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SHORT COMMUNICATION

CYTOKINE mRNA EXPRESSION IN MOUSE COLON: IL-15 mRNA IS OVEREXPRESSED AND IS HIGHLY SENSITIVE TO A FIBRE-LIKE DIETARY COMPONENT (SHORT-CHAIN FRUCTO-OLIGOSACCHARIDES) IN AN *Apc* GENE MANNER

Euphémie Bassonga, Virginie Forest, Fabrice Pierre, Francis Bornet¹,
Pascale Perrin, Khaled Mefflah, Jean Menanteau

On the basis of studies using the *Min* mouse model of colon carcinogenesis, we have recently proposed that a fibre-like food (short-chain fructo-oligosaccharides, sc-FOS) fermented in the colon may stimulate a mechanism of cancer immunosurveillance. In the present paper, we have investigated the expression of cytokines as potential effector molecules. Interleukin (IL-)4, IL-5, IL-13, IL-15 and interferon (INF)- γ mRNAs were detected by a multi-probe ribonuclease protection assay in C57BL/6J and *Min* mouse colons. IL-15 mRNA expression was significantly amplified ($P=0.01$) by the sc-FOS-enriched diet in the colon of *Min* mice.

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We have recently shown that short-chain fructo-oligosaccharides (sc-FOS), a fibre-like food ingredient capable of producing high amounts of butyrate¹ and of changing the colonic flora,² (a regulator of colonic immune functions) was able to reduce the number of colon tumours in *Min* mice,³ a strain bearing an inactivated *Apc* gene (an anti-oncogene, the loss of which is responsible for increased risk of intestinal tumours).⁴ We have further shown that the functionality of the local immune system was necessary for this effect.⁵ We proposed that sc-FOS may interfere directly and/or indirectly (e.g. through the production of butyrate) with the cross-talk between epithelial (colonocytes) and immune cells (intra-epithelial

lymphocytes, IEL)^{6,7} exposed directly to the luminal content. These observations have prompted us to investigate the effector molecules, namely the cytokines, present in the colon. To assess the response of the tissue to the "fibre" and cancerogenesis at the cytokine level, we chose to study the mRNAs since certain cytokines [e.g. interleukin (IL-)15] are frequently not translated or secreted by resting cells.⁶ We used a multiprobe ribonuclease protection assay to study the expression of selected cytokines in the colon of C57BL/6J and *Min* mice fed a low-fibre diet or a sc-FOS-enriched diet.

RESULTS

Five cytokines were consistently detected regardless of the animals or of the diet [IL-4, IL-5, IL-13, IL-15, and interferon (IFN)- γ] (Fig. 1). IL-4, IL-5 and IL-13 were expressed at low but comparable levels and were not sensitive to the modulators used. IL-10, IL-9, IL-6 and IL-2, however, were not detected.

Since mRNAs from C57BL/6J and *Min* mice were run on different gels, quantitative data were analysed separately. In C57BL/6J mice the range of IL-15 mRNA expression was very broad and gave high

From the Human Nutrition Research Center of Nantes, U419 INSERM, Institut de Biologie, 9 Quai Moncousu, F44035 Nantes Cedex 01, France and ¹Eridania Beghin-Say, Vilvoorde Research and Development Center, Nutrition and Health Service, Havenstraat 84, B-1800 Vilvoorde, Belgium
Correspondence to: Dr Jean Menanteau, U.419 INSERM, Institut de Biologie, 9 Quai Moncousu, 44035 Nantes Cedex 01, France.
E-mail: menanteau@nantes.inserm.fr

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Figure 1. Representative multi-probe ribonuclease protection assays for mouse colon cytokine mRNAs.

Total mRNAs were prepared from C57BL/6J mouse fed the control diet (A) or the sc-FOS enriched diet (B) and from *Min* mice fed the control diet (C) or the sc-FOS diet (D).

standard variation values. The lowest expression was found in the group fed the control diet (CD, Fig. 1A) and the highest in the group fed the sc-FOS diet (Fig. 1B). The CD group was heterogeneous (two mice showed very low expression and one mouse had a high expression). The scFOS group was more homogeneous (all mice showed a high expression). The difference in IL-15 mRNA expression between the two groups of C57BL/6J mice (Fig. 2A) was not significant ($P=0.19$, $n=3$) probably because the groups were small and the standard deviation was high (3.3 and 3.4 vs 0.8 and 1.5 in the *Min* family). On the other hand, the IL-15 mRNA was frequently highly expressed in both *Min* groups, (Fig. 1C and D) but to a level significantly higher ($P=0.01$, $n=7$) in the *Min* group fed sc-FOS as compared to the *Min* group fed CD (Fig. 2B). IFN γ mRNA, when detected, showed the same pattern of expression as IL-15.

DISCUSSION

Detected cytokines were similar in all groups except IL-15 and, perhaps, INF- γ . Epithelial cells are a

major source of IL-15 mRNA.⁸ Our results do not imply that functional proteins are produced because IL-15 is regulated at other levels and frequently not detected in the supernatants of cell cultures expressing the mRNA.^{8,9} The physiological significance of a pool of translationally inactive IL-15 mRNAs is not fully understood, but it is proposed that these cells can readily respond to intracellular messages by translating the message into functional IL-15.⁹ The fact that IFN- γ appears to be modulated in the same way as IL-15 supports the hypothesis that IL-15 could be secreted in an active form, since IFN- γ , a cytokine produced by activated T cells and which stimulates cytotoxic activity, is a target of active IL-15.¹⁰

In *Min* mice, individual variations were lower than in C57BL/6J and showed a statistically significant increase in IL-15 mRNA in the mice fed sc-FOS. It is accepted that IL-15 may be a regulator of cellular homeostasis in the intestine.^{11–13} IL-15 may support the tissue-bound lymphocytes that persist in the mucosa, cells which modulate the turn-over of intestinal epithelial cells. The enhancement of IL-15 mRNA (and possibly INF- γ) induced by sc-FOS in *Min* mice correlates with the effects on colon carcinogenesis and the immune response.^{1,2} IL-15 could be a clue for the mechanisms involved in these effects since this cytokine is a key regulator of cellular homeostasis in the intestine and has been shown to exert anti-tumoural activity.^{14,15}

Finally, it is likely that IL-15 is more frequently expressed at higher levels in *Min* mice than in C57BL/6J. In the context of the low fibre diet, and relative to other cytokines, IL-15 was low in 2/3 C57BL/6J but high in 4/7 *Min* mice. If IL-15 expression is truly sensitive to *Apc* gene functionality, it could be a novel target of this gene, opening a new area of research.

MATERIALS AND METHODS

C57BL/6J-*Min*/+ mice and C57BL/6J mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and Charles River (Saint Aubin les Elbeuf, France) respectively, divided randomly into groups and housed in our facilities.

Mice were fed one of the energy-balanced diets previously detailed.¹ The control diet (CD) was a low-fibre diet (2% cellulose, Durieux, Marne-la-Vallée, France). The high fibre diet provided an additional 5.8% (g/100 g) sc-FOS (Actilight P[®]; Beghin Meiji Industries, Neuilly sur Seine, France).

Mice were 7 weeks old at the beginning of the experiment. Each group (three animals investigated in the two C57BL/6J groups and seven in the two *Min* groups) was fed a diet ad libitum in protected feeders that was renewed daily for 42 days.

The mice were sacrificed by cervical dislocation. Colon portions (5 mm in length) were cut in a distal part of the

Figure 2. Quantitative analyses of IL-15 mRNAs expressed in the colon of C57BL/6J (A) and *Min* mice (B) fed either the low-fibre control diet (CD) or the sc-FOS-enriched diet (sc-FOS).

IL-15 mRNA expression is significantly higher ($P=0.01$) in *Min* mice fed sc-FOS than in *Min* mice fed CD.

organ free of lymphoid nodules, frozen in liquid nitrogen and kept at -80°C . Total RNAs were prepared using TRIZOL (Gibco, Paisley, Scotland, UK).²⁴ Multiprobe ribonuclease protection assays were performed with the mCK1 matrix (Pharmingen, San Diego, CA, USA), containing the following probes: IL-4, IL-5, IL-10, IL-13, IL-15, IL-9, IL-2, IL-6, INF- γ . The gels were analyzed using the IPLab Gel Program (Signal Analytics Corporation, Vienna, USA).

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