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Mucosal immunity and type 1 diabetes: looking at the horizon beyond cow's milk

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The etiology of type 1a diabetes (T1aD) is multifactorial with a well-documented genetic component as well as ample evidence for an environmental component. Investigations into the environmental etiologies have studied many diverse exposures that could potentially contribute to the risk of this disease. Among them are gut-related exposures and their subsequent host response. Mucosal immune responses in the gastrointestinal tract are hypothesized to be important contributors to the risk for islet autoimmunity and T1aD. If this proves to be true then effective interventions may be developed that modulate the mucosal immune response in children at risk for type 1 diabetes.

The study published in this issue of *Pediatric Diabetes* (1) investigating antibodies to cow's milk proteins and development of type 1 diabetes touches on a timely theory: that the mucosal immune system of a child who develops type 1 diabetes is systematically different from that of the child who remains disease free. In so doing, the authors raise some points related to this theory that bear discussion such as (i) the role of altered gut permeability in mucosal immunity, (ii) whether or not an aberrant immune response is associated with a specific antigen, a group of antigens found in a specific food, or to general antigenic stimulation, and (iii) the importance of timing of introduction to non-human foods.

The small intestine represents the largest and most extensive interface between the outside world and the internal environment (2). The immune functions of the small intestinal mucosa and submucosa are as varied and sophisticated as would be expected given that the vast majority of antigenic processing and immunologic programming takes place here (3). The physiology of the gastrointestinal immune system and the published evidence have led many to believe that the mucosal immune system holds the etiologic key to type 1 diabetes (4).

In a normally functioning small intestine, the mucosal layer functions as both a physical barrier between the lumen of the intestine and the submucosa, as well as a paracrine tissue that helps orchestrate the physiology of the gut and its immune response (3). When gut permeability is increased as has been shown in the prodrome to type 1 diabetes (5), the integrity of the physical barrier is decreased and therefore the immune system is exposed to more antigens and is possibly more reactive. This increased reactivity may be the result of changes in local signaling, a response to increased antigenic stimulation or a combination of both.

Although this mechanism makes intuitive sense, the question remains as to what incites alterations in gut permeability and an aberrant mucosal immune response. Luopajarvi et al. (1) investigated the potential role that cow's milk has in the pathogenesis of altered gut permeability and subsequent development of T1aD, by characterizing the antibody response to cow's milk proteins in prospectively collected samples in a sub-population of children at increased risk for T1aD who were enrolled in the Trial to Reduce IDDM in the Genetically

at Risk (TRIGR) study. Ninety-four children, all of whom had been exposed to cow's milk (CM) formula before the age of 3 months, were included in this study. The children were followed for 7 yr for the development of diabetes-related autoantibodies and clinical T1aD. The authors found that immunoglobulin A (IgA) antibodies to cow's milk formula at 9 months of age were significantly elevated in children who later developed T1aD compared with those who were auto-antibody negative and disease free at the end of follow up. IgA antibodies to bovine serum albumin also were elevated at 9 months in children who became islet autoantibody positive compared with controls, but they were not elevated in children who subsequently developed diabetes. In the children who later developed T1aD, they also found increased IgG antibodies to bovine lactoglobulin at 6 months of age compared with the other two groups, but not at other time points.

As reviewed by Luopajarvi et al. (1), a number of case-control studies have shown previously that new-onset T1aD patients had elevated levels of antibodies to one or more cow's milk protein compared with healthy children. However, the retrospective nature of these studies made it impossible to determine whether these antibodies were elevated before the diagnosis of T1aD. The current study is the first to examine this question prospectively; and inconsistencies notwithstanding, this is the strongest evidence to date that a dysregulated immune response to milk antigens may precede the development of T1aD.

A remaining question from the case-control studies that the current study (1) could not address fully is whether the enhanced humoral immune response is specific to cow's milk or whether it is a more generalized aberrant response to numerous antigens. The authors attempted to address this question by measuring antibodies to tetanus toxoid in their study subjects. They found lower antibody levels to tetanus in children who later developed islet autoantibodies compared with children who developed T1aD or those who remained autoantibody and disease free. While this was difficult for the authors to interpret, it is possible that this was not actually the most relevant comparison because the toxoid is delivered via an injected vaccine rather than orally and therefore may be measuring a different type of immune response. An analysis of the antibody response to an orally administered vaccine such as the oral polio vaccine may prove more useful to disentangle the differences in antibody responses by different delivery routes.

The mucosal immune response in infancy is likely a function of many factors including dietary exposures, breastfeeding, gut permeability, and genetics (6). The authors of the current study recognized that anomalous immune responses to early exposures are seen in a subgroup of children, but they limit their discussion to cow's milk. It is possible that the immune response to cow's milk is a function of the fact that it is often the first food introduced. A similar response might be expected to any first or early food in individuals who are predisposed to increased immune responsiveness. While the evidence for the elevated immune response seen in children who develop diabetes is compelling, it may be interpreted as an association between increased gut reactivity and the risk for T1aD rather than a risk that is associated with milk antigens specifically.

The already complex interrelationship between gut health, mucosal immunity, and the risk for diabetes may be further modified by the timing of exposure to non-human foods during infancy. The nature of the dietary antigens, the timing of exposure to these antigens, and whether breastfeeding is concurrent at the time of exposure all may play a role in the immune response of individuals.

Four prospective studies of children at increased risk for diabetes have investigated the timing of introduction to milk and foods in the infant diet and risk of islet autoimmunity (7–10). Interestingly, none of these studies found an association between the timing of cow's

milk introduction and risk of islet autoimmunity. And while three of these studies found an increased risk of islet autoimmunity associated with early exposure to foods, the foods themselves differed across studies, with cereals (7), gluten-containing solid foods (8), and fruits, berries and roots (9) all being implicated. These findings may be further evidence that the specific antigenicity of the exposure is less important than the timing of exposure or the immune responsiveness of the individual.

In addition to the sometimes disparate evidence concerning cow's milk, there is evidence that other food antigens such as wheat or gluten are associated with T1aD (11). Investigators have found that antibody production to a wheat storage globulin (Glb1) was higher in T1aD patients compared with their disease-free counterparts (12), and newly diagnosed T1aD patients have enhanced cellular responses to wheat gluten (13). These findings suggest that efforts focused on cow's milk alone (or any other specific food or food antigen) only provide part of the picture.

To thoroughly characterize the association between dietary antigens and the pathogenesis of islet autoimmunity and T1aD, future research should investigate antibodies to dietary antigens from food sources in addition to cow's milk, including cereals [such as wheat (gliadin, Glb1) and oat (avenin)], egg (ovalbumin), and soy (glycinin). If it is consistently found that there is an increased antibody response to a wide range of dietary antigens, it will be further evidence that children who go on to develop T1aD have more reactive gut immune systems. The fact that the authors found antibodies to multiple antigens contained in cow's milk, as opposed to only one that was elevated in future T1aD subjects, suggests that this hyperreactivity is not antigen specific, rather these results would be consistent across a diverse selection of dietary antigens.

Breastfeeding is an essential component of infant diet and important in the normal development of mucosal immunity (14). This is highlighted by the observation that breastfeeding at the time of cereal introduction is associated with decreased risk for islet autoimmunity, regardless of the age at introduction to cereals (7). A similar observation was made for gluten introduction and risk of celiac disease (15).

A generalized humoral response to dietary antigens would be suggestive of increased gut permeability in these children. However, antibody data are only an indirect indicator of gut permeability. To feasibly and directly quantify the degree of gut permeability in populations, identification of minimally invasive biomarkers that reliably reflect the integrity of the gut need to be developed. However, even with recent and future advances in this area, an important caveat remains: can a systemically measured biomarker accurately reflect the local environment in the gut?

The prospective data presented by Luopajarvi et al. (1) suggest that while an aberrant immune response early in life may be important in some cases of T1aD, it does not appear to be important for all diabetes. Identification of genes that regulate or influence gut permeability and/or the mucosal immune response may assist in identifying subgroups at risk for T1aD via a mucosal immunity pathway. And we should continue to investigate other etiologic pathways that lead to T1aD.

The investigation of the role of mucosal immunity in the risk for T1aD is an exciting and expanding area of research. However, as is the case with all relevant research, as new findings are published, more questions arise. If future research determines that mucosal immune responses are important contributors to the risk for T1aD in at least a subgroup of individuals, it is possible that effective T1aD prevention strategies could be developed around strategies to modulating the mucosal immune response.

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