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Adult Mouse Kidneys Become Permissive to Acute Polyomavirus Infection and Reactivate Persistent Infections in Response to Cellular Damage and Regeneration

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Kidneys of newborn (but not adult) mice are normally highly permissive for polyomavirus (Py) infection and readily establish persistent infections. We have proposed that ongoing cellular differentiation, which occurs in newborn mice, may be necessary for a high level of in vivo Py replication (R. Rochford, J. P. Moreno, M. L. Peake, and L. P. Villarreal, J. Virol. 66:3287–3297, 1992). This cellular differentiation requirement may also be necessary for the reactivation of a persistent Py kidney infection and could provide an alternative to the accepted view that reactivation results from immunosuppression. To examine this proposal, the ability of adult BALB/c mouse kidneys to support primary acute Py infection or to reactivate previously established persistent Py infections after kidney-specific damage was investigated. Kidney damage was induced by both chemical (glycerol, cisplatin, or methotrexate) and mechanical (through renal artery clamping to produce unilateral renal ischemia) treatments. We also examined the effects of epidermal growth factor (EGF), which enhances the rate of kidney regeneration, on Py replication. Using histopathologic techniques, in situ hybridization for Py DNA, and immunofluorescence for Py VP1 production, we established that both chemical damage and damage through renal artery clamping of adult kidneys promoted high levels of primary Py replication in these normally nonpermissive cells. This damage also promoted the efficient reactivation of Py replication from persistently infected kidneys, in the absence of immunosuppression. EGF treatment significantly increased acute Py replication and also reactivation in damaged kidneys. These results support the view that ongoing cellular division and differentiation may be needed both for high levels of acute Py replication and for reactivation of persistent infections in vivo.

Murine polyomavirus (Py), like most DNA viruses, has an acute and persistent stage of infection. The acute stage involves a high level of Py replication in the lungs, skin, bone surfaces, salivary glands, and kidneys when mice are infected within 1 day after birth (17). The kidney of the newborn mouse is thought to be a main target for Py, and virus replication here peaks at 6 days postinfection and then rapidly declines to a persistent state during which nondefective, episomal viral DNA is stably maintained at about one copy per cell (17, 45). The precise mechanism of reactivation is not known, nor is it known whether various families of DNA viruses have common strategies or mechanisms for reactivation. A prevailing view is that immune suppression plays a major role in reactivation (for reviews, see references 20 and 41).

Although it has been known for some time that the kidney of the newborn mouse is much more permissive than adult kidney tissue for Py replication, few explanations for this observation have been proposed or tested. One such untested view is that newborn mice are more permissive because they are not fully immunocompetent and that in an adult a functional and rapid immune response limits the primary infection. That some adult tissues can be infected (mammary gland, skin, and bone) would appear to argue against this view, unless the putative rapid immune response can protect in an organ-specific manner (56). Also, a primary infection with Py shows maximal viral DNA replication at 6 days postinfection, before the Py-specific antibodies can be detected (17). In addition, kidneys of nude adult mice remain nonpermissive for Py replication, establishing that the T-cell component of the immune reaction does not contribute to the nonpermissive state of these kidneys (12). There is, therefore, reason to believe that the immune response may not be the main determinant which limits the ability of Py to replicate in adult kidneys.

A similar question of the involvement of the immune system in the reactivation of persistent Py infections can be raised. Although the reactivation of DNA virus production during immunosuppression has been seen after kidney (21, 22) and bone marrow (2) transplantations, during treatment for malignancies (37, 42, 51), in patients with AIDS (28), or in the case of kidney-tropic human Py (BK virus [BKV] and JC virus [JCV]) infection during pregnancy (11), there remain some difficulties with the association of reactivation to immunosuppression. It is puzzling, for example, that persistent BKV infections, which are highly prevalent in the human population, reactivate in only a fraction of immunosuppressed patients (1, 22, 29, 42). In addition, these reactivated infections will resolve after 2 to 3 weeks despite continued immune suppression (2). Also, BKV reactivation is far more common than JCV reactivation after bone marrow transplantation (2), yet both BKV and JCV remain latent in the kidneys after a primary infection (8). Furthermore, JCV, rather than BKV, is more commonly reactivated and excreted during pregnancy (11). Particularly striking, however, is the fact that the patients who show Py reactivation more frequently have conditions, such as diabetes mellitus and ureteral stricture, which are associated with renal damage and with loss of renal function, respectively

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(10, 29). Also, variously induced traumas to the DNA of latently infected tissue, such as UV light irradiation (for a review, see reference 55), are generally associated with reactivation of DNA virus production with no apparent immunosuppression. The results imply that some determinants of Py reactivation other than immunosuppression exist.

What then might restrict the replication of Py in adult kidneys and the reactivation of persistent infections? Some adult kidneys have been observed to replicate high levels of wild-type and enhancer-variant Py in tubular epithelial cells (45). In all of these cases, though, permissive adult mice were clinically ill, severely runted, and showed considerable histopathologic changes and cellular infiltration of the kidneys (45). The inflammation and cellular damage seen in these mice could, by killing cells, induce cellular regeneration and differentiation, possibly making these adult kidneys permissive for Py replication. We have accordingly proposed that ongoing cellular differentiation may be required for high levels of in vivo Py replication (39, 45, 53). If so, the permissiveness of newborn kidneys and lack of kidney (but not mammary, skin, or bone) permissiveness in most adult mice might also be explained. It is known that newborn mouse kidneys are relatively immature and actively differentiate tubule epithelial cells (7, 16, 30), whereas adult kidneys are rather quiescent (6). Thus, if in vivo Py replication requires ongoing cellular differentiation, most newborn (but not adult) kidneys would be permissive. Also, those organs of the adult mouse that remain permissive to acute Py replication, such as the mammary gland, skin, and bone (56), are all nonquiescent tissues that are continually renewing (34, 38, 50). A similar explaination could also apply to the reactivation of persistent Py infection of kidneys. The induction of kidney cell division and differentiation might also be needed for high-level reactivated Py replication. In this case, damage to persistently infected organs could lead to cell regeneration and differentiation to allow virus replication. Some evidence for this view does exist. For example, JCV is associated with progressive multifocal leukoencephalopathy (PML). Reactivation of JCV virus in the glial cells of the brains of patients with AIDS can lead to PML, but in these instances the PML is also associated with neuropathological findings which include intracerebral lesions and gliosis (28). Yet a high incidence of PML is not seen in patients who are immunosuppressed for other reasons (49).

There are, however, some problems with the above proposal. It has been well established that expression of Py or simian virus 40 large T-antigen induces cellular DNA synthesis in cell culture (18, 25). It has been proposed that this activity is involved in compelling the host cell to enter the S phase in preparation for the replication of virus (see reference 54 for early reviews). The view is, therefore, that T-antigen is a *trans*-dominant activator of cellular DNA synthesis. Why should it be required for cells to be already differentiating to replicate Py if T-antigen can induce cellular DNA synthesis? Yet T-antigen expression in vivo is not uniformly associated with increases in cellular or viral DNA synthesis. Transgenic mice can express Py T-antigen in various tissues (testes [3, 4], lens [27], and pancreas [40]), yet these tissues remain nonhyperplastic for months. Along these lines, we have observed that acute infection of newborn mice with Py does not appear to induce cellular DNA synthesis beyond that already present in the newborn (39), so it seems possible that some additional controls exist in vivo which may prevent the induction of cellular DNA synthesis by Py seen in culture. In addition, Py T-antigen expression alone is not sufficient to drive Py DNA replication, because Py DNA replication is often *cis* restricted by the tissue-specific enhancer sequences (43–46). Also, numerous cell lines which are capable of differentiating in culture (erythroblastoma, neuroblastoma, myoblasts, embryonal carcinoma, and stem cells) become much more permissive to Py replication after differentiation (13, 19, 26, 33, 35, 52), but all replicate Py poorly when cell division is high prior to terminal differentiation. Thus it seems possible that there is some general feature of the host cell's differentiation state which is also involved in high levels of Py replication and this feature could be an important determinant in vivo.

In this report we examined whether the in vivo permissiveness of adult kidney tubule epithelial cells both to high-level acute and persistent-reactivated Py replication depends on ongoing cellular regeneration and differentiation. To test this view, we damaged both healthy, uninfected adult mouse kidneys and healthy persistently infected mouse kidneys by injecting the nephrotoxic agents glycerol and cisplatin or the immunosuppressive agent methotrexate. We also induced ischemic kidney damage unilaterally through renal artery clamping (30) and examined the ability of these treatments to affect acute Py replication or reactivation of persistent infection. The effect on Py replication of the presence or absence of epidermal growth factor (EGF) (which enhances tubule DNA synthesis and regeneration [30]) was also examined. Both renal toxicity and renal artery clamping of adult mice kidneys resulted in high levels of acute Py replication and persistent Py reactivation, mainly in the areas and cell types of the kidneys which were most damaged. EGF often increased the fraction of cells permissive for high levels of Py replication.

MATERIALS AND METHODS

Persistently infected mice. BALB/c mice (Bailey strain) were obtained from Jackson Laboratories and bred in the University of California, Irvine, animal care facilities. Animals were maintained and handled in accordance with National Institutes of Health guidelines for laboratory animal care. Mice were persistently infected as newborns with Py after injection with 10^9 PFU of Py intraperitoneally (i.p.) as previously described (17). Only full-sized healthy-appearing mice were used for further analysis.

Timing of acute Py infection. To ensure that Py virus would be present to possibly infect actively regenerating cells, adult mice were inoculated with 10^9 PFU of Py by i.p. injection after kidney damage but 1 day before the maximum amount of cellular damage was induced by each treatment. Py replication was then assayed 6 days after infection. The time required for maximal kidney damage to be achieved by each treatment is indicated below under "EGF administration." Variations in the times after infection needed for maximal Py DNA replication confirmed that the 6-day time point gave maximal levels of Py DNA replication (data not shown).

Nephrotoxic agents and renal clamping. Uninfected adult mice older than 30 days and healthy, persistently infected mice were injected with 200 μl of 50% glycerol intramuscularly, 7 mg of cisplatin (i.p.) (Bristol Laboratories) per kg, or 80 mg of methotrexate (i.p.) (Sigma) per kg to induce various levels of severity of renal damage or with methotrexate to suppress the immune reaction (14, 47, 48). Unilateral ischemic renal injury was induced by clamping one renal artery for 30 min followed by profusion of the kidney (30).

EGF administration. EGF (20 μg; Sigma) was adminis-

tered i.p. to half the treated mice 1 day before the occurrence of maximum tissue damage. The times for maximum induced tissue damage were 2 days for glycerol, 4 days for cisplatin, 4 days for methotrexate, and 2 to 3 days for renal ischemia, as shown previously (14, 30, 32, 47).

Hematoxylin and eosin staining. After sacrifice of the mice, kidneys were removed by dissection and cryogenically protected by saturation in a solution of 19% sucrose in phosphate-buffered saline (PBS) at 4°C. The kidneys were embedded in optimal cutting temperature Tissue-Tek (OCT) from Miles, Inc., and cut into 10-μm sections. The sections were adhered to silane-treated slides by ethanol fixation for 5 min before being stained with hematoxylin and eosin.

In situ hybridization. Py DNA probe was chemically labeled with horseradish peroxidase obtained from Digene, Silver Spring, Md. Mouse kidneys, removed by dissection, were cryostatically protect by saturation in 19% sucrose in PBS overnight at 4°C. The kidneys were then embedded in OCT and sectioned into 10-μm slices onto silane-treated slides. The sections were fixed and permeabilized by 100% ethanol for 5 min and then were air dried. In situ hybridization of the horseradish peroxidase-conjugated Py probe and color development of the substrate diaminobenzidine were performed according to the protocol from Digene, as described by us previously (45).

Detection of VP1 by immunofluorescence. Mouse kidneys, removed by dissection, were cryostatically protect by saturation in 19% sucrose in PBS overnight at 4°C. The kidneys were then embedded in OCT and cut into 10-μm sections onto silane-treated slides. The sections were fixed and permeabilized by 100% methanol for 15 min, followed by rehydration in PBS for 10 min. One percent bovine serum albumin was applied for 15 min to block nonspecific adsorption. The primary antibody to VP1 was a rabbit anti-mouse antibody, a gift from Robert Garsea, Harvard University, and was applied to the section in a 1:200 dilution overnight in a humidified chamber. One percent bovine serum albumin was used as a blocker and was applied to the sections for 15 min. The slides were rinsed in PBS. The secondary antibody was a goat anti-rabbit immunoglobulin G conjugated to fluorescein isothiocyanate and was applied in a 1:100 dilution.

RESULTS

Cytopathology of treated, persistently infected kidneys in the presence of EGF. Hematoxylin and eosin staining of kidney sections after the various damage-inducing treatments is shown in Fig. 1. The unclamped kidney tissue shown (panel A) was from a Py-infected but nonpermissive adult mouse. This section is indistinguishable from that of uninfected mouse kidney or from persistently infected healthy adult mice and served as the noncytopathic control for the appearance of normal adult kidney tissue (45). The sections shown in Fig. 1 were from acute infected mice, but very similar or identical histologic features were seen from similar sections from persistently infected mice (not shown). The most cellular damage and necrosis was seen with ischemia (panel B). Glycerol treatment also induced considerable necrosis and tissue damage (panel C). Hypertonic glycerol causes tubular necrosis by inducing rhabdomyolysis, myoglobinuria, and renal ischemia (48). Both clamping and glycerol treatments induced cytopathologic changes in both the cortical and medullary tubules. Although cisplatin is nephrotoxic to the cortical tubules and interstitial cells, only slight damage was visible (panel D). Cisplatin-treated kidneys have been shown to have toxicity confined to the tubules in the cortex, as well as the interstitial cells, so a less damaged appearance relative to glycerol-treated kidneys is not unexpected (31). As expected, little if any pathologic changes were seen after methotrexate treatment (36) (panel E).

In situ hybridization for Py DNA. Py DNA replication was examined by in situ hybridization, as shown in Fig. 2. Figure 2 is from a persistently infected mouse, but identical in situ hybridization results were obtained from acutely infected mice (not shown). As expected, no Py replication was seen in the unclamped, Py-inoculated, EGF-treated adult kidney (Fig. 2A) (45). In contrast, a high level of Py replication was seen in the ischemic kidney of the same animal (panel B). A number of tubular epithelial cells in both the cortex and medulla showed high levels of Py DNA by in situ hybridization, establishing that an asymmetric acute Py infection or reactivation had occurred only in the clamped kidney. The locations of Py DNA replication appeared to correspond to the cell type and regions of the kidney undergoing regeneration. In addition, the high level of Py replication seen in the tubular epithelial cells of this ischemic kidney is similar to that seen during the acute phase of kidney infection in newborn mice (39, 45). Both primary Py DNA replication and reactivation were also seen at high levels in the tubules in the cortex and medulla of the kidneys of the glycerol-treated mice (panel C). Lower, but still significant levels of Py DNA were seen in the cortical tubules and interstitial cells after cisplatin administration (panel D), although in some experiments, a high level of Py replication occurred (data not shown). With the administration of methotrexate, which is an immunosuppressive agent with little nephrotoxicity, only a very few cells showed evidence of primary Py infection or reactivation of persistent Py infection (panel F).

We had previously established that most healthy persistently infected kidneys will have very low levels of Py DNA (about 1 copy per cell) (17, 45). This level is below the level of sensitivity of our in situ hybridization technique (greater than 10 copies per cell), and as expected, no Py DNA was detected in the unclamped persistently infected kidney (Fig. 2A) (45).

Quantitation, cell type specificity, and EGF dependence of Py replication. Cells positive for Py DNA by in situ hybridization appeared in regions corresponding to the regions of maximum histopathologic change for each treatment. Because the histopathologic changes were focal, positive cells were counted within regions showing damage and tabulated as shown in Tables 1 and 2. One cluster of cells positive for hybridization represents two or more adjacent positive nuclei within one 100× field. As a control, EGF treatment alone, without ischemia or chemical damage, was also examined. Cells positive after acute Py infection with and without EGF are shown in Table 1. EGF treatment with Py inoculation in the absence of any renal damage was also tabulated as a control. No centers of Py replication were observed in the histologically normal undamaged but Py-inoculated adult mouse kidney. In contrast, a high number of Py replication centers were seen in the ischemic kidney from the same mouse, as well as in toxically injured kidneys from the other treatments in other mice. The numbers of replication centers were considerably increased by EGF treatment after renal artery clamping; glycerol or cisplatin induced injury but methotrexate was much less efficient. The effect of EGF was often cell type specific and varied with the type of damage. EGF significantly increased the fraction of cells permissive for high levels of Py replication in the medulla of

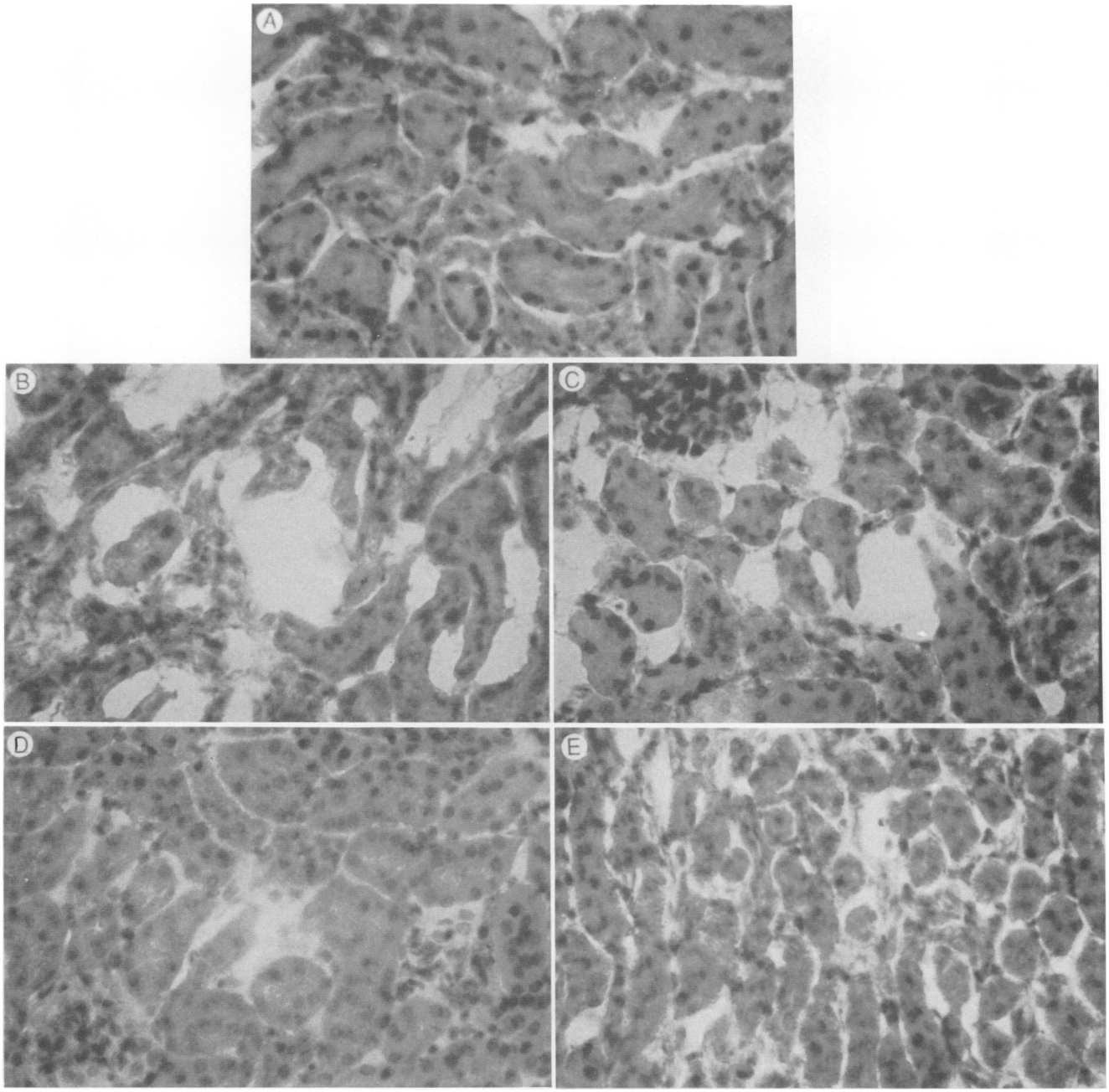

FIG. 1. Hematoxylin and eosin (40× objective) staining of kidney sections from a mouse persistently infected with Py, which had been treated with EGF. Shown is the right kidney (A) from a mouse which underwent unilateral renal ischemia in the left kidney (B). Sections of kidney from persistently infected mice treated with glycerol (C), cisplatin (D), and methotrexate (E) are also shown.

the glycerol-treated kidney and the clamped kidney, while having no effect on the cells in the medulla of the cisplatin- and methotrexate-treated kidneys. In addition, EGF increased Py replication in the cortex of cisplatin- and glycerol-treated kidneys as well as in the clamped kidney.

Table 2 shows the effect of kidney damage and EGF treatment on the reactivation of Py DNA replication from persistently infected mouse kidneys. No reactivation of Py replication was seen in the absence of either ischemia or toxic injury, regardless of EGF administration. Reactivation was clearly apparent after both ischemic and toxic renal injury. The cell type specificity for Py reactivation, as for the acute infection, varied with the specific treatment. Glycerol-treated and clamped ischemic kidneys both showed the tubule epithelial cells to be permissive and the interstitial cells to be nonpermissive in both the cortex and medulla. With cisplatin-treated mice, tubular and interstitial cells which were permissive for reactivated Py replication were primarily in the cortex and the outer strip of the outer medulla. Reactivation, as in the acute infection, was often focal. As in the acute infection, the number of positive replication centers was significantly increased by EGF treat-

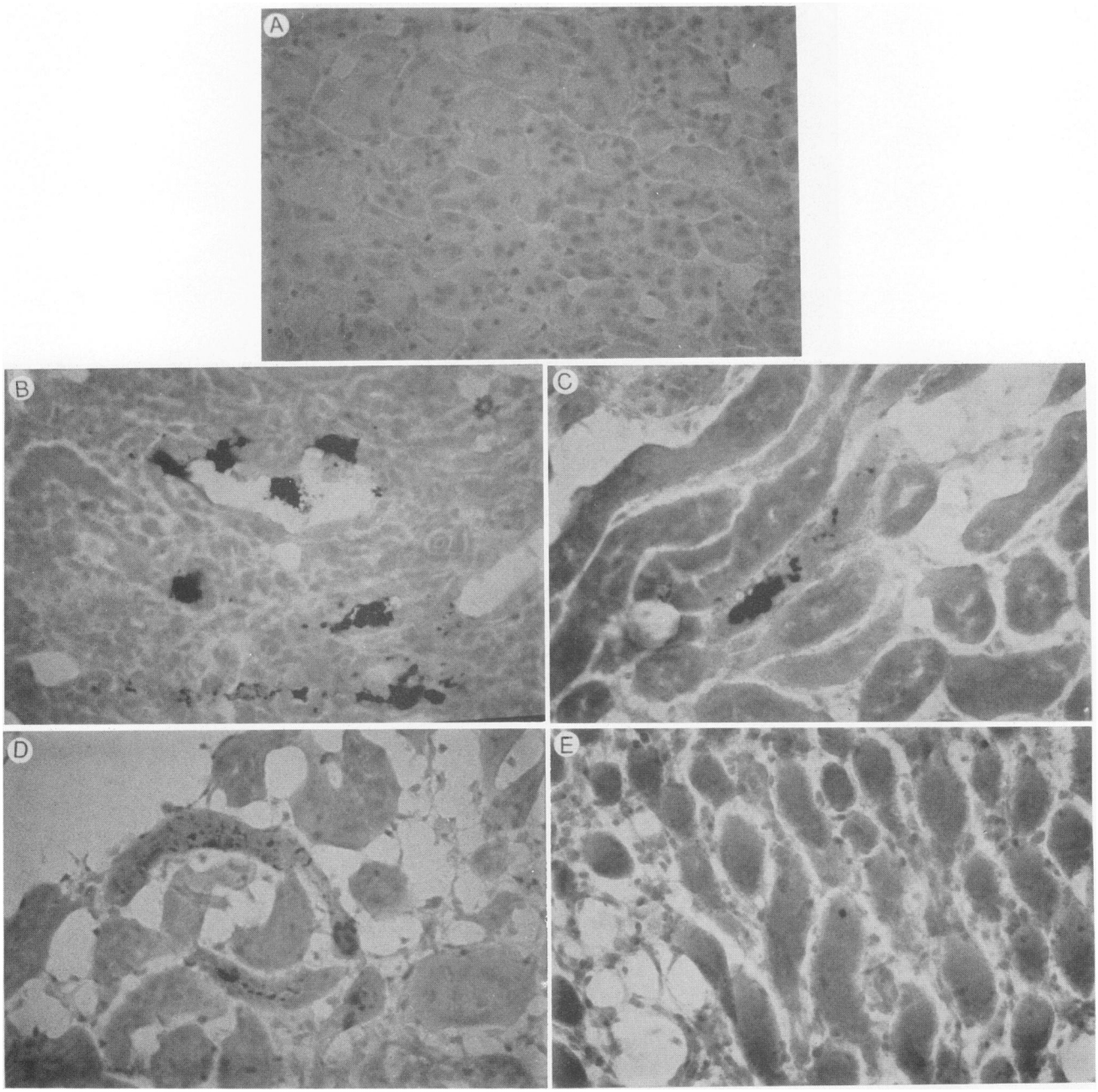

FIG. 2. In situ hybridization (40× objective) and diaminobenzidine staining of kidney sections from a mouse persistently infected with Py, which had been treated with EGF. Shown is the right kidney (A) from a mouse which underwent unilateral renal ischemia in the left kidney (B). Sections of kidney from persistently infected mice treated with glycerol (C), cisplatin (D), and methotrexate (E) are also shown.

ment following ischemia or cisplatin- or glycerol-induced injury but differed as to whether the medulla (renal ischemia or glycerol treatment) or cortex (renal ischemia or cisplatin or glycerol treatment) was involved. The greatest levels of Py reactivation we observed were in the cortex of mice treated with cisplatin plus EGF. These same mice had relatively little reactivation in the medulla of the kidney. In the ischemic model, reactivation was roughly equal in the medulla and cortex and was also enhanced by EGF. Likewise, administration of EGF increased reactivation in both the cortex and medulla in the glycerol-treated renal injury model. Methotrexate treatment induced the least reactivation, albeit above background levels, with little or no EGF effect.

VP1 detection by immunofluorescence. To determine whether the acute and persistent reactivation of high levels of Py DNA replication which we observed were also leading to virus production, equivalent to those sections shown in Fig. 1, sections from nephrotoxin-treated and clamped kidneys were stained with fluorescent antibody against VP1, as described in Materials and Methods. The results are shown in Fig. 3. The kidney sections shown in Fig. 3 are from

TABLE 1. Effect of kidney damage on acute Py replication in adult mouse kidneys

Treatment[a]	No. of positive signals in[b]:		No. of clusters in[c]:	
	Cortex	Medulla	Cortex	Medulla
PY −EGF	0	0	0	0
PY +EGF	0	0	0	0
PY +IC −EGF	20	12	5	3
PY +IC +EGF	80	276	20	23
PY +G1 −EGF	53	20	2	3
PY +G1 +EGF	55	345	11	23
PY +CP −EGF	38	3	8	1
PY +CP +EGF	63	5	15	2
PY +MT −EGF	3	5	1	2
PY +MT +EGF	3	0	1	0

[a] Shown are the effects on Py replication of renal ischemia by renal artery clamping (IC) or by glycerol (G1), cisplatin (CP), or methotrexate (MT) treatments.
[b] Number of cells positive for in situ hybridization in each of the treatments.
[c] Clusters represent two or more cells positive for in situ hybridization within the microscopic field (100×).

persistently infected mice, but identical results were obtained from acutely infected mice (not shown). The production of VP1 was confirmed and correlated with the areas and cell types of the kidney sections seen by in situ hybridization, indicating that complete virus production also occurred in these mice.

DISCUSSION

We have previously proposed that permissive in vivo infections of mouse kidneys with Py require that the host tubule epithelial cells may need to be actively differentiating or regenerating (39, 45, 53). This proposal implies that in vivo (as opposed to in cell culture) acute infection with Py may not compel host cell division in preparation for Py replication and that exogenous stimulation may be needed to make kidney cells permissive. In this report, we have examined a direct prediction of this proposal and induced kidney injury with various agents to allow regeneration of tubular epithelial cells. Such a situation should make the kidney cells permissive for Py replication or reactivation if host cell division and differentiation are required.

TABLE 2. Effect of kidney damage on reactivation of persistently infected adult mouse kidneys

Treatment[a]	No. of positive signals in[b]:		No. of clusters in[c]:	
	Cortex	Medulla	Cortex	Medulla
−EGF	0	0	0	0
+EGF	0	0	0	0
+IC −EGF	53	16	4	3
+IC +EGF	73	60	8	5
+G1 −EGF	15	7	2	1
+G1 +EGF	108	102	10	9
+CP −EGF	106	5	5	2
+CP +EGF	143	5	19	2
+MT −EGF	4	5	2	1
+MT +EGF	9	5	4	1

[a] Shown are the effects on Py replication of renal ischemia by renal artery clamping (IC) or by glycerol (GI), cisplatin (CP), or methotrexate (MT) treatment.
[b] Number of cells positive for in situ hybridization in each of the treatments.
[c] Clusters represent two or more cells positive for in situ hybridization within the microscopic field (100×).

Damaged adult kidneys become permissive to Py replication. In the absence of any kidney damage, none of the examined cells in adult mice was permissive for high levels of acute Py replication, consistent with the normally nonpermissive nature of these cells. Acute Py replication of adult kidney cells appeared to occur only after renal cellular damage and regeneration. In the ischemic animal, Py replication was unilateral in only the clamped, ischemic kidney and not in the normal kidney. The ischemic mouse model results appear to be strong evidence for a primary role of cellular division and differentiation in making host cells permissive for high levels of Py replication. Although the uremia which may follow renal damage could subsequently suppress T-cell immunity, uremia is absent in the ischemic model and any possible immunosuppression should not be unilateral. Also, there was a rough correlation between the types of renal cells injured by each treatment (medullary and cortical tubules for glycerol treatment and renal artery clamping and cortical and interstitial cells for cisplatin treatment) and the cells which became permissive for high levels of Py replication. Cisplatin has been shown in mice to produce morphologic alterations of the S1, S2, and S3 segments of the proximal tubule as well as interstitial fibrosis (31). We observed both tubular and interstitial cells permissive for Py replication, primarily in the cortex and the outer strip of the outer medulla, after cisplatin treatment. In newborns, Py usually replicates in the tubular epithelial cells, not interstitial cells (45), so it appears that cisplatin induced adult interstitial cells to be permissive. It may be that the variability in cell type effects and EGF effects is related to the susceptibility of specific cells to the specific treatments employed. Glycerol treatment and ischemic renal injury both affect the tubule epithelial cells more than interstitial cells in both the cortex and medulla (30, 48), which corresponded to where cells allowed Py replication. That treatment with methotrexate, a potent immunosuppressive agent with little nephrotoxicity, yielded only a very small number of cells permissive for Py also argues against the view that the immune response restricts the permissiveness of adult kidneys to Py replication.

The role of EGF in renal cellular differentiation is inferred both by the role it plays in enhancing tubular cell regeneration after renal insult (30) and by the lack of EGF and tubular cell terminal differentiation in polycystic kidney disease of mice (23, 24). Polycystic kidneys have proximal tubular enlargement with an increase in the tubule mitotic index during the first week after birth, and epithelial hyperplasia is characteristic of this disease (23), implying a role for EGF in kidney epithelial maturation not mitosis. In vitro, the terminal differentiation, but not growth, of normal urothelial cells is also EGF dependent (15). In the ischemic mouse model, EGF has been shown to enhance the regeneration of tubule cells primarily in the medulla (30), which is where we observed the greatest increase in the fraction of cells permissive for Py after EGF treatment. A strong EGF effect on

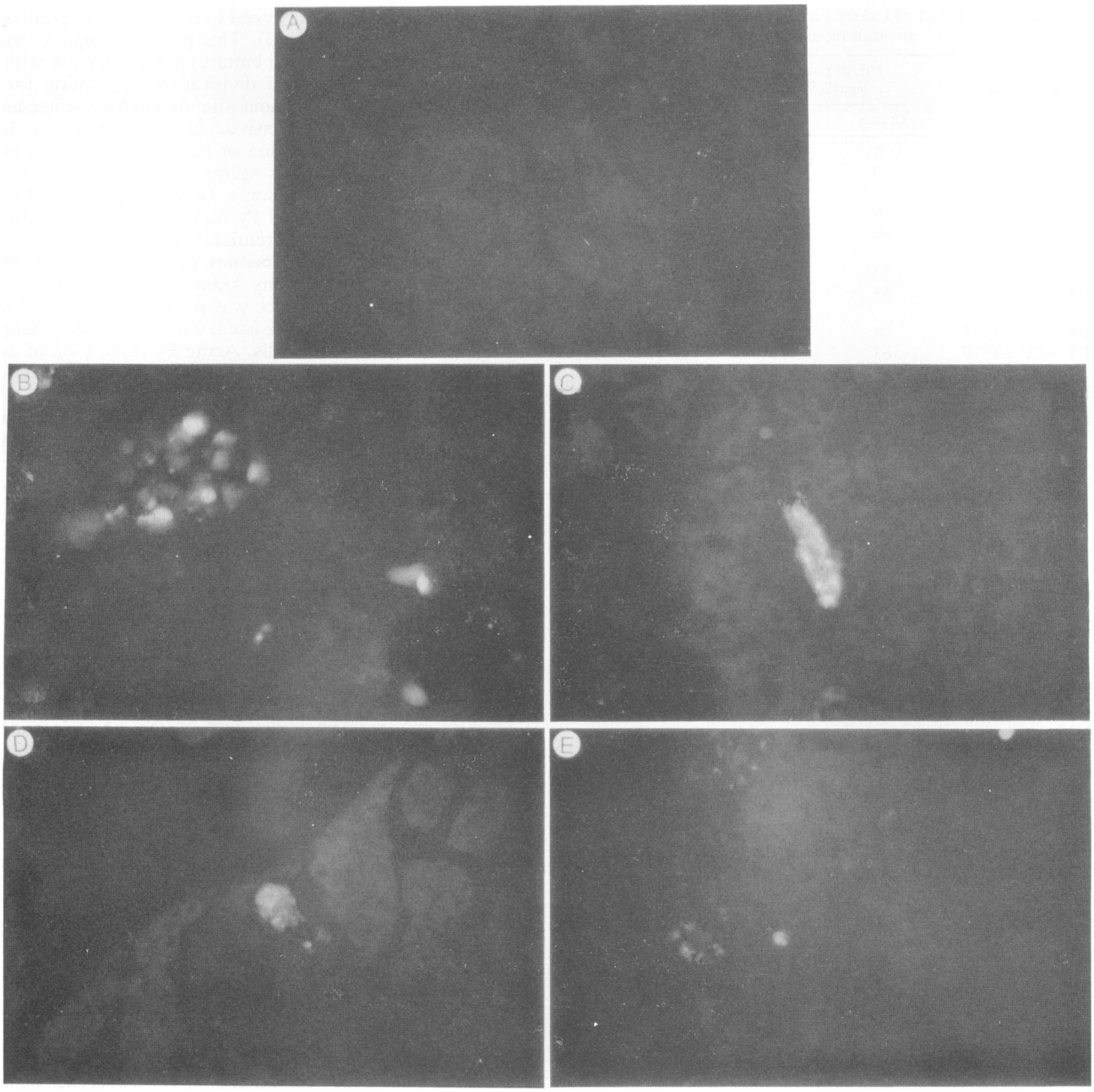

FIG. 3. Immunohistochemistry (40× objective) for VP1 production in kidney sections from a mouse persistently infected with Py, which had been treated with EGF. Shown is the right kidney (A) from a mouse which underwent unilateral renal ischemia in the left kidney (B). Sections of kidney from persistently infected mice treated with glycerol (C), cisplatin (D) and methotrexate (E) are also shown.

acute Py replication by these specific cell types after renal damage and differentiation adds further support to the idea that Py replication becomes permissive in cells in response to cellular differentiation.

These results, if they can be generalized, may also explain why newborn mouse kidneys are permissive to Py replication. The first week after birth, mouse kidneys are immature and produce actively differentiating tubule epithelial cells. Dubensky et al. have in fact reported that newborn kidneys have a notable ridge of tubular precursor cells that differentiate into tubule epithelial cells and that this ridge is also the most active site for Py DNA replication (16). In the adult mouse, those tissues that remain permissive to Py replication (skin, bone, and female mammary gland) also maintain an elevated rate of differentiation relative to the kidney and salivary gland. These observations are therefore consistent with ongoing host cell division and differentiation being necessary for in vivo Py replication.

Damaged adult kidneys reactivate persistent Py infections. If the lack of cellular regeneration and differentiation restricts acute Py replication, it seems logical to also expect a similar restriction on the reactivation of persistent Py infections. Although it has long been thought that immunosuppression was the most important determinant in restricting

Py reactivation, there has been very little direct examination of this view. The very same treatments that damaged the adult mouse kidney and made it permissive to acute Py replication were seen by us to also lead to efficient reactivation of persistent infections. Thus, reactivation of persistent infections appears to be under similar restrictions as the acute adult kidney infection. That Py reactivation in the ischemic mouse was unilateral to only the ischemic and not the normal kidney of the same animal appears to be strong support for the view that cellular regeneration and differentiation and not depressed immunity are the more important determinants of reactivation. Moreover, treatment with methotrexate, a potent immunosuppressive agent with little nephrotoxicity, also yielded the smallest number of cells which reactivated Py, further arguing against an immune restriction to Py reactivation.

The generality of our Py results to other persistent viral infections is currently unknown. It has been established that papillomavirus DNA replication and virus production also appear to be activated from a low-copy episomal state to a high level of DNA replication with the terminal differentiation of basal cells to keratinocytes, so a relationship of DNA viral replication to host cell differentiation is not unique to Py (5, 9). That wild-type Py replication is also induced by the terminal differentiation of keratinocytes (2a), myoblasts (19, 35), erythroblasts (13), neuroblasts (13), embryonal carcinoma cells (33, 52), and embryonal stem cells (26) may indicate a general relationship between host cell differentiation (but not necessarily mitosis) and the activation of Py and other DNA virus replication. If so, this has important implications for the management of persistent infections by Py and possibly other DNA viruses, because conditions which lead to cellular damage and subsequent regeneration could be most important for the reactivation of persistent or latent DNA viruses. We have proposed a theoretical framework for this relationship (53) and suggest that our current results support such a linkage of host cell division and differentiation to Py replication.

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