Reactive Oxygen Species Expose Cryptic Epitopes Associated with Autoimmune Goodpasture Syndrome*

Raghu Kalluri**, Lloyd G. Cantley‡, Dontscho Kerjaschki, and Eric G. Neilson**

Goodpasture syndrome is an autoimmune disease of the kidneys and lungs mediated by antibodies and T-cells directed to cryptic epitopes hidden within basement membrane hexamers rich in \( \alpha_3 \) non-collagenous globular (NC1) domains of type IV collagen. These epitopes are normally invisible to the immune system, but this privilege can be obviated by chemical modification. Endogenous drivers of immune activation consequent to the loss of privilege have long been suspected. We have examined the ability of reactive oxygen species (ROS) to expose Goodpasture epitopes buried within NC1 hexamers obtained from renal glomeruli abundant in \( \alpha_3(IV) \) NC1 epitopes. For some hexameric epitopes, like the Goodpasture epitopes, exposure to ROS specifically enhanced recognition by Goodpasture antibodies in a sequential and time-dependent fashion; control binding of epitopes to \( \alpha_3(IV) \) alloantibodies from renal transplant recipients with Alport syndrome was decreased, whereas epitope binding to heterologous antibodies recognizing all \( \alpha_3 \) NC1 epitopes remained the same. Inhibitors of hydrogen peroxide and hydroxyl radical scavengers were capable of attenuating the effects of ROS in cells and kidney by 30–50%, respectively, thereby keeping the Goodpasture epitopes largely concealed when compared with a 70% maximum inhibition by iron chelators. Hydrogen peroxide administration to rodents was sufficient to expose Goodpasture epitope in vivo and initiate autoantibody production. Our findings collectively suggest that ROS can alter the hexameric structure of type IV collagen to expose or destroy selectively immunologic epitopes embedded in basement membrane. The reasons for autoimmunity in Goodpasture syndrome may lie in an age-dependent deterioration in inhibitor function modulating oxidative damage to structural molecules. ROS therefore may play an important role in shaping post-translational epitope diversity or neoantigen formation in organ tissues.

Type IV collagen is expressed as six distinct \( \alpha \) chains that are assembled selectively into triple helices for incorporation into basement membranes. These \( \alpha \) chains have three domains: the non-collagenous NH\(_2\)-terminal 7 S domain, a middle triple helical region containing a characteristic Gly-X-Y motif, and a COOH-terminal non-collagenous globular (or NC1) domain (1). The type IV collagen protomer is a triple helical molecule composed of three polypeptide \( \alpha \) chains. With six \( \alpha \) chains of type IV collagen, 56 different combinations of type IV collagen protomers are theoretically possible. In basement membrane these protomers interact with their neighbors; four 7 S domains interact to form a tetrad, and each NC1 domain abuts with an adjacent NC1 to form a globular hexamer (1–3). \( \alpha_1 \) and \( \alpha_2 \) chains are present ubiquitously in nearly all basement membranes (1, 4, 5), whereas \( \alpha_3 \), \( \alpha_4 \), \( \alpha_5 \), and \( \alpha_6 \) chains have a much more restricted distribution (1, 4, 5). The \( \alpha_1 \) and \( \alpha_2 \) chains are fetal isoforms in the renal glomerulus, but later in capillary development the glomerular basement membrane (GBM) undergoes an isoform switch and \( \alpha_3 \), \( \alpha_4 \), and \( \alpha_5 \) chains replace and predominate in the adult nephron (6).

Goodpasture syndrome is an autoimmune disease characterized by the presence of rapidly progressive glomerulonephritis, pulmonary hemorrhage, and antibodies to the GBM (4, 7). These anti-GBM antibodies bind primarily to the NC1 domains of the \( \alpha_3 \) chain of type IV collagen (4, 8–16) and are found in all patients with the disease (4, 7–16). Circulating and tissue-bound (kidney and lung) antibodies recognize epitopes on the \( \alpha_3(IV) \) chain located in the NC1 domain (12, 13). Whether these epitopes form a single conformational binding site in their native state is not yet clear.

Goodpasture epitopes in vertebrate tissue are normally invisible to the immune system. Even after biochemical isolation, NC1 hexamers are usually nonreactive with Goodpasture autoantibodies unless their complex structure undergoes a physiochemical change in conformation (7, 13, 17). Exposure of these sites is requisite for the disease, and hydrocarbons or viral infection have been suggested as possible environmental drivers (13, 18). Firm experimental evidence for a physiologic mediator or mechanism of exposure is not available. Because the by-products of aerobic metabolism can alter the structural integrity of macromolecules over time (19), we have evaluated the capacity of reactive oxygen species (ROS) to expose Goodpasture epitopes.

This work was supported in part by Grants DK-51711 and DK-55061 from the National Institutes of Health (to R. K.), the 1998 American Society of Nephrology Carl Gottschalk Award, the 1998 National Kidney Foundation Murry Award (to R. K.), and Grants DK-46282, DK-07006, DK-30280, and DK-45191 from the National Institutes of Health (to G. N.). Grant DK-48871 from the National Institutes of Health (to C. W. C.), and the Austrian Fonds Zur Forderung der Wissenschaftlichen Forschung, Sonderforschungsbereich 5, Project 007 (to D. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. A to whom correspondence should be addressed: Division of Nephrology, Dept. of Medicine, DANA 563a, Beth Israel Deaconess Medical Center, 330 Brookline Ave., Boston, MA 02215. Tel.: 617-667-0445; Fax: 617-975-5663; E-mail: rkalurlure@caregroup.harvard.edu.

1 The abbreviations used are: NC1, non-collagenous globular; GBM, glomerular basal membrane; ROS, reactive oxygen species; \( \text{H}_2\text{O}_2 \), hydrogen peroxide; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay.
FIG. 1. Exposure of Goodpasture epitope by ROS. Panel A, open rectangles denote human Goodpasture autoantibodies (1:50 dilution), open circles denote normal human serum (1:50 dilution), and dark circles denote Alport alloantibodies (1:500 dilution). Panel B, dark circles denote anti-NC1 antibodies (1:50 dilution), dark rectangles denote rabbit anti-α3.26 peptide antibody (1:50 dilution), and open rectangles denote rabbit anti-α3.26 peptide antibody (1:50 dilution). The methods are as described in detail under “Experimental Procedures.”

EXPERIMENTAL PROCEDURES

Basement Membrane Preparations—Normal portions of human kidneys were obtained from nephrectomized kidneys with cancer and/or normal kidneys not used for transplantation. NC1 hexamers were prepared from renal cortex by detergent extractions (47–49).

Reactions to Generate ROS—ROS were generated by an in vitro Fenton reaction (Fe^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH} - + \text{OH}^-) in the presence of human type IV collagen NC1 hexamers. FeCl_3, \text{H}_2\text{O}_2, catalase, dimethylthiourea, and desferrioxamine were purchased from Sigma. The reactions were set up in polyethylene tubes using \text{H}_2\text{O}_2, FeCl_3, 50 mM PBS, and the renal NC1 hexamers. Briefly, 10 μg of human NC1 hexamer was solubilized in 150 μl of 50 mM PBS for each reaction. To this solution, 100 mM FeCl_3 was added to a final concentration of 0.3 mM. 1 min later, 5 μl of \text{H}_2\text{O}_2 was added to a final concentration of 1 mM. The reaction was carried out at 4 °C for 16 min with gentle stirring. A 20-μl aliquot was taken from the reaction at 2-min intervals. Each aliquot of sample was then analyzed by ELISA, gel electrophoresis, and Western blotting described below.

For experiments using inhibitors and scavengers of ROS, we added excess of inhibitors and scavengers to the reaction mixture before the addition of FeCl_3 or \text{H}_2\text{O}_2 (30). Briefly, desferrioxamine and dimethylthiourea were added at four different concentrations from 0.1 mM to 1 mM. Catalase was added at four different concentrations from 0.1 mM to 1 mM. 4–5-μm human kidney sections were also used in selected experiments as substrate, instead of purified hexamers, to expose the Goodpasture epitope by the Fenton reaction. The reaction time for the human kidney sections was 90 min instead of 16 min, and the final concentration of FeCl_3/\text{H}_2\text{O}_2 was used 0.5 μM for these experiments.

Analysis of ROS-treated NC1 Hexamer—For ELISA of treated and control NC1 hexamers, sample material recovered from the Fenton reaction was precipitated with 10 volumes of 95% ethanol, resolubilized in 10 ml of 50 mM sodium carbonate, pH 9.7, and used to coat ELISA plates in triplicate in 200-μl aliquots. Direct ELISA was performed as described previously (9, 10, 12, 13, 50). Goodpasture antibodies (LL), Alport α3(IV) alloantibodies (a particular patient’s antibody with reactivity to α3NC1 domain (20)), α3.36 peptide antibody, α3-n.26 peptide antibody, and α3 NC1 antibody (6, 13, 48) were used as primary antibodies in various experiments. All antibodies were used at a dilution of 1:50, except the Alport alloantibodies and were used at 1:500. For gel electrophoresis, each aliquot sample (after ethanol precipitation) was mixed with an equal volume of gel loading buffer and resolved by nondenaturing gel electrophoresis (11). In some experiments, these gel samples were transferred for Western blotting (11). Goodpasture antibodies (1:50 dilution) and Alport alloantibodies (1:500 dilution) were used as primary antibodies for indirect immunofluorescence staining of human kidney pretreated with the Fenton reaction. Immunofluorescent staining was performed as described previously and washed three times with 10 mM PBS containing 1% bovine serum albumin (11). The staining with primary antibody was developed using fluorescent isothiocyanate-rabbit anti-human IgG (6).

In Vivo Generation of ROS—In vivo generation of ROS was performed via modification of the method of Yoshioka et al. (51). Male Harlan Sprague-Dawley rats weighing between 300 and 400 g were anesthetized with 100 mg/kg Inactin and the abdomen exposed with a midline ventral incision. The right renal artery was cannulated via the superior mesenteric artery with a glass cannula and the right kidney flushed with 1 ml of 0.9% sodium chloride. Perfusion was then performed for 30 min with 70 ml \text{H}_2\text{O}_2 in a buffer containing 50 mM NaPO_4, pH 7.0, at 22 ml/min with confinmation of 150 ml of 0.9% saline/min to maintain arterial patency. Saline-infused rats did not reveal ultrastructural damage, proving that perfusion pressure was not a factor in tissue damage. Control perfusions were performed in the absence of \text{H}_2\text{O}_2 or in the presence of \text{H}_2\text{O}_2 with 100 mM catalase, 100 mM dimethylthiourea, and 100 mM desferrioxamine. After a 30-min \text{H}_2\text{O}_2 perfusion, the kidney was flushed with 0.5 ml of saline and either 10 μg of Goodpasture IgG or 1 mg of sheep anti-rat GBM. IgG was infused into the kidney and allowed to incubate for 1 h in a volume of 0.5 ml of saline. The rat was then sacrificed, and the kidneys surgically removed, transsected, and frozen immediately in liquid nitrogen for immunofluorescence staining as described above.

Anti-α3(IV) NC1 Antibody Production by Hydrogen Peroxide—Three normal mice (BALB/c) weighing 20 g were intravenously (tail vein) injected with 150 mM \text{H}_2\text{O}_2 every 12 h for 14 days. The mice were sacrificed after 14 days, and the blood was collected. The serum samples from these mice were evaluated for circulating antibodies toward the Goodpasture antigen (α3IV NC1 domain) using direct ELISA, as described above. The recombinant α3, α4, and α5 NC1 were generated as described previously (13, 14). These are denatured proteins, and the recombinant α3 NC1 binds to Goodpasture autoantibodies (13, 14). The NC1 hexamer (as described above) is a native structure, and previous studies have shown that Goodpasture autoantibodies bind weakly to this structure and upon denaturation by urea, acid, or ROS (as presented in Fig. 1 in this paper), the binding to Goodpasture autoantibodies increases by severalfold. Placenta type IV collagen was purchased from Sigma.

Statistics—Results are presented as the mean ± S.E. The analysis of variance and significance by Student’s t test are reported where appropriate.

RESULTS

Exposure of Goodpasture Epitope by ROS—In this study we examined the ability of ROS to expose type IV collagen NC1 hexamers containing Goodpasture epitopes on α3 chains. Our results demonstrate that Goodpasture epitopes are not normally visible to Goodpasture autoantibodies in native isolated NC1 hexamers from GBM, but the antibodies will bind to hexamer preparations pretreated with ROS in a time-dependent fashion (Fig. 1A). Briefly, 10 μg of human NC1 hexamer was solubilized in 150 μl of 50 mM PBS for each reaction. To this solution, 100 mM FeCl_3/\text{H}_2\text{O}_2 was added to a final concentration of 0.3 mM. 1 min later, 5 mM \text{H}_2\text{O}_2 was added to a final concentration of 1 mM. The reaction was carried out at 4 °C for 16 min with gentle stirring. A 20-μl aliquot was taken from the reaction at 2-min intervals. Each aliquot of sample was then

... continued...
analyzed by ELISA, gel electrophoresis, and Western blotting. Goodpasture antibodies recognized ROS-modified NC1 hexamers beginning 4 min after initiation of the Fenton reaction with maximum binding achieved by 14 min. Normal human serum did not bind to pretreated hexamers. Alport transplant alloantibodies, which also recognize multiple epitopes on the α3(IV) NC1 domain in some patients (6, 20), initially demonstrated a strong recognition at 2 min but gradually decreased their binding over the lengthening time of treatment with ROS. By 14 min, binding had plateaued by 75%, an effect similar to what was observed previously using 6x guanidine HCl to denature hexamers (13). These results collectively suggest that ROS are capable of altering the epitope structure of NC1 hexamers differentially and that Goodpasture antibodies and the Alport transplant antibodies, whereas recognizing the same NC1 domain, generally bind to different sets of epitopes.

In a second experiment, we kinetically measured the binding of two rabbit polyclonal antibodies raised to peptides containing the putative Goodpasture epitopes (α3-n.26 and α3-c.36) to hexamers pretreated with ROS (Fig. 1B). In the absence of ROS pretreatment, neither α3-n.26 nor α3-c.36 bound substantially to the NC1 hexamer, whereas a polyclonal positive control antibody raised against all visible epitopes expressed by intact NC1 hexamers (160 kDa; α3NC1) did bind NC1 hexamers and did not lose binding capacity over the 16-min exposure of NC1 hexamer to ROS, suggesting that most epitopic structures on these NC1 hexamers were not destroyed by ROS. After a 16-min exposure to ROS, both anti-α3-n.26 peptide antibody directed to the first 26 amino acid residues of the NH2-terminal and anti-α3-c.36 peptide antibody directed to the last 36 residues containing the COOH-terminal epitope in the α3(IV)NC1 bound equally well to the NC1 hexamer and the control antibody, demonstrating that all epitopes were now exposed for binding. However, during the time course of the experiment, anti-α3-n.26 bound earlier and more strongly to the NC1 hexamers than the α3-c.36 antibody. Because the binding titers for all three antibodies were predetermined to be similar and in the linear range of binding (data not shown and (13)), these experiments demonstrate that not all epitopic regions of the α3(IV) NC1 domain are altered by exposure to ROS and that the two principal Goodpasture epitopes in the α3(IV) NC1 domains may open in a preferred order.

Structural Changes Are Induced in the Type IV Collagen NC1 Hexamer by ROS—The changes in the structure, stability, antigenicity, and function of proteins exposed to ROS have been studied in many laboratories from a quantitative perspective (21). To evaluate further the effect of ROS on the structural integrity of NC1 hexamers, aliquots of treated hexamer were analyzed at different time points by gel electrophoresis. These results demonstrate that hexamers remain intact for the first 6 min of the reaction (Fig. 2) but soon undergo a rapid fragmentation and ultimately aggregation which are easily detected by 14 min. Exposure of the Goodpasture epitopes observed between 4 and 6 min before fragmentation/aggregation maybe due to early conformational modifications in the hexamer (secondary or tertiary) prior to shredding.

Inhibition of ROS-mediated Exposure of Goodpasture Epitope—To evaluate the specificity of the ROS exposing the Goodpasture epitopes, we next performed Fenton reactions in the presence of metal chelators, scavengers, and inhibitors. When dimethylthiourea, a scavenger of hydroxyl radical, was used in excess, about a 30% reduction was observed in binding by Goodpasture antibodies to NC1 hexamers (Fig. 3A). These results suggest that hydroxyl radicals may be one of the species contributing to the exposure of Goodpasture epitopes. When catalase, an inhibitor of H2O2, was used in excess, the exposure of Goodpasture epitope was also diminished by more than 50% (Fig. 3B). Finally, use of the iron chelator, desferrioxamine, in the Fenton reaction with NC1 hexamers led to a 70% reduction in the binding of Goodpasture antibodies (Fig. 3A). These results suggest that H2O2 may mediate the exposure of epitope by itself or by the addition of FeCl2-4H2O-dependent species to the microenvironment of the reaction. Although these two species of radicals are substantially culpable, our experiments at this point do not preclude the capacity of superoxide anions to also play a role.

In Vivo Exposure of Goodpasture Epitope by ROS—Several previous reports have suggested that the binding of human Goodpasture antibodies to GBM in tissue sections from normal kidneys first requires denaturation (18, 19, 22–26). To understand this requirement further, we designed experiments to generate ROS in the presence of human kidney sections. ROS generated on these sections were evaluated for their capacity to expose the Goodpasture epitope in tissue. The reaction was carried out on the kidney section for 15 min, after which the sections were rinsed exhaustively using 10 ml PBS containing 1% bovine serum albumin. The sections were then probed with Goodpasture antibodies, Alport alloantibodies, or normal human serum by indirect immunofluorescence. Goodpasture antibodies demonstrated a significant binding to GBM in treated sections (Fig. 4B), whereas only a faint signal was observed on the untreated sections (Fig. 4A). Alport alloantibodies bound well to untreated sections (Fig. 4C), but this signal was reduced in sections pretreated with ROS (Fig. 4D). As an additional control, when acetonite-fixed sections were used (6), the Goodpasture autoantibodies bound strongly to GBM (data not shown). Normal human serum did not bind to the GBM of treated kidney sections (data not shown).

In vivo perfusion of Goodpasture autoantibody in the control rat kidney failed to yield demonstrable binding to the GBM (Fig. 5A), whereas sheep anti-rat GBM polyclonal antibody provided a linear staining under these conditions (Fig. 5B). In vivo generation of ROS via H2O2 perfusion prior to antibody incubation resulted in a marked increase in binding of the Goodpasture autoantibody (Fig. 5C), with no significant alteration in control antibody binding (Fig. 5D). Coinfusion of cat-
alase, dimethylthiourea, and desferrioxamine with H2O2 substantially diminished binding of the Goodpasture autoantibody (Fig. 5E). Thus, generation of ROS in vivo resulted in exposure of Goodpasture epitope in the GBM and subsequent binding of the Goodpasture autoantibody.

Additionally, normal mice treated with 150 mM H2O2 every 12 h (intravenously) for 14 days were analyzed for endogenous circulating anti-GBM collagen antibodies. The GBM collagen is predominantly composed of the α3, α4, and α5 isoforms in mammals (6). Direct ELISA experiments show that H2O2-treated mice develop significant anti-α3(IV)NC1 titers (Fig. 6) in addition to weaker responses α4 and α5 NC1 domain. Some Goodpasture patients also show weaker antibodies to other chains, potentially of insignificant pathological consequence (10). The ROS-initiated anti-α3 antibody was directed to a cryptic epitope within the NC1 hexamer, similar to human Goodpasture autoantibodies (Fig. 6). These results strongly suggest that ROS are capable of exposing Goodpasture epitope, thus initiating a humoral response to this pathogenic antigen.

**DISCUSSION**

Several studies have reported that superoxide, H2O2, and hydrogen radicals can induce proteinuria in rats and mice (27). Superoxide dismutase, an inhibitor of superoxide, is also effective in blocking albumin permeability that has been induced by superoxide in isolated glomeruli (28). Additionally, desferrioxamine, an iron chelator, and dimethylthiourea, a hydroxyl radical scavenger, have been effective in treating proteinuria in anti-GBM disease in rats (29). Dimethylthiourea was also reported to improve proteinuria in passive Heymann’s nephritis...
Student's ELISA was performed as described under "Experimental Procedures." Values are an average of triplicate readings.

The results strongly favor a role for ROS in renal injury. We have now evaluated the capacity of ROS to denature the NC1 hexamer and expose immunologically privileged Goodpasture epitopes through alterations in secondary or tertiary structure, fragmentation, or protein aggregation.

Using bovine serum albumin as a model protein, several studies have observed that exposure to ROS leads to gross structural alterations in the protein (30–36). Moreover, in a recent study of aggregation, fragmentation, and amino acid modifications of 17 proteins by ROS, it was found that all proteins underwent alterations in molecular weight (aggregation and fragmentation) or net electric charge associated with a loss of tryptophan or production of bitirosine (35). It has been suggested that superoxide alone produced no measurable effect on the above mentioned parameters and that hydrogen radicals were probably the initiating species in all cases.

ROS in the current report produced structural changes to the NC1 hexamers and increased the visibility of Goodpasture epitopes within 4 min of the reaction and before fragmentation or aggregation began, as revealed by gel electrophoresis of the NC1 hexamer at 6 min of the reaction. Fragmentation of the NC1 hexamer begins by 6 min of reaction, and eventually aggregation of the protein occurs, some of which remains in the stacking gel. The aggregates could potentially be from the smaller fragments of the NC1 hexamer or NC1 hexamer itself.

It is not clear which of these ROS-affected components of the NC1 hexamer best expose Goodpasture epitopes. Studies using the inhibitors of H2O2 and hydroxyl radicals, and an iron chelator reveal that none of these modifiers individually could decrease the appearance of products from the Fenton reaction exposing relevant epitopes. These experiments suggest that more than one reactive species is likely mediating the exposure. A similar broad range of ROS is thought to contribute to the inflammatory events producing anti-GBM disease in rodents, and more than one reactive species can induce proteinuria in these experiment systems (18, 19, 21, 27).

Several studies have also reported the capacity of epithelial cells and mesangial cells isolated from glomeruli to produce ROS in response to plasma membrane perturbations (30). In response to PMA, for example, rat glomeruli showed a marked increase in ROS production which reached a peak at about 20 min and declined gradually thereafter (37). Results with enzymatic and chemical scavengers of oxygen metabolites suggest a role for superoxide anion, H2O2, and hydrogen radicals. These findings further support the notion that ROS can be generated by resident cells on or near GBM and hence can potentially alter the structural integrity of NC1 hexamers in the basement membrane.

ROS can be produced in response to various normal stimuli such as mediators of inflammation, environmental toxins, de novo respiratory bursts associated with the mitochondria electron transport chain, endoplasmic reticulum and nuclear membrane electron transport systems, the prostaglandin synthase and lipoxygenase systems, and xenobiotics (38, 39). Additionally, ROS may influence GBM degradation by proteolytic enzymes (40). Pretreatment of rat GBM with H2O2 increases its susceptibility to degradation by proteases (41). In this regard, ROS by themselves have been shown to increase the expression of matrix metalloproteinase-9 by the glomerular epithelial cells. Therefore, the presence of ROS in the microenvironment around the GBM can likely activate several pathways of protein modification.

Finally, infusion of H2O2 into the rat renal artery produced local generation of ROS and induce a marked glomerular protein leak within minutes, suggesting an alteration in the integrity of the GBM. In these studies, there was no change in mean arterial pressure, single kidney glomerular filtration rate, or renal plasma flow, suggesting that at least acutely, local generation of ROS by this technique does not result in marked destruction of the normal renal architecture. To determine if H2O2-induced alteration of GBM integrity resulted in unmasking of the Goodpasture epitopes in vivo (as it was found in the in vitro studies using NC1 hexamers and human kidney sections), we infused Goodpasture autoantibodies into the renal artery of either control rats or rats pretreated with H2O2. As predicted, infusion of the Goodpasture autoantibodies into control rat kidneys failed to result in antibody binding, even though a control antibody directed against multiple epitopes in the GBM demonstrated linear binding to the GBM under these conditions. However, infusion of H2O2 into the kidney to induce in vivo generation of ROS resulted in exposure of the Goodpasture epitope and subsequent Goodpasture antibody binding. Additionally, we show that infusion of H2O2 in mice for several days can generate de novo anti-α3(IV) collagen antibodies, further supporting the notion that ROS can be responsible for in vivo generation of Goodpasture autoantibodies.

Although some degree of antigen exposure is essential for immunogenicity, the low frequency of clinical anti-GBM disease (18, 19, 24, 25, 41) suggests that normal exposure of epitopes by self-limited generation of ROS is not sufficient by itself to launch a fatal autoimmune response. Additional permissive factors for disease almost certainly are immunogenetic (18, 19, 24, 25, 41), including susceptibility in humans that maps to the class II HLA-D region (42, 43) and further restricted by the selection of immunoglobulin GM allotypes (43, 44). Recent studies in mice also indicate that immunologic susceptibility to Goodpasture syndrome is closely linked to the emergence of a Th1-dependent, cell-mediated repertoire (43–45).

Because most humans with this disease are in mid-life when they become ill (8), there must be other latent environmental or genetic drivers that finely modulate susceptibility. The findings from our work raise the possibility of an age-dependent deterioration of native ROS inhibitors as an initiator of auto-
immune injury. This hypothesis resonates easily with a more traditional notion of sporadic events that produce structural protein modification in α3(IV) NC1 domains permitting immunologic recognition in a disease-susceptible host (46). It remains to be determined whether ROS scavengers could have an epitope-protective therapeutic effect in Goodpasture patients.

Acknowledgments—We thank Prof. Subba Rao Kalluri and Prof. Sudhir V. Shah for suggestions and helpful discussions during the course of this study. We appreciate greatly the excellent technical assistance of Kate Spokes, Beth Shurtleff, and Michelle C. Werner, and we also thank Meier am Pfarrplatz (Grinzing) for inspiration and support.

REFERENCES

1. Hudson, B. G., Reeder, S. T., and Tryggvason, K. (1993) J. Biol. Chem. 268, 26033–26036
2. Timpl, R., Wiedemann, H., Van Velden, V., Furthmayr, H., and Kuhn, K. (1981) Eur. J. Biochem. 120, 203–211
3. Timpl, R., Oberbaumer, I., von der Mark, H., Bode, W., Wick, G., Weber, S., and Engel, J. (1985) Ann. N. Y. Acad. Sci. 460, 58–72
4. Hudson, B., Kalluri, R., Gunwar, S., Noelken, M., Mariyama, M., and Reeder, S. (1993) Kidney Int. 43, 135–139
5. Tryggvason, K., Soininen, R., Hestikka, S. L., Ganguly, A., Huotari, M., and Prockop, D. J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 9946–9950
6. Kalluri, R., Shield, F. A., Todd, P., Hudson, B. G., and Neilon, E. G. (1997) J. Clin. Invest. 99, 2470–2478
7. Hudson, B. G., Wieslander, J., Wisdom, B. J. J., and Neilon, M. E. (1989) Lab. Invest. 61, 256–260
8. Ryan J. M., Mason, P. J., Pusey, C. D., and Turner, N. (1998) Clin. Exp. Immunol. 113, 17–27
9. Kalluri, R., Gunwar, S., Reeder, S. T., Morrison, K. C., Mariyama, M., Ehser, K. E., Noelken, M. E., and Hudson, B. G. (1991) J. Biol. Chem. 266, 24018–24024
10. Kalluri, R., Wilson, C. B., Weber, M., Gunwar, S., Chonko, A. M., Neilon, E. G., and Hudson, B. G. (1996) J. Am. Soc. Nephrol. 7, 1178–1185
11. Kalluri, R., V. Gattone, V. H., Jr., Noelken, M. E., and Hudson, B. G. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 6201–6205
12. Hellmark, T., Segelmark, M., Unger, C., Burkhardt, H., Saus, J., and Wieslander, J. (1999) Kidney Int. 55, 936–944
13. Kalluri, R., Sun, M. J., Hudson, B. G., and Neilon, E. G. (1996) J. Biol. Chem. 271, 9062–9068
14. Neilon, E. G., Kalluri, R., Sun, M. J., Gunwar, S., Danoff, T., Mariyama, M., Myers, J. C., Reeder, S. T., and Hudson, B. G. (1993) J. Biol. Chem. 268, 8492–8405
15. Turner, N., Mason, P. J., Brown, R., Fox, M., Povey, S., Rees, A., and Pusey, C. D. (1992) J. Clin. Invest. 89, 592–601
16. Hellmark, T., Johannson, C., and Wieslander, J. (1994) Kidney Int. 46, 823–829
17. Wieslander, J., Langeveld, B., Butkowski, R., Jodlowski, M., Noelken, M., and Hudson, B. G. (1985) J. Biol. Chem. 260, 8564–8570
18. Wilson, C. B. (1996) in The Kidney (Brenner, B., ed) pp. 1253–1391, W. B. Saunders, Philadelphia
19. Solal, R. S., and Weindrich, R. (1996) Science 273, 59–63
20. Hudson, B. G., Kalluri, R., Gunwar, S., Weber, M., Ballester, F., Hudson, J. K., Noelken, N. E., Sarrats, M., Richardson, W. R., and Saus, J. (1992) Kidney Int. 42, 179–187
21. Marsden, P., Hall, A., and Brenner, B. (1996) in The Kidney (Brenner, B., ed) pp. 713–781, W. B. Saunders, Philadelphia
22. Wilson, C. B., and Dixon, F. J. (1973) Kidney Int. 3, 74–83
23. Wilson, C. B., Holdsworth, S. R., and Neale, T. J. (1981) Aust. New Zealand J. Med. 11, 94–99
24. Scheiman, J. I., Foidart, J. M., and Michael, A. F. (1980) Lab. Invest. 43, 373–381
25. Couser, W. G. (1988) Am. J. Kidney Dis. 11, 449–458
26. Couser, W. G., Darby, C., Salant, D. J., Adler, S., Stiliman, M. M., and Lowenstein, L. M. (1985) Am. J. Physiol. 249, F241–F250
27. Diamond, J. (1992) Am. J. Kidney Dis. 19, 292–300
28. Dileepan, K., Sharma, R., Stechschulte, D., and Savin, V. (1993) J. Clin. Lab. Med. 121, 797–804
29. Boyce, N., and Holdsworth, S. (1986) Kidney Int. 30, 813–817
30. Shah, S. V. (1989) Kidney Int. 35, 1083–1106
31. Neale, T. J., Ulirich, B., Ojha, P. P., Peczutowski, H., Verhoeven, A. J., and Kerjaschki, D. Proc. Natl. Acad. Sci. U. S. A. 90, 3654–3659
32. Davies, K. J., Lin, S. W., and Pacifici, R. E. (1987) J. Biol. Chem. 262, 9914–9920
33. Davies, K. J., and Delsignore, M. E. (1987) J. Biol. Chem. 262, 9908–9913
34. Davies, K. J., Delsignore, M. E., and Lin, S. W. (1987) J. Biol. Chem. 262, 9902–9907
35. Riedle, B., and Kerjaschki, D. (1997) Am. J. Pathol. 11, 215–231
36. Pacifici, R. E., Lin, S. W., and Davies, K. J. (1988) Basic Life Sci. 49, 531–535
37. Shah, S. (1981) J. Lab. Clin. Med. 98, 46–57
38. McCord, J., and Fridovich, I. (1982) Am. J. Pathol. 113, 418–425
39. Fligel, S., Lee, E., McCoy, J., Johnson, K., and Varani, J. (1984) Am. J. Pathol. 115, 418–425
40. Rees, A. J., Peters, D. K., Amos, N., Welch, K. I., and Batchelor, J. R. (1984) Kidney Int. 26, 444–450
41. Huey, B., McCormick, K., Crapper, J., Ratliff, C., Colombe, B. W., Garovoy, M. R., and Wilson, C. B. (1993) Kidney Int. 44, 307–312
42. Rees, A., Demaine, A., and Welch, K. (1984) Hum. Immunol. 10, 213–220
43. Kalluri, R., Danoff, T. M., Okada, H., and Neilon, E. G. (1997) J. Clin. Invest. 100, 2263–2275
44. Jennette, C., and Falk, R. (1991) Immunogenetics of Autoimmune Renal Disease, pp. 263–281, CRC Press, Boca Raton, FL
45. Wieslander, J., Kataja, M., and Hudson, B. G. (1987) Clin. Exp. Immunol. 69, 332–340
46. Gunwar, S., Ballester, F., Kalluri, R., Timonedo, J., Chonko, A. M., Edwards, S. H., Noelken, M. E., and Hudson, B. G. (1991) J. Biol. Chem. 266, 15318–15324
47. Butkowski, R. J., Wieslander, J., Wisdom, B. J. B., and Hudson, B. G. (1996) J. Biol. Chem. 271, 9062–9068
48. Kalluri, R., Pettrides, S., Wilson, C. B., Tomaszewski, J. E., Palevsky, H., Grippi, M. A., Madaio, M., and Neilon, E. G. (1996) J. Clin. Invest. 99, 813–817
49. Shaffer, F. A., and Foidart, J. M. (1980) Lab. Invest. 43, 373–381
