Adiponectin Inhibits Neutrophil Phagocytosis of Escherichia coli by Inhibition of PKB and ERK 1/2 MAPK Signalling and Mac-1 Activation

Alessandra Rossi, Janet Lord*

Medical Research Council Centre for Immune Regulation, School of Immunity and Infection, University of Birmingham, Birmingham, United Kingdom

Abstract

Full length adiponectin is a potent immune modulatory adipokine, impacting upon the actions of several immune cells. Neutrophil oxidative burst has been shown to decrease in response to adiponectin, and we speculated that it could have other effects on neutrophil function. Here we report that adiponectin reduces the phagocytic ability of human neutrophils, decreasing significantly the ingestion of opsonised E. coli by these cells in whole blood (p<0.05) and as isolated neutrophils (p<0.05). We then determined the mechanisms involved. We observed that the activation of Mac-1, the receptor engaged in complement-mediated phagocytosis, was decreased by adiponectin in response to E. coli stimulation. Moreover, treatment of neutrophils with adiponectin prior to incubation with E. coli significantly inhibited signalling through the PI3K/PKB and ERK 1/2 pathways, with a parallel reduction of F-actin content. Studies with pharmacological inhibitors showed that inhibition of PI3K/PKB, but not ERK 1/2 signalling was able to prevent the activation of Mac-1. In conclusion, we propose that adiponectin negatively affects neutrophil phagocytosis, reducing the uptake of E. coli and inhibiting Mac-1 activation, the latter by blockade of the PI3K/PKB signal pathway.

Introduction

Adipose tissue is the main source of adipokines, circulating molecules that like cytokines are engaged in regulating a variety of physiological and pathological processes. Adiponectin is the most abundant adipokine, reaching concentrations greater than 10 μg/ml in the circulation [1]. Structurally, adiponectin belongs to the C1q/Tumor Necrosis Factor (TNF) superfamily, with its C-terminal domain sharing homology with the complement factor C1q [2]. Different isoforms of adiponectin have been identified: full-length adiponectin, which further oligomerises to form trimers of low molecular weight, hexamers and polymers of high molecular weight [3].

Adiponectin has aroused increasing interest because of its insulin-sensitising [4,5], anti-atherosclerotic [6] and anti-inflammatory properties [7] and its levels have been shown to be inversely correlated with obesity [8] and type 2 diabetes mellitus [9,10]. Adiponectin appears to achieve many of its actions through activation of AMP-activated protein kinase (AMPK), with phosphorylation of AMPK shown to increase following treatment with adiponectin in several cell types including endothelial cells, peripheral blood mononuclear cells (PBMCs) [11] and phagocytes [12]. In relation to its anti-inflammatory role, adiponectin prevents lipopolysaccharide (LPS)-induced acute lung injury (ALI) in mice by inhibiting the production of IL-6 by lung endothelial cells [13] and protects against LPS-induced liver injury in obese mouse models by diminishing TNF-α production [14]. In addition, it has also been shown to inhibit NK cell cytotoxicity [15] and to induce human monocytes to differentiate into alternative the anti-inflammatory M2 macrophage phenotype [16]. Contradictory results have been reported in relation to adiponectin effects on macrophage phagocytosis [17,19] and dendritic cell function [19,20].

Neutrophils are the most abundant immune cell population in the blood, representing the first line of defence against microbial pathogens and with a major pro-inflammatory role. These short-lived cells migrate towards the site of infection where they contribute to the removal and the killing of pathogens through the processes of phagocytosis, degranulation and release of microbicidal peptides, production of reactive oxygen species (ROS) and generation of neutrophil extracellular traps (NETs) [21,22]. Both neutrophil and monocyte ROS production in response to the bacterial product fMLP are reduced by the addition of full-length adiponectin, which inhibits NADPH oxidase activation by decreasing the phosphorylation of the p47phox subunit [12]. In contrast, globular adiponectin has been shown to enhance phagocyte ROS production, favouring NADPH oxidase activation via phosphorylation of the MAPK: ERK 1/2 and p38 [12].

Neutrophil phagocytosis is initiated by ligation of several receptors, including cytokine receptors, pattern recognition receptors (PRRs) such as Toll-like receptor 4 (TLR4), the opsonic Fc-γ receptors FcγRI, FcγRII and FcγRIII (CD16), and the complement receptors CR1 (CD35) and CR3 (CD11b/CD18), alternatively called Mac-1 [23]. Mac-1 undergoes activation by
conformational change in stimulated neutrophils thus achieving a higher affinity and avidity towards its ligands [24]. Following binding to neutrophil membranes, bacterial ingestion is associated with intracellular signalling involving MAPK activation: both ERK 1/2 and p38 MAPK are phosphorylated in response to microbial challenge [25], and activation of the PI3K/PKB pathway has also been shown to be fundamental for cytoskeletal rearrangements during phagocytosis [26,27].

Despite the major pro-inflammatory role of neutrophils the effect exerted by adiponectin on neutrophil phagocytosis has not been investigated, therefore this study aimed to evaluate whether this adipokine could influence the phagocytosis of the bacteria E. coli and the mechanisms involved.

Results
Adiponectin inhibits neutrophil phagocytosis of E. coli
Pre-treating whole blood for one hour with a physiological dose of adiponectin (10 µg/ml) resulted in a significant decrease in the neutrophil phagocytic index for uptake of E. coli (p<0.05; Fig. 1A). We then confirmed that adiponectin directly modulates neutrophil phagocytosis, using isolated neutrophils in serum-free media pre-incubated with adiponectin for one hour before the addition of E. coli (40:1 ratio, E. coli:neutrophils), previously opsonised with autologous serum. Figure 1B shows that adiponectin (0.1, 1 and 10 µg/ml) inhibited neutrophil phagocytosis in a dose-dependent manner. The degree of inhibition was greater than that seen with whole blood possibly due to the presence of adiponectin in the blood. We also determined the stability of the adiponectin effect over time and found the inhibition of phagocytosis was maintained for up to 90 minutes (Figure 1C). Different bacteria to neutrophil ratios (5:1, 20:1) were tested and the decrease in phagocytosis with adiponectin was seen at all ratios (data not shown). We also assessed the surface expression of the two receptors for adiponectin, AdipoR1 and AdipoR2, in 10 donors and we consistently found high expression of both receptors (Figure 1D and E).

Adiponectin reduces Mac-1 activation and bacterial binding to neutrophils
Neutrophils express a broad range of phagocytic receptors which are necessary to promote the ingestion of opsonised microbes. To delineate whether adiponectin could influence the uptake of bacteria through the modulation of phagocytic receptors the surface expression of CD16, CD11b and TLR4 on resting neutrophils was assessed after a one hour treatment with adiponectin. The expression of none of these receptors was affected by adiponectin treatment (data not shown), Mac-1 is a dimer of CD11b and CD18 and is a complement receptor that undergoes activation by conformational change in stimulated neutrophils [24]. Its activation was measured using a specific antibody against the activation induced epitope of Mac-1 after neutrophils were pre-incubated with adiponectin and stimulated with opsonised E. coli for 90 minutes. Levels of activated Mac-1 (expressed as MFI, Figure 2A) and the percentage of cells bearing active Mac-1 (Figure 2B) were both significantly decreased in the presence of adiponectin. As the conformational change of Mac-1 leads to increased receptor affinity towards its ligands [24], we hypothesized that the reduction in the phagocytosis mediated by adiponectin may be due in part to decreased binding of bacteria to Mac-1. Therefore, we evaluated the binding of FITC labelled E. coli to the neutrophil surface at 4°C at 30, 60 and 90 minutes after pre-incubation with adiponectin. The binding of bacteria was significantly reduced by adiponectin at each time point (Figure 2C).

Adiponectin inhibits PKB and ERK signalling
The process of phagocytosis is accompanied by increased activation of PI3K/PKB and MAPK signalling pathways. Hence we evaluated the effect of adiponectin on phosphorylation of PKB, ERK 1/2 and p38 MAPK by western blotting using antibodies specific to the phosphorylated forms of these kinases. E. coli stimulated neutrophils which were pre-treated with adiponectin displayed significantly lower phosphorylation of both PKB and ERK 1/2 compared to control stimulated neutrophils, though the effect was consistently most marked for ERK 1/2 (Fig. 3A, B). p38 phosphorylation was not affected by adiponectin.

To confirm that PI3K/PKB and ERK 1/2 activation was necessary to sustain neutrophil phagocytosis, we studied this function after pharmacologically blocking these two pathways. The inhibitors LY294002 (PI3K inhibitor) and PD98059 (an inhibitor of MEK1 which is directly upstream of ERK 1/2) were added 30 minutes before initiation of the phagocytosis assay. The inhibition of both PI3K/PKB and ERK 1/2 pathways resulted in a reduction in neutrophil phagocytosis (Fig. 3C) in a concentration dependent manner and the efficacy of the two inhibitors was confirmed by western blot (data not shown). The specificity of LY294002 and PD98059 for PI3K and ERK 1/2 is very good and has been reported previously with LY294002 only inhibiting additionally casein kinase 2 even when used at 50µM [28,29]. The concentrations employed here are also widely used [30–34].

As adiponectin signalling results in the activation of AMPK in a variety of cell types, including phagocytes [12], we also evaluated whether this kinase could contribute to decreased neutrophil phagocytosis. We pre-incubated neutrophils with the AMPK activator AICAR (1 mM) for 30 minutes prior to the addition of E. coli but the treatment did not reduce neutrophil phagocytosis (data not shown) therefore adiponectin is unlikely to act through this factor to inhibit neutrophil phagocytosis.

Mac-1 activation depends on PI3K but not ERK 1/2 activation
As adiponectin inhibited both PI3K-PKB and ERK signalling and also Mac-1 activation, we attempted to determine whether these two pathways were involved in Mac-1 conformational change in neutrophils stimulated with bacteria. Our results suggest that PI3K, but not ERK 1/2, contributes to regulating the activation of Mac-1 in response to bacterial stimulation, as pre-treatment with LY294002 (Fig. 4A) but not with PD98059 (Fig. 4B) caused a significant decrease in the activation of Mac-1.

Adiponectin inhibits the F-actin increase in response to E. coli stimulation
Actin polymerisation is required for bacterial uptake [35]. The extension of pseudopods is controlled by PI3K [26,27] and ERK 1/2 has also been shown recently to play a role in regulating cytoskeletal modifications [36,37]. As we found a decrease in PKB phosphorylation in the presence of adiponectin (Figure 3A), we proposed that the reduction in PI3K and ERK 1/2 activation could also have decreased F-actin generation in response to E. coli. F-actin staining with FITC phalloidin confirmed that adiponectin decreased actin polymerization after 5 minutes of stimulation with unlabelled bacteria (Figure 5), indicating a lack of pseudopod maturation and phagosome formation.

Complement C1q does not prevent adiponectin effects on phagocytosis
The adiponectin C-terminal domain shares homology with the serum complement protein C1q and the adiponectin-mediated
inhibition of macrophage phagocytosis has been shown to be blocked by the use of a C1qRp antibody [18]. We therefore assessed whether inclusion of complement protein C1q protein would be able to block the adiponectin effect on phagocytosis.

Neutrophils were pre-incubated with human serum C1q (at the physiological concentration of 100 μg/ml [38]) in order to allow binding to its receptors prior to the addition of adiponectin.

Figure 1. Adiponectin decreases neutrophil phagocytosis. Adiponectin was added to (A) whole blood (n = 10) at a final concentration of 10 μg/ml or (B) isolated human neutrophils (n = 6) at different concentrations (0.1, 1 and 10 μg/ml) prior to addition of opsonized FITC labelled E. coli and assessment of phagocytic index by flow cytometry. C. Time course of neutrophil phagocytosis (30, 60, 90 minutes) with and without the presence of adiponectin (10 μg/ml). Data are mean ± SEM and * indicates p<0.05, ** indicates p<0.02. D. Percentage of human neutrophils expressing adiponectin receptors AdipoR1 and AdipoR2. The bar represents the mean value. E. Representative FACS plots are shown for immunofluorescence staining for the adiponectin receptors AdipoR1 and AdipoR2.

doi:10.1371/journal.pone.0069108.g001

Figure 2. Adiponectin inhibits the activation of Mac-1 and the binding of E. coli to the neutrophil surface. Isolated neutrophils treated with adiponectin for 1 hour and stimulated with opsonized E. coli were immunostained for the activated conformation of Mac-1. (A) Mac-1 expression (MFI) and (B) the percentage of neutrophils expressing active Mac-1 were decreased by adiponectin. Data are mean ± SEM (n = 7). C. The binding of bacteria to the neutrophil cell surface was measured by flow cytometry following pre-treatment with adiponectin and after 30, 60 and 90 minutes of incubation with opsonized FITC labeled E. coli at 4°C. FITC negative cells were considered unbound. Data are expressed as the percentage of cells with no E.coli bound and are mean ± SEM (n=7).

doi:10.1371/journal.pone.0069108.g002
However, the addition of C1q did not prevent adiponectin-mediated inhibition of neutrophil phagocytosis (Fig. 6).

**Discussion**

The process of phagocytosis is central to the anti-microbial effects of neutrophils, representing the first step in the elimination of bacteria and resolution of infection. Full-length adiponectin has already been shown to impair neutrophil ability to kill pathogens by reducing the generation of ROS [12]. Here we have demonstrated that adiponectin also negatively affects neutrophil phagocytic ability in a concentration dependent manner. The inhibitory function of adiponectin was much greater on isolated neutrophils than in whole blood, which is most likely due to the presence of adiponectin in the blood, though we cannot exclude
that the possibility that in whole blood adiponectin's effects are modified by other serum factors or immune cells.

We found that adiponectin did not change the expression of the phagocytic receptors CD11b, CD16 and TLR4 but it did significantly reduce the activation of the complement receptor Mac-1 in response to flow cytometry. Unstimulated and control E. coli stimulated samples were incubated with the highest concentration of carrier (DMSO) used. Data are mean ± SEM (n=6). ** indicates p<0.01, *** p<0.001.

doi:10.1371/journal.pone.0069108.g004

Figure 4. PI3K inhibition reduces Mac-1 activation. A. The PI3K inhibitor LY294002 and (B) the MEK1 inhibitor PD98059 (10 and 50 μM) were added to neutrophils for 30 minutes before stimulating the cells with opsonised E. coli. After 90 minutes the activation of Mac-1 was assessed by flow cytometry. Unstimulated and control E. coli stimulated samples were incubated with the highest concentration of carrier (DMSO) used. Data are mean ± SEM (n=6). ** indicates p<0.01, *** p<0.001.

doi:10.1371/journal.pone.0069108.g004

Figure 5. Adiponectin decreases actin polymerization in response to E. coli stimulation. A. Neutrophils were pre-incubated with adiponectin and stimulated with E. coli for 5 minutes, after which F-actin content was evaluated by staining with FITC phalloidin. Samples were analyzed by flow cytometry; data are mean ± SEM (n=7). * indicates p<0.05. B. Representative images of F-actin in unstimulated and E. coli stimulated neutrophils with and without pre-treatment with adiponectin.

doi:10.1371/journal.pone.0069108.g005

Adiponectin Inhibits Neutrophil Phagocytosis

PI3K phosphorylation represents a downstream measure of PI3K signalling, which sustains the phagocytic process [26,27,42]. ERK 1/2 also has a crucial role in neutrophil phagocytosis [37,43], its activation being both downstream and independent of PI3K activation [27]. We were able to confirm their involvement in neutrophil phagocytosis by using the inhibitors LY29004 and PD98059. PD98059 treatment has been shown to lead to activation of AMPK indirectly by increasing the cellular AMP:ATP ratio [44]. However this should not contribute
Figure 6. Adiponectin-mediated inhibition of neutrophil phagocytosis is not rescued by complement factor C1q. C1q (100 µg/ml) was added to neutrophils 15 minutes before the addition of adiponectin to saturate C1q receptors and phagocytosis of *E. coli* was then measured. Data are mean ± SEM (n=6).

doi:10.1371/journal.pone.0069108.g006

to decreased phagocytic ability as direct activation of this AMPK did not affect phagocytosis.

We also determined that the conformational change of Mac-1 was dependent on PI3K but not ERK 1/2 activation. Previous studies have shown that Mac-1 activation was mediated by cytoskeletal rearrangements [45] and the intracytoplasmic domain of CD16 is phosphorylated in stimulated neutrophils, contributing to the conformational rearrangement of Mac-1 [46]. The PI3K inhibitor LY29004 had a greater effect on neutrophil phagocytosis than it did on Mac-1 activation, suggesting that PI3K is involved in multiple aspects of the phagocytic process. For example, PI3K has also been shown to be indispensable for the correct closure of phagosomes [47].

ERK1/2 has been shown to be indispensable for phagocytosis of *Francisella tularensis*, acting downstream of the tyrosine kinase Syk, whose function is crucial for cytoskeletal rearrangements [37]. Importantly ERK 1/2, together with PI3K and other kinases, phosphorylates cortactin, a monomeric protein involved in actin polymerisation and lamellipodia formation [36]. Consistent with the reduced activation of PI3K and ERK 1/2, we observed a strong reduction in the content of F-actin in response to bacterial stimulation suggesting that adiponectin treatment inhibits the uptake of *E. coli* by blockage of both of these signal pathways.

AMPK is the main kinase previously reported to be activated by adiponectin [11,12], however its activation does not mediate the decrease in phagocytosis induced by adiponectin, as AICAR treatment did not inhibit phagocytosis. This conclusion is supported by a previous report that showed AICAR actually enhanced neutrophilia phagocytosis [48], though we did not see any enhancement of phagocytosis at the dose used here.

Adiponectin might also have inhibited neutrophil phagocytosis through its C-terminal C1q domain, which has been shown to act in an antagonistic manner in macrophages by Yokota and colleagues [18]. C1q is a serum complement factor whose main receptor is C1qR, but it has been proposed to bind to other phagocytic receptors expressed by neutrophils, particularly CR1 [49]. To check whether adiponectin could decrease neutrophil phagocytosis by blocking bacterial binding to these receptors through its C1q-homologous domain, we pre-incubated the cells with a physiological concentration of C1q before the addition of adiponectin and before performing the phagocytosis assay, but we did not observe any improvement in neutrophil phagocytosis. Other groups have shown enhanced phagocytosis of apoptotic bodies mediated by C1q in human macrophages [17] and monocytic cell lines [17,50], suggesting that C1q influences phagocytosis in a cell-specific manner.

In conclusion, these data demonstrate for the first time that adiponectin decreases neutrophil ability to phagocytose *E. coli*, a result observed both in whole blood and in isolated neutrophils. In Figure 7, we propose that adiponectin exerts its effect by decreasing the binding of bacteria to neutrophils through suppression of Mac-1 activation and both the bacterial binding and uptake are mediated by blockage of PI3K and ERK ½ signaling. These data add weight to the literature highlighting an immune modulatory role for adiponectin and importantly indicate that this could help to explain the increased incidence of infections seen in situations of raised circulating adiponectin, such as old age [51,52]. The data are more difficult to interpret in the case of obesity, a complex condition which is characterized by higher risk of infection [53] but lower levels of adiponectin [8].

### Materials and Methods

#### Ethics statement

The study was approved by the Birmingham East North and Solihull Research Ethics Committee (09/H1206/48) and all subjects gave their written informed consent before taking part in the study.

#### Reagents and antibodies

Human recombinant full length adiponectin generated in HEK293 cells (HMW isoforms and hexamers, as specified in the datasheet) was purchased from Enzo Life Sciences (Farmingdale, NY, USA), with contamination from LPS certified to be less than 0.1 EU/µg purified protein. To ensure no artefactual effects from LPS contamination, Polymyxin B (10 µg/ml) (Millipore, MA) was added to the cells 30 min before treatment with adiponectin. Percooll, RPMI 1640 medium, L-glutamine, penicillin-streptomycin, bovine serum albumin (BSA), dimethyl sulfoxide (DMSO), human complement factor C1q, FITC phaloidin, protease inhibitor cocktail and all buffers and salt solutions were purchased from Sigma-Aldrich (Poole, UK). The MEK-1/ERK inhibitor PD98059 was from Cell Signaling Technology (Beverly, MA), the PI3K inhibitor LY29004 was from Millipore and the AMPK activator AICAR was purchased from Enzo Life Sciences. Unconjugated antibodies against AdipoR1 and AdipoR2 were from Phoenix Pharmaceuticals (Burlingame, CA), the unconjugated mouse anti-human CD11b (clone 2LPM19c), FITC-conjugated CD16 (clone DJ130c) and isotype antibody FITC-conjugated mouse IgG1k were obtained from Dako (Ely, UK).

Figure 7. Proposed model for adiponectin inhibition of neutrophil phagocytosis.

doi:10.1371/journal.pone.0069108.g007
FITC conjugated goat anti-rabbit secondary antibody was calculated as the percentage of the cells having ingested bacteria (FITC positive cells), multiplied by the mean fluorescence intensity (MFI) of the FITC positive population and divided by 100. Cytospins of the suspensions were also obtained and visualized using a LEICA DMi 6000 B microscope x63 objective (Leica Microsystems, UK).

To evaluate the binding of bacteria to neutrophil walls, suspensions were incubated at 4°C for 30, 60 and 90 minutes without final addition of quenching solution; samples were analyzed by flow cytometry, FITC negative cells were considered not to have bound bacteria.

Measurement of surface receptor expression
Surface expression of adiponectin receptors (AdipoR1 and AdipoR2) and CD11b was measured by indirect immunofluorescence staining and negative controls consisted of staining with appropriate secondary antibodies alone. Adiponectin receptors were detected with unconjugated rabbit anti-human AdipoR1 and AdipoR2 (5 μg/ml) and CD11b expression was measured with mouse anti-CD11b (10 μg/ml). Non-specific binding sites were blocked by addition of goat serum for 5 minutes before adding the secondary FITC conjugated goat anti-rabbit (2.5 μg/ml) or goat anti-mouse (5 μg/ml) antibodies. Surface expression of the activated form of Mac-1, CD16 and TLR4 were assessed by direct immunofluorescence staining (10 μg/ml for anti-Mac-1 and anti-TLR4, 4 μg/ml for anti-CD16); concentration-matched isotype antibodies mouse FITC IgG1 and mouse APC IgG2a were used as negative controls for Mac-1, CD16 and TLR4. Samples were analyzed by flow cytometry.

Measurement of PI3K-PKB, ERK and p38 MAPK activation
Neutrophils were stimulated with unlabeled, opsonised E. coli for 10 minutes and spun at 4000 rpm for 4 minutes (MSE microcentrifuge) prior to resuspension in lysis buffer (20 mM MOPS, 50 mM NaF, 50 mM β-glycerophosphate, 50 mM Na3VO4, 1% Triton X-100, 1 mM DTT, 1 mM AEBSF and 1% protease inhibitor cocktail). Lysis of neutrophils was performed on ice for 30 minutes with occasional vortexing. The lysate was centrifuged at 13,000 rpm for 1 minute (MSE microcentrifuge) and the supernatant collected. Non-specific protein binding was blocked using 5% BSA. Membranes were incubated with primary antibodies to total PKB (1:1000), phosphorylated PKB (1:1000), total ERK 1/2 (1:1000), phosphorylated ERK 1/2 (1:1000), total PKB (1:1000), phosphorylated PKB (1:1000) overnight at 4°C and with appropriate secondary antibodies (ECL™ anti-rabbit or anti-mouse IgG, GE Healthcare, Sweden) for 1 hour at room temperature. Proteins were visualized by ECL according to manufacturer’s instructions (GeneFlow, UK). Densitometric analyses were performed using Image J software.

Measurement of actin polymerisation
Actin polymerisation was assessed by measuring F actin levels. Neutrophils were stimulated with unlabeled, opsonised E. coli for 5 minutes, after which they were subjected to fixation and permeabilisation using a Fix and Perm® kit (Life technologies, CA) according to the manufacturer’s instructions. Neutrophils were incubated with FITC labeled phalloidin (1 μg/ml) for 30 minutes. After washing in PBS, samples were analysed by flow cytometry. Cytospins were also obtained and nuclei were stained
followed by Tukey's multiple comparison test. Results are expressed as mean ± standard error of the mean (SEM). A p-value of less than 0.05 was accepted as significant.

**Author Contributions**

Conceived and designed the experiments: JML. Performed the experiments: AR. Analyzed the data: AR JML. Wrote the paper: AR JML.

**References**

1. Hofstetter J, Arvidson E, Sjölin E, Wahren K, Arner P (2004) Adipose tissue adiponectin production and adiponectin serum concentration in human obesity and insulin resistance. J Clin Endocrinol Metab 89: 1391–1396.

2. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 270: 26746–26749.

3. Fuki H, Yamasaki T, Kamon J, Ito Y, Uchida S et al. (2003) Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem 278: 40352–40363.

4. Fu Y, Loo K, Klein RL, Garvey WT (2005) Adiponectin promotes adipocyte differentiation, insulin sensitivity, and lipid accumulation. J Lipid Res 46: 1369–1379.

5. Berg AH, Combs TP, Scherer PE (2002) Arcpp30/adiponectin: An adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 13: 84–89.

6. Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y et al. (2002) Adiponectin reduces atherosclerosis in apolipoprotein e-deficient mice. Circulation 106: 2767–2770.

7. Tilk H, Moschen AR (2006) Adipokines: Mediators linking adipose tissue inflammation and infection. Nat Rev Immunol 6: 722–733.

8. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K et al. (1999) Paradoxical decrease of an adipocyte-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 257: 79–83.

9. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M et al. (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arteriosclerosis Thrombosis Vascular Biol 20: 1595–1599.

10. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y et al. (2001) Hypoadiponectinemia in obesity and type 2 diabetes: Close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 86: 1930–1935.

11. Shen YY, Charlesworth JA, Kelly JJ, Loi KW, Peake PW (2007) Up-regulation of adiponectin, its isoforms and receptors in end-stage kidney disease. Nephrol Dial Transplant 22: 171–178.

12. Chedid P, Hurtado-Nedelec M, Marion-Gaber B, Bournier O, Hayem G, et al. (2012) Adiponectin and its globular fragment differentially modulate the oxidative burst of primary human phagocytes. Am J Physiol 180: 692-699.

13. Konter JM, Parker JL, Baze E, Li SZ, Ransche B, et al. (2012) Adiponectin attenuates lipopolysaccharide-induced acute lung injury through suppression of neutrophil cell activation. J Immunol 188: 854–863.

14. Masaki T, Chiba S, Tatsukawa H, Yasuda T, Noguchi H, et al. (2004) Adiponectin protects LPS-induced liver injury through modulation of TNF alpha in kk-ay obese mice. Hepatology 40: 177–184.

15. Kim KY, Kim JH, Han SH, Lim JS, Kim HR, et al. (2006) Adiponectin is a negative regulator of NK cell cytotoxicity. J Immunol 176: 5912–5917.

16. Shen YY, Charlesworth JA, Kelly JJ, Loi KW, Peake PW (2007) Up-regulation of adiponectin, its isoforms and receptors in end-stage kidney disease. Nephrol Dial Transplant 22: 171–178.

17. Takemura Y, Ouchi N, Shihata R, Arapamathin T, Kirker MT, et al. (2007) Adiponectin modulates inflammatory reactions via calreticulin receptor-dependent clearance of early apoptotic bodies. J Cell Sci 119: 1903–1913.

18. Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, et al. (2000) Neutrophil extracellular traps kill bacteria. Science 303: 1532–1535.

19. Rooyakkers AW, Stokes RW (2005) Absence of complement receptor 3 results in constitutive production of nitric oxide. J Leukoc Biol 64: 835–844.

20. Rooney JJ, Fong CC, Bjekic G, Greenberg S (1999) A requirement for phosphatidylinositol 3-kinase in pseudopod extension. J Biol Chem 274: 1240–1247.

21. Garcia-Garcia E, Rosales C (2002) Signal transduction during Fc receptor-mediated phagocytosis. J Leukoc Biol 72: 1092–1101.

22. Davies SP, Reddy H, Gottfried HS (2002) Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem J 351: 95–105.

23. Vlahos CJ, Matter WF, Hsu KY, Brown RF (1994) A specific inhibitor of phosphatidylinositol 3-kinase, 2-4-morpholino-6-phenyl-4-benzopyran-4-one (LY294002). J Biol Chem 269: 5241–5246.

24. Luo J, Xu T, Wang X, Ba X, Feng X, et al. (2010) PI3K is involved in L-selectin- and PSGL-1-mediated neutrophil rolling on E-selectin via Fxactin redistribution and assembly. J Cell Biochem 110: 910–919.

25. Porto BN, Abes LS, Fernandez PL, Dutra TP, Figueredo RT, et al. (2007) Heme induces neutrophil migration and reactive oxygen species generation during signaling pathways characteristic of chemotactic receptors. J Biol Chem 282: 24430–24436.

26. Knaul C, Worthen GS, Johnson GL (1997) Interleukin-1 stimulated phoshatidylinositol 3-kinase activity regulates the migration of human neutrophils independent of extracellular signal-regulated kinase and p38 mitogen-activated protein kinase. Proc Natl Acad Sci U S A 94: 3052–3057.

27. Wang JP, Tsai JJ, Chen YS, Hsu MF (2005) Stimulation of intracellular Ca2+ elevation in neutrophils by thiol-oxidizing phenylsiline oxide. Biochem Pharmacol 69: 1223–1234.

28. Yang PM, Raad I, Derkawi RA, Boussetta T, Paulet MH, et al. (2011) The NAPRF oxidase cytosolic component p67phox is constitutively phosphorylated in human neutrophil: Regulation by a protein tyrosine kinase, MEK1/2 and phophatases 1/2a. Biochem Pharmacol 82: 1143–1152.

29. Hemt M, Heinrich V, Dembo M (2006) Mechanics of neutrophil phagocytosis: Experiments and quantitative models. J Cell Sci 119: 1903–1913.

30. Ammer AG, Weed SA (2000) Cortactin branches out: Roles in regulating actin dynamics. Cell Motil Cytoskeleton 46: 687–697.

31. Pinty C, Courtois M, Richard R, Capron M, Amadio P, et al. (1999) Adenosine 3′,5′-cyclic monophosphate regulates the growth of myelomonocytic progenitors and the functions of macrophages. J Exp Med 189: 705–714.

32. Knall C, Worthen GS, Johnson GL (1997) Interleukin-1 stimulated phosphatidylinositol 3-kinase activity regulates the migration of human neutrophils independent of extracellular signal-regulated kinase and p38 mitogen-activated protein kinase. Proc Natl Acad Sci U S A 94: 3052–3057.

33. Vlahos CJ, Matter WF, Hsu KY, Brown RF (1994) A specific inhibitor of phosphatidylinositol 3-kinase, 2-4-morpholino-6-phenyl-4-benzopyran-4-one (LY294002). J Biol Chem 269: 5241–5246.
47. Araki N, Johnson MT, Swanson JA (1996) A role for phosphoinositide 3-kinase in the completion of macropinocytosis and phagocytosis by macrophages. J Cell Biol 135: 1249–1260.

48. Bae HB, Zmijewski JW, Deshane JS, Tadie JM, Chaplin DD, et al. (2011) AMP-activated protein kinase enhances the phagocytic ability of macrophages and neutrophils. Faseb J 25: 4358–4368.

49. Eggleton P, Reid KB, Temner AJ (1998) C1q – how many functions? How many receptors? Trends Cell Biol 8: 420–431.

50. Ma W, Kai V; Hudson BI, Song F, Schmidt AM, et al. (2012) Rage binds C1q and enhances C1q-mediated phagocytosis. Cell Immunol 274: 72–82.

51. Adamczak M, Rzepka E, Chudek J, Wieck A (2005) Ageing and plasma adiponectin concentration in apparently healthy males and females. Clin Endocrinol 62: 114–118.

52. Gavazzi G, Krause K-H (2002) Ageing and infection. Lancet Infect Dis 2: 659–666.

53. Karlsson EA, Beck MA (2010) The burden of obesity on infectious disease. Exp Biol Med 235: 1412–1424.

54. Jepsen LV, Skottun T (1982) A rapid one-step method for the isolation of human-granulocytes from whole-blood. Scand J Clin Lab Inv 42: 235–238.