The Gut Immune System and Type 1 Diabetes

OUTI VAARALA

Department of Molecular Medicine, National Public Health Institute, Biomedicum, 00251 Helsinki, Finland

ABSTRACT: Accumulating data suggest that the gut immune system plays a role in the development of autoimmune diabetes: (1) Diet modifies the incidence of autoimmune diabetes and the phenotype of the islet-infiltrating T cells in the animal models of human type 1 diabetes; (2) gut-associated homing receptor β7-integrin is found on the islet-infiltrating T cells in both human type 1 diabetes and in the animal models of autoimmune diabetes; (3) mesenterial lymphocytes from young NOD mice are able to transfer diabetes to healthy recipients; (4) autoantigen feeding modifies the disease development in the animal models (prevents or accelerates autoimmune diabetes). In humans, a link between the gut immune system and type 1 diabetes has also been suggested. Early introduction of cow milk formulas in infancy may increase the risk of type 1 diabetes. We have demonstrated that primary immunization to a β cell–specific autoantigen, insulin, occurs in the gut by exposure to cow milk formulas, which contain immunogenic bovine insulin. The induced antibody and T cell responses to bovine insulin cross-react with human insulin. In children at genetic risk who developed β cell autoimmunity, bovine insulin–binding antibodies increased during follow-up in contrast to autoantibody-negative children. This suggests that insulin-specific immune response induced by dietary insulin may not be controlled in children prone to β cell autoimmunity. The gut immune system has a key role in controlling insulin-specific immunity induced by dietary insulin. Indeed, indications for aberrant function of the gut immune system have been reported in type 1 diabetes, such as intestinal immune activation and increased intestinal permeability. Research on the gut immune system in human type 1 diabetes is needed to reveal the role of oral immunity in this disease.

KEYWORDS: gut immune system; type 1 diabetes; cow milk formula and type 1 diabetes; β cell autoimmunity; insulin

INTRODUCTION

Accumulating data indicate that dysregulation of the gut immune system may play a fundamental role in the development of β cell autoimmunity and type 1 diabetes (see TABLE 1) (reviewed in Ref. 1).

The gut immune system has a dual nature: exposure to oral antigens may lead to tolerance and/or immunization.[2] The induction of tolerance by oral autoantigen

Address for correspondence: Outi Vaarala, M.D., Ph.D., Department of Molecular Medicine, National Public Health Institute, Biomedicum, Haartmaninkatu 8, PL 104, 00251 Helsinki, Finland. Fax: +358-9-4744 8960.
outi.vaarala@ktl.fi

Ann. N.Y. Acad. Sci. 958: 39–46 (2002).

TABLE 1. Evidence for the role of the gut immune system in type 1 diabetes

In patients
Enhanced humoral and cellular immunity to several food proteins
Mucosal homing of lymphocytes derived from human diabetic pancreas
Expression of gut homing receptor on GAD-reactive lymphocytes
Markers of immune activation in the gut immunohistology
Increased permeability of the gut
Association of celiac disease with type 1 diabetes
In animal models
Diet modifies the development of autoimmune diabetes in BB rats and NOD mice
Diets with low diabetogenicity induce Th2 type cytokine profile in the islet-infiltrating T cells
The islet-infiltrating T cells express the gut-associated homing receptor β7 integrin
Mesenterial lymphocytes from a young NOD mice transfer diabetes to healthy recipients
Feeding autoantigen may prevent or accelerate autoimmune diabetes

feeding, such as oral insulin, has been used for the prevention of autoimmune diabetes in animal models.[3,4] However, in some circumstances, oral autoantigen feeding has resulted in the induction of cytotoxic autoreactive T cells and acceleration of the autoimmune diabetes.[4–6] The outcome of oral antigen feeding in experimental autoimmune models is influenced by several confounding factors related to the dose of the antigen, the nature of adjuvants used, and characteristics of the host, such as age and genetic factors. In humans, the mechanisms of oral tolerance are poorly known.

Our studies in the infants at genetic risk of type 1 diabetes indicate that primary immunization to insulin occurs by exposure to dietary cow milk insulin.[7–9] Our findings indicate that the insulin-specific immunity originates from the gut immune system in humans. Accordingly, defects in the development of oral tolerance in the gut immune cells may result in the aberrant immune response to insulin, a β cell–specific autoantigen. In this review, the link between the gut immune system and the development of autoimmune diabetes is suggested based on the studies in animal models of type 1 diabetes and in human type 1 diabetes. Accordingly, an intervention targeted at the gut immune system is proposed as a tempting option for the prevention of type 1 diabetes. However, this depends on the understanding of the pathogenic mechanisms of the gut immune system in type 1 diabetes.

A LINK BETWEEN GUT AND AUTOIMMUNE DIABETES IN ANIMAL MODELS

The following evidence from experimental studies suggests that the gut immune system plays a role in the development of autoimmune diabetes: (1) Diet modifies the development of autoimmune diabetes in BB rats and NOD mice.[10,11] Diet of hy-

drolyzed protein results in a lower incidence of autoimmune diabetes than diet containing whole proteins. Diets with low diabetogenicity induce Th2 type cytokine profile in the islet-infiltrating T cells.[11] (2) The islet-infiltrating T cells express the gut-associated homing receptor β7 integrin, and antibodies that block this receptor or its endothelial ligand MadCAM1 inhibit the development of autoimmune diabetes in NOD mice.[12–14] (3) Autoimmune diabetes has been transferred to the recipients by mesenterial lymphocytes from young NOD mice, indicating that diabetogenic T cells are present in the gut immune system.[15] (4) In some studies, feeding autoantigen induced the development of autoreactive cytotoxic lymphocytes and acceleration of autoimmune diabetes.[4–6] These findings point out that an immunological link exists between the gut and pancreas.

COW MILK AND TYPE 1 DIABETES

In humans, a link between the gut immune system and type 1 diabetes has also been suggested. Epidemiological observations indicate that triggering of the gut immune system, such as by early introduction of cow milk formulas and/or short breast feeding in infancy, increases the risk of type 1 diabetes (reviewed in Ref. 16). Especially, the introduction of cow milk formulas during the period of the first two to three months of life seems to imply an increased risk of type 1 diabetes. In a recent prospective Finnish birth cohort study (DIPP study) the early introduction of cow milk formulas was associated with the risk of the development of β cell autoantibodies in children with genetic risk of type 1 diabetes.[17] In some studies the drinking of cow milk later in childhood has also been associated with the increased risk of type 1 diabetes.[18,19] Without understanding the mechanisms by which an epidemiological risk factor could mediate the disease risk, these associations are only suggestive.

In serological studies on patients with newly diagnosed diabetes, enhanced immune responses to several cow milk proteins have been reported.[20–22] It has been suggested that these responses would be cross-reactive with islet cell antigens and would participate in the autoimmune attack targeted against β cells. The mechanisms of molecular mimicry as a cause of β cell destruction have been suggested for several environmental antigens showing similarity with β cell antigens. Bovine serum albumin (BSA) shares a short sequence similarity with ICA69[19] and β casein with glucose transporter 4.[22] Alternatively, the reported hyperreactivity to cow milk antigens could be considered as a failure of tolerance induction.[21] When immunity to other dietary antigens was studied in patients with type 1 diabetes, antibodies to ovalbumin did not differ from the levels seen in healthy children.[23] Other dietary proteins than cow milk proteins, such as ovalbumin, are started later, after the age of 6 months, when the maturation of the gut is more complete. The maturation of the gut with age modifies the characteristics of the immune response to dietary antigens.[24] In humans, the permeability of the gut is higher during the first two months of life than later, as shown by Kuitunen *et al.*[25] Interestingly, the start of cow milk formula may be associated with the increase of gut permeability in infancy.[25] It is possible that the enhanced immune responses to cow milk proteins in type 1 diabetes can be considered as a marker of poor tolerance development during the first months of life, the time when cow milk proteins are introduced.

IS TYPE 1 DIABETES A DISEASE OF THE GUT IMMUNE SYSTEM?

Some evidence suggests that the function of the gut immune system is aberrant in type 1 diabetes. We have shown that GAD-specific T cells in patients with type 1 diabetes express gut-associated homing receptor α4β7 integrin.[26] A similar kind of α4β7 integrin expression was not present in tetanus toxoid–reactive T lymphocytes that are induced by parenteral immunization. Our finding suggests that autoreactive T cells in type 1 diabetes may recirculate between the gut and the pancreas. Supporting our interpretation, it has been shown that in rotavirus infection the virus antigen–specific T cells express α4β7 integrin.[27] This indicates that oral antigen–specific response resides in α4β7 integrin–expressing T cells; in the same lymphocyte population we showed reactivity to GAD.[26] Our data may indicate that the GAD-reactive T cells are induced in the gut. The occurrence of GAD-specific immune response in the α4β7 integrin–expressing T cells also suggests that tolerance in the gut immune system is broken in type 1 diabetes.

Savilahti *et al*. studied the immunohistology of the intestine in 26 patients with type 1 diabetes, 13 of whom had the HLA DQB1*02 gene and increased risk of celiac disease.[28] Despite the normal villous structure and the density of the intraepithelial lymphocytes, the expression of HLA class II antigens (DR and DP) in the villous epithelium was increased in the patients when compared to controls. Also, the patients had increased intensity of α4β7-expressing cells in the lamina propria. The findings were not restricted to the patients who carried the celiac disease HLA-DQB1*02 risk allele, suggesting that activation of the gut immune system may be associated with type 1 diabetes and does not associate only with the genetic risk allele shared with celiac disease. Besides markers of immune activation, increased intestinal permeability to mannitol has been reported in patients with uncomplicated type 1 diabetes.[29]

IS DIETARY INSULIN THE TRIGGER OF β CELL AUTOIMMUNITY?

Insulin is suggested to be the primary autoantigen in type 1 diabetes. It is the only β cell–specific autoantigen in type 1 diabetes; other autoantigens, such as glutamic acid decarboxylase (GAD) and thyrosine phosphatase (IA-2), are also found in tissues other than β cells. In birth cohort studies, insulin autoantibodies often appear as the first sign of the development of β cell autoimmunity.[30] In NOD mice, insulin-specific T cells represent the majority of T cells infiltrating the islets, and insulin-specific T cells transfer diabetes to healthy recipients.[31,32] In the NOD mouse model, insulin-specific cytotoxic diabetogenic T cells have been demonstrated.[33] All these studies emphasize the involvement of insulin-specific autoimmunity in β cell destruction.

We have demonstrated that primary immunization to a β cell–specific autoantigen, insulin, occurs in the gut by exposure to cow milk formulas, which contain bovine insulin.[7–9] Insulin-specific antibodies and T cells were induced in the infants who carried the diabetes-associated HLA risk alleles. Dietary bovine insulin is immunogenic in humans, since it differs from human insulin by three amino acids. This difference is recognized by immune cells, as previously documented. Treatment of patients with bovine insulin induced high levels of insulin-binding antibodies, which

decreased when porcine or human insulin was started.[34] Transgenic mice, which expressed bovine insulin in their β cells, were tolerant to bovine insulin but developed immunity to human insulin.[35] Recently, von Herrath *et al*. have reported that even one amino acid difference in the insulin molecule may affect its tolerogenic properties.[36] Although the inducer of insulin-specific immunity is bovine insulin, both antibody and T cell responses cross-react with human insulin in the infants exposed to cow milk formulas. Involved in this primary immunization to insulin bovine insulin is a "modified" self-antigen that escapes the neonatal tolerance induced in the thymus. The finding that the induction of autoantigen-reactive immune response occurs in the gut immune system during early infancy emphasizes the role of the gut immune system in the development of β cell autoimmunity.

During follow-up, the insulin-binding antibodies and T cell responses seem to decrease in the majority of the children, which reflects the development of tolerance to dietary antigens.[8] In children who developed diabetes-associated autoantibodies during a 18-month follow-up from birth, bovine insulin–binding antibodies detected by EIA increased.[8] This suggests that an insulin-specific immune response induced in early childhood by dietary insulin may not be controlled in the children who are prone to develop autoimmunity. In these children the population of insulin-reactive lymphocytes may be activated when the autoimmune process is triggered. In this respect the regulation of the gut immune system has a key role in controlling the insulin-specific immunity, and, as I suggest, β cell autoimmunity. The development of a harmful immune response to insulin may be related to the functional abnormalities of the gut immune system in children with type 1 diabetes.

Several environmental factors, such as gut microflora, breast milk–derived factors, and enteral infections, control the development of oral tolerance. These factors may either be protective from autoimmunity by supporting tolerance, or they may enhance autoimmunity by abating oral tolerance to insulin. Enterovirus and rotavirus infections have been associated with the development of islet cell autoimmunity.[37,38] Both viruses replicate in the gut and cause stimulation of the gut immune system. The enteric virus infections induce changes in the gut cytokine environment,[39] such as IFN-γ activation, and may activate the insulin-specific immune cells in the gut. Enteric virus infections may also increase the gut permeability and enhance the immunity to dietary insulin.[40]

CONCLUSIONS

Accumulating data indicate that the dysregulation of the gut immune system plays a fundamental role in the development of β cell autoimmunity and type 1 diabetes. Dietary factors modify the incidence of diabetes and the phenotype of islet-infiltrating lymphocytes in animal models of autoimmune diabetes. The expression of gut-associated homing receptors on T lymphocytes in inflamed islets has been reported in both human and animal studies. Our observation that the primary immunization to insulin occurs in the gut by exposure to dietary bovine insulin in infancy emphasizes the role of the gut immune system in the induction of either tolerance or immunity to β cells. This gut-associated lymphocyte population may later be activated when the autoimmune process against pancreatic islets is triggered. Accordingly, the regulation of the gut immune system has a key role in the controlling the

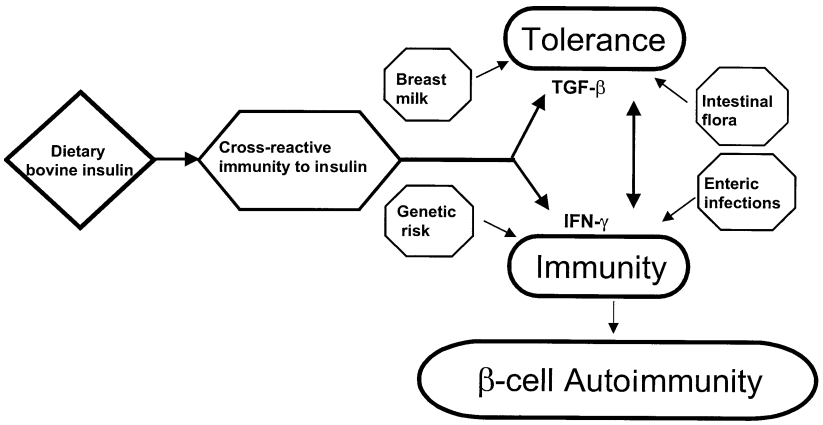

FIGURE 1. The hypothesis that type 1 diabetes is a disease of the gut immune system triggered by dietary insulin is illustrated in this figure. In the hypothesis the primary trigger of insulin-specific immunity is a modified self-antigen, dietary bovine insulin, which breaks neonatal tolerance to self-insulin. The immune response spreads to react with self-insulin. This primary immune response is regulated by the mechanisms of oral tolerance in the gut immune system. Genetic factors control the development of oral tolerance. Also several environmental factors, such as the gut microflora, breast milk–derived factors, and enteral infections, control the development of immune response to insulin. These factors may either be protective from autoimmunity by supporting tolerance, or they may enhance autoimmunity by abating oral tolerance to insulin and switching the insulin-specific immune response towards cytotoxic immunity.

insulin-specific immunity. The identification of the factors that change the balance in the gut immune system—for example, dietary factors, viral infections, and other environmental factors—is the challenge to diabetes research. The hypothesis suggesting that type 1 diabetes is a disease of the gut immune system triggered by dietary bovine insulin is presented in FIGURE 1. The environmental factors that interfere with the function of the gut immune system probably influence the incidence of type 1 diabetes, which shows remarkable variation in different populations at different time periods.

REFERENCES

1. VAARALA, O. 1999. Gut and the induction of immune tolerance in type 1 diabetes. Diabetes Metab. Res. Rev. **15:** 353–361.
2. STROBEL, S. & A. MOWAT. 1998. Immune responses to dietary antigens: oral tolerance. Immunol. Today **19:** 173–181.
3. ZHANG, J.Z. *et al.* 1991. Suppression of diabetes in nonobese mice by oral administration of porcine insulin. Proc. Natl. Acad. Sci. USA **88:** 1052–1056.
4. BERGEROT, I. *et al.* 19994. Oral administration of human insulin to NOD mice generates CD4+ T cells that suppress adoptive transfer of diabetes. J. Autoimmun. **7:** 655–663.
5. BLANAS, E. *et al.* 1996. Induction of autoimmune diabetes by oral administration of autoantigen. Science **274:** 1707–1709.

6. BELLMANN, K. *et al.* 1998. Potential risk of oral insulin with adjuvant for the prevention of type 1 diabetes. A protocol effective in NOD mice may exacerbate disease in BB rats. Diabetes **41:** 844–847.
7. VAARALA, O. *et al.* 1998. Cow milk feeding induces antibodies to insulin in children—a link between cow milk and insulin-dependent diabetes mellitus? Scand. J. Immunol. **47:** 131–135.
8. VAARALA, O. *et al.* 1999. Cow's milk formula feeding induces primary immunization to insulin in infants at genetic risk for type 1 diabetes. Diabetes **48:** 1389–1394.
9. PARONEN, J. *et al.* 2000. The effect of cow milk exposure and maternal type 1 diabetes on cellular and humoral immunization to dietary insulin in infants at genetic risk for type 1 diabetes. Diabetes **49:** 1657–1665.
10. ELLIOTT, R.B. *et al.* 1988. Dietary prevention of diabetes in the non-obese diabetic mouse. Diabetologia **31:** 62–62.
11. SCOTT, F.W. *et al.* 1997. Potential mechanisms by which certain foods promote or inhibit the development of spontaneous diabetes in BB rats: dosage, timing, early effect on islet area, and switch in infiltrate from Th1 to Th2 cells. Diabetes **46:** 589–598.
12. YANG, X-D. *et al.* 1994. A predominant role of integrin a4 in the spontaneous development of autoimmune diabetes in nonobese diabetic mice. Proc. Natl. Acad. Sci. USA 91: 12604–12608.
13. HÄNNINEN, A. *et al.* 1996. Mucosa-associated (beta 7-integrin high) lymphocytes accumulate early in the pancreas of NOD mice and show aberrant recirculation behavior. Diabetes **45:**1173–1180.
14. YANG, X-D. *et al.* 1997. Involvement of β7 integrin and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in the development of diabetes in nonobese diabetic mice. Diabetes **46:**1542–1547.
15. HÄNNINEN, A., I. JAAKKOLA & S. JALKANEN. 1998. Mucosal addressin is required for the development of diabetes in nonobese diabetic mice. J. Immunol. **160:** 6018–6025.
16. ÅKERBLOM, H.K. & M. KNIP. 1998. Putative environmental factors in type 1 diabetes. Diabetes Metab. Rev. **14:** 1–38.
17. KIMPIMÄKI, T. *et al.*. 2001. Short-term exclusive breast-feeding predisposes young children with increased genetic risk of type 1 diabetes to progressive beta-cell autoimmunity. Diabetologia **44:** 63–69.
18. VERGE, C.F. *et al.* 1994. Environmental factors in childhood IDDM. Diabetes Care **17:** 1381–1389.
19. VIRTANEN, S.M. *et al.* 2000. Cow's milk consumption, HLA-DQB1 genotype, and type 1 diabetes mellitus. A nested case-control study of siblings of children with diabetes. Diabetes **49:** 912–917.
20. KARJALAINEN, J. *et al.* 1992. A bovine albumin peptide as a possible trigger of insulin-dependent diabetes mellitus. N. Engl. J. Med. **327:** 302–307.
21. VAARALA, O. *et al.* 1996. Cellular immune response to cow´s milk β-lactoglobulin in patients with newly diagnosed IDDM. Diabetes **45:**178–182.
22. CAVALLO, M.G. *et al.* 1996. Cell-mediated immune response to β casein in recent-onset insulin-dependent diabetes: implications for disease pathogenesis. Lancet **348:** 926–928.
23. SAUKKONEN, T. *et al.* 1994. Children with newly diagnosed IDDM have increased levels of antibodies to bovine serum albumin but not to ovalbumin. Diabetes Care **17:** 970–976.
24. VAARALA, O. *et al*. 1995. Development of immune response to cow milk proteins in infants receiving cow milk formula or hydrolysed formula. J. Allergy Clin. Immunol. **96:** 917–923.
25. KUITUNEN, M., E. SAVILAHTI & A. SARNESTO. 1994. Human alpha-lactalbumin and bovine beta-lactoglobulin absorption in infants. Allergy **49:**354–360.
26. PARONEN, J. *et al.* 1997. Glutamate decarboxylase-reactive peripheral blood lymphocytes from patients with IDDM express gut-specific homing receptor α4β7-integrin. Diabetes **46:** 583–588.
27. ROTT, L.S. *et al.* 1997. Expression of mucosal homing receptor α4β7 by circulating CD4+ cells with memory for intestinal rotavirus. J. Clin. Invest. **100:** 1204–1208.
28. SAVILAHTI, E. *et al.* 1999. Jejuna of patients with insulin-dependent diabetes mellitus show signs of immune activation. Clin. Exp. Immunol. **116:** 70–77.

29. CARRATÙ, R. *et al.* 1999. Altered intestinal permeability to mannitol in diabetes mellitus type 1. J. Ped. Gastroenterol. Nutr. **28:** 264–269.
30. ZIEGLER, A-G., *et al.* 1999. Autoantibody appereance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes. The 2-year analysis of the German BABYDIAB study. Diabetes **48:** 460–468.
31. DANIEL, D. *et al.* 1995. Epitope specificity, cytokine production profile and diabetogenic activity of insulin-specific T cell clones isolated from NOD mice. Eur. J. Immunol. **25:** 1056–1062.
32. WEGMANN, D.R., M. NORBURY-GLASER & D. DANIEL. 1994. Insulin-specific T cells are a predominant component of islet infiltrates in pre-diabetic NOD mice. Eur. J. Immunol. 24**:** 1853–1857.
33. WONG, F.S. *et al.* 1999. Identification of an MHC class I-restricted autoantigen in type 1 diabetes by screening an organ-specific cDNA library. Nature Med. **5:** 1026–1031.
34. KURTZ, A.B., *et al.* 1980. Decrease of antibodies to insulin, proinsulin and contaminating hormones after changing treatment from conventional beef to purified pork insulin. Diabetologia **18:** 147–150.
35. OTTENSEN, J.I. *et al.* 1994. The potential immunogenicity of human insulin and insulin analogues evaluated in a transgenic mouse model. Diabetologia **37:** 1178–1185.
36. HOMANN, D. *et al.* 1999. Insulin in oral immune "tolerance": a one-amino acid change in the B chain makes the difference. J. Immunol. **163:** 1833–1838.
37. LÖNNROT, M. *et al.* 2000. Enterovirus infections as a risk factor for β-cell autoimmunity in a prospectively observed birth cohort: the Finnish Diabetes Prediction and Prevention Study. Diabetes **49:** 1314–1318.
38. HONEYMAN, M.C. *et al.* 2000. Association between rotavirus infection and pancreatic islet autoimmunity in children at risk of developing type 1 diabetes. Diabetes **49:** 1319–1324.
39. TOUGH, D., P. BORROW & J. SPRENT. 1996. Induction of bystander T cell proliferation by viruses and type I interferon in vivo. Science **272:** 1947–1950.
40. JALONEN, T. *et al.* 1991. Increased beta-lactoglobulin absorption during rotavirus enteritis in infants: relationship to sugar permeability. Ped. Res. **30:** 290–293.