ORIGINAL ARTICLE

1,25-Dihydroxyvitamin D3 protects obese rats from metabolic syndrome via promoting regulatory T cell-mediated resolution of inflammation

Wen Jin b, Bing Cui a, Pingping Li a, Fang Hua a, Xiaoxi Lv a, Jichao Zhou a, Zhuowei Hu a, Xiaowei Zhang a*,

a Molecular Immunology and Pharmacology Group, State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China
b Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100193, China

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Abstract  Vitamin D3 has been found to produce therapeutic effects on obesity-associated insulin resistance and dyslipidemia through its potent anti-inflammatory activity, but the precise immunomodulatory mechanism remains poorly understood. In the present study we found that 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], the biologically active form of vitamin D3, significantly attenuated monosodium glutamate (MSG)-induced obesity and insulin resistance as indicated by body weight reduction, oral glucose tolerance improvement, and a glucose infusion rate increase as detected with hyperinsulinemic-euglycemic clamp. Moreover, 1,25(OH)2D3 not only restored pancreatic islet functions but also improved lipid metabolism in insulin-targeted tissues. The protective effects of 1,25(OH)2D3 on glycolipid metabolism were attributed to its ability to inhibit an obesity-activated inflammatory response in insulin secretory and targeted tissues, as indicated by reduced infiltration of macrophages in pancreas islets and adipose tissue while enhancing the expression of Tgf-β1 in liver tissue, which was accompanied by
1. Introduction

Obesity is associated with multiple adverse health outcomes collectively summarized as the “metabolic syndrome”, consisting of insulin resistance, dyslipidemia, and cardiovascular diseases. A linking role of inflammation between obesity and metabolic syndrome has been established which mostly refers to the release of various adipocyte products, such as cytokines, fatty acids, and oxygen free radicals from the white adipose tissue in obese individuals. Many of these products function as molecules of damage-associated molecular patterns (DAMPs) to initiate chronic low-degree inflammation to promote the infiltration of adipose tissue with activated macrophages through the activation of pattern reorganization receptors (PRRs) such as Toll-like receptors (TLRs) expressed on immune cells and residual cells. Indeed, accumulated evidence has indicated that both obesity and metabolic syndrome are inflammatory disorders and inflammatory responses are causally involved in insulin resistance by ultimately suppressing the insulin signaling pathway.

Epidemiological studies indicate that insufficient vitamin D status is a potential contributor to insulin resistance and obesity. Data from a pilot study examining vitamin D deficiency in type 1 and type 2 diabetes suggests that vitamin D insufficiency is more common in type 2 diabetes than in type 1 diabetes, unrelated to age, sex, or insulin treatment. However, studies on the administration of vitamin D supplements to vitamin D-sufficient patients with IGT or type 2 diabetes have yielded conflicting results. Some have reported an improvement, others no effect. One study even showed a worsening of type 2 diabetes: supplementation in three British Asians with vitamin D deficiency and type 2 diabetes led to increased insulin resistance and the deterioration of glycemic control. Interestingly, Wortsman et al. reported that obesity-associated vitamin D deficiency is largely because of the decreased bioavailability of vitamin D3 from cutaneous and dietary sources due to its deposition in body fat compartments. Furthermore, chronic feeding of mice with 1,25(OH)2D3, the biologically active form, suppressed the inflammatory responses in a variety of animal models including experimental asthma, encephalomyelitis, and rheumatoid arthritis. The potential immune modulating effects of 1,25(OH)2D3 on the immune system have been initially derived from in vitro observations following the treatment of dendritic cells (DC) with 1,25(OH)2D3 which could inhibit the maturation of myeloid DC via reducing secretion of IL-12 as well as the expression of co-stimulatory molecules. 1,25(OH)2D3 also enhances the secretion of CCL22 by DC in vitro, which is a chemokine that attracts T cells into the skin. IL-10-producing regulatory T cells can be induced in vitro by 1,25(OH)2D3 in the presence of dexamethasone. In addition, 1,25(OH)2D3 can hamper the secretion of IFN-α from T cells and induce Th2 cell development with increased production of IL-4, IL-5, and IL-10. Nonetheless, the immunoregulatory mechanism of 1,25(OH)2D3 on obesity-associated insulin resistance and dyslipidemia is not fully understood.

In this study, we have investigated the effects and mechanism of 1,25(OH)2D3 in the regulation of insulin sensitivity and glucolipid metabolism in MSG-obese rats. We found that a short course of treatment with 1,25(OH)2D3 improved insulin resistance and glucolipid metabolism disorder of MSG-obese rats by inhibiting the inflammatory responses in primary insulin-targeted tissues including adipose, liver, and muscle, which is largely due to increasing the infiltration of CD4+CD25+FoxP3 regulatory T-cells in these tissues. Our studies indicate that administration of 1,25(OH)2D3 protects MSG-obese rats from the development of obesity and its related metabolic risks.

2. Materials and methods

2.1. Animal model

Wistar rats (newborn) were obtained from Vital River Laboratory Animal Technology (Beijing, China). All animal protocols conformed to the Guidelines for the Care and Use of Laboratory Animals prepared and approved by the Animal Care and Use Committee of the Chinese Academy of Medical Sciences and Peking Union Medical College. Newborn Wistar rats were s.c. injected with monosodium L-glutamate (MSG) at 4 g/kg/day for seven successive days as described previously. In contrast to the normal rats, the MSG rats developed obesity with increased plasma TG, cholesterol, and free fatty acid contents as well as impaired insulin sensitivity in their adulthood. Experimental protocols are outlined in Fig. 1. In protocol A (Fig. 1A), the four-week-aged MSG rats were given s.c. injections of 1 μg/kg 1,25(OH)2D3 twice a week for 16 weeks, and insulin tolerance test (ITT) and the euglycemic hyperinsulinemic clamp were assayed at the end of the experiment to evaluate the incidence of insulin resistance. In protocol B (Fig. 1B), obese MSG rats were sorted into three groups according to body weights and fasting plasma glucose values, and then were treated with 1 μg/kg 1,25(OH)2D3 (twice a week), 4 mg/kg/day rosiglitazone or vehicle for 8 weeks.

2.2. Oral glucose tolerance test

The OGTT and ITT were carried out as previously described. In brief, after fasting for 6 h on the last treatment day, the animals were administrated an oral dose of 2 g/kg glucose. Blood samples were collected at 0, 30, 60, and 120 min after glucose loading. Blood glucose was analyzed by the glucose-oxidase method, and the area under the curve (AUC) was generated from the data of OGTT.
2.5. Semi-quantitative analysis by RT-PCR

The liver tissues were homogenized in Trizol and total RNA was extracted according to the manufacturer's instructions (Invitrogen, USA). cDNA was synthesized and used for PCR amplification. The RNA content was then analysed by agarose gel electrophoresis using the following specific primer sequences: rat Irs-1 (5'-CACCCACTCTCCTCCGCG-3'); (A) 5'-CCCTACTCGTTGTCGTAACAGTCCG-3'; rat Ppar-a (5'-GCAAAAACGTGAAGCACAAATTCT-3'); (A) 5'-AGCTCGTGACGGTGCTCCA-3'; rat Il-6 (5'-CCACTGGCTTCTCTACTTCA-3'; (A) 5'-AACGGAACTCCTCAAGAAGACCA-3'; rat Tgf-β3 (5'-CAGACATTCCGCGCAGCAGTG-3'; (A) 5'-GTTCTATCTGATGAGTTGC-3'; rat β-actin (S) 5'-TGGAACTCTGTCATCCATGAAAC-3'; (A) 5'-TAAACCGCACTCAGTAACAGTCCG-3'. The value was normalized to the values obtained with β-actin.

2.6. Western blot analysis

Briefly, the homogenized tissue extract (40 mg protein) from the liver was fractionated on a 10% SDS-PAGE gel and the proteins were transferred to PMSF membranes. After blocking with 5% nonfat milk, membranes were incubated with antibodies to pNF-κB, NF-κB, STAT3 and pSTAT3 (Cell Signaling Technology, USA) at 4 °C overnight. The membranes were washed and incubated with HRP-conjugated secondary antibodies at room temperature for 1 h. Enhanced chemiluminescence reagents (Amersham, USA) were employed to visualize the protein bands on the membranes. The densities of specific bands were normalized with the β-actin signal and were quantitated by densitometry using gel-pro software.

2.7. Immunolabelling and confocal microscopy

At the end of experiment the liver, pancreas and infrarenal adipose tissues were removed from MSG rats, fixed with 4% polyformalin in phosphate-buffered saline and embedded in paraffin. Liver and adipose tissue sections were stained with hematoxylin-cosin (H&E) and fat cell sizes were measured. The pancreas was fixed in bourin for paraffin embedding and stained with gomori and H&E for analysis of pancreatic β cell mass and islet morphology. Immunostaining was performed using GLUT2 (Bioss, China) and CD68 (BD, USA) antibody. An ABC kit and 3,3-diaminobenzidine (DAB) were used for amplification and staining, respectively. Quantitative analysis of the positive area was performed by image analysis using a computer with Image analyzer (Image Pro-Plus 5.1). Twenty fields were randomly selected from the each section, and the positively stained area was measured and expressed as IOD for each section. Liver, fat and muscle sections were subjected to immunofluorescence for identifying CD4+, CD25+FoxP3+ Tregs. Tissue sections were incubated with CD4+, CD25+ and FoxP3+ primary antibodies (Cell Signaling Technology) at 4 °C overnight. The sections were washed thrice and then incubated with Alexa488-, Alexa647- or Alexa550-conjugated secondary antibodies (Invitrogen) for 30 min. The colocalization of CD4 (green), CD25 (red) and FoxP3 (blue) were acquired using a confocal microscope FV1000 (Olympus Microsystems) and the Tregs were identified by the coexpression of CD4, CD25 and FoxP3 (white dots).
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3. Results

3.1. 1,25(OH)2D3 protected mice from developing MSG-induced insulin resistance

As obesity is the most important determinant of metabolic syndrome which is characterized by insulin resistance, hypertri-
glyceridaemia, hypo-HDL-cholesterolaemia and hypertension, we are firstly interested in analyzing whether 1,25(OH)2D3 protected MSG-induced obese rats from insulin resistance. The male and female MSG rats were treated with 1,25(OH)2D3 (1 g/kg, every other day) from weaning to 5-month age. Insulin resistance was evaluated by ITT and the euglycemic hyper-
insulinemic clamp. (A) The incidence of insulin resistance was identical in male and female rats. (B) 1,25 (OH)2D3 treatment significantly increased the decreased glucose infusion rate (GIR) in MSG obese rats. Data are expressed as mean ± SEM, n = 10. *P < 0.01 vs. normal group; †P < 0.05; ‡P < 0.01 vs. MSG group.

3.2. 1,25(OH)2D3 improved insulin sensitivity of MSG-obese rats

To determine the potential therapeutic significance of 1,25(OH)2D3 on established obesity, the body weight, glucose/lipid metabolism, and insulin sensitivity were then chosen to evaluate the efficacy of 1,25(OH)2D3 in the insulin resistant adult MSG rats (Fig. 1B). After an eight-week treatment, the body weight of the 1,25(OH)2D3-treated MSG rats was significantly lower than that of the vehicle- or rosiglitazone- (a positive control) treated rats (Fig. 3A), and there was no change in serum calcium and serum phosphorus levels (data not shown). As expected, the blood glucose (Fig. 3B) and plasma insulin levels (Fig. 3C) of 1,25(OH)2D3-treated MSG rats were dramatically decreased compared to the vehicle-treated MSG rats. We further examined the effects of 1,25(OH)2D3 on the improvement of insulin resistance in the MSG-obese rats by conducting OGTT and euglycemic-
hyperinsulinemic clamp tests. In the OGTT assay, the integrated blood glucose (AUC) levels of both 1,25(OH)2D3 and rosiglitza-
tone-treated MSG rats significantly decreased at 2 h after glucose loading (Fig. 3D). Using the euglycemic-hyperinsulinemic clamp, we observed that there was significant insulin resistance in MSG-obese rats, characterized by decreased GIR. However, both 1,25(OH)2D3 and rosiglitazone treatment increased the GIR of MSG rats by 107% and 49%, respectively, compared with the vehicle-treated MSG rats (Fig. 3E). Corresponding to decreased insulin sensitivity, the mRNA expression of Irs-1, which plays an important biological function for glucose uptake and augmenting insulin signaling, was dramatically up-regulated in the 1,25(OH)2D3-treated MSG rat livers (Fig. 3F). As accumulated evidence has shown that obesity is characterized by both peripheral insulin resistance and impaired insulin secretion, we are interested to know whether 1,25(OH)2D3 could be beneficial to improve MSG-induced dysfunction of pancreatic islets. In the MSG rats the islets showed substantial fibrosis, whereas there was little fibrosis in the 1,25(OH)2D3-treated MSG rats and the overall morphology of the islets was more regular and rounded (Fig. 3G). GLUT2 is the most important glucose transporter ensuring glucose uptake for most tissues. The suppression of GLUT2 in β-cells is associated with glucose-sensitive insulin secretion and the loss of high-Ka glucose transport. We found GLUT2 loss in MSG rats and 1,25(OH)2D3 normalized the expression of GLUT2 (Fig. 3H). All together, these results indicate that 1,25(OH)2D3 administration leads to an improvement of glucose metabolism and insulin sensitivity in MSG-induced obese rats.

3.3. 1,25(OH)2D3 ameliorated lipid metabolism of MSG-obese rats

As abnormal lipid metabolism is an important consequence and contributing factor in insulin resistance in the pathogenesis of obesity, we investigated the effect of 1,25(OH)2D3 on the regulation of lipid metabolism. We found that 1,25(OH)2D3 treatment significantly reduced the serum TG level of MSG rats (Fig. 4A), but did not change the level of the serum T-CHO and LDL-C (Fig. 4B and C). We further examined the effect of 1,25(OH)2D3 in liver and adipose tissues, both of which are the most important insulin-targeted tissues. PPAR-α is believed to participate in the regulation of fatty acid uptake and β-oxidation in the liver tissue. The mRNA expression of Ppar-α was significantly down-regulated in the vehicle or rosiglitazone-treated MSG rat

Figure 2 1,25(OH)2D3 decreased the MSG-induced incidence of insulin resistance. Male and female MSG rats were treated with 1,25(OH)2D3 (1 g/kg, every other day) from weaning to 5-month age. Insulin resistance was evaluated by ITT and the euglycemic hyper-
insulinemic clamp. (A) The incidence of insulin resistance was identical in male and female rats. (B) 1,25 (OH)2D3 treatment significantly increased the decreased glucose infusion rate (GIR) in MSG obese rats. Data are expressed as mean ± SEM, n = 10. *P < 0.01 vs. normal group; †P < 0.05; ‡P < 0.01 vs. MSG group.

CD4+CD25+FoxP3+ Tregs were identified by methods described previously13. Briefly, single-cell suspensions from spleens and mesentery lymph nodes were stained with antibodies that recognize the following antigens: CD4, CD25 and FoxP3, which were labeled with FITC, APC or PE (eBioscience, USA), respectively. More than 10,000 events were recorded through a CD4+ cell gate, and then CD5+FoxP3+ Tregs were determined using a PARTEC CyFlow (Munich, Germany) and data were analyzed using FCS EXPRESS.

2.8. Flow cytometry analysis

2.9. Statistical analyses

All values are presented as mean ± standard error of mean (SEM). Student's t-test was used for two-group comparisons. Multiple comparisons among three or more groups were performed by one-
way ANOVA. P < 0.05 or P < 0.01 was considered statistically significant.
liver, which was significantly up-regulated by 1,25(OH)₂D₃ (Fig. 4D). Histomorphological analysis of liver tissues using H&E staining revealed severe hepatic steatosis in the MSG rats, characterized by excessive accumulation of fat in hepatic intracellular vesicles. Treatment of the MSG rats with 1,25(OH)₂D₃ completely eliminated the steatosis. Liver slices from the 1,25(OH)₂D₃ treated MSG rats were found to be histologically comparable to those from normal rats (Fig. 4E). Additionally, it has been proved that the accumulation of "dysfunctional" adipose tissue characterized by the presence of "large" lipid-laden adipocytes contributes to the insulin resistance of obese individuals. For adipose tissue, MSG induced a dramatic increase in the fat mass and adipocyte size, while this adipocyte hypertrophy was attenuated by 1,25(OH)₂D₃ treatment (Fig. 4F and G). All of these data suggest that 1,25(OH)₂D₃ has a critical role in the regulation of lipid metabolism in liver and adipose tissues, both of which are the primary insulin-targeted tissues involved in the regulation of lipid metabolism.

3.4. 1,25(OH)₂D₃ exerted anti-inflammatory effects in MSG-obese rats

The macrophage is a major cell-type involved in chronic inflammation, and studies in obese humans and rodents have shown a dramatic activation of macrophages that produce inflammatory factors in pancreatic islets and adipose tissues. MSG-induced dysregulation of glucose and lipid led to high-level expression of CD68, a marker of activated macrophages, in pancreatic islets (Fig. 5A) and adipose tissues (Fig. 5B), indicating more pronounced macrophage infiltration in the insulin-secretory organ and
insulin-targeted tissues. In comparison to the MSG rat, treatment with 1,25(OH)\textsubscript{2}D\textsubscript{3} resulted in significant suppression of macrophage infiltration in both tissues (Fig. 5A and B). Interleukin-6 (IL-6), a typical inflammatory cytokine that is secreted by macrophages and Th2 cells, can alter insulin sensitivity by mediating different steps in the insulin signaling pathway. We found that serum IL-6 was increased in MSG rats (Fig. 5C), and a corresponding increase was also observed on the mRNA expression of Il-6 in MSG rat livers (Fig. 5D). Furthermore, we found that phosphorylation of NF-κB, a nuclear factor playing a central role in inflammatory disease, was increased in MSG rat livers (Fig. 5E). 1,25(OH)\textsubscript{2}D\textsubscript{3} elicited a distinct anti-inflammatory role by decreasing the IL-6 serum level, as well as the infiltration of IL-6 and activation of NF-κB in MSG rat livers (Fig. 5C-E). Additionally, we tested the activity of STAT3, a transcription factor which can suppress the production of pro-inflammatory cytokines. In the livers of MSG rats the phosphorylation of STAT3 was lower than normal rats, which was partly recovered in the livers of MSG rats treated with 1,25(OH)\textsubscript{2}D\textsubscript{3}, but not rosiglitazone (Fig. 5F). Transforming growth factor-β1 (TGF-β1) is an inhibitory inflammatory factor secreted by Treg cells. We found a reduction of Tgf-β1 in MSG rat livers and 1,25(OH)\textsubscript{2}D\textsubscript{3} normalized its expression (Fig. 5G). These results indicate that treatment with 1,25(OH)\textsubscript{2}D\textsubscript{3} inhibits the MSG-enhanced pro-inflammatory response, but restores the inhibitory inflammatory response in adipose and liver tissues of obese rats.

3.5. 1,25(OH)\textsubscript{2}D\textsubscript{3} increased the number of Tregs in spleen and lymph node

Accumulated evidence suggests that regulatory T cells play a key role in controlling the inflammation status. To confirm our above findings that the decreased TGF-β1 in MSG rat livers was markedly restored by treatment of 1,25(OH)\textsubscript{2}D\textsubscript{3}, we performed further studies using flow cytometry to detect the number of Treg cells in secondary immune organs such as spleen and lymph node. We found that the number of CD4\textsuperscript{+}CD25\textsuperscript{+}FoxP3\textsuperscript{+} Tregs did not change in spleen (Fig. 6A) but significantly decreased in the lymph node (Fig. 6B) of MSG rats. However, 1,25(OH)\textsubscript{2}D\textsubscript{3}-treated MSG rats revealed a significant increase of Tregs in both spleen and lymph node, compared to vehicle-treated MSG rats (Fig. 6A and B). Because nonresolving inflammation is known to drive insulin resistant in insulin-targeted tissues, we further examined whether the treatment of 1,25(OH)\textsubscript{2}D\textsubscript{3} could impact the infiltration of Treg in these tissues. Using confocal microscopy, we observed that 1,25(OH)\textsubscript{2}D\textsubscript{3} could dramatically enhance the frequency of CD4\textsuperscript{+}CD25\textsuperscript{+}FoxP3\textsuperscript{+} Tregs in primary insulin-targeted tissues such as liver (Fig. 6C) and muscle (Fig. 6D). These findings indicate that Tregs have a protective role in the pathogenesis of obesity as well as insulin resistant and that 1,25(OH)\textsubscript{2}D\textsubscript{3} may have general therapeutic efficacy for obesity by increasing the secretion or infiltration of Tregs in immune- or insulin-dependent tissues.

4. Discussion

It has become clear that obesity is one of the major risk factors for the onset of metabolic syndrome and type 2 diabetes mellitus. Recent studies have shown that obesity patients and animals appear to be in a chronic low-grade inflammation status characterized by increased plasma level of TNF-α, IL-6, IL-1, IL-8, MCP-1, and PAI-1. The accumulation of lipids and the expansion of the fat mass initiate the production of cytokines and chemokines. The proinflammatory
Factors inducing and maintaining the chronic inflammatory response are the critical inhibitors of insulin action on target organs, especially liver and adipose tissue. It is attributed to their architectural organization in which metabolic cells (hepatocytes or adipocytes) are in close proximity to immune cells (Kupffer cells or macrophages). For instance, co-cultured 3T3-L1 adipocytes and macrophages alter the expression of GLUT4 and insulin receptor substrate-1. Studies in MCP-1 receptor-deficient mice suggest that these mice developed obesity after high-fat feeding but had less macrophage infiltration and weaker insulin resistance than control mice. Macrophages in adipose tissue are suspected to be the major source of inflammatory factors such as TNF-α and IL-6. Corresponding with the Th1/Th2 concept of T-cell activation, a concept of M1/M2 polarization has recently been advanced for macrophages. In adipose tissue macrophages normally are of M2-like phenotype, characterized by expression cytokines involved anti-inflammation including IL-10, TGF-β, IL-4, and IL-13. Consumption of high-fat diet shifts cytokine expression of adipose tissue macrophages from M2- to M1-patterns by decreasing expression of IL-10 and increasing TNF-α and IL-6. Islets isolated from high-fat-fed mice produce much more inflammatory factors, including IL-6, IL-8, chemokine KC, granulocyte colony-stimulating factor, and MIP-1α. In our studies, newborn rats were subjected to monosodium L-glutamate (MSG) s.c. injection which caused chemical ablation of the hypothalamic arcuate nucleus, and as a result induced progressive obesity, insulin resistance, and dyslipidemia. In MSG rats, several cellular lesions have been associated with insulin resistance, including decreased expression of insulin signal protein such as IRS-1 in liver and GLUT2 in pancreas; adipocyte hypertrophy and ECM deposition in pancreas islets. These lesions are concomitant with a chronic inflammatory status involving enhanced macrophage infiltration in adipose tissue and pancreas; an up-regulated IL-6 level and down-regulated TGF-β expression.

Numerous evidence has suggested that inflammation promotes energy expenditure in liver, adipose and skeletal muscle tissues to fight against energy surplus. Over-expression of NF-κB p65, the most crucial pro-inflammatory transcription factor, protects mice from high-fat diet-induced obesity and insulin resistance through...
up-regulation of the expression of TNF-α and IL-6 in adipose tissue and macrophages. In addition, inflammatory cytokines, such as TNF-α, IL-1 and IL-6, can also serve as anti-obesity signals by modifying both energy intake and energy expenditure. However, both obesity and inflammation could be reciprocal causative factors, and breaking the obesity–inflammation cycle with anti-inflammatory reagents seems to be a more effective strategy against obesity from metabolic syndrome and type 2 diabetes. Indeed, IL-1R antagonist and salicylates, a treatment that inhibits the activity of NF-κB, have been used in animal models to improve insulin sensitivity. Agents such as thiazolidinedione (TZD) have been proven to have anti-inflammatory activities that partially contributes to their insulin-sensitizing effects. In this work, we found that rosiglitazone exhibited anti-inflammatory properties by reducing macrophage infiltration in adipose tissue and pancreas. In recent years, 1,25(OH)2D3 has been shown not only to be important for insulin secretion from the pancreatic beta-cell but also for apoptosis of adipose cells, both of

Figure 6 1,25(OH)2D3 enhances the tissue-infiltration of Treg cells in MSG obese rats. (A) and (B) 1, 25(OH)2D3 treatment increased the numbers of Treg in the spleen and lymph node. Treg was detected with flow cytometry as indicated. Moreover, 1,25(OH)2D3 treatment increased the numbers of Treg in the liver (C), adipose tissue (D), and skeleton muscle (E). Treg was detected with the confocal microscope. Data are representative of three independent experiments with identical results. *P<0.05, **P<0.01 vs. normal group; *P<0.05, **P<0.01 vs. MSG group.
which are closely associated with 1,25(OH)2D3-triggered intracellular uptake of calcium38–41. Furthermore, most of the known biological effects of 1,25(OH)2D3 are mediated through the vitamin D receptor (VDR), which can be found in a wide range of immune cells, including lymphocytes, dendritic cells and macrophages. Accumulating research suggests that the immune properties of 1,25(OH)2D3 include inhibition of DC differentiation and maturation, suppression of T cell activation, and enhancement of phagocytosis in monocytes or macrophages.

It has been shown that 1,25(OH)2D3 could reshape immune homeostasis involving a shift of production of T-cell cytokines from predominantly Th1 (IL-2, IFNγ) to Th2 (IL-4, IL-10). This shift is probably due to a direct interference of 1,25(OH)2D3 with the antigen-presenting dendritic cells. Indeed, 1,25(OH)2D3 induces a transformation of dendritic cells towards a tolerogenic phenotype32.

In vitro treatment with 1,25(OH)2D3 inhibited IFNγ-induced phosphorylation of STAT1 and IL-12-triggered phosphorylation of TYK2, JAK2, STAT3, and STAT4 in association with a decrease in T cell proliferation18. In another study Gregori et al.44 found that the analog of 1,25(OH)2D3 inhibited IL-12 production, blocked inhibition of the inflammatory response in metabolic homeostasis. Science 2013;339:172–7.

5. Conclusions

In summary, our studies demonstrate that 1,25(OH)2D3 treatment can reduce body weight, improve glucose and lipid metabolism, decrease the incidence of obesity-induced insulin resistance and improve established insulin resistance in MSG-obese rats. Moreover, the chronic inflammatory status of insulin target tissues was ameliorated and was clearly linked with enhanced CD4+CD25+FoxP3+ regulatory T cells. These findings add substance to the inflammatory theory behind the pathogenesis of obesity and insulin resistance. Furthermore, our study indicates that 1,25(OH)2D3 has potential immunoregulatory efficacy on obesity and insulin resistance, both of which are becoming the leading risk factors for morbidity and mortality in metabolic syndrome and diabetic patients.

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