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Peritoneal Macrophages of Pathogen-Free Rats but not of Conventional Rats Secrete Elastolytic Activity

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Elicited peritoneal macrophages from Sprague-Dawley rats conventionally bred and housed failed, as we have reported, to produce detectable elastolytic activity in culture. They did produce lysozyme and plasminogen activator. We now show that in contrast to these cells, macrophages from pathogen-free, barrier-sustained rats produced readily demonstrable elastolytic activity. Rats raised pathogen-free and subsequently housed conventionally for 2–4 wk appeared to lose the capacity to afford macrophages producing elastase. At the same time they acquired infections with several rat pathogens including Spironucleus muris, Kilham rat virus, sialodacryoadinitis virus, and mycoplasma pulmonis. The acquisition by the rats of one or more of these infections, conditions conducive to infection, or both factors may have suppressed their capacity to yield elastolytic activity.

Key words: rat, macrophage, elastase, environment

INTRODUCTION

There are no known published reports of elastase secretion by rat peritoneal macrophages despite the well-documented secretion of such enzyme activity by mouse macrophages [3,22]. Our past efforts to detect secretion of elastase by thioglycolate-induced rat peritoneal macrophages [8] and similar studies of others (unpublished)

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have failed. Absence of such proteolytic activity from macrophages would be highly significant in light of proposed roles for such enzymes and macrophages in inflammatory tissue-destructive processes [6,13,23,24], many models of which include arthritis and are studied in rats [5,14,19]. It was the purpose of this study to test the hypothesis that rat macrophages can synthesize and secrete elastase but that these activities may be suppressed in rats maintained by conventional housing conditions. The second purpose was to investigate the possibility that the microbial flora of rats may determine this suppression. Elastase secretion by rat peritoneal exudate macrophages was indeed demonstrated, but only if macrophages were obtained from rats maintained free of certain common pathogens associated with conventional rats. The major thrust of this paper is to identify a specific difference with respect to elastase production in thioglycolate-elicited cells from rat population that differed with respect to their microbial environments.

MATERIALS AND METHODS

Animals

Pathogen-free (PF) Sprague-Dawley rats were purchased from the Kingston, N.Y. facility of the Charles River Breeding Laboratories, Inc. (Wilmington, MA.) The PF rats were shipped in filter-protected cartons and housed in either a conventional animal facility (PF-CV rats) or in a barrier facility, which excludes pathogens (PF-BR). Conventional (CV) Sprague-Dawley rats were also purchased from Zivic-Miller Labs, Inc. (Allison Park, PA) and housed in our conventional facility (CV-CV rats). Mice (C57BL/6) were kindly provided by Dr. Joseph Kinkade (Emory University, Atlanta, GA) from his CV mouse colony.

Animal Health Monitoring

Samples of the rats obtained PF from Charles River Breeding Laboratories, Inc., were sacrificed for health evaluation immediately upon receipt from the breeder (PF rats) or after 2 wk and 4 wk in our conventional facility (PF-CV rats). At each time five randomly selected rats were tested for common rodent pathogens, including aerobic bacteria (cultures of nasopharynx, liver, and cecum), mycoplasmas (culture of nasopharynx and the ELISA for IgM and IgG antibody; the latter kindly performed by Dr. Gain H. Cassell, University of Alabama in Birmingham), viruses (hemagglutination inhibition test for Sendai virus, pneumonia virus of mice, Kilham rat virus, and Toolan H-1, and the complement fixation test for rat coronavirus; performed by Microbiological Associates, Bethesda, MD), and endo- and ectoparasites (histology). In addition, sections of all major organs were processed by paraffin methods, stained with hemotoxylin and eosin, and examined by light microscopy for lesions.

Macrophage Collection and Culture

Peritoneal exudates were collected in Dulbecco's phosphate buffered saline (PBS) without calcium or magnesium but with glucose and antibiotic-antimycotic mixture (Gibco, Grand Island, NY) 4 d after intraperitoneal thioglycolate (Brewer's, Difco, Detroit, MI) injection. Resident peritoneal macrophages were obtained for control and baseline purposes in the same fashion from rats of the same shipments as the exudate rats but without thioglycolate or other injection prior to harvest. Because of our repeated observations of close similarity between resident macrophages of PF-

CV rats, PF-BR rats, and CV-CV rats with respect to total cell counts, differential cell counts, or fractionation in Percoll, we have omitted comparisons of those results from our tables (see Fig. 1). Resident peritoneal macrophages are not activated and do not secrete elastase or other activation markers in either rat population. Comparisons of these nonevents would be useless. Peritoneal cells were suspended and plated at 2.5×10^5 macrophages/cm^2 of surface area in plastic dishes with 2.5% acid-treated [21] and heat-inactivated fetal bovine serum (AT-HI-FBS, Flow Labs, Inc., Rockville, MD) in Dulbecco's Modified Eagles Medium (DMEM, Gibco) plus antibiotic and antimycotic agents. After overnight incubation at 37°C in 5% CO_2 in air, cultures were changed to 10% AT-HI-FBS in DMEM for 24 hr. Cultures were washed 3 times in PBS and cultured with DMEM plus 0.2% lactalbumin hydrolysate (LH, Gibco). This adherence and washing procedure gave the best macrophage survival for the relatively poor adherent cells from CV-CV rats. Culture medium (CM) was collected every day, centrifuged to remove cells, and frozen at −20°C prior to assays.

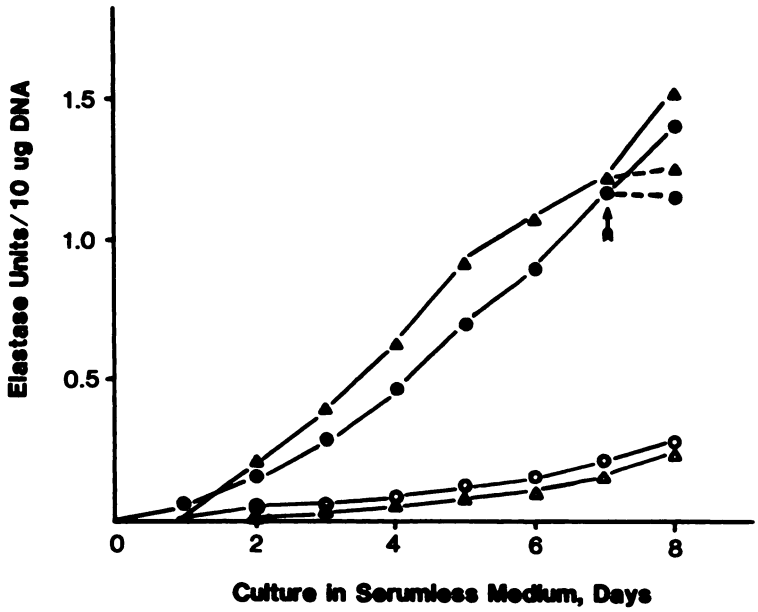

Fig. 1. Elastase secretion by cultured peritoneal macrophages from pathogen-free (PF) Sprague-Dawley rats maintained in conventional (CV) housing. Inhibition with cycloheximide. Thioglycolate-induced induced peritoneal macrophages (●—●) of PF Sprague-Dawley rats actively secreted elastase compared to resident of unstimulated PF macrophages (○—○) that failed to secrete elastase. The rat macrophages secreted plasminogen activator (not shown) in a fashion similar to their secretion of elastase. They also secreted a low level of fibrinolytic and caseinolytic activity (not shown). Cycloheximide (.25 μg/ml) added to cultures on day 7 (arrow) inhibited further accumulation of elastase (●--●) and the other two proteolytic activities, indicating that active protein synthesis was required for those protease secretions. The ordinate represents cumulative enzyme activity released into culture supernatants per 10 μg cellular DNA. Abscissa represents days macrophages were cultured in serum-free medium. Mouse macrophage cultures were used as positive controls for elastase secretion. (▲—▲), thioglycolate-stimulated mouse peritoneal macrophages; (△—△), resident mouse peritoneal macrophages. Elastase cumulative activity following cycloheximide (▲---▲) was also inhibited in mouse macrophage cultures. Points are averages of duplicate values.

Assays for Proteolytic Enzyme

Frozen CM was thawed and dialyzed against 0.01 M Tris-HCl, pH 7.6, plus 0.001 M $CaCl_2$, and then lyophilized. Lyophilized samples were suspended in dH_2O to 20 × concentration for assay. Elastase assays were performed using ^{3}H-elastin particles prepared and used as described by Gordon et al [9]. Briefly, ^{3}H-elastin (100–200 cpm/μg) was washed with distilled water and resuspended at 1 mg/ml in 0.3M-tris buffer pH8, containing .02% NaN_3 and 0.2 mg/ml sodium dodecyl sulfate (SDS). Two hundred milliliters of concentrated (20×) dialyzed culture medium was added to a plastic microcentrifuge tube containing 3 μl 0.5 M $CaCl_2$. Substrate (100 μl) was added to each tube and samples were incubated at 37°C for 24 h. Samples were spun in a microcentrifuge and 100 μl aliquot was counted in scintillation fluid. One unit of elastase is that amount which cleaves 1 μg elastin/hr at 37°C. Nonspecific protease activity was assayed by hydrolysis of ^{3}H-acetyl-casein prepared and used as described by Levine et al [17]. One unit of activity was the amount that hydrolyzed 1 μg casein/hr at 37°C. Plasminogen activator (PA) was assayed with unconcentrated CM by measuring plasminogen (human plasminogen, epsilon-aminocaproate-free, Sigma Chem. Co., St. Louis, MO) dependent solubilization of ^{125}I-fibrin coated onto 16-mm, 24-well plastic dishes (Costar, Cambridge, MA) as described by Gordon et al [9]. One unit of PA activity was plasminogen dependent release of 100 cpm/4 hr at 37°C. Fibrinolytic activity was detected in the absence of added plasminogen.

DNA Assays

Specific enzyme activities in macrophage cultures were based on culture DNA content. DNA was assayed in cell lysates with the fluorescent compound mithramycin as described by Hill and Whatley [11] and modified by Groyer and Robel [10].

Percoll Gradients

Discontinuous gradients of percoll (Pharmacia Fine Chemicals, Piscataway, NJ) were made by layering 3 ml each of 8%, 6%, 4%, and 3% Percoll (made isotonic with PBS, Percoll is 13% w/v) into 15-ml conical centrifuge tubes. Approximately 2×10^7 peritoneal cells were layered on top of these gradients in 3 ml of 1% Percoll. Cell fractions were collected after centrifugation at 400 g for 30 min, 4°C. Cells at the density interfaces were collected, counted, and identified.

RESULTS

Protease Secretion by Macrophages from CV Rats

In previous studies [8] we failed to detect secreted elastase activity in cultures of thioglycolate-elicited rat peritoneal macrophages from CV rats maintained in our CV facility. We also failed to detect elastase in cultures of resident macrophages (see "Materials and Methods"). The absence of elastase activity in rat macrophage (Table 1) was evidently not due to technical problems since elastase activity was easily demonstrated in cocultures of thioglycolate-elicited mouse-plus-rat macrophages (Table 1). The low elastase activity reported for rat cultures could not be distinguished from nonspecific elastin hydrolysis by other proteases. The rat macrophages were synthetically active as shown by accumulating lysozyme activity in cultures. Table 1 demonstrates that co-cultivation of rat macrophages with mouse macrophages does

TABLE 1. Elastase Activity in Co-Cultures of Rat and Mouse Thioglycolate-Induced Peritoneal Macrophages[a]

Cells[b]	Days in culture[c]	Elastase activity total units/culture
Mouse	3	0.59 + .02 (2)
Rat[c]	3	0.04 ± .01 (2)
Rat: mouse (1:1)	3	.66
Rat: mouse (2:1)	3	.88
Mouse	5	0.57 ± .14 (3)
Rat	5	0.02 ± .03 (2)
Rat: mouse (1:1)	5	.58
Rat: mouse (2:1)	5	.60

[a]All rats in these experiments were purchased from conventional colonies and housed in a conventional facility (ie, CV-CV rats). Since resident macrophage did not produce elastase, these negative results are omitted.
[b]Rat and mouse cells cultured in serumless medium at cell ratios of 1:1 or 2:1.
[c]Elastase activity determined on dialyzed, lyophilized culture medium after 3 d and 5 d in culture.

not inhibit accumulating mouse macrophage elastase activity in cocultures. Total activity in cocultures was equivalent to that secreted by equivalent numbers of mouse macrophages alone in control cultures, indicating that all elastase present was secreted by mouse cells alone. Therefore, the presence of an active elastase inhibitor in culture medium of rat macrophages was not shown. That low activities of rat elastase might have been caused by secretion of enzyme bound to an inhibitor as suggested for mouse macrophage elastase in crude conditioned medium [3] seems unlikely since all of our assays were done in the presence of SDS, as described in "Materials and Methods."

Protease Secretion by PF-BR Rats

In our search for rat macrophage elastase activity we tested macrophages from PF-BR rats. Peritoneal macrophages elicited with thioglycolate in these rats secreted elastase (Fig. 1) and in addition lysozyme- and plasminogen-independent fibrinolytic activity (PH) results were shown. Accumulation of these activities in culture medium was inhibited by cycloheximide (Fig. 1), indicating that active enzyme synthesis had been responsible for their accumulation. The elastase activity was optimum at pH 7.5. Inhibitor profiles of protease activity against three protein substrates (results not shown) revealed that elastase activity in crude CM was partially inhibited by both serine protease inhibitors and by EDTA. Purified mouse macrophage elastase has been found to behave as a metallo-protease [3]; however, crude mouse elastase activity in CM is also inhibited by various inhibitors of different specificities [9]. Elastin degradation in assays of crude CM elastase activity may be the result of multiple proteases; thus one might see partial inhibition with several inhibitors and caution should be taken in interpreting such inhibition studies [3].

Comparison of Exudate Cell Populations in PF-BR v CV-CV Rats

Table 2 demonstrates that CV-CV rats produced a weaker exudate response to thioglycolate than did PF-BR rats. In all comparison studies, rats were purchased

TABLE 2. Comparison of Thioglycolate-Induced Peritoneal Exudate Cells in PF-BR v CV-CV Rats[a]

Exudate cells/rat	PF-BR Rats $5.4 \times 10^7 \pm 1.1 \times 10^7$ (5)[b]	CV-CV Rats $3.4 \times 10^7 \pm 0.7 \times 10^7$ (5)
Number of mononuclear phagocytes (% of total exudate)	82 ± 5 (5)	61 ± (5)
Number of large vacuolated macrophage (% ot total exudate)	52 ± 11 (5)	12 ± 5 (5)

[a]PF-BR, Pathogen-free stock, maintained in barrier condition; CV-CV, conventional stock, maintained in a conventional facility. Because no differences were observed between resident macrophage population in PF-BR and CV-CV rats, results relevant to them were omitted from this table.
[b]Values ± SEM. Number of rats in parentheses.

from suppliers and housed for 1 wk prior to injection. Differential cell counts on Wright-stained cytocentrifuged preparations of exudate cells revealed significant morphologic differences between PF-BR and CV-CV macrophages. A larger percentage of total exudate cells were mononuclear phagocytes in PF-BR rats than in CV-CV rats. Perhaps more significant was the predominance of large, vacuolated macrophages in PF-BR exudates compared to the predominance of monocytelike cells in CV-CV exudates.

These results suggested that exudates from CV-CV rats thus morphologically resembled untreated resident peritoneal cell populations. This difference was visible in cultured cells as well. Cultured PF-BR macrophages were well spread on culture dishes and survived well in serumless conditions. Cultured macrophages from CV-CV rats were poorly adherent, rounded, and survived poorly.

Size and density differences in the two exudate populations were demonstrated by separation of exudate cells on discontinous Percoll gradients (Table 3). In such gradients, 90% of PF-BR exudate cells and 90% of total exudate macrophages were found at the 4–6% interface, indicating a very homogeneous cell population. In gradients of CV-CV exudate cells, a heterogeneous distribution of total cells and total macrophages was seen with two major density populations, the major population of macrophages being found at the denser 6–8% interface. The large vacuolation of cells in PF-BR rats, probably due to ingestion of agar from the thioglycolate broth, could acount for their density being less than that of CV-CV rat cells. Thus, there exists a physical difference in the cell populations induced by thioglycolate in PF-BR v CV-CV rats. It is known that unstimulated resident macrophages secrete very little elastase or other protease activity [21,22]. The predominance of residentlike cells in PF-CV and CV-CV rat exudates may be in some way responsible for their low protease secretion.

Effects of CV Housing on PF Rat Macrophages

The only obvious difference in the two groups of Sprague-Dawley rats tested was the commercial source and respective housing conditions. This implied to us that the respective health of the animals as a result of their environments might have been influencing their macrophage responses and functions. In connection with another

TABLE 3. Separation of Macrophage Populations on Discontinuous Percoll Gradients

Percoll fraction	Distribution of cells[a]			
	PF-BR rats[b]		CV-CV rats[b]	
	All cells (% of total)	Mϕ[c] (% of total)	All cells (% of total)	Mϕ[c] (% of total)
1–3% interface	3	2	7	2
3–4% interface	2	2	7	6
4–6% interface	92	95	24	27
6–8% interface	2	1	55	65
Pellet	1	0	7	0

[a]Thioglycolate-induced peritoneal exudates
[b]Rat designations as for Table 2. See also footnote a in Table 2.
[c]Mϕ, macrophages

TABLE 4. Changes in Peritoneal Exudate Cell Populations in PF Rats Following Transfer to CV Housing[a]

Duration of CV[a] housing in weeks	Thioglycolate-induced peritoneal exudates	
	Total Mϕ/rat ($\times 10^7$ + SEM)	Large vacuolated Mϕ (% of total exudate + SEM
0	1.9 + .25 (23)[b]	52 + 5 (24)
1	1.0 + .4 (5)	49 + 11 (5)
2	0.6 + .3 (9)	21 + 9 (9)
3	0.8 + .4 (10)	20 + 8 (10)
4	0.8 + .4 (8)	32 + 13 (8)

[a]PF, pathogen-free; CV, conventional. Because resident Mϕ populations showed no differences, results relevant to them are omitted.
[b]Number of rats

project involving rats housed in the multipurpose conventional animal facility at Emory, we had seen signs of respiratory infections in our animals. Serology on these animals revealed significant serum antibody to Mycoplasma Pulmonis. This led us to our investigation of macrophage responses in PF-BR rats. Successful demonstration of elastase secretion in macrophages from PF-BR rats led us to test if the housing of PF rats in our CV facility (PF-CV rats) would alter their macrophage responses.

For these experiments, PF Sprague-Dawley rats were purchased and shipped in filter-protected cartons. Some of the animals were removed to a barrier facility (PF-BR rats) and some were transferred into our conventional facility (PF-CV rats). Table 4 compares the number of total macrophages and number of large macrophages (percentage of total exudate) present in thioglycolate-elicited exudates. After 2–4 wk in the conventional housing conditions, PF-CV rats showed decreased numbers of total macrophages in thioglycolate exudates and corresponding reductions in the percentage of cells that were large macrophages compared to PF-BR controls. Figure 2 compares the results of elastase assays on cultures of thioglycolate-elicited macrophages collected from either PF-BR rats or PF-CV rats housed for 2–4 wk in the CV

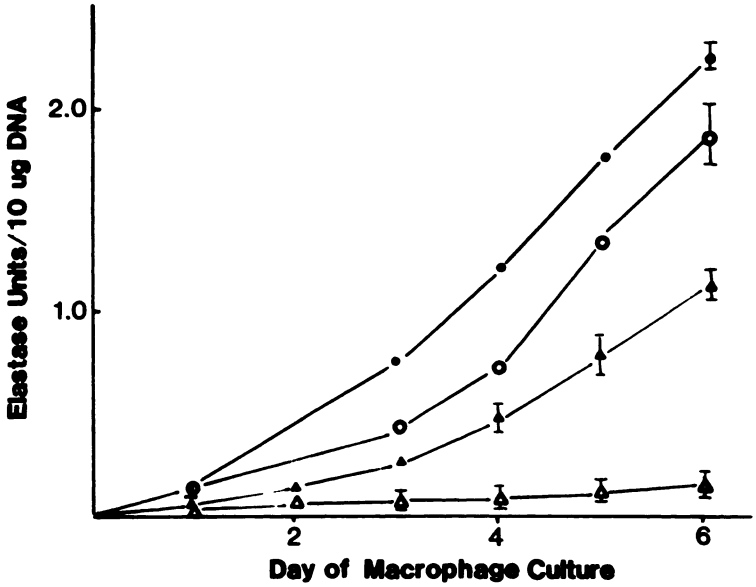

Fig. 2. Elastase secretion by thioglycolate-induced macrophages from pathogen-free (PF) rats transferred to conventional (CV) unquarantined housing. Compared to macrophages from age-matched PF rats, thioglycolate-induced macrophages from PF rats received and transferred to CV conditions at 6 wk of age exhibited significantly depressed elastase secretion in culture. Macrophages from PF rats received and housed 2 wk in the PF-BR facility: ●——●; macrophages from PF rats received and housed 4 wk in the PF-BR facility: ○——○; macrophages from PF rats received and maintained 2 wk in the conventional facility (PF-CV rats): ▲——▲; macrophages from PF rats received and maintained 6 wk in the conventional facility (PF-CV rats): △——△. All rats were age matched and received simultaneously from one supplier. Ordinate and abscissa as for Figure 1. Since resident peritoneal rat macrophages failed to secrete elastase in any of these rat populations (see Fig. 1 for example) results relevant to them were omitted from these graphs. Points represent means of three replicate samples ± standard derivation. Where standard derivation bars are not supplied, less than three samples were available due to technical losses. Values are plotted as values cumulative with time.

facility. Control PF rats were purchased and used within 1 wk of arrival age. They were matched to PF-BR rats. Macrophages from PF-CV rats housed under CV conditions for 2 wk showed depressed elastase secretory activity compared to age-matched PF rats housed solely in BS conditions. This difference was more pronounced after BS rats experienced 4 wk of CV conditions. Thus, the morphologic data and biochemical assays revealed that the sojourn within the CV animal facility modified the rats' intraperitoneal response to thioglycolate and coordinate macrophage secretory activity.

Serology and Histology

The CV rats purchased and housed in our conventional facility (CV-CV rats) for 4 wk were uniformly positive for antibodies to M. pulmonis. In an attempt to show changes in microbial flora, which is known to affect results of animal research [1], microbial testing was performed on PF rats housed in CV conditions (PF-CV rats). Macrophages used in the elastase experiments were obtained from these tested

rats. The organisms most associated with immunomodulation or promotory biochemical alterations of animals and isolated by testing of these rats were as follows: Spironucleus muris, an intestinal parasite that appeared in all rats by 2 wk, and two viruses, Kilham rat virus and sialodacryoadenitis virus, that appeared between 2–4 wk after transfer of PF rats to CV conditions. In a separate experiment, antibodies to Sendai virus increased within 2 wk after housing PF-rats in our CV facility. Most of these agents are known to inhibit in vivo and in vitro biological responses [4,7,12,15,18,20]. Other pathogens detected in individual rats were Pseudomonas sp, pulmonis, Syphacia sp, Giardia muris, and Pasteurella pneumotropica. Whereas the Spironucleus muris appeared by 2 wk, significant antibody titers to the two viruses and Mycoplasma did not appear until 4 wk after PF rats were transferred to CV conditions. Our inference is that these agents being contracted by rats in our CV facility, singly or collectively, altered macrophage responses and secretory activities in vitro.

DISCUSSION

Elastase activity, not detected in previous studies [8], has been demonstrated in cultures of thioglycolate-induced rat peritoneal macrophages. Elastase secretion by rat peritoneal exudate macrophages could be demonstrated only if cells were obtained from PF-BR rats.

Yu and Yoshida [25] detected elastinolytic activity of cell lysates of rat peritoneal cells following intraperitoneal injection of ^{14}C-elastin. They demonstrated in vivo digestion of these particles, however most of the activity in cell lysates appeared to correlate with presence of polymorphonuclear leukocytes. Intracellular and extracellular protease inhibitors were also found. Hence rat peritoneal exudates possess elastinolytic enzymes; however association of such activity with rat macrophages has not been previously described.

An examination of different strains and sources of rats revealed great variation in thioglycolate-induced peritoneal macrophages. The CV Sprague-Dawley rats exhibited inconsistent responses to thioglycolate, and cultured exudate cells from such rats secreted little or no elastase. Examination of conventionally housed rats revealed significant infection with bacterial, viral, and parasitic pathogens. The PF-BR Sprague-Dawley rats responded to thioglycolate injection with macrophage exudates, which actively secreted elastase in culture. The PF rats housed with rats under CV conditions acquired a number of microbial infections and their exudate macrophages no longer secreted elastase. The implication without direct infection studies is that certain microbial infections, however inapparent, markedly influence the in vitro function of macrophages derived from such infected rats. A diversity of physical, chemical, and environmental factors that may have differed between the PF, PF-BR, PF-CV, and CV-CV rats could also have been responsible for these observations [2].

Secretion of proteases is regulated by macrophage activation and by uptake and storage of particulate materials [21,22]. The synthetic controls or secretory mechanisms for elastase must be separate not only from those of constitutively secreted lysozyme [22] but also from those controls on other inducible proteases such as plasminogen activator. Plasminogen activator secretion was always detectable in CV rats in contrast to elastase. Elastase, collagenase, and plasminogen activator are thought to be under coordinate control in mouse macrophages [21,22,26]; however

nonparallel discharge of these inducible enzymes has been shown in mouse macrophages treated with tubulin-binding drugs [26]. Differential synthesis of enzyme inhibitors might also explain differences seen in detectable secreted enzymes [3]. The low levels of elastase found in conditioned medium of macrophages from CV rats is most likely not due to secretions of latent enzyme as proposed for mouse macrophage elastase [3]. Only 10–15% of total secreted mouse macrophage elastase is detectable in crude conditioned medium. Extensive dialysis of samples and use of SDS-treated elastin is necessary to maximize detectable elastase activity in mouse macrophage cultures. Since all rat samples were dialyzed and assayed for hydrolysis of SDS-elastin, presence of an enzyme inhibitor such as that reported for mouse elastase cannot explain the inactivity of our samples. Regardless of the cause, similarly processed samples from PF-BR rats possessed active elastase.

Microbial influences on biological responses are well documented [1,2]. The changes in elastase secretion we have observed, if not due directly to microbial influences, are intimately related to environmental conditions or events associated with acquisition of specific infections by the rats. It seems likely that regulatory systems within the whole animal as a result of environmental or microbial influences inhibited induction of good exudate responses and cell activation by thioglycolate. The poor adherence and poor survival of thioglycolate-induced macrophages from CV rats might be indicative only of their nonactivated state; however viral infection of such macrophage cultures was not tested. Several of the pathogens detected in CV-CV rats are known to alter immune or biological responses. Spironucleus muris infection in rats alters immune responsiveness to certain antigens [20]. In addition, macrophages from mice heavily infected with S. muris have diminished RNA synthesis in response to antigenic stimulation [15]. Kilham rat virus is often latent, but may interfere with in vitro lymphocyte responses [4]. Sendai virus is also known to alter phagocytic function of murine leukocytes [7,12]. Germ-free rats have been shown to be much more susceptible to adjuvant-induced arthritis than are specific-pathogen-free rats or conventional rats [16]. Microbial flora could be modulating the immune responses in such models of inflammatory disease at either the induction or effector stage. Macrophages, which are intimately involved in arthritic responses, may prove to be one component of the immune or inflammatory response that is particularly susceptible to direct or indirect modulation by microbial flora.

Our observations, while perhaps not surprising, are highly significant since much present and past research especially with rats has been conducted with CV housed animals. Absence of elastase secretion in vitro may not correlate with in vivo secretory capacities of macrophages; however, should CV rats have suppressed in vivo secretion of elastase, the role of macrophage elastase in inflammatory diseases would require serious reevaluation. Recognition of the influences of microbial infections on biological responses is essential particularly when studying a cell as adaptive as the macrophage.

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