Dysregulated Serum IL-23 and SIRT1 Activity in Peripheral Blood Mononuclear Cells of Patients with Rheumatoid Arthritis

Daniel Wendling[1,2], Wasim Abbas[2], Marie Godfrin-Valnet[1], Amit Kumar[2], Xavier Guillot[1], Kashif Aziz Khan[2], Claire Vidon[1], Laurie Coquard[2], Eric Toussirot[2,4,5], Clément Prati[1], Georges Herbein[2,3,6]*

1 Department of Rheumatology, Centre Hospitalier Régional Universitaire, Besançon, France, 2 Pathogens & Inflammation Laboratory, University of Franche-Comté, Besançon, France, 3 Department of Virology, Centre Hospitalier Régional Universitaire, Besançon, France, 4 Clinical Investigation Biotherapy Center506, Centre Hospitalier Régional Universitaire, Besançon, France, 5 Department of Therapeutics, University of Franche Comté, Besançon, France, 6 Structure Fédérale de Recherche 4234, University of Franche-Comté, Besançon, France

* georges.herbein@univ-fcomte.fr

Abstract

Sirtuin 1 (Sirt1) is a class III histone deacetylase (HDAC) that modulates gene expression and is involved in the regulation of proinflammatory cytokines. Interleukin-23 (IL-23) is produced by activated macrophages and dendritic cells and could fuel the progression of rheumatoid arthritis (RA). The goal of our study was to evaluate serum IL-23 levels and both Sirt1 activity and expression in peripheral blood mononuclear cells (PBMCs) in patients with RA compared to healthy controls (HC) and to determine the relationship between Sirt1 activity/expression and IL-23 levels. We assessed apoptosis in PBMCs of RA patients and its association with Sirt1 expression and serum IL-23. Serum IL-23 levels were increased in RA patients in comparison with controls. We found a positive correlation between the levels of serum IL-23 and serum IL-6 in RA patients. Decreased cytoplasmic Sirt1 activity was observed in RA patients with severe disease compared to HC. The expression of Sirt1 protein was significantly decreased in PBMCs of RA patients compared to HC using western blotting. Serum IL-23 levels correlated positively with the cytoplasmic Sirt1 activity in RA patients. Apoptosis rate of PBMCs isolated from RA patients was increased compared to HC and correlated negatively with the expression of Sirt1 protein and serum IL-23 levels. Levels of serum IL-23 and Sirt1 activity and expression were disturbed in RA parallel to increased PBMC apoptosis. Our findings might provide the rationale for the development of new therapeutic approaches in RA.

Introduction

Rheumatoid arthritis (RA) is an inflammatory rheumatic disease characterized by chronic inflammation located on the peripheral joints [1]. Active pro-inflammatory cytokines such as

tumor necrosis factor alpha (TNF), interleukin (IL)-1 beta or IL-6 are key players in the synovial inflammation of RA. Recently, the IL-6 related cytokine IL-23 has been reported to display proinflammatory properties [2,3]. Innate immune cells such as dendritic cells or monocytes/ macrophages produce IL-23 [2,4–6]. IL-23 is involved in the development of Th17 response by the main cell type producing IL-17, the type 17 T helper cells (Th17) [4]. IL-17 is present at sites of inflammation and favors synovial inflammation in RA [4]. Additionally, IL-17 blockade limits inflammation and joint erosion [4]. The regulation of proinflammatory cytokines relies on epigenetic modifications that can modulate gene transcription and thus could regulate the production of proinflammatory cytokines [7]. The epigenetic control refers to several biochemical reactions on the DNA without changing its sequence, including histone modifications [8,9]. Methylation, ubiquitination, phosphorylation, sumoylation and acetylation can modify histone proteins. Histone acetylation is catalyzed by histone acetyl transferase (HAT) while deacetylation is influenced by histone deacetylase (HDAC). Based on the sequence homology with yeast, mammalian HDACs have been classified into four groups. Class I HDACs comprises of four members (HDAC 1, 2, 3 and 8). Class II is further divided into two subgroups and has total six members (HDAC 4,5,6,7,9,10). Class III HDACs comprises of seven sirtuins which require nicotinamide adenine dinucleotide (NAD+) for their enzymatic activity. Class IV has only one member (HDAC 11). Sirtuin 1 (Sirt1) is the most studied of the sirtuins with p53, nuclear factor-kappa B (NF-kappaB) and peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1 alpha among others as targets. Sirt1 modulates the expression of genes involved in the regulation of various biological processes (cell survival, apoptosis, gluconeogenesis, adipogenesis, lipolysis), and local and systemic inflammation, as well as in bone and cartilage remodeling [10,11]. A 'protective' role of Sirt1 can be blocked by proinflammatory cytokines such as TNF, leading to inactivation of Sirt1 [12]. Sirt1 may be activated by several compounds [13], including resveratrol [14] and the homeostasis of Sirt1 expression has been reported to favor a long lasting healthy life [15].

The link between Sirt1 expression and activity and the control of proinflammatory cytokines such as IL-23 thus could be considered as a relevant marker for a sustained control of the inflammation process in several diseases including RA. There are limited results on the role of Sirt1 activity and expression and IL-23 production in patients with RA. In this study, therefore we aimed to evaluate both Sirt1 activity and expression in the peripheral blood mononuclear cells (PBMCs) of patients with RA and to analyze the potential association of serum IL-23 levels with Sirt1 activity and spontaneous apoptosis of PBMCs.

Materials and Methods

All the patients and healthy controls (HC) gave their written informed consent to participate in the study according to the Helsinki declaration. The study protocol was approved by the local ethics committee (*Comité de Protection des Personnes EST-II, study registered under the number P/2009/85*).

Patients

We enrolled 27 patients with RA meeting the 1987 American College of Rheumatology criteria. They were all receiving follow-up at the department of Rheumatology in the University Hospital of Besançon, France. Exclusion criteria were the presence of significant comorbidity (diabetes, dyslipidemia). In RA, disease activity and functional impairment were evaluated using the DAS28 score and Health Assessment Questionnaire (HAQ), respectively. Based on DAS28 score, most of the RA patients (60%) had a moderate disease activity ($3.2 \leq DAS28 \leq 5.1$), 25% a high disease activity ($DAS28 > 5.1$) and 15% a low disease activity ($DAS28 < 3.2$). Erythrocyte

sedimentation rate (ESR), C reactive protein (CRP) levels and circulating TNF, IL-6, and IL-8 were used as laboratory parameters to assess inflammation. Positivity for rheumatoid factors (68% of RA patients) and anti-CCP antibodies (76% of RA patients) were also recorded. In the RA group, patients were receiving low dosage corticosteroids (≤10 mg prednisone daily) and/ or traditional DMARDs (methotrexate, leflunomide, sulfasalazine or hydroxychloroquine). At the time of inclusion, no RA patients were receiving TNF blocking agents.

Control subjects

The control group consisted of 24 healthy subjects without inflammatory condition or treatment.

Peripheral blood mononuclear cell isolation and cell lysate preparation

PBMCs were prepared from the peripheral blood of patients and HC and were cultured in RPMI-1640 medium supplemented with 10% (v/v) pooled AB human serum (Sigma), as previously described [16]. Isolation of nuclear and cytoplasmic extracts was performed as previously described [17]. Cells were washed with wash buffer (10 mM HEPES, pH 7.6, 10 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA). Cell pellets were then incubated on ice with cytoplasmic isolation buffer (10 mM HEPES, pH 7.6, 10 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA, 0.02% NP-40). Cytoplasmic extracts were collected by centrifugation and the nuclear pellets were washed twice in wash buffer, spun, and incubated for 15 min on ice with nuclear isolation buffer (20 mM HEPES pH 7.6, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 25% glycerol). Supernatants containing nuclear extracts were collected by centrifugation and stored at—80°C. Protease inhibitors (1 mM DTT, 1 mM PMSF, 1 microg/ml aprotinin, 1 microg/ml leupeptin, 1 microg/ ml pepstatin) were added to all solutions. Protein concentration in nuclear and cytoplasmic extracts was determined by the Bradford method using a BioPhotometer (Eppendorf, Hamburg, Germany). The purity of cytoplasmic and nuclear extracts was further confirmed by the quantification of the expression of beta-actin, a cytoplasmic marker, and TATA-binding protein, a nuclear marker, using western-blotting (data not shown).

Sirt1 activity and Sirt1 protein expression

Sirt1 activity was evaluated from cytoplasmic compartment using a fluorometric assay (SIRT1 fluorimetric kit, BML-AK-555, Enzo Life Sciences, Villeurbanne, France) at the 15 minutes point. Expression of Sirt1 protein was assessed using western blot as previously described [18]. Briefly, 10 microgram of cellular extracts were resolved on 10% SDS-PAGE using a Mini-PRO-TEAN 3 Cell (Bio-Rad Laboratories, Hercules, CA, USA). The proteins were electrotransferred onto a polyvinylidene difluoride membrane (Amersham Biosciences, Saclay, France) using Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad Laboratories). The membranes were probed with primary antibodies followed by horseradish peroxidase-conjugated secondary immunoglobulin raised against the appropriate species; bands were detected using the ECL Plus kit (Amersham Biosciences). The primary antibodies used for western blot are as follows: rabbit anti-Sirt1 antibody (1:1000 dilution; Cell Signaling Technology, Beverly, MA) and mouse anti-beta-actin antibody (1:5000 dilution; Sigma-Aldrich, Saint-Louis, MO). Horseradish peroxidase-conjugated secondary antibodies goat anti-rabbit (1:5000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit anti-mouse (1:5000 dilution; DakoCytomation, Trappes, France) were used.

Measurement of IL-23, TNF, IL-6 and IL-8

Circulating IL-23, TNF, IL-6 and IL-8 were evaluated in sera from patients and HC using ELISA assays (Quantikine assay, R&D Systems, Minneapolis, MN). Sensitivity of the assays for TNF, IL-6, IL-23 and IL-8 detected a few pg/ml.

Detection of apoptosis

The detection of apoptosis by annexin V assay (BD Pharmingen) was performed on PBMCs isolated from RA patients and HC cultured in serum-free medium for 48 hours as previously described [19]. Briefly, PBMCs were washed twice with ice cold phosphate buffered saline (PBS) and resuspended in 1X annexin binding buffer containing annexin V as per manufacturer's instructions (BD Pharmingen). Cells were gently vortexed and incubated at room temperature for 15 minutes in dark. Cell suspension volume was made up to 500µl with 1X annexin V binding buffer and analyzed in flow cytometer (BD FACS Calibur) for annexin V staining.

Statistical analysis

Results were expressed as mean ± standard deviation (SD). The student T-test was used for statistical analysis between the groups (RA and HC). Values of p less than 0.05 were considered significant. The Pearson correlation coefficient was used to measure the strength of a linear association between two variables.

Results

Increased serum IL-23 levels in RA patients

Patients with RA had mostly mild disease activity (DAS28 = 4.27±1.07). The biochemical parameters of inflammation investigated (CRP, ESR) were higher in patients with RA compared to HC (CRP p = 0.001; ESR p = 0.01) (Table 1). Serum IL-8 levels were 2.8-fold higher in RA patients compared to HC (p = 0.01) (Table 1, Fig. 1A). In contrast no significant difference was observed for the levels of IL-6 (p = 0.17) and TNF (p = 0.11) in RA patients compared to HC (Table 1, Fig. 1A). Serum IL-23 levels were 4.4-fold higher in RA compared to HC (mean values 22.7±25.9 pg/ml versus 5.1±7.9 pg/ml, P = 0.03) (Table 1, Fig. 1A). Additionally among RA patients, the highest serum IL-23 levels were observed in patients with mild disease and the lowest in patients with severe disease (Fig. 1B). Serum levels of IL-23 correlated positively with serum IL-6 levels (r = 0.62) (Fig. 2), but neither with TNF nor with IL-8 levels (r = 0.10 and r = 0.06, respectively) (data not shown).

Serum IL-23 levels correlated positively with cytoplasmic Sirt1 activity in PBMCs isolated from RA patients

We did not find significant difference in the cytoplasmic Sirt1 activity in PBMCs of RA patients compared to HC (283818±179670 versus 356069±194855, p = 0.15) (Table 1) as reported previously [20]. Cytoplasmic Sirt1 activity was lower in RA patients with severe disease compared to HC (120063±147394 versus 356069±194855; P = 0.03) (Fig. 3A). Cytoplasmic Sirt1 activity correlated positively with serum IL-23 levels (r = 0.54) (Fig. 3B) and IL-6 levels (r = 0.65) (data not shown), but only very weak or absence of correlation was observed with TNF and IL-8 levels (r = 0.21 and r = -0.09, respectively). The highest serum IL-23 levels and cytoplasmic Sirt1 expression were observed in RA with mild disease (Fig. 3C). To determine whether decreased Sirt1 activity in RA resulted from decreased Sirt1 protein expression, we assessed Sirt1 protein expression in PBMCs isolated from RA and HC using western blotting. The Sirt1 protein

Table 1. Biological data of the studied patients with rheumatoid arthritis and healthy controls.

Mean±SD	Age (years)	Sex ratio (M/F)	Sirt1 Cytopl. activity (AFU)	CRP mg/l	ESR mm/h	DSA28	IL-23 pg/ml	TNF pg/ml	IL-6 pg/ml	IL-8 pg/ml
Control subjects N = 24	54±13	1.12	356069± 194855	2.6± 1.6	8.6± 5.3	na	5.1± 7.9	30.9± 26.2	8.3± 4.9	527.4± 709.6
RA patients N = 27	55±12	0.47	283818± 179670	12.9± 12.3	21.4± 13.7	4.27± 1.07	22.7± 25.9	20.7± 18.1	6.8± 3.5	1503± 1297
P*	0.38	0.10	0.15	0.001	0.01	na	0.03	0.11	0.17	0.01

Abbreviations: AFU, Arbitrary fluorescence units; CRP, C-Reactive Protein; ESR, Erythrocyte sedimentation rate; IL, interleukin; TNF, Tumor necrosis factor; na, not available.

*P value <0.05: significant

doi:10.1371/journal.pone.0119981.t001

expression was reduced by 1.6-fold in PBMCs of RA patients compared to HC (p = 0.03) (Fig. 4A & 4B).

Serum IL-23 levels correlated negatively with apoptosis of PBMCs isolated from RA patients

Since Sirt1 can inhibit apoptosis and promote cell survival in several cell types [21–23], apoptosis was then assessed in PBMCs of RA and HC using annexin-V assay. Rates of apoptosis were 4.3-fold higher in PBMCs of RA patients compared to controls (P<0.001) (Fig. 5A & 5B).
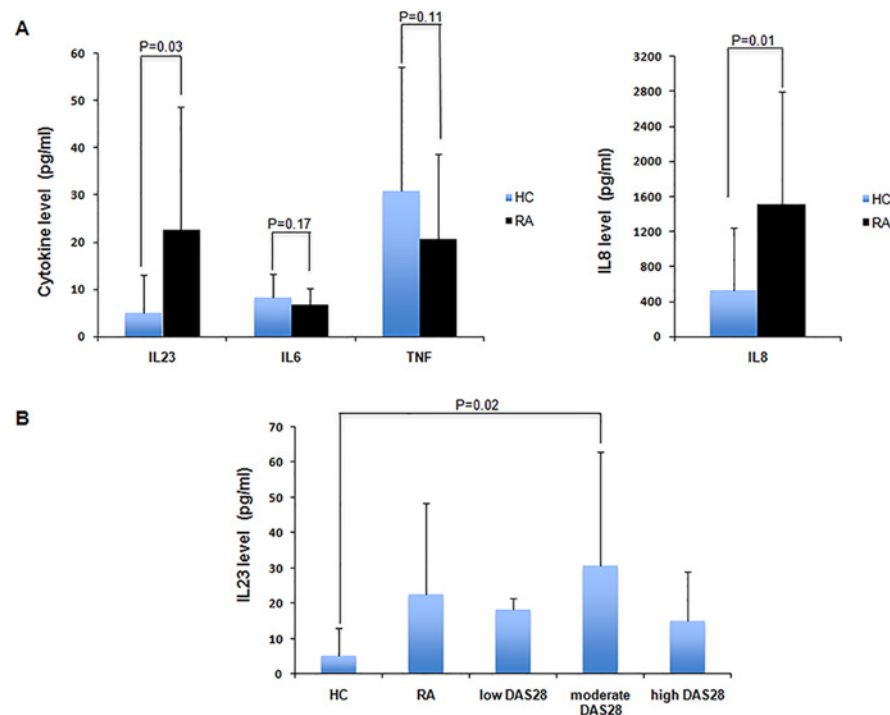

Fig 1. Quantification of levels of IL-23, IL-6, IL-8 and TNF in serum of HC and RA subjects. A. Levels of IL-23, IL-6, IL-8 and TNF in serum of healthy controls and patients with RA. B. Serum levels of IL-23 in subpopulations of RA patients based on DAS 28 score. IL-23, IL-6, IL-8 and TNF were measured by ELISA. Results were expressed as means ± SD. (HC: healthy controls; RA: rheumatoid arthritis).
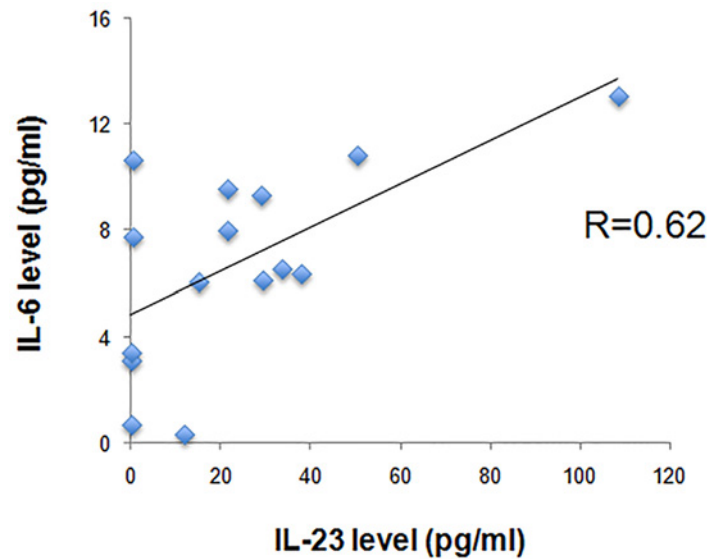
doi:10.1371/journal.pone.0119981.g001

Fig 2. IL-23 levels correlated positively with IL-6 levels in serum of RA patients. Levels of IL-23 and IL-6 were measured in the serum of RA patients. IL-23 levels correlated positively with IL-6 levels in serum of RA patients (r = 0.62).
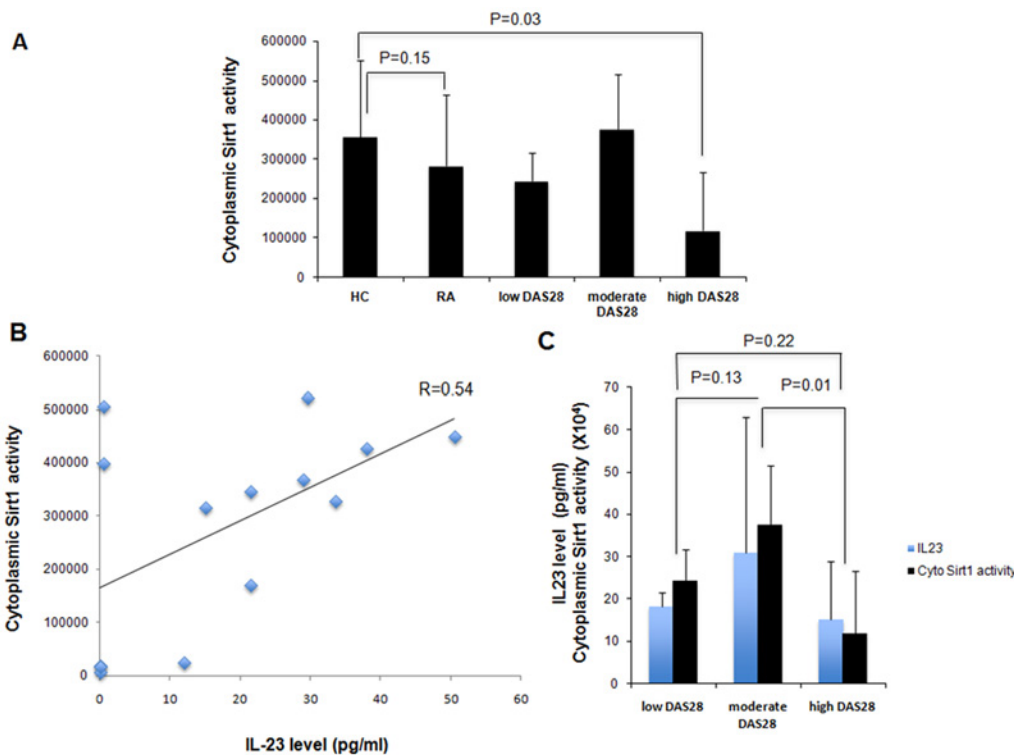
Fig 3. Serum IL-23 levels correlated positively with cytoplasmic Sirt1 activity in PBMCs isolated from RA patients. A. Cytoplasmic Sirt1 activity in PBMCs from subpopulations of RA patients based on DAS 28 score. Cytoplasmic Sirt1 activity was measured in PBMCs of subpopulations of RA patients based on DAS 28 score as described in Materials and Methods. Results were expressed as means ± SD. B. Cytoplasmic Sirt1 activity correlated positively with serum IL-23 levels (r = 0.54). C. The highest serum IL-23 levels and cytoplasmic Sirt1 expression were observed in RA with mild disease. Results were expressed as means ± SD.
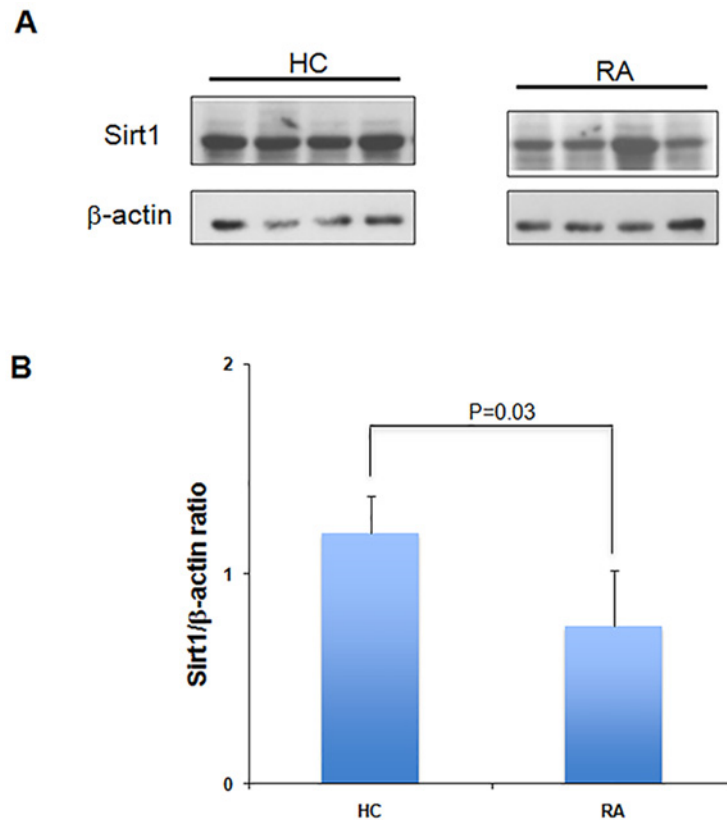
Fig 4. Expression of Sirt1 protein is reduced in PBMCs of RA patients compared to HC. A. Sirt1 protein expression was measured in PBMCs isolated from RA and HC using western blotting as described in Materials and Methods. Beta-actin was used as a loading control. B. Histogram represents Sirt1/beta-actin ratio in PBMCs of healthy controls and patients with RA. Protein levels of Sirt1 and beta-actin were quantified by densitometry using ImageJ 1.40 software. Results were expressed as means ± SD. P = 0.03

doi:10.1371/journal.pone.0119981.g004

Expression of Sirt1 protein correlated negatively with the rate of apoptosis in PBMCs with decreased expression of Sirt1 protein and high rate of apoptosis in PBMCs isolated from RA patients (r = -0.65) (Fig. 5C). Serum levels of IL-23 correlated negatively with apoptosis of PBMCs isolated from RA patients (r = -0.92) (Fig. 5D).

Discussion

Our results indicate that serum IL-23 levels are increased in RA patients compared to HC (Fig. 1). Sirt1 activity was reduced in patients with severe RA (Fig. 3C) and Sirt1 expression was decreased in patients with RA (Fig. 4). We observed a positive correlation between serum IL-23 levels and Sirt1 activity in PBMCs of RA patients (Fig. 3C). Increased apoptosis was detected in PBMCs isolated from RA patients compared to HC. Expression of Sirt1 protein was decreased in PBMCs of RA patients suggesting a negative correlation between rate of apoptosis and Sirt1 expression (Figs. 4–5). Serum IL-23 levels correlated negatively with apoptosis in PBMCs isolated from RA patients. Altogether our results indicate imbalanced IL-23 production, decreased Sirt1 activity and expression in PBMCs from RA patients with increased PBMC apoptosis.

We have shown previously that the measurement of Sirt1 activity in PBMCs drawn from peripheral blood is feasible, allowing an accessible cell subset for investigation [20]. We also
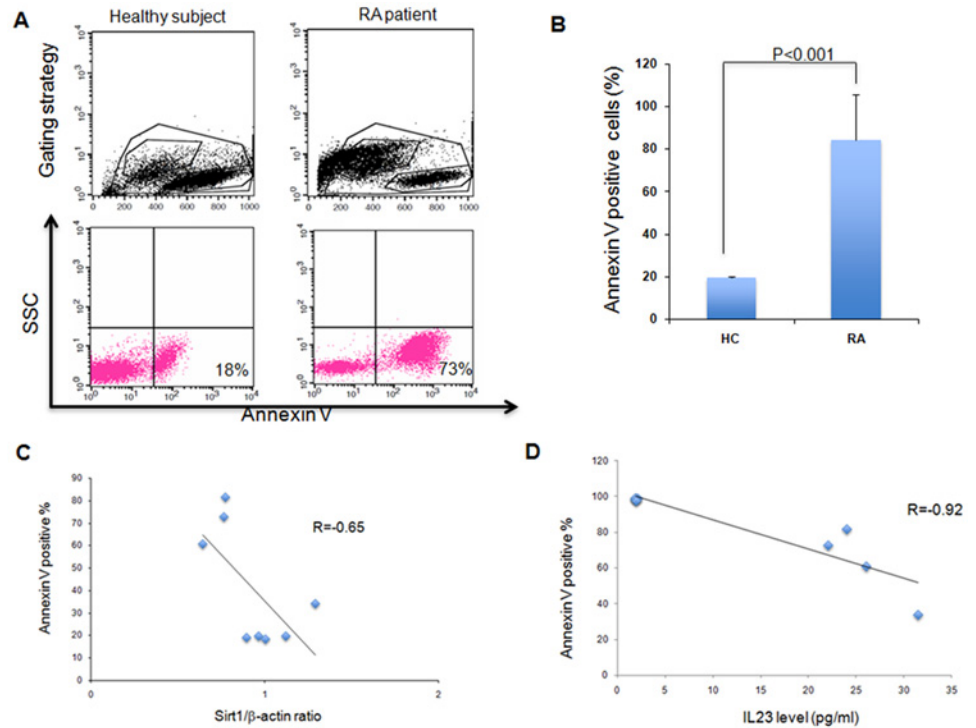
Fig 5. Serum IL-23 levels correlated negatively with spontaneous apoptosis of PBMCs isolated from RA patients. A. B. Spontaneous apoptosis of PBMCs from healthy controls and patients with RA after culture in RPMI without serum for 48 hours. Apoptosis was measured by annexin-V assay as reported in the Materials and Methods chapter. Flow cytometric data are representative of five independent experiments. Percentage of apoptotic cells is indicated. SSC, size scattered count. The histogram summarizes the spontaneous apoptosis of PBMCs from healthy controls and patients with RA after culture in RPMI without serum for 48 hours. The results represent means ± SD. P<0.001. C. Expression of Sirt1 protein correlated negatively with the rate of spontaneous apoptosis in PBMCs isolated from RA patients (r = -0.65). D. Serum levels of IL-23 correlated negatively with apoptosis of PBMCs isolated from RA patients (r = -0.92).

doi:10.1371/journal.pone.0119981.g005

observed in our previous reports that Sirt1 activity is present not only in the nuclear compartment but also in the cytoplasmic one and both are correlated [20]. Therefore in the present study, we performed the measurement of Sirt1 activity solely in the cytoplasmic compartment. An impairment of Sirt1 activity was hypothesized in patients with RA, however no difference was found between patients with RA and controls in regard to cytoplasmic Sirt1 activity as reported previously [20]. Since Sirt1 activity is supposed to decrease with age, we matched the RA patients and HC for age (Table 1). Nevertheless, several other factors including diabetes and obesity have been reported to modulate Sirt1 activity [24]. We found that cytoplasmic Sirt1 activity was lower in RA with severe disease compared to HC, indicating that Sirt1 activity depends on the severity and/or the duration of the disease. Lower Sirt1 activity in RA patients with severe disease could be explained by immunosenescence [25,26]. In agreement with our observation, we reported recently that Sirt1 activity (cytoplasmic and nuclear) was lower in ACPA-positive RA compared to ACPA-negative RA [20].

Although the literature has previously shown mainly the nuclear activity of Sirt1 [14], at least two different mechanisms could account for its biological action. Sirt1 preferentially deacetylates lysine 9 of histone H3 and lysine 16 of histone H4 [27]. Additionally, Sirt1 interacts directly with the p65 subunit of NF-kappa B, leading to deacetylation at lysine 310, culminating in decreased NF-kappa B-associated transcription [28]. Sirt1 deacetylates lysine 310 of RelA/

p65 without affecting the acetylation status of other lysine residues [28]. Following treatment with resveratrol, a Sirt1 activator, the localization of both Sirt1 and RelA/p65 proteins on the gene promoter suggests that Sirt1 may actively repress gene expression by deacetylating RelA/p65 directly on chromatin [27]. In the case of NF-kappa B, the heterodimer composed of RelA/p65 and p50 proteins interacts also with HDAC1, 2 and 3 enzymes, making the regulation of NF-kappa B-dependent genes even more complex [29]. Recently, the cathepsin B-mediated cleavage of Sirt1 by TNF has been reported [12]. Interestingly, other proinflammatory cytokines participating in arthritis, including IL-6, have been reported to activate cathepsin B and therefore could also trigger the cleavage of Sirt1 [30]. IL-23 stimulation has been reported to activate cathepsin K through up-regulation of expression of the receptor activator of NF-B ligand (RANKL) [31]. *In vitro* cleavage of recombinant Sirt1 has been reported with several members of cathepsin family including cathepsin B, L and S [32]. Since we observed that serum IL-23 levels correlated positively with cytoplasmic Sirt1 activity in PBMCs isolated from RA patients, we cannot exclude that cathepsins could be involved in the degradation of Sirt1 mediated by IL-23, thereby providing a negative feed-back loop.

We observed a positive correlation between serum IL-23 levels and Sirt1 activity in PBMCs isolated from RA patients. In agreement with our findings, recent reports indicate that Sirt1 can stimulate proinflammatory cytokine production [23,33]. Additionally, Sirt1 is a key regulator of the interleukin-12 p70/IL-23 balance in human dendritic cells [34]. HDAC inhibitors inhibit IL-6 release by bone marrow-derived macrophages exposed to microbial products such as lipopolysaccharides (LPS) and heat-killed *Escherichia coli* and *Staphylococcus aureus* [35,36] and decrease both IL-6 and TNF production in PBMCs isolated from RA patients [19,37]. Givinostat (originally called ITF2357) is a hydroxamic acid which inhibits class I and class II HDACs and reduces the production and release of proinflammatory cytokines including TNF, IL-1beta and IL-6 from human blood monocytes [38]. HDAC inhibitors suppress inflammatory activation of RA [39,40]. Sirtuin inhibition decreases the production of TNF, IL-6 and RANTES (regulated upon activation normal T cell expressed and secreted) in LPS-stimulated macrophages [33], suggesting that sirtuin activation may favor proinflammatory cytokine production by activated macrophages.

In agreement with this hypothesis, inhibition of Sirt1 enzymatic activity reduces LPS-induced levels of TNF in monocytes of patients with rheumatoid arthritis [23]. We also observed a positive correlation between IL-23 and IL-6 levels in serum of RA patients. IL-23 and IL-6 are two closely related proinflammatory cytokines that are both produced by activated macrophages [4,41,42]. By contrast, other studies indicate that resveratrol inhibits TNF-induced inflammation via Sirt1 [43], and suppresses expression of TNF, IL-6 and IL-8 [44]. Sirt1 deacetylates the p65 subunit of NF-kappaB at lysine 310, attenuating NF-kappa B transcriptional activation, and thereby could decrease proinflammatory cytokine production. Altogether, Sirt1 activity and subsequent proinflammatory cytokine production such as IL-23 may depend on the cell type involved (monocytes or macrophages, or PBMCs in peripheral blood compartment versus cells of the cartilage tissue) and on the activation state of the cell type studied. Recently, a subpopulation of monocytes namely CD56+ monocytes, have been reported to have a dysregulated cytokine response to LPS with enhanced IL-23 production and to accumulate in RA and immunosenescence [25]. Increased plasma IL-23 levels are associated with increased disease activity in patients with early RA [45] and systemic levels of IL-23 are strongly associated with disease activity in RA [46]. We found a peak of serum IL-23 levels in RA patients with mild disease and lower serum IL-23 levels in early and late disease. Future studies will assess whether the lower serum levels of IL-23 observed in severe RA could be explained by some defects in antigen-presenting cells [4, 41]. Sirt1 activity declines with age, potentially due to ROS-mediated depletion of NAD^+, and a compensatory mechanism exists to

increase Sirt1 protein levels to increase the relative success of Sirt1 when competing for the limited NAD$^+$ pool [47]. Additionally IL-23 production is increased during ageing in murine models [48]. Aging dependent upregulation of IL-23p19 gene expression in dendritic cells is associated with differential transcription factor binding and histone modifications [48]. Sirt1 could play a role in IL-23p19 gene expression by modulating histone acetylation. Elevated synthesis and secretion of IL-23 in dendritic cells from aged mice could be one of the possible mechanisms by which T cell maturation is shifted towards a Th17 phenotype with age, increasing autoimmune conditions in the elderly [49]. New treatments such as blocking Th17-polarizing cytokines by HDAC inhibitors could ultimately decrease the production of IL-23/IL-17 cytokines [50,51].

It has been demonstrated that Sirt1 regulates apoptosis- and cartilage-specific gene expression in human chondrocytes and mouse models [52,53]. Mice without Sirt1 activity are characterized by reduced levels of type II collagen, aggrecan, and glycosaminoglycan, elevated levels of matrix metalloproteinases 8, 9 and 13 in the cartilage, and elevated apoptosis of chondrocytes. Sirt1 overexpression in RA synovial fibroblasts protected cells from apoptosis parallel to the production of proinflammatory cytokines IL-6 and IL-8 [23]. In agreement with previous findings [23, 54], we found that expression of Sirt1 protein correlated negatively with the rate of apoptosis in PBMCs isolated from RA patients. Although IL-23 induces apoptosis of self-reactive thymocytes in thymic negative selection [55], we observed that serum levels of IL-23 correlated negatively with apoptosis of PBMCs isolated from RA patients, indicating a possible link between the production of IL-23 by monocytes/macrophages and resistance to apoptosis of PBMCs. Spontaneous apoptosis of CD4+CD25+ T cells from active RA patients has found to be increased in comparison with controls [56]. By contrast, deficient *in vitro* spontaneous apoptosis of monocytes isolated from RA patients has also been reported [57]. Therefore spontaneous apoptosis in PBMCs isolated from RA patients and its regulation by IL-23 might depend on the stage of the disease and on the cell type involved (monocytes versus peripheral blood lymphocytes).

In conclusion, we observed dysregulated IL-23 production, decreased SIRT1 activity and expression in PBMCs from RA patients with increased PBMC apoptosis. The molecular mechanisms underlying IL-23 production, Sirt1 activity and apoptosis in PBMCs of RA patients are complex, and a better understanding of this interplay may lead to new therapeutic approaches.

Author Contributions

Conceived and designed the experiments: DW GH. Performed the experiments: WA KAK AK LC. Analyzed the data: DW GH. Contributed reagents/materials/analysis tools: DW MG-V XG CV CP ET. Wrote the paper: DW GH.

References

1. Viatte S, Plant D, Raychaudhuri S (2013) Genetics and epigenetics of rheumatoid arthritis. Nat Rev Rheumatol 9: 141–153. doi: 10.1038/nrrheum.2012.237 PMID: 23381558

2. Duvallet E, Semerano L, Assier E, Falgarone G, Boissier MC (2011) Interleukin-23: a key cytokine in inflammatory diseases. Ann Med 43: 503–511. doi: 10.3109/07853890.2011.577093 PMID: 21585245

3. Molle C, Zhang T, Ysebrant de Lendonck L, Gueydan C, Andrianne M, Sherer F, et al. (2013) Tristetraprolin regulation of interleukin 23 mRNA stability prevents a spontaneous inflammatory disease. J Exp Med 210: 1675–1684. doi: 10.1084/jem.20120707 PMID: 23940256

4. Burmester GR, Feist E, Dorner T (2014) Emerging cell and cytokine targets in rheumatoid arthritis. Nat RevRheumatol 10: 77–88. doi: 10.1038/nrrheum.2013.168 PMID: 24217582

5. Stamp LK, Easson A, Pettersson L, Highton J, Hessian PA (2009) Monocyte derived interleukin (IL)-23 is an important determinant of synovial IL-17A expression in rheumatoid arthritis. J Rheumatol 36: 2403–2408. doi: 10.3899/jrheum.081304 PMID: 19797506

6. Chang J, Voorhees TJ, Liu Y, Zhao Y, Chang CH (2010) Interleukin-23 production in dendritic cells is negatively regulated by protein phosphatase 2A. Proc Natl Acad Sci U S A 107: 8340–8345. doi: 10.1073/pnas.0914703107 PMID: 20404153

7. Toussirot E, Khan KA, Herbein G (2010) Histone deacetylase inhibitors: a new and promising drug class for the treatment of arthritis? Clin Epigenetics 1: 3–6. doi: 10.1007/s13148-010-0002-6 PMID: 22704084

8. Strietholt S, Maurer B, Peters MA, Pap T, Gay S (2008) Epigenetic modifications in rheumatoid arthritis. Arthritis Res Ther 10: 219. doi: 10.1186/ar2500 PMID: 18947370

9. Huber LC, Brock M, Hemmatazad H, Giger OT, Moritz F, Trenkmann M, et al. (2007) Histone deacetylase/acetylase activity in total synovial tissue derived from rheumatoid arthritis and osteoarthritis patients. Arthritis Rheum 56: 1087–1093. PMID: 17393417

10. Taylor DM, Maxwell MM, Luthi-Carter R, Kazantsev AG (2008) Biological and potential therapeutic roles of sirtuin deacetylases. Cell Mol Life Sci 65: 4000–4018. doi: 10.1007/s00018-008-8357-y PMID: 18820996

11. Zeng L, Chen R, Liang F, Tsuchiya H, Murai H, Nakahashi T, et al. (2009) Silent information regulator, Sirtuin 1, and age-related diseases. Geriatr Gerontol Int 9: 7–15. doi: 10.1111/j.1447-0594.2008.00504.x PMID: 19260974

12. Dvir-Ginzberg M, Gagarina V, Lee EJ, Booth R, Gabay O, Hall DJ (2011) Tumor necrosis factor alpha-mediated cleavage and inactivation of Sirt1 in human osteoarthritic chondrocytes. Arthritis Rheum 63: 2363–2373. doi: 10.1002/art.30279 PMID: 21305533

13. Villalba JM, Alcain FJ (2012) Sirtuin activators and inhibitors. Biofactors 38: 349–359. doi: 10.1002/biof.1032 PMID: 22730114

14. Herbein G, Wendling D (2010) Histone deacetylases in viral infections. Clin Epigenetics 1: 13–24. doi: 10.1007/s13148-010-0003-5 PMID: 22704086

15. Zhang T, Kraus WL (2010) SIRT1-dependent regulation of chromatin and transcription: linking NAD(+) metabolism and signaling to the control of cellular functions. Biochim Biophys Acta 1804: 1666–1675. doi: 10.1016/j.bbapap.2009.10.022 PMID: 19879981

16. Khan KA, Coaquette A, Davrinche C, Herbein G (2009) Bcl-3-regulated transcription from major immediate-early promoter of human cytomegalovirus in monocyte-derived macrophages. J Immunol 182: 7784–7794. doi: 10.4049/jimmunol.0803800 PMID: 19494302

17. Varin A, Manna SK, Quivy V, Decrion AZ, Van Lint C, Herbein G, et al. (2003) Exogenous Nef protein activates NF-kappa B, AP-1, and c-Jun N-terminal kinase and stimulates HIV transcription in promonocytic cells. Role in AIDS pathogenesis. J Biol Chem 278: 2219–2227. PMID: 12419805

18. Wendling D, Abbas W, Godfrin-Valnet M, Guillot X, Khan KA, Cedoz JP, et al. (2013) Resveratrol, a sirtuin 1 activator, increases IL-6 production by peripheral blood mononuclear cells of patients with knee osteoarthritis. Clin Epigenetics 5: 10. doi: 10.1186/1868-7083-5-10 PMID: 23844973

19. Toussirot E, Abbas W, Khan KA, Tissot M, Jeudy A, Baud L, et al. (2013) Imbalance between HAT and HDAC activities in the PBMCs of patients with ankylosing spondylitis or rheumatoid arthritis and influence of HDAC inhibitors on TNF alpha production. PLoS One 8: e70939. doi: 10.1371/journal.pone.0070939 PMID: 24039666

20. Wendling D, Vidon C, Abbas W, Guillot X, Toussirot E, Herbein G (2014) Sirt1 activity in peripheral blood mononuclear cells from patients with rheumatoid arthritis. Joint Bone Spine 81:462–463. doi: 10.1016/j.jbspin.2014.02.006 PMID: 24746477

21. Gabay O, Oppenheimer H, Meir H, Zaal K, Sanchez C, Dvir-Ginzberg M (2012) Increased apoptotic chondrocytes in articular cartilage from adult heterozygous SirT1 mice. Ann Rheum Dis 71: 613–616. doi: 10.1136/ard.2011.200504 PMID: 22258484

22. Takayama K, Ishida K, Matsushita T, Fujita N, Hayashi S, Sasaki K, et al. (2009) SIRT1 regulation of apoptosis of human chondrocytes. Arthritis Rheum 60: 2731–2740. doi: 10.1002/art.24864 PMID: 19714620

23. Niederer F, Ospelt C, Brentano F, Hottiger MO, Gay RE, Gay S, et al. (2011) SIRT1 overexpression in the rheumatoid arthritis synovium contributes to proinflammatory cytokine production and apoptosis resistance. Ann Rheum Dis 70: 1866–1873. doi: 10.1136/ard.2010.148957 PMID: 21742641

24. Kotas ME, Gorecki MC, Gillum MP (2013) Sirtuin-1 is a nutrient-dependent modulator of inflammation. Adipocyte 2: 113–118. doi: 10.4161/adip.23437 PMID: 23805409

25. Krasselt M, Baerwald C, Wagner U, Rossol M (2013) CD56+ monocytes have a dysregulated cytokine response to lipopolysaccharide and accumulate in rheumatoid arthritis and immunosenescence. Arthritis Res Ther 15: R139. doi: 10.1186/ar4321 PMID: 24286519

26. Salminen A, Huuskonen J, Ojala J, Kauppinen A, Kaarniranta K, Suuronen T (2008) Activation of innate immunity system during aging: NF-kB signaling is the molecular culprit of inflamm-aging. Ageing Res Rev 7: 83–105. PMID: 17964225

27. Fusco S, Maulucci G, Pani G (2012) Sirt1: def-eating senescence? Cell Cycle 11: 4135–4146. doi: 10.4161/cc.22074 PMID: 22983125

28. Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, et al. (2004) Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J 23: 2369–2380. PMID: 15152190

29. Ashburner BP, Westerheide SD, Baldwin AS Jr (2001) The p65 (RelA) subunit of NF-kappaB interacts with the histone deacetylase (HDAC) corepressors HDAC1 and HDAC2 to negatively regulate gene expression. Mol Cell Biol 21: 7065–7077. PMID: 11564889

30. Chae HJ, Ha KC, Lee GY, Yang SK, Yun KJ, Kim EC, et al. (2007) Interleukin-6 and cyclic AMP stimulate release of cathepsin B in human osteoblasts. Immuno pharmacol Immunotoxicol 29: 155–172.

31. Ju JH, Cho ML, Moon YM, Oh HJ, Park JS, Yoon CH, et al. (2008) IL-23 induces receptor activator of NF-kappaB ligand expression on CD4+ T cells and promotes osteoclastogenesis in an autoimmune arthritis model. J Immunol 181: 1507–1518. PMID: 18606706

32. Chen J, Xavier S, Moskowitz-Kassai E, Chen R, Lu CY, Sanduski K, et al. (2012) Cathepsin cleavage of sirtuin 1 in endothelial progenitor cells mediates stress-induced premature senescence. Am J Pathol 180: 973–983. doi: 10.1016/j.ajpath.2011.11.033 PMID: 22234173

33. Fernandes CA, Fievez L, Neyrinck AM, Delzenne NM, Bureau F, Vanbever R (2012) Sirtuin inhibition attenuates the production of inflammatory cytokines in lipopolysaccharide-stimulated macrophages. Biochem Biophys Res Commun 420: 857–861. doi: 10.1016/j.bbrc.2012.03.088 PMID: 22469470

34. Alvarez Y, Rodriguez M, Municio C, Hugo E, Alonso S, Ibarrola N, et al. (2012) Sirtuin 1 is a key regulator of the interleukin-12 p70/interleukin-23 balance in human dendritic cells. J Biol Chem 287: 35689–35701. doi: 10.1074/jbc.M112.391839 PMID: 22893703

35. Roger T, Lugrin J, Le Roy D, Goy G, Mombelli M, Koessler T, et al. (2011) Histone deacetylase inhibitors impair innate immune responses to Toll-like receptor agonists and to infection. Blood 117: 1205–1217. doi: 10.1182/blood-2010-05-284711 PMID: 20956800

36. Mombelli M, Lugrin J, Rubino I, Chanson AL, Giddey M, Calandra T, et al (2011) Histone deacetylase inhibitors impair antibacterial defenses of macrophages. J Infect Dis 204: 1367–1374. doi: 10.1093/infdis/jir553 PMID: 21921209

37. Gillespie J, Savic S, Wong C, Hempshall A, Inman M, Emery P, et al (2012) Histone deacetylases are dysregulated in rheumatoid arthritis and a novel histone deacetylase 3-selective inhibitor reduces interleukin-6 production by peripheral blood mononuclear cells from rheumatoid arthritis patients. Arthritis Rheum 64: 418–422. doi: 10.1002/art.33382 PMID: 21952924

38. Vojinovic J, Damjanov N, D'Urzo C, Furlan A, Susic G, Pasic S, et al (2011) Safety and efficacy of an oral histone deacetylase inhibitor in systemic-onset juvenile idiopathic arthritis. Arthritis Rheum 63: 1452–1458. doi: 10.1002/art.30238 PMID: 21538322

39. Grabiec AM, Krausz S, de Jager W, Burakowski T, Groot D, Sanders ME, et al. (2010) Histone deacetylase inhibitors suppress inflammatory activation of rheumatoid arthritis patient synovial macrophages and tissue. J Immunol 184: 2718–2728. doi: 10.4049/jimmunol.0901467 PMID: 20100935

40. Leoni F, Zaliani A, Bertolini G, Porro G, Pagani P, Pozzi P, et al (2002) The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits anti inflammatory properties via suppression of cytokines. Proc Natl Acad Sci U S A 99: 2995–3000. PMID: 11867742

41. Kinne RW, Brauer R, Stuhlmuller B, Palombo-Kinne E, Burmester GR (2000) Macrophages in rheumatoid arthritis. Arthritis Res 2: 189–202. PMID: 11094428

42. Brentano F, Ospelt C, Stanczyk J, Gay RE, Gay S, Kyburz D (2009) Abundant expression of the interleukin (IL)23 subunit p19, but low levels of bioactive IL23 in the rheumatoid synovium: differential expression and Toll-like receptor-(TLR) dependent regulation of the IL23 subunits, p19 and p40, in rheumatoid arthritis. Ann Rheum Dis 68: 143–150. doi: 10.1136/ard.2007.082081 PMID: 18276743

43. Zhu X, Liu Q, Wang M, Liang M, Yang X, Xu X, et al. (2011) Activation of Sirt1 by resveratrol inhibits TNF-alpha induced inflammation in fibroblasts. PLoS One 6: e27081. doi: 10.1371/journal.pone.0027081 PMID: 22069489

44. Kang OH, Jang HJ, Chae HS, Oh YC, Choi JG, Lee YS, et al. (2009) Anti-inflammatory mechanisms of resveratrol in activated HMC-1 cells: pivotal roles of NF-kappaB and MAPK. Pharmacol Res 59: 330–337. doi: 10.1016/j.phrs.2009.01.009 PMID: 19416633

45. Rasmussen TK, Andersen T, Hvid M, Hetland ML, Horslev-Petersen K, Stengaard-Pedersen K, et al. (2010) Increased interleukin 21 (IL-21) and IL-23 are associated with increased disease activity and

with radiographic status in patients with early rheumatoid arthritis. J Rheumatol 37: 2014–2020. doi: 10.3899/jrheum.100259 PMID: 20682664

46. Melis L, Vandooren B, Kruithof E, Jacques P, De Vos M, Mielants H, et al. (2010) Systemic levels of IL-23 are strongly associated with disease activity in rheumatoid arthritis but not spondyloarthritis. Ann Rheum Dis 69: 618–623. doi: 10.1136/ard.2009.107649 PMID: 19196728

47. Braidy N, Guillemin GJ, Mansour H, Chan-Ling T, Poljak A, Grant R (2011) Age related changes in NAD+ metabolism oxidative stress and Sirt1 activity in wistar rats. PLoS One 6:e19194 doi: 10.1371/journal.pone.0019194 PMID: 21541336

48. El Mezayen R, El Gazzar M, Myer R, High KP (2009) Aging-dependent upregulation of IL-23p19 gene expression in dendritic cells is associated with differential transcription factor binding and histone modifications. Aging Cell 8:553–565 doi: 10.1111/j.1474-9726.2009.00502.x PMID: 19624579

49. Brubaker AL, Palmer JL, Kovacs EJ (2011) Age-related dysregulation of inflammation and innate immunity: lessons learned from rodent models. Aging Dis 2:346–360. PMID: 22396887

50. Bosisio D, Vulcano M, Del PA, Sironi M, Salvi V, Salogni L, et al. (2008) Blocking TH17-polarizing cytokines by histone deacetylase inhibitors in vitro and in vivo. J Leukoc Biol 84: 1540–1548. doi: 10.1189/jlb.0708401 PMID: 18780875

51. Toussirot E (2012) The IL23/Th17 pathway as a therapeutic target in chronic inflammatory diseases. Inflamm Allergy Drug Targets 11: 159–168. PMID: 22280236

52. Gabay O, Sanchez C, Dvir-Ginzberg M, Gagarina V, Zaal KJ, Song Y, et al. (2013) Sirtuin 1 enzymatic activity is required for cartilage homeostasis in vivo in a mouse model. Arthritis Rheum 65: 159–166. doi: 10.1002/art.37750 PMID: 23124828

53. Gagarina V, Gabay O, Dvir-Ginzberg M, Lee EJ, Brady JK, Quong MJ, et al. (2010) Sirt1 enhances survival of human osteoarthritic chondrocytes by repressing protein tyrosine phosphatase 1B and activating the insulin-like growth factor receptor pathway. Arthritis Rheum 62: 1383–1392. doi: 10.1002/art.27369 PMID: 20131294

54. Wang W, Yan C, Zhang J, Lin R, Lin Q, Yang L, et al. (2013) SIRT1 inhibits TNF-alpha-induced apoptosis of vascular adventitial fibroblasts partly through the deacetylation of FoxO1. Apoptosis 18: 689–701. doi: 10.1007/s10495-013-0833-7 PMID: 23479127

55. Li H, Wu Q, Yang P, Cua D, Hsu HC, Mountz J (2011) IL-23 induces apoptosis of self-reactive thymocytes in thymic negative selection by an Rorc dependent mechanism. J Immunol 186:64.19.

56. Toubi E, Kessel A, Mahmudov Z, Hallas K, Rozenbaum M, Rosner I (2005). Increased spontaneous apoptosis of CD4+CD25+ T cells in patients with active rheumatoid arthritis is reduced by infliximab. Ann N Y Acad Sci. 1051:506–514. PMID: 16126991

57. Meusch U, Klingner M, Baerwald C, Rossol M, Wagner U (2013). Deficient spontaneous in vitro apoptosis and increased tmTNF reverse signaling-induced apoptosis of monocytes predict suboptimal therapeutic response of rheumatoid arthritis to TNF inhibition. Arthritis Res Ther 15:R219. PMID: 24354986