MEK1/2 inhibitors induce interleukin-5 expression in mouse macrophages and lymphocytes

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Abstract

Uptake of oxidized low-density lipoprotein (oxLDL) by macrophages facilitates the formation of foam cells, the prominent part of atherosclerotic lesions. Interleukin-5 (IL-5) is a cytokine regulating interactions between immune cells. It also activates the production of T15/EO6 IgM antibodies in B-1 cells, which can bind oxLDL thereby demonstrating anti-atherogenic properties. We previously reported that inhibition of extracellular signal-regulated kinases 1 and 2 (ERK1/2) by mitogen-activated protein kinase kinases 1/2 (MEK1/2) inhibitors can reduce atherosclerosis. In this study, we determined the effects of MEK1/2 inhibitors on IL-5 production both *in vitro* and *in vivo*. *In vitro*, MEK1/2 inhibitors (PD98059 and U0126) substantially inhibited phosphorylation of MEK1/2 and ERK1/2. Associated with inhibition of ERK1/2 phosphorylation both *in vitro* and *in vivo*, MEK1/2 inhibitors induced IL-5 protein expression in macrophages (RAW macrophages and peritoneal macrophages) and lymphocytes (EL-4 cells). *In vivo*, administration of mice with MEK1/2 inhibitors increased serum IL-5 levels, and IL-5 protein expression in mouse spleen and liver. At the mechanistic level, we determined that MEK1/2 inhibitors activated IL-5 mRNA expression and IL-5 promoter activity in the liver X receptor (LXR) dependent manner indicating the induction of IL-5 transcription. In addition, we determined that MEK1/2 inhibitors enhanced IL-5 protein stability. Taken together, our study demonstrates that MEK1/2 inhibitors induce IL-5 production which suggests another anti-atherogenic mechanism of MEK1/2 inhibitors.

Keywords: atherosclerosis; interleukin-5; macrophage; MEK1/2 inhibitor; oxLDL

Abbreviations: ERK1/2, extracellular signal-regulated kinases 1/2; IL-5, interleukin-5; LXR, liver X receptor; MEK1/2, mitogen-activated protein kinase kinases 1/2; oxLDL, oxidized low-density lipoprotein.

Introduction

Atherosclerosis is one of the major causes for coronary heart disease, and a chronic pathological process with disorders of lipid biosynthesis/metabolism and inflammation[1,2]. Many independent risk factors for atherosclerosis have been identified, such as hypercholesterolemia, hypertension and smoking[3]. Macrophages can phagocytose cellular debris and multiple pathogens, thereby playing an important role in immune system. Macrophages also express scavenger receptors to uptake modified low-density lipoprotein (LDL). In particular, the uptake of oxidized LDL (oxLDL) by macrophage CD36 can enhance cellular oxLDL accumulation and facilitate formation of lipid-laden foam cells, a prominent part in atherosclerotic lesions[4,5]. Therefore, formation of macrophage/foam cells is the initial and critical step in atherosclerosis.

Interleukins are a group of cytokines secreted by multiple immunocytes and play important roles regulating cell maturation, activation, migration and immune responses. Among the identified interleukins, interleukin-5 (IL-5) can be expressed and secreted mainly by macrophages and T cells[6]. IL-5 has demonstrated to have multiple biological functions, such as promotion of the differentiation of B-1 cells, a subclass of B lymphocytes, into the antibody-secreting cells which can produce and secrete T15/EO6 IgM antibodies[7]. The T15/EO6 IgM antibodies can bind the phosphatidylcholine moiety of the oxidized phospholipids in oxLDL, thereby blocking oxLDL uptake by macrophages which suggests the anti-atherogenic properties of these two antibodies and IL-5[7,8,9]. Indeed, immunization of LDL receptor deficient mice with malondialdehyde-modified LDL (MDA-LDL) resulted in activation of IL-5 and production/secretion of T15/EO6 IgM antibodies, and reduced atherosclerosis[7,10]. Macrophage-specific overexpression of IL-5 reduces while deficiency of IL-5 expression enhances atherosclerosis[7,11]. Overexpression of IL-5 in apoE deficient (apoE^{-/-}) mice markedly inhibits aortic aneurysm formation[12]. In humans, plasma IL-5 levels are negatively correlated to carotid intima-media thickness in women[13].

Extracellular signal-regulated kinases 1 and 2 (ERK1/2) are involved in multiple biological processes including cell proliferation, migration and differentiation[14].

However, the phosphorylation of ERK1/2 by mitogen-activated protein kinase kinases 1/2 (MEK1/2) is pre-required for ERK1/2 activity. Overexpression or constitutive activation of ERK1/2 results in progression of several cancers which suggests that inhibition of ERK1/2 might be a target for cancer therapy[14]. We previously reported that inhibition of ERK1/2 phosphorylation by MEK1/2 inhibitors can reduce atherosclerosis in apoE^{-/-} mice which is related to activation of liver X receptor (LXR) and LXR-mediated ATP-binding cassette transporter A1 (ABCA1) expression and function[15,16]. However, the mechanisms by which MEK1/2 inhibitor reduces atherosclerosis have not been fully understood. In this study, we hypothesized that MEK1/2 inhibitors can activate IL-5 expression, particularly in macrophages, another anti-atherogenic function of MEK1/2 inhibitors.

Materials and methods

Reagents

U0126 and PD98059 were purchased from LC laboratories (Woburn, MA). Rabbit anti-IL-5 polyclonal antibody was obtained from Boster Biologicals (Wuhan, China). Anti-GAPDH polyclonal antibody was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Rabbit anti-ERK1/2, phospho-ERK1/2 (pi-ERK1/2) and phospho-MEK1/2 antibodies were purchased from Cell Signaling Technology (Danvers, MA). Rabbit anti-IFN- γ polyclonal antibodies were purchased from Proteintech Group (Chicago, IL). The IL-5 ELISA kit was purchased from R&D system (Minneapolis, MN).

Cell culture

RAW cells (a murine macrophage cell line) and EL-4 (a murine lymphocyte cell line) were purchased from ATCC (Manassas, VA), and cultured in complete RPMI 1640 medium containing 10% FBS, 50 μ g/ml penicillin/streptomycin and 2 mM glutamine, respectively. Mouse peritoneal macrophages were collected from C57BL/6 wild type mice as described[16] and cultured in complete medium. RAW cells at ~90% confluence, EL-4 cells at a density of $\sim 2 \times 10^5$ cells/ml, and peritoneal macrophages received treatment in serum-free medium.

In vivo study

The protocol for *in vivo* study was granted by the Ethics Committee of Nankai University and conforms to the Guide for the Care and Use of Laboratory Animals published by NIH. C57BL/6 wild type mice (~8 weeks old) were randomly divided into three groups (5/group) and fed normal chow (control), normal chow containing U0126 [5 mg/day/kg bodyweight (mpk)] or PD98059 (20 mpk) for 2 weeks. After treatment, animals were anesthetized and euthanized in a CO₂ chamber followed by collection of blood and tissue samples. After isolation, about 150 μ l serum was diluted with PBS (1:1) and then subjected to the IL-5 ELISA assay (each sample was analyzed in duplicate).

To determine tissue protein expression, ~30 mg tissue was removed and homogenized in a protein lysis buffer (150 mM NaCl , 50 mM Tris pH 7.5, 50 mM sodium fluoride, 1% Triton X-100, 1% sodium deoxycholate, 1 mM PMSF, 1 mM sodium orthovanadate, 50 µg/ml aprotinin/leupeptin). The supernatant of the homogenate was retained as tissue protein extract.

Western blot analysis

After treatment, cells were lysed in the ice-cold lysis buffer described above. Cellular lysate was spun for 20 min at 13,000 rpm at 4°C, and supernatant was collected as cellular protein extract. Expression of pi-ERK1/2, ERK1/2, pi-MEK1/2 and IL-5 protein in cellular or tissue protein extract was determined by Western blot as described[17].

Real time RT-PCR

After treatment, total RNA was extracted from cell followed by cDNA synthesis using a reverse transcription kit (Promega, Madison, WI) and real time PCR with SYBR Green Master Mix (Bio-Rad, Los Angeles, CA) as described[17]. The sequences of primers are listed in Table 1. Expression of IL-5, IL-10 and IL-13 mRNA was normalized with GAPDH mRNA in the corresponding sample.

Preparation of plasmid DNA and determination of IL-5 promoter activity

Mouse normal IL-5 promoters (p1228IL-5, p677IL-5) and the promoter with LXRE mutation (p677IL-5M) or deletion (p677IL-5D) were constructed as described[17]. To analyze promoter activity, ~5×10⁵/well EL-4 cells in 24-well plates were transfected with plasmid for the IL-5 promoters or *Renilla* luciferase (for internal normalization) using Gene Tran (Biomiga, San Diego, CA). After 24 h of transfection plus treatment, cells were lysed and the cellular lysate was used to determine activities of Firefly and *Renilla* luciferases using the dual-luciferase reporter assay system (Promega).

Data Analysis

All of the experiments were repeated at least three times, and the representative results were presented. The bands in all Western blots were subject to scanning and the density of band was statistically analyzed. The statistical analysis was completed with the parametric statistics, post-hoc test of one-way ANOVA if the data were in normal distribution. The data were presented as means $\pm$ standard deviation. A difference was considered to be statistically significant at $P < 0.05$ ($n \geq 3$).

Results

MEK1/2 inhibitors induce IL-5 expression in vitro

IL-5 is produced by macrophages and acts on B-1 cells to generate T15/EO6 IgM, the anti-atherogenic antibodies. Therefore, increased IL-5 can reduce atherosclerosis[12]. Our previous reports demonstrate that inhibition of ERK1/2 phosphorylation by MEK1/2 inhibitors reduced atherosclerosis which is related to LXR activation[15,16]. We also demonstrated that LXR enhances macrophage IL-5 expression[17]. These studies imply that MEK1/2 inhibitors may regulate IL-5 production by inhibition of ERK1/2 phosphorylation. To determine it, we initially determined the efficacy of two MEK1/2 inhibitors, U0126 and PD98059, on the phosphorylation of MEK1/2 (pi-MEK1/2), the enzymes specifically catalyzing ERK1/2 phosphorylation to generate pi-ERK1/2, the active form of ERK1/2. RAW macrophages were treated with U0126 and PD98059 at different concentrations for 6 h. As shown in Figure 1A, both U0126 and PD98059 reduced pi-MEK1/2 with greater effect by U0126 which is consistent with previous reports indicating that PD98059 is a weaker inhibitor for MEK1/2 than U0126. Associated with reduced pi-MEK1/2, PD98059 and U0126 substantially reduced pi-ERK1/2 in a concentration-dependent manner (top panel, Figure 1B). The time course further confirmed PD98059 and U0126 can reduce pi-ERK1/2 (low panel, Figure 1B) although they had little effect on ERK1/2 expression (Figure 1B). In addition, MEK1/2 inhibitors can potently reduce pi-ERK1/2 in peritoneal macrophages (Figure 1C).

To determine if the reduction of pi-ERK1/2 by MEK1/2 inhibitors can regulate macrophage IL-5 expression, we initially determined IL-5 protein expression in RAW cells in response to MEK1/2 inhibitors treatment. The results in the top panel of Figure 1D demonstrate that U0126 induced IL-5 protein expression with the maximum induction at 0.5 μ M. The time course study with 1 μ M U0126 (middle panel, Figure 1D) shows the induction occurred quickly (5 h after treatment) and lasted for 24 h of the treatment. Similarly, we determined that PD98059 also induced IL-5 protein expression with the maximum induction at 10 μ M in RAW macrophages (bottom panel, Figure 1D).

To confirm if the induction of IL-5 expression by MEK1/2 inhibitors can occur to primary cells, peritoneal macrophages isolated from wild type mice were used to conduct both concentration and time course studies. Similar to RAW cells, associated with reduced pi-ERK1/2 in primary macrophages (Figure 1C), both U0126 and PD98059 substantially induced IL-5 protein expression in peritoneal macrophages (Figure 1E).

Besides IL-5, we determined that U0126 and PD98059 can induce IL-13 mRNA expression (left panel), while reduce IL-10 mRNA expression in peritoneal macrophages (right panel). Therefore, MEK1/2 inhibitors can regulate expression of multiple cytokines, either activation or inhibition.

Lymphocytes is another cell type expressing IL-5. To determine if the induction of IL-5 expression by MEK1/2 inhibitor is cell-type independent, we treated EL-4 cells, a murine T lymphocyte cell line, with PD98059 or U0126 at different concentrations overnight. Similar to macrophages, we firstly determined that both U0126 and PD98059 reduced pi-ERK1/2. We then determined that associated with decreased pi-ERK1/2, MEK1/2 inhibitors increased IL-5 expression in concentration-dependent manner (Figures 2B and 2C), suggesting that MEK1/2 inhibitors induce IL-5 expression in different cell types.

MEK1/2 inhibitors activate IL-5 production in vivo

To define the effect of MEK1/2 inhibitors on IL-5 production *in vivo*, we randomly divided C57BL/6 wild type mice into three groups, and fed them a normal chow (control group) or normal chow containing U0126 (5 mpk, U0126 group) or PD98059 (20 mpk, PD98059 group) for 2 weeks. After treatment, we collected mouse blood and tissues (spleen, liver and lung), and then determined serum IL-5 levels by ELISA assay and tissue pi-ERK1/2, ERK1/2 and IL-5 expression by Western blot, respectively. The results in Figure 3A indicate that both U0126 and PD98059 significantly increased serum IL-5 protein levels (18.9 pg/ml in control group vs. 37.2 pg/ml in U0126 group and 35.2 pg/ml in PD98059 group, respectively, $P<0.05$). The results in Figure 3B demonstrate that administration of mice with PD98059

substantially induced IL-5 protein expression in spleen and liver while it mildly increased IL-5 expression in the lung. To confirm if the induction of IL-5 expression by PD98059 is associated with reduced pi-ERK1/2, we determined changes of pi-ERK1/2 in mouse tissues. As shown in Figure 3C, administration of mice with PD98059 significantly reduced pi-ERK1/2 levels in spleen, liver and lung. These data indicate that MEK1/2 inhibitors can induce IL-5 protein production *in vivo* which is associated with inhibition of ERK1/2 phosphorylation.

MEK1/2 inhibitors induce IL-5 expression by activating IL-5 transcription and enhancing IL-5 protein stability

Our above results demonstrate that MEK1/2 inhibitors induce IL-5 expression in a cell-type independent manner *in vitro* while they activate IL-5 production *in vivo*. Furthermore, we investigated the involved mechanisms. Initially we determined macrophage IL-5 mRNA expression in response to U0126 treatment. As shown in Figure 4A, U0126 increased IL-5 mRNA expression in a concentration-dependent (left panel) and a time-dependent manner (right panel) which implies that MEK1/2 inhibitors may activate IL-5 transcription. To determine it, we constructed a natural IL-5 promoter (from -1173 to +45, and defined it as p1218IL-5), and then determined the effect of U0126 on IL-5 promoter activity. Compared with IL-5 promoter alone, treatment of the promoter-transfected cells with U0126 increased p1218IL-5 promoter activity in a dose-dependent manner suggesting the activation of IL-5 transcription (Figure 4B).

We previously reported an LXR response element (LXRE, from -246 to -231 with a sequence of ATGTGGatcaAAGTTG) in the proximal region of IL-5 promoter[17]. To determine the role of this LXRE in the U0126-activated IL-5 transcription, we constructed another natural IL-5 promoter (from -643 to +45) which includes the LXRE and defined this promoter as p677IL-5, and the promoter with LXRE mutation (p677IL-5M) or LXRE deletion (p677IL-5D). We then evaluated the effect of U0126 on the activity of these promoters. The results in Figure 4C indicate that U0126 induced activity of p677IL-5 promoter. However, the mutation or deletion of LXRE totally

abolished the inductive effect of U0126 on IL-5 promoter activity, which suggests that U0126-activated IL-5 transcription is in an LXR/LXRE-dependent manner.

Furthermore, we determined the effects of MEK1/2 inhibitors on IL-5 protein stability. Cycloheximide is an inhibitor of protein biosynthesis in eukaryotic organisms thereby arresting the synthesis of cellular proteins. We treated macrophages with cycloheximide or plus either U0126 or PD98059 (top and middle panels, Figure 4D), and determined IL-5 proteins levels with time of treatment. Inhibition of protein synthesis by cycloheximide resulted in reduced IL-5 protein levels with time. However, in the presence of U0126 or PD98059 (Figure 4D), the decline of IL-5 protein levels by cycloheximide was significantly attenuated, suggesting that MEK1/2 inhibitors can enhance IL-5 protein stability, a post-transcriptional mechanism increasing IL-5 expression.

To determine if MEK1/2 inhibitor-increased IL-5 protein stability is specific or not, we detected changes of IFN- γ protein, another molecule can be regulated by MEK1/2 inhibitors[31], in response to cycloheximide alone or cycloheximide plus U0126 treatment. As shown in Figure 4E, U0126 also significantly attenuated the decline of IFN- γ protein levels by cycloheximide suggesting U0126 is also able to increase IFN- γ protein stability. Thus, the results in Figure 4 indicate that MEK1/2 inhibitor induce IL-5 expression at both transcriptional and post-transcriptional levels.

Discussion

Uptake of oxLDL by macrophages facilitates formation of foam cells, the initial and critical step for the development of atherosclerosis. Antibodies which are able to bind oxLDL can attenuate macrophage oxLDL accumulation thereby inhibiting progression of atherosclerosis[8,9,18]. Clinically, it has been determined that the IgM autoantibodies to MDA-LDL are inversely associated with carotid atherosclerosis[19]. EO6 is an IgM isolated from apoE^{-/-} mouse spleen with a high affinity for oxLDL. In addition, it blocks scavenger receptor activity which can further decrease lipid accumulation in macrophages[18]. Interestingly, EO6 shares a high genetic and structural identity with T15[9], another antibody which is a classic anti-phosphoryl choline B-cell clone, and protects against common infectious pathogens like pneumococci[20]. Similar to EO6, T15 also demonstrates a high affinity for oxLDL. LDLR^{-/-} mice vaccinated with pneumococci can exhibit elevated T15 IgM levels and increased T15 antibody-secreting B cells in the spleen[21]. The production and secretion of T15/EO6 antibodies by B-1 cells can be substantially induced by IL-5. Therefore, IL-5 or/and T15/EO6 can block macrophage oxLDL accumulation and inhibit atherosclerosis[7].

Macrophage cholesterol homeostasis is regulated by cholesterol uptake and its efflux. ABCA1 mediates cellular free cholesterol to extracellular cholesterol acceptor, apoA1, by using the energy of ATP hydrolysis thereby generating nascent high-density lipoprotein (HDL)[22,23]. High expression of ABCA1 reduces atherosclerosis[24]. In contrast, deficiency of ABCA1 expression impairs reverse cholesterol transport, enhances foam cell formation and occurrence of premature coronary heart disease[25,26,27]. Macrophage ABCA1 expression is activated by LXR, a ligand-activated transcription factor[28]. Therefore, LXR ligands have been demonstrated a novel strategy for treatment of atherosclerosis[29,30]. We previously reported that LXR can also induce macrophage IL-5 expression. *In vivo*, LXR ligand (T0901317) increased serum IL-5 and total IgM levels and IL-5 expression in multiple tissues including aortic root[17]. Therefore, our previous study discloses another anti-atherogenic mechanism of LXR.

In our recent report, we determined that MEK1/2 inhibitor can reduce atherosclerosis by mechanisms which are partially attributed to activation of ABCA1 expression and cholesterol efflux[15,16]. In current study, we determined that MEK1/2 inhibitors induced IL-5 expression in both macrophages and lymphocytes (Figures 1 and 2). *In vivo*, administration of mice with MEK1/2 inhibitors increased serum IL-5 levels and IL-5 expression in tissues (Figure 3). Our findings suggest another mechanism by which MEK1/2 inhibitor reduces atherosclerosis. At the mechanistic level, we defined that MEK1/2 inhibitors activated IL-5 transcription and enhanced IL-5 protein stability. In our previous reports, we defined that MEK1/2 inhibitors can activate transcription of several LXR-targeted genes with the mechanisms in which MEK1/2 inhibitors still differ from LXR ligands[16,31]. Similarly, herein we report that MEK1/2 inhibitors induced IL-5 expression, another LXR-targeted gene. We further determined that LXRE deletion or mutation in the proximal region of IL-5 promoter totally abolished U0126-induced IL-5 promoter activity (Figure 4C). Therefore, induction of IL-5 expression by MEK1/2 inhibitors is related to LXR activation. In addition, we determined that MEK1/2 inhibitors activated IL-13 expression but inhibited IL-10 expression (Figure 1F). We previously reported that LXR ligands can increase IL-5 protein stability[17]. In current study, we detected that MEK1/2 inhibitor also increased IL-5 and IFN- γ protein stability. Interestingly, MEK1/2 inhibitor increased macrophage ABCA1 mRNA and protein stability, but not ABCA1 transcription[16]. Therefore, MEK1/2 inhibitors can regulate expression of multiple molecules in different directions by various mechanisms.

Taken together, our studies indicate that MEK1/2 inhibitors can demonstrate anti-atherogenic properties by multiple mechanisms including the induction of IL-5 production. MEK1/2 inhibitors might be potential drugs for treatment of atherosclerosis.

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Figure legends

Figure 1. MEK1/2 inhibitors induce macrophage IL-5 protein expression

A: RAW macrophages were treated with PD98059 and U0126 for 6 h followed by total protein extraction and determination of phosphorylated MEK1/2 (pi-MEK1/2); B, C: RAW macrophage (B) and mouse peritoneal macrophages (C) received treatment of PD98059 or U0126 at the indicated concentration for 6 h, or with a fixed concentration of PD98059 or U0126 for the indicated times. Total cellular protein was used to determine pi-ERK1/2 and total ERK1/2 (ERK1/2), respectively. D, E: RAW macrophages and peritoneal macrophages received the indicated treatment followed by determination of IL-5 protein expression. The experiments were repeated at least 3 times, and the representative results were presented. The Western blot bands were scanned and the density of pi-MEK1/2, pi-ERK1/2 and IL-5 bands was normalized either by GAPDH or by total ERK1/2 bands as indicated. The ratio in control groups was defined as 100%. F: peritoneal macrophages were treated with U0126 or PD98059 at the indicated concentrations for 16 h. Expression of IL-10 and IL-13 mRNA was determined by real time RT-PCR. *: P<0.05; **: P<0.01 vs. control in the corresponding group (n=3).

Figure 2. MEK1/2 inhibitors induce IL-5 protein expression in EL-4 lymphocytes.

EL-4 lymphocytes in serum-free medium were treated with PD98059 or U0126 at the indicated concentrations for 16 h. Expression of pi-ERK1/2, ERK1/2 (A) and IL-5 (B, C) protein was determined by Western blot as indicated, respectively. *: P<0.05 vs. control in the corresponding group (n=3).

Figure 3. MEK1/2 inhibitors activate IL-5 production *in vivo*

C57BL/6 wild type mice were fed with normal chow (control), or normal chow containing U0126 (5 mpk) or PD98059 (20 mpk) for two weeks. After treatment, blood and tissue samples were collected and used for the following assays: A, serum IL-5 levels were determined by ELISA (each sample was analyzed in duplicate). *: P<0.05 vs. control (n=10); B, C, total tissue protein was extracted and used to determine IL-5

(B), pi-ERK1/2 and ERK1/2 (C) protein expression by Western blot. *: $P<0.05$ vs. control (n=5).

Figure 4. MEK1/2 inhibitors activate IL-5 transcription and enhance IL-5 protein stability.

A: peritoneal macrophages were treated with U0126 at the indicated concentrations for 16 h or with 2 μ M U0126 for the indicated times. Total RNA was extracted and used to determine IL-5 mRNA expression by real time RT-PCR; B, C: EL-4 cells were transfected with DNA for pGL4.10 vector or the natural IL-5 promoter (p1218IL-5 or p677IL-5) or IL-5 promoter with LXRE mutation (p677IL-5M) or LXRE deletion (p677IL-5D) as indicated and *Renilla* luciferase (for internal control). After treatment with U0126 at the indicated concentrations, IL-5 promoter activity was detected using dual-luciferase assay kit. *: $P<0.05$, NS: not significantly different; D-E: RAW macrophages were treated with 2 μ g/ml cycloheximide (CHX) or CHX plus 0.5 μ M U0126 or 10 μ M PD98059 for the indicated times. IL-5 (D) and IFN- γ (E) proteins expression were determined by Western blot, and normalized by GAPDH in the corresponding samples. The IL-5/GAPDH or IFN- γ /GAPDH in control sample (time=0) was defined as 100%, respectively. *: $P<0.05$ vs. control in the corresponding groups (n=3).

Table 1. Sequences of primers for real time RT-PCR

<u>Gene</u>	<u>Sense</u>	<u>Anti-sense</u>
<u><i>IL-5</i></u>	<u>5'-AGCACAGTGGTGAAAGAGACCTT-3'</u>	<u>5'-TCCAATGCATAGCTGGTGATTT-3'</u>
<u><i>IL-10</i></u>	<u>5'-GCTCTTACTGACTGGCATGAG-3'</u>	<u>5'-CGCAGCTCTAGGAGCATGTG-3'</u>
<u><i>IL-13</i></u>	<u>5'-AGACCAGACTCCCCTGTGCA-3'</u>	<u>5'-TGGGTCCTGTAGATGGCATTG-3'</u>
<u><i>GAPDH</i></u>	<u>5'-ACCCAGAAGACTGTGGATGG-3'</u>	<u>5'-ACACATTGGGGGTAGGAACA-3'</u>

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