TUMOR NECROSIS FACTOR AND THE PATHOGENESIS OF PICHINDE VIRUS INFECTION IN GUINEA PIGS

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Abstract. Pichinde virus (PIC) is a reticuloendothelial arenavirus of the New World tropics. A guinea pig passage-adapted strain of this virus (adPIC) is uniformly lethal for inbred guinea pigs, while the related, prototype strain (PIC3739) has attenuated virulence. The abilities of adPIC and PIC3739 to induce tumor necrosis factor (TNF) in vivo and in cultured macrophages were compared. Infection with adPIC, but not PIC3739, was associated with detectable serum TNF that peaked in week 2 of infection. Tumor necrosis factor was found in the spleens of adPIC- and PIC3739-infected animals in week 1 of infection; TNF α mRNA levels in spleens and livers of adPIC infected animals increased and remained high throughout infection, whereas PIC3739-infected organs showed down regulation of TNF α mRNA late in infection. Peritoneal macrophages explanted from adPIC-infected animals showed enhanced lipopolysaccharide-inducible TNF production. Altered regulation of TNF production may play a role in the pathogenesis of guinea pig arenavirus disease.

Pichinde virus (PIC) is a New World arenavirus that has been used in inbred guinea pigs as a model for the West African human arenavirus disease known as Lassa fever. A guinea pig passage-adapted PIC strain (adPIC) is highly virulent for inbred guinea pigs compared with the prototype PIC strain (An3739).¹ Since PIC is nonpathogenic for humans, it provides an attractive experimental model. Guinea pig adPIC disease shares certain pathologic and virologic features with severe Lassa fever. Both are characterized by fairly rapid febrile disease courses culminating in shock with only slight and inconstant degrees of hemorrhage. Both adPIC and Lassa virus replicate to very high titers in many extraneural organs.^{1,2} In the PIC system³ and in human Lassa fever,⁴ mortality is correlated closely with viremia levels. Pathologic findings in both human and guinea pig diseases, however, are relatively mild and nonspecific, and do not suggest a pathogenic sequence. The contrast between the severe, fulminating disease course and the relative paucity of histopathologic evidence of direct, organ-specific viral damage has prompted speculation regarding the potential pathogenic role of toxic or pharmacologic mediators since the earliest investigations in Lassa fever.⁵ In the PIC model, tumor necrosis factor- α (TNF α) is a prime candidate for such an endogenous toxic mediator. Pichinde virus infects cells of the reticuloendothelial system,^{3,6,7} which are the major producers of TNF.⁸ Animals infected with adPIC undergo marked wasting^{7,9} that is reminiscent of TNF-induced cachexia of chronic parasitic or malignant diseases. Finally, the terminal vascular collapse seen in human Lassa fever and its counterpart disease in guinea pigs resembles endotoxic shock,¹⁰ for which TNF α is a critical proximal mediator. We report the results of studies aimed at elucidating the role of TNF α in the pathogenesis of PIC infection.

MATERIALS AND METHODS

Virus. Two PIC strains were used. A virulent derivative of PIC strain An4763 was developed by Jahrling and others¹ by 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 Copenhaver (Department of Microbiology, University of Texas Medical Branch, Galveston, TX) in a spleen

homogenate from the 15th guinea pig passage. The less virulent, prototype PIC strain, An3739, was obtained from American Type Culture Collection (Rockville, MD) in a 10% suckling mouse brain suspension. This virus will be designated PIC3739. Viral stocks were generated in strain 13 guinea pigs as previously described.³ The adPIC stocks from the 16th and 17th guinea pig spleen passages and PIC3739 stocks from the first guinea pig spleen passage were used in these experiments; stock viral titers ranged from 3 to 6×10^5 plaque-forming units (PFU)/ml.

Guinea pigs. In some early experiments, adPIC pathogenicity was compared in strain 2, strain 13, and outbred guinea pigs. This comparative pathogenesis approach was subsequently abandoned in favor of the approach based on the two virus strains described above. Strain 13 guinea pigs were used in all subsequent experiments. Strain 2 guinea pigs were obtained from the University of Texas, M.D. Anderson Cancer Center (Bastrop, TX). Strain 13 guinea pigs weighing 350–600 grams were obtained from Crest Caviary (Mariposa, CA). Inbred guinea pigs were free of specific detectable pathogens and were housed in microisolator cages. Outbred Hartley guinea pigs were obtained from Charles River Laboratories (Wilmington, MA).

Guinea pigs were inoculated intraperitoneally with $2-4 \times 10^3$ PFU of virus in spleen homogenate or with diluent alone (complete RPMI 1640 medium; Biofluids, Rockville, MD) on day 0. Weights and rectal temperatures were recorded daily. Guinea pigs were declared moribund and killed when they had lost 25% of their initial body weight and their rectal temperature decreased 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 (30 μ g/ml). Recovered cells were washed once with HBSS and resuspended in RPMI 1640 medium supplemented with 10% fetal bovine serum containing < 0.07 ng of endotoxin/ml (Hyclone Laboratories, Logan, UT), 2 mM L-glutamine, and 30 μ g of gentamicin sulfate/ml. Cells were counted in an electric particle counter (Coulter Inc., Hialeah, FL), and differential leukocyte counts were performed on cytopsin preparations stained with Dif-Quik

(Baxter Scientific, McGaw Park, IL). Cells were cultured in 24-well plates at a concentration of 10^6 cells/ml. After an 8–16-hr incubation at 37°C in a humidified 5% CO₂ atmosphere, nonadherent cells were removed by washing the plates three times with warmed medium. Cultures were refed with supplemented RPMI 1640 medium or medium containing 10 µg of bacterial lipopolysaccharide (LPS)/ml (*Escherichia coli* 011:B4; Difco Laboratories, Detroit, MI). Supernatants were harvested 24 hr later, centrifuged at $2,000 \times g$ for 5 min to pellet dead nonadherent cells, and frozen at -70°C until assayed for TNF. For preparation of spleen macrophage cultures, single-cell suspensions of spleen were made in HBSS and washed once in HBSS. Total and differential cell counts were performed, and cells were plated at a concentration of 10^6 macrophages/ml in complete RPMI 1640 medium. Nonadherent cells were removed by rinsing monolayers with medium after an 8–16-hr incubation at 37°C in a 5% CO₂ atmosphere. Cultures were then treated as described above.

Tumor necrosis factor assay. Macrophage supernatants, serum, or organ homogenates prepared as 1:10 (w/v) suspensions in tissue culture medium were assayed for TNF as previously described.¹¹ Serum was collected in siliconized tubes and was not heated before assaying for TNF. Spleen homogenates stored at -70°C were thawed once and then clarified by centrifugation at $2,000 \times g$ for 5 min before assaying. Briefly, L929 cells were distributed in 96-well plates at a concentration of 4×10^4 cells/well. After incubation overnight to establish monolayers, the medium was removed and replaced with either the desired dilution (in general, serial two-fold increments) of sample to be assayed, or recombinant murine TNF standard (Genzyme, Cambridge, MA). Actinomycin D (Sigma, St. Louis, MO) was added to each well to give a final concentration of 2.5 µg/ml. The plates were placed in an incubator at 37°C for 18–24 hr, and then were washed three times with warmed medium, fixed in 10% formalin, and stained with crystal violet. The stain was extracted with absolute methanol, and absorbance was determined at 540 nm with an enzyme-linked immunosorbent assay plate reader (Biotek Instruments, Winooski, VT). Control wells were treated with tissue culture medium and actinomycin D (2.5 µg/ml) only. Each specimen and dilution was assayed in duplicate. The percent cytotoxicity for each sample was calculated as follows: $100 \times [1 - (\text{mean OD}_{540} \text{ sample wells} / \text{mean OD}_{540} \text{ control wells})]$. The dilution yielding 50% cytotoxicity was derived from a plot of percent cytotoxicity versus log₂ dilution of sample and reported as units of TNF after correction for dilution factors in the assay. Where indicated, units were converted to picograms by comparison with the recombinant murine TNF standard assayed concurrently.

Probes. Uniformly labeled antisense TNFα probe was generated from *Pst* I-linearized PGEM3 plasmid containing the murine TNFα cDNA (generously provided by Bruce Beutler, The University of Texas Southwestern Medical Center, Dallas, TX). This cDNA includes the entire coding region of the murine TNFα gene but lacks the AT-rich repeating octamers in the 3' untranslated region that are common to many cytokine genes.¹² Labeled antisense single-stranded RNA probe was transcribed in the presence of ³²P-UTP using T7 RNA polymerase according to manufacturer's

recommendation (Promega, Madison, WI). Specific activities of the probes were greater than 10^9 counts/min/µg. The β-actin probe was generated in a similar manner using T3 polymerase and *Pst* I-linearized plasmid template pA1, which contains a 1-kilobase (kb) portion of the chicken β-actin cDNA.¹³ This probe hybridizes with actin genes from a variety of diverse species.

Northern blot and slot blot. Total cellular RNA was isolated from organs or cultured cells using the RNazol-B method according to manufacturer's instructions (Biotex Laboratories, Inc., Houston, TX). The amount of RNA in each sample was determined spectrophotometrically. Fifty micrograms of each RNA sample was denatured in loading buffer containing 2.2 M formaldehyde and 50% deionized formamide, separated by formaldehyde agarose gel electrophoresis as described,¹⁴ and transferred to a nylon membrane (Zetabind; Cuno Labs, Meriden, CT) in 1× SSC buffer (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). After transfer, the membrane was quickly rinsed in 2× SSC, and RNA was cross-linked to the filter by baking at 68°C for 2 hr. Prehybridization was carried out in 0.5 M sodium phosphate, 1 mM EDTA, 1% bovine serum albumin (BSA), and 7% sodium dodecyl sulfate (SDS) at 65°C for 2 hr. Blots were hybridized with high specific activity ³²P-labeled riboprobes at 50–80°C in 0.5 M sodium phosphate, 1 mM EDTA, 1% BSA, 7% SDS, and 30% formamide. Stringency conditions were titrated for hybridization of murine probes to guinea pig mRNA; optimal specific hybridization of probe to guinea pig TNF mRNA was achieved in 30% formamide at 68°C for 18 hr. Membranes were washed three times at 65°C in wash buffer (40 mM sodium phosphate, 1 mM EDTA, 1% SDS; pH 7.2), and then exposed to Kodak (Ann Arbor, MI) XAR5 x-ray film using intensifying screens at -80°C.

For slot blots, 25 µg of total cellular RNA was dissolved in 1× SSC, 50% formamide, and 7% formaldehyde and then heated for 5 min at 70°C. Serial two-fold dilutions of each sample were prepared in the above diluent, and transferred to Zetabind nylon filters in a vacuum slot blot filtering apparatus. After samples were applied, filter slots were washed three times with 1× SSC buffer. Filters were air dried and baked at 68°C for 1 hr. Blots were prehybridized and hybridized as above, washed, and exposed to film. Duplicate blots prepared identically were hybridized with β-actin probe under similar conditions. Slot blots were analyzed on a BioImage Visage 60 scanning densitometry system (Kodak). For each RNA sample, computer-integrated optical density for the dilution series of peaks was plotted against log₂ dilution; the dilution yielding a standard reference integrated optical density was derived by linear regression analysis. This reference dilution was used to compare relative amounts of TNFα mRNA among specimens. Values were normalized for actin message content by dividing the relative amount of TNFα mRNA (above) by the similarly derived relative amount of actin mRNA. Because tissues presumably differ in actin message content on a per cell or per gram basis, TNFα/actin mRNA ratios were standardized to that ratio for the same organ from an untreated control animal. Thus, results are reported as the amount of TNFα mRNA in experimental samples (corrected for actin content) relative to corrected TNFα mRNA levels in the same tissue from an uninfected animal or untreated culture.

RESULTS

Tumor necrosis factor-like cytotoxic activity was also detected in homogenates of spleens from PIC-infected animals. This bioactivity displayed a slightly different temporal pattern than that found with serum TNF. Infection with either PIC strain generated TNF activity in the spleen in the first week after infection (Figure 2). Although the small numbers of guinea pigs used in these experiments did not afford statistical significance, the grouping of data points shown in Figure 2 suggests that there was increased TNF bioactivity in PIC3739-infected spleens relative to adPIC-infected spleens at day 6 postinoculation. In these experiments, TNF activity in PIC-infected spleens was normalized to mean TNF activity in spleens from uninfected control animals. The TNF levels in spleens obtained from mock-infected control guinea pigs ranged from below the limits of detection (< 1 pg/ml) to 100 pg/ml. Other investigators have also detected

To substantiate and expand on these bioactivity data, we performed slot blot analysis for TNF α mRNA on organ RNA from PIC-infected guinea pigs. In Northern blot analyses of guinea pig total cellular RNA from various organs, two bands were seen: a major TNF α mRNA species at 1.6 kb and a weaker band at 4.6 kb. These two TNF α mRNA species have been reported previously by many investigators.^{16, 17} Data from a representative slot blot analysis of organ RNA (Figure 3 and Table 1) are reported as relative

FIGURE 2. Tumor necrosis factor (TNF)-like bioactivity in spleens of Pichinde virus-infected guinea pigs. The TNF was assayed in 10% (w/v) homogenates of spleen prepared as described in the Materials and Methods. Data are a compilation of three separate experiments. The TNF values are normalized to mean spleen TNF concentrations from concurrently assayed control uninfected animals for each experiment. Dots represent TNF determinations from individual guinea pigs. Bars show the mean and SD TNF values. adPIC = virulent derivative of Pichinde virus (strain An4763) developed by Jahrling and others;¹ PIC3739 = attenuated strain of Pichinde virus (An3739) with attenuated virulence in guinea pigs.

liver and spleen TNF α mRNA levels in PIC-infected guinea pigs compared with levels in uninfected control animals. Livers and spleens obtained from adPIC-infected guinea pigs displayed consistently higher levels of TNF α mRNA than did organs obtained from PIC3739-infected guinea pigs. Late in PIC infection (day 11), organ TNF α mRNA levels in adPIC- and PIC3739-infected animals diverged. In spleens obtained from adPIC-infected guinea pigs, TNF α mRNA levels were more than twice those of uninfected control guinea pigs. In contrast, TNF α mRNA levels in spleens from PIC3739-infected guinea pigs decreased to 0.2% of the levels in uninfected control animals. Liver TNF α mRNA increased 14-fold over control values for adPIC compared with a two-fold increase for PIC3739 (Table 1). At day 6, TNF α mRNA levels were higher in adPIC-infected spleens than in PIC3739-infected spleens (Table 1). This pattern is the reverse of that for spleen TNF-like cytotoxicity, in which levels were higher in PIC3739 infection than in adPIC infection.

TABLE 1

Tumor necrosis factor α (TNF α) mRNA levels in guinea pig organs during Pichinde (PIC) virus infection*

Fold increase in TNF- α mRNA in PIC-infected organs	Day 6		Day 11	
	adPIC	PIC3739	adPIC	PIC3739
Spleen	19.3	1.8	2.3	0.002
Liver	3.9	0.7	13.6	2.4

* Tabulated slot-blot results are from a representative experiment. Each entry represents the ratio of TNF α mRNA in the indicated organ from a PIC-infected strain 13 guinea pig relative to TNF α mRNA in the same organ from a mock-infected guinea pig. The TNF α mRNA levels were corrected for actin mRNA content as described in the Materials and Methods. adPIC = virulent derivative of PIC virus (An4763) developed by Jahrling and others;¹ PIC3739 = prototype strain of PIC virus (An3739) with attenuated virulence in guinea pigs.

FIGURE 3. Tumor necrosis factor (TNF) mRNA in Pichinde virus-infected guinea pig organs. Slot blot analysis of guinea pig organ TNF mRNA. Representative lanes from a TNF slot blot are shown. Each column represents a serial two-fold dilution series of applied RNA. From the left, lane 1 = (GPM/LPS), guinea pig peritoneal macrophages treated in vitro with 10 μ g of bacterial (*Escherichia coli*) lipopolysaccharide (LPS)/ml; lane 2 (GPM), guinea pig peritoneal macrophages cultured without LPS; lane 3 (adPIC), spleen RNA, day 11 after inoculation with adPIC; lane 4 (PIC3739), spleen RNA day 11 after inoculation with PIC3739. adPIC = virulent derivative of Pichinde virus (strain An4763) developed by Jahrling and others;¹ PIC3739 = prototype strain of Pichinde virus (An3739) with attenuated virulence in guinea pigs.

tion. Increased TNF α mRNA during PIC infection supports the contention that the cytotoxic material in spleen homogenates is at least in part TNF α . Not surprisingly, the liver appears to be a potentially important source of TNF α production.

To identify a cellular source for TNF, we assayed supernatants from splenic or peritoneal macrophages explanted from PIC-infected guinea pigs at various postinoculation intervals. Peritoneal and splenic macrophages removed from PIC-infected guinea pigs and placed in culture without further stimulation did not overproduce TNF compared with uninfected, uninduced control macrophage cultures. However, PIC-infected macrophages responded differently to in vitro stimulation with *E. coli* LPS than did uninfected control macrophages. Peritoneal macrophages removed from adPIC-infected animals six days after infection secreted significantly less TNF after LPS stimulation than did uninfected

FIGURE 4. Tumor necrosis factor (TNF) production by lipopolysaccharide (LPS)-treated peritoneal macrophages in Pichinde virus (PIC) infection. Male strain 13 guinea pigs were inoculated intraperitoneally with 3,000 plaque-forming units of adPIC or PIC3739 or diluent alone (open bar) on day 0. Peritoneal macrophages were isolated by plastic adherence and cultured in the presence of 10 μ g of *Escherichia coli* LPS/ml for 24 hr. Supernatants were harvested and assayed for TNF. Each bar represents the mean TNF value of single or duplicate macrophage cultures from at least three guinea pigs. Dots indicate separate TNF determinations from individual macrophage cultures. The control bar reflects the mean of macrophage cultures from three sham-infected guinea pigs, one each from day 3, day 6, and day 11. * = $P < 0.05$ versus control. adPIC = virulent derivative of Pichinde virus (strain An4763) developed by Jahrling and others;¹ PIC3739 = prototype strain of Pichinde virus (An3739) with attenuated virulence in guinea pigs.

control peritoneal macrophages. The adPIC-infected peritoneal macrophages on day 11, however, showed a striking potentiation of LPS-induced TNF compared with that found in control cultures (Figure 4). A similar pattern of early suppression and subsequent augmentation of LPS-inducible TNF secretion was seen in PIC3739-infected macrophages, but to a lesser degree and without statistical significance. In contrast, splenic macrophages were not similarly primed at any postinfection interval. Instead, their TNF responses to LPS were blunted as compared with findings in control cultures (Figure 5).

DISCUSSION

The pleiotropic monokine TNF α has a host of physiologic, proinflammatory effects, including antiviral activity.^{8, 18, 19} It also possesses potentially pathologic effects that are well-described in several human and experimental infectious diseases.^{20, 21} To explore pathologic and beneficial effects of TNF in this model of arenavirus disease we have compared TNF responses in guinea pigs infected with two PIC strains that differ markedly in pathogenicity. Due to the unavailability of antibodies capable of neutralizing guinea pig TNF in vivo, we have not established definitively a causal link between TNF overproduction and pathologic effects. Nonetheless, this comparative approach lends biological relevance to our findings regarding TNF production in lethal and non-lethal infections. In this system, bioactive TNF was present in the serum of some guinea pigs infected with the uniformly lethal adPIC strain, but undetectable in all sera from guinea

FIGURE 5. Tumor necrosis factor (TNF) production by lipopolysaccharide-treated splenic macrophages in Pichinde virus infection. Splenic macrophage cultures were established from adPIC-infected, PIC3739-infected, or sham-infected control guinea pigs (open bar) as described in the Materials and Methods and Figure 3. Results are expressed as the mean TNF value of cultures from duplicate macrophage cultures from at least three guinea pigs per timepoint. Dots indicate separate TNF determinations from individual macrophage cultures. The control bar reflects the mean TNF value of macrophage cultures from three sham-infected guinea pigs, one animal each from day 3, day 6, and day 11. * = $P < 0.05$ versus control. adPIC = virulent derivative of Pichinde virus (strain An4763) developed by Jahrling and others;¹ PIC3739 = prototype strain of Pichinde virus (An3739) with attenuated virulence in guinea pigs.

pigs infected with the attenuated strain PIC3739. Whereas serum TNF was associated only with lethal (adPIC) infection, TNF was present in spleens from guinea pigs infected with either PIC strain. Tumor necrosis factor activity appeared in the spleens in the first week of infection with either PIC strain, with levels waning later in the disease course. The method of preparation of clarified spleen homogenates in these experiments does not exclude the possibility that cell-associated TNF in membrane fragments accounted for a portion of the measured bioactivity in spleen homogenates.

It is clear from other experimental infectious disease models that TNF α exerts different effects depending on the anatomic compartment in which it is expressed. Gram-negative septic shock is the classic example of an overwhelming TNF α -induced pathologic process with associated high serum levels of TNF α .²² However, localized TNF α secretion is required for protective host responses in human tuberculous pleuritis, murine tuberculosis, and rat bacterial peritonitis.²³⁻²⁵ A cell membrane-associated, 26-29 kD form of TNF α is expressed in organs early in TNF-associated processes and may play an important role in focusing local proinflammatory effects of this potent monokine.^{26, 27} Thus, toxic properties of TNF α may be effected by circulating forms of the monokine, whereas beneficial effects are likely mediated by locally secreted or cell-associated forms of TNF α . Results of the present study support the idea that locally produced TNF α and systemically secreted TNF α also have different effects in PIC infection of guinea pigs. We hypothesize that the expression of secreted or cell-associated forms of TNF α in the spleens of PIC-infected guinea pigs during the period of early rapid viral replication represents

a facet of a physiologic immunoinflammatory response. It is particularly interesting that guinea pigs infected with the attenuated strain (PIC3739) displayed a trend toward slightly higher levels of TNF in the spleen than did guinea pigs infected with the fully virulent strain (adPIC).

Analysis of TNF α -specific mRNA levels in organs from PIC-infected guinea pigs was performed to corroborate TNF bioactivity measurements, particularly since the TNF bioassay does not distinguish between the monokine TNF α and the lymphokine TNF β . Slot blots of organ RNA (Figure 3 and Table 1) and TNF bioassay of spleen homogenates (Figure 2) showed disconjugate levels of TNF α mRNA and TNF-like bioactive material in PIC-infected spleens. It is well-known that there is a large component of post-transcriptional regulation of TNF expression. We believe that altered regulation of TNF expression in adPIC infection may be of pathogenetic significance. Secretion of TNF is tightly regulated in normal and disease states so as to fine tune and harness its potent effects and maintain homeostasis.²⁸⁻³⁰ For example, TNF α gene expression is rapidly upregulated in ascites cells and whole organs in murine endotoxemia and decreases to near or below baseline levels at the time serum TNF α levels peak.^{31,32} The presence of preformed intracellular pools of TNF α transcript allow for rapid responsive TNF α release after LPS stimulation. The expression of TNF α is downregulated by means of autocrine and paracrine mechanisms involving suppressive cytokines such as prostaglandin E₂, and transforming growth factor- β , or systemic hormones such as corticosteroids.^{33,34} Our finding of persistently high levels of TNF α mRNA in spleens and livers from adPIC-infected guinea pigs compared with the levels in PIC3739-infected animals suggests a failure of these normal mechanisms of feedback downregulation of TNF α production in the lethal adPIC infection. A successfully resolved infection with PIC3739 was associated with an early increase in TNF α mRNA and high TNF activity levels in the spleen. The rapid decrease in TNF α mRNA levels by day 11 implies efficient down regulation of this response in PIC3739 infection, minimizing potential harmful effects. In contrast, adPIC infection was characterized by a smaller increase in spleen TNF bioactivity, high levels of spleen and liver TNF α mRNA that remained elevated at the time of death, priming of certain macrophage populations for LPS-induced TNF production (see below), and spillover of TNF into the serum where toxic systemic effects could be exerted. These two patterns of TNF regulation may reflect in part responses to differing extents of viral replication in TNF α -producing target cells.³

The TNF secretion profiles of macrophages explanted from PIC-infected guinea pigs failed to incriminate unequivocally a specific macrophage population as the source for serum TNF. Peritoneal and splenic macrophages removed from PIC-infected guinea pigs and placed in culture without further stimulation did not overproduce TNF compared to uninfected control macrophage cultures. However, LPS-inducible TNF responses of macrophage explants from PIC-infected guinea pigs were strikingly different from those of control macrophage cultures. The PIC-infected spleen macrophages showed blunted LPS-inducible TNF secretion compared with control cultures. There is precedent in the literature for suppression of TNF α production in virus-exposed

macrophages.³⁵ However, this depressed capacity for TNF secretion by splenic macrophages conflicted with the finding of increased TNF-like bioactivity and TNF α mRNA in whole spleen preparations from PIC-infected guinea pigs. There are several possible explanations for this discrepancy in TNF levels between whole organ preparations and macrophages explanted from that organ. First, cytotoxic material in the spleen homogenates may represent some artifactual non-TNF activity. Second, bioactive material in spleen homogenates may include the lymphokine TNF β which would not be produced by isolated macrophages. Third, TNF bioactivity in spleen homogenates may include membrane-associated TNF in cell fragments whereas only secreted TNF would be represented in macrophage culture supernatants. Fourth, macrophages may have altered patterns of monokine secretion after they are removed from the microenvironment of the PIC-infected spleen.

In contrast to spleen macrophages, peritoneal macrophages derived from guinea pigs late in PIC infection were primed for TNF secretion after LPS stimulation. Some viruses, including influenza A, can also stimulate accumulation of TNF α mRNA in infected macrophages, leading to potentiation of LPS-induced TNF α production.³⁶ Although we have not quantitated TNF α mRNA levels in PIC-infected macrophage cultures, our finding of elevated TNF α transcript levels in whole organs from PIC-infected animals suggests that PIC, particularly the virulent adPIC, may have a similar synergistic effect with LPS on TNF α regulation. Augmentation of LPS-inducible TNF α production may be relevant in the pathogenesis of PIC infection in guinea pigs. We have shown previously that mortality is correlated closely with mucosal lesions in the small and large intestine.³ Guinea pigs with breaches in the colonic mucosal barrier (allowing enhanced absorption of endogenous endotoxin), in conjunction with primed macrophage populations (which are hypersensitive to LPS), would be at high risk for developing TNF α -mediated endotoxic shock. The combined insults of intestinal epithelial injury and altered macrophage function have been implicated in the pathophysiologic derangements of murine graft-versus-host disease,³⁷ as well as in Venezuelan equine encephalitis virus infection of hamsters.³⁸

The findings from these descriptive studies raise the possibility that TNF α is important in PIC disease, but do not assign an exact role for this mediator in the pathogenic scheme. The adPIC strain may cause an immunopathologic disease by invoking a hypersensitivity mechanism that is based on systemic cytokine overproduction. On the other hand, circulating TNF may be an epiphenomenal marker of a failed or inefficient immune response with resulting uncontrolled replication of the virulent virus. Therefore, TNF α and other monokines may themselves contribute to and enhance virus-induced cellular pathologic change.³⁹ By altering the regulation of cytokine secretion, virus may contribute to local immunomodulation, promoting viral survival in the host. We believe that our study findings implicate TNF α as a complexly regulated contributor to pathology in guinea pig, and possibly human arenavirus disease.

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