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Paratuberculosis and Type I diabetes Is this the trigger?

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Summary Type 1 diabetes mellitus (T1DM) is an autoimmune disease. The etiology of T1DM is incompletely understood but environmental agent(s) are thought to trigger T1DM in the genetically at risk. Exposure to cow's milk early in life is a recognized risk factor in the development of T1DM. *Mycobacterium avium ss. paratuberculosis* (MAP) is the cause of bovine Johne's disease and also is thought to act as an immune antigen in Crohn's disease and other granulomatous diseases. MAP is shed in cow's milk and has been shown to survive pasteurization. Genetic susceptibilities, epitope homologies and epidemiologic studies are presented that support MAP as a causative agent of T1DM in the genetically at risk.

Introduction

The cause of Type 1 diabetes (TIDM) is unsolved. It thought to be caused by a combination of genetic and environmental factors. It is an autoimmune disease in which *T* lymphocytes infiltrate the islets of the pancreas and destroy the insulin-producing beta cell population [1]. This paper postulates a causative role for *Mycobacterium avium* ss. *paratuberculosis* (MAP), acting as an environmental agent that triggers T1DM in the genetically susceptible individual. Three links are offered to support this postulate:

(1) shared genetic susceptibility to both mycobacterial infection and autoimmune diseases, including T1DM;
(2) epitope homologies between mycobacterial elements and pancreatic glutamic acid decarboxylase (GAD);
(3) an alternative interpretation of the epidemiologic findings that launched a large study the Trial to Reduce Type 1 Diabetes in the Genetically at Risk (TRIGR).

MAP

Mycobacterium avium ss. *paratuberculosis* (MAP) is an obligate intracellular organism that causes a transmural enteric granulomatous disease in

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ruminant animals, Johne's disease [2,3]. MAP is also a suspected to have a causative role in Crohn's disease, an enteric granulomatous disease of humans [4—8]. Traditional methods of detecting bacteria, culture and stain, have largely been ineffective in detecting MAP in humans. The bacteria are very difficult to culture and MAP is able to exist in a spheroplast (cell wall-deficient) form in humans [9—11]. The advent of bacterial DNA detection with polymerase chain reaction (PCR) has greatly aided the detection of mycobacteria [12—14] and the DNA of MAP has been found in greater than 90% of biopsy specimens from individuals with Crohn's disease [15]. Employing newer culture methods Naser reported the detection of MAP bacteremia in a substantial number of patients with Crohn's disease. MAP has been found to survive pasteurization in retail milk [16] and cheese [17]. Additionally, MAP has been found in granulomas of sarcoidosis [18].

Shared genetic susceptibilities

Analysis of multiple populations shows that TIDM is increasing at an incidence of 3% per year since 1960 [19]. Historically, genetic association with TIDM has been established for three chromosomal regions: HLA DQ/DR (IDDM1), INS VNTR (IDDM2) [20] and CTLA-4 (cytotoxic lymphocyte antigen-4) [21].

More recently, susceptibility genes for T1DM have been identified to include the NRAMP-natural resistance-associated macrophage protein gene (also known as SLC11A1) [22,23] as well as the VDR gene (vitamin D receptor) [24,25].

NRAMP

NRAMP (natural resistance-associated macrophage protein) is a gene that encodes a divalent cation transporter in phagosomes of macrophages [26]. NRAMP modulates the cellular environment in response to activation by intracellular pathogens by acidifying the phagosome [27]. As such, it plays a role in host innate immunity [28]. Mutation of NRAMP impairs phagosome acidification yielding a permissive environment for the persistence of intracellular bacteria [29].

VDR

In addition to a role in the regulation of bone and mineral metabolism, Vitamin D is a potent modulator of the immune system [30]. Vitamin D activity occurs via the vitamin D receptor (VDR). VDR is part of the steroid receptor super-family and is widely express in many cell types including lymphocytes, macrophages and the insulin producing pancreatic beta-cells [25]. Vitamin D and its receptor, VDR, have been implicated in the genetic pathogenesis of TIDM: VDR gene polymorphisms have been described in TIDM in Taiwanese [31], Indian Asians [32], Germans [33], Spaniards [34], Japanese [35] and Croatians [36]. Additionally, calcitriol — the hormonal form of vitamin D — prevents or markedly suppress experimental TIDM [37]. In addition to T1DM, NRAMP and VDR polymorphisms also confer susceptibility to other autoimmune diseases [25,37], and to infection — most notably, mycobacterial infection [25,38,39].

Molecular mimicry

It has been proposed that epitope homology between infectious agents and host proteins give rise to molecular mimicry that can induce autoimmune disease [40]. Specific to T1DM is that postulate that cross-reactive microbial antigens in a genetically susceptible host is the critical event leading to the autoimmune destruction of insulin-producing beta-cells of the pancreas [41]. Heat shock proteins are a highly conserved group of chaperone proteins expressed in cells exposed to elevated temperatures or other forms of environmental stress. Hsp65 is a heat shock protein that is unique to mycobacteria [42]. There is an important role for heat shock proteins in autoimmunity and infection; glutamic acid decarboxylase (GAD), the prime antigen of Type 1 diabetes, has similar amino acid sequences to Hsp65 and Hsp65 ''should not be completely discarded as having a possible role in the development of Type 1 diabetes'' [43]. In a study of children newly diagnosed with Type 1 diabetes 47/47 were found to respond to mycobacterial Hsp65 [44].

Epidemiologic evidence

T1DM and milk

Several studies indicate an association between early exposure to dietary cow's milk proteins and an increased risk of TIDM [45—47]. These studies have centered around the observation that children at risk for TIDM who were breast fed exclusively for more than six months were less likely to have TIDM later in life than similar risk children who were weaned onto cow's milk-based formula at an ear-

lier age. This observation spawned a large study, the TRIGR study: Trial to Reduce IDDM in the Genetically at Risk [48]. Driving the study is the postulate that there is something about cow's milk protein that is an immunologic trigger for TIDM and that breaking the protein with hydrolysis will eliminate the trigger.

The TRIGR study is an ongoing, 17-country study enlisting 6200 infants who are genetically at risk to develop TIDM. Children weaned early from breastfeeding are randomized into two groups; one receiving traditional cow's milk-based formula and the other receiving formula in which the protein has been hydrolyzed. This is an ongoing study. MAP has been found in infant formula powder [49].

Discussion

This paper postulates that *Mycobacterium avium* ss. *paratuberculosis* (MAP) acts as an immune antigen in the pathogenesis of T1DM. As the link between MAP and Crohn's disease becomes more compelling, MAP is increasingly recognized for its ability to act as an occult antigen. Genetic evidence suggests that there are states of macrophage dysfunction that promote both T1DM and mycobacterial infection. These states can be viewed as templates of macrophage incompetence that individually or in combination allow obligate intracellular pathogens such as MAP to persist and serve as immune antigens. Viable MAP has been found in commercial milk and infant formula. The epidemiologic association of TIDM with early exposure to cow's milk has prompted the large TRIGR study. The hypothesis offered here is that *Mycobacterium avium* ss. *paratuberculosis* acts as an immune antigen, a trigger, of TIDM.

References

[1] Akerblom HK, Vaarala O, Hyoty H, Ilonen J, Knip M. Environmental factors in the etiology of type 1 diabetes. Am J Med Genet 2002;115(1):18—29 [Review].

[2] Harris NB, Barletta RG. *Mycobacterium avium* subsp. *paratuberculosis* in veterinary medicine. Clin Microbiol Rev 2001;14(3):489—512 [Review].

[3] Collins MT, Sockett DC, Goodger WJ, Conrad TA, Thomas CB, Carr DJ. Herd prevalence and geographic distribution of, and risk factors for, bovine paratuberculosis in Wisconsin. J Am Vet Med Assoc 1994;204(4):636—41.

[4] Hermon-Taylor J. *Mycobacterium avium* subspecies *paratuberculosis* in the causation of Crohn's disease. World J Gastroenterol 2000;6(5):630—2.

[5] Greenstein RJ. Is Crohn's disease caused by a mycobacterium? Comparisons with leprosy, tuberculosis, and Johne's disease. Lancet Infect Dis 2003;3(8):507—14 [Review].

[6] Shafran I, Kugler L, El-Zaatari FA, Naser SA, Sandoval J. Open clinical trial of rifabutin and clarithromycin therapy in Crohn's disease. Digest Liver Dis 2002;34(1):22—8.

[7] Ogura Y, Lala S, Xin W, Smith E, Dowds TA, Chen FF, et al. Expression of NOD2 in Paneth cells: a possible link to Crohn's ileitis. Gut 2003;52(11):1591—7.

[8] Torok HP, Glas J, Lohse P, Folwaczny C. Alterations of the CARD15/NOD2 gene and the impact on management and treatment of Crohn's disease patients. Digest Dis 2003;21(4):339—45.

[9] Sechi LA, Mura M, Tanda E, Lissia A, Fadda G, Zanetti S. *Mycobacterium avium* sub. *paratuberculosis* in tissue samples of Crohn's disease patients. New Microbiol 2004;27(1):75—7.

[10] Wall S, Kunze ZM, Saboor S, Soufleri I, Seechurn P, Chiodini R, et al. Identification of spheroplast-like agents isolated from tissues of patients with Crohn's disease and control tissues by polymerase chain reaction. J Clin Microbiol 1993;31(5):1241—5.

[11] Hines 2nd ME, Styer EL. Preliminary characterization of chemically generated *Mycobacterium avium* subsp. *paratuberculosis* cell wall deficient forms (spheroplasts). Vet Microbiol 2003;95(4):247—58.

[12] Lachnik J, Ackermann B, Bohrssen A, et al. Rapid-cycle PCR and fluorimetry for detection of mycobacteria. J Clin Microbiol 2002;40(9):3364—73.

[13] O'Mahony J, Hill C. A real time PCR assay for the detection and quantitation of *Mycobacterium avium* subsp. *paratuberculosis* using SYBR green and the light cycler. J Microbiol Methods 2002;51(3):283—93.

[14] Bull TJ, McMinn EJ, Sidi-Boumedine K, et al. Detection and verification of *Mycobacterium avium* subsp. *paratuberculosis* in fresh ileocolonic mucosal biopsy specimens from individuals with and without Crohn's disease. J Clin Microbiol 2003;41(7):2915—23.

[15] Naser SA, Ghobrial G, Romero C, Valentine JF. Culture of *Mycobacterium avium* subspecies *paratuberculosis* from the blood of patients with Crohn's disease. Lancet 2004;364(9439):1039—44.

[16] Ellingson JL, Anderson JL, Koziczkowski JJ, Radcliff RP, Sloan SJ, Allen SE, et al. Detection of viable *Mycobacterium avium* subsp. *paratuberculosis* in retail pasteurized whole milk by two culture methods and PCR. J Food Prot 2005;68(5):966—72.

[17] Ikonomopoulos J, Pavlik I, Bartos M, et al. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in retail cheeses from Greece and the Czech Republic. Appl Environ Microbiol 2005;71(12):8934—6.

[18] el-Zaatari FA, Naser SA, Markesich DC, Kalter DC, Engstand L, Graham DY. Identification of Mycobacterium avium complex in sarcoidosis. J Clin Microbiol 1996;34(9):2240—5.

[19] Onkamo P, Vaananen S, Karvonen M, Tuomilehto J. Worldwide increase in incidence of type I diabetes — the analysis of the data on published incidence trends. Diabetologia 1999;42(12):1395—403. Erratum: Diabetologia 2000;43(5):685.

[20] Eerligh P, Koeleman BP, Dudbridge F, Jan Bruining G, Roep BO, Giphart MJ. Functional genetic polymorphisms in cytokines and metabolic genes as additional genetic markers for susceptibility to develop type 1 diabetes. Genes Immun 2004;5(1):36—40.

[21] Hornum L, Markholst H. New autoimmune genes and the pathogenesis of type 1 diabetes. Curr Diab Rep 2004;4(2):135—42.

[22] Bassuny WM, Ihara K, Matsuura N, Ahmed S, Kohno H, Kuromaru R, et al. Association study of the NRAMP1

gene promoter polymorphism and early-onset type 1 diabetes. Immunogenetics 2002;54(4):282—5 [Epub 2002 June 14].
[23] Takahashi K, Satoh J, Kojima Y, Negoro K, Hirai M, Hinokio Y, et al. Promoter polymorphism of SLC11A1 (formerly NRAMP1) confers susceptibility to autoimmune type 1 diabetes mellitus in Japanese. Tissue Antigens 2004;63(3): 231—6.
[24] Motohashi Y, Yamada S, Yanagawa T, et al. Vitamin D receptor gene polymorphism affects onset pattern of type 1 diabetes. J Clin Endocrinol Metab 2003;88(7): 3137—40.
[25] Hayes CE, Nashold FE, Spach KM, Pedersen LB. The immunological functions of the vitamin D endocrine system. Cell Mol Biol (Noisy-le-grand) 2003;49(2):277—300 [Review].
[26] Blackwell JM, Searle S, Goswami T, Miller EN. Understanding the multiple functions of Nramp1. Microbes Infect 2000;2(3):317—21 [Review].
[27] Lapham AS, Phillips ES, Barton CH. Transcriptional control of Nramp1: a paradigm for the repressive action of c-Myc. Biochem Soc Trans 2004;32(Pt 6):1084—6.
[28] Wyllie S, Seu P, Goss JA. The natural resistance-associated macrophage protein 1 Slc11a1 (formerly Nramp1) and iron metabolism in macrophages. Microbes Infect 2002;4(3):351—9 [Review].
[29] Hackam DJ, Rotstein OD, Zhang W, Gruenheid S, Gros P, Grinstein S. Host resistance to intracellular infection: mutation of natural resistance-associated macrophage protein 1 (Nramp1) impairs phagosomal acidification. J Exp Med 1998;188(2):351—64.
[30] Zella JB, DeLuca HF. Vitamin D and autoimmune diabetes. J Cell Biochem 2003;88(2):216—22 [Review].
[31] Chang TJ, Lei HH, Yeh JI, et al. Vitamin D receptor gene polymorphisms influence susceptibility to type 1 diabetes mellitus in the Taiwanese population. Clin Endocrinol (Oxf) 2000;52(5):575—80.
[32] McDermott MF, Ramachandran A, Ogunkolade BW, Aganna E, Curtis D, Boucher BJ, et al. Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians. Diabetologia 1997;40(8):971—5.
[33] Pani MA, Knapp M, Donner H, Braun J, Baur MP, Usadel KH, et al. Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans. Diabetes 2000;49(3):504—7.
[34] Marti G, Audi L, Esteban C, et al. Association of vitamin D receptor gene polymorphism with type 1 diabetes mellitus in two Spanish populations. Med Clin (Barc) 2004;123(8):286—90 [in Spanish].
[35] Ban Y, Taniyama M, Yanagawa T, Yamada S, Maruyama T, Kasuga A, et al. Vitamin D receptor initiation codon polymorphism influences genetic susceptibility to type 1 diabetes mellitus in the Japanese population. BMC Med Genet 2001;2:7 [Epub 2001 June 25].
[36] Skrabic V, Zemunik T, Situm M, Terzic J. Vitamin D receptor polymorphism and susceptibility to type 1 diabetes in the Dalmatian population. Diabetes Res Clin Pract 2003;59(1): 31—5.
[37] Deluca HF, Cantorna MT. Vitamin D: its role and uses in immunology. FASEB J 2001;15(14):2579—85 [Review].
[38] Bellamy R. Susceptibility to mycobacterial infections: the importance of host genetics. Genes Immun 2003;4(1):4—11 [Review].
[39] Hill AV. The immunogenetics of human infectious diseases. Annu Rev Immunol 1998;16:593—617 [Review].
[40] Davies JM. Molecular mimicry: can epitope mimicry induce autoimmune disease? Immunol Cell Biol 1997;75(2):113—26 [Review].
[41] Wucherpfennig KW, Strominger JL. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell 1995;80(5):695—705.
[42] Zugel U, Kaufmann SH. Role of heat shock proteins in protection from and pathogenesis of infectious diseases. Clin Microbiol Rev 1999;12(1):19—39 [Review].
[43] Child DF, Williams CP, Jones RP, Hudson PR, Jones M, Smith CJ. Heat shock protein studies in type 1 and type 2 diabetes and human islet cell culture. Diabetic Med 1995;12(7): 595—9.
[44] Scheinin T, Tran Minh NN, Tuomi T, Miettinen A, Kontiainen S. Islet cell and glutamic acid decarboxylase antibodies and heat-shock protein 65 responses in children with newly diagnosed insulin-dependent diabetes mellitus. Immunol Lett 1996;49(1—2):123—6.
[45] Akerblom HK, Knip M. Putative environmental factors in type 1 diabetes. Diabetes Metab Rev 1998;14(1):31—67 [Review].
[46] Gerstein HC. Cow's milk exposure and type I diabetes mellitus. A critical overview of the clinical literature. Diabetes Care 1994;17(1):13—9 [Review].
[47] Gimeno SG, de Souza JM. IDDM and milk consumption. A case-control study in Sao Paulo, Brazil. Diabetes Care 1997;20(8):1256—60.
[48] The TRIGR study. www.trigr.org; 2006 [accessed 4.07.06].
[49] Pavlik I. *Mycobacterium avium* subsp. *paratuberculosis* in powdered infant milk. In: Proceedings of the 8th international colloquium on paratuberculosis. August 2005, Copenhagen, Denmark [in press]. Available from: http://proc8.paratuberculosis.org/index.html.