Role of probiotics in modulation of host immune response

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Questions

- Do commensal /probiotic microbes have regulatory effects on mucosal immune response?
- Are specific immune mechanisms selectively affected by commensal microbes?
- Is host immune response to commensals stereotyped?
- Do commensal bacteria have a developmental role in the priming of immune response?
- Can oral consumption of probiotic bacteria have a therapeutic effect?
Mucosal immune system

- Mucosal membrane surfaces provide the strategic interface between the internal and external world
- Large and variable antigenic load
  - indigenous mucosal microbiota
  - potential microbial pathogens
  - food antigens
  - environmental allergens
- Highest numbers of macrophages, plasma cells and T cells
Most infectious diseases are acquired by or affect GI, respiratory, or genital tract mucosal surfaces.

Preventive and protective host defense requires mucosal response.

- secretory IgA antibodies
- mucosal cytotoxic T cells
- innate immune response
Mucosal immune response

- **T helper type2 (Th2) skewed response**
  - IL-4
  - IL-5

- **Immune tolerance towards food and environmental antigens**
  - suppression of inflammation
  - diminished cellular immune response
  - prevention of harmful mucosal immune Th1 delayed hypersensitivity reactions

- **Specialized responses:**
  - co existence with commensals
  - containment of potential pathogens
  - Infection control
Do commensal microbes regulate mucosal immune response?

- Microflora stimulate the gut immune system

- Required for development of gut associated lymphoid tissue, GALT.
  

- Induce oligoclonal expansions of intra epithelial CD8 T cells in the gut.
  
Bacterial induction of GALT

- Specific bacteria (Bacteroides fragilis and Bacillus subtilis but not others (E. coli, C. subterminale) induce GALT
- Uptake into M cells necessary but not sufficient.
- GALT development is not consequence of Ag-specific response to bacterial Ags.
- Mechanisms:
  - Somatic B cell receptor /superantigen,
  - TLR/ innate immune cells
  - Requires bacterial stress response pathway
Microbial colonization induces oligoclonal expansion of intraepithelial T cells

Helgeland, L. Eur J Immunol, 2004
Dietary factors stimulate gut flora and modulate the effect of probiotic bacteria on mucosal immune response.

- **Dietary factors that stimulate gut flora affect immune response.**
  
  Fructooligosaccharides stimulate the growth of gut flora leading to increased IgA and immune response. (Hosono, Biosci Biotechnol Biochem 2003)

- **Combining probiotics and prebiotics have different effects than each given separately, not simply additive or synergistic.**
  
  Prebiotic inulin enriched with oligofructose in combination with the probiotics L. rhamnosus and B. lactis modulates intestinal immune functions in rats. (Roller et al., J Nutr. 2004)
Are specific immune mechanisms affected by commensal microbes?
Does the innate immune system respond differently to probiotic bacteria?

Cross et al. FEMS Immunol Med Microbiol 2004
Can non-pathogenic bacteria be differentially recognized?

- In the presence of leucocytes, discriminative activation of CaCO-2 cells was observed between enteropathogenic E coli and non-pathogenic bacteria

Haller, D. et al, Gut, 2000
Is host immune response to commensals stereotyped?

- Germfree mice were colonized with *Lactobacillus johnsonii* or *L. paracasei*. Both have similar growth, survival, and adherence properties, but colonized at different densities.

- Both activated mucosal B-cell responses. However, clear differences in patterns of immunoglobulins were observed.

*Ibnou-Zekri, N, Infect Immun. 2003*
Dendritic cell cytokine response to microbial flora

- Non pathogenic gram negative bacteria induce Th1 response in monocyte derived DCs dependent upon IL-12
- Non pathogenic gram positive bacteria induce IL-12 but did not prime Th1 or Th2

(Smits et al. 2004)
Peripheral blood mononuclear cell cytokine response

- Gram-positives preferentially induce IL-12 and TNF-alpha
- Gram-negatives induce more IL-10, IL-6, and IL-8.
- Monocytes stimulated with Gram-negative species induced more PGE2 than Gram-positive bacteria

Karlsson et al. Inf Immun 2002
Are changes in microflora relevant to emerging diseases?

Increases in allergic/atopic diseases, inflammatory bowel disorders, autoimmunity

- **Hygiene hypothesis of allergy**: decreased exposure to infectious in childhood leads to increased susceptibility

- **Immunoderegulation theory**: reduced exposure to non pathogenic gut flora
  - Th1/Th2 balance
  - T regulatory effects
Effects of Probiotic bacteria on development of immune response

- **Clinical**
  - Delay in the compositional development of gut flora was a general finding in allergic children
  - Perinatal administration of lactobacilli halved the development of atopic eczema during the first 2 years of life.

- **Mechanism**
  - Improve barrier function, reduce antigenic load
  - Increase T regulatory function—inducing IL-10 and TGF beta

*Kalliomaki, Isolauri et al  Curr Opin Aller Clin Immunol 2003*
Hypothesis: Commensal bacteria prime the development of immune response

Bacterial encounter occurs at birth
- potential pathogens
- normal commensals

**ANTIGEN**

- Colonization
- Inflammation/ tolerance
- Infection
Neonatal memory and naïve T cell response to bacterial antigens

\[ p = 0.02 \]
Monocyte Production of IL-6 and IL-8 in Term and Premature Infant Cord Blood
E Coli vs Lactobacillus
Monocyte Production of IL-10 and IL-12 in Term and Premature Infant Cord Blood
E Coli vs Lactobacillus

% Activation

IL-10  IL-12  IL-10  IL-12
E Coli  LP 299v

Term  Preterm
Effect of lactobacillus GG treatment on bacterial species in preterm infants

Growth failure in pediatric HIV disease

- Reduced growth
- Height, weight, & head circumference below fifth percentile for age
- Chronic condition
- Stunting
- Delayed puberty
- Associated with chronic cytokine activation
Weight change in response to oral lactobacillus in HIV-1 infection
Effect of Lp 299v on height

Responders  Non Responders

1: p=0.003
2: p=0.024
3: p=0.05
4: p=0.049
5: p=0.016
Effect of Lp299v on weight

MONTHS ON TREATMENT

RESPONDERS

NON RESPONDERS

* p=0.01
Lp 299v treatment and T cell response to mitogen

HIV-1+ Children

CPM

Pre
Post

P<0.01
Effect of CD4+ T cell level in vivo on response to Lactobacillus in vitro

![Graph showing the effect of CD4+ T cell level on response to Lactobacillus in vitro. The graph compares two groups: HIV+CD4 = 7% and HIV+CD4 = 34%. The y-axis represents CPM (counts per minute) ranging from 0 to 80000, and the x-axis compares Lp299v and PHA.]
NK cell activation in response to *Lactobacillus* in HIV+ children

**CD69 Expression**

![Graph showing NK, NK Bright, and NKT cell activation in controls and HIV+ children. The x-axis represents NK, NK Bright, and NKT cells, while the y-axis represents percent positive cells. The graph compares CD69 expression between controls (red) and HIV+ children (white).](image-url)
Lactobacillus activates both innate (NK) & adaptive (T) immune response infection

HIV+ Children

Percent Activated

CD3  NK  NK Bright  NKT

#1  #2  #3
Conclusions

- Commensal/probiotic microbes have regulatory effects on mucosal immune response
  - Long term effects?
- Specific immune mechanisms selectively affected by commensal microbes?
  - Pathways? Signaling events?
- Host immune response to commensals is species/strain specific
  - Bacterial response element? Immune cell type?
- Commensal bacteria have a developmental role in the priming of immune response
  - Specific? Regulatory?
- Oral consumption of probiotic bacteria can have a therapeutic effect?
  - Specificity? Mechanism? Duration? Safety?
  - Potential use of genetically altered strains?
Collaborators

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