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CD26/DPPIV inhibition alters the expression of immune response-related genes in the thymi of NOD mice

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Abstract

The transmembrane glycoprotein CD26 or dipeptidyl peptidase IV (DPPIV) is a multifunctional protein. In immune system, CD26 plays a role in T-cell function and is also involved in thymic maturation and emigration patterns. In preclinical studies, treatment with DPPIV inhibitors reduces insulitis and delays or even reverses the new - onset of type 1 diabetes (T1D) in *non-obese diabetic* (NOD) mice. However, the specific mechanisms involved in these effects remain unknown. The aim of the present study was to investigate how DPPIV inhibition modifies the expression of genes in the thymus of NOD mice by microarray analysis. Changes in the gene expression of β-cell autoantigens and *Aire* in thymic epithelial cells (TECs) were also evaluated by using qRT-PCR. A DPPIV inhibitor, MK626, was orally administered in the diet for 4 and 6 weeks starting at 6-8 weeks of age. Thymic glands from treated and control mice were obtained for each study checkpoint. Thymus transcriptome analysis revealed that 58 genes were significantly over-expressed in MK626-treated mice after 6 weeks of treatment. Changes in gene expression in the thymus were confined mainly to the immune system, including innate immunity, chemotaxis, antigen presentation and immunoregulation. Most of the genes are implicated in central tolerance mechanisms through several pathways. No differences were observed in the expression of *Aire* and β-cell autoantigens in TECs. In the current study, we demonstrate that treatment with the DPPIV inhibitor MK626 in NOD mice alters the expression of the immune response-related genes in the thymus, especially those related to immunological central tolerance, and may contribute to the prevention of T1D.

Keywords:

Type 1 diabetes prevention

DPPIV/CD26 inhibition

DNA microarray analysis

β-cell autoantigens expression

NOD mice

Gene expression

CD26/DPPIV inhibition alters the expression of immune response-related genes in the thymi of NOD mice

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1. Introduction

Type 1 diabetes (T1D) is a chronic autoimmune disease caused by the selective destruction of pancreatic β cells (Atkinson and Eisenbarth, 2001). The breakdown of immune self-tolerance homeostasis to pancreatic islet β cells is now recognized as the essential cause for the development of the diabetogenic autoimmune response (Geenen, 2012). Therefore, the reestablishment of autoimmune tolerance state toward self-antigens (Ags) is one of the primary objectives for the prevention of autoimmune diseases, including T1D. During the last decade, immunotherapeutic innovative strategies have focused on maintaining and restoring self-tolerance to pancreatic β cells in T1D (Staeva et al., 2013).

CD26, also known as dipeptidyl peptidase IV (CD26/DPPIV), is a multifunctional cell surface glycoprotein expressed on a variety of cell types, including immune cells. This protein is a proteolytic enzyme, receptor and co-stimulatory protein and is involved in adhesion and apoptosis (Boonacker, 2003). CD26/DPPIV's proteolytic activity is capable of cleaving N-terminal dipeptides from polypeptides with either proline or alanine residues in the penultimate position, modulating the activity of biologically relevant peptides such as cytokines, chemokines and incretins, among others. In addition, several studies have highlighted the important role of CD26/DPPIV in T cell activation and its involvement in immune responses (Morimoto and Schlossman, 1998). CD26/DPPIV interacts with molecules such as adenosin deaminase and CD45 and is able to modulate the co-stimulation and proliferation of activated T cells (Ohnuma et al., 2008).

Inhibition of CD26/DPPIV suppresses antigen-stimulated T cell proliferation and cytokine production, thus suggesting a potential application for DPPIV inhibitors as

immunomodulatory drugs in autoimmune diseases (Biton et al., 2011). The effect of treatment with a CD26/DPPIV inhibitor on the immune system has been recently evaluated in several animal models of inflammatory human diseases (Steinbrecher et al., 2011). In relation to T1D, treatment with CD26/DPPIV inhibitors has been shown to delay the onset of the disease as well as even to reverse new-onset diabetes in *non-obese diabetic* (NOD) mice, in both cases with an associated reduction in the islet lymphocyte infiltration (Ding et al., 2014; Jelsing et al., 2012; Tian et al., 2010) although the exact mechanism is unknown. Treatment with a CD26/DPPIV inhibitor was also described to modify T lymphocyte subsets with an increase in the percentage of regulatory T cells (Tregs) in the peripheral and thymic compartments (Tian et al., 2010). Moreover, in the NOD model, treatment with the CD26/DPPIV inhibitor sitagliptin has been reported to preserve islet transplants through a pathway involving modulation of $CD4^+$ T cell migration (Kim et al., 2009). We recently demonstrated that treatment with the CD26/DPPIV inhibitor MK626 decreases the incidence of type 1 diabetes (T1D) by 31% and reduces insulitis in the pre-diabetic NOD mouse model. No differences were observed in the percentage of T cell subsets from peripheral and central compartments between treated and control mice. However, MK626 treatment significantly increased the expression of CD26 in $CD8^+$ T effector memory (T_{EM}) T cells as well as their proliferative capacity and cytokine secretion. *In vitro* assays suggested an immunosuppressive role for $CD8^+$ T_{EM} cell subset that may be involved in the protection against autoimmunity to β pancreatic islets associated to CD26/DPPIV inhibitor treatment (Alonso et al., 2015).

There is now evidence that a failure in thymus-dependent central tolerance to pancreatic β cells plays a primary role in T1D pathogenesis (Geenen, 2012). The thymus is the organ responsible for the establishment of immunological central

tolerance by the deletion of self-reactive T cells through positive and negative selection mechanisms. Defects in the negative selection of self-reactive T cells in the NOD thymus have been reported (Kishimoto and Sprent, 2001). On the other hand, medullary thymic epithelial cells (mTEC) can express a broad range of tissue-restricted Ags (TRAs) (Derbinski et al., 2001; Fornari et al., 2010; Gillard and Farr 2006; Kyewski et al., 2002; Oliveira et al., 2013; Sospedra et al., 1998; Tykocinski et al., 2010), also known as "promiscuous gene expression", that imposes T cell tolerance and protects from autoimmune disease (Sospedra et al., 1998).

In the thymus, CD26/DPPIV has been shown to play a role in the differentiation and maturation of thymocytes, whose impairment has remarkable effects on lymphocyte subsets and thymic architecture (Klemman et al., 2009). Moreover, CD26/DPPIV has been proposed as a mediator of intrathymic lymphocyte migration and may play a role in thymic deletion of emerging clones (Ruiz et al., 1996) thus implying a possible role for CD26/DPPIV in the establishment of central tolerance.

To our knowledge, this is the first report that describes the effect of treatment with a CD26/DPPIV inhibitor on the thymus transcriptome in the NOD mice and hypothesizes its possible involvement in the modification of the expression of genes related to central tolerance mechanisms. Here, we investigated the impact of treatment with the CD26/DPPIV inhibitor MK626 on the thymic gene expression profile of pre-diabetic NOD mice by DNA microarray technique, with particular emphasis on those genes involved in the immune response. We also evaluated the effects of MK626 treatment on islet autoantigens and *Aire* gene expression in thymic epithelial cells by qRT-PCR.

2. Materials and methods

2.1. Mice

Wild-type NOD mice were obtained from our colony established with mice from the Jackson Laboratory (Bar Harbor, ME, USA). Only females were used for this study. Mice were kept under specific pathogen-free conditions and monitored daily for diabetes onset. At the end of the study, mice were sacrificed by cervical dislocation. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Generalitat de Catalunya, Catalan Government. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Germans Trias i Pujol Research Institute (Permit number: DAAM 5928).

2.2. Treatment with MK626

Female NOD/Ltj mice were placed on either a normal chow diet (Research Diets, Inc, New Brunswick, NJ) or the same diet containing the CD26/DPPIV inhibitor MK626 (21 mg/kg of diet), kindly donated by Dr. James Mu (Merck Research Laboratories, New Jersey, USA), for 4 and 6 weeks starting at 6-8 weeks of age (pre-diabetic stage). The CD26/DPPIV inhibitor used in this study, MK626, was a des-fluoroanalog of sitagliptin (Kim et al., 2005). The treatment protocol is based on previous studies published in the literature using DPPIV inhibitors in experimental diabetes (Jelsing J et al., 2012; Kim D et al, 2005; Tian L et al., 2010), and data reported by Merck Research Laboratories. Also, the chosen concentration of the drug was the one able to maximize plasma DPPIV inhibition in order to get full effect. Mice were monitored daily for urine glucose using Glucocard strips during the whole study (Menarini, Barcelona, Spain).

Thymic glands from NOD mice were obtained after 4 weeks (at approximately 10-12 weeks of age, n=5) and 6 weeks (at approximately 12-14 weeks of age, n=5) of treatment with MK626. Thymic glands were also obtained from a NOD mouse control group for each time point (n=10). Samples were snap-frozen in an isopentane/cold acetone bath and were kept at -80 ºC until RNA extraction.

2.3. Microarray experiments

RNA was obtained from the thymi of pre-diabetic treated mice at 4 and 6 weeks of treatment, using RNeasy Micro (QIAGEN, Hilden, Germany). Thymic glands were also obtained from a NOD mouse control group for each time point. RNA quality (2100 Bioanalyzer, Agilent Technologies Inc., Santa Clara, CA) was optimal for microarray experiments (RIN between 6 and 8). cDNA was synthesized with 50-100 ng of total RNA using the WT expression kit (Ambion, Applied Biosystems, CA, USA), fragmented and labeled with the Terminal labeling kit (Affymetrix, Inc. Santa Clara, CA), purified (GeneChip® Sample Cleanup Module, Affymetrix), fragmented and checked to verify its integrity. Mouse Gene1.1 ST 16 array plates (28.853 genes) were hybridized and scanned by an Affymetrix G3000 Gene Array Scanner.

2.4. Statistical analyses of microarrays

Raw expression values obtained from CEL files were pre-processed using the Robust Multiarray Averaging method (Irizarry et al., 2003). These normalized values were used for all subsequent analyses. Experimental data have been uploaded into ArrayExpress for the European Bioinformatics Institute (EBI, www.ebi.ac.uk/aerep/login; E-MTAB-2082). Data were subjected to non-specific filtering to remove low signal and low variability genes. Conservative (low) thresholds

were used to reduce possible false negative results. The selection of differentially expressed genes was based on a linear model analysis with empirical Bayes modification for the variance estimates, as previously described (Smyth, 2004). This method is similar to using a 't-test' with an improved estimate of the variance. To account for the multiple testing probability effects arising when many tests (one per gene) are performed simultaneously, p-values were adjusted to obtain strong control over the false discovery rate using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995). Genes were considered differentially expressed based on the following criteria: genes with an adjusted p-value <0.05 and a logarithmic fold change (log_2FC) $\geq$ 0.8 were considered up-regulated, whereas genes with $log_2FC \leq -0.8$ were considered down-regulated.

Genes were classified into functional categories on the basis of Gene Ontology (GO) nomenclature (www.geneontology.org) and other annotations provided by NCBI Entrez (www.ncbi.nml.nih.gov/gene) database and data from general literature. The Ingenuity Pathway Analysis (IPA) (Ingenuity Systems ®) (www.ingenuity.com) was used to identify the canonical pathways from the IPA library that were most significant to the data sets. Data from the IPA are expressed as a p-value < 0.05 calculated by using the right-tailed Fisher's Exact Test. The Fisher test is used to compare the number of user-specified molecules of interest that participate in a given function, relative to the total number of occurrences of these molecules in all functional annotations in Ingenuity's knowledge base.

2.5. Quantitative RT-PCR

Total RNA from each sample was reverse-transcribed with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). cDNA synthesis reactions were

carried out using random hexamers (0.5 µg/µl, BioTools, Valle de Tobalina, Madrid, Spain) and reverse transcriptase Moloney-murine-Leukaemia-virus (M-MLV) (200 U/µl, Promega, Madison, WI). qRT-PCR assays were performed under Taqman universal assay on a LightCycler® 480 (Roche, Mannheim, Germany) using the following TaqMan assays: *Plunc* (Mm00465064_m1), *Ear1* (Mm03059811_g1), *Reg3g* (Mm01181783_g1), *Ccl11* (Mm00441238_m1), *Nov* (Mm00456855_m1), *Muc1* (Mm00449604_m1), *Ccl6* (Mm01302419_m1), *Sprra1* (Mm00845122_s1), *Gfra2* (Mm00433584_m1), *Clec7a* (Mm01183349_m1), *C3ar1* (Mm01184110_m1), *Lgmn* (Mm01325250_m1), *Ccl21* (Mm03646971_gH), *Ccl3* (Mm00441259_g1), *Ccl9* (Mm00441260_m1), *Cd4* (Mm00442754_m1), *Epcam* (Mm00493214_m1), *Rag2* (Mm01270938_m1), *Dpp4* (Mm00494549_m1) and *Cd3d* (Mm00442746_m1). Relative quantification was performed by normalizing the expression for each gene of interest to that of the housekeeping gene *Gapdh* (Mm99999915_g1), as described in the $2^{-\Delta Ct}$ method (Livak and Schmittgen, 2001), and was referred as arbitrary units.

2.6. Enzyme-linked immunosorbent assays

Protein lysates were obtained from the thymi of pre-diabetic mice at 6 weeks of treatment with MK626. Frozen tissues were crushed with a mortar and pestle in liquid nitrogen and homogenized in phosphate buffered saline with protease inhibitor cocktail (Thermo Scientific, MA, USA) using a 21G needle and syringe at 4 ºC. Homogenates were centrifuged at 5000 × g for 5 min, and supernatants were collected and stored at -80 °C until use. Protein concentration of the lysates was determined using the Bicinchoninic acid (BCA) assay (Thermo Scientific) and the assessment of PLUNC, CCL21 (Abbexa Ltd., Cambridge, UK) and REG3G (Cusabio, Hubei, China) was performed by the corresponding ELISAs. The amount of protein of interest was

normalized to the amount of total protein for each sample, and results were expressed as pg of protein of interest/mg of total protein.

2.7. Isolation of thymic epithelial cells (TECs) and gene expression analysis by qRT-PCR

For the analysis of expression of β-cell autoantigens and *Aire* in TECs, 5 treated under the aforementioned MK626 administration for 6 weeks and 5 control female NOD/LtJ mice were used. Thymic glands of NOD mice, with or without MK626 treatment, were obtained. Extraction and digestion of the individual thymus were performed according to Seach N. et al. (Seach et al., 2012). Thymic cellular suspension was passed through 70-µm nylon mesh filter before staining and sorting. The staining was performed using anti-EpCAM-PE (0.1 µg/ml, BDbioscience) and anti-CD45-APC (2 µg/ml, BDbioscience) and then the cellular suspension was sorted selecting CD45 negative cells and EpCAM positive cells. Collected cells from the sorting were washed twice with a phosphate buffer and then the pelleted cells were snap frozen in liquid nitrogen. RNA extraction was performed using the RNAeasyKit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. cDNA was obtained by retrotranscription with the enzyme MMLV according to manufacturer's instructions (Promega) and using Oligo-dT primers. qRT-PCR assays were performed using TaqMan universal assay conditions and using the following TaqMan assays: *Gad1* (Mm04207432_g1), *Gad2* (Mm00484623_m1), *Ins1* (Mm01950294_s1), *Ins2* (Mm00731595_gH), and *Aire* (Mm00477461_m1). Relative quantification was performed by normalizing the expression for each gene of interest to that of the housekeeping gene *Gapdh* (Mm99999915_g1), as described in the $2^{-\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.8. Statistical analysis

The analyses of the array data were described in the corresponding subsection. Statistics was performed using the Prism 5.0 software (Graph-Pad software Inc., San Diego, CA). Median values between treated and control group were compared using a non-parametric Mann-Whitney test. A p-value < 0.05 was considered significant.

3. Results and Discussion

3.1. Effects of CD26/DPPIV inhibition in thymic gene expression profile

A microarray analysis was performed on the thymi of 5 treated and 5 control NOD mice for each checkpoint (4 and 6 weeks of treatment). One of the samples from treated group was discarded because quality control post-hybridization was not optimal for microarray experiments. A total of 58 genes out of the 28,853 mouse genes represented on the gene chip were differentially expressed in treated mice after 6 weeks of treatment with MK626 using an adjusted p-value < 0.05. Strikingly, all of these 58 differentially expressed genes were up-regulated. In contrast, after 4 weeks of treatment, no significant differences in thymic gene expression were found between groups. The heatmap analysis is represented in Figure 1.

3.2. Validation of the microarray results by qRT–PCR and ELISA

Validation by qRT-PCR of the most selected targets confirmed the microarray findings (Fig. 2). Only minor discrepancies were found: The difference observed in two over-expressed genes in MK626-treated mice, *Sprr1a* and *Gfra2*, do not reach statistical significance and *Ear1* gene showed an inverse tendency with respect the microarray results, although without significant differences.

As a control, we also validated the gene expression of some alternative thymus-related gene such as *Rag2*, *Cd4*, *Cd3d*, *Epcam* and *Dpp4* genes by qRT-PCR. Although microarray data did not show statistical difference between treated and controls for the expression of the aforementioned genes, qRT-PCR technique showed a significant increased expression of *Epcam* ($p<0.01$), *Dpp4* ($p<0.01$) and *Cd3d* ($p<0.05$) in MK626-treated mice (Fig. 2). Moreover, *Cd4* and *Rag2* gene expression tended to increase in the treated mice, although the difference was not statistically significant.

Additionally, the validation of gene expression microarray results was also achieved at protein level. Quantification of the protein levels of some over-expressed genes (*Plunc, Reg3g and Ccl21*) was performed in the thymi of MK626-treated mice by ELISA technique. Results showed that PLUNC, REG3G and CCL21 protein levels tend to be increased in treated mice compared to control mice, although the difference was not statistically significant (Fig.3).

3.3. Analysis of differentially expressed genes

The differentially expressed genes mainly belonged to categories linked to the immune system, inflammation and other biological cellular processes according to gene ontology (GO) categories and data provided by NCBI Entrez database and by the literature. Genes selected by functional categories are displayed in Table 1. Within the immune system, the most over-represented categories included innate immunity, chemotaxis, immunoregulation and antigen presentation.

The Ingenuity Pathway analysis (IPA) identified 5 canonical pathways (Table 2). Altered pathways in the thymi from treated mice were primarily related to the immune system. Moreover, IPA analysis also indicated that the most over-expressed genes encode molecules belonging to inflammatory and immune responses, including those

linked to immune cell trafficking (Table 3). Gene interactions involved in immune and inflammation responses (Fig. 4) are represented by the interaction network analysis.

Remarkably, a number of genes that were differentially affected by CD26/DPPIV inhibition are involved in processes related to differentiation or the activation of T cells and/or are implicated in the maintenance of immune tolerance. The discussion section of the present study will focus on those genes implicated in immunological central tolerance.

CD26/DPPIV inhibition increases the expression of innate immunity genes in the thymus of NOD mice.

Treatment with the CD26/DPPIV inhibitor MK626 was associated with higher expression of genes involved in the innate immunity, such as pattern recognition receptors (*Clec7a, Clec9a, Reg3g)* and other anti-bacterial genes (*Muc1, Plunc, Lbp, Csf1r, EARs). Plunc* was the most induced gene in our microarray analysis, and although the exact biological function of this gene is not clearly defined, it may play a role in innate immunity in the upper respiratory tract (Bartlett et al., 2011). In the murine thymus, the expression of *Plunc* gene in the medullary compartment has been reported (LeClair et al., 2001).

The role of innate immunity in preventing autoimmunity against pancreatic β cells in T1D is gaining importance. It has been described that a combination of toll–like receptor 2 (TLR2) tolerization and CD26/DPPIV inhibition can reverse early-onset diabetes in NOD mice (Kim et al., 2012). In addition, recent studies have reported a key role of CLEC7a (Dectin-1) protein, which belongs to the C-type lectin-domain superfamily of pattern recognition receptors, in the establishment of immune tolerance and in preventing autoimmune diseases, such as T1D. It has been recently demonstrated

306 that treatment with zymosan, a fungal wall cell component, protects NOD from T1D.
307 Zymosan interacts with TLR2 and CLEC7a to induce suppressor cytokines (IL-10,
308 TGF-β) by antigen presenting cells (APCs), thus promoting the activation and
309 expansion of Tregs (Dillon et al., 2006; Karumuthil-Melethil et al., 2008). It is
310 noteworthy that in the present study, *Clec7a* gene was found to be over-expressed in the
311 thymi of treated mice.

312 Another up-regulated gene expression in MK626-treated mice was *Muc1*. This
313 gene encodes a transmembrane glycoprotein expressed on the surface of most types of
314 epithelial cells that plays an essential role in the protection of mucosal barriers
315 (Mockensturm-Gardner et al., 1996). In the immune system, MUC1 protein (or mucin)
316 is expressed on the surface of dendritic cells (DCs), monocytes and activated T cells.
317 MUC1 was shown to inhibit T cell proliferative response inducing an anergy-like state.
318 MUC1 also acts in lymphocyte trafficking due to its adhesion and/or anti-adhesion
319 properties (Agrawal and Longenecker, 2005). In addition, the promiscuous expression
320 of MUC1 by mTECs has been described to confer a state of immune tolerance (Acres et
321 al., 2000; Cloosen et al., 2007). More recently, there has been a report of increased
322 expression of MUC1 on Tregs after CD3 stimulation and that CD3/MUC1 co-
323 stimulation leads to Treg expansion (Konowalchuk and Agrawal, 2001). Hence, MUC1
324 may have implications for immune tolerance through its involvement in the modulation
325 of T cell responses and Tregs proliferation.

326 Data obtained in the present study suggest a possible role of CD26/DPPIV
327 inhibition in the innate immune response and support the hypothesis that innate
328 immunity has a protective role in T1D. The targeting of CD26/DPPIV by MK626
329 increases transcripts of genes related to innate immunity and may improve

immunological central tolerance through the impairment of T cell responses and the enhancement of Tregs expansion.

Chemotaxis genes were over-expressed in the thymus by MK626 treatment.

The microarray data revealed elevated expression of genes encoding some chemokines in treated NOD mice (Table 1). This group represented one of most over-expressed categories affected by CD26/DPPIV inhibition. Several chemokines expressed in the thymus have been described as substrates of the peptidase activity of CD26/DPPIV, which either inactivates or alters the chemotactic activity of these chemokines. In the microarray analysis, the expression levels of genes encoding several DPPIV-processed chemokines, such as CCL11 and CCL3, were increased in MK626-treated mice. However, genes that encode other chemokines not targeted by CD26/DPPIV activity, such as CCL21, CCL9, CCL6 and CXCL16, were also over-expressed, suggesting that these differential effects in gene expression of these chemokines may be independent of CD26/DPPIV enzymatic activity.

The migration of developing thymocytes within the thymus is crucial for T cell repertoire selection and requires complex interactions between thymocytes and the surrounding microenvironment (Ruiz et al., 1996). It is well known that chemokines are key elements in intrathymic organization and the migration of thymocytes during their maturation, thus contributing to the sorting of positively and negatively selected thymocytes (Annunziato et al., 2001). Therefore, the up-regulation of the *Ccl21* gene expression represents one of the most important differential effects caused by CD26/DPPIV inhibition in this category due to its effects on thymocyte migration processes. The chemokine CCL21 is chemotactic agent for thymocytes and naïve T cells and binds to chemokine receptor 7 (CCR7). CCR7 and its ligands, CCL21 and

CCL19, play an important role in lymphoid cell trafficking and the structural organization of lymphoid tissues, and they contribute to both immunity and tolerance (Förster et al., 2008). Several studies have demonstrated the role of the CCR7 axis in thymic compartmentalization by coordinating migratory events during T cell development (Ueno et al., 2004). In the thymus, the chemotactic interaction between CCR7 expressed by positively selected $CD4^+$ and $CD8^+$ thymocytes and CCL21 produced by mTECs is essential for the migration of these thymocytes from the cortex to the medulla. This thymocyte migration process mediated by the CCR7 axis contributes to the negative selection of self-reactive thymocytes and is crucial in the establishment of a self-tolerant T cell repertoire (Nitta et al., 2009). A deficiency of CCR7 or its ligands increases the risk for the development of autoimmune diseases, including T1D (Misslitz et al., 2007). The present microarray analysis revealed that gene expression levels of *Ccr7* and *Ccl19* were also up-regulated in treated NOD mice, but the differences were not statistically significant.

The *Ccl11* gene was also significantly over-expressed in treated NOD mice. This chemokine, also known as eotaxin, is constitutively expressed in the thymus (Rothenberg et al., 1995) and is cleaved by CD26/DPPIV proteolytic enzyme (Struyf et al., 1999) affecting its biological activity. CCL11 displays a chemotactic selective activity for eosinophils, and it has been reported that the inhibition of CD26/DPPIV induces an *in vivo* recruitment of human eosinophils (Forssmann et al., 2008). Moreover, a recent report described an increase in the secretion of eosinophil-associated RNAases (EARs) from mouse eosinophils due to eotaxin (Shamri et al., 2012). EARs from intracellular granules are eosinophil ribonucleases and represent the major source of eosinophilic secretory effector protein participating in allergic diseases and host immunity (Rosenberg et al., 2001). It is of note that the gene expression levels of

several EAR genes (*Ear1, Ear10, Ear11)* were increased in the present microarray study. Therefore, the elevated expression of *Ccl11* and *Ear* genes in the thymus may reflect an increased number of eosinophils due to CD26/DPPIV inhibition. Notably, eosinophils have been shown to be recruited in the thymus during MHC class I-restricted T cell selection, implying an immunomodulatory role for these cells (Throsby et al., 2000). In addition, it is known that self-reactive T cells are depleted by apoptosis during intrathymic selection. The clearance of millions of these apoptotic thymocytes is important for thymic development and is achieved by thymus resident macrophages and DCs (Esashi et al., 2003). However, recent data suggest a direct contribution of eosinophils and neutrophils, which are recruited to the sites of extensive apoptosis to maximize the efficiency of apoptotic cell removal (Kim et al., 2010). Moreover, it has been demonstrated that the rapid removal of apoptotic cells is crucial for preventing inflammatory and autoimmune responses (Sarang et al., 2013).

Expression levels of other genes encoding chemokines were also significantly increased in our treated mice. These chemokines included CCL3 (MIP-1α), CCL6 (C10/MRP-1) and CCL9 (MIP-1γ/MRP-2), which have also been shown to have chemotactic activity, primarily for monocyte-macrophages (CCL3, CCL6, CCL19), as well as for eosinophils (CCL3, CCL6) and neutrophils (CCL3, CCL9) (Coelho et al., 2007; Maurer and von Stebut, 2004). Thus, these chemokines may also be involved in apoptotic cell clearance.

Taken together, our data suggest that CD26/DPPIV inhibition by MK626 treatment may have a key role in thymocyte trafficking through the modification of the expression profile of thymic microenvironmental chemokines contributing to the enhancement of negative selection. These data support the results obtained in the IPA analysis in which 20 molecules were found to be involved in immune cell trafficking

(Table 3). Alternately, MK626 treatment may enhance the clearance of apoptotic cells generated during thymocyte selection through the recruitment of eosinophils.

Targeting CD26/DPPIV induces the expression of antigen presentation- and immunoregulation-related genes in the thymus of treated mice.

As shown in Table 1, treated NOD mice showed a higher expression of genes involved in antigen presentation and the regulation of immune responses, notably *Fcgr2b* and *Lilrb4* genes.

The protein encoded by *Fcgr2b* (Fc receptor, IgG, low affinity IIb) gene belongs to the family of low affinity receptors for the Fc portion of immunoglobulin gamma complex. These receptors are involved in a variety of effector and regulatory functions such as the phagocytosis of immune-complexes from the circulation and the modulation of antibody production by B cells. In contrast to other members of this family, FcγRIIB acts as a negative regulator of the immune response, limiting T cell activation by inhibiting both antigen processing and DC activation (Desai et al., 2007) as well as by suppressing the activation of autoreactive B cells (Nimmerjahn and Ravetch, 2007). A deficiency in the inhibitory FcγRIIB leads to imbalanced immune responses that result in the development of autoimmune diseases (Nimmerjahn and Ravetch, 2006). Therefore, FcγRIIB potentially may contribute to the maintenance of tolerance and protection from autoimmune diseases.

Another significantly over-expressed gene was *Lilrb4*. The encoded protein, also known as immunoglobulin-like transcript 3 (ILT-3), is a member of the leukocyte Ig-like receptor family (LIR), which is selectively expressed by APCs such as DCs, where it binds to MHC class I molecules and transduces a negative signal that inhibits stimulation of the immune response (Kim-Chulze et al., 2006). It is known that

inhibitory activity of Tregs is determinant in the prevention of autoimmune disorders. A distinct population of these lymphocytes with a $CD8^+$ $CD28^-$ phenotype has been characterized and is referred to as T suppressor cells (Chang et al., 2002). These lymphocytes are $FOXP3^+$ and MHC class I-restricted, and they tolerize APCs by inducing the up-regulation of inhibitory receptors, such as LILRB4/ILT-3 and LILRB/ILT-4, as well as by inhibiting CD40 signaling in APCs. These tolerogenic LILRB4/ILT-3 DCs induce an anergic state in $CD4^+$ T cells, resulting in the loss of their proliferative and differentiation capacity and the transformation of these cells into Tregs, which continue the cascade of suppression by tolerizing other DCs (Kim-Chulze et al., 2006; Manavalan et al., 2003). Therefore, the up-regulation of LILRB4/ILT-3 appears to be a general characteristic of tolerogenic DCs and may be important for induction of antigen-specific tolerance.

Notably, the IPA analysis identified an altered pathway implicated in immunoregulation: IL-10 signaling. IL-10 is an anti-inflammatory and immunoregulatory cytokine. It is expressed by many immune cell types of both the adaptive and innate immune systems, supporting its crucial role as a regulator of immune responses. The main producers of IL-10 are Tregs, and this cytokine enhances the differentiation of these IL-10-secreting Tregs cells, thus providing a positive regulatory feedback (Saraiva and O'Garra, 2010). In addition, IL-10 can be induced by CLEC7a (dectin-1) stimuli. Remarkably, the *Clec7a* gene expression was up-regulated in the present microarray analysis. A potent effect for IL-10 in preventing autoimmunity has been described, although its role in the development of diabetes is controversial. In NOD mice, some studies have demonstrated that IL-10 is important in establishing immune tolerance, whereas others have demonstrated accelerated diabetogenic autoimmune responses (Saraiva and O'Garra, 2010; Tai et al., 2011). In our present

455 study, the lack of IL-10 gene over-expression in the thymi of treated mice can be
456 attributed to the low sensitivity of the arrays used to detect cytokines (Park and Stegall,
457 2007).

458 Thus, CD26/DPPIV inhibition may exert a potent immunosuppressive activity
459 on T cells by inducing tolerogenic DCs through the over-expression of inhibitory
460 receptors, such as FcγRIIB and LILRB4/ILT-3. Remarkably, these encoded proteins are
461 not cleaved by CD26/DPPIV, and these results support the hypothesis that the
462 proteolytic activity of DDPIV is not essential for its co-stimulatory function in T cell
463 response (Boonacker et al., 2002).

464

465 **MK626 treatment increases the expression of genes related to other cell functions**

466 Several genes regulating biological processes, such as cell adhesion, cell
467 migration and cell proliferation, were more highly expressed in treated mice.

468 The interactions between thymocytes and the microenvironment are essential for
469 the intrathymic migration of thymocytes. Interestingly, the impairment of thymocyte
470 migration through the thymic microenvironment has been reported in NOD mice (Cotta-
471 de-Almedia et al., 2004). The enzymatic activities of metalloproteinases are involved in
472 thymocyte-stroma interactions by remodeling of the extracellular matrix (ECM). A role
473 for metalloproteinases in thymic T cell development has been recently demonstrated
474 using Adam8-deficient mice (Gossens et al., 2010). ADAM8 is a member of the
475 Disintegrin and Metalloproteinase (ADAM) family of proteins. A variety of biological
476 processes involving cell-cell and cell-matrix interactions have been implicated. In the
477 thymus, ADAM8 is expressed by TECs, principally by mTECs, and is involved in
478 intrathymic T cell migration through the aforementioned remodeling of the ECM.
479 Interestingly, data from the present microarray experiments revealed increased

transcripts of *Adam8* gene in treated mice and strongly support the contention that CD26/DPPIV inhibition may enhance the migration of positively selected thymocytes to the medulla, which is essential for the establishment of central tolerance.

3.4. Role of MK626 treatment in altering the expression of β-cell autoantigens and Aire in the thymi of NOD mice.

To determine the potential mechanisms by which the CD26/DPPIV inhibition is involved in T1D prevention, we next investigated whether MK626 treatment alters the expression of genes encoding islet autoantigens, such as *Gad1, Gad2, Ins1* and *Ins2* as well as the autoimmune regulator *Aire*, in purified TECs obtained at 6 weeks using qRT-PCR.

MK626 treatment does not alter β-cell autoantigens and *Aire* expression in TECs

As shown in Figure 5, no differences were observed in the gene expression of *Gad1 (*glutamic acid decarboxylase), *Ins2* (proinsulin) and *Aire* (transcription factor autoimmune regulator) in TECs between MK626-treated and control mice. *Gad2* and *Ins1* showed a very low expression in either treated and control animal. These findings are consistent with data reported in the literature. INS2 is the major isoform recognized by T cells in NOD mouse and is expressed in both β cells and the thymus, while the expression of *Ins1* in the thymi has been debated (Thébault-Baumont et al., 2003).

The thymus is involved in the establishment of tolerance to peripheral Ags. The expression of a broad repertoire of TRAs within the thymus has been termed promiscuous gene expression (Sospedra et al., 1998). Data suggests that this expression of TRAs by TECs, mainly mTECs, play a role in thymic central tolerance through clonal deletion of self-reactive thymocytes (Derbinski et al., 2001; Kyewski et al., 2002). In T1D, insulin represents one of the principal targets in the development of

diabetogenic autoimmunity and their presentation in the thymus promotes the deletion of self-reactive thymocytes (Kent et al., 2005; Nakayama et al., 2005). Aire is responsible for mediating central tolerance of peripheral self-Ags because it induces the expression of many of these TRAs by TECs, which are processed and then presented on surface to MHC/HLA (Mathis and Benoist, 2009; Rizzi et al., 2006). It has been reported that the expression of *Aire* and TRAs by TECs in NOD model is much lower than normal (Balb/c) TECs (Chen et al., 2008) and its expression varies with age and with the onset of T1D (Fornari et al., 2010; Oliveira et al., 2013). So, recent data suggest that thymic down-regulation of *Aire* in young NOD mice (pre-diabetic checkpoint) precedes the onset of T1D (Fornari et al., 2010), whereas *Aire* expression during the perinatal period is important to prevent autoimmunity in this model (Guerau-de-Arellano et al., 2009).

Taken together, we found that gene expression of islet autoantigens and the autoimmune regulator *Aire* is not influenced by CD26/DPPIV inhibitor treatment, unless in the late preclinical stage of the disease in NOD mice. Although further studies with more amount of thymic tissue analysed are required to confirm our findings, it maybe also needed to investigate whether MK626 affects the expression of these genes in a much earlier phase of the pre-diabetic state, including the perinatal period.

4. Conclusion

In a previous study, our group found that the CD26/DPPIV inhibitor MK626 decreased the incidence of T1D and reduced islet lymphocyte infiltration in pre-diabetic NOD mice. Moreover, in peripheral compartment, MK626 increases CD26 expression in the $CD8^+$ T_{EM} T cells. Indeed, *in vitro* assays revealed an immunosuppressive role for $CD8^+$ T_{EM} cell subset that may be involved in the protection against autoimmunity to β-cells. In the current study, we demonstrate for the first time that the expression of immune-related genes, especially those potentially involved in central tolerance, is modified in the thymus of MK626-treated mice. Our data from microarray analysis suggest that targeting CD26/DPPIV may affect immunological central tolerance mechanisms through several possible pathways, including the following: 1) contributing to the migration of thymocytes to the thymic medulla, thus enhancing negative selection; 2) increasing the efficiency of clearance of apoptotic cell generated during positive and negative thymic selection; 3) limiting T cell activation and responses; 4) inducing tolerogenic DCs; and 5) enhancing natural Tregs generation and function. Altogether, the inhibition of CD26/DPPIV may enhance the efficiency of deleting self-reactive thymocytes and modulate T cell responses and, consequently, may reduce the diabetogenic autoimmune response. However, the effects of DPPIV inhibition regarding T1D prevention do not seem to involve modifications of *Aire* and β-cell autoantigens expression.

Our results provide more insight into the understanding of the mechanisms through which targeting CD26/DPPIV prevents and even reverses T1D in NOD mice. However, further functional studies are needed to confirm the present findings.

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889 Table 1.Functional categorization of transcripts that were differentially expressed
890 in treated NODmice
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Function	Gene	Locus ID	Protein name	Log_2 FC	adj *p*-value	al/prol*	Other functions
Innate immunity	***Plunc***	18843	BPI fold containing family A, member 1	4.8334	0.0108	No*	Inflammatory response
	Reg3g	19695	Regenerating islet derived protein 3γ·	2.8724	0.0174	No	Inflammatory response
	Ear11	93726	Eosinophil-associated, ribonuclease A family, member 11	1.9618	0.0298	No	Inflammatory response; Chemotaxis
	Ear10	93725	Eosinophil-associated, ribonuclease A family, member 10	1.5329	0.0174	No	Inflammatory response
	Ear1	13586	Eosinophil-associated, ribonuclease A family, member1/ Eosinophil cationic protein 1	1.4553	0.0159	No	Inflammatory response
	Clec7a	56644	C-type lectin domain family 7, member A /Dectin-1	1.3757	0.0298	No	Inflammatory response; Immunoregulation; Phagocytosis; Cell adhesion; Signaling
	Muc1	17829	Mucin -1	1.2602	0.0174	No	Inflammatory response; Cell adhesion; Cell growth; Signaling; Apoptosis
	Lbp	16803	Lipopolysaccharide binding protein	1.2689	0.0478	No	Inflammatory response; Phagocytosis; Chemotaxis; Cell motility; Cell migration; Signaling
	Ifitm1	68713	Interferon-induced transmembrane protein 1	1.1397	0.049	Yes	Cell adhesion; Cell migration; Cell proliferation
	Clec9a	232414	C-type lectin domain family 9 member A	0.8924	0.0439	No	Antigen presentation; Immunoregulation
	Csf1r	12978	Macrophage colony-stimulating factor 1 receptor	0.864	0.0437	Yes	Inflammatory response; Cell proliferation; Cell migration; Cell motility; Cell adhesion; Signaling
Chemotaxis	***Ccl11***	20292	Chemokine (C-C motif) ligand 11/Eotaxin	1.6042	0.0174	Yes	Cytokine activity; Inflammatory response; Cell migration; Cell motility; Cell growth; Cell proliferation; Signaling
	Ccl6	20305	C-C motif chemokine 6	1.4969	0.0177	No	Cytokine activity; Inflammatory response; Cell migration; Cell motility
	Ccl27	20301	Chemokine ligand 27	1.618	0.0177	Yes	Cytokine activity
	C3ar1	12267	C3a anaphylatoxin chemotactic receptor	1.559	0.0298	No	Inflammatory response; Antigen presentation; Phagocytosis; Cell migration; Cell motility; Signaling
	Ccl21	18829	Chemokine ligand 21a	1.1009	0.0298	No	Cytokine activity; Inflammatory response; Immunoregulation; Cell migration;

	Ccl3	20302	C-C motif chemokine 3, MIP1α	1.1966	0.0298	Yes	Cytokine activity; Inflammatory response; Cell migration; Cell motility; Signaling; Apoptosis
	Ccl9	20308	C-C motif chemokine 9, MIP1γ	1.1829	0.0311	No	Cytokine activity; Inflammatory response
	Cxcl16	66102	C-X-C motif chemokine 16	0.9195	0.0437	No	Cytokine activity; Innate immunity; Antigen presentation; Cell migration; Cell motility; Cell growth
Immuno regulation	***Fcgr2b***	14130	Low affinity Ig gamma Fc region receptor IIB	0.9484	0.0339	No	Inflammatory response; Antigen presentation; Phagocytosis; Cell proliferation; Signaling; Apoptosis
	Lilrb4	14728	Leukocyte Ig-like receptor subfamily B member 4	0.9461	0.0341	No	Antigen presentation; Signaling
Antigen presentation	***Lgmn***	19141	Legumin	0.9826	0.0298	Yes	Cell growth; Apoptosis
Inflammation	***Scgb3a1***	68662	Secretoglobin family 3A member 1	4.4231	0.0241	No	Cytokine activity; Cell proliferation
	Sgb1a1	22287	Uteroglobin	4.2748	0.0241	No	Cytokine activity; Immunoregulation; Cell proliferation; Signaling
	Retnla	57262	Resistin-like alpha	2.2577	0.0341	No	
	Fstl1	14314	Follistatin related protein	1.1499	0.0298	No	Innate immunity; Autoantigen; Cell proliferation
Cell adhesion	***Itga8***	241226	Integrin alpha 8	1.7714	0.0177	Yes	Metanephric and Nervous system development, Extracellular matrix organization; Signaling
	Postn	50706	Periostin	1.3371	0.0174	No	Inflammatory response; Extracellular matrix organization
	Mfap4	76293	Microfibril-associated glycoprotein 4	1.1654	0.0311	Yes	Extracellular matrix organization; Signaling
	Adam8	11501	Disintegrin and metalloproteinase domain-containing protein 8	1.1554	0.033	No	Inflammatory response; Innate immunity; Chemotaxis; Cell migration; Cell motility; Signaling; Apoptosis; Extracellular matrix organization
	Col6a2	12834	Collagen alpha-2 (IV) chain	1.1455	0.0298	No	Extracellular matrix organization
	Tgfbi	21810	Transforming growth factor-beta-induced protein ig-h3	1.0326	0.0341	Yes	Signaling; Extracellular matrix organization
Cell proliferation /Growth	***Nov***	18133	Nephroblastoma overexpressed gene/Protein NOV homolog	1.6978	0.0174	No	Cell growth
	Tppp3	67971	Tubulin polymerization promoting protein family member 3	1.42	0.0298	Yes	
	Smpd3	58994	sphingomyelinphosphodiesterase 3	1.1967	0.0174	No	Signaling; Apoptosis
	Atf3	11910	Activating transcription factor 3/Cyclic AMP-dependent transcription factor 3	0.8808	0.0298	No	Inflammatory response; Transcription factor; Apoptosis

Apoptotic process	***Timp1***	21857	Metalloproteinase inhibitor 1	1.4433	0.0341	No	Erythrocyte maturation; Cell proliferation
	Clca2	80797	Chloride channel calcium activated 2	1.324	0.0413	No	
Nervous system	***Gabrp***	216643	Gamma-aminobutyric acid (GABA) A receptor, pi	1.9095	0.0298	No	
	Efhd1	98363	EF-hand domain-containing protein D1	1.3264	0.0341	No	
	Gas7	14457	Growth arrest specific protein 7	1.1794	0.0435	Yes	Cytoskeleton
	Duoxa 1	213696	Dual oxidase maturation factor 1	1.12	0.0298	Yes	Cell adhesion
	Gfra2	14589	Glial cell line derived neurotrophic factor family receptor alpha2	1.0545	0.0298	No	Signal transduction
	Mtap1b	17755	Microtubule-associated protein 1B	0.9402	0.0413	No	Cytoskeleton
Miscellaneous							
Ion binding	***Cyp2f2***	13107	Cytochrome P450, family 2, subfamily f, polypeptide 2	4.07	0,.0311	No	
	Cyp4a12b	13118	Cytochrome P450, family 4, subfamily a, polypeptide 12	3.0037	0.0298	No	
	Cyp2a5	13087	Cytochrome P450, family 2, subfamily a, polypeptide 5	2.3177	0.0159	No	
	Cyp2a4	13086	Cytochrome P450, family 2, subfamily a, polypeptide 4	1.9005	0.0298	No	
	Mt2	17750	Metallothionein 2	1.4602	0.0405	No	
Olfaction	***Olfr111***	545205	Olfactory receptor 111	1.5254	0.0298	No	Signal transduction
Keratinization	***Sprr2a1***	20755	Small proline-rich protein 2A1	1.5064	0.0298	No	
	Sprr1a	20753	Small proline-rich protein 1A/Cornifin-A	1.1202	0.0298	No	
Protein transport	***Slc15a3***	65221	Solute carrier family 15	0.89	0.0298	No	
Bio mineralization	***Aspn***	66695	Asporin	1.1079	0.0298	Yes	
Blood coagulation	***F3***	14066	Coagulation factor III /Tissue factor	1.1209	0.0477	No	
Cytoskeleton	***Acta2***	11475	Actin, aortic smooth muscle	1.3261	0.0413	No	
	Tagln	21345	Transgelin	0.9713	0.0311	No	
Histidine metabolism	***Hal***	15109	Histidine ammonia lyase	1.11209	0.0298	Yes	

892

Genes significantly up-regulated by targeting CD26/DPPIV are listed, with the mean difference between the groups (Fold Change; FC) and the false discovery rate estimated by the Benjamini-Hochberg method (adjusted *p*-value: < 0.05). *Al/prol is referenced to the presence in peptide of alanine and proline in the penultimate position.

Table 2. Top over-represented pathways identified in treated NOD mice by Ingenuity Pathway Analysis **(**IPA)

Top IPA canonical pathways (*p*-value)
Agranulocyte adhesion and diapedesis (1.29E-07)
Granulocyte adhesion and diapedesis (1.23E-06)
Hepatic fibrosis/Hepatic stellate Cell Activation (1.58E-03)
Communication between innate and adaptive immune cells (2.07E-02)
IL- 10 signaling (2.4E-02)

Immune Canonical Pathways among the "Top 20" biological canonical pathways resulting from IPA analysis. p-value calculated by the Ingenuity algorithm is given.

Table 3. Top biological functions identified in treated NOD mice by Ingenuity Pathway Analysis (IPA)

Disease and disorders		
Name	P-value	Molecules
Inflammatory response	3.49E-09 – 1.83E-02	27
Immunological disease	7.32E-08 – 1.73E-02	21
Gastrointestinal disease	9.48E-08 – 1.83E-02	21
Ophthalmic disease	9.48E-08 – 7.38E-03	11
Respiratory disease	5.27E-06 – 1.47E-02	18
Molecular and cellular functions		
Name	P-value	Molecules
Cellular Movement	5.31E-10 – 1.83E-02	31
Cell-To-Cell Signaling and Interaction	2.98E-08 – 1.83E-02	24
Cellular Function and Maintenance	2.19E-06 – 1.47E-02	22
Cellular Assembly and Organization	2.85E-05 – 1.83E-02	17
Cellular Development	6.68E-05 – 1.83E-02	24
Physiological System Development and Function		
Name	P-value	Molecules
Immune Cell Trafficking	6.08E-10 – 1.83E-02	20
Hematological System Development and Function	1.67E-08 – 1.83E-02	28
Tissue Morphology	1.67E-08 – 1.47E-02	27
Tissue Development	2.85E-05 – 1.83E-02	25

Numbers of molecules involved in each biological function are shown. Data are expressed as a p-value < 0.05.

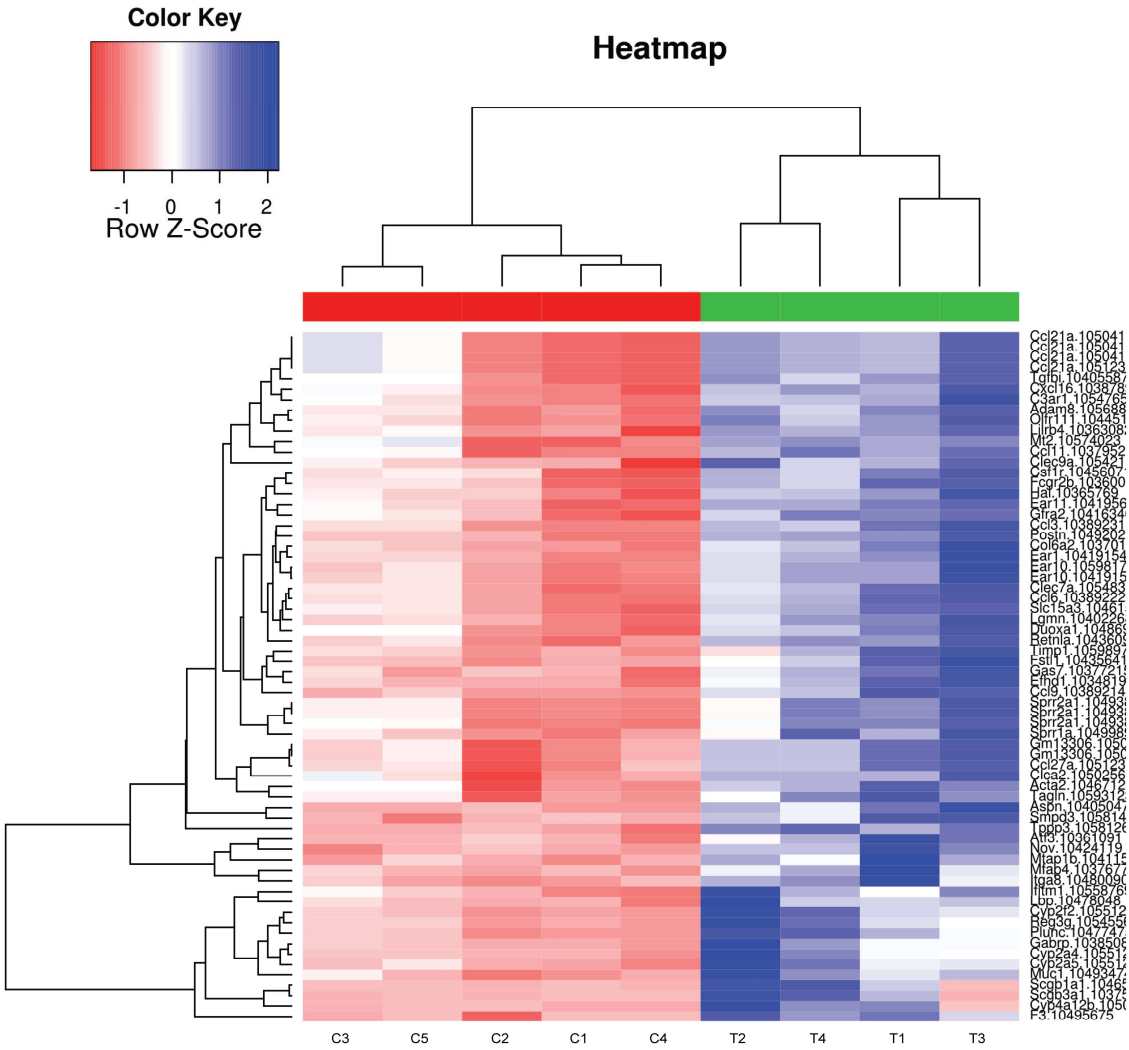
Figure 1. Heatmap of thymic gene expression profile from NOD mice. Rows correspond to differentially expressed genes, and columns represent each thymus sample from control (red columns) and treated (blue columns) NOD mice. Data were row-centered by subtracting the row-mean from each value so that under- and overexpression are indicated by negative (red) and positive (blue) values. C: Control group; T: Treated group.

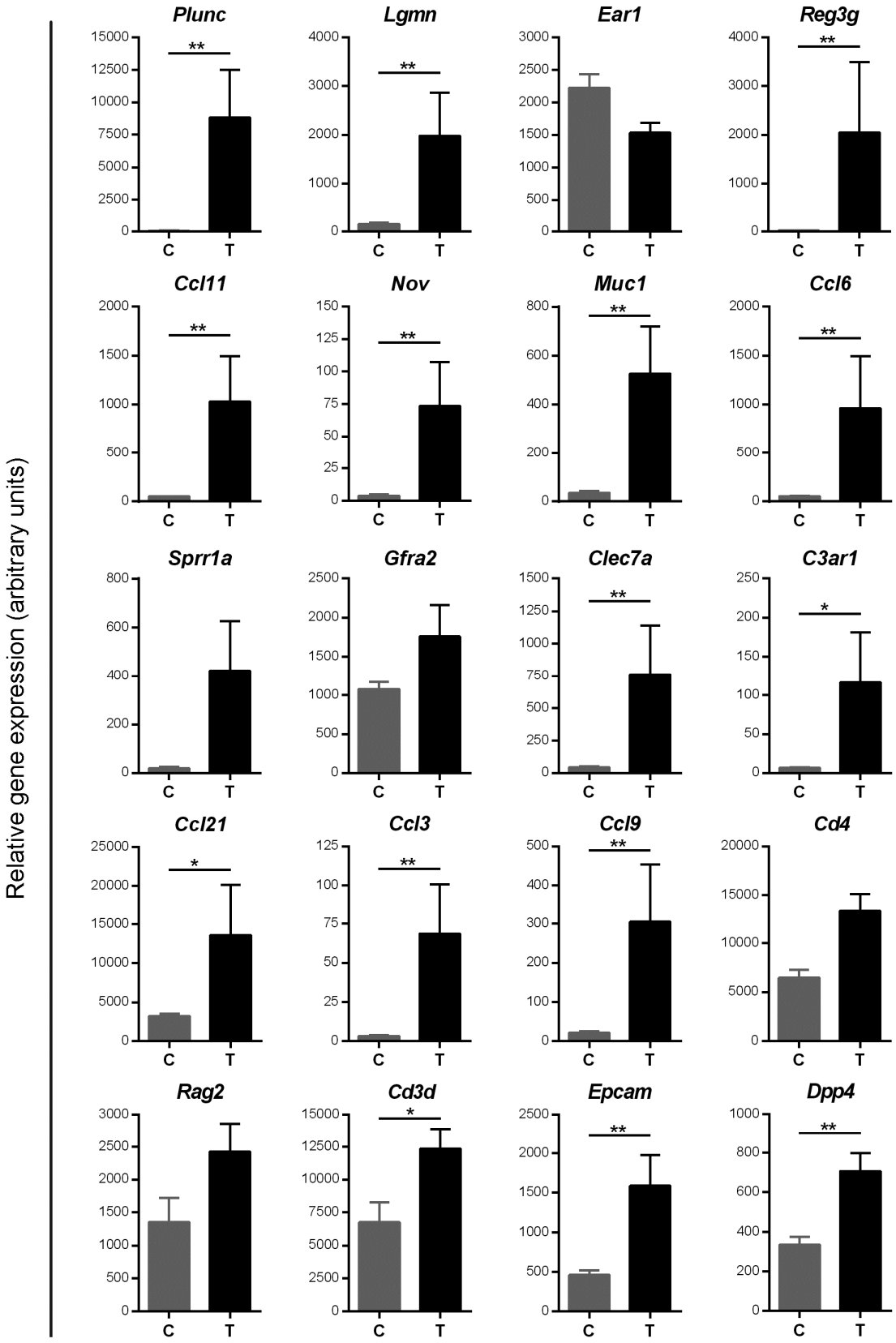
Figure 2. Validation of the gene expression profile. Relative expression levels measured by qRT-PCR of selected genes in control (C, n=5) and treated (T, n=5) groups. Results are expressed as mean + SEM. Non parametric Mann-Whitney test was used for the evaluation of statistical significance. Significance levels, *$p < 0.05$, **$p < 0.01$.

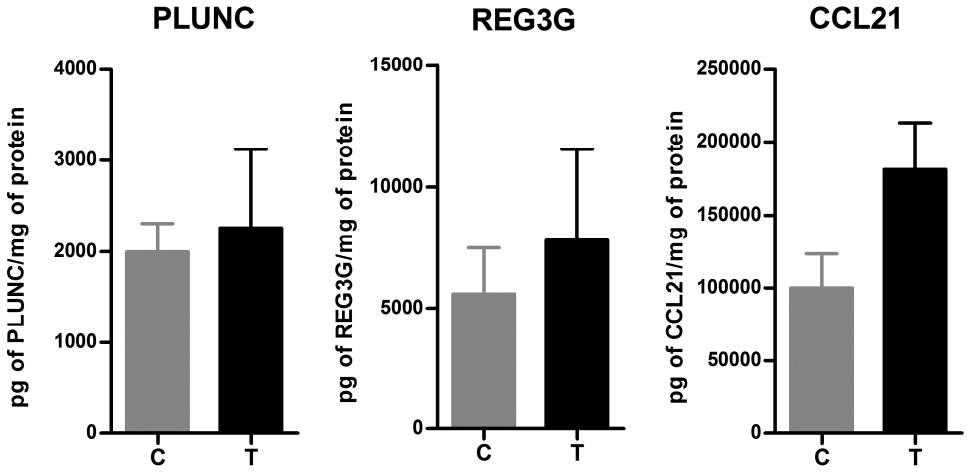
Figure 3. Analysis of the gene expression profile at protein level. Levels of PLUNC, CCL21 and REG3G measured by ELISAs in control (C, n=5) and treated (T, n=5) groups. Results are expressed as pg of protein of interest/mg of total protein (mean + SEM). Non parametric Mann-Whitney test was used for the evaluation of statistical significance.

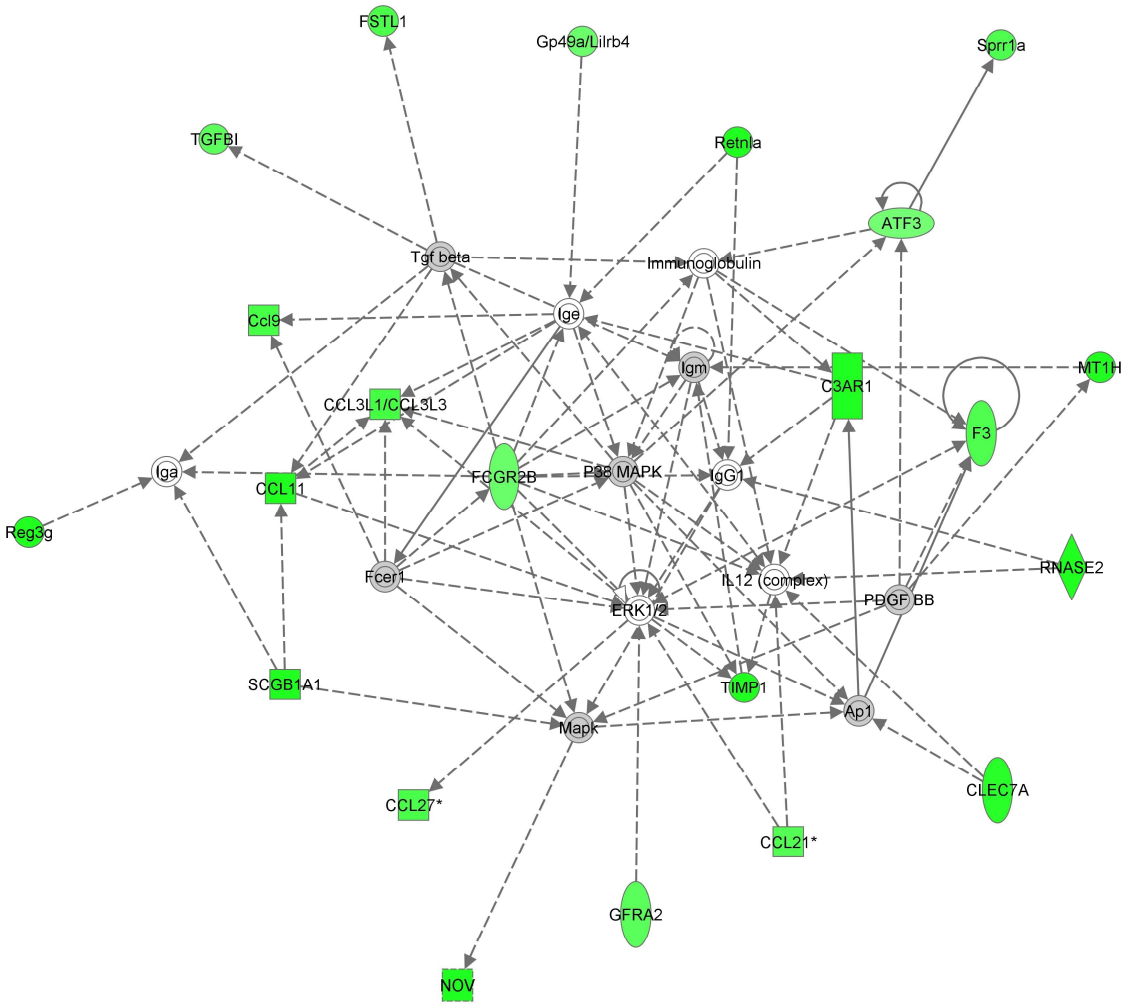
Figure 4. Gene network generated by Ingenuity Pathway Analysis (IPA). IPA was applied to create an inter-related gene network. Gene interactions linked to the immune and inflammatory responses are represented. Interacting nodes are defined by either direct relationships (solid arrows) or indirect relationships (dashed arrows). The direction of the arrows shows the direction of the interaction. Green molecules indicate higher expression in treated NOD mice than in control mice at the gene level.

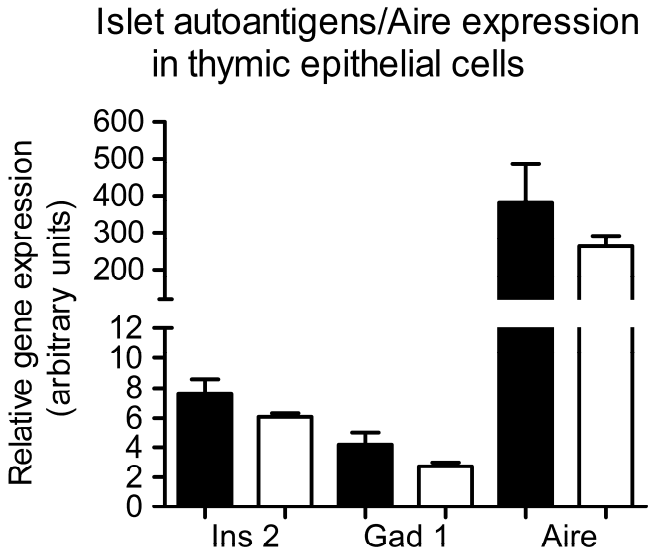
Figure 5. Gene expression levels of islet autoantigens and *Aire* in thymic epithelial cells (TECs) from MK626-treated and control mice. TECs obtained from treated and control group (≈ 12 weeks old) were purified after 6 weeks of treatment. Quantitative qRT- PCR results for *Gad1, Ins2* and *Aire* genes in treated (black bars) and in control mice (white bars). Gene expression signals were normalized to *Gapdh*. Data are presented as the mean + SEM. Non parametric Mann-Whitney test test was used for the evaluation of statistical significance.




Relative gene expression (arbitrary units)
Plunc
Lgmn
Ear1
Reg3g
Ccl11
Nov
Muc1
Ccl6
Sprr1a
Gfra2
Clec7a
C3ar1
Ccl21
Ccl3
Ccl9
Cd4
Rag2
Cd3d
Epcam
Dpp4
C
T







Highlights

- Treatment with DPPIV inhibitors may contribute to the prevention of T1D.
- Expression of immune –related genes is modified in the thymi of NOD mice by MK626.
- MK626 treatment increases the expression of genes involved in central tolerance.
- DPPIV inhibitor treatment does not alter β-cell autoantigens and *Aire* expression in TECs.