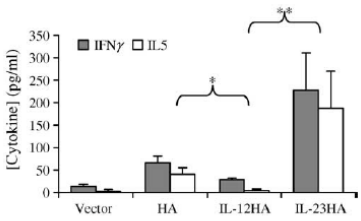

Fig. 5. Splenocytes from mice vaccinated with the constructs encoding the HA gene or IL-23HA only produced levels of both IFNγ and IL-5 that were significantly higher than both vector and IL-12HA. $^{*}P<0.05$, $^{**}P<0.01$. Splenocytes from individual DNA vaccinated mice were restimulated in vitro for 72 h with heat inactivated influenza virus and cytokine production was measured by ELISA. Data shows the mean and standard error from eight mice per group and is representative of two individual experiments.

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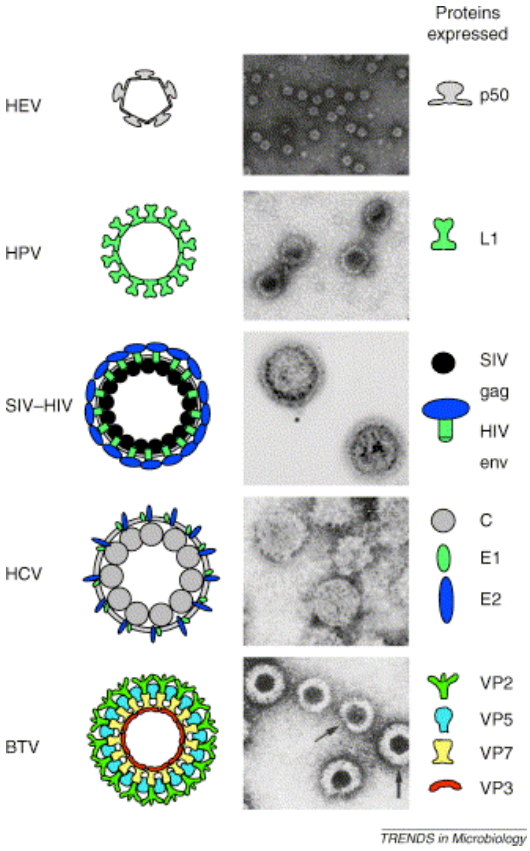
Virus-like particles

- Pseudo-virion: complexe protéique # structure virale, # taille
- Sans génome viral: déficients pour la réplication
- Auto-assemblage « particulaire » des protéines de la capside virales:
  - Hépatite B
  - Papillomavirus (HPV)
  - Parvovirus
- Système de production:
  - E.Coli
  - Levure
  - Baculovirus
  - Cellules mammifères
- Ag particulaires : +++ réponses immunitaires
- Ag=Protéines d'assemblage ou accrochage de l'Ag aux protéines d'Env



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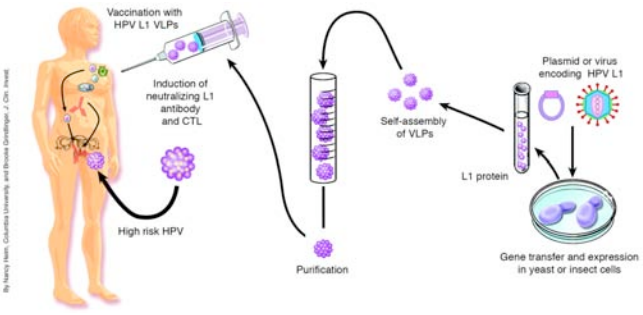
Vaccin avec VLP homologues

- Intérêts:
  - sécurité
  - présentation dans contexte naturel; bonne immunogénicité (*Bachmann M; Science, 1993*)
  - Réponses AC neutralisants
- Résultats pour vaccin préventif :
  - Human papilloma virus (HPV-6, 11, 16, 18)
  - 20% pop HPV-16 sero+
  - Risque associé de cancer du col de l'utérus (2nd cause de mortalité dans les cas de cancer chez la femme
  - Essai vaccinal: VLP formées par la capside L1 du virus papillomavirus de type 16
  - Schéma thérapeutique: 40mg VLP à J0, 2 et 6 mois vs placebo
  - Résultats:
    - Phase II: *New England Journal Medecine 2002; 347, 1645 (VLP-16) n=1200*
    - Phase II: *Villa LL; Lancet Onc 2005 (VLP 6, 11, 16, 18) n=277*
    - Phase III: 7 oct 2005, USA
  - Commercialisation oct 2005 du vaccin **Gardasil** (développé par Merck et Sanofi-Pasteur)



Inclus	Vaccination	Cas d'infection HPV-16	Cas de cancer
2400 femmes	Placebo: N=1200	41 nouveaux cas	N=9
	VLP HPV-16: N=1200		N=0

•*New England Journal Medecine 2002; 347, 1645*

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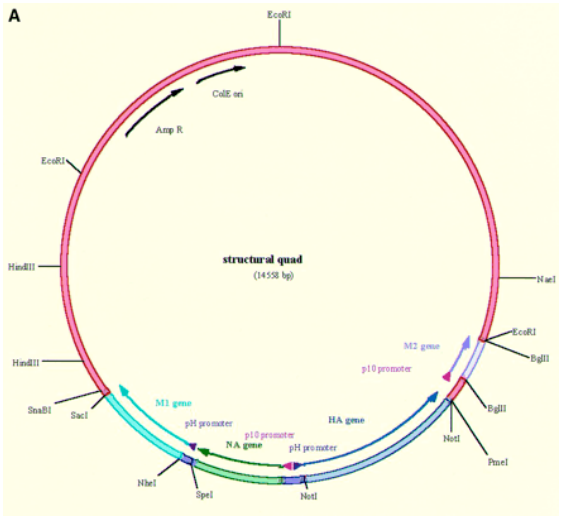
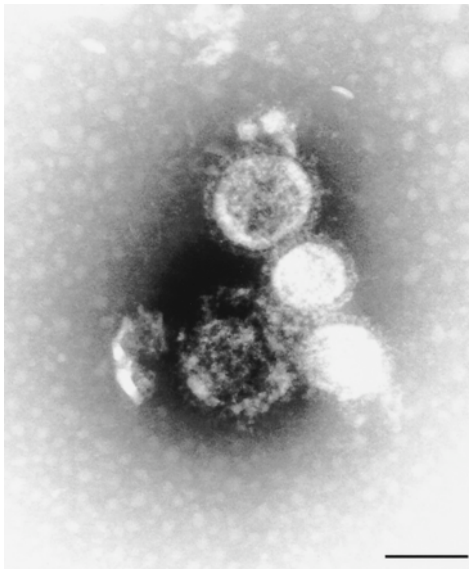
VLP Influenza

Formation of Wild-Type and Chimeric Influenza Virus-Like Particles following Simultaneous Expression of Only Four Structural Proteins

Theresa Latham and Jose M. Galarza*

JVI 2001





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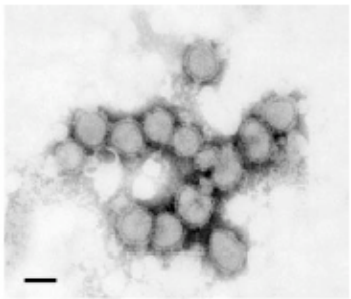
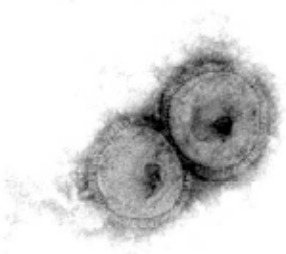
Influenza virus-like particles comprised of the HA, NA, and M1 proteins of H9N2 influenza virus induce protective immune responses in BALB/c mice

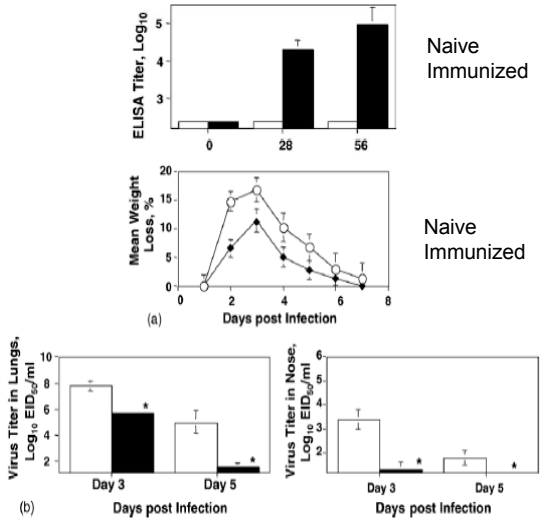
Peter Pushko [a,*], Terrence M. Tumpey [b], Fang Bu [b], John Knell [a], Robin Robinson [a], Gale Smith [a]

[a] *Novavax, Inc., Vaccine Technologies, 1 Taft Court, Rockville, MD 20850, USA*
[b] *Influenza Branch, Centers for Disease Control and Prevention, Atlanta, 1600 Clifton Road, GA 30333, USA*

Received 25 April 2005; accepted 28 July 2005
Available online 15 August 2005







5. Protective response in BALB/c mice vaccinated with VLPs derived from influenza A/Hong Kong/1073/99 (H9N2) virus. (a) Weight loss in VLP-cinated (black diamonds) vs. non-vaccinated (open circles) mice (8 mice/group). (b) Titers of replicating influenza A/Hong Kong/1073/99 virus in lungs and e tissues of VLP-vaccinated (filled bars) vs. non-vaccinated (open bars) mice (5 mice/group). Detection limit was 1.2 $\log_{10}$ EID_{50}/ml of virus. Also shown are standard deviation values. Standard deviation for day 3 in lungs of the VLP-vaccinated animals was 0.2 (not visible at this scale). An asterisk indicates the VLP-vaccinated group was significantly ($p < 0.05$) different from the unimmunized-control group by using analysis of variance (ANOVA).

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Virus-Like Particle Vaccine Conferred Complete Protection Against a Lethal Influenza Virus Challenge

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tein. In this work, we present data on the immunogenicity and protective efficacy afforded by VLPs (formed by M1 and HA) after immunization of mice. VLP vaccine (~1 μg HA) were formulated with or without IL-12 as adjuvant and administered twice, at 2-week intervals, by either intranasal instillation or intramuscular injection. All VLP-vaccinated and influenza-immunized control mice demonstrated high antibody titers to the HA protein; however, intranasal instillation of VLPs elicited

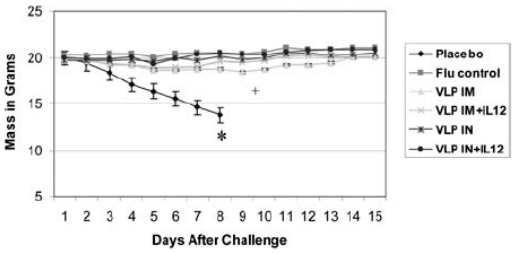

FIG. 4. Body weight was monitored for all the groups and weight average was plotted versus the days after virus challenge. All mice in the placebo control group (*) died between days 7 and 8. Also, one mouse in the VLP IN+IL-12 group (+) died at day 9. Abbreviations as in Fig. 2.

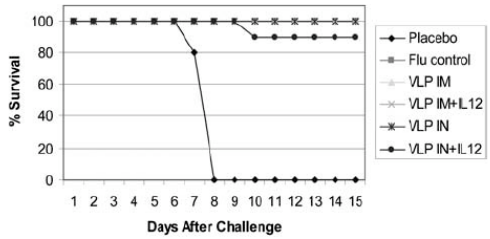

FIG. 3. Control and VLP-immunized mice were challenged with 5LD50 of a mouse-adapted influenza A/Hong Kong/68 (H3N2) virus. All mice immunized intramuscularly with VLP vaccine formulated with or without IL-12 survived the virus challenge. Also, intranasal instillation of VLP alone conferred complete protection, whereas VLP+IL12 protected 90% of the vaccinated mice. All control mice died between days 7 and 8. Abbreviations as in Fig. 2.

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Caractéristiques des nouveaux vaccins

	ADN	Vecteurs viraux	VLP
Réponse immunitaire	CTL Ac (Ag sécrété ou mbr)	CTL Ac (Ag sécrété ou mb)	Ac Ac neutralisant CTL (cross-p)
Immunogénicité	++ CpG	+++	+++
Sécurité	++	+	+++
Production	+++	++	+

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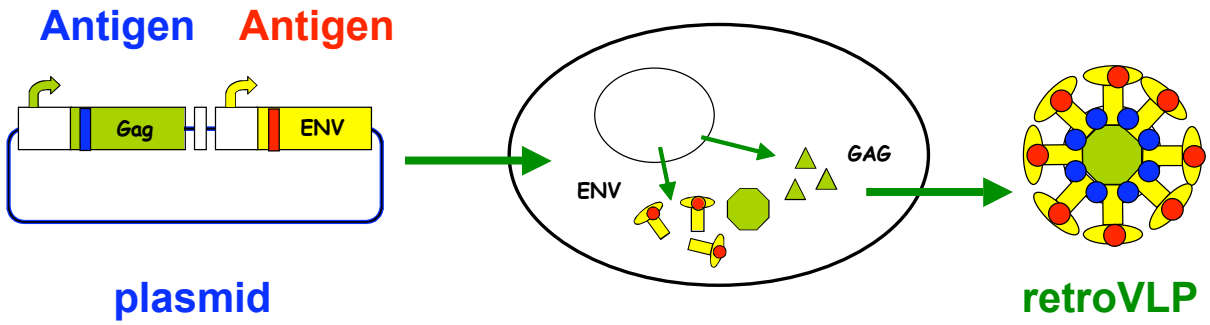
Plasmo-VLP

strategy to combine the advantages of DNA vaccines and VLP vaccines



Antigen plateform

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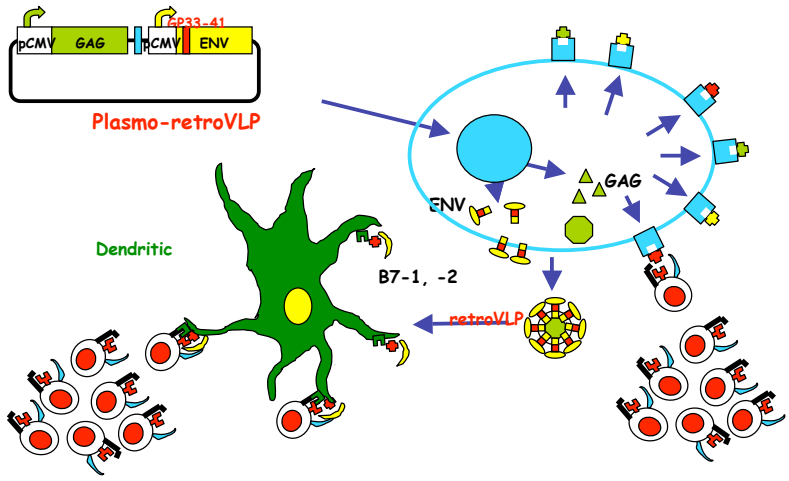
plasmoVLP



Présentation particulaire:
Augmente poids moléculaire de l'Ag
Favorise capture par les DC
Présentation épitopes issus de cross-présentation
Présentation antigénique double
Signal de danger: augmente capacité de présentation des APC

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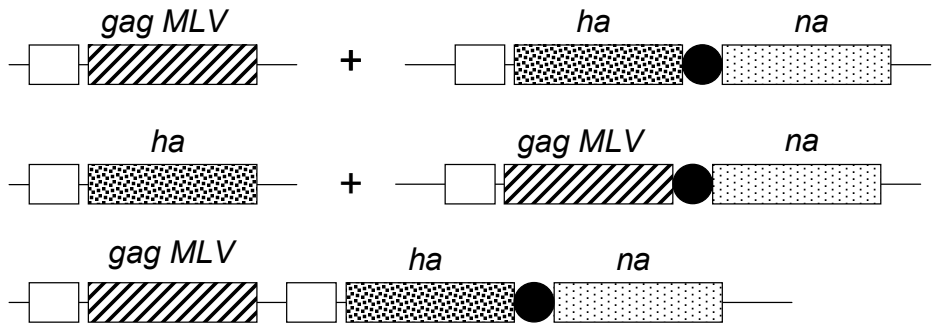
Application à la grippe aviaire

- DNA plasmids coding :
  - GAG from MLV oncoretrovirus
  - HA from H5N1
  - NA from H5N1



CMV promotor

IRES

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Bilan des vaccins

Type de vaccin	Aptitude selon le critère précité	Disponibilité		Observation
Virus inactivé	+ à +++ selon standardisation de la concentration de protéines virales (hémagglutinine)	H_5 Continent américain	H_7 Europe	- Interférence avec le diagnostic sauf artifice (neuraminidase différente) - Autres inconvénients tels que risque élevé dans la phase de production et durée de protection inconnue
Vaccin recombinant Pox aviaire (exprimant HA)	HA H_5 + à ++++ selon degré hétérologie avec souche sauvage HA H_7 à prouver	USA	Non	Aucune efficacité si immunité antivariolique préexistante
Vaccin sous-unitaire HA recombinante exprimée en baculovirus	HA H_5 ou H_7 ++	(USA)	(USA)	Production arrêtée semble-t'il ?
Vaccin ADN HA	HA H_5 ou H_7 +++	Non	Non	Pas économiquement viable pour le moment

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http://www3.niaid.nih.gov/news/focuson/flu/default.htm