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CATIONIC POLYELECTROLYTES: A New Look at Their Possible Roles as Opsonins, as Stimulators of Respiratory Burst in Leukocytes, in Bacteriolysis, and as Modulators of Immune-Complex Diseases (A Review Hypothesis)[1]

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INTRODUCTION

Voluminous literature exists today on the involvement of cationic polyelectrolytes (CPs) in host and parasite interrelationships. It has been shown that CPs of neutrophil (1–14), eosinophil (15, 16), macrophage (17), and platelet (18) origins function as distinct microbicidal agents. These probably constitute a "secondary" defense line supplementary to the main oxygen-dependent microbicidal systems of "professional" phagocytes. CPs have, however, also been implicated as modulators of blood clotting (19) and fibrinolysis (20), as a permeability-enhancing factors (21–23), in mast cell degranulation and in histamine release (24), as pyrogenic agents (25), as enhancers of complement-mediated lysis (26), as modulators of PMN adherence (27–29) and chemotaxis (30–34), and as modulators of endocytosis (35–45) to list only several of the properties ascribed to these agents. Since the effects of CPs probably involve the interaction, through electrostatic forces, with negatively charged sites on

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target cells (36), it is plausible that the complex polyelectrolytic milieu found in infectious and inflammatory sites might function to modulate and regulate several important interactions of the host with invaders (46–48).

Although CPs are primarily recognized for their distinct killing properties (10–13), recent studies have suggested that CPs might also be involved in a variety of additional biological, biochemical, and immunopathological phenomena which are seldom discussed in the general context of host and parasite interrelationships.

The present review deals primarily with the possible involvement of CPs (1) in endocytosis and in cell adherence, (2) as activators of the respiratory burst in "professional" phagocytes, (3) as activators of the autolytic wall enzymes in certain microbial species and its relation to bacteriolysis and to the pathogenesis of chronic inflammation induced by bacterial cell walls, and (4) as agents capable of modulating the pathogenicity of immune complexes.

It was felt that a discussion of these "other" properties of CPs is timely as it may shed a new light on the role of surface charge in cell-to-cell interactions as seen in inflammatory and infectious sites.

ROLE OF CPs IN ENDOCYTOSIS

The very extensive literature on the role played by receptor-mediated phagocytosis by "professional" phagocytes in host defense against foreign invaders has been recently reviewed (49–52). In addition to immunoglobulins (Ig) and complement ligands, endocytosis may also be mediated by nonspecific recognition mechanisms (53), by "primitive agglutinins" known to be found in invertebrates (54), by lectins and natural peptides (55), as well as by extracellular matrix proteins (56). Both electrostatic and hydrophobic interactions have been implicated in the internalization (endocytosis) of both soluble and particulate agents (35–45, 51, 57, 58).

The opsonic properties of the CPs were not necessarily related to their high bactericidal effects (36–38), as nonviable bacteria are also taken up by phagocytes. The efficiency of the CPs as opsonizing agents depended on their binding to the particle surface, on the length of the polymers, and on their net cationic charge (36). Usually, polymers with a molecular weight smaller than 4000 were not toxic and poorly opsonic. It is of interest, however, that nuclear histone at nontoxic concentrations proved to be an excellent opsonin despite the fact that only about 30% of its amino acids are basic (see next section). Since CPs very efficiently opsonized highly encapsulated group C streptococci and *Klebsiella pneumoniae*, as determined by the capacity of the coated cells to induce chemiluminescence in PMNs (59), and since nonopsonized bacteria were not phagocytosed, it was suggested that CPs might also function as auxiliary opsonins

in vivo. Although no direct proof to this effect is available in the published literature, preliminary observations (Ginsburg and Polatchek) suggested that *Cryptococcus neoformans*, which had been preopsonized in vitro by nonlethal concentrations of histone, caused a much milder infection in mice as compared with mice which had been infected by untreated yeasts. It is suggested that the cationized yeast cells were "recognized" by the mononuclear phagocytic system and were removed by phagocytosis.

An unexpected observation on the role of CPs in endocytosis of yeast and bacteria by "nonprofessional" phagocytic cells was described in detail by Ginsburg et al. (42). *Candida albicans*, which had been preopsonized either with histone or with poly-*L*-arginine (PARG), adhered, within seconds, to monolayers of mouse L-fibroblasts, to human buccal epitelial cells, to HeLa cells, and even to beating rat heart cells. The yeast cells which attached themselves to the cell membrane were later seen to migrate and localize in a rosette form around the nucleus. A few hours after attachment, endocytosis was observed. Electron microscope analysis of the cells revealed the presence of *Candida* within tight phagosomes. No fusion between phagosomes and lysosomes was evident, however. In many instances, a partial degradation of the yeast cytoplasm was seen, however, suggesting that lysosomal enzymes of the fibroblasts might have been responsible for this phenomenon (42). Nonopsonized *Candida* were never seen either attached to the cells or intracellularly. Under similar conditions, both histone and PARG-opsonized group A streptococci and *M. lepraemurium* were also avidly phagocytosed by mouse fibroblasts, but no signs of bacterial degradation were evident (Ginsburg, unpublished results).

Nuclei of human U266 myeolma cells that had been preopsonized either with histone or with PARG were also avidly taken up by mouse L-929 fibroblasts in culture (64). The nuclei that were found within tight phagosomes had undergone a partial degradation 48 h following phagocytosis. As in the case of *Candida*, the nuclei first adhered very rapidly to the cell surfaces, but phagocytosis was evident only several hours later.

Entamoeba histolytica, which failed to engulf either group A streptococci or *Candida albicans*, nevertheless avidly took up these microorganisms following in vitro opsonization by histone (65). Since fibroblasts have been shown to avidly take up (phagocytose) mast cell granules in culture (66), it is tempting to speculate that cationic agents released from the mast cells might have been involved in this process.

More recent studies (Ginsburg and Varani, in preparation) have also shown that *Candida albicans* (Figure 1), group A streptococci (Figure 2) or *E. coli* which had been preopsonized either with nuclear histone or with poly-L-arginine were avidly internalized by fibrosarcoma cells in culture. Monolayers of fibrosarcoma cells which had been treated for short periods with cationized microorganisms to allow their adherence to the tumor cells and then challenged

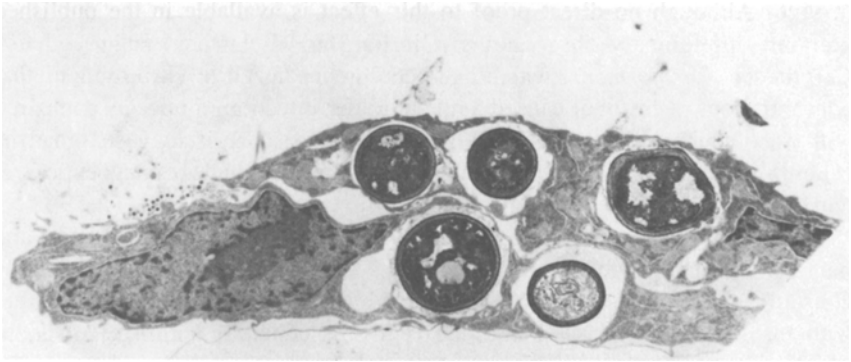

Fig. 1. Phagocytosis of *Candida albicans* by mouse fibrosarcoma cells in culture. Note the presence of yeast within phagocytic vacuoles (Giemsa stain, ×10,000).

either by human or by rat neutrophils had peeled off the surfaces of the plastic dishes. The tumor cells, however, were not killed. Since scavengers of oxygen radicals did not prevent the breakdown of the tumor monolayers, but a variety of proteinase inhibitors did, it is suggested that proteinases released from the activated neutrophils were responsible for sloughing off the cultures. This phenomenon might explain how activated neutrophils could perhaps enhance tumor spreading.

Tumor cells which had been treated with cationized streptococci and then challenged with neutrophils in the presence of sodium azide were readily killed. This suggests that inhibition of neutrophil catalase facilitated the accumulation of hydrogen peroxide which might have been responsible for killing of the tumor cells.

A variety of CPs were found to inhibit the binding of human IgG to Fc receptors upon group A streptococci (67). While protamine and arginine-rich histone markedly inhibited the binding of IgG, neither lysine-rich histone nor egg-white lysozyme had any effect. This suggested that the arginine moieties of the CPs were more effective than the lysine ones. We also found similar results concerning the chemiluminescence-inducing properties of histone (68). CPs might, therefore, interfere with the uptake of Fc-bearing streptococci by phagocytes.

The role played by CPs derived from PMNs in cell adherence and aggregation has also been discussed (27–29). Since adherence of PMNs to endothelial cells was found to be accompanied by capillary wall destruction due to both oxidative (69) and nonoxidative mechanisms (70), it might be postulated that cationic agents serve as ligands which enhance the adherence of PMNs to such surfaces (see below).

Nuclear histone has been found to interfere with the binding of both LPS and lipoteichoic acid (LTA) to human red blood cells (71). This was, however, strongly inhibited by trypsin and by neutrophil extracts. Thus passive immune

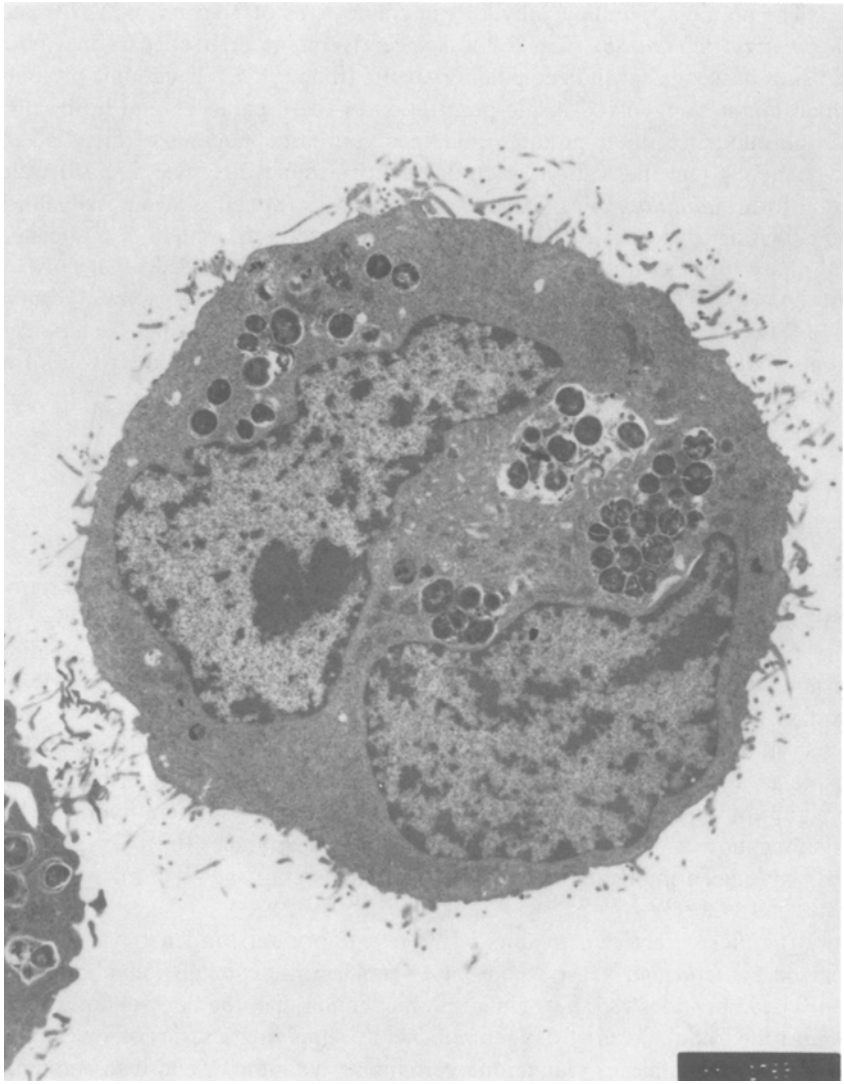

Fig. 2. Phagocytosis of histone-opsonized group A streptococci by mouse fibrosarcoma cells in culture. Note the large numbers of streptococci in the cytoplasm of the tumor cell (electron microscopy, ×9000).

kill induced by the interaction of antibodies with cells sensitized by LPS or by LTA might be regulated. Since both LTA and LPS might function as ligands aiding in the anchoring of bacteria to cell surfaces (72), the role of CPs as modulators of the early events of bacterial colonization should also be considered.

The possible role played by CPs in endocytosis of *T. gondii* (73, 74) and *Plasmodium falciparum* (75, 76) was recently reported. It appears that both parasites possess a specialized polar organelle (rhoptry) rich in cationic proteins which might be involved in the penetration of these parasites into host cells. The plasmodial cationic proteins contained very large amounts of histidine. It is of interest that the histidine-rich polycations found in saliva (77, 78) were lethal for *Candida albicans* (79) and *Strep. mutans* (80). It is also possible that the clumping properties of saliva towards oral bacteria (81) might be associated with these types of proteins. It has been reported (82) and (82A) that poly-L-histidine (PHSTD) is one of the most potent activators of the respiratory burst in PMNs (see next section). Thus CPs serve not only as distinct bactericidal agents, but also as important ligands capable of enhancing endocytosis of a variety of particles.

CPs AS ACTIVATORS OF RESPIRATORY BURST IN LEUKOCYTES

It is well accepted that the most important microbicidal effects of neutrophils are linked with their capacity to generate toxic oxygen radicals (83–91). The formation of chlorine compounds by the myeloperoxidase–H_2O_2–halide system is believed to kill microorganisms following phagocytosis (85). NADPH oxidase associated with the leukocyte membrane is thought to be the key enzyme involved in the generation of superoxide (O_2^-) (86) which could further dismutate to H_2O_2 either spontaneously or via superoxide dismutase. The interaction of superoxide with hydrogen peroxide might also yield hydroxyradicals thought to mediate peroxidation of lipids in membranes (69). Oxygen radicals have been implicated as distinct tissue-damaging agents in many disease states (69, 92, 93).

The most effective activators of the respiratory burst in leukocytes are opsonized bacteria and yeasts, phorbol esters, lectins, calcium ionophore, and formylated peptides (85–87). Under normal conditions, the body defends itself against the toxic effects of oxygen radicals by supplying a series of scavangers such as SOD, catalase, glutathione peroxidase, vitamin E, and beta-carotene, all widely distributed in tissues (83–87, 90, 91).

The consideration that CPs might also function as activators of the respiratory burst in PMNs and in macrophages was based on our observations on the opsonic properties of these agents as described in detail in the preceding section.

Streptococci, staphylococci, bacilli, yeasts, and a variety of gram-negative rods which had been preopsonized by histone induced the formation of very intense luminol-dependent chemiluminescence (LDCL) by human PMNs as well as in mouse peritoneal macrophages (59, 68, 82). Arginine-rich histone was

superior to lysine-rich histone as a stimulator of LDCL when employed to opsonize particles (see preceding section). PARG was also found to be more effective than PLYS, poly-L-ornithine, or PHSTD as a stimulator of LDCL when employed in the absence of a carrier particle (82). It is of interest that most of the highly bactericidal cationic proteins (LCPs) derived from neutrophil lysosomes are also arginine-rich (12), pointing to the higher efficiency of the arginine structure as a perturber of membranes of bacteria and of mammalian cells. The stimulation of oxygen radical generation by PLYS was also recently reported (94).

The properties of the synthetic CPs as stimulators of LDCL depended on their molecular weights. Polymers with a molecular weight smaller than 5000 were inactive. On the other hand, polymers with a molecular weight greater than 10,000 were invariably toxic to PMNs. Toxicity, however, did not interfere with their capacity to stimulate neutrophils to release oxygen radicals (95, 96).

PARG, but not PLYS or PHSTD, collaborated with the chemotactic peptide FMLP and with phytohemagglutinin to trigger very intense and synergistic LDCL responses (95). This "cocktail" also induced the generation of large amounts of O_2^- (95, 96).

Acid extracts, derived from human buffy-coat cells as well as from purified PMNs following freezing and thawing, which contained cationic proteins were also capable of "opsonizing" group A streptococci to induce enhanced O_2^- generation. This was further boosted by cytochalasin B (CYB). More recent studies (82, 82A) showed that PHSTD was a very effective stimulator of O_2^- generation by PMNs. Unlike PARG, PHSTD acted both as a soluble agent and as a particle which was internalized by phagocytosis as demonstrated by electron microscopy. PARG, in collaboration with PHA and with CYB, could also reactivate the O_2^--generating capacities of human PMNs which had been partially lysed by saponin, digitonin, or lysolecithin. This occurred provided that NADPH (lost by diffusing out through the injured membrane) was replenished (96). Since exposure of the PMNs to PARG for more than 5 min killed most of the cells and since the same amounts of PARG triggered O_2^- generation immediately upon addition to the cells, it was postulated that PARG acted both as a cytolytic agent and as a "primer" of the NADPH oxidase involved in the generation of O_2^- (87, 96).

The mechanisms by which CPs triggered the generation of oxygen radicals by PMNs is not fully known. It may, however, be postulated that, like other ligands, the CPs interacted with the cell surface to induce a transmembranal signal which aided in the assembly of certain of the components known to be needed to activate the membrane-associated NADPH oxidase (86, 87, 90, 91). It is also tempting to speculate that CPs might be involved in two bursts of oxygen radical generation following their interaction with PMNs. The first burst might occur when the CP was bound to a particle and interacted, by electrostatic

forces, with complementary negatively charged sites on the cell membrane leading to the activation of the NADPH oxidase (see also reference 82A). A secondary burst might, however, also take place inside the phagosome when bactericidal cationic proteins, released from lysosomes, interacted with the particle surface. Thus CPs, might function not only as opsonins and as killing agents, but also as potent modulators of oxygen radical generation.

ROLE OF CPs IN BACTERIOLYSIS

The importance of the lysosomal apparatus of "professional" phagocytes in the killing and biodegradation of microbial cells, both in vitro and in vivo, has been discussed in great detail (10–13, 46–48, 98, 99, 102). It stands to reason that the rich arsenal of PMN hydrolases, including the key muralytic enzyme, lysozyme, should, theoretically, be capable of biodegrading the complex structures of microbial cells leading to the rupture of the protoplast and to the elimination of the highly phlogistic peptidoglycan–polysaccharide (PPG–PS) complexes from infectious and inflamed sites. Surprisingly, however, the majority of pathogenic microorganisms appear to be highly refractory to degradation by leukocytes both in vitro and in vivo (10, 11, 102). Thus, the role played by lysozyme in bacteriolysis is controversial (see below). Lysozyme may, however, act indirectly by collaborating with serum complement in the killing, and perhaps also in the biodegradation, of gram-negative rods (100, 101).

There is, however, some confusion in the literature concerning the definition of a bactericidal versus a bacteriolytic event. While extensive degradation of PPG–PS complexes of the cell walls may lead to a bactericidal reaction (lack of replication), the killing of bacteria either by the oxygen-dependent or oxygen-independent microbicidal systems of leukocytes might not necessarily be accompanied by a drastic degradation their cell-wall structures (10, 11) (bacteriolysis) (102). Thus one should categorically differentiate between the two terminologies. It was observed that apparently intact microbial cell-wall components persisted for long periods, within macrophages, in sites of chronic inflammation in animal models (reviewed in 46–48, 102). This suggested that the extreme resistance of the cell walls to degradation was due either to the specialized highly crosslinked structures of the murein or to the inactivation, by the inflammatory exudates, of the lysosomal enzymes involved in microbial degradation. It is also possible that both reasons contributed to the "storage" of phlogistic cell-wall components in tissues.

In recent studies (7, 46–48, 102–124) it has become apparent that a tight collaboration between leukocyte cationic proteins (LCP) and the bacteria's own autolytic wall enzymes might have been needed to secure the enzymatic biodegradation of the cell walls of certain microbial species. Most of these studies have been made with *Staph. aureus* and with *Strep. faecalis.*

Staph. aureus, which had been labeled with [^{14}C]*N*-acetylglucosamine (NAGA) and which had been incubated either with a variety of CPs, including LCP, or with highly diluted human serum or synovial fluid, were observed to undergo a gradual lysis. Bacteriolysis was assessed by determining the solubilization of labeled NAGA and by electron microscopy. While a substantial solubilization of the labeled NAGA occurred 5–15 h folowing the addition of the various agents, practically no lysis took place if heated bacteria (65°C for 10 min) were employed instead. Since bacteriolysis could be fully regained if freshly harvested unlabeled staphylococci were added to heated [^{14}C]staphylococci, in the presence of the CP (114, 120), it was assumed that heat-labile endogenous bacterial factor(s) collaborated with the CPs to induce cell-wall degradation. The labile staphylococcal agents were assumed to be linked with the autolytic wall enzymes which regulated normal cell division (127) and which were tightly controlled by lipoteichoic acid (LTA) (128, 129). Our results suggested, therefore, that the CP probably upset the regulation of the autolytic wall enzymes by binding to LTA (109, 111). This led to the degradation of the rigid cell walls from within (suicide) and to bacteriolysis.

The cell-wall degradation induced by LCP, by the synthetic CPs (PARG, PLYS), as well as by the diluted sera always assumed bell-shaped curves (113, 114). At very low concentrations (a few micrograms of protein in the case of extracts containing LCP, serum, or synovial fluids, and about 10^{-7} M of the synthetic CP) a very extensive cell-wall degradation took place; higher concentrations totally shut down bacteriolysis. The data suggest that either the CP inactivated the autolysins, due to their high toxicity for the protoplast membrane (36), or that the crude LCP, sera, and synovial fluids contained inhibitors of the putative activators (CP). Since a variety of anionic polyelectrolytes [heparin, chondroitin sulfate, dextran sulfate, suramine, polyanethole sulfonate (liquoid)] very markedly inhibited bacteriolysis which had been initiated by the CPs (107, 110, 112–114, 115, 130) and since bacteria which had been cultivated in the presence of polyanions also became highly refractory to bacteriolysis, it appeared that polyanions might function as regulators of the autolytic wall enzymes (130). The question whether lysozyme functioned as a distinct muralytic enzyme, as a cationic protein, or as both was also investigated. Surprisingly, heat-activated egg-white lysozyme, which had totally lost its lytic effect on *M. lysodeikticus*, nevertheless still continued to activate autolysis in staphylococci. Our findings were recently corroborated with *Bacillus cereus* (131), *Strep. pneumoniae* (132), as well as with *Strep. faecalis* and *Strep. sanguis* (133), further supporting our assumption that CPs might trigger bacteriolysis. The potent bactericidal–permeability-increasing cationic proteins (BPI) which permeated the outer membranes of gram-negative rods also induced some degradation of bacterial phospholipids and peptidoglycan. This also suggested the involvement of autolytic wall enzymes in the lysis of gram-negative bacteria (10, 11).

The massive digestion of cytoplasmic constituents of bacteria by elastase and by other proteases (134, 135) did not necessarily lead to the degradation of the cell walls (46–48). The possible role of proteases in the inactivation of autolysins should, however, also be considered. It was demonstrated (136, 137) that trypsin inactivated the autolysins of *M. lysodeikticus*. Proteases might, however, also digest CPs derived from leukocytes and thus function as regulatory agents in bacteriolysis.

The role of oxygen radicals as modulators of bacteriolysis should, however, also be considered. The exposure of staphylococci either to a superoxide-generating system (xanthine–xanthine oxidase) or to hydrogen peroxide inactivated their autolysins (Ginsburg and Lahav, in preparation), pointing to the vulnerability of the autolytic wall enzymes in the inflammatory milieu.

These phenomena might also explain, in part, the reasons for the long persistence of undegraded microbial cell walls in inflammatory sites (46–48, 102).

Our observations that liquoid and other polyanions markedly inhibited lysozyme action on *M. lysodeikticus* and the lysis of staphylococci by lysostaphin (130) [known to contain glycosidase, muramidase, peptidase, and amidase activities (138)], further shed light on the role that may be played by anionic polyelectrolytes as regulators of CP-induced bacteriolysis. It is also important to mention, in this context, that a variety of glycosaminoglycans (heparin, chondroitin sulfate, and hyaluronate) were found to inhibit 21 different glycosidases, including lysozyme, present in PMN lysosomes (139). This lends further support to our findings on the inhibition by liquoid of lysostaphin action (130). Very surprisingly, however, a variety of polycations also markedly inhibited the lysis of *M. lysodeikticus* by lysozyme, suggesting that the cationic agents blocked the sites of attachment of lysozyme upon the bacterial cells (Ginsburg, unpublished results). This phenomenon could, however, be similar to the findings that spermine (a polyamine) protected the lysis of *M. lysodeikticus* by lysozyme presumably by stabilizing the protoplasts generated following the lysis of the murein (139A). Further support for the assumption that polyanions might regulate autolytic enzymes came from our studies on penicillin-induced lysis in staphylococci (118). It is thought that bacteriolytic concentrations of penicillin might lyse cells by releasing LTA, believed to be the regulator of autolysins in gram-positive bacteria (128, 129).

The activity of LTA (a polyanion) might, however, also be regulated by CPs shown by us to bind to and release LTA from streptococci (109).

Since we have shown (130) that liquoid inhibited the lysis of staphylococci treated with CPs, presumably by interfering with the autolysins, we postulated that liquoid might also inhibit penicillin lysis. Indeed the addition of liquoid to growing staphylococci in the presence of bacteriolytic concentrations of penicillin allowed the bacteria to continue growing. Electron microscope analysis of such cultures, however, revealed abnormal cell division. Many cross-walls

appeared in a single coccus, suggesting an interference with the deposition and separation of the cell walls. This phenomenon might be typically caused by inhibition of the normal activity of autolytic wall enzymes. It is also of interest that penicillin-grown staphylococci and streptococci were degraded much faster than controls following treatment either with CPs from leukocytes or with lysozyme (108).

Although it is appealing to think that degradation of microbial cell walls might involve activation of the autolytic wall enzymes by CPs, this phenomenon has been shown only in in vitro studies and with a selective group of gram-positive (102, 110, 114, 131–133) and perhaps some gram-negative rods (11). No evidence exists that autolysins are involved in the biodegradation of streptococci, mycobacteria, nocardia, pathogenic yeasts, or fungi. It is well known that cell walls of these microorganisms are involved in the generation of granulomatous inflammation (46–48) (see below). Furthermore, the possible involvement of the autolytic wall system in the killing (140) or degradation of pneumococci by PMNs (141) has recently been challenged. Pneumococcal mutants deficient in autolysins have been shown to undergo the same rate of killing and lysis as the parent strain. Thus the question of the participation of autolysins in microbial killing and degradation is still open for further scrutiny (see also references 10, 11, 102).

Further insight into the problem of the biodegradation of microbial cell-wall components in vivo came from extensive studies on the persistence of microbial cell-wall components in inflamed sites (46–48, 124, 142–149). The persistence of nonbiodegradable microbial cell walls in vivo is probably central in the initiation of chronic inflammatory sequellae. The activation of macrophages by bacterial PPG–PS complexes (149) has been suggested to be the major cause of tissue damage seen in infectious granulomas (142–149). Even penicillin-grown bacteria (121, 147) persisted with apparently intact cell walls within macrophages in joint (121) and liver (147) lesions for long periods. Resistance of the PPG–PS complexes to degradation in vivo could therefore also be a result of the selective inactivation either of leukocyte hydrolases and CPs or of the bacterial autolysins by proteinase, glycosaminoglycans, and oxygen radicals, known to accumulate in large amounts in inflammatory and infectious foci (46–48, 143–149).

ROLE OF CATIONIC AGENTS IN IMMUNE COMPLEX DISEASE

The role of cationic agents as modulators of the functions of immune complexes has recently emerged from studies on experimental glomerulonephritis and arthritis. It is well established that sialloglycolipids and heparan sulfate proteoglycans are responsible for the negative charges of the glomerular capil-

lary wall (150, 151) and the synovial tissues (152). In the kidney, these polyanions (PAs) have been shown to affect glomerular permeability to proteins bearing different charges. Any reduction of the glomerular charges may, therefore, contribute to proteinuria. In the synovium, the PAs might trap cationic agents (152). The net charge of the capillary wall in the kidney is also important as it may facilitate the deposition of cationic proteins that might function as planted antigens to initiate immune complex formation and glomerular injury. Polycation treatment in vivo could also reduce the charge barrier and reduce deposition of cationized antibodies, thereby contributing to the modulation of the severity of pathological alterations seen in kidney diseases.

This novel approach to the study of the pathogenesis of immune complex disease has been dealt with in great detail in a series of publications over the last five years (153–169). Most of the experimental models reported have employed antigens which had been cationized by the method of Danon et al. (170), using carbodiimide as activator and *N*,*N*-dimethyl-1,3-propandiamine as a nucleophile to replace carboxyl groups in the protein.

A significant fixation of protein antigens in the kidney took place when their isoelectric point (PI) exceeded a threshold level of 8.5–9.5. It was also found that cationized IgG could bind to rat glomerular basement membrane where it acted as a planted antigen and induced the in situ formation of complexes which was accompanied by severe glomerulonephritis (153–173).

The possible therapeutic value in the prevention of immune complex nephritis induced by cationized antigens has recently been reported (161). Animals that had been pretreated with the relatively nonimmunogenic protamine sulfate had significantly fewer renal lesions as compared with controls. It was postulated that protamine, which bound to PA in the glomeruli through electrostatic forces, blocked the binding of the cationized antigen, thereby limiting the severity of kidney pathology. Cationization of antigens and antibodies by naturally occurring CPs might, however, shed more authentic light on the pathogenesis of human nephritis, arthritis, and perhaps other immune complex diseases. Cationic proteins derived from human platelets were shown to bind to the glomerular capillary wall of rat kidney as well as to the mesangium and peritubular capillaries (171). The interaction was ionic in nature, and treatment of the kidney sections with heparin abrogated the binding of the cationic proteins to the glomerular structures. The glomerular deposits of the CPs were associated with the loss of glomerular PA. The binding of the polycations to PAs in the kidney could explain the increase in glomerular permeability. The localization of neutrophil-derived CPs in 14 patients with systemic lupus erythematosus (SLE) nephritis was studied (172). Employing antibodies to the cationic agents, it was found that CPs were localized in the capillary walls of kidneys in seven of the patients. This was accompanied by proteinuria and severe nephrotic syndrome. A loss of glomerular PA was also observed. The PMNs present in the peripheral blood of the patients were markedly depleted

of their CPs, suggesting that the release in vivo of CP was directly connected to the development of glomerular injury.

The perfusion of rats with avidin, a native basic protein, followed by the administration of anti-rat avidin serum, resulted in the deposition of rat IgG along the glomerular capillary loop. This suggested that avidin was bound to anionic sites in the kidney where it acted as a planted antigen. This might result in in situ immune complex formation (173).

Cationic antigens elaborated by nephritogenic streptococci might also be implicated as planted antigens instrumental in the development of poststreptococcal glomerulonephritis (AGN) (174). Cationic antigens from streptococci were detected in eight of 18 renal biopsies taken from patients with AGN. The antigens were found to be bound to both IgG and IgM antibodies. Since at the time of biopsy the patients' sera contained antibodies to the streptococcal extracellular antigens, it was postulated that the cationic antigens released during infection were planted in the kidney by electrostatic interactions. This might lead to the development of immune complex disease.

The possible role played by cationized antigens in the pathogenesis of experimental arthritis has also been reported (175, 176). While native BSA with a PI of 4.5 was unable to induce chronic joint inflammation upon intraarticular injection into preimmunized mice, long persisting arthritis occurred if amidated BSA (PI = 8.5) was administered to the preimmunized animals. This was followed by a long persistence of antigen in the joints. Also unlike in animals injected with native antigen, large amounts of cationized BSA were retained in all the collagenous structures of the joints with deep penetration into the dense hyaline cartilage. This study suggested that the chronicity of joint inflammation seen in many diseases might be determined by the degree of cationization of the antigens. Ideal candidates for such antigens might be bacterial cell wall components which might adsorb on their surfaces CPs derived from leukocytes. This might lead to the enhancement of their pathogenicity.

The role played by bacterial cell walls in the initiation of chronic arthritis in experimental animals has been extensively reviewed (46–48, 143, 146–148, 177–180). Chronic arthritis is thought to be caused mainly by activated cell-wall-laden macrophages which secrete lysosomal enzymes and oxygen radicals shown to induce severe tissue damage (92, 93, 149).

Recent studies (181) have also examined the role played by cationized oxygen scavengers as potential modulators of joint inflammation. Cationized superoxide dismutase (SOD), catalase, or peroxidase were found to be more effective than the native enzymes as depressors of chronic joint inflammation in the mouse. These findings suggested that the "fixation" of the cationized enzymes in the tissues contributed to their longer survival, thus increasing their scavenging efficiencies. Thus cationic enzymes may offer a novel approach to therapeutics.

Recent studies (Ginsburg, Warren, Johnson, and Ward, submitted for pub-

lication) demonstrated that BSA–anti-BSA complexes (CX) which had been cationized by histone, PARG, or PHSTD induced the formation of larger amounts of O_2^- by PMNs as compared with the amounts induced by the unaltered CX. Very surprisingly, however, CX treated with polyanethole sulfonate (liquoid) were even more effective than the cationized CX in O_2^- generation. Since liquoid was found to bind strongly to streptococci and to induce intense LDCL in human PMNs (68), it appears that strongly charged macromolecules, when bound to particles, could perturb the leukocyte membrane. We also found that liquoid complexed either with histone, PARG, or PHSTD also induced the generation of large amounts of O_2^- by PMNs which were markedly enhanced by cytochalasins.

The role of polyelectrolytes as amplifiers of the reversed Arthus phenomenon was also studied. Anti-BSA IgG which had been cationized either with histone, PARG, or PHSTD was injected intracutaneously into rats. This was followed 2 h later by intravenous injection of radiolabeled BSA. It was found that both the cationized and anionized antibodies induced a marked enhancement of the severity of the inflammatory process in the skin as determined by the accumulation of radioactivity at the injected sites. It might be postulated that the cationized antibodies were "planted" in the tissues and facilitated the interaction of the complexes with the antigen. The severity of the skin lesion might also be enhanced by the release of excessive oxygen radicals by PMNs. The generation of oxygen radicals following the stimulation of rat PMNs by immune complexes has been described (182). It might also be postulated that cationic agents of neutrophil, eosinophil, or platelet sources might act in vivo in a similar manner. The presence of eosinophil (15) and neutrophil (172) cationic proteins in tissues of patients points to the release of such agents in vivo. Finally, complexes formed between hyaluronate and poly-L-lysine induced the generation of chronic arthritis when injected intraarticularly to rabbits (183). On the other hand, neither PLYS nor hyaluronate alone induced chronic inflammation (183). It appears that PLYS might have been protected the hyaluronate, and vice versa, against enzymatic cleavage by proteases and hyaluronidase. This contributed to the long survival of these non-immune complexes in the tissues. Such complexes might be phagocytosed by both PMNs and macrophages to induce exocytosis of enzymes capable of breaking down the connective tissue elements in a manner similar to that found following the phagocytosis of immune complexes. Such non-immune complexes might also stimulate the generation of oxygen radicals (82A).

COMMENTS

The present review deals with experimental models which point to the possible role played by cationic polyelectrolytes (CPs) as modulators of several basic phenomena related to the interaction of the host with foreign invaders.

The capacity of CPs to interact, by electrostatic forces, with both prokaryotic and eukaryotic cells as well as with immune complexes, and the ability of anionic polyelectrolytes (APs) to modulate such interactions, suggest that the complex polyelectrolytic milieu of the inflammatory bed might affect the outcome of the interactions of hosts with their parasites.

It appears that the common denominator in the activity of the various CPs is their capacity to interact by electrostatic forces with negatively charged domains on the surface of cells, leading to the activation of the metabolic patterns of both eukaryotic and prokaryotic cells.

Based on the experimental models presented, it might be postulated that the following series of events, involving both CPs and APs, might take place following the assault of the tissues of a host by pathogenic microorganisms.

CPs might already be involved in the very early phases of bacterial adherence to epithelial cells. This might be mediated by highly negatively charged surface macromolecular complexes such as LPS and LTA. The accumulation of PMNs attracted either by complement-derived chemotactic agents or by chemotactic agents from bacteria might also be altered by CPs. These might either block, in a nonspecific fashion, the membrane receptors or interact directly with the chemotactic agents. PMNs, eosinophils, and platelets, attracted to the inflammatory sites, might also release large amounts of bactericidal and tissue-damaging CPs. Furthermore, CPs might also "opsonize" both target cells and leukocytes to enhance their mutual adherence. This might facilitate target cell killing. PMNs which had become activated by CP-opsonized bacteria or by immune complexes might release proteolytic enzymes capable of disintegrating solid tumors. This might enhance tumor spreading and metastasis.

CPs might also act both as procoagulants and as anticoagulants (184–187) by virtue of their interaction with fibrinogen. This might alter the early phases of inflammation. Arginine-rich CPs might also enhance the release of histamine from mast cells (188) and lysosomal enzymes from PMNs (189), thus contributing to tissue damage.

CPs from eosinophils or from PMNs might also function as suppressors of lymphocyte functions (190) and thus regulate immunological reactions.

CPs and oxygen radicals of leukocyte origin might also deregulate autolytic wall enzymes in bacteria. This might lead, on the one hand, to bacteriolysis and to the release of highly phlogistic peptidoglycan–polysaccharide complexes LTA and LPS, but on the other hand, to the persistence of nondegraded cell-wall components. CPs might also promote the adherence of PMNs to endothelial cells and their migration into tissues.

CP-opsonized bacteria might stimulate PMNs and macrophages to generate oxygen radicals and lysosomal hydrolases injurious to tissues. CPs might also stimulate phagocytosis of microorganisms and pinocytosis of soluble macromolecules by "nonprofessional" phagocytic cells like fibroblasts, epithelial cells, etc. If such phagocytosis indeed took place in vivo, it might contribute to a longer survival of microbial cell wall components in cells that possess a

poor lysosomal apparatus and which totally lack lysozyme, the key muramidase. The survival and persistence of PPG–PS within activated macrophages is thought to be the major cause of tissue damage seen in infectious granulomas.

The interaction of CP with both antigens and antibodies might cationize the proteins which might now become "planted" upon polyanions on the surface of the capillary wall, the basement membrane of the kidney, and the synovial membrane. The planted cationized agents might then be involved in the pathogenesis of immune complex phenomena.

By virtue of their high cationic charges, CPs might function to enhance the uptake of liposomes, laden with drugs, by cells (Ginsburg, Gatt, and Bernholtz, in preparation). CPs might also enhance the fusion of phospholipid membranes and vesicles (191–193), thus contributing to rearrangement of membrane structures.

Cationized scavengers of oxygen radicals might be retained in inflamed tissues for longer periods to afford a better protection against oxygen toxicity.

It should, however, also be considered that polyanions which are likely to accumulate in infectious and inflammatory sites, due to the degradation of the connective tissue elements, could also form complexes with the CPs to neutralize their bactericidal, bacteriolytic, and other biological activities. Such complexes might also mimic the effects of immune complexes in their ability to stimulate the generation of oxygen radicals by leukocytes (82A). CPs, however, might inhibit the adherence of CP-opsonized bacteria and immune complexes and function as regulators of leukocyte stimulations.

It is plausible, therefore, that a vicious circle, involving a multiplicity of polyelectrolytic factors, might occur in tissues of hosts assaulted by pathogens.

Although many of the models described in this review employed cationization of proteins by chemical means, it is highly likely that naturally occurring cationic agents of cell and tissue origin might function in a similar way to modulate antigens, antibodies, and cell surfaces.

It is hoped that the models and approaches presented in this review will shed new light on several still controversial phenomena which accompany the complex interactions between hosts and parasites as seen in human disease.

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