Food, microbial ecology and mucosal immunity

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Introduction

Food, which is not absorbed by the small intestine, is food for the colon. It is metabolised by a consortium of bacteria which convert the substrate predominantly to short chain fatty acids (acetate, proprionate, butyrate), inert gases (hydrogen, carbon dioxide, methane), ammonia, hydrogen sulphide and related compounds. Such food is known variously as prebiotic or fibre or colonic food. Its components, which include non-starch polysaccharide, resistant starch, tartrate, dextrins etc, can influence not only the mass of the bacterial consortium in the colon but also its microecology (1).

The growth of juxtamucosal bacteria may be influenced more by mucosal factors, which are less directly affected by diet. The experimental data presented in this paper concerns interrelationships between the juxtamucosal bacteria and the immune system in humans. Ex vivo data are presented which indicate that there is an active immune reponse by $CD8^+$ T cells to heterologous colonic bacteria. This is suppressed by a subset of autologous colonic bacteria.

Methods

A brief overview of the methods is given. Juxtamucosal bacteria were grown from human colonic biopsies in broth cultures over 48 h, and sonicated. Supernatants were filtered to remove bacteria and membranous vesicles and then stored at -70°C ready to use as antigen to stimulate cultures of lamina propria mononuclear cells in various combinations. Supernatants derived from self bacteria are termed autologous; supernatants derived from hosts different from the cell cultures are termed heterologous.

Lamina propria cells were isolated from colonic biopsies (about 20 per experiment) by first removing epithelial cells in an EDTA, calcium-free digest, then digesting with collagenase, elastase, DNAse for 1 hour, then centrifuging through a Ficoll Hypaque density gradient.

In some experiments, cells were sorted using a FACS Vantage to isolate T cells ($CD3^+$, $CD19^-$), helper T cells ($CD4^+$), cytotoxic T cells ($CD8^+$), non-T cells ($CD3^-$) and dendritic cells before reconstituting in 96 well culture plates.

Cultures were stimulated by heterologous antigen (1-1000 nM) or autologous antigen or by various combinations of heterologous and autolgous antigen subsets. Negative controls were antigen-free cultures. Positive controls used the nonspecific mitogen PHA.

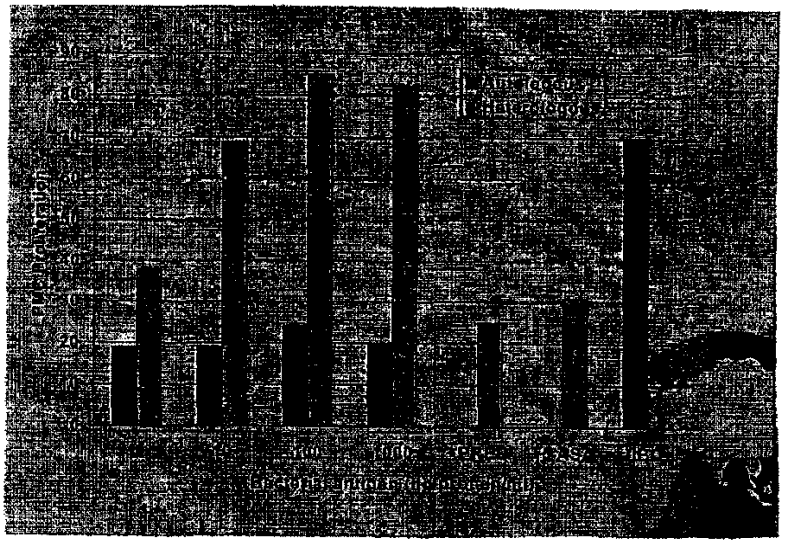
Endpoints measured after five days culture were blast formation (FACS analysis on forward and side scatter), DNA replication (3H-thymidine) or cell activation using FACS analysis of $CD69^+$, which is a marker of cell activation.

The work was approved by the Mater Hospitals' Ethics Committee.

Results

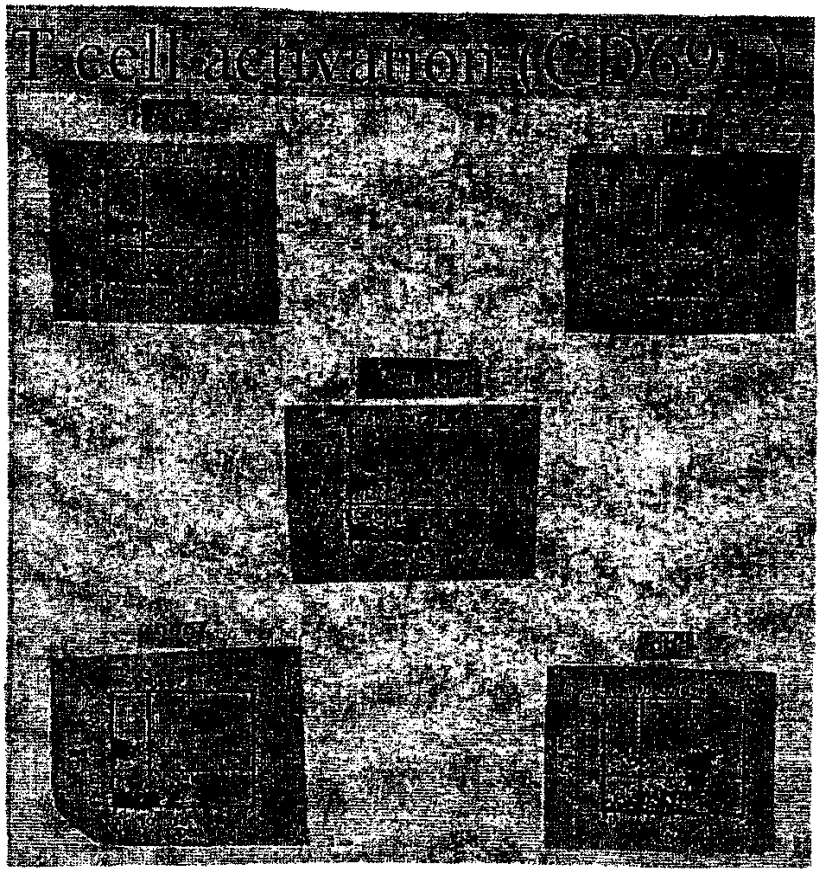
There was a dose-related proliferation to heterologous bacterial antigen (1-1000 nM) but not to autologous bacterial antigen in ex vivo cultures derived from healthy humans. Drugs used to treat inflammatory bowel diseases suppressed the normal heterologous immune proliferative response. There was a proliferative response in autologous cultures derived from inflamed

inflammatory bowel disease colon but not from those derived from uninflamed colon. (Figure 1) (2).



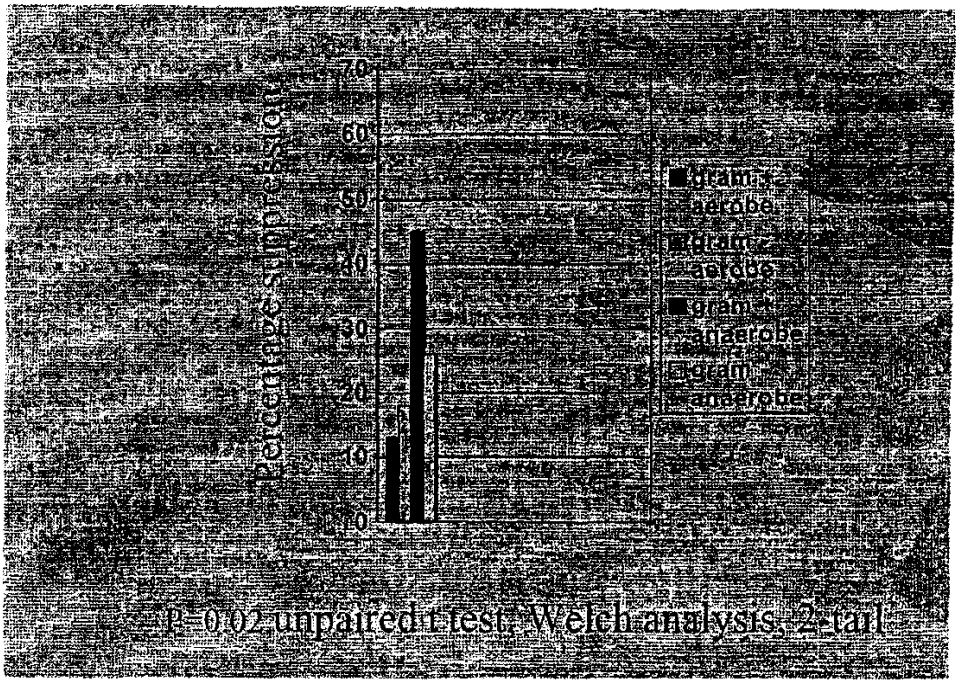
Khalil D, Radford-Smith GL, Florin THJ. Immunol Cell Biol 1997

The proliferative cell was $CD8^{+}$. This was paralleled by blast formation and cell activation of $CD8^{+}$ cells in heterologous cultures but not in autologous cultures or cultures with autologous and heterolgous antigen (Figure 2).



Suppression by autologous antigen was derived mainly from gram positive anaerobic bacteria (Figure 3).

Bacterial antigen partition experiment



Discussion

Immune tolerance to one's own colonic bacteria was first muted in the 1960s and 70s (3). This study provides experimental evidence to support this and is in agreement with Duchmann et al (4).

The data indicate that the responder cell in the ex vivo culture is $CD8^+$, which is consistent with either a suppressor or cytotoxic cell phenotype. Suppression of this response is mediated primarily by juxtamucosal gram positive anaerobic bacteria.

It is apparent that this phenomenon may explain the phenomenon of oral tolerance (5) generally. We are currently engaged in a four-pronged attack to further elucidate the experimental model by characterising the responder cell, the antigen presentation, the autologous antigen and environmental factors which determine the juxtamucosal bacteria.

References

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