

# T cell contact-mediated activation of respiratory burst in human polymorphonuclear leukocytes is inhibited by high-density lipoproteins and involves CD18

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**Abstract:** Polymorphonuclear neutrophils (PMN) are recruited to sites of inflammation, where they are in close vicinity with other immune cell types. The present study demonstrates that direct cell-cell contact with stimulated T cells activates PMN respiratory burst. To discard interferences with soluble products, membranes isolated from human T lymphocytes (msT) or the monocytic cell line HUT-78 (msHUT) were used to mimic cellular contact. msT and msHUT induced a dose-dependent production of radical oxygen species (ROS) in PMN, as detected by chemiluminescence. Similar results were obtained with fixed, stimulated T cells, confirming that ROS production was a result of cell-surface molecules and not to soluble products of T cells. ROS production was mainly intracellular, suggesting that ROS may take part in intracellular processes. High-density lipoproteins (HDL), which had previously been shown to inhibit T cell contact-induced cytokine production in monocyte-macrophages, potently reduced ROS production induced in PMN upon contact with stimulated T cells. This supports the emerging role of HDL as immunomodulators in inflammatory diseases. Furthermore, monoclonal antibodies to CD18 inhibited 60% of the PMN respiratory burst induced by msT, suggesting that CD18 contributed to PMN activation. The present results emphasize the importance of direct cell-cell contact with stimulated T cells in inflammatory processes. *J. Leukoc. Biol.* 77: 000–000; 2005.

**Key Words:** oxidative metabolism · lymphocyte activation · neutrophil

## INTRODUCTION

During the inflammatory response, polymorphonuclear leukocytes (PMN) are recruited to the sites of inflammation, where they produce reactive oxygen species (ROS), release a variety of proteolytic enzymes, and phagocytose pathogens or fragments of damaged tissue. In inflammatory processes, infiltrating cells such as PMN, mononuclear phagocytes, and T and B

lymphocytes are in close vicinity together with resident cells and can therefore communicate through cell-cell contact. PMN are the predominant and earliest infiltrating cells in many acute and chronic inflammatory conditions, and the regulation of the immunoinflammatory processes depends to a large extent on the control of ROS generation [1]. Indeed, although it contributes to the elimination of pathogenic microorganisms, PMN respiratory burst could be harmful by causing membrane lipidic peroxidation, which results in cell and tissue destruction at the sites of inflammation [2]. We demonstrated previously that cellular contact with stimulated T cells can prime PMN for further activation by N-formyl-methionyl-leucyl-phenylalanine (fMLP) [3, 4]. It is interesting that this activity correlated with the ability of stimulated T cells to induce interleukin-1 $\beta$  (IL-1 $\beta$ ) in monocyte-macrophages [4]. Indeed, direct cell-cell contact between T cells and monocyte-macrophages is considered a major mechanism that triggers the production of inflammatory cytokines such as IL-1 $\beta$  and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), which play an important part in chronic inflammatory diseases (reviewed in ref. [5]). The identity of the surface molecule(s) involved in contact-mediated activation of monocyte-macrophages is still elusive. Some studies have shown that CD40-CD40 ligand (CD40L) might be involved to some extent in proinflammatory cytokine production [6, 7]. CD69 and  $\beta$ 2-integrins, i.e., lymphocyte function-associated antigen-1 (CD18/CD11a), membrane-activated complex-1 (CD18/CD11b), and p153,95 (CD18/CD11c), as well as intercellular adhesion molecule-1 (ICAM-1), have also been shown to participate in the cell contact-mediated activation of monocyte-macrophages by stimulated T cells [8–10]. However, blocking antibodies to the latter molecules and to others (CD2, CD18, CD23, CD29, CD95), as well as specific inhibitors of inflammatory cytokines such as TNF-soluble receptors and IL-1 receptor antagonist, failed to abolish this activation, suggesting that one or several other factors expressed at the surface of stimulated T cells are primarily involved in monocyte-macrophage activation.

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The induction of cytokine production in monocyte-macrophages upon direct cell–cell contact with stimulated T cells was strongly inhibited by high-density lipoproteins (HDL), which are likely to interact through their major protein component, apolipoprotein A-I, with a ligand at the surface of T cells [11], thus inhibiting specifically the activation of monocyte-macrophages. This study shows that direct cell–cell contact with stimulated T cells induces the production of ROS in PMN and that this mechanism is inhibited by HDL and involves CD18 engagement.

## MATERIALS AND METHODS

### Materials and reagents

The following reagents were purchased from the designated suppliers: fMLP, phorbol myristate acetate (PMA), type II horseradish peroxidase (HRP), luminol (5-amino-2,3-dihydro-1,4-phtalazindione), nitro blue tetrazolium (NBT), superoxide dismutase (SOD), and catalase (Sigma Chemical Co., St. Louis, MO); phosphate-buffered saline (PBS), RPMI 1640, L-glutamine, penicillin, and streptomycin (Gibco, Paisley, Scotland); fetal calf serum (FCS; Flow Laboratories, Meckenheim, Germany); purified phytohemagglutinin (PHA; EY Laboratories, San Marco, CA); and Ficoll-Paque (Pharmacia, Uppsala, Sweden).

### Monoclonal antibodies (mAb)

Dr. Peter E. Lipsky (National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD) provided anti-CD40L mAb (Clone 5C8). Mouse mAb to CD11a [immunoglobulin G (IgG)2a, Clone 38, ANC-158-020], CD11b (IgG1, Clone ICRF44, ANC 159-020), CD11c (IgG1, Clone 3.9, ANC-160-020), to CD18 (IgG2a, Clone IB4, ANC-167-020) and CD69 (IgG2a, Clone HP-4B3, ANC-221-020) were purchased from Alexis Corporation (Läufelfingen, Switzerland). None of the antibody preparations contained azide.

### T cells

The human T cell line HUT-78, obtained from American Type Culture Collection (Manassas, VA), was maintained in RPMI-1640 medium supplemented with 10% heat-inactivated FCS, 50  $\mu$ g/ml streptomycin, 50 U/ml penicillin, and 2 mM L-glutamine in a 5% CO<sub>2</sub> air-humidified atmosphere at 37°C. T lymphocytes were isolated from human peripheral blood of healthy donors as described previously [8]. Isolated T lymphocytes ( $4 \times 10^6$  cells/ml) were stimulated by 1  $\mu$ g/ml PHA and 5 ng/ml PMA. After 48 h of culture in 5% CO<sub>2</sub> air-humidified atmosphere at 37°C, stimulated T lymphocytes were washed in PBS, and their membranes (msT) were isolated as described previously [12]. HUT-78 cells ( $1 \times 10^6$  cells/ml) were stimulated for 6 h with 1  $\mu$ g/ml PHA and 5 ng/ml PMA and washed, and their membranes were isolated [13]. Alternatively, HUT-78 cells were fixed with paraformaldehyde as described previously [8]. Plasma membranes of stimulated T cells (msHUT) were used to activate PMN, considering that their activity in human monocytes was similar to that of living T cells (i.e., in cocultures) or fixed T cells as described previously [11]. The involvement of a putative carryover of PMA and PHA by T cell membranes in target cell activation was previously ruled out [3, 8].

### PMN preparation

PMN were isolated from heparinized blood collected from healthy donors as described previously [14]. Cells ( $1 \times 10^7$  cells/ml) were resuspended into external solution (ES) without Ca<sup>2+</sup>, consisting of 10 mM Hepes, pH 7.2, containing 140 mM NaCl, 5 mM KCl, 1 mM MgCl<sub>2</sub>, and 0.1% (10 mM) glucose, kept on melting ice, and used within 2–4 h.

### HDL isolation

Human serum HDL were isolated by high-density ultracentrifugation as described previously [11]. To optimize the inhibitory effect of HDL, they were consistently added together with the stimulus.

## Chemiluminescence measurement

PMN ( $2 \times 10^6$  cells/200  $\mu$ l/well, i.e.,  $1 \times 10^7$  cells/ml) were stimulated by the indicated stimulus in ES containing 2 mM CaCl<sub>2</sub>, 5 U/ml HRP type II, and 50  $\mu$ M luminol [15]. Chemiluminescence was determined on a Luminoskan Ascent (Labsystems, Franklin, MA), using 96-well white plastic plates (Cliniplate, Bioconcept, Switzerland). To assess intracellular production of ROS, chemiluminescence was tested in the presence of 200 U/ml SOD and 2000 U/ml catalase to scavenge extracellular ROS.

## Detection of ROS by NBT

NBT test was performed as described previously [16]. Briefly, PMN ( $1 \times 10^7$  cells/ml) were incubated at 37°C with fixed HUT-78 cells in ES containing 2 mM CaCl<sub>2</sub> in the presence of 1.7 mg/ml NBT (yellow) for the indicated time. In the presence of oxidative metabolites, NBT is reduced to formazan, which forms a blue precipitate, detected by light microscopy.

## RESULTS

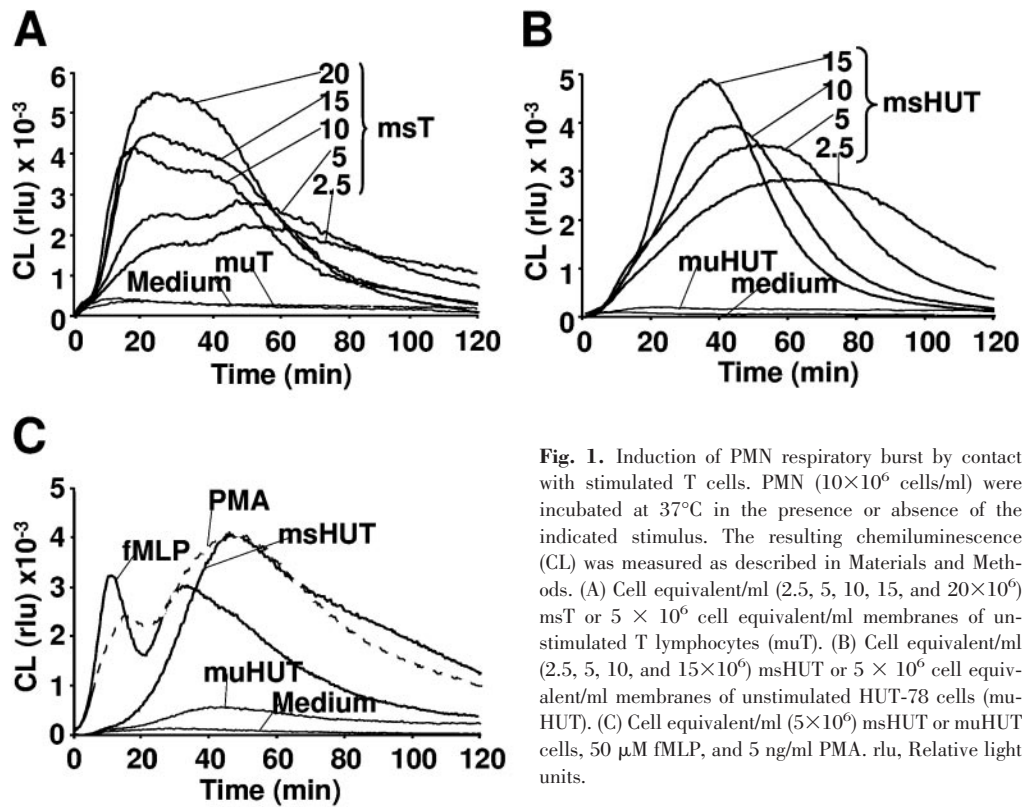
### Membranes of stimulated T cells activate PMN respiratory burst

To ascertain whether direct contact with stimulated T cells would trigger PMN respiratory burst, membranes of PHA/PMA-stimulated T lymphocytes (msT) were added to PMN in the presence of luminol, which detects intra- and extracellular ROS. As shown in **Figure 1**, increasing amounts of msT resulted in a dose-dependent enhancement of rate and level of production of reactive oxidant in PMN (Fig. 1A). Similar results were obtained with membranes isolated from msHUT (Fig. 1B). In contrast, membranes isolated from unstimulated T cells (T lymphocytes or HUT-78 cells) did not induce PMN respiratory burst (Fig. 1, A and B).

To determine whether the respiratory burst induced by cellular contact was of significant magnitude, the chemiluminescence observed upon stimulation by msHUT was compared with stimulation by commonly used activators of PMN respiratory burst, i.e., fMLP and PMA. As shown in Figure 1C, msHUT induced PMN respiratory burst to an extent that was comparable with that triggered by optimal concentrations of fMLP and PMA, although some variations in patterns were observed. Indeed, activation by fMLP and PMA resulted in a two-phase ROS production (Fig. 1C), the second phase observed with PMA being more sustained. The activation of PMN by msHUT was slower, and although it lacked the first rapid induction phase, it displayed a sustained activation similar to the second activation phase by PMA (Fig. 1C). Together, the results shown in Figure 1 demonstrate that membrane-associated molecules at the surface of T cells (T lymphocytes and HUT-78 cells) were potent stimulators of PMN respiratory burst.

### PMN activation is a result of cell–cell surface contact with stimulated T cells

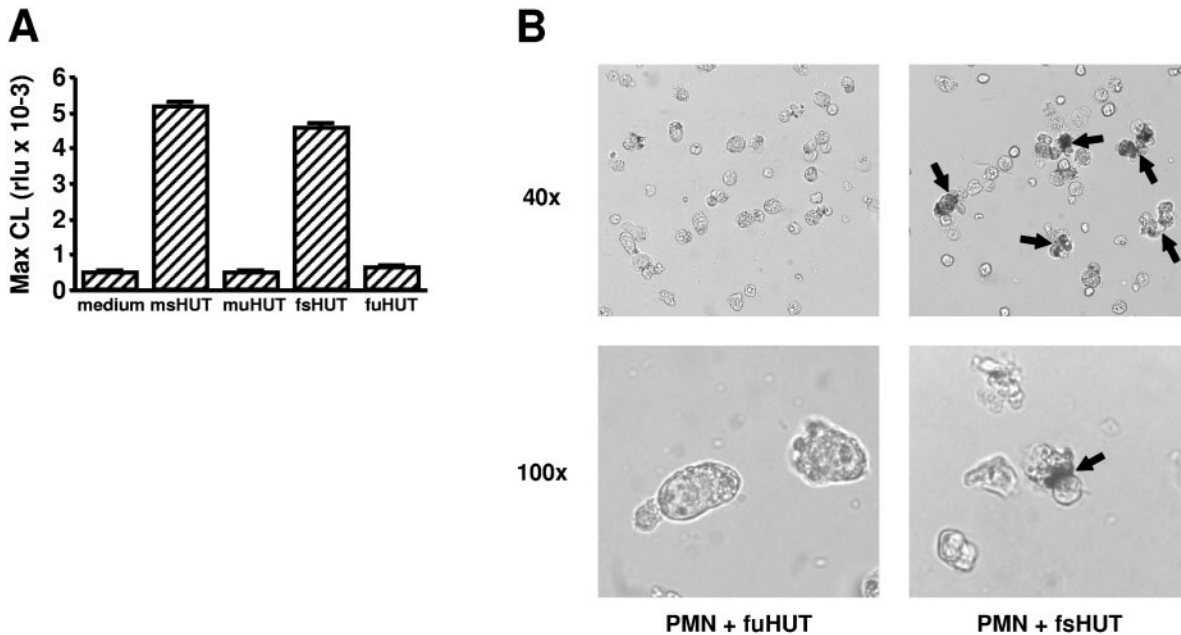
Considering that membrane preparations might expose intracellular components and contain fragments of intracellular structures, fixed, stimulated T cells were used to determine whether ROS production was induced in PMN by molecules at the surface of T cells. Fixed, stimulated, but not unstimulated, HUT-78 cells triggered respiratory burst in PMN in a similar



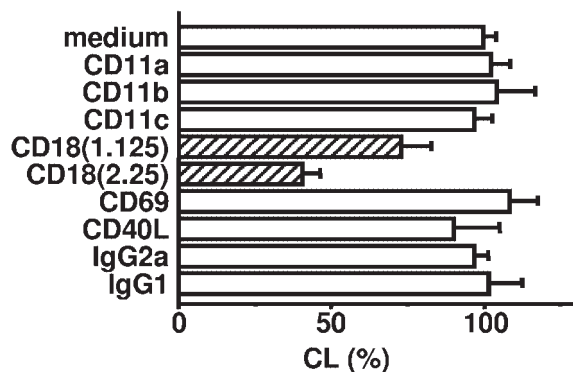
**Fig. 1.** Induction of PMN respiratory burst by contact with stimulated T cells. PMN ( $10 \times 10^6$  cells/ml) were incubated at  $37^\circ\text{C}$  in the presence or absence of the indicated stimulus. The resulting chemiluminescence (CL) was measured as described in Materials and Methods. (A) Cell equivalent/ml ( $2.5, 5, 10, 15,$  and  $20 \times 10^6$ ) msT or  $5 \times 10^6$  cell equivalent/ml membranes of unstimulated T lymphocytes (muT). (B) Cell equivalent/ml ( $2.5, 5, 10,$  and  $15 \times 10^6$ ) msHUT or  $5 \times 10^6$  cell equivalent/ml membranes of unstimulated HUT-78 cells (muHUT). (C) Cell equivalent/ml ( $5 \times 10^6$ ) msHUT or muHUT cells,  $50 \mu\text{M}$  fMLP, and  $5 \text{ ng/ml}$  PMA. rlu, Relative light units.

way to membranes isolated from HUT-78 cells (Fig. 2A). To ensure that contact with stimulated T cells was directly activating PMN, the latter cells were incubated in the presence of fixed HUT-78 cells and NBT to visualize ROS production in

living cells simultaneously. As shown in Figure 2B, stimulated and unstimulated HUT-78 cells were in close contact with PMN. ROS production occurred mainly with fixed, stimulated HUT-78 cells, resulting in a marked activation of 24% of total



**Fig. 2.** Fixed, stimulated HUT-78 cells displayed similar activity to membranes of the latter cells. (A) PMN ( $10 \times 10^6$  cells/ml) were incubated at  $37^\circ\text{C}$  with fixed, stimulated (fsHUT) or unstimulated (fuHUT) HUT-78 cells ( $4 \times 10^6$  cells/ml) or membranes of the latter cells, i.e., msHUT and muHUT, respectively. Results are expressed as maximum chemiluminescence (Max CL; arbitrary units). (B) PMN ( $10 \times 10^6$  cells/ml) were incubated at  $37^\circ\text{C}$  with  $5 \times 10^6$  cells/ml fuHUT or  $5 \times 10^6$  cells/ml fsHUT cells in the presence of  $1.7 \text{ mg/ml}$  NBT. Pictures were taken after 50 min of incubation at various magnifications (upper panels,  $40 \times$  original; lower panels,  $100 \times$  original). Solid arrows indicate the formation of blue (dark) formazan upon yellow NBT reduction.



**Fig. 3.** Induction of ROS production in PMN by membranes of stimulated HUT-78 was partially a result of CD18. PMN ( $10 \times 10^6$  cells/ml) were incubated at  $37^\circ\text{C}$  with membranes of stimulated HUT-78 cells ( $1.25 \times 10^6$  cell equivalent/ml) in the presence of antibody to the indicated surface molecule or control IgG isotype; anti-CD11a, -b, or -c ( $10 \mu\text{g/ml}$ ), anti-CD18 (1.125 or 2.25  $\mu\text{g/ml}$ ), anti-CD69 ( $10 \mu\text{g/ml}$ ), anti-CD40L ( $10 \mu\text{g/ml}$ ), IgG2a ( $10 \mu\text{g/ml}$ ), and IgG1 ( $10 \mu\text{g/ml}$ ). Results are expressed as percentage of maximum chemiluminescence (CL) observed in the absence of antibodies (medium), mean  $\pm$  SD,  $n = 3$ .

PMN, and in the presence of unstimulated HUT-78 cells, only 7% of PMN was slightly activated after 50 min. Furthermore, there was no engulfment of T cells by PMN after 50 min. This demonstrates that ROS production was related to specific molecules on the surface of stimulated T cells and not to soluble factors or nonspecific contact.

#### CD18 but not CD69 and CD40L was involved in inducing ROS production in PMN upon contact with stimulated T cells

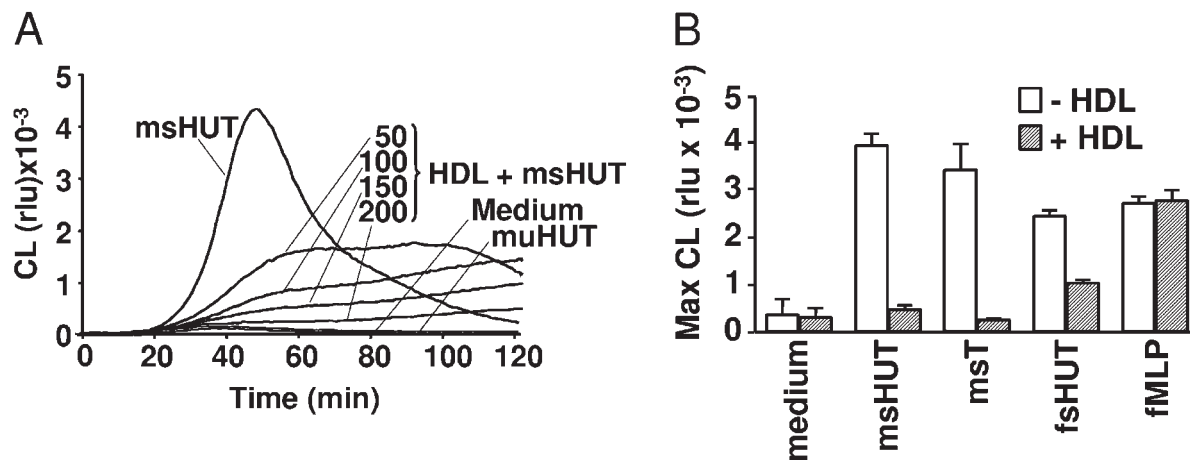
T cell membrane-associated molecules such as  $\beta 2$ -integrins, CD40L, and CD69 were partially involved in monocyte activation by direct cell-cell contact [7, 9]. To determine whether these molecules played a part in PMN activation by T cells, the induction of ROS in PMN by msHUT was measured in the

presence of blocking mAb to CD11a, CD11b, CD11c, CD18, CD40L, and CD69. Only anti-CD18 mAb inhibited the activation of PMN oxidative metabolism by msHUT (**Fig. 3**). None of the other blocking antibodies affected contact-induced PMN respiratory burst. The activation observed in the presence of anti-CD18 antibodies reached  $73.1 \pm 13\%$  and  $39.7 \pm 7.3\%$  (mean  $\pm$  SEM,  $n=3$ ) of maximal chemiluminescence with 1.125  $\mu\text{g/ml}$  and 2.25  $\mu\text{g/ml}$  antibody, respectively, showing that the inhibition displayed by anti-CD18 antibodies was dose-dependent.

The effect of anti-CD18 was similar when using msT (data not shown). These data suggest that the  $\beta$ -chain of  $\beta 2$ -integrin (i.e., CD18) is involved in PMN activation by cellular contact with stimulated T cells.

#### HDL inhibit PMN activation induced by T cell contact

As HDL specifically inhibited contact-mediated cytokine induction in monocytes [11], the effect of HDL on ROS production by PMN was assessed. To determine whether HDL would inhibit PMN respiratory burst induced by T cell membranes, PMN were activated by msHUT in the presence of increasing concentrations of HDL or in its absence. At low concentrations (25  $\mu\text{g/ml}$ ), HDL inhibited by 50% the activation of PMN respiratory burst induced by msHUT. The inhibition of chemiluminescence increased proportionally to HDL concentrations and reached 100% at 200  $\mu\text{g/ml}$  HDL (**Fig. 4A**). The inhibitory effect of purified HDL was effective on msHUT and msT, as well as on fixed, stimulated HUT-78 cells (**Fig. 4B**). In contrast, HDL did not inhibit fMLP-induced ROS production, indicating that HDL did not exert a general inhibitory activity to ROS production by PMN. This suggests that HDL might preferentially interact with T cell membranes. Low-density lipoproteins or albumin used as controls had no inhibitory effect on PMN oxidative metabolism (data not shown).



**Fig. 4.** Purified HDL inhibited ROS production in PMN activated by membranes of stimulated T cells. (A) PMN ( $10 \times 10^6$  cells/ml) were incubated at  $37^\circ\text{C}$  with membranes of stimulated HUT-78 cells ( $5 \times 10^6$  cell equivalent/ml) in the presence or absence of increasing concentrations of purified HDL (50, 100, 150, and 200  $\mu\text{g/ml}$ ). (B) PMN ( $10 \times 10^6$  cells/ml) were incubated at  $37^\circ\text{C}$  with msHUT cells (12  $\mu\text{g/ml}$  proteins,  $5 \times 10^6$  cell equivalent/ml), msT cells (10  $\mu\text{g/ml}$  proteins), fsHUT cells ( $5 \times 10^6$  cells/ml), and fMLP (50  $\mu\text{M}$ ) in the presence (shaded bars) or absence (open bars) of 200  $\mu\text{g/ml}$  HDL. Results are expressed as maximum chemiluminescence (Max CL; arbitrary units), mean  $\pm$  SD,  $n = 3$ .

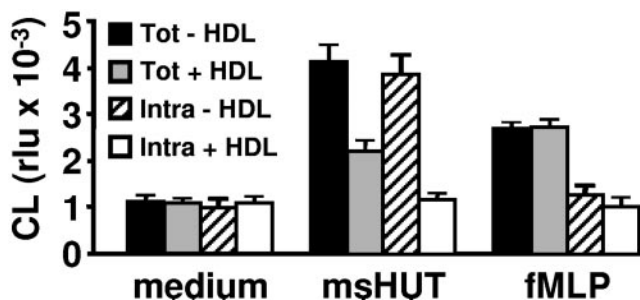
## Contact with stimulated T cells mainly induces intracellular ROS in PMN

To determine whether ROS production induced by cellular contact with stimulated T cells would be preferentially intra- or extracellular, we compared chemiluminescence induced in the presence or absence of SOD and catalase, i.e., scavenging enzymes that eliminate extracellular ROS. PMN incubated in the absence of stimulus displayed similar basal levels of chemiluminescence, whether HDL were added or not (Fig. 5). In PMN activated by msHUT, levels of chemiluminescence were similar in the presence and absence of SOD and catalase (Fig. 5), suggesting that ROS production was mainly intracellular. This contrasts with PMN activated by fMLP, which mainly produced extracellular ROS. In the presence of HDL, the intracellular ROS production induced by msHUT reverted to basal level, i.e., the chemiluminescence levels observed in the absence of stimulus (Fig. 5). In the presence of HDL, total and intracellular ROS production was inhibited, intracellular ROS production being abolished. HDL had no effect on ROS induction by fMLP, whether intra- or extracellular (Figs. 4 and 5).

## DISCUSSION

This study demonstrates that direct cellular contact with stimulated T cells induces respiratory burst in human PMN at levels comparable with those triggered by PMA and fMLP. This mechanism involves the engagement of CD18 and one or more unidentified HDL ligands. This emphasizes the importance of contact-mediated activation of neighboring cells by stimulated T cells in inflammatory processes [1] and substantiates previous results demonstrating the priming and activation of respiratory burst by fixed T cells [3, 4].

Activation by contact with T cell membranes induced mainly intracellular ROS production in PMN, as the levels of chemiluminescence measured in the presence or absence of SOD and catalase were similar. This might explain why in previous studies, we found only a slight, direct activation of PMN by cellular contact with stimulated T cells and essentially the



**Fig. 5.** Contact with stimulated T cells mainly induced intracellular ROS in PMN ( $10 \times 10^6$  cells/ml), which were incubated at  $37^\circ\text{C}$  with the indicated stimulus in the presence or absence of SOD and catalase to measure intracellular (Intra) and total (Tot) ROS production in the presence (+) or absence (-) of  $200 \mu\text{g/ml}$  HDL. msHUT were at  $5 \times 10^6$  cell equivalent/ml and fMLP, at  $50 \mu\text{M}$ . Results are expressed as maximum chemiluminescence (CL; arbitrary units), mean  $\pm$  SD,  $n = 3$ .

priming of PMN to respond to fMLP [3]. In our attempts at the time to detect chemiluminescence induced by ROS, we used lucigenin, which recently proved to be less sensitive than luminol in detecting intracellular ROS generation [17]. However, in this former study, extracellular generation of ROS was measured by cytochrome c reduction upon contact with intact T cells. In contrast, in the present study, this extracellular probe failed to detect ROS production upon PMN stimulation by msHUT (data not shown), which may be a result of differences between fixed T cells and membrane preparations as far as the configuration of the molecules involved are concerned and consequently, their ability to stimulate PMN. It is also possible that soluble products (IFN- $\gamma$  or TNF) leaking from fixed T cells were responsible for the stimulation of the extracellular generation of ROS. The use of T cell membranes precludes interference with soluble products and is more appropriate for examining the mechanism of PMN oxidative metabolism activation upon T cell contact. This approach gave rise to results that strongly suggest that ROS generation is intracellular.

ROS generation is often related to phagocytosis and destruction of pathogens, which in this case, usually occur in phagolysosome [18]. However, particle-mediated cross-linking of CD18 on the neutrophil surface mediates intracellular production of ROS without phagosome formation [16]. Multiple ligands can bind to  $\beta 2$ -integrins and induce signals in PMN, including extracellular matrix proteins, receptors of the Ig superfamily such as ICAM-1 and ICAM-2, blood coagulation proteins, and microorganism products [19]. Although our results did not warrant a conclusion as to whether the CD18 molecules implicated in T cell contact-mediated activation of ROS production in PMN are located on T cells, PMN, or both, by analogy with the previous study [16], it is likely that a putative CD18 ligand at the surface of T cells initiates a signaling cascade, leading to the generation of intracellular ROS without extracellular release of ROS. In this regard, some studies suggest that oxygen species may also act as intracellular messengers by activating transcription factors and protein phosphorylation [20–22]. Whether the intracellular generation of ROS in response to activated T cell contact is part of a signal-transduction cascade deserves further investigation.

We demonstrated previously that the capacity of T cell clones to prime PMN oxidative metabolism correlates with their capacity to induce cytokine production in monocytic cells, suggesting that similar molecules at the surface of T cells are involved in the activation of PMN and monocytes [4]. The present data show that similar to contact-mediated activation of monocyte-macrophages, HDL indeed inhibits contact-mediated induction of PMN respiratory burst. This further suggests that analogous molecules at the surface of T cells are likely to be involved in the activation of monocyte-macrophages and PMN. However, there are differences in the involvement of other surface molecules, which might be considered coactivators. According to the present data, CD18 is involved in the induction of PMN respiratory burst by cellular contact with stimulated T cells. This is reminiscent of numerous other studies demonstrating the involvement of  $\beta 2$ -integrins in activation, adhesion, and transendothelial migration of PMN [16, 23–29]. The involvement of CD18 in the induction of respira-

tory burst in nonadherent PMN upon contact with stimulated T cells differs from that observed with monocyte-macrophages, whose activation by contact with T cells was not affected by blocking antibodies to CD18 [8, 9]. However, contact-mediated activation of monocyte-macrophages involves the engagement of  $\beta$ 2-integrins, as it is partially inhibited by antibodies to CD11a, CD11b, and CD11c [8], which did not affect PMN activation. Together, these results demonstrate that  $\beta$ 2-integrins are involved in T cell contact-mediated activation of PMN and monocyte-macrophages but that the use of  $\beta$ 2-integrin chains varies depending on the target cell type. Another difference between monocyte-macrophages and PMN is the involvement of CD69, which is not implicated in the activation of PMN but in that of monocyte-macrophages [9].

Similar to the induction of proinflammatory cytokines in monocyte-macrophages, HDL potently inhibited T cell contact-induced ROS production in PMN. HDL had no effect on ROS induction by fMLP, whether intra- or extracellular, confirming that HDL did not exert a general inhibitory activity to ROS production by PMN, but rather specifically interfered with cell-cell contact induction of ROS in PMN by interacting with stimulated T cell membranes. It is interesting that other authors have demonstrated that lipid-free apolipoprotein A-I, the main protein constituent of HDL, inhibits IgG-induced activation of PMN, i.e., degranulation and superoxide production [30]. However, the authors failed to detect an inhibitory effect of HDL, which is consistent with the hypothesis of a specific inhibitory activity of HDL to the contact-mediated activation of PMN.

Our observation suggests that the well-known antiatherogenic function of HDL [31–34], mainly attributed to their role in reverse cholesterol transport, may also be a result of the inhibition of immune cell activation (PMN and monocyte-macrophages) involved in atherosclerosis [35, 36]. Indeed, it has recently been postulated that PMN play an important part in the pathogenesis of thrombosis and atherosclerosis as a result of the release of ROS, which in turn induces lipid oxidation and thus contributes to the production of tissue factor [11, 37]. Taking into account the present data, the inhibition of ROS production by PMN upon activation by T lymphocytes may also contribute to the protective effect of HDL in atherosclerosis. This is sustained by clinical observations showing that the incidence of atherosclerosis is enhanced in patients with rheumatoid arthritis and systemic lupus erythematosus, whose serum HDL levels are decreased [1, 38–40]. Therefore, the ability of T cells to activate PMN by cellular contact in a HDL ligand-dependent manner constitutes an additional link between inflammation and lipid metabolism.

In conclusion, by demonstrating the direct induction of PMN respiratory burst by cellular contact with stimulated T cells, the present study substantiates previous observations, pointing out that the direct cellular contact within the inflammatory site is a potent signal to stimulate the release of inflammatory mediators. The potency of contact-mediated induction of IL-1 $\beta$  and TNF- $\alpha$  production in monocyte-macrophages and its inhibition by HDL are well established [5, 11]. The triggering effect of stimulated T cells on PMN respiratory burst and its inhibition by HDL further demonstrate that similar molecules are involved in the activation of both target cells. However,

various integrins or other molecules may function as coactivators depending on the target cell type. Furthermore, HDL might play a protective role overall by inhibiting the inappropriate activation of circulating monocytes and PMN by activated T lymphocytes in the bloodstream.

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