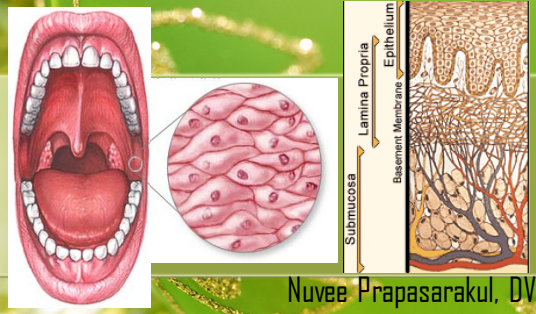
THE MUCOSAL SURFACE:

The front line of antibacterial defense



Nuvee Prapasarakul, DVM, Ph.D.

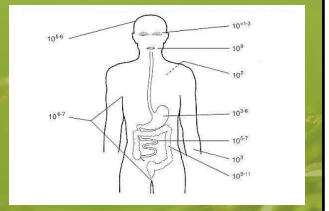
Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University.

OUTLINE

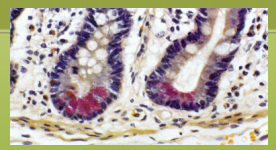
- Mucosal structure.
  - M-cell, Paneth cell, IELs, Goblet cell.
- Mucosal bacterial defense.
  - Physical barrier
  - Antibacterial chemicals (mucosae)
  - Antibacterial Biomolecules
- Mucosal Immunity.
- Commensal against Pathogen.

ANTIBACTERIAL BIOMOLECULES

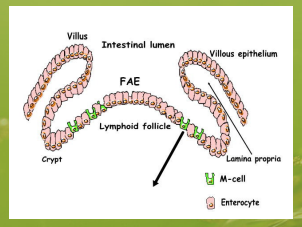
- Mucin
- Lysozyme
- Lactoferrin
- Lactoperoxidase
- Secretory phospholipase A2
- Secretory leucocyte protease inhibitor
- Trefoil peptides
- Acute phase protein
- Collectins
- Secretory IgA
- Antimicrobial peptides



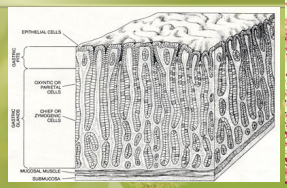
CELL POPULATION IN MUCOSAL EPITHELIUM

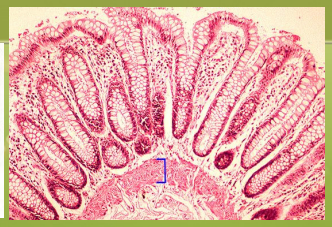


- M cells
- Paneth cells
- Intra-epithelium lymphocytes
- Goblets cells
- Enteroendocrine cells
- Tuft cells
- Cup cells

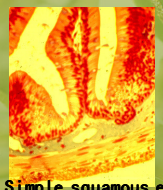


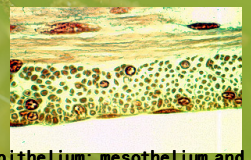
Mucosal structure





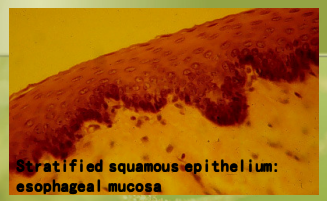
Cross section of mucosa in the stomach body shows arrangement of gastric cells.





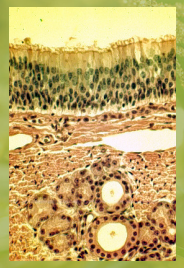
Simple squamous epithelium: mesothelium and endothelium from ileum

Mucosal Structure

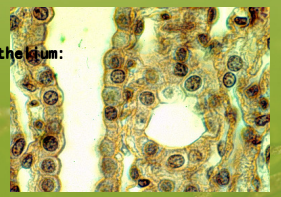


Stratified squamous epithelium: esophageal mucosa

Transitional epithelium: mucosa of urinary bladder



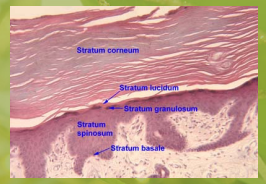
Simple cuboidal epithelium: renal tubules

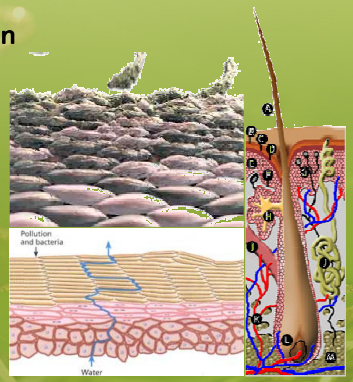


Pseudostratified ciliated columnar epithelium with goblet cell: respiratory mucosa

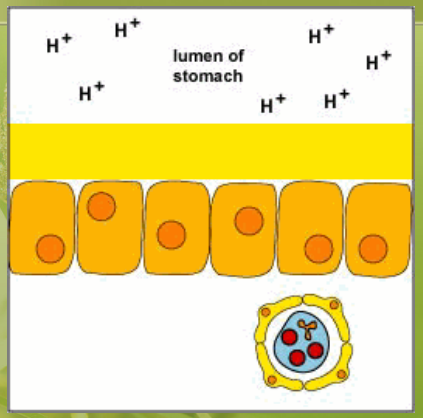
Mucosal Bacterial Defense.

- Physical barrier
  - Cell tight junction
  - Sweat, Sebum
  - Lysozyme
  - Skin shedding



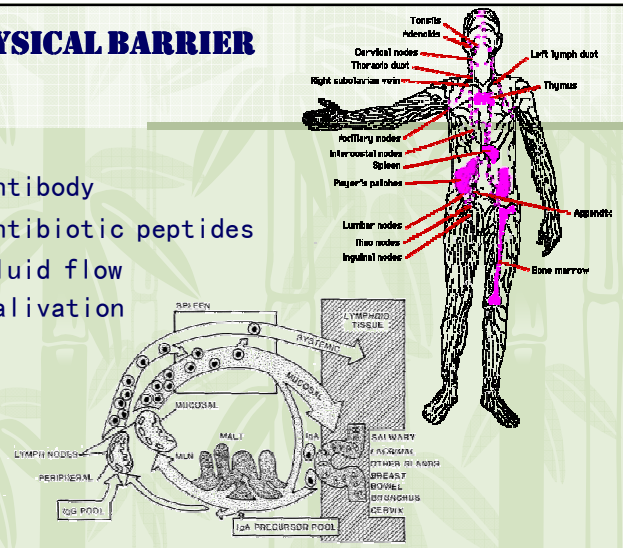


Helicobacter pylori-Induced Ulceration of the Stomach

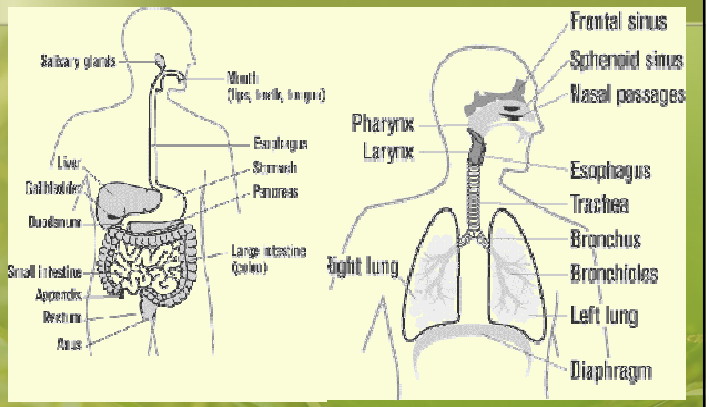


❖PHYSICAL BARRIER

❖Antibody
❖Antibiotic peptides
❖Fluid flow
❖Salivation

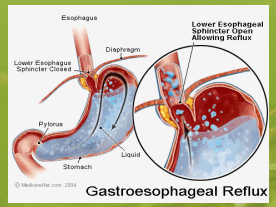


MUCOSAL BARRIER



MUCOSAL ANTIBACTERIAL CHEMICALS

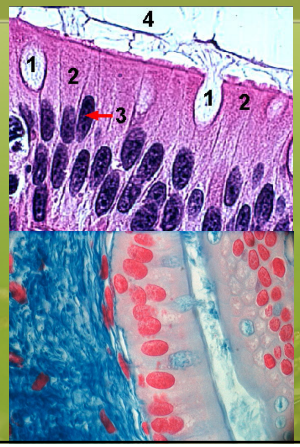
- Skin : Sweat → Lactic acid
- Vagina : Bacteria → Lactic acid
- Stomach : Acid substance
- Lower intestine : Alkaline



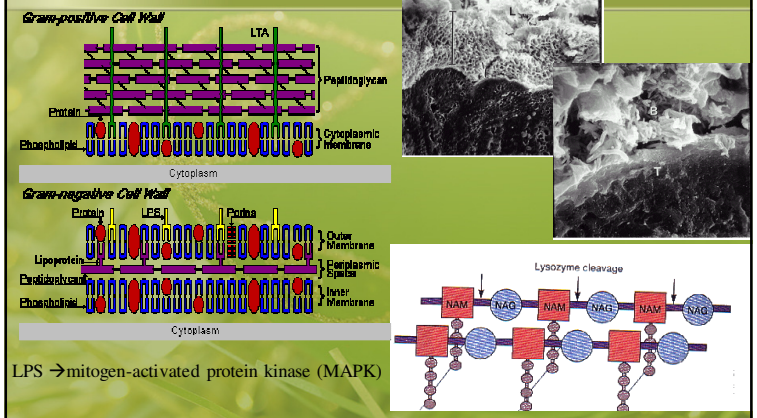
Gastroesophageal Reflux

ANTIBACTERIAL BIOMOLECULES

- Mucin
  - Keeping moist
  - Lubricant
  - Trapping bacteria
  - Antibacterial activity



Lysozyme



LPS →mitogen-activated protein kinase (MAPK)

Porcine Beta-Defensin 1: An Innate Immunity

INFECTION AND IMMUNITY, Apr. 2006, p. 2338–2352 Vol. 74, No. 4
0019-9567/06/$08.00+0 doi:10.1128/IAI.74.4.2338–2352.2006

The Host Defense Peptide Beta-Defensin 1 Confers Protection against *Bordetella pertussis* in Newborn Piglets

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Received 2 November 2005/Returned for modification 21 December 2005/Accepted 13 January 2006

Innate immunity plays an important role in protection against respiratory infections in humans and animals. Host defense peptides such as beta-defensins represent major components of innate immunity. We recently developed a novel porcine model of pertussis, an important respiratory disease of young children and infants worldwide. Here, we investigated the role of porcine beta-defensin 1 (pBD-1), a porcine defensin homologue of human beta-defensin 2, in conferring protection against respiratory infection with *Bordetella pertussis*. In this model, newborn piglets were fully susceptible to infection and developed severe bronchopneumonia. In contrast, piglets older than 4 weeks of age were protected against infection with *B. pertussis*. Protection was associated with the expression of pBD-1 in the upper respiratory tract. In fact, pBD-1 expression was developmentally regulated, and the absence of pBD-1 was thought to contribute to the increased susceptibility of newborn piglets to infection with *B. pertussis*. Bronchoalveolar lavage specimens collected from older animals as well as chemically synthesized pBD-1 displayed strong antimicrobial activity against *B. pertussis* in vitro. Furthermore, in vivo treatment of newborn piglets with only 500 μg pBD-1 at the time of challenge conferred protection against infection with *B. pertussis*. Interestingly, pBD-1 displayed no bactericidal activity in vitro against *Bordetella bronchiseptica*, a closely related natural pathogen of pigs. Our results demonstrate that host defense peptides play an important role in protection against pertussis and are essential in modulating innate immune responses against respiratory infections.

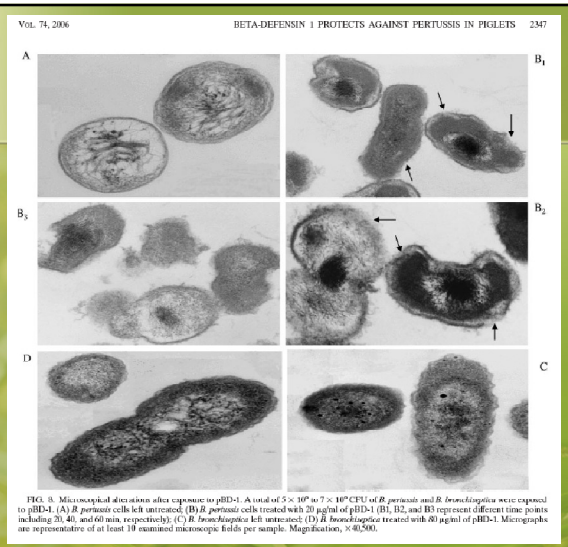
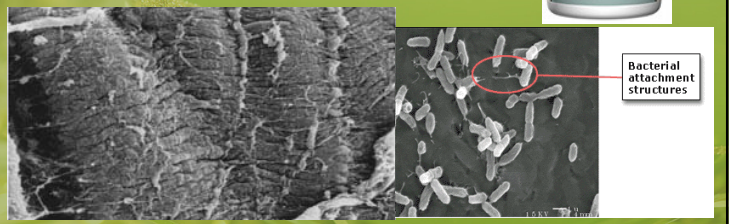


FIG. 8. Microscopical alterations after exposure to pBD-1. A total of 5×10^6 to 7×10^6 CFU of *B. pertussis* and *B. bronchiseptica* were exposed to pBD-1. (A) *B. pertussis* cells left untreated; (B) *B. pertussis* cells treated with 20 μg/ml of pBD-1 (B1, B2, and B3 represent different time points including 20, 40, and 60 min, respectively); (C) *B. bronchiseptica* left untreated; (D) *B. bronchiseptica* treated with 40 μg/ml of pBD-1. Micrographs are representative of at least 10 examined microscopic fields per sample. Magnification, ×40,500.

Lactoferrin

- Glycoprotein binding iron
- Epithelium and PMN produced
- Synergism with lysozyme
- Lactoferricin



HOW BACTERIA CAN USE Fe^{++}

$$3H_2O + Fe^{2+} \Leftrightarrow Fe^{3+} + 3OH^- + e^- + 3H^+ \quad (2)$$

INFECTION AND IMMUNITY, June 1993, p. 2698–2702 Vol. 61, No
0019-9567/93/062698-05$02.00/0

A Siderophore Production Mutant of *Bordetella bronchiseptica* Cannot Use Lactoferrin as an Iron Source

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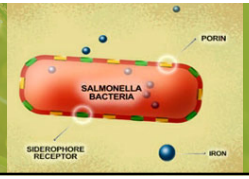
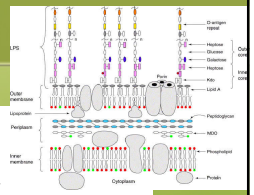
Received 16 February 1993/Accepted 5 April 1993

Bordetella bronchiseptica **secreted a hydroxamate siderophore when grown in Fe-depleted medium. A Tn*5lac* insertion mutant of *B. bronchiseptica*, DBB22, did not produce this hydroxamate siderophore and was incapable of using lactoferrin as an Fe source. Our data suggest that *B. bronchiseptica* uses a siderophore for removal of Fe from lactoferrin and transferrin rather than relying upon a receptor for these host Fe-binding proteins.**



LF. DECREASE LPS-INDUCED CYTOKINES RELEASE BY MONOCYTE.



INFECTION AND IMMUNITY, June 1994, p. 2628–2632
0019-9567/94/$04.00+0

Lactoferrin Is a Lipid A-Binding Protein

BEN J. APPELMELK,[1]* YUN-QING AN,[1]† MARLIEKE GEERTS,[2] BERT G. THIJS,[3] HERMAN A. DE BOER,[4] DAVID M. MACLAREN,[1] JOHANNES DE GRAAFF,[1] AND JAN H. NUIJENS[2]

Department of Medical Microbiology[1] and Medical Intensive Care Unit,[3] Vrije Universiteit, 1081 BT Amsterdam, and Gene Pharming Europe B.V.,[2] and Department of Biotechnology, Leiden Institute of Chemistry, Leiden University,[4] Leiden, The Netherlands

Received 6 December 1993/Returned for modification 24 January 1994/Accepted 29 March 1994

Lactoferrin (LF), a cationic 80-kDa protein present in polymorphonuclear leukocytes and in mucosal secretions, is known to have antibacterial effects on gram-negative bacteria, with a concomitant release of lipopolysaccharides (LPS, endotoxin). In addition, LF is known to decrease LPS-induced cytokine release by monocytes and LPS priming of polymorphonuclear leukocytes. Its mechanism of action is incompletely understood. We have now demonstrated by in vitro-binding studies that LF binds directly to isolated lipid A and intact LPS of clinically relevant serotypes of the species which most frequently cause bacteremia (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*), as well as to lipid A and LPS of mucosal pathogens (among others, *Neisseria meningitides* and *Haemophilus influenzae*). Binding to LPS was inhibitable by lipid A and polymyxin B but not by KDO (3-deoxy-D-*manno*-octulosonate), a glycoside residue present in the inner core of LPS. Binding of LF to lipid A was saturable, and an affinity constant of $2 \times 10^9\ M^{-1}$ was calculated for the LF-lipid A interaction. Our data may explain, in part, the mechanism whereby LF exerts its antibacterial and anti-endotoxic effects. Further studies on the ability of LF to block the detrimental effects of LPS, both in vitro and in vivo, are warranted.

+SECRETORY PHOSPHOLIPASE A2

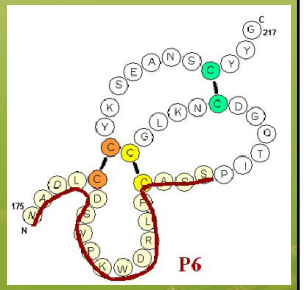
- •A low-molecular-mass secreted phospholipase
- •Hydrolyses the acyl of phospholipids
- •Bacterial cell membrane damage
- •Tear SPLA2 VS Gram +
- •Paneth cell produced VS Gram –

LACTOPEROXIDASE

- Ingredients: Glycerin, Purified Water, Propylene Glycol, Benzyl Alcohol, Sodium Benzoate, Zinc Gluconate, Sodium Laurel Sarcosinate, Aloe Vera, Glucose Oxidase, Lactoferrin, Lactoperoxidase, Lysozyme, Potassium Iodide.

TREFOIL PEPTIDE

- Epithelium Cell produced mucin associated.
- Intestine and lung
- Stimulate movement of epithelium.
- Mucosal re-epithelization

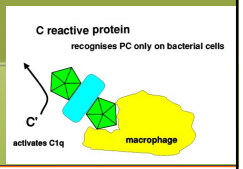


RESEARCH LITERATURES

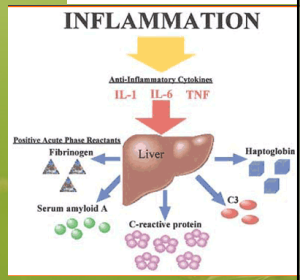
- Knock out TFF → cystic fibrosis
  - → Atrophic gastric mucosa
  - → Impair healing of intestinal mucosa
- TFF3 induced ciliogenesis → epithelium differentiation (Lung)
- TFF2 induced mucous secretion via IFN

ACUTE PHASE PROTEIN

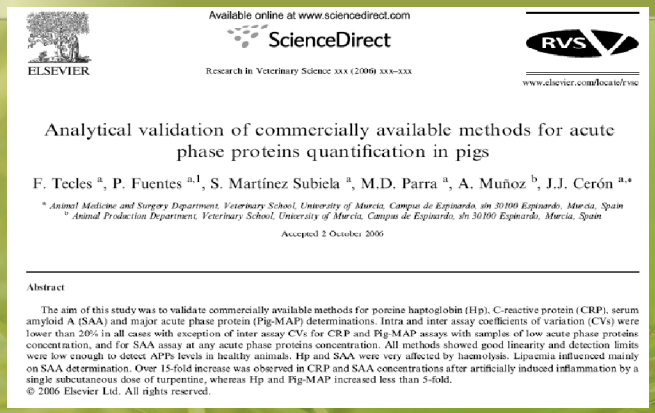
: Support opsonise bacteria



- Pentraxin
  - C Reactive Protein.
  - Serum amyloid
- C-type lectin
  - Mannose binding protein
  - Other Lectin



SAA, CRP >> HP, PIG-MAP



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Research in Veterinary Science xxx (2006) xxx–xxx

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Analytical validation of commercially available methods for acute phase proteins quantification in pigs

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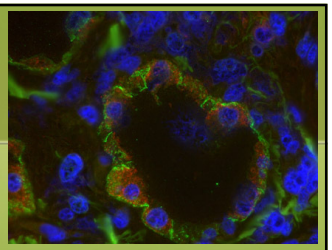
Accepted 2 October 2006

Abstract

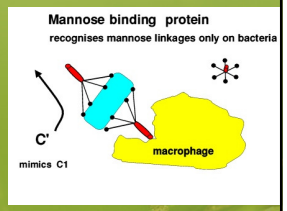
The aim of this study was to validate commercially available methods for porcine haptoglobin (Hp), C-reactive protein (CRP), serum amyloid A (SAA) and major acute phase protein (Pig-MAP) determinations. Intra and inter assay coefficients of variation (CVs) were lower than 20% in all cases with exception of inter assay CVs for CRP and Pig-MAP assays with samples of low acute phase proteins concentration, and for SAA assay at any acute phase proteins concentration. All methods showed good linearity and detection limits were low enough to detect APPs levels in healthy animals. Hp and SAA were very affected by haemolysis. Lipaemia influenced mainly on SAA determination. Over 15-fold increase was observed in CRP and SAA concentrations after artificially induced inflammation by a single subcutaneous dose of turpentine, whereas Hp and Pig-MAP increased less than 5-fold.

COLLECTIN



Lectin binding CHO

- Collagen analogue
- Surfactant protein A and D
- Action
  - Bacterial Agglutination
  - Induce Phagocytosis PMN
  - Induce Cytokine
  - Induce NO Production



MBL BINDING O ANTIGEN

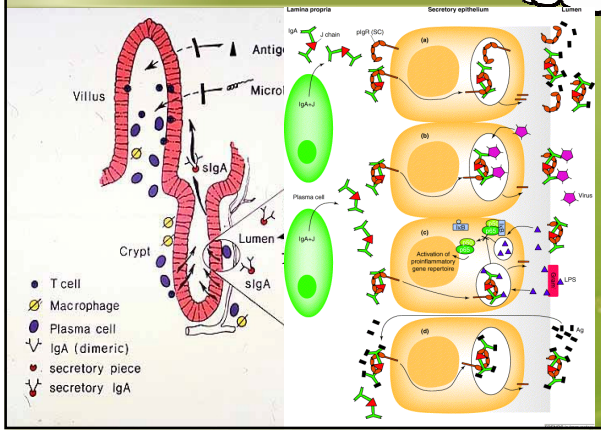
Mannan-binding lectin activates C3 and the alternative complement pathway without involvement of C2

Barbro Selander,[1] Ulla Mårtensson,[1] Andrej Weintraub,[2] Eva Holmström,[1] Misao Matsushita,[3] Steffen Thiel,[4] Jens C. Jensenius,[4] Lennart Truedsson,[1] and Anders G. Sjöholm[1]

[1]Institute of Laboratory Medicine, Section of Microbiology, Immunology, and Glycobiology, Lund University, Lund, Sweden.
[2]Department of Laboratory Medicine, Division of Clinical Bacteriology, Karolinska Institutet, Karolinska University Hospital, Huddinge, Sweden.
[3]Institute of Glycotechnology and Department of Applied Biochemistry, Tokai University, Hiratsuka, Japan.
[4]Department of Medical Microbiology and Immunology, University of Aarhus, Aarhus, Denmark.

Lectin pathway activation of C3 is known to involve target recognition by mannan-binding lectin (MBL) or ficolins and generation of classical pathway C3 convertase via cleavage of C4 and C2 by MBL-associated serine protease 2 (MASP-2). We investigated C3 activation in C2-deficient human sera and in sera with other defined defects of complement to assess other mechanisms through which MBL might recruit complement. The capacity of serum to support C3 deposition was examined by ELISA using microtiter plates coated with O antigen–specific oligosaccharides derived from *Salmonella typhimurium*, *S. thompson*, and *S. enteritidis* corresponding to serogroups B, C, and D (BO, CO, and DO). MBL bound to CO, but not to BO and DO, and efficiently supported C3 deposition in the absence of C2, C4, or MASP-2. The existence of an MBL-dependent C2 bypass mechanism for alternative pathway–mediated C3 activation was clearly demonstrated using CO, solid-phase mannan, and *E. coli* LPS. MASP-1 might contribute, but was not required for C3 deposition in the model used. Independent of MBL, specific antibodies to CO supported C3 deposition through classical and alternative pathways. MBL-dependent C2 bypass activation could be particularly important in various inherited and acquired complement deficiency states.

SECRETORY IGA



ANTIBACTERIAL PEPTIDES

- Possess in all organisms; polymyxin B, bactericin, tachyplesin and etc.
- 4 Groups Classification
  1. Peptide without cysteine; cecropins, temporin, magainins, dermaseptin.
  2-3. Peptide with cysteine; tachyplesin, a , b defensin
  4. Specific amino acid; proline, arginine, tryptophan such as apidaecin

EXAMPLE: CFTR, PR39

CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Nov. 1997, p. 774–777 Vol. 4, No. 6
1071-412X/97/$04.00+0

NOTES

Salmonella Infection Increases Porcine Antibacterial Peptide Concentrations in Serum†

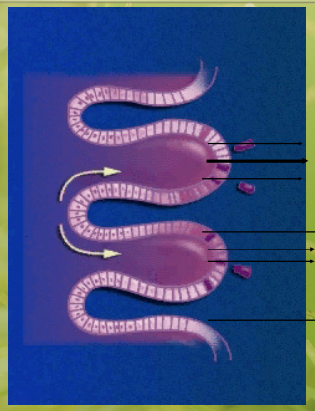
GUOLONG ZHANG,[1] CHRISTOPHER R. ROSS,[1] STEVEN S. DRITZ,[2] JEROME C. NIETFELD,[2] AND FRANK BLECHA[1]*

Departments of Anatomy and Physiology[1] and Diagnostic Medicine/Pathobiology[3] and the Food Animal Health and Management Center,[2] Kansas State University, Manhattan, Kansas 66506

Received 9 April 1997/Accepted 28 July 1997

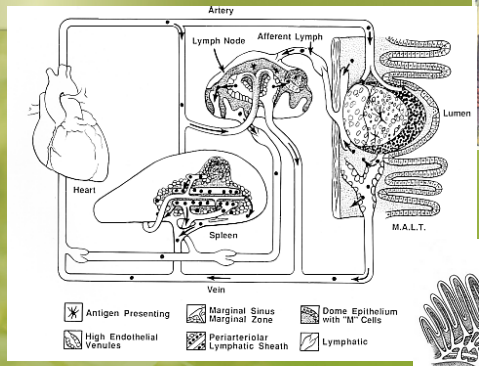
PR-39 is a multifunctional neutrophil peptide involved in host defense and inflammation. To investigate the involvement of PR-39 in a *Salmonella choleraesuis* infection, a PR-39 enzyme immunoassay was developed. The concentrations of PR-39 in serum were 13.6 ± 1.9 ng/ml before challenge and increased ($P < 0.01$) threefold by 10 to 14 days postinfection. Peripheral blood neutrophil counts paralleled the changes in the concentrations of PR-39 in serum, both returned to basal values by 4 weeks postinfection. These findings suggest that the concentrations of serum PR-39 reflect the involvement of this antibacterial peptide in the host's response to an *S. choleraesuis* infection.

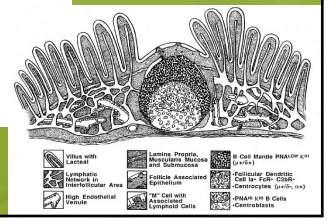
Summary of the secretion of antibacterial components



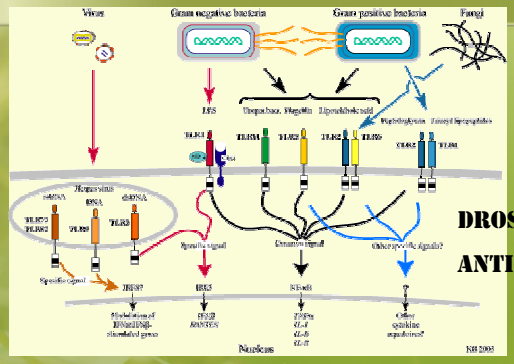
- Mucus
- Lysozyme
- Lactoferrin
- Lactoperoxidase
- SLPI
- APP
- Trefoil peptide
- Collectin
- Secretory PLA2
- Antibacterial Peptides
- Secretory IgA
- Antifungal peptides

Cinderella of Immunity





Schematic of TLR recognition



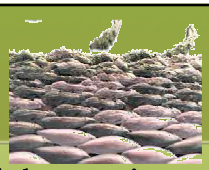
DROSOMYCIN

ANTIFUNGAL

DEFENSIN, CECROPIN, ATTACIN, DIPTERICIN, DROSOCIN

ANTIBACTERIAL

Mucosal epithelium as shedding surface



- Reduce adherent and removal bacteria
- Control commensal population
- External Factors eg.
  Cytolethal Distending Toxin (CDT)

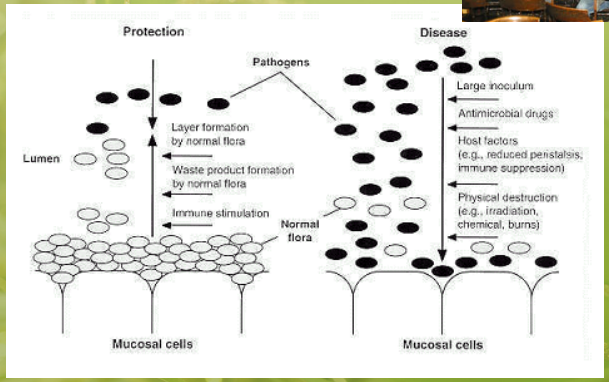
SYNERGY !!!!!!!!!!!!!!!!!!

Lysozyme + Lactoferrin

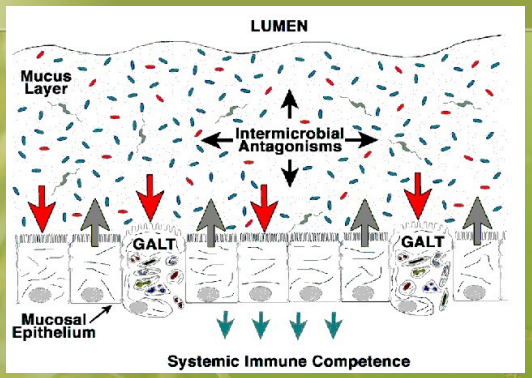
Lysozyme + Secretory Leucocyte Protease Inhibitor

SLPI + Lactoferrin

Normal Flora as Antimicrobial agent



Bacterial Interference

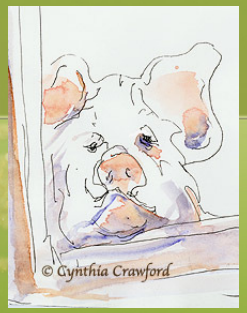


Bacteriocin: H_2S gas, Short chain volatile fatty acid.

Table 7 Available vaccines listed by year of first vaccine development or licensure in the United States, 1700–2002

Period	Year	Vaccine	Recommended route of administration	
			Mucosal	Systemic
1700	1798	Small pox	–	0
1800	1885	Rabies	–	+
	1896	Typhoid	–	+
	1896	Cholera	–	+
	1897	Plague	–	+
1900–59	1923	Diphtheria	–	+
	1926	Pertussis	–	+
	1927	Tetanus	–	+
	1927	Tuberculosis	–	+
	1945	Influenza	–	+
	1953	Yellow Fever	–	+
	1955	Poliomyelitis (IPV)	–	+
1960–2002	1960	Poliomyelitis (OPV)	+	–
	1963	Measles	–	+
	1969	Mumps	–	+
	1969	Rubella	–	+
	1970	Anthrax	–	+
	1975	Meningococcus (Aac)	–	+
	1977	*Streptococcus pneumoniae*	–	+
	1980	Adenovirus	+*	–
	1981	Hepatitis B	–	+
	1985	*Hemophilus influenzae* B	–	+
	1992	Japanese encephalitis	–	+
	1995	Hepatitis A	–	+
	1995	Varicella-zoster	–	+
	1998	Lyme disease	–	+
	1998	Rotavirus	+†	–
	1999	Typhoid	+*	–
	1999	Cholera	+*	
	2001	Influenza A	+‡	

* Not available for routine use; † Discontinued; ‡ Recently developed vaccines.



Odd things: animals. All dogs look up to you. All cats look down to you. Only the pig looks at you as an equal.
- Sir Winston Leonard Spencer Churchill
(1874–1965) British prime minister, WW2, orator, writer, Nobel Prize, literature 1953