

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

Mucosal immunity and host defense

- **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**

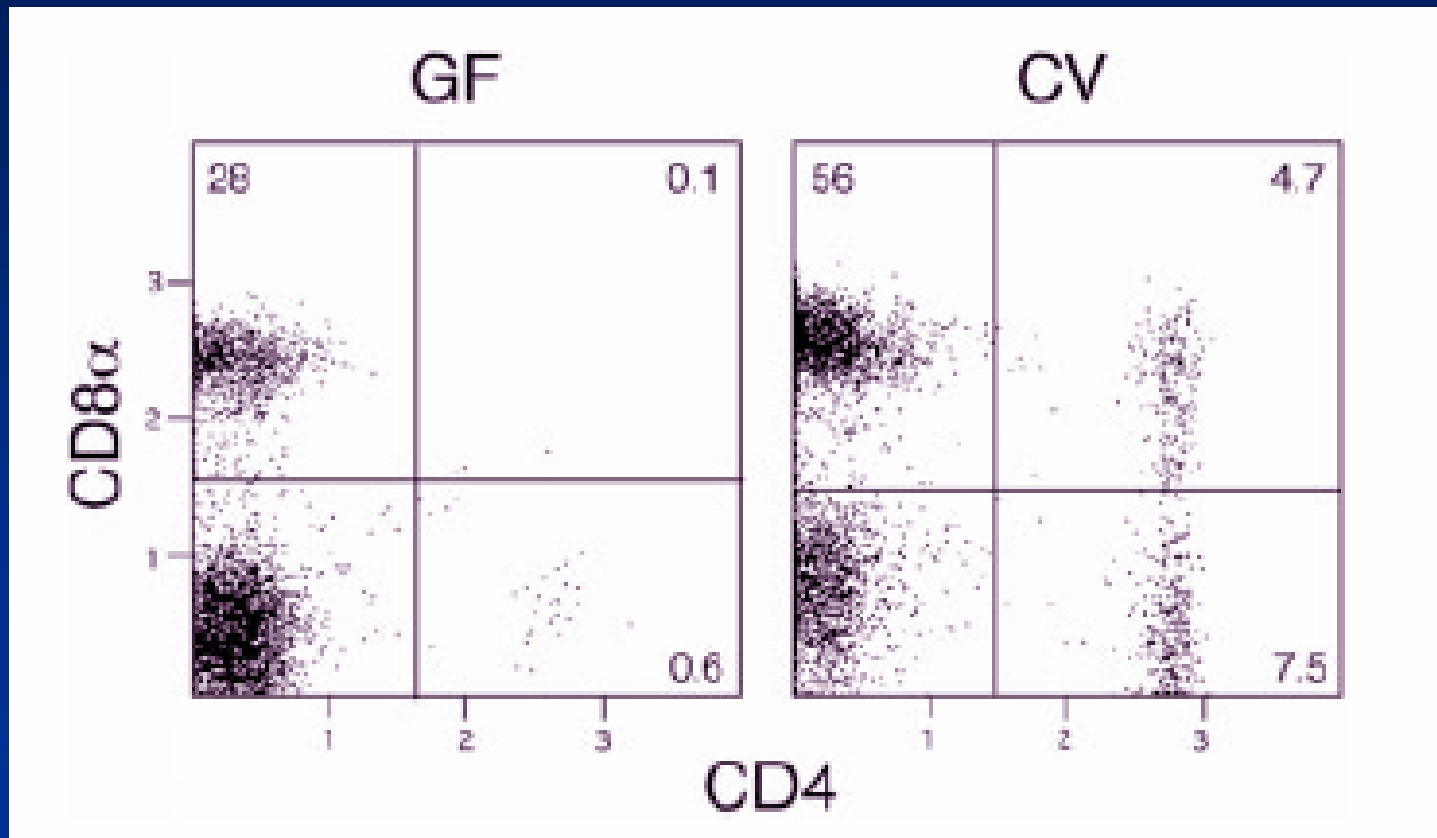
Do commensal microbes regulate mucosal immune response?

- **Microflora stimulate the gut immune system**
 - **Required for development of gut associated lymphoid tissue, GALT.**
(Rhee et al. , J. Immunol., 2004, Jexp Med, 2005)
 - **Induce oligoclonal expansions of intra epithelial CD8 T cells in the gut.**
(Helgeland, L., et al. , Eur J Immunol , 2004)

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.**

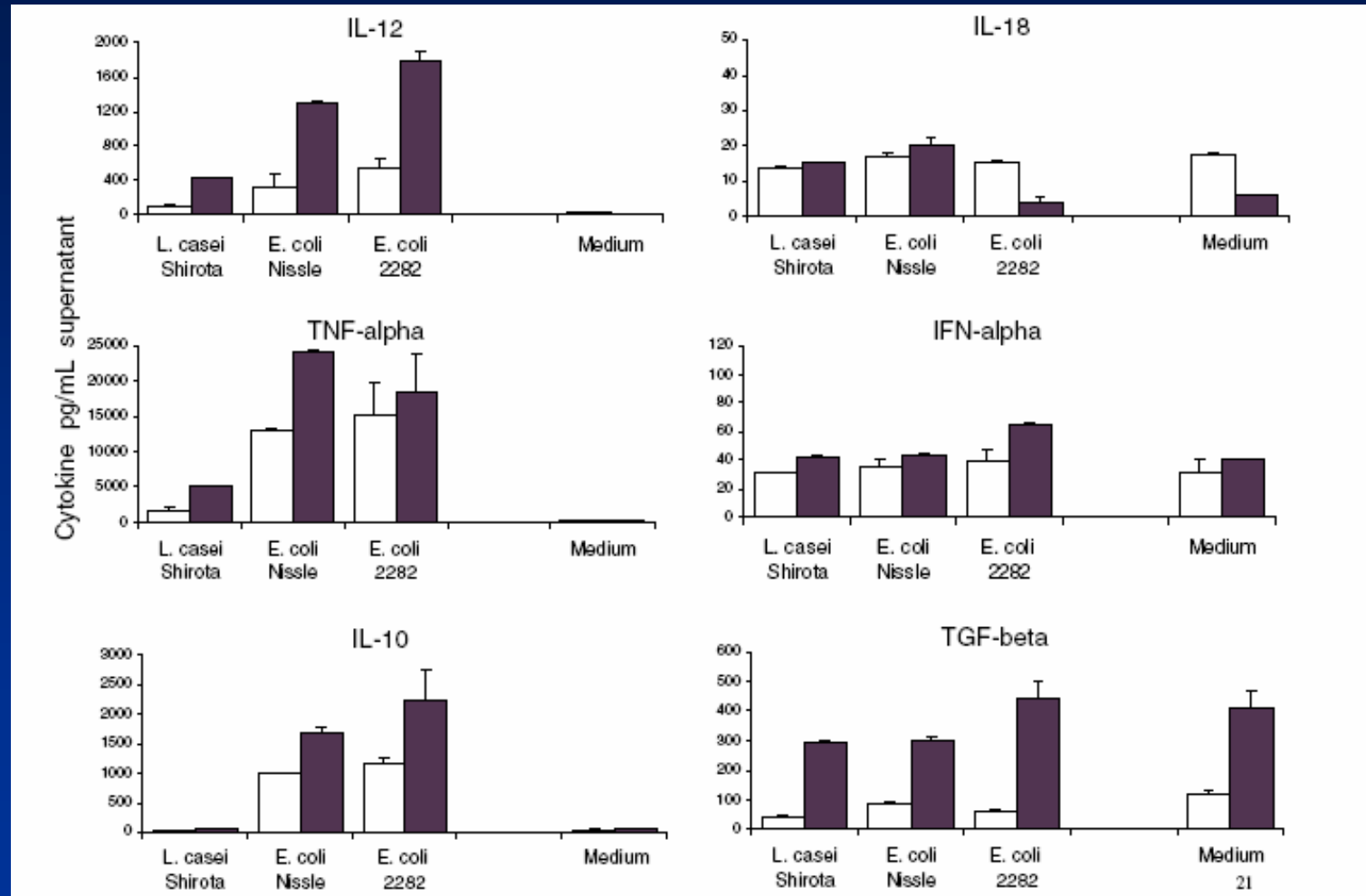
Fructooligosaccharides stimulate the growth of gut flora leading to increased IgA and immune response. (Hosono, Biosci Biotechnol Biochem 2003)

- **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)*

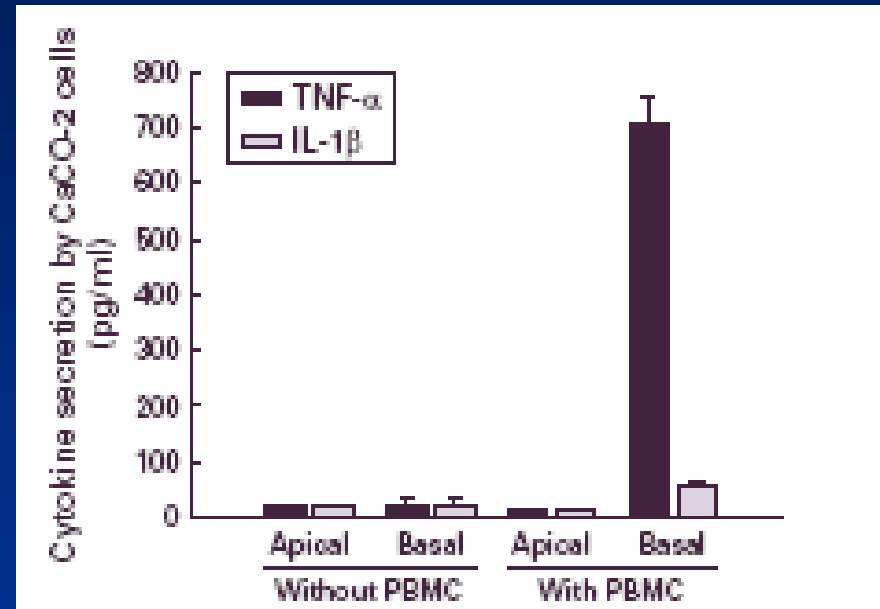
**Are specific immune mechanisms
affected by commensal microbes ?**

Does the innate immune system respond differently to probiotic bacteria?



Can non-pathogenic bacteria be differentially recognized?

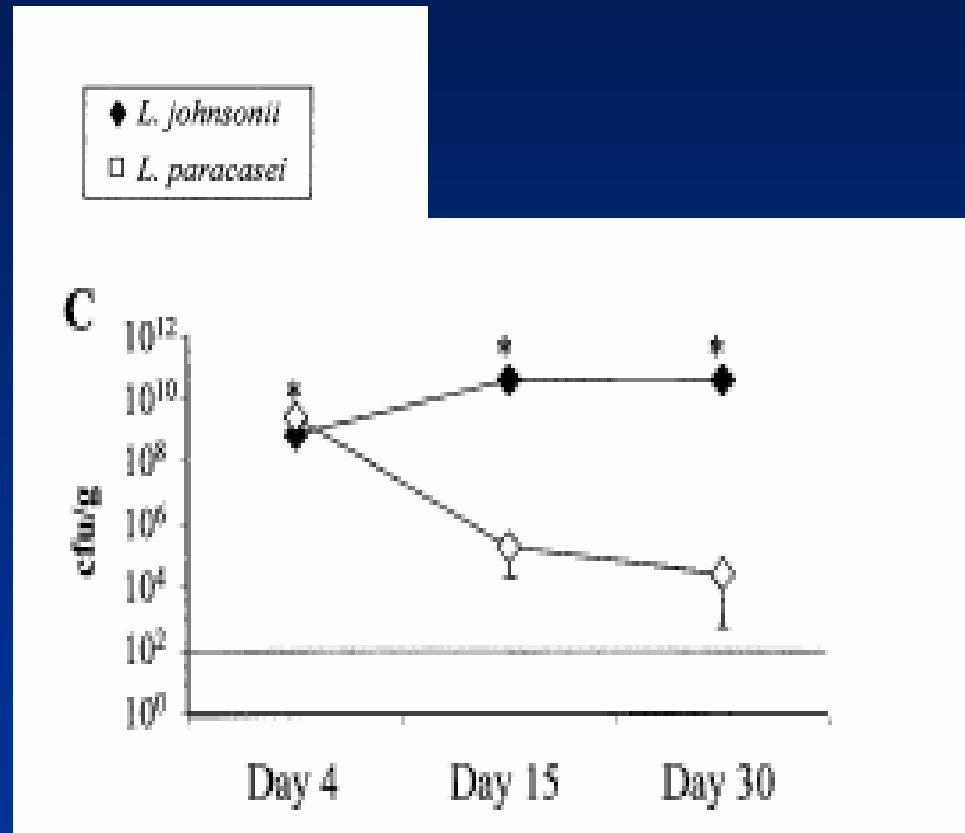
- In the presence of leucocytes, discriminative activation of CaCO-2 cells was observed between enteropathogenic *E coli* and non-pathogenic bacteria



Haller, D. et al, Gut, 2000

Is host immune response to commensals stereotyped?

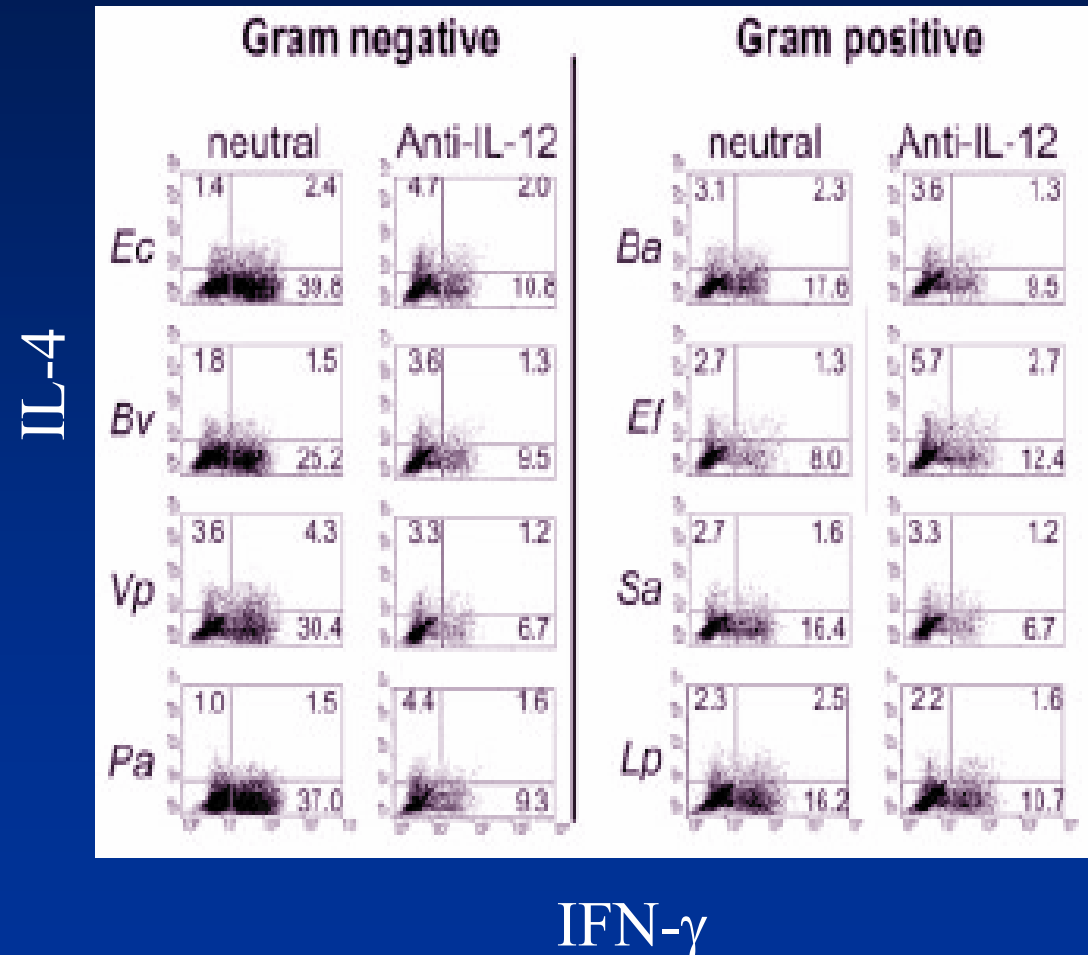
- Germfree mice were colonized with *Lactobacillus johnsonii* or *L. paracasei*. Both have similar growth, survival, and adherence properties, but colonized at different densities
- Both activated mucosal B-cell responses. However, clear differences in patterns of immunoglobulins were observed.



Dendritic cell cytokine response to microbial flora

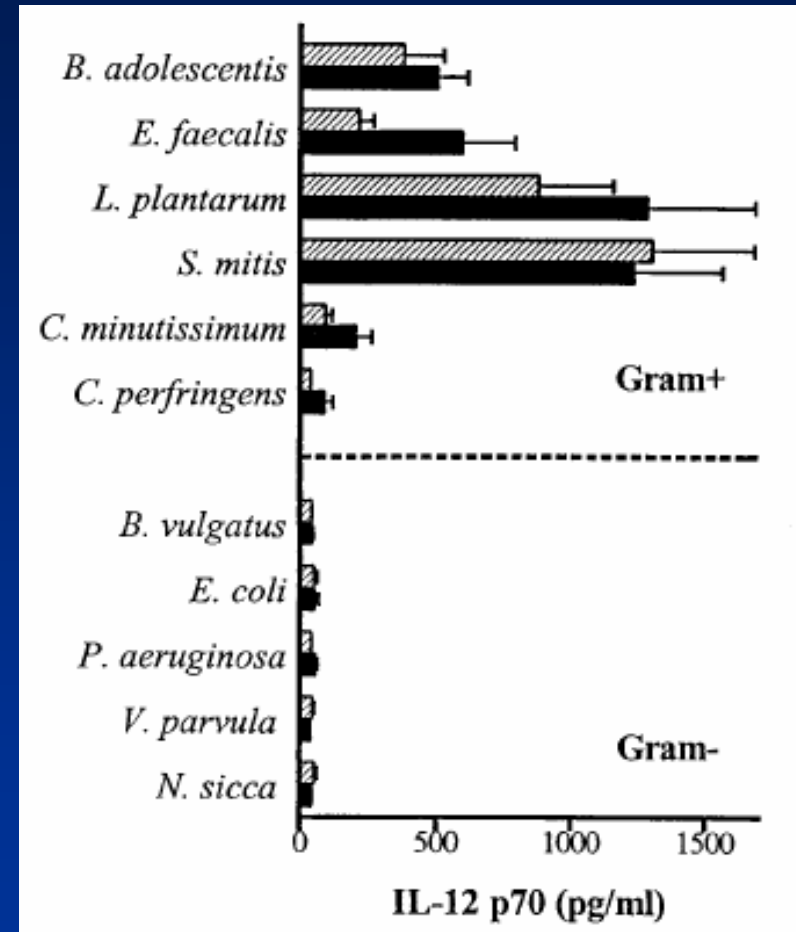
- Non pathogenic gram negative bacteria induce Th1 response in monocyte derived DCs dependent upon IL-12
- Non pathogenic gram positive bacteria induce IL-12 but did not prime Th1 or Th2

(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



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

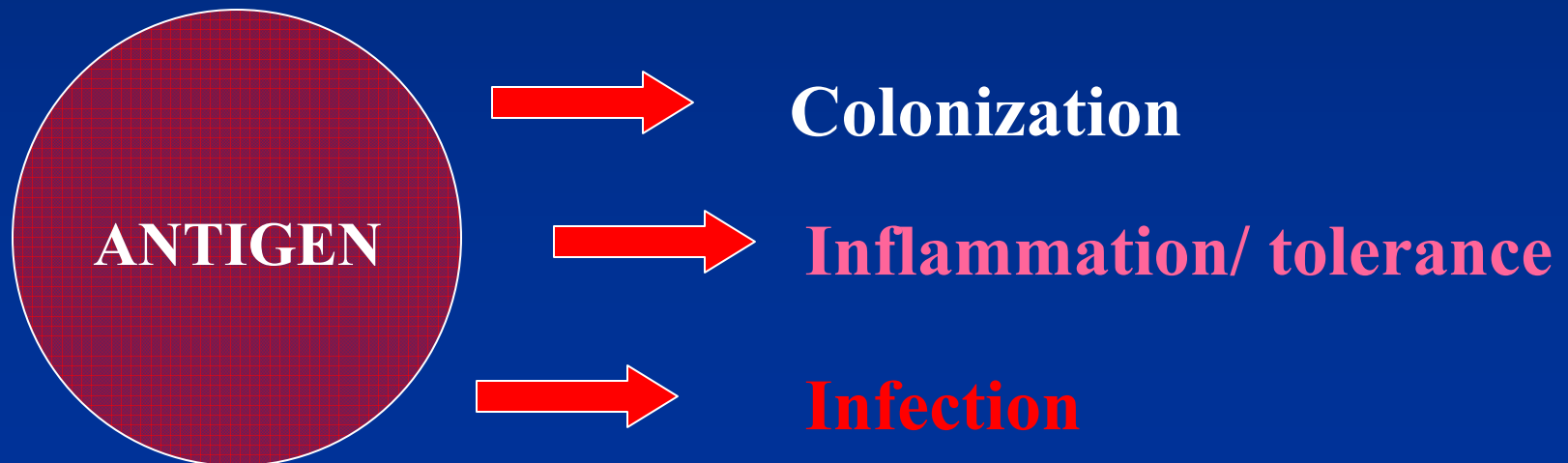
- Improve barrier function, reduce antigenic load
- Increase T regulatory function- inducing IL-10 and TGF beta

Kalliomaki, Isolauri et al Curr Opin Allerg Clin Immunol 2003

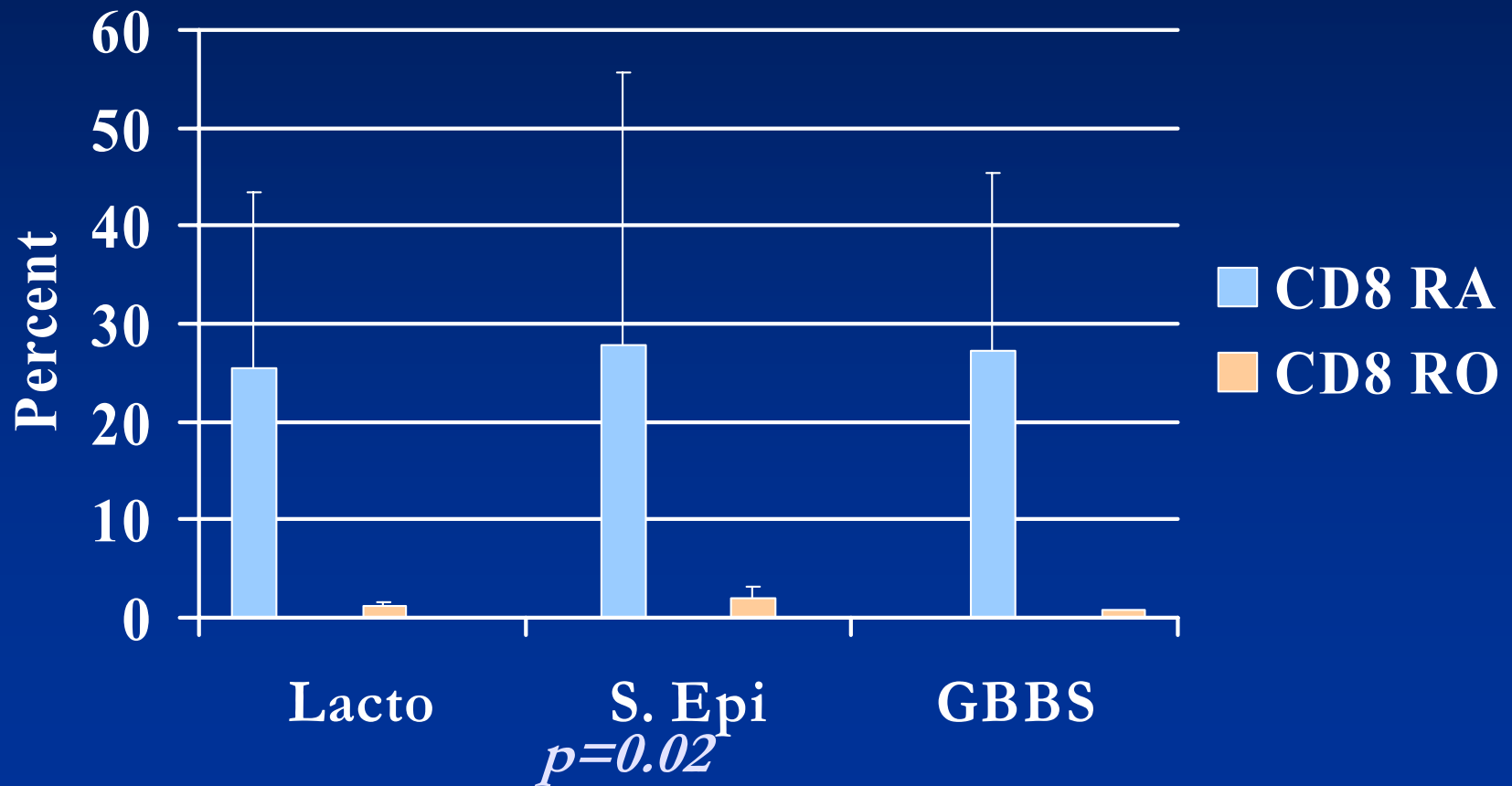
Hypothesis: Commensal bacteria prime the development of immune response

Bacterial encounter occurs at birth

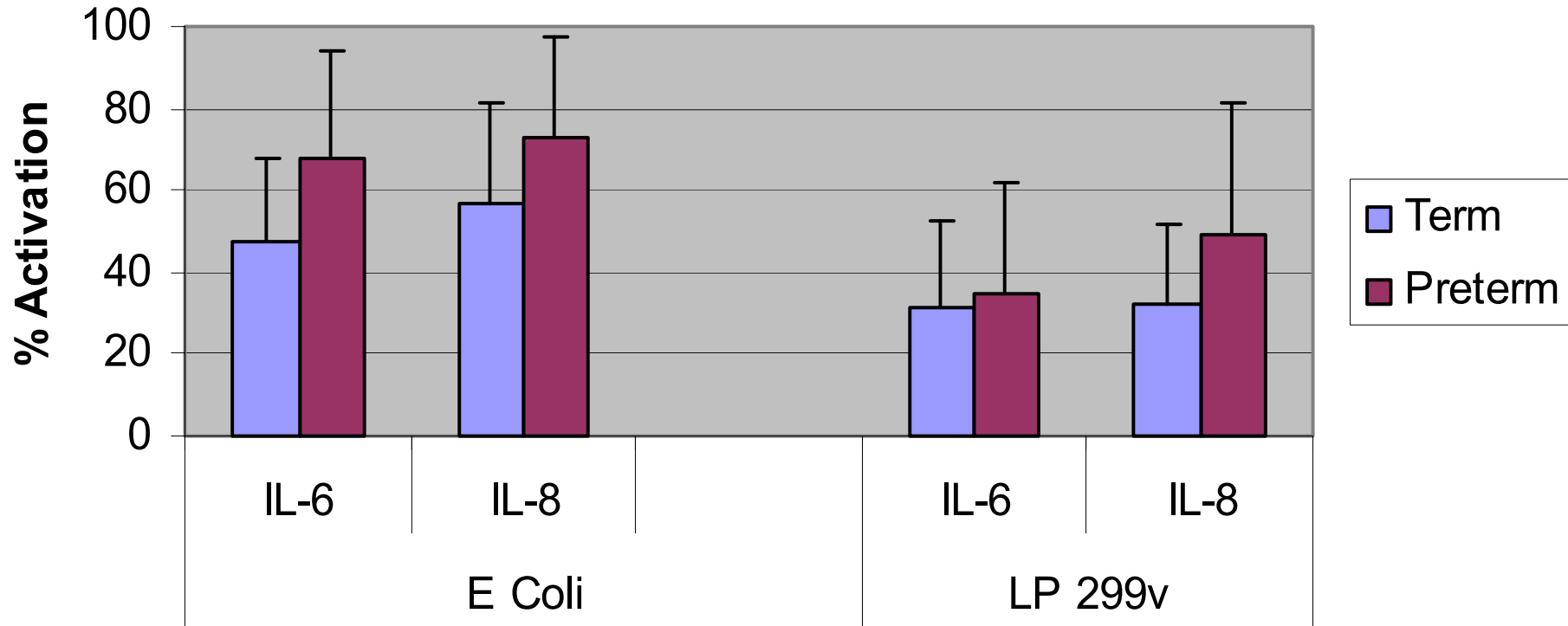
- potential pathogens
- normal commensals



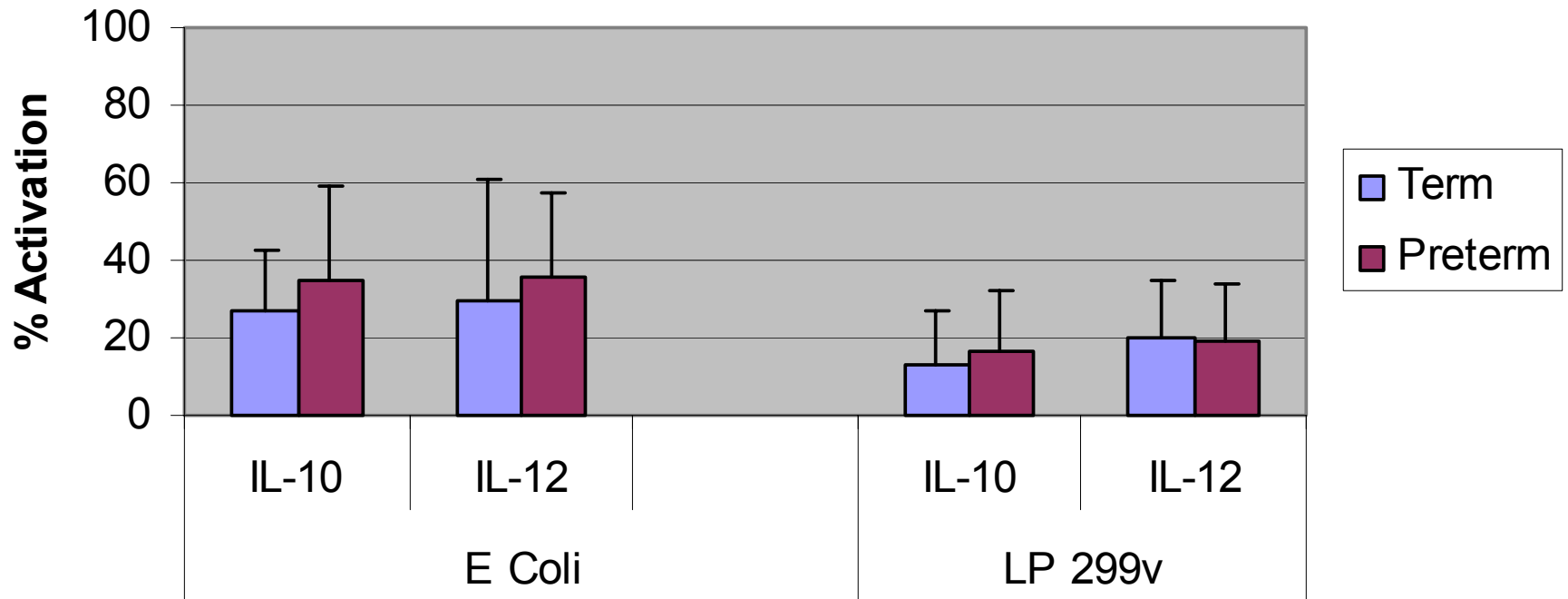
Neonatal memory and naïve T cell response to bacterial antigens



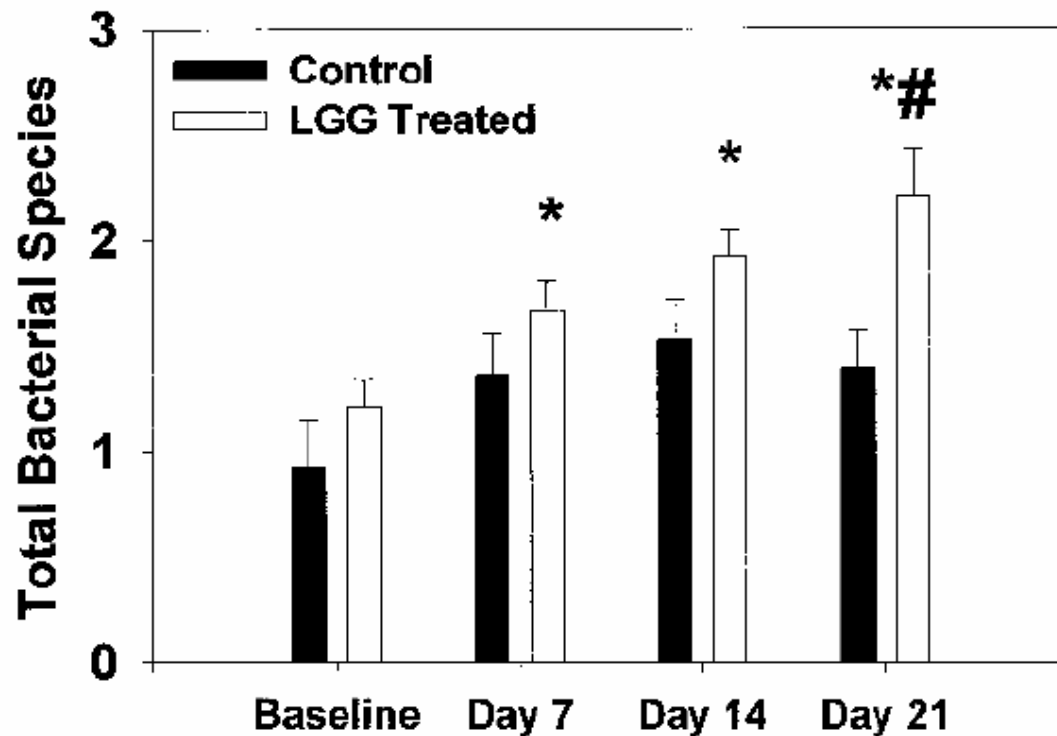
Monocyte Production of IL-6 and IL-8 in Term and Premature Infant Cord Blood E Coli vs Lactobacillus



Monocyte Production of IL-10 and IL-12 in Term and Premature Infant Cord Blood E Coli vs Lactobacillus



Effect of lactobacillus GG treatment on bacterial species in preterm infants

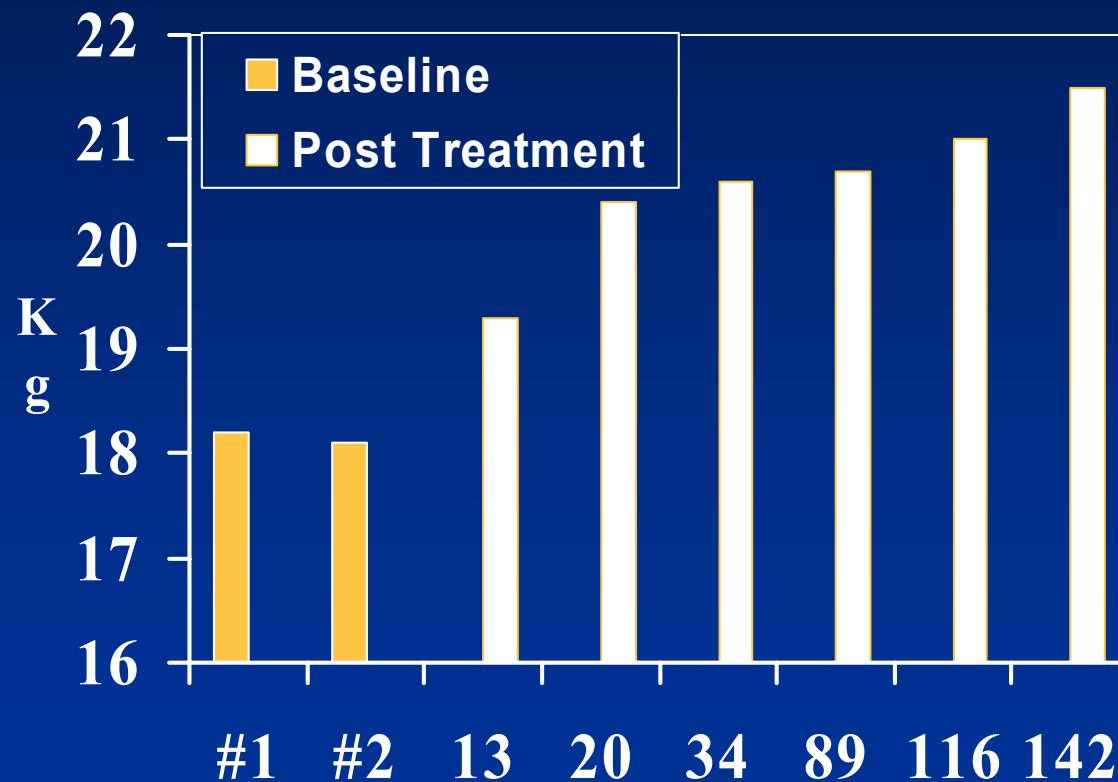


Agarwal R, J Pediatr Gastroenterol Nutr. 2003

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**

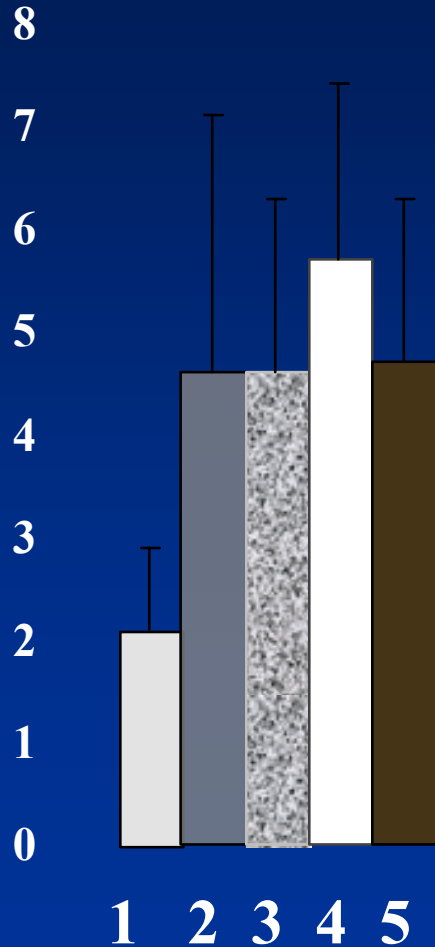
Weight change in response to oral lactobacillus in HIV-1 infection



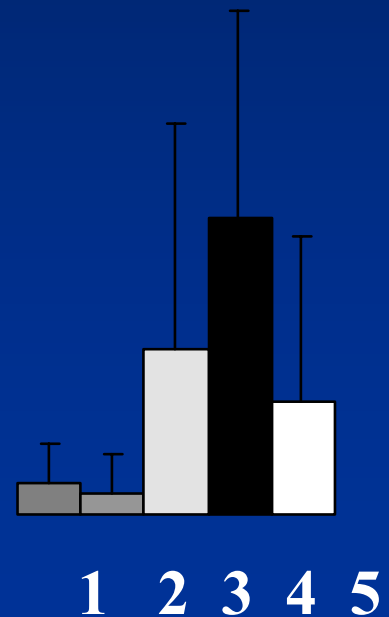
Effect of Lp 299v on height

Responders

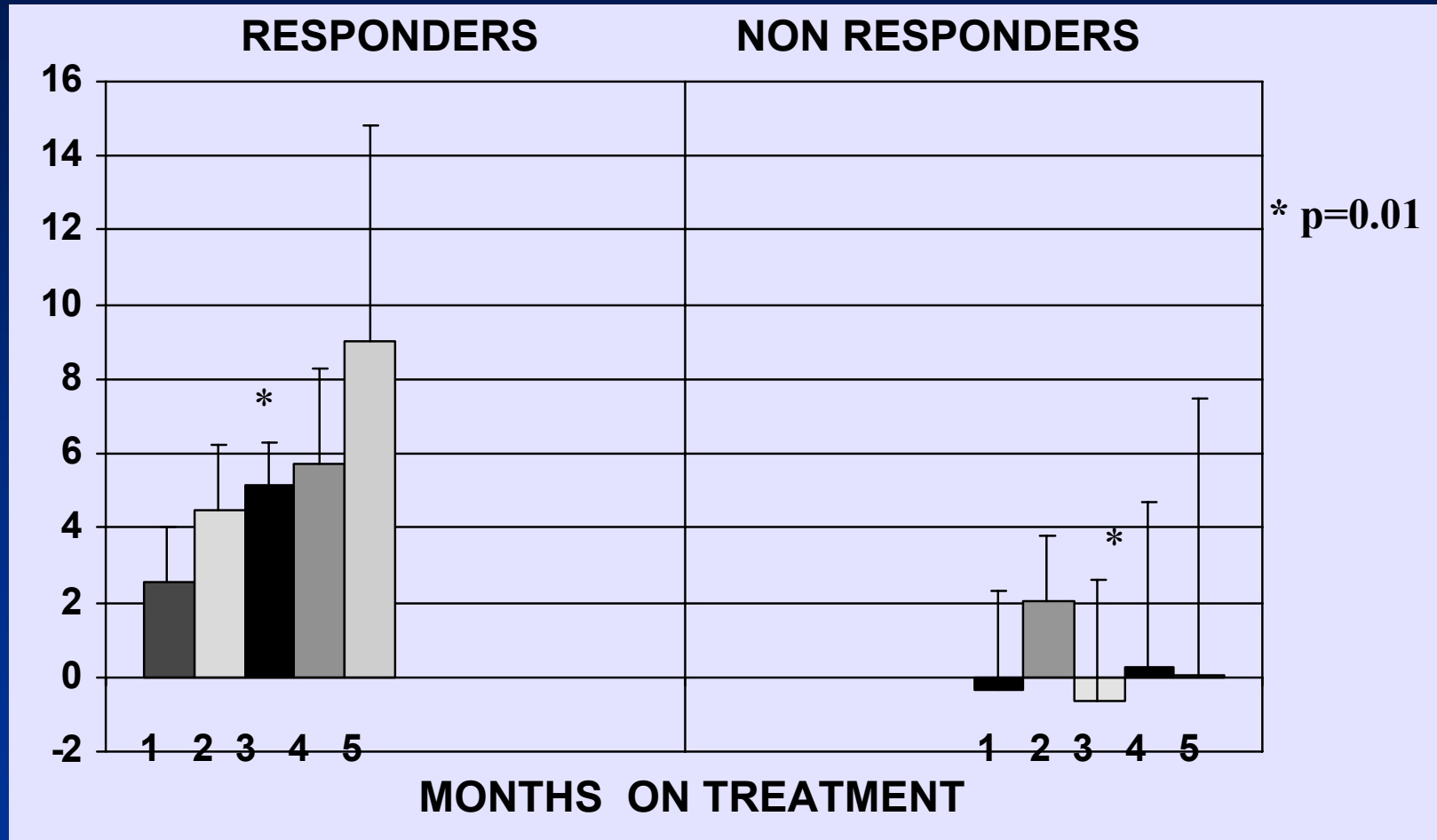
Non Responders



1: $p=0.003$
2: $p=0.024$
3: $p=0.05$
4: $p=0.049$
5: $p=0.016$

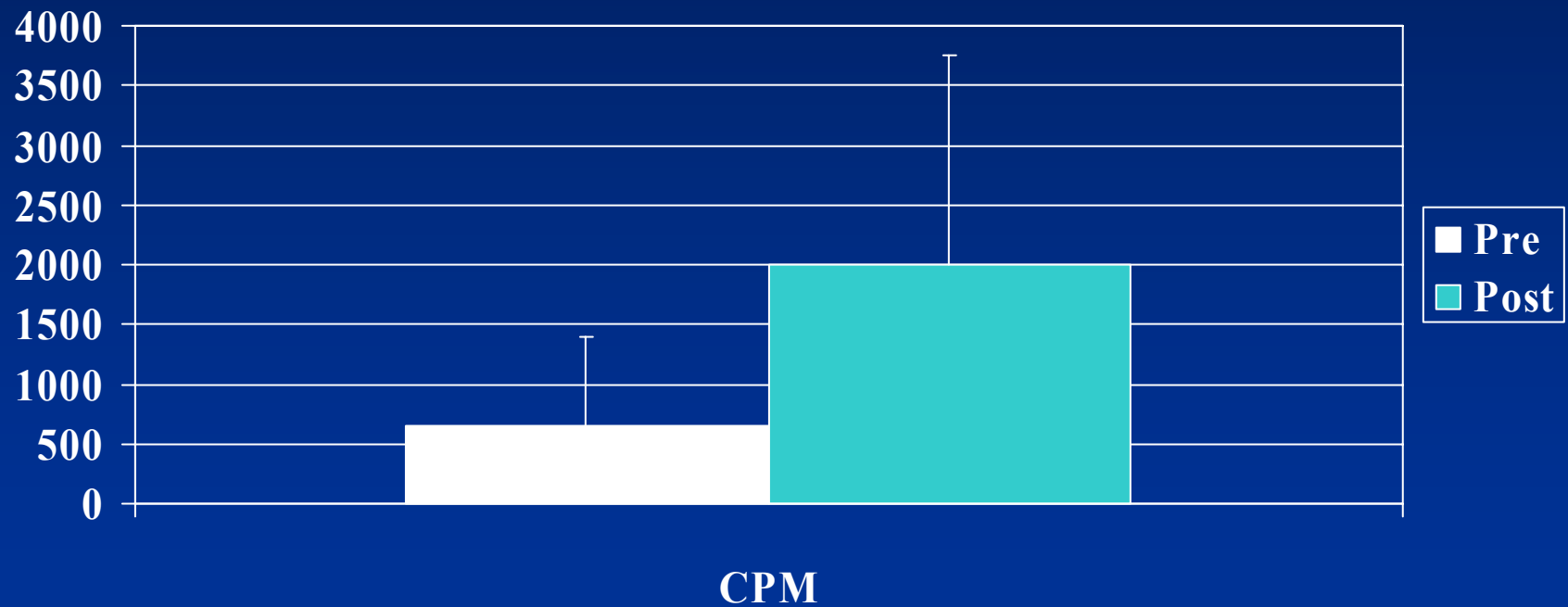


Effect of Lp299v on weight



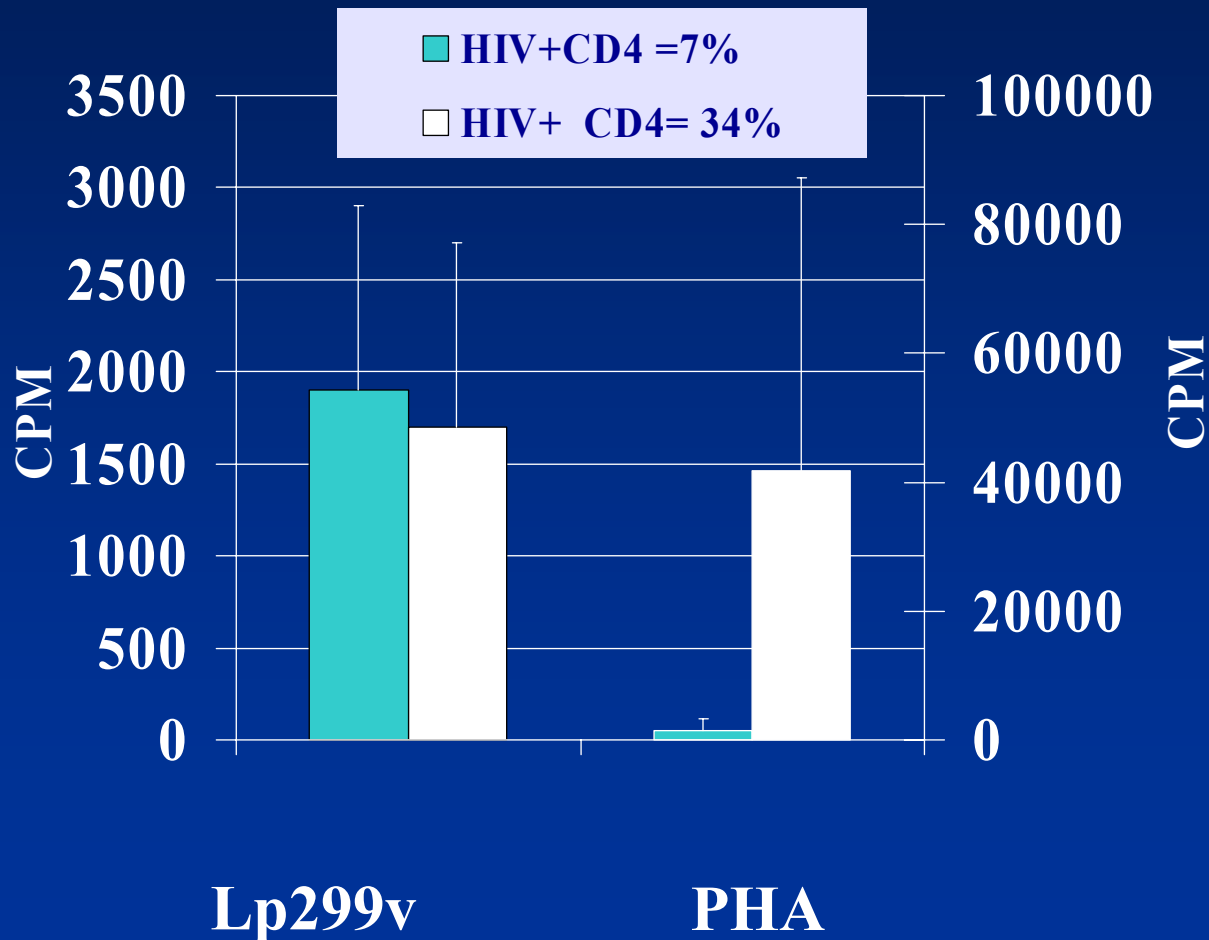
Lp 299v treatment and T cell response to mitogen

HIV-1+ Children



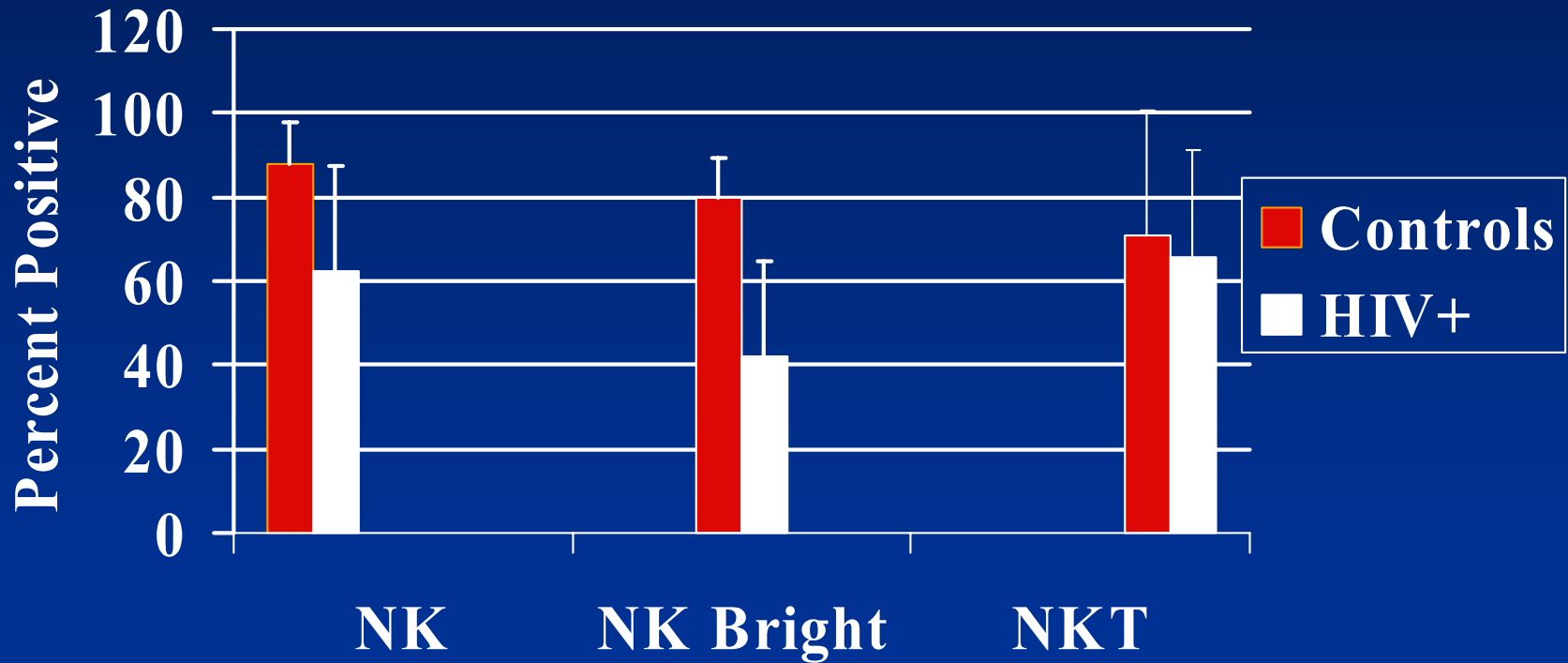
$P < 0.01$

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



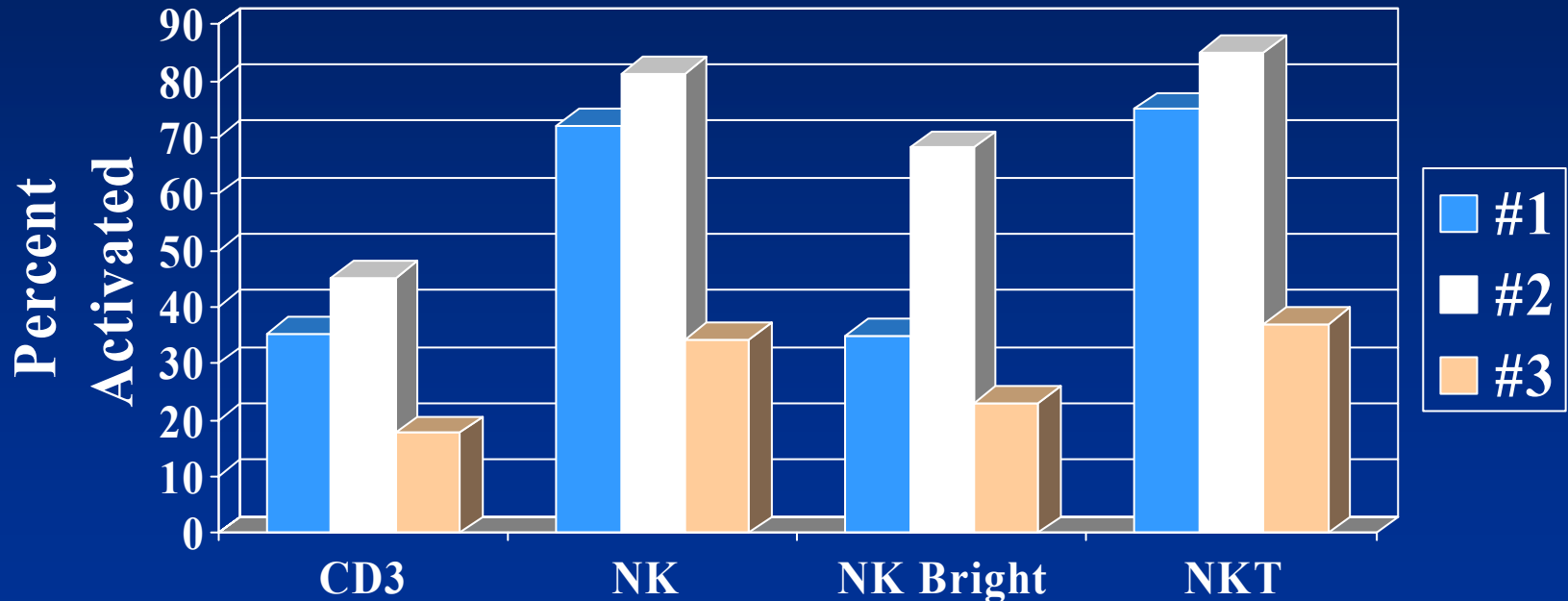
NK cell activation in response to *Lactobacillus* in HIV+ children

CD69 Expression



Lactobacillus activates both innate(NK) & adaptive (T) immune response infection

HIV+ Children



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

NYC: Neonatal Studies

- **Mirjana Negin, MD**
- **AMF Tatad, MD (Fellow, Neonatology)**
- **John Peoples, BS (CUMC Class of 2005)**
- **S.Cheung, BS**
- **Mohamed Mohamed, MD (Fellow, Neonatology)**
- **Maciej Simm, BS**
- **A. Krauss, MD**

NYC: HIV Studies

- **Florence Marshall, MD**
- **Ann Margaret Dunn, CNP**
- **Lily Chan, CNP**
- **Manuel Cotilla, MD**
- **Joseph Stavola, MD**

Lund: Microbiology Studies

- **Siv Ahrne', PhD**
- **Marie Louise Johansson, PhD**