Beta cell DNA damage response promotes islet inflammation in type 1 diabetes

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Abstract

Type 1 diabetes (T1D) is an autoimmune disease, where pancreatic beta-cells are destroyed by islet infiltrating T-cells. While a role for beta-cell defects has been suspected, beta-cell abnormalities are difficult to demonstrate. We show a beta-cell DNA damage response (DDR), presented by activation of the 53BP1 protein and accumulation of p53, in biopsy and autopsy material from recently diagnosed T1D patients as well as a rat model of human T1D. The beta-cell DDR is more frequent in islets infiltrated by CD45+ immune cells, suggesting a link to islet inflammation. The beta-cell toxin streptozotocin (STZ) elicits DDR in islets, in vivo and ex vivo, and causes elevation of the pro-inflammatory molecules IL-1β and Cxcl10. Beta-cell specific inactivation of the master DNA repair gene Ataxia Telangiectasia Mutated (ATM) in STZ-treated mice decreases the expression of pro-inflammatory cytokines in islets and attenuates the development of hyperglycemia. Together, these data suggest that beta-cell DDR is an early event in T1D, possibly contributing to autoimmunity.
Introduction

Type 1 diabetes (T1D) is a metabolic disorder that develops following an autoimmune attack on pancreatic beta-cells. Emerging evidence suggests that beta-cell defects may have a causal or enabling role in the development of T1D. Analysis of islets from recently diagnosed T1D patients revealed that many islets retain an intact structure with minimal evidence of immune cell infiltration, and preservation of as much as 36% of beta-cell mass at disease onset [1]. Beta-cell dysfunction in such patients may therefore result from a functional defect, rather than only immune destruction [2,3]. However, there is currently scarce evidence for early beta-cell defects in T1D, in part due to limited access to material from recently diagnosed patients.

We have previously reported that metabolic stress, driven by hyperglycolysis, activates a DNA damage response (DDR) in beta-cells, in mouse models as well as in patients with type 2 diabetes (T2D) [4]. While the findings suggested DNA damage as a novel player in beta-cell dysfunction, the functional impact of this phenomenon remains unclear. Genetic disruption of some DDR components in mice does impact beta-cell biology [5]. For example, mutation in DNA ligase 4 led to spontaneous beta-cell apoptosis and insulin dependent hyperglycemia [6]. On the other hand, mice deficient for the Ku70 DNA damage sensor showed increased beta-cell proliferation and were hypoglycemic [7]. In addition, a recent study has suggested that genetic polymorphisms in genes participating in DNA double strand break repair (XRCC4 and DNA ligase 4) may predispose to beta-cell dysfunction in T1D [8]. Given this body of evidence and the established, bidirectional links between DNA damage and the immune system activation in cancer [9], we set out to assess the beta-cell DNA damage response in recent onset human and rodent T1D, and the potential contribution of DDR to islet inflammation.

Methods

Immunostaining

Sections from formalin-fixed, paraffin embedded donor-derived pancreata were obtained from the nPOD repository, the DiViD study and the tissue bank of Dr. Alvin Powers at Vanderbilt University (including non-diabetic age matched controls). Full details on all donors are given in Supplemental Table S1. Paraffin sections were rehydrated, and antigen retrieval was performed in a decloaking chamber (Biocare Medical) in 50mM citrate buffer pH=6. The following primary antibodies were used: Guinea Pig anti-insulin (1:400; DAKO), Mouse anti-glucagon (1:200; Abcam), Rabbit anti-53BP1 (1:200, Bethyl), Mouse anti-γH2AX ser139 (1:3000, Millipore), Mouse anti-CD45 (1:100, DAKO), rabbit anti-phosphorylated-Kap1 (1:100, Bethyl), Rabbit anti-p53 (1:400, Novocastra), Rat anti-CD3 (1:300, Millipore), and Rabbit anti human Growth Hormone (1:200, Abcam). Fluorophore conjugated secondary antibodies used were Donkey anti Guinea Pig alexa-488, Donkey anti Rabbit Cy3/Cy5 and Donkey anti Mouse Alexa488/Cy3 (Jackson Laboratories). DAPI (Invitrogen) was used as a nuclei marker.
HRP conjugated secondary antibody was Donkey anti-Rabbit (Histofine, Nichirei Biosciences). Diaminobenzidine (DAB, Lab Vision) was used as chromogen. Fluorescent images were taken on a Nikon C1 confocal microscope at 400× magnification. Bright field images were taken on Olympus BX53 at 400× magnification. Image quantification was performed using the ImageJ software.

Statistics

Data was analyzed using a paired two tails Student’s T-Test (for two group comparisons), two-way ANOVA for repeated measurements and Log-Rank for Kaplan-Meier analyses. Data was processed using Microsoft Excel 2010. Graphs were generated using GraphPad Prism 5.0 and Excel. Throughout the work, error bars represent 1 standard error of the mean [10].

Animal studies

Animal experiments were approved by the Institutional Animal Care and Use Committee of the Hebrew University. The Hebrew University is an AAALAC International accredited institute. NOD mice were purchased from Harlan. Floxed Atm mice [31] and Rip-Cre mice [12] were purchased from the Jackson Laboratories and housed in the Hebrew University SPF facility. The genetic background was mixed ICR and SV129. For the ex vivo and STZ-caffeine experiments, we used 2 months old male ICR mice purchased from Envigo. STZ (Sigma) was dissolved in citrate buffer (pH=4.5) immediately prior to injections and was administrated intraperitonealy for five consecutive days to mice that were starved for 4-6 hours prior to injection. Different STZ doses were tested, selected doses were the highest that did not result with hyperglycemic mice during injection days (30mg/kg for females and 20mg/kg for males). Acute single dose STZ was administered at 200mg/kg. Caffeine (Sigma) was dissolved in saline (50mg/Kg) and administered I.P. along with STZ (20mg/kg). Irradiation of mice in a confined Perspex cage was performed in a DBX 6X machine (Varian) with an Eclipse 13.0 TPS system. Serum insulin was measured using an ELISA kit (Crystal Chem). DNA damage response in LEW.1AR1-iddm rats [13-15] was assessed using archived histological material.

Mouse islets

Islet isolation was performed as described [16]. Briefly, mice were euthanized and the bile duct was clamped at the duodenal entry. Collagenase P (0.4mg/ml, Roche) was perfused into the bile duct. Pancreata were then removed and incubated for 15 minutes at 37°C. Samples were washed with RPMI medium (Gibco) and were then loaded on Ficoll (1.119g/ml density) and a layer of Ficoll (1.079g/ml Ficoll) was added above. Samples were centrifuged at 1,250xG for 20 minutes. Islets floating in the upper phase were pipetted out of the gradient, washed in RPMI and manually picked under a binocular. For ex vivo experiments, islets were kept in RPMI media. Islets were treated with STZ (5mM, Sigma), NCS (0.5µg/ml, Sigma) and the ATM inhibitor KU55933 (10µM, Biovision) or Mouse TNFα (aa 80-235,1000 IU/ml, R&D systems).
Human islets

Live human islets from pancreata of brain-dead subjects were obtained from Prodo Laboratories Inc., providing islets isolated from donor pancreases obtained from deceased individuals with research consent from Organ Procurement Organizations (OPOs) or from the Clinical Islet Transplant Program, Alberta Health Services, Edmonton, Canada, as previously described(45), under approval of the Health Research Ethics Board of the University of Alberta and following informed consent. Islets were cultured in PIM (Prodo) or CMRL (Edmonton) media and were treated similarly to mouse islets. Subject details are presented in Supplementary Table S1.

DNA and RNA analyses

DNA was produced with a Qiagen DNeasy Kit. DNA PCR reactions were performed with GoTaq Green master mix on a PTC-200 thermal cycler (MJ Research). DNA primers sequences are: ATM 57F3GCTAAGCCTGACCACCTGAG, ATM58F3CTTTAATGTGCCTCCCTTCG, ATM58R3GAAGGATCTTCCCCTGTTCA, Cre F-TGCCACGACCAAGTGACAGC, Cre R-CCAGGTACGGATATAGTTCATG. PCR products were electrophoresed on 2% agarose (Invitrogen) gels and were imaged with ethidium bromide (Amresco) and were documented using a UVP BioDoc-It Benchtop UV transilluminator.

RNA was isolated with Trizol (Invitrogen). Reverse transcription was performed with the qScript kit (Quanta). Quantitative PCR was performed on a CFX384 thermal cycler with PerfeCTa SYBR (Quanta). Primer sequences for qPCR are listed in Supplemental Table S2.

Results

DNA damage response in beta-cells shortly after T1D diagnosis

To explore changes in beta-cells during the development of human T1D, we used histological specimens from the pancreata of donors with a different duration of T1D. We assessed the DNA damage response in samples from T1D-prone, auto antibody-positive individuals (AAB+); recently diagnosed T1D patients (1 to 9 weeks from diagnosis, median 4 weeks); patients with established T1D (only samples in which insulin-positive cells could still be observed, 0.6-32.5 years from diagnosis); and healthy donors serving as controls. The recently diagnosed T1D group included material from cadaveric donors obtained from nPOD (n=3, diagnosed around one week prior to death) [17], and, importantly, samples taken from pancreatic tail resections of 6 live patients included in the DiViD study [1,18]. The latter are of exceptional histological quality, as the time from tissue sampling to fixation was minimal. However, we were able to detect significant numbers of beta-cells only in 4 of these samples. The clinical data of all donors is summarized in Table 1 and in Supplemental Table S1.
We first examined the staining pattern of 53BP1, an established marker of the DDR which is recruited to sites of double strand breaks in the DNA [19]. We determined the staining pattern of 53BP1 in beta-cells of mice exposed to high dose, sub-lethal gamma irradiation (5 Grey) 8 hours prior to sacrifice. As shown in Figure 1A and Supplemental Figure S1A, beta-cells of irradiated mice showed a typical accumulation of 53BP1 in a single, large nuclear focus. This pattern is reminiscent of the large nuclear foci previously reported to mark unreparable DNA damage (13). These findings support the idea that the 53BP1 foci mark the cellular DDR and their presence indicates the presence of DNA double strand breaks.

While most beta-cells in human samples showed a diffuse pattern of nuclear 53BP1, a minority showed a single large nuclear focus of 53BP1 like those observed in mice after gamma irradiation (Figure 1A). We quantified the effect, by scoring the fraction of insulin-stained cells that have a large 53BP1 nuclear focus (Figure 1B). Compared with the healthy control group (nuclear foci in 0.658±0.15% of beta-cells), we found a significant increase of the 53BP1+ fraction in both the AAB+ group (1.52±0.15% of beta-cells, p<0.01) and in the established T1D group (1.536±0.28%, p<0.05).

Remarkably, samples from recently diagnosed T1D patients showed the most significant elevation in the proportion of beta-cells that scored positive (2.99±0.52%, p<0.0001), suggesting that the beta-cell DDR may peak near diagnosis. The extent of 53BP1 activation in beta-cells did not correlate with age, gender, c-peptide levels, BMI, duration of disease or the number of autoantibody types (data not shown). As our samples originated from different sources (different centers and from both live and cadaveric donors) we performed a comparison of samples belonging to the same clinical group. We found significant differences between nPOD samples and Powers lab samples in the healthy controls group (Supplemental Figure S1B) and between nPOD and the DiViD samples in the recently diagnosed T1D group (Supplemental Figure S1C). The differences may result from the condition of donors before tissue fixation (live biopsies vs. cadaveric samples). However, significant differences between controls and patients were observed even when examining only the subset of samples originating from nPOD (Supplemental Figure S1D). We conclude that inter-source differences exist, but do not mask the differences between controls and patients.

To determine the cell specificity of the DDR in T1D, we assessed the activation of 53BP1 in other pancreatic cell types. First we examined cells of the exocrine pancreas, given evidence for the involvement of the exocrine pancreas in T1D (14; 15). We observed a large variation among individuals recently diagnosed with T1D, but the difference between controls and recently diagnosed patients did not reach statistical significance (controls, 1.59±0.578% positive acinar cells, recent T1D 3.66±1.12% labeled acinar cells; p=0.09). The fraction of labeled acinar cells in recent onset T1D did appear to be higher than in samples from established T1D (0.88±0.24%, p<0.05, Supplemental Figure 2A). There were no significant differences in the 53BP1 pattern among glucagon-expressing alpha-cells from healthy controls and recently diagnosed T1D patients (2.88±0.49%, and 2.42±0.40, Supplemental Figure S2B). These results suggest that there is a heightened
DDR in the pancreas of recently diagnosed T1D patients, and that the elevated response is largely restricted to beta-cells.

Consistent with heightened DDR, we observed in recent T1D a significant increase in the fraction of islet cells that stained for p53, a transcription factor that is stabilized following cellular insults including DNA damage (control islets, 0.288±0.07% p53+ cells; islets of recent T1D, 0.78±0.17% p53+ cells; p<0.01, Figure1C, D). These findings further support the idea that beta-cells in the early stages of T1D have excessive DNA breaks and a subsequent cellular response. Other markers of DDR activation including the phosphorylated forms of ATM, Kap1 and H2Ax were very scarce in the human T1D samples (data not shown) potentially due to the short half-life of these phosphorylation events in beta-cells following DNA damage (Supplemental Figure S1A and data not shown).

**The beta-cell DNA damage response is associated with islet inflammation**

The islets of recently diagnosed T1D patients have a heterogeneous morphology. Some islets are heavily infiltrated by immune cells (insulitis), while other islets from the same individual and in the same histological section appear intact, presumably captured in the midst of the pathogenic process. We took advantage of this heterogeneity to ask if immune infiltration status is locally correlated to beta-cell DDR. Within the recent T1D group, islets or islet remnants with a higher index of 53BP1 tended to be surrounded by larger masses of cells with small nuclei (Figure 2A). Coimmunostaining of 53BP1 with the pan-leukocyte marker CD45 confirmed that infiltrating cells were leukocytes (Figure 2A, inset). To quantify the link between DDR in beta-cells and immune cell infiltration, we assigned to each islet an immune infiltration grade according to the pattern of the CD45 staining (no infiltration, minor, mild, moderate and severe insulitis, Figure 2B and supplemental figure S2C). We then quantified the extent of activated 53BP1 in beta-cells of recent onset T1D patients according to this classification (with the scoring of each islet done while blinded to the score of the other parameter). The percentage of beta-cells with a 53BP1 nuclear focus was higher in islets with a more severe immune infiltration (Figure 2C), and the differences reached statistical significance, with as much as 10.5% of beta-cells stained positive for 53BP1 in islets scored as severely infiltrated. These findings reveal a positive association between the DNA damage response in beta-cells and islet immune infiltration.
Assessing physical proximity between immune cells and beta-cells with DNA damage response

DNA damage and inflammation may trigger each other [23]. The innate and adaptive immune systems can inflict DNA damage to target cells, including beta-cells (17), typically via direct cell-cell interactions (18; 19). On the other hand, DNA damage may trigger an inflammatory immune response, typically via secreted factors (20-23). We assessed the spatial relationship between 53BP1 activated beta-cells and islet-infiltrating cells by counting the fraction of 53BP1+ beta-cells that were in direct contact with CD45+ cells, or separated from immune cells by at least one islet cell layer (Figure 2D). Only a minority of beta-cells with activated 53BP1 was in physical proximity to cells of immune origin (20.17% of 53BP1+ cells physically adjacent to immune infiltrating cells; Figure 2E). These findings suggest that the beta-cell DDR in recent T1D is unlikely a mere response to factors from immediately adjacent immune cells.

DDR signaling in rodent models of T1D

To assess the generality of DDR in autoimmune diabetes, we turned to rodent models. One classic model of diabetes in mice is based on treatment with streptozotocin (STZ), a beta-cell-selective toxin that induces DNA damage through both alkylation and the generation of reactive oxygen and nitrogen species [31]. We used the Multiple Low Dose STZ (MLDS) model, where 5 daily sublethal injections of STZ trigger hyperglycemia within two weeks (28). To examine if the MLDS model recapitulated the beta-cell DDR observed in recently diagnosed T1D patients, we quantified 53BP1 in islets of wild type mice injected with a low dose of STZ. Within 8 hours of STZ, there was a marked increase in the percentage of beta-cells that had nuclear 53BP1 foci (from 2.77±0.02 to 19.67±1.2, p<0.01, Figure 3A). Moreover, mice treated with STZ showed islet staining for phospho-Kap1 (serine 824), a histone modifier whose phosphorylation is a hallmark of ATM activity [32], which was similar to the pattern observed in the pancreas (both endocrine and exocrine) after gamma irradiation (Figure 3B).

We next examined DDR in beta-cells from the non-obese diabetic (NOD) [20] mice, a strain that spontaneously develops autoimmune diabetes [15]. We documented 53BP1 patterns in NOD pancreas sections at 2, 7 and 10 weeks of age, and immediately following the first blood glucose measurement that exceeded 200mg/dL (15-18 weeks). Age matched outbred ICR mice were used as wild type controls. Although NOD mice show insulitis from a very early age, we did not detect significant differences in the extent of 53BP1 activation between these mice and ICR controls at any age (Figure 3C). Therefore, the NOD model does not recapitulate the increased DDR observed in beta-cells from human T1D patients. The lack of evidence for beta-cell DDR activation in NOD mice suggests that that DDR is not obligatory for islet inflammation to occur, and that islet inflammation by itself does not necessarily lead to activation of a beta-cell DNA damage response.
Finally, we studied the LEW.1AR1-iddm rat model of spontaneous T1D [15]. In this model, a mutation in the gene encoding for Dock8 leads to an immune attack on islet cells culminating in insulin dependent diabetes in 20% of the animals around the age of 60 days [13]. We examined beta-cell 53BP1 activation in tissue sections from pre-diabetic LEW.1AR1-iddm rats and healthy controls (Figure 3D). We found, similarly to T1D human patients, that activated 53BP1 and gamma-H2AX are more frequent in beta-cells from iddm compared with non-diabetic controls (Figure 3D and data not shown). Therefore, a beta-cell DDR in vivo in the form of 53BP1 activation occurs in human T1D patients, in spontaneous rat T1D, and upon MLDS treatment of mice.

DNA damage induces proinflammatory gene expression in islets ex vivo and in vivo

To explore the link between DDR and inflammatory signaling in islet cells, we exposed cultured murine islets to STZ [31]. One hour following treatment, we isolated RNA from the islets and measured the expression of the pro-inflammatory molecules Ccl3, Ccl4, Ccl5, Ccl11, Cxcl10, II-1β, II-6 and TNFα. Most of these cytokines have been reported to be elevated in islets of recently diagnosed T1D patients [1]. As a positive control we applied recombinant TNFα, a strong inducer of many of these cytokines [33]. TNFα and Cxcl10, a factor implicated in islet inflammation and T1D [34-37], were most strongly elevated upon STZ and TNFα treatment (Figure 4A). We also monitored the expression of cytokines in islets from MLDS mice immediately after the fifth injection and found increased levels of several proinflammatory cytokines including Cxcl10 and IL1-β (Figure 4B). These results show that DNA damage in mouse islets can evoke an immune response in the form of cytokine expression.

ATM mediates DNA damage-induced cytokine expression in islets

A possible mediator of inflammatory cytokine transcription upon DNA damage is the double strand break response factor Ataxia Telangiectasia Mutated (ATM) [38]. We tested whether attenuation of ATM activation with a pharmacologic inhibitor (KU55933, herein ATMi) could affect the response of islets to STZ ex vivo. We incubated wild type murine islets with STZ alone or with ATMi for 48 hours and measured Cxcl10 expression and secretion. Cxcl10 was elevated upon exposure to STZ in both the mRNA (25.7±2.43 fold, p<0.001) and secreted protein levels (from 73.00±2.50 to 124.6±11.53 pg/100 islets, p<0.05), and the response could be abrogated by coincubation with ATMi (1.68±0.83 fold, p<0.001 and 89.56±5.02 pg/100 islets, p<0.05) as shown in Figure 4C.

To assess whether DDR plays a role in vivo in STZ mediated pro-inflammatory gene expression, we applied the MLDS protocol together with general inhibition of the DDR through injections of the ATM/ATR general inhibitor caffeine (30). We found that mice co-injected with STZ and caffeine had a blunted induction of both Cxcl10 (3.04±0.5 fold, p<0.05 to NT, p<0.05 to STZ only), and IL-1β (0.98±0.2 fold, p<0.01 to STZ only), as
shown in Figure 4D. These results suggest a link between DNA damage (induced by STZ) and an islet inflammatory response (Cxc110 and IL-1β elevation) via a DDR response factor (ATM).

To define more precisely the role of ATM in the response of islets to DNA damage, we generated mice with beta-cell-specific deletion of the gene for Atm by crossbreeding mice with a conditional Atm allele (loxP-flanked exons 57-58, encoding the core PIKK kinase domain) (31) with Insulin-Cre mice [12]. PCR analysis confirmed the deletion of the two ATM exons in islets from compound Insulin-Cre; ATMlox/lox mice (termed ATMABC herein) (Supplemental Figure S3). mRNA levels of Atm in total islet lysates of the ATMABC were significantly lower than in littermate controls (an average decrease of ~50%, p<0.0001, Supplemental Figure S3), with remaining ATM likely derived from non-beta-cells in the islets. To confirm effective inactivation of ATM in beta-cells, we stained pancreatic sections of mice sacrificed at one hour after gamma irradiation (5Gy) for phospho-Kap1. As expected, irradiation led to a rapid accumulation of p-Kap1 in both the exocrine and endocrine pancreas (Supplemental Figure S3). In irradiated ATMABC mice phosho-Kap1 was missing specifically in islet cells, demonstrating the beta-cell-specific ablation of ATM activity (Supplemental Figure S3).

Untreated ATMABC mice displayed normal pancreas and islet morphology, and a mild glucose intolerance upon challenge (not shown), consistent with a previous report on impaired insulin secretion in mice globally deficient for ATM (33). We subjected mice to the MLDS model, and evaluated the mRNA levels of Cxc110 and IL-1β in islets of ATMABC and control mice. We observed an elevation of Cxc110 (5.15±0.4 fold, p<0.001) and IL-1β (2.43±0.3 fold, p<0.05) in control MLDS islets, which was blunted in ATMABC mice (Cxc110, 1.94±0.6 fold, p<0.05; IL-1β, 0.56±0.2 fold, p<0.05) (Figure 4E). These results provide genetic evidence that DDR signaling through ATM contributes to the development of insulitis under the MLDS model.

**DDR signaling contributes to streptozotocin-induced diabetes in mice**

We next asked whether reduced DDR in ATM mutants affected the development of diabetes induced by STZ. Strikingly, ATMABC mice exposed to MLDS demonstrated blunted development of hyperglycemia compared to control mice (Figure 5A,B, two Way ANOVA p=0.0058). Moreover, a time course follow up of mice with persistent hyperglycemia (over 250mg/dL) showed that ATMABC mice were largely resistant to MLDS diabetes induced hyperglycemia (8 days median time to diabetes in controls, undefined median in ATMABC mice, Log-Rank p=0.0071, Figure 5C). Mice carrying the Rip-Cre transgene alone (ATM proficient) responded to MLDS similarly to wild type mice, ruling our non-specific effects of the Cre transgene (Supplemental Figure S3). It was also claimed that this driver transgene expresses the human growth hormone (hGH) [41]. However, we found that pancreatic tissue slides from Rip-Cre mice were negative for hGH staining (Supplemental Figure S3).
To test whether the milder response to MLDS in ATM-deficient mice is related to an autoimmune process, we subjected ATM^{ABC} mice again to MLDS, this time sacrificing the cohort 5 days after the last injection. We immunostained pancreatic tissue from these mice for the T-cell marker CD3 (Figure 5D). Quantifying the fraction of islets stained positive for CD3 revealed a marked difference between ATM^{ABC} mice and sibling controls. The area stained positive for CD3 in islets from the control group was 8.85±1.2%, compared with only 3.67±0.2% in ATM^{ABC} mice (Figure 5E). The reduced T-cell infiltration is consistent with decreased islet expression of the T-cell chemo-attractant Cxcl10 in ATM^{ABC} mice (Figure 4E), and supports the idea that DDR inhibition under the MLDS model can affect the extent of islet immune cell infiltration.

We then studied whether beta-cell ATM deficiency prevents beta-cell loss in MLDS. The insulin/glucose ratio, a measure of beta-cell function, was reduced in wild type MLDS mice, but not in ATM^{ABC} MLDS mice, compared with untreated controls (untreated mice, 1±0.13; wild type STZ, 0.32±0.07; ATM^{ABC} STZ, 0.95±0.22; p<0.05; Figure 5F). ATM^{ABC} STZ mice also displayed a higher proportion of area stained for insulin (1.18±0.1%) compared with similarly treated wild type sibling control mice (0.62±0.1%, p<0.01, Figure 5G,H) and a higher number of islets per section (wild type STZ, 9.6±1.34; ATM^{ABC} STZ, 15.2±1.43; p<0.05; Figure 5I). Thus, beta-cell-specific ablation of ATM protects mice from experimental diabetes induced by MLDS.

Lastly, we analyzed beta-cell 53BP1 activation as a function of islet T-Cell infiltration in ATM^{ABC} and control mice, 5 days after the first STZ injection (Figure 6A). Wild type MLDS mice revealed an association between beta-cell 53BP1 nuclear foci and the extent of islet T-Cell infiltration (Figure 6B), with non-infiltrated islets showing the lowest levels of 53BP1 activation, and islets with a more extensive CD3^{+} infiltrate having a higher index of 53BP1 activation. This result is in agreement with the findings in samples from recently diagnosed human T1D patients. Strikingly, the association between DDR and immune cell infiltration was abrogated by ATM deletion. ATM^{ABC} mice had higher levels of islet 53BP1 activation with all the different degrees of infiltration (Figure 6B), yet the overall presence of insulitis was lower. This suggests that an ATM-dependent beta-cell DDR is important for T-Cell recruitment. We further observed that the general activation of 53BP1 (all islets combined regardless of infiltration levels) was higher in the ATM^{ABC} group (Figure 6C). This shows that in the MLDS model, DNA damage to beta-cells can signal through ATM to recruit T-Cells. We conclude that ATM-dependent DDR in beta-cells promotes proinflammatory cytokine expression, islet T-Cell infiltration and diabetes.

**Discussion**

We have identified a DNA damage response as an early beta-cell-specific injury in human T1D. Our analyses suggest that this phenomenon peaks around the clinical onset of the disease, a time in which islet inflammation is most prevalent. In addition to the
temporal coincidence between DDR and islet inflammation, we found a physical association between insulitis and beta-cell DDR. Our experiments in mice with genetic and pharmacologic inhibition of ATM implicate DDR as a factor that may accelerate islet inflammation. While islet inflammation is undoubtedly mediated by a complex molecular network, this work reveals the pro-inflammatory cytokines Cxcl10 and IL-1β as two potential mediators of DNA damage-induced islet inflammation. Interestingly, elevated Cxcl10 was recently reported in a cohort of pancreas samples from newly diagnosed T1D patients (26). Cxcl10 is a potent chemoattractant for T-cells (25; 34) and indeed that study reported an increase in the presence of immune cells expressing Cxcr3 (the Cxcl10 receptor) [36]. These data suggest a pathway leading from beta-cell DNA damage, via ATM-dependent cytokine expression to islet inflammation and autoimmunity.

What is driving the beta-cell DDR in T1D? The marked heterogeneity of DDR within beta-cells of the same individual suggests that systemic hyperglycemia is unlikely a sole driver of the effect. The increased 53BP1 activation in islets of normoglycemic AAB+ donors confirms that it is not hyperglycemia that leads to this elevation. It is possible that beta-cell heterogeneity (e.g. beta-cells having different aging phenotypes, or different sensitivities to metabolic stress) (35-37) results in a differential cellular response to systemic insults. It was previously shown that reactive nitrogen species can lead to DNA breaks in isolated islets [45], and that iNOS inhibition can prevent hyperglycemia following the MLDS protocol [46,47]. Therefore, NO is a possible factor leading to beta-cell DNA damage at early stages of T1D. Alternatively, it is possible that beta-cell DDR is triggered by islet-infiltrating cells, although the lack of tight physical association between 53BP1+ beta cells and immune cells argues against this possibility as an exclusive mechanism. Moreover, the increase in 53BP1+ beta-cells in AAB+ donors also argues against induction of DDR by immune cells as insulitis was scarce in these samples. We hypothesize that both metabolic and inflammatory injuries may contribute to DNA damage in beta-cells. In turn, the DDR may lead to further aggravation of beta-cell dysfunction and immune attack, thus contributing to a vicious cycle ending with T1D (see model, Figure 6D). Another potential contributor to DDR in human T1D is viral infection. We have previously demonstrated a low grade, persistent enterovirus infection in the same resection samples analyzed here (38), and others have shown that viral infection may trigger the DDR (23; 39). Low grade viral infection may give rise to cell stress and DNA damage, inducing both inflammation and beta-cell death. Further experiments are needed to examine the potential effect of viral infection on DDR in human T1D.

While it is difficult to determine the effect of DNA damage using histologic specimens of human T1D, our experiments with mouse islets ex vivo and with mice treated with STZ do suggest a causal role for DDR in triggering islet inflammation and autoimmune diabetes. When ATM was absent in beta-cells or inhibited pharmacologically, STZ-induced expression of proinflammatory cytokines such as Cxcl10 and IL-1β was blunted, as were islet infiltration and hyperglycemia, while islet cell mass and beta-cell function were preserved. This suggests that the DNA damage response in beta-cells is inducing the
expression and secretion of inflammatory cytokines, potentially contributing to autoimmune destruction of beta-cells.

Our conclusions are consistent with several lines of evidence that have previously implicated beta-cell DDR in T1D. First, the established diabetogenic beta-cell toxin STZ is a DNA alkylating agent [50]. Second, mice deficient for p53 (the activation of which during DNA damage depends on ATM) are resistant to STZ-induced autoimmunity (40), further supporting a chain of events leading from DNA damage, through p53 and ATM, to immune system activation. In support of the relevance of the MLDS induced diabetes [51]. Third, genetic polymorphisms in the DNA double strand break repair genes XRCC4 and DNA ligase 4 were reported to predispose to beta-cell dysfunction in T1D [8].

We note the similarity between the cytokines observed in human T1D [1], in the LEW.1AR1-iddm rat model of human T1D [14,15] and the cytokines induced in a DDR-dependent manner in STZ treated mice; both processes appear to share the involvement of NF-κB and its targets, in particular the key pro-inflammatory cytokine IL-1β (41). Indeed, it was previously shown in the context of cancer that DNA damage can lead, via ATM, to the activation of NF-κB and its targets including IL-1β (42; 43). Moreover, NF-κB was shown to control the induction of Cxcl10 following STZ administration and beta-cell NF-κB inhibition attenuated diabetes under MLDS [55].

In conclusion, our findings suggest that a DNA damage response in beta-cells may contribute to islet autoimmunity, beta-cell death and T1D.

Author contributions

EH, BG and YD designed the study. EH, SZ, AS, MF, TL, TD, NH, NCW and AK performed experiments. LK, KDJ, MB and ACP contributed human material. AJ and SL contributed rat material. EH, AS, AK, BG and YD wrote the paper. YD is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgements

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NIH (DK104216), The Helmsley Charitable Trust, the European Union (project ELASTISLET), BIRAX, the Israel Science Foundation, the DON foundation and by a pilot grant from the Leona M. and Harry B. Helmsley Charitable Trust George S. Eisenbarth nPOD Award for Team Science (to YD). Work in the Powers group at Vanderbilt University was supported by grants from the NIH (DK89572, DK104211, DK108120, DK106755, DK20593), the JDRF, and the Department of Veterans Affairs. Dr. Elad Horwitz is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. No potential conflicts of interest relevant to this article were reported.

References


25. Corrado A, Ferrari SM, Ferri C, Ferrannini E, Antonelli A, Fallahi P: Type 1 diabetes and (C-X-C motif) ligand (CXCL) 10 chemokine. La Clinica terapeutica 2014;165:e181-185


36. Weir GC, Bonner-Weir S: Glucose Driven Changes in Beta Cell Identity Are Important for Function and Possibly Autoimmune Vulnerability during the Progression of Type 1 Diabetes. Front Genet 2017;8:2


**Figure legends**

**Table 1: A summary of the clinical data of the donors from the different groups.**

**Figure 1: DNA damage response in beta-cells of recently diagnosed T1D patients.**

A) Immunofluorescent staining of 53BP1 in beta-cells in vivo. Upper panel, the pattern of nuclear 53BP1 (red) in beta-cells (stained for insulin, green) of wild type mice, untreated or 8 hours after sub-lethal gamma irradiation. Note the typical accumulation of a large, single nuclear focus of 53BP1 in irradiated mice (white arrow). Lower panel shows two adjacent beta-cells from recently diagnosed T1D patients, with the normal or DNA damage typical pattern of nuclear 53BP1 (white arrow). Scale Bars, 2µm.

B) The fraction of beta-cells with activated 53BP1 in healthy donors (n=12), people at risk for T1D (AAB+, n=20), recently diagnosed T1D patients (n=7) and established T1D patients (n=18). Each dot represents an individual case.

C) Representative images from immunostaining for p53 in islets of control and recent T1D donors. Nuclear accumulation of signal signifies p53 activation. Scale Bars, 20µm.

D) Quantification of nuclear p53 in islets from healthy controls (N=10) and recently diagnosed T1D donors (N=7). Each dot represents an individual case.

**Figure 2: association of human beta-cell DNA damage with islet immune infiltration.**

A) Co-staining for beta-cell DNA damage and immune infiltration in a recently diagnosed T1D patient. A beta-cell (stained for insulin, green) with a 53BP1 nuclear focus is surrounded by a mass of small cells with compact nuclei, that stain positive for the pan leukocyte marker CD45 (white, see inset). Scale Bar, 50µm.

B) Classification of islets from recently diagnosed T1D patients according to the extent of inflammatory infiltrate. Immunofluorescent staining for beta-cells (insulin, green) and immune cells (CD45, red). The degree of infiltrate is determined by both the amount of CD45 cells surrounding the islet and their level of islet penetrance. Scale bars, 10µm.

C) Scores of 53BP1 foci in islets from recently diagnosed T1D donors, according to the islet infiltration score. Shown are combined results from all recently diagnosed T1D samples that had insulin staining.

D) High magnification images demonstrating 53BP1+ beta-cells (insulin, green cytoplasm and 53BP1, red nuclear focus) that are immediately adjacent to leukocytes (CD45, blue) or are physically separated from such cells. Scale bars, 5µm.

E) Quantification of immunofluorescence images as those in in panel D, showing the distribution of beta cells with activated 53BP1 according to proximity to CD45+ cells (bordering and non-bordering).
Figure 3: DDR in rodent models of diabetes.

A) Left, immunostaining for 53BP1 in mouse beta cells in vivo after a single dose of 30 mg/kg STZ to wild type mice, Red, 53BP1; insulin, green. Scale Bars, 20µm. Right, quantification of the percentage of beta-cell nuclei with nuclear 53BP1 foci. NT, n=2; STZ injected (40mg/Kg), n=3. Bars represent one SEM.

B) Representative images from immunostaining for phosphorylated Kap1 in islets of control, single acute STZ injected (200mg/Kg) and 5Gy irradiated mice. Nuclear accumulation of signal signifies Kap1 phosphorylation. Scale Bar, 20µm.

C) Quantification of 53BP1 nuclear foci in islets of NOD and ICR control mice at different ages.

D) Immunostaining for 53BP1 in healthy control rats and in pre-diabetic LEW.1AR1-iddm rats, and quantification of the 53BP1 nuclear foci in beta-cells. Red, 53BP1; insulin, green. Scale Bars, 20µm.

Figure 4: DNA damage response via ATM induces proinflammatory cytokine expression in islets.

A) Heatmap presentation of qPCR of T1D-associated cytokines. Cultured mouse islets treated with 5mM STZ, or TNFα for one hour (pools of three different mice each). T-test p-values are listed for each gene.

B) Heatmap presentation of qPCR of T1D associated cytokines. Islets were harvested and analyzed following five daily STZ injections (20mg/kg). T-test p-values are listed for each gene.

C) Left, qPCR analysis of CXCL10 performed on isolated wild type mouse islets treated with 5mM STZ and 10µM KU55933 (ATMi) for 48 hours. Bars represent 1 SEM. Only statistically significant relations are connected. Right, ELISA analysis of IP-10 performed on medium supernatants from the same experiment. Bars represent 1 SEM. Only statistically significant relations are connected.

D) qPCR analyses of IL-1β and Cxcl10 performed on islets from wild type mice, untreated (NT, saline injections), injected with caffeine (50mg/kg), injected with streptozotocin (STZ, 20mg/Kg), and co-injected with STZ and caffeine (STZ-Caffeine). All treatments were given for 5 consecutive days. Mice were sacrificed following last injection. Bars represent 1 SEM. Only statistically significant relations are connected.

E) qPCR analyses of IL-1β and Cxcl10 performed on islets from untreated wild type mice (NT, saline injections), wild type mice injected with streptozotocin (STZ-WT), and ATM^BC mice injected with STZ (STZ-ATM^BC). STZ (20mg/Kg) was injected for 5 consecutive days. Mice were sacrificed following last injection. Bars represent 1 SEM. Only statistically significant relations are connected.

Figure 5: Attenuated development of STZ diabetes in mice with ATM-deficient beta-cells.
A) Average blood glucose levels of control (CTL, n=9) and ATM$^{ABC}$ (n=12) mice subjected to MLD-STZ. Graph starts with the first day of 5 consecutive daily low dose STZ injections. Shown are the combined results of three different iterations of the experiment. Bars represent one SEM.

B) Average area under the curve (AUC) of the glucose measurements in A (CTL, n=9 and ATM$^{ABC}$, n=12).

C) Hyperglycemia-free survival curve of control (CTL, n=9) and ATM$^{ABC}$ (n=12) mice subjected to MLDS. Hyperglycemic mice were defined by >2 consecutive measurements of glucose >250mg/dL.

D) Representative images from immunostaining for CD3. Scale Bar, 20µm.

E) Quantification of the immunostaining in D (control, n=2, ATM$^{ABC}$, n=3). Bars represent one SEM.

F) Insulin/glucose ratio in the blood of non-treated (control n=3, ATM$^{ABC}$ n=3), STZ-injected control (n=7) and STZ-injected ATM$^{ABC}$ (n=9) mice. Blue, normoglycemic mice; red, hyperglycemic mice.

G) Insulin immunostaining in STZ-treated control (CTL) and ATM$^{ABC}$ mice. Scale bar, 1mm.

H) Quantification of the staining in E. Blue, normoglycemic mice; red, hyperglycemic mice.

I) Number of islets per section, determined by inspection of H&E stained sections of STZ-injected controls (n=9) and STZ-injected ATM$^{ABC}$ (n=12) mice. Within each group, normoglycemic mice are marked blue and hyperglycemic mice are marked red.

**Figure 6: ATM ablation disrupts the DDR-insulitis association.**

A) Immunostaining for 53BP1 and CD3 in mouse beta-cells in vivo. Mice received 5 daily injections of STZ (30mg/kg) and were sacrificed 5 days after the last injection. Red, 53BP1; insulin, green; blue, CD3. Scale Bars, 20µm.

B) Scoring for 53BP1 foci in islets from STZ injected wild type and ATM$^{ABC}$ mice, according to the islet CD3 infiltration score.

C) Overall percentage of beta-cell 53BP1 nuclear foci in STZ injected wild type and ATM$^{ABC}$ mice.

D) Model. Beta-cell DNA damage caused by metabolic stress, inflammation, viral infection or other injury leads to a DNA damage response that triggers a pro-inflammatory response. This may lead to islet dysfunction and exacerbation of islet inflammation.
Table 1: donor details

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Figure 1

190x275mm (96 x 96 DPI)
Figure 4

A. STZ ex vivo

B. MLDS in vivo

C. Relative expression of Cxcl10

D. Relative expression of Il-1β and Cxcl10

E. Relative expression of Il-1β and Cxcl10

190x275mm (96 x 96 DPI)
Figure 5

190x275mm (96 x 96 DPI)
Figure 6

190x275mm (96 x 96 DPI)
Supplemental Figure S1: DNA damage response in pancreata of recently diagnosed T1D patients.

A. The activation pattern of DDR proteins in mice following gamma irradiation. Wild type mice were subjected to 5 grey gamma irradiation and were sacrificed at different time points. Changes in markers of DDR were monitored using immunofluorescence to γH2AX and 53BP1. An atypical large focal form of γH2AX (white arrows) is found in beta cells regardless of a genotoxic condition. Note that these foci disperse at 4h past gamma irradiation and later reform.

B. The fraction of beta cells with activated 53BP1 in healthy donors originating in the nPOD repository (black dots, n=7) or at the Powers group (green squares, n=5). Each dot represents an individual case.

C. The fraction of beta cells with activated 53BP1 in recently diagnosed T1D donors originating in the nPOD repository (black dots, n=3) or at the DiViD study (pink triangles, n=4). Each dot represents an individual case.

D. The fraction of beta cells with activated 53BP1 in samples originating solely from the nPOD repository. Samples groups are healthy donors (n=7), people at risk for T1D (AAB+, n=20), recently diagnosed T1D patients (n=3) and established T1D patients (n=18). Each dot represents an individual case, p-values inside brackets are from include all three tissue sources (nPOD, Powers and DiViD).
Supplemental Figure S2: Classification of islets from recently diagnosed T1D patients according to the extent of inflammatory infiltrate.

A. S3BP1 foci in cells of the exocrine pancreas. S3BP1 pattern was assessed on samples from recently diagnosed T1D patients (n=8), long standing T1D (N=12) and healthy control donors (n=13). Each dot represents an individual case.

B. Quantification of S3BP1 nuclear foci in glucagon positive cells on pancreas samples from recent T1D (n=5) and healthy control donors (n=11). Each dot represents an individual case.

C. Immunofluorescent staining for beta cells (insulin, green) and immune cells (CD45, red). The degree of infiltrate is determined by both the amount of CD45 cells surrounding the islet and their level of islet penetrance. Infiltrate definitions were: minor - up to three CD45+ cells in the islet periphery; mild - spread CD45+ cells (up to ∼20) with some intra-islet infiltration; moderate - aggregated CD45+ cells (∼20 cell or more) with intra-islet infiltration; severe - large aggregations of CD45+ cells with intra-islet infiltration. Scale bars, 10μm.

D. qPCR analysis of islets produced from mice injected with STZ (five daily doses, 30mg/kg).

E. qPCR analysis of IL-1β performed on isolated human islets from different donors. Islets were cultured with STZ or TNFα for four hours (values are compared to non-treated samples from each donor).
Supplemental Figure S3

A. Western blot analysis of islet DNA showing LoxP-flanked ATM, RIP-CRE, and Deleted ATM in WT, CTL, and ATM\(^{ΔBC}\) samples.

B. Bar chart showing ATM mRNA relative expression with statistical significance.

C. IHC images of phospho-Kap1 in CTL NT, ATM\(^{ΔBC}\) NT, CTL 5Gy, and ATM\(^{ΔBC}\) 5Gy samples.

D. Graph depicting the fraction of diabetes-free mice over days for CTL and Rip-Cre groups.

E. Line graph showing blood glucose levels over days for CTL and Rip-Cre groups.

F. Histological images of P.G., CTL, and Rip-Cre samples.
Supplemental Figure S3: Beta cell conditional deletion of the gene for Atm, and lack of effect in RIP-Cre single transgenic mice.

A. ATM genomic deletion in islets of ATM^{f/f}; insulin-Cre mice. PCR was performed of DNA from islets of wild type (WT), control (CTL, ATM^{f/f}) and Insulin-Cre; ATM^{f/f} (ATM^{ΔBC}) mice. A deletion product can be found only in the presence of Cre recombinase and the floxed ATM allele. Arrow indicate the location of genotyping PCR primers for each panel. Recombination deletes exons 57+58 of ATM.

B. qPCR analysis of the Atn transcript in control (CTL) and ATM^{ΔBC} mice. RNA was isolated from whole islets.

C. Immunostaining for the phosphorylated form of the ATM substrate Kap1. Control and mutant mice were non-treated (NT) or gamma irradiated (5Gy), and sacrificed 1 hour later. Note that in the mutants, acinar cells stain positive but islet cells are negative, consistent with lack of ATM. Scale bars, 20µm.

D. Hyperglycemia-free survival curve of control (CTL, n=5) and Rip-Cre (n=4) mice subjected to MLDS.

E. Average blood glucose levels of control (CTL, n=5) and Rip-Cre (n=4) mice subjected to MLDS. Day 0 is the first day of 5 consecutive daily low dose STZ injections. Bars represent one SEM.

F. Growth hormone immunostaining of pituitary gland (PG), and the pancreas of control and RIP-Cre mice.
## Supplemental Table S1 – donor details

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<td>TTTCATCTCATTGACCTGGTCC</td>
</tr>
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