Acquired Chemotactic Inhibitors During Infection with Guinea Pig Cytomegalovirus

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Factors involved in neutrophil and monocyte migrations were serially studied in strain 2 guinea pigs undergoing initial cytomegalovirus infection and sham-inoculated controls. All studies remained unchanged in uninfected animals. Monocyte migrations and neutrophil spontaneous migration remained unchanged in infected animals. However, transient abnormalities occurred early in infection, comprising a decrease in neutrophil-directed migration towards C5-derived chemotactic fractions (C_{5-fr}) and a decrease in the chemotactic activity of zymosan-activated plasma. Consequently, the presence of neutrophil- and chemotaxin-directed inhibitors in plasma was investigated. Normal neutrophils, C_{5-fr}, *Escherichia coli*-derived bacterial factor, and the synthetic peptide F-met-leu-phe were first incubated with control or infected plasmas and then assayed for directed migration and lysosomal enzyme release. Results indicated the de novo appearance of both neutrophil- and chemotaxin-directed inhibitory activities in plasma during early infection. The neutrophil-directed inhibition was heat stable (56°C for 120 min) and nonspecific (responses to all chemotaxins were inhibited). The chemotaxin-directed inhibition was heat stable and C_{5-fr} specific. The cytomegalovirus-induced inhibitors may be important in the enhanced susceptibility to concurrent opportunistic infections.

Clinical and laboratory observations suggest that cytomegalovirus (CMV) infections have a synergistic effect on infections by other organisms. For example, the mortality for infections due to both fungi and bacteria are increased in bone marrow and kidney transplant recipients during concurrent infection with CMV (12, 16, 26-28). In mice, mortality and morbidity due to *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* are enhanced by murine CMV infection (4, 15). In addition, the unusual incidence of opportunistic infections (*Pneumocystis carinii* pneumonia, bacterial infections, and fungal infections) and Kaposi's sarcoma among male homosexuals (10, 13, 20, 24, 34) may be related to CMV infections.

The synergistic effects on the frequency and severity of opportunists imply an alteration in immune function. Thus, alterations of cellular immune responses are suggested by changes of lymphocyte mitogenic responses and suppressor/helper T-cell ratios in both humans and laboratory animals (7, 22, 29). The role of altered phagocytic cell functions during CMV infection

has recently attracted attention. Although phagocytosis, Nitro Blue Tetrazolium reduction, and chemotaxis were normal in three patients during the acute stages of CMV mononucleosis (30), abnormal neutrophil chemotaxis has been reported in a child with disseminated CMV (30). During infection with guinea pig CMV (gpCMV), infection was associated with moderate neutropenia, decreased H₂O₂ release, decreased bacterial killing, and a reduction in the number of neutrophils migrating into the peritoneal cavity (38), which might have resulted from either the neutropenia or a neutrophil migratory defect. In the murine model, decreased neutrophil chemotaxis in the absence of neutropenia (3, 4) suggests altered directed migration as the probable defect, although the mechanisms of such a defect remain undefined. Our studies were aimed at defining neutrophil migrations, monocyte migrations, and plasma chemotactic factor generation during gpCMV infection. The data indicated that both the directed migration of neutrophils and the chemotactic factor generation in plasma were decreased during the initial stages of infection.

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MATERIALS AND METHODS

Animals. Young adult male (475 to 645 g) strain 2 guinea pigs (Biological Systems, Toms River, N.J.) were utilized. The animals were housed in a facility separate from other guinea pigs until inoculation. After inoculation, infected animals were housed in the general animal care facility in individual cages, whereas control animals were maintained separately.

Tissue culture. First-passage fetal guinea pig tissue cultures were utilized. Primary cultures of these tissues were prepared from 4-week gestation outbred guinea pigs by previously described techniques (25). When confluent, monolayers were trypsinized, and cell suspensions (2×10^6 /ml) were stored frozen in Eagle basal medium containing 20% fetal bovine serum and 8% dimethyl sulfoxide until use. Uninoculated primary, first-passage, and second-passage fetal guinea pig tissue cultures were observed for at least 60 days for the occurrence of spontaneous degeneration.

Virus. gpCMV (VR683; American Type Culture Collection, Rockville, Md.) was maintained in liquid nitrogen as a seed pool after three successive passages in fetal guinea pig tissue cultures. Infected whole cells were used for the subculture of virus (25).

Animal inocula. Animals were inoculated by the intramuscular route with 0.5 ml of infected or uninfected cell suspensions (10^5 cells per ml) in Eagle basal medium containing 5% fetal bovine serum. Infected inocula contained $10^{4.5}$ 50% tissue culture infective doses per ml.

Viral cultures. Nasal wash cultures were performed as previously described (25). Blood was obtained via cardiac puncture without anesthesia in syringes containing 3.5% sodium citrate (1 volume of citrate per 9 volumes of blood). Just confluent (3 days) tubes of fetal guinea pig tissue cultures were inoculated with 1.0 ml of a 1:10 dilution of blood in Puck saline A and adsorbed for 45 min at 36°C. After adsorption, the cultures were washed three times with 2.0 ml of maintenance medium consisting of Eagle basal medium supplemented with 5% fetal bovine serum, 50 µg of penicillin per ml, 50 µg of streptomycin per ml, and 1 µg of amphotericin B per ml. The cultures were observed for 30 days. gpCMV was identified by characteristic cytopathic effects.

Serology. Antibody to gpCMV was measured on serum that had been heat-inactivated for 30 min at 56°C, using an enzyme-linked immunosorbent assay (25). Sera were tested at a 1:5 dilution.

Neutrophil and monocyte cell suspensions. Citrated blood (0.38%, wt/vol) was kept on ice for a maximum of 15 min before cell separation. The blood was centrifuged at 4°C for 10 min at 800 rpm, and the buffy coat was collected. Mononuclear and neutrophil leukocytes were separated by the method of Böyum (5). Contaminating erythrocytes were eliminated by hypotonic lysis. When destined for the chemotactic assays, all cell suspensions were suspended in Gey balanced salt solution supplemented with 2% bovine serum albumin at 5×10^7 cells per ml. Neutrophil suspensions contained >95% neutrophils. Mononuclear cell suspensions contained $17 \pm 6\%$ monocytes (mean $\pm$ standard deviation). Cell viability was routinely tested by dye exclusion. When destined for the enzyme-releasing and enzyme release inhibition assays, the

leukocytes were suspended in Hanks balanced salt solution supplemented with 2% bovine serum albumin (HBSS) at 10^7 cells per ml.

Chemotactic factors. Zymosan-activated plasma (ZAP) was prepared from guinea pig plasma mixed with zymosan (1 mg of zymosan per 1 ml of plasma) and incubated at 37°C for 60 min. After centrifugation the supernatant was heated at 56°C for 30 min.

C5-derived chemotactic fractions (C_{5-fr}) were obtained from normal guinea pig ZAP that had been activated in the presence of 1 M *E*-amino-*n*-caproic acid as previously described (37). A pool of C_{5-fr} was prepared and standardized by defining the quantities capable of inducing maximal chemotaxis and lysosomal enzyme release responses of neutrophils and mononuclear cells. A single C_{5-fr} pool was utilized throughout the study.

Bacterial chemotactic factor was prepared from a pathogenic *E. coli* isolate obtained from a neonate with *E. coli* septicemia, after an overnight culture in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.). The supernatant was neutralized with 1 N NaOH and standardized by defining the quantities capable of inducing maximal chemotaxis and lysosomal enzyme release.

The synthetic peptide F-met-leu-phe (FMLP; Sigma Chemical Co., St. Louis, Mo.) was diluted in normal saline at 10^{-7} M.

Neutrophil migration assays. A modified chemotaxis-under-agarose test was used as previously described (36). Briefly, three wells were cut into a 0.5% agarose gel. The lateral wells received 10-µl cell suspensions containing 5×10^5 cells. The medial wells received 20 µl of Gey solution containing 0 to 16 µl of C_{5-fr} . After 3 h of incubation at 37°C, the migration distances of neutrophils towards the medial wells were measured with a micrometer to determine spontaneous migration (cell migration towards wells with Gey solution alone) and chemotaxis (cell migration towards wells containing C_{5-fr}). Directed migration was determined by the chemotactic differential (chemotaxis minus spontaneous migration). All assays were run in quadruplicate.

Monocyte migration assays. Similarly, monocyte migrations were performed under agarose, using 20 µl of Gey solution containing 0 to 18 µl of C_{5-fr} , 10-µl mononuclear cell suspensions containing 5×10^5 cells, and an incubation time of 12 h. The migrations were determined by the number of cells that migrated towards the medial well.

Chemotactic activity of ZAP. To determine chemotactic factor generation, neutrophils were obtained from normal uninoculated strain 2 guinea pigs. The medial wells received 20 µl of Gey solution containing 0 to 16 µl of Gey solution plus 0 to 16 µl of ZAP, and the chemotactic activity was determined by the chemotactic differential.

Chemotactic inhibition assays. The neutrophil migration assay was modified to detect and quantify chemotactic inhibitors in plasma. Neutrophil-directed inhibition was measured by preincubation of normal neutrophils with plasma for 0 to 30 min at 37°C before measuring their directed migration towards C_{5-fr} , bacterial factor, and FMLP. In some experiments the plasmas were heated at 56°C for 120 min before mixing with the neutrophils to determine whether the inhibitory activity was heat labile. Chemotaxis-directed inhibition was measured by preincubation of the vari-

ous chemotaxins with plasma before measuring their chemotactic activity towards normal neutrophils.

Lysosomal enzyme-releasing assays. A previously described enzyme-releasing assay was used, based upon the observation that C_{5-f_r} induce the release of lysosomal enzymes by neutrophils in the presence of cytochalasin B, whereas cytoplasmic enzymes such as lactic dehydrogenase are not released (11, 37). A 100- μ l cell suspension (containing 10^6 cells) was mixed with 150 μ l of HBSS containing 0 to 70 μ l of C_{5-f_r} . The mixture was incubated at 37°C for 5 min and then centrifuged (500 rpm) for 10 min at 4°C to remove the neutrophils. Finally, the activity of the lysosomal enzyme *N*-acetyl- β -D-glucosaminidase was measured in the supernatant, using *N*-acetyl- β -D-glucosaminide as substrate. Similar assays were performed with the synthetic peptide and the bacterial chemotactic factor.

Enzyme release inhibition assays. An enzyme release inhibition assay (11, 37) was used with some modifications. To detect the presence of and to quantify the C_{5-f_r} -directed inhibitory activity in plasma, 70 μ l of C_{5-f_r} ($1.75 \times 50\%$ effective dose, the quantity of C_{5-f_r} shown to induce maximal glucosaminidase release) was incubated at 37°C for 0 to 30 min with 0 to 10 μ l of plasma in 80 μ l of HBSS. After incubation, 100- μ l normal neutrophil suspensions were added and enzyme release was determined. Controls included reaction mixtures without C_{5-f_r} and after the plasma had been preheated at 56°C for 120 min. To determine the presence of cell-directed inhibitors, the assay was further modified as follows: 100- μ l normal neutrophil suspensions were preincubated at 37°C for 0 to 30 min with 50 μ l of HBSS supplemented with 0 to 10 μ l of plasma. After incubation, 70 μ l of C_{5-f_r} in 100 μ l of HBSS was added and enzyme release was determined. Controls also included reaction mixtures without C_{5-f_r} and after the plasma had been preheated at 56°C for 120 min.

Similar enzyme release inhibition assays were used to measure the plasma inhibitory activity against FMLP and bacterial factor and the plasma ability to inhibit normal neutrophil response to FMLP and bacterial factor.

Study design. The migration and inhibitory studies were performed without knowledge of the infection status of the animals. Four infected and three control animals were studied by serial intracardiac puncture on days 0 (preinoculation) through 10 and then twice weekly for another 2 weeks.

Statistical analyses. Reproducibility of the neutrophil migration, monocyte migration, and lysosomal enzyme release inhibition assays were studied by intraclass correlation coefficients to estimate the correlation between two observations made on the same sample. A small correlation coefficient (close to 0) indicates poor reproducibility, whereas a large coefficient (close to 1) indicates excellent reproducibility. These intraclass correlation coefficients were 0.67, 0.87, and 0.96, respectively, for neutrophil migrations, monocyte migrations, and enzyme release inhibition.

Because infected and control animals were studied serially, multiple linear regression was used to fit time curves for both the infected and the control groups. Polynomials were used to fit the time changes, and terms were included in the model which allowed for separate curves for the two groups of animals. Comparing the coefficients of the polynomials to zero

supplied tests for significant changes with time in each group. Tests for the equality of the curves between infected and control animals were generated by testing for the equality of the coefficients of the polynomials, using the *t*-test.

RESULTS

Viremia, viral shedding, and serological studies. The susceptibility of all guinea pigs to CMV infection was determined by the absence of CMV antibodies (titers < 1/5). Viremia was demonstrated on post-inoculation day 2 in three guinea pigs, and all four animals were viremic on day 3. The viremia persisted through days 9 to 10. Nasopharyngeal viral shedding was demonstrated on day 2 in two animals, on day 3 in the other two, and persisted through day 18 in one. All infected animals seroconverted on day 18.

Neutrophil spontaneous migration. Neutrophil spontaneous migration was unaffected by the experimental procedures. Before inoculation, mean $\pm$ standard deviation neutrophil spontaneous migration was not significantly different between control and infected animals (12.7 ± 1.8 versus 12.3 ± 2.0 , respectively). After inoculation, neutrophil spontaneous migration remained similar in both groups of animals and failed to show any significant changes with time.

C_{5-f_r} -induced neutrophil-directed migration (chemotaxis). Chemotaxis of control and infected guinea pig neutrophils was induced by C_{5-f_r} . Dose-response curves were routinely performed, using 10, 12, 14, and 16 μ l of C_{5-f_r} . Before inoculation, the dose-response curves were comparable in control and infected animals (Fig. 1A and B) and similar to those previously noted in human studies (37). Post-inoculation, the dose-response curves were analyzed for changes in either position or amplitude. Position and amplitude of neutrophil chemotactic dose-response curves did not change with time in the control group. The maximum chemotactic response occurred with 14 μ l of C_{5-f_r} , and the 50% effective dose C_{5-f_r} chemotactic concentration was 8.5 to 9 μ l of C_{5-f_r} . Position also remained unchanged in the infected group; however, amplitude of the dose-response curves decreased progressively between days 1 and 4 and then increased progressively to attain the preinoculation levels by days 9 to 10 (Fig. 1). These findings suggested that the observed chemotactic defect was probably due to a decrease in neutrophil responsiveness to C_{5-f_r} (18).

Time-related changes in neutrophil chemotaxis were determined by comparing the maximum chemotactic responses in both animal groups, using the *t*-test. Before inoculation, maximum neutrophil chemotaxis was similar in both groups (18.1 ± 4 in controls, 16.8 ± 3.6 in infected animals; $P = 0.5$). After inoculation, no change occurred in controls, whereas maximum

FIG. 1. Typical changes in neutrophil (PMN)-directed migration dose-response curve during early gpCMV infection. (A) and (B) illustrate the serial studies performed on a single infected animal and one concurrent control curve.

chemotaxis decreased significantly in the infected group ($P < 0.001$), the nadir of response (7.5 ± 2.3) occurring on day 4. When the results in the infected group were expressed as percentages of the concurrent controls (Fig. 2), time-related changes were equally significant ($P < 0.001$). The mean maximal chemotactic response on day 4 was 49% of controls.

Monocyte spontaneous migration. Before inoculation, monocyte spontaneous migration was similar in both groups. Post-inoculation, no significant time-related changes or intergroup differences were noted.

Monocyte chemotactic response to C5-fr . Dose-response chemotactic curves were determined with 10, 12, 14, 16, and 18 μL of C5-fr . As in the case of neutrophils the curves were bell-shaped, but maximal responses occurred with 16 μL of C5-fr . Before inoculation, monocyte chemotaxis was similar in both groups. After inoculation, no significant time-related changes or intergroup differences were seen. Thus, neither spontaneous nor directed monocyte migrations were affected by gpCMV infection.

FIG. 2. Maximum neutrophil (PMN)-directed migration during gpCMV infection. Each curve represents serial studies in one infected animal. Viremia occurred between days 2 and 10.

Chemotactic inhibitors in plasma. The enzyme release inhibition assay was used to determine the presence of C5-fr or neutrophil-directed inhibitors or both. C5-fr -directed inhibition was determined by preincubating 70 μL of C5-fr with 0.01 μL of plasma in 50 μL of HBSS at 37°C for 30 min before testing for glucosaminidase release. The inhibitory activity of the plasma was measured by the percent decrease in glucosaminidase release (Fig. 3). Preinoculation control plasmas inhibited the glucosaminidase release by $4 \pm 2\%$. No significant changes in the plasma-inhibiting activity was noted in this group after inoculation. The preinoculation inhibitory activity in the infected group ($4.5 \pm 2\%$) was similar to that in the controls but increased significantly after inoculation ($P < 0.001$). The inhibitory

FIG. 3. Plasma-induced inhibition of C5-fr enzyme-releasing activity in control (◇) and infected (⊠) plasmas.

FIG. 4. Correlation between neutrophil (PMN)-directed migration (Fig. 2) and plasma C_{5-fr} -directed inhibition (Fig. 3) in gpCMV-infected guinea pigs.

activity increased to a maximum by post-inoculation day 4 and then decreased progressively and attained control levels by days 9 to 10.

The C_{5-fr} -directed inhibitory activity in infected guinea pig plasmas correlated with the chemotactic defect in their neutrophils (Fig. 4) ($r = -0.96$), suggesting that the inhibitory activity in the plasma was directed against the neutrophils rather than against C_{5-fr} . Further studies were performed to define the characteristics of the inhibitory activity in the plasmas.

Heat stability was determined by preheating the plasma samples at 56°C for 120 min before incubation with C_{5-fr} . Control plasmas lost 97% of their inhibitory activity compared with a 1% loss in infected plasmas (Table 1). To determine whether the inhibitory activity was time dependent, enzyme release inhibition was determined without prior incubation of the plasma- C_{5-fr} mixture at 37°C . No significant inhibitory activity was noted in either the infected or the control plasmas in the absence of preincubation. Thus, the inhibitory activity in controls was heat labile

and time dependent (features characteristic of the chemotactic factor inactivator [CFI] activity) (37). The inhibitory activity in the infected plasmas was heat stable and time dependent, thus distinct from CFI activity. That C_{5-fr} inhibition required preincubation with plasma before adding the neutrophils implied the presence of a C_{5-fr} -directed inhibitor in gpCMV-infected plasmas.

To determine whether the enzyme-releasing activities of other chemotactic factors were also affected by the infected plasmas, similar studies were performed with the bacterial factor and FMLP. Infected plasmas were less inhibitory than control plasmas against both the bacterial factor and FMLP (Table 2). In contrast to C_{5-fr} inhibition, the inhibitory activities against the bacterial factor and FMLP were heat labile and time dependent; thus, they were related to a CFI-like activity.

The presence of a neutrophil-directed inhibitor(s) in the plasmas was determined through modification of the enzyme release inhibition assay. Neutrophils were mixed with 10 μl of plasma and preincubated at 37°C for 0 or 30 min before adding C_{5-fr} . Normal plasmas failed to inhibit neutrophil glucosaminidase release, whereas significant inhibition occurred with infected plasmas ($P < 0.001$) (Table 3). The inhibitory activity was time dependent and resisted heating at 56°C for 120 min. In addition, incubation of normal neutrophils with infected plasmas inhibited neutrophil response to both the bacterial factor and FMLP.

Chemotactic factor generation, as determined by the chemotactic differential of ZAP (Table 4), was decreased in infected animals (18 ± 5 versus 95 ± 2 in controls; $P < 0.001$).

Table 4 also summarizes the chemotactic inhibitory studies of infected and uninfected plasmas. The results were similar to those obtained with the enzyme release inhibition assays in Tables 1 and 2. Infected plasmas were more inhibitory against C_{5-fr} but less inhibitory against

TABLE 1. Plasma inhibition of C_{5-fr} -induced enzyme release

Initial reaction mixture (in 150 μl of HBSS)	Incubation time at 37°C (min) before adding normal neutrophils	Enzyme release inhibition (%)		<i>P</i> (<i>t</i> -test)
		Control plasma (<i>n</i> = 3)	Infected plasma (<i>n</i> = 4)	
70 μl of C_{5-fr} + 0.01 μl of plasma	30	4 ± 0.05^a	23 ± 2	<0.05
70 μl of C_{5-fr} + 10 μl of plasma	30	100 ± 1	100 ± 5	
70 μl of C_{5-fr} + 10 μl of heated plasma ^b	30	1 ± 0.2	99 ± 1	<0.01
70 μl of C_{5-fr} + 10 μl of plasma	0	3 ± 0.5	1 ± 0.4	NS ^c

^a Mean $\pm$ standard deviation.

^b Plasma was heated at 56°C for 120 min.

^c NS, Not significant.

TABLE 2. Plasma inhibition of bacterial factor- and FMLP-induced enzyme release

Initial reaction mixture (in 150 μ l of HBSS)	Incubation time at 37°C (min) before adding normal neutrophils	Enzyme release inhibition (%)		<i>P</i> (<i>t</i> -test)
		Control plasma (<i>n</i> = 3)	Infected plasma (<i>n</i> = 4)	
110 μ l of bacterial factor + 20 μ l of plasma	30	96 $\pm$ 2 ^a	31 $\pm$ 7	<0.01
110 μ l of bacterial factor + 20 μ l of plasma	0	1 $\pm$ 0.5	2 $\pm$ 0.8	NS ^b
110 μ l of bacterial factor + 20 μ l of heated plasma ^c	30	1 $\pm$ 0.4	2 $\pm$ 1	NS
80 μ l of FMLP + 20 μ l of plasma	30	38 $\pm$ 9	12 $\pm$ 9	<0.05
80 μ l of FMLP + 20 μ l of plasma	0	5 $\pm$ 2	2 $\pm$ 0.5	NS
80 μ l of FMLP + 20 μ l of heated plasma	30	2 $\pm$ 1	1 $\pm$ 1	NS

^a Mean $\pm$ standard deviation.^b NS, Not significant.^c Heating at 56°C for 120 min.TABLE 3. Plasma inhibition of neutrophil enzyme release response^a

Chemotactic factor	Incubation time at 37°C (min) before adding chemotactic factor	Inhibition of lysosomal enzyme release (%)		<i>P</i> (<i>t</i> -test)
		Control plasma	Infected plasma	
70 μ l of C ₅ -fr	30	5.5 $\pm$ 1 ^b	35 $\pm$ 4	<0.01
	0	1 $\pm$ 0.5	7.5 $\pm$ 1	NS ^c
110 μ l of bacterial factor	30	2.5 $\pm$ 2	19 $\pm$ 4	<0.01
	0	1 $\pm$ 1	1 $\pm$ 1	NS
80 μ l of FMLP	30	8 $\pm$ 3	27 $\pm$ 10	<0.05
	0	1.5 $\pm$ 1	3 $\pm$ 2	NS

^a The initial reaction mixture (in 150 μ l of HBSS) was a 100- μ l neutrophil suspension plus 10 μ l of plasma.^b Mean $\pm$ standard deviation.^c NS, Not significant.TABLE 4. Chemotactic factor generation and chemotactic factor inhibition^a

Chemotactic mixture (in 24 μ l of Gey soln)	Chemotactic differential (% control; mean $\pm$ SD)
Chemotactic factor generation	
14 μ l of uninfected ZAP	95 $\pm$ 2
14 μ l of infected ZAP	18 $\pm$ 5 (<i>P</i> < 0.001)
Chemotactic inhibition	
14 μ l of C ₅ -fr + 0.02 μ l of infected plasma	99 $\pm$ 0.5
14 μ l of C ₅ -fr + 0.02 μ l of control plasma	53 $\pm$ 3 (<i>P</i> < 0.01)
18 μ l of bacterial factor + 4 μ l of infected plasma	13 $\pm$ 3
18 μ l of bacterial factor + 4 μ l of control plasma	65 $\pm$ 5 (<i>P</i> < 0.01)
16 μ l of FMLP + 4 μ l of infected plasma	54 $\pm$ 15
16 μ l of FMLP + 4 μ l of control plasma	57 $\pm$ 14 (NS) ^b

^a The plasmas were obtained from four infected and three control guinea pigs on post-inoculation day 3.^b NS, Not significant.

the bacterial factor and FMLP. Further studies (not shown) also confirmed the heat stability of the chemotactic inhibitory activity.

DISCUSSION

Our studies were aimed at defining whether monocyte and neutrophil migratory defects are acquired during gpCMV infection and at studying the pathogenesis of any such defects. Infected and control animals underwent similar procedures (intramuscular injection of fetal guinea pig tissue cultures, blood drawing, etc.). Results were analyzed for time-related changes as well as intergroup differences. No procedure-related changes were identified.

Monocyte and neutrophil migrations are affected by cell concentrations, functional cell integrity, presence and concentration of chemotaxins, presence of CFIs, and presence of cell- or chemotaxin-directed inhibitors. CFIs are heat-labile plasma components that destroy chemotaxins irreversibly in a time- and dose-dependent manner. The inactivators have no direct inhibitory effect on leukocytes; however, inactivated chemotaxins can inhibit leukocyte responses (37). Other chemotaxin-directed inhibitors also lack direct effects on leukocytes and can be distinguished from the inactivators by their physical characteristics, heat lability, specificity, etc. Cell-directed inhibitors act directly on the leukocytes. Differentiation between cell- and chemotaxin-directed inhibitors is usually demonstrated by the higher inhibitory activity induced by the plasma when present in the cellular compartment of chemotactic chambers; however, results are inconclusive when both cell- and chemotaxin-directed inhibitors are present. As we discuss below, we used heat lability, time dependency, and specificity to characterize and identify various inhibitors.

In vitro studies allowed us to study each of the various factors involved in leukocyte chemotaxis independently. Monocytes and neutrophils were separated, standardized, washed, and then tested for responses to C_{5-f_r} from normal (non-study) guinea pigs; thus, the possible effects of variation in cell concentration and of chemotaxin-directed plasma factors were eliminated. Monocyte migrations were unaffected by gpCMV infection, as was neutrophil spontaneous migration. In contrast, neutrophil-directed migration towards C_{5-f_r} decreased. Similarly, activated plasmas from experimental animals were tested for chemotactic activity to normal neutrophils and exhibited a transient defect during early infection. Further studies were aimed at determining whether the abnormalities in neutrophils and in chemotactic factor generation were related to inhibitors in plasma. Plasma from infected animals inhibited the directed mi-

gration of normal neutrophils towards C_{5-f_r} , bacterial factor, and FMLP. Inhibition was time dependent; i.e., incubation was necessary before adding the chemotaxins, strongly suggesting a cell-directed inhibition. Because the plasmas of infected animals were inhibitory against normal neutrophils, it is reasonable to assume that the decrease in directed migration of infected guinea pig neutrophils was due to the same plasma inhibitor(s) rather than some intrinsic abnormalities.

Incubation of various chemotaxins with the plasmas inhibited C_{5-f_r} chemotactic and enzyme-releasing activities in a time-dependent fashion. Contrary to the above-mentioned results, the activities of bacterial factor and FMLP were unaffected, clearly suggesting a different, C_{5-f_r} -specific, inhibitory system. This finding is in agreement with the decrease in chemotactic activity in ZAP during infection since C_{5-f_r} account for most of the chemotactic activity in ZAP. Thus, our studies strongly suggested the presence of distinct neutrophil- and C_{5-f_r} -directed inhibitors during early gpCMV infection. Both inhibitory activities were heat stable, thus unrelated to the CFI system.

Because studies of infected guinea pig monocytes revealed no cellular abnormalities, a search for monocyte-directed inhibitors was not done. Monocyte-directed migration may, however, be impaired indirectly, since C_{5-f_r} is chemotactic for monocytes as well.

Peritoneal macrophage chemiluminescence is enhanced during murine CMV infections, and lung macrophage bacterial phagocytosis is decreased after incubation with murine CMV (32, 33). We are unaware of any previous migration studies of monocytes. Decreased neutrophil migration during the period of viremia was previously reported by Yourtee et al. (38) and Bale et al. (3, 4), and the virus appears to be primarily neutrophil associated (38). However, only a small proportion of neutrophils contain the virus (38), the chemotactic defect improved before the termination of viremia (Fig. 2), and the inhibiting activity was detected in plasma which has been shown to be free of virus (38). Thus, the in vivo acquired cellular defect is more likely related to the inhibitors in plasma than to a direct effect of the virus on neutrophils.

Human studies failed to demonstrate a decrease in neutrophil migration (7); however, the chemotactic defects observed in guinea pig and murine CMV models occur early in infection and are transient, whereas human studies were performed later in CMV infection and similar defects may have been missed.

Abnormal monocyte and neutrophil migrations are seen in association with other viral illnesses: monocyte chemotaxis is decreased

and random migration is increased in patients during acute influenza infection (19). Neutrophil chemotaxis is depressed during hepatitis B and measles (2, 23), and spontaneous migration is decreased during measles infection (2). In all cases the observed defect is transient. Similar defects are seen in chinchillas during influenza A infection (1). Direct effects of the viral agents on monocytes and neutrophils are routinely suggested as pathogenesis. Such a possibility is suggested by a decrease in neutrophil chemotaxis after *in vitro* incubation with influenza viruses and echovirus (6, 21, 31).

To our knowledge, acquired inhibitors of chemotaxis have not been described during acute viral infection. Anderson et al. (2) sought, but did not detect, chemotactic inhibitors during infection with measles. However, a neutrophil chemotactic defect and two serum inhibitors (one of which is CFI-like and the other of which is heat stable and apparently directed against neutrophils) are present in some patients with bacterial sepsis (8). Additional studies will be required to further define these inhibitors and to clarify their role in the enhanced susceptibility to concurrent infections.

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