Activation of Extracellular Signal-regulated Kinase in Proliferative Glomerulonephritis in Rats

Dirk Bokemeyer, Kelly E. Guglielmi, Ann McGinty, Andrey Sorokin, Elias A. Lianos, and Michael J. Dunn
Department of Medicine, Division of Nephrology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Abstract

Multiple extracellular mitogens are involved in the pathogenesis of proliferative forms of glomerulonephritis (GN). In vitro studies demonstrate the pivotal role of extracellular signal-regulated kinase (ERK) in the regulation of cellular proliferation in response to extracellular mitogens. In this study, we examined whether this kinase, as a convergence point of mitogenic stimuli, is activated in proliferative GN in vivo. Two different proliferative forms of anti–glomerular basal membrane (GBM) GN in rats were induced and whole cortical tissue as well as isolated glomeruli examined using kinase activity assays and Western blot analysis. Administration of rabbit anti-rat GBM serum to rats, preimmunized with rabbit IgG, induced an accelerated crescentic anti-GBM GN. A significant increase in cortical, and more dramatically glomerular ERK activity was detected at 1, 3, and 7 d after induction of GN. Immunization of Wistar-Kyoto rats with bovine GBM also induced a crescentic anti-GBM GN with an increase of renal cortical ERK activity after 4, 6, and 8 wk. ERK is phosphorylated and activated by the MAP kinase/ERK kinase (MEK). We detected a significant increase in the expression of glomerular MEK in the accelerated form of anti-GBM GN, providing a possible mechanism of long-term activation of ERK in this disease model. In contrast to ERK, activation of stress-activated protein kinase was only detectable at early stages of proliferative GN, indicating these related kinases to serve distinct roles in the pathogenesis of GN. Our observations point to ERK as a putative mediator of the proliferative response to immune injury in GN and suggest that upregulation of MEK is involved in the long-term regulation of ERK in vivo. (*J. Clin. Invest.* 1997. 100:582–588.) Key words: inflammation • proliferation • MAP kinase • ERK • MEK

Introduction

Cellular proliferation accompanies a wide variety of renal diseases. This is especially true for proliferative forms of glomerulonephritis (GN).[1] Renal mesangial cells, glomerular epithelial cells as well as leukocytes are thought to account for the hypercellularity in proliferative GN. A major focus for research into the pathogenesis of GN has been to define extracellular growth promoting factors that induce mitogenesis in the glomerulus in the course of GN. Several cytokines have been implicated in the pathogenesis of proliferative GN and are thought to account for hypercellularity. Cytokines implicated include: PDGF, TGF-β, IL-1, IL-6, and TNF (1). A common feature of these stimuli is their ability to activate extracellular signal-regulated kinase (ERK) in vitro in a variety of cell lines (1–7). Since ERK is known to be an intracellular check point of cellular mitogenesis, ERK activation might be a crucial mediator of cellular proliferation in renal diseases mediated by the extracellular stimuli listed above.

ERK, the best described mammalian mitogen-activated protein (MAP) kinase, belongs to the group of serine/threonine kinases and regulates the expression of many genes by phosphorylation of several transcription factors namely c-*myc*, NF-IL6, Stat, c-Jun, ATF2, and ELK-1 (8–11). Binding of extracellular stimuli to G-protein coupled receptors or protein-tyrosine kinase receptors induces the formation of GTP-Ras, which in turn induces the sequential activation of cytoplasmic protein kinases leading to phosphorylation and activation of MAP kinase/ERK kinase (MEK) (9). MEK, the specific activator of ERK, is a dual specificity protein kinase that phosphorylates both threonine and tyrosine regulatory sites in ERK (12). Studies that use dominant interfering mutants or constitutively active mutants of kinases of the ERK cascade established the pivotal role of this signaling pathway in the control of cellular proliferation (13–16). Although an extensive body of data describes the ERK cascade in vitro little is known about the role of ERK in physiologic or pathophysiologic conditions or its activation in vivo.

In the present study, we examined the activation of ERK in proliferative GN in rats and in addition investigated potential mechanisms of its regulation. We demonstrate a rapid and sustained activation of ERK in an accelerated heterologous as well as in an autoimmune model of anti-GBM antibody-induced crescentic GN and show an upregulation of MEK protein levels in the former. This upregulation of the upstream kinase of ERK might be an underlying mechanism to the observed activation of ERK. Therefore, the present study provides new insights into the role of ERK in vivo and suggests that activation of the ERK cascade is linked to the cellular proliferation accompanying crescentic GN.

Address correspondence to Michael J. Dunn, M.D., The Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226-0509. Phone: 414-456-8213; FAX: 414-257-0449. The present address of Dr. Bokemeyer is Medizinische Poliklinik, University of Bonn, Germany.

Received for publication 24 October 1996 and accepted in revised form 22 April 1997.

J. Clin. Invest.

0021-9738/97/08/0582/07 $2.00
Volume 100, Number 3, August 1997, 582–588
http://www.jci.org

1. *Abbreviations used in this paper:* ERK, extracellular signal-regulated kinase; GBM, glomerular basal membrane; GN, glomerulonephritis; MAP kinase, mitogen-activated protein kinase; MEK, MAP kinase/ERK kinase; MKP-1, MAP kinase phosphatase-1; SAPK, stress-activated protein kinase.

Methods

Materials. The enhanced chemiluminescense system was obtained from Amersham Corp. (Arlington Heights, IL) and [^{32}P]dCTP from New England Nuclear (Boston, MA). Myelin basic protein, complete Freund's adjuvant, PMSF, leupeptin, aprotinin and all other reagents were obtained from Sigma Chemical Co. (St. Louis, MO). Protein A-sepharose was obtained from Pharmacia Biotech. Inc. (Piscataway, NJ). GST-ATF2 (1-55) was purchased from Santa Cruz Biotechnologies, Inc. (Santa Cruz, CA).

Induction of accelerated proliferative GN. Male Sprague-Dawley rats weighing 180–200 g were immunized intraperitoneally with 1 mg rabbit IgG emulsified in complete Freund's adjuvant and given as a total volume of 0.5 ml. 5 d after this immunization, animals were injected in the tail vein with a subnephritogenic dose of rabbit immune serum raised against rat particulate GBM (17). This dose of anti-rat GBM serum (0.15 ml/100 g body wt) was insufficient by itself to cause significant proteinuria when given to rats not preimmunized with rabbit IgG (subnephritogenic). The intravenous injection of the anti-rat GBM serum was repeated 24 h after the first injection. The rabbit anti-rat GBM immune serum was heat inactivated and filtered through 1.2-μm filters before each intravenous injection. Control rats were preimmunized with rabbit IgG in complete Freund's adjuvant and subsequently given two intravenous injections of nonimmune rabbit serum. Studies were performed on days 1, 3, and 7 following the second injection of anti-rat GBM serum or of nonimmune rabbit serum. The day before killing animals were placed in metabolic cages for urine collection to assess urinary protein excretion. Upon completion of this collection, animals were killed and nephrectomized. A renal cortical section was obtained for light microscopy using routine methods of fixation and processing. Another cortical section was directly lysed in Triton X-100 buffer while the rest of the cortical tissue (~ 80% of the whole cortex) was used to generate a preparation of glomeruli using standard sieving methods (18) before lysis in Triton X-100 buffer.

Leukocyte depletion studies. Glomerular leukocytes were depleted by the use of a whole animal x-irradiation protocol as previously described (19). Specifically, animals were subjected to 250 Kvp orthovoltage x rays with a half value of 1 mm Cu at a dose rate of 133 rad/min for a total dose of 900-1100 rad using parallel opposed fields. Kidneys were shielded with 6-mm thick lead blocks that covered the kidneys within 5-mm margins. Dosimetry was done in a plexiglass phantom using a Farmer-type ionization chamber. The effect of x-irradiation was assessed on peripheral leukocyte counts, determined by an automated hematology analyzer, 2 and 5 d after x-irradiation. After preimmunization, as described above, rats were x-irradiated 24 h before the first injection of anti-GBM antiserum or normal rabbit serum. Thereafter, animals were treated as described above.

Induction of an autoimmune model of proliferative GN. Male Wistar-Kyoto rats weighing 260–300 g were subcutaneously injected at multiple sites over the posterior neck with 130 mg wet wt of bovine GBM homogenate (20), diluted to a volume of 0.4 ml in 0.9% normal saline, and then emulsified with 0.4 ml complete Freund's adjuvant to a total volume of 0.8 ml. The rats were subsequently injected intradermally at a separate site with 50 μl of pertussis toxin (5×10^9 cells). Control animals received the injections of complete Freund's adjuvant, without bovine GBM, and of the pertussis vaccine. Rats were killed 4, 6, and 8 wk postinjection and whole cortical tissue processed as described above.

Western blot analysis. Whole cortical tissue or isolated glomeruli were homogenized in 2 ml of Triton X-100 lysis buffer (50 mM Hepes pH 7.5, 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 10% glycerol, 1% Triton X-100, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM PMSF, 0.1 mM sodium orthovanadate) at 4°C. After incubation for 5 min, lysates were centrifuged at 4°C for 15 min at 10,000 *g*. The soluble lysates were mixed 1:4 with 5× Laemmli buffer and heated for 5 min at 95°C. Soluble lysates (30 μg) were loaded per lane and separated by SDS-PAGE using 4 and 10% acrylamide for stacking and resolving gels, respectively. Protein was transferred to nitrocellulose (pore size: 0.45 μm, Schleicher and Schuell, Keene, NH) and probed with polyclonal antibodies against the COOH-terminal peptide of either p42 ERK (21), p46 SAPK (Santa Cruz Biotechnology) or with monoclonal antibodies against MEK-1 (Transduction Laboratories, Lexington, KY). The primary antibodies (diluted 1/1,000) were detected using horseradish peroxidase (HRP)–conjugated rabbit anti-mouse IgG or HRP-conjugated protein A and visualized by Amersham ECL system after extensive washing of the membranes.

Kinase activity assays. 200 μg of soluble lysates (as described above) was incubated for 90 min with 2 μl of polyclonal antibody recognizing p42 ERK (21) or 2 μg of polyclonal antibody recognizing p46 SAPK. Immune complexes were adsorbed to protein A-sepharose, washed twice with lysis buffer and twice with kinase buffer (10 mM $MgCl_2$, 20 mM Hepes, pH 7.4, containing 200 μM Na-orthovanadate) and resuspended in 60 μl of kinase buffer containing 50 μM ATP, and 5 μCi [γ-^{32}P]ATP. The final reaction buffer also contained 15 μg of myelin basic protein (MBP) or 4 μg of GST-ATF2 for ERK or SAPK activity assay, respectively. The reaction was initiated by incubation at 30°C for 15 min. Thereafter, 20 μl of 4× Laemmli buffer was added to terminate the reaction and samples were subjected to SDS-PAGE.

Results

Activation of ERK in two independent models of proliferative GN. Cellular proliferation is a characteristic feature of crescentic GN. We evaluated the in vivo activity of ERK both in the renal cortex and in isolated glomeruli in two models of crescentic GN. Sprague-Dawley rats preimmunized with rabbit IgG and subsequently injected with rabbit anti-rat GBM antibodies developed an accelerated GN with heavy proteinuria detected as early as 1 d after the anti-GBM antibody injections (Fig. 1). There were prominent lesions of proliferation and crescent formation as early as day 3 (Fig. 2, *A* and *B*) and marked hypercellularity with prominent crescents at day 7 (Fig. 2 *C*). Most animals died by days 9–11. We examined the activation of ERK at days 1, 3, and 7 in whole cortical tissue as well as in isolated glomeruli. We used anti-ERK Western blot analysis that separates unphosphorylated from phosphorylated protein forms (bands with delayed mobility indicate phosphorylated ERK) as well as immunocomplex ERK activity assays, to detect a significant increase in the activation of this kinase at day 1 with even greater increases at days 3 and 7 (Fig. 3 *A*). Quantification of MBP phosphorylation by ERK immu-

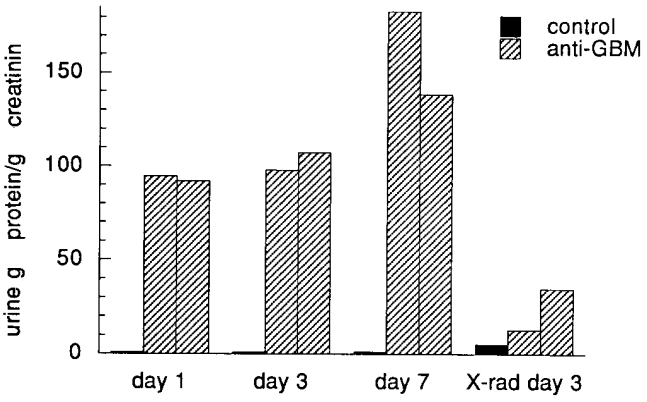

Figure 1. Urinary protein excretion in rats with accelerated anti-GBM antibody-induced nephritis. Each column represents one animal.

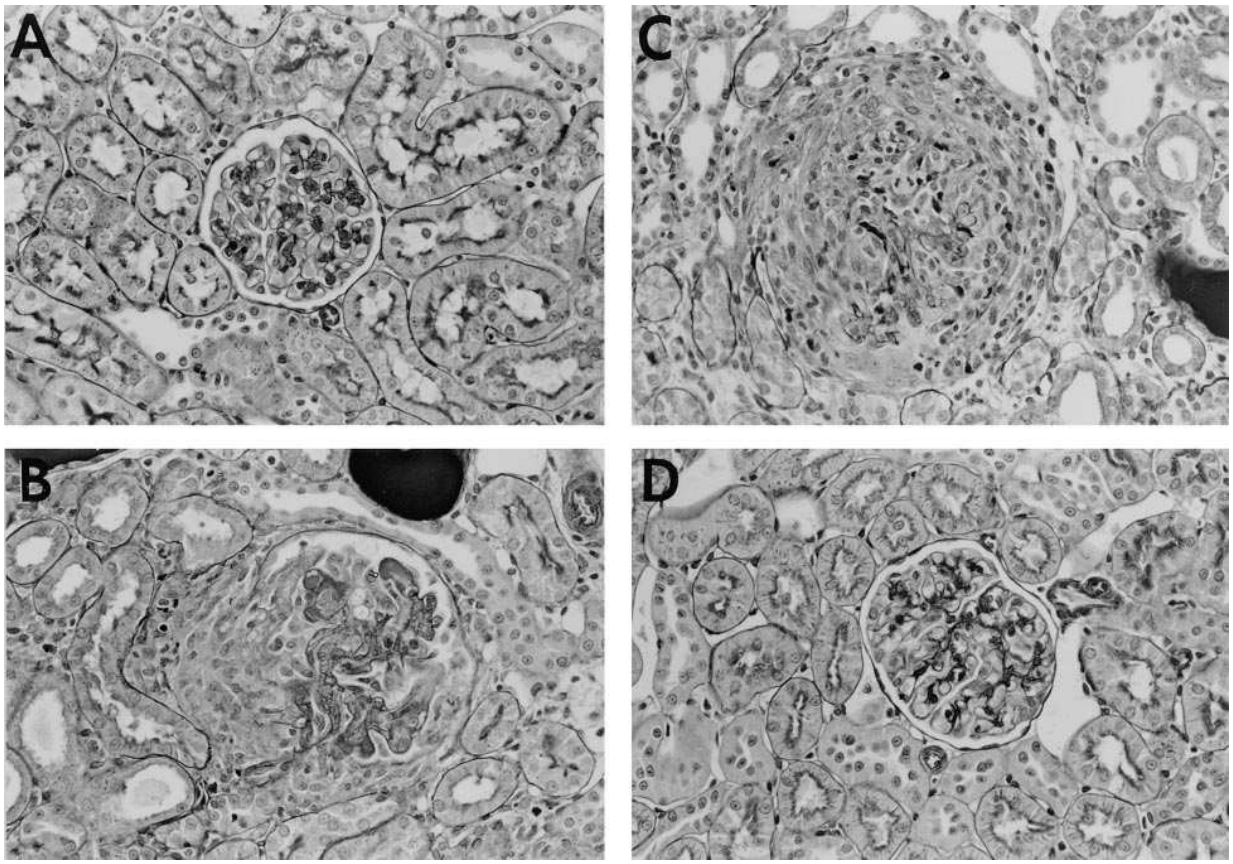

Figure 2. Light microscopy of renal cortical sections stained with periodic acid-Schiff. (*A*) (×40): Glomerulus of a rat 3 d after injection of normal rabbit serum. (*B*) (×40): Glomerulus of a rat 3 d after injection of anti-GBM serum. (*C*) (×40): Glomerulus of a rat 7 d after injection of anti-GBM serum. (*D*) (×40): Glomerulus of an x-irradiated (macrophage depleted) rat 3 d after injection of anti-GBM serum.

noprecipitated from cortical tissue revealed a 2.6- and 3.3-fold increase in activity in the rats injected with anti-GBM and studied at day 3. More significantly, in isolated glomeruli an even more pronounced increase in ERK activity 5.4- and 7.5-fold was detected on day 3, indicating that the glomeruli were the major source of the increased cortical ERK activity in anti-GBM GN (Fig. 3 *B*). These results are in agreement with the histological finding that glomeruli rather than the interstitium were the areas with prominent lesions (Fig. 2, *B* and *C*). The data shown in Figs. 2 and 3 were reproduced in an independent experiment with one control and two experimental animals.

To examine whether the described changes in ERK activity occur in other forms of anti-GBM antibody associated proliferative GN, we used an autologous model of proliferative anti-GBM GN in Wistar-Kyoto rats immunized with bovine GBM. In this form of the disease we observed progressive development of proliferation and crescent formation as previously described (20) (Fig. 4 *A*). Affected animals developed severe proteinuria 4 wk after immunization with bovine GBM and this was maintained throughout the course of the study (8 wk). We examined activation of ERK in whole cortical tissue and detected, as shown in Fig. 4 *B*, a significant activation of ERK (5.0- and 6.3-fold increases in activity were evident at 4 wk). This finding supports ERK activation as a common feature of two distinct models of proliferative anti-GBM GN.

As both models, in particular the accelerated form of anti-GBM GN, are characterized by prominent glomerular infiltration by activated macrophages, we addressed the extent to which infiltrating macrophages contribute to the activation of ERK observed in the accelerated model of anti-GBM GN. 5 d after total body x-irradiation, the levels of circulating leukocytes were reduced to levels below 500 cells/ml, indicating the development of bone marrow depletion. As shown in Fig. 2, the proliferative lesions were notably absent in x-irradiated animals studied 3 d following injection of anti-GBM serum (compare Fig. 2 *D* with Fig. 2 *B*). Furthermore, only moderate proteinuria was detectable in these animals (Fig. 1). This mild nephropathy was accompanied by a marginal increase in ERK activity in isolated glomeruli (1.2- and 2.2-fold) compared with the control x-irradiated animal (Fig. 5).

Induction of MEK expression in proliferative anti-GBM GN. We (22) and others (23) have previously suggested that an upregulation of MEK protein levels, the upstream kinase of ERK, to be important in the long-term regulation of ERK in cultured glomerular mesangial cells. To examine whether this mechanism is also involved in regulating ERK activation in vivo, we assayed MEK protein by Western blot analysis in the accelerated form of anti-GBM serum induced proliferative GN. As shown in Fig. 6, the changes of MEK protein levels in whole cortex samples were not significant. However, in isolated glomeruli, there was a dramatic increase in MEK protein in rats injected with anti-GBM antiserum. Thus, in a manner

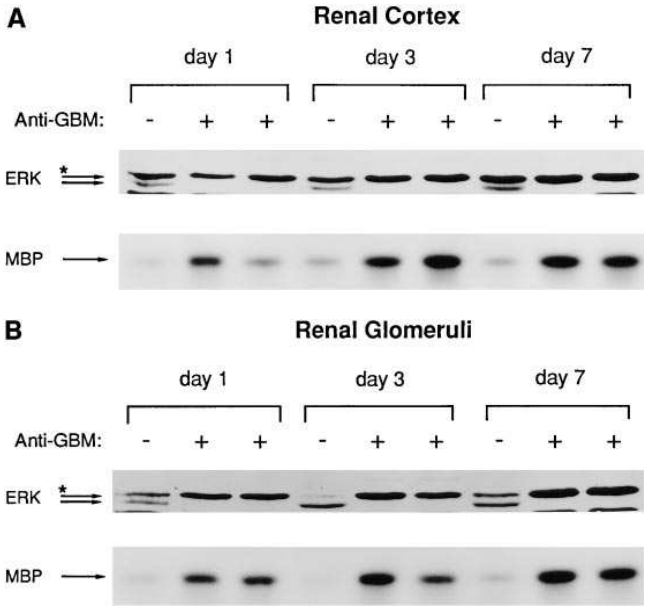

Figure 3. Activation of ERK in the accelerated model of anti-GBM GN. In the upper panels, ERK2 (p42 MAP kinase) is detected in whole tissue lysates by Western blot analysis. At each time point one control and two experimental animals were studied. Rats were killed at the indicated times following the second injection of anti-GBM serum. Activation of ERK is identifiable by an increase of phosphorylated protein forms, detected by bands with delayed mobility (indicated by stars), compared to the unshifted unphosphorylated ERK forms. In addition to the bands of p42 ERK a band with the size of 39–40 kD was detectable. The significance of this band remained unclear. The lower panels show ERK activity assayed by the ability of immunoprecipitated ERK to phosphorylate MBP. Data shown were reproduced in two independent experiments. (*A*) ERK activity in whole cortical tissue. (*B*) ERK activity in isolated glomeruli.

similar to ERK activation, MEK protein levels were found elevated principally in glomeruli.

The dual specificity phosphatase MAP kinase phosphatase-1 (MKP-1) has been implicated in the inactivation of ERK in vitro (11, 24, 25). MKP-1 is capable of dephosphorylating both regulatory tyrosine and threonine residues on ERK (24). To assess whether changes in MKP-1 accompany those of ERK in the course of anti-GBM disease, we used two different anti–MKP-1 antisera (26, 27) for immunoprecipitation and Western blot analysis of MKP-1 expression. No significant levels of MKP-1 were detected in either whole cortical tissue or isolated glomeruli (data not shown).

Activation of stress-activated protein kinase at early time points of anti-GBM GN. Stress-activated protein kinase (SAPK) is a recently described mammalian MAP kinase. Little is known about the physiological relevance of SAPK. However, SAPK has recently been suggested to play a role in the induction of cellular apoptosis (28). In cultured cells SAPK can be activated by a wide variety of intra- and extracellular stress stimuli (11) as well as primary inflammatory cytokines such as IL-1 (29) or TNF-α (30) that are implicated in the pathogenesis of proliferative GN. As shown in Fig. 7, we detected in isolated glomeruli activation of SAPK at days 1 and 3 after injection of anti-GBM serum but no significant SAPK activation at day 7. Measurements in whole cortical tissue did not show significant changes of SAPK activity, pointing to the glomeruli as the major site of SAPK activation during early stages of proliferative GN. The difference in the time curves of ERK and SAPK activation suggests these kinases play different roles in the pathogenesis of proliferative GN.

Discussion

In cultured cell lines mitogenic stimulation by various extracellular agonists correlates with activation of ERK (11). More important dominant negative interfering mutants of Ras or Raf-1, components upstream of MEK in the ERK signaling cascade, were shown to inhibit growth factor induced cell proliferation (13, 31), whereas constitutively activated Raf-1 induces cell proliferation (13). Furthermore, dominant negative or constitutively active mutants of MEK inhibit or accelerate cell proliferation of NIH3T3 cells, respectively (14, 15), and constitutively active MEK has been shown to induce cellular transformation (32). Finally, catalytically inactive mutants of ERK and its antisense cDNA inhibit proliferation (16). These data point to the pivotal role of the ERK cascade in the control of cellular proliferation. However, most of our current knowledge about the regulation and physiological relevance of the MAP kinase ERK has come from in vitro studies using cultured cells. In this regard, activation of ERK in proliferative GN, as shown in the present study, is a novel finding and points to ERK as a putative regulator of the proliferative response to immune injury in vivo. This conclusion is further supported by recent data, demonstrating an increase in ERK activity in tissue samples of human renal cell carcinoma (33). Cellular proliferation is thought to be pivotal for the development of crescent formation and progression of proliferative GN to end-stage renal disease. The proliferative response to injury in GN may be augmented by a convergence of multiple cytokines on ERK, inducing its activation. Since inhibition of ERK in cultured cells potently diminishes cellular proliferation, it is tempting to speculate that an inhibition of ERK activation in vivo could limit the extent of glomerular cell proliferation in proliferative GN, thereby providing a novel strategy for the treatment of rapidly progressive forms of GN.

Our observations indicate that infiltrating macrophages significantly contribute to the enhanced ERK activity in proliferative GN, since macrophage depletion using total body x-irradiation potently inhibited the progression of proliferative GN and significantly inhibited the activation of ERK. The release of multiple cytokines by infiltrating macrophages is thought to be a pivotal step in the pathogenesis of proliferative GN (34). Therefore, it is tempting to speculate that either infiltrating macrophages themselves are a cellular site of ERK activity or macrophages are causing the activation of ERK within intrinsic glomerular cells in a paracrine manner. Furthermore, these experiments employing total body x-irradiation demonstrate a close correlation of the extent of renal damage to the activation of ERK in proliferative GN.

Binding of cytokines to their receptors induces the interaction of multiple intracellular signaling molecules and sequential induction of cytoplasmatic protein kinases leading to the activation of the highly conserved MEK-ERK module. Recent in vitro studies suggest that the protein levels of MEK are tightly regulated and are involved in the long-term regulation of ERK (22, 23). The present study demonstrates an upregula-

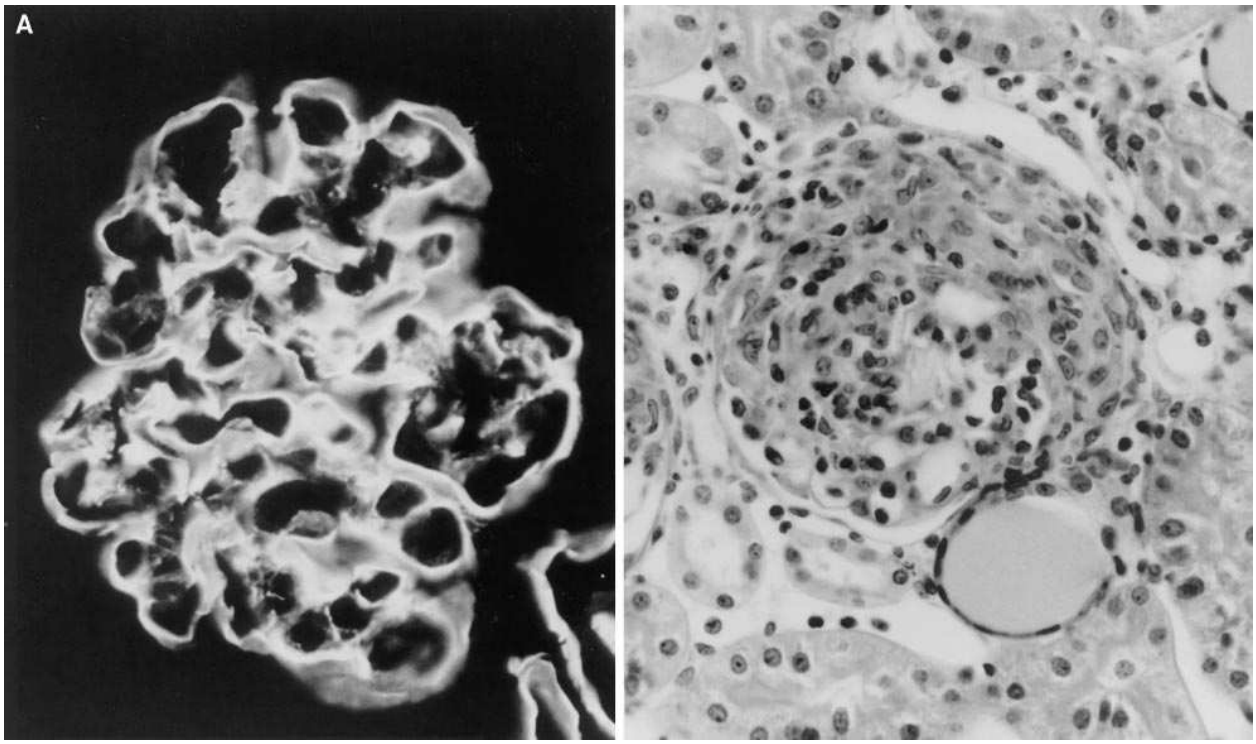

Figure 4. Activation of ERK in the autoimmune model of anti-GBM GN. (*A*) Autoimmune glomerulonephritis 6 wk after immunization with bovine GBM. Shown are deposition of rat IgG on the GBM (*left*) and a proliferative lesion including crescent formation (*right*). The urine protein excretion was 22.7 mg protein/mg creatinine in comparison to 1.5 mg protein/mg creatinine in control animals. (*B*) Activation of ERK is detected by Western blot analysis (*upper panel*), as described in the legend of Fig. 1 (phosphorylated protein forms are indicated by stars), and by ERK immune complex assay.

tion of MEK protein levels in rats with experimental proliferative GN, thereby increasing the amount of MEK protein available for phosphorylation and activation by the upstream kinase Raf. The elevation of MEK protein levels was accompanied by increased ERK activity. It is therefore likely, that the elevated MEK protein levels are contributing to ERK activation in vivo.

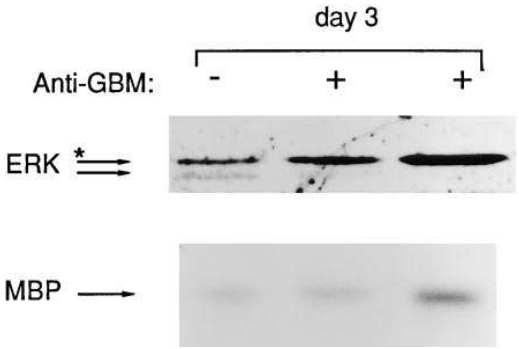

Figure 5. Effect of x-irradiation (macrophage depletion) on activation of ERK in accelerated anti-GBM GN. Animals were x-irradiated 24 h before injection of anti-GBM serum or normal rabbit serum and killed on day 3 following completion of anti-GBM serum injections. Activation of ERK is detected by Western blot analysis (*upper panel*), as described above (phosphorylated protein forms indicated by stars), and by ERK immune complex assay.

Another molecule directly involved in the regulation of ERK is the MAP kinase phosphatase-1 (MKP-1), capable of dephosphorylating and inactivating ERK in vitro (11, 24). However, we did not detect significant levels of MKP-1 expression or changes in MKP-1 expression during the time course of either model of proliferative GN. These data suggest MKP-1 not to take part in the regulation of ERK in the presented experimental setting. Recently, MKP-1 has been implicated in a very early phase of anti-GBM GN (35). Injection of anti-GBM antibodies to rats induced an increase in MKP-1 expression as early as 30 min, that returned to basal levels after 24 h (35). In the present study we measured MKP-1 expression no earlier than 24 h following induction of anti-GBM disease and detected therefore in accordance with the study mentioned above (35), no increase in MKP-1 expression.

The physiological relevance of the recently described MAP kinase SAPK remains to be established. The SAPK signaling cascade like the ERK pathway displays a high level of evolutionary conservation (11). In the present study we found activation of SAPK with a time course different to that of ERK activation. This indicates that these two MAP kinases may

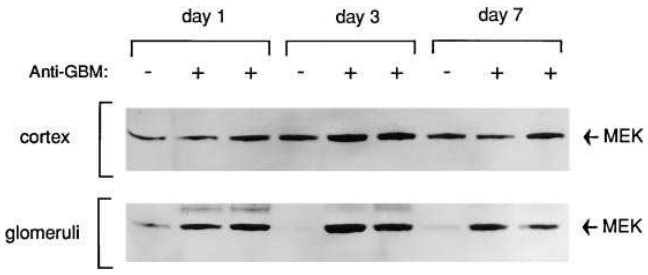

Figure 6. Expression of MEK protein detected by Western blot analysis of whole tissue lysates using monoclonal antibodies. The celluloses were reprobed with the housekeeping gene product GRB2 demonstrating even loading. Similar results were obtained in two independent experiments.

play distinct roles at different stages of proliferative GN. SAPK activation was detected only on days 1 and 3 after induction of accelerated proliferative GN. This implicates SAPK only at early stages in the pathogenesis of this disease model. Recently, mesangial cell apoptosis was shown to occur during anti-Thy1 GN and suggested to account for the resolution of glomerular hypercellularity in this disease model (36). However, apoptosis did also accompany the proliferative phase of anti-Thy1 GN (36). Since activation of SAPK has recently been implicated in the induction of apoptosis (28), the observed SAPK activation might account for a similar induction of apoptosis in proliferative GN. However, further studies will be needed for a better understanding of the role of SAPK in crescentic GN.

In conclusion, the present study provides new insights into the pathophysiology of proliferative GN. Activation of the MAP kinase ERK points to ERK as a regulator of cellular proliferation in proliferative GN. Moreover, upregulation of MEK protein levels suggests that this kinase is involved in the long-term regulation of ERK in the course of GN.

Acknowledgments

This work was supported by National Institutes of Health Grants HL-22563 and DK-41684 (to M.J. Dunn), by a grant of the Deutsche Forschungsgemeinschaft BO 1288/3-1 (to D. Bokemeyer) and a Veterans Affairs merit review grant (to E.A. Lianos).

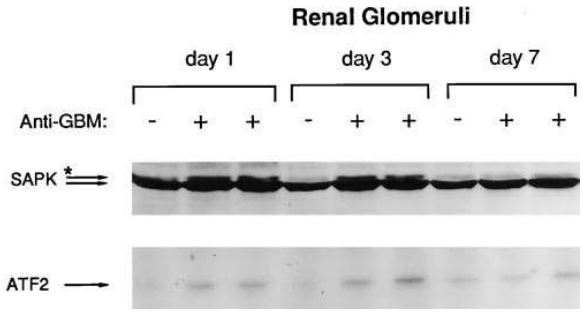

Figure 7. Activation of SAPK in anti-GBM GN. The upper panel shows a Western blot analysis of isolated glomeruli using polyclonal anti-SAPK antiserum. SAPK activation is detected by the appearance of protein forms with delayed mobility indicating phosphorylated protein forms (indicated by star). The lower panel shows SAPK immune complex assay using ATF2 as a substrate.

References

1. Abboud, H.E. 1993. Growth factors in glomerulonephritis. *Kidney Int.* 43:252–267.

2. Johnson, R.J., J. Floege, W.G. Couser, and C.E. Alpers. 1993. Role of platelet-derived growth factor in glomerular disease. *Am. J. Soc. Nephrol.* 4: 119–128.

3. Bruijn, J.A., A. Roos, B. deGeus, and E. deHeer. 1994. Transforming growth factor-β and the glomerular extracellular matrix in renal pathology. *J. Lab. Clin. Med.* 123:34–47.

4. Border, W.A., and N.A. Noble. 1993. From serum sickness to cytokines: Advances in understanding the molecular pathogenesis of kidney disease. *Lab. Invest.* 68:125–128.

5. Ahlers, A., C. Belka, M. Gaestel, N. Lamping, C. Sott, F. Hermann, and M.A. Brach. 1994. Interleukin-1-induced intracellular signaling pathways converge in the activation of mitogen-activated protein kinase and mitogen activated protein kinase-activated protein kinase 2 and the subsequent phosphorylation of the 27-kilodalton heat shock protein in monocytic cells. *Mol. Pharmacol.* 46:1077–1083.

6. Daeipour, M., G. Kumar, M.C. Amaral, and A.E. Nel. 1993. Recombinant IL-6 activates p42 and p44 mitogen-activated protein kinases in the IL-6 responsive B cell line, AF-10. *J. Immunol.* 150:4743–4753.

7. Berra, E., M.T. Diaz-Meco, J. Lozano, S. Frutos, M.M. Munico, P. Sanchez, L. Sanz, and J. Moscat. 1995. Evidence for a role of MEK and MAPK during signal transduction by protein kinase zeta. *EMBO J.* 14:6157–6163.

8. Hill, C.S., and R. Treisman. 1995. Transcriptional regulation by extracellular signals: mechanisms and specificity. *Cell.* 80:199–211.

9. Johnson, G.L., and R.R. Vaillancourt. 1994. Sequential protein kinase reactions controlling cell growth and differentiation. *Curr. Opin. Cell Biol.* 6:230–238.

10. Seger, R., and E.G. Krebs. 1995. The MAPK signaling cascade. 1995. *FASEB J.* 9:726–735.

11. Bokemeyer, D., A. Sorokin, and M.J. Dunn. 1996. Multiple intracellular MAP kinase signaling cascades. *Kidney Int.* 49:1187–1198.

12. Payne, D.M., H.J. Rossomando, P. Martino, A.K. Erickson, J.-H. Her, J. Schbanowitz, D.F. Hunt, M.J. Weber, and T.W. Sturgill. 1991. Identification of the regulatory phosphorylation sites in pp42/mitogen-activated protein kinase (MAP kinase). *EMBO J.* 10:885–892.

13. Miltenberger, R.J., J. Cortner, and P.J. Farnham. 1993. An inhibitory Raf-1 mutant suppresses expression of a subset of v-raf-activated genes. *J. Biol. Chem.* 268:15674–15680.

14. Seger, R., D. Seger, A.A. Reszka, E.S. Munar, H. Eldar-Finkelman, G. Dobrowolska, A.M. Jensen, J.S. Campbell, E.H. Fischer, and E.G. Krebs. 1994. Overexpression of mitogen-activated protein kinase kinase (MAPKK) and its mutants in NIH 3T3 cells. *J. Biol. Chem.* 269:25699–25709.

15. Brunet, A., G. Pages, and J. Pouysségur. 1994. Constitutively active mutants of MAP kinase kinase (MEK1) induce growth factor-relaxation and oncogenicity when expressed in fibroblasts. *Oncogene.* 9:3379–3387.

16. Pages, G., P. Lenormand, G. L'Allemain, J.C. Chambard, S. Meloche, and J. Pouysségur. 1993. Mitogen-activated protein kinase $p42^{mapk}$ and $p44^{mapk}$ are required for fibroblast proliferation. *Proc. Natl. Acad. Sci. USA.* 90:8319–8323.

17. Nishikawa, K., Y.-J. Guo, M. Mikasaka, T. Tamatani, A.B. Collins, M.-S. Sy, R.T. McCluskey, and G. Andres. 1993. Antibodies to intercellular adhesion molecule 1/lymphocyte function-associated antigen 1 prevent crescent formation in rat autoimmune glomerulonephritis. *J. Exp. Med.* 177:667–677.

18. Misra, R.P. 1972. Isolation of glomeruli from mammalian kidney by graded sieving. *Am. J. Clin. Pathol.* 58:135–139.

19. Schreiner, G., and E.R. Unanue. 1984. Origin of the rat mesangial phagocyte and expression of the leukocyte common antigen. *Lab. Invest.* 51: 515–523.

20. Krakower, C.A., and S.A. Greenspon. 1951. Localization of the nephrotoxic antigen within the isolated renal glomerulus. *Arch. Pathol.* 51:629.

21. Wang, Y., M.S. Simonson, J. Pouysségur, and M.J. Dunn. 1992. Endothelin rapidly stimulates mitogen-activated protein kinase activity in rat mesangial cells. *Biochem. J.* 287:589–594.

22. Schramek, H., A. Sorokin, R.D. Watson, and M.J. Dunn. 1996. Differential long-term regulation of MEK and of p42 MAPK in rat glomerular mesangial cells. *Am. J. Physiol.* 270:C40–C48.

23. Huweiler, A., and J. Pfeilschifter. 1994. Transforming growth factor β2 stimulates acute and chronic activation of the mitogen-activated protein kinase cascade in rat mesangial cells. *FEBS Lett.* 354:255–258.

24. Sun, H., C.H. Charles, L.F. Lau, and N.K. Tonks. 1993. MPK-1 β3CH134), an immediate early gene product, is a dual specificity phosphatase that dephosphorylates MAP kinase in vivo. *Cell.* 75:487–493.

25. Bokemeyer, D., A. Sorokin, M. Yan, N.G. Ahn, D.J. Templeton, and M.J. Dunn. 1996. Induction of mitogen-activated protein kinase phosphatase 1 by the stress-activated protein kinase signaling pathway but not by extracellular

signal-regulated kinase in fibroblasts. *J. Biol. Chem.* 271:639–642.

26. Bokemeyer, D., A. Sorokin, and M.J. Dunn. 1996. Differential regulation of the dual specificity protein-tyrosine phosphatases CL100, B23 and PAC1 in mesangial cells. *J. Am. Soc. Nephrol.* 8:40–50.

27. Brondello, J.M., F.R. McKenzie, H. Sun, N.K. Tonks, and J. Pouysségur. 1995. Constitutive MAP kinase phosphatase (MKP-1) expression blocks G1 specific gene transcription and S-phase entry in fibroblasts. *Oncogene.* 10:1895–1904.

28. Xia, Z., M. Dickens, J. Raingeaud, R.J. Davis, and M.E. Greenberg. 1995. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science (Wash. DC).* 270:1326–1331.

29. Bird, T.A., J.M. Kyriakis, L. Tyshler, M. Gayle, A. Milne, and G.D. Virca. 1994. Interleukin-1 activates p54 mitogen-activated protein (MAP) kinase/stress-activated protein kinase by a pathway that is independent of p21ras, Raf-1, and MAP kinase kinase. *J. Biol. Chem.* 269:31836–31844.

30. Minden, A., A. Lin, M. McMahon, C. Lange-Carter, B. Derijard, R.J. Davis, G.L. Johnson, and M. Karin. 1994. Differential activation of ERK and JNK mitogen-activated protein kinases by Raf-1 and MEKK. *Science (Wash. DC).* 266:1719–1723.

31. Pronk, G.J., and J.L. Bos. 1994. The role of $p21^{ras}$ in receptor tyrosine kinase signaling. *Biochim. Biophys. Acta.* 1198:131–147.

32. Mansour, S.J., W.T. Matten, A.S. Hermann, J.M. Candia, S. Rong, K. Fukasawa, G.F. Vande Woude, and N.G. Ahn. 1994. Transformation of mammalian cells by constitutively active MAP kinase kinase. *Science (Wash. DC).* 265:966–970.

33. Oka, H., Y. Chatani, R. Hoshino, O. Ogawa, Y. Kakehi, T. Terachi, Y. Okada, M. Kawaichi, M. Kohono, and O. Yoshida. 1995. Constitutive activation of mitogen-activated protein (MAP) kinases in human renal cell carcinoma. *Cancer Res.* 55:4182–4187.

34. Lianos, E.A., B.A. Bresnahan, and C. Pan. 1991. Mesangial cell immune injury. Synthesis, origin and role of eicosanoids. *J. Clin. Invest.* 88:623–631.

35. Feng, L., X. Yiyang, D. Seifert, and C.B. Wilson. 1995. Oxidative stress-inducible protein tyrosine phosphatase in glomerulonephritis. *Kidney Int.* 48: 1920–1928.

36. Baker, A.J., A. Mooney, J. Hughes, D. Lombardi, R.J. Johnson, and J. Savill. 1994. Mesangial cell apoptosis. The major mechanism for resolution of glomerular hypercellularity in experimental proliferative nephritis. *J. Clin. Invest.* 94:2105–2116.