

HYPOTHESIS

Fluoride toxicity and new-onset diabetes in Finland: a hypothesis

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Key Words

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Abstract

The incidence of type 1 diabetes (T1D) has increased substantially in Finland, but the exact trigger for the onset of T1D is still unknown. We know that use of amoxicillin and anti-cariogenic fluoride tablets is a common practice for children in Finland. It seems that beta-cell destruction is initiated by modification of the proinsulin by combined effects of fluoride (F₂) and amoxicillin. Amoxicillin especially when used together with clavulanic acid results in an acid environment around the beta-cells that promotes the conversion of F₂ to hydrogen fluoride (HF). Unlike F₂, HF can diffuse easily into the beta-cell cytosol. Because the cytosol has a neutral pH, virtually all HF reverts to F₂ in the cytosol and F₂ cannot easily diffuse out of the cell. Exposure to excess F₂ promotes proinsulin covalent dimerization and simultaneously hyperexpression of MHC Class I molecules. Proinsulin dimers then migrate to the cell membrane with MHC class I molecules, accumulate at the beta-cell membrane and produce a powerful immunogenic stimulus for the cytotoxic T-cells. Production of cytotoxic cytokines from the infiltrating T-cells initiates the destruction of beta-cells. In Finnish children, this might be helped along by a higher beta-cell activity and by a reactive thymus-dependent immune system induced by higher levels of thyroid hormones and calcitonin respectively. After repeated similar attacks, more and more effector T-cells are raised and more and more beta-cells are destroyed, and clinical diabetes occurs.

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INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disease wherein insulin-producing pancreatic beta (β)-cells are attacked and destroyed by T lymphocytes [1]. During the natural history of T1D, T-cell activity develops against more and more β-cell epitopes, which is often referred to as antigen spreading [2]. The presence of both effector T-cell reactions and autoantibodies can be detected, however the β-cell destruction is mediated largely by T-lymphocytes [2, 3]. Resting β-cells display less antigenicity and are less sensitive to immune destruction. Growing evidence suggests that the functional state of the β-cells plays a role in the pathogenesis of T1D [4, 5]. They might be especially sensitive to autoimmune diseases due to the fact that these cells open themselves up during the insulin secretion. It might well be imagined that not every single molecule out of several billions produced is totally correct, and therefore could elicit an antigenic reaction. The possible mechanisms behind the β-cell sensitivity as a function of their activity are: increased susceptibility to the toxicity of diabetogenic agents and increased antigen expression in β-cells with high activity which could activate the destruction caused by the immune system [6].

AUTOANTIGEN PRESENTATION TO CYTOTOXIC T LYMPHOCYTES

The destruction of the β-cells is mediated by cellular immune responses [7]. But, the detailed mechanisms of how the autoimmune response is initiated remain unclear. The major cell type that destroys β-cells in T1D is the CD8⁺ cytotoxic T lymphocyte (CTL) that directly recognizes peptide antigens presented by class I major histocompatibility complex (MHC) proteins on the surface of β-cells [8]. T lymphocytes have the unique property of recognizing and responding only to peptide antigens that are present on the surfaces of other cells. Although the expression levels vary, all nucleated cells within the body use class I MHC molecules to present antigen to CTLs. Such antigens are derived from peptides produced by intracellular degradation of target molecules and, by this way, a cell can present to the immune system any marker indicative of abnormal function [9]. In contrast, CD4⁺ T cells recognize antigen only in the context of class II MHC, which is normally expressed exclusively on antigen-presenting cells (APCs). In this case, the antigen is usually derived from the breakdown of proteins that the APCs have endocytosed from their environment [9]. Hyperexpression of MHC class I molecules by β-cells is a feature unique to T1D

whereas increased expression of MHC II molecules have not been seen consistently on β -cells [10]. The hyperexpression of class I MHC by islet endocrine cells in human T1D appears to precede insulinitis [10-13] and insulinitis is never observed in the absence of class I MHC hyperexpression [14]. Hyperexpression of MHC class I molecules on islet cells renders them more susceptible to CTL killing as a result of the increased surface density of autoantigenic peptide with MHC molecules [14]. It has been argued that β -cells might, themselves, represent the source of the signal that results in the hyperexpression of class I MHC antigens within islets [11, 15]. Some class I MHC-binding peptides may be generated by proteolytic enzymes resident in the endoplasmic reticulum (ER) and MHC class I binding to self-peptides occurs within the endoplasmic reticulum [16]. For example, peptides from secretory proteins with hydrophobic signal sequences are often found associated with class I MHC molecules. These proteins bind directly to class I MHC complex in the ER [16]. Class II MHC antigens have been shown to be aberrantly expressed in the pancreas in T1D [10-12, 17] and it occurs after hyperexpression of MHC class I within a given islet [12], but the signal that initiates the aberrant class II MHC expression in β -cells has yet to be determined [18].

PROINSULIN DIMER AS AUTOANTIGEN

We thought that post-translational modifications of β -cell peptides could contribute to the interaction between peptides, MHC molecules and the autoreactive T-cells. In this respect, a conformationally altered form of native proinsulin may play such a role in the T1D process [19-22]. It was suggested that disulfide cross-linked dimers of proinsulin could provide the autoantigenic stimulus, since their abnormal tertiary structure would not be recognized as self by the immune system [23]. Proinsulin is present in a soluble aggregate state in the ER but may form dimers due to abnormalities of microenvironment induced by toxic compounds. Exposure of proinsulin monomers to halogens such as iodine and chlorine was reported to result in disulfide cross-linked dimers [24, 25]. Dimerization, being post-translational and not under direct enzymatic control, would then result in autoantigenicity by virtue of the altered tertiary structure. Dimeric proinsulin would then migrate to pancreatic β -cell membranes together with MHC class I molecules to be presented to cytotoxic T lymphocytes. This abnormal dimer would not be recognized as self by the immune system, triggering a selective destruction of pancreatic β -cells, resulting in T1D [26].

T1D is a complicated disease that is difficult to understand; the question of what causes T1D is still not fully answered [2]. Environmental factors, such as diet,

and toxic compounds may potentially trigger the onset of autoimmune diabetes [9]. It is in good accordance with the partially un-inherited nature of T1D that the incidence of the disease during the last 3-4 decades has increased substantially in Finland; T1D is seen in up to 2% of all individuals during their life-time [2]. This is an unusually high incidence for a potentially deadly disease. However, for the vast majority of T1D patients no direct β -cell toxic compound has been identified yet. In the present study, we present a hypothesis in which multiple pathogenetic factors related to fluoride, amoxicillin, calcitonin, thyroid hormones, β -cell activity and T-cells, act in concert for the development of T1D.

FLUORIDE TOXICITY

Fluoride (F₂) is another halogen like iodine and chlorine and it is used as anti-cariogenic in drinking water, oral tablets and dentifrices [27]. However, chronic exposure to high dose F₂ can result in dental fluorosis [28]. Fluorosis is found in cities with a fluoridated water supply and higher incidence of T1D was reported in a number of these countries [29, 30]. However, in Finland children have fluorosis despite the absence of fluoridated water supply [30]. Use of fluoride tablets is the only significant contributory factor for fluorosis in Finland [30] and fluorosis is more common among children who take amoxicillin during the first 2 years of life [31]. It seems that dental ameloblasts are exposed to an acid environment with the use of amoxicillin [27]. The low extracellular pH surrounding the maturation stage ameloblasts promotes the conversion of F₂ to hydrogen fluoride (HF). Unlike F₂, HF can diffuse easily into the cell cytosol. Because the cytosol has a neutral pH, virtually all HF reverts to F₂ in cytosol and F₂ cannot easily diffuse out of the cell. Over the course of months, the F₂ concentration within an ameloblast could rise to many times that present in the extracellular matrix. Excess F₂ can then compromise the protein synthesis [32, 33], disrupt the export of secretory granules from the ER, and lead to the formation of autophagosomes in cytosol thus generating the clinical manifestations of dental fluorosis [34]. Fluoride was also reported to alter the activity and morphology of pancreatic cells [35-38], resulting in the decrease in insulin secretion and hyperglycemia, thus indicating the diabetogenic effect of fluoride [36].

CONTRIBUTION OF THYROID HORMONES AND CALCITONIN

Increased linear growth, as measured by attained childhood height, is associated with an increased risk for T1D, especially at young ages [39-41]. Rapid

growth observed in infants and young children [42] is partly a continuation of the fetal growth under the effect of thyroid hormones [43]. Growth becomes thyroid hormone dependent immediately after birth [44], and excessive thyroid hormone in this period was reported to enhance body height in humans [45]. Growth velocity in this period is different between populations [46, 47], and Finnish infants showed significantly higher growth rate and higher thyroid hormone serum levels than all other ethnic groups [48], but the differences do not seem to correlate with thyrotropin levels [49, 50]. The control of the thyroid hormone secretion in this period was suggested to come from parafollicular (C) cells which were reported to stimulate the follicular cells in a paracrine way [51], and calcitonin was suggested to be responsible for the population differences in thyroid physiology [52]. Therefore, thyroid hormone dependent rapid growth observed after birth is controlled by calcitonin; and higher thyroid hormone levels and higher growth rate in the early postnatal period seem to result from elevated calcitonin levels in this period [53].

Thyroid hormones increase the rate of absorption of carbohydrate from the gastrointestinal tract and they also accelerate the degradation of insulin [54]. With elevated levels of thyroid hormones, therefore, the blood glucose level rises rapidly after a carbohydrate meal, sometimes exceeding the renal threshold [55]. Higher thyroid hormone levels and higher growth velocity observed in Finnish children may thus result in greater insulin secretion which may increase demands on the beta cells and make the beta cells vulnerable to autoimmune attack. This view supports the overload hypothesis for the onset of diabetes in Finland.

High levels of calcitonin receptor are expressed by normal human T lymphocytes and binding of the receptor with calcitonin leads to proliferation and cytokine production in lymphocytes [56-59]. Serum calcitonin concentration is significantly elevated in the patients with T1D indicating a role of calcitonin in the pathogenesis of diabetes [60-65]. The link between calcitonin and T1D may involve increased numbers of T-cells and higher levels of cytokines secreted by lymphocytes in the pancreatic islets [66].

Previous considerations has led us to suggest that childhood rapid growth in Finland trigger the autoimmunity under the combined effect of thyroid hormone and calcitonin by inducing higher insulin production from the pancreas, which may make the β -cell more active and more visible to the immune system and by inducing proliferation and cytokine production in T-cells already performing autoimmune attack in the islets.

A SCENARIO FOR THE DEVELOPMENT OF TYPE 1 DIABETES IN FINNISH CHILDREN

We suggest that β -cell destruction in T1D progresses through a number of stages:

-Stage 1 is initiated by modification of the proinsulin (dimer formation) by combined effects of fluoride and amoxicillin. Amoxicillin especially when used together with clavulanic acid results in an acid environment around the β -cells that can dip below pH 6. The low extracellular pH surrounding the β -cells promotes the conversion of F2 to HF. Concentration of HF increases as the pH falls. After the pH of the extracellular matrix gets lower than that of the cell cytoplasm, an intracellular-extracellular pH gradient is maintained that continuously drives HF into the cell. Unlike F2, HF can diffuse easily into the β -cell cytosol. Because the cytosol has a neutral pH, virtually all HF reverts to F2 and F2 cannot easily diffuse out of the cell. Over the course of months, the F2 concentration within a β -cell rises to many times that present in the extracellular matrix. Exposure to excess F2 promotes disulfide bond instability thereby allowing the formation of novel disulfide cross-links between two proinsulin molecules in the ER, thus leading proinsulin dimers and simultaneously hyperexpression of MHC class I molecules. The abnormal products of proinsulin then migrate to the cell membrane with MHC class I molecules, and immunoreactivity due to the changed conformation of proinsulin molecules initiates a destructive autoimmune process against the islets. Shedding dimeric proinsulin molecules from beta cells in combination with hyperexpression of MHC molecules is a powerful immunogenic stimulus for the cytotoxic T-cells.

-Stage 2 commences with infiltration of the islets by the activated T-cells. Production of cytokines from the infiltrating cells induces further upregulation of MHC molecules in β -cells. The final stage encompasses autoimmune-mediated destruction of the β -cells by the targeted delivery of cytotoxic cytokines and other mediators. In Finnish children, this might be helped along by high β -cell activity and by a reactive thymus-dependent immune system induced by thyroid hormones and calcitonin respectively. Once the islets have become infiltrated and highly populated with T-cells and macrophages, they communicate via antigen presentation and can, in turn, activate each other via cytokines and direct cell communication through surface receptors. With such a high population of immune cells centered in one distinct area, activation signals can travel fast, initiating a destructive cascade easily. CTLs (CD8+) can directly kill β -cells, whereas CD4+ effector T-cells activated possibly by β -cells initiates the activation of B lymphocytes, thus prompting autoantibody production. At this stage, various amounts of β -cell antibodies are present, but

the process may still be reversible. However, after repeated similar attacks more and more effector T-cells are raised and more and more β -cells are destroyed, and a point of no return is passed. The insulinitis process perpetuates by itself and clinical diabetes will occur. Consequently, Finnish children will have healthy teeth at the expense of T1D.

FUTURE CONSIDERATIONS

T1D seems to develop if all the pathogenetic factors related to fluoride, amoxicillin, thyroid hormones, calcitonin, beta-cell activity and T-cells act in concert to some degree, and that if any of the factors are neutralized, inhibited, or acted against, T1D would not occur. The fluoride compounds in drinking water are completely absorbed from the gastrointestinal tract [67] and while 60% of the absorbed fluoride is retained in adults, this level rises to 80-90% in infants [35]. As a result, drinking water should also be considered as the potential source of fluoride that causes T1D in children. In addition, dental products are other common sources of overexposures today, particularly dentifrices, because of their relatively high fluoride concentrations, pleasant flavors, and their presence in non-secure locations in most homes [68]. Therefore, it should be kept in mind that ingestion of a little standard fluoridated dentifrice by a child delivers enough fluoride to reach the toxic dose.

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COMPETING INTERESTS

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