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PROVAXS

Mucosal vaccines in Veterinary Species

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LABORATORY OF IMMUNOLOGY

Why mucosal immunity?

- Protection at mucosal surfaces, the entry site of most pathogens
- Reduces pathogen concentration and spread in the environment by preventing infection instead of preventing disease: production of naïve livestock and danger for epizootic outbreaks
- Animal/human(child) friendly
- Less labour-intensive for mass vaccination

Current mucosal vaccines

Human mucosal vaccines

Holmgren and Svennerholm, 2012. Current Opinion in Immunology

Polio		
-Live attenuated vaccine trivalent	Oral	OPV (Novartis, BIBCOL, Biofarma,)
	Oral	Orimune (Medimmune)
-Live attenuated bivalent	Oral	Poliomyelitis type 1 & 3 (Sanofi)
-Live attenuated monovalent	Oral	Poliomyelitis type 1 (Sanofi, Novartis, GSK)
Cholera		
-Cholera toxin B subunit (CTB) +**inact.** *V. cholerae* (10^{11} heat-killed or formalin killed El Tor O1 Inaba & Ogawa biotypes)	Oral	Dukoral (Crucell)
-**inact**. *V. cholerae* (same as above+ $5x10^5$ formalin-killed O139, no CTB)	Oral	Shanchol (Santa Biotechnics)
-CVD 103-HgR live recombinant V. cholerae O1 strain lacking CTA	Oral	Orochol (Crucell)
-Live attenuated *Vibrio cholerae* serogroup O1 (CVD 103-HgR)	Oral	Vaxchora (PaxVax)
-Bivalent **inact**. *V. cholerae (O1 and O139)*	Oral	Euvichol-Plus (EuBiologics)
Typhoid		
-*Salmonella typhi* Ty21a live attenuated vaccine	Oral	Vivotif (Crucell)
- Salmonella typhi Ty21	Oral	TypBar TCV (Bharat Biotech)
Rotavirus		
-Live attenuated monovalent human rotavirus strain G1P	Oral	RotaRix (GSK)
-Multivalent human-bovine reassortant (G1-G4 and P1A)	Oral	RotaTeq (Merck)
-Live attenuated monovalent human rotavirus strain (116E BUK)	Oral	RotaVac (Bharat Biotech)
-Pentavalent human-bovine reassortant (G1-G4 and G9)	Oral	BRV-Penta (Serum Inst. Inde)
Influenza		
-Live attenuated cold-adapted influenza virus reassortant strains	Nasal	FluMist (MedImmune)
-H1N1 influenzavirus (swine)	Nasal	Nasovac (Inst Exp Med)
Adenovirus type 4 and Type 7		
	Oral	Teva Women's Health, Inc
Enterotoxigenic E. coli		
-Cholera toxin B subunit (CTB) +**inact.** *V. cholerae*	Oral	Dukoral (Crucell)

Oral vaccines in pigs

Porcine proliferative enteropathy

Att. Lawsonia intracellularis MS B3903	Enterisol Ileitis®	Boehringer Ingelheim

Salmonella choleraesuis

Avir. live *Salmonella cholerae suis* var. Kurzendorf SC-54	EnterisolSC-54®	Boehringer Ingelheim

Enterotoxigenic E. coli

Avirulent live F4 E. coli strain	Coliprotec F4®	Prevtec microbia
Avirulent live F4 and F18 E. coli strain	Coliprotec F4/F18®	Prevtec microbia
Avirulent live F4(K88) E. coli strain	Entero vac	Arko Laboratories

Verotoxigenic E. coli

Avirulent live F18 E. coli strain	Edema vac F18	Arko Laboratories

Erysipelothrix Rhusiopathiae

Avirulent live Er. rhusiopathiae	Ingelvax® ERY-ALC	Boehringer Ingelheim
Avirulent live Er. rhusiopathiae	Suvaxin® E-oral	Zoetis

Rotavirus

Bivalent modified live G serotype 5 & 4 serogroup A	ProSystem® Rota	Merck Animal Health

dogs

Bordetella bronchiseptica

Recombitek Oral Bordetella (Live attenuated; Merial: one oral dose)
Bronchi-Shield oral (Boehringer Ingelheim)
Vanguard B Oral (Zoetis)

wildlife

Rabies

Raboral V-RG
Rabitec (live attenuated)

nasal vaccination

Pig

Porcine influenza

Live attenuated influenza	H1N1 & H3N2	Ingelvac Provenza	Boehringer Ingelheim

Cattle

Infectious bovine rhinotracheitis

-Attenuated bovine herpes virus type 1 (DIFIVAC $gE^{-)}$	Rispoval IBR-marker Vivum	Zoetis
-Attenuated bovine herpes virus type 1 (Stam GK/D gE^{-})		MSD

Dogs

Bordetella bronchiseptica + canine parainfluenza virus + canine adenovirus

-Live attenuated vaccine 3-way	Vanguard Rapid Resp	Zoetis

Bordetella bronchiseptica + canine parainfluenza virus

-Live att bivalent	Nobivac BbPi	MSD

Bordetella bronchiseptica

-Live att. monovalent	Bronchi-Shield	Zoetis

Mucosal vaccines in birds

Ocular, oculonasal (spray, droplets), oral (drink water)
Live attenuated or deletion mutation

Newcastle disease virus
Infectious bronchitis virus
Gumboro disease virus
Infectious laryngotracheitis virus
Avian rhinotracheitis virus
Coccidiose (Eimeria sp.)
Avian Escherichia coli
Salmonella enteritidis
Salmonella typhimurium
Mycoplasma synoviae

Mucosal vaccines in fish

Nervous Necrosis virus
Insect larvae containing the virus Oral Vaksea

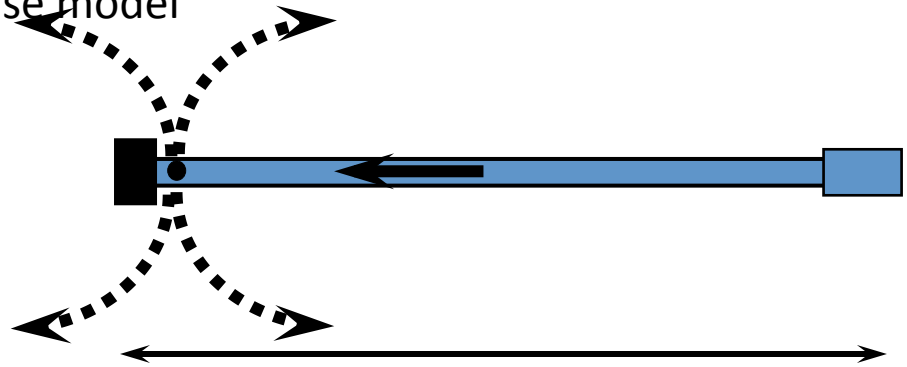
Challenges of mucosal vaccination

- Identification of protective **antigens**
- Efficient **delivery** of protective antigens to mucosa-associated lymphoid tissue
  - delivery systems
- Activation of protective immune mechanisms, often neutralizing **IgA**
  - adjuvants
- **Escaping** maternal (passive) immunity
  - Passive milk antibodies (lactogenic immunity)
  - Passive serum antibodies (placental-colostral immunity)

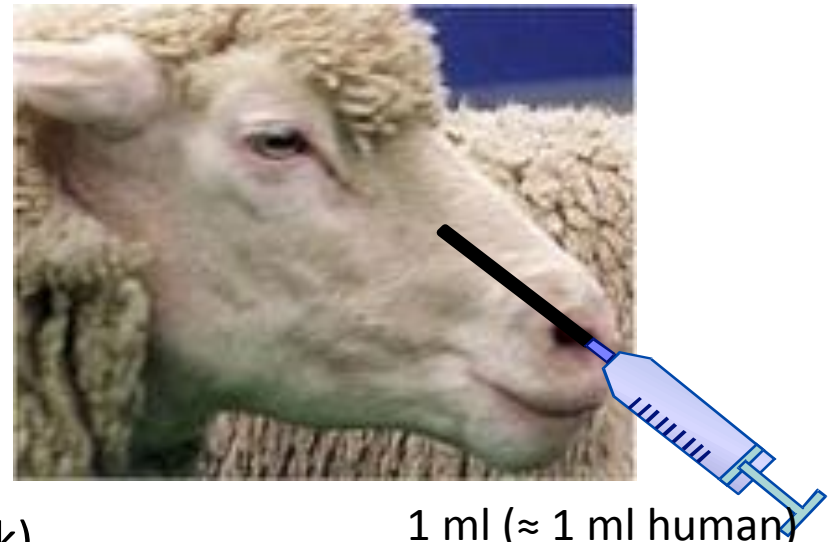
Intra-nasal vaccination

The mouse model

50 µl ≈ 200 ml in a human



10 cm

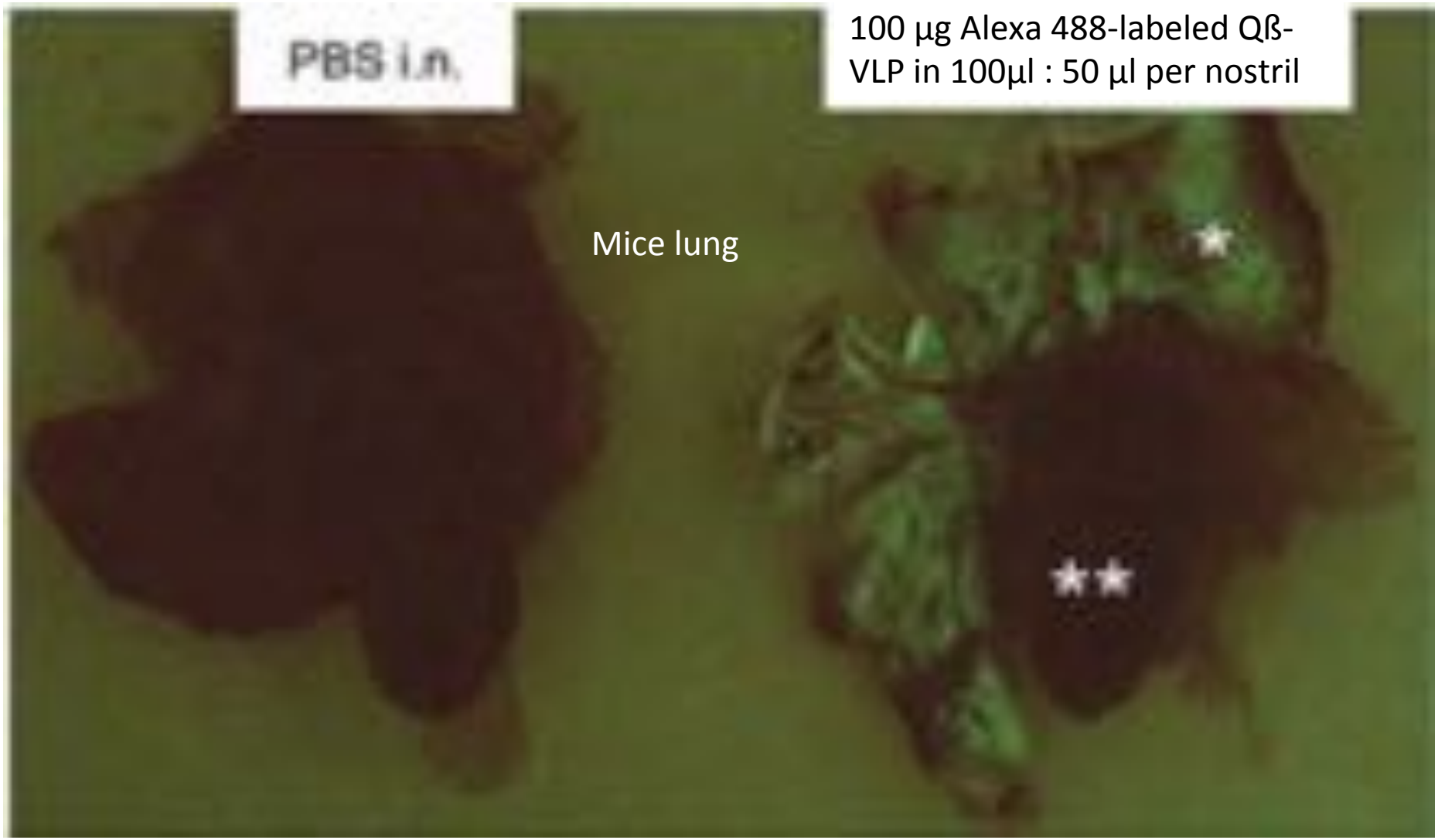


1 ml (≈ 1 ml human)

- Intra-nasal in mice, is “intra-mouse”:
(i.e. also intra-lung, intra-gastrointestinal track)

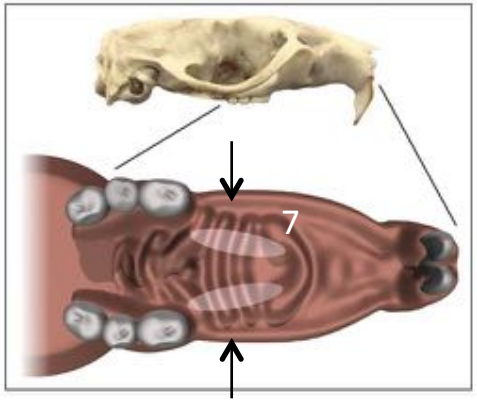
- Unlike mice, sheep nasal associated lymphoid tissue is similar to human structure.

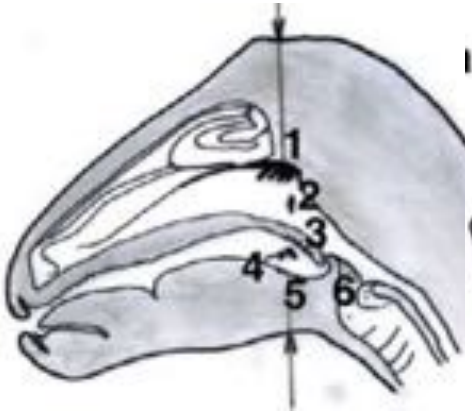
Vujanic et al., 2012. Vet Imm Immunopath
JP Scheerlinck, University of Melbourne, personal communication

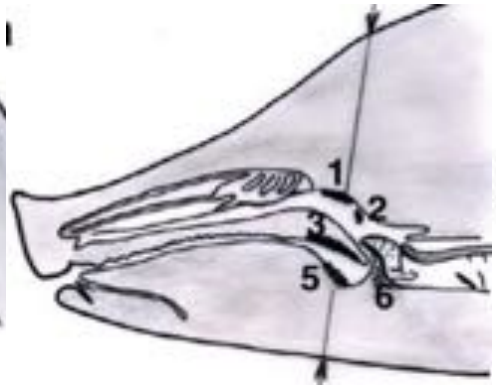


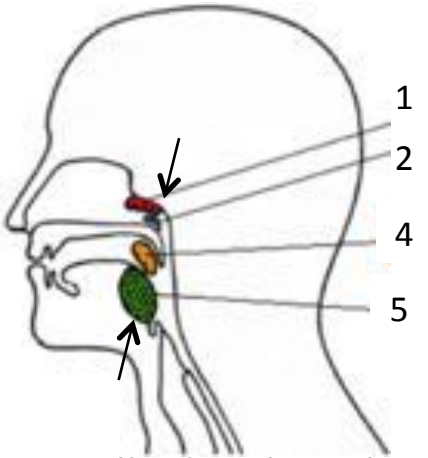
Bessa et al., 2008. J. Eur. Imm.

	5 Lingual tonsil	4 Palatine tonsil	6 Paraepiglottic tonsil	1 Pharyngeal tonsil	2 Tubal tonsil	3 Tonsil of the soft palate	7 NALT
Rabbit	–	+++	–	–	–	–	++
Rat	–	–	–	–	–	–	+
Mice	–	–	–	–	–	–	+
Sheep	±	+++	+	+++	+	+	–
Pig	++	–	+	++	+	+++	–
Human	++	+++	–	++	+	–	–

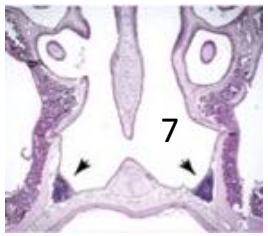


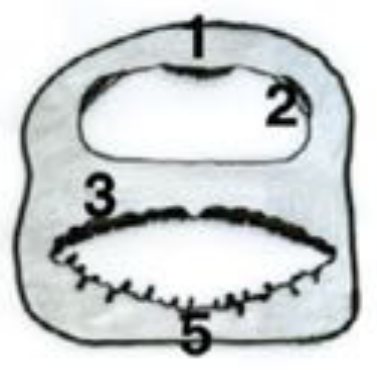


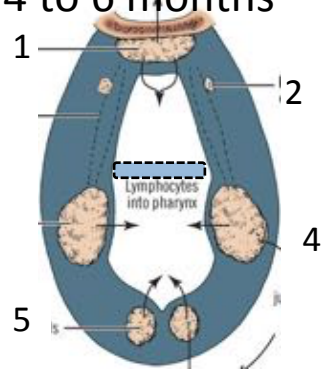




Fully developed at 4 to 6 months

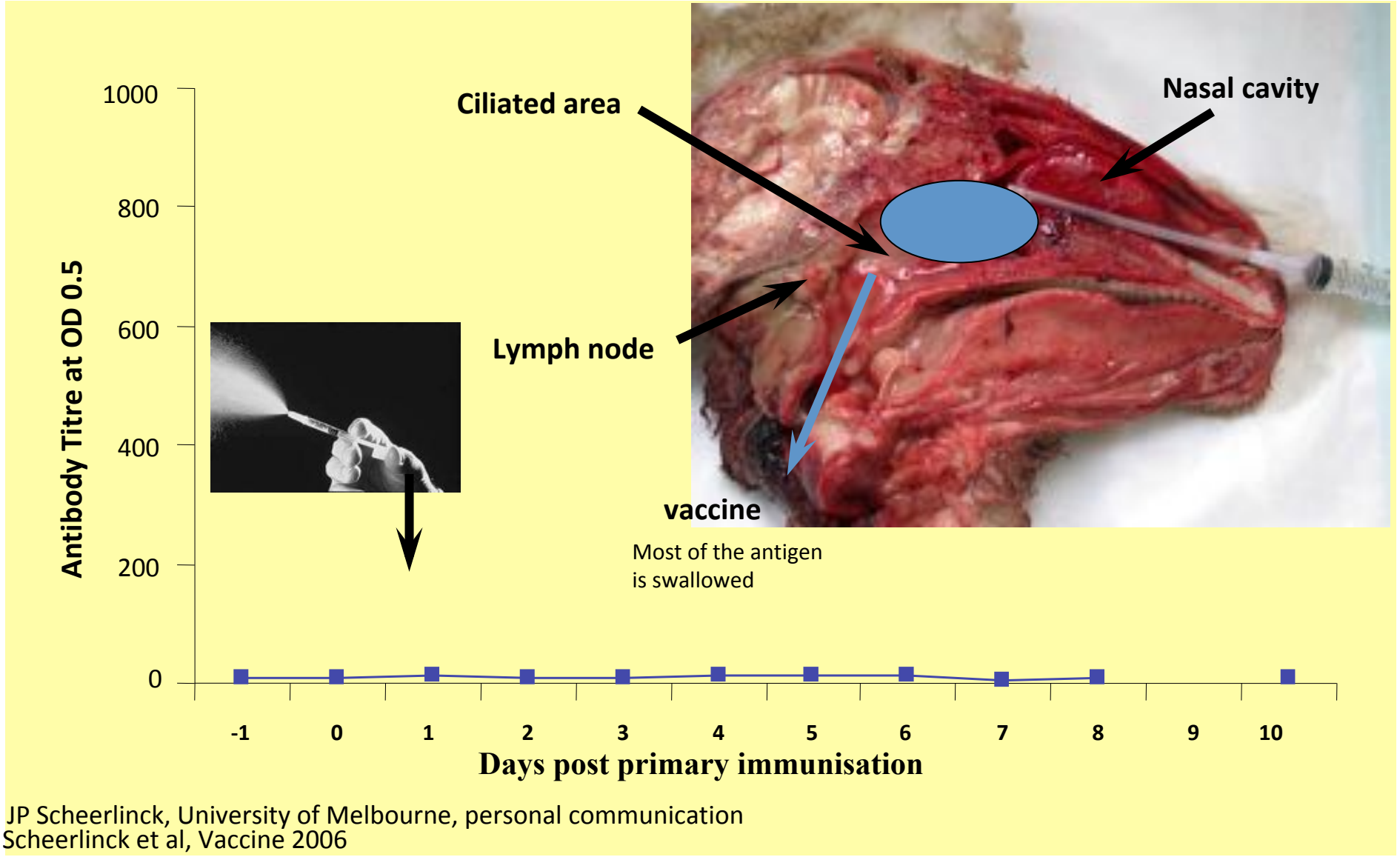






Liebler et al., 2006. Vet Res
G. Kraal, 2005. Mucosal Imm.

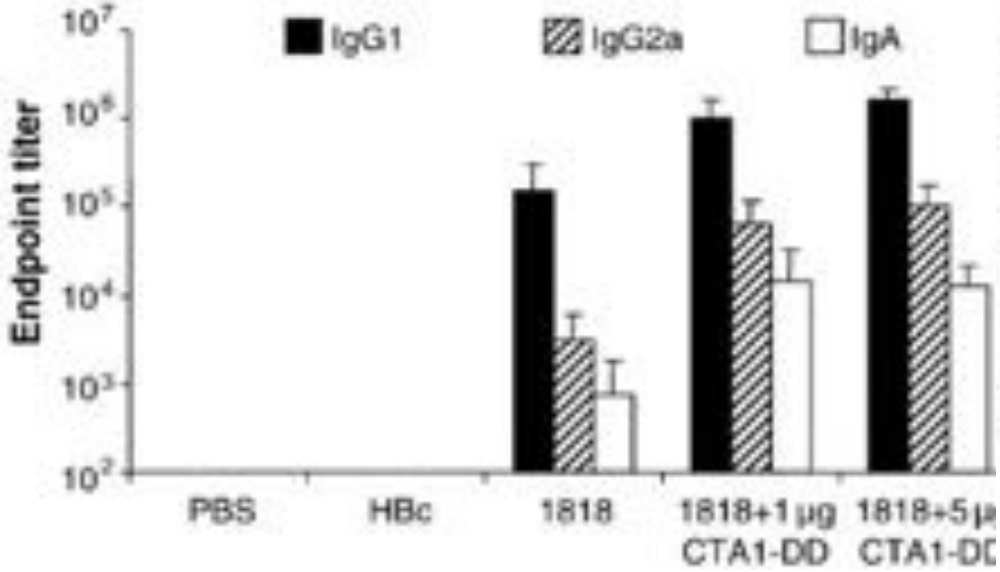
Immune responses in lymph following intranasal spray



JP Scheerlinck, University of Melbourne, personal communication
Scheerlinck et al, Vaccine 2006

Mucosal adjuvant CTA1-DD in intranasal immunisation

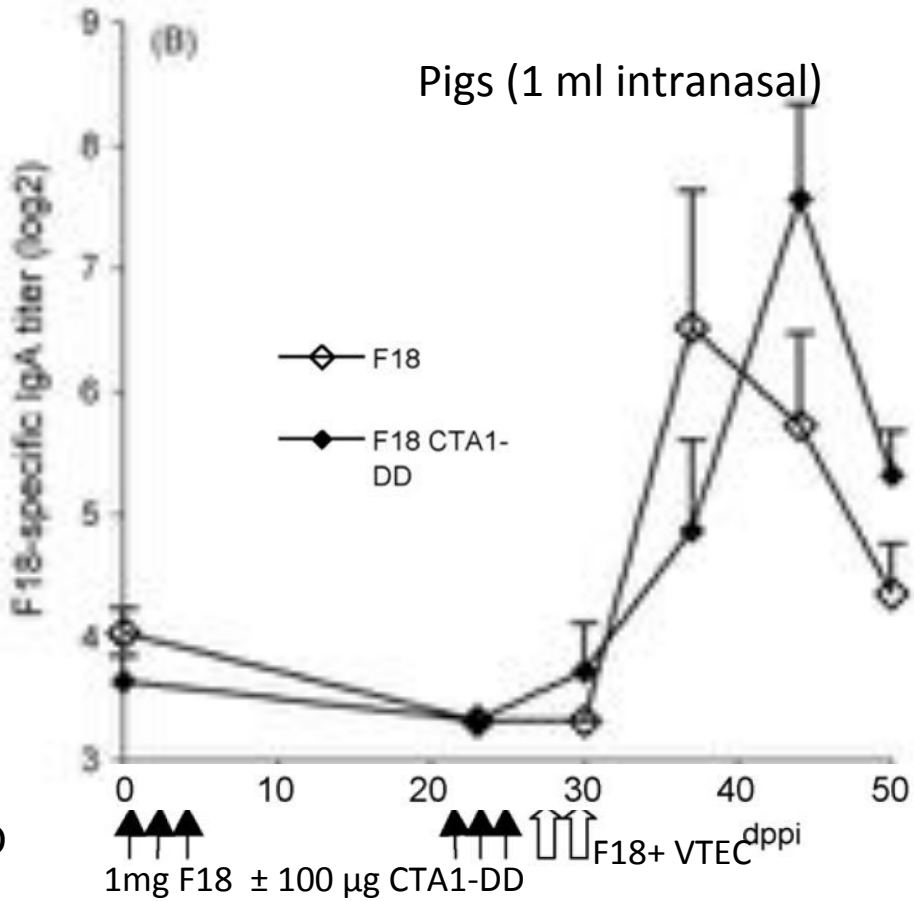
Mice (50 µl intranasal)



Enhanced efficacy of the M2e-HBc vaccine by combination with the mucosal adjuvant CTA1-DD

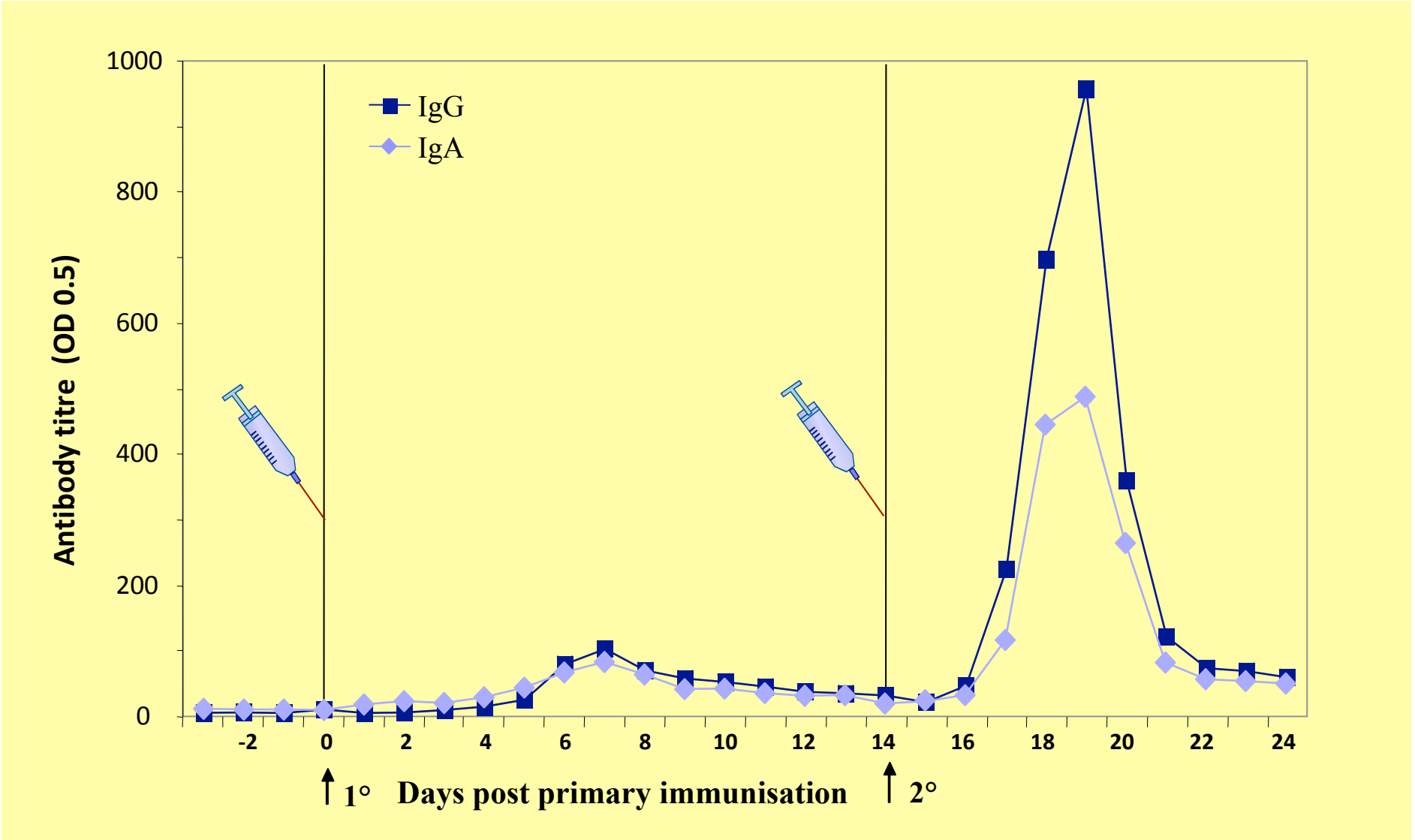
Filette et al., 2006. Vaccine

Pigs (1 ml intranasal)



Verdonck et al., 2006. Vet. Imm. Immunopath

Immune responses in lymph following intranasal injection



JP Scheerlinck, University of Melbourne, personal communication

Oral vaccination

Virulence

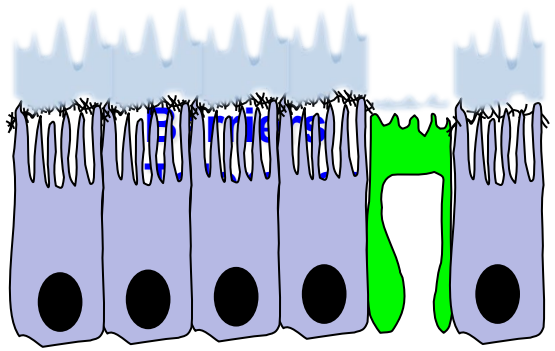
VACCINE

alive ⟷ dead

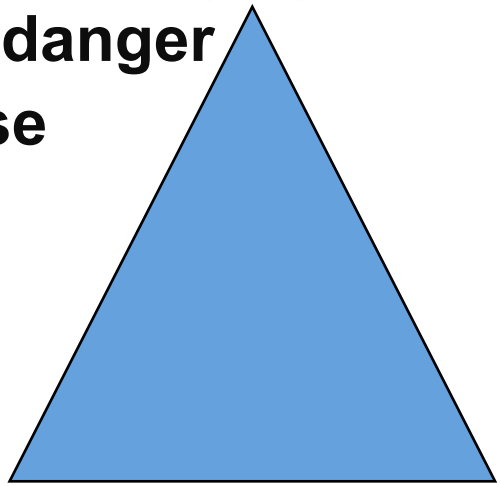
Too attenuated => no danger

Too virulent => disease

Expresses virulence factors



Intestinal Mucosa



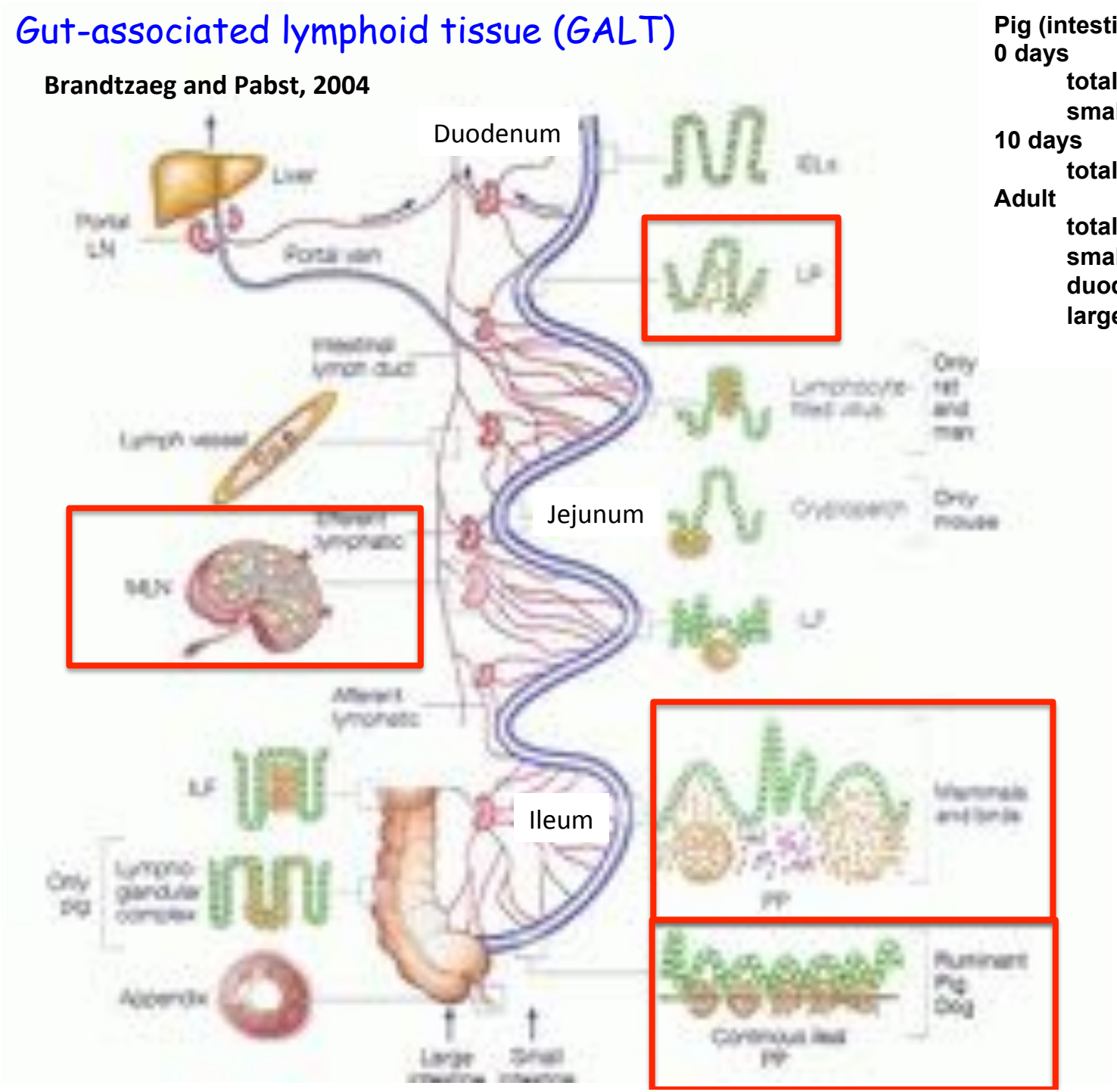
Preferred ROUTE for intestinal immunity

AGE passive ⟷ active

Passive immunity
-milk antibodies
-placental (humans) or colostral antibodies (animals)

Gut-associated lymphoid tissue (GALT)

Brandtzaeg and Pabst, 2004



Pig (intestinal length)
0 days
- **total ≈ 7.4 m**
- **small intestine ≈ 3.4 m**

10 days
- **total ≈ 8.5 m**

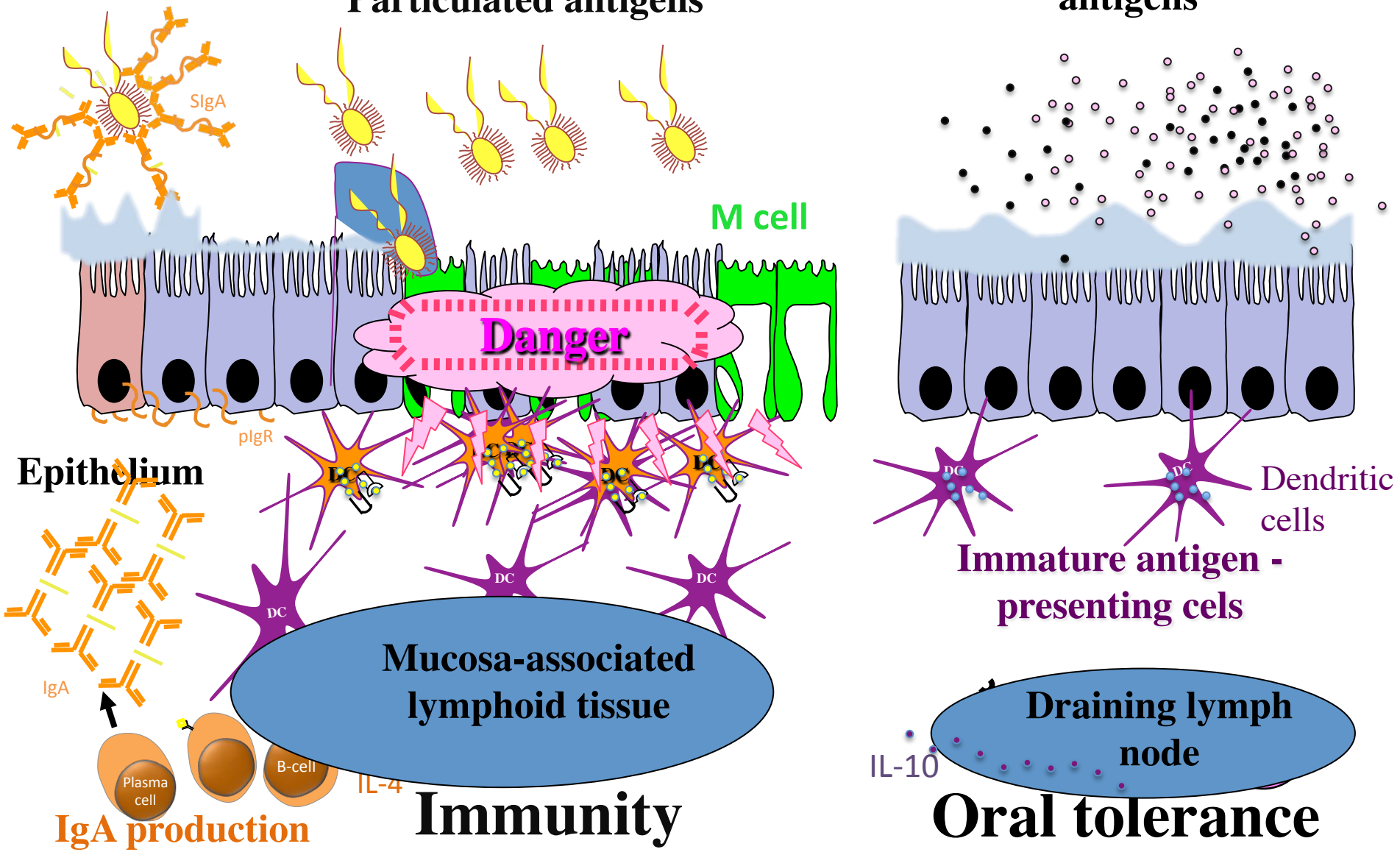
Adult
- **total ≈ 19 – 25 m**
- **small intestine ≈ 15 - 20 m**
- **duodenum ≈ 60 cm**
- **large intestine ≈ 4-5 m**

FOLLICLE-ASSOCIATED EPITHELIUM

MUCOSA

Pathogens
Particulated antigens

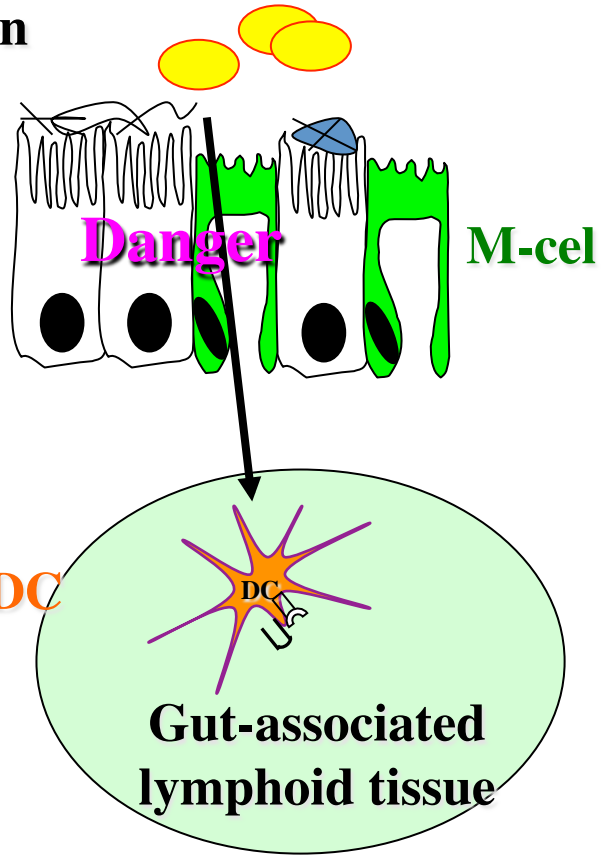
Non-replicating soluble antigens



Oral vaccination

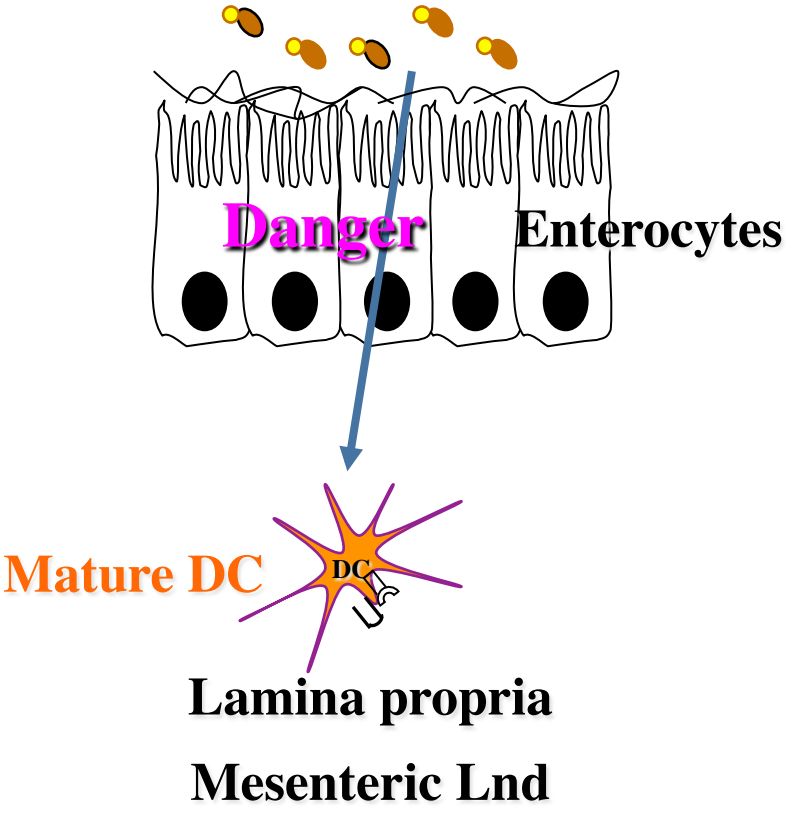
Follicle-associated epithelium
M-cells

Particulated antigen



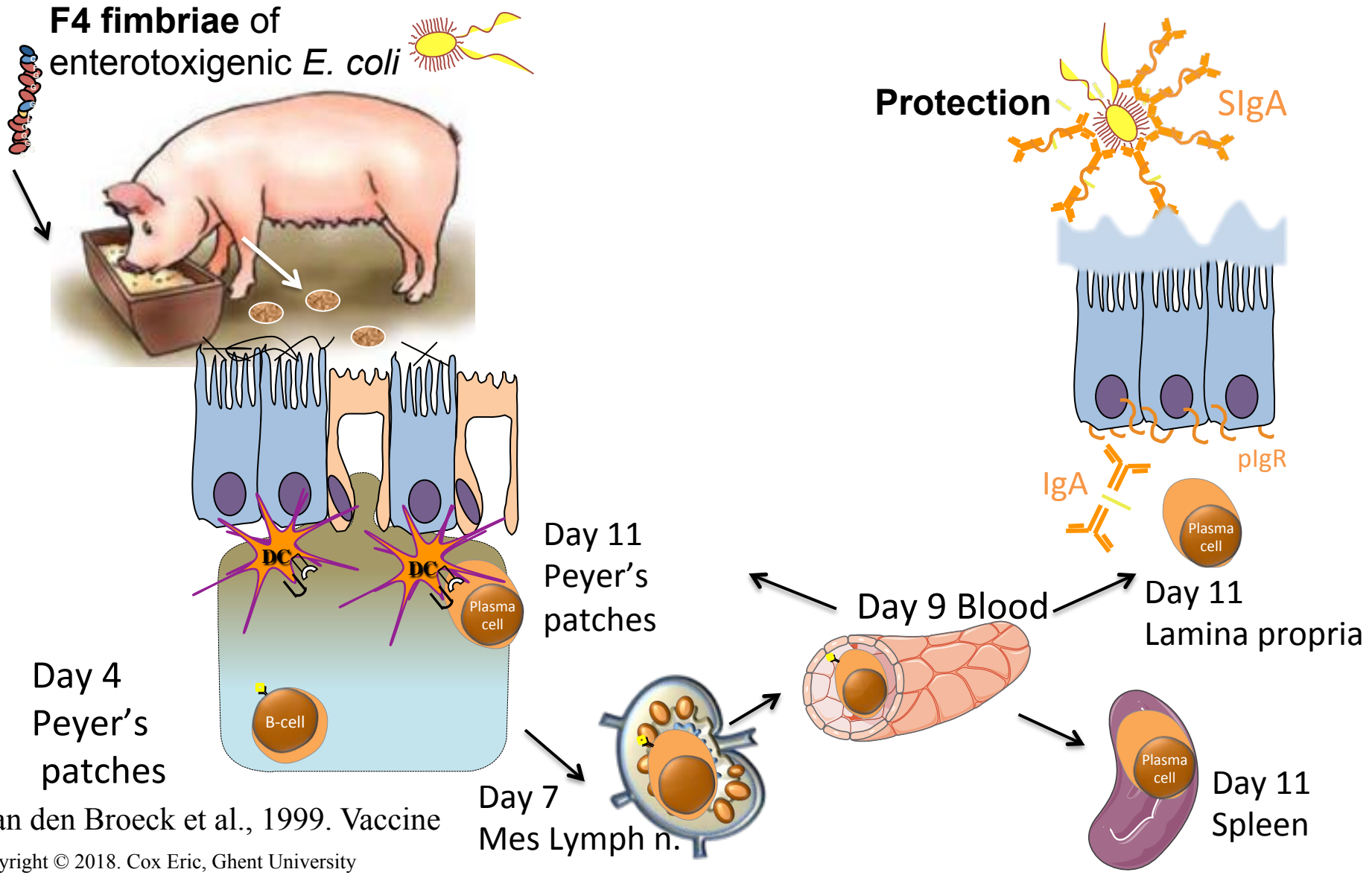
Enterocytes

Few soluble antigens
Virulence factors



Immunity

F4 as a tool to study the immune response following oral immunization

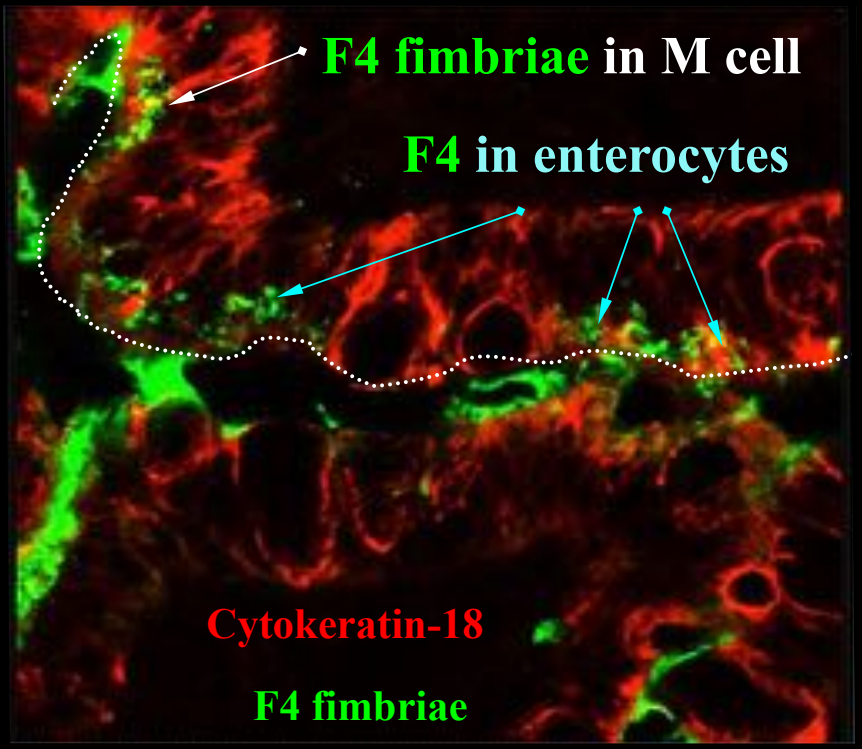


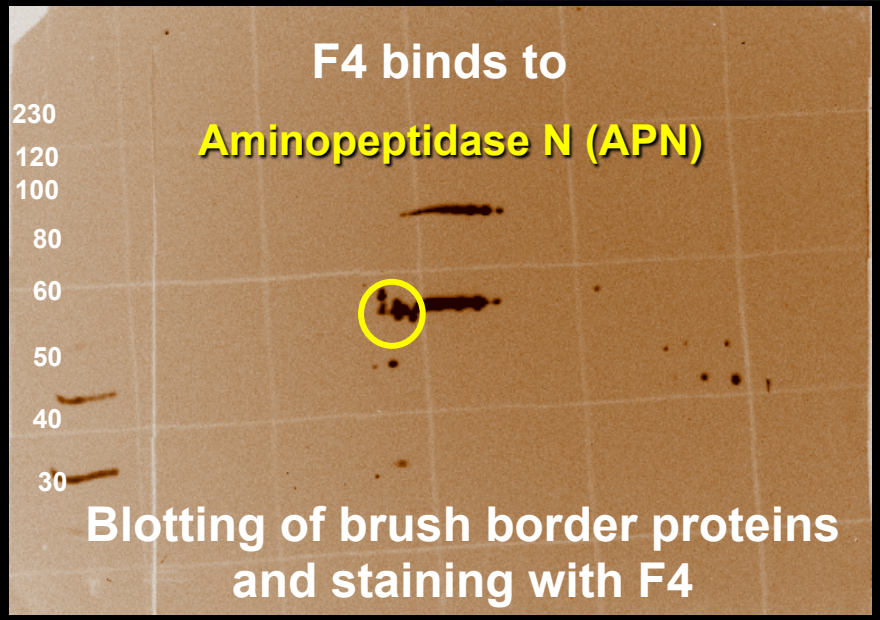
Van den Broeck et al., 1999. Vaccine

Binding and uptake of F4 fimbriae

Ligated loops injected with F4

Snoeck et al., 2008. Vet Imm Immunopath.





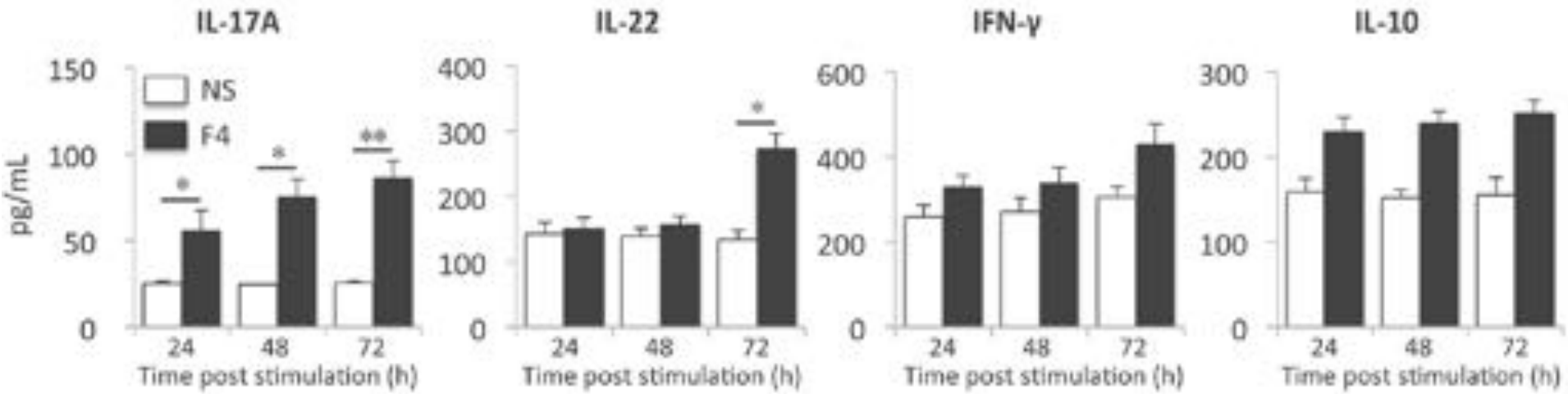
Duodenum
Jejunum
Ileum

Melkebeek et al., 2012. Mucosal Immunity

F4 fimbriae can induce IL17A in naïve PBMCs

Endotoxin-free F4 fimbriae (5 µg/mL) or medium for 72 h.

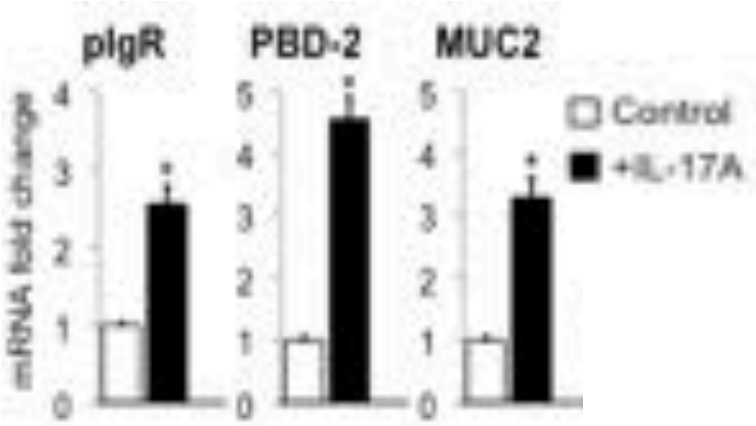
Concentrations of cytokines in supernatant

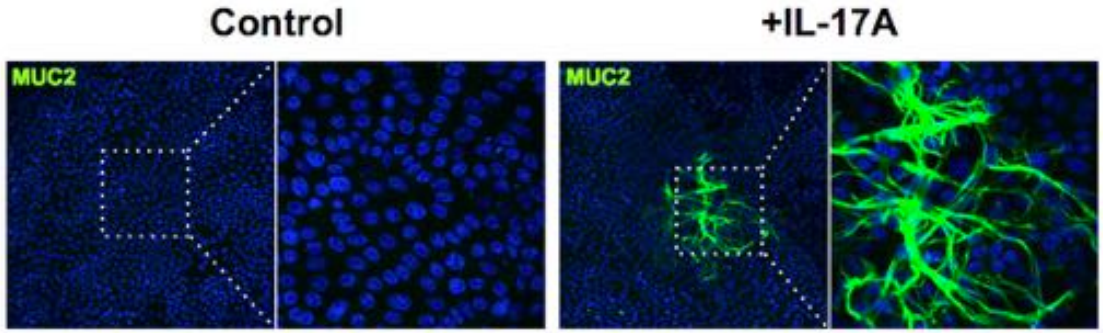


Luo, 2016. PhD UGent

What is the role of IL-17A?

IPEC-J2 cells stimulated with **IL-17A** (24 h)





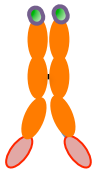
Intestinal barrier becomes fortified

Luo, 2016. PhD UGent

Different strategies are followed

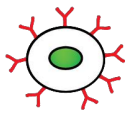
F4 fimbriae

- Carrier (Tiels et al, 2008. Vaccine)
- Aminopeptidase N (APN) is F4R (Melkebeek et al, 2012. Muc Imm.)
- Th17 dominated response (Yu et al., 2015. Vet. Research)



Anti-APN antibodies targeting soluble antigen to APN and the GALT

- Polyclonal Ab Induce IgA (Melkebeek et al, 2012. Muc. Imm.)
- Monoclonal Ab (in development)



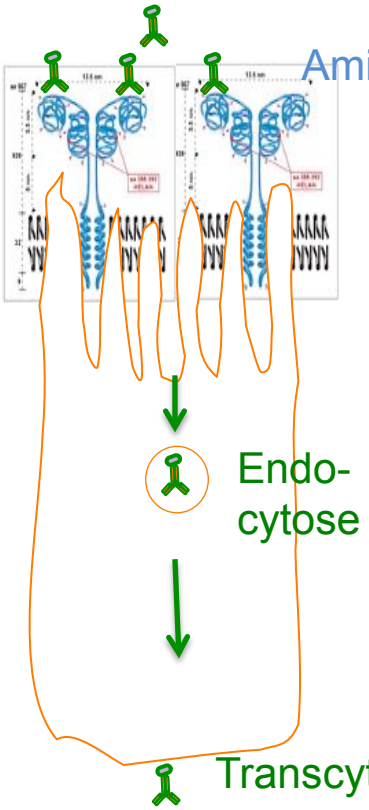
Yeast ghosts targeted to the F4 receptor

- Enhanced antibody responses (Baert et al., 2015. J. Contr. Rel.)
- B. Devriendt, Poster 132

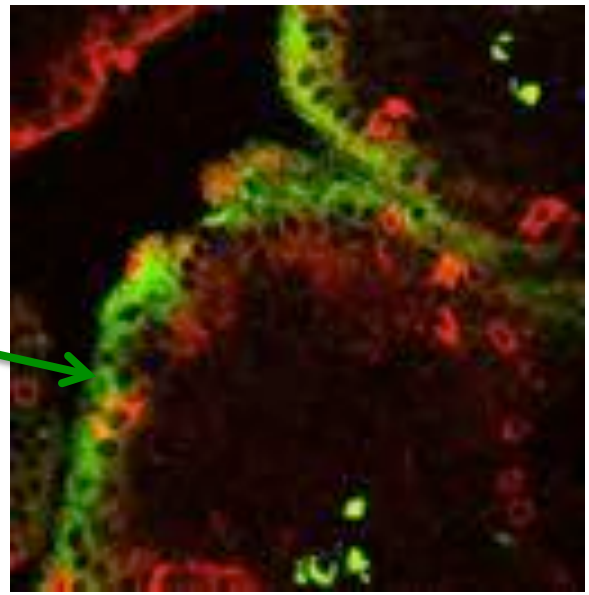
Soluble Antigen

Aminopeptidase N targeting antibodies

Antibodies target antigen to APN

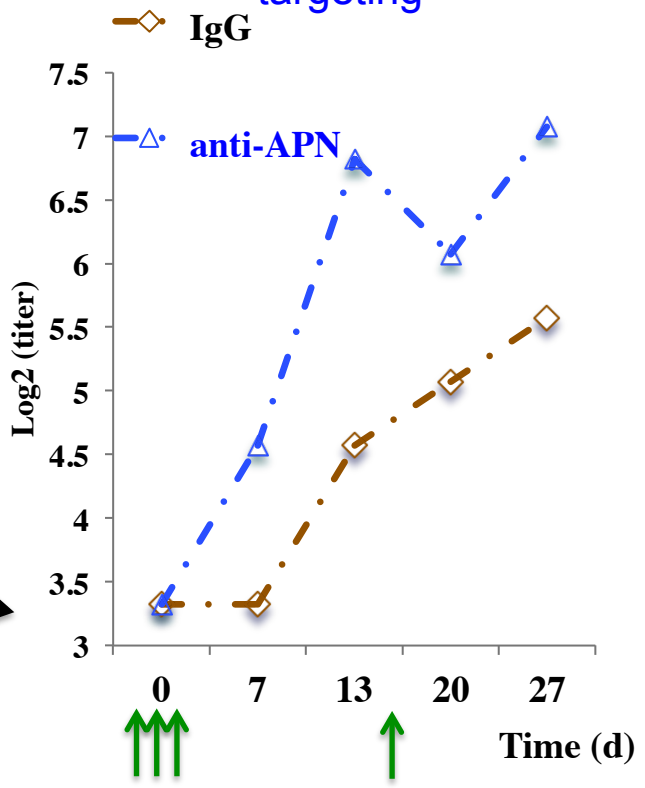


ANPEP targeting antibodies
15 minutes after contact



Antigen reaches antigen-presenting cells in the lamina propria

IgA response in pigs following APN targeting

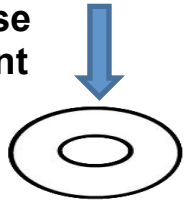


Oral immunization with 1mg

Particles carrying antigen targeted to APN

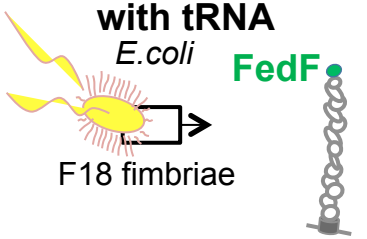
Saccharomyces cerevisiae

Acid/base treatment

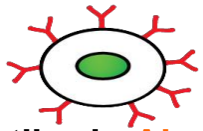


Load with antigen complexed with tRNA

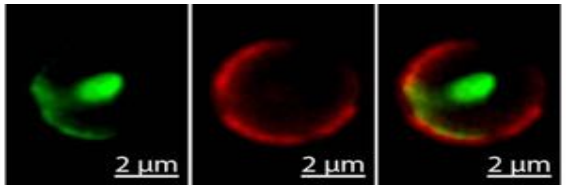
Adjuvant
ß-glucan
.....

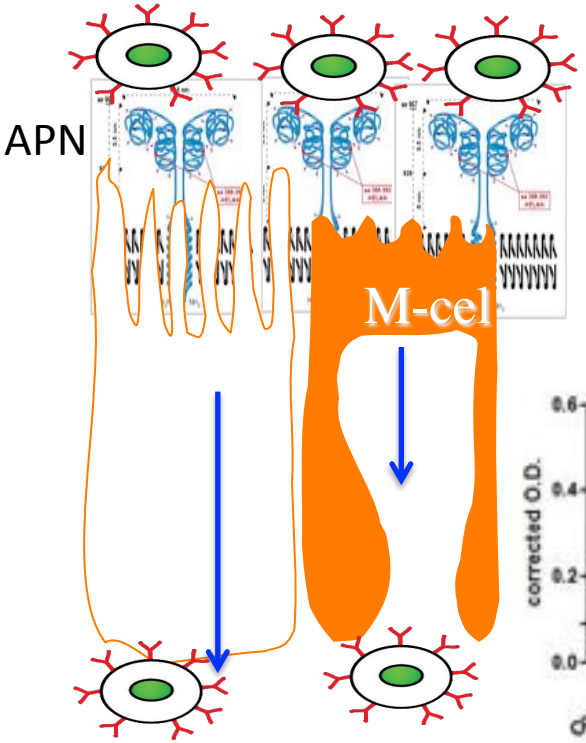


Conjugate APN-targeting antibody

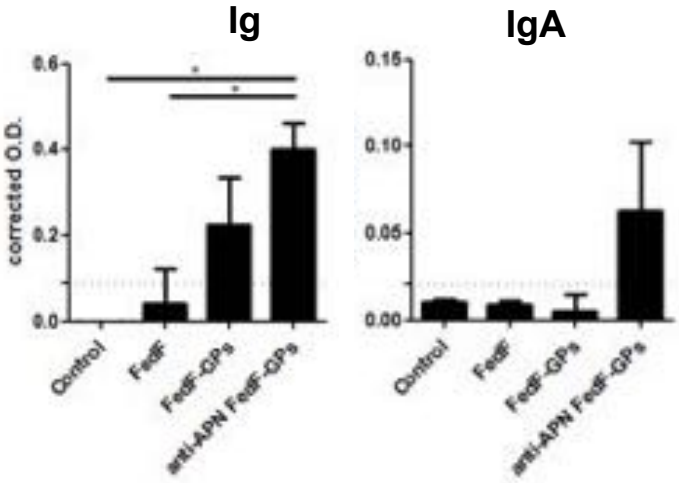


APN targeting antibody-AlexaFluor 64

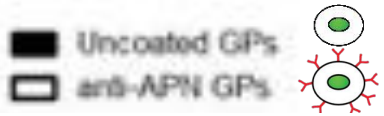


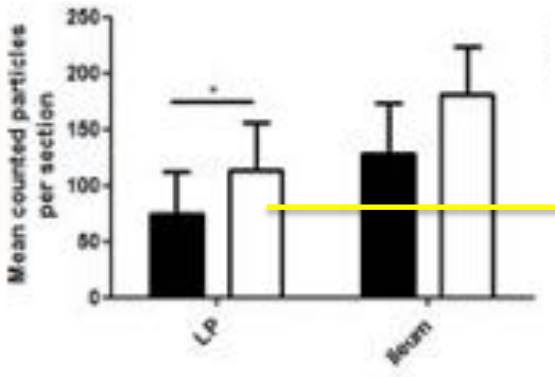


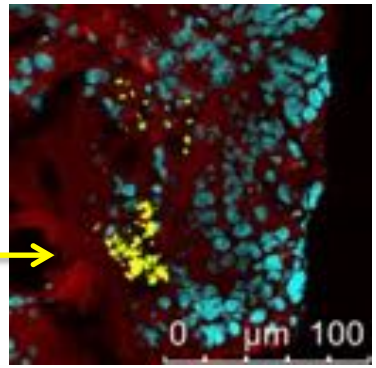
Serum antibody response 14 days after 2 immunisations



1 hour



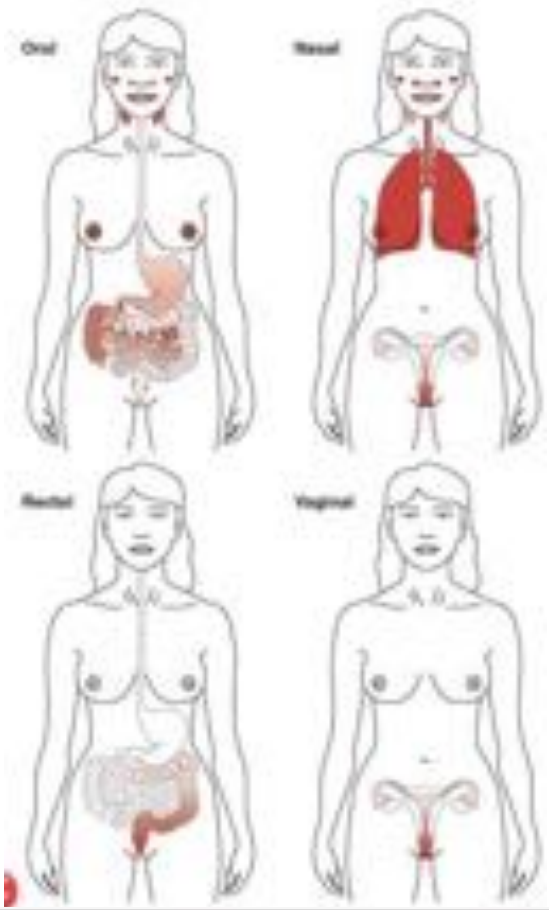




Lamina propria

Baert et al., 2015. J Contr Rel
Baert et al., 2016. Int J Nanomedicine

What tissues do you protect if you vaccinate mucosal



The common mucosal immune system is not so common

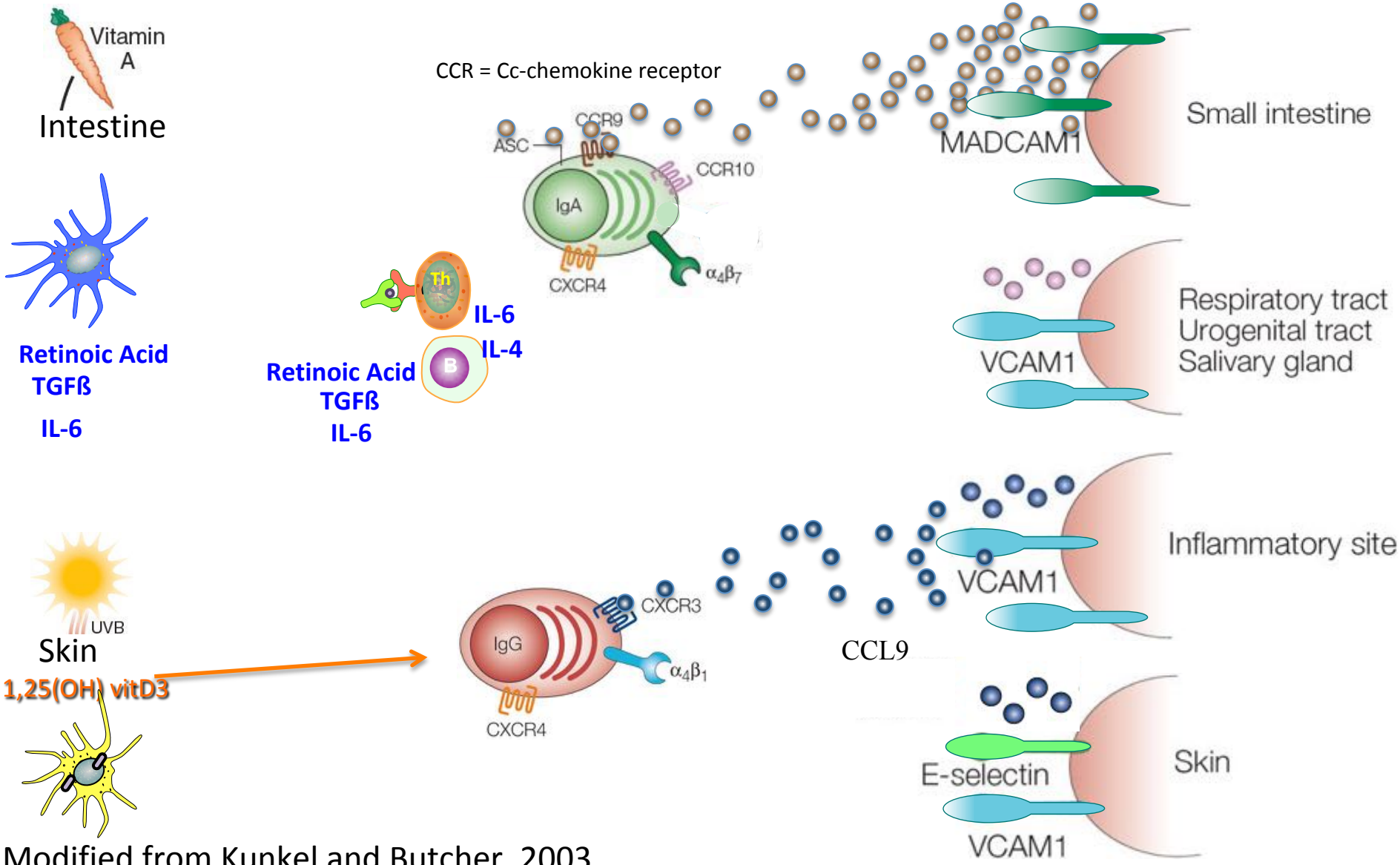
Holmgren and Czerkinsky, 2005. Nat. Medicine
Czerkinsky, Holmgren, 2010. Mucosal Immunol.

	Nasal	Subling	Oral	Rectal	Vaginal	Trans-dermal
Upper respiratory	+ + +	+ + +	–	–	–	+ + +
Lower respiratory	+ to + + +	+ + +	–	–	–	+ + +
Stomach	–	+/+ + +	+/+ + +	–	–	?
Small intestine	–	+ + +	+ + +	–	–	+
Colon	–	?	+	+ +	–	+
Rectum	–	?	(+)	+ + +	–	?
Reproductive tract	+ + +	+ + +	–	–	+ +/+ + +	?
Blood	+ + +	+ + +	+(+)	+(+)	+(+)	+ + +

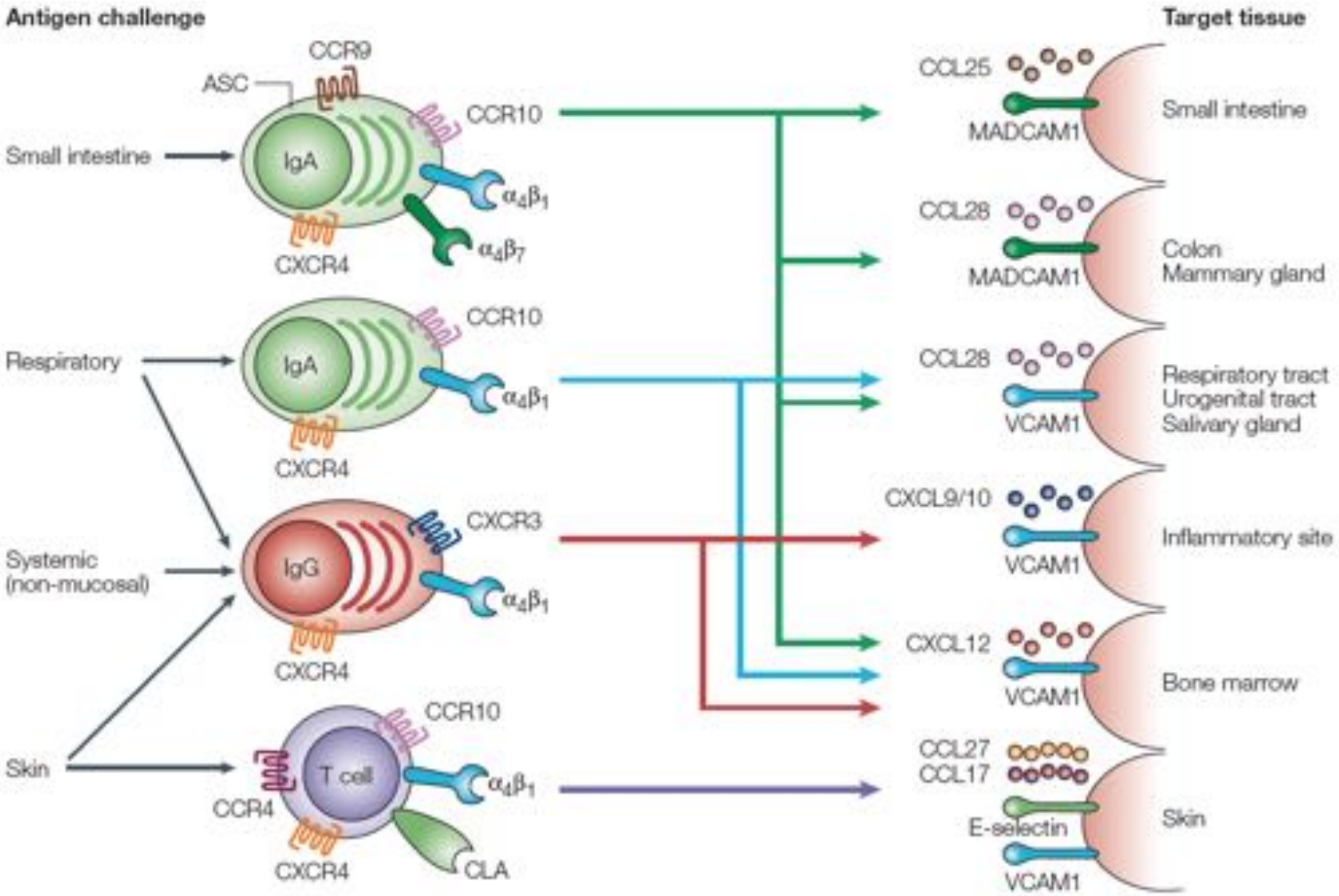
Why not so common?

Induction site | Lymph node | Homing receptors | Tissues

CCL = CC Chemokine ligand



Modified from Kunkel and Butcher, 2003



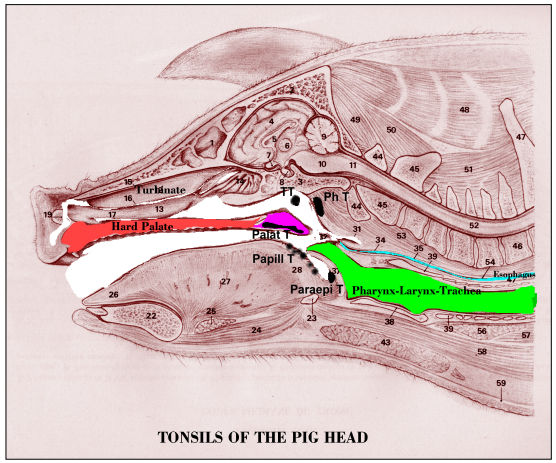
Kunkel and Butcher, 2003. Nature review Immunol

INDUCTOR SITE(S)

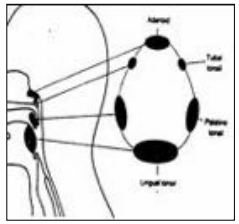
EFFECTIVE SITES

NEAR

DISTANT



Upper respiratory tract



Tonsils

•Nose,

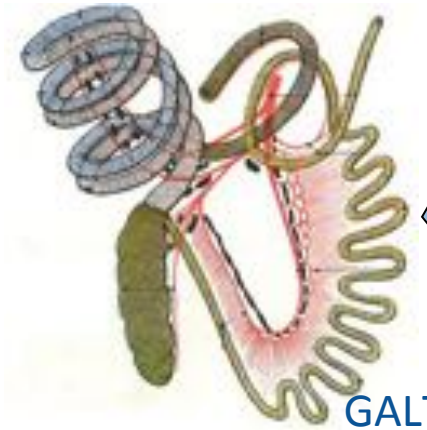
•Trachea, bronchia

IgA/IgG

Blood

Dissemination to Mammary Gland

Lactating mammary gland



GALT

Digestive tract

IgA

Salmon et al

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E. coli and the Mucosal Immune System

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