Dietary short chain fatty acids protect against type 1 diabetes

Li Wen [1] & F. Susan Wong [2]

[1]Section of Endocrinology, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06520, USA

[2]Division of Infection and Immunity, Cardiff University School of Medicine, Cardiff, CF14 4XN, UK

Correspondence to Li Wen: li.wen@yale.edu

Dr. Wen is supported by NIH (DK092882, DK100500, P30DK045735), JDRF (1-INO-2015-136) and ADA (1-14-BS-222). Dr. Wong is supported by UK Medical Research Council (MR/K021141/1), Diabetes UK, European Foundation for the Study of Diabetes, JDRF and the British Council.

Short chain fatty acids (SCFAs), acetate and butyrate, which are released by specialized diets by gut microbiobes, protect non-obese diabetic (NOD) mice against insulitis and slow the progression of diabetes.

Increasing evidence emphasizes the important role of gut microbiota in type 1 diabetes development. It is clear that the composition of gut microbiota changes prior to and after diabetes onset; however, it is not clear how these change in gut microbiota affect the disease development. Some of these effects may relate to the metabolites produced by the microbiota.

Using the Non Obese Diabetic (NOD) mouse that develops spontaneous diabetes with considerable similarities to human type 1 diabetes, Marino and colleagues report a comprehensive study on the importance of the microbial metabolites acetate and butyrate, which are short-chain fatty acids (SCFAs), in autoimmune diabetes[1]. They build on the knowledge that the progression of diabetes correlates inversely with blood and fecal concentrations of these SCFAs, designing diets to release acetate and butyrate. Their data show that these diets boost the concentrations of acetate or butyrate respectively, in the feces, hepatic and peripheral blood. The mice on both diets exhibit a significant reduction of insulitis and diabetes development, accompanied by an increase in gut integrity, with a marked synergy in diabetes protection when a combined diet is used.

Interestingly, the SCFA-rich diets have a strong impact on the immune system through different modes of action. Whereas the acetate diet reduces splenic B cells, especially transitional and marginal zone (MZ) B cells, which are required for autoreactive T cell proliferation, the butyrate diet increases regulatory T cell number and function (Fig.1). Marino and colleagues further test the immune regulatory effect of SCFA-rich diets in the T cell antigen receptor (TCR) transgenic NY8.3 NOD mouse, which is an accelerated diabetes model. NY8.3 TCR recognizes an islet protein peptide, islet-specific glucose-6- phosphatase catalytic subunit-related protein (IGRP). In these NY8.3 mice, the majority of the T cells are diabetogenic $CD8^+$ T cells and often 100% of these mice develop diabetes by about 12 weeks of age. Impressively, the butyrate and acetate rich diets delay~~ed~~ diabetes development by 19 and 25 weeks, respectively. The authors find that the acetate-rich diet considerably reduces IGRP-reactive CD8 T cells in the periphery, possibly by inhibiting the proliferation of NY8.3 $CD8^+$ T cells. However, since the suppression is surprisingly strong, it would be interesting to learn whether acetate has any effect on the

generation of these NY8.3 $CD8^+$ T cells in the thymus.

NOD mice deficient in the signaling adaptor protein MyD88, which governs innate immunity, are completely protected from diabetes development. The diabetes protection, in this model, is mediated by altered gut microbiota[2]. However, removing commensal bacteria from MyD88-deficient NOD mice by re-deriving them into a germ-free environment abolishes the disease protection[2]. Marino and colleagues find that diabetes protection in MyD88-deficient NOD mice housed in specific pathogen-free conditions is associated with high concentrations of SCFAs in the circulation[1]. It is worth mentioning that this observation occurs are on a conventional diet. This result is ~~was~~ also observed in wild-type NOD mice, in which diabetes development is negatively correlated with the concentration of SCFAs contributed by the gut microbiota. Taken together, these findings are exciting in indicating the potential to develop a natural therapy that could be used to correct immunological defects that may contribute to disease.

Type 1 diabetes and other autoimmune diseases have polygenic determinants of susceptibility. However, it is clear that the disease onset requires other factors either to initiate or perpetuate the autoimmune process. Studies in identical twins in type 1 diabetes have provided strong evidence for these other factors, as the diabetes concordance rate is less than 50%[3]. While we have learned that environmental factors may interact with genetics and lead to disease onset, there is no clear consensus as to the environmental causes. The gut is an obvious interface with the environment. Indeed, the gut mucosal immune system is vital for the maturation of immune system and the generation of immune tolerance to food and other foreign antigens, via the oral-gastrointestinal route. Research on the microbiota in health and disease has significantly expanded in recent years, including the investigation of the contribution of the microbiota to the pathogenesis of type 1 diabetes. Studies in both diabetic NOD mice and humans suggest differences in the gut microbiota between those that develop diabetes, as compared with those who do not. Such differences include a reduction of diversity in the composition of gut microbiota and this reduction is more evident before the time of diabetes onset[4]. A recent study reported that Russian children, who have a low incidence of type 1 diabetes, have a low abundance of Bacteroides species compared with a high abundance of these bacteria in children from Finland and Estonia, where the incidence of type 1 diabetes is also much higher[5].

Treatment and prevention of this complex autoimmune disease, other than replacement of insulin, has been elusive. A number of strategies for immunotherapy as treatment for type 1 diabetes have now been trialed in individuals who have already developed diabetes, and these include non-antigen-specific therapies that target T or B cells[6]. All of these require intravenous administration and have shown some efficacy in the short term, but for a number of reasons, are not yet viable current treatment options. Antigen-specific therapy has also been attempted, but this approach is still in its infancy[6]. It would be of considerable importance to identify a treatment modality that could be given safely and easily administered, which had a local effect and that could potentially be repeated. Additionally, the possibility of primary prevention of type 1 diabetes with a diet-based treatment would be of considerable interest[7].

The beneficial effects of SCFAs have been known for some time. A number of inflammatory diseases including allergic airways disease[8], and type 2 diabetes[9] can be improved in mouse models by boosting SCFAs. On the other hand, it will be important to ensure that, in reducing inflammation and boosting regulation, treatments do not increase the risk of cancers as a side effect; for example, acetate has been shown to be a good substrate for glucose-starved tumors *in vitro* and in an animal model[10].

Most of the studies showing an immune regulatory effect of SCFAs have concentrated on the induction of regulatory T cells[11]. It is very interesting that Marino and colleagues find that SCFA-enriched diets also have a clear effect on B cells, especially marginal zone (MZ) B cells, which are markedly reduced in number and function. It is not clear whether SCFAs have any effect on plasma cells, as MZ B cells can become long-lived plasma cells that secrete both T-dependent and independent antibodies[12,13]. It would be interesting to know if SCFAs affect mucosal IgA- producing B cells.

Given the current information on the diversity of the gut microbiota, it is unlikely that we will find one therapeutic combination of bacteria for type 1 diabetes that will be universally beneficial. The evidence so far suggests that groups of bacteria have more or less beneficial effects in diabetes and other diseases, and these findings extend the importance of bacteria that produce these SCFA metabolites. What is particularly important here is that these findings, and the observations of others, suggest that understanding which bacteria produce specific classes of metabolites may be more

informative than the individual bacteria themselves. By the same token, identifying the bacterial products that promote diabetes development is also crucial[5,14] as not all gut microbiota are beneficial for the health of the host. However, boosting beneficial bacterial metabolites, including SCFAs, by incorporating them into the diet would be a very attractive strategy. The different but synergistic effects that individual SCFAs have on the immune system would allow for an improved outcome. These beneficial effects, which include reduction in pathogenic cells and an increase in regulatory type cells would be of particular importance, not only for autoimmune diabetes, but potentially other autoimmune diseases, which may occur in part because of deficient function in regulatory cells. However, a cautionary note should be added, as although most of the studies suggest that SCFAs promote immunoregulation and ameliorate autoimmune diseases, SCFAs can also exacerbate some autoimmune disorders[15]. Thus, more work is required to understand the further effects of these short chain fatty acids as provided by diets, both beneficial and potentially deleterious in a human context.

Competing financial interests: The authors declare no competing financial interests.

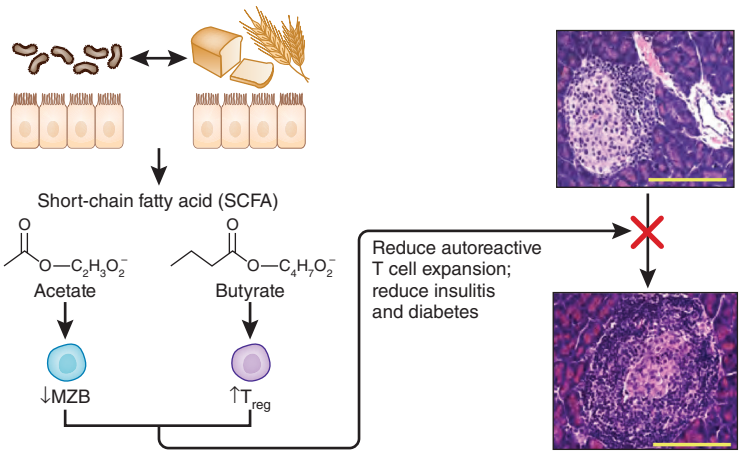

Fig.1. Specialized diets protect against type 1 diabetes.

Diet can affect the composition of gut microbiota and the gut microbiota affect metabolites that can be derived from the diet. High-amylose maize is high-fiber starch and promotes the generation of short chain fatty acids (SCFAs) by gut microbiota in the process of fermentation. Acetate and butyrate are major SCFAs. Diets with acetylated or butyrylated high-amylose maize starch further increase the concentrations of SCFAs in the body of the host. Acetate reduces marginal zone B (MZB) cells, which facilitate the proliferation of islet-autoantigen-reactive T cells, whereas butyrate increases the number and function of regulatory T (T_{reg}) cell. Both of these effects reduce insulitis and diabetes in NOD mice. Hematoxylin and eosin histopathology images (right) from Marino *et al*[1].

References

1 Marino, E. *et al*. *Nat*. *Immunol*. **18**, 18:484-486. doi:10.1038/ni.3730 (2017).

2 Wen, L. *et al. Nature* **455**, 1109-1113, doi:10.1038/nature07336 (2008).
3 Hyttinen, V., Kaprio, J., Kinnunen, L., Koskenvuo, M. & Tuomilehto, J. *Diabetes* **52**, 1052-1055(2003).
4 Kostic, A. D. *et al. Cell Host Microbe* **17**, 260-273,doi:10.1016/j.chom.2015.01.001 (2015).
5 Vatanen, T. *et al.-Cell* **165**, 842-853, doi:10.1016/j.cell.2016.04.007 (2016).
6 Atkinson, M. A., von Herrath, M., Powers, A. C. & Clare-Salzler, M. *Diabetes Care* **38**, 979-988, doi:10.2337/dc15-0144 (2015).
7 Ziegler, A. G. *et al. Mol Metab* **5**, 255-262, doi:10.1016/j.molmet.2016.02.003 (2016).
8 Trompette, A. *et al. Nat Med* **20**, 159-166, doi:10.1038/nm.3444 (2014).
9 Canfora, E. E., Jocken, J. W. & Blaak, E. E. *Nat Rev Endocrinol* **11**, 577-591, doi:10.1038/nrendo.2015.128 (2015).
10 Lakhter, A. J. *et al. J Biol Chem* **291**, 21869-21879, doi:10.1074/jbc.M115.712166 (2016).
11 Zeng, H. & Chi, H. *Trends Immunol* **36**, 3-12, doi:10.1016/j.it.2014.08.003 (2015).
12 Martin, F., Oliver, A. M. & Kearney, J. F. *Immunity* **14**, 617-629 (2001).
13 Song, H. & Cerny, J.-*J Exp Med* **198**,-1923-1935, doi:10.1084/jem.20031498 (2003).
14 Tai, N. *et al. J Exp Med* **213**, 2129-2146, doi:10.1084/jem.20160526 (2016).
15 Mizuno, M., Noto, D., Kaga, N., Chiba, A. & Miyake, S. *PLoS One* **12**, e0173032, doi:10.1371/journal.pone.0173032 (2017).