CONCISE COMMUNICATIONS

Induction of Glomerular Lesions in the Kidneys of Mice Infected with Vero Toxin–Producing Escherichia coli by Lipopolysaccharide Injection

Keiko Shimizu, Yuji Aiba, Kazuo Tanaka, Akira Akatsuka, Masayuki Endoh, and Yasuhiro Koga

Lipopolysaccharide was injected into germ-free mice after they had been infected with Vero toxin–producing Escherichia coli. Microscopic examination of the kidneys of these mice showed an increased number of mesangial cells and a vacancy in the glomerular capillary lumen. A significant elevation in the expression level of interferon (IFN)-γ in the kidney may have played a key role in the induction of glomerular lesions, because the administration of neutralizing antibody to IFN-γ markedly alleviated such lesions.

Materials and Methods

Mice. Male germ-free BALB/c mice from Nippon Clea (Tokyo) were inoculated intragastrically at age 4 weeks with 10^7 cfu of bacteria. Seven days after infection, the mice were injected intraperitoneally (ip) with 50 μg of LPS (E. coli O55:B5; Difco Laboratories, Detroit).

Bacteria. For the VTEC strain, O157:H7 (TI001), which produces both VT1 and VT2, was used (Johnson et al. [5], Laboratory Centre for Disease Control, Ottawa, Canada). For inoculation into mice, bacteria were grown in brain-heart infusion broth at 37°C overnight and then used immediately. VT2 was purified from TI001 strain as reported previously [6].

Histologic studies. The mice were killed 10 days after infection; their kidneys were removed and processed for light microscopy and transmission electron microscopy (TEM) [7]. In order to quantify the empty area of Bowman’s capsule, which is the space between its outer envelope and the capillary network of glomerular lobules, we measured the area of the capsule and of the whole glomerulus in a visual field with a computer image analyzer (VIDAS; Carl Zeiss, Tokyo). The percent ratio of these areas was used as the index to assess the narrowing of the empty space. Statistical analysis was done by Student’s t test.

Passive antibody transfer. VTEC-infected mice were injected ip with 100 μg of monoclonal hamster anti-murine interferon (IFN)-γ antibody or 3.0 × 10^6 U of polyclonal rabbit anti-mouse TNF-α antibody (both Genzyme, Boston) 6 h before ip challenge with LPS. Normal serum was used as the control for IFN-γ and immunoglobulin as the control for anti-TNF-α.

RNA polymerase chain reaction (PCR) and Southern blot analysis. RNA PCR was performed by use of an RNA PCR Kit (Takara, Ohitsu, Japan) by the method reported elsewhere [8]. The primers and probes for β-actin, TNF-α, IFN-γ, and iNOS were purchased from Clontech (Palo Alto, CA). The nucleotide sequences of cDNA for these genes have been described [8]. The standard PCR reaction containing 10 amol of each cDNA as a control was loaded onto a lane. The Southern transfer was done on a transfer membrane. Hybridization was performed for 2 h at...
Figure 1. Microscope images of mouse kidney sections. A–D, Light microscope images of hematoxylin-eosin stain; E, F, transmission electron images. Sections are from mice treated as follows: A, E, noninfected and not lipopolysaccharide (LPS) injected; B, F, VTEC infected and LPS injected; C, VT2 and LPS injected; D, VTEC infected and LPS injected (pretreated with anti-interferon-γ).

42°C with the probe tailed at the 3′ end with fluorescein dUTP, which was then detected by chemiluminescence with the ECL system (Amersham, Buckinghamshire, UK).

Results

Germ-free mice were inoculated intragastrically with 10⁷ cfu of VTEC. About 5 days after inoculation, the mice developed acute systemic symptoms such as lethargy and dehydration, but they did not have bloody diarrhea. The symptoms were followed by death in ~50% of the mice 5–7 days after infection; the rest recovered. Bacteria reached 10⁹ cfu/g of feces after 1 day and remained at this level. VT1 and VT2 titers in feces rose rapidly after inoculation: >100 ng of VT1 and 1000 ng of VT2 had accumulated in 1 g of feces 2 days after infection. A histologic examination of the gut 5 days after infection revealed no erosion or bleeding, indicating resistance of murine gut epithelium to VT. In the kidney, no particular lesion was found.
Figure 2. RNA polymerase chain reaction (PCR) and Southern blot analysis of kidneys from infected and noninfected mice. All mice (except 0 h group, as indicated above the lanes) were injected intraperitoneally 7 days earlier with lipopolysaccharide (LPS) and killed 3 or 8 h after LPS injection to remove kidneys and prepare them for RNA analysis. PCR products of A, β-actin; B, tumor necrosis factor-α, C, interferon-γ, and D, iNOS. Each lane is sample from different mouse. Lane S in each panel was loaded with standard PCR product.

in the glomeruli. To compensate for a possible insufficiency for the entrance of bacterial products from the gut, the mice were injected with LPS 7 days after infection.

Light microscopy of the mouse kidneys revealed a marked reduction in the empty area of Bowman’s capsule in a distorted glomerulus (figure 1B). Enlargement of the glomerular lobules or shrinkage of the outer envelope of Bowman’s capsule are thought to cause the empty area in the glomerulus to disappear. The crucial involvement of both VT and LPS was suggested by such glomerular lesions. To test this hypothesis, germ-free mice were first injected ip with 2 ng of VT2 (LD50 = 2 ng). Surviving mice were treated with LPS 7 days after the VT2 injection. As expected, the VT2 + LPS group had a marked reduction in the empty area of Bowman’s capsule (figure 1C); this did not occur in either the VT2 alone or the LPS alone groups (data not shown). A quantitative analysis of such empty areas confirmed that a narrowing of the empty area was more common in the VTEC + LPS (17.6% ± 4.9%) and the VT2 + LPS (13.3% ± 2.5%) groups than in the others: noninfected (figure 1A; 30.58% ± 3.6%), VT2 alone (28.1% ± 8.0%), and LPS alone (30.6% ± 3.8%). There were 10 mice in all groups.

TEM showed a significant increase in the number of mesangial cells and a vacancy in the capillary lumen of the glomerulus in the VTEC + LPS group (figure 1F) but not in the noninfected group (figure 1E) or in the LPS alone group (data not shown). No swelling or detachment of endothelial cells, which are characteristic features of the glomerulus in human HUS, was seen in these specimens, which indicates that HUS is not established in this model.

The expression level of proinflammatory cytokines was examined in the kidney (figure 2). TNF-α was significantly expressed not only in the VTEC + LPS group (figure 2B), which indicates that this cytokine is not directly involved in the formation of the glomerular lesions in the VTEC + LPS group. IFN-γ seems to be an important pathogenic cytokine. Its expression level was markedly higher and lasted longer in the VTEC + LPS group than in the LPS alone group (figure 2C). The expression level of iNOS was also very high in the VTEC + LPS group (figure 2D), suggesting a crucial role for nitric oxide (NO) in the pathologic events. To determine if the cytokines that are highly expressed in the kidney are really responsible for such glomerular lesions, the
VTEC-infected mice were injected with anti-TNF-α or anti-IFN-γ neutralizing antibody before the LPS treatment. The survival rate of these mice improved profoundly compared with control groups: anti-TNF-α (88% [n = 8] vs. 29% [n = 7]) or anti-IFN-γ (100% [n = 5] vs. 33% [n = 6]). Light microscopy of the glomerular histopathology showed that there was a significant restoration in the empty area of Bowman’s capsule in the IFN-γ-treated group (figure 1D) (29.1% ± 3.3% [n = 5] vs. 11.6% ± 2.0% [n = 6]; P < .0001). No such improvement was found in the anti-TNF-α-treated group: 10.9% ± 1.7% (n = 8) vs. 9.6% ± 2.9% (n = 7) in control groups (P > .5). Therefore, IFN-γ seems to be entirely involved in producing this glomerular lesion, but TNF-α is not, although both cytokines cause wasting disease that results in death.

Discussion

In the kidney of our murine model, we found a marked reduction in the empty area of Bowman’s capsule in the glomeruli. This occurred primarily in the VTEC-infected mice after LPS injection. The empty area in Bowman’s capsule represents the space between the outer envelope of Bowman’s capsule and the capillary network of glomerular lobules. This space is usually filled with urine formed from the renal blood flow by filtration across the glomerular capillary wall. Therefore, the disappearance of this space suggests an enlargement of glomerular lobules, the contents of Bowman’s capsule, and/or the shrinkage of the outer envelope of Bowman’s capsule. However, TEM revealed an increase of mesangial cells in the glomerular lobules, which perhaps causes the enlargement of the lobules and the collapse of the capillary lumen and suggests a marked decrease in the renal blood flow.

The mesangial cells are enclosed in the mesangium, which consists of a spongelike meshwork of basement membrane. IL-1 promotes mesangial cell proliferation [9], and TNF-α augments this pathologic process by acting synergistically with IL-1 [10]. Karpman et al. [11] also reported the focal proliferation of glomerular mesangial cells and increased deposition of mesangial matrix in O157-infected mice. Such expansion of mesangium was also found in gnotobiotic mice infected with O157 and treated with TNF-α [12], which suggests a crucial role for these cytokines in this kidney lesion.

In the present study, a significant alleviation of the glomerular lesion occurred after administration of anti-IFN-γ antibody, which indicates that this proinflammatory cytokine plays a key role in the pathogenesis of the present model. The failure of anti-TNF-α to improve this glomerular lesion suggests that IFN-γ, not TNF-α, is predominantly involved in pathogenesis in our model, probably owing to a strong insult to the host caused by the additional LPS injection to the VTEC-infected mice.

When combined with IFN-γ, bacterial LPS can profoundly augment the expression of iNOS [13]. A marked elevation of iNOS in the kidney of VTEC + LPS mice is likely caused by further stimulation with LPS in the presence of IFN-γ and may participate in such IFN-γ-mediated glomerular injury through the production of NO.

References