Wound healing to longevity: Microbe-induced immune proficiency in human health

Human Microbiome Science
Vision for the Future
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Can microbe-based restructuring of immune networks remedy our public health inflammatory disease crisis?
Healthful longevity

Th-1  Th-2  Th-17  T\textsubscript{REG}
Gut microbiota community restructuring in human subjects

Christina Clarke-Dur
CPIC & Stanford University

Eric J Alm
Massachusetts Institute of Technology & Broad Institute

David A Hafler
Yale University & Broad Institute


California Teacher Study
60,000+ women
Does maternal and infant microbial ecology offer opportunity to impart good health to future generations?
Why use animal models?
NIEHS  P30 ES002109.  [P30 PIs: Leona Samson → John Essigmann]
Perinatal microbe exposures.  Pilot project co-PIs: SE Erdman & EJ Alm
2011 – 2013
Grandma’s microbial ecology may put grandchild at risk for cancer?

Preliminary cancer outcomes in ‘grandchildren’ mice:
- 1/3 liver cancer (hepatocellular carcinoma)
- 2/3 lymphoma (high grade)
- 3/3 lung cancer (bronchoalveolar adenocarcinoma)

Bronchoalveolar adenocarcinoma in 3/3 ‘grandchild’ outbred Swiss mice (age = 5 months)
Gut microbe-triggered systemic events

(modified from Coombs, et al 2005; Fiona Powrie lab)
Gut microbe-triggered systemic events

Dendritic Cells

Migration and maturation

CD40-CD40L

Macrophages

Inflammatory cytokines

Neutrophil recruitment

(modified from Coombs, et al 2005; Fiona Powrie lab)
Adoptive Cell Transfer Paradigm

Transplantable anti-inflammatory CD4+CD45RBloCD25+ lymphocytes

adoptive cell transfer

Cells extracted from immune-competent donor mouse

Wild type mouse donor

Cells injected into recipient without its own lymphocytes

Rag2-KO mouse recipient
Cells extracted from immune-competent donor mouse

Wild type mouse donor

Sham

pro-inflammatory CD4+CD45RB^{hi}CD25-

Co-transfer anti-inflammatory CD4+CD45RB^{lo}CD25+

Cells injected into Rag2-KO recipient without its own lymphocytes
Cells extracted from immune-competent donor mouse

Wild type mouse donor

Sham

Transfer anti-inflammatory CD4+CD45RBloCD25+

Helicobacter hepaticus

Microbe infection into Rag2-KO recipient without its own lymphocytes

We thank Bruce H Horwitz & James G Fox
H. hepaticus-infected cecum colon

Uninfected

H. hepaticus-infected

3-4 wks

Cells of adaptive immunity suppress IBD and carcinoma

Innate immunity is sufficient for IBD and carcinoma

Rag2-KO (Erdman et al 2003)
Gut microbe-triggered systemic events

- Neutrophil recruitment
- Macrophages
- Inflammatory cytokines
- Neutrophil recruitment

Migration and maturation

CD40-CD40L

(modified from Coombs, et al 2005; Fiona Powrie lab)
Neutrophil recruitment

Macrophages

Inflammatory cytokines

Migration and maturation

Dendritic Cells

IL10

TGFβ

IL10

Macrophages

Inflammatory cytokines

Neutrophil recruitment

CD40-CD40L

TR

IL10

TGFβ

IL10

TR

TGFβ

IL10

TR

Gut microbe-triggered systemic events

(modified from Coombs, et al 2005; Fiona Powrie lab)
Invasive colonic carcinoma in *H. hepaticus*-infected Rag2-/- mice

Colonic carcinoma in \textit{H. hepaticus}-infected Rag2-/- mice


Mucinous carcinoma

Poorly-differentiated carcinoma

\textit{H. hepaticus}-infected Rag2-/-

uninfected Rag2-/-

Rag2-KO

Start 3 6 9 12 15 18 21 24

24 wks
Rag2-KO

Start 3 wks 6 9 12 15 18 21 24 wks 24 wks
**H. hepaticus** infection up-regulates pro-inflammatory cytokines

* = p<0.05  
** = p<0.01  
*** = p<0.001

- **IL-6**
- **TNF-α**
- **IL-17**

Fold change in expression
Reciprocal relationships exist between Interleukin (IL)-10 and IL-17

IL-17 *in situ* using IHC in mesenteric lymph node of mice.

[Hh+ 129 Rag2<sup>−/−</sup>] [Hh+ 129 WT] [Hh+ 129 IL10<sup>−/−</sup>]

We thank Theofilos Poutahidis

Erdman et al 2010
Blocking inflammation leads to total remission of established invasive colonic carcinoma

mucinous CRC with peritoneal neoplastic invasion

- anti-TNFα
- or
- IL-10
- or
- T_{REG}

restoration of normal colonic epithelia after anti-inflammatory treatment
Interleukin-10

\(T_{REG}\)

Pro-inflammatory cells & cytokines → Tumor growth
Inter-related roles for IL-10 and TGF-β in colon cancer

NIH  R01 CA108854

[2005 – 2015]

PI = SE Erdman
Prior microbe exposures convey health benefits transplantable via purified immune cells

*H. hepaticus* dosed orally by gastric gavage

adoptive cell transfer

Transplantable anti-inflammatory CD4+CD45RBloCD25+ lymphocytes

*H. hepaticus*-fed donor

Cells injected into recipient without its own lymphocytes

Rag2-KO mouse recipient
A Dominant Mutation That Predisposes to Multiple Intestinal Neoplasia in the Mouse

Amy Rapaich Moser,* Henry C. Pitot, William F. Dove

In a pedigree derived from a mouse treated with the mutagen ethylnitrosourea, a mutation has been identified that predisposes to spontaneous intestinal cancer. The mutant gene was found to be dominantly expressed and fully penetrant. Affected mice developed multiple adenomas throughout the entire intestinal tract at an early age.

19 JANUARY 1990 SCIENCE, VOL. 247
Luminex (serum protein) assay reveals that serum levels of cytokine TNFα and IL17 were significantly increased in aged Min mice at high risk of intestinal polyposis. Serum cytokine levels in pg/ml. Statistics using 2-tailed Student’s t-test; ns, not significant * = p > 0.05. ** = P > 0.01.
Significant differences exist between microbiota in Min mice and their co-housed wild type littermates.

Min mouse microbiomes deviate from co-housed wild type littermate mice.

We thank Mark Burnham-Smith (EJ Alm lab) for microbiome analyses.
Adoptive Cell Transfer Paradigm

Transplantable anti-inflammatory CD4+CD45RBloCD25+ lymphocytes

Adoptive cell transfer

Cells extracted from immune-competent donor mouse

Wild type litter mate mouse donor

Cells injected into recipient

Apc Min/+ mouse recipient
**T**\textsubscript{REG} cells require IL-10 to prevent intestinal adenomas in \textit{Apc}^{Min/+} mice

Erdman, et al, 2005

We thank David B Schauer
ApcMin

Start 3 wks 6 9 12 15 18 21 24 wks

2-5 days

Sham-treated ApcMin

ApcMin + 300K wt Treg cells
T_{REG} cells induce regression of intestinal adenomas in Apc^{Min/+} mice

untreated Apc^{Min/+}

μ = 58 adenomas

Apc^{Min/+} plus T_{REG} cells

μ = 12 adenomas

N = 14; total intestinal adenomas μ = 58 (37-91)  N = 14; total intestinal adenomas μ = 12(6-43)  (p<0.01)

Age at treatment 4.5 - 6 months (μ age = 5.6 mos)
T$_{\text{REG}}$ cells induce regression of intestinal adenomas in Apc$^{\text{Min/+}}$ mice

Erdman, et al, 2005
$T_{\text{REG}}$ cells induce apoptosis within intestinal adenomas

Erdman, et al, 2005
Cells extracted from immune-competent wt donor mouse

Wild type mouse donor

Transfer anti-inflammatory CD4+CD45RBloCD25+

Sham

Microbe infection in Min or Min/Rag2-KO recipients

Helicobacter hepaticus
Cells extracted from immune-competent wt donor mouse

Wild type mouse donor

Sham

Transfer anti-inflammatory CD4+CD45RBloCD25+

Cells of adaptive immunity suppress IBD and carcinoma

Innate immunity is sufficient for IBD and carcinoma

Microbe infection in Min or Min/Rag2-KO recipients
*H. hepaticus*-infected Apc\(^{Min/+}\) mice rapidly develop mammary tumors.
Innate Immune Inflammatory Response against Enteric Bacteria *Helicobacter hepaticus* Induces Mammary Adenocarcinoma in Mice

Varada P. Rao,¹ Theofilos Poutahidis,¹,³ Zhongming Ge,¹ Prashant R. Nambiar,¹ Chakib Boussahmain,¹ Yan Yan Wang,² Bruce H. Horwitz,² James G. Fox,¹ and Susan E. Erdman¹

*Cancer Res* 2006; 66: (15). August 1, 2006
DOD Award W81XWH-05-1-0460.
Anti-inflammatory regulatory cells and breast cancer.
Breast Cancer: Should Gastrointestinal Bacteria Be on Our Radar Screen?

Varada P. Rao,¹ Theofilos Poutahidis,¹,² James G. Fox,¹ and Susan E. Erdman¹

¹Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, Massachusetts and ²Laboratory of Pathology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece
IL1β → MDSC → IL6 → cancer

TC Wang (Columbia)  
SE Erdman and EJ Alm (MIT)  
C Clarke-Dur (CPIC)  
DA Hafler (Yale)
*H. hepaticus* triggers accumulation of MDSCs

\[2.67\pm0.49 \quad 4.91\pm0.30 \quad 0.88\pm0.21 \quad 0.96\pm0.12\]

\[3.44\pm0.56 \quad 2.73\pm0.38 \quad \]

Spleen  Mammary gland LN  Mesenteric LN

\[\text{Apc}^{\text{Min/+}}\]

\[\text{Apc}^{\text{Min/+}}\]

\[H. \text{hepaticus}\]

\[(\text{we thank TC Wang})\]
Apc$^{\text{Min/+}}$ sham-dosed mammary gland

Apc$^{\text{Min/+}}$ + Hh mammary gland (tumor)

Apc$^{\text{Min/+}}$ sham-dosed mammary gland

Apc$^{\text{Min/+}}$ + Hh mammary gland

Apc$^{\text{Min/+}}$ sham-dosed mammary LN

Apc$^{\text{Min/+}}$ + Hh mammary LN

H. hepaticus infection promotes mammary tumorigenesis in C57BL/6 Apc$^{\text{Min/+}}$ mice

(we thank TC Wang)
Adoptive Cell Transfer Paradigm

- **Bacteria ingestion**
- **Microbe-fed wt donor**
- **adoptive cell transfer**
- **Cells extracted from immune-competent donor mouse**
- **Transplantable anti-inflammatory CD4+CD45RB\(^{lo}\)CD25+ lymphocytes**
- **Cells injected into recipient**
- **Microbial benefits**
- **RagMin or Min recipient**
Interleukin-10

\[ T_{\text{REG}} \]

Pro-inflammatory cells & cytokines → Tumor growth
Earlier exposures to gut microbes impact potency of CD4+ lymphocytes to modulate extra-intestinal cancers.
Modern Hygiene Practices

From the above discussion, it is clear that pathogenic gut bacteria may pose a trigger for breast cancer. However, this seems to be only half the story. It does not explain why breast cancer risk is increasing in developed countries with more rigorous hygiene practices, or answer how chronic use of prescribed antibiotics enhances the risk for breast cancer in women (4). The “hygiene hypothesis” is based on the observation that early childhood infections reduce the incidence of allergies (24). A later counter-regulatory model of the hygiene hypothesis, forwarded by Wills-Karp et al. (24), postulates that microbial infections have a beneficial role in the developing immune system and that the anti-inflammatory cytokine interleukin 10 (IL-10), produced by cells of both innate and adaptive immune
We thank Mark Burnham-Smith (EJ Alm lab) for microbiome analyses.

Significant changes occur in Min mice after adoptive transfer of ‘hygienic’ T cells

Increased polyposis in Min mice after adoptive transfer of ‘hygienic’ T cells

\[ p < 0.05 \]
Microbe-educated lymphocytes impart longevity

Survival Curve by Treatment in Female Min Mice

(Erdman et al 2010)
IL1β → MDSC → IL6 → cancer

TC Wang (Columbia)  C Clarke-Dur (CPIC)

SE Erdman and EJ Alm (MIT)  DA Hafler (Yale)
Hygienic rearing & GI tract dysbiosis (Christina Clarke-Dur)

- Inflammation
- $T_{REG}$
- Breast cancer
- Ovarian cancer
- Endometrial cancer
- Thyroid cancer
- Melanoma
Gut microbiota community restructuring in human subjects after novel exposures
disorders in later life. Whether the increased protection against cancer involves only regulatory T cells of thymic origin (19) or also peripherally recruited IL-10–dependent regulatory subsets is not well understood. We speculate that immune competency may be suboptimal in individuals with more stringent hygiene practices, and when combined with other known risk factors of Western lifestyle this contributes to the paradoxical increase in inflammation-associated cancers seen in developed countries. Likewise, antibiotics may deplete intestinal bacteria directly or indirectly essential for enteric homeostasis, thereby leading to increased risk of breast cancer in women undergoing chronic antimicrobial therapy (4). Interestingly, it seems that the long-term health benefits imparted by intestinal bacterial infections early in life may also be achieved in other ways. Recently, probiotic bacteria were shown to reduce IBD in mice through an IL-10–dependent regulatory lymphocyte–mediated mechanism (26), and clinical
Microbe benefits are transplantable via immune cells

* Lactic Acid Bacteria
* Probiotic-fed donor
* Rag2-KO recipient

Transplantable CD4+gfp-Foxp3+ lymphocytes

Probiotic benefits

* We thank James Versalovic for the gift of *Lactobacillus reuteri* ATCC 6475
Growing luxuriant hair

SHAVED

PURIFIED PROBIOTIC, 5 DAYS AFTER SHAVING
Hair follicles (HF) in subcutis
**Glow of health**

**Hair follicles (HF) in subcutis**

- Counts per x4 image:
  - Control: 0
  - Probiotic: 20

- Chi-Square test: P < 0.0001

**Hair Cycle Stages**

- Telogen
- Anagen IIa
- Anagen VI
- Catagen II

- Chi-Square test: P < 0.0001

- Number of HF/Stage:
  - Telogen: 30
  - Anagen: 20
  - Catagen: 10

- Control
- Probiotic
Staying slender

Probiotics help reduce body fat – even while eating a ‘fast food’ diet.
Fast food chow

Fast food chow + *L. reuteri*

**a**

Epididymal fat

**b**

Subcutaneous fat histology

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Birth 3 wks 6 9 12 15 18 21 24

20 wks
Gfp-Foxp3+ cells

Cells injected into recipient without its own lymphocytes

Probiotic benefits transplantable via lymphocytes

Cell transfer 1  2  3  4  5  6  7  8 wks

a

Rag 2−/− recipient
Wildtype donor

Rag 2−/− recipient
Wildtype + L. reuteri donor

Subcutaneous fat histology

SF
Obesity

Frequent 'fast food'

Commensal bacteria

Interleukin (II)-6

Probiotic yogurt

Probiotic bacteria

Interleukin-10

T helper (Th)-17
Pro-Inflammatory response

Regulatory (Treg) cells
Anti-inflammatory response

Leaner physique

Inflammation
Interleukin-10

T_{REG}

Pro-inflammatory cells & cytokines → Obesity
Changes in Diet and Lifestyle and Long-Term Weight Gain in Women and Men

Dariush Mozaffarian, M.D., Dr.P.H., Tao Hao, M.P.H., Eric B. Rimm, Sc.D., Walter C. Willett, M.D., Dr.P.H., and Frank B. Hu, M.D., Ph.D.
Division of Cardiovascular Medicine (D.M.) and Channing Laboratory (D.M., E.B.R., W.C.W., F.B.H.), Brigham and Women’s Hospital and Harvard Medical School; and the Departments of Epidemiology (D.M., T.H., E.B.R., W.C.W., F.B.H.) and Nutrition (D.M., E.B.R., W.C.W., F.B.H.), Harvard School of Public Health — all in Boston
Humans Eating at Fast Food Restaurants

* P<0.05

percentage of IL-17A positive CD4+ T cells

frequent = at least once a week up to daily
These mice are wild type litter mate brothers .....  

The one that eats probiotics daily, left, is slimmer and shinier than his brother
Serum thyroid hormone (free T4) levels remain in high-normal range even with increasing age.
Mice That Eat Yogurt Have Larger Testicles

Probiotics may endow rodents with a "mouse swagger"

By Elie Dolgin
L. reuteri-treated mouse paired-testes weights
Survival to wean (control) = 67% (351/563)
(L. reuteri) = 98% (622/630)
Reproductive Fitness

“my bacteria made me do it”
Importance of wound healing capability

Stages of Normal Cutaneous Wound Healing

Skin wounds heal twice-as-fast when mice are eating L. reuteri
Skin wounds heal twice-as-fast when mice are eating L. reuteri.
Lactobacillus reuteri-induced phenotypes are transplantable

Probiotic-fed donor

adoptive cell transfer

Cells extracted from gfp donor mouse

Transplantation of CD4+Foxp3+gfp lymphocytes

Cells injected into Rag-KO recipient without its own lymphocytes

Rag2-KO recipient

Probiotic benefits
Gfp-Foxp3+ cells

Cells injected into recipient without its own lymphocytes

Probiotic benefits transplantable via lymphocytes

Biopsy 1 day 2 3 4 5 6 7 8 days

B6 Rag2+ L. reuteri Foxp3-gfp cells
B6 Rag2+ untreated Foxp3-gfp cells
Interleukin-10

\( T_{\text{REG}} \)

Pro-inflammatory cells & cytokines

\[ \rightarrow \]

Tumor growth
“my bacteria made me do it”
L. reuteri-induced improvement in wound repair requires oxytocin

![Graph and images](image)

**a.**
- oxt- WT + L. reuteri (D6)
- oxt- KO + L. reuteri (D6)

**Morphometric Analysis**

- Wound Area Size
  - oxt- WT + L. reuteri
  - oxt- KO + L. reuteri
  - p = 0.007

**b.**
- Histopathology
- Day 6 Re-epithelialization
  - oxt- WT + L. reuteri
  - oxt- KO + L. reuteri
  - p = 0.004
NIEHS P30 ES002109. [P30 PIs: Leona Samson ➔ John Essigmann]
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Does human maternal and infant microbial ecology offer opportunity to impart good health to future generations?
Can microbe-based restructuring of immune networks improve our public health?
Harnessing microbes for public health

Do lactic acid bacteria offer an immediate palatable public health remedy?
Harnessing microbes for public health

Prospective longitudinal studies to identify hygienic signatures for risk assessment and microbial rescue strategies?
Harnessing microbes for public health

Probe epigenetic/genetic impact of microbes upon host progeny and their offspring
Thank you!

- Eric Alm
- Karen Sue Anderson
- Christina Clarke-Dur
- Bevin Engelward
- John Essigmann
- James G Fox
- David A Hafler
- Bruce H Horwitz
- Theofilos Poutahidis
- Leona D Samson
- David B Schauer
- James Versalovic
- Jerrold Ward
- Timothy C Wang