Role of Dexamethasone on immune dysregulation mediated through Th1/Th2 cytokine balance in mice challenged with type 1 diabetes and allergic asthma

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ABSTRACT

Dysregulated equilibrium between T helper 1 (Th1) and T helper 2 (Th2) immune responses has been implicated in the pathogenesis of type 1 diabetes (T1D) and asthma. Conflicting evidence exist explaining the association between T1D and asthma and is still a point of debate. In the present study, our objective was to investigate the influence of associated T1Dco morbid condition on the induction of experimental asthma in mice and also to evaluate the efficacy of Dexamethasone (0.5 mg/kg, s.c.ly) in these mice. Type 1 diabetes was induced by a single intravenous injection of alloxan (80 mg/kg) in Balb/c mice. Following diabetes induction, mice were sensitized with an intraperitoneal injection of 50 μg ovalbumin (Ova) emulsified in 2.5 mg aluminum hydroxide on days 3 and 8. From day 13 to day 15, animals were challenged intranasally with 100 μg Ova in 25 μl of sterile saline. Dexamethasone treatment was initiated on sensitization day and continued once in 2 days thereafter until day 15. Control animals received only saline without Ova. On day 16, mice were subjected to nasal hyperresponsiveness (NHR) immediately after the Ova challenge. Bronchioalveolar lavage fluid (BALF), blood, and lungs were collected 1h post completion of NHR for further analysis. Alloxan diabetic mice showed significantly lower levels of eosinophils in BALF and blood with the corresponding decrease in inflammatory cells around airways in hematoxylin & eosin-stained lung sections, but with no change in NHR than in non-diabetics after Ova sensitization and challenge. The presence of T1D featured a unique, yet the intermediary stage of asthma induction and also presented an altered magnitude of Dexamethasone efficacy compared to the absence of T1D in the murine model of Ova induced asthma.

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INTRODUCTION

Immune dysregulation especially T helper 1/ T helper 2 (Th1/Th2) imbalance is associated with the pathogenesis of various chronic immune-mediated diseases and the dominance of Th1 can down-regulate Th2 and vice versa (Kidd, 2003). Type 1 diabetes (T1D) and allergic asthma are mediated through the autoimmune responses of Th1 and Th2 cells, respectively (Metsälä *et al.*, 1981). Converging evidence exists on the involvement of Th1 in exacerbations of severe asthma and likewise Th2 in the pathogenesis of T1D. The co-existence of T1D and asthma is still provocative due to their

opposing regulatory dependence of Th1 and Th2 on each other (Kidd, 2003; Rachmiel *et al.*, 2006). Conflicting results are available from both clinical and nonclinical studies on the association between T1D and asthma. A meta-analysis of clinical literature reported a decrease in the occurrence of asthma in T1D patients (Cardwell *et al.*, 2003). In contrast, a nationwide population-based study in Taiwan showed a 47% higher rate of asthma incidence in the T1D cohort than in a control cohort (Hsiao *et al.*, 2015). Also, a population study in Europe and elsewhere showed a strong positive relationship between the occurrence of T1D and asthma (Stene and Nafstad, 2001). In addition, diabetes-prone autoimmune NOD mice demonstrated exacerbated asthma symptoms compared to normal Balb/c mice indicating a positive association of T1D and asthma (Araujo *et al.*, 2004). However, a recent study in diabetic A/J mice induced by alloxan confirmed the down-regulation of allergic inflammation in the lungs (Carvalho *et al.*, 2018).

Congregating data designate that cytokine disparity due to overexpression of either autoreactive Th1 or Th2 T cells coupled to defective regulatory T cells play a key role in occurrence of these diseases (Araujo *et al.*, 2004; Chou *et al.*, 2013; Anderson and Bluestone, 2005; Bach and Chatenoud, 2001; Holgate, 2012). Functionally, Th1, and Th2 cells are distinguished by their cytokine profile and activities. Interferon-gamma (IFN-γ) is a major cellular immune response cytokine produced by Th1 cells, whereas, interleukin-4 (IL-4) is a principle humoral immune response cytokine produced by Th2 cells (Vaseghi and Jadali, 2016). Although several inflammatory cells like mast cells, eosinophils, macrophages, Th2 lymphocytes, dendritic cells, etc., are involved in the pathophysiology of asthma, clear evidence are available indicating the critical role of cytokines in asthma exacerbations (Mahajan and Mehta, 2006). There is accumulating experimental data that indicate the increased IFN-γ levels and Th1/Th2 ratio in T1D and over secretion of IL-4, IL-5 and IL-13 in asthma (Xiangyang *et al.*, 2006; Guo *et al.*, 2018; Park *et al.*, 2013; Mazzarella *et al.*, 2000; Stewart and Levine, 2010). A unique cytokine phenotype and Th1/Th2 ratio is observed in patients with both T1D and asthma (Rachmiel *et al.*, 2006). Data paucity is present from animal studies that indicate the differences in the Th1/Th2 paradigm and cytokine profile under co-morbid conditions of these two diseases versus asthma alone. It is relevant to understand the immune dysregulation in these conditions for identifying the immune-modulatory agents that may be effective at treating asthma patients with associated autoimmune dia-

betes.

In this study, it was aimed to understand the incidence of allergen-induced experimental asthma in diabetic mice, especially focusing on immune profile changes and also to evaluate the immuno modulatory effect of Dexamethasone in these mice.

MATERIALS AND METHODS

Animals

Male adult Balb/c mice (6-8 weeks old) of Taconic origin were obtained from the Vivo Biotech breeding facility (Vivo Biotech Ltd, India). All experimental procedures involving laboratory animal's use and care in this study were examined and approved by the Institutional Animal Ethics Committee (IAEC) of Vivo Biotech Ltd. bearing protocol number VB/IAEC/07/2019/360/Mouse/Balb/c. Mice were maintained in a controlled environment of the experimental animal room with a temperature of 19-25^{0}C, a relative humidity of 30-70%, a light/dark cycle of 12 hours and 15-20 fresh air changes per hour. Mice were provided with food and water *ad libitum* and housed individually throughout the study period.

Treatment protocol

Animals were randomly assigned into 4 groups with n=6 in each group as follows,

Group 1 received vehicle and was induced with asthma (non-diabetic asthma),

Group 2 received Dexamethasone (0.5 mg/kg, s.c.) and was induced with asthma (non-diabetic asthma+DEX),

Group 3 received vehicle and was induced with diabetes and asthma (diabetic asthma), and Group 4 received Dexamethasone (0.5 mg/kg, s.c.) and was induced with diabetes and asthma (diabetic asthma+DEX).

A negative control (control) with n=6 was also included as a disease control group. In addition, a separate set of 8 satellite animals were randomly assigned into 2 groups as follows: non-diabetic (n=4) and diabetic (n=4) for estimation of differential leukocyte and cytokine levels.

Induction of Diabetes

Type 1diabetes was induced as described previously with slight modification (Pettersson *et al.*, 2011). Briefly, a single intravenous (i.v) injection of 80 mg/kg of alloxan monohydrate (Sigma-Aldrich) dissolved in sterile water was given to mice after 3h of fasting. Control mice were injected with sterile water only. After 24h of alloxan injection, the

Table 1: Hyperglycaemia and immune profile in satellite animals at 72h post-alloxan-induced type 1 diabetes

Group	RBG (mg/d	Blood leucocyte count (x10³/mm3)				Serum level (pg/ml)		
		Neu	Eos	Lym	Total	IFN-γ	IL-4	IL-10
Non-diabetic	109.75 ± 4.07	1.63 ± 0.21	0.08 ± 0.02	4.56 ± 0.77	6.43 ± 0.99	17.31 ± 0.57	12.05 ± 0.27	42.27 ± 1.43
Diabetic	392.25 ± 47.96**	1.97 ± 0.30	0.07 ± 0.02	3.41 ± 0.39	5.58 ± 0.69	22.53 ± 3.52	12.67 ± 0.07	65.57 ± 10.95

Data are represented as mean ± SEM (n=4).
**p<0.01 versus non-diabetic
RBG- random blood glucose, Neu-neutrophils, Eos- eosinophils, Lym- lymphocytes, IFN-γ interferon-gamma, IL-4- interleukin-4,IL-10- interleukin-10.

incidence of hyperglycaemia was verified by blood glucose level >250 mg/dl, and blood glucose levels were estimated with blood glucometer (AccuCheck Aviva, Roche Diagnostics) in samples obtained by retro-orbital plexus method. Diabetic groups of the experiment were randomized based on their random blood glucose values.

Induction of Asthma

Diabetic and non-diabetic mice were sensitized on day 3, and 8 with an intraperitoneal (i.p) injection of 50 μg ovalbumin (Ova) emulsified in 2.5 mg aluminum hydroxide (Al(OH)$_3$)to a total volume of 0.2 ml in PBS, followed by intranasal challenge with 100 μg Ova/25 μl of sterile saline (4% *w/v* Ova) on days 13, 14 and 15 as described previously with modifications (Carvalho *et al.*, 2018; Fernandez-Rodriguez *et al.*, 2008). Control mice received only sterile saline without Ova.

Nasal hyperresponsiveness (NHR) evaluation

Following asthma induction, on day 16, the frequency of nose rubbing as a measure of upper airway hyperresponsiveness was counted for each mouse immediately for 10 minutes after Ova instillation as previously described (Zhang *et al.*, 2016).

Hematology

Blood samples were collected in ethylene diamine tetra acetic acid (EDTA) tubes by retro-orbital plexus method from satellite animals on day 3 after diabetes induction and from study groups on day 16 after NHR evaluation and hematology parameters were assessed using hematology analyzer (Medonic CA620). A blood smear was made, stained with Wright-Giemsa and counted for the differential leukocyte cells manually. Absolute differential leukocyte count (DLC) was calculated by multiplying the percentage of DLC with a total leukocyte count. An aliquot of blood was collected separately on the days mentioned above; serum was separated and

stored at -80 ^{0}C for further analysis.

Bronchoalveolar lavage fluid (BALF)

Following NHR, mice were euthanized immediately after blood collection by CO_2 asphyxiation, and the chest cavity was exposed to allow for expansion. Through tracheal cannula, 2x0.8 ml of PBS was infused into the lungs, and BALF was collected. The BALF was centrifuged at 2500 rpm for 10 minutes at 4 ^{0}C, and the supernatant was collected and stored at -80 ^{0}C for further analysis. The lavage pellet was suspended in 250 μl of PBS and then counted with Medonic CA620 hematology analyzer.

Histology of lung

After NHR, the lungs of 3 mice in each group were collected in 10% formalin, embedded in paraffin blocks. A 4 μm sections were made, stained with hematoxylin & eosin (H & E) and all histological sections were evaluated by a pathologist in a blinded manner and counted for perivascular aggregates of inflammatory cells and bronchioles number in each lung. And also, airway inflammation and goblet cell metaplasia were determined.

ELISA of Th1/Th2 cytokines in serum and BALF

The cytokine expression of IFN-γ, IL-4, and IL-10 were assessed in both serum and BALF by ELISA method, following kit's manual (Invitrogen Mouse Th1/Th2 Elisa kit, cat # 88-7711-44) and using Microplate reader (Thermo scientific; Mutiskan Go). The standard curve was constructed using concentration range as follows: mouse IFN-γ, IL-4, and IL-10 between 15-2000, 4-500, and 30-4000 pg/ml, respectively.

Statistical analysis

All statistical analysis was done, and graphs were generated by using Graph Pad Prism 8 software. Data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test or

unpaired student's t-test wherever applicable, and p<0.05 was considered as significant.

RESULTS AND DISCUSSION

Acute immune features of alloxan-induced type 1 diabetic mice

Balb/c mice injected with a single intravenous dose of alloxan featured a significant increase in blood glucose levels at 72 h after alloxan injection, indicating a diabetic state. The immune profile of these mice indicated unaltered leukocyte count and serum levels of Th1 and Th2 cytokines at the acute diabetic state versus non-diabetic state (Table 1).

Alloxan-diabetes showed intermediary asthma development in a mouse model of Ova-induced allergic airway inflammation

In order to understand the effect of T1D comorbid condition, the nasal hyperresponsiveness was first evaluated by measuring the frequency of nasal rubbings in each mouse immediately after the last Ova challenge. The frequency of nasal rubbings in sensitized diabetic mice was significantly higher than in control mice. However, there was a slight but insignificant decrease in the incidence of nasal hyper responsiveness in diabetic mice with asthma compared to mice with asthma alone (Figure 1).

Next, the effect of the T1D comorbid condition was examined on inflammatory cell infiltration triggered by Ova in sensitized mice. One hour after the last Ova challenge, the inflammatory cell number in BALF and blood smear was estimated. In BALF, the differential leukocyte count obtained indicated a significant increase in neutrophil, eosinophil, and total leukocyte cells after Ova sensitization and challenge in non-diabetic asthma animals compared to control animals. The mononuclear cell number in these animals was decreased to an insignificant level compared to control animals. Nevertheless, the presence of diabetes condition showed a significant increase only in eosinophil cells compared to control but showed a significant decrease in both eosinophil and total leukocyte cells compared to non-diabetic asthma groups. Also, a significant decrease in mononuclear cells was observed in diabetic asthma animals compared to control animals (Figure 2 a). In line with BALF observations, a significant decrease in blood eosinophils was also noticed in diabetic asthma mice compared to non-diabetic asthma mice that were sensitized and challenged with Ova (Figure 2 b).

Histopathological observation of lung sections stained with H & E, revealed significant inflammatory cell infiltration and mucus cell metaplasia

in the perivascular and peribronchiolar regions respectively, of non-diabetic asthma mice (Figure 2 d) compared to control mice (Figure 2 c). Lung sections of Ovasensitized and challenged diabetic mice (Figure 2 e) showed a reduced number of perivascular inflammatory aggregates and mucus cell metaplasia compared to non-diabetic asthma mice (Figure 2 d). However, a substantial lung airway infiltration of cells was noticed in diabetic mice (Figure 2 e) after the Ova challenge compared to control mice (Figure 2 c) challenged with saline.

Further, we estimated IFN-γ as Th1 responsive cytokine and IL-4 & IL-10 as Th2 responsive cytokines in both BALF and serum after the last Ova challenge in these mice. The cytokine levels in BALF and serum indicated insignificant downtrend of Th1 responsive cytokine (IFN-γ) and significant up-trend of Th2 responsive cytokine (IL-4) after asthma induction in non-diabetic mice, but observed insignificant changes in these cytokines in diabetic mice (Figure 3 a &Figure 3 b) compared to control mice. Asthma responsive IL-4 cytokine levels of BALF and serum were significantly lower in diabetic compared to non-diabetic mice when subjected to Ova-induced asthma (Figure 3 a & Figure 3 b).In the case of IL-10, an insignificant downtrend was observed in BALF, but significant uptrend in the serum of both non-diabetic and diabetic mice after asthma induction with Ova (Figure 3 a & Figure 3 b). The Th1/Th2 (IFN-γ/IL-4) ratio in BALF and serum was significantly less in both non-diabetic and diabetic mice indicating increased Th2 responses to Ova asthma compared to control (Figure 3 c & Figure 3 d), though the Th1/Th2 ratio in serum was significantly high in diabetic versus non-diabetic (Figure 3 d). Pearson correlation analysis revealed a linear relationship between IL-4 and eosinophil responses to Ova asthma in these mice in both BALF (Pearson r=0.8558,Figure 3 e) and blood (Pearson r=0.8743, Figure 3 f).

Dexamethasone showed the altered magnitude of anti-asthmatic activity in diabetic asthma

In order to understand the immunomodulatory effects of Dexamethasone in asthma treatment under diabetes comorbid conditions, its efficacy was evaluated on Ova-induced airway inflammation in diabetic and non-diabetic mice. Evaluation of allergic nasal hyperresponsiveness symptoms like nasal rubbings evidenced that dexamethasone showed a significant reduction only in non-diabetic asthma mice than in diabetic mice (Figure 4 a). Dexamethasone treated non-diabetic and diabetic mice showed significantly reduced numbers of eosinophils in both BALF and blood after Ova asthma induction (Fig-
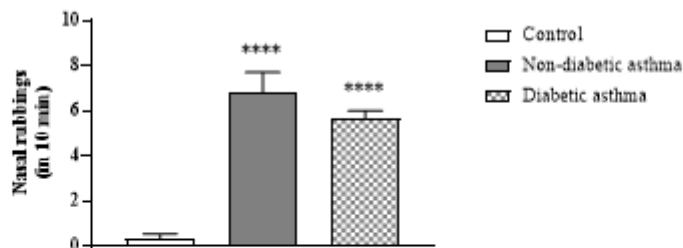
The number of nasal rubbings per mice was counted for 10 minutes immediately after the last Ova-challenge.
Data are represented as mean ± SEM (n=6)
****p<0.0001 versus control

Figure 1: Frequency of allergic airway responses in Ova-induced allergic asthma in mice

ure 4 b &Figure 4 c). Moreover, histological examination of lung sections from Dexamethasone treated non-diabetic mice (Figure 4 e) showed significantly reduced inflammatory aggregates and mucus cell metaplasia around airways versus untreated (Figure 4 d). Also, similar observations were noticed in Dexamethasone treated diabetic mice (Figure 4 g) versus untreated (Figure 4 f) but to a lesser magnitude. Dexamethasone treatment showed no significant change in random blood glucose in these mice compared to untreated (data not shown).

Further, Dexamethasone treated non-diabetic and diabetic asthma mice showed an insignificant decrease in IFN-γ (Figure 5 a), a significant decrease in IL-4 (Figure 5 b) and in IL-10 (Figure 5 c)compared to untreated in BALF. Also, serum IL-4 levels were significantly decreased in Dexamethasone treated non-diabetic and diabetic asthma mice compared to untreated mice (Figure 5e). However, its treatment did not show significant changes in serum IFN-γ (Figure 5 d) and IL-10 (Figure 5 f) in these mice.

Discussion

In recent decades, the incidence of both asthma and T1D had increased and is still rising. A multifaceted interaction of acquaintance to numerous environmental factors and genetic predisposition had been suggested to play an important role in the pathogenesis of both diseases (Hörtenhuber *et al.*, 2018; Long *et al.*, 2012). However, the association between these two chronically mediated immune diseases is still pointed of discussion as some reported higher and few others showed lesser incidences of asthma in T1D patients (Cardwell *et al.*, 2003; Hsiao *et al.*, 2015; Stene and Nafstad, 2001; Huang and Hitchcock, 2002). It is unclear that which immune characteristics of autoimmune T1D patients make them susceptible or resistant to asthma induction. Partly,

this mixed relationship between these two diseases can be attributed to early-life exposure to environmental factors or genetic risk factors that regulate the Th1/Th2 balance for the development of either of diseases (Hsiao *et al.*, 2015). Gene expression analysis indicated a shift of Th1 towards Th2 type of immune response in adult T1D patients with longer duration of disease (Vaseghi and Jadali, 2016). Nonetheless, it is important to understand the immune responses in associated comorbid conditions of T1D and asthma so that newer immune-modulatory agents can be identified for its treatment.

In the present study, the incidence of experimental allergic airway inflammation after ovalbumin sensitization and challenge under associated alloxan diabetic conditions in Balb/c mice was explored. It was observed that significant infiltration and accumulation of inflammatory cells around lung airways with an associated increase of eosinophils in BALF and blood of both non-diabetic and alloxan diabetic mice after they were sensitized and challenged with Ova. However, the severity of airway inflammation in lung sections and Th2-mediated eosinophilic response were diminished in alloxan diabetic mice compared to non-diabetic mice after the Ova challenge. (Carvalho *et al.*, 2018), reported similar findings in alloxan diabetic A/J mice after Ova sensitization and challenge. These results were also correlated with decreased secretions of Th2 responsive IL-4 in both BALF and serum of diabetic asthma mice compared to non-diabetic asthma mice. Although diabetic asthma mice presented less severe airway inflammation and Th2 cytokine-mediated responses, the frequency of NHR to Ova challenge was not different than in non-diabetic asthma mice. Our study used acute T1D mice that presented only a modest increase in Th1 cytokine
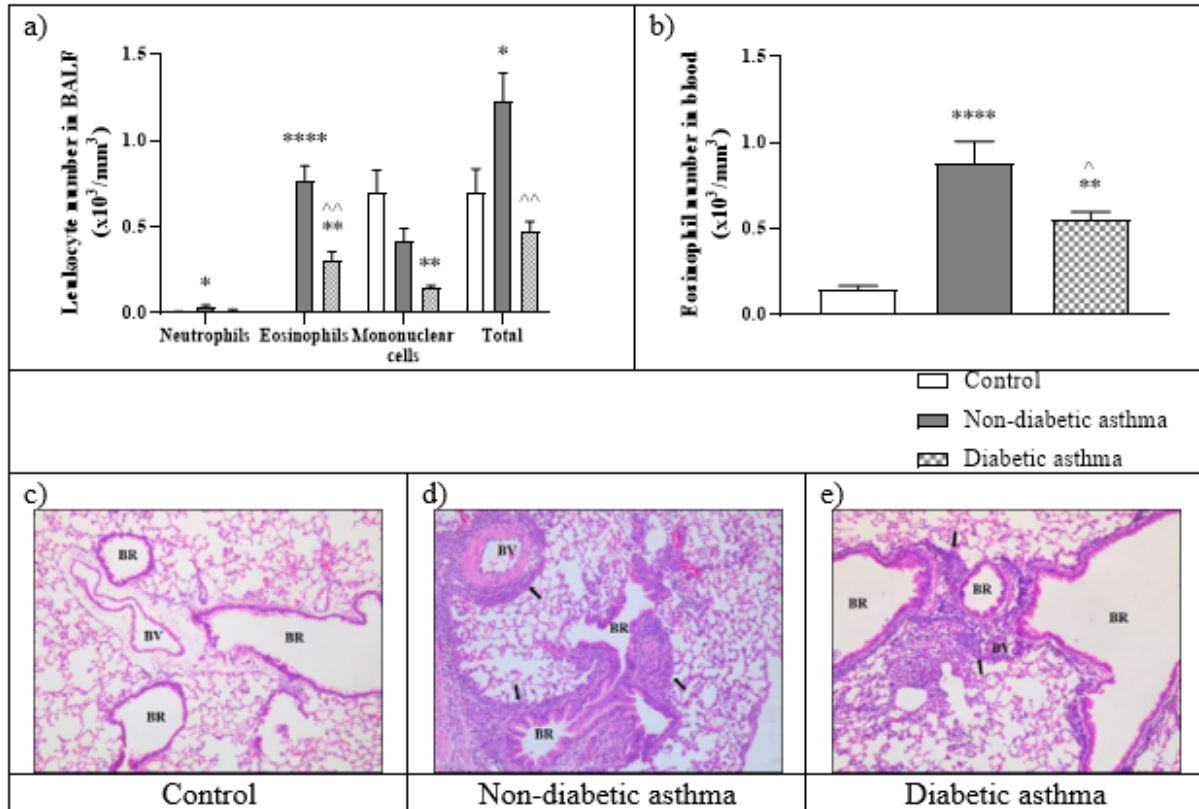
Inflammatory cell profile in a) BALF, b) blood, and c-e) lung sections obtained 1h after last Ova-challenge from mice with no disease (control), asthma alone (non-diabetic asthma) and diabetes plus asthma (diabetic asthma).
Differential leukocyte numbers in BALF and blood were determined by Giesma staining. Lung tissue inflammation was assessed by H & E staining of lung sections (magnification, x100).
Inflammatory cell aggregation around a blood vessel (BV) and mucus cell metaplasia of bronchiole (BR) in lung sections was shown by an arrow.
Data are represented as mean±SEM (n=4-6).
*$p < 0.05$, **$p < 0.01$ and ****$p < 0.0001$ versus control; ^$p < 0.05$ and ^^$p < 0.01$ versus non-diabetic asthma

Figure 2: Decreased infiltration of inflammatory cells in diabetic mice sensitized and challenged with ovalbumin

secretion at 72h post alloxan injection. The decrease in BALF and serum cytokines in diabetic mice compared to non-diabetic post-challenge with Ova may be attributed to a general dampening of spontaneous cytokine secretions observed at diagnosis of T1D in children (Rydén *et al.*, 2014).

Further, diabetic mice showed a significant increase in blood glucose at 72 h after alloxan injection with a concomitant decrease in body weights (data not shown) indicating the presence of T1D. Also, these alloxan-induced acute diabetic mice presented an insignificant increase in neutrophil number in blood

with a modest increase in Th1 responsive cytokine IFN-γ secretion. This modest increase in IFN-γ in alloxan diabetic mice may also be related to a modest increase in IL-10 that may have controlled the release of IFN-γ release. Increased levels of IL-10 in alloxan-treated mice liver has shown to inhibit Th1 cytokine release (Fan *et al.*, 2016).

Besides an increase in eosinophil number in non-diabetic asthma mice after Ova sensitization and challenge, neutrophils were also increased in BALF and blood (blood data not shown). This increase in the accumulation of neutrophils may be linked
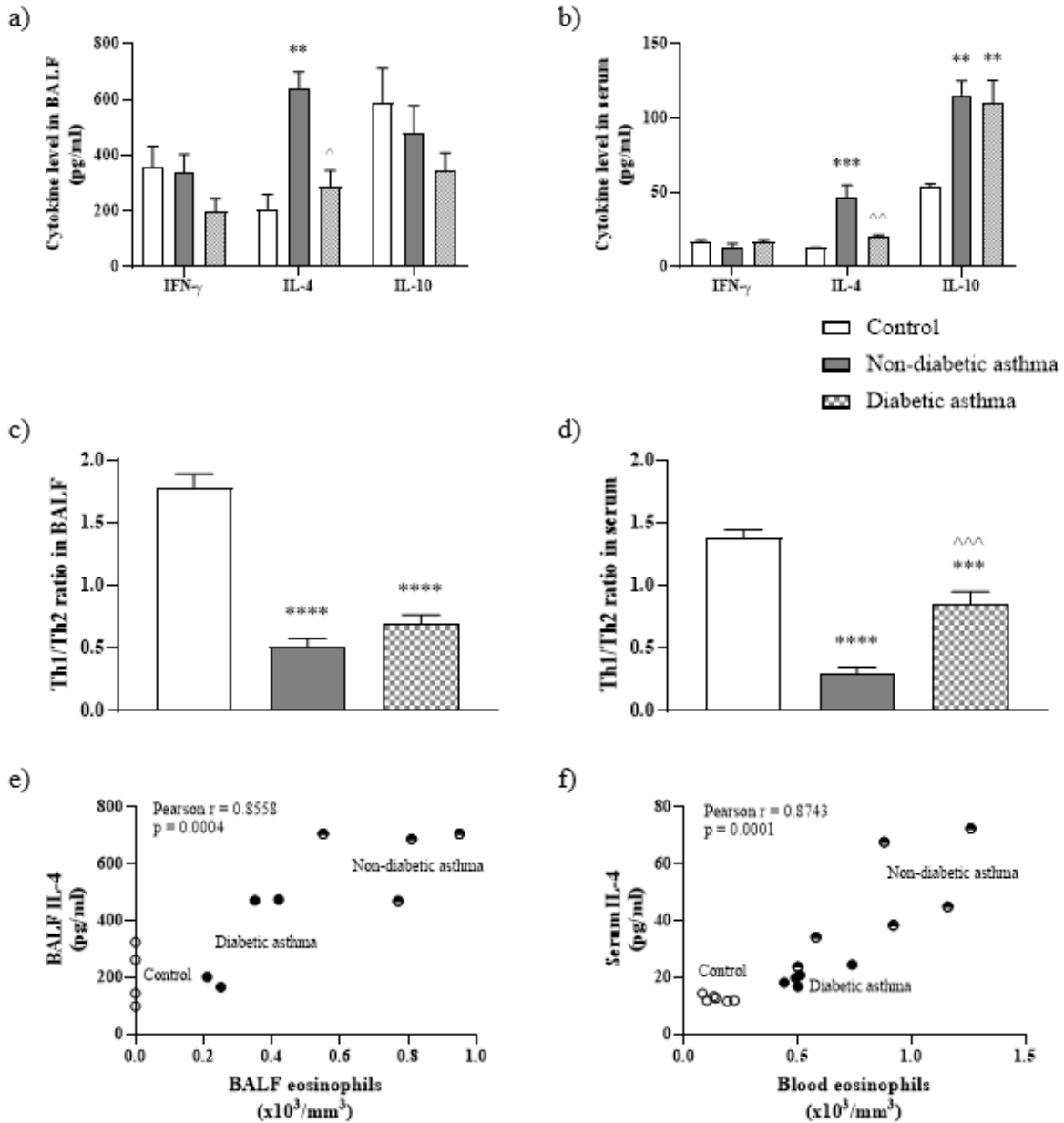
a & b) Profile of Th1 and Th2 inflammatory cytokines in BALF & serum, respectively. c & d) Th1/Th2 ratio in BALF & serum, respectively. e & f) Pearson correlation coefficient between IL-4 versus eosinophil number in BALF & serum, respectively.

Samples were obtained 1h after last Ova-challenge and analyzed using mouse Th1/Th2 ELISA kit.

Data are represented as mean ± SEM (n=4-6).

**p<0.01, ***p<0.001 and ****p<0.0001 versus control; ^p<0.05, ^^p<0.01 and ^^^p<0.001 versus non-diabetic asthma

Figure 3: Intermediary Th1/Th2 ratio observed in diabetic mice sensitized and challenged with ovalbumin
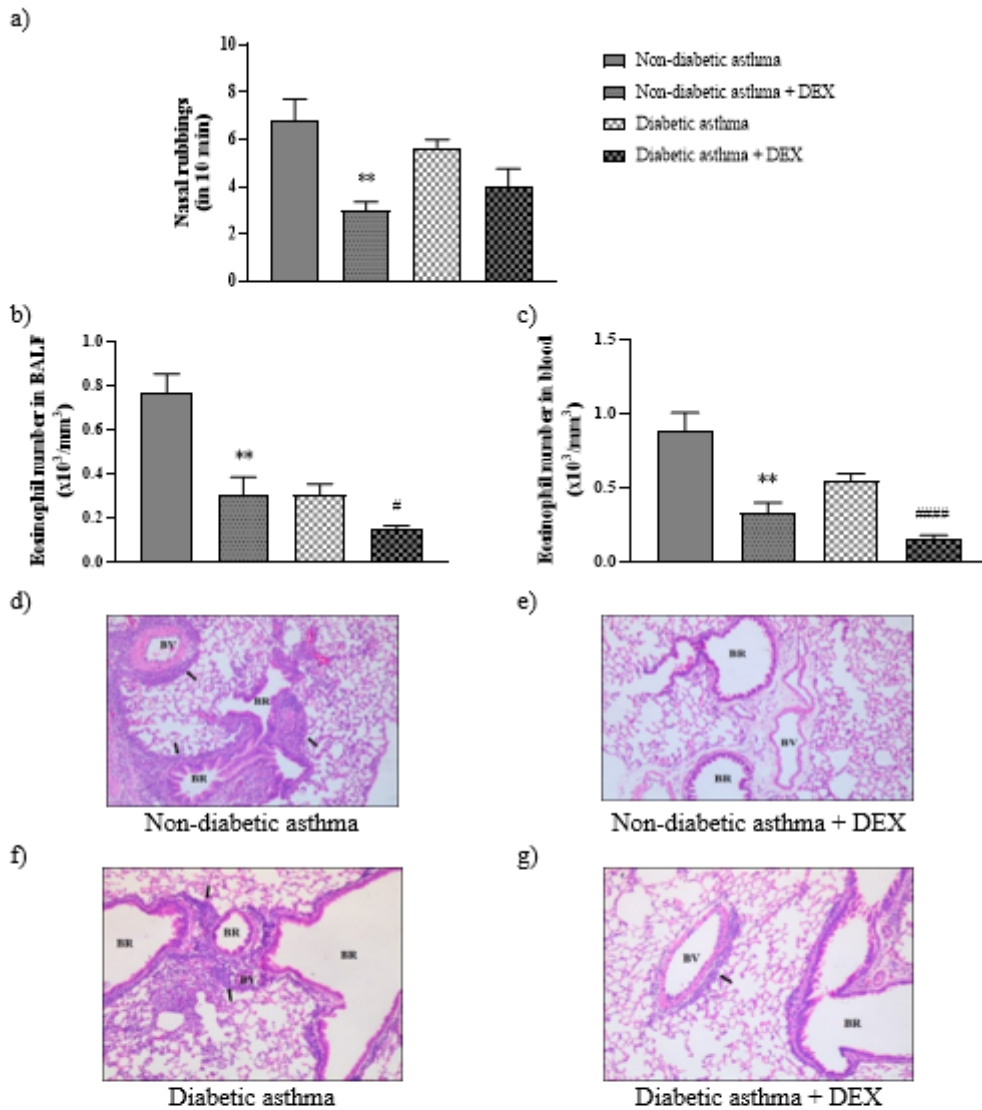
a) A number of nasal rubbings. b & c) Inflammatory eosinophil number in BALF & blood, respectively. d-g) Lung sections obtained 1h after last Ova-challenge from mice with asthma alone (non-diabetic asthma), non-diabetic asthma plus dexamethasone (Non-diabetic asthma + DEX), diabetes plus asthma (diabetic asthma) and diabetes plus asthma plus dexamethasone (diabetic asthma + DEX). Eosinophil number in BALF and blood was determined by Giesma staining.

Lung tissue inflammation was assessed by H & E staining of lung sections (magnification, x100). Arrow represents inflammatory cell aggregation around a blood vessel (BV) and mucus cell metaplasia of bronchiole (BR) in lung sections.

Data are represented as mean ± SEM (n=4-6). **$p<0.01$ versus non-diabetic asthma; #$p<0.05$ and ###$p<0.01$ versus diabetic asthma.

Figure 4: Dexamethasone showed altered magnitude of anti-asthmatic activity in diabetic mice sensitized and challenged with ovalbumin
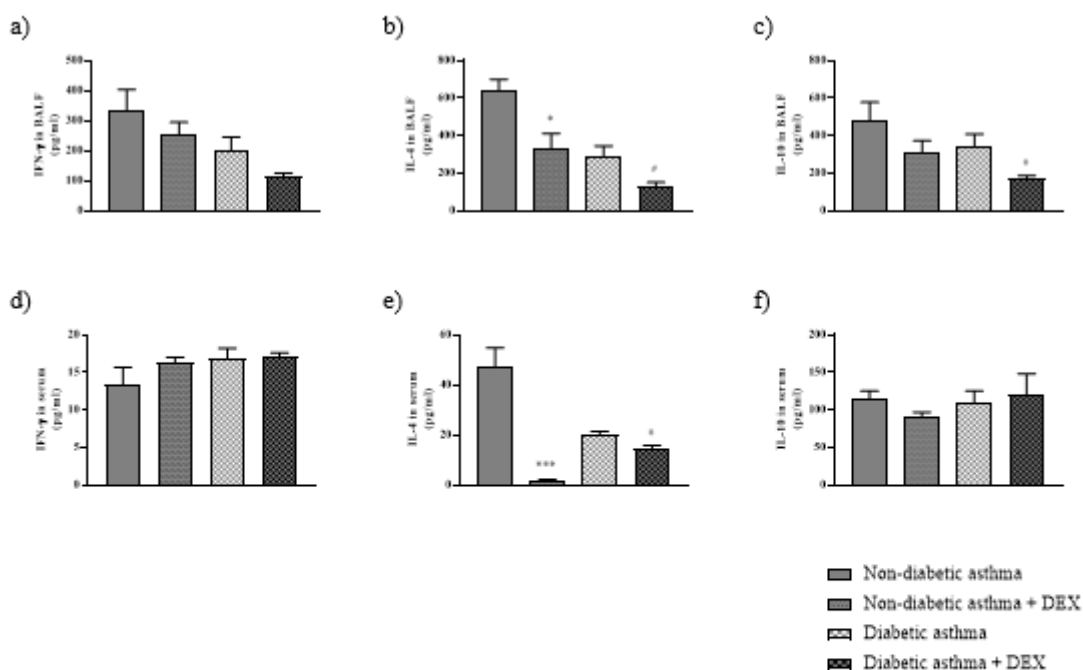
a-c) Secretion levels of IFN-γ, IL-4, IL-10 respectively in BALF at 1h after last Ova-challenge. d-f) Serum levels of IFN-γ, IL-4, IL-10 respectively at 1h after last Ova-challenge.
Data are represented as mean ± SEM (n=4-6).
*p<0.05 and ***p<0.001 versus non-diabetic asthma; #p<0.05 versus diabetic asthma

Figure 5: Th1 and Th2 cytokine responses to dexamethasone treatment in non-diabetic and diabetic mice sensitized and challenged with ovalbumin

to increased cell recruitment and decreased apoptosis, as seen in asthma patients (Tian *et al.*, 2014; Simon, 2009). Yet, our results showed a decrease in neutrophil numbers in both BALF and blood of sensitized diabetic mice when challenged with Ova. The possible explanation for this finding may be due to the increased accumulation of neutrophils in the pancreas after alloxan-induced β-cell damage and general peripheral consumption. Recent evidence shows that peripheral neutrophil numbers are decreased in β-cell-specific autoimmune T1D patients due to site-specific accumulation of neutrophils in the pancreas (Huang *et al.*, 2016). Also, a reduced number of mononuclear cells was observed in BALF of both non-diabetic asthma and diabetic asthma mice compared to control after the last ova challenge. Only diabetic mice demonstrated a significant reduction in mononuclear cells. Evidence suggests that there is marked a reduction in peripheral lymphocytes because of thymocyte depletion, increased apoptosis and decreased proliferation in diabetes conditions (Barreto *et al.*, 2005; Jung *et al.*, 1999; Otton *et al.*, 2004; Satoh and Iwasaki, 2011).

Moreover, the lung histology data demonstrated that the mucus cell metaplasia and perivascular inflammatory cell aggregates were less in sensitized diabetic mice compared to sensitized non-diabetic mice after the Ova challenge. Our results are in line with the observations seen in diabetic animals and could be possibly explained by the decrease in mast cell degranulation and mast cell numbers in these animals (Cavalher-Machado, 2004; Carvalho *et al.*, 2005). It is notable that diabetic animals present decreased inflammatory cellular infiltration in lung airway spaces after allergen challenge (Carvalho *et al.*, 2018; Kolahian *et al.*, 2011).

Furthermore, ELISA analysis meant a significant decrease in Th2 responsive IL-4 levels in BALF and serum of sensitized diabetic versus sensitized non-diabetic mice after challenged with Ova. There was a marked increase in IL-4 levels in non-diabetic mice after asthma induction, and the results are in general acceptance that inflammatory condition observed in asthma is Th2 dominant (Kidd, 2003; Mahajan and Mehta, 2006). Additionally, this increase in IL-4 in BALF and serum of non-diabetic asthma mice can

be correlated to an increase in eosinophils. Pearson correlation coefficient of IL-4 versus eosinophil numbers in both BALF and blood suggested a significant linear relationship between these two levels. Alternatively, there could be the possibility of mandatory IL-4 secretion from accessory cells like mast cells in these mice (Holgate, 2012). An intermediary Th2 dominance was observed in the case of diabetic asthma after the Ova challenge that was higher than in control but lower than in non-diabetic mice. The decrease in lung inflammatory cell infiltration and mucus cell metaplasia in diabetic asthma mice can be enlightened by the lower IL-4 levels. Evidences indicate that alloxan diabetic mice display the lower levels of IL-4 that can be reversed by insulin treatment and suggesting the role of insulin in IL-4 secretion (Casagrande et al., 2018; Novoselova et al., 2016). Supplementary to increased Th2 responsive IL-4 levels in BALF and serum of both non-diabetic and diabetic mice sensitized and challenged with Ova, an insignificant decrease in Th1 responsive cytokine IFN-γ was noticed, suggesting the counter relationship of Th1 and Th2 immune responses. The Th1/Th2 (IFN-γ/IL-4) ratio revealed decreased value in the case of both non-diabetic and diabetic mice when compared to control, suggesting the dominance of Th2 response in these mice after asthma induction. Diabetic asthma animals displayed an intermediary Th1/Th2 ratio compared to non-diabetic and control animals. These observations are consistent with the findings seen in patients with both T1D and asthma (Rachmiel et al., 2006). In the case of IL-10, there was no significant change in its levels in BALF, but a significant increase in IL-10 levels in the blood of both non-diabetic and diabetic mice was observed compared to control. The IL-10 levels in asthma are controversial, as some studies showed more levels, and others showed fewer levels in asthma patients than in control patients (Broeke et al., 2006). Interleukin-10 as s general inhibitor of immune response plays a role in peripheral tolerance to allergens (Soyer et al., 2013) and elevated serum IL-10 levels are seen in patients and animals with asthma (Zhang et al., 2013). The increased serum IL-10 levels in our study thus may explain a complementary peripheral tolerance mechanism to Ova induced immune activation in both non-diabetic and diabetic animals.

The antigen-induced NHR is characterized by allergic symptoms like nasal rubbing, sneezing and nasal congestion and is frequently accompanied with increased Th2 immune response (Zhang et al., 2016; Nishimura et al., 2018). On this basis, the influence of T1D condition on the frequency of nasal rubbings as the NHR induction parameter was studied and observed that the NHR was significantly increased in Ova sensitized and challenged mice with or without diabetes. Several independent and concomitant pathways are shown to be responsible for the induction of hyperresponsiveness to allergen-induced asthma, and studies in mice indicated no change in airway hyperresponsiveness to allergen despite the decrease in eosinophilia (Corry, 1996; Kobayashi et al., 2000). This probably explains our observation of similar NHR response in diabetic mice despite decreased eosinophils and cytokine levels in these mice compared to non-diabetic mice after Ova challenge. In the present study, an attempt was made to understand the immunomodulatory effects of Dexamethasone on asthma, and it was found that Dexamethasone showed a lower magnitude of anti-asthmatic activity in diabetic asthma mice than in non-diabetic asthma mice. In contrast to its inhibitory activity on eosinophilia, cytokine secretion, and inflammatory cell infiltration, there was no significant reduction in NHR in diabetic mice immediately after the Ova challenge. Glucocorticoids represent the most effective anti-inflammatory medications for the treatment of asthma (Bateman et al., 2008; Shefrin and Goldman, 2009). Experimental studies in mice indicate a significant reduction in airway hyperresponsiveness, inflammatory cell infiltration, goblet cell metaplasia and Th2 cytokine secretions in allergen-induced asthma models (Nishimura et al., 2018; Mushaben et al., 2013). However, it is unknown about their efficacy in asthma associated with diabetes. Glucocorticoids are known to increase glycaemia and represent one of the classes of drugs inducing diabetes (Lansang and Hustak, 2011; Liu et al., 2013). Hence, in our present study, Dexamethasone was evaluated for its efficacy in diabetic asthma mice and noted that it significantly lowered the eosinophil numbers in BALF and serum with the corresponding decrease in IL-4 levels, but showed the lesser magnitude of efficacy compared to its efficacy in non-diabetic asthma mice. Also, an insignificant reduction was noticed in the NHR of diabetic asthma mice than in non-diabetic asthma mice with Dexamethasone treatment. Additionally, no increase in glycaemia was observed with Dexamethasone treatment, but decreased health condition in terms of loss of body weight was observed in diabetic asthma animals than in untreated animals (data not shown).

CONCLUSION

Our results in this study indicate that Ova sensitization and challenge leads to a significant increase in inflammatory eosinophils, Th2 responsive cytokine

levels, airway inflammatory cell infiltration, and NHR in both non-diabetic and diabetic mice. But an intermediate level of asthma induction was observed in diabetic mice compared to control and non-diabetic mice after the Ova challenge. Further, the Dexamethasone treatment significantly reduced the airway inflammation in Ova sensitized and challenged mice that are presented with or without diabetes. However, a lesser magnitude of efficacy that may account for the altered immunomodulatory activity was observed in diabetic asthma than in non-diabetic asthma.

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