

THE DEVELOPMENT AND FUNCTIONAL ACTIVITY OF T-CELLS IN HUMAN FOETAL SMALL INTESTINE

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INTRODUCTION

The normal human and rodent small intestine is densely populated with T-cells, the function of whom is unknown. The study of the lymphocytes in human foetal small intestine gives the opportunity to investigate the development of gut associated lymphoid tissue

in a sterile germfree environment. In addition, through use of an organ culture technique, we can investigate the function of foetal gut T-cells following activation. This article reviews some of the major findings.

MATERIALS AND METHODS

Tissue

Small intestine (jejunum and ileum) from therapeutically aborted fetuses was snap frozen and stored at -70°C.

Organ culture of foetal human small intestine

Small intestine from fetuses was placed in a petridish in serum-free CMRL-1066 medium (Flow) modified as described (*MacDonald and Spencer, 1988*). The intestine was cut into pieces 2-3 mm². Five pieces of tissue were cultured in 7 ml modified CMRL-1066 medium in 5 cm diameter tissue culture dishes (Sterilin). The cultures were incubated at 37°C in a 95% oxygen, 5% CO atmosphere with or without pokeweed mitogen. At the end of the culture period the 5 explants in each dish were carefully removed from the culture dish and placed on top of one another on a piece of filter paper to absorb excess

moisture. The tissues were then snap-frozen in liquid nitrogen and stored at -70°C. Frozen sections profiling each of the explants were then cut and stained immunohistochemically using the peroxidase technique.

Monoclonal antibodies used were Ki67 (Dako Ltd., High Wycombe, Bucks) which identifies a nuclear antigen in all dividing cells and the anti-T-cell antibodies anti-CD3, CD4, CD8 and CD25 purchased from Becton-Dickinson, and anti-HLA-DR (a gift from Dr. K. Adams).

Quantification of IEL

Differential counts of villus or crypt epithelial cells and peroxidase-stained intra-epithelial lymphocytes in each explant were made (*Ferguson and Murray, 1971*). Results are expressed as the percentage IEL stained with any particular antibody per 100 epithelial

Table 1: Number of CD3⁺ IEL, CD4⁺ IEL and CD8⁺ IEL per 100 epithelial cells in the tissues of different ages

Age of Tissue (weeks)	Stained cells per 100 epithelial cells		
	CD3	CD8	CD4
11	0.0	0.0	0.0
14	1.8	0.8	0.3
16	2.9	1.5	0.3
18	3.2	2.1	0.9
22	4.2	3.9	0.1

cells. Crypt cell proliferation was measured by counting the number of crypt epithelial cell nuclei staining immunohistochemically with Ki67 (MacDonald and Spencer, 1988).

Induction of HLA-DR on HT-29 cells with organ culture supernatants to measure interferon gamma

The transformed intestinal epithelial cell line HT-29 was obtained from the European Collection of Animal Cell Cultures, Porton Down, and was grown and passaged in RPMI-1640 culture medium containing 10% foetal calf serum. 50,000 HT-29 cells were added to microculture wells on plastic slides (Labtech tissue culture chamber slides, Miles Laboratories, Naperville, Illinois) in a volume of 0.2 ml and allowed to

adhere overnight. The next day the adherent cells were washed and were treated with dilutions of organ culture supernatants or recombinant gamma interferon (a gift from Dr. Allan Morris, University of Warwick). Twenty-four hours later the cells were washed and stained immunohistochemically with anti-HLA-DR. Each culture supernatant was tested in duplicate. The person reading the slides was unaware of the origin of the supernatant added to the HT-29 cells. The percentage cells expressing HLA-DR was taken as the mean of the duplicate tests of each supernatant dilution.

Sheep anti-recombinant interferon gamma (a gift from Dr. A. Meager, British Institute for Biological Standards, Potters Bar) was added to some of the wells.

RESULTS

Development of T-cells in human small intestine

Twenty-two foetal small intestines were examined, ranging in age from 11 to 22 weeks. The number of CD3⁺ IEL, CD4⁺ IEL, and CD8⁺ IEL per 100 epithelial cells in the tissues of different ages are shown in Table 1.

The results clearly show an increase in intra-epithelial lymphocytes with increasing gestation. Interestingly, most

of the cells are CD8⁺, as in adult intestine. This would indicate that exogenous bacterial or dietary antigen is not responsible for the appearance of CD8⁺ cells in the gut epithelium.

As well as an increase in IEL there was also an increase in lamina propria T-cells, but these were CD4⁺, as is also the case in post-natal intestine.

Organised aggregates of lymphoid tissue (Peyer's patches) were not seen



Figure 1: CD3⁺ cells in a Peyer's patch from 19 week-old foetal human small intestine.

until 19 weeks gestation. Figure 1 illustrates the appearance of a foetal Peyer's patch stained immunohistochemically with anti-CD3 to identify the T-cell zones.

T-cell activation in organ cultures of foetal intestine

Explants of human foetal intestine can be maintained in organ culture for several weeks with retention of gut structure and epithelial cell function (MacDonald and Spencer, 1988). We therefore thus attempted to directly stimulate mucosal T-cells in situ in explants of human small intestine.

Preliminary experiments indicated that the T-cell mitogens phytohaemagglutinin and wheatgerm agglutinin added directly to the cultures activated mucosal T-cells in the explants as measured by the appearance of CD25⁺ cells, however the most striking effects were seen with pokeweed mitogen (PWM).

Associated with the appearance of CD25⁺ cells there was also dramatic changes in mucosal morphology in the

older tissues (an 18 week-old foetal gut cultured for 3 days with PWM is shown in Figure 2). At the onset of culture, villous morphology was good and there was epithelial cell division in the crypts, highlighted using the monoclonal antibody Ki67 and the peroxidase technique. After 3 days in culture the villi had become swollen as a result of transporting water across the epithelium and there were still epithelial cells in division in the crypts. However in the presence of PWM, there is increased cellularity in the lamina propria, villous atrophy, and a dramatic crypt epithelial cell hyperplasia. This effect was age-dependent in that in 14 week-old specimens PWM had no effect and in specimens aged 16-17 weeks the effect was less striking than that shown in Figure 2.

Interferon-gamma production by activated T-cells in foetal small intestine in organ culture

To determine if the T-cells activated with PWM were secreting lymphokines, the organ culture supernatants were

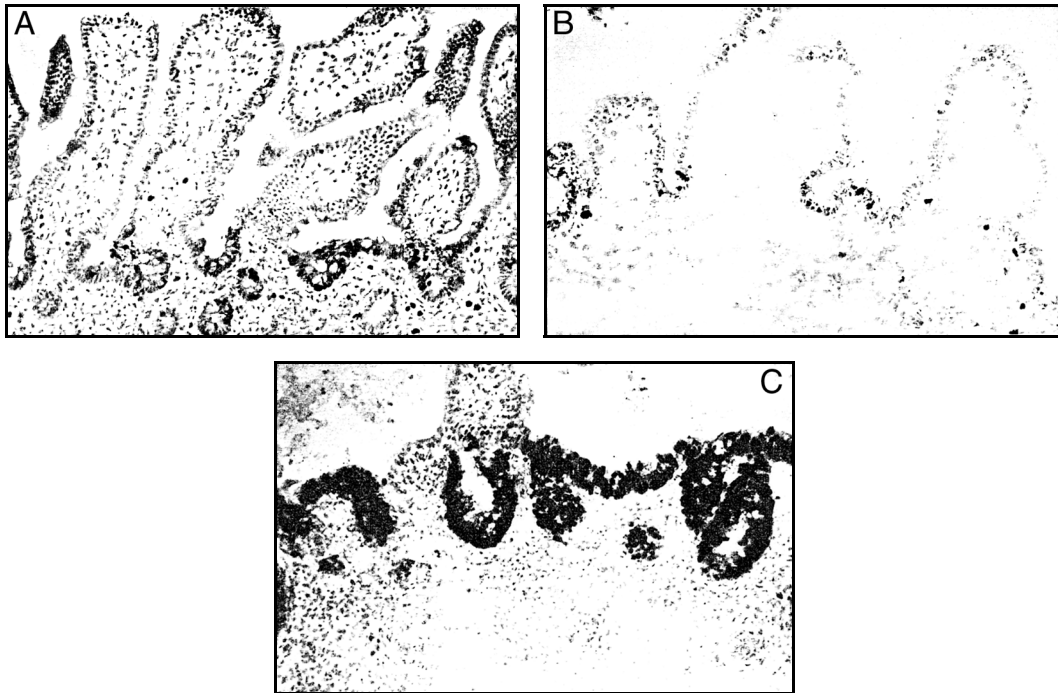


Figure 2: The immunohistochemical appearance of 18-week-old foetal gut on day 0 (A), after 3 days in culture in medium alone (B), and after 3 days in culture with PWM (15 mg/ml, C). The sections were stained with Ki67 to highlight dividing cells in the crypts.

tested for interferon-gamma. This was done by using the ability of gamma-interferon to induce *de novo* the expression of Class II MHC molecules on an epithelial cell line (MacDonald et al., 1988).

Less than 1% of HT-29 cells cultured in medium alone was HLA-DR⁺. However treatment with recombinant interferon-gamma increased the number

of HLA-DR⁺ cells to 18% (Figure 3). This effect titrated out with decreasing amounts of gamma-interferon and was completely inhibited by sheep anti-gamma-interferon. Organ culture supernatants from PWM-treated explants (but not control cultures) also increased HLA-DR positivity on the HT-29 cells, and this effect was also inhibited with sheep anti-interferon-gamma.

SUMMARY AND CONCLUSIONS

These studies show that lymphocytes populate human small intestine in an antigen-independent fashion. Most noticeably, CD8⁺ T-cells show a marked tropism for gut epithelium, even in the absence of antigen. Peyer's patches also form in the absence of antigen. In organ culture T-cells in foetal small intestinal lamina propria can be activated with

PWM to secrete lymphokines.

A consequence of T-cell activation is a rapid increase in the rate of intestinal crypt cell proliferation. Thus although the function of T-cells in health remains unknown, these results indicate that T-cells may play an important role in the development of enteropathy.

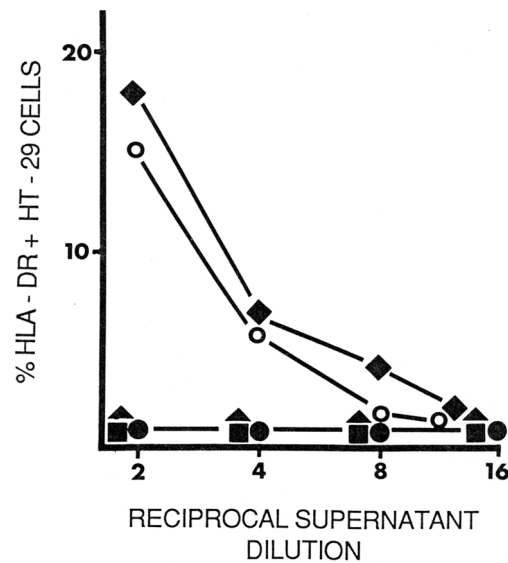


Figure 3: The induction of HLA-DR on HT-29 cells with recombinant gamma interferon and organ culture supernatants. Each point is the mean of duplicate observations per dilution of culture supernatant or recombinant interferon-gamma. In this representative experiment recombinant gamma-interferon (200 units/ml, ◆—◆) was titrated out and gave a dose dependent increase in HT-29 HLA-DR expression. Organ culture supernatants (○—○) from explant cultures of a 22 week-old foetal gut cultured for 3 days with PWM also caused an increase in HLA-DR expression. Control supernatants not stimulated with PWM (●—●) had no effect on HLA-DR expression. In the presence of sheep anti-interferon gamma (final dilution in the wells-1:200) the HLA-DR inducing effects of recombinant interferon-gamma (■—■) and the PWM-treated organ culture supernatant (▲—▲) was completely neutralised.

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