Selective Sensitization to Tumor Necrosis Factor-α-induced Apoptosis by Blockade of NF-κB in Primary Glomerular Mesangial Cells*

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Recent data have implicated nuclear factor κB (NF-κB) in the prevention of apoptosis in transformed cell lines exposed to tumor necrosis factor α (TNF-α). However, it is obscure whether NF-κB plays an anti-apoptotic role in nontransformed cells, and it is not clear whether NF-κB inhibits apoptosis triggered by other mediators. We investigated the effect of specific inhibition of NF-κB on cytokine-induced apoptosis of glomerular mesangial cells, which is important in determining the outcome of glomerulonephritis. Cultured rat mesangial cells were stably transfected with the dominant negative mutant inhibitor of NF-κB (IκBoM). IκBoM was resistant to stimulus-dependent degradation and suppressed NF-κB activation induced by TNF-α (10 ng/ml) or IL-1β (10 ng/ml). IκBoM significantly sensitized mesangial cells to TNF-α-induced apoptosis in a dose- and time-dependent manner but had no significant effects on the level of apoptosis in the presence of proinflammatory or apoptosis-inducing stimuli including Fas ligand, IL-1α, IL-1β, hydrogen peroxide, lipopolysaccharide, cycloheximide, or serum deprivation. Moreover, IκBoM-mediated sensitization to TNF-α overcame the protective effect of mesangial cell survival factors present in serum, which usually inhibit killing of mesangial cells by the proapoptotic stimuli used. These data show that inhibition of NF-κB selectively sensitizes primary adult glomerular mesangial cells to TNF-α-induced apoptosis but not to other mediators of cell death including the Fas ligand.

Nuclear factor-κB (NF-κB)† is an inducible transcription factor of well established importance in cytokine-mediated inflammation (1). After stimulation by cytokines, the IκB kinase complex phosphorylates and degrades the inhibitory protein (IκB), and then NF-κB is translocated to the nucleus (2). Here the transcription factor promotes expression of a wide range of genes encoding proinflammatory mediators including TNF-α, IL-1α and -β, IL-2, -3, -6, -8, -12, granulocyte-, macrophage-, granulocyte-macrophage-colony stimulating factor, macrophage chemotactic protein-1, intracellular adhesion molecule-1, vascular-cell adhesion molecule-1, and E-selectin (3). Consequently, NF-κB has attracted attention as a therapeutic target in disorders characterized by persistent inflammation, such as rheumatoid arthritis. Indeed, recent work from Foxwell et al. (4) demonstrated that it is possible to suppress production of TNF-α, a proinflammatory cytokine of key importance in inflammatory diseases, in isolated human macrophages or human rheumatoid joint cell cultures using adenviral-mediated overexpression of IκBo.

However, inhibition of NF-κB at inflamed sites could have additional and probably undesirable consequences. In particular, in a range of transformed/cancer/embryonic cell lines, a blockade of NF-κB sensitized such cells to TNF-α-induced apoptosis, establishing a potentially “anti-apoptotic” role for the transcription factor (5–7). This was further emphasized by studies of mice in which expression of NF-κB components was deleted, because knockout mice lacking the RelA component of NF-κB died before birth by extensive apoptosis of liver cells at days 15–16 of gestation (8), and embryonic fibroblasts taken from RelA knockout mice were highly sensitive to TNF-α-mediated killing (9). Consequently, inhibition of NF-κB might undesirably increase deletion by apoptosis of cells at inflamed sites, promoting unscheduled cell loss, scarring, and loss of organ function (10). Nevertheless, an important finding in the studies of adenviral-mediated IκBo gene transfer by Foxwell et al. (4) was that infected macrophages were not induced to undergo apoptosis or sensitized to TNF-α, whereas transformed HeLa cells were. This intriguing observation emphasizes that very little is known of the consequences of inhibiting NF-κB in primary cell types resident at inflamed sites and suggests that the NF-κB blockade may not sensitize such cells to proapoptotic stimuli.

Therefore, we set out to study the effects of the NF-κB blockade in a primary cell type relevant to inflammation at various sites. Glomerular mesangial cells play crucial roles in glomerular inflammation, regulating blood flow, secreting chemotactic cytokines, and depositing an extracellular matrix. Accumulation of mesangial cells threatens progression of glomerular injury to a functionless scar, but when glomerular disease resolves excess mesangial cells are deleted by apoptosis (11). Importantly, mesangial cells in diseased glomeruli and in culture assume the phenotype of myofibroblasts, a tissue cell type that accumulates at sites of inflammation or injury in a range of tissues including the skin, liver, and gut (12–14) and is believed to promote formation of scar tissue at these sites. To achieve specific inhibition of NF-κB, we transfected cultured

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† The abbreviations used are: NF-κB, nuclear factor-κB; IκB, inhibitor of NF-κB; IκBoM, mutant IκBo; EMSA, electrophoretic mobility shift assay; FasL, Fas ligand; LPS, lipopolysaccharide; CHX, cycloheximide; Ab, antibody; IL, interleukin; TNF, tumor necrosis factor.

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rats mesangial cells with the dominant negative mutant IκBα (IκBαM), which inhibits NF-κB translocation to the nucleus. IκBαM has both N- and C-terminal mutations and thus is resistant to both basal and stimulus-dependent degradation (7, 15, 16).

We now report for the first time that 1) inhibition of NF-κB in a primary adult resident tissue cell type relevant to inflammatory disease does sensitize such cells to apoptosis; 2) sensitization of mesangial cells is remarkably selective to TNF-α, not being observed with a range of other proapoptotic stimuli; and 3) TNF-α can trigger apoptosis in IκBαM-transfected cells in the presence of serum-derived survival factors, which normally protect myofibroblast-like mesangial cells from a range of proapoptotic stimuli. The data raise the intriguing concept that when NF-κB is inhibited, TNF-α could serve as a “molecular knife” for removal of excess resident cells from inflamed sites.

**EXPERIMENTAL PROCEDURES**

*Tissue Culture Materials and Reagents—* All reagents were obtained from Sigma unless otherwise stated and include lipopolysaccharide (LPS), 3-methyladenine (3-MCA), and H2O2. Culture media (Dulbecco’s modified Eagle’s medium nutrient mix F-12 with glutamax-1) and supplements (100 units/ml penicillin, 100 μg/ml streptomycin, and heat-inactivated fetal bovine serum) were purchased from Life Technologies Inc. Recombinant TNF-α and Fas ligand (FasL) were purchased from Genzyme (Cambridge, MA) and Oncogene Research Products (Cambridge, MA), respectively. Recombinant IL-1α and IL-1β were gifts from Professor Saklatvala (Kennedy Institute, Charing Cross Hospital, London, United Kingdom). Rabbit polyclonal anti-IκBα and anti-IκBβ Ab were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA).

*Establishment of Stably Transfected Mesangial Cells—* Mesangial cells were cultured from isolated glomeruli of normal kidneys of a male Harlan Sprague-Dawley rat. Clonal cells were established by a limiting dilutional method and identified as being of mesangial cell phenotype, as described previously (17). Mesangial cells overexpressing IκBαM were created as follows. pLIXIκBαMN (a gift from Dr. I. Verma, Salk Institute) (7) comprising IκBαM cDNA and a neomycin phosphotransferase gene (neo) were transfected into a helper-free ecotropic packaging line HE8 (18). Stable transfectants were selected in the presence of the neomycin analogue G418 (500 μg/ml). Conditioned media of the transfectants were used as sources of the IκBαM retrovirus. In the presence of 10 μg/ml of polybrene, mesangial cells were exposed to a diluted retrovirus, as described before (17). Stable transfecants were selected in the presence of G418 (750 μg/ml). Stable transfectants were established. As control cells, mock-transfected mesangial cells that express neo alone were created (19). By light microscopy, the IκBαM and control transfectants had an elongated and stellate morphology and exhibited “hillocks” characteristic of untransfected parental cells. By Northern blot analysis, they expressed mRNA for IκBα and exhibited “hillocks” characteristic of untransfected parental cells. By Northern blot analysis, they expressed mRNA for IκBα and exhibited “hillocks” characteristic of untransfected parental cells. By Northern blot analysis, they expressed mRNA for IκBα and exhibited “hillocks” characteristic of untransfected parental cells.

*Induction of Apoptosis in Cultured Rat Mesangial Cells—* Rat mesangial cells were seeded at 4000 cells/well in 96-well plates for acridine orange (final concentration, 10 μg/ml in phosphate-buffered saline) to culture wells as described previously (11, 20). This assay has been extensively validated against biochemical, flow cytometric, and electron microscopic assays of apoptosis (11, 20).

**FIG. 1.** A, cultured rat mesangial cells transfected with IκBαM constitutively overexpress IκBα proteins in the cytosol. B, the specificity of TNF-induced NF-κB DNA binding in nuclear extracts from rat mesangial cells transfected with control vector. A, Western blot for detection of IκB proteins. Rat mesangial cells were transfected with control vector (CV) or IκBαM. Equal amounts (50 μg) of cytosolic proteins from transfected or untransfected (UT) cells were electrophoresed. IκBα and IκBβ proteins were visualized with specific Ab. The specificity of primary Ab binding was examined by competition study with synthetic blocking peptide (lanes 4, 7, and 10) and by a negative control study without primary Ab (lanes 7–9). The positions of IκBα (37 kDa) and IκBβ (43 kDa) proteins in UT (lanes 1, 4, and 7), CV-transfected (lanes 2, 5, and 8), or IκBαM-transfected rat mesangial cells (lanes 3, 6, and 9) are indicated by arrows. The size of molecular markers (kDa) is shown at the right. B, EMSA for detection of NF-κB DNA-protein complexes. Control vector-transfected rat mesangial cells were incubated for 24 h in medium containing 10 ng/ml TNF-α without serum. Equal amounts of nuclear extracts (15 μg) were analyzed for NF-κB-specific DNA binding using the 32P-labeled consensus or mutant NF-κB probe. Negative control without nuclear extracts (lane 2) and competition assay with 100-fold excess of unlabeled consensus, mutant NF-κB, or unrelated oligonucleotides (lanes 3, 4, and 5, respectively) were performed to establish the specificity of the reaction. NF-κB-specific bands (arrows 1), nonspecific bands (arrows 2 and 3), and free radiolabeled probe are indicated.

**Extraction of Cytosolic and Nuclear Protein—*Cell extracts were prepared according to published methods with some modification (21). Mesangial cells were centrifuged, resuspended in chilled hypotonic buffer (buffer A: 10 mM HEPES, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 50 μM dithiothreitol, 100 μM phenanthrolime, 1 μg/ml pepstatin, 100 μM trans-epoxysuccinyl-l-leucylamido(4-guanidino)butane, 100 μM 3,4-dichloroisocoumarin, 10 mM NaF, 100 mM sodium orthovanadate, 25 mM β-glycerophosphate), and centrifuged at 10,000 × g for 5 min at 4 °C. They were lysed in a solution of buffer A containing 0.2% (v/v) Nonidet P-40 for 10 min on ice and centrifuged at 10,000 × g for 10 min at 4 °C. The supernatant was then collected as cytosolic extract. The remaining pellets were resuspended in extraction buffer (buffer C: 20 mM HEPES, pH 7.9, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 25% (v/v) glycerol, 100 μM 3,4-dichloroisocoumarin), incubated for 15 min at 4 °C, and centrifuged at 10,000 × g for 10 min at 4 °C. The supernant containing soluble nuclear protein was collected as nuclear extract. Protein con-
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**Fig. 2.** IκBαM is (A) resistant to stimulus-dependent degradation and (B) blocks NF-κB activation induced by TNF-α or IL-1β in cultured rat mesangial cells. Rat mesangial cells transfected with control vector or IκBαM were incubated for up to 48 h in serum-free medium (SFM) (lanes 1–6) with or without cytokine (IL-1β or TNF-α, respectively) at the concentrations shown (lanes 7–12 and 13–18, respectively). Cytosolic and nuclear extracts were prepared from the same cells at 0, 24, and 48 h. A, Western blot. Equal amounts (50 μg) of cytosolic proteins were electrophoresed, and IκBα and IκBβ proteins were detected with specific Ab. The positions of IκBα (37 kDa) and IκBβ (43 kDa) proteins are indicated by arrows. The size of molecular markers (kDa) is shown on the right. An immunoblot representative of three experiments is shown. B, EMSA. Nuclear extracts (15 μg) from the control vector-transfected or IκBαM-transfected rat mesangial cells were analyzed using a 32P-labeled consensus NF-κB probe. The position of the specific NF-κB complex is indicated by an arrow. An autoradiogram representative of three experiments is shown.

**Western Blot Analysis**—Equal amounts of cytosolic extracts (50 μg) were analyzed by SDS-polyacrylamide gel electrophoresis followed by Western blotting (21). The gels were stained with Coomassie Brilliant Blue to confirm whether the protein extracts had been loaded evenly. Antibody-labeled proteins on the nitrocellulose membrane were detected by enhanced chemiluminescence (ECL) (Amersham Pharmacia Biotech). For the competition study, synthetic blocking peptides (IκBα and IκBβ, Santa Cruz Biotechnology Inc.) were coincubated with the labeled consensus NF-κB probe. The specificity of the reaction was confirmed by using blocking peptides of IκBα and IκBβ containing the epitope of the corresponding Ab completely neutralized. The specificity of immunoblotting under the conditions employed was demonstrated by enhanced chemiluminescence (ECL) (Amersham Pharmacia Biotech). The positions of IκBα (37 kDa) and IκBβ (43 kDa) proteins were detected after incubation with pooled anti-IκBα and anti-IκBβ Ab (Fig. 1A, lane 1). In control transfectants, IκBα and IκBβ proteins were detected at similar levels to those in untreated cells (Fig. 1A, lane 2). As expected on the basis of similar electrophoretic mobility of IκBα and IκBαM, when IκBαM was stably overexpressed in transfected mesangial cells there was an increase in the intensity of IκBα staining relative to IκBβ (Fig. 1A, lane 3). Synthetic blocking peptides of IκBα and IκBβ containing the epitope of the corresponding Ab completely neutralized the binding of the primary Ab at a ratio of 10:1 (peptide:Ab, μg, respectively) (Fig. 1A, lanes 4–6). Incubation without the primary Ab in the presence of the secondary Ab alone also entirely abolished labeling (Fig. 1A, lanes 7–9), demonstrating the specificity of immunoblotting under the conditions employed.

We next examined the level of NF-κB in mesangial cell nuclear extracts by EMSA (Fig. 1B). After stimulation by 10 ng/ml TNF-α without serum for 24 h, extracts from control transfectants revealed three DNA-protein complexes when incubated with the labeled consensus NF-κB probe (Fig. 1B, lane 1, arrows 1–3). Incubation with the labeled mutant NF-κB probe abrogated the binding to the upper complex, but not to the lower two faster migrating complexes, indicating that only the upper complex was specific for NF-κB (Fig. 1B, lane 6, arrow). The consensus NF-κB probe did not detect any complexes in the absence of the extracts (Fig. 1B, lane 2). Competition studies with cold NF-κB, cold mutant, or unrelated oligonucleotides confirmed the specificity of DNA-protein binding (Fig. 1B, lanes 3–5).

**Mutant IκBα Can be Stably Overexpressed in Cultured Rat Mesangial Cells**—To inhibit cytokine-induced NF-κB translocation to the nucleus, we transfected rat mesangial cells with the dominant negative IκBαM cDNA, which is not susceptible to phosphorylation at N-terminal serines 32 and 36 and is therefore resistant to subsequent degradation. In Western blot analysis of the cytosolic extracts from untransfected rat mesangial cells, the expected 37- and 43-kDa polypeptides were detected after incubation with pooled anti-IκBα and anti-IκBβ Ab (Fig. 1A, lane 1). In control transfectants, IκBα and IκBβ proteins were expressed at similar levels to those in untreated cells (Fig. 1A, lane 2). As expected on the basis of similar electrophoretic mobility of IκBα and IκBαM, when IκBαM was stably overexpressed in transfected mesangial cells there was an increase in the intensity of IκBα staining relative to IκBβ (Fig. 1A, lane 3). Synthetic blocking peptides of IκBα and IκBβ containing the epitope of the corresponding Ab completely neutralized the binding of the primary Ab at a ratio of 10:1 (peptide:Ab, μg, respectively) (Fig. 1A, lanes 4–6). Incubation without the primary Ab in the presence of the secondary Ab alone also entirely abolished labeling (Fig. 1A, lanes 7–9), demonstrating the specificity of immunoblotting under the conditions employed.

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**Mutant IκBα Is Resistant to Stimulus-dependent Degradation and Blocks Inducible Activation of NF-κB by TNF-α or IL-1β**—We examined the level of expression and degree of modification of cytosolic IκBα and IκBβ in transfected rat mesangial cells after stimulation by TNF-α or IL-1β, two cytokines known both to activate NF-κB in a range of cell types and to play key regulatory roles in inflammatory responses (24–26). Western blot analysis showed time-dependent degradation of IκBα proteins both in TNF-α- and in IL-1β-treated control transfectants for periods up to 48 h (Fig. 2A, lanes 7–9 and

**Electrophoretic Mobility Shift Assay (EMSA)—**Nuclear extracts (15 μg) were analyzed by EMSA as described previously with some modifications (21, 23). The double-stranded wild-type NF-κB (5′-AGT TGA GAC TTT CCC AGG C-3′) and mutant NF-κB (5′-ACT TGA GCC GAC TTT CCC AGG C-3′) oligonucleotides were obtained from Promega (Madison, WI) and radiolabeled with [γ-32P]ATP (Amersham Pharmacia Biotech) by incubation with 10 units of T4 polynucleotide kinase (Promega). After DNA-binding reaction and electrophoresis, gels were dried and autoradiographed (23). To assess the significance of the reaction, competition assays were performed with 100-fold excess of unlabeled wild type, mutant NF-κB, or unrelated oligonucleotides (AP-1, obtained from Promega). The unlabeled probes were added to the binding reaction 5 min before the addition of the labeled probe.

**Statistical Analysis**—Results were expressed as means ± S.E. Differences were evaluated by a nonparametric test (Mann-Whitney U test). A level of p < 0.05 was considered statistically significant.

**RESULTS**

**Mutant IκBα Can be Stably Overexpressed in Cultured Rat Mesangial Cells**—To inhibit cytokine-induced NF-κB translocation to the nucleus, we transfected rat mesangial cells with the dominant negative IκBαM cDNA, which is not susceptible to phosphorylation at N-terminal serines 32 and 36 and is therefore resistant to subsequent degradation. In Western blot analysis of the cytosolic extracts from untransfected rat mesangial cells, the expected 37- and 43-kDa polypeptides were detected after incubation with pooled anti-IκBα and anti-IκBβ Ab (Fig. 1A, lane 1). In control transfectants, IκBα and IκBβ proteins were expressed at similar levels to those in untrans-
13–15). However, in IxBα transfectants IxBα was stably overexpressed and resistant to stimulus-dependent degradation by TNF-α or IL-1β (Fig. 2A, lanes 10–12 and 16–18). IxBβ proteins were degraded in IxBα transfectants after stimulation by the cytokine, suggesting some NF-κB may be available for translocation to the nucleus (Fig. 2A, upper bands in lanes 10–12 and 16–18). IxBβ proteins were not degraded without exposure to cytokines either in control or IxBα transfectants (Fig. 2A, lanes 1–6).

Nuclear extracts were obtained from the same cells as analyzed by Western blot in Fig. 2A. EMSA studies performed over 48 h demonstrated that NF-κB activation induced by TNF-α or IL-1β was significantly suppressed in IxBα transfectants (Fig. 2B, lanes 7–18). NF-κB was not induced without exposure to cytokines either in control or IxBα transfectants (Fig. 2B, lanes 1–6).

Mutant IxBα Significantly Sensitizes Mesangial Cells to Apoptosis Induced by TNF-α but Not to IL-1β or IL-1α—Our previous work had established that serum deprivation (withdrawal of survival factors) induces apoptosis in cultured mesangial cells, as assessed by morphological examination by light, fluorescence, and electron microscopy and by biochemical evidence of oligonucleosomal DNA fragmentation in agarose gel electrophoresis (11, 20). We included TNF-α or IL-1β in our system for induction of apoptosis by serum deprivation and compared the effects of cytokines on apoptosis of mesangial cells constitutively overexpressing IxBα or control vector (Fig. 3A). Both cytokines increased the level of apoptosis to some extent as compared with serum deprivation alone in control transfectants, although only the effect of TNF-α (10–100 ng/ml) at 48 h was statistically significant (Fig. 3A). There were no significant effects of IxBα overexpression on mesangial cell apoptosis in cultures treated with IL-1β for periods up to 48 h (Fig. 3A); however, TNF-α (10 ng/ml) significantly increased apoptotic cell death in IxBα transfectants as compared with control transfectants at 24 h (Fig. 3A, 46.9% in-
crease in apoptosis). Moreover, the effect of TNF-α on apoptosis in IxBoM was dose- and time-dependent, being greatest at 100 ng/ml TNF-α for 48 h (Fig. 3A). We further examined the effect of IL-1α (10–100 ng/ml), another member of the IL-1 family, on mesangial cell apoptosis, but there were no significant differences in apoptosis between control (24.2 ± 0.8%) and IxBoM transfectants (26.4 ± 3.8%) (IL-1α 10 ng/ml at 24 h, n = 4). Overexpression of IxBoM also had no significant effects on the basal level of mesangial cell apoptosis induced by serum deprivation (Fig. 3A).

**TNF-α-mediated Killing Overcomes the Survival Effect of Serum on Mutant IxBo Transfectants**—We examined the protective effect of serum against cytokine-mediated apoptosis in control or IxBoM transfectants (Fig. 3B). In the presence of 10% serum in the medium, less than 2% of mesangial cells were apoptotic, a background level of cell turnover compatible with previous studies (11). TNF-α (10 ng/ml) or IL-1β (10 ng/ml) did not significantly increase apoptosis in control transfectants in the presence of 10% serum. However, IxBoM significantly sensitized mesangial cells to TNF-α-induced apoptosis even in the presence of serum containing survival factors for these cells (Fig. 3B). IxBoM significantly inhibited NF-κB activation induced by TNF-α or IL-1β in the presence of serum as judged by EMSA (data not shown).

**Mutant IxBo Does Not Sensitize Mesangial Cells to Apoptosis Induced by Other Stimuli**—Because our data indicated that a blockade of NF-κB by IxBoM sensitized mesangial cells to apoptosis induced by TNF-α but had no sensitizing effect toward proapoptotic serum deprivation or to IL-1β and IL-1α, which can induce mesangial cell apoptosis under particular conditions (11, 27), we went on to examine whether IxBoM might sensitize mesangial cells to apoptosis induced by other stimuli. An impressive body of data has implicated reactive oxygen species in the pathogenesis of glomerulonephritis (28), in activation of NF-κB in some cell types (29), and in triggering apoptosis (30). Thus, earlier studies reported that reactive oxygen species generated by H2O2 induced apoptosis in cultured mesangial cells (31–33). H2O2 increased apoptosis both in control and in IxBoM transfectants in a dose-dependent manner at 24 h. IxBoM did not significantly promote or suppress oxidant-induced apoptosis either in the presence or absence of serum (Fig. 4A).

**Discussion**

This study provides important new insights into the effects of inhibiting NF-κB in a primary adult resident tissue cell type known to play important roles in inflammatory disease. Indeed, because glomerular mesangial cells adopt a myofibroblastic phenotype under the conditions employed, the data may be relevant to regulation of myofibroblast numbers at inflamed sites elsewhere. For the first time it has been shown that, by contrast with macrophages (4), the blockade of NF-κB sensitizes primary tissue cells from the mesangium of the glomerulus to TNF-α-induced apoptosis. Furthermore, this sensitization by the NF-κB blockade was selective in that we observed no increase in susceptibility to other proapoptotic stimuli, including H2O2, FasL, IL-1β, IL-1α, LPS, and cycloheximide. Lastly, despite the generally protective effects of mesangial cell survival factors in serum, which include insulin-like growth factor-I, insulin-like growth factor-II and basic fibroblast growth factor (20) and which inhibit induction of apoptosis by various stimuli, serum did not inhibit TNF-α-induced apoptosis in mesangial cells transfected with IxBoM.

To date, studies of the regulatory effects of NF-κB activation upon cell survival and apoptosis have been restricted to transformed/malignant/embryonic cells (5–7) or to leukocytes (4, 38). The effects of the NF-κB blockade in primary adult tissue cells have been unknown, but were important to determine because...
of a growing interest in NF-κB as a therapeutic target in inflammatory disease (1). Indeed, glomerular cells from the adult kidney were of particular interest in view of a report that rather than an anti-apoptotic role, NF-κB activation induces apoptosis in the human 293 embryonic kidney cell line (39). Our finding that primary adult mesangial cells are indeed rendered susceptible to TNF-α-induced apoptosis by a blockade of NF-κB, consistent with an anti-apoptotic role for NF-κB, should not obscure the fact that NF-κB inhibition failed to affect apoptosis induced by H₂O₂ or FasL, indicating that apoptosis triggered in mesangial cells by such stimuli does not involve a role for NF-κB; these data are supported by lack of demonstrable NF-κB activation in such circumstances.

Perhaps the most intriguing findings in this study were that the blockade of NF-κB selectively sensitized mesangial cells to TNF-α-induced apoptosis and that this sensitization was still demonstrable in the presence of survival factors in serum, which are known to protect mesangial cells against many pro-apoptotic stimuli. Although highly speculative, the possibility is raised that selective sensitization to TNF-α might enable the combination of this cytokine and the NF-κB blockade to be used as a molecular knife to pare away excess mesangial tissue cells where these threaten accumulation of abnormal extracellular matrix and progression of glomerular inflammation to a functionless scar. However, because mesangial cell apoptosis may be a double-edged sword (40), being beneficial in the early hypercellular stage of inflammatory injury (11) but deleterious once scarring ensues because of undesirable loss of resident cells (10), the data also emphasize that attempts to ameliorate inflammation by inhibition of NF-κB could have undesirable consequences. Consequently, it follows that future work on NF-κB-mediated protection of primary cells from the proapoptotic effects of TNF-α should address whether regulatory pathways defined in transformed cells are relevant (41). Roles for the inhibitor of apoptosis proteins 1 and 2 and TNF receptor-associated factors 1 and 2 should be sought, because these might represent therapeutic targets much more specifically involved in the protection against TNF-α-induced apoptosis than NF-κB itself.

To conclude, this study extends known anti-apoptotic roles of NF-κB to primary tissue cells and emphasizes that the blockade of NF-κB can selectively sensitize such cells to apoptosis induced by TNF-α, even in the presence of protective survival factors.

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