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Suppression of dendritic cell activation by diabetes autoantigens linked to the cholera toxin B subunit

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ABSTRACT

Antigen presenting cells, specifically dendritic cells (DCs) are a focal point in the delicate balance between T cell tolerance and immune responses contributing to the onset of type I diabetes (T1D). Weak adjuvant proteins like the cholera toxin B subunit when linked to autoantigens may sufficiently alter the balance of this initial immune response to suppress the development of autoimmunity. To assess adjuvant enhancement of autoantigen mediated immune suppression of Type 1 diabetes, we examined the cholera toxin B subunit (CTB)-proinsulin fusion protein (CTB-INS) activation of immature dendritic cells (iDC) at the earliest detectable stage of the human immune response. In this study, Incubation of human umbilical cord blood monocyte-derived immature DCs with CTB-INS autoantigen fusion protein increased the surface membrane expression of DC Toll-like receptor (TLR-2) while no significant upregulation in TLR-4 expression was detected. Inoculation of iDCs with CTB stimulated the biosynthesis of both CD86 and CD83 co-stimulatory factors demonstrating an immunostimulatory role for CTB in both DC activation and maturation. In contrast, incubation of iDCs with proinsulin partially suppressed CD86 co-stimulatory factor mediated DC activation, while incubation of iDCs with CTB-INS fusion protein completely suppressed iDC biosynthesis of both CD86 and CD83 costimulatory factors. The incubation of iDCs with increasing amounts of insulin did not increase the level of immune suppression but rather activated DC maturation by stimulating increased biosynthesis of both CD86 and CD83 costimulatory factors. Inoculation of iDCs with CTB-INS fusion protein dramatically increased secretion of the immunosuppressive cytokine IL-10 and suppressed synthesis of the pro-inflammatory cytokine IL12/23 p40 subunit protein suggesting that linkage of CTB to insulin (INS) may play an important role in mediating DC guidance of cognate naïve Th0 cell development into immunosuppressive T lymphocytes. Taken together, the experimental data suggests Toll like receptor 2 (TLR-2) plays a dominant role in CTB mediated INS inhibition of DC induced type 1 diabetes onset in human Type 1 diabetes autoimmunity. Further, fusion of CTB to the autoantigen was found to be essential for enhancement of immune suppression as co-delivery of CTB and insulin did not significantly inhibit DC costimulatory factor biosynthesis. The experimental data presented supports the hypotheses that adjuvant enhancement of autoantigen mediated suppression of islet beta cell inflammation is dependent on CTB stimulation of dendritic cell TLR2 receptor activation and co-processing of both CTB and the autoantigen in the same dendritic cell.

Introduction

Insulin dependent diabetes mellitus, or Type 1 diabetes (T1D), is the most destructive metabolic disease of children. T1D is caused by autoreactive lymphocyte destruction of insulin-producing islet beta cells of the pancreas (Eisenbarth 1986; Tisch and McDevitt 1996). The progressive loss of islet β-cell function results in an increasing deficiency of insulin production resulting in elevated blood sugar levels (hyperglycemia). The inability to transfer glucose from the blood into the cells of the body results in increased levels of cellular oxidative stress which leads to chronic inflammation throughout the body resulting in an increased and premature risk for secondary neural and circulatory health problems, including amputation of extremities, blindness, heart attack and stroke (Libby et al. 2005). Young T1D patients must inject insulin several times a day for the rest of their lives or risk diabetic shock and death (Lodinova-Zadnikova et al. 2004).

The first step in diabetes mediated breakdown of immunological homeostasis leading to islet β-cell mortality is initiated by autoantigen stimulated maturation of antigen-presenting cells

Abbreviations: DC, dendritic cell; TLR, Toll like receptor; T1D, type 1 diabetes; PMA, phorbol myristate acetate; LPS, lipopolysaccharide.

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(APCs), largely dendritic cells (DC). Maturation of DCs induces the development of autoreactive $CD8^+$, and $CD4^+$ T helper (Th1) cells as well as B cell production of auto-antigen specific antibodies (Atkinson and Maclaren 1994; Han et al. 2005; Tang et al. 2006; Tisch and McDevitt 1996). Following autoreactive $CD4^+$ Th1 cell infiltration of pancreatic islets in NOD mice, autoreactive Th1 lymphocytes were shown to secrete the inflammatory cytokines IFN-gamma and IL-2. These diabetes autoantibodies are known to stimulate macrophage and CTL secretion of oxidative compounds NO, O_2, H_2O_2) in addition to inflammatory cytokines (IL-1 beta, TNF-alpha, TNF-beta, IFN-gamma) (Atkinson and Maclaren 1994; Tang et al. 2006; Han et al. 2005). The persistence of these immune responses induces chronic pancreatic inflammation (insulitis), which results in the induction of apoptosis of approximately 90% of the islet insulin-producing β-cells leading to insufficient levels of insulin production (Piccinni et al. 1998). A variety of immune cell types including B cells, dendritic cells, macrophages and natural killer (NK) cells were shown to be involved in the onset of diabetes pathogenesis (Cardell 2006; Kent et al. 2005; Silveira and Grey 2006; Tian et al. 2006, 2009; Wang et al. 2005; Yoon and Jun 2005). Specifically, dendritic cells (DCs) were shown to play a primary role in antigen priming of naïve T helper cells (Th0) and in the modulation of their development into autoreactive Th1 lymphocytes or immunosuppressive Th2 cells critical for maintenance of immunological homeostasis (Itano et al. 2003; Pulendran et al. 1999, 2001). Immuno-cytochemical analyses showed that oral inoculation results in auto-antigen uptake through M cells of the intestinal epithelium into peripheral DCs via several routes that may aid in the establishment of immune suppression (Yoon and Jun 2005). Autoantigens (AutoAg) are taken up and processed by immature DC subsets (Figdor et al. 2004). Following DC activation by autoantigens, the DCs migrate to adjacent lymph nodes, where they present antigen peptides on MHCII receptors, synthesize co-stimulatory molecules and secrete IL-12 which guides the development of naïve cognate Th0 cells into Ag-specific inflammatory Th1 lymphocytes. In contrast, oral inoculation with small amounts of autoantigen was shown to induce DC production of the anti-inflammatory cytokine IL-10 which stimulates the development of naïve Th0 lymphocytes into anti-inflammatory Th2 lymphocytes, or alternatively into IL-10 or TGF-beta producing $CD4^+CD25^+$ Tr1 or Th3 regulatory T cells (D'Ambrosio et al. 2008; Kapsenberg 2003). Thus, interactions between autoantigens, DCs and T cells in the gut associated lymphoid tissues, may dictate the onset of inflammatory or tolerogenic outcomes following initial autoAg presentation. In addition, DCs residing in lymphoid follicles and the Peyer's patches shown to synthesize IL-10 were found to down-regulate Th1 cell mediated autoimmunity (Steinbrink et al. 1997). Further, immature or peripheral DCs (iDCs), that displayed low levels of co-stimulatory molecule expression and that secreted cytokine IL-10, were shown to remain in the periphery and were found to induce Th2 lymphocyte mediated immunological tolerance (Holmgren et al. 2005; Li et al. 2006; Liu et al. 2001; Rissoan et al. 1999). In an alternative set of experiments, the addition of IL-12 to immature dendritic cells (iDCs), induced autoreactive Th1 cell morphogenesis and accelerated type 1 diabetes in NOD mice (Trembleau et al. 1995). In composite, the available experimental data suggests that initial stages of type 1 diabetes progression may be largely under DC control and may set the stage for anti-inflammatory or inflammatory disease outcomes (Shinomiya et al. 1999).

Oral administration of auto-antigens has shown promise for prevention of spontaneous autoimmune diabetes (Trentham et al. 1993; Zhang et al. 1991). However, the need for repeated autoantigen administration over an extended period of time poses a limitation to such therapy. Further, a low efficiency of immune suppression was reported in previously sensitized hosts (Arakawa et al. 1998a,b). These limitations were largely overcome through application of the non-toxic B subunit of the cholera enterotoxin (CTB) from *Vibrio cholera.* The CTB molecule was shown to be a strong immunomodulator for induction of oral tolerance when used as a carrier molecule for conjugated autoantigens (Sun et al. 1994, 1996, 2000a,b). The bacterial AB enterotoxin from *Vibrio cholerae,* cholera toxin (CTX) contains a toxic ADP-ribosyltransferase subunit A1 (CTA1), linked through a small helical (A2) peptide to a pentamer of non-toxic B carrier subunits (CTB). The CTB subunits were shown to be required for binding the toxin to monosialoganglioside receptor molecules embedded in gut epithelial cell membranes facilitating entry of the holotoxin into the cell (Eriksson and Holmgren 2002). The CTB subunit was shown to bind specifically to GM1-ganglioside, a receptor molecule found in common on the membrane of most types of epidermal cells. Thus, CTB can provide an efficient transmucosal carrier molecule for autoantigen induction of peripheral tolerance (Shreedhar et al. 2003; Sun et al. 1994).

In previous studies, oral delivery of CTB conjugated to specific autoantigens was shown to enhance autoantigen mediated protection of mice against several organ-specific autoimmune diseases including autoimmune encephalomyelitis (Sun et al. 2000a,b) autoimmune chondritis (Kim et al. 2001) and uveitis (Phipps et al. 2003). In addition, CTB-INS conjugates were shown to substantially suppress diabetes in NOD mice (Arakawa et al. 1998a,b; Bergerot et al. 1997). The observed suppression of diabetes onset was associated with a reduction in Th1 cell IFN-γ production and the migration of Tr1 regulatory T cells into pancreatic islets (Aspord and Thivolet 2002; Roncarolo et al. 2001). Further, the fusion of CTB to insulin was shown to provide up to a 10,000-fold reduction in autoantigen amounts required for immuno-tolerization (Arakawa et al. 1998a,b; George-Chandy et al. 2001). Mechanisms underlying CTB modulated immune suppression of T1D may include the inhibition of DC maturation, inhibition of autoreactive T cell development and/or induction of Th2 and regulatory T cell (iTreg) proliferation and activation (Lavelle et al. 2003, 2004; Marinaro et al. 1995).

Recent immunotherapy and vaccination strategies strongly target receptors that mediate immune cell activation. Pathogen recognition receptors, especially APC Toll-like receptors (TLRs) have received increasing attention because activation of innate immunity through pathogen protein, nucleic acid and lipopolysaccharide pattern recognition has been increasingly identified as an essential first line of immunological defense (Hemmi et al. 2000; Schnare et al. 2001; Takeda et al. 2003). In general, TLRs interact with a variety of microbial structures widely expressed by fungi, bacteria, protozoa and viruses conferring a high degree of specificity to the immune response (Takeda et al. 2003). Recent developments support the concept that activation of immunity by microbial molecules may involve cooperative interaction with multiple host receptors within the membrane lipid raft (Beutler et al. 2006; Hoebe et al. 2006; Triantafilou et al. 2002). That is, TLRs are usually present as preformed homodimers with the exception of TLR2 which preferentially forms heterodimers with either TLR1 or TLR6 (Akira and Takeda 2004). Toll like receptors TLR2/TLR1 and TLR2/TLR6 were shown to be activated in response to agonists such as lipoteichoic acid and lipoproteins, while other APC surface and internal TLRs respond to a variety of bacterial and virus DNA and lipopolysaccharide immunostimulatory molecules (Akira and Hemmi 2003; Kanzler et al. 2007; Roger et al. 2005; Takeda et al. 2003). Recently, the type II heat-labile enterotoxin from *Escherichia coli* which has an AB_5 subunit structure similar to cholera toxin was found to stimulate cytokine release in mouse and human cells through interactions with TLR2. However, up to the present, the mechanism of CTB mediated TLR activation is only poorly understood. An increased understanding of the initial interactions between CTB and its fusion proteins with TLRs is predicted to improve our understanding of mechanisms by which these molecules exert their immunomodulatory activities.