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Rapid Letter

Adiponectin Inhibits Superoxide Generation by Human Neutrophils

ULYSSES J. MAGALANG, RAMYA RAJAPPAN, MELISSA G. HUNTER, VIJAY K. KUTALA, PERIANNAN KUPPUSAMY, MARK D. WEWERS, CLAY B. MARSH, and NARASIMHAM L. PARINANDI

ABSTRACT

Adiponectin (Ad), a member of the adipocytokine family, has been reported to possess antiinflammatory properties. We investigated the effects of full-length human Ad (hAd) on phorbol 12-myristate 13-acetate (PMA)-induced $O_2^{-\cdot}$ generation by human neutrophils. hAd, even at the lowest tested concentration of 0.001 μg/ml, after 30-min pretreatment of cells, significantly inhibited $O_2^{-\cdot}$ generation by neutrophils stimulated with PMA (100 n*M*). However, no relation between the dose of hAd and extent of inhibition of PMA-induced $O_2^{-\cdot}$ generation was observed with increasing the concentration of hAd up to 1 μg/ml. hAd also significantly inhibited neutrophil $O_2^{-\cdot}$ generation stimulated by *N*-formyl-methionyl-leucyl-phenylalanine (100 μ*M*) and diacylglycerol (500 n*M*), as well as the PMA-induced neutrophil nitroblue tetrazolium reduction and H_2O_2 formation. Pretreatment of neutrophils with pronase-digested hAd failed to inhibit the PMA-induced $O_2^{-\cdot}$ generation. For the first time, this study revealed that Ad inhibited $O_2^{-\cdot}$ generation by neutrophils, possibly through regulation of NADPH oxidase. *Antioxid. Redox Signal.* 8, 2179–2186.

INTRODUCTION

White adipose tissue (WAT) is not merely a passive reservoir of fat for energy reserves but is emerging as an endocrine organ that hosts and operates critical but complex metabolic functions in normal physiology and disease states (19). The WAT secretes a wide variety of bioactive peptides and proteins, collectively called "adipokines" or "adipocytokines," that are involved in lipid metabolism, insulin sensitivity, alternate complement system, hemostasis, regulation of blood pressure, angiogenesis, and regulation of energy balance (26).

Adiponectin (Ad), an adipocytokine secreted abundantly by adipose tissue (6), has recently received considerable attention, given its pleiotropic actions that include antidiabetic, antiatherogenic, and antiinflammatory effects (18). Inflammation is associated with the generation of neutrophil-derived reactive oxygen species (ROS) (13). NADPH oxidase, a multicomponent enzyme complex, catalyzes the generation of ROS ($O_2^{-\cdot}$ and H_2O_2) by the neutrophils (2). Ad suppresses the oxidized low-density lipoprotein (oxLDL)-induced $O_2^{-\cdot}$ generation by endothelial cells (ECs) that is attributed to the regulation of endothelial NAD(P)H oxidase activity (21). Although Ad has been shown to act as an antiinflammatory adipocytokine (22), the modulatory effects of Ad on neutrophil-generated ROS have not been reported so far. Therefore, we hypothesized that Ad would inhibit the generation of ROS by neutrophils. Hence, in the current study, we studied the effects of human recombinant full-length Ad (hAd) on the phorbol 12-myristate 13-acetate (PMA)-, *N*-formyl-methionyl-leucyl-phenylalanine (fMLP)-, and diacylglycerol (DAG)-induced and NADPH oxidase–mediated $O_2^{-\cdot}$ generation by human neutrophils *in vitro*. For the first time, the results of the current study demonstrated that Ad inhib-

Division of Pulmonary, Critical Care, and Sleep Medicine, Dorothy M. Davis Heart and Lung Research Institute, College of Medicine and Public Health, The Ohio State University, Columbus, Ohio.

ited $O_2^{-\bullet}$ generation by neutrophils through possible regulation of NADPH oxidase and further suggested the antiinflammatory role of Ad at the neutrophil level.

MATERIALS AND METHODS

Materials

Recombinant full-length human Ad (hAd) was purchased from Biovision (Mountain View, CA). Dextran sulfate sodium salt was obtained from Amersham Biosciences (Piscataway, NJ). Ammonium chloride lysing reagent was purchased from BD Biosciences-Pharmingen (San Diego, CA). Amplex red hydrogen peroxide assay kit was obtained from Molecular Probes (Eugene, OR). Ferricytochrome *c*, human erythrocyte superoxide dismutase (SOD), PMA, fMLP, *p*-nitroblue tetrazolium chloride (NBT), diphenylene iodonium (DPI), diethylenetriamine pentaacetic acid (DTPA), heparin, Ficoll-Hypaque, and other reagents of highest purity were all obtained from Sigma Chemical Company (St. Louis, MO). DAG (*sn*-1,2-dioctanoylglycerol) was obtained from Avanti Polar Lipids (Alabaster, AL). Pronase was obtained from Roche Applied Science (Indianapolis, IN). DMPO (5,5-dimethylpyrroline-*N*-oxide) was procured from Dojindo Laboratories (Gaithersburg, MD).

Isolation of neutrophils

Neutrophils were freshly isolated from the whole blood of human volunteers by using the Ficoll-Hypaque gradient centrifugation method. Blood (150 ml) was treated with heparin (20 units/50 ml of blood). A volume of 25 ml of blood was mixed with 15 ml of phosphate-buffered saline (PBS) and layered on 10 ml of Ficoll-Hypaque at a density of 1.077 and centrifuged for 15 min at room temperature at 720 *g*. After centrifugation, the top layer containing plasma and lymphocytes was aspirated, and the pellet containing erythrocytes and neutrophils was mixed with 20 ml of 3% dextran sulfate, allowed to sit at room temperature for 1 h, and the top layer was collected and centrifuged for 10 min at 405 *g*. The resultant pellet was resuspended in 20 ml of PBS and washed twice by centrifuging at 405 *g* for 10 min. The erythrocytes in the pellet were lysed with ammonium chloride lysis reagent and centrifuged for 10 min at 405 *g*. The resultant neutrophil pellet was washed with PBS by centrifuging at 405 *g* for 10 min. The viability of freshly prepared neutrophils was checked by the trypan blue exclusion method, and the cells were used within 4 h of isolation.

Buffers

For stimulation with PMA, neutrophils were incubated in PBS containing glucose (0.1%) and DTPA (0.001%) (pH 7.4). For stimulation with fMLP, neutrophils were incubated in modified PBS containing $CaCl_2$ (0.9 m*M*), $MgCl_2$ (0.5 m*M*), glucose (0.1%), and DTPA (0.001%) (pH 7.35). For NBT reduction, Krebs–Henseleit buffer (pH 7.35) containing NaCl (117.3 m*M*), KCl (4.7 m*M*), $NaHCO_3$ (25 m*M*), $MgSO_4$ (1.3 m*M*), KH_2PO_4 (1.2 m*M*), $CaCl_2$ (1.23 m*M*), and glucose (11.1 m*M*) was used. For H_2O_2 determination, Krebs–Ringer phosphate buffer (pH 7.35) containing NaCl (145 m*M*), NaH_2PO_4 (5.7 m*M*), KCl (4.86 m*M*), $CaCl_2$ (54 m*M*), $MgSO_4$ (1.22 m*M*), and glucose (5.5 m*M*) was used.

Treatment of neutrophils with agonists, Ad, and pharmacologic agents

Neutrophils were pretreated with Ad or DPI in PBS or Krebs–Henseleit buffer or Krebs–Ringer buffer, wherever required, for 30 min at 37°C in a shaker water bath with 80 oscillations/min. Neutrophils, without and with Ad and DPI pretreatment, were treated with agonists (PMA, fMLP, DAG) for desired periods in PBS or Krebs–Henseleit buffer or Krebs–Ringer buffer, wherever required, for 30 min at 37°C in a shaker water bath with 80 oscillations/min.

$O_2^{-\bullet}$ determination by cytochrome c reduction method

Generation of $O_2^{-\bullet}$ by neutrophils was determined spectrophotometrically by the SOD-inhibitable cytochrome *c* method (23). The incubation medium was PBS or modified PBS containing ferricytochrome *c* (75 μ*M*) in the absence and presence of SOD (200 U/ml). Neutrophils were incubated at 37°C for the desired lengths of time after pretreatment with mAd or hAd in absence or presence of the agonist (PMA, fMLP, and DAG) in a shaker water bath with 80 oscillations/min. At the end of the incubation, the medium was withdrawn, and absorbance was measured at 550 nm against appropriate blanks. The amount of $O_2^{-\bullet}$ generated by neutrophils was calculated by using the micromolar absorption coefficient (19.1) of cytochrome *c* and expressed as nmoles of $O_2^{-\bullet}$/cell number.

Detection of oxygen radicals by electron paramagnetic resonance spectroscopy

Electron paramagnetic resonance (EPR) spectroscopic detection of oxygen radicals was done according to Crowther *et al.* (7). Human neutrophils (10^5 cells) without or with hAd pretreatment in PBS (1 ml final volume) containing glucose (1%), DTPA (0.001%), and DMPO (50 m*M*) were treated with PMA (100 n*M*). Controls without PMA + hAd and with PMA + SOD (200 U) were simultaneously established under identical conditions. The incubation mixture was drawn into a flat cell and subjected to EPR spectroscopy measurements for 20 min. The EPR measurements were carried out by using a Bruker X-band (9.8 GHz) spectrometer (Bruker Instruments, Karlsruhe, Germany) equipped with a TM110 cavity. EPR spectral acquisitions were performed by using custom-developed data-acquisition software, which is capable of fully automated data acquisition and processing.

H_2O_2 determination

The amount of H_2O_2 released by neutrophils was determined according to the method devised by Mohanty *et al*. (20) by using Amplex Red H_2O_2 assay kit. Neutrophils, of desired cell number in 96-well sterile plates, were incubated at 37°C in Krebs–Ringer phosphate buffer (pH 7.35) for the designated lengths of time after pretreatment with hAd or

pharmacologic inhibitors and subsequent treatment with PMA. At the end of the incubation, the fluorescence was measured on a fluorescence plate reader with an excitation and emission set at 560 and 590 nm, respectively, by using appropriate blanks. The amount of H_2O_2 generated by neutrophils was calculated from a standard curve prepared with known concentrations of H_2O_2 and expressed as μmol H_2O_2 formed/cell number.

Determination of NBT reduction

The extent of reduction of NBT by neutrophils was determined according to Katamoto *et al.* (15), with a slight modification. Neutrophils (5×10^6 cells) after pretreatment without and with hAd were incubated in Krebs–Henseleit buffer (pH 7.35) containing PMA and NBT (0.5 mg/ml) in a final volume of 1 ml for 30 min at 37°C in a shaker water bath with 80 oscillations/min. At the end of incubation, the reaction was stopped by adding 1 ml of 0.5N HCl, centrifuged at 1,000 g for 10 min, and the supernatant was discarded. The precipitate was dissolved in 3 ml of dimethyl sulfoxide, heated in boiling water bath for 5 min, cooled to room temperature, centrifuged at 500 *g* for 5 min, and the absorbance was measured at 565 nm against an appropriate blank. The extent of NBT reduction by neutrophils was expressed as absorbance units.

Denaturation of adiponectin

Ad (5 μg/ml) was treated with 25 μg of pronase at 37°C for 1 h in PBS or modified PBS or Krebs–Ringer buffer or Krebs–Henseleit buffer and then heat-denatured in boiling-water bath for 1 h. The preparation was cooled to room temperature and used for pretreatment of neutrophils in the chosen buffer in absence or presence of PMA and other agonists.

Statistical analysis

All the experiments were done in triplicate, and the standard deviation (SD) for each data point was calculated. Data were subjected to analysis of variance (ANOVA) by using SigmaStat (Jandel). The level of statistical significance was taken as $p < 0.05$.

RESULTS

Effect of dose of adiponectin on PMA-induced $O_2^{-\bullet}$ generation by human neutrophils

We determined the effects of different doses of hAd on PMA-induced $O_2^{-\bullet}$ generation by human neutrophils. Cells were pretreated with hAd (0.001–1.0 μg/ml) for 30 min and then were treated with PMA for an additional 30 min. PMA alone significantly caused a 20-fold increase in $O_2^{-\bullet}$ generation by neutrophils as compared with that in the vehicle-treated control cells (Fig. 1A). hAd, even at the lowest tested dose of 0.001 μg/ml, caused a significant inhibition of PMA-induced $O_2^{-\bullet}$ generation by neutrophils. Further increase in the dose of hAd up to 1.0 μg/ml did not alter its effect in inhibiting the PMA-induced $O_2^{-\bullet}$ generation by neutrophils. We also tested the lowest possible doses (0.00025–0.0005 μg/ml) of hAd and still observed an extent of inhibition of PMA-induced $O_2^{-\bullet}$ generation by neutrophils similar to that exerted by the higher doses of hAd (data not shown). Under the current experimental conditions, no clear-cut relation between the dose of hAd and inhibition of PMA-induced $O_2^{-\bullet}$ generation by human neutrophils was evident.

Adiponectin inhibits PMA-induced oxygen radical formation by human neutrophils

By using EPR spectroscopy, we investigated whether hAd would cause inhibition of PMA-induced oxygen radical formation by human neutrophils. Cells were pretreated with hAd for 30 min before exposure to PMA for 20 min. The results clearly showed that in neutrophils (a) PMA induced formation of oxygen radicals, as revealed from the characteristic oxygen radical EPR spectrum; (b) hAd markedly inhibited PMA-induced oxygen radical formation, as evident from the reduction in the amplitude of the EPR spectrum; and (c) SOD completely abolished the formation of PMA-induced oxygen radical formation (Fig. 1B).

Adiponectin inhibits PMA-induced H_2O_2 formation by human neutrophils

$O_2^{-\bullet}$, the initial product of NADPH oxidase, dismutates to generate H_2O_2 (12). Therefore, we also investigated the effect of hAd on PMA-induced H_2O_2 generation by human neutrophils. Cells were pretreated with hAd or DPI for 30 min, and then were treated with PMA for 10–30 min. Pretreatment of cells with hAd significantly inhibited PMA-induced H_2O_2 generation by neutrophils at 10, 20, and 30 min of incubation. The flavoenzyme inhibitor, DPI, used widely to inhibit NADPH oxidase, also significantly inhibited PMA-induced generation of H_2O_2 by neutrophils (Fig. 1C). The results also revealed that the potency of hAd in inhibiting PMA-induced H_2O_2 generation by neutrophils was almost the same as that of DPI at the tested concentrations of both agents.

Adiponectin inhibits PMA-induced NBT reduction by human neutrophils

Oxidative burst by neutrophils is also determined by the widely used NBT reduction assay (17). Neutrophils were pretreated with hAd for 30 min before treatment with PMA for 30 min. The results revealed that hAd caused a significant inhibition of the PMA-induced NBT reduction by neutrophils (Fig. 1D).

Adiponectin inhibits fMLP-, DAG-, and PMA-induced $O_2^{-\bullet}$ generation by human neutrophils

We further conducted studies to establish whether hAd would also inhibit $O_2^{-\bullet}$ generation induced by other widely used agonists to stimulate the neutrophil oxidative burst. Neutrophils were pretreated with hAd for 30 min, after which $O_2^{-\bullet}$ generation was induced by treating the cells with fMLP, DAG, and PMA for an additional period of 30 min. Our results showed that hAd pretreatment of cells significantly caused inhibition of fMLP-, DAG-, and PMA-induced generation of $O_2^{-\bullet}$ by neutrophils (Fig. 2).

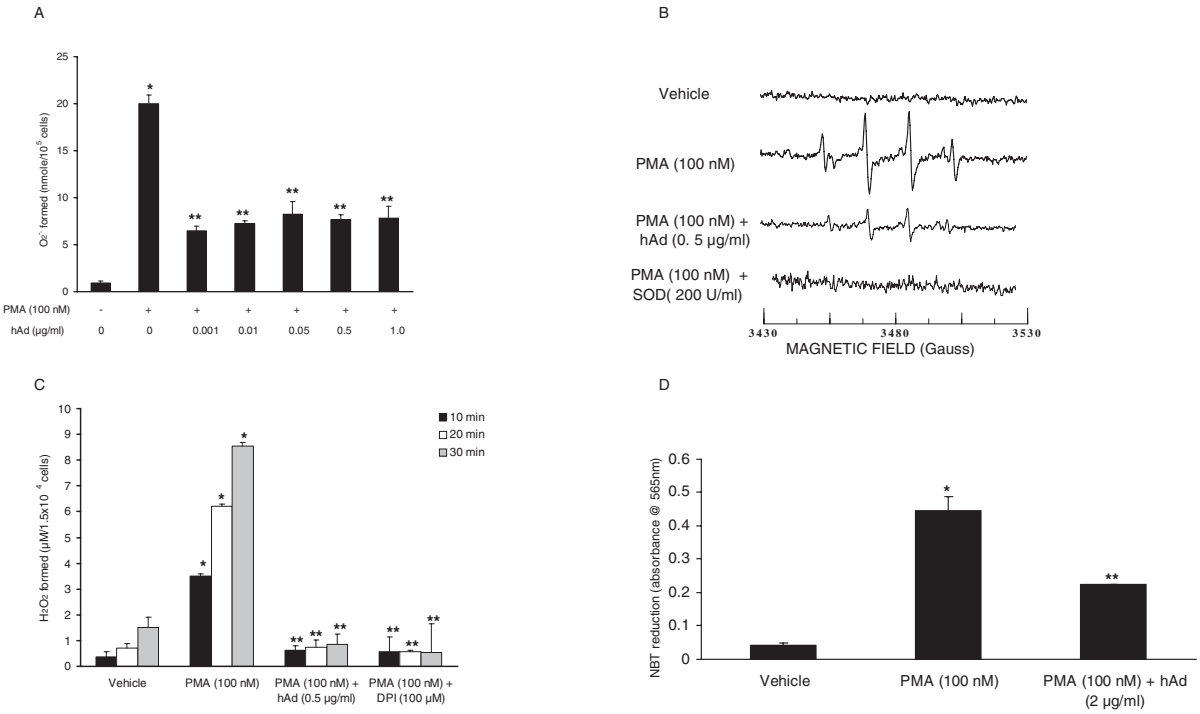

FIG. 1. Effect of adiponectin on neutrophil ROS generation. (**A**) Effect of dose of adiponectin on inhibition of PMA-induced $O_2^{-\cdot}$ generation as determined by SOD-inhibitable cytochrome *c* reduction method.. Human neutrophils, following pretreatment without or with hAd (0.001–1.0 µg/ml) for 30 min, were treated with PMA (100 n*M*). (**B**) Adiponectin inhibits PMA-induced oxygen radical formation by human neutrophils as determined by EPR spectroscopy. Human neutrophils (10^5 cells) without or with hAd pretreatment (0.5 µg/ml for 30 min) in 1 ml volume of PBS containing DMPO (50 m*M*) were treated with PMA (100 n*M*). Controls without PMA + hAd and with PMA (100 n*M*) + SOD (200 U/ml) were established under identical conditions. Each spectrum is a representative of three independent experiments. (**C**) Adiponectin inhibits PMA-induced H_2O_2 formation by human neutrophils. Human neutrophils, after pretreatment without or with hAd (0.5 µg/ml) or DPI (100 µ*M*) for 30 min, were treated with PMA (100 n*M*) in a 100-µl volume of Krebs–Ringer phosphate buffer (pH 7.35) for different lengths of time. Extracellular generation of H_2O_2 by neutrophils was determined fluorimetrically. (**D**) Adiponectin inhibits PMA-induced NBT reduction by human neutrophils. Human neutrophils (10^6 cells), after pretreatment without or with hAd (2 µg/ml) for 30 min, were treated with PMA (100 n*M*) in a 1-ml volume of Krebs–Henseleit buffer (pH 7.35) containing NBT for 30 min. NBT reduction by neutrophils was determined spectrophotometrically as described in Materials and Methods. Each histogram is an average of three independent determinations. *Significantly different from vehicle-treated control at $p < 0.05$. **Significantly different from PMA-treated cells at $p < 0.05$.

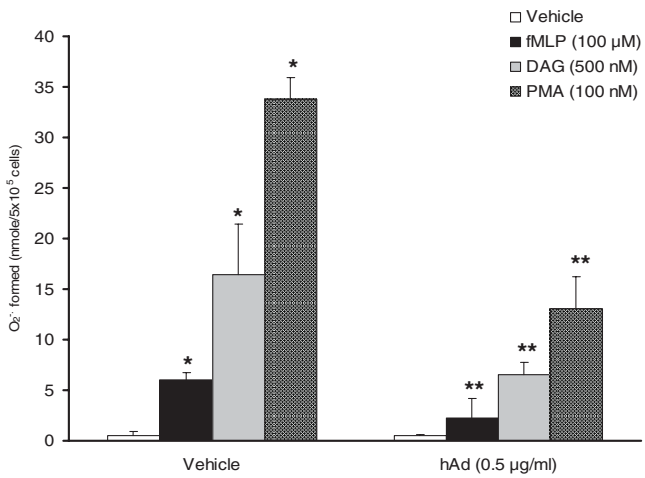

FIG. 2. Adiponectin inhibits fMLP-, DAG-, and PMA-induced $O_2^{-\cdot}$ generation by human neutrophils. Human neutrophils, after pretreatment without or with hAd (0.5 µg/ml) for 30 min at 37°C, were treated with fMLP (100 µ*M*) or DAG (500 n*M*) or PMA (100 n*M*) in a 1-ml volume of PBS (pH 7.4) at 37°C for 30 min. Extracellular generation of $O_2^{-\cdot}$ by neutrophils was determined spectrophotometrically by the SOD-inhibitable cytochrome *c* reduction method as described in Materials and Methods. Each histogram is an average of three independent determinations. *Significantly different from vehicle-treated control at $p < 0.05$. **Significantly different from agonist-treated cells at $p < 0.05$.

Effect of pre- and cotreatment of with adiponectin and pronase-digested and denatured adiponectin on PMA-induced $O_2^{-\bullet}$ generation by human neutrophils

We compared the potency of hAd under conditions of pretreatment and cotreatment in causing inhibition of PMA-induced generation of $O_2^{-\bullet}$ by human neutrophils. Cotreatment with hAd did not significantly inhibit the PMA-induced generation of $O_2^{-\bullet}$ by human neutrophils, whereas the same was markedly inhibited by hAd pretreatment for 30 min before exposure of cells to PMA (Fig. 3A). These results clearly demonstrated that pretreatment of cells with hAd was essential for its maximal inhibitory effect on PMA-induced generation of $O_2^{-\bullet}$ by human neutrophils.

We further conducted studies to show that intact full-length hAd was essential to exhibit its inhibitory effect on PMA-induced $O_2^{-\bullet}$ generation by neutrophils. Cells were pretreated with intact hAd or pronase-digested and denatured hAd for 30 min before exposure to PMA for 30 min. The results revealed that intact hAd significantly inhibited PMA-induced $O_2^{-\bullet}$ generation by neutrophils, whereas pronase-digested and denatured hAd failed to cause significant inhibition of the same (Fig. 3B). Pronase (25 μg) alone, after denaturation, did not alter the basal $O_2^{-\bullet}$ generation by neutrophils (data not shown). These results clearly established that only intact hAd was effective in causing inhibition of PMA-induced $O_2^{-\bullet}$ generation by neutrophils, as opposed to its pronase-catalyzed proteolytic products.

Time course of adiponectin-mediated inhibition of PMA-induced $O_2^{-\bullet}$ generation by human neutrophils

We further determined the time course of hAd inhibition of PMA-induced $O_2^{-\bullet}$ generation by neutrophils. Neutrophils treated with PMA alone exhibited a linear and significant increase in $O_2^{-\bullet}$ generation with time (0–30 min), whereas control cells without PMA + hAd treatments showed a slightly measurable release of $O_2^{-\bullet}$ at 20 and 30 min of incubation (Fig. 4). Conversely, neutrophils pretreated with hAd followed by exposure to PMA showed significant decrease in $O_2^{-\bullet}$ generation. These results showed that after pretreatment of human neutrophils with hAd, the extent of inhibition of PMA-induced generation of $O_2^{-\bullet}$ was time dependent.

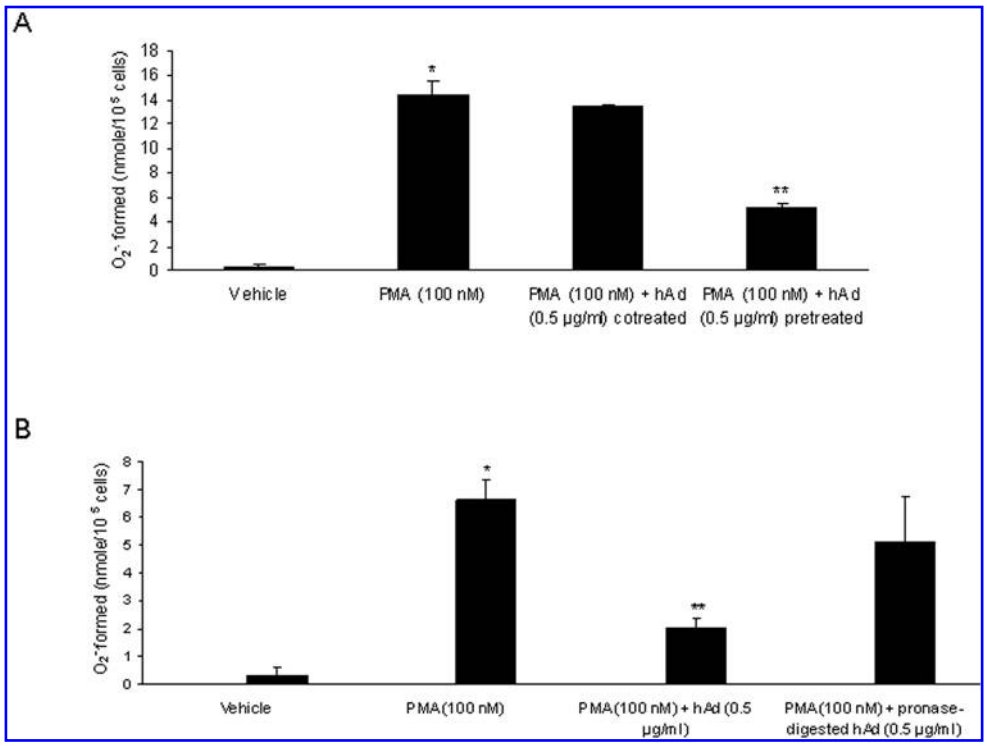

FIG. 3. Effects of pre- and cotreatment with adiponectin and pronase-digested adiponectin on PMA-induced $O_2^{-\bullet}$ generation by human neutrophils. Human neutrophils were treated with PMA (100 n*M*) in a 1-ml volume of PBS (pH 7.4) at 37°C for 30 min after (**A**) pretreatment with hAd (0.5 μg/ml) for 30 min at 37°C or cotreatment with hAd (0.5 μg/ml), and (**B**) pretreatment with hAd (0.5 μg/ml) or pronase-digested and denatured hAd (0.5 μg/ml) for 30 min at 37°C. Extracellular generation of $O_2^{-\bullet}$ by neutrophils was determined spectrophotometrically by the SOD-inhibitable cytochrome *c* reduction method, as described in Materials and Methods. Simultaneous controls were established without PMA treatment. Each histogram is an average of three independent determinations. *Significantly different from vehicle-treated control at $p < 0.05$. **Significantly different from PMA-treated cells at $p < 0.05$.

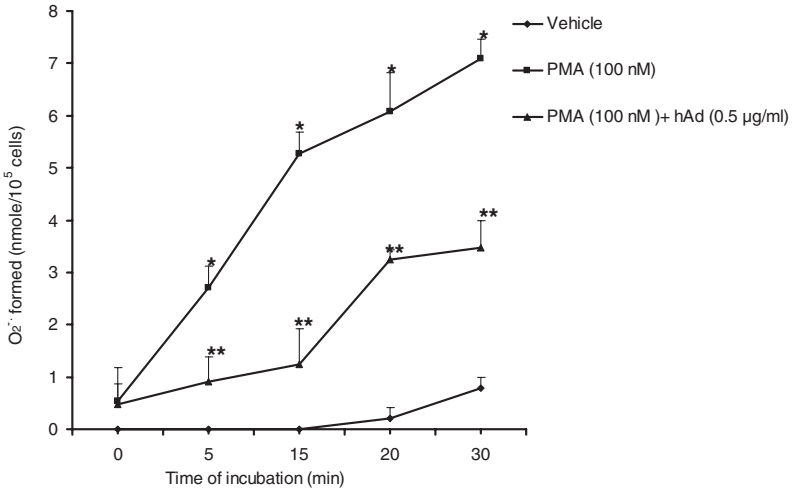

FIG. 4. Time course of adiponectin-mediated inhibition of PMA-induced $O_2^{-\bullet}$ generation by human neutrophils. Human neutrophils, after pretreatment without or with hAd (0.5 µg/ml) for 30 min at 37°C, were treated with PMA (100 n*M*) in a 1-ml volume of PBS (pH 7.4) at 37°C for 0–30 min. Extracellular generation of $O_2^{-\bullet}$ by neutrophils was determined spectrophotometrically by the SOD-inhibitable cytochrome *c* reduction method, as described in Materials and Methods. Each point is an average of three independent determinations. *Significantly different from vehicle-treated control at $p < 0.05$. **Significantly different from PMA-treated cells at $p < 0.05$.

DISCUSSION

The results of the present study revealed that pretreatment of neutrophils with recombinant full-length hAd caused a significant inhibition of agonist-induced generation of ROS independent of hAd dose. The results also showed that intact hAd in its native form was required to cause the observed attenuation of ROS generation by human neutrophils. The results further suggested that the efficacy of hAd to inhibit agonist-induced ROS formation by human neutrophils was not due to its antioxidant actions. Overall, for the first time, the results of the present study demonstrated that hAd inhibited PMA-induced ROS formation by human neutrophils.

Besides its antidiabetic and antiatherogenic actions, Ad has also been shown to possess antiinflammatory properties (18). Ad has been shown to suppress leukocyte colony formation, decrease phagocytosis, and attenuate secretion of TNF-α by macrophages (30). Circulating Ad exists as both its full-length form (fAd) and as a proteolytic protein fragment with the globular C-terminal domain (gAd) (9). gAd has been shown to inhibit oxidized low-density lipoprotein (oxLDL)-induced proliferation and $O_2^{-\bullet}$ formation in vascular ECs, wherein the suppression of oxLDL-induced $O_2^{-\bullet}$ generation has been attributed to its regulation of endothelial NAD(P)H oxidase activity (21). Agonists such as PMA, fMLP, and DAG activate NADPH oxidase of neutrophils and induce release of ROS through complex signaling events involving several agonist-specific signal mediators, with protein kinase C (PKC) as the common signaling kinase (3, 8, 14, 16). The present study showed that the full-length form of hAd, after pretreatment of cells, effectively inhibited the agonist-induced ROS generation in human neutrophils, indicating the modulation of crucial signaling events upstream of ROS generation by Ad. Also, as shown in the present study, failure to observe the inhibition of PMA-induced $O_2^{-\bullet}$ generation by neutrophils during hAd cotreatment with PMA supported the absence of nonspecific and antioxidant actions of hAd. Nevertheless, the exact site of action of hAd in exerting its inhibitory effect on ROS generation by human neutrophils, through regulation of PKC or NADPH oxidase or both, is a subject for further study.

Two distinct forms of receptors for Ad (AdipoR1 and AdipoR2) have been identified and cloned; the former has very high affinity for gAd and very low affinity for fAd, and the latter has intermediate affinity for both fAd and gAd (28). We concur with the earlier plausible mechanism proposed by Goldstein and Scalia (10) that AdipoR1 and AdipoR2 likely regulate the vascular endothelial NAD(P)H oxidase activity, and the same mechanism may also explain the hAd-mediated inhibition of ROS generation by human neutrophils, as observed in the present study. Furthermore, as shown by the present study, the lack of a dose-dependent inhibition of PMA-induced $O_2^{-\bullet}$ generation by hAd and the significant inhibition of the same by hAd in human neutrophils prompted us also to suggest the Ad receptor–mediated suppression of ROS generation by neutrophils.

Ad has also been shown to mediate crucial cellular signaling steps in organs involving the activation of the pleiotropic enzyme, AMP-activated protein kinase (AMP kinase) (10, 25, 27, 29). Activation of AMP kinase has been shown to attenuate significantly the NADPH oxidase–mediated $O_2^{-\bullet}$ release in human neutrophils that involves PKC (1). These reports also suggest that concerted signal events among AdipoR1 and AdipoR2 and downstream AMP kinase and other protein kinases are most likely involved in the regulation of NADPH oxidase activity and ROS generation by hAd in human neutrophils. Oxidative-redox operations are critical regulators of intracellular signaling pathways in many physiologic and pathophysiologic states involving key oxygen- and redox-sensitive protein kinases and transcription factors (mitogen-activated protein kinases and nuclear factor-κB) (4, 5, 11, 24). Hence, the possibilities that Ad may very well act through these pathways in exerting its modulatory effects on ROS signaling cascades are not ruled out at present. Overall, the results of the present study further support that Ad exerts its antiinflammatory activity by inhibiting the neutrophil-derived ROS as well.

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ABBREVIATIONS

Ad, adiponectin; AdipoR1, adiponectin receptor 1; AdipoR2, adiponectin receptor 2; AMP kinase, 5′-adenosine monophosphate-activated protein kinase; ANOVA, analysis of variance; DAG, diacylglycerol; DMPO, 5,5-dimethylpyrroline-*N*-oxide; DPI, diphenylene iodonium; DTPA, diethylenetriamine pentaacetic acid; EPR, electron paramagnetic resonance; fAd, full-length adiponectin; fMLP, *N*-formyl-methionyl-leucyl-phenylalanine; gAd, globular adiponectin; hAd, human adiponectin; NBF, nitroblue formazan; NBT, *p*-nitroblue tetrazolium; $O_2^{-\bullet}$, superoxide anion; oxLDL, oxidized low-density lipoprotein; PBS, phosphate-buffered saline; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α; WAT, white adipose tissue.

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Address reprint requests to:
Ulysses J. Magalang, M.D.
Division of Pulmonary, Critical Care, and Sleep Medicine
Dorothy M. Davis Heart and Lung Research Institute
473 W. 12th Ave, Room 201
The Ohio State University
Columbus, OH 43210

E-mail: magalang.1@osu.edu

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