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Pathological and Virological Features of Arenavirus Disease in Guinea Pigs

Comparison of Two Pichinde Virus Strains

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A guinea pig passage-adapted strain of the arenavirus Pichinde (adPIC) is highly virulent in inbred guinea pigs, whereas the related strain PIC3739 is attenuated. Both viruses were macrophage tropic and infected peritoneal, splenic, liver, and alveolar macrophages during experimental Pichinde virus infection. Infection with the virulent strain was associated with unlimited viral replication in the face of exaggerated delayed-type hypersensitivity response, manifested by the macrophage disappearance reaction. Histopathological lesions unique to adPIC-infected guinea pigs included intestinal villus blunting with mucosal infiltration by pyknotic debris-laden macrophages and apoptosis of crypt epithelial cells. Splenic red pulp necrosis was also significantly associated with adPIC infection but not PIC3739 infection. These findings may provide clues to the pathogenesis of a group of poorly understood human viral hemorrhagic fevers. (Am J Pathol 1994, 145:228–235)

Human viral hemorrhagic fevers are potentially fulminating illnesses caused by a diverse range of geographically restricted viruses, including those of the families *Arenaviridae, Flaviviridae, Bunyaviridae*, and *Filoviridae*.[1] Experimental approaches to elucidating the pathogenesis of these dramatic diseases have been in general limited by the virulence of the causative viruses for human investigators. However, Lassa fever, a human arenavirus hemorrhagic fever endemic in West Africa, is amenable to study via a guinea pig model system developed by Jahrling et al.[2] This model is based on a passage-adapted strain of the New World arenavirus Pichinde (adPIC), which has the advantage of being nonpathogenic for humans. This model has significant similarities with human Lassa fever. Shared features include fulminating disease courses with terminal shock (in which hemorrhage is not a major component) and pantropism of virus with high virus titers found in blood and virtually all extraneural tissues examined.[2,3] In both diseases, histopathological findings are generally unimpressive and do not suggest a mode of death.[2–4] Although the principal target cells of Lassa virus are not known, adPIC viral antigens are localized predominantly in cells of the reticuloendothelial system.[5,6]

Prompted by these various pathological and virological clues, we undertook studies of the role of tumor necrosis factor (TNF) in guinea pig arenavirus disease. Those experiments (Aronson JF, Herzog NK, Jerrells TR, manuscript submitted for publication) exploited the comparison of passage-adapted PIC (adPIC) with the less virulent prototype PIC strain, An3739 (PIC3739). PIC3739 and the parental strain ofthe passage-adapted variant, An4763, are serologically related, demonstrating cross-reactivity in complement fixation assays.[7] Partial sequence analysis of the genomes of PIC3739 and adapted PIC indicate that the two strains share similar sequences for the first 120 nucleotides of the S segment of the viral RNA and the first 50 nucleotides of the L segment.[8] Additional comparative sequence information to our knowledge is currently unavailable. We and others[2] (Aronson JF, Herzog NK, Jerrells TR, manuscript submitted for publication) have found that these two related PIC strains have different virulence

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properties in inbred guinea pigs. As an outgrowth of comparative TNF studies, we obtained data concerning histopathological features, viral spread and replication in the host, and quantitative changes in macrophage populations for the two PIC strains. We report observations extending the existing descriptive work done with this arenavirus disease model, with the belief that morphological clues can help direct and define experimental approaches to understanding arenavirus pathogenic mechanisms.

Materials and Methods

Virus

Two PIC strains were used. A virulent derivative of PIC An4763 was derived by Jahrling et al[2] after serial guinea pig passages, this strain is hereafter designated adPIC. The adPIC stock, originally derived from the passage-adapted strain of Jahrling, was obtained from Dr. Dorian Coppenhaver (Department of Microbiology, University of Texas Medical Branch, Galveston, TX) as spleen homogenate from the 15th guinea pig passage. The prototype PIC strain, An3739, was obtained from American Type Culture Collection (Rockville, MD) as 10% (weight/volume) suckling mouse brain suspension. This strain is hereafter designated PIC3739. Virus stocks were generated in strain 13 guinea pigs as follows. Strain 13 guinea pigs were inoculated intraperitoneally with approximately 10^3 to 10^4 plaque forming units (pfu) of the 15th animal passage of adPIC or 10% suckling mouse brain suspension of PIC3739. Six days after inoculation, spleens from infected animals were removed aseptically, homogenized in a 10% (weight/volume) mixture with supplemented RPMI 1640 (Biofluids, Inc., Rockville, MD), and then stored in aliquots at −70 C. The adPIC stocks from the 16th and 17th spleen passages and PIC3739 stocks from the first guinea pig passage were used in these experiments; stock virus titers ranged from 3 to 6 × 10^5 pfu per milliliter.

Viral Plaque Assay

Virus was quantitated in a standard plaque assay on Vero cells as described previously,[4] except that Vero cells were exposed to 2500 rads before plating.

Guinea Pigs

Strain 13 guinea pigs (weight, 350 to 600 g) were obtained from Crest Caviary (Mariposa, CA). Animals were free of specific detectable pathogens and were housed in microisolator cages. Guinea pigs were inoculated intraperitoneally with 2 to 4 × 10^3 pfu of virus in spleen homogenate or with diluent alone on day 0. Weights and rectal temperatures were recorded daily. Guinea pigs were declared moribund and euthanized when their body weight decreased by 25% and their rectal temperatures fell below 39.8 C.

Macrophage Culture

Resident peritoneal macrophages were harvested by aseptic lavage of the peritoneal cavity with ice-cold, calcium- and magnesium-free Hanks' balanced salt solution (HBSS) supplemented with gentamicin sulfate. Recovered cells were washed once with HBSS and resuspended in RPMI 1640 supplemented with 10% fetal bovine serum (containing <0.07 ng of endotoxin per milliliter; HyClone Laboratories, Inc., Logan, UT), 2 mM L-glutamine, and 30 μg of gentamicin sulfate per milliliter. For preparation of spleen macrophage cultures, single cell suspensions of spleen were made in HBSS, washed once in HBSS, and resuspended in complete RPMI. Cells were counted in a electronic particle counter (Coulter, Inc., Hialeah, FL) and differential leukocyte counts were performed on cytospin preparations stained with Dif-Quik (Baxter Scientific, McGaw Park, IL). Histochemical staining for myeloperoxidase was performed on acetone-ethanol-fixed cytospin preparations by a modification of Kaplow's method.[9] Smears were counterstained with Dif-Quik-modified Wright-Giemsa stain. Cells with morphological features of monocytes/macrophages and granular cytoplasmic peroxidase staining were scored as positive and distinguished from cells with darkly staining cytoplasmic inclusions, indicative of phagocytosed neutrophils.

Infectious Center Assay

Macrophages from infected or uninfected (control) animals were plated in 24-well plates at varying concentrations. Macrophage cultures were washed to remove nonadherent cells and free virus and the proportion of adherent macrophages was estimated visually for each separate culture. Macrophages were then overlaid with 2 × 10^5 Vero cells previously treated with 2500 rads. After overlay monolayers were established, medium containing 0.5% agarose was added to each well. Wells were refed on day 6 with an agarose overlay containing 0.01% neutral red and plaques were counted 12 hours later. Results are reported as plaques (infectious centers) per 100 adherent cells, on the basis of the visual estimate of

adherent cells. Plaque lifts were achieved by transferring putative viral antigens (in plaque-containing monolayers) to nitrocellulose disks. Immunostaining for absorbed viral protein was performed by using a guinea pig anti-adPIC antiserum; signal was amplified and developed using a Vectastain ABC peroxidase kit (Vector Laboratories, Inc., Burlingame, CA). With the use of this technique, it was confirmed that plaques contained viral antigen and were not the result of a spurious cytotoxic effect of activated macrophages.

Histology

Guinea pig tissues were immersion fixed in 10% neutral-buffered formalin, processed through graded alcohols and xylene, embedded in paraffin, and sectioned at 5 μ. Sections were stained with hematoxylin and eosin or phosphotungstic acid hematoxylin and then examined. Grading of histological lesions was performed as described in Table 1.

Statistics

Statistical significance of differences between proportions of animals showing lesions was assessed using one-tailed, 2 × 2 contingency table analysis. Elsewhere where indicated statistical significance was determined using the Mann-Whitney *U* test.

Results

Mortality curves for guinea pigs infected with adPIC and PIC3739 are shown in Figure 1. AdPIC caused 100% mortality within 12 days after intraperitoneal inoculation of 3000 pfu, whereas the equivalent infecting dose of PIC3739 resulted in less than 15% mortality. The increased mortality rates in adPIC infection were associated with 10- to 100-fold higher viral titers in blood and spleens of adPIC-infected guinea pigs compared with PIC3739-infected animals (Figure 2). Infectious center assays performed on peritoneal or splenic macrophages explanted from PIC-infected guinea pigs showed that macrophages were viral targets in experimental PIC infection (Figure 3). On day 6, 2.5% of peritoneal macrophages from adPIC-infected animals contained infectious virus compared with 0.5% of peritoneal macrophages from PIC3739-infected animals. Similarly, 0.7% of adPIC splenic macrophages were productively infected compared with 0.2% of PIC3739 splenic macrophages. These trends were also seen at day 11, although differences in rates of infection of macrophages between the two strains were not statistically significant at this time point. Macrophages from liver and lung were also productively infected in adPIC and PIC3739 infection (data not shown).

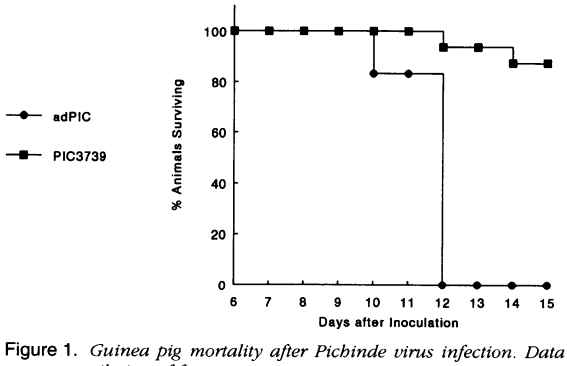

Figure 1. *Guinea pig mortality after Pichinde virus infection. Data are a compilation of five experiments. Strain 13 guinea pigs were inoculated with 3000 pfu of adPIC or PIC3739. They were euthanized when moribund (loss of >25% of original body weight, loss of febrile response). The postinoculation interval at which these criteria were achieved was counted as the day of death. N = at least 12 for all points, except for adPIC on day 12 in which only five animals were kept until day 12. adPIC = virulent derivative of Pichinde virus (strain An4763) developed by Jahrling et al;[2] PIC3739 = attenuated strain (An3739) of Pichinde virus.*

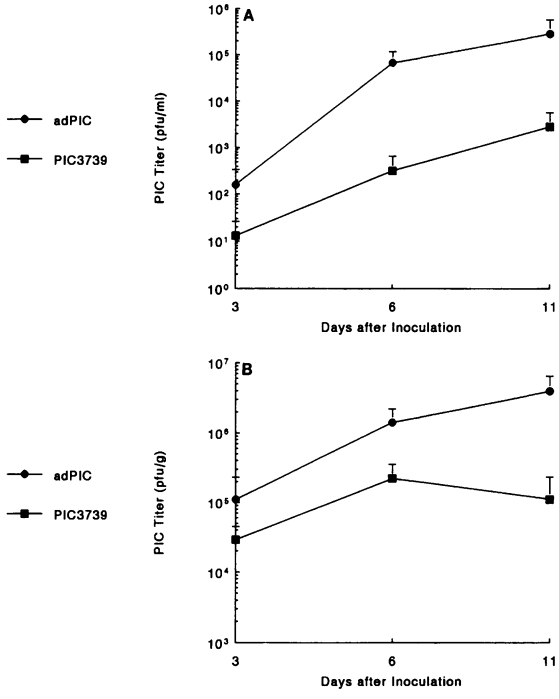

Figure 2. *Pichinde virus (PIC) titers in blood and spleen. Male strain 13 guinea pigs were inoculated intraperitoneally with 3000 pfu of either adPIC or PIC3739. On the indicated day after inoculation, three animals from each group (or four from day 6, PIC3739) were killed. Viral quantitations were performed on whole anticoagulated blood (A) or 1:10 (weight/volume) homogenates of spleen in tissue culture medium (B). adPIC = virulent derivative of Pichinde virus (strain An4763) developed by Jahrling et al;[2] PIC3739 = attenuated strain (An3739) of Pichinde virus.*

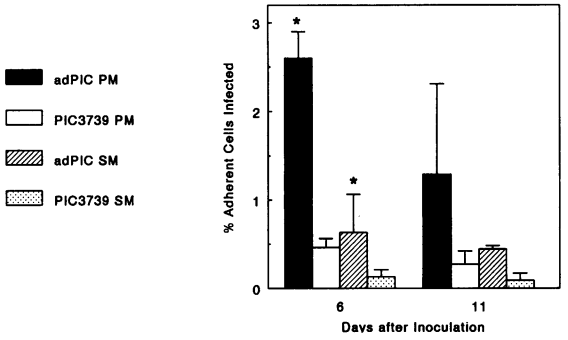

Figure 3. *Pichinde virus (PIC) infection of macrophages:* ex vivo *infectious center assay. Infectious center assays were performed as described in Materials and Methods. Data from a representative experiment are shown. Each bar represents the mean and standard deviation of separate cultures from individual animals in which n = 3 for adPIC day-6 cultures, n = 6 for PIC3739 day-6 cultures, and n = 2 for all day-11 cultures. *Statistically significant difference between strains, P < 0.05 (Mann-Whitney* U *test). adPIC = virulent derivative of Pichinde virus (strain An4763) developed by Jahrling et al;[2] PIC3739 = attenuated strain (An3739) of Pichinde virus; SM = splenic macrophages; PM = peritoneal macrophages.*

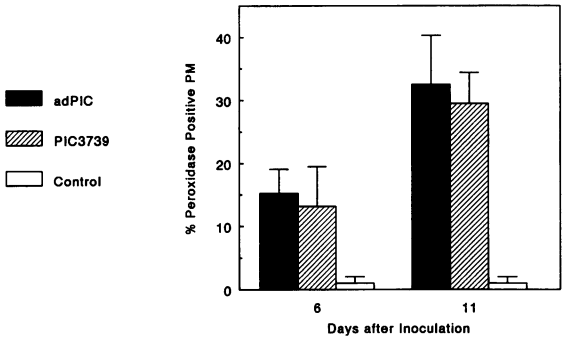

Figure 5. *Peroxidase-positive peritoneal macrophages in PIC infection. Histochemical staining for peroxidase was performed on peritoneal exudate cells as described in Materials and Methods. Each bar represents the mean value from two to six animals from one experiment. adPIC = virulent derivative of Pichinde virus (strain An4763) developed by Jahrling et al;[2] PIC3739 = attenuated (An3739) of Pichinde virus.*

Infections with adPIC and PIC3739 were associated with strikingly different patterns of visceral macrophage distribution. There was a consistent, profound decrease in the number of recoverable macrophages from unprepared peritoneal cavities of guinea pigs inoculated intraperitoneally with adPIC compared with PIC3739 (Figure 4). Peritoneal exudate cells were stained for peroxidase to determine whether reduced influx of monocytes into the peritoneal cavity accounted for the decreased peritoneal macrophage numbers in adPIC-infected guinea pigs. Peroxidase is a histochemical marker of monocytes that have recently entered a site of inflammation; this enzyme is lost in mature, resident tissue phagocytes.[10] A parallel rise in the proportion of peroxidase-containing macrophages from peritoneal cavities of adPIC- and PIC3739-infected guinea pigs was observed (Figure 5).

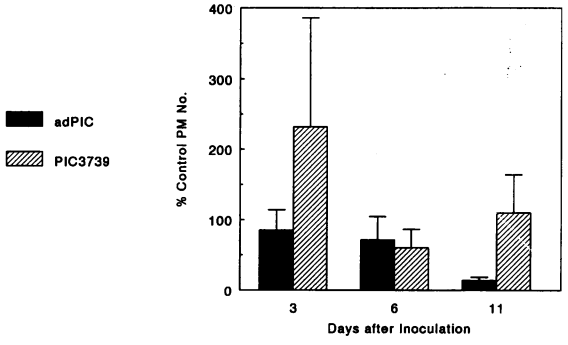

Figure 4. *Peritoneal macrophage numbers during Pichinde virus (PIC) infection. Data are a compilation from two separate experiments in which the two PIC strains were compared directly. Peritoneal exudate cells were obtained as described and the proportion of macrophages was determined by examination of Dif-Quik-stained cytospin preparations. Recovery was calculated as a percentage of the mean number of peritoneal macrophage obtained from five sham-injected guinea pigs. adPIC = virulent derivative of Pichinde virus (strain An4763) developed by Jahrling et al;[2] PIC3739 = attenuated strain (An3739) of Pichinde virus.*

Histopathological features of guinea pig adPIC and PIC3739 infection are compared in Table 1. Tissues were examined from animals in the late phase of adPIC infection (day 11 or 12 after inoculation) and the corresponding period for the nonlethal PIC3739 infection (day 11, 12, or 15). Splenic and intestinal lesions were significantly associated with infection with

Table 1. *Comparative Histopathological Features of adPIC and PIC3739 Infection*

Organ	Lesion	adPIC	PIC3739	Statistics
Spleen	MZN	5/5	7/13	NS
	RPN > 1^+	5/5	1/13	$P < 0.005$
Liver	F > 1^+	4/5	7/10	NS
	PI > 1^+	0/5	5/10	NS
	LI > 2^+	1/5	6/10	NS
Gut	FM > 1^+	5/5	4/10	$P < 0.05$
	VF > 1^+	5/5	1/10	$P < 0.005$
	LPN > 1^+	4/5	0/10	$P < 0.005$
Lung	IP > 1^+	1/4	2/10	NS

Strain 13 guinea pigs were inoculated with 3000 pfu of adPIC or PIC3739 and then killed on day 11, 12, or 15. Fractions are expressed as the number of animals displaying the indicated lesion divided by the number of animals from which a tissue was examined. Lesions were catalogued and graded as follows: Spleen MZN = marginal zone necrosis/fibrinoid change—1–3$^+$; RPN = red pulp necrosis—1–3$^+$; Liver F = fatty change—1–2$^+$ mild, midzonal, 3$^+$ severe, panlobular; PI = portal inflammation—1$^+$ mild, portal tract not expanded, 2$^+$ moderate, portal tract expanded, 3$^+$ severe, expansion of portal tract and piecemeal necrosis of limiting plate; LI = lobular inflammation with hepatocyte necrosis—1$^+$ >1 focus per 5 10× fields, 2$^+$ 1 to 5 foci per 10× field, 3$^+$ >5 foci per 10× field. Gut (small intestine) FM = foamy macrophages in the lamina propria—1$^+$ lamina propria not expanded, 2 to 3$^+$ lamina propria expanded, villi broad; LPN = lamina propria necrosis and cytoclastic debris in lamina propria macrophages—1–3$^+$; VF = villus flattening—1$^+$ slight broadening, 2$^+$ intermediate, 3$^+$ flat villi; Lung IP = interstitial pneumonitis, 1 to 3$^+$; NS = not significant (one-tailed 2 × 2 contingency table analysis).

the virulent adPIC strain. The spleens from all adPIC-infected animals and only 1 of 15 PIC3739-infected animals showed necrosis of the red pulp (Table 1, Figure 6). Marginal zone necrosis and deposition of fibrinoid material was a consistent feature of adPIC infection but also was seen in more than one-half of PIC3739-infected animals. Late in infection (days 12 to 15), the majority of PIC3739-infected animals showed splenomegaly with corresponding reactive and hyperplastic histological changes in the red and white pulp (spleen weights: PIC3739, 1.04 ± 0.26 g; adPIC, 0.74 ± 0.11 g; uninfected control, 0.60 ± 0.02 g).

Small intestines from adPIC-infected guinea pigs showed atrophy and broadening of mucosal villi with evidence of epithelial damage and regeneration. The lamina propria demonstrated focal necrosis and infiltration by foamy macrophages containing pyknotic nuclear debris and golden-brown pigment (Figure 7, Table 1). In addition, there was a striking degree of apopotosis (individual cell necrosis) among colonic crypt epithelial cells. Liver lesions including steatosis, portal inflammation, and lobular inflammation were seen in both adPIC- and PIC3739-infected guinea pigs. Mild, nonspecific lung lesions were also seen in small numbers of guinea pigs from both groups. Features associated with adPIC infection (splenic necrosis, intestinal lesions, and lack of splenomegaly) were also evident in both PIC3739-infected guinea pigs, which demonstrated criteria of lethal infection at the time of sacrifice on day 15 (ie, loss of greater than 25% of original body weight, loss of a febrile response). Thus, gastrointestinal and splenic lesions were associated with lethal outcome, regardless of the infecting virus strain.

Discussion

The relationship between viremia levels and disease outcome is a well described feature of human and experimental Lassa virus disease. Blood viral titers are an accurate predictor of lethality in human Lassa fever.[11] In both rhesus macaque and guinea pig Lassa virus infections, lethality likewise parallels level of viremia.[12,13] The correlation between the amount of circulating arenavirus and lethal outcome also applied in the PIC model system. The virulent adPIC strain consistently achieved higher titers in strain 13 guinea pig organs than did PIC3739. Discrepant viremia levels were seen as early as day 3 after infection, suggesting that adPIC may have some inherent replication advantage in target cells or that host nonspecific immune mechanisms cannot impede its spread. In support of the former possibility, adPIC replicated faster and to higher titers than PIC3739 in peritoneal macrophages infected *in vitro* over a range of multiplicities of infection (Aronson JF, unpublished observations).

Immunostaining for viral antigens has indicated that macrophages are a major cellular target of infection for PIC.[5,6] Several investigators have demonstrated that macrophages and monocytic cell lines support PIC infection *in vitro*.[14–16] We have shown that macrophages explanted from several sites from PIC-infected guinea pigs harbor infectious virus. Both peritoneal and splenic macrophages were infected at slightly higher rates in adPIC than in PIC3739 infection. The marginal zone of the spleen is an important homing site for recirculated peritoneal macrophages.[17,18] Thus, peritoneal and splenic macro-

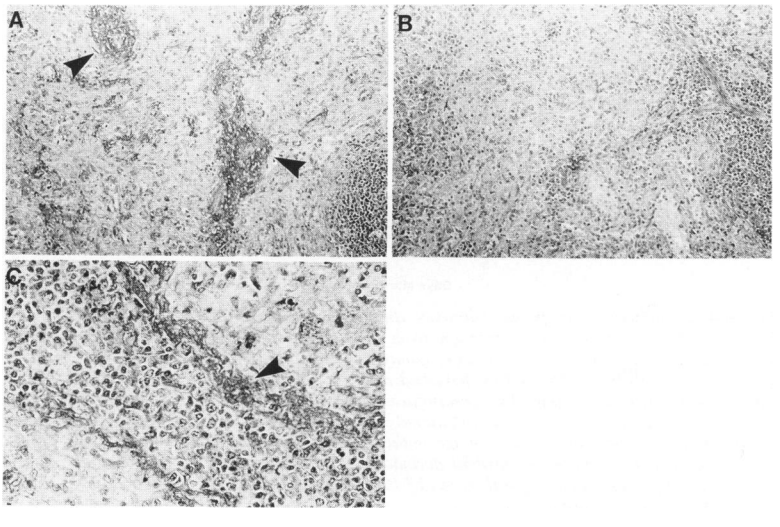

Figure 6. *Histopathological features of the spleen in guinea pigs infected with Pichinde virus (PIC).* **A:** *Spleen, day 11, adPIC infection showing expansion of the red pulp and deposition of fibrinoid material in marginal zones and around central arteries (arrows). Phosphotungstic acid hematoxylin (PTAH), ×80.* **B:** *Spleen, day 11, PIC3739 infection. Red pulp is expanded but fibrinoid necrosis is lacking. PTAH, ×80.* **C:** *Spleen, day 11, adPIC infection. Fibrinoid material deposited in marginal zone (arrow). PTAH, ×200. adPIC = virulent derivative of Pichinde virus (strain An4763) developed by Jahrling et al;[2] PIC3739 = attenuated strain (An3739) of Pichinde virus.*

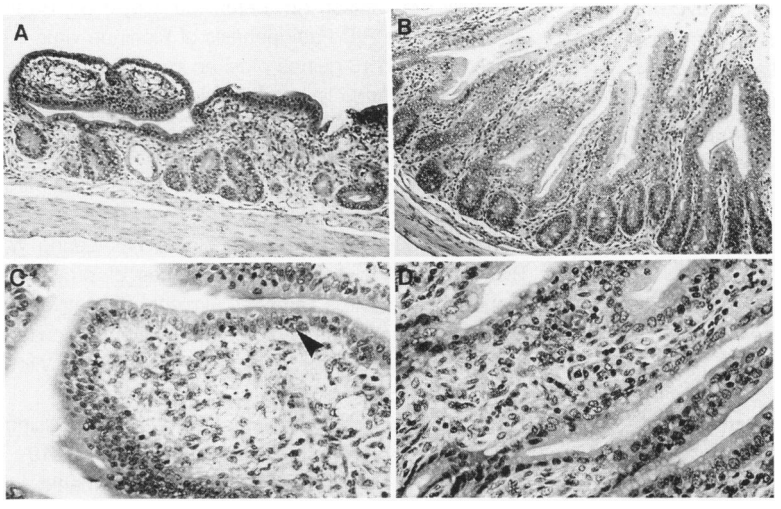

Figure 7. *Histopathological features of the small intestine in guinea pigs infected with Pichinde virus.* **A:** *Small intestine, day 11, adPIC infection. The villus architecture is effaced and surface and crypt epithelial cells show evidence of injury and regenerative change. Hematoxylin and eosin (H&E) ×80.* **B:** *Small intestine, day 11, PIC3739 infection. Well-preserved villi with mild artifactual autolytic changes. H&E, ×80.* **C:** *Small intestine, day 11, adPIC infection. High power of broad mucosal villus showing infiltration of the lamina propria by foamy, slightly pigmented macrophages containing nuclear debris. Arrow indicates an apoptotic epithelial cell. H&E, ×80.* **D:** *Small intestine, day 11, PIC3739. Mucosa shows a moderate mononuclear cell inflammatory infiltrate. Evidence of cell necrosis is lacking. H&E, ×200. adPIC = virulent derivative of Pichinde virus (strain An4763) developed by Jahrling et al;[2] PIC3739 = attenuated strain (An3739) of Pichinde virus.*

phages may be among the first cellular populations to encounter PIC after intraperitoneal inoculation. Our findings suggest that PIC3739 is less efficient at initiating and sustaining productive infection in target cells than adPIC.

A striking feature of lethal PIC infection was the loss of recoverable macrophages from the peritoneal cavity late in adPIC infection. It seems unlikely that this was attributable to outright killing of macrophages by PIC, because we were unable to document cytopathic effect of PIC on elicited peritoneal macrophages infected *in vitro* at a range of multiplicities of infection (Aronson JF, unpublished observations). Other investigators have likewise found that PIC is noncytopathic for macrophages.[15] It is instead possible that adPIC infection somehow altered the flux of macrophages into or out of the peritoneal cavity. Certain systemic influences (eg, glucocorticoids) decrease the influx of peripheral blood monocytes into an inflamed peritoneal cavity.[19] Both adPIC- and PIC3739-infected animals demonstrated increased proportions of peroxidase-positive macrophages in their peritoneal exudates compared with controls, indicating an active inflammatory response in this site. The presence of equivalent proportions of peroxidase-positive macrophages in the peritoneal exudates of the two groups implied that the decrease in total numbers of peritoneal macrophages in adPIC infection was not solely attributable to a failure of recruitment of peripheral blood monocytes to the peritoneal cavity. That the rate of peroxidase positivity among adPIC peritoneal macrophages was not increased relative to PIC3739 also suggested that there was not an uncompensated loss of mature macrophages from this site.

The peritoneal macrophage disappearance reaction has been well described in immunologically primed guinea pigs and is believed to be a manifestation of delayed-type hypersensitivity.[20–22] Nelson et al[20] demonstrated that the macrophage disappearance reaction reflects increased clumping and adherence of macrophages to the mesothelial lining of the peritoneal cavity rather than macrophage damage or migration to another organ. Thus, just as the classical skin test measures macrophage and mononuclear cell accumulation at the cutaneous injection site in sensitized animals, the macrophage disappearance reaction reflects lymphokine-induced increased adhesive properties of fully activated macrophages. Peritoneal exudate changes observed in PIC-infected animals may represent a version of the macrophage disappearance reaction. In this case, peritoneal macrophages in adPIC-infected guinea pigs appear to be more activated than those of PIC3739-infected guinea pigs. We have shown previously (Aronson JF, Herzog NK, and Jerrells TR, manuscript submitted for publication) that these peritoneal macrophages from adPIC-infected guinea pigs are primed to overproduce TNF after stimulation with bacterial lipopolysaccharide, a classical correlate of macrophage activation. Elicited peritoneal macrophages infected *in vitro* with adPIC also constitutively overproduced IL-6 (Jerrells TR, unpublished observations). These findings suggest that lethal PIC infection is associated with a potentially pathological hypersensitivity state, at least in terms of macrophage activation.

Striking pathological changes in the mucosae of the large and small intestine were uniquely associated with adPIC infection and lethal outcome. Connolly et al[6] recently reported similar lesions in the intestines of adPIC-infected strain 13 guinea pigs and described localization of viral antigens in infiltrating mucosal macrophages, smooth muscle cells of the

muscularis mucosa, and periglandular stromal cells. Intramucosal macrophages function as scavengers of migrating or apoptotic crypt epithelial cells, lymphocytes, and sheath fibroblasts.[26] Pigmented macrophages accumulate in the intestinal mucosa of guinea pigs in several reactive and disease states characterized by increased cell turnover and epithelial injury.[26–28]

The pathogenesis of intestinal epithelial injury in PIC infection is not clear, because epithelial cells are apparently not infected by PIC.[6] It is possible that monokines secreted by PIC-infected macrophages in the lamina propria damage the epithelium.[29] Epithelial injury and mucosal atrophy may cause malabsorption and contribute to the wasting diathesis seen in adPIC infection. In addition, loss of mucosal integrity in the colon may lead to increased translocation of endogenous lipopolysaccharide and increased susceptibility to endotoxic shock. This mechanism has been proposed for Venezuelan equine encephalitis virus infection in hamsters,[30] as well as in murine graft-*versus*-host disease.[29]

Having further substantiated the validity of the PIC model of human Lassa fever in virological terms, we have presented evidence that begins to address issues of arenavirus pathogenic mechanisms. We believe that this comparative approach using viral strains with different pathogenic potentials will prove useful in investigations of arenavirus disease. Future molecular genetic characterizations of such strain pairs will provide more valuable, specific pathogenetic insight into this model system and its human arenavirus disease counterpart.

References

1. Cosgriff TM: Viruses and hemostasis. Rev Infect Dis 1989, 11 (Suppl 4):S672–S688
2. Jahrling PB, Hesse RA, Rhoderick JB, Elwell MA, Moe JB: Pathogenesis of a Pichinde virus strain adapted to produce lethal infections in guinea pigs. Infect Immun 1981, 32:872–880
3. Walker DH, McCormick JB, Johnson KM, Webb PA, Komba-Kono G, Elliott LH, Gardner JJ: Pathologic and virologic study of fatal Lassa fever in man. Am J Pathol 1982, 107:349–356
4. Lucia HL, Coppenhaver DH, Harrison RL, Baron S: The effect of an arenavirus infection on liver morphology and function. Am J Trop Med Hyg 1990, 43: 93–98
5. Murphy FA, Buchmeier MJ, Rawls WE: The reticuloendothelium as the target in a virus infection: Pichinde virus pathogenesis in two strains of hamsters. Lab Invest 1977, 37:502–515
6. Connolly BM, Jenson AB, Peters CJ, Geyer SJ, Barth JF, McPherson RA: Pathogenesis of Pichinde virus infection in strain 13 guinea pigs: an immunocytochemical, virologic, and clinical chemistry study. Am J Trop Med Hyg 1993, 49:10–24
7. Trapido H, Sanmartin C: Pichinde virus: a new virus of the Tacaribe group from Colombia. Am J Trop Med Hyg 1971, 20:631–641
8. Auperin D, Dimock K, Cash P, Rawls WE, Leung W, Bishop DH: Analyses of the genomes of prototype Pichinde arenavirus and a virulent derivative of Pichinde Munchique: evidence for sequence conservation at the 3′ termini of their viral RNA species. Virology 1982, 116:363–367
9. Kaplow LS: Simplified myeloperoxidase stain using benzidine dihydrochloride. Blood 1965, 26:215–219
10. Van Furth R, Diesselhoff-Den Dulk MC, Mattie H: Quantitative study on the production and kinetics of mononuclear phagocytes during an acute inflammatory reaction. J Exp Med 1973, 138:1314–1330
11. Johnson KM, McCormick JB, Webb PA, Smith ES, Elliott LH, King IJ: Clinical virology of Lassa fever in hospitalized patients. J Infect Dis 1987, 155:456–464
12. Peters CJ, Liu CT, Anderson GW Jr, Morrill JC, Jahrling PB: Pathogenesis of viral hemorrhagic fevers: Rift Valley fever and Lassa fever contrasted. Rev Infect Dis 1989, 11 (Suppl 4):S743–S749
13. Jahrling PB, Frame JD, Smith SB, Monson MH: Endemic Lassa fever in Liberia. III. Characterization of Lassa virus isolates. Trans R Soc Trop Med Hyg 1985, 79:374–379
14. Polyak SJ, Rawls WE, Harnish DG: Characterization of Pichinde virus infection of cells of the monocytic lineage. J Virol 1991, 65:3575–3582
15. Friedlander AM, Jahrling PB, Merrill P, Tobery S: Inhibition of mouse peritoneal macrophage DNA synthesis by infection with the arenavirus Pichinde. Infect Immun 1984, 43:283–288
16. Buchmeier MJ, Rawls WE: Variation between strains of hamsters in the lethality of Pichinde virus infections. Infect Immun 1977, 16:413–421
17. Rosen H, Gordon S: Adoptive transfer of fluorescence-labeled cells shows that resident peritoneal macrophages are able to migrate into specialized lymphoid organs and inflammatory sites in the mouse. Eur J Immunol 1990, 20:1251–1258
18. Roser, B: The migration of macrophages In Vivo. In Mononuclear Phagocytes. Edited by Van Furth R. Oxford, UK, Blackwell Scientific, 1970, pp 166–177
19. Van Furth R, Hirsch JG, Fedorko ME: Morphology and peroxidase cytochemistry of mouse promonocytes, monocytes, and macrophages. J Exp Med 1970, 132: 794–805
20. Nelson DS, North RJ: The fate of peritoneal macrophages after the injection of antigen into guinea pigs with delayed-type hypersensitivity. Lab Invest 1965, 14:89–101

21. Sonozaki H, Cohen S: The macrophage disappearance reaction: mediation by a soluble lymphocyte-derived factor. Cell Immunol 1971, 2:341–352
22. D'Silva H, Yoshida T, Cohen S: The effect of lymphokines on macrophage accumulation and disappearance in the peritoneal cavity. J Exp Pathol 1983, 1:61–69
23. Sarrat H, Camain R, Baum J, Robin Y: Diagnostic histopathologique des hepatites dues au virus Lassa. Bull Soc Pathol 1972, 5:642–650
24. McCormick JB, Walker DH, King IJ, Webb PA, Elliott LH, Whitfield SG, Johnson KM: Lassa virus hepatitis: a study of fatal Lassa fever in humans. Am J Trop Med Hyg 1986, 35:401–407
25. Winn WC Jr, Walker DH: The pathology of human Lassa fever. Bull WHO 1975, 52:535–545
26. Sawicki W, Kucharczyk K, Szymanska K, Kujawa M: Lamina propria macrophages of intestine of the guinea pig: possible role in phagocytosis of migrating cells. Gastroenterology 1977, 73:1340–1344
27. Takahashi M, Iwata S, Uchida K, Fujiwara H: The pathological study of enterosiderosis in guinea pigs. Jikken Dobutsu 1988, 37:171–177
28. Walker NI, Bennett RE, Axelsen RA: Melanosis coli: a consequence of anthraquinone-induced apoptosis of colonic epithelial cells. Am J Pathol 1988, 131:465–476
29. Nestel FR, Price KS, Seemayer TA, Lapp WS: Macrophage priming and lipopolysaccharide-triggered release of tumor necrosis factor alpha during graft-versus-host disease. J Exp Med 1992, 175:405–413
30. Gorelkin L, Jahrling PB: Virus-initiated septic shock: acute death of Venezuelan encephalitis virus-infected hamsters. Lab Invest 1975, 32:78–85