



Tellurium Compounds Prevent and Reverse Type-1 Diabetes in NOD Mice by Modulating $\alpha 4\beta 7$ Integrin Activity, IL-1 β , and T Regulatory Cells

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Tom Eitan Yossipof^{1†}, Ziva Roy Bazak^{1†}, Dvora Kenigsbuch-Sredni², Rachel R. Caspi³, Yona Kalechman¹ and Benjamin Sredni^{1*}

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National Defense Medical
Center, Taiwan

*Correspondence:

Benjamin Sredni
srednib@biu.ac.il

[†]These authors have contributed
equally to this work

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¹ The Mina & Everard Goodman Faculty of Life Sciences, The Safdiè AIDS and Immunology Research Center, C.A.I.R. Institute, Ramat Gan, Israel, ² Interdisciplinary Department, Bar-Ilan University, Ramat Gan, Israel, ³ Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, MD, United States

The study shows that treatment of NOD mice with either of two tellurium-based small molecules, AS101 [ammonium trichloro(dioxoethylene-o,o')tellurate] or SAS [octa-O-bis-(R,R)-tartarate ditellurane] could preserve β cells function and mass. These beneficial effects were reflected in decreased incidence of diabetes, improved glucose clearance, preservation of body weight, and increased survival. The normal glucose levels were associated with increased insulin levels, preservation of β cell mass and increased islet size. Importantly, this protective activity could be demonstrated when the compounds were administered either at the early pre-diabetic phase with no or initial insulinitis, at the pre-diabetic stage with advanced insulinitis, or even at the advanced, overtly diabetic stage. We further demonstrate that both tellurium compounds prevent migration of autoimmune lymphocytes to the pancreas, via inhibition of the $\alpha 4\beta 7$ integrin activity. Indeed, the decreased migration resulted in diminished pancreatic islets damage both with respect to their size, β cell function, and caspase-3 activity, the hallmark of apoptosis. Most importantly, AS101 and SAS significantly elevated the number of T regulatory cells in the pancreas, thus potentially controlling the autoimmune process. We show that the compounds inhibit pancreatic caspase-1 activity followed by decreased levels of the inflammatory cytokines IL-1 β and IL-17 in the pancreas. These properties enable the compounds to increase the proportion of Tregs in the pancreatic lymph nodes. AS101 and SAS have been previously shown to regulate specific integrins through a unique redox mechanism. Our current results suggest that amelioration of disease in NOD mice by this unique mechanism is due to decreased infiltration of pancreatic islets combined with increased immune regulation, leading to decreased inflammation within the islets. As these tellurium compounds show remarkable lack of toxicity in clinical trials (AS101) and pre-clinical studies (SAS), they may be suitable for the treatment of type-1 diabetes.

Keywords: diabetes, tellurium, integrin, inflammation, IL-1 β , Tregs

INTRODUCTION

Type 1 diabetes mellitus (T1D) develops as a result of pancreatic islet β -cells loss, mainly by means of CD4T cells and other leukocytes action. The autoimmune etiology of T1D is affected by both genetic and environmental factors. Nevertheless, the exact mechanisms of disease initiation remain largely unknown.

There is no proven means to avoid the development of diabetes, nor to control the anti- β -cell autoimmune response after the disease has been diagnosed. Furthermore, the progressive loss of self-tolerance to islet β -cell antigens in human patients and animal models, is one of the most important fundamental contributing factors to T1D development. One of the main primary factors controlling such self-tolerance is the activity of Regulatory T cells (Tregs) (1).

Upon immunological challenge, lymphocytes migrate from the bloodstream into secondary lymphoid tissues such as lymph nodes (LNs) and Peyer's patches. Infiltration of immune cells is essential for organ and antigen-specific adoptive immunity against pathogens and antigens, including autoimmunity.

This migration is controlled in part by adhesion molecules and chemokine receptors on lymphocytes and their ligands on blood vessel high endothelial venules (HEVs) in LNs and Peyer's patches (2–4).

In T1D, activated CD4⁺ and CD8⁺ T lymphocytes destroy the insulin-producing β -cells of the pancreatic islets. In recent years it was recognized that B cells in pancreatic LNs are crucial antigen presenting cells (APCs) not only to initiate and optimize priming of naive autoreactive CD4⁺ T cells by β cell antigens, but also to contribute to epitope spreading of T cell autoimmunity among β cell antigens (5). Furthermore, the $\alpha 4\beta 7$ integrin/MAdCAM-1 pathway has been found crucial for the migration of B cells from the bloodstream into pancreatic lymph nodes (PLN) in NOD mice (6). Importantly, an $\alpha 4$ -integrin-VCAM-1 interaction contributes to T cell entry into the pancreatic islets and in the pathogenesis of T1D (7).

AS101 is a potent tellurium-based immunomodulator, which has demonstrated activity for several indications, both *in vitro* and *in vivo*, and has a large number of potential therapeutic applications (8, 9). The compound is non-toxic and is currently undergoing phase II/III testing in patients with cervical tumors, and phase I/II testing for treatment of patients with age-related macular degeneration. Much of the biological activity of AS101 can be attributed to its chemical redox interactions with vicinal thiols in the exofacial domain of VLA-4 (10); these modifications enable this agent to mediate diverse effects including abrogation of acquired drug resistance in acute myelogenous leukemia (10), and improvement of symptoms in experimental autoimmune encephalomyelitis (11) and uveitis (12). The specific redox-modulating activities of AS101 result in a variety of beneficial biological effects, demonstrated in diverse preclinical and clinical studies (13). The anti-inflammatory properties were found to be crucial for the clinical activities of AS101, including the protective effects of AS101 in autoimmune diseases (14–17) and in septic mice (18). The same thiol–redox interactions of AS101 enabled it to exert beneficial effects in a variety of tumor

models in mice and humans where AS101 had clear antitumor effects (10, 19, 20).

The second generation tellurium IV compound SAS synthesized by us, has also been shown to possess a unique Te^{IV}-thiol chemistry and exert biological activities similar to those of AS101 (13).

Based on our previous data showing that AS101 inhibits the $\alpha 4\beta 7$ activity in mesenteric lymph node cells of DSS-induced murine colitis (14), we hypothesized that both tellurium compounds may have the potential to suppress the activity of the $\alpha 4\beta 7$ integrin on lymphocytes from NOD mice, and consequently prevent their infiltration into pancreatic LNs, potentially affecting the pathogenesis of T1D.

We demonstrate that AS101 and SAS are able to prevent as well as inverse new onset type 1 diabetes in the NOD mouse model, and exhibit effects on $\alpha 4\beta 7$ activity and lymphocyte migration, as well as inhibition of pancreatic IL-1 β and IL-17 production and enrichment of T-regulatory (Treg) cells in the islets. Our data thus suggest that tellurium compounds may have potential in the treatment of type 1 diabetes.

METHODS

Antibodies and Reagents

FITC anti-mouse CD3; PE anti-human/mouse Integrin $\beta 7$ (Biolegend, San Diego, CA, USA); APC anti-mouse CD45R/B220; anti-Mouse/Rat Foxp3 PE; anti-Mouse CD4 FITC; anti-Mouse CD25 APC; rat IgG2a Isotype Control PE (eBioscience, Carlsbad, CA, USA); Alexa 594 anti-mouse; Alexa 488 anti-rabbit; anti-glucagon; anti-insulin (Innovex Biosciences, Richmond, CA, USA); recombinant mouse MAdCAM-1 Fc chimera (R&D BioSystems, Minneapolis, MN, USA); murine TNF- α (Peprotech, Rocky Hill, NJ, USA); mouse IL-1 β , IFN γ , and IL-17 ELISA kits (R&D Biosystems). AS101 and SAS were supplied by M. Albeck, Bar-Ilan University, Ramat-Gan, Israel, and were synthesized at Bar-Ilan University.

Mice

Three to Four week old NOD/ShiLtJ (strain 001976) female mice were purchased from the Jackson Laboratory (Bar Harbor, ME), bred, and housed in individual metabolic cages with unlimited access to food and water. Experiments conformed to approved institutional protocols and were approved by the Institutional Animal Care and Use Committee.

Cells

SVEC-4 murine endothelial cells were obtained from the American Type Culture Collection. Cells were cultured in DMEM containing 10% fetal calf serum at 37°C with 5% CO₂ and 95% air.

Purification of Tregs and T Effector Cells

CD4⁺CD25⁺ Treg were purified from 3 months old NOD mice using a CD4⁺CD25⁺ Treg purification kit (Miltenyi Biotec) according to the manufacturer's instructions, with slight modifications. The CS columns were used for depleting non-T cells, and LS columns were employed to positively select

CD4⁺CD25⁺ cells. Non-CD4 cells were magnetically labeled using a cocktail of biotin-conjugated antibodies recognizing CD8, CD11b, CD45R, CD49b, Ter-119, followed by incubation with anti-biotin micro beads. The labeled cells were then depleted over a column. To attain maximum purity, the CD25 positive selection was performed twice. CD4⁺CD25⁻ T cells were isolated from the CD25-depleted fraction using CD4 microbeads and LS columns.

Cell Migration Assays

Cells (2.5×10^5) were incubated for 1 h in the presence of mobilized MadCAM-1 (100 μ g/ml) medium containing MnCl₂ (0.25 mM) and various concentrations of SAS or AS101. The cells were washed twice with PBS, and equal numbers of cells were loaded onto 8 mm polycarbonate membrane inserts. The bottom chambers were filled with RPMI-1640 (800 μ l) with FBS (20%), containing MadCAM-1 (100 μ g/ml) as a chemoattractant. Cell migration was quantified after 24 h by Trypan Blue exclusion test using a hemacytometer.

FACS Analysis

For evaluation of integrin expression on lymphocyte suspensions, single cells from spleens, peripheral LNs, and PLNs from 8 week old NOD mice were stained with FITC-conjugated anti CD3, PE-conjugated anti β 7, and APC-conjugated anti B220. Cells were then analyzed on a BD FACSCalibur flow cytometer. The proportion of B and T cells that express the integrin was evaluated on 1×10^4 B220 B or CD3 T cells in the lymphocyte forward scatter/side scatter gate. For evaluation of the proportion of Tregs, lymphocytes from PLNs from 20 w old treated mice were isolated and stained for membranar CD4 (FITC) and CD25 (APC) and nuclear Foxp3 (PE). Cells positive for both CD4 and CD25 were gated, and the expression of Foxp3 on this subpopulation was evaluated. The percentage of CD4⁺CD25⁺Foxp3⁺ cells from total lymphocytes was evaluated.

Quantitation of Caspase Activity

Isolated pancreatic extracts from treated mice were prepared in Tris/acetate buffer (pH 7.5) at 30°C. The extracts were centrifuged at 12,000 \times g for 10 min, and the supernatant was collected. A volume of supernatant equivalent to 100 μ g of protein was assayed for caspase-1 or caspase-3 activity using colorimetric caspase-1 and-3 assay kits (R&D Biosystems).

Quantitation of Cytokine Levels

IL-1 β , IFN γ , and IL-17 ELISA kits were used for the quantitative measurement of these cytokines in pancreas extracts of treated mice.

Attachment Assay for Evaluation of the α 4 β 7 Activity

For testing attachment to MadCAM-1, 96-well plates were coated with 80 μ L of MAdCAM1 (100 μ g/ml), or BSA. Cells were incubated in the wells for 2 h in the presence or absence of AS101 or SAS followed by extensive (3x) washing. The proportion of cells that remained attached to the wells was

determined by the colorimetric XTT (2,3-bis[2-methoxy-4-nitro-S-sulphophenyl]H-tetrazolium-5-carboxanilide) assay at 450 nm.

For testing attachment to endothelial cells, SVEC-4 endothelial cells were cultured on 6 well plates until 80% confluency. Next, 10 μ l of rTNF α (20 ng/ml) was added to the wells to induce MadCAM-1 expression, and then washed. Lymphocytes at 1×10^6 /well were then added in the presence or absence of AS101 or SAS and incubated o.n.. Wells were washed with PBS. The remaining attached cells were stained with anti CD3 and anti B220. Endothelial cells are not stained by either antibody (**Supplementary Figure 1**). The proportion of T or B cells in the total cell population was recorded.

Islet Histology

Resected pancreas heads were fixed in 4% formalin and then paraffinized. To prepare histological sections, 5 μ M sections were cut from each paraffin block, and stained with hematoxylin-eosin for detection of insulinitis.

The level of insulinitis was determined according to the following scale: Grade 0 - no insulinitis (no infiltration); Grade 1- pre-insulinitis (<25% of the islet area infiltrated); Grade 2- mild insulinitis (<50% of the islet area infiltrated); Grade 3 - severe insulinitis (>50% of the islet area infiltrated).

Immunohistochemistry: Insulin and Glucagon Staining

Fixed paraffin embedded pancreas head sections were deparaffinized and rehydrated. After antigen retrieval, slides were incubated with anti-mouse insulin Ab and anti-glucagon Ab o.n. at 4°C, followed by secondary Alexa 594-conjugated and Alexa 488-conjugated secondary antibodies for 1 h at RT. This was followed by Hoechst staining. Ten slides/mouse were visualized by fluorescence microscopy.

I.P Glucose Tolerance Test

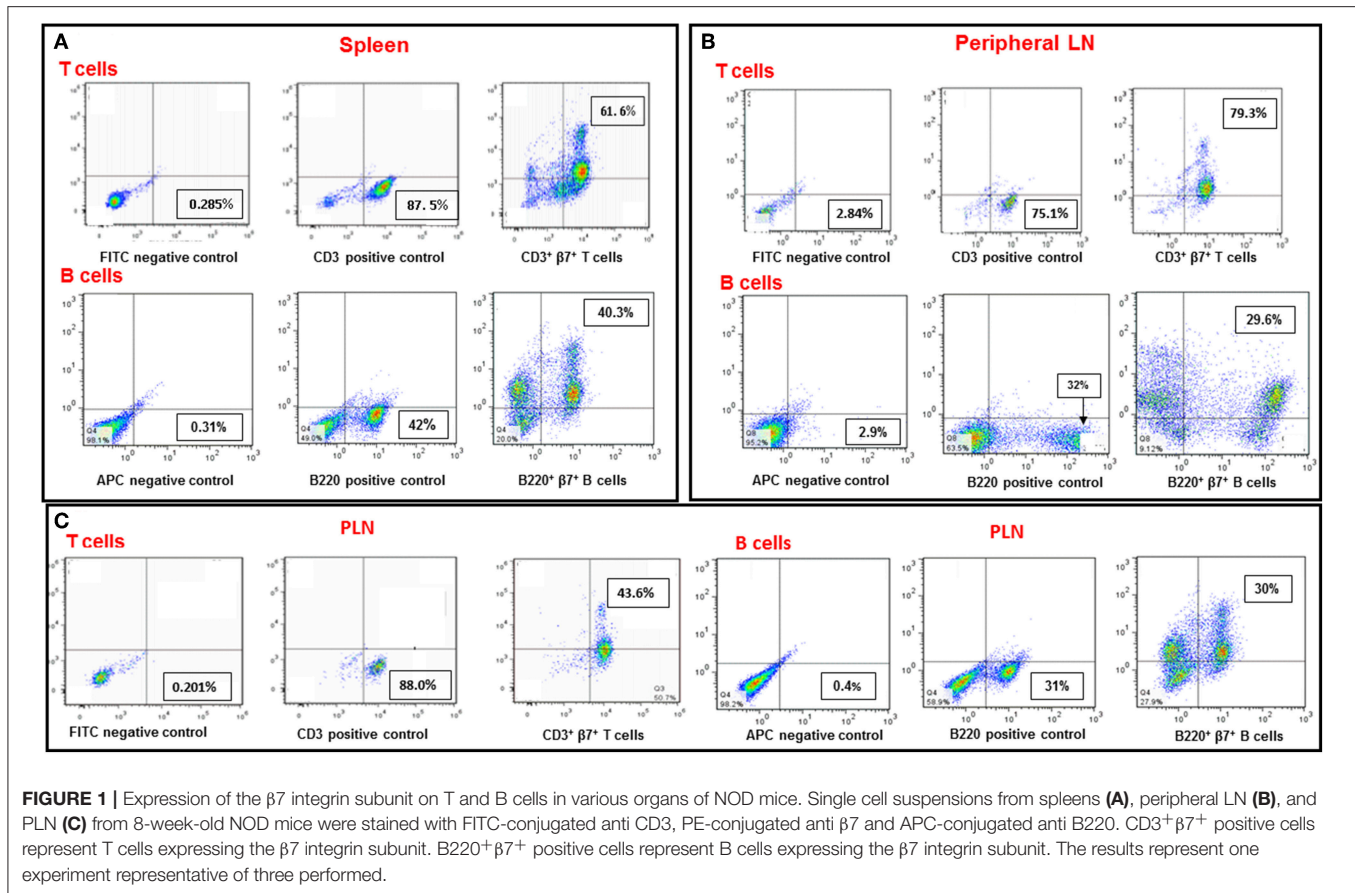
After 12 h fast, mice were administered an i.p bolus of 2 g/kg glucose. Blood glucose concentrations were measured by tail bleed at the indicated time points before and after glucose administration. All glucose measurements were performed using a Free Style Freedom (Alameda, CA, USA) glucometer.

Adoptive Transfer

NOD mice (5 w old) were treated with PBS or with 1 mg/kg AS101 every other day. Mice were mated, and treatment continued during gestation and during lactation, until the offspring reached an age of 3 weeks. At that time treatment stopped. Some of the male offspring of treated mothers were irradiated at 6w of age with 550 rads and were injected 24 h later with 20×10^6 splenocytes from diabetic female NOD mice.

Statistics

Data are presented as means \pm SE. For comparisons between groups in the *in vitro* and in some *in vivo* studies, we used the one- or two-way ANOVA. For *in vivo* studies (weight, blood glucose, glucose tolerance test, insulinitis), ANOVA multiple comparisons with repeated measures



with Bonferroni corrections, were applied. For analysis of incidence of diabetes and survival, the Kaplan Meier survival analysis tests were applied. The software used for all statistical analysis was IBM SPSS Statistics 21. $p < 0.05$ was considered statistically significant.

RESULTS

AS101 and SAS Attenuate $\alpha 4\beta 7$ Activity in Mononuclear Cells Isolated From NOD Mice

The $\alpha 4\beta 7$ integrin, MAdCAM-1 pathway is essential for infiltration of lymphocytes into pancreatic islets, and for the initiation of T1D in NOD mice (21). Therefore, regulation of this pathway has the potential to ameliorate the course of the disease. For this purpose, we first analyzed $\beta 7$ integrin expression on T and B cells from various organs of NOD mice. The results shown in **Figure 1** demonstrate that most spleen (**Figure 1A**) and peripheral lymph node (**Figure 1B**) T and B cells express the $\alpha 4\beta 7$ integrin, while about 50% of pancreatic T cells (**Figure 1C**), but most of B cells in this organ still express this integrin. We then analyzed the ability of AS101 and SAS to inhibit the activity of $\alpha 4\beta 7$ on T and B cells from various organs of NOD mice. This was performed by testing the level of attached cells to the $\alpha 4\beta 7$ specific ligand, MAdCAM-1. **Figure 2** shows a

dose dependent and significant inhibition of the $\alpha 4\beta 7$ integrin activity by both compounds. This was evident in both total spleen cells (**Figure 2A**), peripheral lymph node cells (**Figure 2B**), and pancreatic lymph node cells (**Figure 2C**). Moreover, SAS significantly inhibited the activity of the integrin in sorted T (**Figure 3A**), and B (**Figure 3B**) splenocytes (**Figure 3**).

To further substantiate the inhibitory effect of SAS on integrins, we examined the extent of lymphocyte adhesion, to high endothelial venular ("HEV") cells. MAdCAM-1 is expressed on approximately 95% of HEVs on the lymph nodes of young NOD mice (6). The interaction between MAdCAM-1 on HEVs in PLN and $\alpha 4\beta 7$ on lymphocytes enables their trafficking to PLN. For these experiments, we used the SVEC-4 endothelial cell line which represents HEV cells and highly expresses MAdCAM-1 after exposure to TNF α (22). **Figure 4** shows that treatment with SAS significantly inhibits attachment of both T and B cells from NOD mice to endothelial cells, and that the effect is dose-dependent. Collectively, these results suggest that by inhibiting the interaction between $\alpha 4\beta 7$ on T or B lymphocytes from NOD mice, and MAdCAM-1 on endothelial cells, AS101 and SAS might affect attachment and penetration of autoreactive T and B cells through pancreatic high endothelial venules, potentially affecting the course of T1D. For this purpose, we used the NOD mouse model of T1D using AS101 and SAS in various concentrations and different timing of administration.

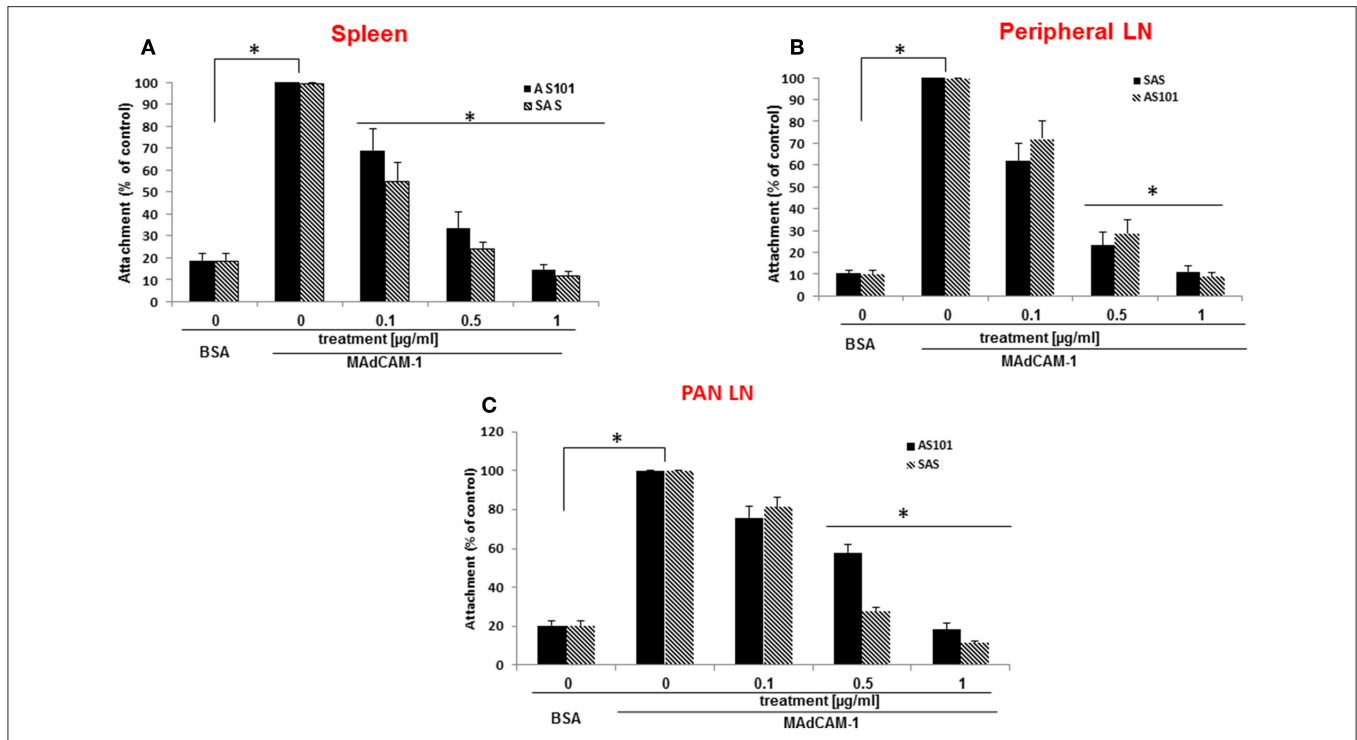


FIGURE 2 | AS101 and SAS inhibit *in vitro* the activity of the $\alpha 4\beta 7$ integrin in various organs of NOD mice. 5×10^5 single cells from spleens (A), peripheral LN (B), and PLN (C) from 10 week old NOD mice were cultured on BSA or MAdCAM-1-coated micro wells for 2 h in the presence or absence of different concentrations of SAS or AS101. The cells were washed twice and then subjected to XTT reagent according to the manufacturer's instructions. The results represent means \pm SE of three different experiments. * $p < 0.001$ decrease vs. MAdCAM-1 without AS101 or SAS (SAS 0).

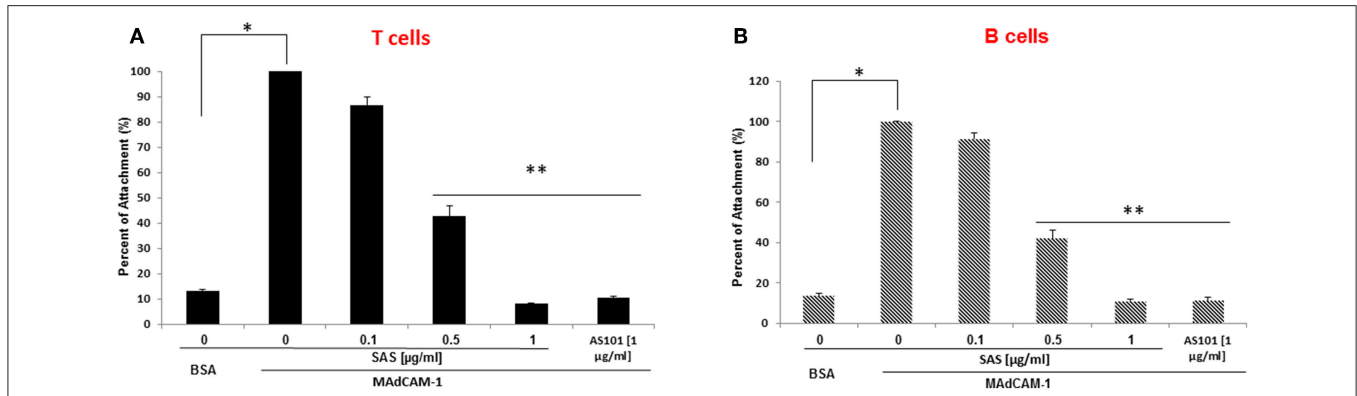
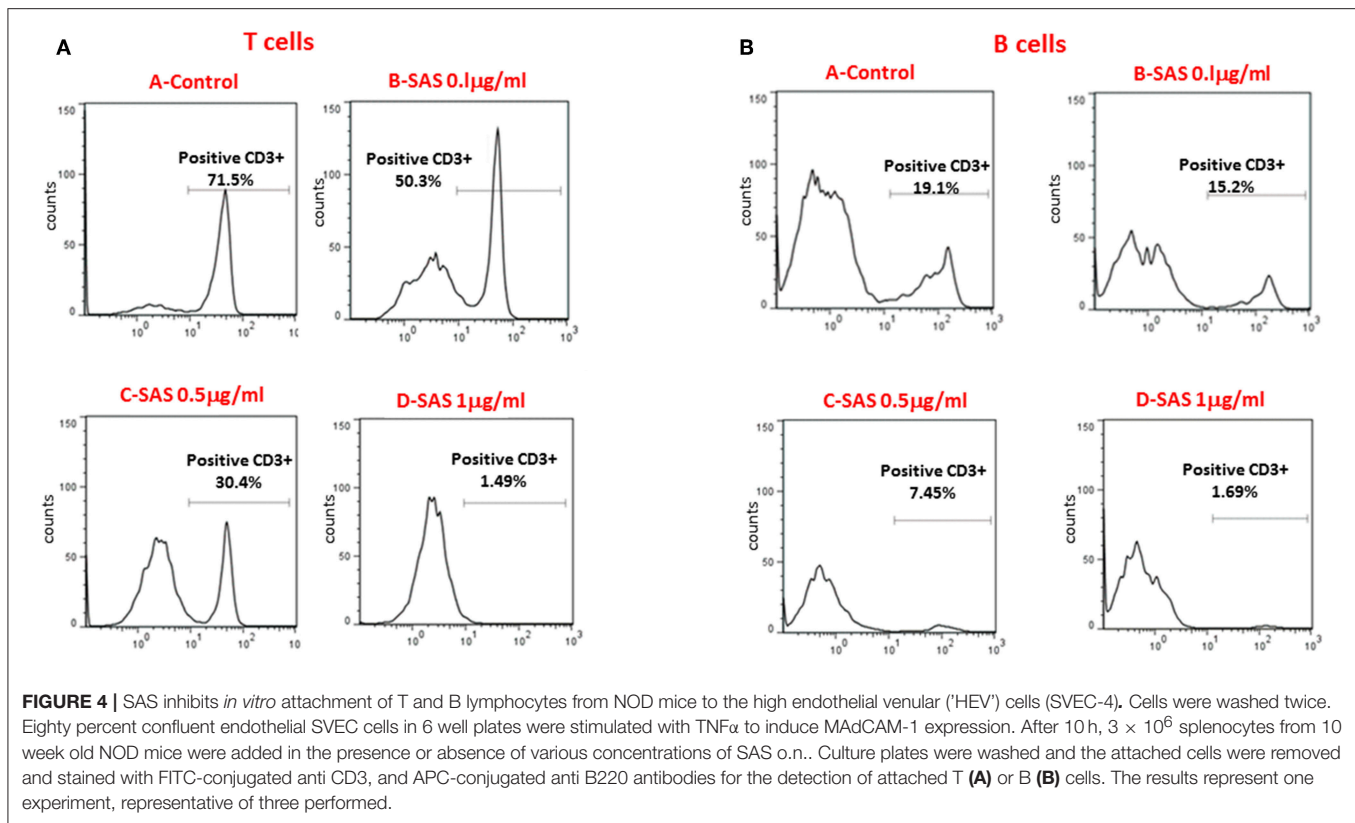


FIGURE 3 | AS101 and SAS inhibit *in vitro* the activity of the $\alpha 4\beta 7$ integrin on both T and B spleen cells of NOD mice. T and B spleen cells from 10 week old mice were sorted by FACS, using anti CD3 or anti B220 antibodies. Single cells (5×10^5) of each lineage (A= T cells; B= B cells) were cultured on BSA or MAdCAM-1-coated microwells for 2 h in the presence or absence of different concentrations of SAS or 1 μ g/ml AS101. The cells were washed twice and then subjected to the XTT reagents according to the manufacturer's instructions. The results represent means \pm SE from three different experiments. * $p < 0.01$; ** $p < 0.001$ decrease vs. MAdCAM-1 without AS101 or SAS (SAS 0).

Treatment of NOD Mice With AS101 Alleviates Clinical Symptoms of Diabetes

Figure 5A shows that extended treatment of NOD mice with an optimal dose of AS101 starting at 5–6 weeks (before the development of insulinitis), resulted in the delay and prevention of diabetes incidence in a dose-dependent fashion,

showing a narrow effective dose range (Figure 5). The optimal concentration of AS101 (0.5 mg/kg/injection) prevented disease in 90% of mice. Moreover, sub or supra-optimal concentrations of AS101 marginally delayed the onset of the disease, though these effects did not reach statistical significance. This effect was accompanied by increased body weight (Figure 5B), and



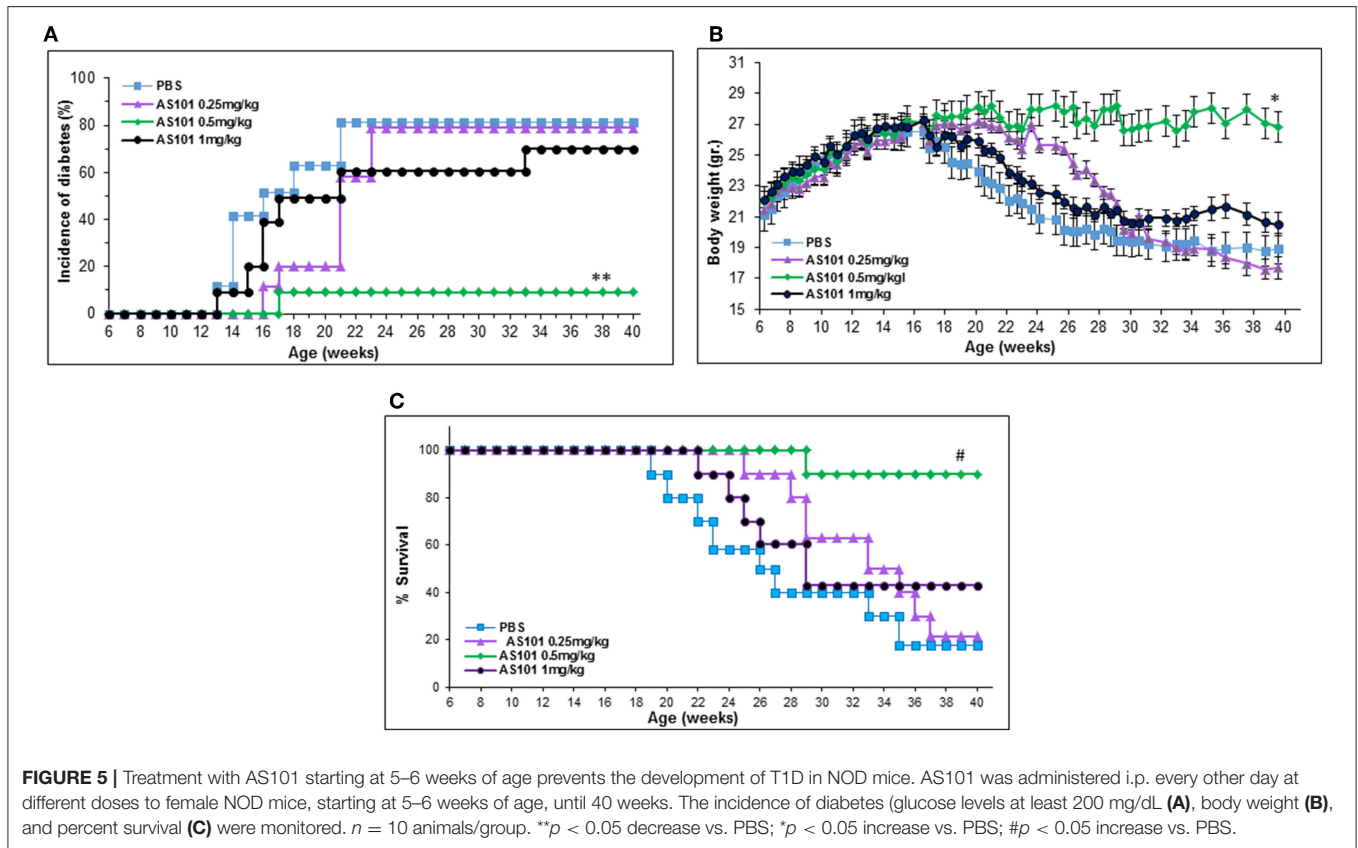
increased survival (Figure 5C) in mice receiving the optimal dose of 0.5 mg/kg. When treatment with AS101 at 0.5 mg/kg/mouse was stopped at 40 weeks of age, no change occurred with respect to percent incidence of diabetes, percent survival, or body weight until at least 52 weeks, suggesting a lasting and significant remission in the course of the disease. The average weight of mice at 52 weeks was 30 ± 77 gr, and 100% of mice surviving at 40 weeks, were also surviving with no diabetes at 52 weeks. None of the mice in the PBS control group survived to 52 weeks of age (data not shown). Importantly, even when AS101 was administered at an advanced stage of insulinitis (12 weeks), the incidence of diabetes in treated mice until 40 weeks of age was only 10%, whereas that of control mice reached 90% at that time point (Figure 6A). This timing of AS101 administration resulted in preserved body weight (Figure 6B) and increased survival (Figure 6C). Importantly, even when treatment with AS101 at 0.5 mg/kg/mouse was started at 12 weeks and stopped at 40 weeks, all mice were remained alive, diabetes-free, and maintained their body weight at 52 weeks (average body weight of 26.88 ± 1.01 grams at 52 weeks). None of the mice in the control group were still alive at that time (data not shown). Moreover, not only could AS101 prevent development of diabetes when administered to pre-diabetic mice with different degrees of insulinitis, but AS101 increased survival and body weight as well when administered to 14 w old mice that already developed symptoms of diabetes (all untreated NOD mice were diabetic at this time point, not shown) (Figures 6D,E).

Treatment of NOD Mice With SAS Alleviates Clinical Symptoms of Diabetes

The encouraging results obtained regarding the beneficial effects of AS101 treatment on the course of T1D led us to investigate the potential effect of the new generation tellurium compound, SAS, shown here to inhibit the activity of $\alpha 4\beta 7$ (Figures 2–4), on the intensity of disease. Treatment with SAS was compared to that with AS101 (the optimal dose of the specific batch of AS101 at that time was 1 mg/kg/injection).

Figure 7 shows that extended treatment with the tellurium compounds, AS101 and SAS of NOD mice starting at 5–6 weeks (Figures 7A–D) or at 3–4 weeks (Figures 7E–H) [initiation of the autoimmune process (23)], decreased levels of blood glucose (Figures 7A,E), decreased incidence of diabetes (Figures 7B,F), decreased weight loss (Figures 7C,G) and eventually increased survival (Figures 7D,H) (Figure 7). The results show that treatment with the tellurium compounds delay the onset of the disease, and even result in its prevention with increased efficiency when treatment is started earlier.

Pretreatment with AS101 or SAS preserved optimal glucose tolerance (Figure 8). Treatment with AS101 and SAS resulted in normal levels of glucose in intraperitoneal glucose tolerance test (IPGTT), either when the test was performed at 12 w of age (Figure 8A), or at 20 w (Figure 8B), suggesting that preservation of pancreatic islets may have occurred in AS101 or SAS-treated mice early after treatment. The fact that in treated mice, 1 h after glucose loading, glucose levels amounted to only



~100 mg/dL, considered to be normal levels, suggests that treatment with tellurium compounds prevents islet destruction, enabling normal function of β cells. Indeed, the results show that the level of insulinitis was dramatically and significantly decreased in treated mice (Figures 8C–H) both with respect to the percentage of infiltrated islets (Figure 8G) and the percentage of cells with severe insulinitis score (Figure 8H). Moreover, the function of the islets was preserved in treated mice, producing normal insulin in pancreatic islets (Figure 9A), as compared to mice in a pre-diabetic state. More importantly, in addition to the normal morphology and size of islets of treated mice (Figure 9B), caspase-3 activity, the classical hallmark of apoptosis, was significantly decreased compared to control mice, and not significantly different from that of pre-diabetic mice (Figure 9C).

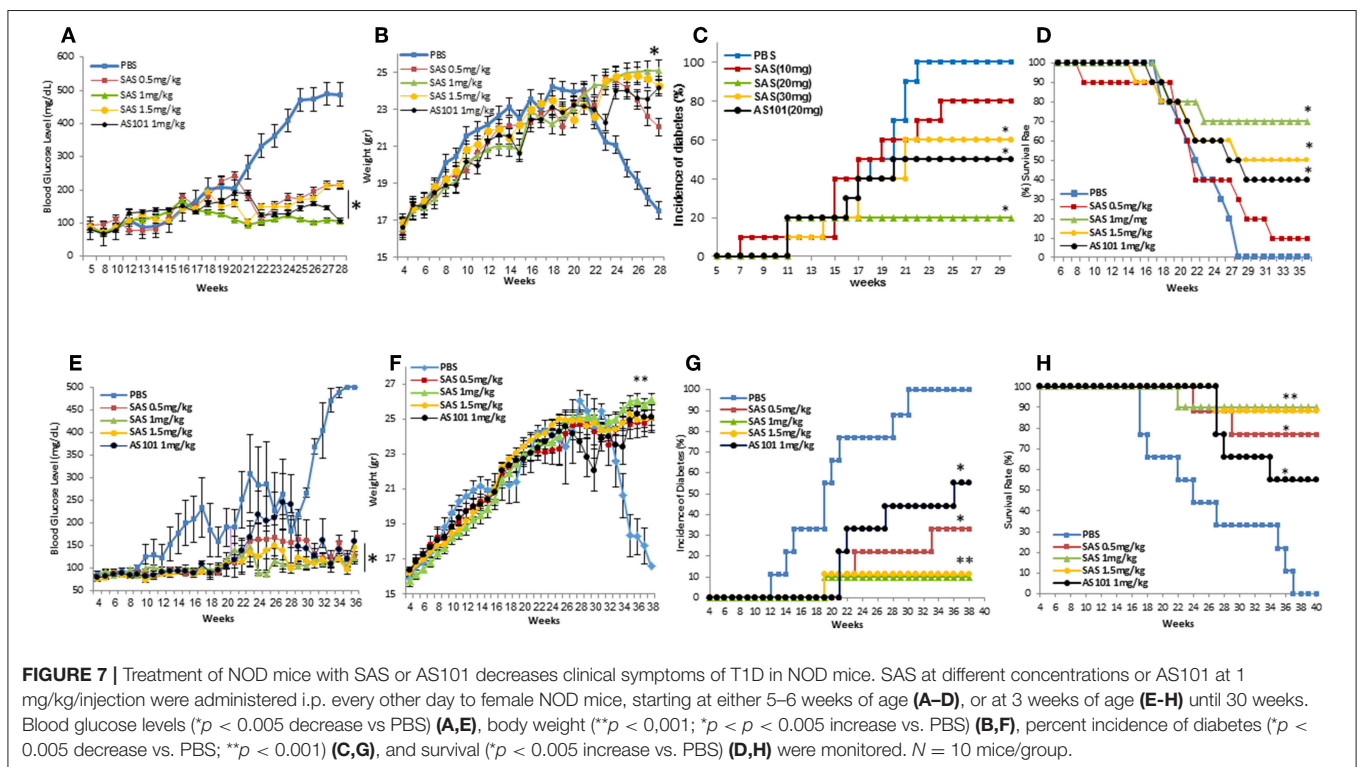
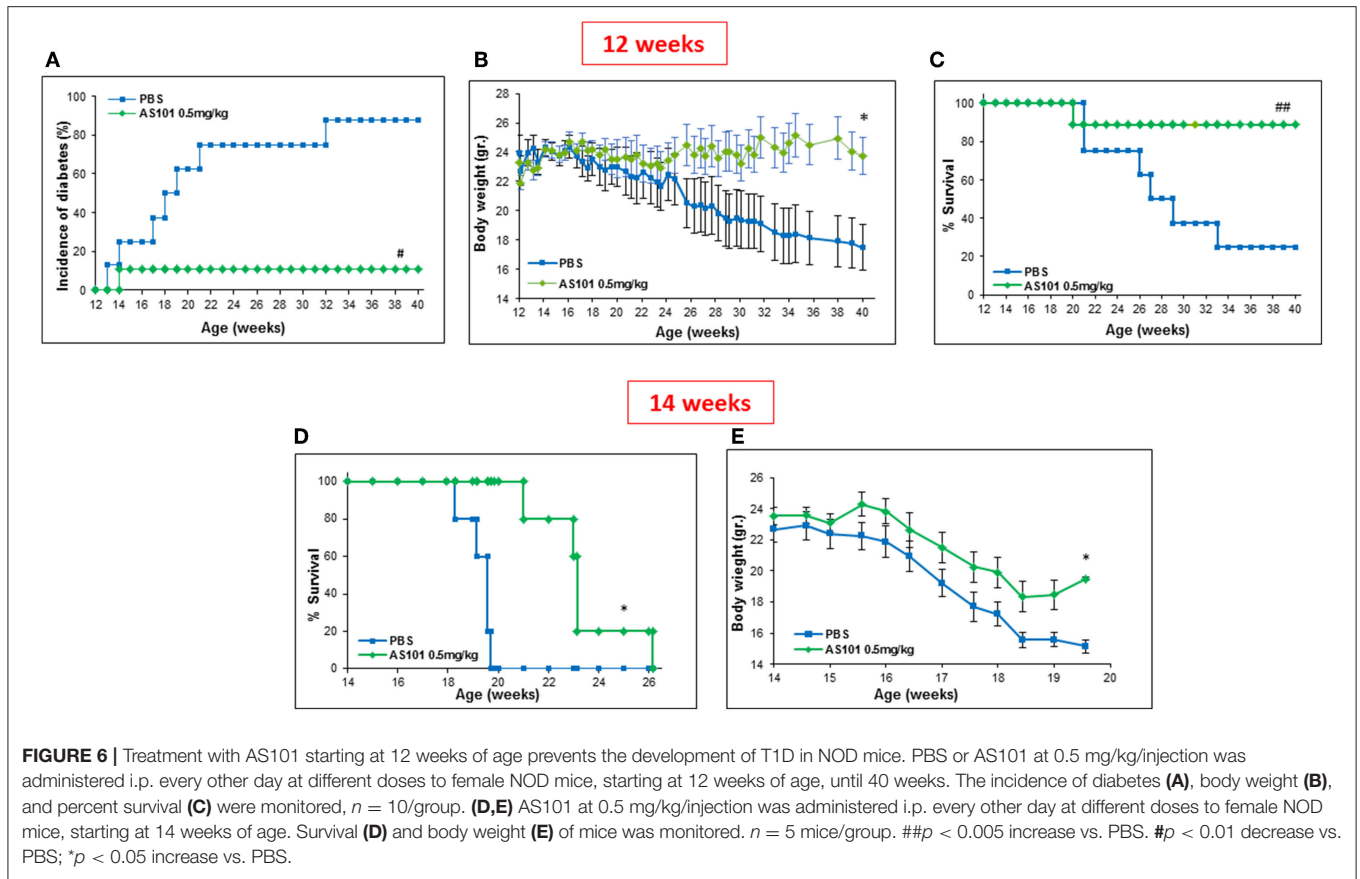
AS101 and SAS Attenuate Pancreatic Inflammation and Increase the Frequency of Tregs

In order to gain insight into the mechanism of the beneficial effects of both tellurium compounds in T1D, we first examined whether integrin $\alpha 4\beta 7$ activity in PAN lymph nodes was inhibited following treatment. As can be seen in Figure 10A, the integrin activity in PLN lymphocytes from NOD mice treated with the compounds from 3 to 20 w was significantly inhibited. This was manifested by diminished attachment of the cells to the $\alpha 4\beta 7$

specific ligand, MADCAM-1. This inactivation might prevent the infiltration of autoreactive cells to the pancreas.

We next examined protein expression of IL-1 β , a key proinflammatory cytokine, which drives several autoimmune diseases. IL-1 is a pleiotropic cytokine, which attenuates CD4⁺CD25⁺FoxP3⁺ regulatory T cell function, and allows the CD4⁺CD25⁻ autoreactive effector subset to escape from suppression (24). IL-1 β has been previously shown to be inhibited by AS101 in various pre-clinical studies (14, 16, 25). Figure 10B shows that both AS101 and SAS significantly inhibited pancreatic IL-1 protein levels at 20 weeks. Furthermore, caspase-1 activity, previously shown to be inhibited by AS101 (16, 25) resulting in decreased IL-1 β , was also inhibited in this study in PAN LN by AS101 and SAS-treated mice (Figure 10C). Importantly, the pro-inflammatory cytokine IL-17, which is implicated as essential in the development of T1D (26), was also inhibited in treated mice (Figure 10D). Furthermore, the Th1 cytokine IFN γ , playing a dominant role in the pathogenesis of autoimmune diabetes (27), was also significantly decreased in SAS and AS101-treated mice (Figure 10E). These results prompted us to evaluate the proportion of CD4⁺CD25⁺FoxP3⁺ regulatory T cells, known to be affected by these two inflammatory cytokines (IL-1 β and IL-17). Figures 11A–D shows a significant increase in the proportion of Tregs among PAN LN cells.

The increase in Tregs following treatment by SAS or AS101 prompted us to evaluate if the tellurium compounds affect the $\alpha 4\beta 7$ integrin activity in Tregs vs. T effector cells and to



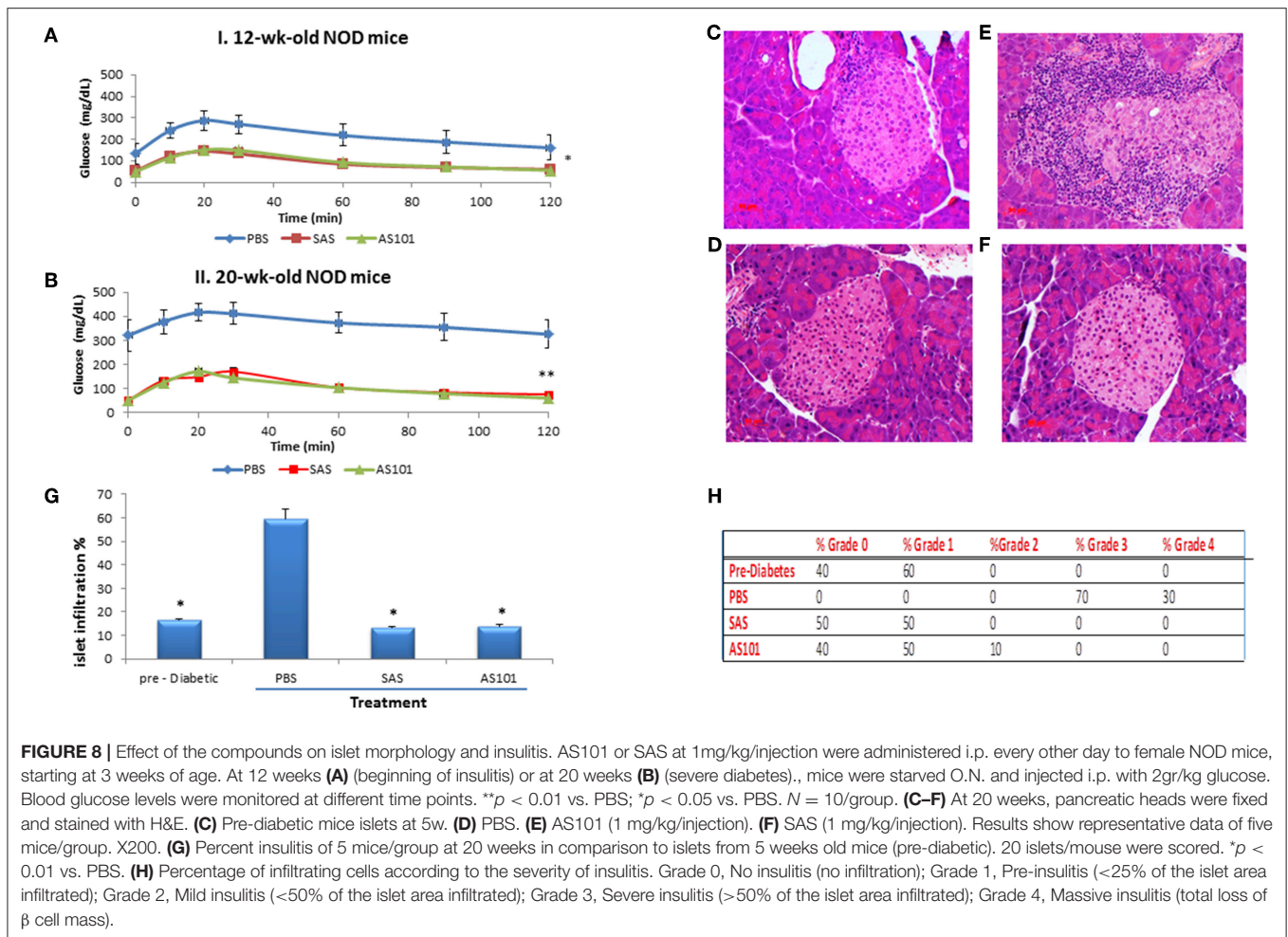


FIGURE 8 | Effect of the compounds on islet morphology and insulinitis. AS101 or SAS at 1 mg/kg/injection were administered i.p. every other day to female NOD mice, starting at 3 weeks of age. At 12 weeks (A) (beginning of insulinitis) or at 20 weeks (B) (severe diabetes), mice were starved O.N. and injected i.p. with 2gr/kg glucose. Blood glucose levels were monitored at different time points. ** $p < 0.01$ vs. PBS; * $p < 0.05$ vs. PBS. $N = 10$ /group. (C–F) At 20 weeks, pancreatic heads were fixed and stained with H&E. (C) Pre-diabetic mice islets at 5w. (D) PBS. (E) AS101 (1 mg/kg/injection). (F) SAS (1 mg/kg/injection). Results show representative data of five mice/group. X200. (G) Percent insulinitis of 5 mice/group at 20 weeks in comparison to islets from 5 weeks old mice (pre-diabetic). 20 islets/mouse were scored. * $p < 0.01$ vs. PBS. (H) Percentage of infiltrating cells according to the severity of insulinitis. Grade 0, No insulinitis (no infiltration); Grade 1, Pre-insulinitis (<25% of the islet area infiltrated); Grade 2, Mild insulinitis (<50% of the islet area infiltrated); Grade 3, Severe insulinitis (>50% of the islet area infiltrated); Grade 4, Massive insulinitis (total loss of β cell mass).

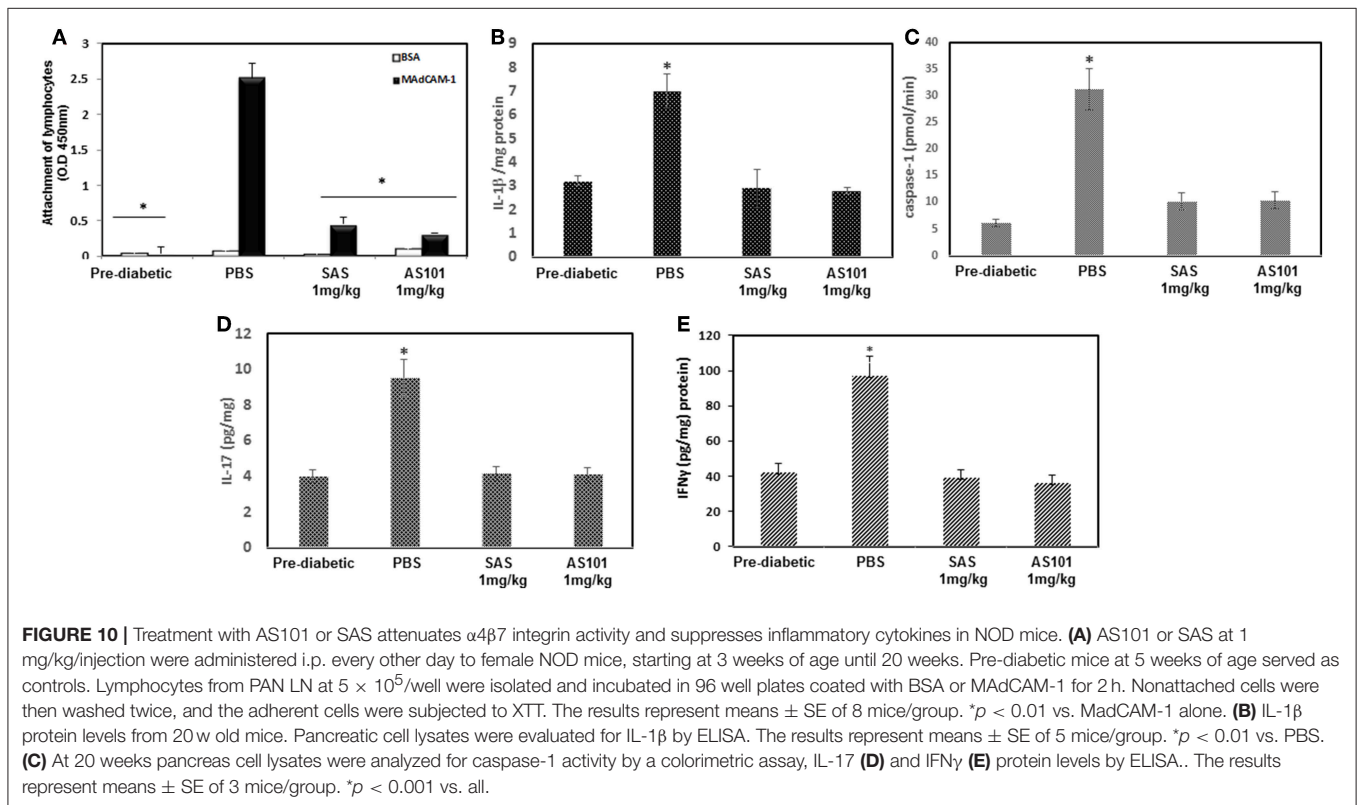
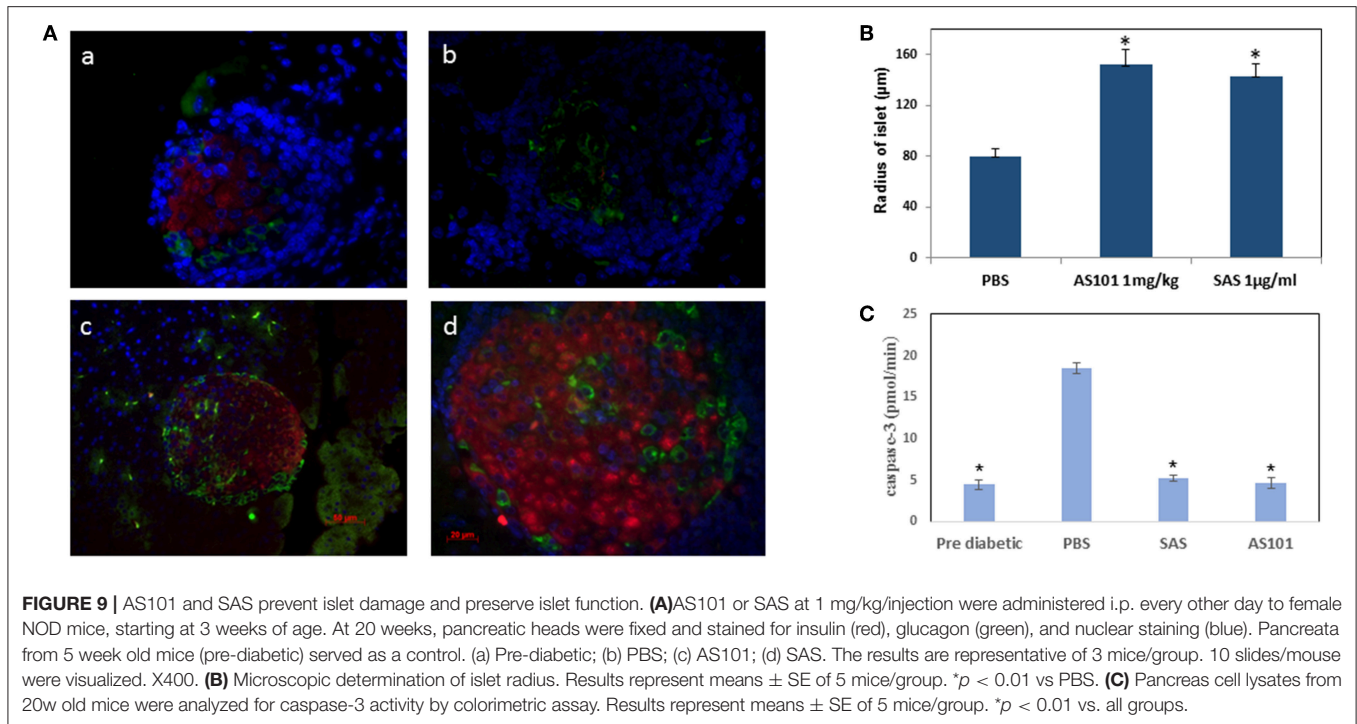
explore the effects and if this property affects the migration of each subset of cells. Figures 12A,B shows that SAS and AS101 inhibits the activity of the $\alpha 4\beta 7$ integrin in both isolated T effector cells (Figure 12A) and regulatory cells (Figure 12B); nevertheless, the extent of inhibition is higher in T effector cells. Moreover, the $\alpha 4\beta 7$ integrin-dependent migration of both T cell subsets was also inhibited by both compounds, however that of T effector cells was more intense. Furthermore, The extent of control T regulatory cells migration was significantly lower than that of T effector cells (Figure 12C). Interestingly, the expression of $\alpha 4\beta 7$ in T regulstoty cells was lower than that of T effector cells (Figure 12D). This might explain the lower $\alpha 4\beta 7$ -dependent of Tregs and their decreased sensitivity to tellurium compound inhibition.

Finally, we wished to evaluate if offspring of AS101-treated NOD mothers acquire some resistance to T1D development. Female NOD mice were treated with AS101 starting 2 weeks prior to mating, during pregnancy and throughout breast feeding. When the pups were analyzed, they exhibited decreased incidence of diabetes (Supplementary Figure 1A) and increased survival (Supplementary Figure 1B). Moreover, offspring of treated mothers were relatively more resistant to adoptive transfer of autoreactive cells as expressed by

decreased incidence of diabetes (Supplementary Figure 1C) and increased survival (Supplementary Figure 1D). These results suggest that AS101 can significantly protect the fetus from future diabetes development when the mother is treated during pregnancy. Furthermore, the fetus might acquire both diminished autoreactive cells and increased regulatory mechanisms.

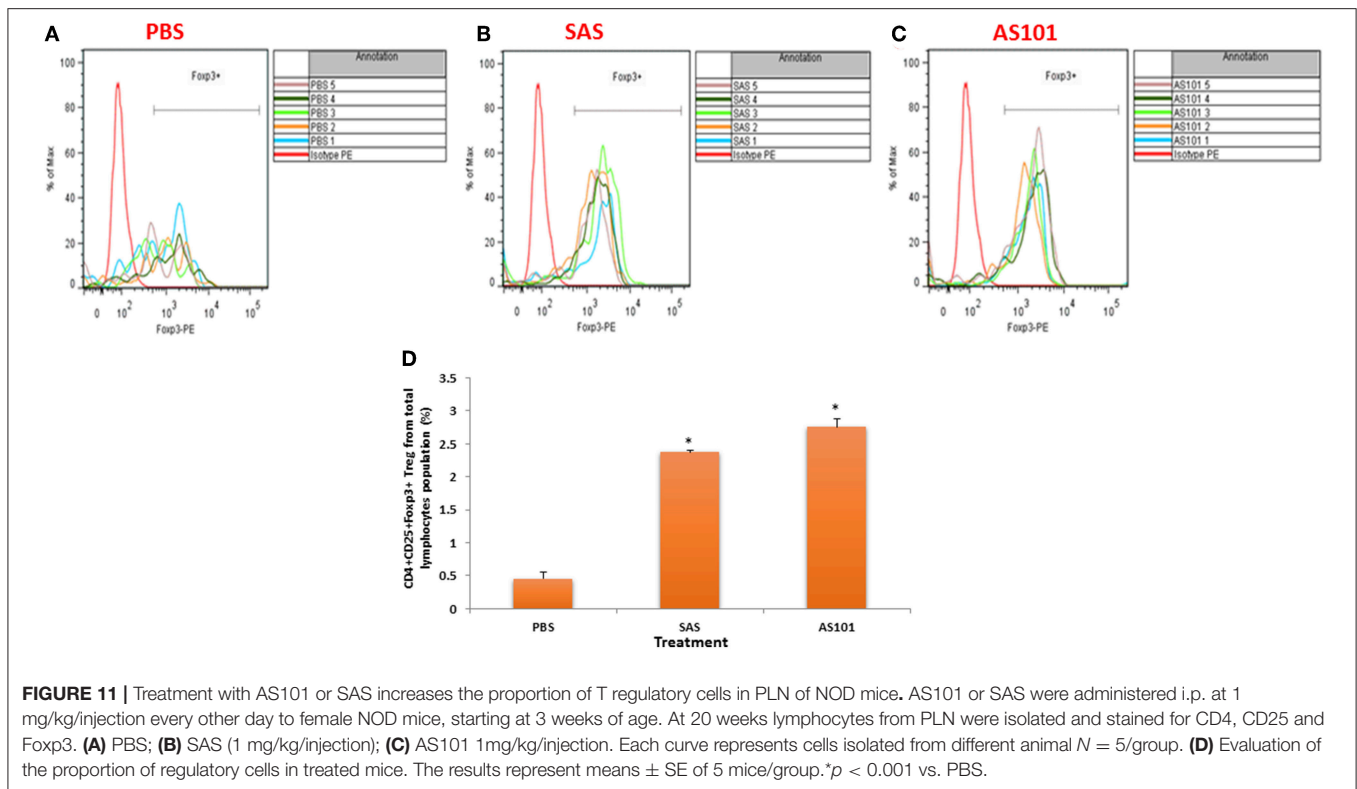
DISCUSSION

The present study presents evidence demonstrating the beneficial effects of the two non-toxic tellurium small molecules AS101 and SAS in a mouse model of T1D. Systemic treatment with these compounds could preserve β cells function and mass. These beneficial effects were reflected in decreased incidence of diabetes, decreased hyperglycemia, improved glucose clearance, preservation of body weight and increased survival. The normal glucose levels were associated with increased insulin levels, preservation of β cell mass and increased islet size compared to controls. Importantly, this protective activity could be demonstrated when the compounds were administered either at the early pre-diabetic phase with either no or early insulinitis, at the pre-diabetic stage with advanced insulinitis, or even at the



advanced, overtly diabetic stage. Moreover, delaying the onset of diabetes or its prevention was dose-dependent, showing an optimum curve.

Our results suggest that both tellurium compounds prevent migration of autoimmune lymphocytes to the pancreas, i.e., prevent insulinitis, by inhibiting $\alpha 4 \beta 7$ integrin activity. Indeed,

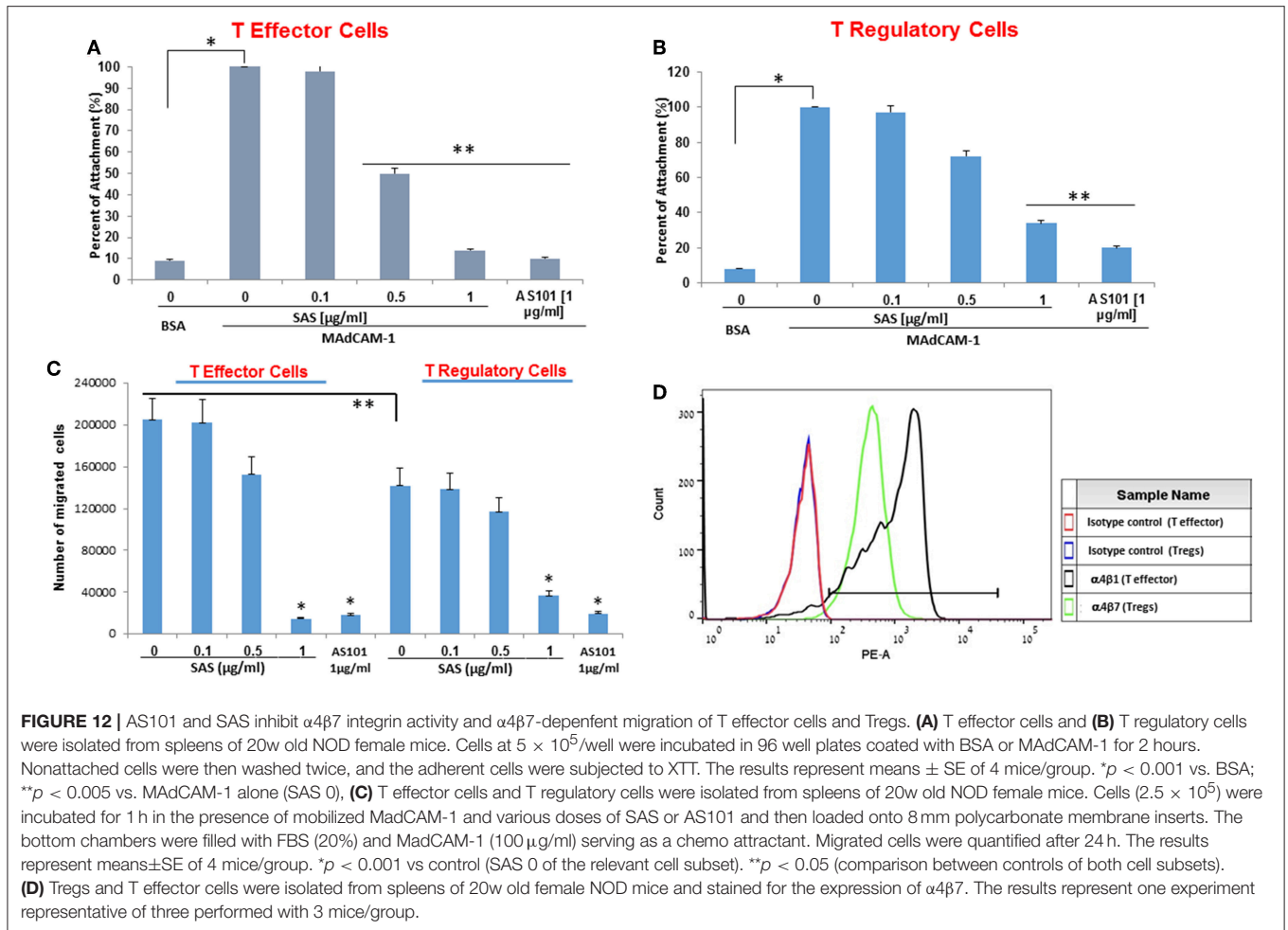


the decreased infiltration resulted in a significantly diminished extent of pancreatic islet damage, both with respect to their size, β cell function (insulin production), and caspase-3 activity, the hallmark of apoptosis. Most importantly AS101 and SAS significantly elevated the frequency of T regulatory cells in the pancreas, thus potentially controlling the autoimmune process.

We have shown in our previous studies that much of the biological activity of AS101 and SAS is directly mediated by their unique chemical interactions with specific cysteine thiol residues. The formation of such TeIV-thiol bonds may induce conformational changes or alter disulfide bond formation in specific proteins, possibly resulting in the loss of their biologic activity, in cases where the thiol residue is essential for a particular function (28, 29). Much of the biological activity of AS101 may be directly attributed to its redox interactions with vicinal thiols in the exofacial of the $\alpha 4\beta 1$ integrin VLA-4 (10), residing within the α but not the β chain of the integrin. We thus suggested that the $\alpha 4\beta 7$ integrin sharing the same $\alpha 4$ chain might be similarly inhibited by the tellurium compounds. This also implies that the specific thiol residues on the $\alpha 4$ chain of the $\alpha 4\beta 7$ integrin are essential for the $\alpha 4\beta 7$ integrin activity. Indeed, we previously showed that AS101 inhibits the $\alpha 4\beta 7$ activity in mesenteric lymph node cells of DSS-induced murine colitis (14). This study shows for the first time that both tellurium compounds suppress the activity of the $\alpha 4\beta 7$ integrin in pancreatic LNs, impacting lymphocyte infiltration into PanLN, and consequently affecting the pathogenesis of T1D.

It was previously shown that the mucosal vascular addressin MAdCAM-1, the ligand for $\alpha 4\beta 7$, is also constitutively expressed

at low levels on pancreatic vasculature and, in conjunction with the appearance of lymphocyte infiltrates (insulinitis) in pancreatic islets, becomes strongly induced on islet vessels (30–32). Indeed, we show in the present work that both tellurium compounds inhibit the attachment of lymphocytes from pancreatic lymph nodes of NOD mice to either rMAdCAM-1 or to MAdCAM-1 expressed on endothelial cells after exposure to TNF α . This inhibition was demonstrated both *in vitro* and *in vivo*. MAdCAM - 1 is expressed on the surface of endothelial cells in response to secretion of inflammatory cytokines such as to secretion of inflammatory cytokines, including Tumor necrosis alpha (TNF- α) and Interleukin-1 beta (IL-1 β). We found that mice successfully treated with AS101 or SAS, showed a significant decrease in pancreatic IL-1 β , a cytokine that plays an important role in many autoimmune diseases (33). The decrease in IL-1 β suggests that AS101 and SAS prevent insulinitis not only by inhibiting the activity of the $\alpha 4\beta 7$ integrin but also possibly by indirectly inhibiting the expression MAdCAM-1 on pancreatic vasculature via inhibition of IL-1 β . This cytokine has been previously shown to be inhibited by the tellurium compounds in various preclinical studies due to inhibition of caspase-1 activity (14, 16, 26). Interestingly, we have recently shown that modulation of caspase-1 activity is dependent on VLA-4 inactivation and is mediated through VLA-4 inactivation, and underlies the anti-inflammatory activity of AS101 (16). In line with this, the present study shows a significant decrease in pancreatic caspase-1 activity. The inhibition of $\alpha 4\beta 7$ activity by both Tellurium compounds was most evident both in T effector cells and Treg cells, although that of Tregs was lower. This might



be explained by the diminished expression of the $\alpha 4\beta 7$ integrin in Tregs.

It has been previously reported that IL-1 β induces proliferation and cytokine production by CD4⁺CD25⁺FoxP3⁻ effector/memory T cells, attenuates CD4⁺CD25⁺FoxP3⁺ regulatory T cell function, and releases CD4⁺CD25⁻ autoreactive effectors from physiological suppression (24). Thus, inflammation or constitutive overexpression of IL-1 β in a genetically predisposed host can promote activation and expansion of autoreactive effector T cells, thereby interfering with the ability of Treg cells to maintain self-tolerance. Thus, inhibition of IL-1 β by AS101 and SAS might explain the upregulation of Tregs by the compounds.

Multiple studies support an essential role of Th17 cells together with defects in T regulatory (Treg) cell function in the development of T1D in both animal models and in humans (34). The decrease in pancreatic Th17 in AS101 or SAS treated mice can be explained in two ways. IL-1 β is known to promote IL-17 production by memory CD4⁺ T cells (35). Hence, its inhibition by the tellurium compounds might affect IL-17 levels. Conversely, the increase in regulatory cells could regulate Th17 cells. The potential pathways through which Treg cells regulate

the Th17 cell response have been elucidated in several studies. In mice, CD4⁺ Treg cells restrain the Th17 immune response via Foxp3 binding to STAT3, a key factor in the initiation of Th17 differentiation (36). Overexpression of Foxp3 results in a strong reduction of IL17A gene expression by inhibiting ROR γ t-mediated IL-17A mRNA transcription (37). On the other hand, Th17 cells counteract the Treg cells, to expand and allow the development of T1D (26). Thus, the homeostasis between Th17 and Tregs is important in keeping autoimmunity in check. Our results showing a delay of the onset of diabetes by AS101 and SAS combined with their effect on IL-17 and Tregs imply they have the ability to preserve this homeostasis and regulate autoimmunity. Interestingly, although the tellurium compounds inhibited $\alpha 4\beta 7$ -dependent migration of both effector cells and Tregs, the proportion of Tregs in PLNs was increased in treated mice. We assume that this may be explained by our results showing that the extent of inhibition is lower in Tregs as opposed to T effector cells, possibly because of the lower expression of $\alpha 4\beta 7$ on Tregs (**Figure 12D**). Furthermore, even if less Tregs arrived to pancreatic lymph nodes, their number would be much increased with time within their low IL-1 β and IL-17 environment.

Several clinically relevant observations emerged from this study:

1. When treatment with AS101 was stopped at 40 weeks of age, no change occurred with respect to incidence of diabetes, survival, or body weight at least until 52 weeks, suggesting that this compound had a long lasting effect on preventing T1D.
2. AS101 could alleviate symptoms of the disease even when treatment was started at relatively advanced stages of the disease (12 or 14 weeks of age). While T1D can be predicted in human patients based on the presence of antibodies recognizing autoantigens, such as insulin, GAD65 (glutamic acid decarboxylase), IA-2 (islet antigen 2), and ZnT8 (islet zinc transporter) (38), there is no known way to prevent or arrest the development of this disease, nor has any treatment been demonstrated that can reduce the anti- β -cell autoimmune response following the diagnosis of diabetes. Therefore, our positive results in the early treatment protocol (starting at 3 weeks of age) are applicable to individuals at risk, in which the disease is predicted by the presence of autoantibodies. Conversely, at more advanced stages of disease, AS101 might enable regeneration of β cells or their preservation. Indeed, it has been previously suggested that β cell regeneration can be induced in animal models provided a sufficient endogenous β cell mass remains (39, 40).
3. The increased resistance to diabetes followed by the increased survival could be transferred to offspring of 5w old female NOD mice treated with AS101 starting 2 weeks prior to gestation, during pregnancy and throughout breast feeding (**Supplementary Figure 1**). These results suggest that AS101 can significantly protect the fetus from future diabetes development when the mother is treated during pregnancy. The fact that decreased incidence of diabetes occurs in offspring of treated mothers undergoing adoptive transfer of autoreactive cells suggests that these offspring acquire from the mother regulatory means to fight the autoimmune cells injected. Thus, not only the fetus but the newborn as well can benefit from treatment of his mother. It has been recently reported that breastfeeding transfers to the infant not only protective antibodies but also maternal immune cells (41). The mechanisms underlying these important results are now the focus of our laboratory studies.

REFERENCES

1. Wing K, Sakaguchi S. Regulatory T cells exert checks and balances on self-tolerance and autoimmunity. *Nat Immunol.* (2010) 11:7–13. doi: 10.1038/ni.1818
2. Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. *Science.* (1996) 272:60–6.
3. Campbell DJ, Kim, C.H, Butcher EC. Chemokines in the systemic organization of immunity. *Immunol. Rev.* (2003) 195:58–71.
4. von Andrian UH, Mempel TR. Homing and cellular traffic in lymph nodes. *Nat. Rev. Immunol.* (2003) 3:867–78. doi: 10.1038/nri1222
5. Wong FS, Wen L, Tang M, Ramanathan M, Visintin I, Daugherty J, et al. Investigation of the role of B-cells in type 1 diabetes in the NOD mouse. *Diabetes.* (2004) 53:2581–7. doi: 10.2337/diabetes.53.10.2581

In summary, we introduce in the present study two non-toxic small molecules that, by virtue of their unique mechanism of action, may both prevent trafficking of autoreactive B and T cells to the pancreas and regulate the activity of those that have infiltrated the tissue. As AS101 demonstrated an excellent safety profile in clinical trials for other indications, these compounds may serve as good candidates for the treatment of T1D.

ETHICS STATEMENT

Experiments conformed to approved institutional protocols and were approved by the Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

TY and ZB performed and analyzed the experiments. DK-S provided technical assistance. YK, RC, and BS designed the study, discussed the results, and wrote the paper. All the authors discussed the results and commented on the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2019.00979/full#supplementary-material>

Supplementary Figure 1 | The decreased incidence of diabetes followed by the increased survival can be transferred to offspring of treated female NOD mice. 5w old female mice were treated with PBS or with various concentrations of AS101. Treatment continued during gestation and pregnancy and during breast feeding until the offspring aged 3 weeks. At that time treatment stopped and female offspring were evaluated for % incidence of diabetes (**A**) and for % survival (**B**) from 10 to 40 weeks. $N = 15$ offspring mice/group. * $p < 0.05$; ** $p < 0.005$ vs. PBS. Some of the male offspring of treated mothers were irradiated at 6w of age with 550Gy and were injected 24h later with 20×10^6 splenocytes from diabetic female NOD mice. The incidence of diabetes (**C**) and the percentage of survival (**D**) were monitored until 30 weeks. * $p < 0.05$ vs. PBS. N-9.

6. Xu B, Cook RE, Michie SA. $\alpha 4\beta 7$ integrin/MAdCAM-1 adhesion pathway is crucial for B cell migration into pancreatic lymph nodes in nonobese diabetic mice. *J. Autoimmun.* (2010) 35:124–9. doi: 10.1016/j.jaut.2010.04.002
7. Baron JL, Reich EP, Visintin I, Janeway, CA Jr. The pathogenesis of adoptive murine autoimmune diabetes requires an interaction between alpha 4-integrins and vascular cell adhesion molecule-1. *J. Clin. Invest.* (1994) 93:1700–8. doi: 10.1172/JCI117153
8. Sredni B, Caspi RR, Klein A, Kalechman Y, Danziger Y, Ben Ya'akov M, et al. New immunomodulating compound (AS-101) with potential therapeutic application. *Nature.* (1987) 330:173–6. doi: 10.1038/330173a0
9. Sredni B, Xu RH, Albeck M, Gafter U, Gal R, Shani A, et al. The protective role of the immunomodulator AS101 against chemotherapy-induced alopecia studies on human and animal models. *Int. J. Cancer.* (1996) 65:97–103.

10. Layani-Bazar A, Skornick I, Berrebi A, Pauker MH, Noy E, Silberman A, et al. Redox modulation of adjacent thiols in VLA-4 by AS101 converts myeloid leukemia cells from a drug-resistant to drug-sensitive state. *Cancer Res.* (2014) 74:3092–103. doi: 10.1158/0008-5472.CAN-13-2159
11. Lee JH, Halperin-Sheinfeld M, Baatar D, Mughal MR, Tae HJ, Kim JW, et al. Tellurium compound AS101 ameliorates experimental autoimmune encephalomyelitis by VLA-4 inhibition and suppression of monocyte and T cell infiltration into the CNS. *Neuromolecular Med.* (2014) 16:292–307. doi: 10.1007/s12017-013-8277-3
12. Bing SJ, Shemesh I, Chong WP, Horai R, Jittayasothorn Y, Silver PB, et al. AS101 ameliorates experimental autoimmune uveitis by regulating Th1 and Th17 responses and inducing Treg cells. *J Autoimmun.* (2019) 100:52–61. doi: 10.1016/j.jaut.2019.02.006
13. Sredni B. Immunomodulating tellurium compounds as anti-cancer agents. *Semin Cancer Biol.* (2012) 22:60–69. doi: 10.1016/j.semcancer.2011.12.003
14. Halpert G, Eitan T, Voronov E, Apte RN, Rath-Wolfson L, Albeck M, et al. Multifunctional activity of a small tellurium redox immunomodulator compound, AS101, on dextran sodium sulfate-induced murine colitis. *J Biol Chem.* (2014) 289:17215–27. doi: 10.1074/jbc.M113.536664
15. Kalechman Y, Gafter U, Da JP, Albeck M, Alarcon-Segovia D, Sredni B. Delay in the onset of systemic lupus erythematosus following treatment with the immunomodulator AS101: association with IL-10 inhibition and increase in TNF-alpha levels. *J Immunol.* (1997) 159:2658–67.
16. Hachmo Y, Kalechman Y, Skornick I, Gafter U, Caspi RR, Sredni B. The small tellurium compound AS101 ameliorates rat crescentic glomerulonephritis: Association with inhibition of macrophage caspase-1 activity via very late antigen-4 inactivation. *Front Immunol.* (2017) 8:240. doi: 10.3389/fimmu.2017.00240
17. Halpert G, Sredni B. *Autoimmun Rev.* (2014) 13:1230–5. doi: 10.1016/j.autrev.2014.08.003
18. Kalechman Y, Gafter U, Gal R, Rushkin G, Yan D, Albeck M, et al. Anti-IL-10 therapeutic strategy using the immunomodulator AS101 in protecting mice from sepsis-induced death: dependence on timing of immunomodulating intervention. *J Immunol.* (2002) 169:384–92. doi: 10.4049/jimmunol.169.1.384
19. Danoch H, Kalechman Y, Albeck M, Longo DL, Sredni B. Sensitizing B- and T- cell lymphoma cells to paclitaxel/abraxane-induced death by AS101 via inhibition of the VLA-4-IL10-survivin axis. *Mol. Cancer Res.* (2015) 13:411–22. doi: 10.1158/1541-7786.MCR-14-0459
20. Sredni B, Weil M, Khomenok G, Lebenthal I, Teitz S, Mardor Y, et al. Ammonium trichloro(dioxoethylene-o,o')tellurate (AS101) sensitizes tumors to chemotherapy by inhibiting the tumor interleukin 10 autocrine loop. *Cancer Res.* (2004) 64:1843–52.
21. Yang, XD., Sytwu HK, McDevitt HO, Michie SA. Involvement of $\beta 7$ integrin and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in the development of diabetes in obese diabetic mice. *Diabetes.* (1997) 46:1542–7.
22. Oshima T, Pavlick KP, Laroux FS, Verma SK, Jordan P, Grisham MB, et al. Regulation and distribution of MAdCAM-1 in endothelial cells *in vitro*. *Am J Physiol Cell Physiol.* (2001) 281:C1096–1105. doi: 10.1152/ajpcell.2001.281.4.C1096
23. Högglund P, Minter J, Waltzinger C, Heath W, Benoist C, Mathis D. Initiation of autoimmune diabetes by developmentally regulated presentation of islet cell antigens in the pancreatic lymph nodes. *J Exp Med.* (1999) 189:331–9.
24. O'Sullivan BJ, Thomas HE, Pai S, Santamaria P, Iwakura Y., Steptoe RJ, et al. IL-1 beta breaks tolerance through expansion of CD25+ effector T cells. *J Immunol.* (2006) 176:7278–7. doi: 10.4049/jimmunol.176.12.7278
25. Sredni, Geffen-Aricha R, Duan W, Albeck M, Shalit F, Lander HM, et al. Multifunctional tellurium molecule protects and restores dopaminergic neurons in Parkinson's disease models. *FASEB J.* (2007) 21:1870–83. doi: 10.1096/fj.06-7500com
26. Emamaullee JA, Davis J, Merani S, Toso C, Elliott JF, Thiesen A, et al. Inhibition of Th17 cells regulates autoimmune diabetes in NOD mice. *Diabetes.* (2009) 58:1302–11. doi: 10.2337/db08-1113
27. Sobel D, Ahvazi B, Pontzer C. The role of type I interferon subtypes and interferon-gamma in type I interferon diabetes inhibitory activity in the NOD mouse. *J Interferon Cytokine Res.* (2016) 36:238–46. doi: 10.1089/jir.2014.0232
28. Kalechman Y, Longo DL, Catane R, Shani A, Albeck M, Sredni B. Synergistic antitumoral effect of paclitaxel (Taxol)+AS101 in a murine model of B16 melanoma: association with ras-dependent signal-transduction pathways. *Int J Cancer.* (2000) 86:281–8.
29. Yosef S, Brodsky M, Sredni B, Albeck A, Albeck M. *ChemMedChem.* (2007) 2:1601–6. doi: 10.1002/cmcd.200700155
30. Hänninen A, Taylor C, Streeter PR, Stark LS, Sarte JM, Shizuru JA, et al. Vascular addressins are induced on islet vessels during insulinitis in nonobese diabetic mice and are involved in lymphoid cell binding to islet endothelium. *J Clin Invest.* (1993) 92:2509–15. doi: 10.1172/JCI116859
31. Yang XD, Michie SA, Tisch R, Karin N, Steinman L, McDevitt HO. A predominant role of integrin $\alpha 4$ in the spontaneous development of autoimmune diabetes in nonobese diabetic mice. *Proc Natl Acad Sci USA.* (1994) 91:12604–08. doi: 10.1073/pnas.91.26.12604
32. Hänninen A, Jaakkola I, Jalkanen S. Mucosal addressin is required for the development of diabetes in nonobese diabetic mice. *J Immunol.* (1998) 160:6018–25.
33. Dinarello CA, van der Meer JW. Treating inflammation by blocking interleukin-1 in humans. *Semin. Immunol.* (2013) 25:469–84. doi: 10.1016/j.smim.2013.10.008
34. Li Y, Liu Y, Chu CQ. Th17 cells in type 1 diabetes: Role in the pathogenesis and regulation by gut microbiome. *Mediators Inflamm.* (2015) 2015:638470–77. doi: 10.1155/2015/638470
35. Bradshaw EM, Raddassi K, Elyaman W, Orban T, Gottlieb PA, Kent SC, et al. Monocytes from patients with type 1 diabetes spontaneously secrete proinflammatory cytokines inducing Th17 cells. *J Immunol.* (2009) 183:4432–9. doi: 10.4049/jimmunol.0900576
36. Chaudhry A, Rudra D, Treuting P, Samstein RM, Liang Y, Kas A, et al. CD4+ regulatory T cells control TH17 responses in a stat3-dependent manner. *Science.* (2009) 326:986–91. doi: 10.1126/science.1172702
37. Ichiyama K, Yoshida H, Wakabayashi Y, Chinen, T., Saeki, K., Nakaya M, et al. Foxp3 inhibits ROR γt -mediated IL-17A mRNA transcription through direct interaction with ROR γt . *J Biol Chem.* (2008) 283:17003–8. doi: 10.1074/jbc.M801286200
38. Stadinski B, Kappler J, Eisenbarth GS. Molecular targeting of islet autoantigens. *Immunity.* (2010) 32: 446–6. doi: 10.1016/j.immuni.2010.04.008
39. Chong AS, Shen J, Tao J, Yin D, Kuznetsov A, Hara M, et al. Reversal of diabetes in non-obese diabetic mice without spleen cell-derived β cell regeneration. *Science.* (2006) 311:1774–5. doi: 10.1126/science.1123510
40. Pospisilik JA, Martin J, Doty T, Ehses JA, Pamir N, Lynn FC, et al. Dipeptidyl peptidase IV inhibitor treatment stimulates β -cell survival and islet neogenesis in streptozotocin-induced diabetic rats. *Diabetes.* (2003) 52:741–50. doi: 10.2337/diabetes.52.3.741
41. Cabinian A, Sinsimer D, Tang M, Zumba O, Mehta H, Toma A, et al. Transfer of maternal immune cells by breastfeeding: Maternal cytotoxic T lymphocytes present in breast milk localize in the Peyer's patches of the nursed infant. *PLoS ONE.* (2016) 11:e0156762. doi: 10.1371/journal.pone.0156762

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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