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LETTER

Comment on: Brugman S et al. (2006) Antibiotic treatment partially protects against type 1 diabetes in the Bio-Breeding diabetes-prone rat. Is the gut flora involved in the development of type 1 diabetes? Diabetologia 49:2105–2108

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To the Editor:

In the recent report by Brugman et al. [1], investigators utilised the Bio-Breeding (BB) rat model for type 1 diabetes to test a hypothesis relating the development of type 1 diabetes to composition of intestinal flora. Support for such a notion can readily be found in an accumulating body of evidence suggesting potential roles of diet, microbial constituents and, more recently, innate immunity in the pathogenesis of type 1 diabetes [2]. Indeed, changes in exposure to some of these parameters over time have been postulated to represent major contributors to the well-described worldwide increase in the frequency of autoimmune and allergic disorders [3]. Furthermore, with a majority of the immune system residing within the gut, a potential role for this organ in modulating the development of type 1 diabetes has been noted [4]. As a consequence, large clinical investigations have been conducted or are ongoing, with a view to (1) ascertaining the ability of oral tolerance to prevent type 1 diabetes, (2) evaluating the relationship between this disorder and coeliac disease, and (3) assessing the impact of diet (e.g. breast feeding, cow's milk, infant cereal, omega 3 fatty acids).

To explain the potential mechanistic relationship between gut immune responses and the development of type 1 diabetes, a series of hypotheses has been generated. These concepts have largely focused on the presence or absence of environmental agents, the timing of their introduction to the body's dietary and/or immune system, and 'leakiness' of the gut to agents capable of inducing autoimmune responses against beta cells, among others. It is important to note that these individual hypotheses need not be mutually exclusive but rather, may be overlapping and synergistic.

To this, we would like to describe the following experience. We recently performed a series of investigations using the non-obese diabetic (NOD) mouse model of type 1 diabetes, in which animals were provided oral doxycycline, an analogue of the antibiotic tetracycline, as a means of inducing (in vivo) the transgenic expression of a variety of experimental compounds under control of a doxycycline-inducible promoter. As part of those efforts, a series of standard NOD mice was provided free access to chow containing doxycycline (200 mg/kg) from 4 to 50 weeks of age. These 'control' animals were monitored on a weekly basis for diabetes development. By a fortunate coincidence, we unexpectedly observed that oral doxycycline delivery had a significant effect on the frequency of type 1 diabetes. Specifically, at the end of this study, the frequency of disease in female NOD mice provided standard chow (i.e. without doxycycline) was significantly higher (75%; 6/8) than in animals provided chow containing doxycycline (20%; 2/10; P=0.05 [two-tailed Fisher's exact test]; Fig. 1). This outcome was not due to the simple factor of chow preference as differences in body weight were not seen between the two groups throughout the period of observation (P=NS).

While much in the way of additional experimentation is required to understand the exact mechanisms underlying this observation (e.g. alterations in gut flora, stimulation of innate immune responses, effect on immune regulation,

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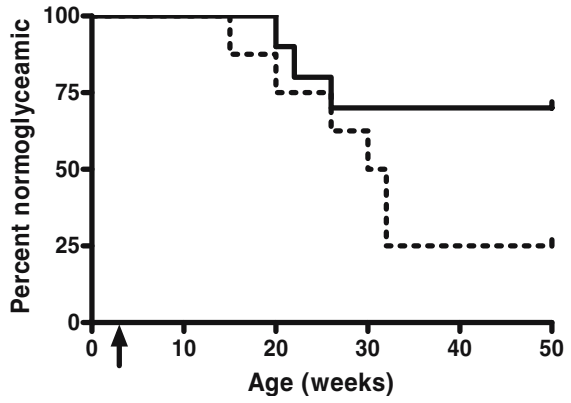

Fig. 1 Life table depicting NOD mice on conventional diet (*dashed line*) or doxycycline 200 mg/kg modified diet (*solid line*). The doxycycline diet was started at 4 weeks of age (*arrow*). The animals were followed for diabetes development until 50 weeks of age, with the presence of blood glucose concentrations >13.2 mmol/l (240 mg/dl) on two consecutive days considered as onset of diabetes

etc.), we believe these observations support the hypothesis put forward by Brugman et al. highlighting the potential significance of antibiotics in modulating the frequency of type 1 diabetes. It is important to note not only that the two model systems are different (i.e. NOD mice versus BB rats), but also, in addition, that we used a single agent in contrast to the antibiotic combination used by Brugman's team.

While experimental confirmation may be a good thing, confirmation in-and-of-itself does not necessarily clarify knowledge, as we not only viewed our initial observations in NOD mice as surprising, but also confusing. Both antibiotic usage [5], as well as the incidence of type 1 diabetes are on the rise. If applied to animal models, one would have presumed that exposure to antibiotics should have accelerated type 1 diabetes, yet in these two studies the opposite was observed. Therein lies a paradox, but one which may provide key information to further understanding the complex linkage between environmental exposures in the gut, their influence on the immune system, and the subsequent development of an autoimmune disease, given a permissive (i.e. at-risk) genetic background. Indeed, additional studies ascertaining the specificity for reduction of faecal flora and/or effects on the immune system should be performed to bring further clarification to this area.

It is important to note that our observations vary, in part, from recent work by Millet et al., where doxycycline treatment did not influence disease outcomes in NOD mice [6]. However, the reported end-point of that observation was 28 weeks of age, and interestingly, we note that the survival curves in our study (Fig. 1) are almost identical with those reported by Millet et al. until that age: only thereafter was the protective effect of doxycycline observed.

These investigations are potentially important on many fronts, not only for the information they could provide as to the observed changes in frequency of type 1 diabetes, but in addition because they identify a possible novel means of disease intervention. An overly permeable gut has been described in BB rats and humans with type 1 diabetes [7]. This physiological environment may allow for translocation of microbes or microbial products, which in turn are responsible for initiating an immune response. Selective decontamination of the gastrointestinal tract may prevent this response in a similar manner in which it prevents translocation of bacteria. Accordingly, we are seeing an increase in investigational efforts involving the use of probiotics [8] to modulate type 1 diabetes. In addition, in the context of antibiotic usage, the identification of unquestionable linkages between an autoimmune disorder, gut flora and antibiotics would add important information to the ongoing debate regarding appropriate versus spurious use of these agents.

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