Impact of age and intestinal microbiota on the expression of avian defensins in the chicken gut

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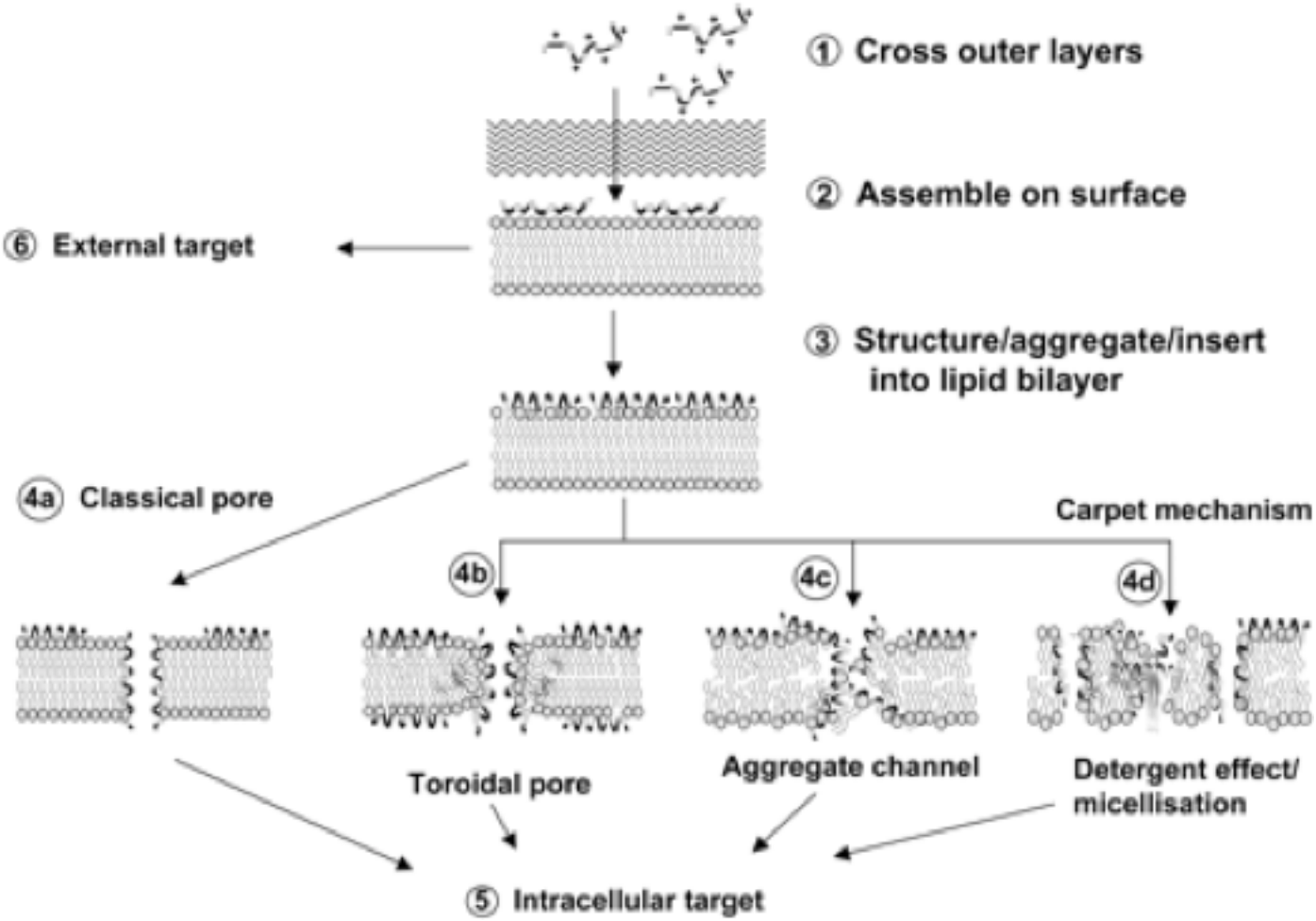
Defensins

- Cationic antimicrobial peptides, with 6 cysteins
- Highly conserved (animal and vegetal kingdoms)
- Three families: α (mammals)
  β
  θ (primates)
- Key factors of innate immunity (skin and mucosal) : epithelium and phagocytes arsenal

Defensins

- Antimicrobial activity: membrane disruptors

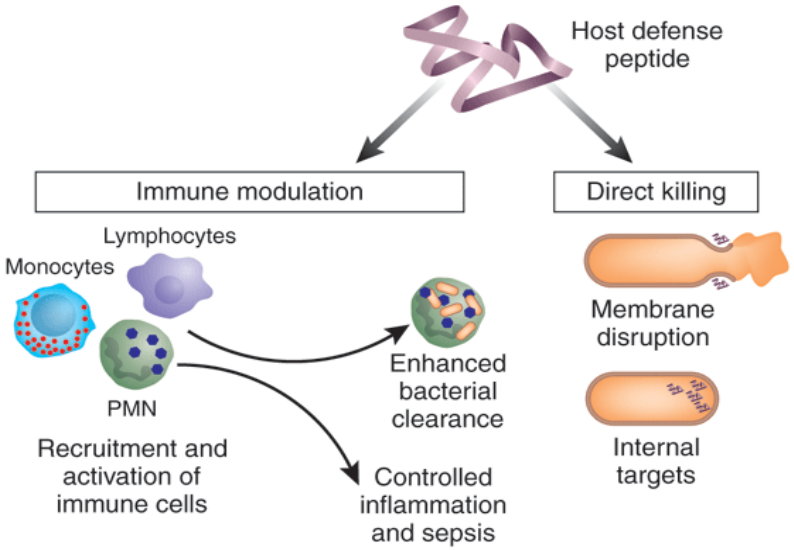


Defensins

- Biological activity: more than microbicidal

chemotactism (hBD2/CCL20)
-> DC, T cells (Yang et al. 2002, Trends in Immunology)

activation of monocytes, neutrophils
APC, through TLR1, 2 or 4
(Biragyn et al, Science, 2002; Funderburg et al, PNAS, 2007)



functions of β-defensins
from R. E. W. Hancock & H. G. Sahl, 2006

Pleiotropic : on mastocytes, fibroblasts, tissue repair, enzyme inhibition…

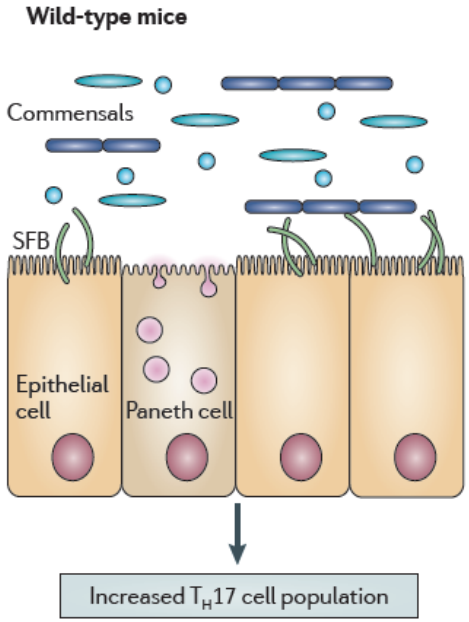
Defensins

- Link with microbiota, lessons from mammals:

the human α-defensin hD5 transgenic murine model (Salzman et al. 2009, Nature Immunology)

hD5 TG +/+ ➡ Bacteroidetes > Firmicutes (loss of SFB)

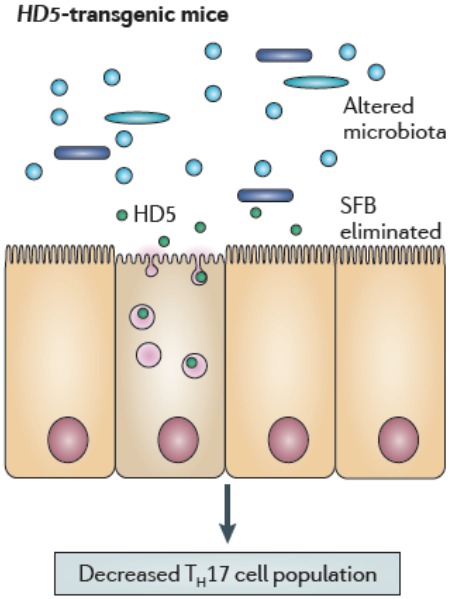
MMP7 KO -/- ➡ Firmicutes > Bacteroidetes



SFB of Firmicutes upregulate TH17

↓

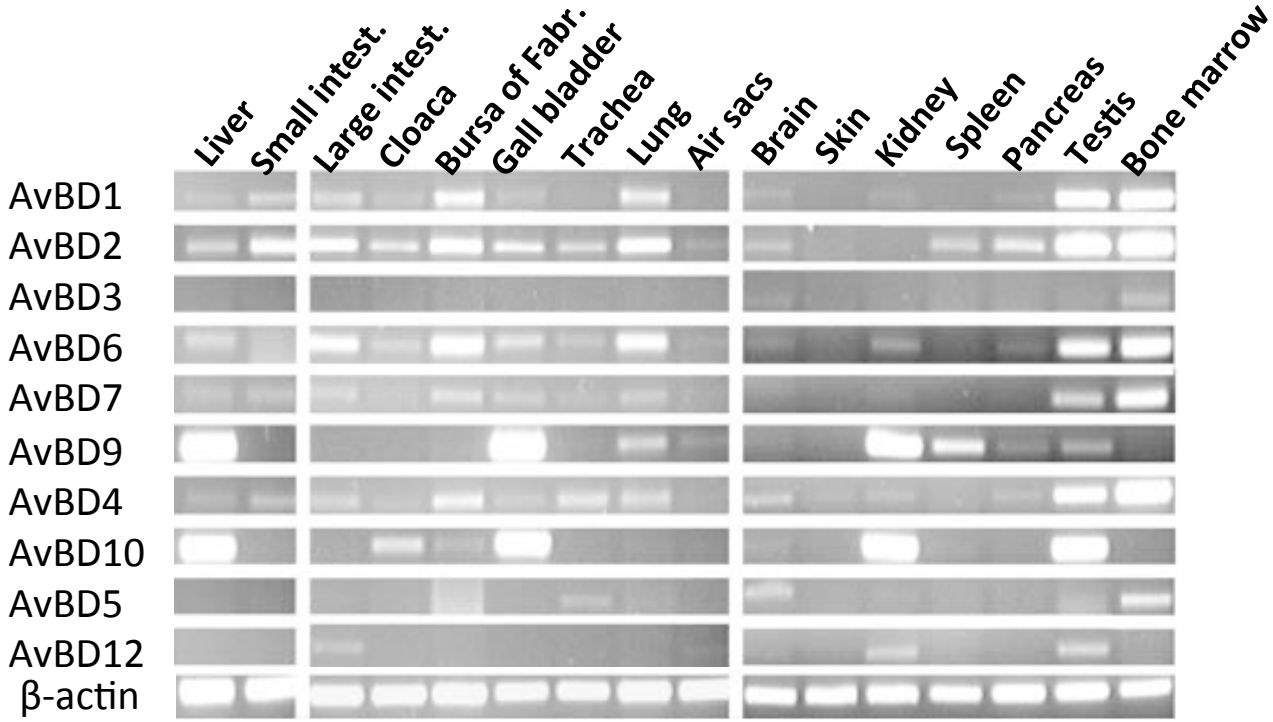
IL17, IL22



Birds defensins

- Avian β-defensins (AvBD), 14 members (for review: van Dijk et al, Vet Immunol Immunopathol, 2008)

- Transcriptomic analysis in tissues :

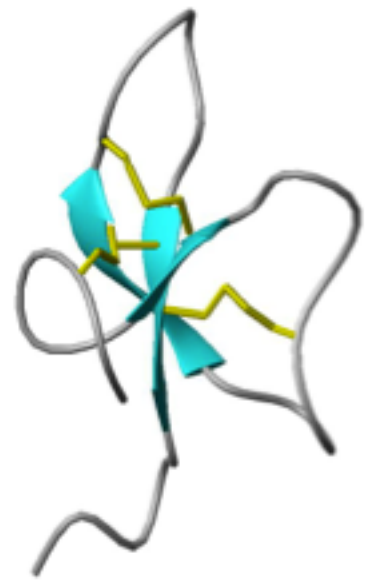


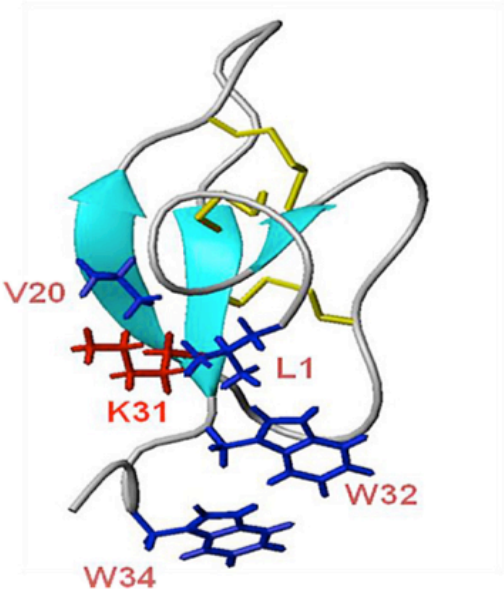
Male chickens, 3 weeks old (broilers), from Lynn et al, Immunogenetics, 2004

Birds defensins

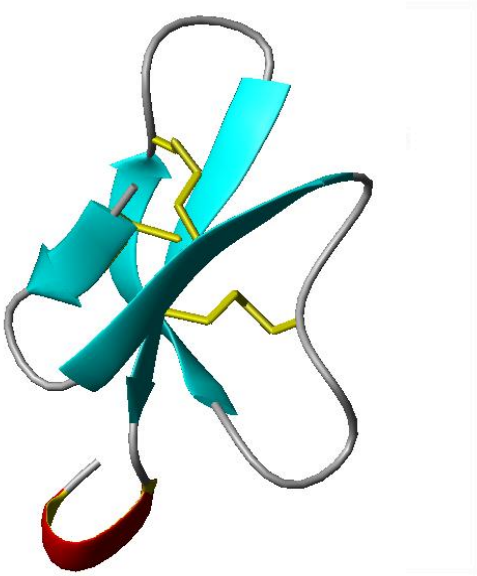
- Structural features

AvBD2 3D structure (Derache et al, J Biol Chem, 2012) solved by NMR (collab. A Delmas and C Landon, CBM, CNRS Orléans)





AvBD2



AvBD2 K31A

Birds defensins

- Antibacterial activity spectrum: minimal inhibitory concentration

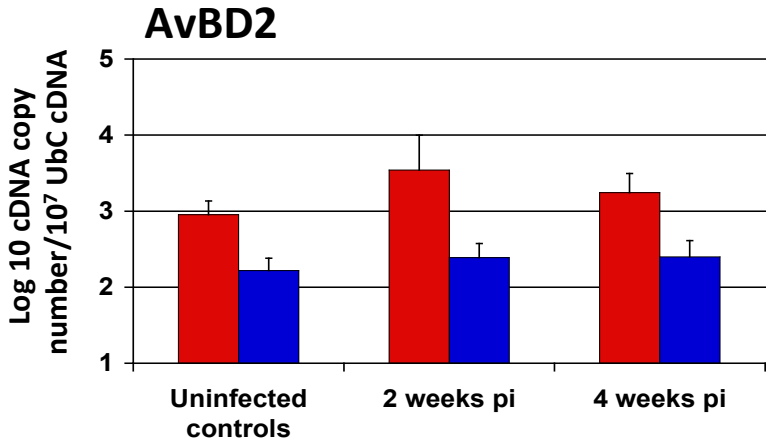
Bacterial strains	MIC in µM 95% confidence interval				
	L-AvBD2	D-AvBD2	L-AvBD2-Acm	AvBD2-K31A	AvBD2-K31A-Acm
Gram +					
B. cereus	**0.31** 0.19-0.47	**0.34** 0.19-0.53	**3.47**[a] 2.12-4.87	**0.75**[b] 0.56-0.97	**>58.26**
L. monocytogenes	**0.20** 0.09-0.36	**0.20** 0.11-0.34	**3.21**[a] 0.98-6.13	**1.55**[c] 1.05-2.14	**23,3-58,26**
S. aureus	**0.58** 0.05-1.79	**1.37** 0.35-3.03	**>114.99**	**6.56**[c] 0-32.5	**>116.52**
Gram -					
E. coli	**1.09** 0.68-1.57	**1.05** 0.73-1.40	**1.23** 0.32-2.49	**2.07**[d] 0.11-5.20	**23,3-58,26**
S. Enteritidis	**0.48** 0.20-0.86	**0.52** 0.26-0.87	**1.31**[c] 0.64-2.13	**2.55**[c] 0.77-4.64	**>58.26**
S. Typhimurium	**3.67** 1.73-5.99	**1.59** 1.83-5.96	**5.18**[a] 0-6.06	**7.08**[c] 0.57-10.23	**>58.26**

[a], P=0.0002, [b], P=0.0003, [c], P<0.0001, [d], P=0.016.

(Derache et al, J Biol Chem, 2012)

Birds defensins

- Involvement in the control of intestinal colonization by *Salmonella* Enteritidis



Bacterial load in caecum			
	Line 6 (R):	+/-	+/-
	Line 15 (S):	++	++

Gene expression of AvBD1 and 2 linked to the phenotype of R/S to *Salmonella* carriage (Sadeyen et al., Microbes &Infection, 2004, 2006)

Birds defensins

- Producing cells *in vivo* :

heterophils granulocytes (Harwig et al, FEBS Lett, 1994; Evans et al, J Leukoc Biol, 1994)

Intact cell mass spectrometry (ICM-MS): Collab. V Labas, PAIB, INRA Tours.
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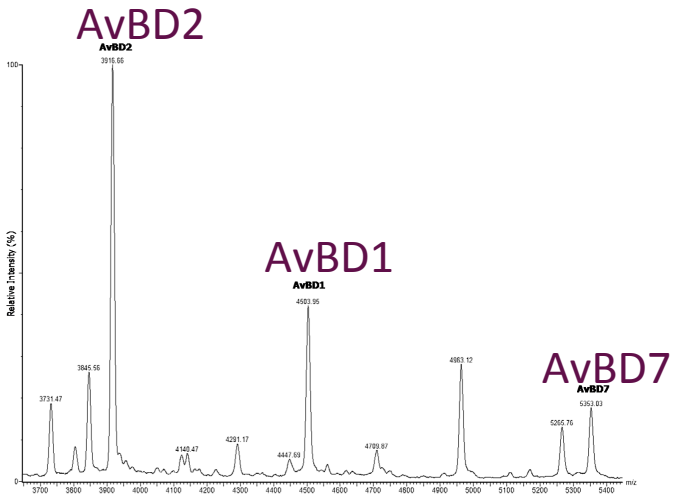

Birds defensins

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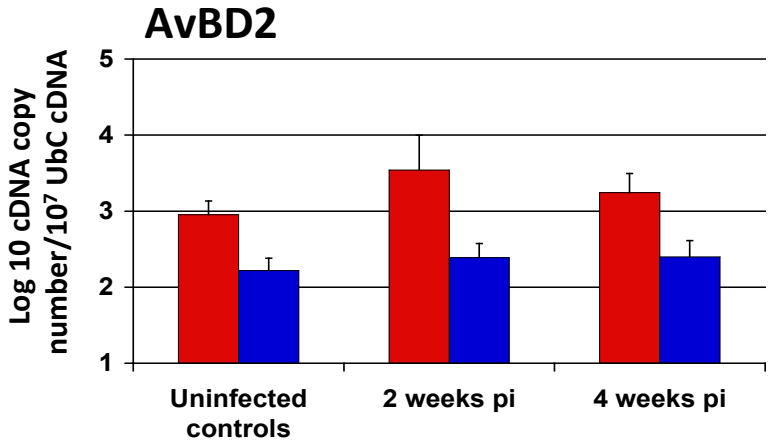

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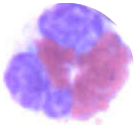

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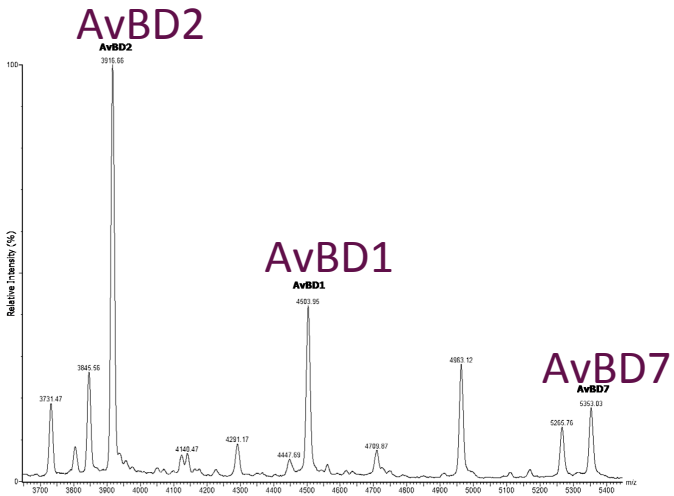
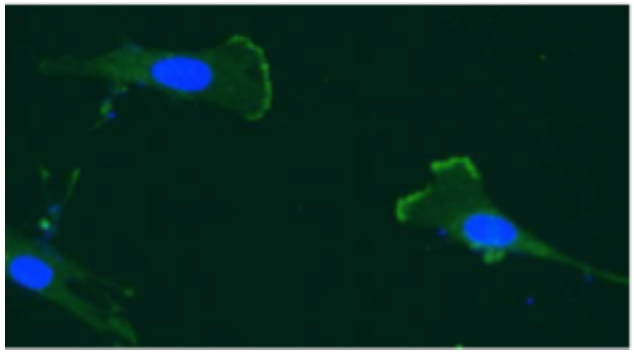


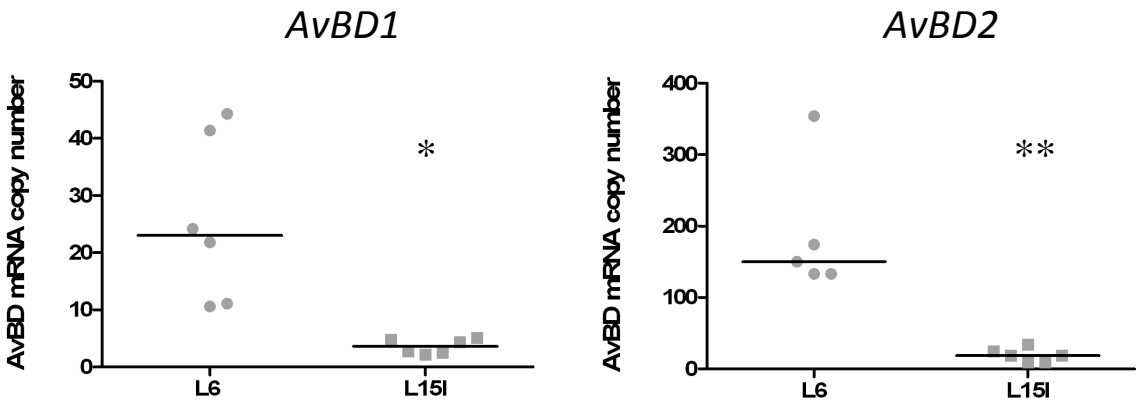
Birds defensins

- Producing cells *in vivo* :

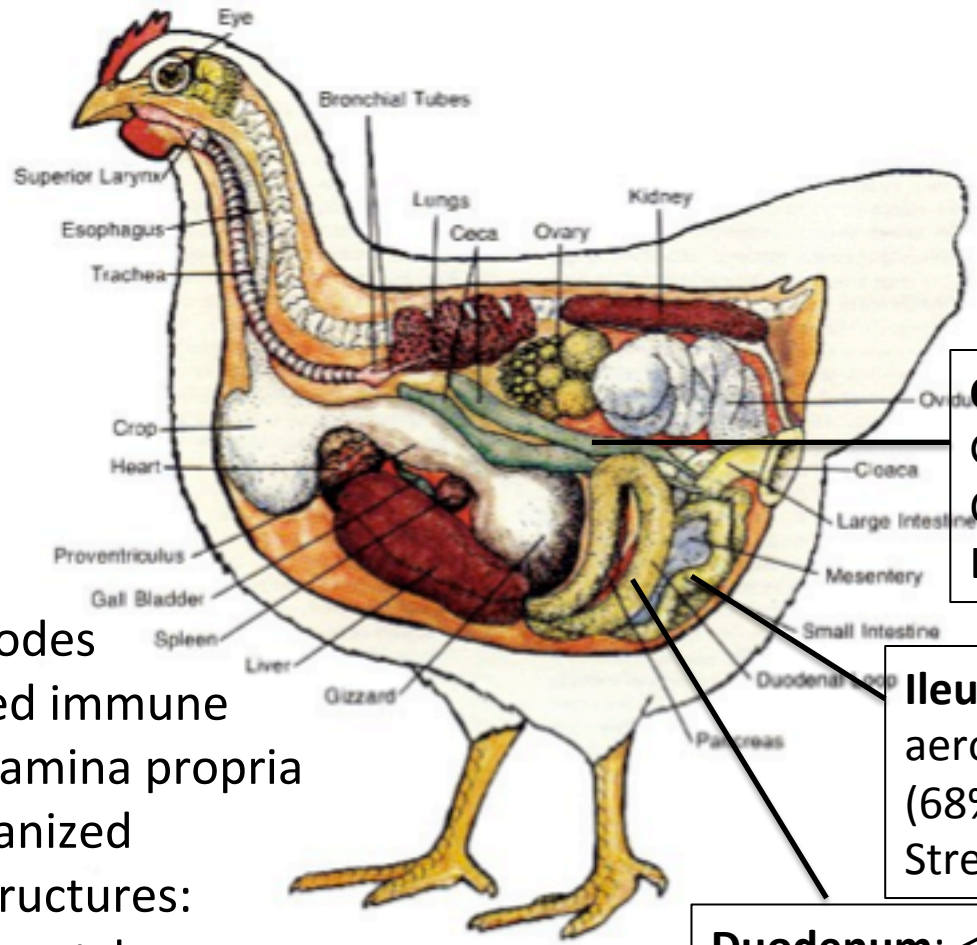
epithelial cells

(Derache et al, Dev Comp Immunol, 2009)





Birds digestive tract



Caecum: ≈10^{11} CFU/g
Gram+/-, strict anaerobes, Clostridiaceae (65%), Fuso., Lacto.,*Bacteroides*…

Ileum: ≈10^{9} CFU/g, Gram+, aero-anaerobes, *Lactobacillus* (68%), *Clostridium*, Strepto., Entero., …

Duodenum: <10^{8} CFU/g

Lu et al, Appl Environ Microbiol, 2003

Peculiarities:

- No lymph nodes
- Disseminated immune cells in the lamina propria
- Specific organized lymphoid structures:
  - Peyers patches
  - Bursa of Fabricius
  - Caecal tonsils

Birds digestive tract

Question Are AvBD1 and 2 regulated by the microbiota?

Expression level influenced by microbiota

Producing-cells influenced by microbiota

Approach Comparison of axenic versus conventional chicks

Transcriptomic analysis

Histological analysis

Birds digestive tract

- Kinetic analysis of AvBD1 and AvBD2 expression

Axenic versus Conventional chickens

- Similar patterns of expression:

  early for AvBD2 then downregulated after one week of age

  induced for AvBD1 after one week of age

- Independently of the microbial status and of the intestinal segment

Birds digestive tract

- Comparative analysis of AvBD1 expression between axenic and conventional birds

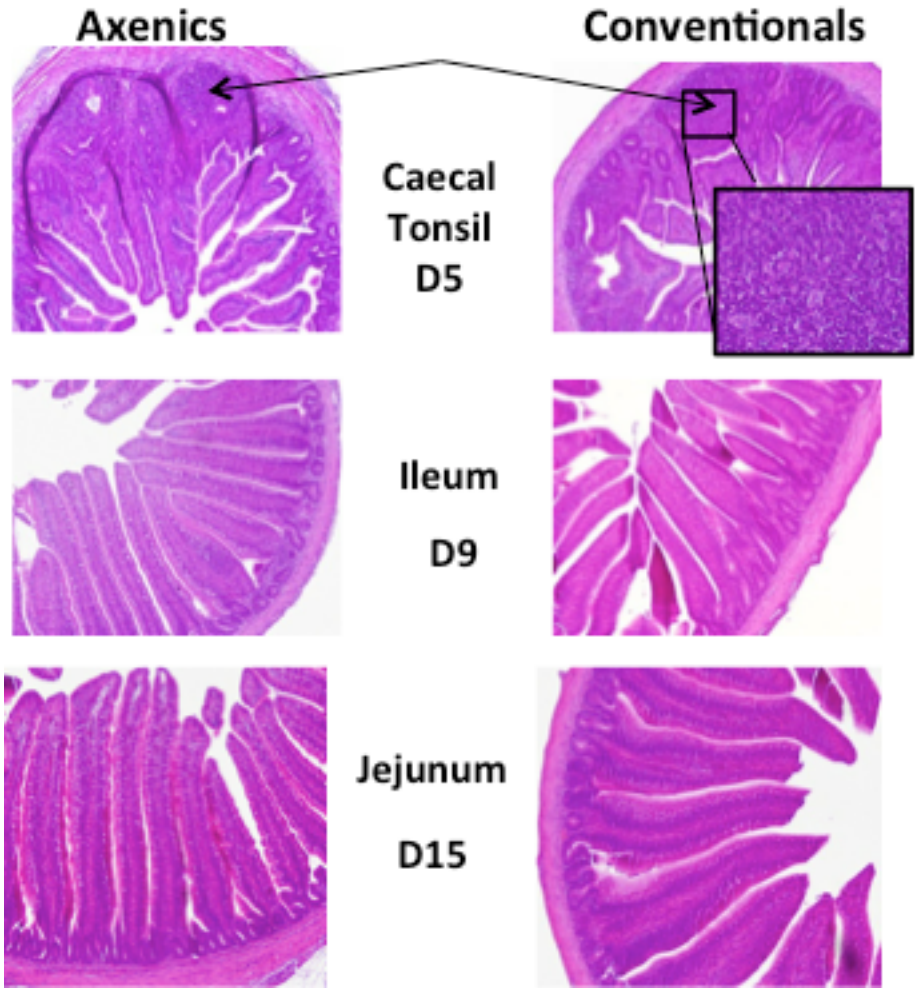
At day 15 of age
In the presence of microbiota:

- Increased expression of AvBD1 in the ileum

- Decreased expression of AvBD1 in the caecum and caecal tonsils

Birds digestive tract

- Comparative analysis of tissue architecture



Presence of lymphoid structures

- as soon as age 5 days
- independently of the flora

Lamina propria

- thickness reduced in absence of flora
- No granulocytes infiltration observed

Birds digestive tract and avian defensins
In conclusion

- Similar profiles of AvBD1 and 2 expression between axenic and conventional birds: AvBD2 followed by AvBD1

- Regulatory effect of the microbiota on AvBD1 expression:
  Positive in the ileum
  Negative in the caecum

- Variations of AvBD1: non related to variations in granulocytes infiltration

Perspectives

- **Questions to be addressed:**

  - ✧ Commensal bacteria phylum involved in AvBDs regulation
  - ✧ Effect of AvBDs on more various bacterial isolates (field strains, MDR strains, commensal strains…), synergistic effect
  - ✧ Orientation of the defensins production in situ
  - ✧ Susceptibility to proteases
  - ✧ Chemotactic activity
  - ✧ Involvement of avian defensins in other mucosal barriers (lung…)

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