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2 **Cross-talk between minimally-primed HL-60 cells and resting HUVEC reveals a crucial**
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4 **role for adhesion over extracellularly-released oxidants.**
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Abstract

1
2 This study demonstrates that a long-lasting co-culture of neutrophil surrogates (HL-60 cells),
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4 minimally-primed by platelet activating factor (PAF), and resting endothelial cells (EC)
5
6 results in the elaboration of an hyper-adhesive endothelial surface, as measured by the
7
8 increase in the expression of endothelial adhesion molecules E-Selectin, VCAM-1, and
9
10 ICAM-1. This endothelial dysfunction is mediated by the activation of the redox-sensitive
11
12 transcription factor NF- κ B through an exclusive adhesion-driven mechanism active in the
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14 endothelial cell: reactive oxygen and nitrogen species, extracellularly-released by minimally-
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16 primed HL-60 cells, are not involved in the induction of the endothelial dysfunction.
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24 By exploring for the first time the potential for minimally-primed neutrophil surrogates to
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26 induce endothelial dysfunction, this study suggests a novel mechanism which may be
27
28 operative in pathologies, mediated by minimally-primed neutrophils, such as
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30 hyperdyslipidemia and cardiovascular complications.
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39 **Keywords:** neutrophils, priming, endothelial cells, inflammation, adhesion, oxidants.
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1. Introduction

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2 Given the impressive array of their harmful weapons, neutrophils (PMNs) have long been
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4 considered effective but indiscriminate attackers with a low control of their responses hence
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6 with a great potential for damaging the host tissue, should these weapons become
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8 uncontrolled and/or misdirected [1]. In recent years, however, it has become increasingly
9
10 clear that the PMN response to pro-inflammatory stimuli is finely tuned to provide an
11
12 appropriate level of reactivity. One of the regulatory mechanisms to control neutrophil
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14 responses is priming [1]. PMN activation, indeed, is not an all-or-none process; rather it can
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16 be modeled as a continuum from a nonresponsive to a fully activated phenotype. Within this
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18 continuum, PMN can exist in one of three functional states: quiescent, primed or activated
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24 [1].

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28 Under non-infectious conditions PMNs are quiescent, exhibiting little or no release of
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30 oxidants; in this state, when first encountering a stimulus they reach a *primed status*. Upon
31
32 encountering a second appropriate stimulus, PMNs would then proceed to full activation
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34 releasing oxidants, granule contents and inflammatory mediators in the surrounding milieu.
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36 Noteworthy, if the PMN has been primed, its functional response (ROS production,
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38 chemotaxis) to the second stimulus will be amplified, in comparison to that of unprimed
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40 (quiescent) cells. Importantly, the priming agent itself does not cause any noticeable
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42 functional response [1-3].
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51 Interaction between PMNs and endothelial cells (EC) has been extensively investigated due to
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53 the crucial role these cells play in vascular biology and inflammatory processes. Indeed PMNs
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55 may trigger an endothelial injury seriously compromising the function of vasculature in
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1 several inflammatory pathologies as well as in relation to the inflammatory processes
2 operative in atherosclerosis, diabetes and ischemia-reperfusion injury [4-6].
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7 While it has extensively been demonstrated that fully activated PMNs do induce endothelial
8 dysfunction [7], it is now becoming evident that also primed cells can be direct mediators of
9 EC dysfunction. For instance, PMNs from patients with risk of developing atherosclerosis and
10 cardiovascular complications (e.g. essential hypertension, diabetes mellitus type II or chronic
11 renal failure) display a certain degree of priming that correlates positively with both the
12 magnitude and type of the disease, leading to an oxidative imbalance within the endothelium
13 [8-14]. This has led to propose that loose - but continuous - interaction between primed
14 neutrophils and EC may induce a chronic threshold injury to the endothelium and a systemic
15 oxidative stress able to initiate atherosclerosis and cardiovascular complications [8-10].
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17 Conversely, EC-derived factors can also contribute to maintain and/or increment PMN
18 activation.
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36 Most of the studies have so far focused on the dysfunctioning effect produced in EC by fully
37 activated or maximally primed PMNs [8, 10, 13]. While the evidence of an endothelial
38 damage by maximally primed PMN can support a pathogenic role for these cells,
39 investigating the interaction between minimally-primed neutrophils and resting EC may help
40 to understand the very early events promoting endothelial injury, possibly occurring before
41 overt EC damage.
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53 With this in mind, we have created a novel experimental model of resting EC/minimally-
54 primed PMN interaction to investigate whether, and to what extent, this cross-talk can lead to
55 endothelial activation.
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2. Materials and methods

2.1. Chemicals

Unless otherwise stated, all chemicals were from Sigma-Aldrich (Poole, UK), and of the highest available purity grade.

2.2. Cell cultures

Primary human umbilical vein ECs (HUVEC) were obtained from Lonza (Wokingham, UK) and cultured in Endothelial Growth Medium (Lonza, UK) at 37°C in a humidified atmosphere with 5% CO₂; cells were sub-cultured by trypsinization and used for the investigation up to passage four. HL-60 cells were obtained from European Type Culture Collection (ECACC, Sigma-Aldrich, Poole, UK) and maintained in RPMI-1640 with 10% heat-inactivated foetal bovine serum (Invitrogen, Paisley, UK), 1% L-glutamine, 1% non essential amino acids, 0.5% Gentamicin in a 5% CO₂ atmosphere at 37°C; cellular density was maintained between 2 and 9x10⁵ cells/ml. Cell number and viability were determined by Trypan blue exclusion. Differentiated HL-60 (dHL-60) cells were obtained by 5-day culturing in a medium containing 1% (v/v) dimethyl sulfoxide, as previously reported [15]. On day five, cells were harvested and re-suspended in fresh medium.

2.3. Assessment of HL-60 cell differentiation into neutrophil-like cells

CD11b expression was evaluated as marker of HL-60 cell differentiation into PMN-like cells [16]. Both HL-60 and dHL-60 cells were washed with a buffer containing PBS and 0.1% bovine serum albumin. Aliquots (1-5 x10⁴ cells in 20µl) were added to a 96-well plate and incubated at 4°C with 3µg/well of a mouse anti-human CD11b phycoerythrin-conjugated monoclonal antibody (Immunotech, Marseille, France). After 1 h at 4°C, cells were washed and analyzed for fluorescence on a Becton Dickinson FACScan (NJ, USA) using a Cell Quest

1 software. At least 10,000 events were analyzed for each sample. Data are expressed as median
2 fluorescence intensity units measured in the FL2 (red) channel with a 488 nm (excitation) and
3
4 585 nm (emission) filter set.
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7 8 9 2.4. Assessment of dHL-60 cell priming

10 Platelet activator factor (PAF) is a lipid mediator produced by enzymatic cleavage of
11 membrane phospholipids [1]. In addition to its activating effects on neutrophils (at a
12 concentration $> 10^{-6}$ M), PAF may also exert many priming-type actions, evident at
13 concentrations between 10^{-7} - 10^{-9} M, without stimulating PMN to produce significant amounts
14 of oxidants [17]. Thus, differentiated HL-60 cells were pre-treated with PAF (10^{-10} - 10^{-5} M) for
15 30 min at 37°C and then activated with phorbol 12-myristate 13-acetate (PMA, 0.3×10^{-7} M at
16 37°C for 60 min). Superoxide release was evaluated to assess the degree of priming.
17 Measurements of the rate of superoxide release are based on SOD inhibitable reduction of
18 80 μM cytochrome *C* to its ferrous form. The rate of $\text{O}_2^{\cdot -}$ release was monitored from 10^6
19 either HL-60 or dHL-60 cells at 22°C up to 90 min, applying a protocol previously described
20 in detail [11].
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41 2.5. HUVEC dysfunction

42 Given the minimal priming state of PMN, a long lasting co-culture was required to visualize
43 changes in cell phenotype. Primary PMN are end-stage cells that cannot be cultured for long
44 periods of time (>4 h) without losing responsiveness to various stimuli or becoming apoptotic
45 [18]. HL-60 cells are widely reported to be similar to human neutrophils in morphology,
46 expression of receptors, superoxide generation, chemotaxis and, unlike primary neutrophils,
47 can be cultured for long periods of time [18].
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1 HUVEC were grown on 6-well plates and when confluent incubated with or without 1×10^6
2 dHL-60 cells for 16 h, with or without 1×10^{-7} M PAF. When required, superoxide dismutase
3 (SOD) and catalase (CAT) were added in combination (200 U/ml each enzyme) to the culture
4 medium. In the experiments employing cell-permeable antioxidant, HUVEC were pretreated
5 for 2 h with N-acetyl-cysteine (NAC, 30 mM), washed with fresh medium and then co-
6 cultured for 16 h with dHL-60 cells as above described. In the experiments designed to
7 prevent dHL-60 cell adhesion to HUVEC, Transwell™ polycarbonate membranes inserts (24
8 mm diameter, 0.4 μ m pore size, Corning B.V. Life Sciences (Amsterdam, The Netherlands)
9 were employed: confluent HUVEC were co-cultured for 16 h with PAF-primed dHL-60 cells
10 added in the Transwell™ insert.
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27 In all cases, at the end of the incubation time HUVEC were washed with PBS, harvested with
28 cell dissociation medium and diluted in a washing buffer containing PBS, 0.1% bovine serum
29 albumin and 1 mM CaCl_2 . Aliquots (0.1 to 0.5×10^5 cells in 20μ l) were added to a 96-well
30 plate and incubated with 2μ g/well of a mouse anti-human ICAM-1 (CD54), PECAM-1
31 (CD31) or E-selectin (CD62E) monoclonal antibody (clones 15.2, 13.5 and 18.6, respectively;
32 AbD Serotec, Kidlington, UK). After 1 h at 4°C , cells were washed and incubated with 20μ l
33 of a rabbit anti-mouse F(ab')_2 polyclonal FITC-conjugated secondary antibody (Serotec,
34 Kidlington, UK) for a further 1 h. Finally, samples were washed and analyzed for
35 fluorescence on a Becton Dickinson FACScan using a Cell Quest software. HUVEC and
36 dHL-60 cells were clearly distinguished for their forward and side scatter characteristics. At
37 least 20,000 events were analyzed for each sample. Data are expressed as median
38 fluorescence intensity units, measured in the FL1 (green) channel with a 488 nm (excitation)
39 and 530 nm (emission) filter set.
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2.6. Detection of intracellular reactive oxygen and nitrogen species

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2 Intracellular levels of ROS and RNS were assessed using the non-fluorescent probe 2',7'-
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4 dichlorodihydrofluorescein diacetate (DCDHF-DA) as previously reported [19]. In brief,
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6 either EC or dHL-60 cells were cultured as described above. At selected time points, cells
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8 were washed with warm PBS, 100 μ M DCDHF-DA (Molecular Probes, OR, USA) was added
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10 to the culture system and cells were incubated at 37°C in a humidified atmosphere with 5%
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12 CO₂. After 45 minutes cells were washed with PBS, harvested, diluted in a FACS buffer
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14 containing PBS and 0.1% bovine serum albumin, and analyzed for fluorescence on a Becton
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16 Dickinson FACScan using a Cell Quest software. At least 20,000 events were analyzed for
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18 each sample. Data are expressed as median fluorescence intensity units as measured in the
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20 FL1 channel.
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2.7. NF- κ B luciferase assay

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30 NF- κ B activity was examined by transfecting HUVEC with pNF- κ B-Luc luciferase construct
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32 (Stratagene, CA, USA). Transfections were carried out using Amaxa Nucleofector
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34 Technology (Lonza, UK). Briefly, HUVEC were passaged two days before nucleofection and,
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36 on the day of the experiment, 100 μ l of a cell suspension at 0.5×10^6 cell/ml was combined
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38 with 3 μ g of pNF- κ B-Luc luciferase construct, transfected according to the manufacturer
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40 protocol and incubated for 12 h in a 5% CO₂ atmosphere at 37°C. Transfected HUVEC were
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42 thereafter co-cultured with PAF-primed dHL-60 cells and the specific treatments applied as
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44 detailed above. After 6 h, cells were washed with PBS and lysed for 5 min at 4°C using a lysis
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46 buffer according to the manufacturer's instructions (Promega, WI, USA). Luciferase activity
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48 is expressed as relative luminescence units (RLU) $\times 10^3$ following reading in a TD/2020
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50 luminometer (Turner Biosystems, CA, USA).
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2.8. Statistical Analysis

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2 Data are reported as mean \pm SEM of n experiments performed in triplicate or quadruplicate.
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4 Differences among experimental groups were assessed using one-way analysis of variance
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6 and, once significant, applying the Dunnett test to selected experimental groups. A probability
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8 value less than 0.05 was considered significant.
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3. Results

3.1. Priming effect of PAF on dHL-60 cells.

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21 Primed PMN are pre-activated cells such that exposure to a subsequent inflammatory
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23 stimulus generates an enhanced response with respect to resting cells. Preliminary assays
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25 were carried out to establish the concentration of PAF required to yield minimally-primed
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27 dHL-60 cells. As shown in **Figure 1**, stimulation with PAF $< 10^{-7}$ M did not prime cells, as
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29 they did not release any significant amount of $O_2^{\cdot-}$ above the levels of control cells exposed to
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31 PMA. In contrast, PAF concentrations between 10^{-7} - 10^{-5} M primed dHL-60 cells in a
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33 concentration-dependent fashion, as judged by the increment of PMA-stimulated superoxide
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35 release (**Figure 1**). No release of $O_2^{\cdot-}$ was measured when cells were not treated with PMA,
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37 irrespective of the PAF concentration used for priming (not shown). Thus, 10^{-7} M PAF was
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39 regarded to be the minimal concentration required to significantly prime, without activating,
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41 dHL-60 cells.
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3.2. Co-culture of minimally-primed dHL-60 cells with resting HUVEC causes EC dysfunction.

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56 Next we investigated the effects of a long-lasting co-culture of minimally-primed dHL-60
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58 cells with resting HUVEC on the expression of the major EC adhesion molecules. Flow
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cytometry allowed endothelial cells and adherent leukocytes to be distinguished in view of their different scatter profiles: HUVEC showed a spread distribution with high FSC and SSC values (**Figure 2A** and **B**) whereas dHL-60 cells appeared as a lower and more compact population (**Figure 2B**). Co-incubation of minimally-primed dHL60 cells with HUVEC for 16 h induced a significant increase of E-Selectin, VCAM-1 and ICAM-1 levels ($P < 0.01$) (**Figure 1D-F**, respectively) whereas the expression of PECAM-1 did not show any significant change (**Figure 1C**), demonstrating that the HUVEC dysfunction was not consequent to non-specific loss of cell viability or phenotype.

3.3. Minimally-primed dHL-60 cell-induced endothelial dysfunction is mediated by a redox-dependent mechanism.

We then investigated the mechanisms by which minimally-primed dHL-60 cells might cause the HUVEC activation. Since changes in endothelial adhesion molecule expression can be induced by changes of the endocellular redox milieu [20], oxidative stress inside EC was then evaluated. Co-incubation of minimally-primed dHL-60 cells with resting HUVEC induced a time-dependent increase of reactive species (ROS/RNS) inside EC that peaked at 4 h, returning to basal levels >12 hours (**Figure 3A**).

Closer monitoring of the time-course of E-Selectin, VCAM-1 and ICAM-1 expression in the same conditions, provided evidence that the increase of the adhesion molecule expression started only after a critical ROS/RNS level was achieved (i.e. 4 h), with a maximum expression at 16 h (Figure 3A, inset). Consistent with this, pretreatment of HUVEC with N-acetyl-cysteine (NAC), a cell-permeable thiol radical scavenger widely used to assess the role of reactive species in gene expression [21], abrogated the expression of ICAM-1, VCAM-1 and E-selectin induced by minimally-primed dHL60 (**Figure 3B**).

3.4. Activation of NF- κ B in HUVEC by minimally-primed dHL-60 cells

It is well established that endothelial adhesion molecule expression is under control of several redox-regulated transcription factors, including the master minder NF- κ B [22]. To determine whether this possible cascade of events was indeed occurring in our experimental settings, we transfected HUVEC with a NF- κ B reporter construct prior to co-culture with minimally-primed dHL-60 cells. Luciferase activity in HUVEC co-cultured with control un-primed dHL-60 cells was not significantly different than that observed in HUVEC alone (not shown). Conversely, co-culture of HUVEC with minimally-primed dHL-60 cells caused a significant increase (~ 3-fold) in the activation of this transcription factor within the EC (**Figure 4A**).

3.5. HUVEC dysfunction is mediated by cell-cell interaction and not by extracellularly-released oxidants.

It has been reported that PMN-induced EC activation can be triggered by oxidants released by adherent PMN, adhesion molecule ligation, or both [7]. In order to investigate which event was to be considered crucial for the EC activation measured in our system we determined the effect of superoxide dismutase and catalase on NF- κ B activation and ICAM-1 upregulation in HUVEC. No differences in both NF- κ B activation and ICAM-1 up-regulation were observed between HUVEC stimulated in the absence or in the presence of these two antioxidant enzymes (**Figure 4B**), suggesting that released oxidants, whenever released by adherent PMN, were ineffective on the modulation of redox-regulated signaling pathways.

To investigate the role of physical adhesion between HUVEC and minimally-primed dHL-60 cells on the process leading to endothelial dysfunction, HUVEC were co-cultured with minimally-primed dHL-60 cells, either in the absence or in the presence of a Transwell™

1 insert, a device that allows EC and dHL-60 cells to exchange soluble molecules, but prevents
2 the physical contact between the two cell types. As shown in **Figure 4B**, physical separation
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4 between cells abolished both NF- κ B activation and ICAM-1 up-regulation, indicating that cell
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6 adhesion is necessary and sufficient to trigger the signaling events conducive to EC
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8 dysfunction.
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11 3.6. Cross-talk between minimally-primed dHL-60 cells and resting HUVEC.

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Though priming pre-disposes neutrophils to a prompt activation, primed PMN by themselves are not capable of producing reactive species. When co-cultured with resting HUVEC for 16h, minimally-primed dHL-60 cells showed a significantly increase in the endocellular levels of ROS/RNS, peaking at 4h. On the other hand, when they were prevented to adhere to EC, by being cultured inside a TranswellTM inserts, no intracellular production of ROS/RNS was detected (**Figure 5**). This provides evidence that adhesion-driven signals establish a real cross-talk between EC and dHL-60 cells, yielding EC activation. In our experimental settings cell adhesion represents the “second stimulus” that leads primed-neutrophils to full activation.

41 **4. Discussion**

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This study investigate endothelial dysfunction mediated by primed PMN, and explores mechanistic aspects of minimally-primed neutrophil surrogates to potentially induce EC activation. Our results support previous studies on the pathogenic role of primed neutrophils [1, 8-14] where it has been shown that minimally-primed neutrophils are able to induce over-expression of E-Selectin, VCAM-1 and ICAM-1, thus promoting an activating phenotype of EC. However, several novel observations were made here.

1 First, minimally-primed neutrophil surrogates can activate EC as long as they are co-cultured
2 for a time period considerably higher (16 h) than that reported in studies employing highly-
3 primed neutrophils from hemodialysis, diabetic or hyperlipidemic patients (15 min) [10, 13,
4 14]. Secondly, minimally-primed dHL-60 cells neither alter EC viability nor induce
5 irreversible lethal events that would result in cell detachment and apoptosis, outcomes
6 reported when highly primed PMN are used [10, 13, 14]. This is coherent with the threshold
7 degree of priming employed here, at variance from that used in previously published studies.
8 At a mechanistic level, our data demonstrate for the first time that minimally-primed HL-60
9 generate endothelial intracellular signals, namely variation in the endothelial redox milieu and
10 downstream activation of NF- κ B, resulting in EC dysfunction.
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27 In endothelial cells, the activation of NF- κ B by oxygen radicals is a cardinal event leading to
28 up-regulation of several adhesion molecules including ICAM-1 and VCAM-1. Furthermore,
29 treatment of TNF- α -stimulated cells with antioxidants such as NAC and pyrrolidine
30 dithiocarbamate reduces Ser32 phosphorylation leading to a reduction in NF- κ B activity in
31 endothelial cells. Our results are consistent with these findings and show a strong reduction of
32 E-selectin, ICAM-1 and VCAM-1 upregulation in HUVEC co-cultured with minimally-
33 primed dHL-60 in presence of NAC providing a direct link between activation of NF- κ B and
34 the increased expression of these adhesion molecules.
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49 More importantly, if integrated in the wider frame of knowledge related to the pathogenic role
50 of primed neutrophils, our results also suggest that EC dysfunction can be the result of a
51 continuous interaction of minimally-primed PMN with endothelium, finally leading to a clear
52 injury. Moreover, we suggest that the extent of neutrophil priming is crucial to influence the
53 way the EC response can be triggered. Indeed, we show that when neutrophils are minimally-
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1 primed, EC activation is achieved through adhesion-driven signals whereas, as elsewhere
2 reported [10], soluble factors released by these cells overcome direct adhesive effects when
3 highly-primed PMN are used.
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10 Finally, a pivotal function for physical adhesion between EC and minimally-primed dHL60
11 was found. Adhesion to the monolayers led to production of reactive species in the
12 neutrophil-like cells too, stimulating a real cross-talk between these two cell types instead of a
13 mere “one-way” activation of EC.
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22 In conclusion, the present study reveals a function for intracellular, but not extracellularly-
23 released, oxidants and, more importantly, describes how a minimal degree of leukocyte
24 activation might be sufficient to elicit these intracellular responses which would, then, impact
25 on the cell phenotype. Based on these results we propose a new mechanism of EC
26 dysfunction that maybe relevant to clinical states where a persistent threshold level of
27 neutrophil activation occurs, without overt cell activation or neutrophil-dependent cell injury.
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29 Indeed, since initial endothelial dysfunction is a fundamental event in hyperdyslipidemia-
30 associated and inflammation-based diseases such as atherosclerosis and cardiometabolic
31 complications, our findings may possibly suggest new pharmacological targets helpful to the
32 development of preventive strategies.
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Figure legends

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2 **Figure 1. Effect of PAF on dHL-60 cell-released $O_2^{\cdot-}$.** Cell were exposed to the indicated
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4 concentration of PAF immediately prior to addition of PMA (as indicated in the Method
5
6 section); the rate of $O_2^{\cdot-}$ release is measured as nmol/ 10^6 cell/h. Data are means \pm SE of 3
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8 distinct experiments with different cell preparations. * $P < 0.05$ vs. control.
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14 **Figure 2. Flow cytometry analysis of endothelial PECAM-1, E-selectin, VCAM-1 and**
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16 **ICAM-1 expression induced by PAF-primed dHL-60 cells.** (A, B) Dot plots of the scatter
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18 characteristic of HUVEC alone (panel A) and co-cultured with PAF-primed dHL-60 cells
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20 (panel B), representative of five separate experiments. Solid lines represent gating on
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22 HUVEC. (C-F) Endothelial PECAM-1, E-selectin, VCAM-1 and ICAM-1 expression induced
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24 by PAF-primed dHL-60 cells. HUVEC were cultured either in the absence (CTRL) or in the
25
26 presence of PAF-primed dHL-60 cells (HL-60). Data are means \pm SEM of 5 separate
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28 experiments. * $P < 0.05$ vs. control.
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36 **Figure 3. Time-course of EC dysfunction.** (A) Kinetics of endothelial ROS/RNS increase
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38 and E-selectin, VCAM-1 and ICAM-1 expression (inset) induced by PAF-primed dHL-60
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40 cells. (B) Effect of NAC on E-selectin, VCAM-1 and ICAM-1 expression induced by PAF-
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42 primed dHL-60 cells. HUVEC were either left untreated (CTRL) or co-cultured with PAF-
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44 primed dHL-60 cells (HL-60/NONE) or pretreated with NAC and then co-cultured with PAF-
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46 activated dHL-60 cells (HL-60/NAC). Data are means \pm SE of 3 separate experiments.
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51 * $P < 0.05$ vs. control.
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56 **Figure 4. NF- κ B activation in EC.** (A) Effect of PAF-primed dHL-60 cells on endothelial
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58 NF- κ B activation. HUVEC were either left untreated (CTRL) or co-cultured with PAF-
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1 primed dHL-60 cells (HL-60) for 6 hours. (B) Effect of cell-impermeable antioxidants or
2 Transwell[®] inserts on NF- κ B activation and ICAM-1 expression induced by PAF-primed
3 dHL-60 cells. HUVEC were co-cultured with PAF-primed dHL-60 cells either in the absence
4 (NONE) or presence of superoxide dismutase and catalase (SOD/CAT; 200 U/ml) or
5 employing Transwell[®] inserts (TW). Untreated HUVEC were also tested (CTRL). Data are
6 means \pm SE of 3 separate experiments. * P <0.05 vs. control.
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17 **Figure 5. Time course of intracellular ROS/RNS generation in dHL-60 cells.**

18 Differentiated HL-60 cells were primed with 10^{-7} M PAF and either left alone (●), or co-
19 cultured with HUVEC in the absence (▽) or in the presence (◆) of Transwell[®] inserts. At
20 selected time points, ROS/RNS generation inside dHL-60 was evaluated. Data are mean \pm SE
21 of 3 separate experiments. * P <0.05 vs. dHL-60 alone.
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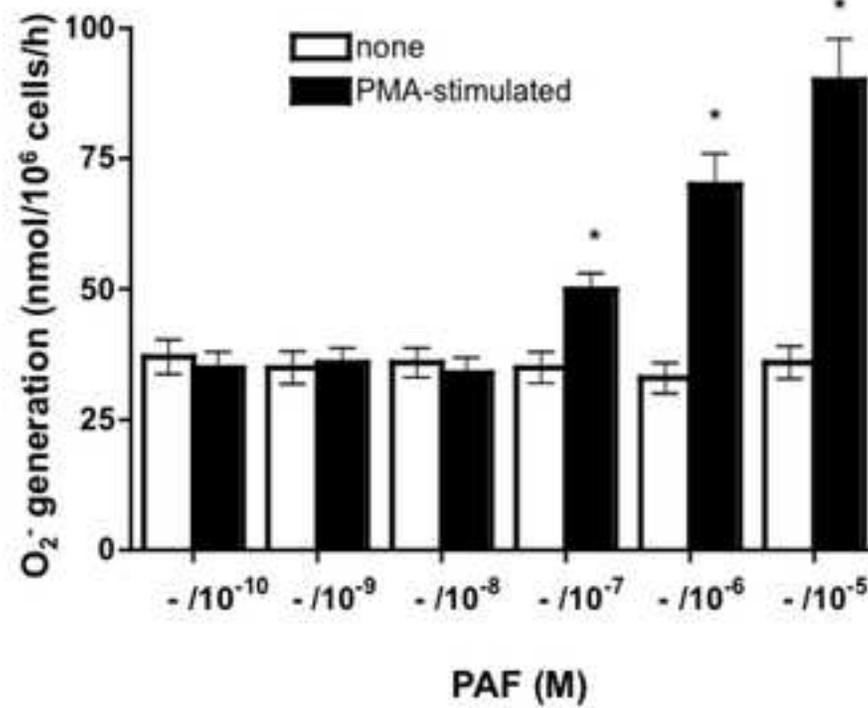
34 **Acknowledgements**

35 This study was supported by the William Harvey Research Foundation.
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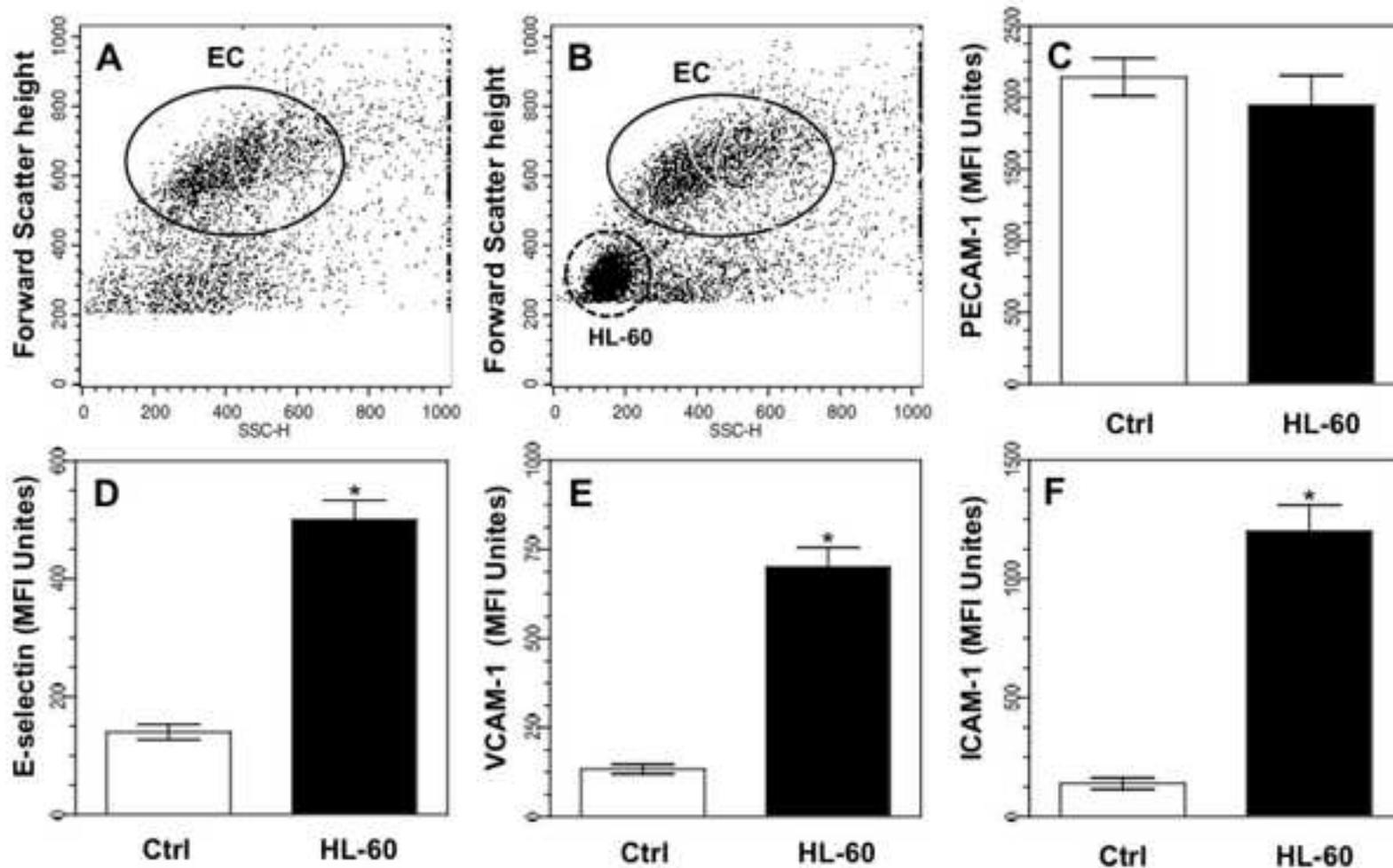
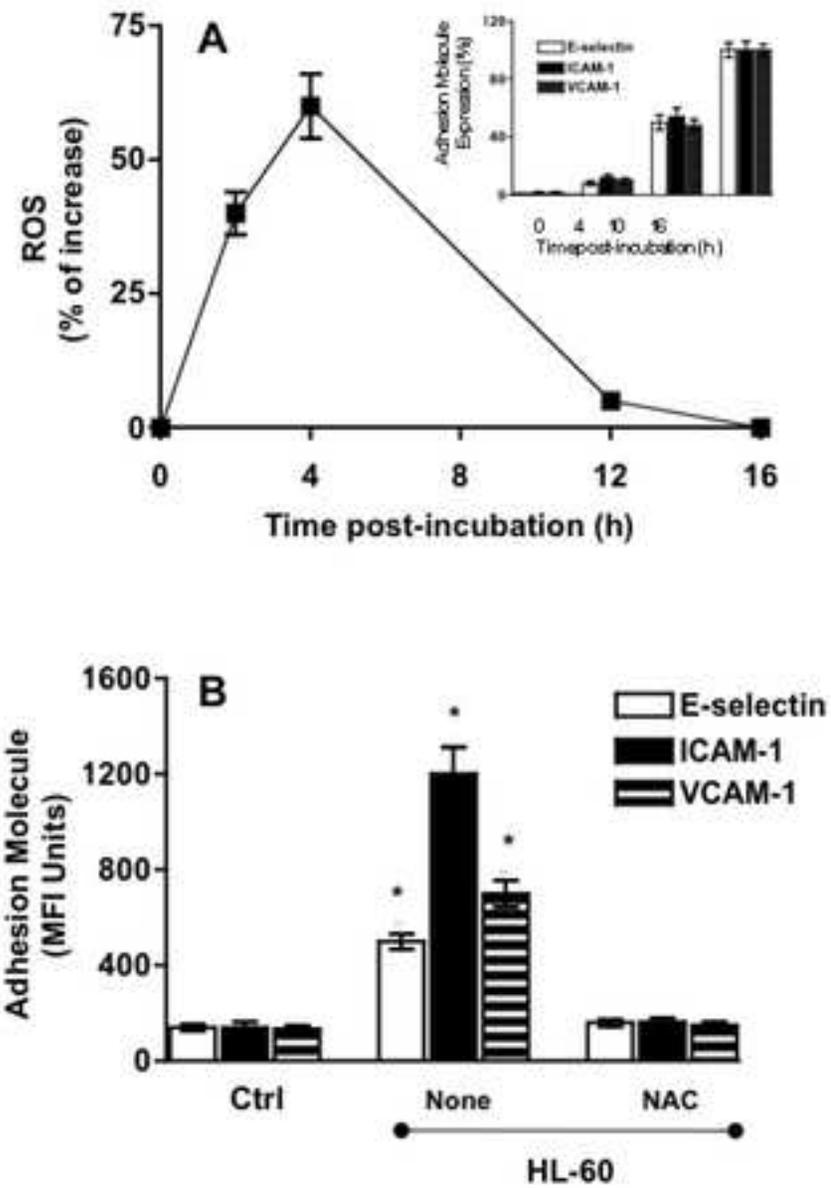


Figure 3
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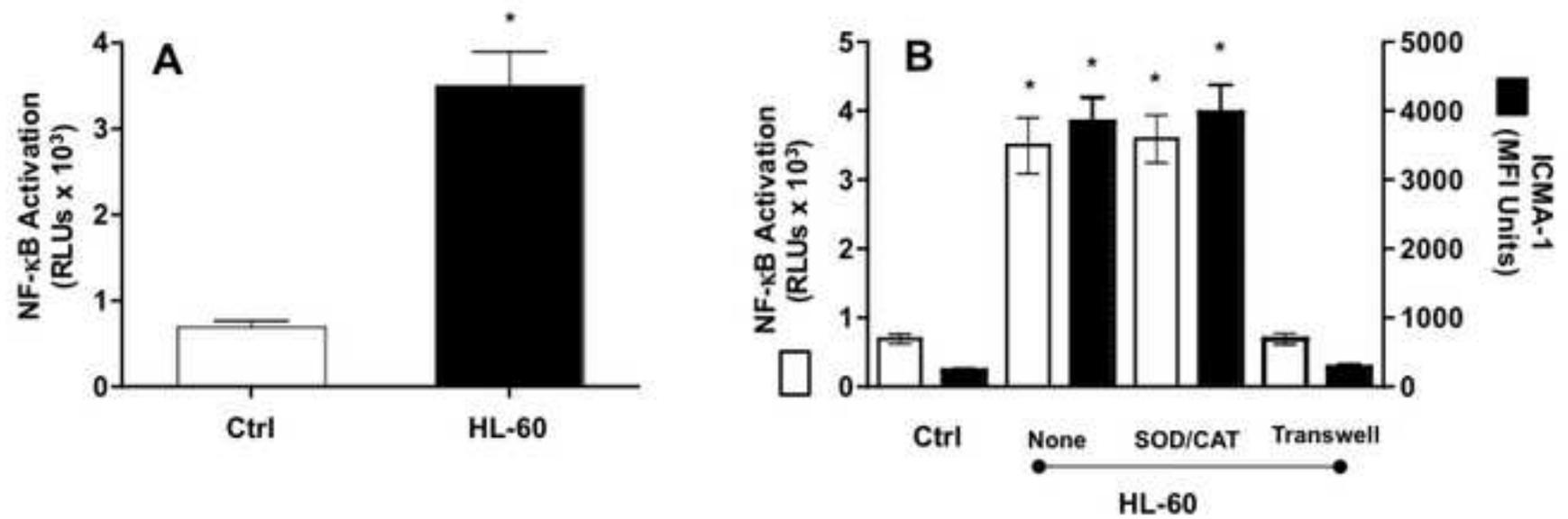
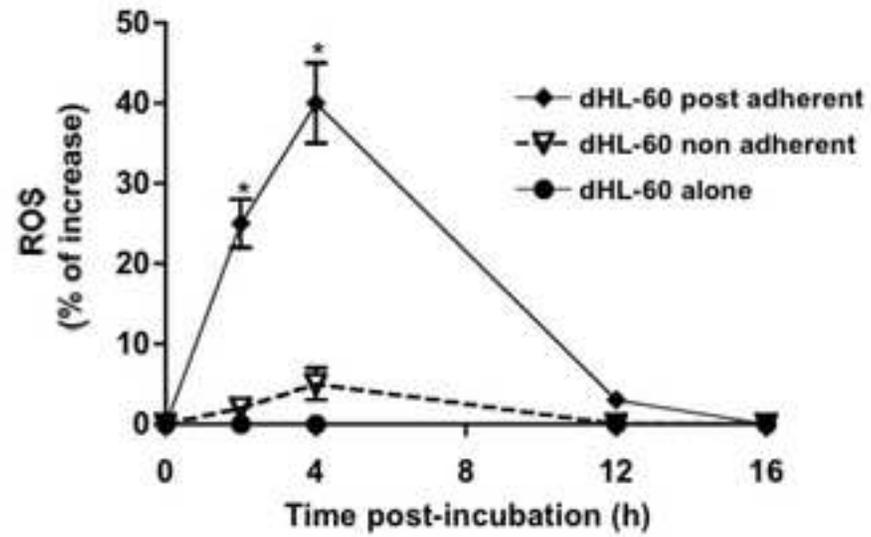


Figure 5
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Resting Leukocyte

- No adhesion
- No EC activation

PAF-primed Leukocyte

- Adhesion to EC
- EC activation → ↑Adhesion molecule expression
- EC dysfunction

