Defence against Pathogens and Tolerance of Commensal Microbiota as well as of Dietary Antigens in the Early Stage: Challenging Steps to avoid Immune Deviances Later on.

Langhendries JP, MD, Pharmed. CHC – Site St Vincent - NICU, 4000 Rocourt-Liège Belgium
The Bacterial Colonisation and the Feeding in the Early Stage: Why it matters?
The Bacterial Colonisation and the Feeding in the Early Stage: Why it matters?

- It initiates the mucosal immune system rendering it able to favour the HOST DEFENCE but in the same time the DIETARY ANTIGEN TOLERANCE
The Bacterial Colonisation and the Feeding in the Early Stage: Why it matters?

- It initiates the mucosal immune system rendering it able to favour the HOST DEFENCE but in the same time the DIETARY ANTIGEN TOLERANCE.

- An adequate presentation of the dietary antigen to the mucosa in the early stage is likely to be a CRUCIAL STEP in optimising this tolerance to the DIETARY ANTIGEN.
The Bacterial Colonisation of the Neonatal Intestine is mandatory to get Diet Antigen Tolerance
The Fetus and its Immunological protection....
Maternal TH-1⁺, TH-2⁺
No fetal rejection

IL-4, 13

IL-10, TGF-β

Warner JO
Arch Dis Child
2004;89:97
Circulating allergens and maternal Ig-E to amniotic fluid

Fetal TH-2 biased allergen sensitisation

IL-4, 13

IL-10, TGF-β

Fetal swallowing
The Process of Birth triggers a dramatic Immune Induction at the Sterile Intestine Mucosal level...

....a challenging step initiated by the Invasive Microbiota....
Physiological Fetal Immune Imbalance to be corrected by invasive bacteria in the Early Stage

Th1, Th17 impulse and up-regulation of CD4+ iT_{reg} cells (Bystander Suppression) in a progressively increased TGF-β immune climate.
Crucial role of bacteria to induce **HOST DEFENCE** but also in the same time to get **DIET ANTIGEN TOLERANCE**……

……but, how can it be possible to get this opposite effect at the mucosal level ....

....how does it work ?

which **Actors** are involved in the process of the dietary antigen tolerance ?
From experimental studies but which tend to be recently confirmed in HUMANS .........

......... 3 Challenging Steps (a, b, c) to Get the Dietary Antigen Tolerance......
Diet and microbial antigens

- Dietary antigen = PP
- LPS antigen = LPS antigen

- TGF-β = defence
- TGF-β = tolerance

Mucosal Cell
Mucin rich Glycocalyx

MHC Class I

M cell

Diet and microbial antigens
Diet and microbial antigens

PP = dietary antigen
LPS = LPS antigen

TGF-β = defence

MHC Class I
TCR
CD8+ γδ

Tolerance via:

Dose-dependent induction of T-cell-mediated suppression

Mucosal Cell
Mucin rich Glycocalyx

Epithelial cell
MHC Class I
(or CD1)

Peptide

TCR
CD8 γδ + Tcell

Diet Antigen Tolerance via:

(a)
Diet and microbial antigens

CD8+ γδ

γδ γδ γδ γδ

MHC Class II

NO Costimulation

= dietary antigen

= LPS antigen

= tolerance

= defence

Mucosal Cell

Mucin rich Glycocalyx

Epithelial cell

MHC Class I (or CD1)

Peptide

TCR

CD8 γδ

Tcell

MHC Class I

Dose-dependent induction of T-cell-mediated suppression

T-cell deletion

T-cell anergy

Apoptosis

CD103+ CX3CR1+ or CD11b+ CX3CR1+

IDO

CD28

CTLA-4

B7-1/2

COX2's

Diet Antigen Tolerance via :

(a)

(b)

APC (myeloid or plasmacytoid)
Diet and microbial antigens

MHC Class I

MHC Class II

Costimulation

Diet Antigen Tolerance via:

(a) Epithelial cell

MHC Class I (or CD1)

Peptide

TCR

CD8 γδ

Tcell

Dose-dependent induction of T-cell-mediated suppression

(b) APC (myeloid or plasmacytoid)

MHC

Peptide

IDO

COX2's

PGE2's (+)

TGF-β

Bystander Suppression

CD4+ Naïve Tcell

CD4+CD25+

IT-reg's

CD4+CD103+

CD11b+

CD8 γδ

γδ

B7-1/2

CD28

CTLA-4

TGF-β

Foxp3

PGE2's (+)
Diet and microbial antigens

Mucosal Cell

Mucin rich Glycocalyx

MHC Class I

CD8+ γδ

APC

Diet Antigen Tolerance via:

- IDO
- COX2's PGE2's (+)
- CTLA-4
- CD103
- CX3CR1
- or
- CD11b
- CX3CR1

B7-1/2

CD28

Costimulation

CD4+ naïve T cell

Active immunity

TLR's 2,4,5,9

dectin-1

TGF-β
Diet and microbial antigens

Diet Antigen Tolerance via:

IMMUNITY

Th subsets

Control of DTH

CD8+ αβ

T-bet

Th1

IFN-γ
Il-2

Th2

GATA-3
Il-4
Il-5
Il-6

sIgA

B

CD4+

Naïve Tcell

Costimulation

IFN-γ
Il-12

PGE2’s (+)

CD103 CX CR1

or

CD11b CX CR1

IDO

COX2

B7-1/2

APC (myeloid or plasmacytoid)

CD1a

CD28

CTLA-4

CD163

TLR’s 2,4,5,9
dectin-1

TGF-β

MHC Class I

MHC Class II

Mucin rich Glycocalyx

Mucosal Cell

Dietary Antigen

LPS Antigen

= dietary antigen

= LPS antigen

= tolerance

= defence
Emigration of memory cell

Regional lymph nodes

Peripheral blood circulation

Mucosal effector sites

Diffuse lymphoid tissue

Mucosal lamina propria

and exocrine (BREAST....) glands

Endothelial cells

IgM

IgA

CD4

NK

Eosinophil

APC

Gut lumen

Intraepithelial lymphocytes

From P.BRANDTZAEG

JPL, NICU, Rocourt
Diet and microbial antigens

CD8+ γδ

APC (myeloid or plasmacytoid)

MHC Class I

Diet Antigen Tolerance via:

IMMUNITY

Th subsets

Mucin rich Glycocalyx

Mucosal Cell

Control of DTH

CD8+ αβ

T-bet

Th1

IFN-γ

II-2

Th2

GATA-3

II-4

II-5

II-6

CD8+

sIgA

Naïve T cell

CD4+

Naïve T cell

IFN-γ

II-12

B7-1/2

Costimulation

CTLA-4

CD28

APC (myeloid or plasmacytoid)

TLR’s 2,4,5,9
dectin-1

COX2’s

PGE2’s (+)

IDO

CD103+ CX3CR1 or CD11b+ CX3CR1

TGF-β

= dietary antigen

= LPS antigen

= defence

= tolerance

DTH
Diet and microbial antigens

PP

M cell

CD8+

γδ γδ γδ γδ

APC

(myeloid or plasmacytoid)

CD28

CD4+

Naïve T cell

MHC Class II

Th1

IFN-γ

IL-2

B

sIgA

Mucin rich Glycocalyx

Mucosal Cell

Th2

GATA-3

IFN-γ

IL-4

IL-5

IL-6

Th17

ROR-γt

IFN-γ

IL-17

IL-22

TGF-β

Th1

IFN-γ

IL-12

CD8+

αβ

control of DTH

Immunity

Th subsets

TLR's

2, 4, 5, 9
dectin-1

APC (myeloid or plasmacytoid)

B7-1/2

Costimulation

TGF-β

CD4+

Naïve T cell

PGE2's (+)

CTLA-4

CD103

CD11b

CD11b

CX3

CR1

- or

+ CD103

CX3

CR1

IDO

COX2

PGE2's (+)

CD103


Diet Antigen Tolerance via:

= dietary antigen

= LPS antigen

= tolerance

= defence
Diet and microbial antigens

PP M cell

CD8+ γδ γδ γδ γδ

APC (myeloid or plasmacytoid)

B7-1/2

CD4+

Naïve T cell

IFN-γ

IL-2

IL-4

IL-5

IL-6

Th1

T-bet

GATA-3

ROR-γ

IL-17

IL-22

Th2

GATA-3

II-4

II-5

II-6

Th17

TGF-β

II-6

II-21

Diet Antigen Tolerance via:

IMMUNITY

Th subsets

Control of DTH

CD8+ αβ

T-bet

IFN-γ

II-2

II-12

IFN-γ

PGE2’s (+)

B7-1/2

Costimulation

CD4+

Naïve T cell

CD8+

B

sIgA

Mucin rich Glycocalyx

Mucosal Cell

MHC Class I

MHC Class II

TLR’s 2, 4, 5, 9, dectin-1

sTLR’s (2, 4, 5, 9) + sCD14’s IFN-γ PGE2’s

IDO

COX2

PGE2’s (+)

Wnts ↔ β-catenin/E-catherin

sCD14’s

IFN-γ

PGE2’s

(-)

Retinoic acid

(+)

IDO

COX2

PGE2’s (+)

MHC Class II

CTLA-4

CD103

CD11b

+ CX3 CR1

- or + CX3 CR1

Diet Antigen Tolerance via:

= dietary antigen

= LPS antigen

= tolerance

= defence

TGF-β

Wnts ↔ β-catenin/E-catherin

CTLA-4

CD103

CD11b

+ CX3 CR1

- or + CX3 CR1

Retinoic acid

(+)

IDO

COX2

PGE2’s (+)

MHC Class II

CTLA-4

CD103

CD11b

+ CX3 CR1

- or + CX3 CR1

Retinoic acid

(+)

IDO

COX2

PGE2’s (+)

MHC Class II

CTLA-4

CD103

CD11b

+ CX3 CR1

- or + CX3 CR1

Retinoic acid

(+)

IDO

COX2

PGE2’s (+)

MHC Class II

CTLA-4

CD103

CD11b

+ CX3 CR1

- or + CX3 CR1

Retinoic acid

(+)

IDO

COX2

PGE2’s (+)

MHC Class II

CTLA-4

CD103

CD11b

+ CX3 CR1

- or + CX3 CR1

Retinoic acid

(+)

IDO

COX2

PGE2’s (+)
Diet and microbial antigens

Mcell

CD8+ γδ γδ γδ γδ

APC (myeloid or plasmacytoid)

B7-1/2

CD28

CD4+

Naïve T cell

Th1

Th2

Th17

Diet Antigen Tolerance via:

TLR’s 2,4,5,9 + sCD14’s IFN-γ PGE2’s

TGF-β

CTLA-4

CD103 or CD11b + CX3 CR1

Wnts ↔ β-catenin/E-catherin

MHC Class I

MHC Class II

Mucin rich Glycocalyx

Mucosal Cell

Diet Antigen Tolerance via:

= dietary antigen

= LPS antigen

= defence

= tolerance

Control of DTH

IFN-γ
Il-2
Il-12

COX2

PGE2’s (+)

TGF-β

Retinoic acid (+)

CD4+ Naïve T cell

sIgA

Mucosal Cell

B

GATA-3

Il-4
Il-5
Il-6

ROR-γt

Il-17
Il-22

IMMUNITY

Th subsets

T-bet

TGF-β
Immune Equilibrium: to be got at the sub-Mucosa Level

Immunity

Th subsets: 1, 2, 17

1. Inflammatory process
2. Oral antigen intolerance and allergic diseases
3. Auto-immune diseases
4. Cancer

Tolerance

1. LPS antigen
2. Diet antigen
3. Retinoic acid

TLR’s (2,4,5,9); sCD14’s; PGE2’s; INF-γ

When in excess:
- Infectious tolerance and chronic infections
- Chronic inflammatory tolerance
- Cancer

CD103*CD11b* (myeloïd or plasmacytoid)

B7-1/2

CD103*CXCR3-CR1*

CD11b*CXCR3-CR1*

DO

CD4+CD25+

CD8

TGF-β

IL-2

sCD14’s

TGF-β

Il-4

TGF-β + Il-6

IL-12, INF-γ

IL-10

IL-21

IL-22

IL-23

PGE2’s

Naïve Th cell

CD28

CTLA-4

Dectin-1

COX2’s

TLR’s 2,4,5,9

sCD14’s

PGE2’s

TGF-β

IL-12, INF-γ

IL-10

IL-21

IL-22

IL-23

sCD14’s

PGE2’s

TGF-β

IL-10

IL-27

TGF-β

IL-2

sTLR’s

sCD14’s

PGE2’s

TGF-β

IL-10

IL-27

TGF-β

IL-2

sTLR’s

sCD14’s

PGE2’s

TGF-β

IL-10

IL-27
Cyclooxygenase-2-dependent arachidonic acid metabolites are essential modulators of the intestinal immune response to dietary antigen

Rodney D. Newberry¹,², William F. Stenson¹,² & Robin G. Lorenz¹,³

Department of Internal Medicine¹, Division of Gastroenterology², Center for Immunology, Department of Pathology³, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110, USA

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Intestinal inflammatory diseases are mediated by dysregulated immune responses to undefined luminal antigens. Feeding hen egg-white lysozyme to mice expressing a transgenic T-cell receptor that recognizes hen egg-white lysozyme peptide 46–61 resulted in no intestinal pathology; however, simultaneous administration of cyclooxygenase-2 Inhibitors and dietary hen egg-white lysozyme resulted in increased proliferation of lamina propria mononuclear cells and crypt epithelial cells, crypt expansion and villus blunting. Lamina propria mononuclear cells produce high levels of cyclooxygenase-2-dependent arachidonic acid metabolites, which act as immunomodulators in the immune response to dietary antigen. These findings establish that cyclooxygenase-2-dependent arachidonic acid metabolites are essential in the development and maintenance of intestinal immune homeostasis.
FOXP3\(^+\)CD4\(^+\)CD25\(^+\) Adaptive Regulatory T Cells Express Cyclooxygenase-2 and Suppress Effector T Cells by a Prostaglandin E\(_2\)-Dependent Mechanism\(^1\)

Milada Mahic,* Sheraz Yaqub,* C. Christian Johansson,\(^2\)* Kjetil Taskén,\(^3\)* and Einar M. Aandalh*†

CD4\(^+\)CD25\(^+\) regulatory T (T\(_R\)) cells suppress effector T cells by partly unknown mechanisms. In this study, we describe a population of human suppressive CD4\(^+\)CD25\(^+\) adaptive T\(_R\) (T\(_R\)\(^{adap}t\)) cells induced in vitro that express cyclooxygenase 2 (COX-2) and the transcription factor FOXP3. T\(_R\)\(^{adap}t\) cells produce PGE\(_2\) and suppress effector T cell responses in a manner that is reversed by COX inhibitors and PGE\(_2\) receptor-specific antagonists. In resting CD4\(^+\)CD25\(^-\) T cells, treatment with PGE\(_2\) induced FOXP3 expression. Thus, autocrine and paracrine effects of PGE\(_2\), produced by COX-2-positive T\(_R\)\(^{adap}t\) cells may be responsible for both the FOXP3\(^+\) phenotype and the mechanism used by these cells to suppress effector T cells. *The Journal of Immunology, 2006, 177: 246–254.*
Cyclooxygenase-2 in mucosal DC mediates induction of regulatory T cells in the intestine through suppression of IL-4

F Broere¹,²,³, MF du Pré²,⁷, LA van Berkel², J Garssen³,⁴, CB Schmidt-Weber⁵, BN Lambrecht⁶, RW Hendriks⁶, EES Nieuwenhuis², G Kraal¹ and JN Samsom²

Oral intake of protein leads to tolerance through the induction of regulatory T cells (Tr cells) in mesenteric lymph nodes (MLNs). Here we show that the inhibition of cyclooxygenase-2 (COX-2) in vivo suppressed oral tolerance and was associated with enhanced differentiation of interleukin (IL)-4-producing T cells and reduced Foxp3⁺ Tr-cell differentiation in MLN. As a result, the functional suppressive capacity of these differentiated mucosal T cells was lost, IL-4 was causally related to loss of tolerance as treatment of mice with anti-IL-4 antibodies during COX-2 inhibition restored tolerance. Dendritic cells (DCs) in the MLN differentially expressed COX-2 and reductionist experiments revealed that selective inhibition of the enzyme in these cells inhibited Foxp3⁺ Tr-cell differentiation in vitro. Importantly, the inhibition of COX-2 in MLN-DC caused increased GATA-3 expression and enhanced IL-4 release by T cells, which was directly related to impaired Tr-cell differentiation. These data provide crucial insights into the mechanisms driving de novo Tr-cell induction and tolerance in the intestine.

Chen W
Nature Immunol
2011;12:809
Immunological Immaturity in Early Life

- Limitations of the Innate immune response
  - Immature APC function ($CD103^{+}CX_{3}CR1^{-}$ or $CD11b^{+}CX_{3}CR1^{+}$)
  - Adenosine antagonises TLR-mediated cytokines production

- Limitations of early life antibody responses
  - Limited responses to protein antigens, to PS and LPS antigens
  - Influence of maternal antibodies

- Limitations of T cell responses
  - Reduced expression of MHC class II
  - Defective IFN-gamma secretion and low Th-17 activation
  - $CD4^{+}/CD25^{+}$ T reg’s fully functional and abundant

- Limitations in mucosal immunity
  - Deficiency in BPI (bactericidal/permeability-increasing protein)
  - Limited sIgA’s synthesis in the first months (IgM’s)
IEL
Epithelial cell
Professional MHC class I/II Positive APC

Gut

MHC I/II +

CD8 +

>>

CD4 +

many

suppression

Airway

MHC I/II +

CD4 +

few

CD8 +

<<

CD4 +

help

From P.BRANDTZAEG, 1996
Our modern perinatal ways of care badly interfere with the bacterial colonisation at birth..... and could favour immune deviances....
Percentage of *Bifidobacterium*-like bacteria (BLB) colonization in infants

Dietary Antigen load

Factors interfering with microbial exposure in the early stage (mode of delivery, Low Breast-feeding rate, AB’s,....)

Suboptimal Bystander Suppression Persistent Immune Imbalance

Incidence of allergic and auto-immune diseases

Genetics

Modes of nutrition

Air Pollution

Pharmacological Factors (?) (anti-COX’s-2)

Dietary Antigen load

Langhendries JP
adapted from E.Isolauri

Birth Physiological Immune Imbalance

Th1

Th2

JPL, NICU, Rocourt
Exclusive Breast-Feeding: Optimising Bacterial/Mucosal Interface

**Bifidogenic Factors**

- Glycoproteins \{κ Casein (N-Acetyl-Glucosamine)\}
- Mono-oligosaccharides, GOS, ....
- Low protein level
- High lactose concentration
- Low phosphate concentration

**Immunomodulating Components**

- Virtually all known immune components/nutriments found in HM are relevant for specific protective action on the epithelial cell
- Of outstanding interest: sCD14, IL-10, TGF-β, (S)IgA.

**Biologically active Components**

- Whey proteins (Lactoferrin, Lysozyme, Defensins, EGF, PAF-AH.
- Osteoprotegerin, adiponectin,
- PUFA’s
Jones et al

Breast milk sCD14 (ng/ml)

- **Eczema (n = 8)**
- **No Eczema (n = 21)**

\[ p = 0.003 \]
THE BEST WAY TO PROGRESS WITH THE INFANT FEEDING IN ORDER TO FAVOUR THE DIETARY ANTIGEN TOLERANCE: NEW DATA FROM EXPERIMENTAL STUDIES
• Exclusive Breast-feeding is the best way by which the Antigen Epitopes are presented to the infant intestinal mucosa (Verhasselt et al, *Nature Immunol* 2008;14:170)

• Whatever the postnatal age (preferably not before 4 months), the dietary diversification should progress ahead according to the 4 points rule: 1) antigen in very slow amount; 2) daily repeated; 3) increased; 4) very progressively (Friedman A. *Ann NY Acad* 1996;778:103; Williamson et al *J Immunol* 2002;169:3606; Mahic et al *Eur J Immunol* 2008;38:6406;
Breast milk–mediated transfer of an antigen induces tolerance and protection from allergic asthma

Valérie Verhasselt¹, Valérie Milcent¹, Julie Cazareth², Akira Kanda³, Sébastien Fleury³, David Dombrowicz³, Nicolas Glaichenhaus¹ & Valérie Julia¹ Nature Immunol 2008; 14: 170.
Breast milk–mediated transfer of an antigen induces tolerance and protection from allergic asthma.

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Breast milk-mediated transfer of an antigen to the neonate results in oral tolerance induction leading to antigen-specific protection from allergic airway disease.
Breast milk–mediated transfer of an antigen induces tolerance and protection from allergic asthma.

Breast milk-mediated transfer of an antigen to the neonate results in oral tolerance induction leading to antigen-specific protection from allergic airway disease. The presence of TGF-β in breast milk together with the antigen was needed and mandatory to get this tolerance.
Hypothesis emerging from Verhasselt’s studies: to be confirmed in humans
This study may pave the way for the design of new strategies to prevent the development of allergic diseases...... such as deliberate exposure of mothers to allergens during breastfeeding .... {to try enhancing their tolerance to the progeny}.

THE BEST WAY TO PROGRESS WITH THE INFANT FEEDING IN ORDER TO FAVOUR THE DIETARY ANTIGEN TOLERANCE: NEW DATA FROM EXPERIMENTAL STUDIES

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**Child Protection of upper respiratory tract mucosa and gastrointestinal mucosa, oral dietary antigen tolerance**

- *sIGA, sIgM*
- *Natural defense factors (lactoferrin,..)*
- *Controlled Bacterial translocation*
- *Maternal Dietary Atg Epitopes*
- *Immuno regulatory factors (TGF-β,..)*

- Microbes
- Food antigens
- Breast milk
- Peripheral blood
- GALT

*adapted from P.BRANDTZAEG, 1996*
Recommendations are needed to ameliorate Infant Bacterial Colonisation of the Intestine as well as the Mode of Presentation of the Dietary Antigen to the Intestine Mucosa = PUBLIC HEALTH IMPACT IN ALLERGY PREVENTION

• Prefer vaginal delivery when possible

• Exclusive breast feeding as long as possible
  ⇒ optimal immune response after optimal mucosal microbial stimulation
  ⇒ allows low early diet antigen stimulation on immature mucosa

• Progressive introduction of complementary foods
  = not before four or six months = according to the 4 points rule

• Rationale use of antibiotics and anti-COX’s:
  ⇒ restriction in the early stage when possible
  ⇒ avoid excessive use of broad spectrum AB (esp. in prophylaxis)
Factors interfering with microbial exposure in the early stage (*mode of delivery, mode of feeding, AB’s,....*)

Suboptimal Tregs Suppression Function in the Early Stage
<p>| Factors interfering with microbial exposure in the early stage (<em>mode of delivery, mode of feeding, AB’s,....</em>) | Dietary Factors (low Breast-feeding habits, inadequate diversification,...) in the Early Stage |
| Suboptimal Tregs Suppression Function in the Early Stage |</p>
<table>
<thead>
<tr>
<th>Factors interfering with microbial exposure in the early stage (mode of delivery, mode of feeding, AB’s,....)</th>
<th>Dietary Factors (low Breast-feeding habits, inadequate diversification,....) in the Early Stage</th>
</tr>
</thead>
</table>
| Suboptimal Tregs Suppression Function in the Early Stage | - Genetics  
- Air pollution  
- Allergic load  
- AB’s overuse and modification of microbial pressure on the submucosa area  
- Pharmacological factors (anti-COX ’s overuse) (??) |
Factors interfering with microbial exposure in the early stage *(mode of delivery, mode of feeding, AB’s,...)*

Suboptimal Tregs Suppression Function in the Early Stage

Dietary Factors (low Breast-feeding habits, inadequate diversification,...) in the Early Stage

- Genetics
- Air pollution
- Allergic load
- AB’s overuse and modification of microbial pressure on the submucosa area
- Pharmacological factors (anti-COX ’s overuse) (??)

EPIGENETIC MODIFICATIONS (GENE EXPRESSION) in the EARLY STAGE
EPIGENETIC MODIFICATIONS (GENE EXPRESSION) INCREASE INCIDENCE OF IMMUNE DEVIANCES LATER ON
Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man

Burkhard Hinz, *1 Olga Cheremina, † and Kay Brune †

*Institute of Toxicology and Pharmacology, University of Rostock, Rostock, Germany; and †Institute of Experimental and Clinical Pharmacology and Toxicology, Friedrich Alexander University Erlangen-Nürnberg, Erlangen, Germany
Epigenetics can be defined as mitotically and meiotically heritable changes in gene expression that do not involve a change in the DNA sequence.
The control of 3 epigenetic stable processes (environmental factors):

- RNA
- miRNA’s
- Histone Modification
- DNA Methylation
- HERITABLE

- / +
- / +
+ / -
Epigenetic Gene Expression stable process maybe dysregulated

Repeated Environmental Factors

RNA

miRNA’s

HERITABLE Silencing

Histone Modification

DNA Methylation

Epigenetic Gene Expression stable process maybe dysregulated
Early life environment

“Stress” response signaling

Epigenetic changes

Inter-individual epigenetic

Gene expression programming variation

Phenotypic variation

Health disease and behavioral pathologies

EPIGENETIC MODIFICATIONS could be issued from an inadequate bacterial interface and/or diversity at the intestinal sub-mucosal level in the early stage ..... leading to IMMUNE DEVIANCES later on.
**Immunology Diagram**

**Tolerogenic Pathway**
- **Tregs** and iTregs subsets
- CD4+CD25+
- IL-10
- TGF-β

**Costimulation or not**
- Th1
  - IFN-γ
  - IL-2
  - IL-12
- Th2
  - IL-4
  - GATA-3
- Th17
  - ROR-γ
  - IL-17
  - IL-22
- Th3
  - IL-10
  - TGF-β

**Defensive Pathway**
- **APC** (myeloid or plasmacytoid)
- B7-1/2
- CD11b
- CX3CR1

**Hypersensitive Site to Epigenetics Modifications**
- CD103
- TLR’s 2,4,5,9
- Dectin-1

**Tolerance Pathway**
- nT-regs and iT-regs subsets
- CD4+
- IL-10

**Immunity Pathway**
- Naïve T cell
- TGF-β
- TGF-β
- IL-6
- IL-21
- ROR-γ
- Th17
- IL-17
- IL-22

**Diet Antigen**
- = diet antigen

**LPS Antigen**
- = LPS antigen

**TLR’s**
- 2, 4, 5, 9
- Dectin-1
Hypersensitive sites to Epigenetic modifications in the Interferon gamma gene

Lee et al. *Immunology* 2006;24:369.
Naïve T-cell IFNγ gene demethylation and allergic disease

Naïve T-cell IFNγ gene demethylation and allergic disease

High IFNγ gene methylation in the T helper cell

Low IFNγ gene methylation in the T helper cell

Attenuated IFNγ response capacity

Increased IFNγ response capacity

1st year of life

Birth

Risk of allergic disease

High

Low

Microbial exposure

Naïve T cell in the peripheral circulation

CD103^+ CX^3 CR1^− or CD11b^+ CX^3 CR1^+

APC (myeloid or plasmacytoid)

MHC

CD4^+ Naïve Tcell

B7-1/2

IL-17 IL-22

Il-17 Il-21

CD4^+CD25^+ T-reg's

ROR-γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ γ gamma
Competitive antagonism between FOXP3 and ROR family members

The FOXp3 locus subjected to epigenetic control

Huehn et al
Nat Rev Immunol
2008.
Competitive antagonism between FOXP3 and ROR family members

Weaver CT, Hatton RD
Th1 - Th17 - Th2 balance according to the age

**Immune Immaturity**

- Th1 > Th17, Th2 immaturity
- Early microbial stimulation, (microbial paucity, but high pro-inflammatory immune induction)
- Very prog. diet antigen stimul.

**Tolerance**

- Increased risk of NEC

**SENSITIZATION**

- in utero Th2 environment
- Perinatal early stage AB’s
- Delayed microbial stimulation
- No breastfeeding
- Inadequate dietary diversification
- Genetic factors

**Atopic Disease**

- Early microbial stimulation
- No breastfeeding
- Inadequate dietary diversification
- Genetic factors

**Immune Maturity**

- Tregs equilibrium

**Entero-bacteriaceae**

- Th1, Th17-Th2 balance

**LAB**

- Th1 Th17
- Th2

**Birth**

- Early post-natal age

**Later Post-Natal Age**

- Late post-natal age
- Decreased microbial stimulation
- No breastfeeding
- Inadequate dietary diversification

**Langhendries JP, 2009**
The oral administration of bacterial extracts prevents asthma via the recruitment of regulatory T cells to the airways

The prevalence of asthma has steadily increased during the last decade, probably as the result of changes in the environment, including reduced microbial exposure during infancy. Accordingly, experimental studies have shown that deliberate infections with live pathogens prevent the development of allergic airway diseases in mice. Bacterial extracts are currently used in children suffering from repeated upper respiratory tract infections. In the present study, we have investigated whether bacterial extracts, commercially available as Broncho-Vaxom (BV), could prevent allergic airway disease in mice. Oral treatment with BV suppressed airway inflammation through interleukin-10 (IL-10)-dependent and MyD88 (myeloid differentiation primary response gene 88)-dependent mechanisms and induced the conversion of FoxP3 (forkhead box P3)- T cells into FoxP3+ regulatory T cells. Furthermore, CD4+ T cells purified from the trachea of BV-treated mice conferred protection against airway inflammation when adoptively transferred into sensitized mice. Therefore, treatment with BV could possibly be a safe and efficient strategy to prevent the development of allergic diseases in children.

Navarro et al *Mucosal Immunol* 2011;4:63