INFECTION AND IMMUNITY, Oct. 1976, p. 982-989

Vol. 14, No. 4
Printed in U.S.A.

Synergistic Effect on Mortality in Mice with Murine Cytomegalovirus and *Pseudomonas aeruginosa*, *Staphylococcus aureus*, or *Candida albicans* infections

JOHN R. HAMILTON, JAMES C. OVERALL, JR.,* AND LOWELL A. GLASGOW

Department of Microbiology, Division of Infectious Diseases, and Department of Pediatrics, University of Utah College of Medicine, Salt Lake City, Utah 84132*

Received for publication 6 May 1976

A synergistic effect on mortality was demonstrated in a combined infection of mice with murine cytomegalovirus (MCMV) and *Pseudomonas aeruginosa*, *Staphylococcus aureus*, or *Candida albicans*. Mice infected intraperitoneally with a 0 to 20% lethal dose inoculum of MCMV 3 days prior to the intravenous injection of a 0 to 20% lethal dose inoculum of either the bacteria or fungus demonstrated a striking enhancement of mortality. MCMV-infected mice given *Pseudomonas* or *Staphylococcus* exhibited a 90 to 100% mortality within 24 to 48 h, whereas 80% of viral-infected animals injected with *Candida* died in 5 days. Injection of the bacteria or fungus at various times during the MCMV infection resulted in enhanced mortality on days 0, 1, 2, and 3 of the viral infection. Greatest synergism was observed on day 3, with a progressive decline in death rates thereafter. Immunization with MCMV abrogated the synergistic effect on mortality in all three combined infections. Immunization with *Pseudomonas* reduced mortality in the combined MCMV-*Pseudomonas* infection. These results indicate that mice exhibit a markedly enhanced susceptibility to bacterial and fungal infections during the course of the MCMV infection and suggest that the enhancement may be related to viral-induced alterations in host resistance.

Cytomegalovirus (CMV) is a common cause of infection in humans, with over 50% of the population demonstrating complement-fixing antibodies by age 35 (33, 37, 38). Although CMV infections are usually asymptomatic, clinical manifestations may include microcephaly and mental retardation in the congenital infections (32), a mononucleosis-like syndrome in normal adults (15, 19-21), and pneumonia and hepatitis in immunosuppressed patients (1, 6, 8, 10, 15, 20, 23, 24, 26). CMV infection occurs in renal transplant patients at a higher frequency than any other viral infection (2). In only a minority of the cases, however, is the infection felt to be of clinical significance (8). The most common cause of death in renal transplant patients is infection due to bacteria and/or fungi, presumably because of depressed host defenses secondary to the immunosuppressive therapy. Since several investigators have demonstrated that immunosuppression or alteration of host defenses may occur during the course of a viral infection (16, 18; S. Lauteria, G. B. Kantzler, R. Ganguly, C. L. Cusumano, and R. H. Waldman, Clin. Res. 22:29A, 1974), we postulated that the CMV infection might also contribute to the enhanced susceptibility to bacterial and fungal infections in renal transplant patients.

To test this hypothesis, we utilized an experimental model infection of mice with murine CMV (MCMV), which is characterized by a disseminated infection involving many organs and an associated depression of both the humoral and cellular immune response (11, 12, 28, 29). More specifically, the model was used to determine the susceptibility of mice, during the course of an acute MCMV infection, to a secondary infection with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, or *Candida albicans*.

(This work was part of a dissertation submitted by John R. Hamilton in partial fulfillment of the requirements for the Ph.D. degree in Microbiology from the University of Utah, 1976.)

MATERIALS AND METHODS

Animals. Six-week-old female Swiss Webster mice were obtained from Simonsen Breeding Laboratories (Gilroy, Calif.) and maintained in a controlled environment for 1 week before use.

Virus. The Smith strain of MCMV originally obtained from June Osborn was used to prepare a salivary gland virus pool in 3- to 4-week-old Swiss

Webster mice. After an intraperitoneal (i.p.) inoculation of MCMV, which resulted in a 40 to 60% mortality, the salivary glands were aseptically removed from the surviving animals on day 12 to 15 of the infection. A 10% (wt/vol) homogenate of the tissue was prepared in minimal essential medium containing glutamine (300 μg/ml) and amino acids, 10% fetal calf serum, streptomycin (50 μg/ml), and penicillin (100 U/ml). After centrifugation at 2,000 $\times$ *g* for 10 min at room temperature, the supernatant was dispensed in samples and stored at -70°C. A normal salivary gland pool was prepared in the same manner using uninfected 6- to 7-week-old mice. The MCMV pool regularly titered 2×10^8 plaque-forming units (PFU)/ml when assayed on mouse embryo fibroblasts cells using procedures described previously (17). Tests of the normal salivary gland pool revealed no evidence of infectious virus.

The mouse is the natural host for lactic dehydrogenase virus, which has been shown to persist in the serum and tissues of infected mice and to alter host immune function (27). Lactic dehydrogenase virus is detected by inoculating normal mice with infected serum or tissues and then assaying plasma obtained 96 h after inoculation for the presence of elevated levels of lactic dehydrogenase enzyme (27, 39). To determine whether our MCMV pools were contaminated with lactic dehydrogenase virus, samples of the MCMV salivary gland pool, the normal salivary gland pool, and serum obtained from normal mice used in our experiments were injected into mice known to be free of lactic dehydrogenase virus. Heparinized blood was obtained 96 h later, and the plasma was analyzed for the presence of lactic dehydrogenase activity. The absence of any rise in lactic dehydrogenase activity indicates that neither the salivary gland homogenates nor the animals utilized in our experiments were infected with lactic dehydrogenase virus.

Bacteria and fungus. The isolates of *P. aeruginosa*, *S. aureus* (coagulase positive), and *C. albicans* were obtained from clinical specimens submitted to the University of Utah Medical Center Diagnostic Bacteriology Laboratory. All organisms were identified by appropriate criteria for genus and species determination. The bacteria were maintained on stock agar slants, and the yeast was maintained on Sabouraud dextrose (BBL, Cockeysville, Md.) slants at room temperature and subcultured frequently to preserve viability.

Before animal inoculation, each organism was subcultured from the agar slants to 5% sheep blood agar plates (BBL blood agar base plus whole blood), incubated for 18 to 22 h at 37°C, and harvested by repeated washings with phosphate-buffered saline, pH 7.2. The turbid suspensions were washed twice by centrifugation at 2,000 $\times$ *g* and then diluted in phosphate-buffered saline to appropriate concentrations, using optical density readings in a Klett-Summerson photoelectric colorimeter with a blue filter (400- to 465-nm wavelength) (Klett Manufacturing Co., Inc., New York). The Klett readings were shown to correlate directly and reproducibly with the number of colony-forming units (CFU) of organisms per milliliter using standard melted agar pour plate assay methods and specific plating media. *S. aureus* was plated on mannitol salt agar (BBL) and *P. aeruginosa* was plated on MacConkey agar (BBL). *C. albicans* was plated on Sabrouraud dextrose agar (BBL) supplemented with 100 μg of reagent-grade gentamicin per ml (Roche Laboratories).

Animal inoculation. The virus was administered by the i.p. route in a standard volume of 0.1 ml of an appropriate dilution of the virus to produce the desired mortality. In the combined infection, inoculation of MCMV was followed by injection of either *Pseudomonas*, *Staphylococcus*, or *Candida* on day 3 of the virus infection, except where indicated otherwise. The bacterial and fungal suspensions were injected in a volume of 0.1 ml through a 25-gauge needle into the tail vein of unanesthetized mice. Animals receiving only partial intravenous (i.v.) injections due to technical problems were discarded from all studies. Controls for the combined infection included inoculation of animals with: (i) each infectious agent alone (MCMV, *Pseudomonas*, *Staphylococcus*, *Candida*); (ii) normal salivary gland followed by the injection of bacteria or fungus; and (iii) MCMV followed by i.v. phosphate-buffered saline.

RESULTS

Establishment of the synergistic infection. By inoculation of mice i.p. with serial dilutions of the MCMV pool, the 0 to 20% lethal dose (LD_{0-20}) inoculum was established as 2×10^6 PFU/mouse. After challenge with this dose of virus, mice developed hunching, ruffled fur, and diminished activity from day 3 to day 5 or 6. By day 7 to 8 surviving animals appeared normal. Deaths, when they occurred, were on days 5 to 8. Day 3 of the MCMV infection, the first day of observable clinical illness, was chosen as the time for injection of the bacteria or the fungus in the first set of experiments.

Representative results obtained from several independent experiments in which groups of 10 animals were infected with MCMV, *Pseudomonas*, or the combination of the two organisms are illustrated in Fig. 1. Mice inoculated i.p. with 2×10^6 PFU of MCMV alone exhibited moderate signs of illness, with a 20% mortality in 7 days, whereas mice injected i.v. with 1.8×10^6 CFU of *Pseudomonas* demonstrated minimal signs of illness, with a 20% mortality by 12 days. In contrast, the combined infection with these two agents (*Pseudomonas* injected 3 days after MCMV inoculation) produced a rapidly lethal infection. Within 48 h after the injection of *Pseudomonas*, 90% of the animals died, a marked increase when compared with either of the individual infections.

Almost identical results were obtained with the individual and combined infections with MCMV and *Staphylococcus*. Animals inoculated with either MCMV or 8×10^6 CFU of

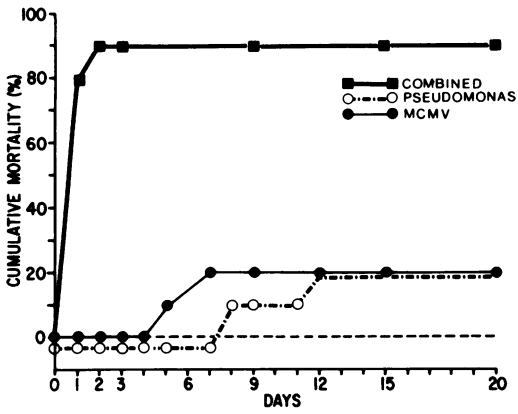

FIG. 1. *Cumulative mortality rates in groups of 10 mice infected with 2 × 10⁶ PFU of MCMV or 1.8 × 10⁶ CFU of Pseudomonas, or both. Mortality in the combined infection is shown as days after the injection of Pseudomonas on day 3 of MCMV infection.*

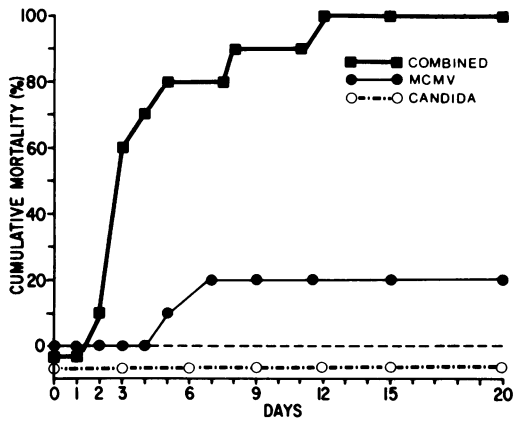

FIG. 2. *Cumulative mortality rates in groups of 10 mice infected with 2 × 10⁶ PFU of MCMV or 7.6 × 10³ CFU of Candida, or both. Mortality in the combined infection is shown as days after injection of Candida on day 3 of MCMV infection.*

staphylococci had a mortality of 20 or 10%, respectively, within 6 days. In the combined infection (staphylococci injected 3 days after MCMV challenge), 90% of the mice died within 24 h.

Results from the combined infection of mice with MCMV and *Candida* are presented in Fig. 2. The individual MCMV infection resulted in a 20% mortality, whereas the individual *Candida* infection produced no signs of illness or mortality. In the combined infection, 100% of the mice died by 12 days, with the majority of deaths occurring 5 days after the *Candida* injection. It is apparent from these results that MCMV infection strikingly enhanced the mortality from an injection of *Pseudomonas*, *Staphylococcus*, or *Candida*.

Effect of the inoculum size of the infectious agents on mortality in the combined infection. In the previous experiments the synergistic effect on mortality was demonstrated with a single concentration of either the virus or the bacteria or fungus. To determine the optimum inoculum of organisms required for enhanced mortality in the combined infection, groups of mice were given serial dilutions of MCMV followed 3 days later by decreasing concentrations of *Pseudomonas*, *Staphylococcus*, or *Candida*. The data from one representative experiment are presented in Table 1. The inoculation of 2 × 10^6 PFU of MCMV alone produced signs of severe illness, with a 20% mortality and a mean day of death (MDD) of 6 days. Although lower concentrations of MCMV produced no mortality, mice showed signs of severe illness after the inoculation of 4 × 10^5 PFU. No alteration of mortality resulted when phosphate-buffered saline was injected into MCMV-infected mice. Injection of the largest inoculum of *Pseudomonas* or *Staphylococcus* alone resulted in only 20 and 10% mortality, respectively. Animals showed little signs of illness with either of these two organisms. In contrast, the larger inocula of *Candida*, 6.2 × 10^5 and 7.1 × 10^4 CFU, produced a mortality of 70 and 20%, respectively. The lowest inoculum, 7.6 × 10^3 CFU, resulted in no mortality. No enhancement in mortality was observed in mice inoculated with a normal salivary gland dilution followed by injection of either the bacteria or fungus. The greatest synergism (highest final mortality, shortest MDD) occurred when the largest inoculum of virus was administered with the largest inoculum of *Pseudomonas*, *Staphylococcus*, or *Candida*. With decreasing concentrations of virus, as well as with decreasing concentrations of bacteria, the mortality rate decreased and/or the MDD was prolonged. From these data it can be concluded that an inoculum of 2 × 10^6 PFU of MCMV resulted in the greatest synergistic effect on final mortality and MDD over the widest range of concentrations of bacteria and fungi.

Period during MCMV infection that synergism was observed. The previous experiments were performed by inoculating the bacteria or the fungus on day 3 of the MCMV infection. To determine the time during the MCMV infection when synergism was maximal, the following experiments were performed. Groups of 10 mice each were inoculated with 2 × 10^6 PFU of MCMV i.p. followed by 1.6 × 10^6 CFU of *Pseudomonas*, 3.0 × 10^6 CFU of *Staphylococcus*, or

TABLE 1. *Effect of decreasing concentration of the infectious agents on the synergistic effect on mortality*

Bacterial or fungal inoculum (i.v.) (CFU)	MCMV inoculum (i.p.) (PFU)							
	No virus[a]		2×10^6		4×10^5		2×10^4	
	Mortality (%)[b]	MDD[c]	Mortality (%)	MDD	Mortality (%)	MDD	Mortality (%)	MDD
None[d]	0		20	6.0	0		0	
Pseudomonas								
1.6×10^6	20	10.0	90	1.1	80	1.6	90	3.4
2.3×10^5	0		90	1.5	40	2.3	0	
2.4×10^4	0		80	2.3	20	3.0	10	12
Staphylococcus								
1.6×10^7	10	5.0	90	1.0	60	5.3	60	4.8
2.0×10^6	0		90	1.8	50	5.8	40	5.8
2.6×10^5	0		0		0		0	
Candida								
6.2×10^5	70	6.0	100	2.3	100	2.7	100	3.5
7.1×10^4	20	1.0	90	3.8	90	7.2	50	11.4
7.6×10^3	0		100	4.6	80	8.7	10	12.0

[a] Salivary gland homogenate.
[b] Each group contained 10 animals.
[c] MDD of the animals that died over a 20-day period.
[d] Phosphate-buffered saline.

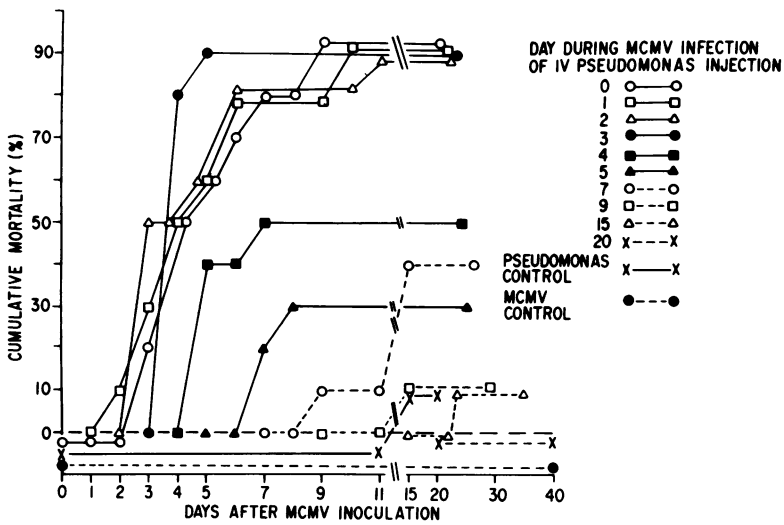

FIG. 3. *Cumulative mortality rates in mice injected i.v. with 1.8 × 10⁶ CFU of Pseudomonas on various days after i.p. inoculation of 2 × 10⁶ CFU of MCMV.*

7.6×10^3 CFU of *Candida* i.v. on days 0 (immediately after), 1, 2, 3, 4, 5, 7, 9, 15, and 20 of the viral infection and observed for mortality during the 20 days after the inoculation of the bacteria or the fungus. The results with *Pseudomonas* and *Candida* are shown in Fig. 3 and 4. Results with *Staphylococcus* were almost identical to those illustrated for *Pseudomonas*. The striking enhancement in mortality was observed only after injection of either the bacteria or fungus on days 0, 1, 2, and 3. A progressive decline in mortality to control levels was observed after injection on days 4 and 5 and thereafter. Maximal synergism (highest final mortality with the shortest MDD) occurred after injection of either bacteria or fun-

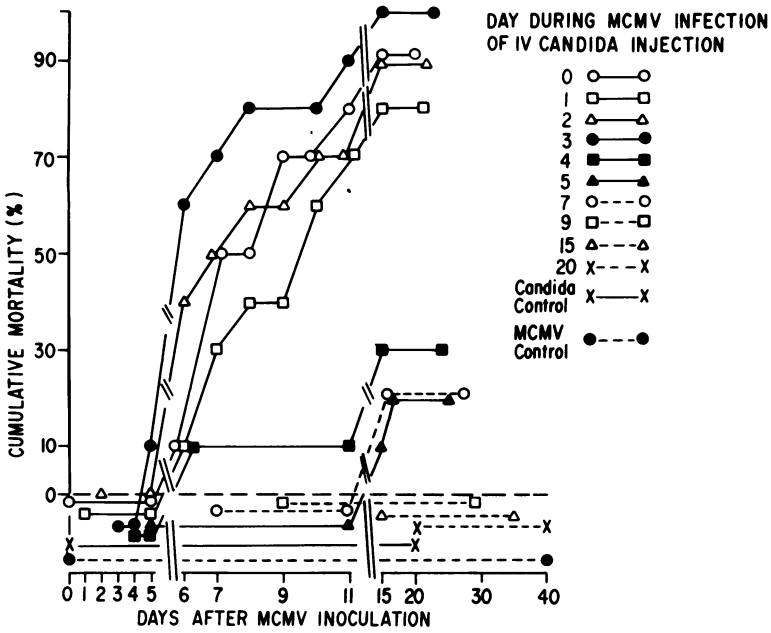

FIG. 4. *Cumulative mortality rates in mice injected i.v. with 7.6 × 10^3 CFU of Candida on various days after i.p. inoculation of 2 × 10^6 PFU of MCMV.*

gus on day 3 of the MCMV infection. Mice inoculated with *Pseudomonas* or *Staphylococcus* on day 3 began to die within 1 day (day 4 of the viral infection), whereas animals inoculated with either of the bacteria on days 0, 1, or 2 did not begin to die for 2 to 5 days (day 3 to 6 of the viral infection). Mice inoculated with *Candida* on day 3 of the MCMV infection began to die within 2 to 5 days (day 5 to 8 of the viral infection), whereas animals inoculated with the fungus on days 0, 1, or 2 did not begin to die for 3 to 11 days (day 6 to 11 of the viral infection). Since maximal synergism occurred on day 3, this day was chosen for the i.v. injection of the bacteria or the fungus in all subsequent experiments.

Effect of immunization on mortality in the combined infection. Degre and Glasgow (5) demonstrated that, in a synergistic infection of mice with Sendai virus and *Haemophilus influenzae*, prior immunization with either agent eliminated the synergistic effect. Osborn and Walker (31) showed that mice that had previously received an inoculum of attenuated MCMV were resistant to challenge with a lethal inoculum of the wild-type virus. We initially determined the effect of prior immunization with MCMV on the synergistic activity in the combined infection. Groups of mice were injected i.p. with 2 × 10^6 PFU of MCMV (immunizing dose), and 28 days later the survivors were challenged i.p. with 10^7 PFU of MCMV alone (lethal dose) or with 2 × 10^6 PFU of virus followed by the bacteria or the fungus as above. Normal mice were used as nonimmune controls and were given the individual or the combined infection according to the same protocol as above. The results of this experiment are shown in Table 2. No mortality was observed with the lethal viral inoculum (10^7 PFU) in the MCMV-immune mice, whereas 80% of the nonimmune mice died. In nonimmune control mice, injection of *Pseudomonas*, *Staphylococcus*, or *Candida* on day 3 of the virus infection produced a mortality rate of 80% or greater. In contrast, MCMV-immune mice exhibited no mortality when challenged with the bacteria or fungus. These data indicate that prior immunization with MCMV eliminated the enhanced susceptibility to the bacteria or the fungus observed during the MCMV infection and suggest that an acute primary infection with MCMV is necessary for the synergistic effect.

Since Jones and Dyster reported that mice immunized with *Pseudomonas* were protected against a lethal infection (14), and since there is conflicting data on the capacity of mice to be immunized against *Staphylococcus* or *Candida* infection, we then determined whether mice immune to *Pseudomonas* might be protected against the combined MCMV-*Pseudomonas* infection. Groups of mice were immunized with an i.p. injection of a formalin-killed vaccine containing 5 × 10^8 CFU of *Pseudomonas*. A nonimmune control group of mice was given an

injection of phosphate-buffered saline alone. Four days after immunization, both groups of mice were inoculated i.p. with an LD_{20} dose of MCMV, followed 3 days later by an i.v. injection with 1.6×10^6 CFU of *Pseudomonas*. No mortality was observed in the *Pseudomonas*-immune mice, whereas in the nonimmune mice 60% of the animals died. The results show that immunization with *Pseudomonas* was able to protect the mice against the lethal effect of the secondary bacterial infection.

DISCUSSION

Mice infected i.p. with an LD_{0-20} inoculum of MCMV exhibited a markedly enhanced susceptibility to a subsequent i.v. injection with an LD_{0-20} inoculum of *Pseudomonas*, *Staphylococcus*, or *Candida*, as evidenced by an increase in final mortality (90 to 100%) and a decrease in the MDD. The synergistic effect was observed only when the bacteria or the fungus was injected during the early phases of the viral infection on days 0, 1, 2, and 3, with maximal synergism occurring on day 3. The synergistic activity was observed over a wide range of bacterial and fungal, as well as viral, concentrations when animals were challenged on day 3 of the MCMV infection.

Most of the previously described animal models of viral-bacterial synergism have investigated the effect of a respiratory virus infection on local defenses against bacterial pneumonias (22). Synergism was usually demonstrated by decreased clearance of bacteria from pulmonary tissue and was not always associated with enhanced mortality. The enhancement of susceptibility paralleled the appearance of viral-induced pulmonary pathology. In the few animal models used to examine synergism associated with systemic infections with viruses and bacteria (7, 9, 13), authors have usually found enhanced susceptibility to the bacterial, but not the viral, infection. Although an enhancement in the time of death was observed in mice injected i.p. with *Streptococcus pneumoniae*, *P. aeruginosa*, or *Salmonella enteritidis* ser. *typhimurium* at various times before or after i.v. injection of Newcastle disease virus, final mortality was not always appreciably different (7). Mice injected i.v. with Newcastle disease virus 8 h before an i.v. injection of *S. typhimurium* demonstrated decreased clearance of the bacteria from spleens in the ensuing 3 days (13). In these latter experiments, no data concerning the effect of the viral infection on mortality are reported. Mice infected with adenovirus, herpes simplex virus, or vaccinia virus demonstrated an enhanced susceptibility to *Escherichia coli* pyelonephritis, as evidenced by histological changes (9). Injection of the bacteria i.v. 6 days

TABLE 2. *Effect of immunization with MCMV on mortality in the combined infection*

Immune status	Type of infection	Agent inoculated		Mortality	
		Agent	Dose	No.	%
Nonimmune	Individual	MCMV	2×10^6 PFU[a]	2/20	20
		MCMV	1×10^7 PFU[b]	8/10	80
		P. aeruginosa	1.6×10^6 CFU	0/10	0
		S. aureus	3.0×10^6 CFU	0/10	0
		C. albicans	7.6×10^3 CFU	0/10	0
	Combined[c]	MCMV *P. aeruginosa*	2×10^6 PFU 1.6×10^6 CFU	9/10	90
		MCMV *S. aureus*	2×10^6 PFU 3.0×10^6 CFU	9/10	90
		MCMV *C. albicans*	2×10^6 PFU 7.6×10^3 CFU	8/10	80
MCMV immune[d]	Individual	MCMV	1×10^7 PFU	0/10	0
	Combined[c]	MCMV *P. aeruginosa*	2×10^6 PFU 1.6×10^6 CFU	0/10	0
		MCMV *S. aureus*	2×10^6 PFU 3.0×10^6 CFU	0/10	0
		MCMV *C. albicans*	2×10^6 PFU 7.6×10^3 CFU	0/10	0

[a] Immunizing dose of MCMV.
[b] Challenge dose of MCMV.
[c] Bacteria or fungus injected i.v. on day 3 of the MCMV infection.
[d] Animals challenged 28 days after an immunizing dose of MCMV.

after the i.p. viral inoculation resulted in the most significant enhancement of the nonlethal pyelonephritis. In contrast to these models of a systemic synergistic infection, the model system presented in this study was characterized by: (i) a dramatic enhancement of final mortality in the combined infection, which was paralleled by a decreased MDD; (ii) synergism between a herpesvirus and two different bacteria; (iii) enhanced susceptibility to a fungus during the course of a viral infection, an observation which, to our knowledge, has not been reported previously; (iv) a synergistic effect on mortality which was markedly influenced by the time during the viral infection when the bacteria or the fungus was inoculated; and (v) an enhanced susceptibility associated with a broad range of viral and bacterial or fungal concentrations.

There are several possible explanations for the markedly enhanced mortality observed in the combined infection. First, foci of viral-induced tissue destruction could serve as a nidus of localization and subsequent growth of the bacteria or the fungus. Viral-induced tissue pathology has been thought to play a major role in bacterial growth in renal tissue (9) and in pulmonary tissues (23), since the degree of tissue destruction appeared to parallel enhanced susceptibility to the bacteria. In CMV infection of mice, extensive foci of inclusion-bearing cells have been described in the liver, spleen, lung, and kidney (25, 30, 34, 35). The fact that mice exhibited progressively diminishing susceptibility to the bacteria or the fungus after day 3 of the MCMV infection, a time when the mice were still showing clinical evidence of illness and when significant tissue pathology was still present, would tend to suggest that viral-induced tissue damage is not the primary mechanism. Second, the MCMV infection could alter host defenses known to be important in the recovery from bacterial and fungal infections. MCMV infection has been shown to result in a leukopenia (30) and to cause suppression of both humoral (12, 28, 29) and cellular (11) immune response.

Immunization with MCMV eliminated the synergistic effect on mortality in the combined infection with both the bacteria and the fungus. In previous studies of the effect of immunization on MCMV infection in mice, decreased titers of virus were noted in all organs except salivary glands (31, 36). Similar alterations in the pathogenesis of the MCMV infection probably occurred in our model system, since no immune animals died after an LD_{80} MCMV inoculum. This would suggest that replication of MCMV to appreciable titers in critical organs is necessary for synergistic activity to occur.

Immunization with *Pseudomonas* 4 days before MCMV inoculation also prevented mortality upon rechallenge with the bacteria. Jones and Dyster (14) postulated that mice immunized with *P. aeruginosa* 3 to 7 days before challenge with a lethal inoculum of bacteria were protected by the presence of specific antibody. Antibody, in conjunction with the complement system, enhanced phagocytosis and intracellular killing of the bacteria by granulocytes and macrophages (3, 4). Sufficient amounts of circulating opsonizing antibody could have been induced by the *Pseudomonas* immunization in the MCMV-infected animals to augment the host defenses suppressed by the virus infection, and thereby confer protection on the animals.

These studies provide evidence that MCMV infection plays an important role in enhancing the susceptibility of mice to infection with *Pseudomonas*, *Staphylococcus*, and *Candida*. This experimental infection offers a model to further elucidate the major determinants of host defense, which are important in resistance to primary bacterial and fungal infections. In addition, these studies may have clinical implications for the possible role of CMV infections in predisposing to bacterial and fungal infections in renal transplant patients.

ACKNOWLEDGMENTS

These investigations were supported by Public Health Service grant no. AI 10217 from the National Institute of Allergy and Infectious Diseases. J. C. O. is an investigator of the Howard Hughes Medical Institute.

LITERATURE CITED

1. **Armstrong, D., L. Saidapet, S. L. Balakrishnan, L. Steger, B. Yu, and K. H. Stenzel.** 971. Infections with viremia following renal transplantation. Arch. Intern. Med. 127:111-115.
2. **Balakrishnan, S. L., D. Armstrong, A. L. Rubin, and K. H. Stenzel.** 1969. Cytomegalovirus infection after renal transplantation. J. Am. Med. Assoc. 207:1712-1714.
3. **Bjornson, A. B., and J. G. Michael.** 1971. Contribution of humoral and cellular factors to the resistance to experimental infection by *Pseudomonas aeruginosa* in mice. I. Interaction between immunoglobulins, heat-labile serum factors, and phagocytic cells in the killing of bacteria. Infect. Immun. 4:462-467.
4. **Bjornson, A. B., and J. G. Michael.** 1972. Contribution of humoral and cellular factors to the resistance to experimental infection by *Pseudomonas aeruginosa* in mice. II. Opsonic, agglutinative, and protective capacities of immunoglobulin G anti-*Pseudomonas* antibodies. Infect. Immun. 5:775-782.
5. **Degre, M., and L. A. Glasgow.** 1968. Synergistic effect in viral-bacterial infection. I. Combined infection of the respiratory tract in mice with parainfluenza virus and *Haemophilus influenzae*. J. Infect. Dis. 118:449-462.
6. **Emodi, G., and M. Just.** 1974. Impaired interferon response of children with congenital cytomegalovirus disease. Acta Pediatr. Scand. 63:183-187.
7. **Eyckmans, L, and A. Billiau.** 1972. Inhibition of bacte-

ricidal capacity in mice after administration of Newcastle disease virus. Scand. J. Infect. Dis. 4:101-104.
8. Fine, R. N., C. M. Grushkin, M. Malekzadeh, and H. T. Wright. 1972. Cytomegalovirus syndrome following renal transplantation. Arch. Surg. 105:564-570.
9. Ginder, D. R. 1964. Increased susceptibility of mice infected with mouse adenovirus to *E. coli*-induced pyelonephritis. J. Exp. Med. 120:1117-1128.
10. Hanshaw, J. B., R. F. Betts, G. Simm, and R. C. Boynton. 1965. Acquired cytomegalovirus infection: association with hepatomegaly and abnormal liver function tests. N. Engl. J. Med. 272:602-608.
11. Howard, R. J., J. Miller, and J. S. Najarian. 1974. Cytomegalovirus-induced immune suppression. II. Cell-mediated immunity. Clin. Exp. Immunol. 18:119-126.
12. Howard, R. J., and J. S. Najarian. 1974. Cytomegalovirus-induced immune suppression. I. Humoral immunity. Clin. Exp. Immunol. 18:109-118.
13. Hugh, R., K. Huang, and T. B. Elliott. 1971. Enhancement of bacterial infections in mice by Newcastle disease virus. Infect. Immun. 3:488-493.
14. Jones, R. J., and R. E. Dyster. 1973. The role of polymorphonuclear leucocytes in protecting mice vaccinated against *Pseudomonas aeruginosa* infections. Br. J. Exp. Pathol. 54:416-421.
15. Jordan, M. C., W. E. Rousseau, J. A. Stewart, G. R. Noble, and T. D. Y. Chin. 1973. Spontaneous cytomegalovirus mononucleosis: clinical and laboratory observations in 9 cases. Ann. Intern. Med. 79:153-160.
16. Kauffman, C. A., J. P. Phair, C. C. Linnemann, Jr., and G. M. Schiff. 1974. Cell-mediated immunity in humans during viral infection. I. Effect of rubella on dermal hypersensitivity, phytohemoagglutinin response, and T-lymphocyte numbers. Infect. Immun. 10:212-215.
17. Kern, E. R., J. C. Overall, Jr., and L. A. Glasgow. 1973. Herpesvirus hominis infection in newborn mice. I. An experimental model and therapy with iododeoxyuridine. J. Infect. Dis. 128:290-299.
18. Kleinerman, E. S., R. Snyderman, and C. A. Daniels. 1974. Depression of human monocyte chemotaxis by herpes simplex and influenza viruses. J. Immunol. 113:1562-1567.
19. Klemola, E. 1973. Cytomegalovirus infection in previously healthy adults. Ann. Intern. Med. 79:267-278.
20. Klemola, E., and L. Kaariainen. 1965. Cytomegalovirus as a possible cause of a disease resembling infectious mononucleosis. Br. Med. J. 2:1099-1102.
21. Klemola, E., R. von Essen, O. Wager, K. Haltia, A. Koivuniemi, and I. Salmi. 1969. CMV mononucleosis in previously healthy individuals, 5 new cases and follow-up of 13 previously published cases. Ann. Intern. Med. 71:11-19.
22. Loosli, C. G. 1973. Influenza and the interactions of viruses and bacteria in respiratory infections. Medicine 52:369-384.
23. Lopez, C., R. L. Simmons, S. M. Mauer, J. S. Najarian, and R. A. Good. 1974. Association of renal allograft rejection with virus infections. Am. J. Med. 56:280-289.
24. Luby, J. P., W. Burnett, A. R. Hull, A. J. Ware, J. W. Shorey, and P. C. Peters. 1974. Relationship between cytomegalovirus and hepatic function abnormalities in the period after renal transplant. J. Infect. Dis. 129:511-518.
25. MacCordock, M. A., and M. G. Smith. 1936. The visceral lesions produced in mice by the salivary gland virus of mice. J. Exp. Med. 63:303-310.
26. Millard, P. R., B. M. Herbertson, J. Hagington, and D. B. Evans. 1973. The morphological consequences and the significance of cytomegalovirus infection in renal transplant patients. Q. J. Med. 167:585-596.
27. Notkins, A. L. 1971. Enzymatic and immunologic alterations in mice infected with lactic dehydrogenase virus. Am. J. Pathol. 64:733-746.
28. Osborn, J. E., A. A. Blazkovec, and D. L. Walker. 1968. Immunosuppression during acute murine cytomegalovirus infection. J. Immunol. 100:835-844.
29. Osborn, J. E., and D. N. Medearis, Jr. 1967. Suppression of interferon and antibody and multiplication of Newcastle disease virus in cytomegalovirus infected mice. Proc. Soc. Exp. Biol. Med. 124:347-353.
30. Osborn, J. E., and N. T. Shahidi. 1973. Thrombocytopenia in murine cytomegalovirus infection. J. Lab. Clin. Med. 81:53-63.
31. Osborn, J. E., and D. L. Walker. 1970. Virulence and attenuation of murine cytomegalovirus. Infect. Immun. 3:228-236.
32. Overall, J. C., Jr., and L. A. Glasgow. 1970. Virus infections of the fetus and newborn infant. J. Pediatr. 77:315-333.
33. Rowe, W. P., J. W. Haretly, S. Waterman, H. C. Turner, and R. J. Huebner. 1956. Cytomegalic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids. Proc. Soc. Exp. Biol. N.Y. 92:418-424.
34. Ruebner, B. H., T. Hirano, R. Slusser, J. E. Osborn, and D. N. Medearis. 1966. Cytomegalovirus infection: viral ultrastructure with particular reference to the relationship of lysosomes to cytoplasmic inclusions. Am. J. Pathol. 48:971-989.
35. Ruebner, B. H., K. Miyai, R. J. Slusser, P. Wedemeyer, and D. N. Medearis, Jr. 1964. Mouse cytomegalovirus infection. An electron microscopic study of hepatic parenchymal cells. Am. J. Pathol. 44:799-821.
36. Selgrade, M. K., and J. E. Osborn. 1974. Role of macrophages in resistance to murine cytomegalovirus. Infect. Immun. 10:1383-1390.
37. Stern, H., and S. D. Elek. 1965. The incidence of infection with CMV in a normal population. J. Hygiene 63:79-87.
38. Weller, T. E. 1971. The cytomegaloviruses: ubiquitous agents with protean clinical manifestations, parts 1 and 2. N. Engl. J. Med. 285:203, 267.
39. Wroblewski, F., and J. S. LaDue. 1955. Lactic dehydrogenase activity in blood. Proc. Soc. Exp. Biol. Med. 90:210-213.