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Review

Oxidative stress and coxsackievirus infections as mediators of beta cell damage: A review

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Type I diabetes (T1D) is an autoimmune disease resulting in gradual cell-mediated destruction of insulin producing beta cells in the pancreatic islets of Langerhans. The most plausible environmental triggers to launch and/or accelerate this process in a genetically predisposed organism including enterovirus infections and oxidative stress. Among other enteroviruses the group B of coxsackieviruses is associated with potential beta cell toxicity. Beta cells are weak in antioxidative defense, which makes them hypersensitive to oxidative stress. Acknowledging the inhibitory potential of *in vivo* conditions scientists have developed two models resembling a slowly progressing coxsackievirus infection first, by restricting the production of viral progeny with a selective inhibitor of viral RNA replication and second, by means of lowering the multiplicity of infection. Hydrogen peroxide has been established as the oxidative stressor. Recent studies reflect that a productive CVB-infection results in lytic beta cell death. When pharmacologically restricted by guanidine-HCl, the viability increases dramatically through decreased necrosis and associates with simultaneous stimulation of apoptotic death. In summary, the review introduces potential mechanistic models for enterovirus infections in beta cells.

Key words: Oxidative stress, type 1 diabetes, coxsackievirus infection, beta cells, human leukocyte antigen.

INTRODUCTION

T1D is considered to represent a multifactorial disorder based on genetic predisposition triggered by an environmental factor e.g. virus infection. The effector stage, insulitis, is characterized by gradual invasion of macrophages, T-cells and the produced cytokines and oxidative radicals into the islets (Rasilainen et al., 2004). The pre-diabetic period is usually long and characterized by formation of islet cell autoantibodies even years before the development of diabetic symptoms. Enterovirus infections are epidemiologically linked to the pathogenesis of T1D (Rasilainen et al., 2004). Beta cells exhibit poor intracellular antioxidative capacity, which renders them vulnerable to oxidative stress. Thus, both enterovirus infections and oxdative stress are considered to represent particularly potential triggers and/or accelerators of beta cell destruction**.**

It is generally known that the amount of viral particles needed to establish a local infection is low and all viruses invading a physiological environment face natural resistance by factors of the hosting immune system (Rasilainen et al*.,* 2002). Considering that coxsackievirus infections most often remain subclinical *in vivo*, the progression of infection and amount of virus are possibly limited and submaximal (Roivainen et al., 2000). The present review is a comprehension of information on experi-

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Abbreviations: APC, antigen presenting cell; CAR, coxackie/adenoviral receptor;
CVA, coxackievirus A strain; GAD, glutamic acid decarboxylase; GPx, glutathione peroxidase; HLA, human leukocyte antigen; IFN, interferon; IL-1β, interleukin 1 beta; MHC, major histocompatibility complex; NF-κB, nuclear factor-kappa B transcription factor; NO, nitric oxide; NOD mouse, spontaneously diabetic non-obese; ROS, reactive oxygen species; SOD, super oxide dismutase; TNF – α, tumor necrosis factor alpha.

mental models of slowly progressing enterovirus infection and reports regarding the patterns of mechanisms of virus induced beta cell damage. Further, this scientific report includes: characterizing the mechanisms of oxidative beta cell injury; counteracting it by means of antioxidative agents and studying the effects of virus infection on intracellular redox balance.

Background of type I diabetes (T1D)

Juvenile, insulin-dependent diabetes mellitus (Type I diabetes, T1D) is a consequence of selective autoimmune destruction of the insulin-producing beta cells in the pancreatic islets of Langerhans (Bach, 1994; Schranz and Lernmark, 1998). The resulting insulin deficiency leads to a chronic metabolic derangement associated with significant secondary morbidity. According to the traditional opinion consistent within most autoimmune diseases, both a genetically predisposed individual and a suitable environmental trigger are needed for the destructive process, in the case of T1D against beta cells, to begin. Nowadays, the group of human leukocyte antigen (HLA) genes is considered to account for the major genetic risk and virus infections to represent prime environmental regulator. Various lines of evidence suggest that the autoimmune destruction is launched by a local insult to islet(s) exciting a pool of immune cells, dominantly T-cells and macrophages, to invade the islets resulting in insulitis (Anastasi et al., 2005) Considering the pathogennesis, most results are obtained from studies in spontaneously diabetic non-obese (NOD) mice, whose pathogen-nesis mimics human type I diabetes (Anita et al., 2006). Most probably both cytotoxic (CD8+) and helper (CD4+) T-cells are required for both the primary attack and the later overt destruction, respectively, to progress (Bendelac et al., 1987; Hanafusa et al., 1988; Jarpe et al., 1990; Jarpe et al., 1991; Miller et al., 1988; Sumida et al., 1994; Wicker et al., 1994; Wong et al., 1996). Besides, a pool of antigen presenting cells (APC) is indispensable in the vicinity of pancreatic islets to introduce the islet antigen to the autoreactive T-cells. According to current opinion, programmed cell death (apoptosis) represents the dominant mechanism of beta cell death during immune mediated T1D. The immune reaction related proinflammatory cytokines, especially interferon gamma (IFN-γ), interleukine 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α), are thought to play a primary role in activating signal transduction pathways leading to beta cell dysfunction and apoptosis (Delaney et al., 1997; Mandrup, 1996; Rabinovitch, 1998). The direct killing through the granzyme-perforin pathway by cytotoxic T cells is also believed to be a key mechanism ultimately leading to apoptotic beta cell death.

Finland has the highest incidence of T1D in under 15-year-old children worldwide. It has gradually increased during the last 43 years from 12/100000 (1953) to 45/100000 (1996); the average relative annual increase thus being 3.4 % between 1965 and 1996 (Somersalo, 1955; Tuomilehto et al., 1999). The incidence is not only geographically, but also seasonally distributed peaking in winter (Levy-Marchal et al., 1995). Finland and some other Nordic high-incidence countries have also shown gender-impact with a slight male predominance (Padaiga et al., 1997). Approximately 10% of newly diagnosed T1D patients have an affected first degree relative (Dahlquist et al., 1985; Tuomilehto et al., 1992). The genetic risk and familial clustering of T1D have traditionally been concidered to originate from the class II major histocompatibility complex (MHC) genes on chromosome 6p21, including HLA DP, DQ and DR (Campbell and Trowsdale, 1993; Davies et al., 1994;). These genes encode heterodimeric proteins expressed on APC-cells and have a function in presenting antigenic peptides to CD4+ T cells. HLA class II genes have a high degree of polymorphism and marked linkage disequilibrium that potentiate the risk to form certain high-risk allele-pairs. Furthermore, these susceptibility alleles may then choose potential autoreactive T-cells during thymic sorting, and enhance the disease probability. Many recent studies have demonstrated the DQ-locus to primarily harbor the susceptibility for T1D (Dorman and Bunker, 2000; Heimberg et al., 1992; Thorsby and Ronningen, 1993). Specifically, worldwide approximately 30% of T1D patients are heterozygous for the high risk HLA-DQA1*0501-DQB1*0201 / DQA1*0301-DQB1*0302 alleles (previously referred to as HLA-DR3/4 or HLADQ2/ DQ8). In addition lack of the protective HLA-DQA1*0102-DQB1*0602 allele (HLADR2 or HLA-DQ6) increases T1D susceptibility through potentiating the impacts of predisposing alleles (Pociot and McDermott, 2002). Genes present in the HLA-region, including genes worthy for structure and function of both the HLA-DQ and DR, are considered capable of accounting for less than 50% of the inherited disease risk (Pociot and McDermott, 2002). Largely by means of genome 13 scan dependent linkage studies of affected sib-pair families at least 17 non-HLA T1D susceptibility loci have also been identified (Pociot and McDermott, 2002). Among these, the insulin gene locus (IDDM2) is the strongest and most consistent (Bennett et al., 1995).

Necrosis of cell death and their association to T1D

Various routes and mechanisms have been described for a cell to die. The specific pathway chosen depends largely on the trigger, but the ultimate result may vary according to the cellular capacity of defense and the net status of the host (age, inflammation etc.). A general divi- sion is traditionally done by the resulting morphological characteristics: apoptotic, pyknotic, necrotic (Figure 1).

Necrotic cell death is an accidental, traumatic event always associated with specific pathology. It is an unregulated process and follows no specific pattern. The salient features include cytoplasmic and mitochondrial swelling and breakdown of cell to cell junctions and com-

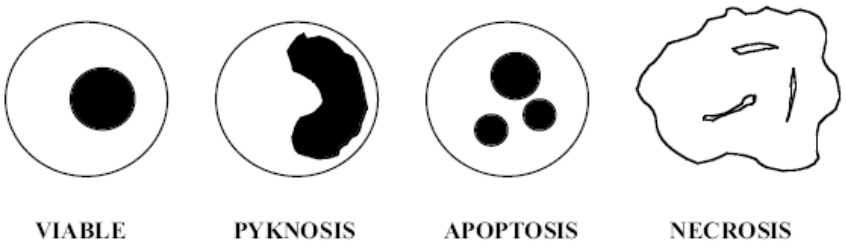

Figure 1. Illustration of nuclear morphology associated with different forms of cell death.

munication that lead to early rupture of cellular membranes and thereafter leakage of the contents to the extracellular space provoking inflammation (Buja et al., 1993; Lemasters, 1999). Considering beta cells, comprehensive studies on cytokine-induced stress in rat islets have revealed nitric oxide (NO) to mediate functional deterioration and mostly necrotic beta cell death (Hoorens et al., 2001). More recently, necrotic beta cell damage was shown to dominate the pattern of spontaneous, NO-induced and streptozotocin (STZ)-induced diabetes in diabetes prone BB rats (Fehsel et al., 2003). Particularly considering cell culture models, environmental factors such as high cell density may also lead the outcome of cell death towards necrosis.

The term apoptosis descends from the *Greek* words: *'apo'* (away) + *'ptosis'* (drop) and characterizes a cellular suicide (Ueda and Shah, 1994). Apoptosis is a physiological, energy-requiring, orderly phenomenon used by multicellular organisms to control the size of cell populations and to eliminate damaged, infected or mutated cells. It is thus important for proper embryonic morphogenesis, development, and adult cellular homeostasis. The apoptotic machinery is constitutively expressed in all nucleated animal cells and is strictly regulated by a set of evolutionarily conserved genes (Horvitz, 1999; Vaux et al., 1992). The apoptotic process can be divided in three stages: a) initiation: a cell receives an apoptotic stimulus; b) execution: enzymatic events, and 3) degeneration: disintergration and elimination of the cell. In generally apoptotic stimuli may activate two different pathways: an extrinsic death-receptor pathway or an intrinsic pathway based on mitochondrial dysfunction (Hengartner, 2000). The extrinsic trail is launched by triggering of a death receptor on cell surface, the most common of which belong to the family of tumor necrosis factor receptors (TNF-R) (Armitage, 1994; Baker and Reddy, 1998). These receptors include a death domain (Tartaglia et al., 1993), which is responsible for the activation of the downstream cascade: the employment of intracellular adapter proteins (FADD, TRADD, RAIDD) and the following activation of caspase (cysteine aspartic acidspecific proteases) 8 and possibly caspase 2 (Alnemri et al., 1996; Ashkenazi and Dixit, 1998). T-cells use alternative extrinsic routes by activating either Fas (discussed later) or granzyme B leading to stimulation of either caspase 8 or the intrinsic pathway (Pinkoski et al, 2001). Commonly the intrinsic route is activated by stress factors including DNA damage, cell cycle perturbation or growth factor deprivation. They engage pro-apoptotic members of the Bcl-2-family (Bak, Bax) to translocate to mitochondria and form pores to permit cytochrome c to release from the mitochondrial intermembrane space and to compose the apoptosome with Apaf-1 and procaspase 9 (Rodriguez and Lazebnik, 1999; Zou et al., 1999;). Recently, a new mitochondrial flavoprotein (apoptosis inducing factor; AIF) was observed to translocate to nucleus in response to e.g. oxidative apoptotic stimuli and launch caspase- (in) dependent chromatin condensation and DNA cleavage (Susin et al., 1999). At the last, both routes activate the effector caspases (3, 6 and 7) that proteolytically activate other downstream caspases (Nicholson and Thornberry, 1997; Slee et al., 2001). Caspase 3 activates DFF/CAD, which results in DNA cleavage to n x 180 bp fragments (Enari et al., 1998; Liu et al., 1997). This step is called 'point of no return', as the proteolytic cascade irreversibly results in to cellular collapse featured by the characteristic morphology: cell shrinkage, chromatin condensation, blebbing of the plasma membrane and formation of apoptotic bodies that become eliminated by neighboring phagocytes (Kerr et al., 1972; Wyllie, 1981).

Apoptotic death mediated by immune effector cells is considered to dominate beta cell death. Several molecular pathways may be activated for this outcome to occur. Macrophages and activated T-cells produce and secrete proinflammatory cytokines, IL-6, IL-2, IL-10, IL-1β, TNF-α and IFN-γ, the last three of which are known for their potential to induce functional and structural beta cell damage and to provoke apoptosis (Liu et al., 2000a; Marselli et al., 2000; Saldeen, 2000; Zumsteg et al., 2000). A recent microarray study on cytokine-stressed rat islets revealed the transcription factor NF-κB to be a core regulator of cytokine induced signaling pathways (Cardozo et al., 2001). By inhibiting its activity the cytokine induced beta cell apoptosis could be prevented (Heimberg et al., 2001). Attracted T-cells express Fas-ligand (FasL) on cell surface, which by binding to the target cell Fas receptor results in apoptosis (Kagi et al., 1994). In spontaneously diabetic NOD mice the proinflammatory cytokines may induce Fas expression in beta cells and thus contribute to beta cell death through interaction with FasL on the sur-

Table 1. Species and subgroup division of enteroviruses.

Species	Subgroup and species
Poliovirus	Polioviruses 1-3
Human enterovirus A	coxsackievirus A 2-8, 10, 12, 14, 16
Human enterovirus B	coxsackievirus A9
	coxsackievirus B 1-6
	echovirus 1-7, 9, 11-21, 24-27, 29-33
	echovirus 69
Human enterovirus C	coxsackievirus A 1, 11, 13, 15, 17-22, 24
Human enterovirus D	echovirus 68, 70

face of the neighboring CD4+ T-cells (Augstein et al., 2003; Nakayama et al., 2002; Petrovsky et al., 2002; Suarez-Pinzon et al., 1999). Also human beta cells in pancreatic biopsies from newly diagnosed diabetics have been reported to express Fas and to contain apoptotic beta cells (Moriwaki et al., 1999). As mentioned earlier CD8+ T-cells, suggested to have a critical role in the early stages of beta cell destruction, may mediate apoptosis also through the perforin-granzyme pathway, dependent on perforin-built channels on target cell membrane allowing granzyme entry and caspase activation (Garcia-Sanz et al., 1987; Kagi et al., 1994; Yoon and Jun, 1999).

Beside the well-known apoptotic and necrotic pathways, a pyknotic form of cell death has also been differentiated. It morphologically resembles apoptosis with an intact plasma membrane, but is characterized by condensed but intact chromatin, unlike the apoptotic fragmented DNA, demarcated at the margins of the nucleus (Agol et al., 1998; Tolskaya et al., 1995). Pyknotic morphology is typically seen in the early phase of a cytocidal virus infection when the first virus-induced alterations in host cell membrane organization and cytoskeleton have on set (Koch and Koch, 1985). This change is often ireversible and results in lytic cell death. A comparable phenomenon has also been observed during an apoptotic process, which due to a sudden ATP depletion or some other disability was interrupted and turned into secondary necrosis (Hirsch et al., 1997; McCarthy et al., 1997).

Enteroviruses

Picornaviruses are a diverse group of small, non-enveloped animal viruses with single-stranded RNA genome of positive polarity ('pico' *Greek*: very small – RNA viruses) (Racaniello, 2001). The first infections, later pinpointed to have been caused by a specific picorna-virus, were described over 3000 years ago (poliomyelitis in a temple record from Egypt ca. 1400 BC). Nowadays they comprise the most common infections of humans in the developed world (Rotbart, 2002). Picornaviruses were previously classified according to their physicochemical properties (particle density, pH-sensitivity) and serological relatedness. More recently, the classification has been based on nucleotide sequence comparisons. The picornavirus genera infecting man include enteroviruses, hepatoviruses, kobuviruses, parechoviruses and rhinoviruses. The enterovirus genus is further divided into five species, which are listed in Table 1 together with the previous subgroups (based on pathogenesis in experimental animals) and serotypes (Hyypiä et al., 1997; King et al., 1999).

Enteroviral genome and viral replication cycle

In order to multiply, the virus must first bind to its specific receptor on the surface of a target cell. Several receptors have been demonstrated for enteroviruses: coxsackieadenovirus receptor (CAR) is recognized by CVB 1-6 and decay-accelerating factor (DAF, CD55) by CVB 1, 3, 5 (Bergelson et al., 1995, 1997, 1998). In addition, CVA 9 has been shown to use alpha v beta 3 integrin, the vitronectin receptor, to penetrate host cells (Roivainen et al., 1994). In studies using neutralizing antibodies, both alpha and beta cells in human islets were observed to express CAR (Chehadeh et al., 2000). After contacting the receptor, the virus should successfully enter the cell, which commonly requires some conformational changes of the viral capsid. During entry or immediately afterwards, the viral genomic material is released into the host's cytoplasm in a process called uncoating (Figure 2). Once released in the cytoplasm, the viral genomic RNA, about 7500 nucleotides in length, functions directly as a template for the production of viral proteins. The single open reading frame encodes a large precursor poly-protein. This precursor includes two proteolytic sequences (2A and 3C), which cleave the precursor into intermediate products P1, P2 and P3 (Kitamura et al., 1981). P1 is further digested into capsid (structural) proteins VP0, VP3 and VP1 (Korant, 1973). P2 and P3 are in the sequential steps cleaved into seven non-structural proteins functioning in the replication and encapsidation of the viral RNA and in the processing of the proteins. One specific product is the virus RNA-dependent RNA polymerase (3D). It duplicate the genomic RNA into a negative sense strand, which then acts as a template for positive -strand

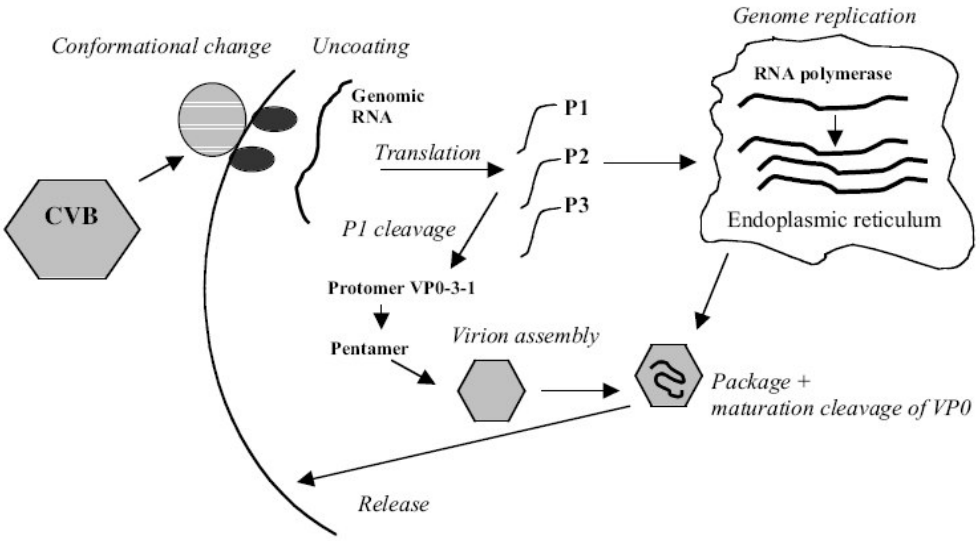

Figure 2. The replication cycle of enteroviruses.

RNA synthesis. Finally, the newly pro-duced genomic material is packaged into the procapsids formed by twelve pentameric structures, each of which contains five protomers (VP0-VP3-VP1 –heterotrimers) (Korant, 1973). During this RNA-encapsidation, the VP0-protein is further cleaved into VP2 and VP4 (maturation cleavage; relevant for enteroviruses, but not for all picornaviruses (Hyypiä et al., 1992; Yamashita et al., 1998)) stabilizing the procapsid.

Host Vs virus infection

As suggested earlier, the outcome of an enterovirus infection may vary between aggressive fulminant and acute subclinical (Ramsingh, 1997b; Yoon et al., 1979). In suitable conditions persistence may develop (Adachi et al., 1996; Chehadeh et al., 2000; Conaldi et al., 1997a; 1997b Klingel et al., 1992; Tam and Messner, 1999). In general, a viral invasion into an organism supplied with a properly functioning immune system always attracts it to respond. The innate arm of immunity composed of natural killer cells, natural (nonspecific) antibodies, the complement system, phagocytes, antimicrobial peptides and interferons (IFNs) together constitute the first line of defense. IFNs may mediate the antiviral response through many routes. Firstly, through binding to type I IFN receptor (Platanias et al., 1996) they stimulate protein tyrosine kinases (JAK 1, TYK2) which further act to stimulate STAT-, Crk- and IRS- pathways (Ahmad et al., 1997; Darnell et al., 1994; Uddin et al., 2000). These routes finally lead to the induction of anti-viral interferon stimulated genes (ISG) (Bose and Banerjee, 2003). Interferons are also able to induce the production of MHC-molecules on virus-infected cells and on antigen presenting cells required for CD4+ T-cell action. Furthermore, they may initiate or contribute to the production of antigen specific antibodies and thus activate the second line of antiviral host defense, the adaptive or cell-mediated arm of immunity (Kadowaki et al., 2000). Recent observation in human islets concluded CVB-infection to stimulate INF-α production selectively in beta cells, which by inhibiting the efficiency of viral replication prevents the infection from disseminating and maintains its persistence (Chehadeh et al., 2000). Furthermore, infection–induced IFN-α has been speculated to participate in the initiation of beta cell autoimmunity (Chakrabarti et al., 1996; Chehadeh et al., 2000; Stewart et al., 1993). It was recently found that efforts of the host to inhibit viral dissemination and persistence often involve induction of apoptosis through either perforin-granzyme or death receptor mediated pathways (Ashkenazi and Dixit, 1998; Froelich et al., 1998). IFNs are reported to sensitize infected cells to apoptosis (Balachandran et al., 2000; Tanaka et al., 1998). As and when beta cells are stimulated with IFNs in combination with double-stranded viral RNA or the cytokine IL-1ß, a synergistic apoptotic effect is observed (Liu et al., 2002). To defend them-selves, many viruses have evolved to counteract the host responses. To secure the replicative potential and production of progeny virions, it is favorable for the virus to maintain the host cell viable and for this purpose many routes of the host signaling machinery may be manipulated. For example, various steps of the IFN cascade may be blocked by several different viruses (Katze, 1995; Komatsu et al., 2000; Munoz-Jordan et al., 2003; Ronco et al., 1998). Also cleavage of p21(ras) GTPase-activating protein (RasGAP) and activation of the MAPK family members ERK 1/2, registered essential for CVB3 replication, could be affected (Huber et al., 1999a; Luo et al., 2002; Opavsky et al., 2002). After stimulation of the innate arm of immunity a cell-mediated immune response is launched.

The dendritic cells, stimulated during the innate res-

ponse by cytokines and the pathogen itself, present antigen to naive T-cells, which start to develop differential markers. Specifically the Th1 type CD4+ T-cells secrete proinflammatory cytokines, which enhance virus-specific host cell lysis by CD8+ T-cells and stimulate the MCH upregulation on APC cells, that further helps in activating antibody production by B-cells. Other interleukines secreted by Th2 type CD4+ T-cells promote this B-cell activation (Salusto et al., 1998). The host responsiveness to virus and the outcome of the infection have been observed to furthermore depend on the status of the host cell cycle. Several studies have revealed actively dividing cells, like T-cells, to be more susceptible to virus and to efficiently produce viral progeny (Liu et al., 2000b; Molina et al., 1992). Quiescent cells (in G0 phase) on the other hand do not support viral multiplication, but may harbor infective viruses thus creating persistence (Feuer et al., 2002). These theories may partially explain the individual susceptibility of cells to infection. Some studies further indicate several viruses capable of forcing the host cell into a favored phase of cell cycle (Op De Beeck and Caillet-Fauquet, 1997; Swanton and Jones, 2001). Viral mRNA, containing an internal ribosome entry site (IRES) instead of the eukaryotic 5' cap, may also redirect the cellular translation pattern and favor viral protein synthesis, particularly in enterovirus-infected cells where cap-dependent translation is specifically inhibited (Kuyumcu-Martinez et al., 2002; Marissen et al., 2000).

Infections and clinical evidence

Enteroviruses are predominantly transmitted by the fecal-oral route into the human body, although transmission via either upper respiratory tract or the conjunctiva of the eye is also possible. They normally replicate in the respiratory and gastrointestinal mucosa, where the infection may remain subclinical or result in mild symptoms. In a proportion of cases, the virus spreads through the lymphatics into the circulation, and after a brief viraemic phase may establish secondary replication sites in specific tissues and organs. Polioviruses may proverbially enter the central nervous system, replicate in the motor neurons and in about 1% of cases with the most virulent strains result in flaccid paralysis (Melnick, 1996). Coxsackie A viruses typically induce diseases with mucosal and skin lesions (e.g. herpangina, hand, foot, and mouth disease) (Bendig and Fleming, 1996; Itagaki et al., 1983; Seddon and Duff, 1971). In addition to the milder disorders, coxsackie B viruses are also associated with more severe and possibly chronic diseases including meningitis, myopericarditis, epidemic pleurodynia and T1D (Beck et al., 1990; Gauntt and Huber, 2003; Muir and van Loon, 1997; Rotbart, 1995; Tracy et al., 2000). They are together with echoviruses responsible for severe neonatal viral infections (Chiou et al., 1998; Sawyer, 1999).

Enteroviruses Vs T1D

Enterovirus infections could initiate or accelerate beta cell damage many years before the clinical onset of T1D. Seroepidemiological studies exist to support this at least after early CVB and rubella exposures (Hyöty et al., 1995; Lönnrot et al., 1998, 2000; Salminen et al., 2003). Some mini case reports show evidence for intrauterine viral exposure, a maternal enterovirus infection, as a risk factor for future development of T1D (Dahlquist et al., 1995, Hyöty et al., 1995). In a larger birth cohort study (Funchtenbusch et al., 2001) contradictory results were obtained. A recent survey reveals that while the incidence of T1D has doubled within the last 40 years in Finland, the incidence of enterovirus infections has simultaneously decreased (Karvonen et al., 1999; Viskari et al., 2002). Despite the fact that the maternal enterovirus antibody levels have decreased (Viskari et al., 2002), the low maternal antibody status could boost the viral exposure of the fetus/newborn and the rarer incidence furthermore exposes the child to catch the infection later, often in the absence of protecting maternal antibodies.

This peculiar phenomenon could partially explain the increase-ing number of patients with type I diabetes and supports the role of enteroviruses as possible triggers. Coxsackie B viruses have long been associated with the pathogenesis of T1D (Andreoletti et al., 1997; Banatvala et al., 1985; Yoon, 1990). Groundbreaking findings were made in 1979. When the autopsy specimens of a 10-year-old patient with diabetes showed lymphocytic infiltration of the islets and beta cell necrosis which lead to the detection of a diabetogenic variant of CVB-4 from the pancreatic cultures (Yoon et al., 1979). This very serotype was further observed to induce T1D in mouse. However, in a series of 88 pancreas-specimens from patients who died soon after onset of T1D, coxsackie-viruses were not detected in the islets (Foulis et al., 1990). Therefore, it should be considered unlikely that an acute CVB infection in pancreatic islets would frequently associate with the onset of T1D. However, this does not exclude the possibility that CVB could have been present in the islets at an earlier stage of T1D pathogenesis. In recent seroepidemiological studies several enterovirus serotypes have been shown to share the association with T1D (Frisk et al., 1992; Helfand et al., 1995; Roivainen et al.,1998; Otonkoski 2000; Vreugdenhil et al., 2000). In fact, it has been proposed that any serotype could potentially possess diabetogenicity. Furthermore, aggre-ssive, cytolytic isolates can develop from originally benign enterovirus serotypes (Roivainen et al., 2002).

Mechanisms of beta cell obliteration

T1D is an immune-mediated disease in which a specific immune response to islet beta cells is induced. In the animal model of T1D, the NOD mouse, diabetes may be transferred only with cells of the immune system. Also in

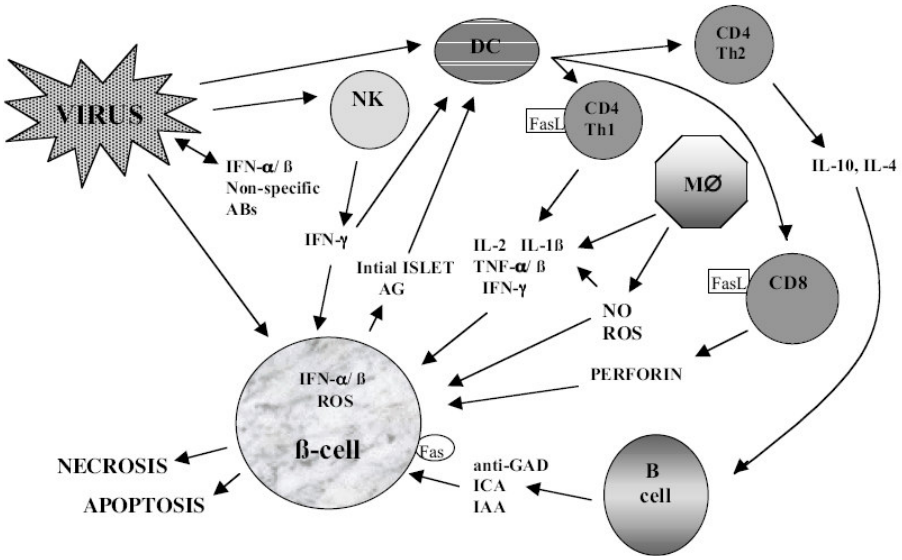

Figure 3. The putative pathways involved in virus induced beta cell death. A potential course of the process: Virus infects the beta cell and stimulates intracellular IFNα/ ß and MHC I production and the innate arm (IFN, nonspecific antibodies and NK cells) of the hosting immune system. The resulting initial beta cell damage provokes the activation and infiltration of macrophages and T cells, which secrete cytokines and finally stimulate antibody production. The produced cytokines, interferons, nitric oxide, ROS and perforin contribute to beta cell death through apoptosis or necrosis.

humans T1D has been adoptively transferred via bone-marrow transplantation from a diabetic donor. However, there are several mechanisms through which a virus could induce immune mediated destruction of islet beta-cells, as shown in Figure 3.

Cell lysis

Lytic cell death is an aggressive, rapid process featured by breakdown of the cell's structural integrity. It equals necrosis, which is used generally to describe cell death of aggressive nature. As mentioned earlier viruses often induce host cell pyknosis in the initial phase of infection (Koch and Koch, 1985), which may in later stages turn into lysis. In a recent study (Roivainen et al., 2002) a large group of prototype enteroviruses from different genetic subgroups were characterized according to their cytolytic activity. Echovirus 6, 7, 11, CVA 13, CVB 1, 3, 4, 5, 6 and poliovirus type 1/Mahoney were detected to cause beta cell lysis. Furthermore an echovirus 9 isolate, from a 6-week-old baby with acute onset T1D was found with destructive features unlike its corresponding prototype virus. These variations of outcomes might have to do with donor-related characteristics, thus the possibility that a certain HLA-type or antibody-positivity could confer resistance or susceptibility for destructive beta cell death remains.

Programmed cell death (Apoptosis)

In order to maintain an efficient multiplication environment, viruses may prevent or delay host cell death through various routes. Especially the abilities to block p53 tumor suppressor -dependent and Bax/Bak/Bik –dependent apoptosis are well characterized (Afonso et al., 1996; Bargonetti et al., 1992; Chen et al., 1996; Henderson et al., 1993; Dobner et al., 1996; Scheffner et al., 1993). On the other hand the same pathways may be used in the opposite direction to induce host cell apoptosis and safely release new viral particles in enveloped bodies to be engulfed by other target cells (Debbas and White, 1993; Sastry et al., 1996; Westendorp et al., 1995).

Additionally, by this mechanism the inflammatory /immune reactions following lytic cell death or free virus exposure are avoided and contact with neutralizing antibodies is prevented, thus potentiating the survival of the virus and uninterrupted dissemination of infection (Gliedman et al., 1975; Jeurissen et al., 1992). Concerning non-enveloped viruses, into which category enteroviruses belong, the mechanisms of exit from a dying host cell are still obscure. Although cell lysis is thought to play a major role, a recent report presents host cell apoptosis as a potential new explanation. It would result in the formation of membrane-bound bodies enabling viral exit (Teodoro and Branton, 1997). In the group of picornaviruses, apoptosis has been extensively studied by the model of CVB3 induced myocarditis, during which persistence often develops and apoptotic myocardial death is evident (Carthy et al., 1998; Huber et al., 1999b). Also poliovirus infection or individual poliovirus proteases alone are reported to be able to induce apoptotic death in selected

cell types (Barco et al., 2000; Girard et al., 1999; Goldstaub et al., 2000; Lopez-Guerrero et al., 2000; Tolskaya et al., 1995).

Considering development of T1D, virus infections are regarded as the leading environmental triggers and apoptosis the leading form of beta cell death (Eizirik and Mandrup-Poulsen, 2001). Their possible association is difficult to study in clinical materials. Experimentally, recent studies on rat islets stressed with synthetic double stranded RNA, a general product of viral replication; reveal enhanced susceptibility to cytokine induced islet cell apoptosis by Fas-FasL interaction (Liu et al., 2002). Virus infections generally induce an inflammatory reaction mediated in part by proinflammatory cytokines. In addition to their direct proapoptotic potential, cytokines may also launch the expression of MHC class I molecules in beta cells followed by their appearance on plasma membranes, which exposes beta cells to T cell mediated death through Fas or perforin dependent pathways (Kim et al., 2002; Seewaldt et al., 2000).

Enteroviruses as key players of autoimmunity in T1D

Molecular mimicry

Molecular mimicry is based on a sequential and structural homology between a foreign antigen and a host protein enabling an immunologic attack against the pathogen to cross-react with the host molecule (Davies, 1997). Glutamic acid decarboxylase GAD65, an enzyme synthesizing the inhibitory neurotransmitter gamma amino butyric acid (GABA), is one of the important islet cell auto-anti-gens in T1D. The sequences of GAD65 and the non-structural protein 2C of CVB-like enteroviruses were shown to share a similar motif (PEVKEK) and to bind the same groove in a MHC-molecule in an exactly similar position (Kaufman et al., 1992). In later studies with synthetic peptides, the binding was restricted to HLA-DR3 molecule (Vreugdenhil et al., 1998). Furthermore, GAD65-reactive T-cells are present and circulating in both T1D patients and healthy normal people, with the difference of functioning like pre-activated memory cells in patients and thus being highly more susceptible to enter clonal expansion. These data have given evidence for molecular mimicry between enteroviral and self-fac-tors as a possible mechanism of beta cell destruction, although contradictory observations have also been made (Atkinson and Maclaren, 1994; Horwitz et al., 1998; Marttila et al., 2001; Schloot et al., 2001). Accordingly, molecular mimicry could also exist between GAD and cytomegalovirus on T-cell level (Roep et al., 2002).

Local inflammatory destruction and bystander progression

The model of locally (intra-pancreatically) launched beta cell autoimmunity and destruction has been widely studied by Horwitz et al. (1999). Their recent report concluded that the primary role of an enterovirus infection (pancreatrophic CVB4) in the pathogenesis of T1D is to specifically infect and damage beta cells leading to release of sequestered islet antigen and stimulation of a local inflammatory response. The intra-pancreatic antigen presenting cells introduce the antigen to the population of resting beta-cell-autoreactive T-cells resulting in initiation of the disease process. Unless a critical threshold level of these precursor autoreactive T-cells exist, the destructive process will not be triggered (Horwitz et al., 1998). They also observed the critical role of beta cell damage as the driving force of virus induced T1D, since a non-specific cytokine-attack did not precipitate T1D in mice with a diabetogenic T cell repertoire, while exposure to the beta-cell toxin streptozotozin was able to do it (Horwitz et al., 2002). CVB infections have previously been reported to lead to necrotic cell death in both rodent and human exocrine pancreatic tissue (Arnesjo et al., 1976; Lansdown, 1976; Ozsvar et al., 1992; Ramsingh, 1997a; Vella et al., 1992). The inflammatory mediators produced during this infective process have been speculated to activate the bystanding beta-cell-autoreactive T-cell pool and thus possibly accelerate T1D development (Serreze et al., 2000).

Oxidative stress

Oxidative metabolites Vs apoptosis

Oxygen derived reactive metabolites (reactive oxygen species, ROS) appear abundantly in the human body (Figure 4). They form as a consequence of incomplete reduction of molecular oxygen by the mitochondrial respiratory (electron-transport) chain (Fernandez-Checa et al., 1998) or through some reactions of cellular metabolism by oxidizing enzymes including xanthine oxidase, P450 mono-oxygenase, cyclooxygenase, lipoxygenase, monoamine oxidase etc (Boveris and Chance, 1973; Forman, 1982; Maeda et al., 1999; Siraki et al., 2002). The most common intracellular ROS molecules include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (-OH) (Fridovich, 1998; DiGuiseppi and Fridovich, 1984). ROS react with biological molecules such as proteins, lipids, carbohydrates and DNA threatening their integrity and exposing the organism to toxic, mutagenic and carcinogenic assaults (Stadtman and Levine, 2000; Steinberg, 1997; Marnett, 2000). Low molecular weight GTPases Ras and Rac1, for example, have been shown to be ROS targets leading to altered signal transduction (Irani et al., 1997; Sundaresan et al., 1996). Except for -OH, which is mostly noxious, ROS also have beneficial functions mainly as stimulators and mediators of intra-cellular signaling cascades (Krieger-Brauer and Kather, 1992; Lo and Cruz, 1995; Ohba et al., 1994; Sundaresan et al., 1995). They also function in microbial killing during infection, for example through inactivating viral enzymes by nitrosylation (Adams et al., 1990; Babior, 1978a,b;

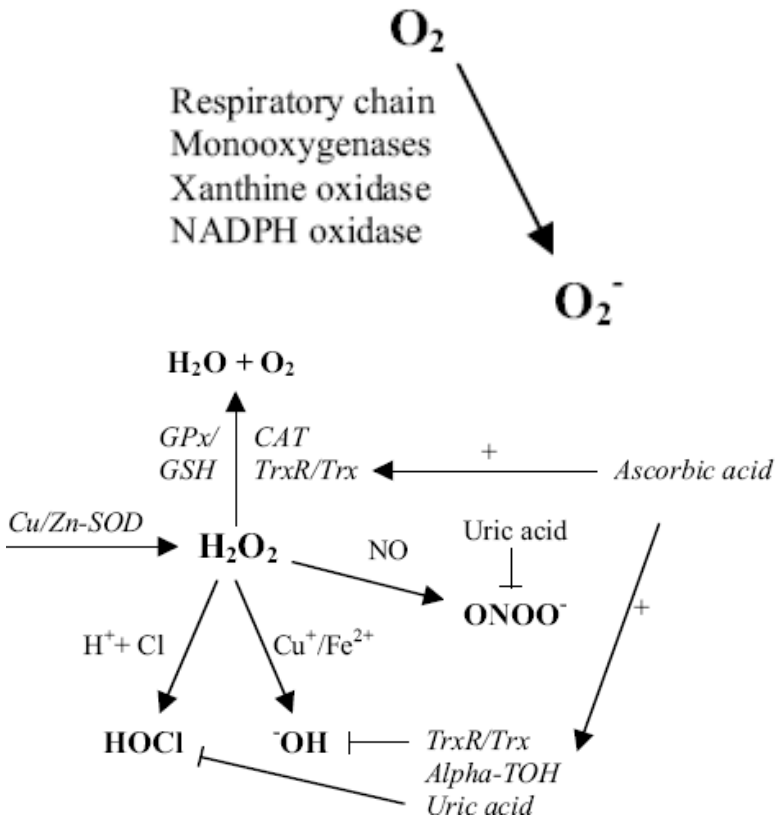

Figure 4. The major pathways of ROS generation and antioxidant defense (modified from Jacobson MD 1996).

Colasanti et al., 1999). Their properties often shift into pathologic in response to increasing concentrations, which might be due to the metabolic state (Di Meo and Venditti, 2001; Gambelunghe et al., 2001), pH, oxygen partial pressure (pO_2) and ADP availability. Very commonly, overproduction of ROS is triggered by nonphysiological states like inflammation or exogenous toxins, which stimulate phagocytic cells to produce ROS. In a variety of disease processes associated with either acute or chronic inflammation, increased oxidative stress appears to be involved. Depending on the surrounding milieu, ROS molecules may also react together. Specifically, in a reaction between O_2^- and NO highly cytotoxic peroxynitrite, $ONOO^-$ is formed (Beckman and Koppenol, 1996), which may further react with carbon dioxide (CO_2) to form peroxocarboxylate ($ONOOCO_2^-$). H_2O_2 may react with $H^+ + Cl^-$ or Cu^+/Fe^{2+} forming either highly reactive HOCl (hypochlorus acid) or a hydroxyl radical, respecttively (Babior, 2000).

Highlighting the dual role of ROS in both physiological and disease states, recent data show concentration dependent stimulation of either cell growth or death. Moderate levels of pro-oxidants may promote mitogenic stimuli (Burdon and Rice-Evans, 1989) e.g. by affecting kinase or proto-oncogene activities (Cerutti and Trump, 1991; Larsson and Cerutti, 1988). At slightly increased levels ROS are reported at various different experimental conditions to be able to induce apoptotic cell death either directly or in combination with antioxidant, mainly glutathione (GSH), depletion (Hampton and Orrenius, 1997; Lennon et al., 1991; Macho et al., 1997). The stimulatory mechanisms often involve exposing to or triggering the mitochondrial membrane permeability transition which leads to the release of apoptogenic factors and thus stimulation of the downstream apoptotic cascade (Armstrong and Jones,2002; Armstrong et al., 2002; Costantini et al., 1996;. Datta et al., 2002; Dypbukt et al., 1994; Fleury et al., 2002; Petronilli et al., 1994; Ueda et al., 2002; Wei and Lee, 2002) Also, HIV-infection mediated apoptosis of CD4+ T-cells is preceded by ROS production and antioxidant depletion. This process has recently been associated with p53, NF-κB and AP-1, pro-apoptotic redox-active factors, that also support expression of viral genes and pro-inflammatory cytokines (Perl et al., 2002). p53, independent of the activating factor, may further stimulate ROS production and induce apoptosis (Sawada et al., 2001; Li et al., 1999).

Moreover, studies evidencing antioxidants' capacity to inhibit apoptosis and recent observations on antiapoptotic molecules with antioxidative properties further argue for a role for ROS in stimulating apoptosis (Kelso et al., 2001; Melnick, 1996; Sato et al., 2002). Additionally, oxidants

may direct an apoptotic process into a necrotic one by inactivating caspases or impairing the mitochondrial energy production resulting in subsequent ATP depletion (Leist et al., 1999; Samali et al., 1999). Although possible in the other direction, overwhelming oxidative stress usually always leads to necrosis leaving no possibilities to inhibit or re-regulate the process towards apoptosis.

Antioxidative system

In an attempt to prevent ROS-mediated damage, cells have developed an antioxidative defense system (Benzie, 2000). The main goal is to maintain the intracellular milieu in a reduced and stable state. This antioxidative machinery consists of several components including heme- or thiol-based enzymatic systems and non-enzymatic scavengers. The most ubiquitous and abundant of them is the glutathione (GSH) system (Anderson, 1998; Deneke and Fanburg, 1989). GSH is formed from the aminoacids glutamate, cysteine and glycine in two ATP-dependent enzymatic reactions by gamma-glutamyl-cysteine synthetase and glutathione synthetase (Griffith and Mulcahy, 1999). GSH itself functions mainly as a sulfhydryl buffer and helps to detoxify xenobiotics in conjugation reactions catalyzed by glutathione S-transferase. Most importantly glutathione peroxidases, selenocysteine-containing enzymes, use GSH as substrate in the elimination of e.g. hydroxyl radical, peroxynitrite, hydroperoxides and reactive electrophiles. In these reactions two GSH-molecules are oxidized to GSSG, which is converted back to reduced GSH in an NADPH-dependent reaction either by glutathione reductase or the thioredoxin system, described below. Furthermore, as a thiol-containing reductant, GSH maintains so-called thiol-enzymes in their catalytically active forms, and low molecular weight antioxidants, vitamins C and E in their biologically active forms (Fridovich, 1999; Havivi et al., 1991; Livingstone and Davis, 2007). Other major antioxidants include catalase (CAT) and superoxide dismutase (SOD). As referred to by its name, the latter inhibits ROS-mediated damage by scavenging O_2^- (Fridovich, 1995, 1999). Two isoenzymes exist: an essential mitochondrial Mn-SOD and a less essential cytosolic Cu/Zn-SOD. They metabolize two O2 $^-$ molecules to O_2 and H_2O_2; thus another ROS is formed. The general and ubiquitous mechanism to remove H_2O_2 is by peroxisomal heme-containing CAT, which converts two H_2O_2 molecules to O_2 and 2 x H_2O (Kirkman and Gaetani, 1984; Kirkman et al., 1999). Moreover, CAT detoxifies e.g. phenols and alcohols via coupled reactions with H_2O_2. Another thiol-containing and ubiquitous reducing enzyme system is the thioredoxin (Trx) machinery. Trx functions as a hydrogen donor for other catalytic enzymes (e.g. glutathione peroxidase) and reduces disulfide bonds of diverse proteins (Holmgren, 1984). It protects particularly against peroxide induced stress (Nakamura et al., 1994; Spector et al., 1988) and has also been reported to have anti-apoptotic power (Saitoh et al., 1998). Like the GSH system, oxidized Trx is converted back to reduce form by a flavoenzyme thioredoxin reductase (TrxR) in an NADPH-dependent reaction (Holmgren, 1985). In addition to Trx, TrxR reduces lipid peroxides, diverse antioxidative selenium containing compounds and converts vitamin C back to its active reduced form.

A distinct, non-enzymatic group of antioxidants of low molecular weight include vitamins E and C, several selenium-containing compounds, lipoic acid, and ubiquinones. Ascorbic acid (AA; vitamin C) and alpha-tocopherol (alpha-TOH; vitamin E) constitute the major water-soluble and lipid-soluble small antioxidants, respectively (Buettner, 1993). As alpha-TOH reduces e.g. peroxyl radicals by its OH group and converts into tocopheroxyl (chromanoxyl) radical, vitamin C, as its main function, reduces it back to alpha-TOH, the active vitamin E (Sies et al., 1992). Additionally, vitamin C acts as an electron donor for some transmembrane enzymes with oxidoreductase activity (May et al., 1995). As previously mentioned, TrxR reduces oxidized vitamin C (Mendiratta et al., 1998), but its major recycler is GSH (May et al., 1996). Heme (Fe protoporphyrin IX) is an integral protein to life delivering oxygen into cells. It circulates incorporated in hemoproteins and occurs unbound (free) only in pathologies sometimes associated with tissue accumulation. Free heme as such is a powerful oxidative molecule and thus a system for its degradation exists. It consists of an oxidative stress–inducible protein HO-1 (heme oxygenase-1, HSP32) and the constitutive isozyme HO-2, which catalyze the oxidation of heme to biologically active molecules: free iron, a gene regulator (Ferris et al. 1999), biliverdin, an antioxidant and carbon monoxide, a heme ligand (Maines, 1997). Uric acid is produced in liver and represents the end product of the purine metabolism reaction chain. The enzyme xanthine oxidase catalyses the last two reactions from hypoxanthine through xanthine to uric acid as byproducts of which two molecules of H_2O_2 are created. Uric acid is considered a powerful antioxidant (Ames et al., 1981) especially because of its potency in peroxynitrite scavenging (Balavoine and Geletii, 1999; Regoli and Winston, 1999; Whiteman et al., 2002) but also in its ability to chelate transition metal ions and stabilize reactive hypochlorus acid and hydroxyl radical (Becker, 1993). On the other hand, its increased production indicates increased H_2O_2 production, which may further react with peroxynitrite and form new aggressive oxidative metabolites (Santos et al., 1999; Skinner et al., 1998; Vasquez-Vivar et al., 1996) including alloxan and a nitrite derivative capable of NO release. These both are known for their direct or indirect beta cell toxicity (Lenzen and Panten, 1988). NO may further stimulate peroxynitrate production by reacting with superoxide, thus contributing to beta cell injury (Suarez-Pinzon et al., 1997, 2001). However, there is evidence that uric acid might further react with and scavenge also these newly formed radicals (Kooy et al., 1994; Whiteman and Halliwell,

1996), which implies to a dual role for this molecule in controlling/affecting the cellular redox status.

Redox status in beta cells Vs T1D

It is well recognized that type 2 diabetes is a progressive condition with insulin production tending to fall with time as the beta cell mass fails and the cells synthesize less insulin. Hyperglycaemia is recognized to have toxic effects on beta cell, so-called 'glucotoxicity' leading to reduced insulin gene expression, impaired insulin secretion and ultimately cell death (Robertson, 2004). Recently it has been hypothesized that chronic oxidative stress as a consequence of hyperglycaemia is an important mechanism for glucotoxicity (Robertson et al., 2003). The mechanisms whereby glucose can lead to the production of relative oxidative species (ROS) in beta cells are well defined. Beta cells appear to be vulnerable to oxidative stress as they contain relatively low levels of glutathione peroxidase (GPx) and other protective enzymes compared to other cells (Grankvist et al., 1981). Moreover, studies on pancreatic islets and beta cell lines observed the cells to be incapable of increasing their antioxidant enzyme expression in response to cellular stress induced by exposure to glucose (Tiedge et al., 1997). There is evidence that ROS are also involved in the pathogenesis of peripheral insulin resistance (Haber et al., 2003). Nitrosative stress is also thought to contribute to the pathogenesis of beta cell apoptosis (Bast et al., 2002). Over time these factors increase the burden on beta cells, promoting the development of diabetes.

Nevertheless, cellular damage in diabetes is associated with various biochemical pathways. There is some evidence of trace element and vitamin deficiencies in diabetes which may contribute to beta cell damage (Aruoma, 1998; Havivi et al., 1991), concomitant with oxi-dative mechanisms participating in the pathogenesis of both beta cell destruction at the prediabetic period and of vascular damage and endothelial dysfunction during the development of further disease complications. According to recent knowledge, the latter are mostly due to increased mitochondrial production of superoxide anion stimulated by prolonged hyperglycemia (Nishikawa et al., 2000). The earlier mentioned NF-κB, an important mediator of cytokine induced pathways of beta cell damage, is also redox-sensitive and assumably also activated by the minor amounts of ROS produced at the initial stage of beta cell destruction. NF-κB is further reported to increase the expression level of iNOS (among other genes) and then through NO-signaling to down regulate the expression levels of beta cell specific genes including Pdx-1, Glut-2, and Isl-1 and also to stimulate the production of cytokines and chemotactic agents amplifying the production of ROS. This cascade then leads to beta cell dysfunction and death through apoptosis or necrosis depending on the overall stress (Cardozo et al., 2001; Ho and Bray, 1999). A chain of events resembling this is also considered to characterize insulitis, during which T-cells and macrophages invade the islets and act as sources of inflammatory mediators and ROS (Rabinovitch et al., 1996b). Many studies have discovered the general antioxidative capacity to be defective in diabetic patients (Maxwell et al., 1997; Santini et al., 1997; Tsai et al., 1994). Depending on the design of the study, decreased levels of vitamin C, vitamin E, uric acid, GSH and GSH-related enzymes have been detected in diabetics compared to control subjects (Courderot-Masuyer et al., 2000; Hoeldtke et al., 2002; Jain and McVie, 1994; Marra et al., 2002; Maxwell et al., 1997; Ruiz et al., 1999; Seghieri et al., 1998; Seghrouchni et al., 2002; Sharma et al., 2000). Controversial results also exist and imply the need for critical evaluation of the markers measured to assess oxidative stress (Leinonen et al., 1998; VanderJagt et al., 2001; Vessby et al., 2002). Importantly, beta cells themselves have a poorer antioxidative defense system in comparison to other cell types. Specifically, the expression levels and activities of antioxidants are low and the adaptive properties to increase antioxidant enzyme production during stress are limited, thus rende-ring beta cells extremely vulnerable to oxidative damage (Grankvist et al., 1981; Lenzen et al., 1996; Tiedge et al., 1997). Further evidence for these defects have been obtained from substitution studies on insulin producing cells showing effective protection against ROS- or cytokine mediated cell death by a cocktail of antioxidants namely CAT+Gpx+SOD (Loetz et al.,2000; Tiedge et al., 1998). Beta cells' own properties are also considered to affect their survival, exemplified by exaggerated ROS-sensitivity during low glucose levels and slow mitochon- drial metabolic rate. Thus, normal kinetics of glucose metabolism may protect beta cells against ROS-induced damage (Pipeleers et al., 2001).

The role of Trx has been studied in nonobese diabetic mice overexpressing Trx specifically in beta cells. The incidences of both spontaneous autoimmune and drug-(streptozotocin, a ROS generating agent) induced T1D were reduced (Hotta et al., 1998). Also after major injury (partial pancreatectomy) or transplantation, antioxidative molecules have been shown to increase beta-cell survival through e.g. attenuated apoptotic beta cell death and increased viability (Ribeiro et al., 2003; Laybutt et al., 2002; Bottino et al., 2002; Gunther et al., 2002; Pileggi et al., 2001). Overall, the existing data indicates oxidative molecules to be important mediators of beta cell damage and thus suggests a role for antioxidant supplementation in diabetes. Due to the complexity and non-specificity of the machinery controlling redox status, this balance is of crucial importance.

Interaction between oxidative stress and Virus infections:

Malnutrition is known to result in defective and inefficient antioxidative capacity and aggravated exposure to oxida-

tive stress triggered by various pathogens, e.g. viruses (Sofic et al., 2002). According to the traditional view, malnutrition could predispose an individual to infections by weakening the host's immune system and thus allowing the pathogen to multiply and disseminate in the organism. Today it is generally known that many virus infections (e.g. influenzavirus induced pneumonia, HIV, coxsackievirus myocarditis) exert many kinds of oxidative stress in the host (Maeda and Akaike, 1998; Schwarz; 1996; Xie et al., 2002b). Phagocytes become activated and produce ROS (Peterhans et al., 1987) and pro-oxidative cytokines (TNF-γ, IL-1β, which further poten-tiate oxidative and other viral damage through e.g. increased virus multiplication, impaired mitochondrial function and reactive iron accumulation (Polla et al., 1996; Schulze-Osthoff et al., 1992; Schreck et al., 1992; Klempner et al., 1978). Various studies have demonst-rated the effect of these cytokines to provoke ROS, especially NO, production within the islets, both in macrophages and beta cells (Rabinovitch and Suarez-Pinzon, 1998; Eizirik et al., 1996; Rabinovitch et al., 1996a; Mandrup-Poulsen et al., 1990). Similarly, the pathogenesis of influenza virus induced pneumonia has been reported to be mostly due to IFN-γ mediated NOS and iNOS activation resulting in NO and further peroxy-nitrate production (Akaike et al., 1996). The previously mentioned antioxidant-substitution studies confirm ROS production to mediate IL-1β+ TNF-α + IFN-γ stimulated beta cell damage (Lortz et al., 2000). These interactions are further strengthened by the knowledge of NF-κB as a mediator of both ROS and cytokine induced apoptosis and the fact that also ROS mediate p53- dependent apoptosis, the pathway commonly used by viruses (Datta et al., 2002; Armstrong et al., 2002). The exposure to radical stress is further intensified by virus-mediated impairment of host's antioxidative defenses through decreasing concentrations of several ROS scavengers (Allard et al., 1998; Bannister et al., 1986; Hennet et al., 1992; Staal et al., 1992; Xie et al., 2002a). Many *in vitro* studies have characterized this phenomenon and observed reparative effects by supplementing antioxidants. *In vivo* the effect of antioxidant therapy is usually tested as a supplement to some specific antiviral treatment, because of the antioxidants weak efficiency alone. In some combinations, antioxidative supplement has resulted in improved outcome of the specific therapy for influenzavirus and HIV infections, although the specific mechanisms of protection still remain poorly understood (Allard et al., 1998; Oda et al., 1989). Concerning the immune system, ROS may act as immunomodulators allowing or activating T-cell proliferation, an essential phenomenon in cell-mediated immune response. This idea is based on observations on the ability of several antioxidants to directly inhibit T-cell proliferation or the activation of transcription factors involved in T-cell activation (Chaudhri et al., 1986; Hunt, 1994). On the other hand, the fatality of HIV infection lies on the exact opposite: increased apoptosis of CD4+ T-cells potentiated or possibly even triggered by the changed redox status (Banki et al., 1998; Romero-Alvira and Roche, 1998).

At last but not least, it has been shown that the interacttions extend further malnutrition and deficiency of antioxidants are capable of affecting not only the host, but also the pathogen by increasing its virulence. In a series of experiments Beck et al. (1997) first observed that an avirulent strain of CVB3 (3/0), which did not cause any damage in normally fed control mice, established moderate myocardial lesions in both selenium and vitamin E deficient mice. When this avirulent strain was further passaged in a selenium or vitamin E deficient mouse and then reinoculated into a control mouse, severe myocardial damage was provoked. This was shown to be due to six point mutations in the viral genome, which changed an originally avirulent strain into virulent (Beck, 1997). As an example of another virus, ROS are reported to activate the binding of NF-κB to the viral promoter region of HIV resulting in increased viral replication and production of Tax protein. Tax again stimulates NF-κB (Baruchel and Wainberg, 1992) thus creating a vicious cycle for the benefit of the dissemination of the infection.

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