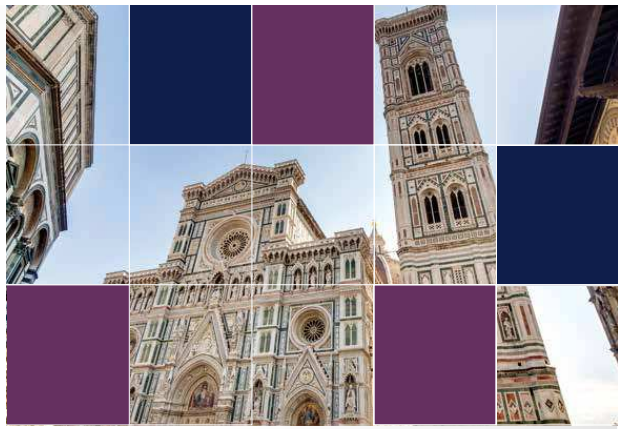

XXX CONGRESSO NAZIONALE

SIAAIC

Società Italiana di Allergologia, Asma ed Immunologia Clinica

FIRENZE 6/9 APRILE 2017 | WWW.SIAAIC2017.ORG

Nanoparticelle e vaccini

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Vice-President of SIAAIC

ICOH-SC on "Allergy and Immunotoxicology"

Secretary of the Italian Society of Nanotoxicology

Nanoparticle/immune system interaction

Nano as hapten? Antigenicity?

Immunodulation? Adjuvant properties?

Cytonecrosis? Immunosuppression? Cancer?

Nanoparticles can be engineered to either avoid interaction or to specifically interact with the immune system

Immunostimulation

Desirable

- Vaccine efficacy
- Antitumoral effects

Undesirable

- Hypersensitivity reactions
- Inflammation
- Anaphylaxis

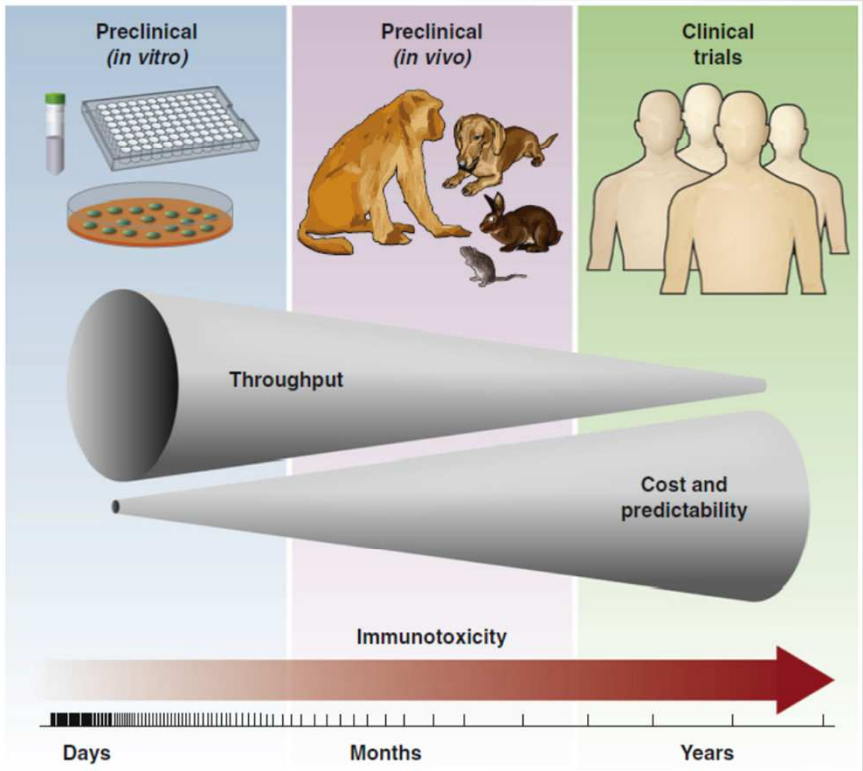
Immunosuppression

Desirable

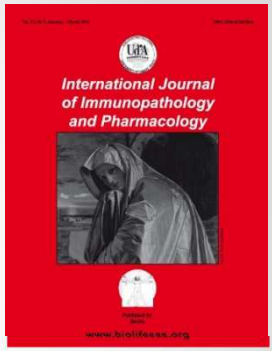
- Treatment of inflammatory disorders and autoimmmune disease
- Prevention of allergic responses
- Transplant acceptance

Undesirable

- Lower body's response to infected and cancerous cells
- Myelosuppression and thymic supression

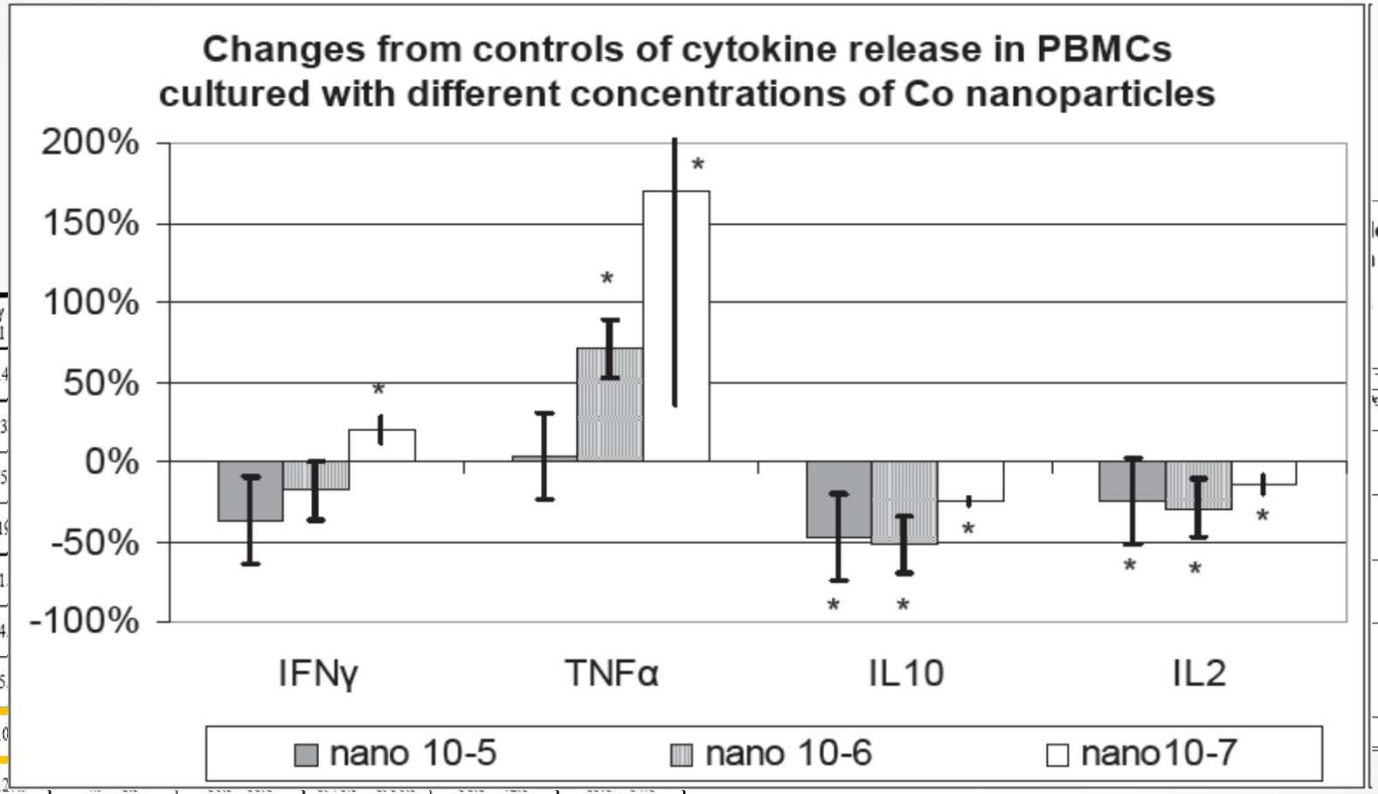


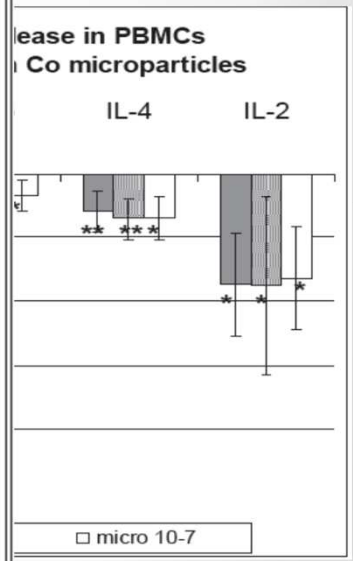
Cobalt nanoparticles modulate cytokine in vitro release by human mononuclear cells, mimicking autoimmune disease.



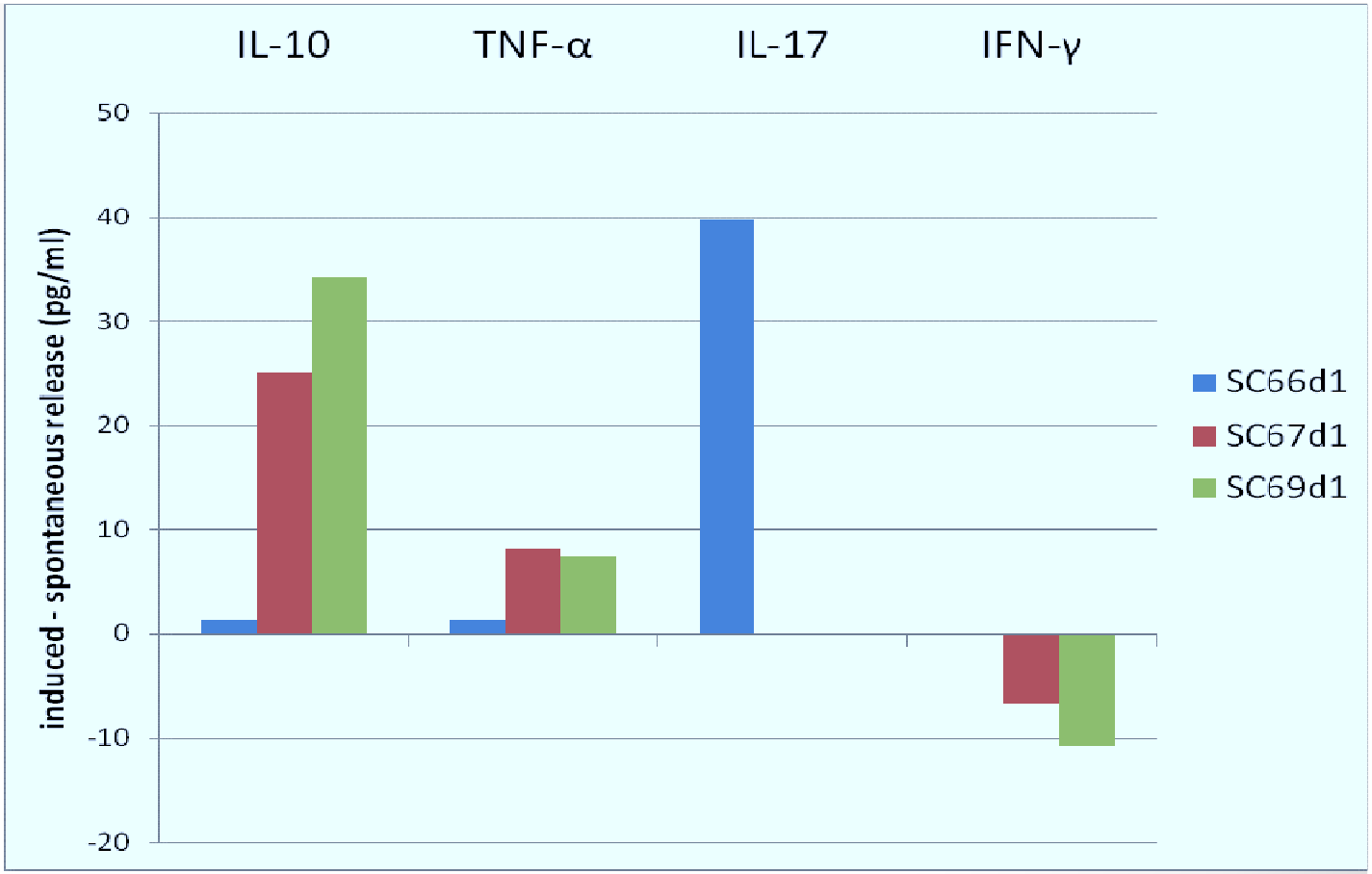
Di Gioacchino et al 2006

	IFN γ ng/ml					
control cells	159.5 ± 14					
Co nano 10^{-5}	73.2 ± 3					
Co nano 10^{-6}	110 ± 75					
Co nano 10^{-7}	199.7 ± 19					
Co micro 10^{-5}	50.4 ± 11					
Co micro 10^{-6}	43.4 ± 14					
Co micro 10^{-7}	55.7 ± 25					
$CoCl_2$ 10^{-5}	59.8 ± 10					
$CoCl_2$ 10^{-6}	146.7 ± 12					
$CoCl_2$ 10^{-7}	105.5 ± 43.8	4.9 ± 1.5	3.9 ± 1.9	719.7 ± 501.7	27.5 ± 16.6	15.1 ± 4.2



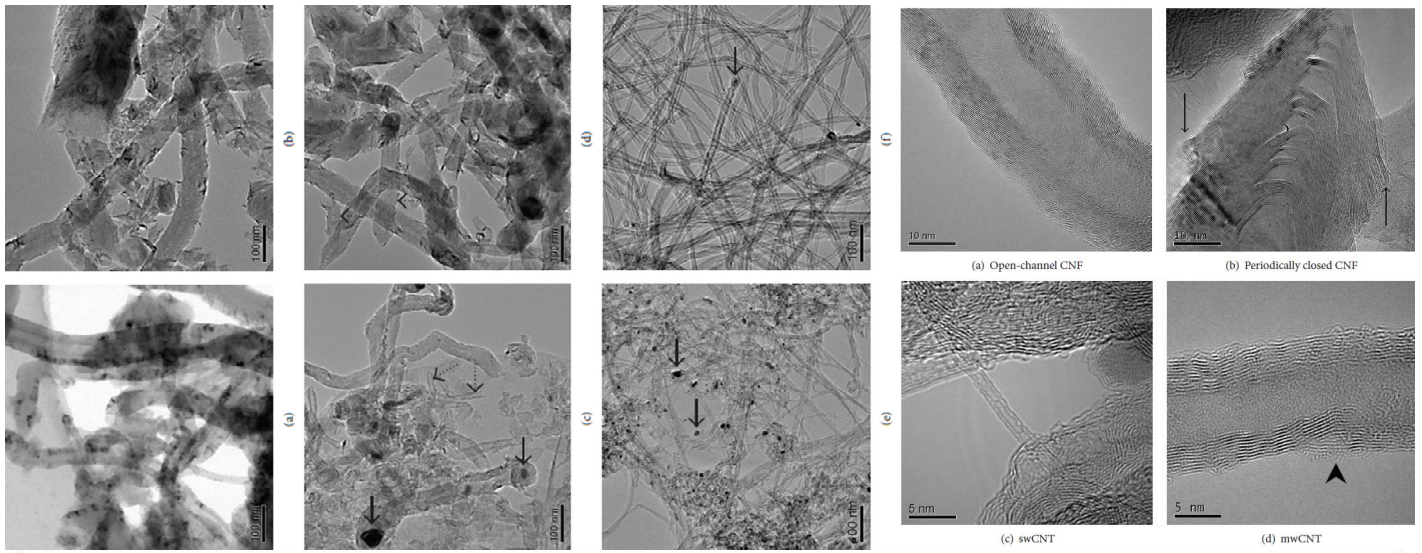


Nano Pd induce stem cells to produce different pattern of cytokines in relation to their differentiation state



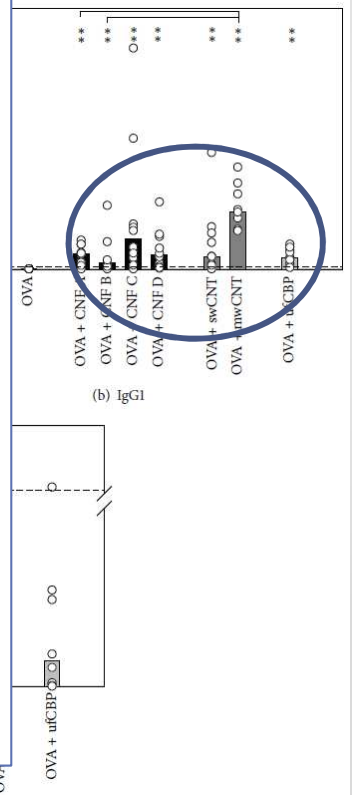
Cytokine production by stem cells

Di Gioacchino et al, in press



	Main carbon structure	Fraction of fibers/tubes versus disordered material	Fiber/tube width (nm)	Surface area (m^2/g)	Structural defects ($R = I_D/I_G$)	Metallic contaminants	Allergy-promoting capacity
CNF A	***Fibers, open channels***	***Medium***	***37.01 ± 1.57 [11.25–108.69]***	***103***	**1.7**	**High, mainly Ni**	***Medium***
CNF B	**Fibers, periodically closed channels**	**High**	83.14 ± 4.18 [18.90–302.21]	61	0.7	Traces	***Medium***
CNF C	***Fibers, open channels***	Low	***35.82 ± 2.13 [14.46–185.75]***	***124***	***0.9***	**High, mainly Ni**	***Medium***
CNF D	Fibers, periodically closed channels	***Medium***	70.57 ± 2.68 [18.52–286.85]	56	0.6	Traces	***Medium***
swCNT	**Tubes, open channels**	***Medium***	**4.05 ± 0.23 [1.41–10.91 nm]**	**543**	n.a.	**High, mainly Co**	**High**
mwCNT	**Tubes, open channels**	**High**	**15.04 ± 0.47 [7.62–29.01 nm]**	***140***	n.a.	***Less, mainly Ni(Fe)***	**High**
ufCBP	Spherical	Not relevant	Not relevant	**321**	***1.2***	Traces	***Medium***

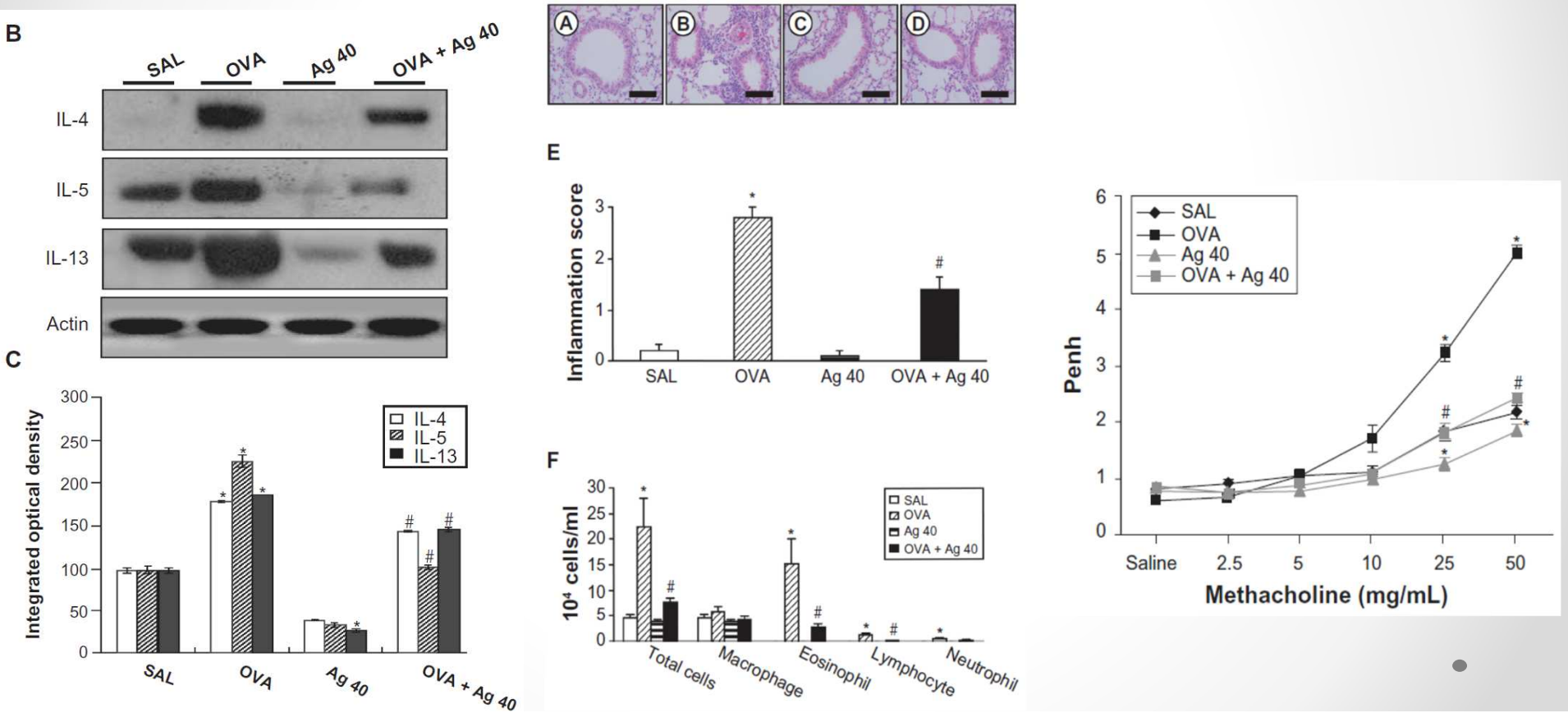
IgG



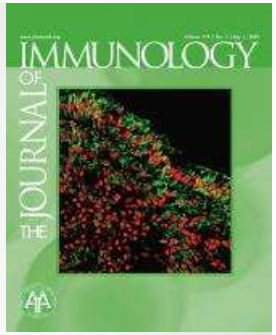
Attenuation of allergic airway inflammation and hyperresponsiveness …. by silver nanoparticles

the international journal of nanomedicine
Dovepress

Park et al. 2010



Fullerens and mast cells

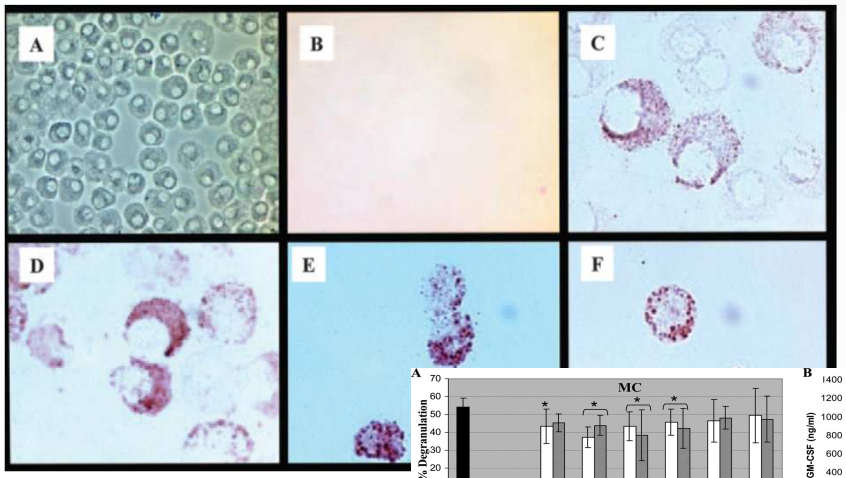


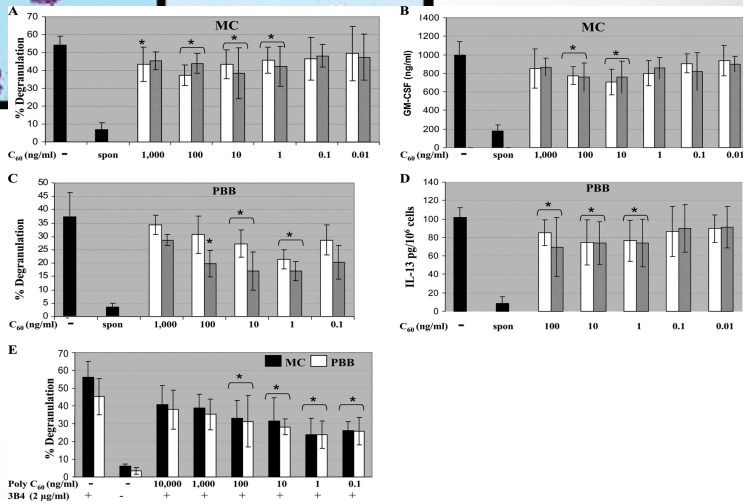
Ryan et al, 2007

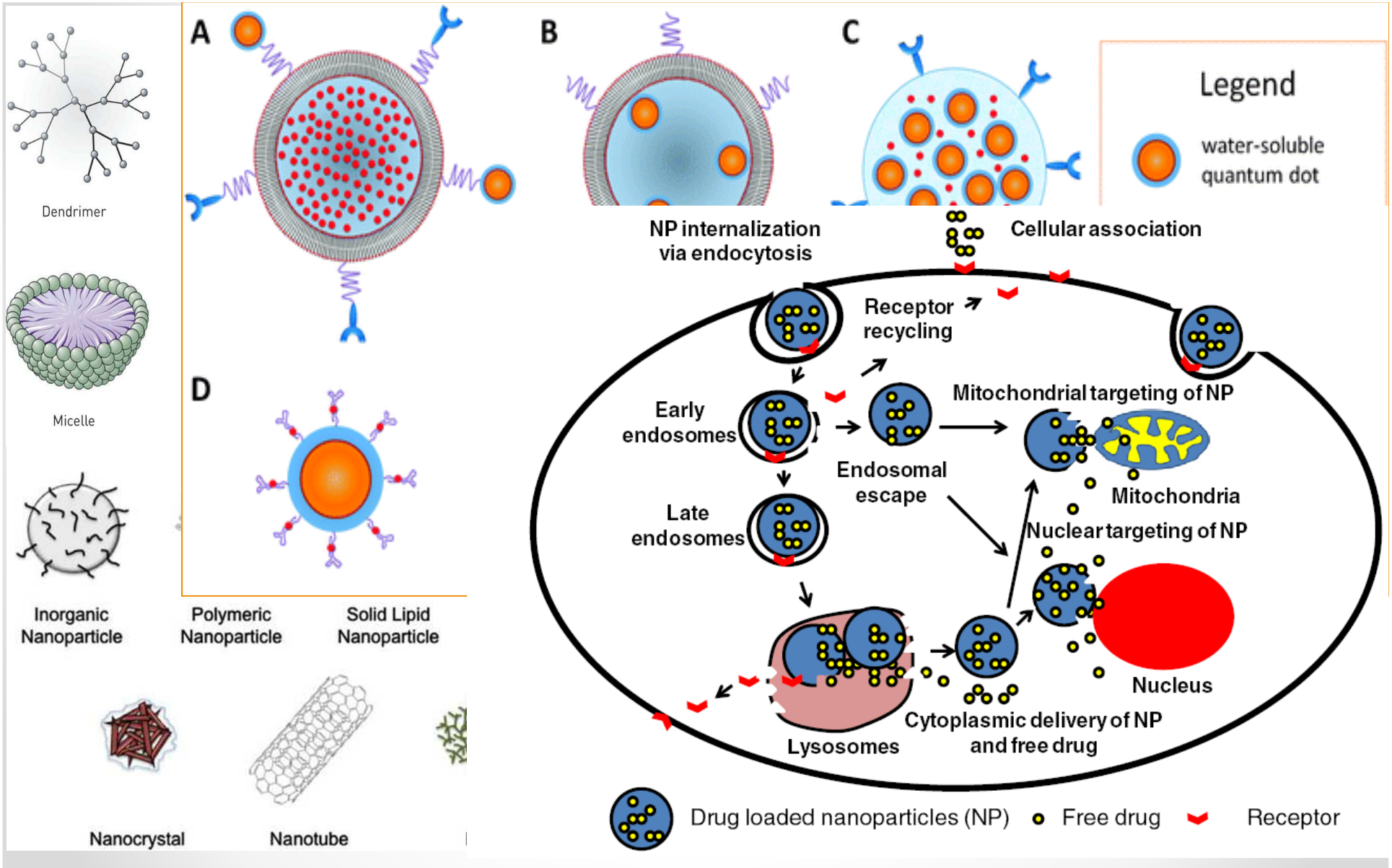
- Inhibition of degranulation
- Inhibition of cytokine release
- Inhibition of IgE response
- Inhibition of PGD2 production
- Inhibition of IgE and

not IgE mediated mediatory release

-





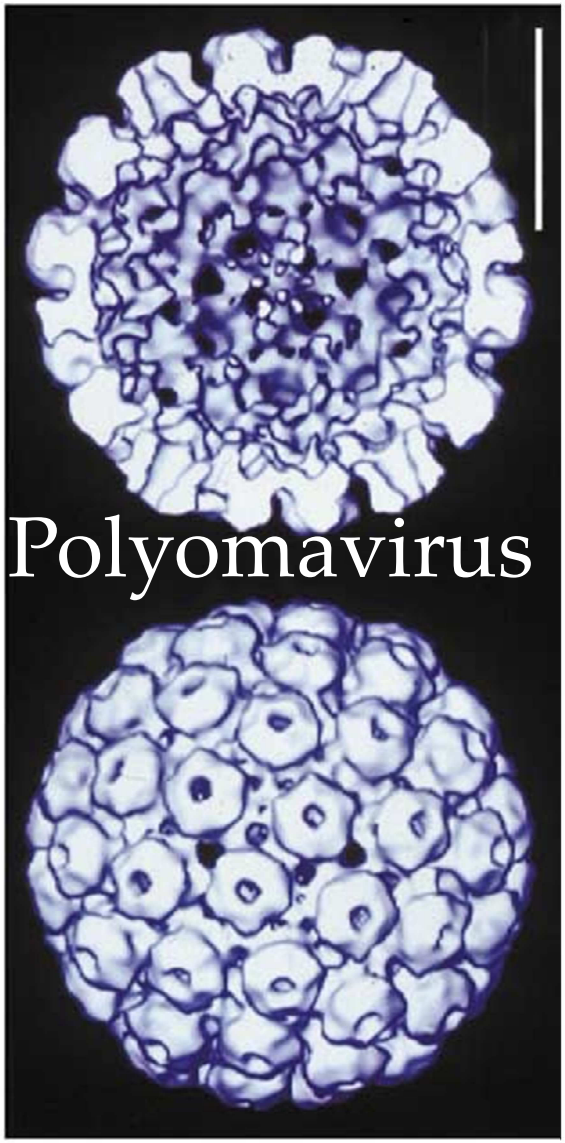

Dendrimer
Micelle
Inorganic Nanoparticle
A
D
Polymeric Nanoparticle
Solid Lipid Nanoparticle
Nanocrystal
Nanotube
B
C
Legend
water-soluble quantum dot
NP internalization via endocytosis
Cellular association
Receptor recycling
Early endosomes
Endosomal escape
Late endosomes
Mitochondrial targeting of NP
Mitochondria
Nuclear targeting of NP
Nucleus
Lysosomes
Cytoplasmic delivery of NP and free drug
Drug loaded nanoparticles (NP)
Free drug
Receptor

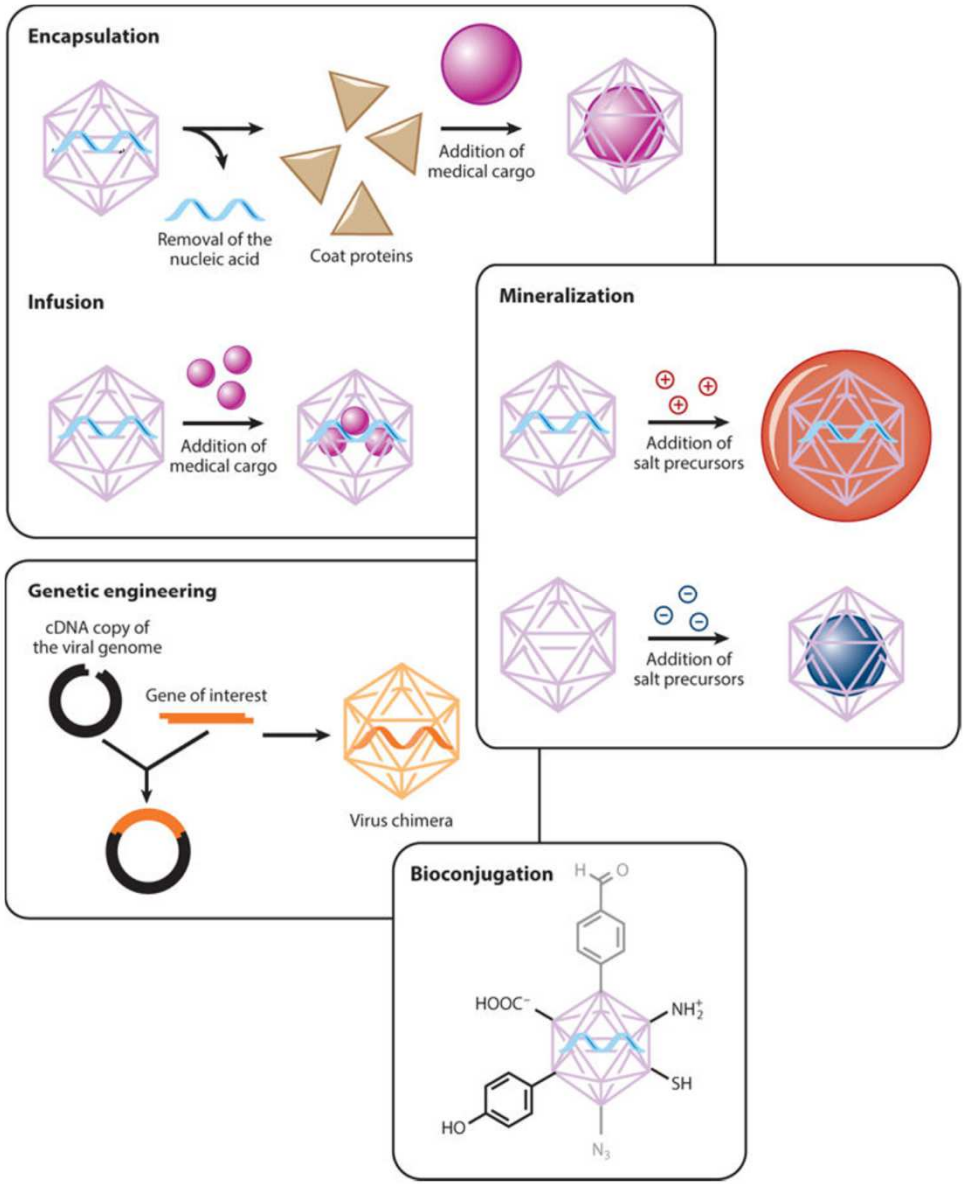
Examples of VLPs used for vaccines and vaccine development

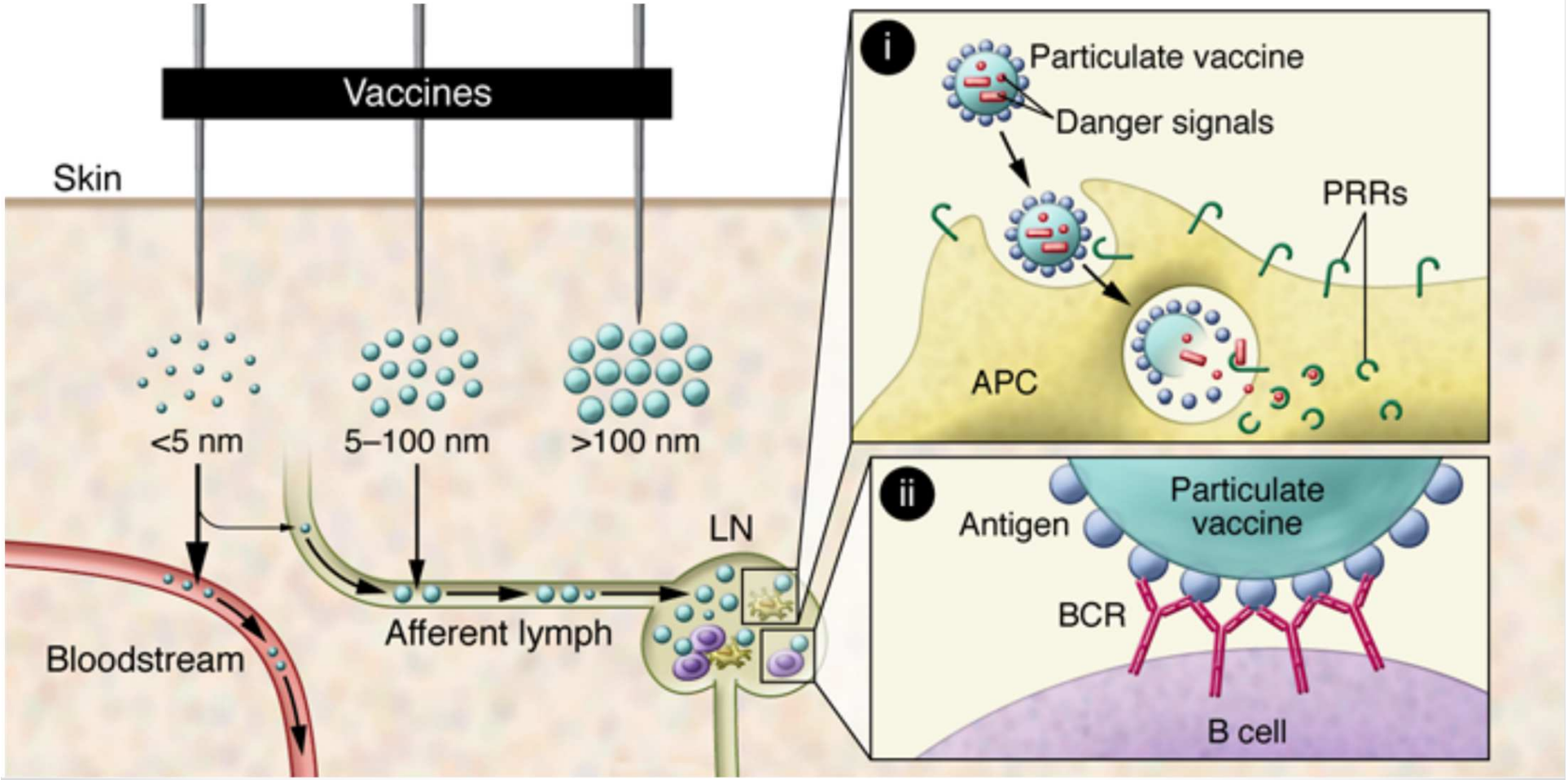
Virus	Particle composition	Type/expression system	Size	Vaccine status	References
HBV	Small envelope protein (HBsAg)	rec VLP (yeast) (Recombivax-HB; Engerix-B)	22 nm	Licensed	[46,47]
	Small envelope protein (HBsAg)	rec VLP (potato)	17 nm	Preclinical	[48]
	PreS1+2 and HBsAg	rec VLP (CHO cells) (Sci-B-Vac; BioHepB)	22 nm	Licensed	[9,10,12,13]
	HBsAg	Native SVP (plasma)	22 nm	Licensed (developing world)	[49]
HPV	L1, major capsid protein	recVLP (mammalian cells; baculovirus; yeast) Gardasil, Cervarix	40–50 nm	Licensed	[50–53]
HEV	Truncated major capsid protein (ORF2)	rec VLP (baculovirus)	23.7 nm		[54–56] [57] (review)
Influenza	HA, NA, matrix	recVLP (baculovirus)	80–120 nm	Preclinical	[14–16]
HCV	Core, E1, E2	recVLP (baculovirus)	40–60 nm	Preclinical	[58–61]
Poliovirus	Capsid (VP0,1,3)	recVLP (baculovirus)	27 nm	None	[3]
HIV	Pr55gag, envelope	recVLP (baculovirus; mammalian cells; yeast)	100–120 nm	Preclinical	[62,63] [64,65] (review) [18] (review)
Ebola virus; Marburg virus	Glycoprotein (GP) and matrix (VP40)	recVLP (mammalian cells)	Filovirus-like particle	Preclinical	[66–68]
Norwalk virus	capsid	rec VLP (baculovirus; transgenic potatoes)	38 nm	Phase1	[69,44,70]
Rotavirus	VP2,VP6,VP7	recVLP (baculovirus)	70–75 nm	Preclinical	[5,71,72]
SARS coronavirus	S, E and M	rec VLP (baculovirus)	100 nm	Preclinical	[73]

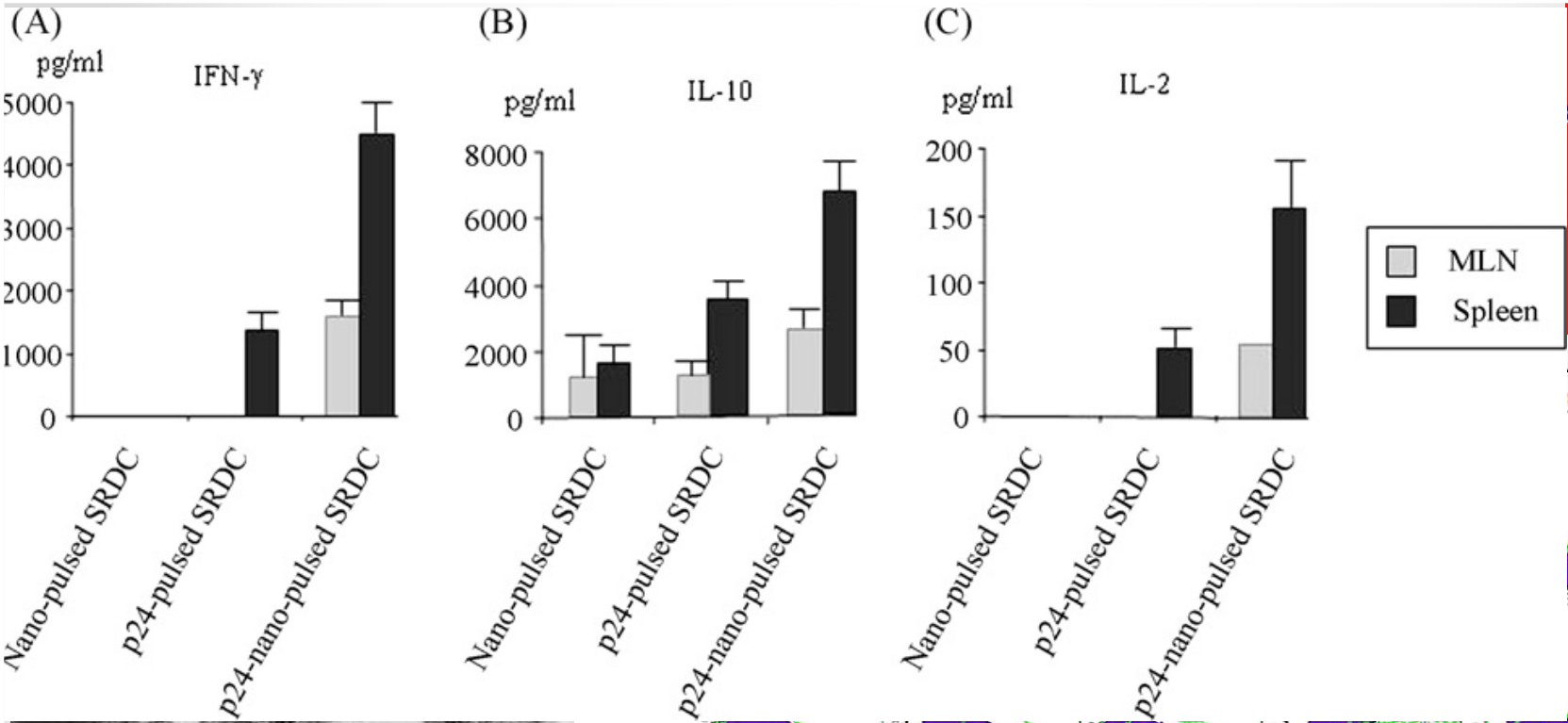
Abbreviations: HBV, hepatitis B virus; HPV, human papilloma virus; HEV, hepatitis E virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; SARS, severe acute respiratory syndrome.

Virus like particles

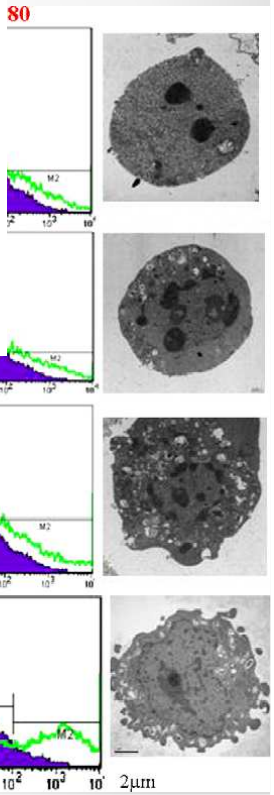


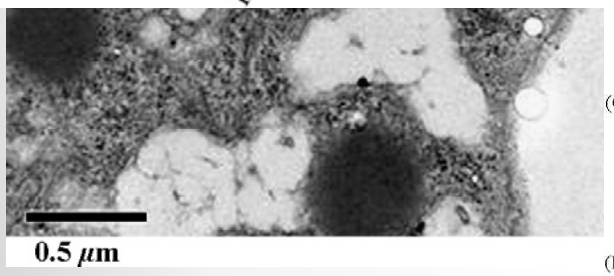



Vaccines
Skin
<5 nm
5–100 nm
>100 nm
LN
Afferent lymph
Bloodstream
i
Particulate vaccine
Danger signals
PRRs
APC
ii
Antigen
Particulate vaccine
BCR
B cell



Aline et al,2009





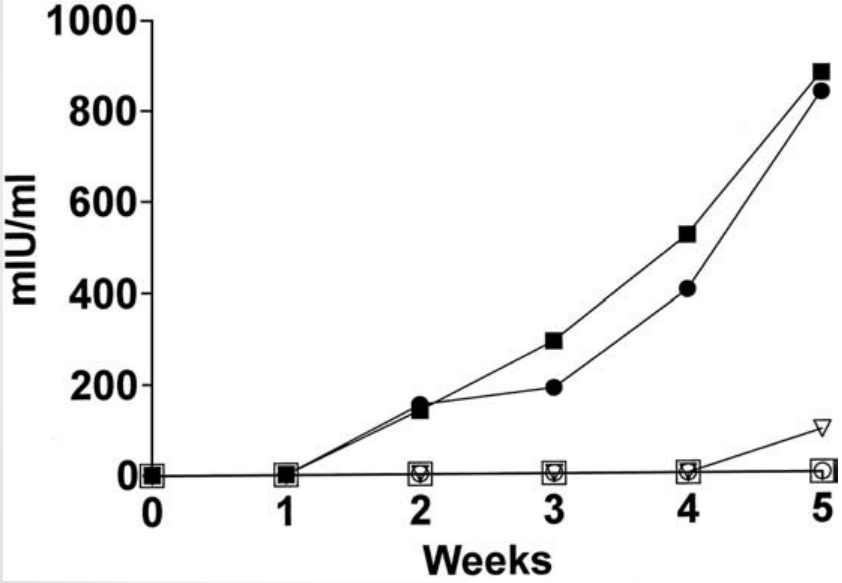
DC phenotyping

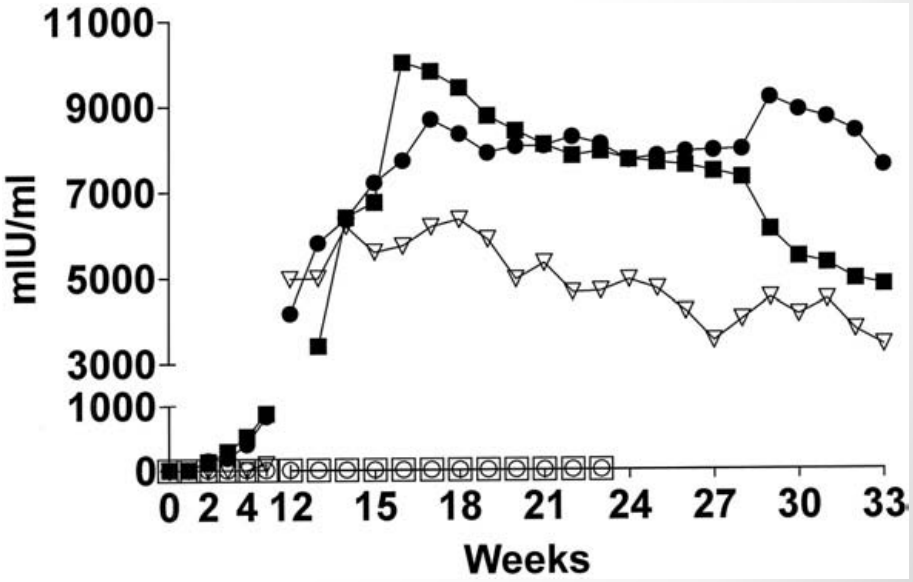
HIV p24 proteins adsorb

Novel nanoparticles for the delivery of recombinant hepatitis B vaccine

nanomedicine

Dhruba et al, 2008





- Recombinant HBsAg (▽)
- HBsAg-PEG5000-PLGA5000 NP (●)
- HBsAg-PEG1000-PLGA5000 NP ( )
- Empty (□ - ○)

Nanoparticles-Based Allergen-Delivery Systems

- *Biodegradable Polymeric Nanoparticles*.
  - *Polyesters*
    - *Polylactides* - PLA and PLGA (Bet v 1 – Ole e 1 – ragweed - mites)
    - *Poly(ε-Caprolactone) – PCL (mostly used for bacterial vaccine)*
  - *Poly(Anhydrides) - (PVMA (Gantrex nanoparticles) (Grass)*
  - *Poly(Gamma-Glutamic Acid) - γ-PGA (Grass)*
  - *Poly(Vinylpyrrolidone) - PVP (aspergillus fumigatus)*
  - *Polysaccharides – Chitosan (mites - peanut)*
- *Nondegradable Polymeric Nanoparticles*
  - Latex, gold, silica, or polystyrene (in vivo clearance?)

Gantrex nanoparticles (methyl vinyl ether and maleic anhydride - PVMA)

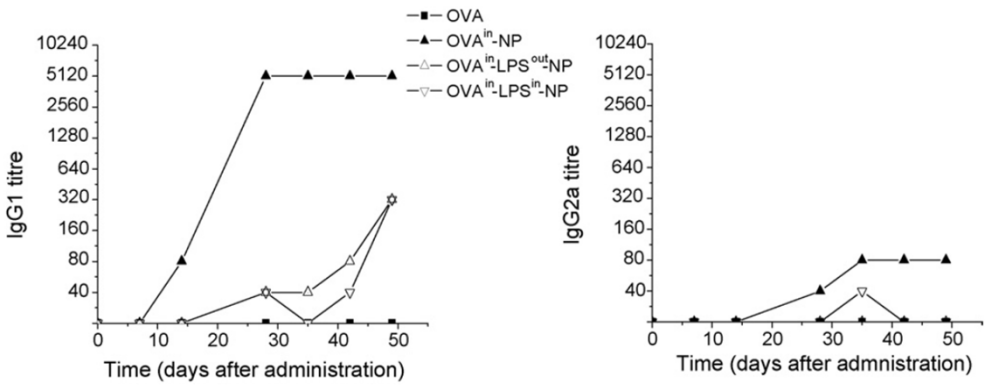
Gomez 2006 - 2010

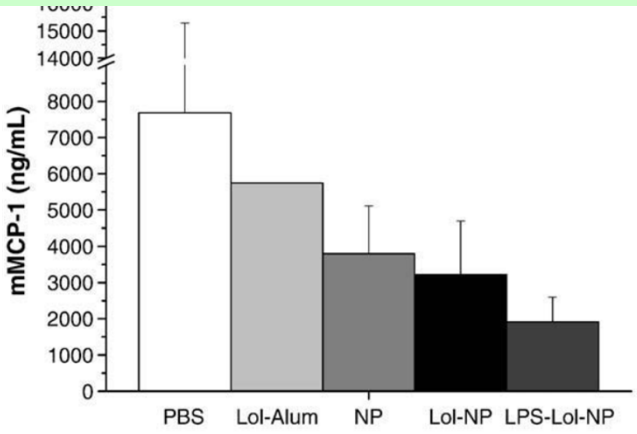
Symptoms score after the challenge with OVA i.p.

Treatment	Temperature decrease (°C)	Piloerection	Mobility	Cyanosis	Survival rate (%)
OVA	7.2 ± 1.1	+	Low	+++	40
OVA^{in}-NP	7.3 ± 0.2	+	Normal	++	100
OVA^{in}–LPS^{out}-NP	4.5 ± 3.6	++	Low	++	20
OVA^{in}–LPS^{in}-NP	6.1 ± 2.2	+	Normal	++	80

Decrease in specific IgE

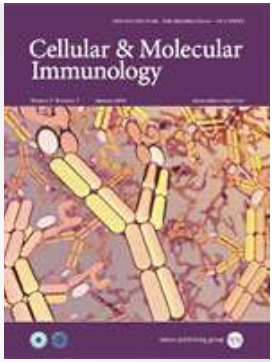
Increase in IgG(2a) isotype





Reduced mortality rate and mMCP-1 levels in challenge experiment

Induction of Th1-Type Immune Response by Chitosan Nanoparticles Containing Plasmid DNA Encoding House Dust Mite Allergen Der p 2 for Oral Vaccination in Mice



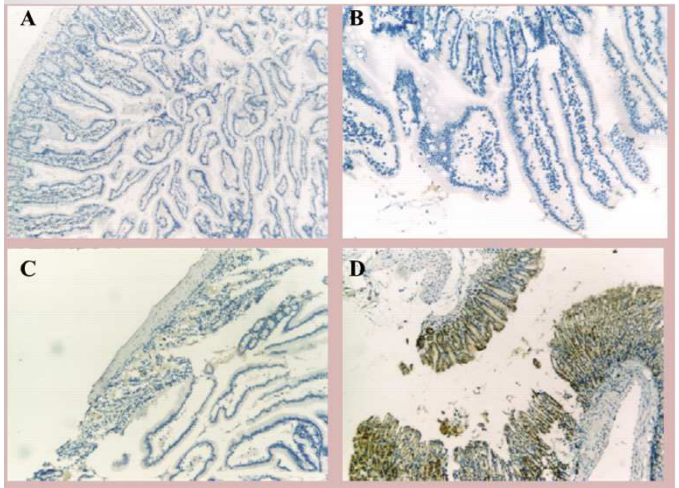
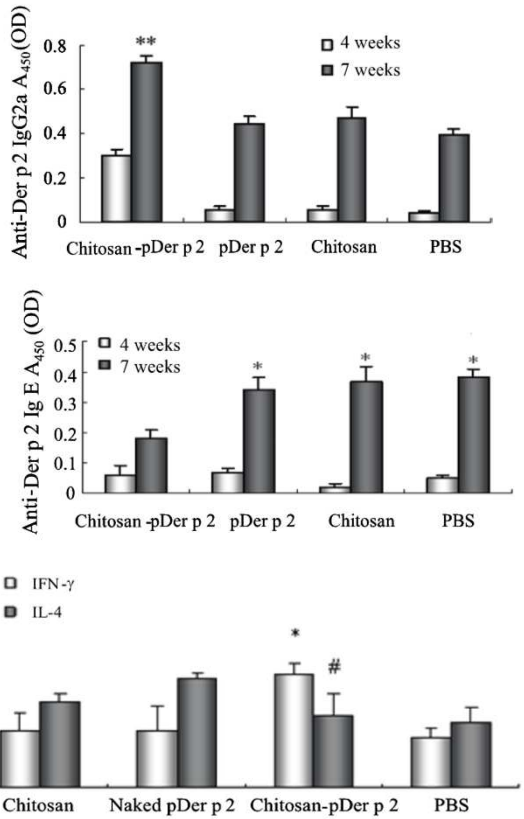
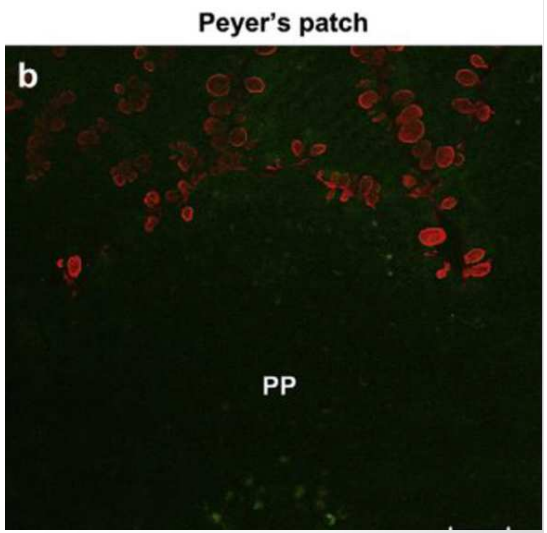
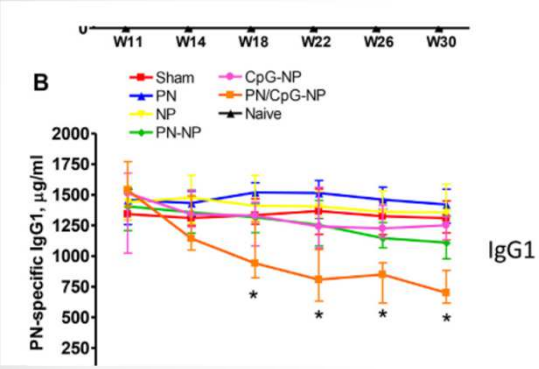


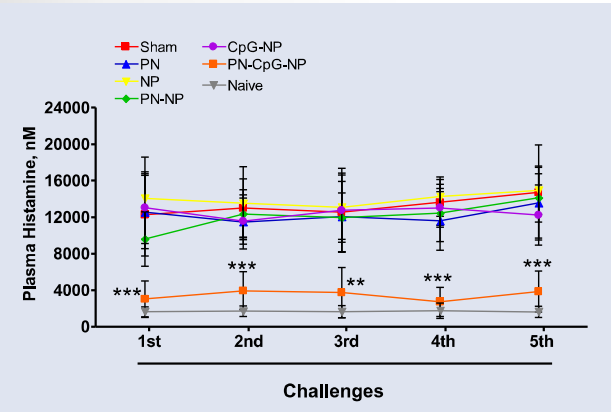
Figure 5. Immunohistochemical examination of Der p 2 expression in mouse stomach and small intestine 3 days after oral delivery of DNA nanoparticles. (A) Intestine from oral PBS (×100). (B) Intestine from oral naked DNA (pcDNA3.1) (×100). (C) Intestine from oral naked DNA (pDer p 2) (×100). (D) Intestine from oral chitosan-pDer p 2 nanospheres (×100).

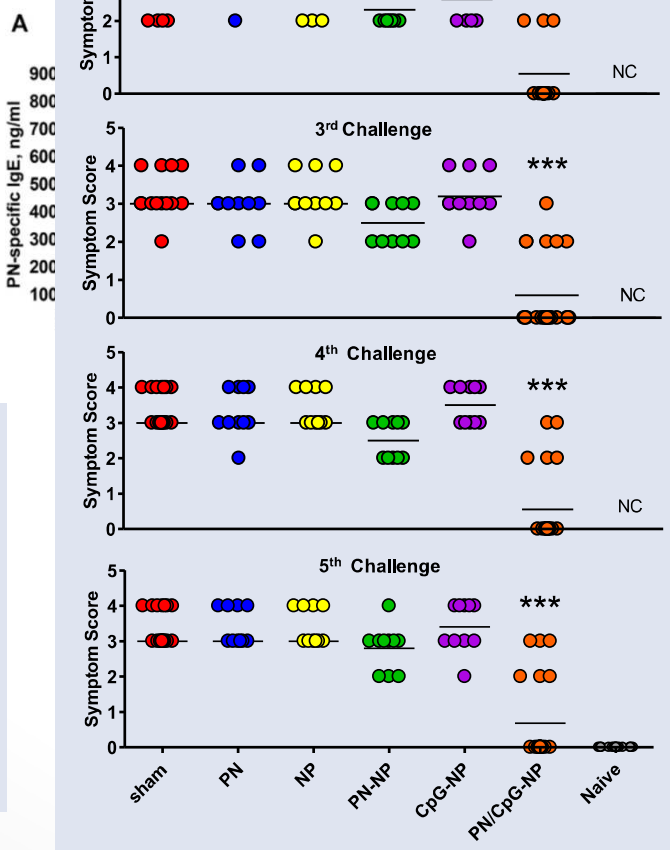


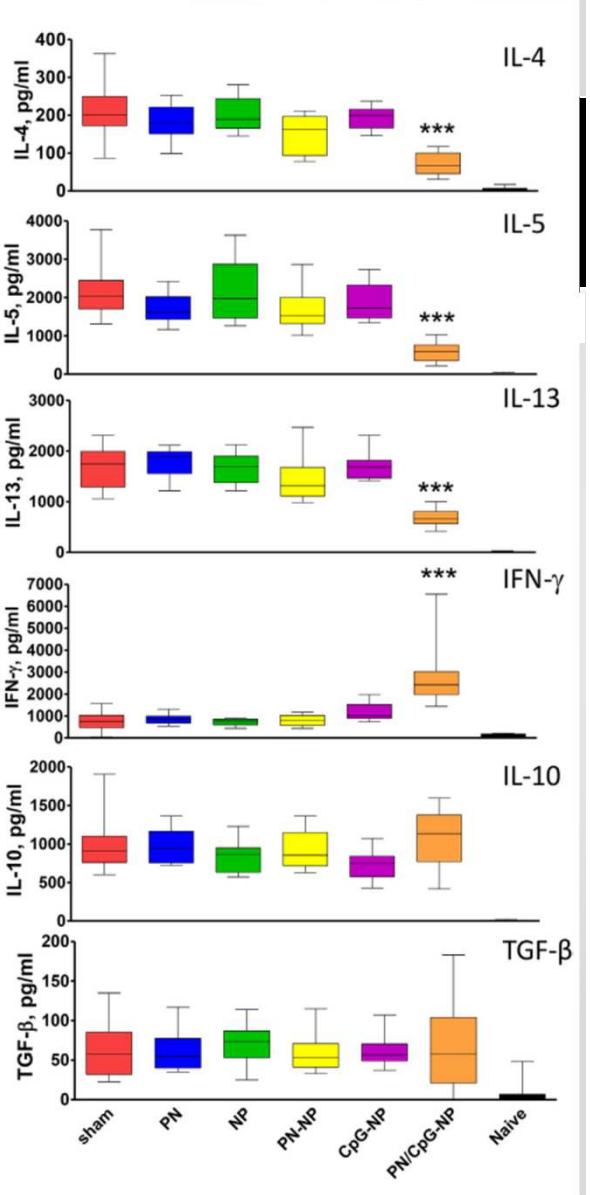


Investigation of pea… a… with CpG/peanut n… i… model of peanut all…

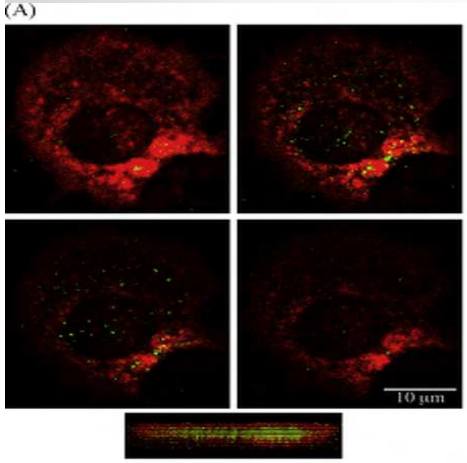


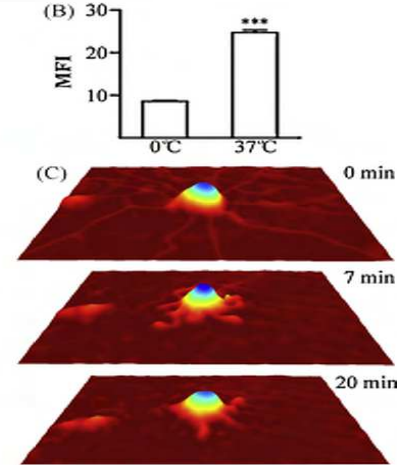


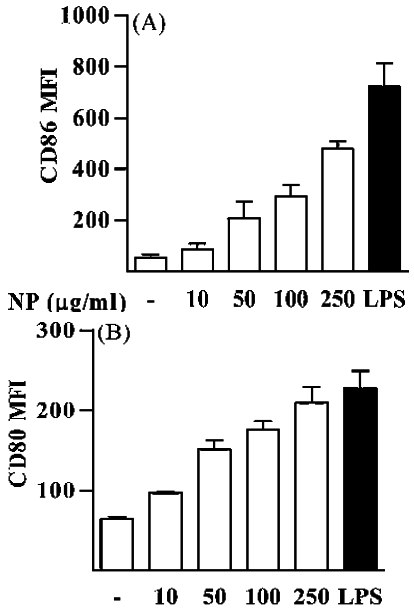


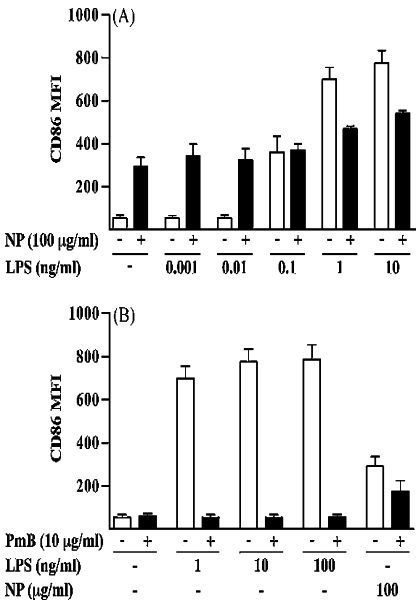


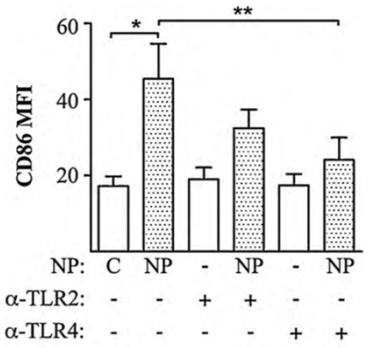
Immunomodulatory nanoparticles as adjuvants and allergen-delivery system to human dendritic cells: Implications for specific immunotherapy

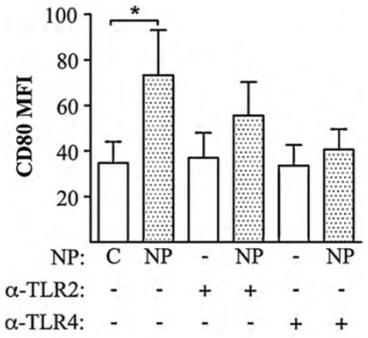


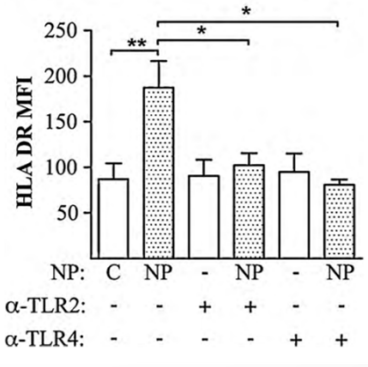


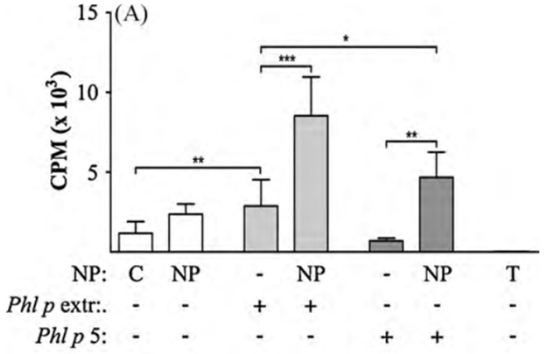


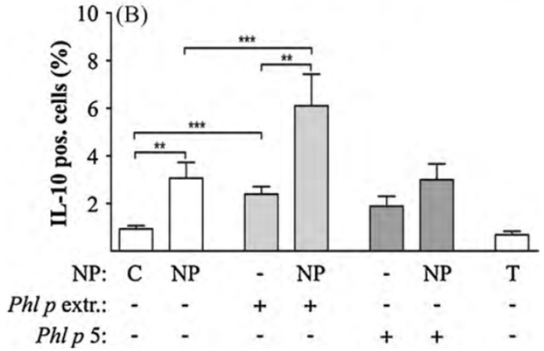












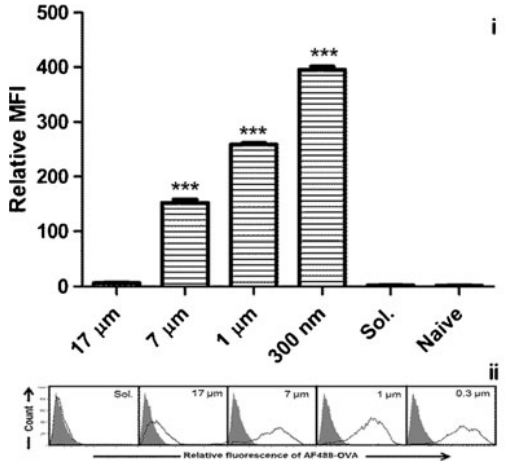
Mannan nanoparticles

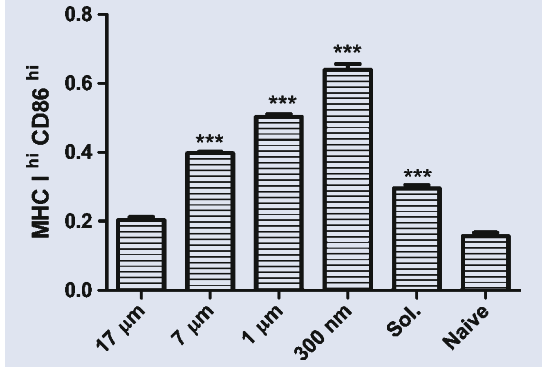
Modulation of DCs co-stimulatory molecules, soluble mediators, and receptors after treatment with different NPs

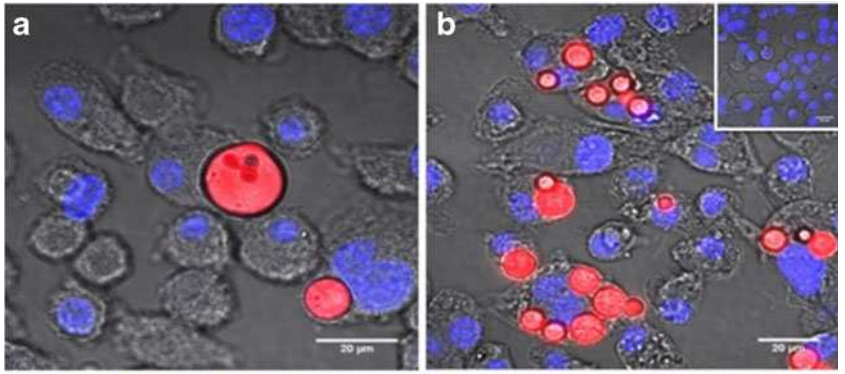
NPs	Size (nm)	Cell type	Co-stimulatory molecules	Soluble mediators	Receptors involved
Poly(γ-glutamic acid)	~210	Spleen DCs	CD40 CD80 CD86	TNF-α IL-6 RANTES MIP-1α	MHC I
Carbon black	14–56	Bone marrow DCs	DEC205 CD80 CD86		MHC II
Mannosylated dendrimers ovalbumin		Bone marrow DCs	CD40 CD80 CD86		
Poly(d,l-lactide-co-glycolide)	500–1000	Bone marrow DCs		IL-2	
siRNA nanocomplexes	40–60	Tumor-associated DCs	CD80		MHC I MHC II TLRs

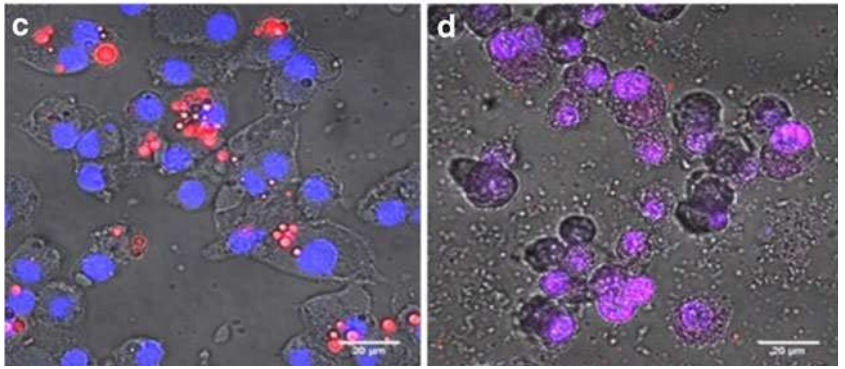
Biodegradable Particles as Vaccine Delivery Systems: Size Matters

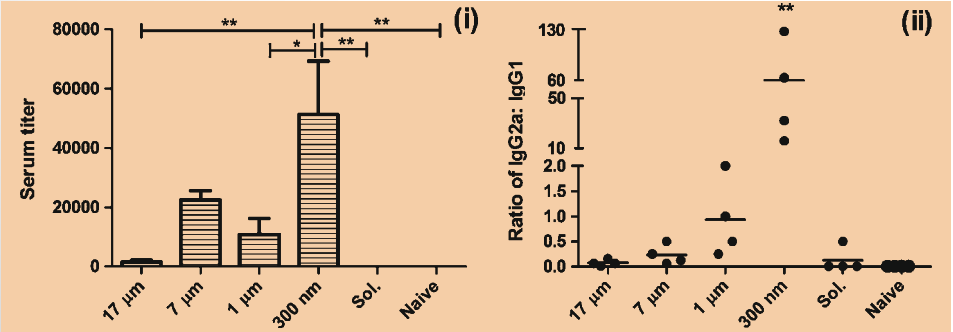
2013



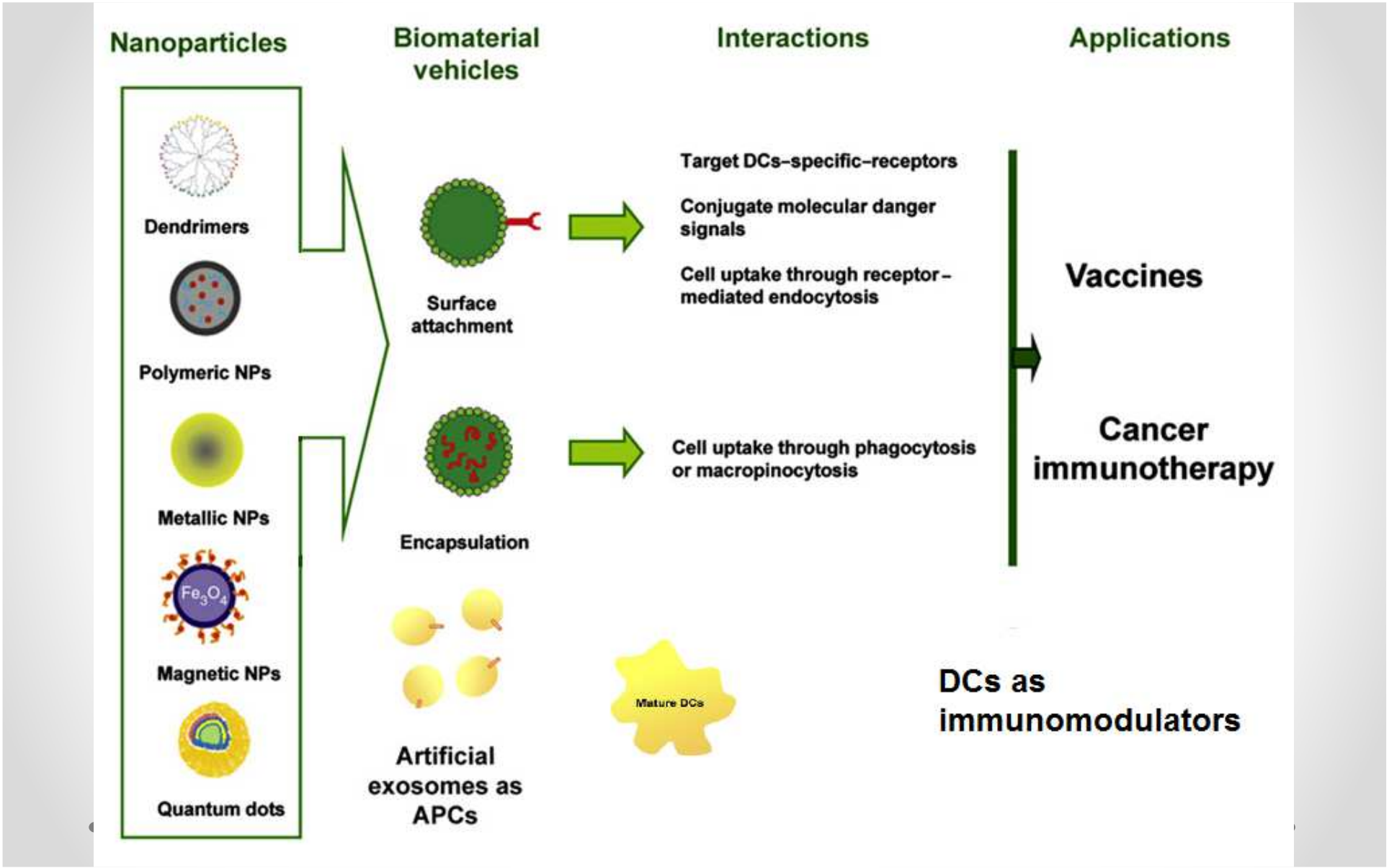


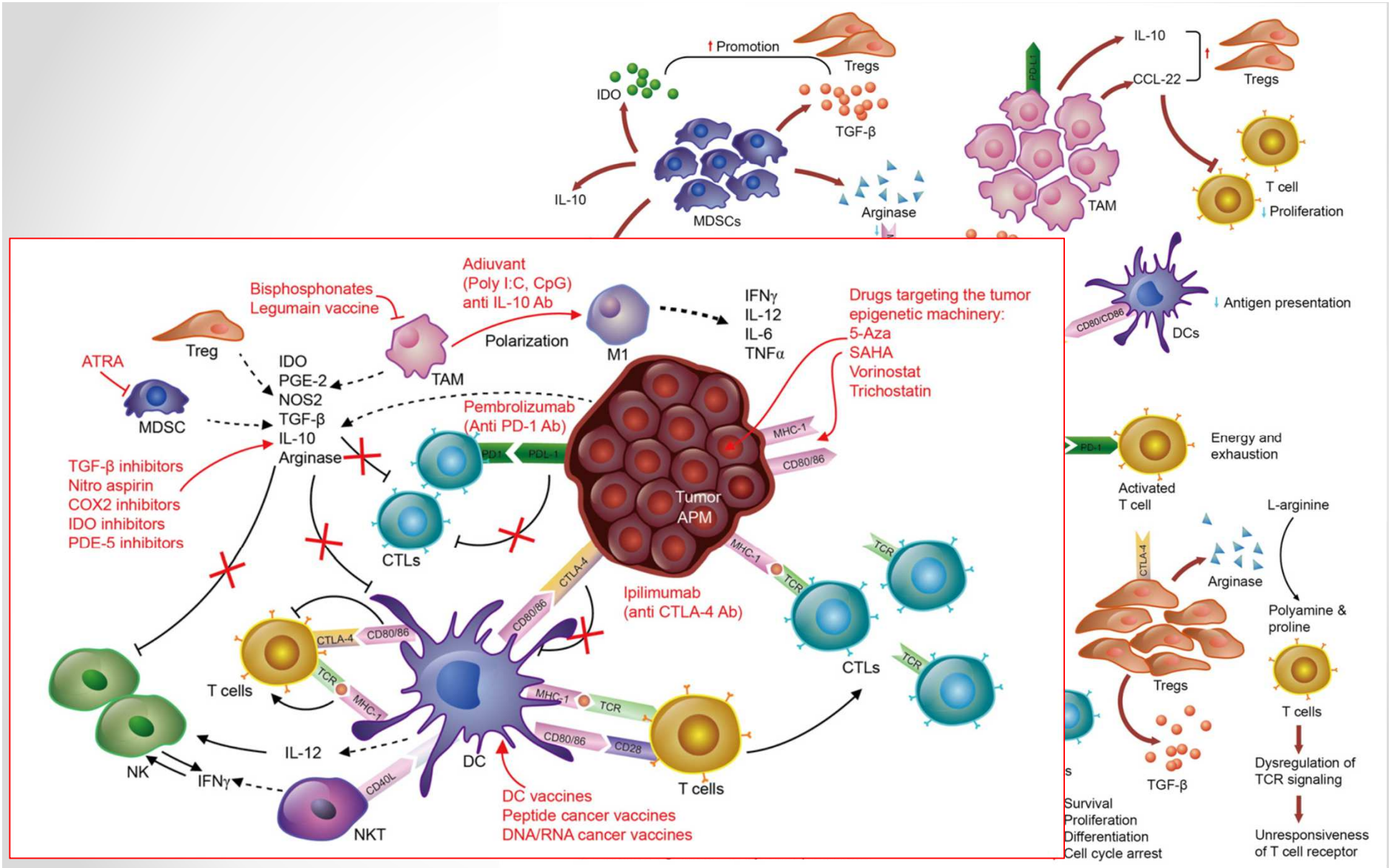




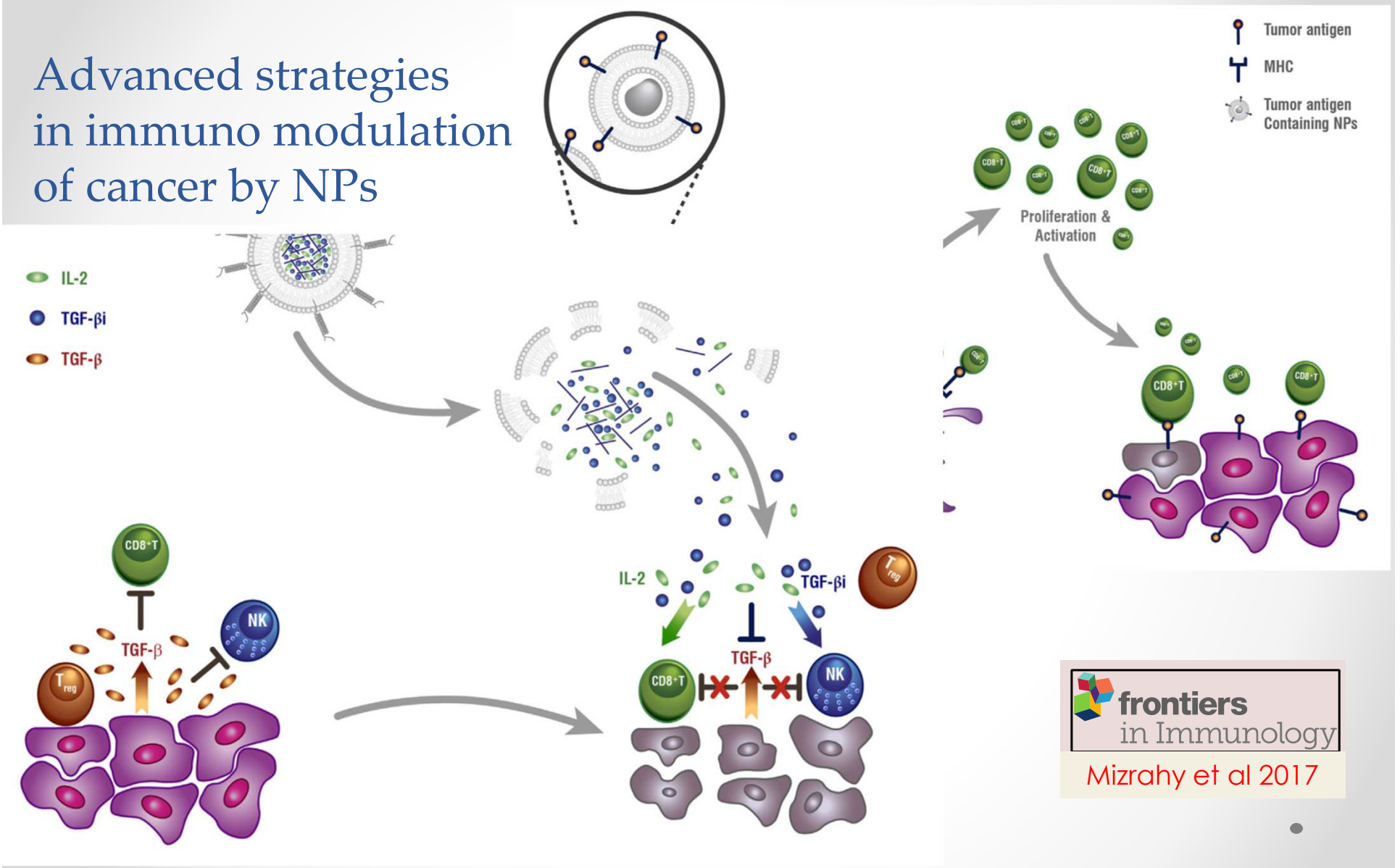


- Nanoparticles can be designed to provoke an immune response, by either direct immunostimulation of antigen presenting cells or delivering antigens to specific cellular compartments
- For the obtention of the desired therapeutic response, size particle control is important since microparticles are rapidly cleared by reticuloendothelial system, while nanoparticles have prolonged circulation time and are efficient drug, enzyme, and protein carriers by any route of administration
- These biodegradable polymers can be either natural (chitosan, alginate, carrageenan, albumin, gelatin, collagen, among others) or synthetic [poly(lactic acids), PLA), poly(lactide-co-glycolic acids), PLGA), poly(methyl meth- acrylate), PMMA), poly(ε-caprolactone), PCL), poly(alkyl- cyanoacrylates), PACA), and copolymers].
- The former generally provide a relatively quick drug release, while the latter enable extended drug release over periods from days to several weeks


Nanoparticles
Biomaterial vehicles
Interactions
Applications
Dendrimers
Polymeric NPs
Metallic NPs
Magnetic NPs
Quantum dots
Surface attachment
Encapsulation
Artificial exosomes as APCs
Target DCs-specific-receptors
Conjugate molecular danger signals
Cell uptake through receptor-mediated endocytosis
Cell uptake through phagocytosis or macropinocytosis
Vaccines
Cancer immunotherapy
Mature DCs
DCs as immunomodulators


↑ Promotion
Tregs
IDO
TGF-β
IL-10
MDSCs
Arginase
IL-10
CCL-22
Tregs
PD-L1
TAM
T cell
↓ Proliferation
Adiuvant
(Poly I:C, CpG)
anti IL-10 Ab
Bisphosphonates
Legumain vaccine
Polarization
M1
IFNγ
IL-12
IL-6
TNFα
Drugs targeting the tumor
epigenetic machinery:
5-Aza
SAHA
Vorinostat
Trichostatin
Treg
ATRA
IDO
PGE-2
NOS2
TGF-β
IL-10
Arginase
TAM
MDSC
Pembrolizumab
(Anti PD-1 Ab)
PD1
PDL-1
MHC-1
CD80/86
Tumor
APM
CTLs
TGF-β inhibitors
Nitro aspirin
COX2 inhibitors
IDO inhibitors
PDE-5 inhibitors
CTLA-4
CD80/86
Ipilimumab
(anti CTLA-4 Ab)
TCR
MHC-1
CTLs
T cells
NK
IL-12
IFNγ
DC
CD40L
NKT
CD28
T cells
DC vaccines
Peptide cancer vaccines
DNA/RNA cancer vaccines
CD80/CD86
DCs
↓ Antigen presentation
PD-1
Energy and
exhaustion
Activated
T cell
L-arginine
CTLA-4
Arginase
Polyamine &
proline
Tregs
T cells
TGF-β
Dysregulation of
TCR signaling
Unresponsiveness
of T cell receptor
Survival
Proliferation
Differentiation
Cell cycle arrest

Advanced strategies in immuno modulation of cancer by NPs



frontiers in Immunology

Mizrahy et al 2017

Nanoparticle assisted

The key advantages of using nanoparticulate carriers:

- Improved solubility and bioavailability of the cargo;
- Possibility to be loaded with a variety of cargos such as siRNA, peptides, proteins, and small molecule therapeutics.
- The cargo can be protected from degradation, which can increase its half-life, enhancing potential efficacy.
- NPs can be modified for targeted site-specific delivery, mitigating systemic toxicity issues.
- To date, there are 45 NPs formulations approved for clinical use


Demons ?
Angels ?
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