*Kidney International, Vol. 54 (1998), pp. 1967–1975*

# Expression of CD27 and ischemia/reperfusion-induced expression of its ligand Siva in rat kidneys

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**Expression of CD27 and ischemia/reperfusion-induced expression of its ligand Siva in rat kidneys.**

*Background.* Studies identifying genes that are differentially expressed following induction of acute ischemic injury have been useful in delineating the pathophysiology of acute renal failure.

*Methods.* A differential cDNA library screening technique was used to identify genes that are differentially expressed in rat kidney following induction of acute ischemic renal injury.

*Results.* Levels of mRNA with a high homology to that coding for Siva, a human proapoptotic protein, were increased approximately 4.5-fold in kidneys obtained from rats within 12 hours following ischemia, compared to kidneys from sham-operated rats. A partial cDNA sequence for the rat protein (rat Siva) was determined that overlaps 92% of the human open reading frame. The cDNA sequence predicts a protein 177 amino acids in length with 76% homology to human Siva. Levels of rat Siva in kidneys were elevated at one, five and seven days post-ischemia. However, levels in kidneys obtained two days post-ischemia were not different from those in kidneys from sham-operated controls. *In situ* hybridization demonstrated that rat Siva mRNA was expressed in cells lining damaged sections in the $S_3$ segment of the proximal tubule at 12 hours and one day post-ischemia. At five and seven days, Siva mRNA was located in epithelial cells of regenerating tubules including in papillary proliferations. TdT-mediated dUTP-biotin nick end-labeling (TUNEL)-positive cells colocalized with cells containing Siva mRNA. CD27, the receptor for Siva was localized by immunohistochemistry to sloughed cells in the lumens of damaged $S_3$ segments at 12 hours post-ischemia and to cells within papillary proliferations at five days post-injury.

*Conclusions.* Siva that is produced within the kidney could be a mediator of apoptosis post-ischemia via an interaction with CD27.

CD27 is a member of the tumor necrosis factor receptor (TNFR) superfamily. Members of this family which include tumor necrosis factor receptor 1 (TNFR1) and Fas, are important regulators of cell growth and cell survival. In addition to providing co-stimulatory signals for proliferation, the binding of ligands to TNFR1 or Fas can result in apoptosis [1]. The mechanism of TNFR1 or Fas-induced apoptosis requires that an intact 80 amino acid sequence, termed the death domain, be present in their cytoplasmic tails [2].

CD27 is not known to be expressed in kidney [1, 2]. However, the expressions of other members of the TNFR superfamily, such as TNFR1 and Fas, have been described in renal tissue. Fas is not detected in normal human kidneys and Fas mRNA is expressed at low levels in normal mouse kidneys. However, Fas is highly expressed in tubular epithelial cells of diseased renal tissue such as transplanted human kidneys undergoing acute rejection. In this setting, its expression is observed in association with apoptosis of tubular cells [3]. Levels of Fas mRNA and protein are increased in kidneys of mice rendered endotoxemic, and also in association with apoptosis along nephron segments [4].

CD27 is known to be expressed in T and B lymphocytes. The interaction of CD27 with its ligand CD70, also expressed in T and B cells, can provide costimulatory signals for T and B cell proliferation and for immunoglobulin production in B cells [2, 5]. Unlike TNFR1 and Fas, the cytoploasmic tail of CD27 lacks a death domain. However, the interaction between CD70 and CD27 can induce apoptosis [2].

Recently Prasad et al, using the yeast two-hybrid system, cloned a novel ligand for CD27 from a HeLa cell cDNA library, which they designated Siva. Siva has a death domain-like region that binds to the CD27 cytoplasmic tail *in vitro*. In addition, Siva has a B-box-like ring finger domain [2]. Overexpression of Siva in a number of cell lines induces apoptosis in the absence of CD70. Presumably, the association of CD27 in the cell membrane with intracellular Siva results in apoptosis [2]. Unlike CD70, Siva is present in a number of non-lymphatic tissues such as prostate, testis, ovary and colon [2].

We report here, the cloning and sequence characterization of rat Siva, its time course of expression, and the

**Key words:** apoptosis, ischemia/reperfusion injury, regeneration, tumor necrosis factor, death domain, Fas.

Received for publication March 17, 1998
and in revised form July 8, 1998
Accepted for publication July 9, 1998

spacial expression pattern of Siva and its receptor CD27 in ischemic kidney. The findings represent the initial cloning of rat Siva and the first demonstrations of Siva and CD27 expression in the kidney. It is possible that an interaction between the death domain of Siva and CD27 mediates apoptosis of renal cells following ischemic/reperfusion injury.

## METHODS

### Animals

Male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) weighing approximately 250 g were housed with 12:12 hours light/dark cycle and food and water available *ad libitum*.

### Induction of acute renal failure

Acute renal failure was induced by 60 minutes of bilateral renal artery clamping as described previously [6, 7] with sham-operated controls included at each time point. To control for the extent of renal injury during the procedure, animals were selected such that the level of serum creatinine measured 24 hours following injury fell into the range used in previous studies [6–8]. Levels in groups of rats (mean ± SE, $N = 3$ rats) were 4.2 ± 0.3; 3.2 ± 0.5; 3.0 ± 0.3; and 3.5 ± 0.1 mg/dl in groups of rats used to obtain kidneys one, two, five, and seven days post-ischemia, respectively. Levels were 1.0 ± 0.1 and 2.6 ± 0.1 mg/dl ($N = 3$ rats) at 1 and 12 hours post-injury in the groups of rats that were sacrificed at these times. Levels of serum creatinine in sham-operated rats were measured at the time of sacrifice and averaged 0.6 ± 0.01 mg/dl ($N = 36$). These levels did not differ significantly from group to group.

Levels of serum creatinine at the time of sacrifice of rats that had been rendered ischemic were 2.0 ± 1.2, 0.8 ± 0.1, and 0.7 ± 0.1 mg/dl at two, five, and seven days post-injury, respectively.

### Isolation of kidney RNA

At the time points indicated above, rats were anesthetized with ketamine and pentobarbital. Their kidneys were perfused with sterile phosphate-buffered saline to remove blood from the organs [7], quickly excised, frozen in liquid nitrogen and stored at −70°C. Total RNA was isolated using the Ultraspec RNA isolation solution (Biotecx, Houston, TX, USA).

### Differential library screening

A small fraction of a cDNA library generated from kidneys of adult rats rendered ischemic [8, 9] was plated on a Nunc bioassay dish (Fisher, Pittsburgh, PA, USA). Replica filters were made from the plates on zeta probe membranes (BioRad, Hercules, CA, USA). Total RNA was isolated from kidneys of sham-operated rats and rats rendered ischemic at 24 hours post-surgery. For differential library screening, first strand $^{32}$P-labeled cDNA was synthesized from 5 μg of total RNA using superscript reverse transcriptase (BRL, Bethesda, MD, USA). One of the replica filters was then hybridized to the cDNA probe from sham-operated kidney, and the second filter was hybridized to the probe from ischemic kidney. The hybridization and washing conditions used were those recommended by the manufacturer of the zeta probe. The hybridized filters were then exposed to X-ray films for two days at −70°C and the autoradiograms aligned with each other. The plaques that exhibited differential hybridization were isolated and their differential expression confirmed by Northern blot hybridizations.

### DNA sequencing

Both strands of the cDNA were completely sequenced by primer walking on an automated ABI377 sequencing machine. Sequences were searched against the GenBank nucleotide database using the BLASTN program [10] and through a non-redundant protein database using the BLASTX program.

### Northern blot analysis

Northern blots were probed as described before [8, 9] using a fragment containing bases −26 to 630 of the rat Siva cDNA (Fig. 1). To confirm that equal quantities of RNA were loaded onto each lane, 18S and 28S bands on an agarose gel were stained using eithidium bromide, and it was determined that staining was the same in each lane. The band intensity after hybridization was measured using a Phosphorimager and the statistical analysis performed as before [8, 9]. Comparison between gels was accomplished by normalizing data to the value of the respective band originating from a kidney of a sham-operated rat.

Statistical analysis was performed using the Student's *t*-test for unpaired sample means. $P < 0.05$ for two-tailed analysis was considered significant.

### *In situ* hybridization

*In situ* hybridization was performed on tissue sections originating from sham operated rats and rats rendered ischemic as previously described [8, 9]. A rat Siva cDNA cloned in plasmid pBK-CMV (Stratagene, La Jolla, CA, USA) was used as the template to generate digoxigenin-11-dUTP labeled sense and antisense riboprobes. A fragment containing bases from −26 to 630 was used as the sense and antisense probes.

### Reverse transcriptase-polymerase chain reaction (RT-PCR)

Deoxyribonuclease treated total RNA was reverse transcribed using a random oligonucleotide primer using Superscript reverse transcriptase (BRL, Bethesda, MD, USA) in a 40 μl reaction volume. Twenty microliters of the reaction was then PCR amplified using CD27 receptor

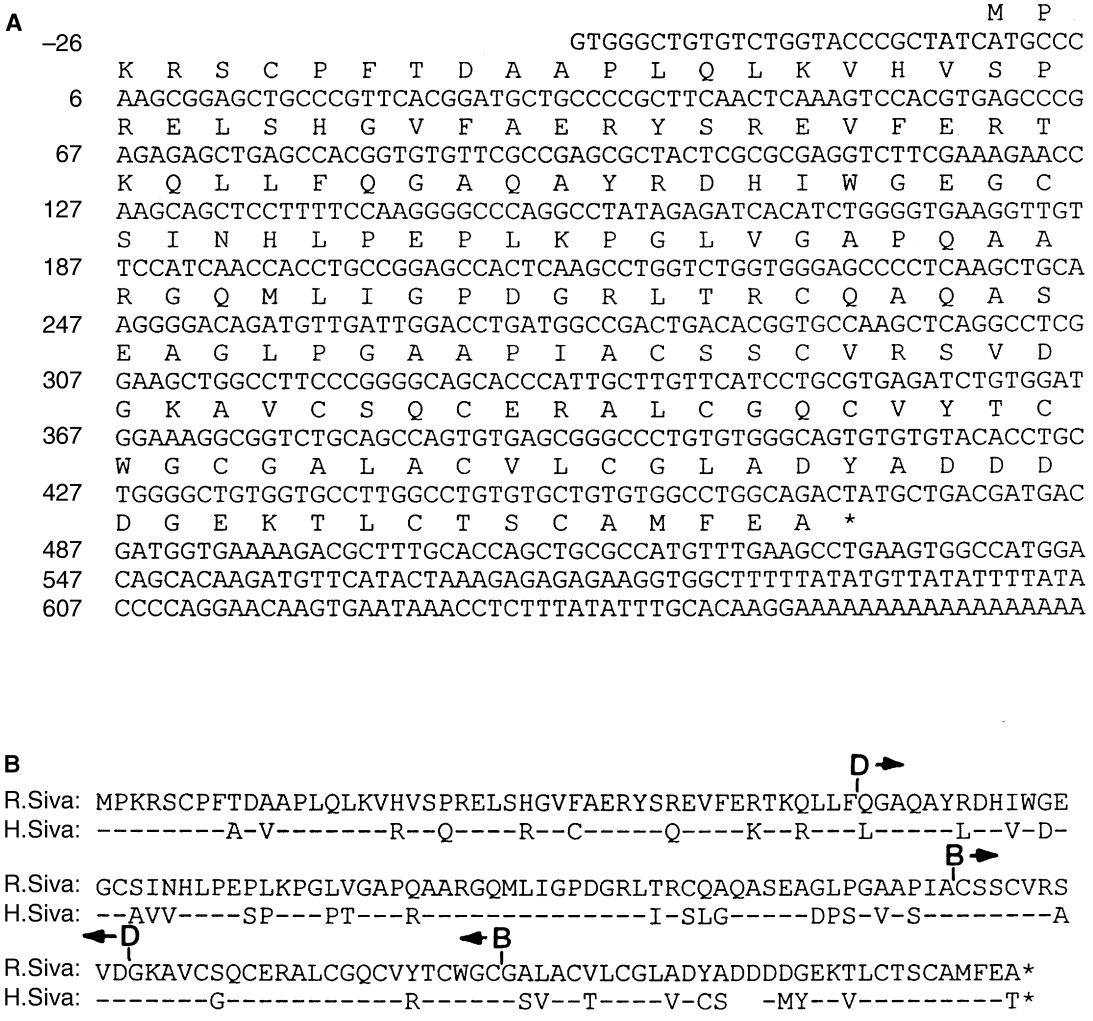


**Fig. 1. (*A*) cDNA and deduced amino acid sequence for rat Siva, and (B) comparison between rat (R) Siva and human (H) Siva.** D defines the death domain and B defines the B-box-like region.

specific oligonucleotides: 5′-CACCTCCCTACTGGCTCTG and 5′-CACTCTGTACATTCCTGGTC. The oligonucleotide sequences were derived from the published mouse sequence [11]. The 348 bp PCR fragment amplified from the rat RNA was cloned into the plasmid vector pGEM-T (Promega, Madison, WI, USA) and confirmed to represent the rat CD27 homologue by sequence characterization. Control PCR amplifications were performed in the absence of RT.

### Western blotting

Whole kidneys from rats were homogenized in cold buffer (20 mM Tris-HCl, pH 7.5, 250 mM sucrose, 10 mM EGTA, 1 mM EDTA, 1 mM PMSF, 20 $\mu$g/ml leupeptin, 1% Nonidet P40). Homogenates were centrifuged at 1000 $\times$ g for 10 minutes at 4°C to remove the nuclear fraction and debris. The supernatant was centrifuged at 100,000 $\times$ g for one hour at 4°C to separate the soluble (cytosolic) fraction from the membrane fraction. The membrane fraction was resuspended to half the original volume using the homogenization buffer to which Triton X-100 was added to a final concentration to 0.3%. Twenty micrograms of protein was fractionated on a 4 to 20% gradient polyacrylamide gel and transferred to a nitrocellulose membrane. The membranes were incubated in 3% non-fat milk in Tris-buffered saline containing 0.05% Triton X-100 for one hour, then at 4°C overnight with, M-20, a CD27-specific primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in Tris-buffered saline. M-20 is an affinity-purified goat polyclonal antibody raised against a peptide corresponding to amino acids 230 to 249 mapping at the carboxy terminus of the mouse CD27 precursor. M-20 reacts with CD27 of mouse and rat origin and is not cross-reactive with other TNFR family members (Santa Cruz Biotechnology; unpublished observations).

The membranes were washed and incubated with horseradish peroxidase conjugated anti-goat antibody (Pierce, Rockford, IL, USA) for one hour at room temperature.

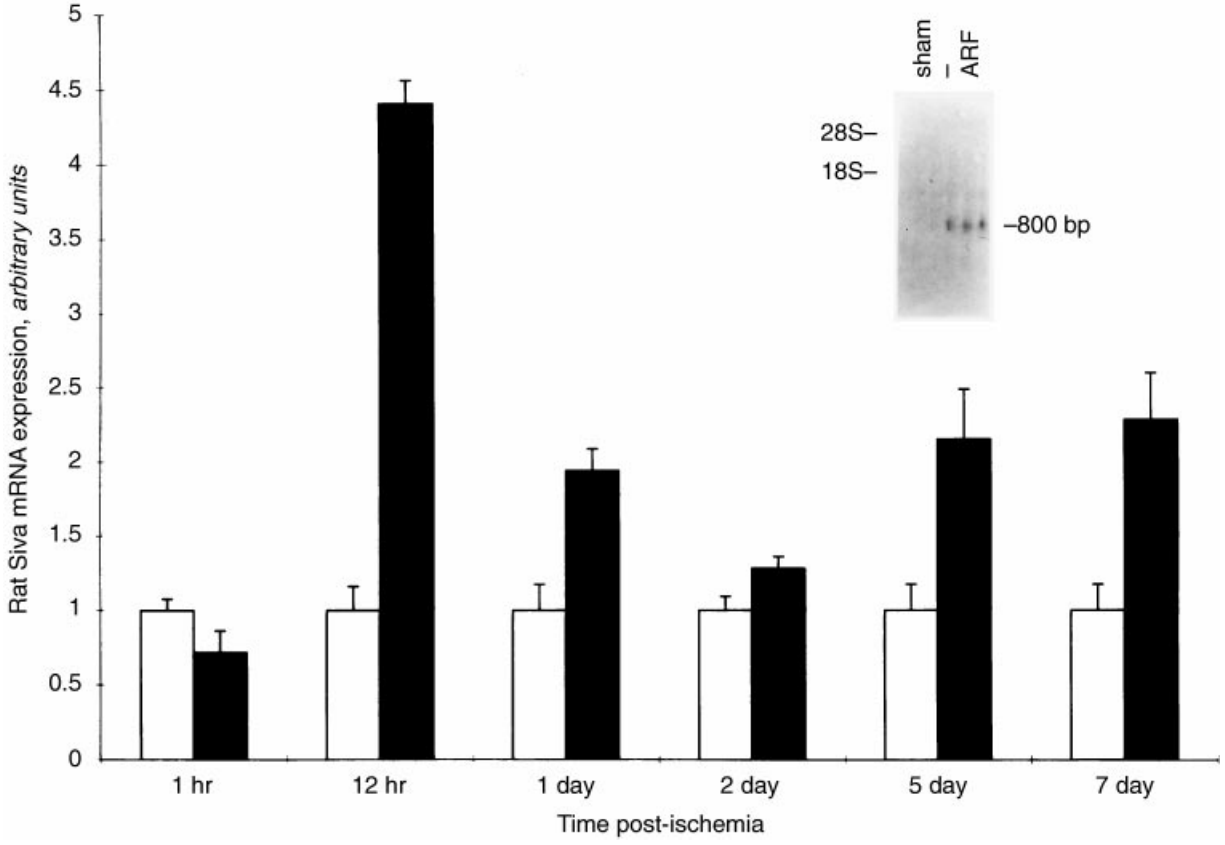


**Fig. 2. Northern assay for Siva mRNA and the effect of ischemia on renal Siva mRNA levels.** (*Inset*) A representative autoradiogram of a Northern blot generated using RNA from kidneys of three rats that underwent sham-surgery and three rats rendered ischemic five days previously. The size of the RNA species (800 bp) is shown. Also shown are results from three Northern assays quantified using a phosphorimager. $N = 3$ rats in each group. Data are mean ± SE. ARF > sham at 12 hours, and one, five and seven days ($P < 0.05$, Student's *t*-test). Symbols are: (□) sham; (■) ARF.

The reaction products were visualized using ECL-chemiluminescence kit (Amersham, Arlington Heights, IL, USA).

### Tissue staining

Localization of CD27 was performed by immunofluorescent staining of 5 μm Bouins-fixed paraffin-embedded sections as described previously [12]. The primary antibodies were M20, obtained from Santa Cruz Biotechnology, Inc. The sections were deparaffinized in xylene, rehydrated in graded ethanol, washed in phosphate-buffered saline (PBS) followed by blocking in a PBS buffer containing 10% rabbit serum and 0.2% triton X-100 for two hours. The sections were incubated overnight at 4°C with the primary antibodies (2 μg/ml) in 1% rabbit serum and 0.2% triton X-100. Cy3 linked donkey anti-goat IgG was used as secondary antibodies. For blocking experiments, 2 μg of CD27 antibody was resuspended in 900 μl of PBS and incubated overnight at 4°C with 10 μg of blocking peptide (SC1743p) or a scrambled peptide (SC1744p; Santa Cruz Biotechnology). TdT-mediated dUTP-biotin nick end-labeling (TUNEL) staining was used to identify apoptotic cells as before [12].

## RESULTS

To identify genes that are expressed following ischemia/reperfusion injury, we performed differential library screening. A cDNA library generated from kidneys from rats rendered ischemic one day previously was screened using cDNA probes generated from kidneys of either sham operated rats or rats rendered ischemic. Several plaques were identified that: (*1*) either failed to hybridize or hybridized weakly to the probe originating from sham-operated rats; and (*2*) hybridized strongly to the probe from ischemic rats. One clone, upon sequence characterization was found to have high homology to the human proapoptotic protein, Siva cDNA.

Translation of the partial rat Siva open reading frame revealed a primary sequence 177 amino acids long (Fig. 1A) with high homology (76%) to the comparable portions of human Siva (Fig. 1B), which is 189 amino acids long [2]. Fifty-three of 75 amino acids are conserved within the death domain (71% homology; Fig. 1B). Therefore, the homology between the death domains of rat and human Siva is higher than that between human Siva and the death

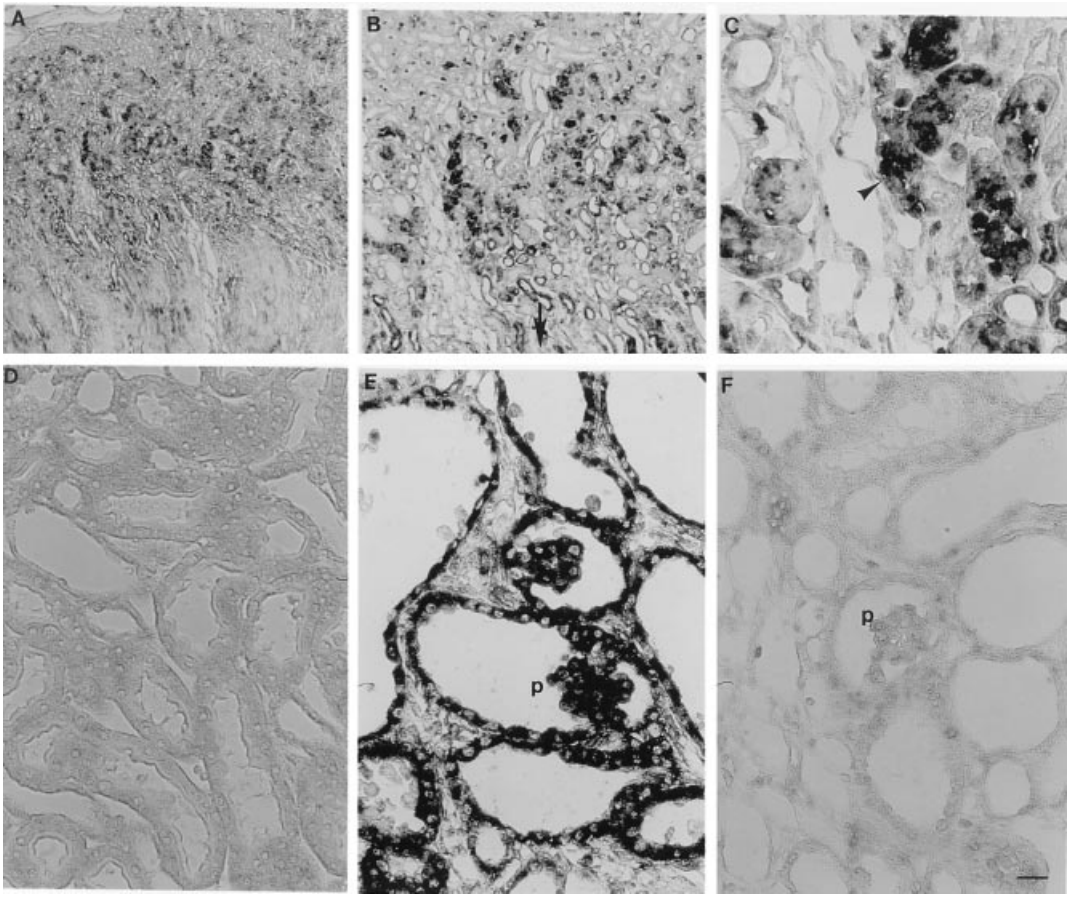


**Fig. 3. Localization of renal Siva mRNA by non-isotopic *in situ* hybridization.** Siva mRNA in kidneys from rats subjected to sham-surgery (*D*) or rendered ischemic (*A-C, E, F*) 12 hours (A-C) or five days prior to sacrifice (E, F), was localized using a digoxigenin labeled antisense riboprobe (A-E) or sense probe (F). Arrow points towards renal papilla (B). Arrowhead shows ischemic proximal tubule (C). P, papillary proliferation (E,F). Sections are representative of $> 5$ experiments. A→C are progressive enlargements of the same section. Magnification (15 $\mu$m) is shown for C-F (F).

domains of the human proteins FADD or RIP (40%) [2]. Twenty-nine of 32 animo acids in the B-box-like ring finger region [2] of rat Siva are homologous to those in human Siva (Fig. 1B).

To characterize the time course of renal rat Siva expression post-ischemic injury, kidneys were obtained from sham-operated rats and rats rendered ischemic, one and 12 hours, and one, two, five, and seven days post-sham surgery or post-ischemia, and Northern assays were performed using RNA extracted from the kidneys. A representative autoradiogram of a gel from a Northern assay originating from mRNA extracted from three kidneys obtained five days post-ischemia or sham surgery is shown in Figure 2 (upper right). Rat Siva mRNA (800 bp band) was visibly increased in kidneys from rats rendered ischemic relative to levels in kidneys from sham-operated animals.

Levels of rat Siva mRNA in kidneys of three groups of rats subjected to sham-surgery or ischemic injury at 12 hours, and one, two, five or seven days prior to sacrifice determined by Northern analysis were quantified using a phosphorimager. The level of rat Siva mRNA in kidneys from rats subjected to ischemic injury was not significantly increased compared to that in kidneys from sham-operated rats at one hour post-injury. However, by 12 hours, the increase in levels of rat Siva mRNA was approximately 4.5-fold. Levels of rat Siva mRNA in kidneys from rats subjected to injury were elevated significantly ($P < 0.05$) compared to levels in kidneys from sham-operated rats at 12 hours, and one, five and seven days post-injury, but not at two days post-injury (Fig. 2).

Rat Siva mRNA expression in kidney was localized using *in situ* hybridization. The pattern of expression did not differ among sham-operated rats sacrificed at varying times post-surgery (not shown). Figures 3 and 4 show photomicrograph sections originating from the inner cortex and outer medulla of kidneys in sham-operated rats (Fig. 3D) or ischemic rats (Fig. 3 A-C, E, F) sacrificed at 12 hours (Fig. 3 A-C), one day (Fig. 4 A-C), five days (Fig. 3 D-F), or seven days (Fig. 4 D, E) post-surgery. Little or no hybridization is observed when a sense control probe is used at 12 hours (not shown) or five days post-ischemia (Fig. 3F). Little rat Siva mRNA is present in tubules of

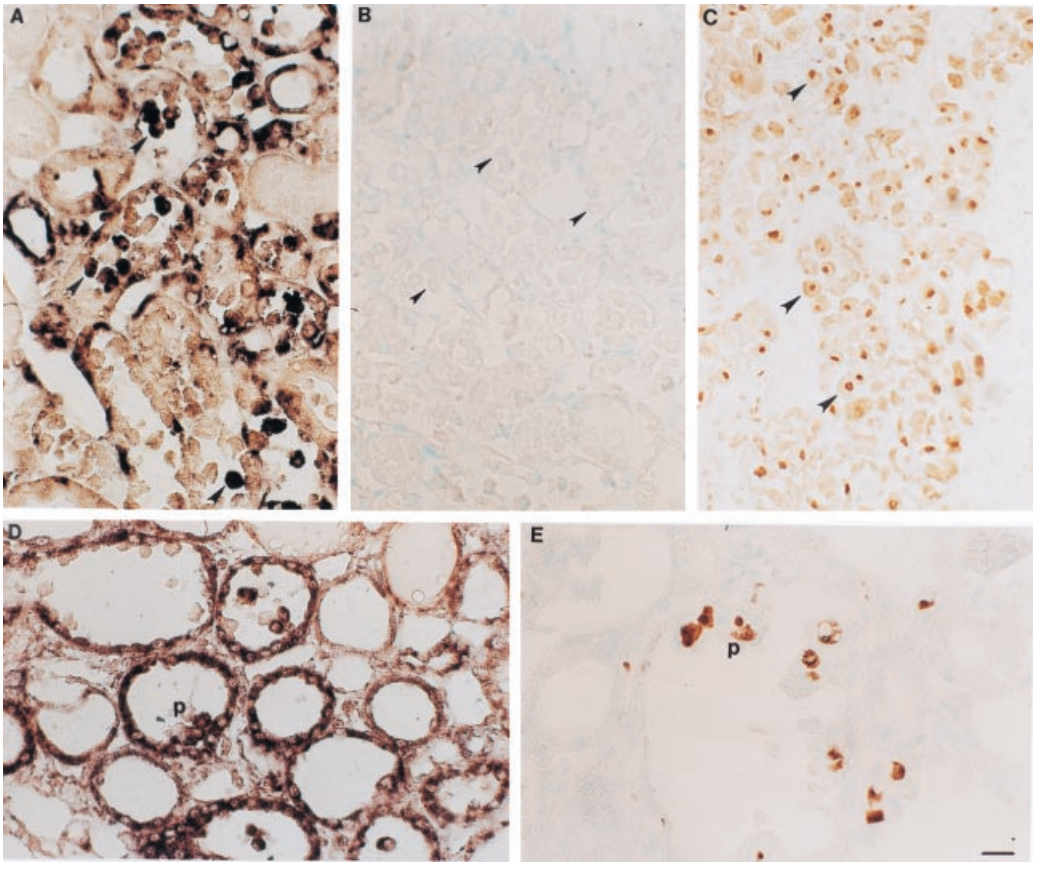


**Fig. 4. TUNEL staining and localization of renal Siva mRNA by non-isotopic *in situ* hybridization.** TUNEL staining was performed in kidneys from rats rendered ischemic one day (*B, C*) or seven days (*E*) prior to sacrifice. TdT was omitted in the experiment illustrated in B. Siva mRNA in kidneys from rats rendered ischemic one day (*A*) or seven days prior to sacrifice (*D*) was localized as in Figure 3. Arrowheads show cells sloughed within ischemic renal tubules (A-C). P, papillary proliferation (D, E). Non-serial sections are shown representative of > 5 experiments. Magnification (10 $\mu$m) is shown (E).

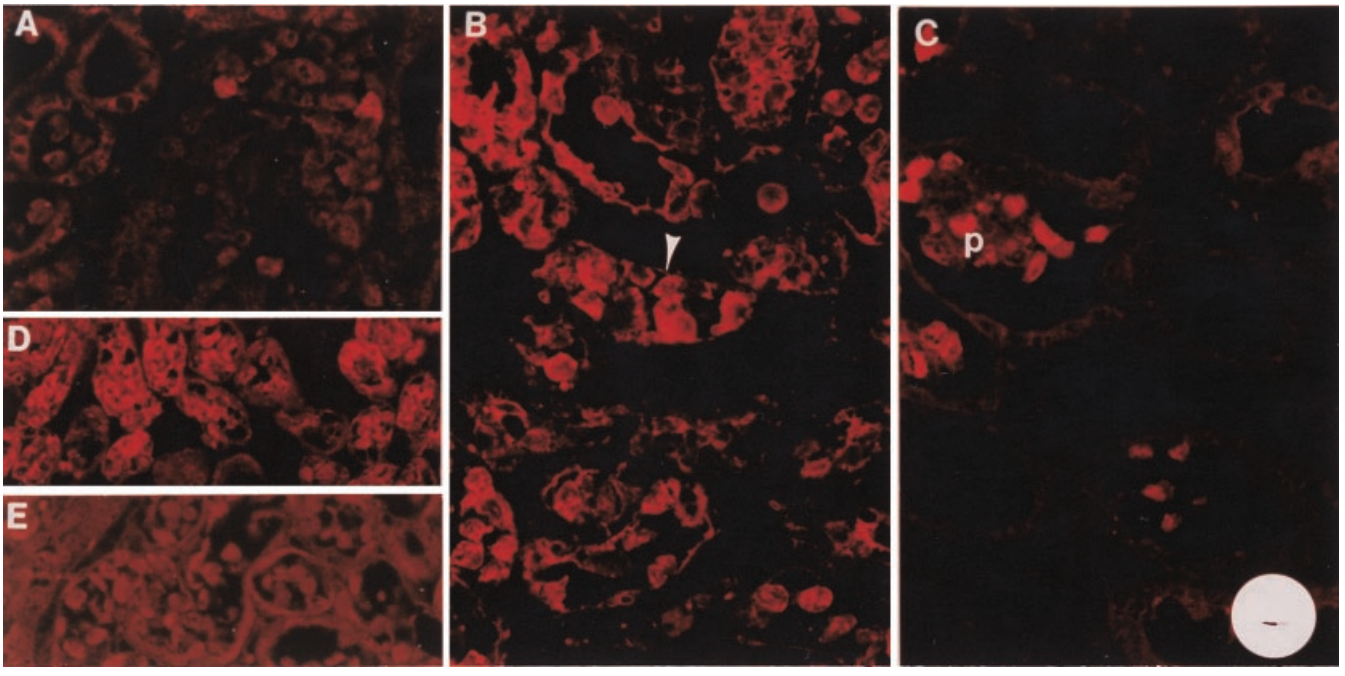


**Fig. 6. Immunohistochemical localization of CD27 in kidneys.** CD27 peptide in kidneys from rats rendered ischemic 12 hours (*A, B*) 24 hours (*D, E*) or five days (*C*) prior to sacrifice was localized using an antibody generated against CD27 (B, C), anti-CD27 together with scrambled peptide (D), blocking peptide (E) or control antibody (A). Arrowhead points out sloughed cells within the lumen of tubules (B). Papillary proliferations are shown (p) (C). Sections are representative of > 5 experiments. Magnification (10 $\mu$m) is shown (C).

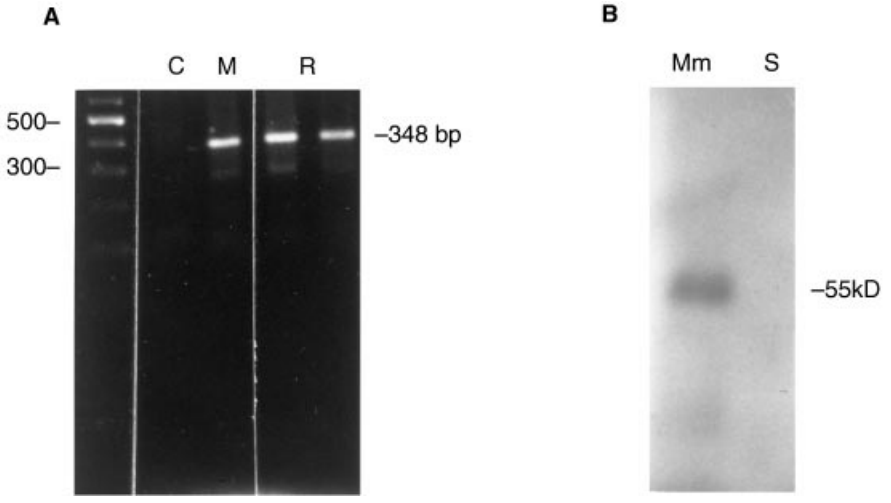


**Fig. 5. (*A*) Reverse transcribed-polymerase chain reaction (RT-PCR) of rat kidney RNA extract and (*B*) Western blot of rat kidney extracts.** (A) Size markers (left) and the size of the amplified CD27 cDNA product is shown (right). Abbreviations are: C, control (no reverse transcriptase); M, mouse kidney; R, rat kidney. (*B*) The size of the CD27 monomer is shown (right). Abbreviations are: Mm, membrane fraction; S, soluble extract.

kidneys from sham-operated rats (Fig. 3D). Rat Siva mRNA is localized to cells within ischemic tubules of the $S_3$ proximal segment at 12 hours post-injury (Figs. 3 A-C), and at one day post-injury (Fig. 4A) and in papillary proliferations (p) within regenerating tubules at five days post-injury (Fig. 3E), and seven days post-injury (Fig. 4D). In addition to papillary proliferations, rat Siva mRNA is expressed in simplified (flattened) epithelial cells lining dilated regenerating segments (Fig. 3E). Siva mRNA is expressed at no other locations in the kidney. A sense probe was used in Figure 3F.

In non-serial sections, TUNEL positive cells were present at the same locations as were cells staining for Siva mRNA that had been sloughed into ischemic tubules in the $S_3$ segment, or that were present in papillary proliferations. This is illustrated in photomicrographs of kidneys obtained from rats one day post-ischemia (Fig. 4 A, C) or seven days post-ischemia (Fig. 4 D, E). Figure 4B shows the results of a control experiment performed without TdT [12].

To determine whether CD27, the ligand for Siva, is expressed in rat kidney, we amplified cDNAs in reverse-transcribed extracts of mouse or rat kidneys using PCR and CD27-specific primers, and performed Western blots of rat renal extracts.

As shown in Figure 5A, CD27 mRNA was present in mouse (M) and rat (R) kidney extracts. The size of the amplified cDNA product (348 bp) was identical. No band was observed if reverse transcriptase was omitted from the reaction (C). Western blots of proteins extracted from rat kidneys showed antibody binding to a single 55 kD band in membrane (Mm), but not in soluble (S) fractions of rat kidney (Fig. 5B). These results show that CD27 is present in kidney as a transmembrane dimer composed of 55 kD monomers, as expected [13].

The localization of CD27 protein in kidney is shown in Figure 6. In sections originating in kidneys obtained 12 hours (Fig. 6B) and five days (Fig. 6C) post-ischemia, immunoreactivity is observed only in sloughed cells within the $S_3$ segments of the proximal tubule (Fig. 6B) and in papillary proliferations (p) (Fig. 6C). Unlike rat Siva mRNA (Fig. 3E), CD27 protein is absent from epithelial cells lining regenerating tubules (Fig. 6C). Figure 6 A, D, and E show tissue for which the control antibody was substituted for anti-CD27 (Fig. 6A), or tissue exposed to anti-CD27 together with CD27 scrambled peptide (Fig. 6D), or blocking peptide (Fig. 6E). Staining was inhibited by blocking peptide (Fig. 6E), but not scrambled peptide (Fig. 6D).

## DISCUSSION

Apoptosis is an unusual event in normal mature kidney. However, following induction of acute ischemic renal injury in the rat, apoptosis is observed in regenerating tubules [14, 15]. Apoptotic cells at the site of regeneration have been identified by light microscopy [14], electron microscopy [15] and, to our knowledge, for the first time using TUNEL-staining (Fig. 4).

Two peaks of apoptosis have been described following ischemia. The first peak coincides with a burst of proliferative activity that is maximal at two to three days post-injury [15]. Between days 3 and 6 post-ischemia, the percentage of cells undergoing apoptosis falls. However, the incidence of apoptosis increases again on day 7 and is maximal on day 8 following injury, after which the incidence again declines slowly over a period of weeks [15]. Hyperproliferation during days 2 to 3 results in a stratification of hyperplastic epithelial cells that is demonstrable two to four days post-injury and in a hyperplasia of circumferential epithelial cells with focal papillary projections that are evident at 5 to 7 days [15, 16]. Epithelial cells in papillary clusters desquamate into the tubular lumen in the early repair phase and this process appears to reflect the first peak of apoptosis. The hyperplastic tubules are returned to their original cellularity and papillary proliferations are remodeled through a wave of apoptosis that is reflected by the second peak [15].

The existence of a predictable pattern of apoptosis in kidney during recovery following ischemic injury indicates that this process is an important one. However, relatively little is known about its regulation. One approach to provide insight into its regulation post-ischemia is to define changes within kidney in the expression of factors known to regulate programmed cell death. We have shown that the expressions of the regulatory protooncogenes, bcl-2 and bax, are enhanced in regenerating $S_3$ segments following ischemic injury in the rat [16]. The changes in bcl-2 and bax expression that we describe post-ischemia may or may not control apoptosis in regenerating $S_3$ segments. However, bcl-2 is known to play a major anti-apoptotic role during kidney development [17]. Since the process of renal regeneration post-ischemia is similar in many ways to development, a role for bcl-2, and perhaps for the related protein, bax, in the former process would not be unexpected [16, 17].

Presently, we show that a rat homologue of the human gene Siva is expressed in the $S_3$ segment of proximal tubules and in papillary proliferations at times (12 hr to 1 day and 5 to 7 days post-injury, respectively) that roughly parallel the two peaks of apoptosis that occur at this site post-ischemia [15]. In contrast, enhanced expression is not observed at an intermediate time (2 days post-injury). CD27, a ligand for Siva, is expressed in injured kidney tissue at 12 hours and five days post-injury within sloughed cells (12 hr) and cells within papillary proliferations (5 days; Fig. 6). In each of these locations at each of these times, its ligand, Siva, is also expressed (Fig. 3). These are the cell populations known to be undergoing death via apoptosis or necrosis at these times (Fig. 4) [14–16]. Our findings are consistent with a role of Siva acting through CD27 in the apoptosis that occurs in the rat proximal tubule following ischemic injury, although they do not establish such a role.

Enhanced expression of members of the TNFR superfamily and ligands for these receptors occurs in ischemic kidney [3, 4] and in ischemic organs in addition to kidney. For example, hepatic ischemia/reperfusion injury in rats results in tumor necrosis factor (TNF) production in liver. Pretreatment of rats rendered ischemic with anti-TNF antiserum attenuates the injury [18].

Also in rats, TNF is expressed in neurons, glial cells, endothelial cells and infiltrating polymorphonuclear leukocytes within ipsilateral cerebral cortex, within four to six hours after permanent occlusion of the middle cerebral artery. In addition, within six hours of artery occlusion, the expressions of TNFR1 and TNFR2 are enhanced in neurons within ischemic cortex. Immunoreactive TNFRs are present in neurons undergoing apoptosis [19].

It is proposed that the relative overproduction of TNF amplifies and extends the severity of cell death during brain ischemia. Therapeutic implications for this proposal are demonstrated by the attenuation of brain damage post-ischemia following the intra-cerebroventricular administration of anti-TNF antibodies or receptor constructs [19]. As is the case for TNF-TNFR, antibodies have been generated against CD27 that modify effects of its ligation [20].

To our knowledge, ours is the first report of Siva and CD27 expression in kidney. The ligation of CD27 with Siva can induce apoptosis *in vitro* [2]. However, the the mechanism by which CD27 ligation with Siva induces apoptosis *in vitro* is unknown. Furthermore, the existence of CD27-Siva protein complexes *in vivo* and the role of Siva in CD27-mediated apoptosis *in vivo* are unproven. Our demonstration that Siva and CD27 are expressed in kidney following renal ischemia does not define a causative role of Siva-CD27 for renal apoptosis. Nonetheless, as is the case in brain for TNF and TNFRs, it is possible that strategies directed at modifying CD27-mediated renal apoptosis will impact positively on the course of acute ischemic renal injury. Their success will depend on an expanded understanding of the role that apoptosis plays in the regenerative process, as they could be aimed at accelerating the programmed death of damaged cells [21, 22], or at limiting it [22, 23].

## ACKNOWLEDGMENTS

B.J.P. and M.R.H. were supported by a grant from Bayer Corporation and by grant DK45181 from NIH. A.J.P.L. was supported by Barnes-Jewish Hospital, St. Louis, MO.

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