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Depression of Monocyte and Polymorphonuclear Leukocyte Oxidative Metabolism and Bactericidal Capacity by Influenza A Virus

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Decreased host defense against bacterial disease associated with influenza infection may be related to virus-induced changes in phagocytic cell function. Influenza A virus initiates the respiratory burst in peripheral blood monocytes and polymorphonuclear leukocytes, with a peak chemiluminescent response approximately 3 min after virus is added to the cells in vitro. Electron micrographs of phagocytic cells incubated with influenza virus demonstrated virus attached to the cell membrane and within phagocytic vacuoles. After 20 min of incubation of the virus with phagocytic cells, the chemiluminescent response to opsonized zymosan or phorbol myristate acetate was decreased by 30 to 90%. Phagocytic activity of monocytes and polymorphonuclear leukocytes incubated with influenza virus was normal, but the bactericidal activity was significantly depressed. Influenza A virus therefore stimulates an oxidative burst in monocytes as well as polymorphonuclear leukocytes, leading to a subsequent depression of the oxidative metabolic response and bactericidal capacity of the phagocytic cells.

Human influenza virus infection is associated with an increased susceptibility to bacterial and fungal infections (10, 13, 17, 23, 33, 36). Influenza virus has been demonstrated to stimulate the respiratory burst in polymorphonuclear leukocytes (PMNs) (18) and, in both in vivo and in vitro systems, has been shown to induce abnormalities of PMN and macrophage function, including depression of oxidative, chemotactic, phagocytic, and bactericidal activities (1, 8, 11, 12, 15, 16, 28–30). Influenza-induced changes in phagocytic cell function may predispose the host to secondary bacterial or fungal invasion, since these cells are crucial to host defense against these microbes.

The present report describes the effect of influenza A virus on initiation of the respiratory burst in peripheral blood mononuclear leukocytes (MNs) and PMNs and the subsequent oxidative, phagocytic, and bactericidal responses of these cells. Influenza virus initiates a respiratory burst in MNs as well as PMNs. After incubation of these phagocytic cells with influenza virus for 20 min, there is depression of the chemiluminescent response to zymosan and phorbol myristate acetate (PMA) and of bactericidal activity against *Staphylococcus aureus*, but phagocytic activity is normal. The study shows that influenza virus can induce metabolic dysfunction in both the peripheral MNs and PMNs which is temporally related to initiation of the phagocytic cell respiratory burst by the virus.

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MATERIALS AND METHODS

Preparation of reagents. Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma Chemical Co., St. Louis, Mo.) and zymosan (Sigma) were prepared as previously described (19). PMA was prepared as a stock solution (0.2 mg/ml in dimethyl sulfoxide) and stored at −70°C until just before use.

Preparation of microbes. Influenza virus type A, Texas 77 (H_3N_2), obtained courtesy of N. Masurel (Rotterdam, The Netherlands), was harvested from allantoic fluid as described previously (18). The stock virus was clarified by centrifugation at 1,000 × *g* for 30 min and stored at −70°C until just before use. The virus had a hemagglutination titer of 1:640 and a 50% egg infective dose of 10^8/ml.

Bacteria. *Staphylococcus aureus* 502A was maintained on solid nutrient agar. Bacteria were grown overnight in broth (antibiotic medium 3; Difco Laboratories, Detroit, Mich.) at 37°C and harvested at an early stationary phase (18 h). The bacteria were centrifuged at 700 × *g* for 10 min, washed three times in phosphate-buffered saline, and kept at 4°C until used.

Leukocyte separation. Heparinized blood (10 U of heparin per ml of blood) obtained from human volunteers was sedimented on 6% dextran (Cutter Laboratories, Berkeley, Calif.). The buffy coat was layered onto lymphocyte separation media (Bionetics, Inc., Bethesda, Md.) and centrifuged at 375 × *g* for 25 min, and the mononuclear cell layer was removed at the

interface. Residual erythrocytes in the erythrocyte-neutrophil pellet were lysed with 0.87% NH_4Cl. The PMN cell preparation was sedimented at 160 × *g* for 5 min, washed twice with Hanks balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.) with 1% gelatin without phenol red (gel-HBSS), resuspended in gel-HBSS, counted by a Coulter Counter (model ZBI; Coulter Electronics, Inc., Hialeah, Fla.), and adjusted to the desired concentration. This preparation contained ≥97% PMNs. Separation of MNs was done by adherence of MNs to baby hamster kidney cell flasks (BHK-21; American Type Tissue Culture, Inc., Rockville, Md.) as described by Ackerman and Douglas (2), and this preparation contained ≥95% MNs.

Chemiluminescent activity. Chemiluminescent activity was measured by the method of Mills et al. (20). To each dark-adapted scintillation vial, 4.08 ml of gel-HBSS, 20 μl of luminol, and 2.5×10^4 MNs or PMNs per ml were added, and background counts were determined in a liquid scintillation counter (LS 150; Beckman Instruments, Inc., Irvine, Calif.). At zero time 0.1 ml of nonopsonized influenza A virus or gel-HBSS was added to duplicate vials. The vials were agitated for 30 s, and each vial was counted for 30 s every 3 min for a total of 20 min at 22°C. In experiments in which a second stimulus was used, 0.4 ml of zymosan (preopsonized in 75% pooled human serum), 55 μl of PMA, or 0.1 ml of virus was added to the vials, each vial was agitated for 30 s, and counts were recorded for an additional 20 min. Preliminary experiments had demonstrated that the dose of each stimulus used in the present report maximally stimulated the chemiluminescence reaction and that allantoid fluid taken from non-virus-infected eggs did not initiate chemiluminescent activity or affect the chemiluminescent response to subsequent stimuli. Each experiment was done on three separate occasions, and chemiluminescent activity was expressed as peak counts per minute per cell.

Phagocytic and bactericidal capacities. MNs and PMNs were incubated with 0.1 ml of influenza A virus or gel-HBSS in the absence of serum for 20 min at 22°C. For some experiments the cells were centrifuged at 160 × *g* for 5 min, washed twice with gel-HBSS, and resuspended to the desired concentration. The phagocytic activity of MNs and PMNs was studied by the method of Peterson et al. (22). Radiolabeled *S. aureus* (10^7/ml) which had been preopsonized with 10% pooled human serum for 30 min was incubated with the cells at ratios of 10:1 and 100:1. The phagocytic mixture was apportioned into polypropylene vials (Bio-Vials; Beckman) and gently agitated at 37°C. At 5 and 30 min, cold phosphate-buffered saline was added to quadruplicate vials. Two vials were subjected to differential centrifugation at 160 × *g* for 5 min to separate cells from bacteria, and the other two vials were centrifuged at 1,000 × *g* for 15 min to sediment all bacteria and cells. Scintillation counting was done to calculate the percentage of bacteria phagocytized by the cells at the specified times.

For the bactericidal assay, preopsonized *S. aureus* (10^7/ml) was added to MNs or PMNs at a ratio of 10:1 and incubated with gentle agitation at 37°C as previously described (25). At 0, 15, and 60 min, 0.9 ml of cold sterile water was added to stop the bactericidal reaction. Serial dilutions were made for measurement of viable colony-forming units after overnight incubation at 37°C on agar plates (antibiotic medium 2; Difco). Duplicate determinations were done, and the percentage of bacteria killed was calculated according to the following formula: [1 − (number of viable colony-forming units at specified time/number of viable colony-forming units at zero time)] × 100.

Electron microscopy. MNs (5×10^6/ml) and PMNs (1.5×10^7/ml) were incubated with 0.3 ml of influenza virus or gel-HBSS in the absence of serum for 20 min at 22°C with gentle agitation, and the cell suspension was then prepared for electron microscopy by previously described methods (6). In other experiments PMNs were incubated with *S. aureus* (bacteria-to-cell ratio was 10:1) for 40 min after prior incubation of the cells with influenza virus for 20 min and prepared for electron microscopy.

Statistical evaluation was performed by the Student's *t* test for independent samples ($P > 0.05$, not significant). All experiments, except those for electron microscopy, were performed in duplicate on two or more separate occasions.

RESULTS

Viability of phagocytic cells was determined after the cells had been incubated with either influenza virus or gel-HBSS for 20 min, and cell viability was greater than 95% as measured by trypan blue exclusion.

Chemiluminescent activity. Influenza virus in the absence of serum elicited chemiluminescent activity in both MNs and PMNs. The peak chemiluminescent activity occurred between 3 and 6 min after influenza virus was added to the cells. Peak chemiluminescent activity of these cells in response to influenza virus was approximately one-half that elicited by stimulation with opsonized zymosan or PMA and was 30- to 60-fold greater than the background activity with buffer (Fig. 1).

The chemiluminescent activity of MNs and PMNs in response to the particulate stimulus zymosan and the soluble stimulus PMA was significantly depressed after the cells were preincubated with influenza virus for 20 min, compared with cells exposed to buffer alone (Fig. 2). Phagocytic cells also had a decreased response to additional influenza A virus stimulation after previous exposure to the virus for 20 min. When MNs and PMNs were initially stimulated with opsonized zymosan or PMA for 20 min and then subsequently stimulated with zymosan, PMA, or influenza virus, there was no chemiluminescent activity (unpublished data). The variation in peak counts per minute for the control cells (Fig. 2) is due to the fact that leukocytes from different donors were used in each set of experiments.

Phagocytic and bactericidal capacities. The phagocytic activity of MNs and PMNs for *S. aureus* was equivalent in cells pretreated with influenza virus or with buffer for 20 min. MNs and PMNs pretreated with virus phagocytized 30 ± 3 and 42 ± 2% of the *S. aureus*, respective-

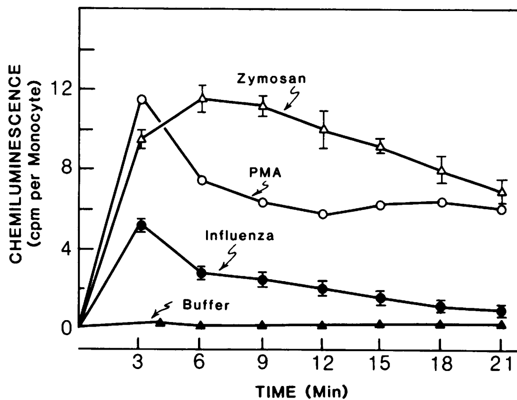

FIG. 1. Production of chemiluminescence by peripheral blood MNs in response to opsonized zymosan, PMA, unopsonized influenza A virus, and buffer. Chemiluminescent activity is expressed as mean counts per minute (cpm) per monocyte; the bars indicate the standard error of the mean of duplicate samples. This graph is a representative example of five separate experiments with each stimulus.

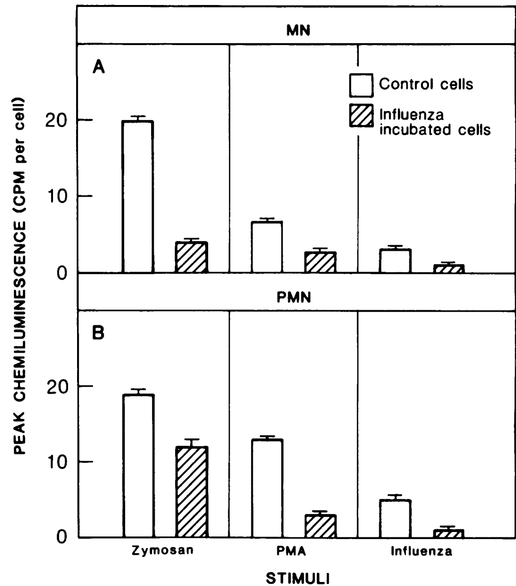

FIG. 2. Peak chemiluminescent activity of influenza virus-incubated and control MNs (A) and PMNs (B) in response to subsequent stimulation with zymosan, PMA, and influenza virus. Results are expressed as mean counts per minute (cpm) per cell; the bars indicate the standard error of the mean of three separate experiments. The influenza virus-incubated MNs and PMNs had significantly less chemiluminescent activity in response to each of the three stimuli than did control cells ($P < 0.05$).

ly, after 5 min of incubation of the cells and bacteria, compared with 28 ± 4 and 41 ± 4%, respectively, for control MNs and PMNs ($P > 0.5$). Equivalent phagocytosis also occurred in influenza virus-incubated MNs and PMNs compared with control cells after 30 min of incubation of cells and bacteria. When the ratio of bacteria to cells was increased from 10:1 to 100:1, there was no difference between the phagocytic activity of virus-treated and control cells. The bactericidal activity of MNs and PMNs was significantly depressed after pretreatment of the cells with influenza virus. After 15 and 60 min of incubation of MNs with *S. aureus*, a greater percentage of viable bacteria was found in the influenza virus-treated MNs than in controls. The bactericidal activity of influenza virus-treated PMNs was also significantly depressed (Table 1). Washing the phagocytic cells after they had been incubated with influenza virus for 20 min did not affect the results of either the phagocytic or bactericidal assays.

Electron microscopy. Electron micrographs of MNs and PMNs incubated with influenza A virus showed virus attached to the cellular membrane and within intracellular spaces (Fig. 3). This virus had a 110-nm external diameter, which is consistent with its being an influenza virus. The numbers of *S. aureus* within PMNs pretreated with influenza virus and within controls appeared to be equivalent. Influenza virus and *S. aureus* were observed within the same phagocytic cell and within the same phagocytic vacuole (Fig. 4).

DISCUSSION

We have recently shown that unopsonized influenza virus type A, Texas 77, can initiate PMN oxygen consumption, superoxide production, and chemiluminescence (18). The data pre-

TABLE 1. Bactericidal activity of influenza A virus-infected leukocytes against *S. aureus*[a]

Expt	Cell type	Cell treatment	% Bacteria killed	
			15 min	60 min
1	PMN	HBSS	71 ± 0[b]	91 ± 0.7
		Influenza	27 ± 1.4[c]	51 ± 7[c]
2		HBSS	83 ± 0	92 ± 4
		Influenza	48 ± 2.4[c]	50 ± 6[c]
1	MN	HBSS	42 ± 2.8	57 ± 8
		Influenza	21 ± 1.4[c]	38 ± 11
2		HBSS	36 ± 1.4	53 ± 2
		Influenza	18 ± 0.7[c]	39 ± 0.7[c]

[a] Bactericidal assay used a bacteria-to-cell ratio of 10:1.

[b] Mean ± one standard deviation of duplicate samples.

[c] $P < 0.02$ by two-tailed Student's *t* test.

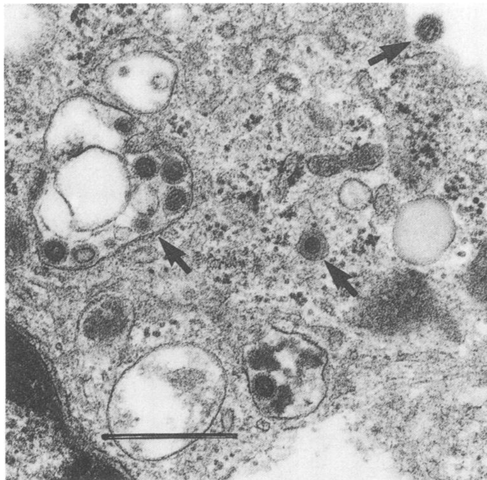

FIG. 3. Electron micrograph of a peripheral blood MN incubated with influenza A virus for 20 min at 22°C. Arrows indicate representative virus within phagocytic vacuoles and attached to the outer plasma membrane. The bar is equivalent to 0.5 μm.

sented in this study demonstrate that this same influenza virus can activate the respiratory burst in peripheral MNs and that MNs and PMNs preincubated with influenza virus for 20 min have depressed oxidative and bactericidal activities when subsequently challenged with various agents. There was little change in cell viability, and the phagocytic activity of these MNs and PMNs did not appear to be affected by prior treatment of the cells with virus, suggesting that the depressed metabolic and microbicidal cellular function could not be attributed to a defect in particle ingestion. Whereas the phagocytic assay did not allow us to differentiate between adherence of the bacteria to the leukocyte and phagocytosis, the results from the electron microscopy experiments suggested that there was equivalent uptake of *S. aureus* by influenza virus- and buffer-treated cells. The decreased chemiluminescent response of virus-treated MNs and PMNs to a soluble stimulus such as PMA further suggests that the virus-induced phagocytic cell dysfunction is independent of phagocytosis and is due to intrinsic cellular dysfunction.

Previous reports of the effect of influenza virus on leukocyte phagocytic and metabolic activities have produced conflicting results. Human PMNs incubated with influenza B virus have shown decreased phagocytic activity for *S. aureus*, whereas the hexose monophosphate shunt and chemiluminescent activities were normal (16). When influenza virus type A, Texas 77 (H_3N_2), was incubated with PMNs for 18 h, depressed chemiluminescent and phagocytic activities were observed (8). The candicidal activity of PMNs was decreased during the acute phase of human influenza A infection (7), whereas enhanced PMN phagocytic activity was noted during the convalescent stage of illness (27). Studies of the influence of influenza virus on phagocytic cell function in animals have demonstrated an interspecies as well as an intercellular variability. Guinea pig alveolar macrophages have depressed bactericidal activity and normal phagocytic capacity for *Klebsiella pneumoniae* after infection with influenza A virus (32). Guinea pigs and mice infected with influenza A and B viruses had reduced PMN glycolytic and phagocytic activities for *Streptococcus pneumoniae*, whereas PMNs from similarly infected rats had depressed glycolytic activity and normal phagocytic activity (29). Granulocytes from chinchillas inoculated intranasally with influenza A virus had depressed chemiluminescent and bactericidal activities but had intact phagocytic activity (1).

The chemiluminescence assay is a sensitive method for detecting oxygen radicals generated in stimulated phagocytic cells (5, 9, 24). These oxygen radicals play a critical role in phagocytic cell killing of bacteria and fungi (3, 4). As has been previously noted, there is a direct correlation between chemiluminescent and bactericidal activities (1, 20). The demonstration by electron microscopy of influenza virus attached to MN and PMN membranes and within phagocytic vacuoles supports the chemiluminescence data showing that influenza virus can initiate an oxidative metabolic response and oxygen radical

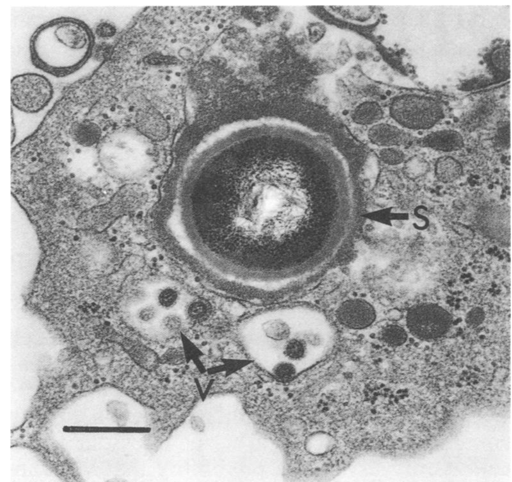

FIG. 4. Electron micrograph of a PMN incubated with influenza A virus for 20 min followed by incubation with *S. aureus* for 40 min at 22°C. Arrows (V) indicate representative virus within phagocytic vacuoles. Arrow (S) indicates *S. aureus* within phagocytic vacuoles. The bar is equivalent to 0.5 μm.

formation. The role of these oxygen radicals in the killing of influenza virus remains to be determined.

The effect of influenza virus on peripheral blood MN oxidative, phagocytic, and bactericidal activities has not been previously reported to our knowledge. In the present study, MNs as well as PMNs pretreated with influenza A virus had a decreased chemiluminescent response to the particulate stimulus zymosan and to the soluble stimulus PMA. The bactericidal activity of the cells was also decreased, but the phagocytic activity remained intact at bacteria-to-cell ratios ranging from 10:1 to 100:1. If the peripheral MNs have an important role in directly killing the invading organism, influenza-induced depression of MN oxidative and bactericidal activities, as well as the PMN dysfunction noted in our study, may be involved in the pathogenesis of secondary microbial infection.

The mechanisms of MN and PMN dysfunction after influenza A virus incubation have not been established, but the generation of oxygen radicals by the virus may play an important role. We have demonstrated that oxygen radicals are produced early on during incubation of phagocytic cells with influenza virus, and upon subsequent stimulation of these cells, fewer oxygen radicals are produced and bactericidal activity is depressed. The initial virus-induced respiratory burst could produce cellular exhaustion, which results in decreased production of oxygen radicals and other products involved in bactericidal activity. Alternatively, the decreased bactericidal activity noted after phagocytic cell incubation with influenza virus could be due to toxic effects on the phagocytic cell from the oxygen radicals produced during initial stimulation with the virus. Voetman et al. (34) have shown that oxygen radicals produced during PMN stimulation with zymosan or PMA partially inactivate lysosomal enzymes which are involved in microbicidal activity. In addition, the influenza virus may also interfere with the fusion of granule membranes with phagocytic vacuoles, a phenomenon which has been described in Sendai virus-infected PMNs and macrophages (14). Products from influenza virus-induced phagocytic cell degranulation could also contribute to phagocytic cell dysfunction. Although influenza viremia has been difficult to demonstrate, several reports have noted influenza viral antigens in human lymphocytes and several tissue sites during infection, suggesting the possibility that virus invasion of peripheral leukocytes may occur (21, 26, 31, 35, 37).

The findings in this study show that the MN and PMN dysfunction induced by influenza virus is temporally related to initiation of the respiratory burst by the virus and could be due to injury by cellular oxygen radicals or products of degranulation. The present study did not establish a causal relationship between oxygen radicals and phagocytic cell dysfunction, nor did it examine the potential of the virus to induce cellular degranulation. Although the exact mechanism by which influenza virus induces phagocytic cell dysfunction remains to be determined, the data in our study support the hypothesis that influenza virus can interfere with phagocytic cell function and thereby increase the susceptibility of the host to secondary microbial infections.

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LITERATURE CITED

1. **Abramson, J. S., G. S. Giebink, E. L. Mills, and P. G. Quie.** 1981. Polymorphonuclear leukocyte dysfunction during influenza virus infection in chinchillas. J. Infect. Dis. **143**:836–845.
2. **Ackerman, S. K., and S. D. Douglas.** 1978. Purification of human monocytes on microexudate-coated surfaces. J. Immunol. **120**:1372–1374.
3. **Babior, B. M.** 1978. Oxygen-dependent microbial killing by phagocytes. N. Engl. J. Med. **298**:659–668.
4. **Badwey, J. A., and M. L. Karnovsky.** 1980. Active oxygen species and the functions of phagocytic leukocytes. Annu. Rev. Biochem. **49**:695–726.
5. **Beall, G. D., J. E. Repine, J. R. Hoidal, and F. L. Rasp.** 1977. Chemiluminescence by human alveolar macrophages: stimulation with heat-killed bacteria or phorbol myristate acetate. Infect. Immun. **17**:117–120.
6. **Clawson, C. C.** 1972. Platelet interaction with bacteria. III. Ultrastructure. Am. J. Pathol. **70**:449–464.
7. **Craft, A. W., and M. M. Reid.** 1976. Effect of virus infections on polymorph function in children. Br. Med. J. **1**:1570.
8. **Debets-Ossenkopp, Y., W. C. Van Dijk, E. L. Mills, H. A. Verbrugh, and J. Verhoef.** 1980. The effect of influenza virus on polymorphonuclear leukocytes. Antonie van Leeuwenhoek. J. Microbiol. Serol. **46**:103.
9. **Faden, H., P. Sutyla, and P. L. Ogra.** 1979. Effect of viruses on luminol-dependent chemiluminescence of human neutrophils. Infect. Immun. **24**:673–678.
10. **Fischer, J. J., and D. H. Walker.** 1979. Invasive pulmonary aspergillosis associated with influenza. J. Am. Med. Assoc. **241**:1493–1494.
11. **Frances, T., and M. V. de Torregrosa.** 1945. Combined infection of mice with *H. influenzae* and influenza virus by the intranasal route. J. Infect. Dis. **76**:71–77.
12. **Gerone, P. J., T. G. Ward, and W. A. Chappell.** 1957. Combined infections in mice with influenza virus and *Diplococcus pneumoniae*. Am. J. Hyg. **66**:331–341.
13. **Hers, J. F., W. R. Goslings, N. Masurel, and J. Mulder.** 1957. Death from Asiatic influenza in the Netherlands. Lancet **ii**:1164–1165.
14. **Jakab, G. J., G. A. Warr, and P. L. Sannes.** 1980. Alveolar macrophage ingestion and phagosome-lysosome fusion defect associated with virus pneumonia. Infect. Immun. **27**:960–968.

15. **Larson, H. E., and R. Blades.** 1976. Impairment of human polymorphonuclear leukocyte function by influenza virus. Lancet i:285.
16. **Larson, H. E., R. P. Parry, C. Gilchrist, A. Luquetti, and D. A. Tyrall.** 1977. Influenza viruses and staphylococci *in vitro*: some interactions with polymorphonuclear leukocytes and epithelial cells. Br. J. Exp. Pathol. **58**:281–288.
17. **Loosli, C. L.** 1973. Influenza and the interaction of viruses and bacteria in respiratory infections. Medicine (Baltimore) **52**:360–384.
18. **Mills, E. L., Y. Debets-Ossenkopp, H. A. Verbrugh, and J. Verhoef.** 1981. Initiation of the respiratory burst of human neutrophils by influenza virus. Infect. Immun. **32**:1200–1205.
19. **Mills, E. L., K. S. Rholl, and P. G. Quie.** 1980. X-linked inheritance in females with chronic granulomatous disease. J. Clin. Invest. **66**:332–340.
20. **Mills, E. L., K. S. Rholl, and P. G. Quie.** 1980. Luminol-amplified chemiluminescence: a sensitive method for detecting the carrier state in chronic granulomatous disease. J. Clin. Microbiol. **12**:52–56.
21. **Oseasohn, R., L. Adelson, and M. Kayi.** 1947. Clinicopathologic study of thirty-three fatal cases of Asian influenza. N. Engl. J. Med. **260**:509–518.
22. **Peterson, P. K., B. J. Wilkinson, Y. Kim, D. Schmeling, and P. G. Quie.** 1978. Influence of encapsulation on staphylococcal opsonization and phagocytosis by human polymorphonuclear leukocytes. Infect. Immun. **19**:943–949.
23. **Public Health Laboratory Service.** 1957. Deaths from Asian influenza. Br. Med. J. **2**:915–919.
24. **Quie, P. G., and E. L. Mills.** 1979. Bactericidal and metabolic function of polymorphonuclear leukocytes. Pediatrics **64**:719–721.
25. **Quie, P. G., J. G. White, B. Holmes, and R. A. Good.** 1967. *In vitro* bactericidal capacity of human polymorphonuclear leukocytes: diminished activity in chronic granulomatous disease of childhood. J. Clin. Invest. **46**:668–679.
26. **Rukavishnikova, G. E., and A. K. Alekseeva.** 1977. Influenza virus antigens in human leukocytes after oral administration of live tissue culture influenza A monovaccine. Acta Virol. (Prague) (Engl. Ed.) **21**:391–396.
27. **Ruutu, P., and T. U. Kosunen.** 1971. Phagocytic activity of neutrophilic leukocytes of A_2 influenza patients. Acta Pathol. Microbiol. Scand. **79**:67–72.
28. **Ruutu, P., A. Vaheri, and T. U. Kosunen.** 1977. Depression of human neutrophil motility by influenza virus *in vitro*. Scand. J. Immunol. **6**:897–905.
29. **Sawyer, W. D.** 1969. Interaction of influenza virus with leukocytes and its effect on phagocytosis. J. Infect. Dis. **119**:541–556.
30. **Sellers, T. F., J. Schulman, C. Bouvier, R. McCune, and E. D. Kilbourne.** 1961. The influence of influenza virus infection on exogenous staphylococcal and endogenous murine bacterial infection in the bronchopulmonary tissue of mice. J. Exp. Med. **114**:237–256.
31. **Stanley, E. D., and G. G. Jackson.** 1966. Viremia in Asian influenza. Trans. Assoc. Am. Physicians **79**:376–387.
32. **Taylor, R. N., T. M. Dietz, K. W. Maxwell, and S. Marcus.** 1974. Effect of influenza virus infection on phagocytic and cytopeptic capabilities of guinea pig macrophages. Immunol. Commun. **3**:439–455.
33. **Tyrrell, D. A.** 1952. The pulmonary complications of influenza as seen in Sheffield in 1949. Q. J. Med. **21**:291–306.
34. **Voetman, A. A., R. S. Weening, M. N. Hamers, L. J. Meerhof, A. M. Bot, and D. Roos.** 1981. Phagocytosing human neutrophils inactivate their own granular enzymes. J. Clin. Invest. **67**:1541–1549.
35. **Wilson, A. B., D. N. Planterose, J. Naginton, J. R. Park, R. D. Barry, and K. K. Coombs.** 1976. Influenza A antigens on human lymphocytes *in vitro* and probably *in vivo*. Nature (London) **259**:582–584.
36. **Young, L. S., M. LaForce, J. J. Head, B. S. Fealey, and J. V. Bennet.** 1972. A simultaneous outbreak of meningococcal and influenza infections. N. Engl. J. Med. **287**:5–9.
37. **Zakstelskaya, L. Y.** 1953. Recovery of the virus from the urine of patients with epidemic influenza. Gupp: OKYDR Trans. Ob'jed. Sess. Fnst. AMN USSR, Moscow, 72.