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INVITED REVIEW

CATIONIC POLYELECTROLYTES: POTENT OPSONIC AGENTS WHICH ACTIVATE THE RESPIRATORY BURST IN LEUKOCYTES[1]

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Bacteria and yeasts which are "opsonized" with cationic polyelectrolytes (poly-L-arginine, poly-L-histidine and arginine-rich histone) are avidly endocytosed by both "professional" and "non-professional" phagocytes. The cationized particles also strongly activate the respiratory burst in neutrophils and in macrophages leading to the generation of chemiluminescence, superoxide and hydrogen peroxide. On the other hand, lysine and ornithine-rich polymers are poor opsonic agents. Poly L-arginine is unique in its capacity to act synergistically with lectins, with chemotactic peptides and with cytochalasin B to generate large amounts of chemiluminescence and superoxide in human neutrophils. Unlike polyarginine, polyhistidine, in the absence of carrier particles, is one of the most potent stimulators of superoxide generations, known. Neutrophils treated with cetyltrimethylammonium bromide fail to generate superoxide, but generate strong luminol-dependent chemiluminescence which is totally inhibited by sodium azide and by thiourea. Neutrophils injured by cytolytic agents (saponin, digitonin, lysolecithin) lose their chemiluminescence and superoxide-generating capacities upon stimulation by a variety of ligands. These activities are however regained by the addition of NADPH. Lysolecithin can replace polyarginine in a "cocktail" also containing lectins and cytochalasin B, which strongly activate the respiratory burst. This suggests that polyarginine acts both as a cytolytic agent and as a ligand. Arginine and histidine-rich polyelectrolytes enhance the pathogenic effects of immune complexes *in vivo* (reversed Arthus phenomenon) presumably by "glueing" them to tissues. Polyhistidine complexed to catalase or to superoxide dismutase, markedly enhances their efficiency as antioxidants. On the other hand polyhistidine complexed to glucose oxidase markedly enhances injury to endothelial cells suggesting that the close association of the cationized enzyme with the plasma membrane facilitates the interaction of hydrogen peroxide with the targets. A variety of cationic agents (histone, polyarginine, polyhistidine, polymyxin B) and membrane-active agents (lysophosphatides, microbial hemolysins) act synergistically with glucose oxidase or with reagent hydrogen peroxide to kill target cells. The mechanisms by which arginine-and histidine-rich polyelectrolytes activate the respiratory burst in neutrophils might involve interaction with G-proteins, the activation of arachidonic acid metabolism and phospholipase A2, or the interaction with myeloperoxidase. Naturally-occuring cationic proteins might modulate several important functions of leukocytes and the course and outcome of the inflammatory process.

KEY WORDS: Cationic polyelectrolytes, opsonic agents, leukocytes

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INTRODUCTION

The biochemical pathways leading to the activation of the respiratory burst in "professional" phagocytes and the role played by oxygen radicals in both the protection and the destruction of tissues in infectious and inflammatory sites is the focus of current intense investigation.[1-8]

Activators of the respiratory burst range from antibody and complement-opsonized particles to a series of soluble agents (i.e. phorbol esters, C5a, formylated peptides, platelet activating factor, leukotriene B4, lectins, calcium ionophores, lipopolysaccharides, cytochalasins, digitonin, phospholipase C, NaF, Lanthanum, fatty acid, and TNF).[2,3] Although most of these agents do not seem to have a common chemical structure, many of them share common properties of binding to cell membranes, of changing their fluidity and polarization and of transmitting membrane signals which lead to the activation of NADPH-oxidase. Generation of reactive oxygen intermediates after cell activation is responsible only for killing microorganisms but also for the degradation of macromolecular tissue structures and for the destruction of mammalian cells.[1-8]

Interest in the possible role played by cationic polyelectrolytes (CPS) as "novel" activators of the respiratory burst in neutrophils (PMNs) and macrophages (MQs) originated from the findings that such agents functioned as potent agglutinins,[9] and as bactericidal agents[2,4,10-17] and as such might mimic the effects of antibodies. The present review describes the properties of both synthetic and naturally-occurring polycations as 1) opsonins, 2) activators of the respiratory burst in "professional" phagocytes and 3) as modulators of the interactions of scavengers of oxygen radicals and of membrane-active agents, with target cells.

ROLE OF CATIONIC POLYELECTROLYTES AS STIMULATORS OF ENDOCYTOSIS

Starch particles[18] soluble albumin,[19] ferritin and horseradish peroxidase[20,21] which had been "opsonized" with poly-L-lysine (PLYS) or with poly-L-arginine (PARG) were avidly taken up by PMNs, sarcoma S-180 cells and by smooth muscle cells, respectively. Synthetic cationic polymers also facilitated phagocytosis of latex particles,[22] a variety of microbial species[23-27] and LPS-coated paraffin droplets[25] by PMNs and macrophages and of streptococci and yeasts by *Entamoeba histolytica*.[29]

Paradoxically, and unexpectedly, histones, PARG, and poly L-Histidine (PHSTD) very effectively opsonized *Candida albicans*, group A streptococci, cell nuclei and *E. coli* (Figure 1) to phagocytosis by "non-professional" phagocytes such as fibroblasts, epithelial cells, fibrosarcoma cells, endothelial cells and even by beating heart cultures.[24,28-30] Even human spermatoza and a variety of liposomes could be opsonized, by polycations, for phagocytosis by fibroblasts and macrophages, respectively. (unpublished results).

Once the cationized particles adhered to the cell membranes, even potent polyanions (i.e. polyanethilesulphonate, dextran sulphate) failed to detach them. Such polyanions, however totally inhibited phagocytosis when premixed with cationized particles prior to their addition to the cells suggesting that the polycation-coated particles interacted electrostatically with negatively-charged domains on the cell surfaces. Thus polycations proved to be potent non-specific stimulators of endocytosis by mammalian cells

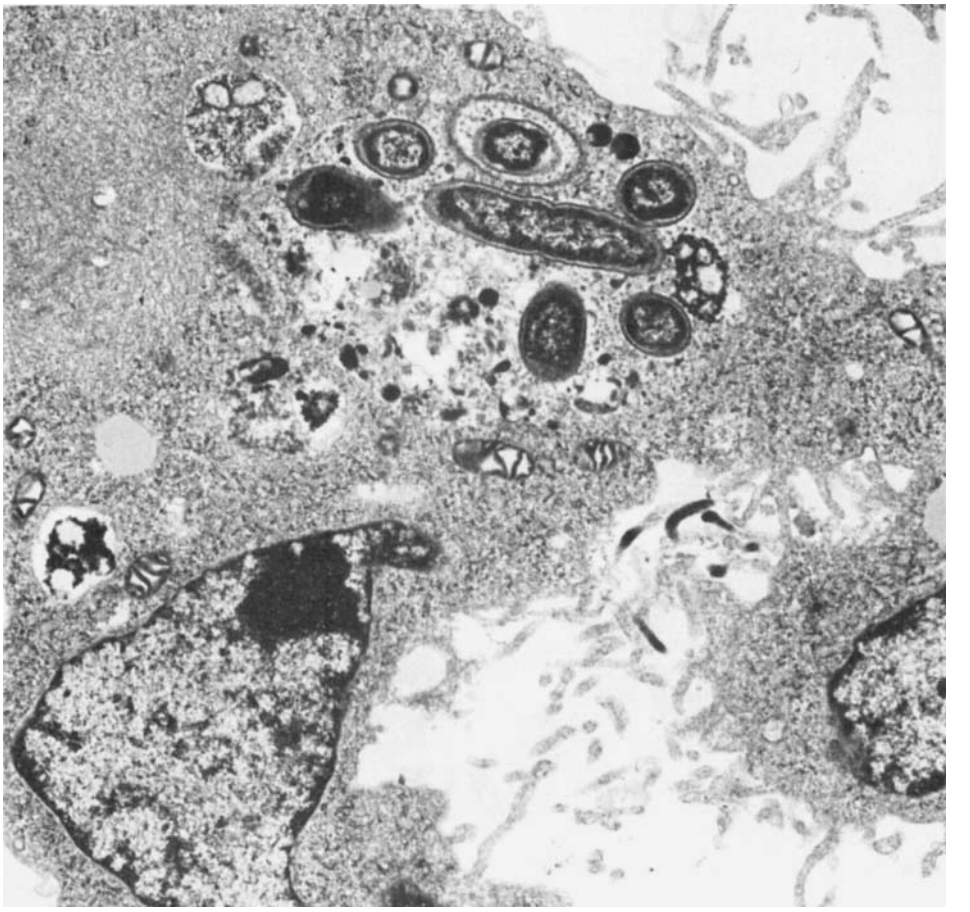

FIGURE 1 Phagocytosis of histone-opsonized *E. coli* by human skin fibroblasts. Note the presence of undigested rods within a large vacuolar structure. E.M. × 13,950.

ROLE OF POLYCATIONS AS ACTIVATORS OF THE RESPIRATORY BURST

1) Generation of luminol-dependent chemiluminescence (LDCL)

Both PMNs and macrophages emit light when stimulated with particulate or soluble agents known to activate NADPH oxidase.[31,32] Light emission can be markedly potentiated by luminol. Although the exact nature of the oxygen intermediate responsible for light emission is still not fully known, superoxide, hydroxyl radicals and singlet oxygen have been considered responsible for the generation of light.[32] Luminol-dependent chemiluminescence (LDCL), however, is strongly linked with myeloperoxidase as both luminol and non luminol-dependent chemiluminescence were blocked by azide.[2,32,33]

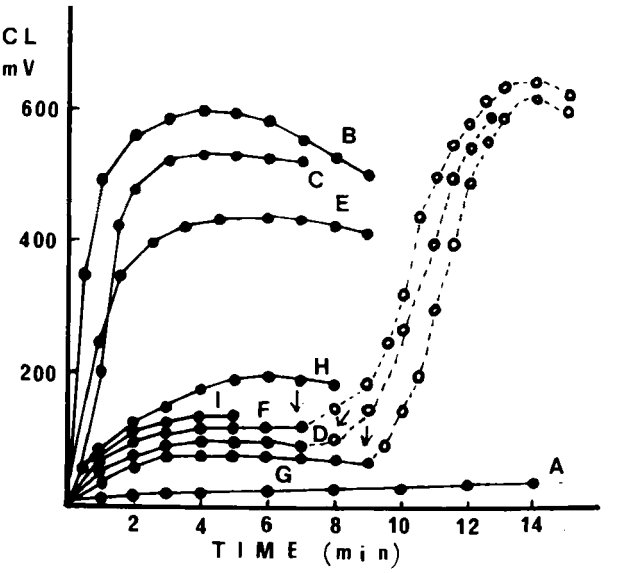

FIGURE 2 Luminol-dependent chemiluminescence induced in phagocytes (1×10^6/ml by polycations-preopsonized streptococci. (A) Streptococci (50μl) of a 2.0 optical density/ml (550 nm): B) streptococci opsonized with PARG (M.W 44,000) 10^{-5}M; (C) streptococci opsonized with arginine-rich histone (M.W approx 10,000) 10^{-5}M; (D) streptococci opsonized with lysine-rich histone (M.W approx 10,000) 10^{-5}M. PARG(10^{-6}M) added at 7 min. Note the secondary CL response (Arrow) (E) Streptococci opsonized with PHSTD (M.W 13,900) 10^{-5}M; (F) Streptococci opsonized with PLYS (M.W 25,000) 10^{-5}M, PARG(10^{-6}M) was added at 7 min. Note the secondary CL response (Arrow); (G) Streptococci opsonized with PLYS (M.W 14,000)10^{-5}M. PARG (10^{-6}M) was added 9 min later (Arrow). Note the secondary CL response; (H) Streptococci opsonized with PLYS (M.W. 65,000) 10^{-5}M; (I) Streptococci opsonized with PORNT (M.W 10,000)10^{-5}M (From *Inflammation*, **9**, 245–271, 1985).

Bacteria and yeasts which had been either properly opsonized with histone, PARG or with PHSTD or simply mixed with these substances triggered very intense LDCL responses by both PMNs and macrophages.[33–36] Arginine-rich histone was superior to lysine-rich polymers as triggers of CL[34,35] (Figure 2). PARG, but not PLYS, also approved effective as a chemiluminescence-stimulating agent when employed in the absence of a carrier particle. Cytochalasin B which markedly enhanced O_2^- generation by various agonists[37] inhibited LCDL which had been induced by histone-opsonized particles. Under similar conditions,neither protamine sulphate, lysozyme, spermine nor spermidine (all cationic in nature) had any LDCL-stimulating capacities when employed together with particles. The only polyanions which also triggered LCDL generation were polyanetholesulphonate and dextran sulphate[33] (see below). LCDL induced by the polycations was only partially inhibited by SOD, catalase and by histidine, but was nearly totally blocked by sodium azide and by aminotriazole, suggesting the central involvement of myeloperoxidase in this reaction.[2,32,33] Other inhibitory agents were deoxyglucose, sodium benzoate histamine, [38] and cimetidine (Ginsburg, Misgav and Kohen to be published).

2) Generation of superoxide by polycations

A) Role of polyarginine and polylysine Streptococci and *Candida albicans* which are

simply mixed with either histone, PARG or with PHSTD (see below) trigger very marked generation of both O_2^- and H_2O_2 by PMNs.[34–36,38,39] On the other hand, Poly-L-lysine and poly L-ornithine were inferior to the arginine or histidine polymers as stimulators of O_2^- generation. As in the case of chemotactic peptides and immune complexes,[37] CYB also markedly enhanced O_2^- generation by the polycation-opsonized particles, suggesting that phagocytosis was not essential for O_2^- generation.[34–36,38,39] This was also verified by electron microscopy.[38] As in the case of LDCL, neither polyarginine, polylysine nor polyornithine were effective stimulators of O_2^- generation in the absence of a carrier particle. All these polycations were highly cytolytic for PMNs at concentrations greater than 10^{-7}M (see below).

B) Role of PHSTD Paradoxically PHSTD, which failed to induce any appreciable generation of LCDL, proved to be one of the most potent stimulators of O_2^- generation known, when employed in the absence of a carrier particle[36,38] ((Figure 3). Generation of O_2^- by PHSTD (at 4–6 x 10^{-5}M) started after a lag period of approximately 25 sec and at a rate of 160 nmol/10^7 PMNs/10 min. PHSTD was also avidly phagocytosed by the PMNs. Since CYB only very slightly depressed O_2^-

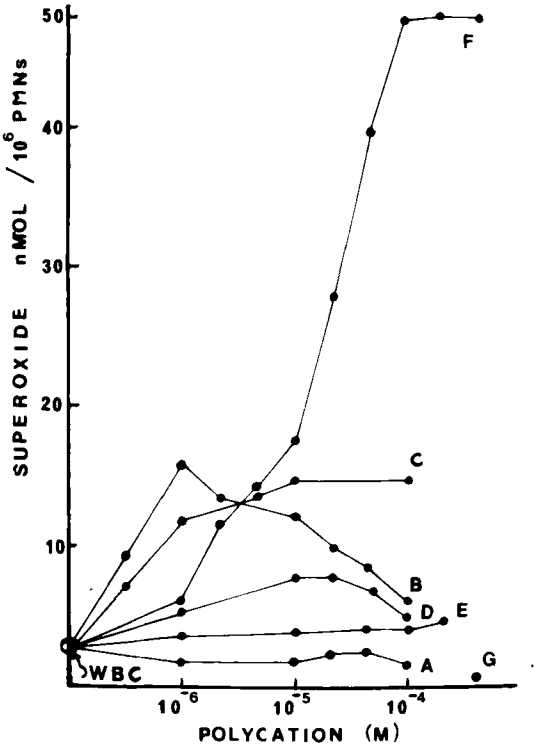

FIGURE 3 Superoxide generation by leukocytes stimulated by soluble cationic polyelectrolytes. WBC (1×10^6) were incubated for 15 min at 37°C with $10^{-4} - 10^{-6}$M polyelectrolytes in the presence of 80 μM cytochrome c. (A) poly-L-lysine (M.W 25,000); (B) poly-L-arginine (M.W 40,000); (C) poly-L-arginine (M.W 120,000); (D) arginine-rich histone (M.W 120,000). (E) monomeric L-histidine HCl ; (F) poly-L-histidine (M.W 13,900); (G) poly-L-histidine plus superoxide dismutase (30 μg/ml). Note the very intense generation of O_2^- by polyhistidine. (From *Inflammation*, **9**, 245–271, 1985).

generation by PHSTD the role of phagocytosis in its production seems to be insignificant. Generation of O_2^- by PHSTD was dependent on extracellular calcium and magnesium. It induced a steep rise of intracellular calcium as determined by the Quin-2 fluorescence technique.[38] Its activity was however depressed by calcium channel blockers, by trifluroperazine and by nordihydroguaiaretic acid (a lipoxygenase inhibitor).[38] Stimulation of O_2^- generation by PHSTD was not accompanied by release of lactate dehydrogenase (LDH) indicating that O_2^- generation was not accompanied by permeabilization of the membrane (see below).

POLY ARGININE SYNERGIZES WITH SOLUBLE AGONISTS TO ENHANCE THE RESPIRATORY BURST

PMNs which had been pretreated ("primed") for 30 seconds with PARG followed by the addition of lectins (phytohemagglutinin-PHA, or with Con-A) generated very

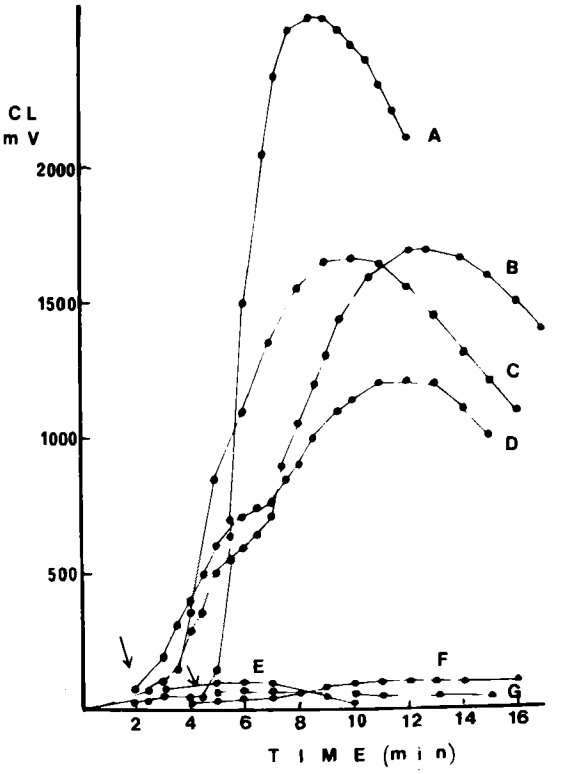

FIGURE 4 LDCL induced by mixtures of fMLP, PARG and PHA. WBC(3×10^6/ml) were pretreated for 1.5 min at 37°C with (A) fMLP (10^{-6}M) followed 1.5 min later by the addition of PARG (50 μg/ml) followed 1.5 min later by PHA (40 μg/ml) (sequence = fMLP, PARG, PHA); (B) with the sequence fMLP → PHA → PARG. (C) with the sequence PHA → PARG → PARG. (D) with the sequence PHA → fMLP → PARG; (E) with PARG alone (F) with fMLP alone. (G) with PHA alone. Note that maximal LDCL is obtained with the sequence fMLP → PARG → PHA. (From *Chemical Chemiluminescence* Volume II, edited by K.Van Dyke and V. Castranove, CRC Press pp 120–156, 1987)

intense synergetic LDCL responses.[39] On the other hand, when the PMNs were first treated with a lectin followed by the addition of PARG a smaller response was recorded suggesting that the sequence of addition of the different agents to the cells was important to secure maximal cell responses. PMNs which had been treated either simultaneously or sequentially with sub-stimulatory concentrations of fMLP, PARG and PHA (or Con-A) also generated very intense synergistic LDCL signals (Figure 4).[35,39] O_2^- generation by the mixture of agents was also much greater than that induced individually by the different agonists.[35,39] PARG also synergized with LPS to generate enhanced amounts of LDCL by murine macrophages,[35] suggesting that both PMNs and macrophages respond in a similar way to the same cationic agents. Such "priming effects are similar to those reported by McPhail *et al.*[40,41] PARG was also found to synergize with PMA to induce LDCL and O_2^- but only when the cells were first treated with PMA. A very strong synergy between PHA and fMLP was also found.[39] Synergy among the various agent depended on the presence of extracellular calcium and magnesium ions. Neither polylysine, polyornithine nor poly-L-asparagine could mimic the effect of polyarginine as synergizing agents.[39]

GENERATION OF O_2^- AND CL BY LEUKOCYTES (PMNs) INJURED BY CYTOLYTIC AGENTS

The marked toxicity of PARG to PMNs and the role played by this polycation in O_2^- generation were further studied by first exposing the cells to PARG prior to the addition of a "cocktail" of other agonists.[42] Table 1 shows that PMNs exposed to PARG for more than 7 minutes totally lost their O_2^--generating capacities when further treated with PHA and CYB. On the other hand cells pre-exposed to PHA and CYB generated normal amounts of O_2^- when challenged with Polyarginine. Since very large amounts of O_2^- were generated when all 3 agents were simultaneously added to PMNs it appeared that PARG participated in the activation of NADPH oxidase as soon as it was added to the cells and that its toxic effects were expressed at a later

TABLE I
Effect of Sequence of Stimulation by Soluble Ligands on O_2^- Generation

Leukocytes pretreated with[a]	Followed by[b]	Superoxide[c] (nmol/10 min)
None	None	6.3 ± 1.2
PARG (10^{-6}M)	None	2.5 ± 0.8
PHA (50 μg/ml)	None	3.8 ± 0.6
Cytochalasin B (CYB) 5×10^{-6}M	None	6.5 ± 0.8
PARG	PHA + CYB	3.5 ± 0.4
CYB	PARG + PHA	59.6 ± 7.8
PHA	PARG + CYB	58.3 ± 5.9
None	PARG PHA + CYB	63.3 ± 9.3
PARG + PHA	CYB	13.3 ± 1.6
PARG + PHA + CYB	None	6.6 ± 0.9

[a]Human blood leukocytes (3×10^6) were pretreated with the various agents for 7 min at 37°C.
[b]Various agents plus cytochrome c, 80 μM, were then added to the reaction mixtures and were further incubated for 10 min at 37°C.
[c]The data are the average ± standard deviation of the mean of four different experiments.
From: *Inflammation* **9**, 341–363 (1985).

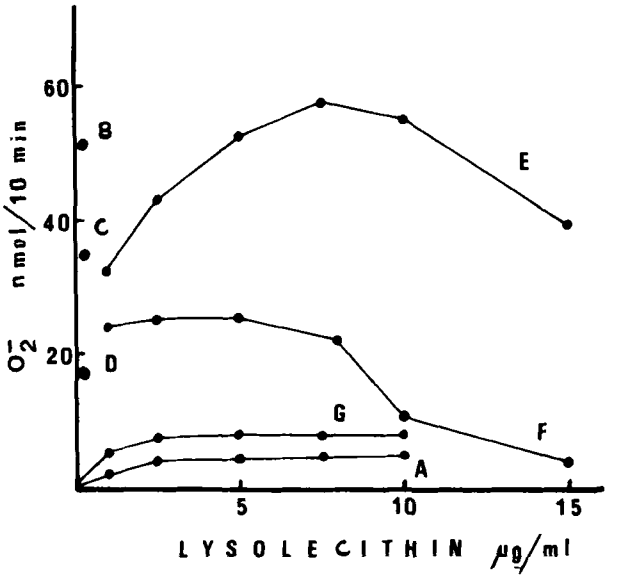

FIGURE 5 Collaboration between lysolecithin, PHA and CYB in O_2^- generation:
Leukocytes (3×10^6/ml) suspended in HBSS containing NaN_3 (1 mM) and 80 μM cytochrome c, were incubated for 10 min at 37°C with: (A) increasing concentrations of lysolecithin (LL); (B) with a mixture of PARG (M.W 40,000, 10^{-6}M), phytohemagglutinin (PHA, 40 μg/ml), and cytochalasin B(CYB 5×10^{-6}M); (C) PHA + CYB; (D) PARGH + CYB; (E) increasing concentrations of LL plus constant amounts of PHA + CYB; (F) increasing concentrations of LL + constant amounts of PARG + CYB; (G) increasing concentrations of LL + constant amounts of PHA + CYB + SOD (30 μg/ml). Note that sub-stimulating concentrations of LL acted synergistically with PHA + CYB to generate O_2^-. Thus, LL totally replaced PARG as an ingredient of the "cocktail". (From *Inflammation* **9**, 341–363, 1985.)

stage. PMNs which had been exposed to hypotonic solutions gradually lost their O_2^--generating capacities.[42] By analogy, PMNs which had been incubated for short periods with increasing concentrations of the cytotytic agents, saponin, lysophosphatidylcholine or with digitonin also progressively lost their capacity to generate O_2^- and LCDL upon stimulation with a "cocktail" comprised of PARG, PHA and CYB. Generation of O_2^- was, however, restored to a large extent by the addition of NADPH but not of NADH, suggesting that the loss of NADPH by the leaky cells was responsible for the low O_2^- generation obtained.[42] Since PARG+PHA +CYB induced the formation of large amounts of O_2^- we also tested the possibility that other membrane-active agents might replace PARG in the cocktail. Indeed both lysolecithin (Figure 5) and saponin (not shown), which are poor O_2^--generating agents, nevertheless collaborated effectively with PHA and CYB to generate the same amounts of O_2^- as produced by a mixture comprised of PARG+PHA+CYB. Thus one cytolytic agent might effectively replace another to trigger maximal generation of O_2^-. Essentially similar replacement of PARG by digitonin also took place. Here, however, digitonin alone at concentrations greater than 5 μg/ml induced generation of progressive amounts of O_2^-.[43] Thus synergy with PHA and CYB was observed only over a narrow range of digitonin concentrations.

MECHANISMS OF ACTION OF POLYCATIONS

The mechanisms by which arginine and histidine-rich polymers induced membrane changes which lead to the generation of large amounts of O_2^-, and luminol-dependent chemiluminescence, are not fully known. The effect of polyarginine and polyhistidine as potent stimulators of the respiratory burst cannot be simply attributed to their cationic properties as neither polylysine, polyornithine, protamine sulphate, or lysozyme had any appreciable ability to activate NADPH oxidase in PMNs and macrophages. Polyarginine and polyhistidine might, however, interact with specific receptors upon the cells. The findings that histamine and cimetidine (structural analogue of histidine) markedly interfered with O_2^- generation induced by PHSTD support this assumption. Another possibility is that leukocyte membranes respond either to the hydrophobic domains in PARG and PHSTD or to their membrane-fusion properties.[47] Recent studies[44,45] however showed that the cytolytic effect of polyarginine on neutrophils and the resulting release of lactate dehydrogenase from such treated cells was totally inhibited by guanosine 5-O-(3-thiotriphosphate) (GTP γ S) suggesting that polyarginine toxicity might be mediated by guanine nucleotide-binding structures. GTP γ S also synergized with polyarginine to generate O_2^-.[45] The involvement of guanine nucleotides in the interaction of polyarginine with membranes links this effect to G-proteins,[46] which have been implicated in signal transduction from receptors for chemotactic agents.[46] Most of our studies on the role played by charge in the activation of the respiratory burst were focused mainly on polycations (see above). Paradoxically, however, we also observed that streptococci which had either been coated by or grown with polyanetholesulphonate liquoid or dextran sulphate (M.W 500,000) also generated very intense LDCL and O_2^- responses in human PMNs.[36] Both liquoid alone, and liquoid complexed with polyhistidine,[38] also initiated a strong generation of O_2^- by PMNs, which was boosted by CYB.[38] In addition to its antiphagocytic, antichemotactic and anticoagulant activities,[36] this polyanion is an activator of the oxidation of glucose (C1) and formate[48] and also affects the iodination of bacteria.[49] These reports serve to indicate that certain negatively-charged agents might also

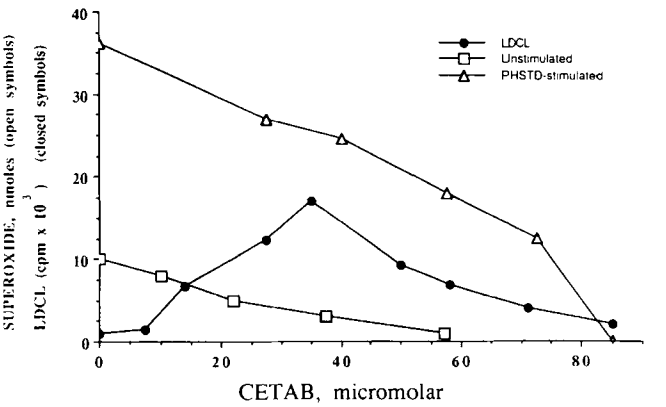

FIGURE 6 Effect of CETAB on O_2^- generation. PMNs (1×10^6/ml) were treated with increasing concentrations of CETAB. Note that while both the spontaneous and the PHSTD-induced O_2 generation were depressed by CETAB this cationic agent markedly stimulated the generation of LDCL.

activate the respiratory burst in PMNs. Further insight into the possible role played by charge in the regulation and activation of NADPH oxidase came from recent studies with cationic lipophilic alkyl compounds.[51-53] While cetylamine, CETAB, and sphinganine markedly inhibited O_2^- generation induced either by PMA or by fMLP, the anionic agent myristate totally reversed the inhibitory effect of the cationic agents even in the presence of inhibitors of protein kinase c. Sodium dodecyl sulphate also activated the generation of O_2^- induced by combining the membrane and cytosolic fractions of macrophages.[50]

Recent studies from our laboratory have paradoxically shown that while cetyltrimethylammonium bromide (CETAB) totally fails to trigger the generation of any detectable amounts of O_2^- by PMNs, this lipophilic cationic agent induces the generation of strong luminol-dependent chemiluminescence (Figure 6). Furthermore, CETAB markedly depressed O_2^- generation induced either by PHSTD (Figure 6) or by polycation-opsonized streptococci (not shown) These latter results are essentially similar to those describing the effects of cetylamine and CETAB on O_2^- generation induced by PMA.[51,52] We have found that either histone-opsonized streptococci or mixtures of PARG and PHA[39] (see above) also stimulated PMNs to generate lucigenin-dependent chemiluminescence (LUCDCL). On the other hand, CETAB totally failed to generate LUCDCL. Since SOD which only partially inhibited LDCL generation (induced by CETAB, opsonized bacteria, PARG+PHA) totally inhibited LUCDCL generation induced by opsonized bacteria or by polyarginine +phytohemagglutinin, the failure of CETAB to induce O_2^- generation might now also be corroborated by measuring LUCDCL, which most probably exclusively measures O_2^-.

Stimulation of LDCL bt CETAB depended on the presence of calcium and magnesium ions. It was totally inhibited by azide, partially by SOD and very markedly by adrenaline, histidine, cimetidine, heparin, nordihydroguaiaretic acid, bromophenylacyl bromide (a phospholipase A2 inhibitor), chloroquine and the compound H-7 (a protein kinase c inhibitor). It was however totally unaffected either by catalase or by desferal. On the other hand, the non-luminol dependent CL was markedly inhibited only by azide. The apparent opposite effects of cationic lipophilic agents on CL and on O_2^- generation is intriguing. One possible explanation for this paradoxical finding is that while CL is totally dependent on the azide-sensitive myeloperoxidase, generation of O_2^- and H_2O_2 proceeds unabated in the presence of azide. Thus CETAB might perhaps interact directly with MPO to trigger the generation of hypochlorous acid which might be involved in light emission. Further studies on the comparison between polycations and lipophilic cationic agents as modulators of the respiratory burst, are underway.

NATURALLY-OCCURING CATIONIC AGENTS WHICH ACTIVATE THE RESPIRATORY BURST

Cationic proteins extracted from human PMNs markedly inhibited O_2^- generation induced during phagocytosis of *E. coli* but markedly enhanced O_2^- generation induced by IgG-coated latex particles.[54] The O_2^- -enhancing agent was not identical with either lysozyme or with chymotrypsin. The basic tetrapeptide tuftsin (Thr-Lys-Pro-Arg) which was found to affect many of the known functions of phagocytic cells (phagocytosis, bactericidal activity, motility, cytotoxicity towards tumor cells) rapidly binds to the surfaces of macrophages and also augments superoxide generation by such

cells.[56,57] Stimulation of O_2^- generation by tuftsin was found to be directly proportional to the activity of adenosine deaminase,[57] but did not correlate with its phagocytosis-stimulating properties. It was concluded that while there is a general consensus that NADPH oxidase is the major source of O_2^-, the purine salvage pathway should be considered as an additional source of O_2^- generation in PMNs. Despite the reports that tuftsin also stimulated O_2^- generation by PMNs, repeated attempts by us to show this effect employing a variety of both particulate and soluble agonists, failed. (Ginsburg, unpublished observations). Conversely, we found that human PMNs which had been preincubated for 2 min with tuftsin (50–150 – μg/ml) generated 40–60% less chemiluminescence upon stimulation either by PMA, histone-opsonized streptococci, PARG or by CETAB suggesting that tuftsin might down-regulate the respiratory burst.

Spermine and spermidine (polyamines), which failed to induce O_2 generation in human PMBNs,[33] markedly inhibited hexose monophosphate shunt activity in PMNs but only in the presence of polyamine oxidase-rich sera (bovine, human pregnancy and inflammatory[69]). The inhibitory activity of polyamine-oxidase on leukocyte metabolism essential for the activation of NADPH-oxidase is probably linked with the generation of reactive amino aldehydes.

POLYCATIONS MODULATE IMMUNE-COMPLEX-INDUCED TISSUE DAMAGE

The recent interest in the role played by charges in the deposition of immune complexes in tissues has focused mostly on kidney diseases.[36,58] Experimentally, immunoglobulins cationized by the carbodiimide method (Danon *et al.*[59]) and which had a PI above 8.5. avidly bound to negatively-charged basement membrane of the kidney. Such planted cationic immunoglobulins then interacted with antigen to induce kidney damage (reviewed in[36]). *In vitro* studies have also shown that cationization of BSA-anti-BSA complexes either with nuclear histone or with PARG markedly enhanced their O_2^--generating capacities and also enhanced lysosomal enzyme release from human and rat PMNs.[60] *In vivo* studies have also shown that the intracutaneous injection of anti-BSA complexed with histone or with PARG, followed by the intravenous injection of iodine-labeled BSA (reversed Arthus phenomenon), resulted in a marked augmentation of the inflammatory skin responses.[60] This effect could be suppressed by PEG-SOD, supporting the assumption that SOD might increase H_2O_2, which might have been responsible for the initiation of more severe skin lesions.

POLYCATIONS MODULATE BINDING OF SOD, CATALASE AND GLUCOSE OXIDASE TO MAMMALIAN CELLS

The very short half-lives of SOD and catalase after their administration, *in vivo*, to combat the deleterious effects of oxygen reactive species, greatly limits their beneficial effects. Cationization of both catalase and SOD by the carbodiimide method[59] and the elevation of the isoelectric point of these modified agents above 8.5, facilitated the persistence of these scavengers in joint tissues of animals which developed experimental arthritis.[61,62] More recently, catalase was complexed with PHSTD.[63] Monolayers of endothelial cells which had been treated with cationized catalase were protected

against the toxic effects either of reagent H_2O_2 or of peroxide which had been generated with glucose oxidase. We observed that the insoluble PHSTD-catalase complexes strongly bound to cell surfaces and unlike the native enzyme could not be washed away. Human neutrophils which had been precoated with PHSTD-SOD complexes and then washed failed to generate any detectable amounts of O_2^- when stimulated with PMA suggesting that the close proximity of the cationized scavenger to the O_2^--generating sites was responsible for this effect. Non-cationized SOD failed to induce this effect (Ginsburg, Gibbs, Ward and Varani, unpublished).

Enhanced killing of endothelial cells occured when PHSTD-glucose oxidase complexes were applied to monolayers of endothelial cells (Ginsburg, Gibbs, Ward and Varani, unpublished), suggesting that H_2O_2 which had been generated by glucose oxidase acted close to the cell membrane to cause enhanced permeability and cell death.

POLYCATIONS SYNERGIZE WITH MEMBRANE-ACTIVE AGENTS TO KILL TARGET CELLS

PHSTD synergized with a variety of lysophosphatides to generate enhanced amounts of O_2^- by PMNs[64] and to kill endothelial cells in culture.[65] Subtoxic concentrations either of histone, PARG or of polymyxin B also synergized with subtoxic concentrations of H_2O_2 to kill endothelial cells. This effect was totally abolished either by adding catalase or heparin to the reaction mixtures.[65] Both H_2O_2 and the cationic agents had to be present simultaneously to secure tissue damage.[65] Similar synergy between the cationic peptides and reagent H_2O_2 in target cell killing was also reported.[66,67] These findings suggest that perturbation of the cell membrane by a cationic agent prepared the ground for the effect of H_2O_2. PHSTD which had been complexed with soybean trypsin inhibitor was firmly bound to endothelial cells and protected them against the effect of trypsin (Ginsburg, Gibbs, Varani and Ward unpublished). Since trypsin, elastase and plasmin also synergized with H_2O_2 to kill target cells,[68] the cationic SBTI complex might aid in the protection of cells against proteases.

Finally, streptococci or *Candida albicans* which had been pre-opsonized with histone markedly enhanced the generation of IL-1 by T-cell hybridoma,[70] and of IL-2 by macrophages (Ginsburg, Barak, Treves unpublished).

SUMMARY AND CONCLUSIONS

Arginine and histidine-rich polypeptides, which function as potent "opsonins" for phagocytosis by both "professional" and "non-professional" phagocytes, might also be added to the long list of activators of the respiratory burst in PMNs and macrophages. While both histone and PARG functioned optimally as activators of the oxygen metabolism of leukocytes only when attached to particles (bacteria, yeasts), poly-L histidine (PHSTD) and to a much lesser extent poly-L-arginine are the only polycations capable of activating the respiratory burst in the absence of a carrier particle. On the other hand, lysine and ornithine-rich polypeptides were very weak stimulators of the respiratory burst even when bound to particles.

Polyarginine, however, possesses a unique ability to synergize with lectins, with chemotactic peptides and with cytochalasins to cause generation of large amounts of

O_2^- and of chemiluminescence. Polyarginine, which proved to be cytolytic, could however be totally replaced in O_2^--generating "cocktails" (also containing PHA and CYB) by other cytolytic agents (i.e. saponin, digitonin, lysolecithin) suggesting that polyarginine functions both as an agonist and as a cytotoxin.

The mechanisms by which arginine and histidine-rich polypeptides function to activate NADPH oxidase is still not fully understood. Since, however, the cytolytic effects of PARG can be inhibited by GTP γ S and since this compound synergized with polyarginine to generate O_2^-, its NADPH-oxidase-activating properties might be linked to G-proteins. PARG and PHSTD might, however, also function due to their hydrophobic domains and to their membrane fusion capacities. The recent paradoxical findings that cationic lipophilic alkyl compounds shut down O_2^- generation, while anionic lipids, polyanetholesulphonate and dextran sulphate, proved potent stimulators of O_2^- generation, are intriguing. Our recent findings have however shown that while the cationic agent CETAB totally fails to activate O_2^- generation as measured either by cytochrome c reduction or by lucigenin-dependent CL and also markedly inhibits O_2^- generation induced either by PHSTD or by histone-opsonized bacteria, it markedly stimulates the generation of LDCL. Since LDCL might measure primarily hydroxyl radical and singlet oxygen, the selective effect of cationic lipids as a potent stimulators of LDCL but not O_2^-, points to their ability to bypass protein kinase c and perhaps to interact with myeloperoxidase.

Arginine and histidine-rich polymers can also modulate immune complex-induced tissue injury. They probably function to "glue" the immunoglobulin to negatively-charged tissue components. Tissue injury is then initiated by interaction of the antigen with the planted immunoglobulin. Polycations are also capable of "opsonizing" catalase, superoxide dismutase, glucose oxidase and soybean trypsin inhibitor, which can then avidly bind to cell surfaces, either to protect them against oxidant stress or against proteases or to enhance their toxic effects. Polycations also synergized with membrane-active agents and with H_2O_2 to enhance target cell killing. Polycations might also enhance the generation of interleukins by mammalian cells.

Finally, it is tempting to speculate that cationic proteins which are released after stimulation of PMNs and eosinophils either by immune complexes or by chemotactic peptides, might "opsonize" microorganisms for phagocytosis by bystander PMNs or by macrophages in the absence of anti-bacterial antibodies. This might also result in the relaese of enhanced amounts of oxygen radicals and H_2O_2 which together with lysophospholipids might enhance tissue injury. Also the intra-phagosomal accumulation of leukocyte cationic proteins after phagocytosis of microorganisms might trigger a secondary oxygen burst due to intraphagosomal "opsonization" of the engulfed bacteria leading to excessive accumulation of toxic oxygen species. Further insight into the role played by naturally-occuring cationic agents, of leukocyte origin, not only as distinct bactericidal agents but also as agents capable of enhancing phagocytosis by both professional and non-professional phagocytes, might shed more light on post-phagocytic events and on the complex processes which underlie the interactions of parasites with hosts, leading to tissue injury.

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