NANO ET MICRO TECHNOLOGIES POUR LA CONCEPTION DE VACCINS

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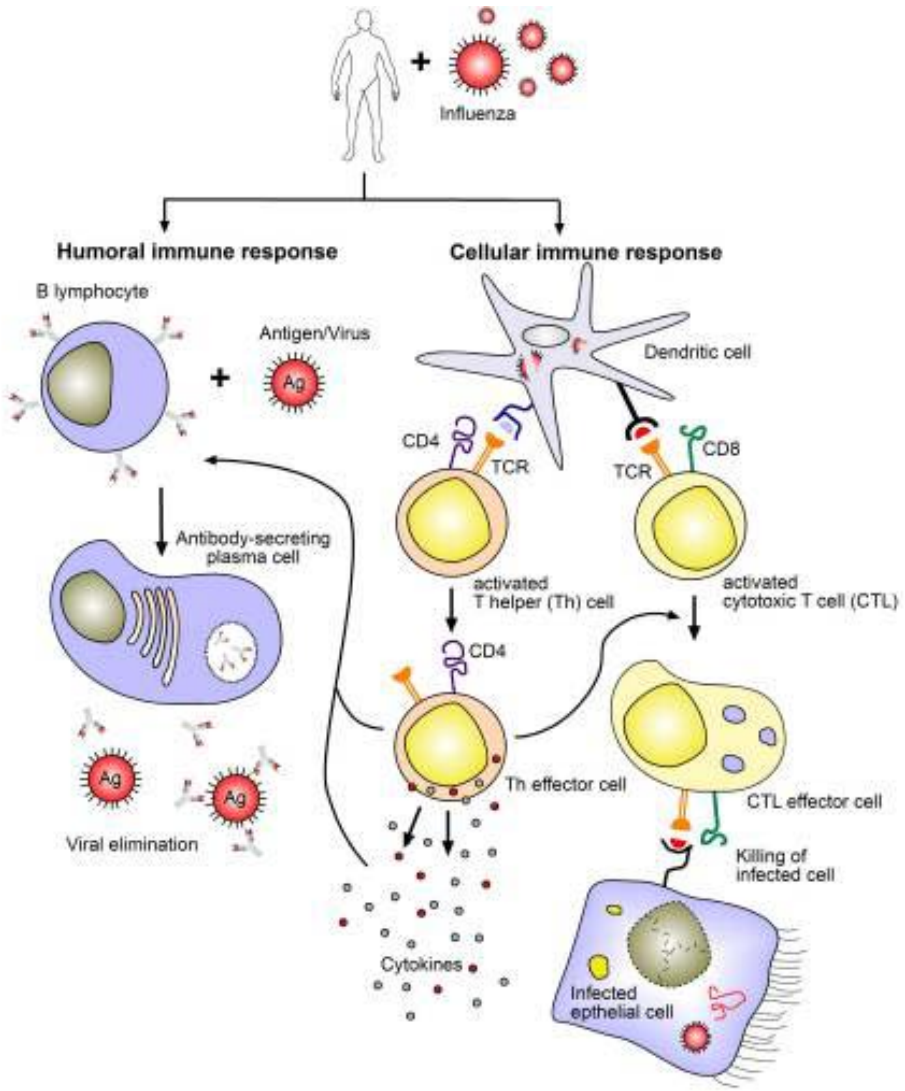
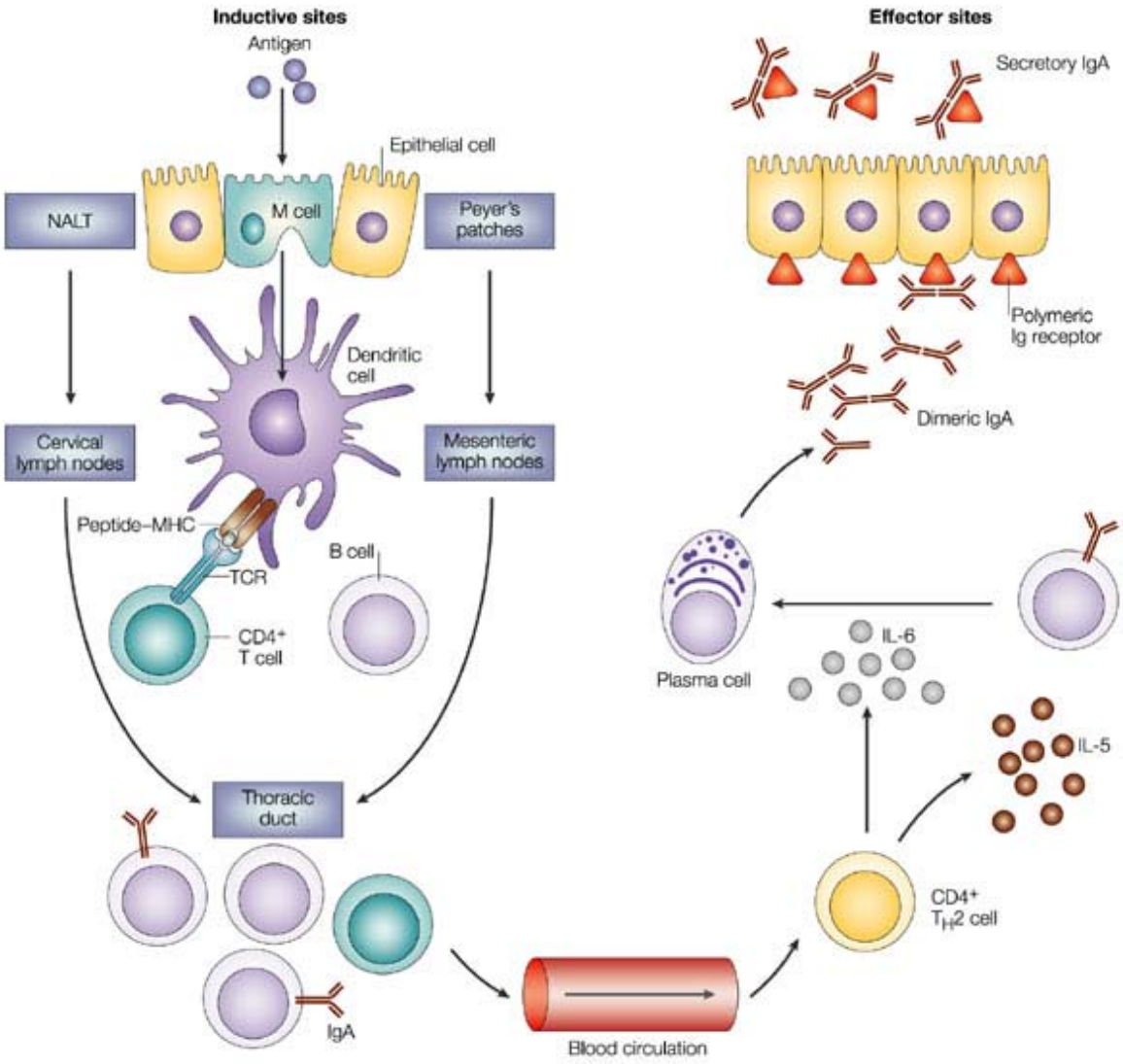
HUMORAL AND CELLULAR IMMUNE RESPONSE



MUCOSAL IMMUNITY



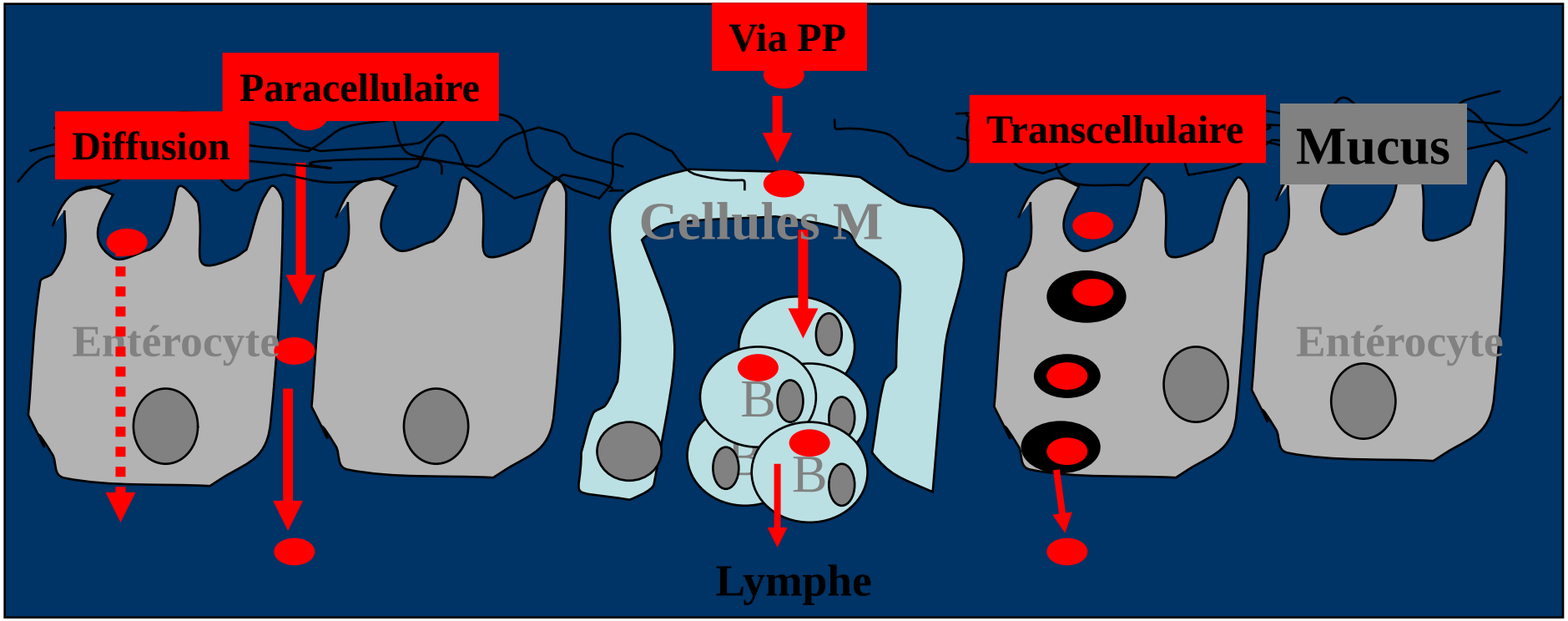
WHY NANO AND MICROTECHNOLOGIES FOR VACCINATION ?

- Micro and nanoparticles may protect the antigen from the degradation
- They allow the antigen to be released gradually, thereby extending the time possible for the antigen to interact with antigen presenting cells and lymphocytes
- Particles smaller than 10 μM are more easily phogocytosed by macrophages and dendritic cells
- Micro and nanoparticles are thought to stimulate the immune system through cytokine production

MICRO AND NANOPARTICLES HAVE A SIZE SIMILAR TO MANY PATHOGENS THAT THE IMMUNE SYSTEM IS EQUIPPED TO ATTACK

WHY USING MICRO AND NANOPARTICLES FOR MUCOSAL IMMUNITY ?

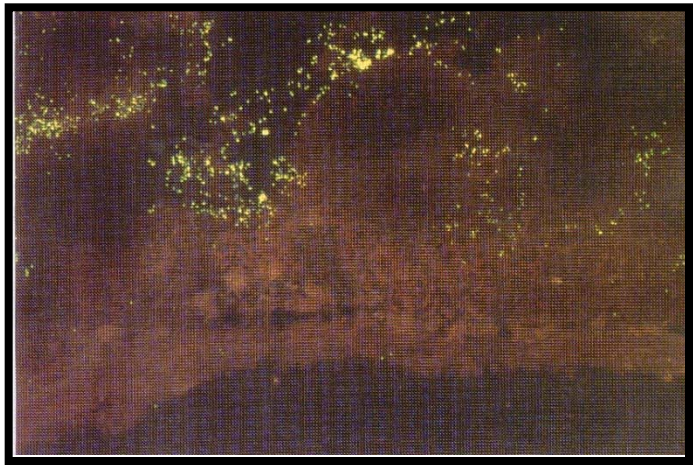
- Macromolecules, microparticles and microorganisms are taken up by epithelial M cells present in the Peyer's patch
- M cells show little transport specificity in that a wide variety of substances and microbes are transported
- M cells are not equipped with degradative lysosomal enzymes
- Hydrophobic surfaces can enhance interaction with M cells

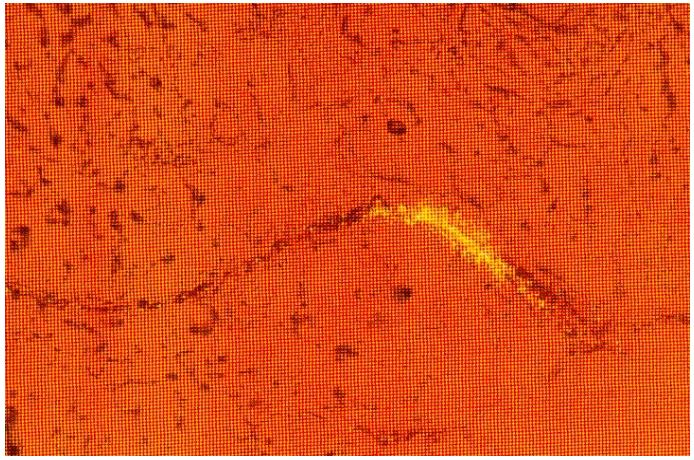


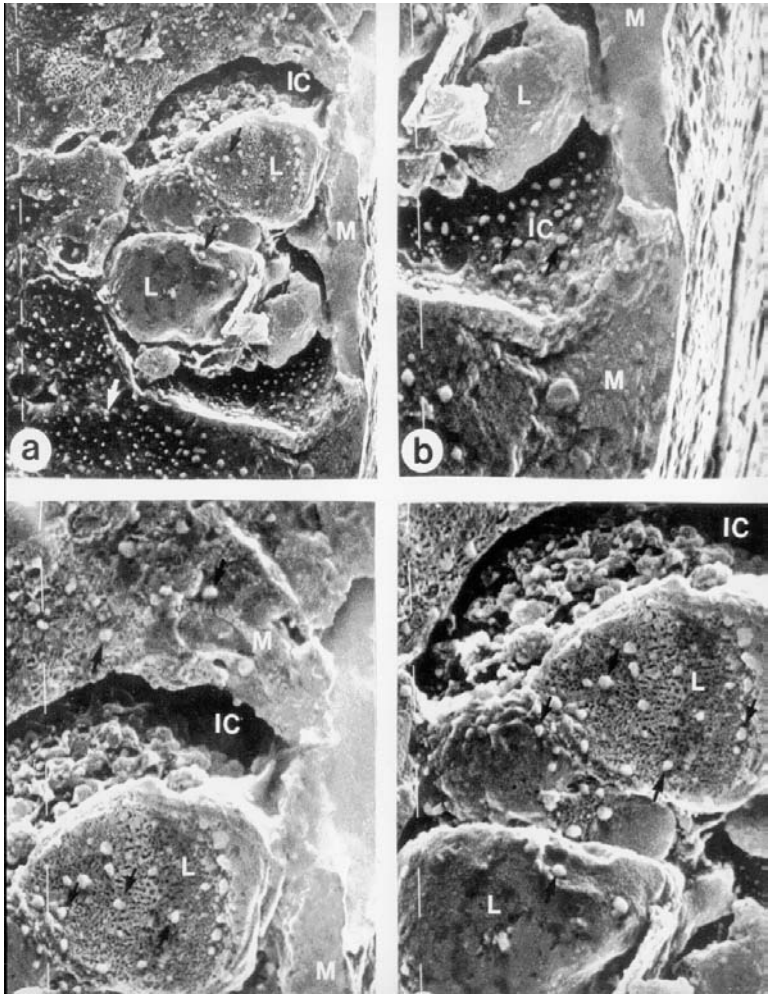
PEYER'S PATCH UPTAKE OF MICRO AND NANOPARTICLES

A.T. Florence, N. Hussain, Adv. Drug Del. Rev., 2001

1μM IS BELIEVED TO BE THE OPTIMAL SIZE





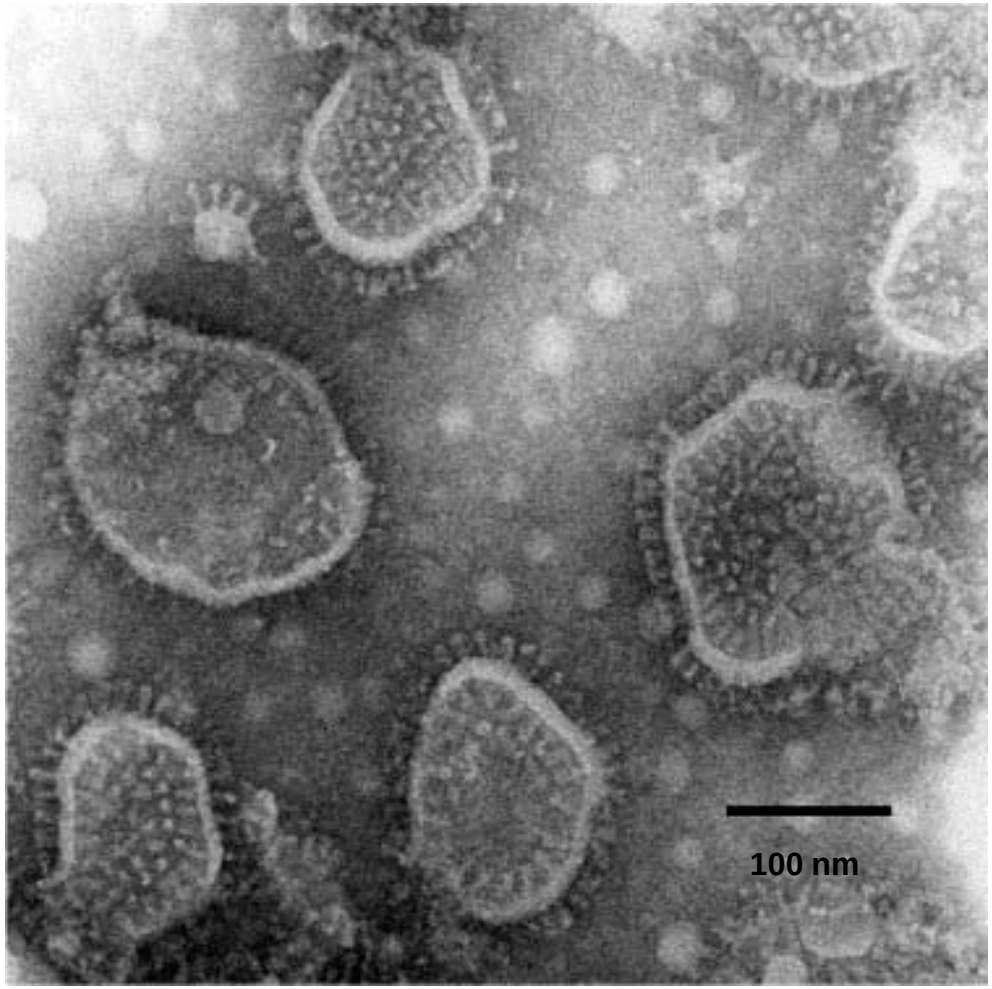


NANOCARRIERS FOR VACCINATION

-Virosomes
-Proteosomes
-Iscoms
-Nanoemulsions/Nanodroplets
-Liposomes
-Nanoparticles

VIROSOMES

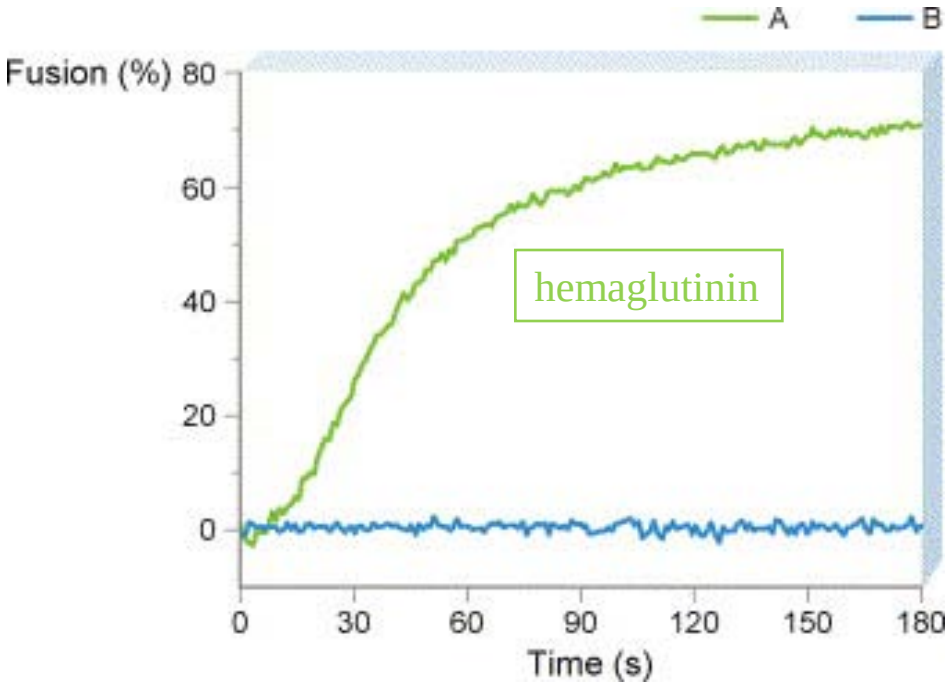
Anke Huckriede et al., Vaccine,23S1, 26-38, 2005

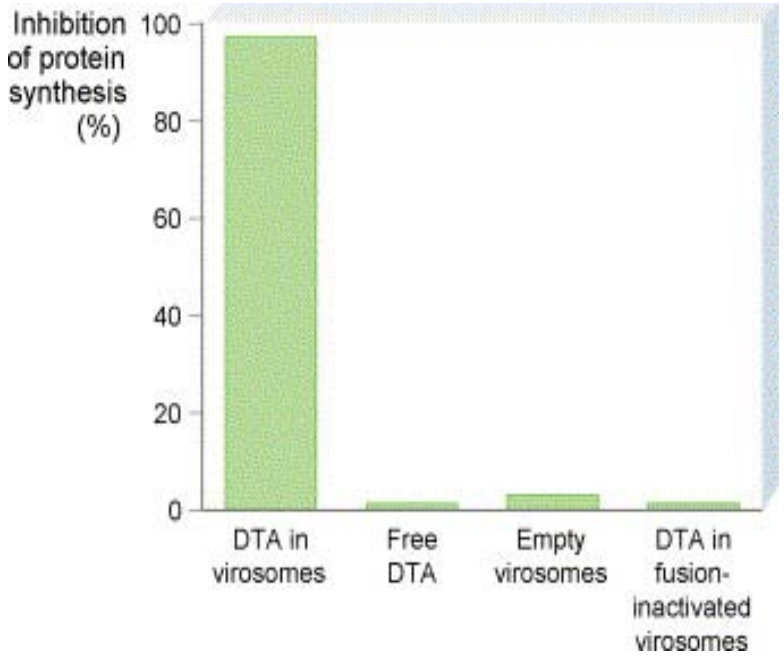


Virosomes are virus-like particles consisting
In reconstituted viral envelope lacking the viral
genetic material. They are prepared either by:
-Detergent solubilisation of virus and reconstitution
-Or by preparing SUV liposomes enriched with virus membrane proteins

VIROSOMES ARE SIMILAR TO ORIGINAL VIRUS IN TERMS OF MEMBRANE FUSION AND CELL ENTRY CHARACTERISTICES

Anke Huckriede et al., Vaccine,23S1, 26-38, 2005



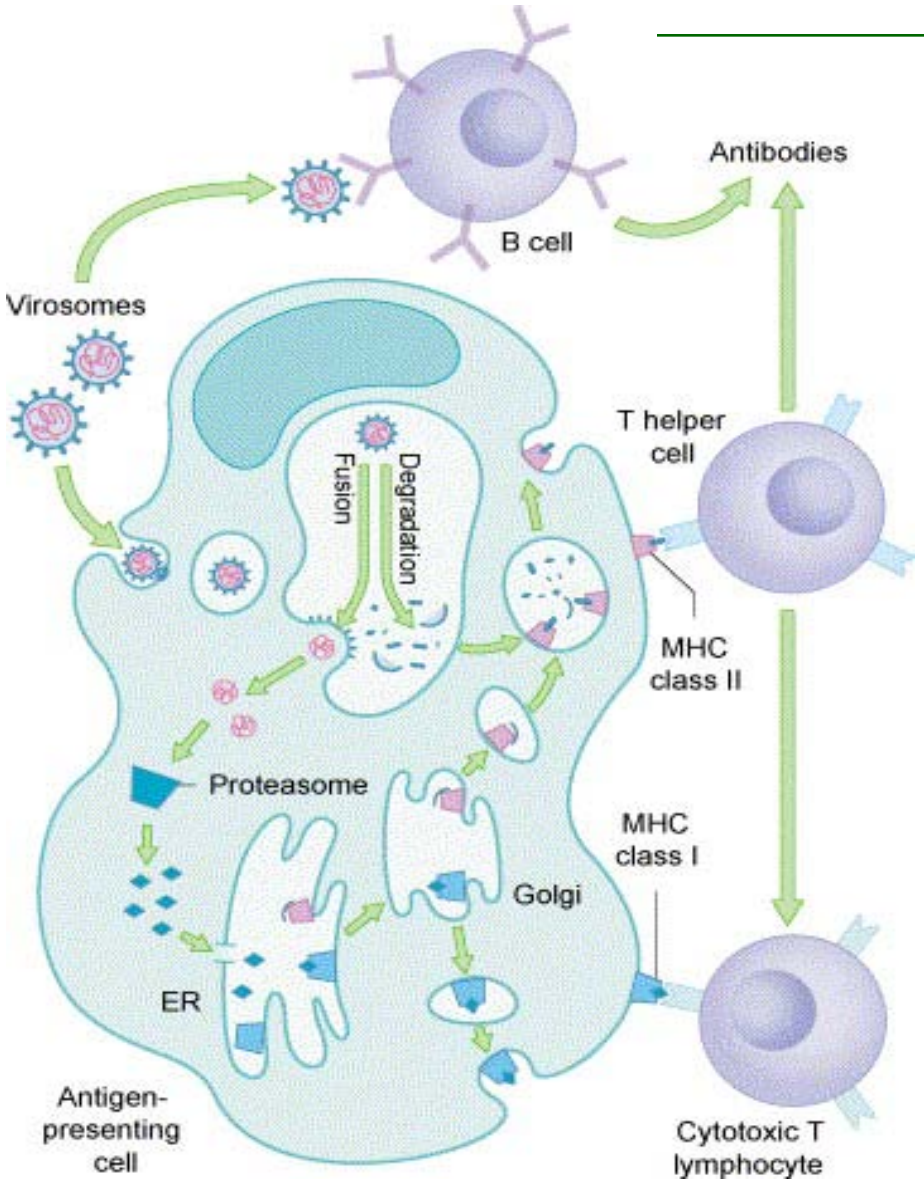


Fusion activity of influenza virosomes determined by a **fluorescence lipid mixing assay using erythrocyte ghosts** as target membranes. At pH 5.5, the optimal pH for fusion of A/Panama influenza virus (H3N2), influenza virosomes derived from this virus strain exhibit a high extent of fusion (A). When the virosomes are neutralized and then used in the fusion assay at pH 5.5, fusion activity is completely abolished (B).

Virosomal delivery of the **A subunit of diphtheria toxin (DTA)**. In the absence of the receptor-binding B subunit DTA cannot enter cells. Virosome-encapsulated DTA is efficiently delivered to the cell cytosol, resulting in complete inhibition of cellular protein synthesis.

INTERACTIONS OF VIROSOMES WITH CELLS OF THE IMMUNE SYSTEM

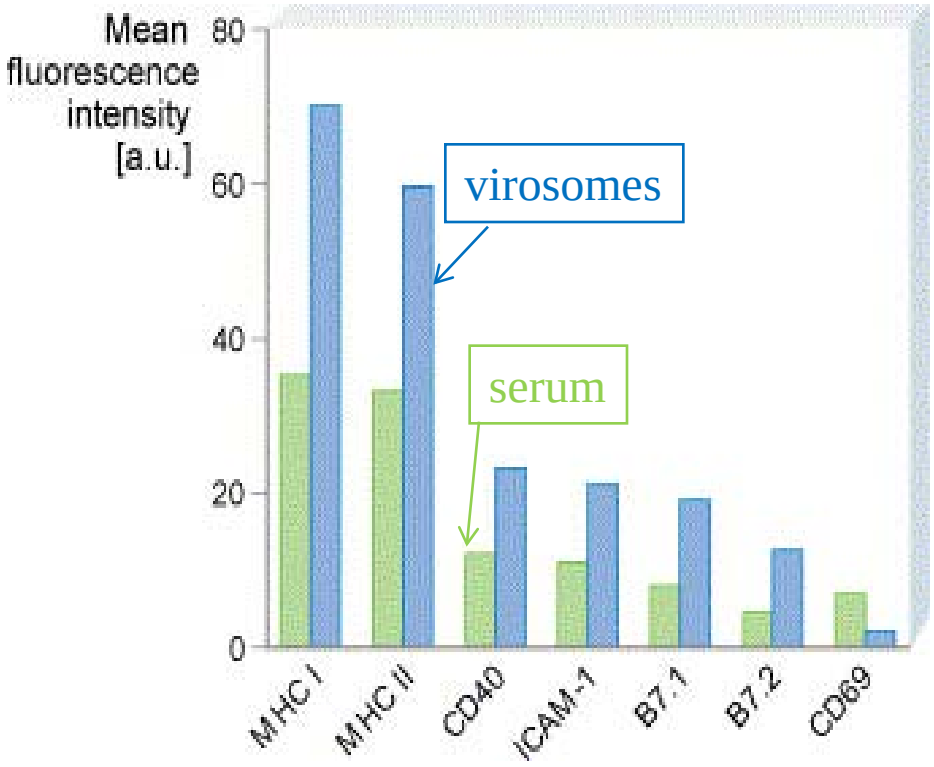
Anke Huckriede et al., Vaccine,23S1, 26-38, 2005



By virtue of the repetitive arrangement of haemagglutinin on the virosome surface, virosomes interact efficiently with immunoglobulin receptors on B lymphocytes. Virosomes are also taken up avidly by antigen-presenting cells, particularly dendritic cells. Antigens on the virosome surface, as well as antigens derived from degraded virosomes, enter the MHC class II pathway, activating T helper cells. Antigens inside the virosomes, through fusion of the virosomes, access the cytosolic MHC class I presentation pathway, activating cytotoxic T lymphocytes (CTL)

MATURATION OF DENDRITIC CELLS INDUCED BY VIROSOMES

Huckriede A, et al., Vaccine 21, 925–31 (2003)



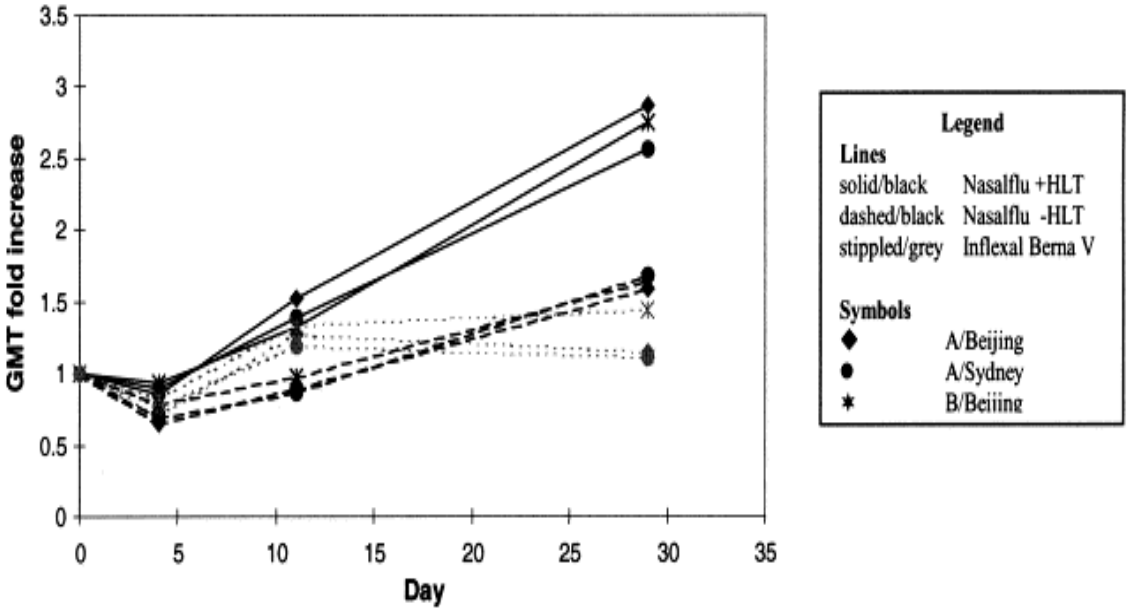
Dendritic cells were incubated with 10 nM of OVA in fusion-active virosomes (blue bars). Cells were incubated with supplemented cell culture medium as a negative control (green bars).

After 24 h of incubation, the cells were washed and cultured for an additional 24 h in supplemented cell culture medium before **staining with marker-specific monoclonal antibodies and flow cytometry.**

Maturation of dendritic cells induced by virosomes: upregulation of maturation markers. Influenza virosomes stimulate expression of MHC class I and II, CD40, ICAM-1, B7.1 and B7.2 on dendritic cells.

NASAL VACCINATION WITH VIROSOMES CONTAINING INFLUENZA A AND B HEMAGGLUTININ

Glueck R et al., ADDR, 51, 203-211, 2001



Geometric mean titer (GMT) increase of influenza-specific IgA antibodies in nasal lavage after nasal immunization with influenza A and B hemagluttinin virosomes (Nasalflu), supplemented or not with Escherichia coli heat-labile toxin (HLT). Comparison with Inflexal. In humans.

Vaccine group	Serum (IgG) anti-HA antibody response No. with a ≥4-fold rise/total (%)			Mucosal (IgA) antibody response in saliva % with a rise of ≥4-fold		
	Strain H1N1	Strain H3N2	Strain B	Strain H1N1	Strain H3N2	Strain B
A	8/20 (41)	17/20 (85)*	12/20 (60)*	73**	73**	80**
B	5/20 (25)	0/20 (0)	4/20 (20)	14	14	0
C	7/20 (35)	7/20 (35)	4/20 (20)	58	39	33
D	12/20 (60)**	12/20 (60)	9/20 (45)*	25	38	27

Humoral (anti HA) and mucosal (IgA) antibody responses after intranasal or intramuscular vaccination. Superiority of group A.

A Two doses with HLT intranasal

B two doses without HLT intranasal

C one dose with HLT intranasal

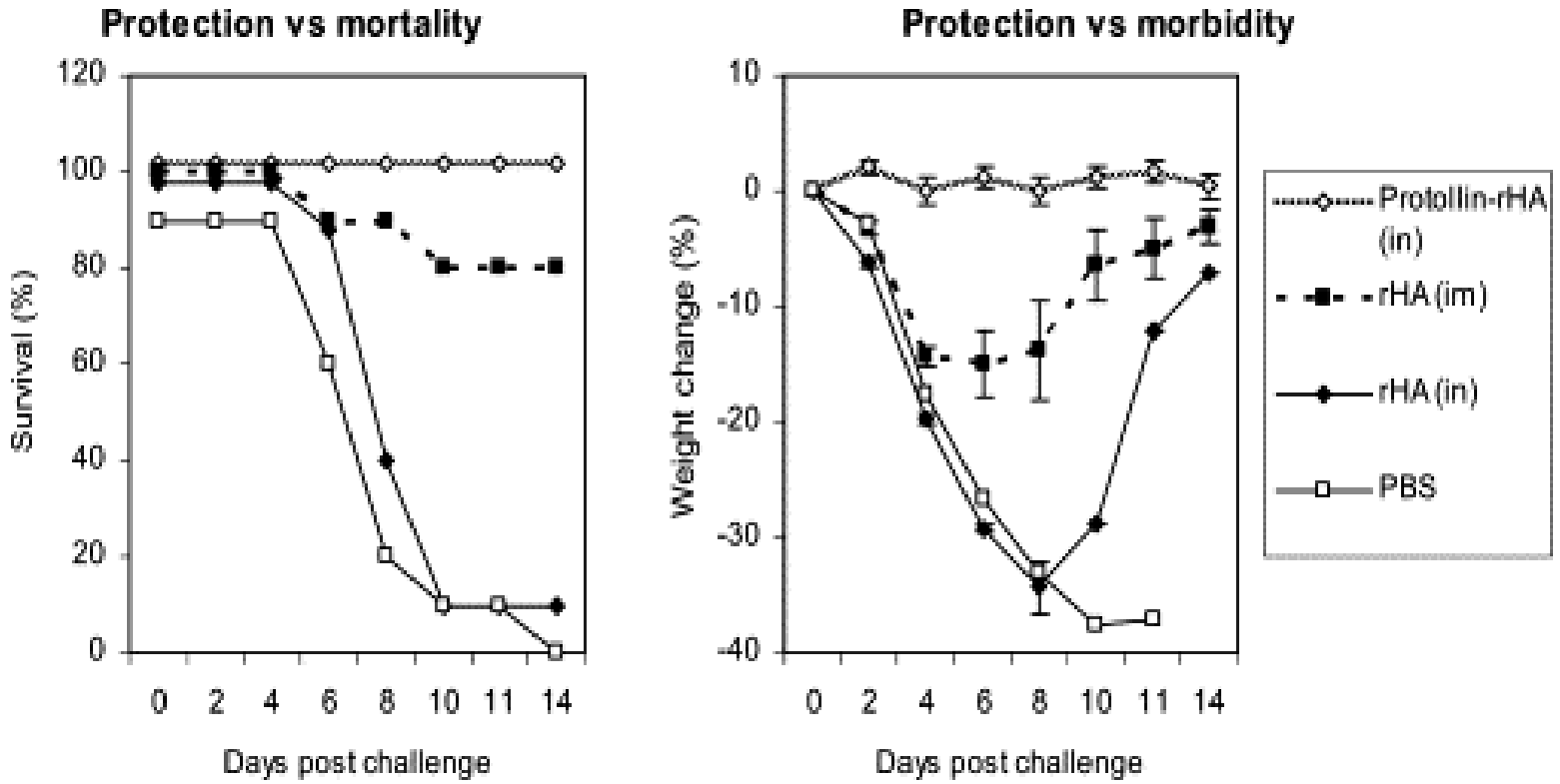
D one dose without HLT intramuscular

PROTEOSOMES FOR NASAL IMMUNIZATION

Jones T et al., Vaccine 22, 3691-3697, 2004

Proteosomes = hydrophobic outer membrane (OMP) protein derived from *N. meningitidis* non-covalently complexed with lipopolysaccharide (LPS)s isolated from *Shigella flexneri* or *Pleisiomonas shigelloides.*

Detergent + OMPs + LPS ⟹ *nanoparticles comprising Proteosome porins and LPS*

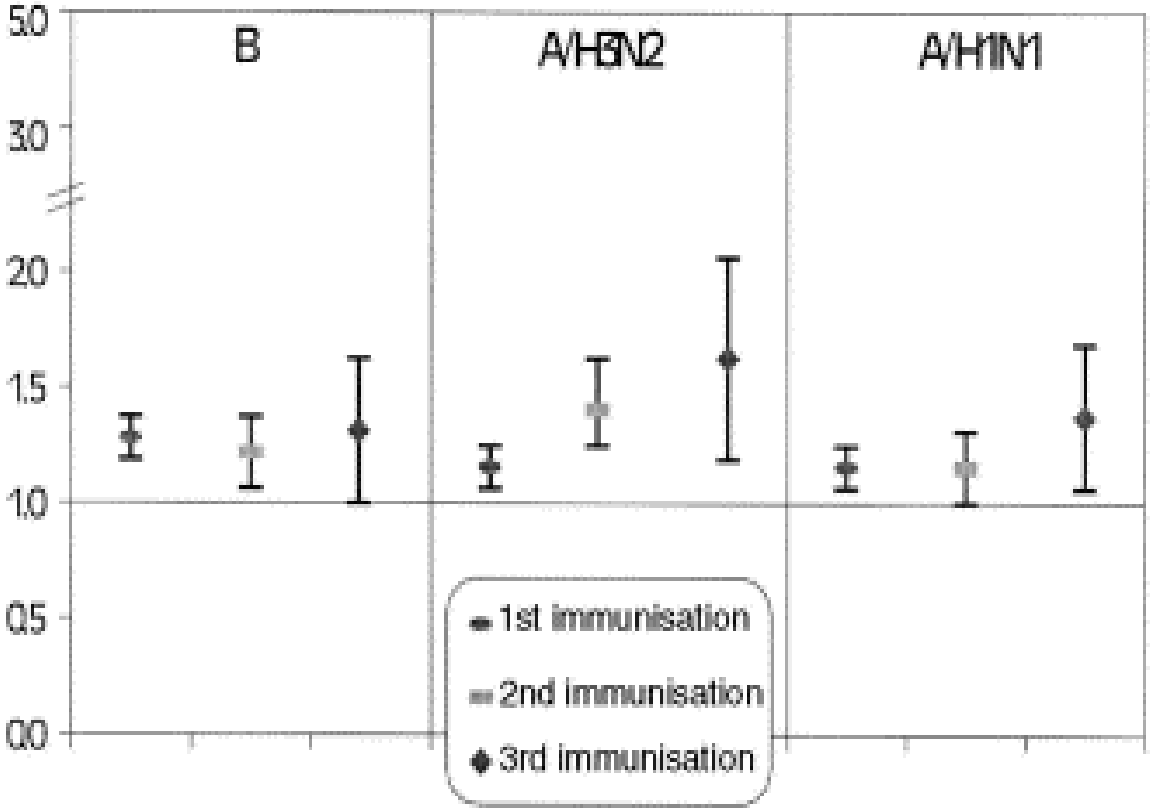


rHA = viral antigene

Survival and weigth loss of mice after lethal challenge with live, homotypic influenza virus

MF59 OIL NANODROPLETS

A. Podda,Vaccine, 19, 2673-2680, 2001

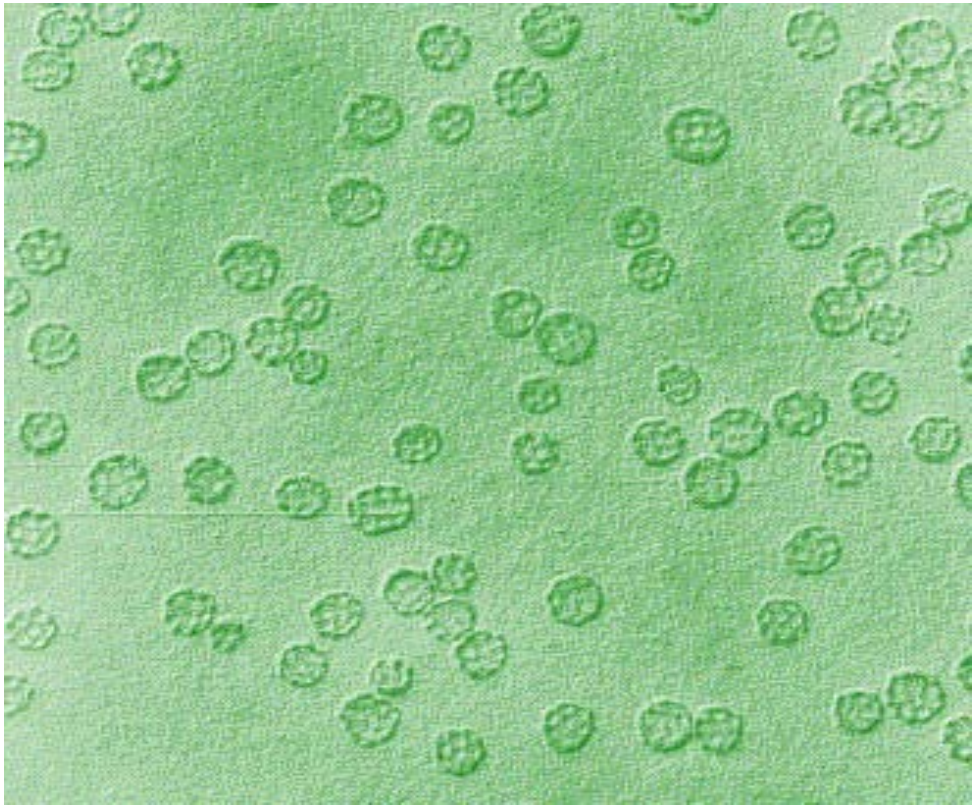


MF59 ARE NANODROPLETS (250 NM) OF A W/O NANOEMULSION CONTAINING
- **SQUALENE (4.3%)**
- **POLYSORBATE TWEEN 80 (0.5%)**
- **SORBITAN TRIOLEATE (0.5%)**

Influenza FLUAD™MF59 compared to classical flu vaccine (1.0) post-immunisation GMT ratios and 95% confidence intervals for the B, A/H3N2, A/H1N1 antigens after the first, the second and the third immunisation (humans)

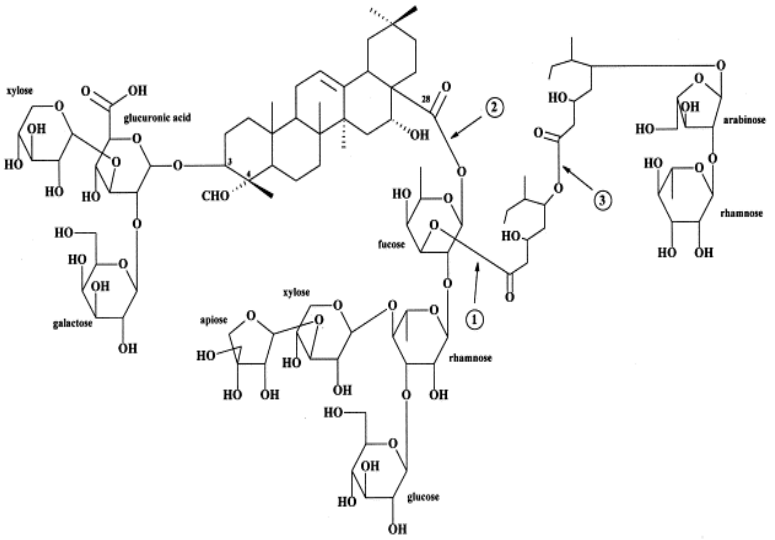
IMMUNOSTIMULATING COMPLEXES (ISCOMS)

Martin J Pearse et al., ADDR, 57, 465-474, 2005



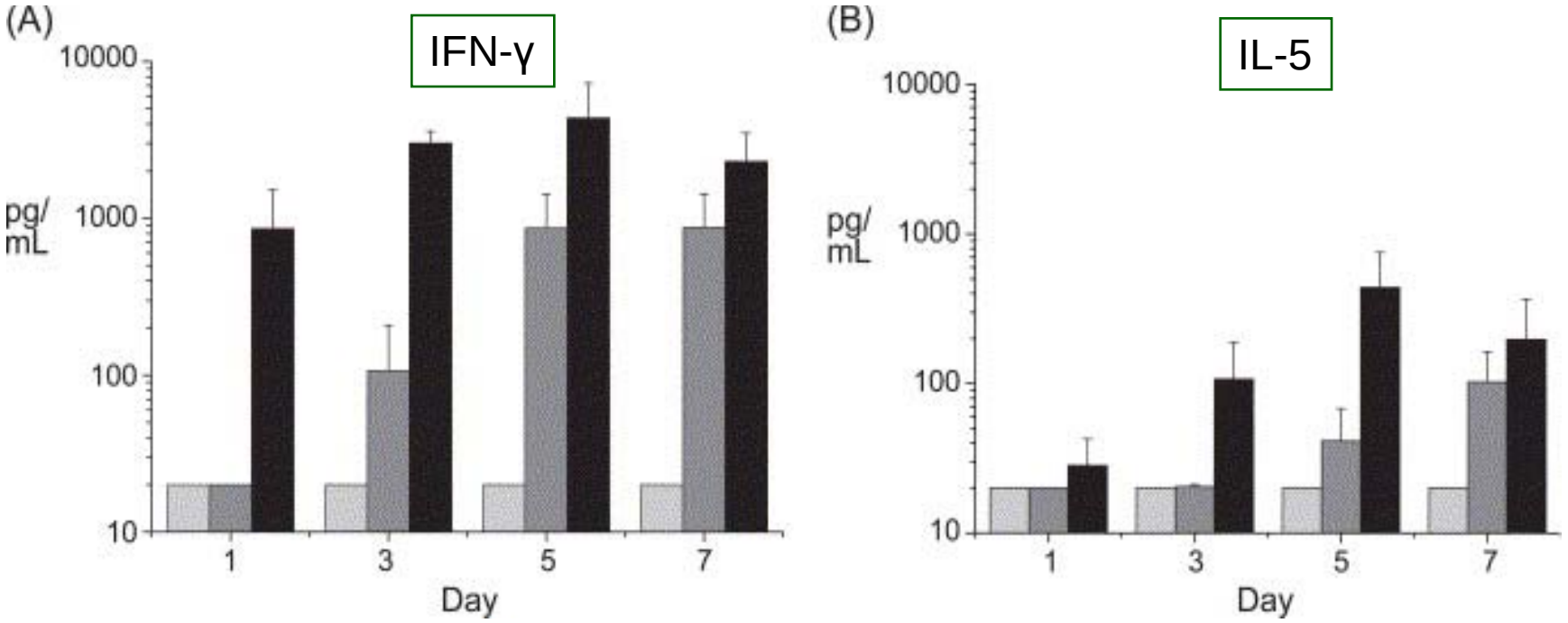
Transmission electron micrograph of negatively stained ISCOMATRIX® adjuvant demonstrating the typical 40nm cage-like structures.

ISCOMS ARE 40 NM CAGE-LIKE STRUCTURES COMPOSED OF
- A PROTEIN ANTIGEN
- CHOLESTEROL
- PHOSPHOLIPID
-SAPONIN ADJUVANT QUIL A



ISCOMS GENERATE T-CELLS PRODUCING INF-γ AND IL-2 (Th1 AND Th2 CYTOKINES)

Martin J Pearse et al., ADDR, 57, 465-474, 2005



IFN-γ (A) and IL-5 (B) responses in naïve mice (light grey), and mice vaccinated with NY-ESO-1 protein (dark grey) or NY-ESO-1 ISCOMATRIX™ vaccine (black).

MICE INTRANASAL VACCINATION WITH A/PR8/34 ISCOMS

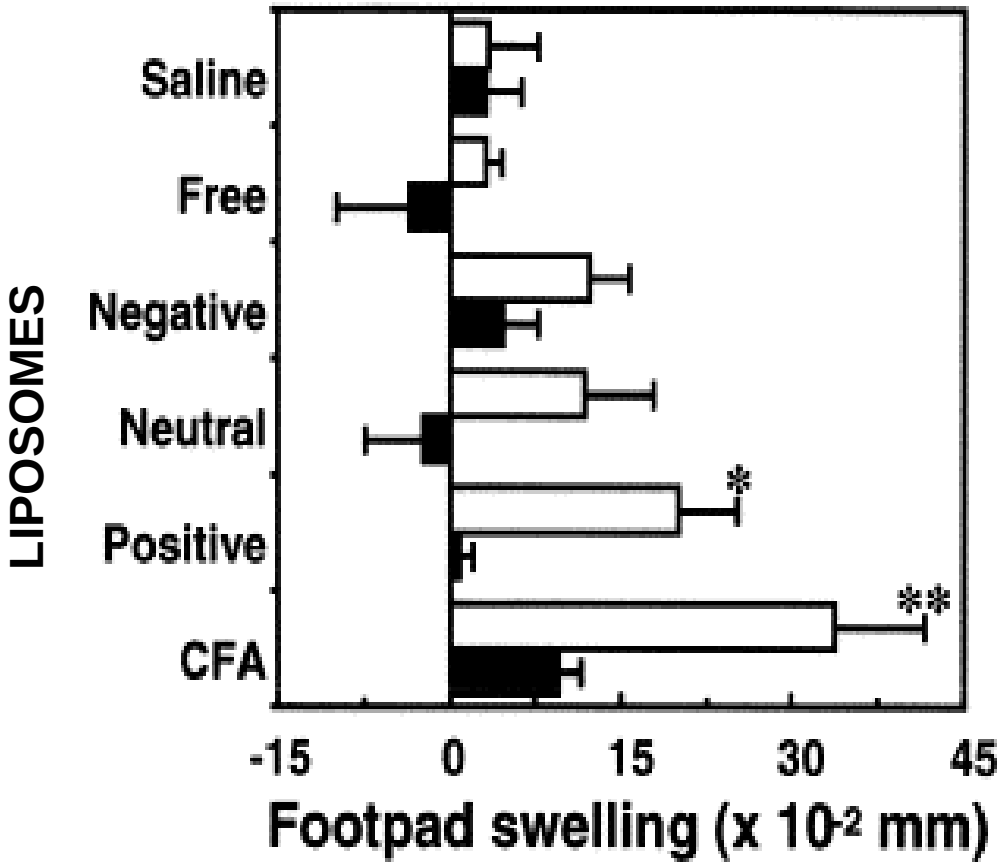
Barr IK et al, ADDR, 32, 247-271, 1998

Gp	Treatment[a]	HA dose µg[b]	Serum IgG GMT[c]	Serum IgA GMT[c]	Following live influenza challenge[d]	
					Weight change%	Survivors %
1	Flu-ISCOMs	5	2202	171	−2.2	90
2	Flu	5	137	< 100	−22.4	40
3	Flu-ISCOMs	1	131	107	−25.9	10
4	Flu	1	< 100	< 100	−22.8	0
5	PBS	0	< 100	< 100	−22.6	0
6	Flu-ISCOMs[e] (sc)	0.5	6961	< 100	−0.2	100
		$p < 0.05$	*gp*1 > 2,3,4,5	N.S	*gp* 1 < 2,3,4,5	*gp* 1 > 5

Table: Intranasal vaccination of unanaesthetised mice with A/PR8/34 influenza-ISCOMs

LIPOSOMAL VACCINATION

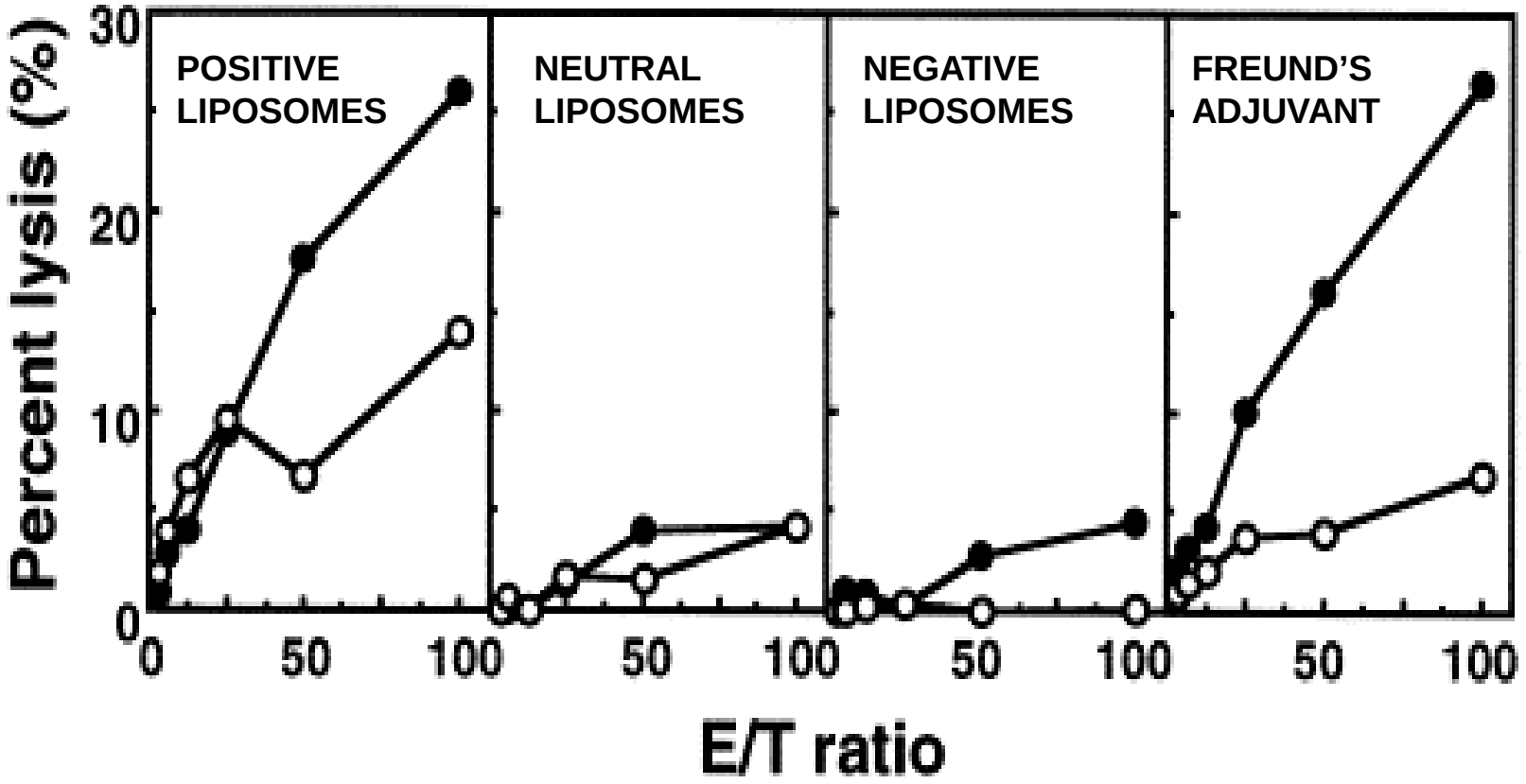
Nakanishi T et al., J. Control. Rel., 61, 233-240, 1999



Footpad swelling measured **24 h (open columns) and 48 h (solid columns)** after challenge with 2% heat-aggregated OVA in the rear footpad of mice primed 8 days previously with 50 µg of various formulations of OVA.

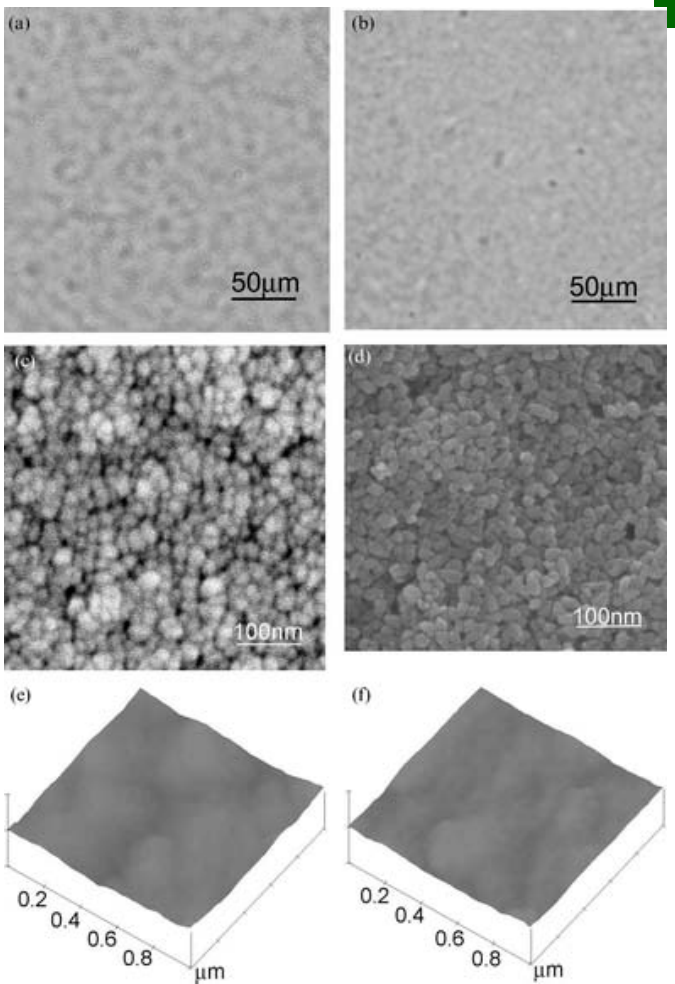
LIPOSOMAL VACCINATION CTL RESPONSE

Nakanishi T et al., J. Control. Rel., 61, 233-240, 1999



β-Gal -specific CTL response after **SUBCUTANEOUS** priming with various formulations of β-gal. Spleen cells were assayed for cytotoxic activity against the 51Cr-labeled targets: (•) P13.1 and (○) P815 cells.

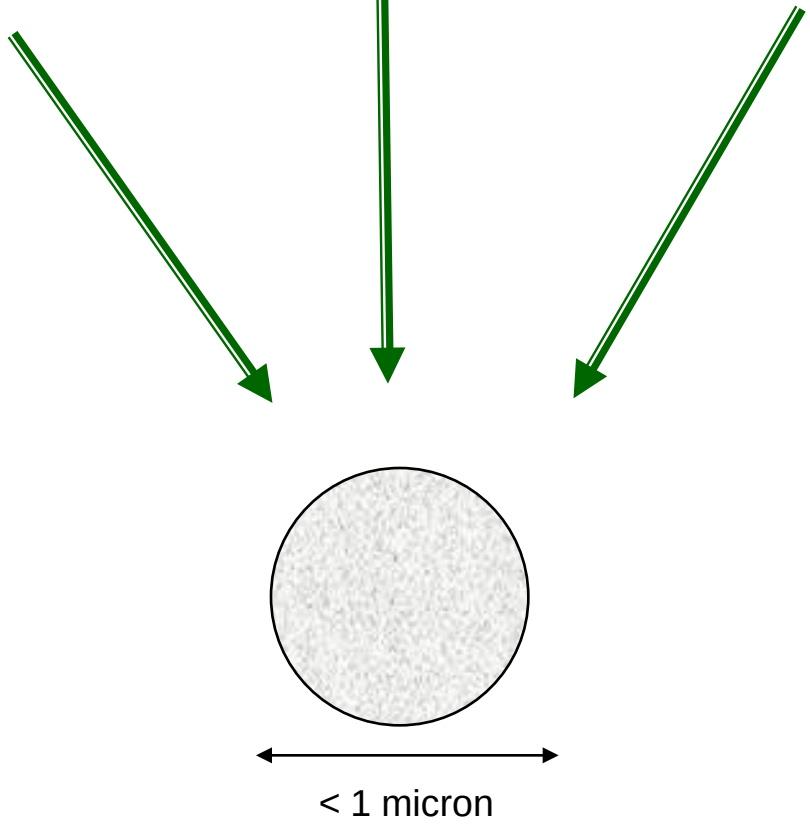
CALCIUM PHOSPHATE NANOPARTICLES



1mg Herpes simplex virus (HSV) type 2 protein in calcium Chloride 12.5 mM (7.5 ml)

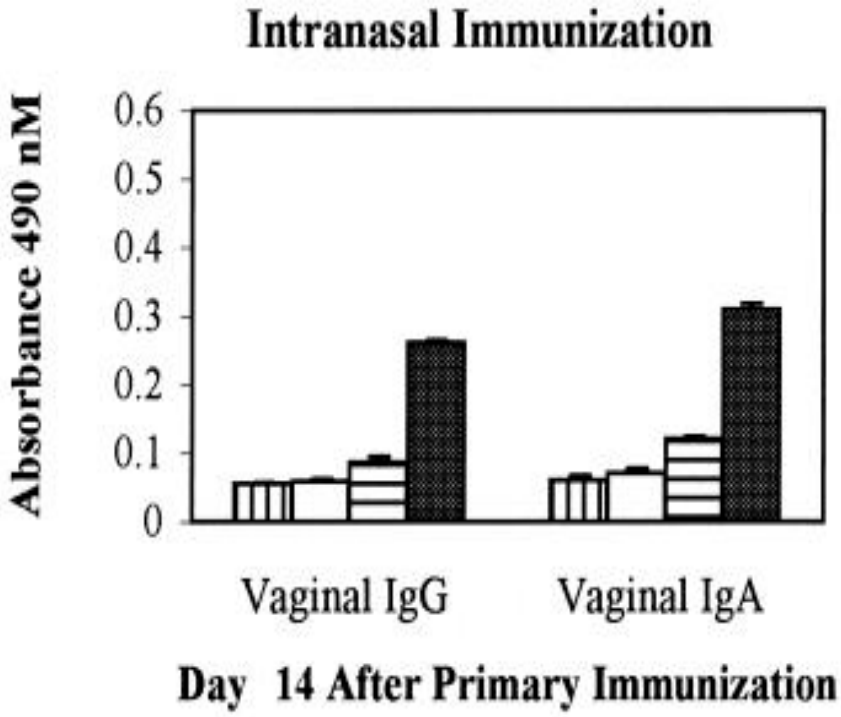
Na_2HPO_4 12.5 mM (7.5 ml)

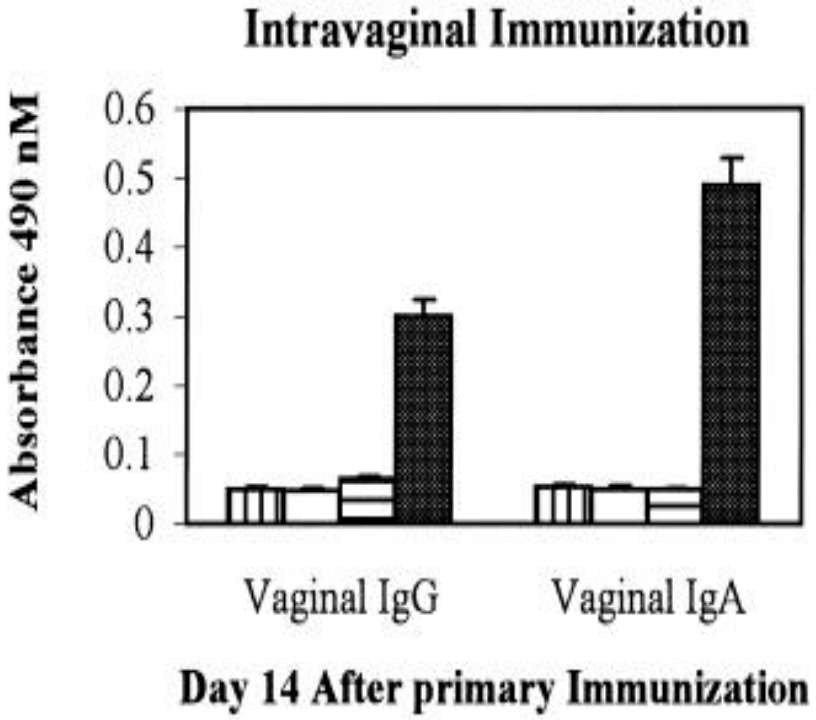
15.6 mM Calcium Citrate (1.5 ml)



IgA RESPONSE AFTER INTRANASAL AND INTRAVAGINAL IMMUNIZATION BY CALCIUM PHOSPHATE NANOPARTICLES (CAP)

He Q et al, Clin Diagn Lab Immunol. 9(5) 1021–1024, 2002

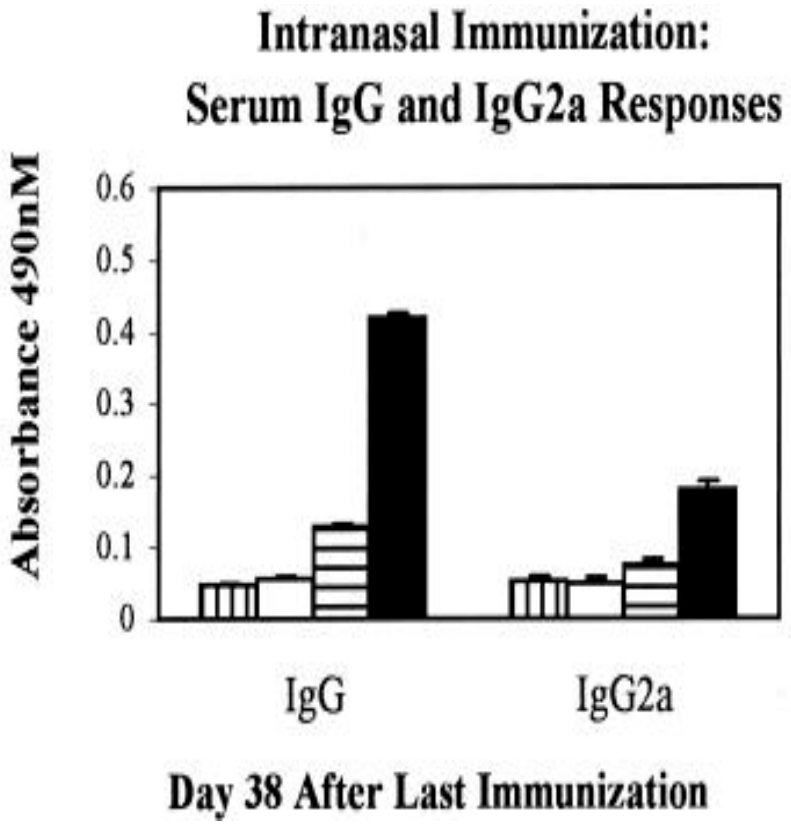


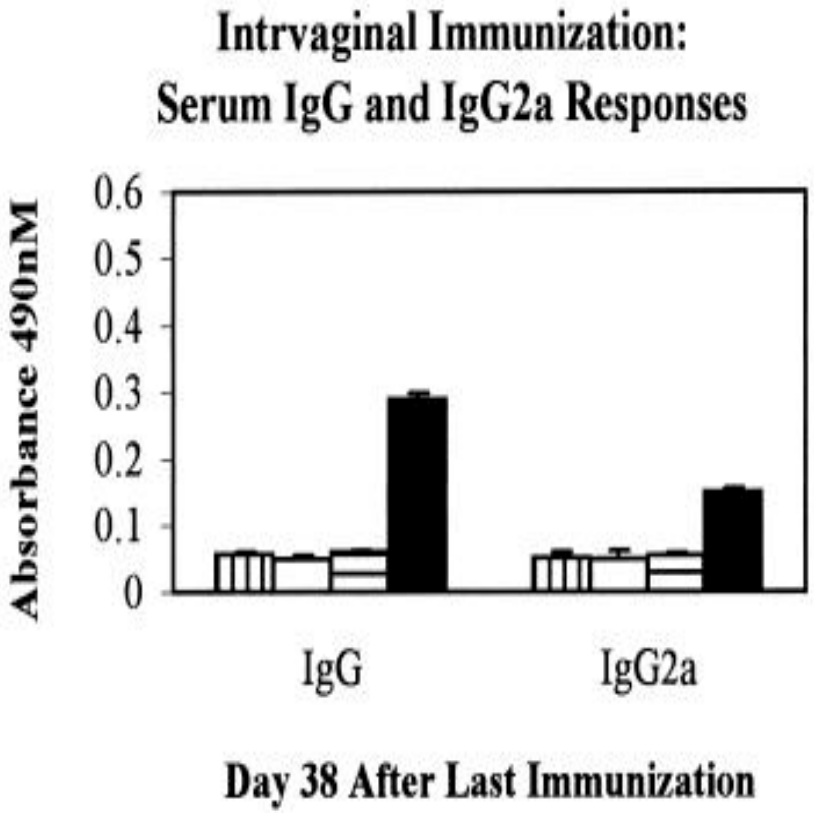


BALB/c mice were immunized on days 0 and 7 by intranasal or intravaginal delivery of PBS (vertically striped bars), CAP alone (open bars), herpes simplex virus type-2 HSV-2 antigen alone (horizontally striped bars), or HSV-2+CAP (solid bars).

IgG RESPONSE AFTER INTRANASAL AND INTRAVAGINAL IMMUNIZATION BY CALCIUM PHOSPHATE NANOPARTICLES (CAP)

He Q et al, Clin Diagn Lab Immunol. 9(5) 1021–1024, 2002

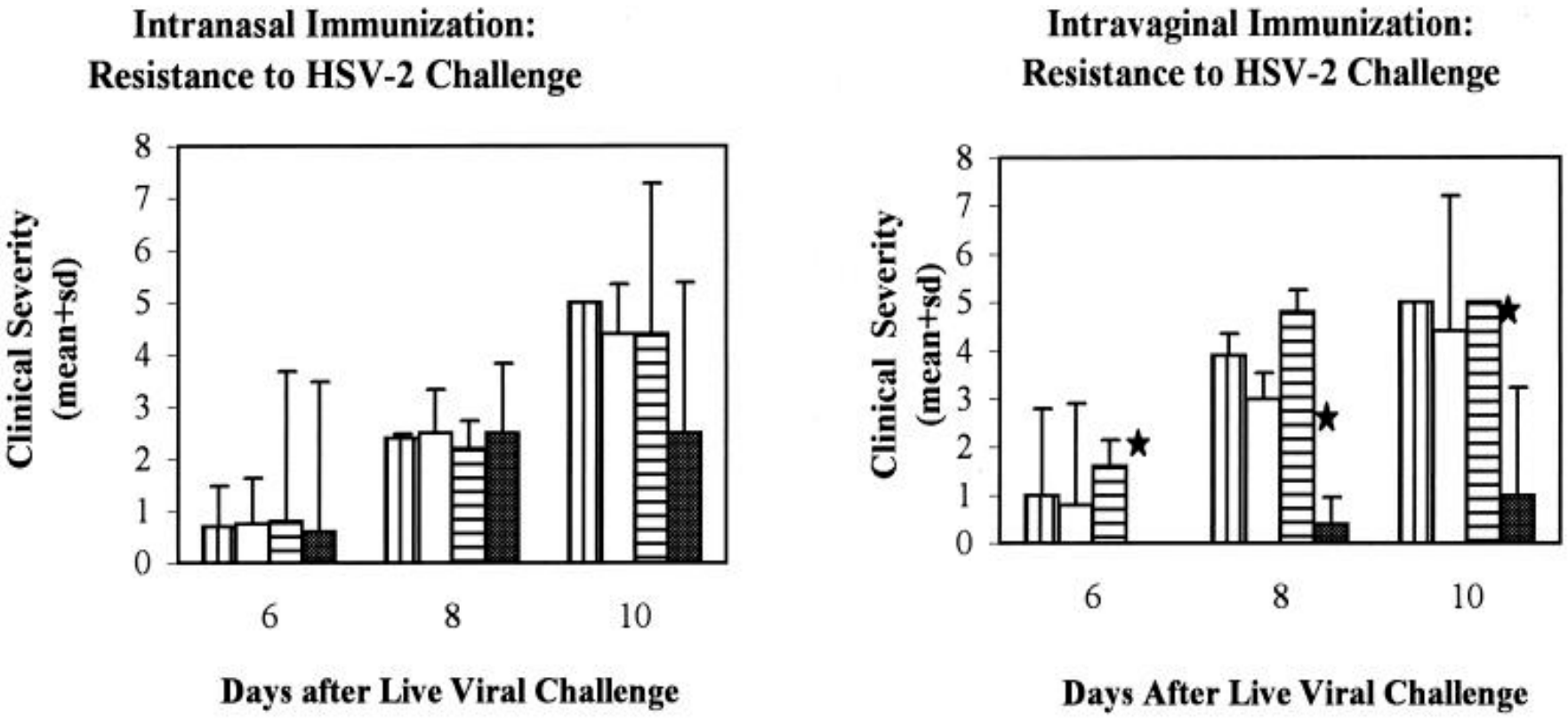




Each bar represents the group mean antibody level for mice immunized intranasally or intravaginally with PBS (vertically striped bars), CAP alone (open bars), HSV-2 alone (horizontally striped bars), or HSV-2+CAP (solid bars).

CLINICAL SEVERITY AFTER INTRANASAL AND INTRAVAGINAL IMMUNIZATION BY CALCIUM PHOSPHATE NANOPARTICLES (CAP)

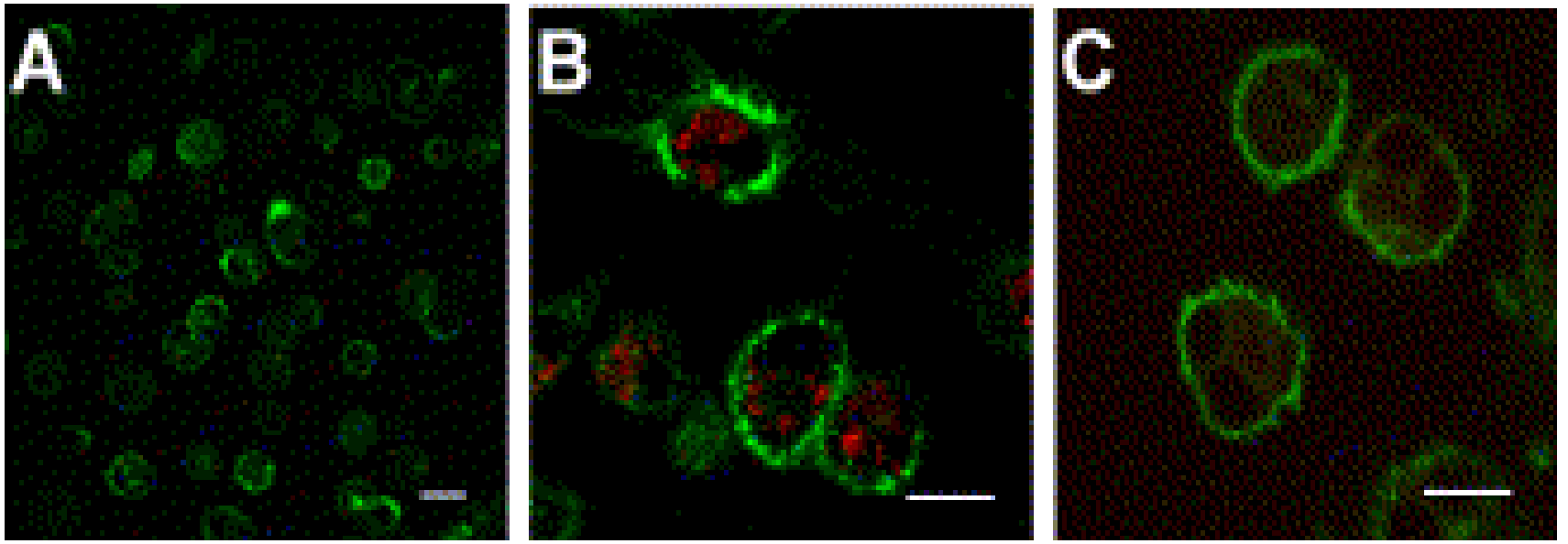
He Q et al, Clin Diagn Lab Immunol. 9(5) 1021–1024, 2002



Five BALB/c mice per group were immunized intranasally or intravaginally with PBS (vertically striped bars), CAP alone (open bars), HSV-2 alone (horizontally striped bars), or HSV-2+CAP (solid bars) and challenged intravaginally with 106 PFU of HSV-2 at 43 days after the last immunization. 0 = no apparent infection 1= slight redness of external vagina 2= severe redness and swelling of vagina 3= genital ulceration 4= severe genital ulceration + paralysis 5= dead

PLGA NANOPARTICLES: INTERACTION WITH DENDRITIC CELLS

Elamanchili P et al, Vaccine, 22, 2406-2412, 2004



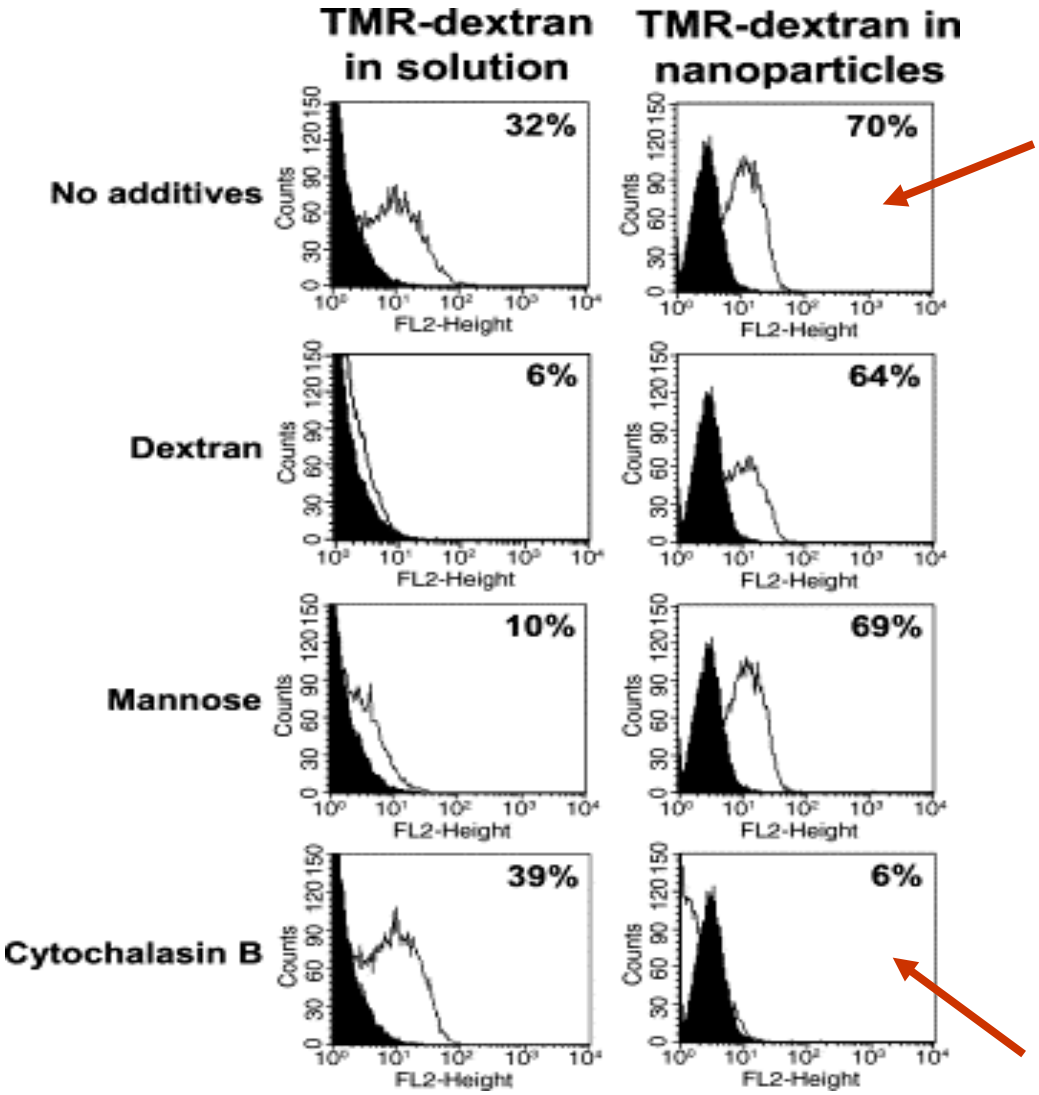
CONTROL

RHODAMINE-DEXTRAN CONTAINING NP

RHODAMINE-DEXTRAN CONTAINING NP + CYTOCHALASIN B

EFFECT ON NANOPARTICLE UPTAKE BY DENDRITIC CELLS IN THE PRESENCE OF MANNOSE RECEPTORS SUBSTRATE OR ENDOCYTOSIS INHIBITOR

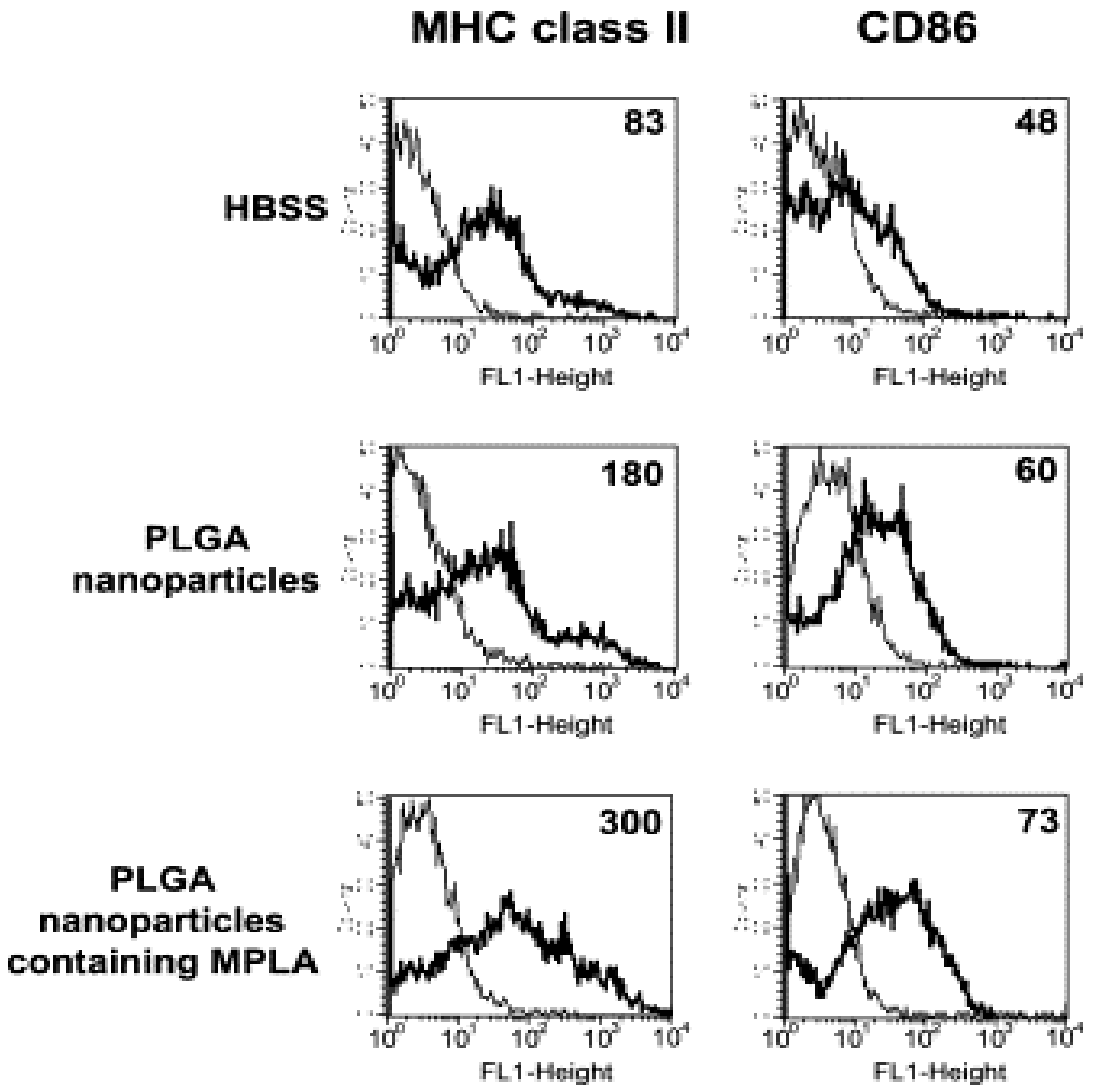
Elamanchili P et al, Vaccine, 22, 2406-2412, 2004



Effect on nanoparticle uptake by DCs in the presence of mannose receptor substrates (dextran and mannose) and phagocytosis inhibitor (Cytochalasin B). The TMR-dextran+ cell population was analyzed by flow cytometry. Only a weak signal (<5% DCs) was obtained for the soluble probe Solid black areas in the histogram plot indicate the background fluorescence after pulsing the cells either with empty nanoparticles (for nanoparticle uptake studies) or HBSS (for soluble formulation uptake studies).

ENHANCED EXPRESSION OF MHC class II and CD86 ON DENDRITIC CELLS AFTER UPTAKE OF PLGA NANOPARTICLES LOADED WITH MONOPHOSPHORYL LIPID A (MPLA)

Elamanchili P et al, Vaccine, 22, 2406-2412, 2004



Enhanced expression of MHC class II and CD86 on DCs after uptake of PLGA nanoparticles. DCs were incubated with nanoparticles (with or without MPLA) for 24 h and analyzed for expression levels of MHC class II and CD86. Control groups were treated with HBSS. Thin black line in the histogram plot represents the background staining with isotype controls.

MICROPARTICLES FOR VACCINATION

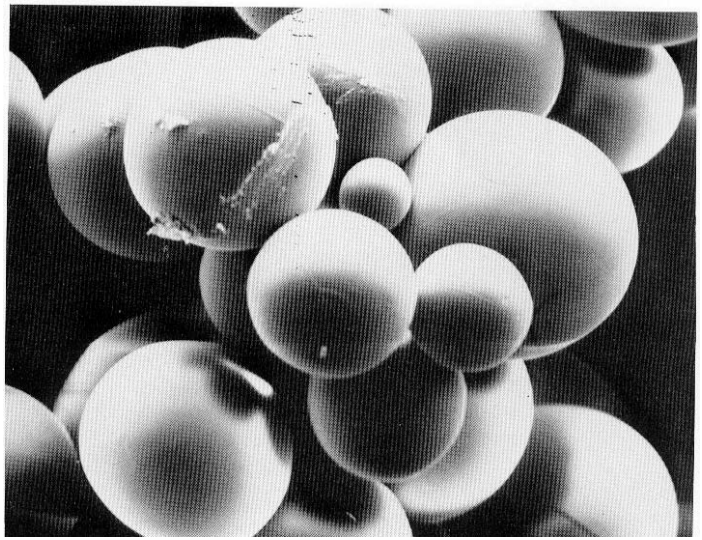
-PLGA microspheres

-Hyaluronic acid bioadhesive PLGA microspheres

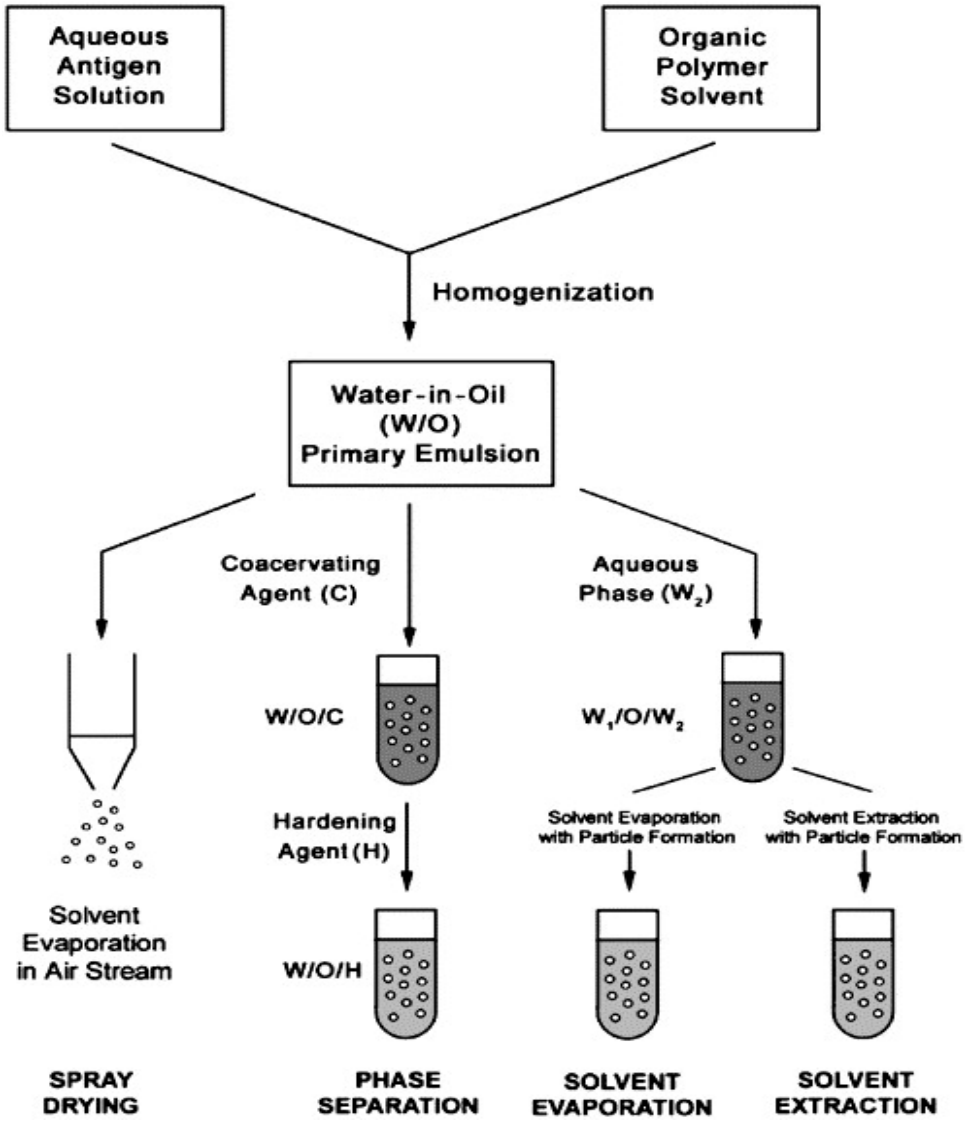
-Cationic PLGA microspheres

MICROSPHERES PREPARATION FOR VACCINATION

A significant problem with PLGA micoencapsulation is the possibility of antigen denaturation due to exposure to organic solvants and high shear

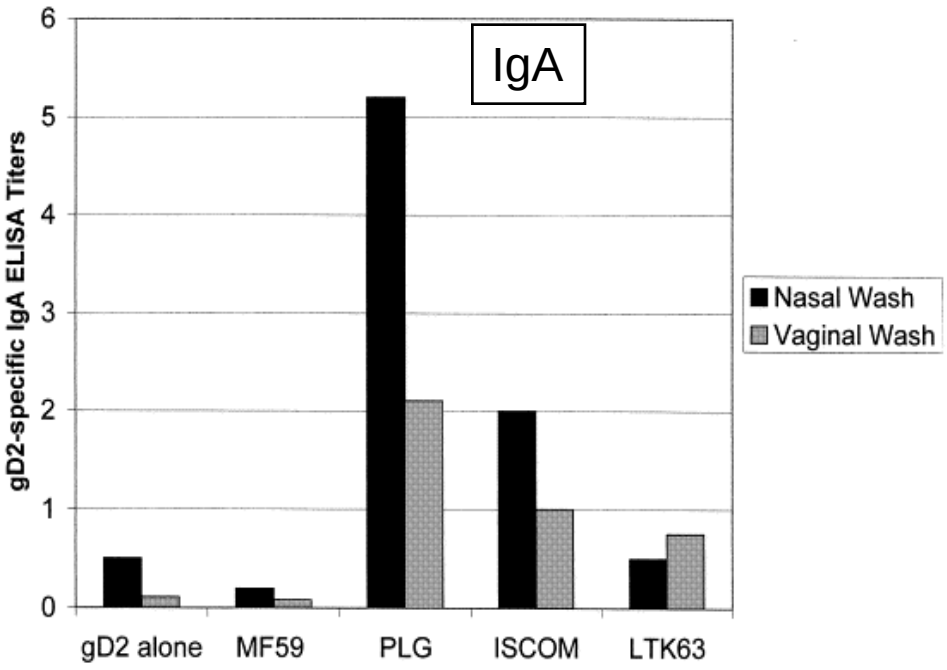


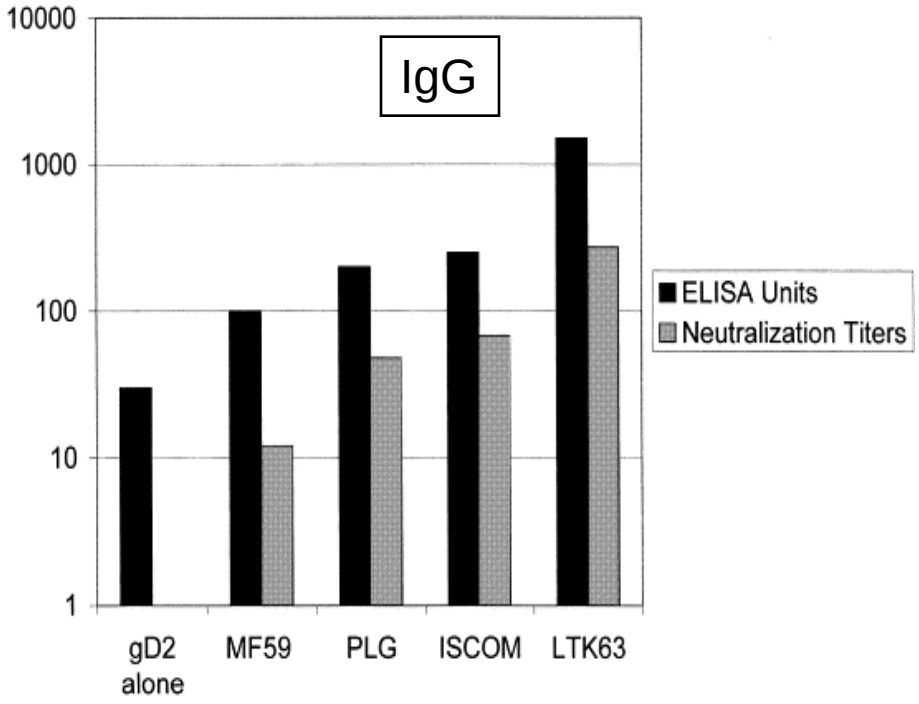
Microspheres of polylactic acid
Sizing a few microns



INTRANASAL IMMUNIZATION WITH PLA MICROSPHERES ADMINISTERED INTRANASALLY (IN)

Ugozzoli M. et al., Immunology, 93, 563-571, 1998



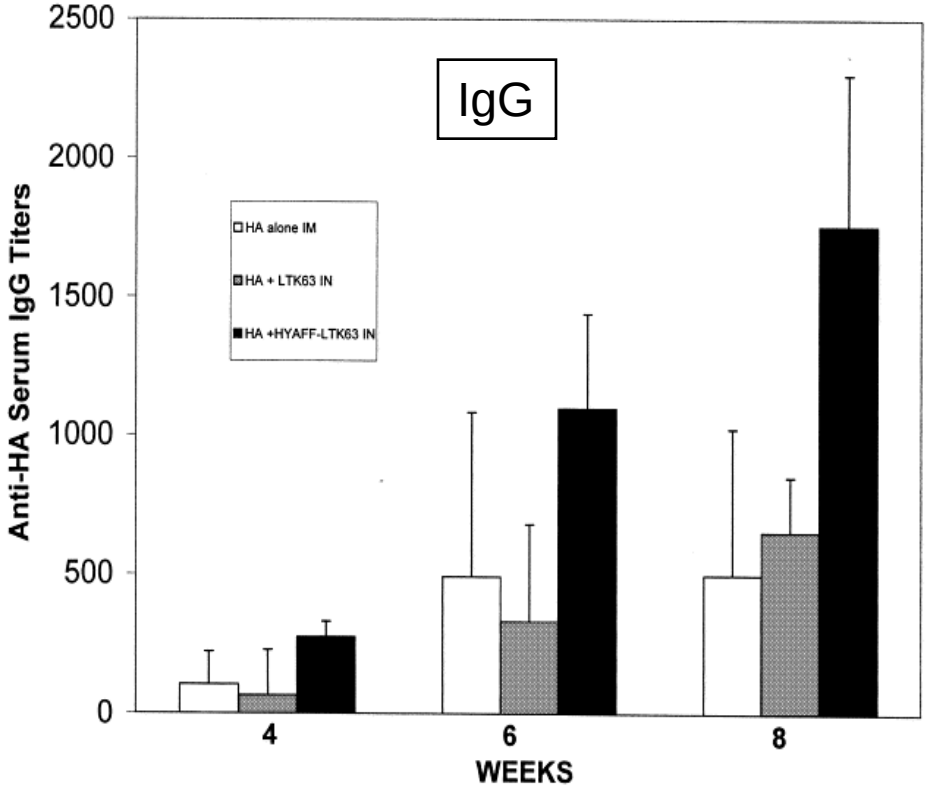


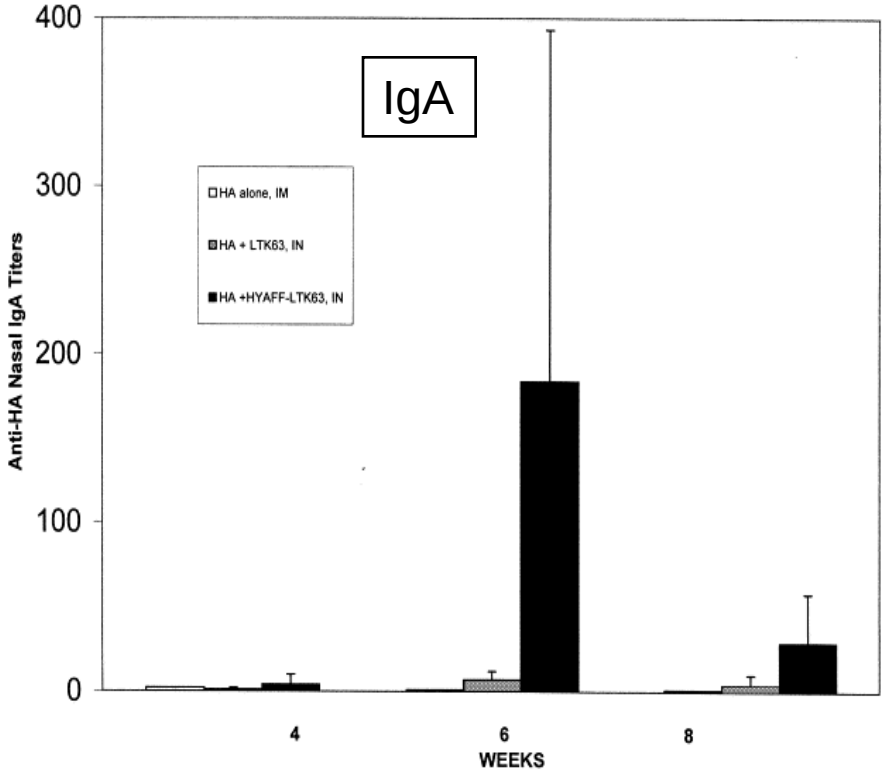
Specific IgA responses in mucosal secretions 2 weeks following IN immunizations with 10 µg **Herpes simplex virus** antigene gD2 in combination with MF59 emulsion, PLG microparticles, ISCOMs or LTK63 adjuvants (mutated non toxic enterotoxine).

Serum antibody responses following IN immunizations with 10 µg Herpes simplex virus antigene gD2 in combination with MF59 emulsion, PLG microparticles, ISCOMs or LTK63 adjuvants.

HYALURONIC BIOADHESIVE PLGA MICROSPHERES LOADED WITH HAEMAGGLUTININ AS NASAL VACCINE

Vajdy M et al., ADDR 51, 127-141,2001



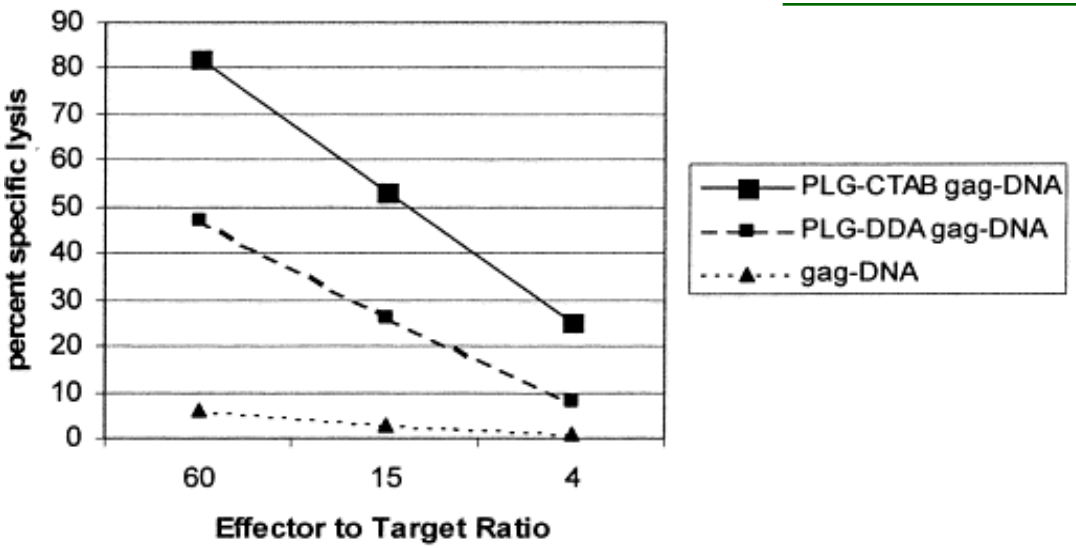


Anti-HA IgG serum titers in three groups of pigs immunized with either haemagglutinin (HA) alone IM, HA+LTK63 IN or HA+LTK63+HYAFF (bioadhesive microspheres) INmean for each group.

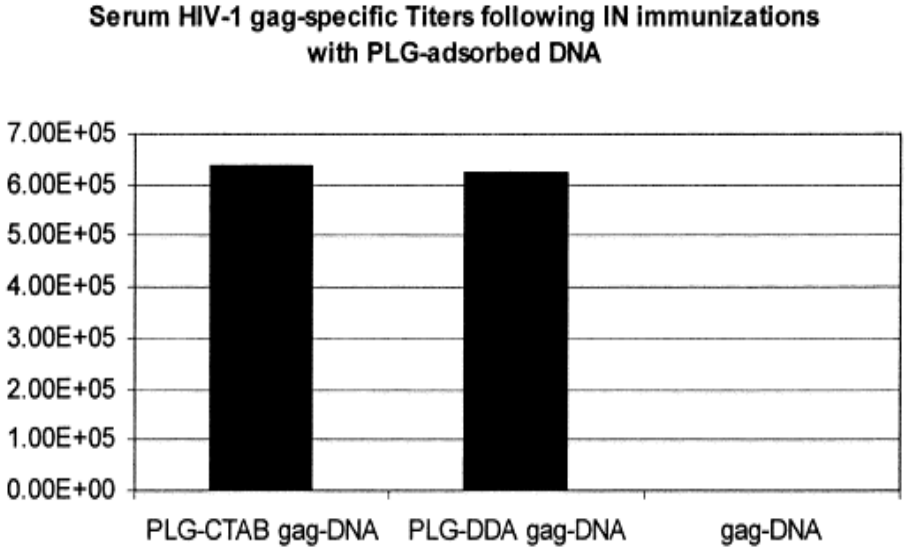
Anti-hemagglutinin (HA) IgA titers (nasal wash) in three groups of pigs immunized with either HA alone IM, HA+LTK63 IN or HA+LTK63+HYAFF (bioadhesive microsphers) IN

DNA INTRANASAL VACCINATION USING POSITIVELY CHARGED CTAB OR DDA OVERCOATED PLGA MICROSPHERES

Singh M et al., PNAS, 97, 811-816, 2000



Induction of systemic CTL responses in splenocytes following IN immunizations with DNA encoding HIV-1 gag adsorbed onto cationic PLGA microspheres, prepared using either CTAB or DDA, compared to naked plasmid DNA as measured by a 51Cr-release assay.



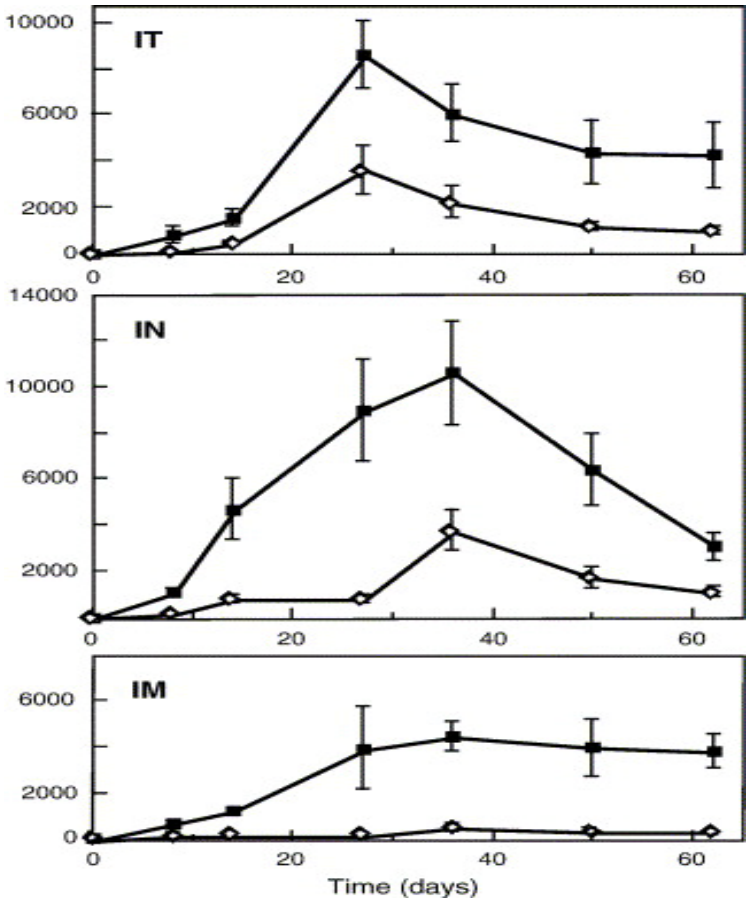
Induction of systemic humoral immune responses following IN immunizations with DNA encoding HIV-1 gag adsorbed onto cationic PLG microparticles, prepared using either CTAB or DDA, compared to naked plasmid DNA as measured by ELISA

BIODEFENSE APPLICATIONS OF VACCINATION WITH PLGA MICROSPHERES

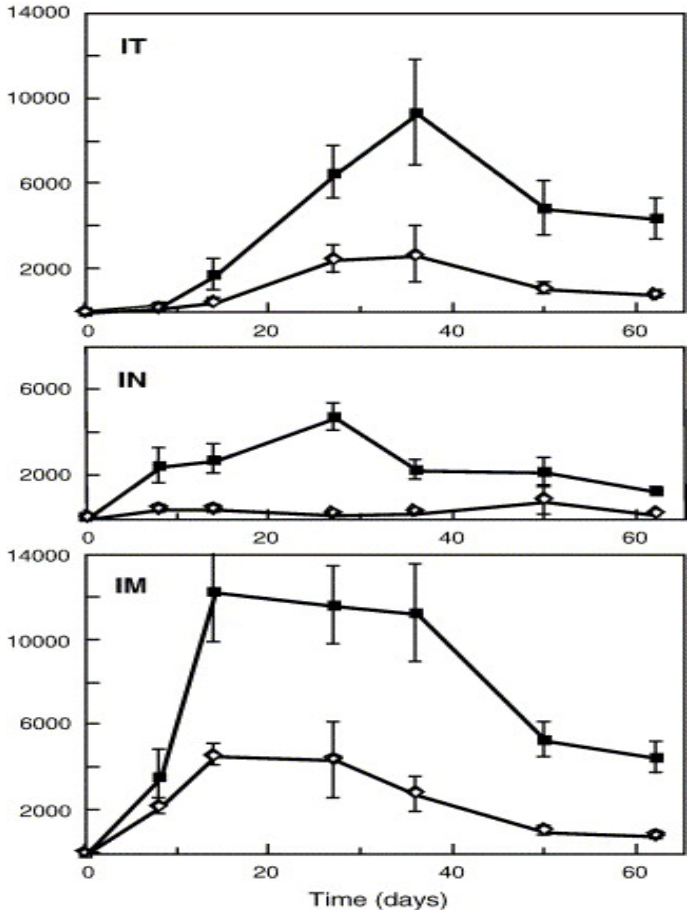
YERSINIA PESTIS

- The fraction F1 antigen is a capsular protein that has antiphagocytic properties
- Virulence V antigen is a protein secreted to regulate delivery of *Yersinia* outer proteins

SYSTEMIC IMMUNITY FOLLOWING ADMINISTRATION OF PLGA MICROSPHERES LOADED WITH YERSINIA PESTIS ANTIGENS



Anti-V IgG titres following intratracheal (IT) intranasal (IN) and intramuscular (IM) administration of microsphere coencapsulated V and F1 antigenes (■) or soluble antigenes (◇) V (3 µg) and F1 (0.47 µg);



Serum anti-F1 IgG titres following IT, IN and IM administration of microsphere coencapsulated V and F1 antigenes (■) or soluble antigenes (◇)

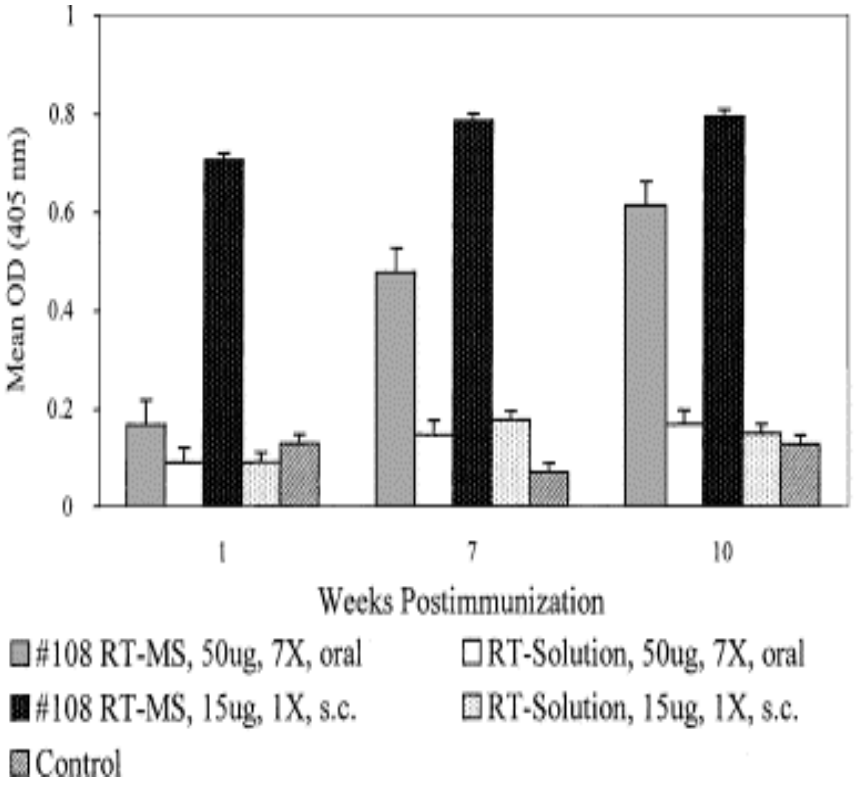
Eyles JE et al., Vaccine, 16, 2000-2009, 1998

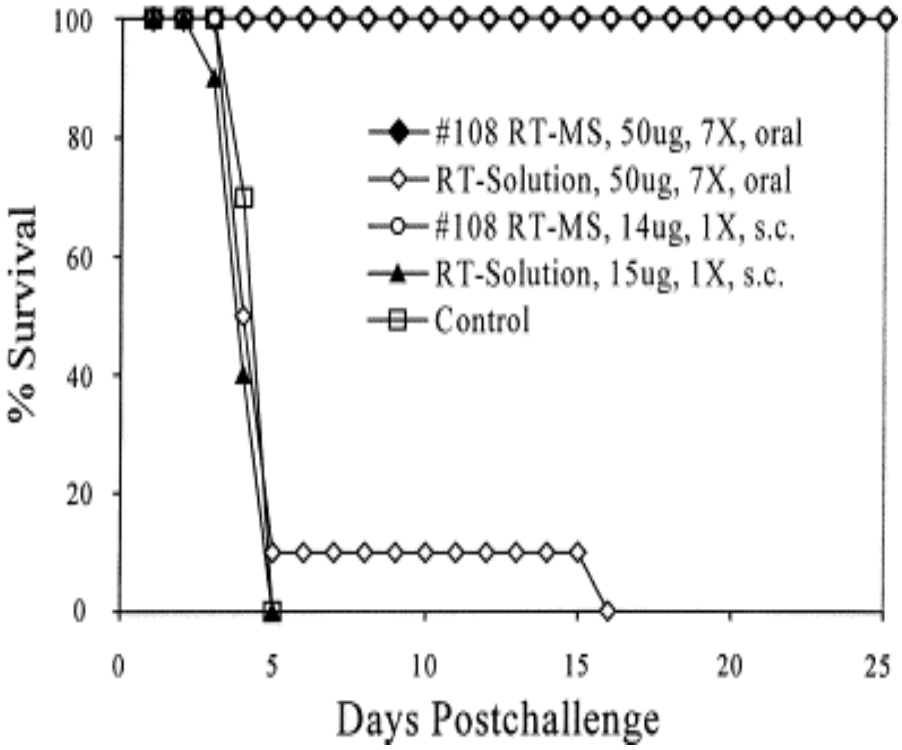
RICIN TOXINE

- One of the most potent plant toxins, easier to produce than anthrax or botulinum
- Thus, it is a convenient, potent and available toxin that may potentially be used as an agent for biological threat or terrorism
- Aerosol may be employed

PROTECTION OF RICIN TOXIN BY ORAL VACCINATION WITH PLGA MICROSPHERES

Kende M et al., Vaccine, 20, 1681-1691, 2002





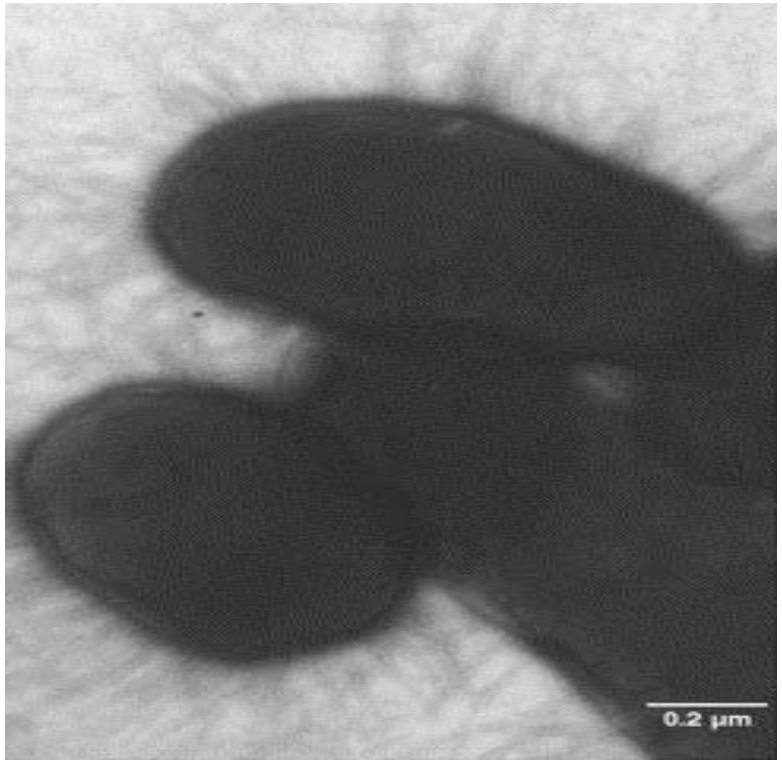
Anti-ricin serum IgG (mean OD) in mice immunized orally with 50 µg of RT in PLGA Microspheres (RT-MS) or in aqueous solution on days 1, 2, 3, 28, 29, 30 and 49; or s.c. on day 1 with 15 µg of RT-MS or aqueous vaccine (*n*=6).

Mice survival to aerosol ricin challenge 10 weeks postimmunization elicited orally with 50 µg of RT in MS (RT-MS) or in aqueous solution on days 1, 2, 3, 28, 29, 30 and 49; or s.c. on day 1 with 15 µg of RT-MS or aqueous vaccine (*n*=9–10).

ENTEROTOXIGENIC *ESCHERICHIA COLI*

Byrd W et al, ADDR, 57, 1362-1380, 2005

- Escherichia coli is very common in the aeras where fecal contamination of water and food occur
- Mortality by diarrhea estimated at 800,000 per year worldwide
- Important medical problem for military personnel deployed in developing countries
- During operation Desert Shield diarrhea was reported by 57% troops and 20% work inability
- ETEC and shigella were the predominant pathogens



ORAL IMMUNIZATION OF ADULT VOLONTEERS WITH MICROENCAPSULATED ENTEROTOXIGENIC ESCHERICHIA COLI CS6 ANTIGEN

Katz DE et al., Vaccine, 21, 341-346, 2003

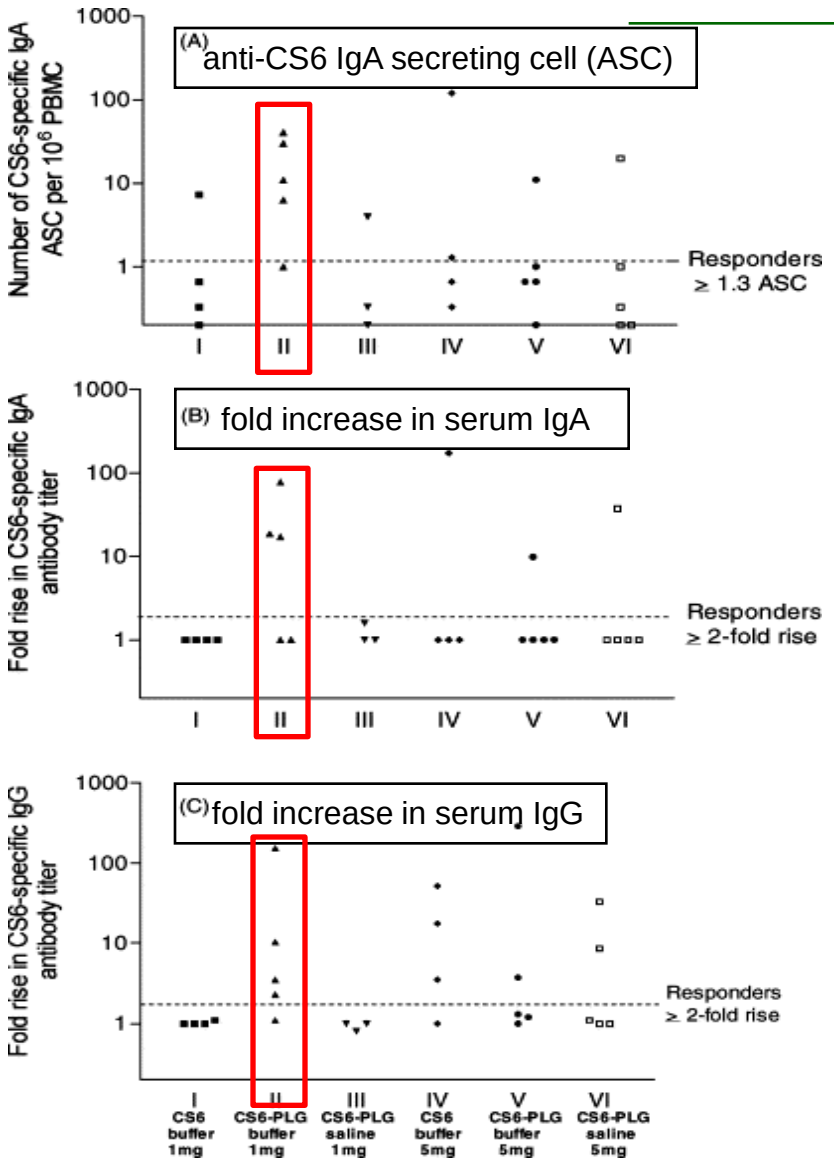


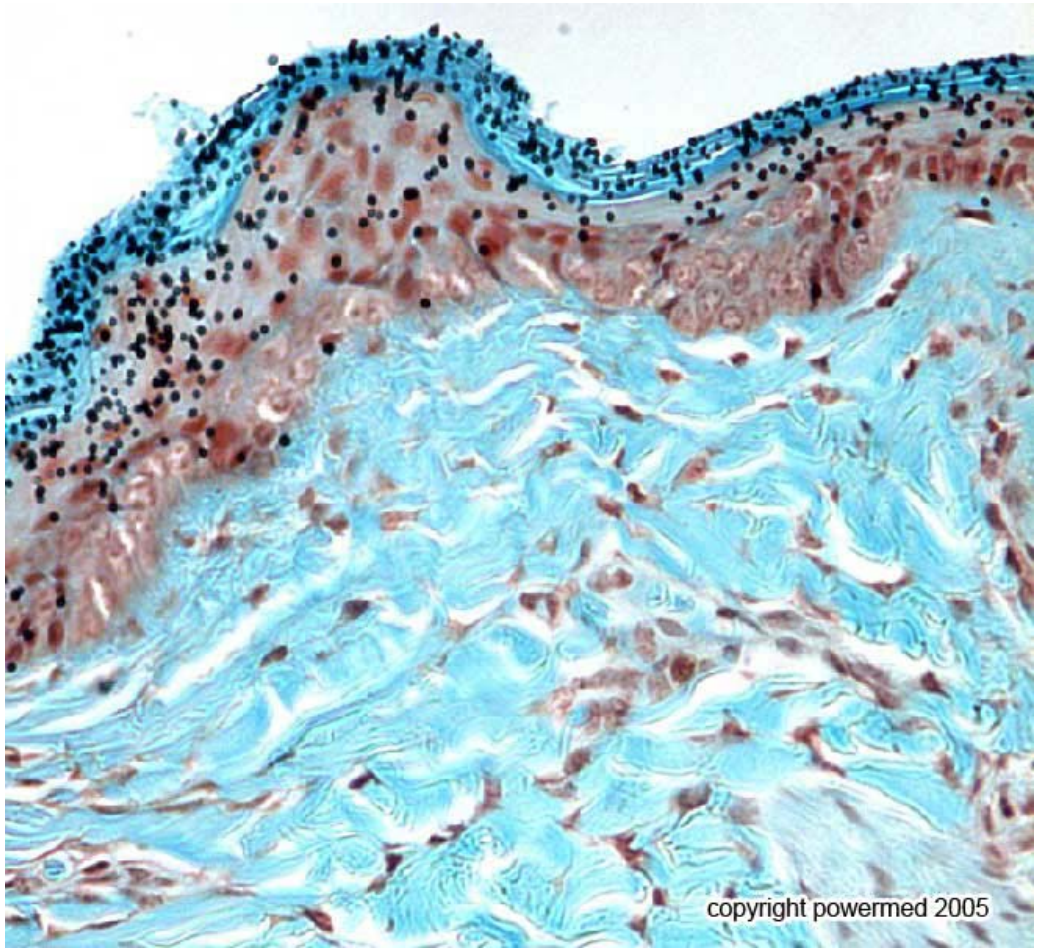
Table 1 Study groups

Group	Form	Buffer	Dose	Number
I	Non-encapsulated	Yes	1 mg CS6	4
II	PLG	Yes	1 mg CS6	5
III	PLG	No	1 mg CS6	3
IV	Non-encapsulated	Yes	5 mg CS6	4
V	PLG	Yes	5 mg CS6	5
VI	PLG	No	5 mg CS6	5

VACCINATION BY PHYSICAL FORCES

- « shot guns » with gold particles
- Electroporation

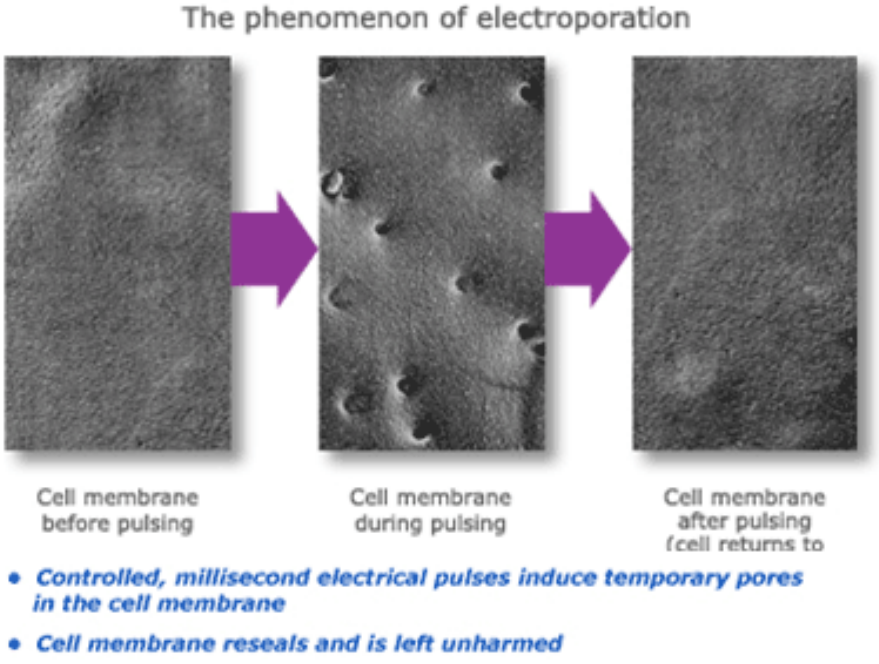
PARTICLE MEDIATED EPIDERMAL DELIVERY OF DNA

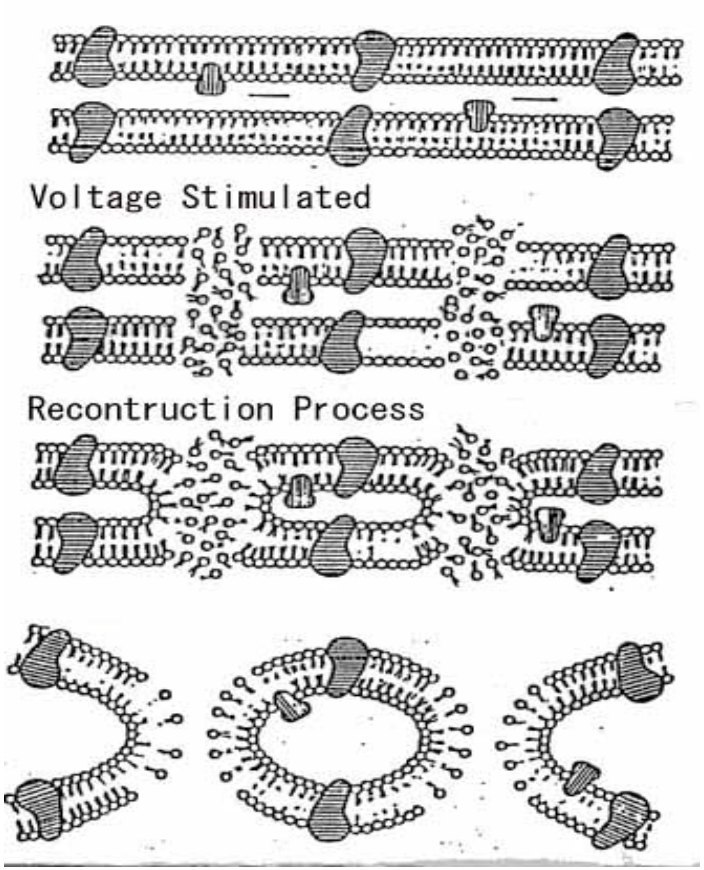


The PMED system involves the precipitation of DNA onto microscopic gold particles that are then propelled by helium gas into the epidermis. The DNA-coated gold particles are delivered into the antigen-presenting cells (APCs) of the epidermis and once inside the nuclei of the APCs, the DNA elutes off the gold and becomes transcriptionally active, producing encoded protein. This protein is then presented by the APCs to the lymphocytes to induce a T-cell-mediated immune response.

ELECTROPORATION

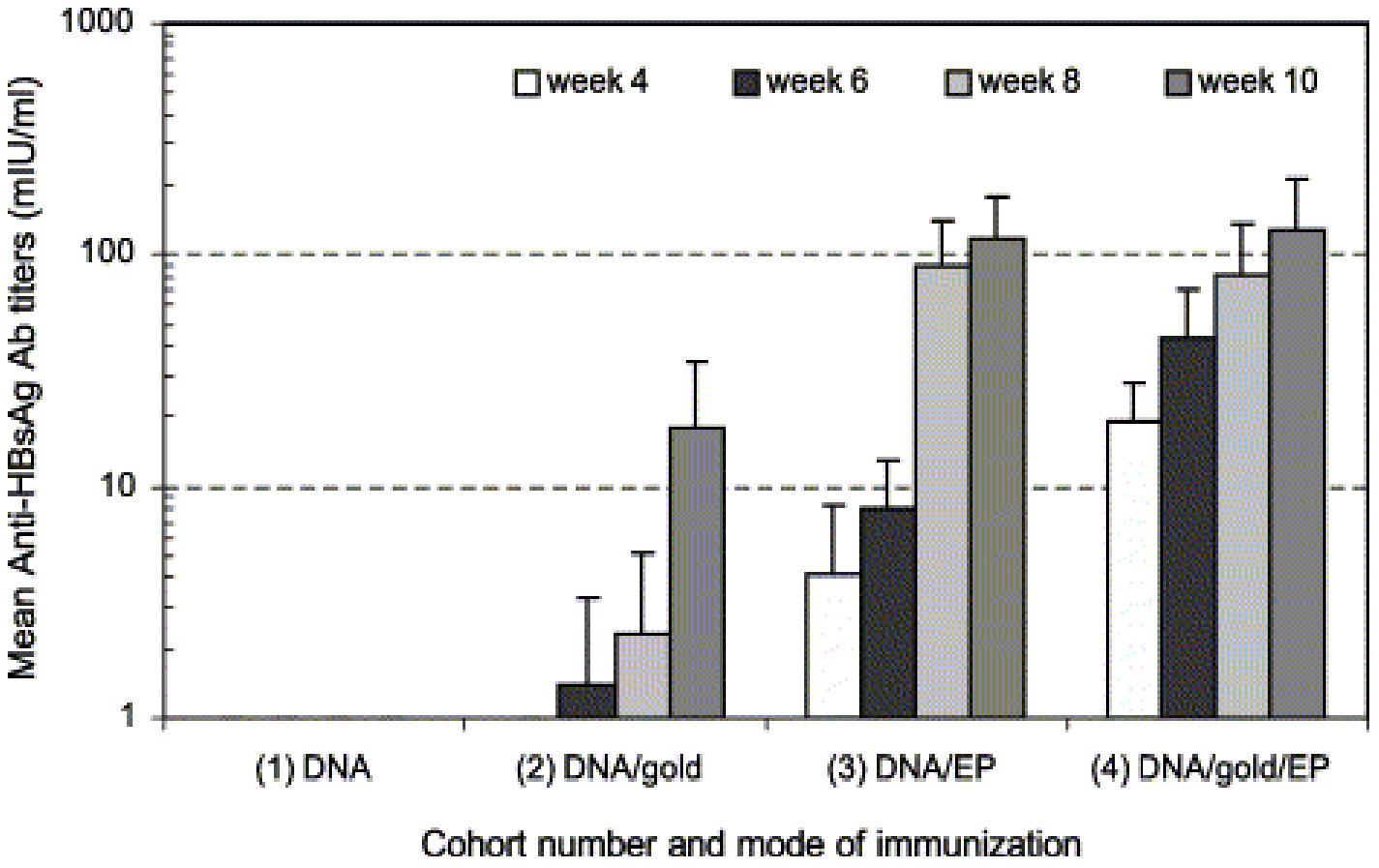
- Electroporation is the application of short electrical pulses to the target tissue render the cell membrane transiently permeable to DNA and other molecules





DNA VACCINATION USING PMED + ELECTROPORATION

Zhang L. et al.,Bioelectrochemistry, 63, 369-373, 2004



Balb/c mice were injected intradermally with **Hepatitis B surface antigen DNA**. For cohorts 2 and 4, gold particles ("gold") were suspended in the DNA solution. Cohorts 3 and 4 received electroporation pulses (EP) at the injection sites immediately following DNA±particle injection. At the end of weeks 4 and 8, boost immunizations identical to the primary immunization were administered. (*n*=6).

LES MICRO ET NANOTECHNOLOGIES

-Représentent de nouveaux adjuvants de l'immunité
- Capables de « booster » la réponse immunitaire
- au niveau sérique et surtout humorale