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Mouse Neutrophils Lack Defensins

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Defensins are broad-spectrum antimicrobial peptides that are abundant in human, rat, and rabbit neutrophils. We now report that neutrophils from nine strains of mice lacked appreciable defensin content. Mice may therefore be imperfect experimental surrogates for humans or rats in models of infection in which neutrophil function is significant.

The use of mice in experimental models of human infection, a central practice in infectious disease research, is based on the assumption that the cellular mechanisms of host defense are similar in mice and humans. The ability of human neutrophils (PMN) to kill infectious organisms arises from two general mechanisms, often called oxygen dependent and oxygen independent. The former derive from the NADPH oxidase-catalyzed respiratory burst, which produces superoxide anions, hydrogen peroxide, and other, more potent antimicrobial oxidants (6). The latter are mediated by antimicrobial peptides and proteins stored within the PMN's cytoplasmic granules and delivered to its phagolysosomes consequent to postphagocytic degranulation (7). Defensins, a family of cysteine-rich antimicrobial peptides of relatively low molecular weight (M_r < 4,000), comprise 30 to 50% of the total protein of the human PMN's primary (azurophil) granules (5). Defensins are also abundant in PMN of rabbits (11), rats (2, 3), and guinea pigs (10).

The following mouse strains, all specific pathogen free, were obtained from Jackson Laboratories, Bar Harbor, Maine: A/HeJ, AKR/J, BALB/cJ, CBA/J, C3H/HeJ, C57BL/10J, DBA/2J, and SWR/J. Swiss Webster outbred mice were obtained from Harlan-Sprague Dawley, San Diego, Calif. All animals were female and were at least 8 weeks old. Human PMN granules were prepared from the peripheral blood of normal volunteer donors as previously described (5), under a protocol approved by our institutional review board. Female Sprague-Dawley rats weighing approximately 200 g were purchased from Charles River Breeding Laboratories. Rat PMN granules were purified from 6-h sterile peritoneal exudates, induced with sodium caseinate as previously described (3).

Mouse PMN were harvested from sterile peritoneal exudates 4 h after the intraperitoneal instillation of 3% Bacto NIH thioglycolate broth. They were collected and washed once in phosphate-buffered saline containing 5 U of heparin per ml, subjected to hypotonic lysis to remove any erythrocytes, and resuspended in 0.34 M sucrose at a concentration of 10^8 cells per ml. On average, approximately 75% (range, 62.4 to 90.0%) of the recovered cells were neutrophils, and approximately 2×10^8 cells were recovered per 25 mice.

The cells were disrupted with two cycles of homogenization in a motor-driven Teflon-glass (Potter-Elvejhem) homogenizer. The homogenate was centrifuged at approximately 250 × *g* for 10 min at 4°C to deposit unbroken cells and nuclei, and the supernatants were centrifuged at 27,000 × *g* to deposit the cytoplasmic granules. All PMN granules (mouse, rat, and human) were stored at −70°C until they were extracted with 5% acetic acid for 18 h at 4°C, in the presence of a mixture of protease inhibitors (phenylmethylsulfonyl fluoride, leupeptin, pepstatin, *N*-ethylmaleimide, and EDTA) as previously described (3). The granule extracts were dialyzed extensively against 5% acetic acid in Spectra/por 3 membranes (Spectrum Medical Industries, Los Angeles, Calif.) and lyophilized. The lyophilates were dissolved in 0.01% acetic acid for further testing. Protein concentrations were measured by the BCA assay (Pierce Chemical, Rockford, Ill.). Sodium dodecyl sulfate (SDS)-polyacrylamide minigels were processed with a Hoefer model SE 250 electrophoresis unit (Hoefer Scientific, San Francisco, Calif.) and were silver stained (14). Prestained molecular weight standards were purchased from Sigma (St. Louis, Mo.).

Figure 1 shows an SDS-gel containing granule extracts (approximately 3 μg of protein per lane) derived from human, rat, and mouse (CBA/J, C57BL/10J, and DBA/2J) PMN. Whereas the human and rat defensins were evident as a prominent 3.5- to 4.0-kDa band, no such band was present in the mouse extracts, whose smallest abundant component had a molecular mass of approximately 7.5 kDa. Figure 2 shows a more heavily loaded gel (approximately 12 μg of protein per lane) containing extracts of PMN obtained from seven strains of mice. In addition to demonstrating the absence of any appreciable defensin in any of these strains, the gel showed that PMN from C3H/HeJ mice also had low levels of several ca. 29-kDa components.

Given the prominence of defensins in human and rat PMN, their absence in murine PMN was somewhat surprising. It may be that this defensin deficiency resulted from unnatural selection, since many laboratory mouse strains have been selected for increased susceptibility to disease, often infections or neoplasms (12). Although this inference is conjectural, it would be more likely if the PMN of nondomesticated wild mice proved to have defensins, a possibility that has not yet been tested. Alternatively, it may be that during evolution, the mouse and rat have diverged in their patterns of tissue specific expression of defensins. Indeed, specialized epithelial cells (Paneth cells) of mouse small intestine were recently shown to produce large amounts of a prodefensin mRNA (8), and we have recently purified three homologous defensins from the small intestines of Swiss Webster mice (3a). The existence of analogous small-intestinal defensins in rats or humans has not yet been demonstrated.

In human and rabbit leukocytes, defensins are synthesized as 93- to 95-amino-acid prepropeptides that require proteo-

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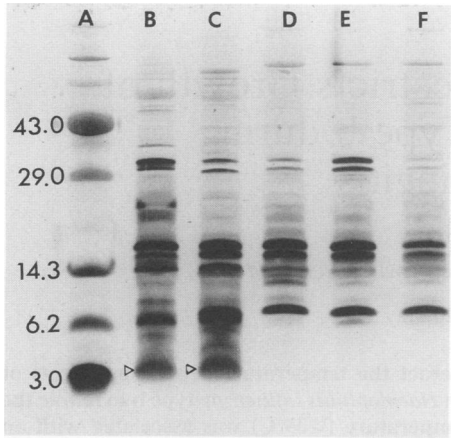

FIG. 1. Defensin content of human rat and mouse PMN. Lane A of the silver-stained SDS-polyacrylamide minigel contained molecular weight standards (indicated in kilodaltons). Each of the other lanes contained 3 μg of protein extracted from PMN granules from the following sources: B, human; C, rat; D, CBA/J mice; E, C57BL/10J mice; and F, DBA/2J mice. Defensins (arrowheads) form a prominent 3.5- to 4-kDa band in lanes B and C that is absent in lanes D to F. Note also that the 29-kDa proteins vary considerably among the mouse strains.

lytic processing to generate the mature 3.5- to 4.0-kDa mature forms (1, 4, 13). Consequently, the absence of mature defensins in mouse PMN might reflect a lack of posttranslational processing rather than absence of synthesis. Although not yet absolutely excluded, this possibility is unlikely because the murine 32D myeloid cell line, transduced with mRNA encoding a 95-amino-acid human prepro-

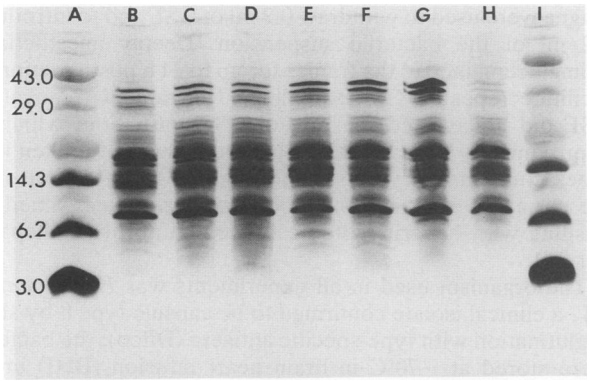

FIG. 2. Defensin-deficient PMN in seven mouse strains. Lanes A and I of the silver-stained SDS-polyacrylamide minigel contained molecular weight standards (indicated in kilodaltons). Each of the remaining lanes contained 12 μg of protein extracted from the PMN of the following mice: B, A/HeJ; C, AKR/J; D, BALB/cJ; E, SWR/J; F, Swiss Webster outbred; G, C57BL/10J; and H, C3H/HeJ. Note also that the C3H/HeJ PMN were markedly deficient in ca. 29-kDa components, which were relatively increased in PMN granules obtained from C57BL/10J mice.

defensin, processed the precursor to a mature defensin (5a). Additionally, murine bone marrow cells were reported to lack mRNA encoding the defensins expressed by murine small-intestinal Paneth cells (9).

Whatever its genesis, the profoundly different composition of murine and human PMN with respect to their endogenous antimicrobial peptides should be considered when results obtained from murine models are extrapolated to human disease.

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