American Journal of Pathology, Vol. 133, No. 3, December 1988
Copyright © American Association of Pathologists

Interferon-Induced Glomerular Basement Membrane and Endothelial Cell Lesions in Mice

An Immunogold Ultrastructural Study of Basement Membrane Components

J. MOSS, PhD,* I. SHORE, BSc,*
D. WOODROW, MD,* and
I. GRESSER, MD†

From the Department of Histopathology, Charing Cross and Westminster Medical School, London, England, and the Laboratoire d'Oncologie Virale, Groupe de Laboratoires de l'Institut de Recherches Scientifiques sur le Cancer, Villejuif, France†*

Newborn Swiss mice were injected daily for the first week of life with mouse interferon α/β. This treatment resulted in a delay in the maturation of the kidney and the development of glomerular abnormalities. The width of the glomerular basement membrane (GBM) was increased up to tenfold and was characterized by a marked thickening of the endothelial aspect of the GBM. The endothelial cells lining the capillary loops were also abnormal with many dilated regions of the rough endoplasmic reticulum that contained amorphous electron-opaque material. Immunogold studies showed that type IV collagen and laminin/entactin were distributed throughout the thickened GBM, and also within the dilated rough endoplasmic reticulum of the endothelial cells. These results show that the interferon-induced lesion within the glomerulus is associated with an accumulation of normal GBM components and that endothelial cells are involved in this pathologic process. (Am J Pathol 1988, 133:557–563)

IT HAS BEEN SHOWN PREVIOUSLY that daily administration of potent mouse or rat interferon α/β to newborn mice[1–5] or rats[6] resulted in a marked inhibition of growth and the development of a crescentic "immune complex type" glomerulonephritis.[2,3,5] Ultrastructural examination of the kidneys from 8-day-old interferon-treated mice showed that the predominant lesion was a gross thickening of the endothelial aspect of the glomerular basement membrane (GBM), which contained an accumulation of amorphous material having the appearance of basement membrane.[5] We present here the results of an immunofluorescence and ultrastructural immunogold study using antibodies to type IV collagen and laminin/entactin designed to elucidate the composition of the interferon-induced GBM lesion and to evaluate the roles of epithelial, mesangial, and endothelial cells in the genesis of these lesions.

Materials and Methods

Mice

Swiss mice were provided from a specified pathogen-free colony at the Charing Cross and Westminster Medical School, London.

Interferon

Mouse interferon α/β was prepared from suspension cultures of C-243 cells induced with Newcastle disease virus and was concentrated, partially purified, and assayed as described previously.[7]

Experimental Plan

Mouse interferon α/β (4×10^4 international reference units in 0.05 ml phosphate buffered saline [PBS]) was injected in the interscapular region daily for the first 10 days of life. Control mice consisted of littermates injected with 0.05 ml of PBS. Previous experiments had shown that a variety of control preparations did not induce glomerulonephritis.[2,3] Mice were

Supported in part by the National Kidney Research Fund, UK, The C.E.E. (contracts ST2J-0043-2 and ST2J-0317), the Fondation de France (contract 87-1897) and I.N.S.E.R.M. (contract 87 1008).

Accepted for publication August 4, 1988.

Address reprint requests to J. Moss, Department of Histopathology, Charing Cross and Westminster Medical School, Fulham Palace Rd., London W6 8RF, England.

killed at 8 or 10 days and the kidneys removed for light microscopy, immunofluorescence, electron microscopy, and immunoelectron microscopy.

Antisera

The rabbit antibodies to GBM components were as follows: antibodies to mouse laminin/entactin (a gift from Dr. B. Hogan, National Institute for Medical Research, London, UK) were raised against the native complex of these two proteins purified from the Reichert's membrane of mouse embryos, and characterized as described previously.[8] Antiserum to mouse type IV collagen (obtained from the Institut Pasteur de Lyon, France) was raised against this component extracted and purified from mouse EHS sarcoma. The specificity and cross reactivity was checked by the suppliers, using immunofluorescence, ELISA, and radioimmunoassay.

The antibody to laminin/entactin reacts by immunoblotting with laminin and entactin but not with fibronectin or type IV procollagen. Antibody to type IV collagen reacts 100% with mouse type IV collagen, <0.1% for type I and type III collagen, <0.1% for heparan sulphate proteoglycans, and 12.5% for mouse laminin. The antisera to laminin/entactin and type IV collagen were diluted 1:3 and 1:99 in phosphate-buffered saline (PBS) respectively.

Light Microscopy

Portions of mouse kidney from interferon-treated and control mice were fixed in Duboscq-Brazil solution and embedded in paraffin wax. Sections were stained by hematoxylin and eosin (H & E), and periodic acid-Schiff (PAS).

Immunofluorescence

Samples of kidney were snap-frozen in isopentane cooled to −165 C by liquid nitrogen and were stored at −70 C. Cryostat sections were fixed briefly in acetone at room temperature and immunofluorescence carried out by an indirect technique. FITC-labeled sheep anti-rabbit immunoglobulin was obtained from Wellcome Research Laboratories, UK.

Electron Microscopy

Small pieces of kidney cortex were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 2% osmium tetroxide, dehydrated through ascending grades of alcohol, and embedded in Spurr's resin. Ultrathin sections were stained with aqueous uranyl acetate and Reynold's lead citrate.

Immunoelectron Microscopy

Specimens were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2, dissected into 1 cu mm pieces left for 3 hours at 4 C, and stored in 1% paraformaldehyde at 4 C for 1 week. The samples were rinsed briefly in buffer at 4 C. All further processing was carried out at approximately −25 C. The tissue was rapidly dehydrated through ascending grades of ethanol over 30 minutes, infiltrated with a 1:1 followed by a 1:2 mixture of ethanol with Lowicryl K4M resin (Taab Laboratories Ltd., Aldermaston, UK) for 30 minutes each, and left for 1 hour and then overnight in 2 changes of Lowicryl K4M. The samples were embedded in Lowicryl in brimming, sealed gelatin capsules and polymerized under ultraviolet light for 2 days at −20 C, and then for 2 days at room temperature.

Gold-colored ultrathin sections were collected on uncoated nickel grids. The immunogold technique was performed by immersing the grids in drops of the following solutions at room temperature: 1% ovalbumin (Grade V, Sigma, Poole, UK) in modified PBS for 25 minutes, primary antiserum in PBS for 2 hours, 5 drops of PBS and 5 drops of modified tris-buffered saline (TBS) over 15 minutes, goat anti-rabbit IgG conjugated with 10 nm gold (Janssen Life Sciences Products, Wantage, UK) diluted 1 in 10 with TBS for 1 hour, 5 drops of TBS over 10 minutes, and a final wash in several drops of distilled water. The sections were stained for 20 minutes in 1% aqueous uranyl acetate and Reynold's lead citrate for 3 minutes. The modified buffers used were PBS, pH 7.2, or 0.2 M TBS, pH 8.2, each containing 0.05% Tween 20, 0.1% BSA (Sigma), and 0.02 M sodium azide. Controls consisted of omitting the primary antiserum, or replacing the primary antiserum with nonimmune rabbit serum.

Morphometry

Electron micrographs of immunogold localizations of the basement membrane components in two control and two interferon-treated mice were placed on a coordinate digitizer tablet connected to an image analysis system, and areas of GBM were measured. The gold particles were then counted and expressed as numbers per unit area of basement membrane.

Results

Characterization of the Interferon-Induced Glomerular Lesions

Light Microscopy, Immunofluorescence, and Electron Microscopy

The most conspicuous abnormalities seen in the kidneys of interferon-treated suckling mice were the

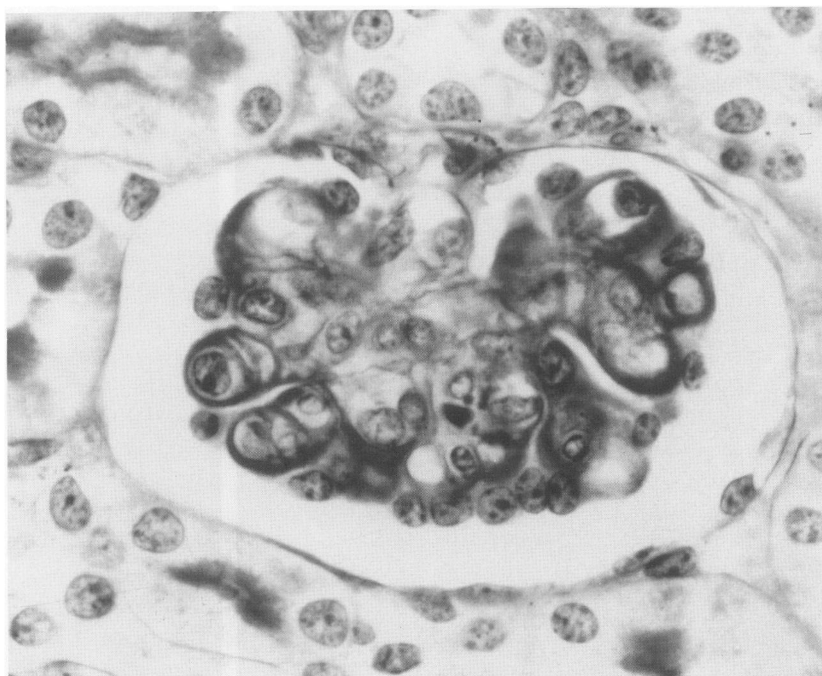

Figure 1—Glomerulus from an 8-day-old interferon-treated Swiss mouse. Note thickened capillary loops. PAS stain, ×900

marked thickening of glomerular capillary loops (Figure 1), and an overall increase in the mesangium but no apparent increase in the number of mesangial cells. These abnormalities were not seen in any control mice.

In interferon-treated mice the expanded mesangial region stained intensely with antibodies to laminin/entactin while most capillary loops showed focal, thickened areas of staining (Figure 2b). The basement membranes of the Bowman's capsule and tubules, and the matrix around the arterioles all stained as in control mice (Figure 2a). Interferon-treated mice also showed a conspicuous increased glomerular staining within the expanded mesangium and thickened capillary loops using antibody to type IV collagen.

Ultrastructurally, the glomeruli of interferon-treated mice were grossly abnormal. The GBM measured up to about ten times the width of the normal mouse GBM due to a marked thickening of the endothelial aspect of the GBM that showed areas of rarefaction, electron-dense material, small granules, and fine fibrils (Figure 3a). The lamina densa was a clearly defined region just beneath the lamina rara externa. There was also an increase in the mesangial matrix. In addition, in interferon-treated mice some glomerular endothelial cells showed conspicuous dilated cisternae of the rough endoplasmic reticulum that contained electron-opaque material (Figure 3b). No such lesions were seen in mesangial or epithelial cells.

Immunoelectron Microscopy

In control mice the gold particles showing the distribution of laminin/entactin were seen in singlets and also in small clusters across the full width of the basement membrane (Figure 4a). Laminin/entactin was also seen distributed throughout the mesangial matrix, but was not present in any amount over other areas of the section. The distribution of type IV collagen was similar to that of laminin/entactin.

In interferon-treated mice antigenic sites for laminin/entactin and type IV collagen, as demonstrated by immunogold particles, were present across the whole of the grossly thickened GBM (Figure 4b, c). Both GBM components were also distributed throughout the mesangial matrix.

Counts of the number of gold particles per square micron of GBM, using antibodies to laminin/entactin and type IV collagen, in control and interferon-treated mice showed that the density of particles in the thickened or nonthickened areas of the GBM in these mice was approximately the same (data not shown). Thus, the thickening of the GBM in interferon-treated mice was not due to oedema which would have resulted in a lower density of gold particles/sq μ.

Examination of the rough endoplasmic reticulum of glomerular endothelial cells of interferon-treated mice showed that the dilated cisternae contained gold particles using antibodies to laminin/entactin (Figure 5a, b), and also to type IV collagen. Immunogold particles were seen only in the dilated cisternae of the rough endoplasmic reticulum of endothelial cells, in which case all the dilated cisternae were labeled. Particles were not observed in the nondilated regions of the rough endoplasmic reticulum of endothelial cells, epithelial cells, or mesangial cells.

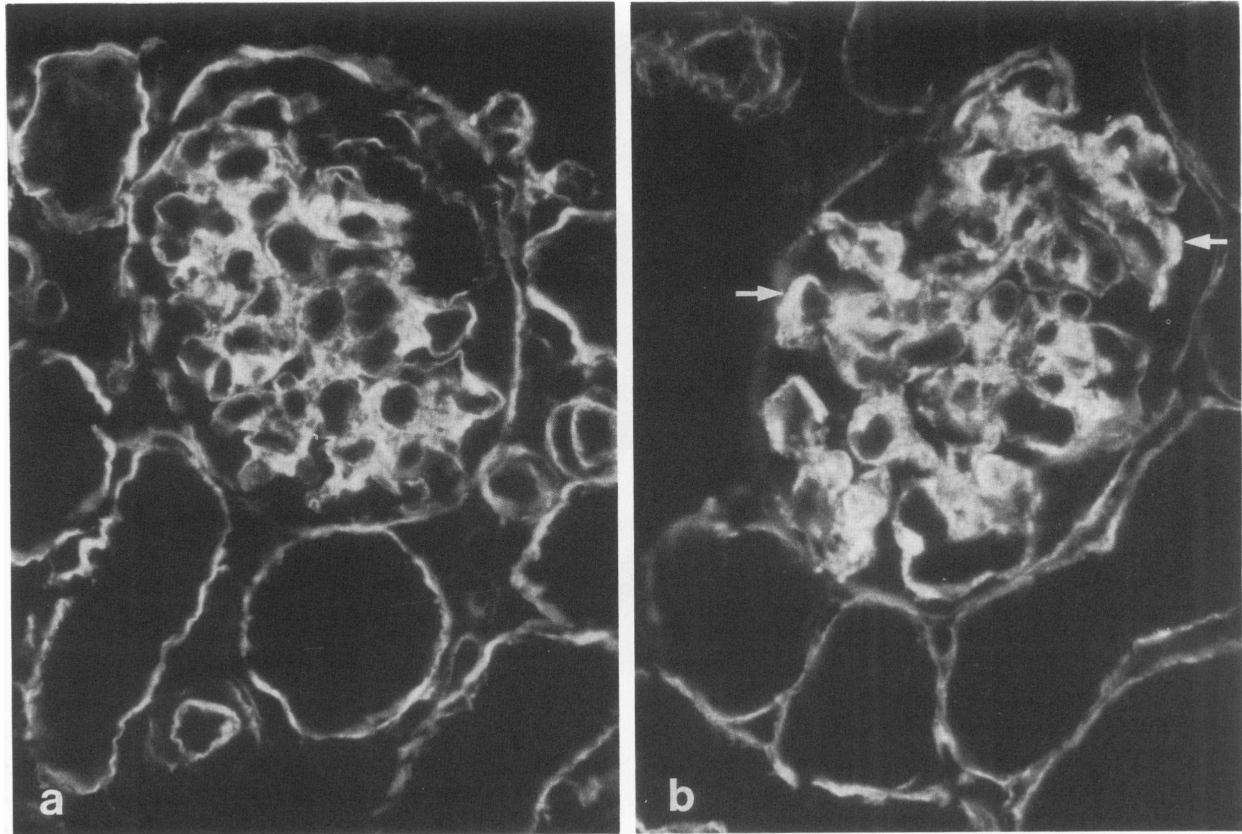

Figure 2—Immunofluorescence showing the presence of laminin/entactin in glomeruli from 8-day-old mice. There is linear staining of normal capillary loops (**a**), and intense staining of the thickened loops (arrow) of the interferon-treated mouse (**b**). ×500

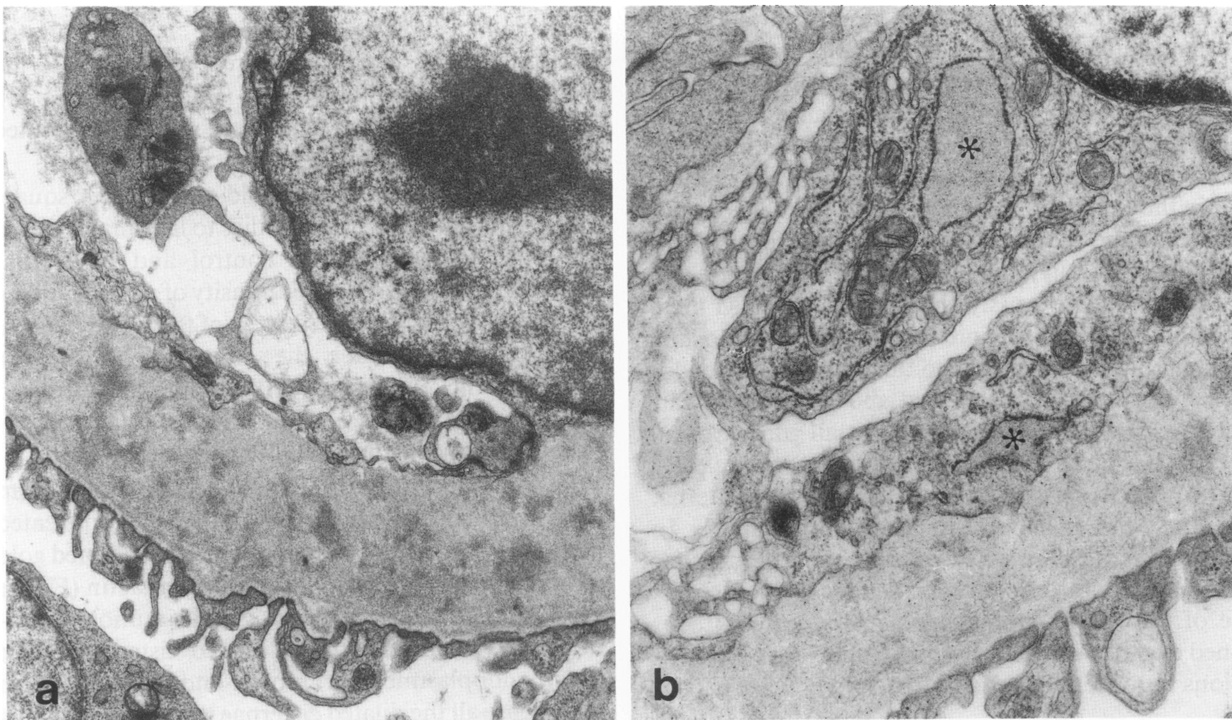

Figure 3a—Electron micrographs showing the markedly thickened lamina rara interna of GBM in 8-day-old interferon-treated mice. **b**—Dilated cisternae (*) of the rough endoplasmic reticulum of endothelial cells. a, ×13,000, b, ×24,000

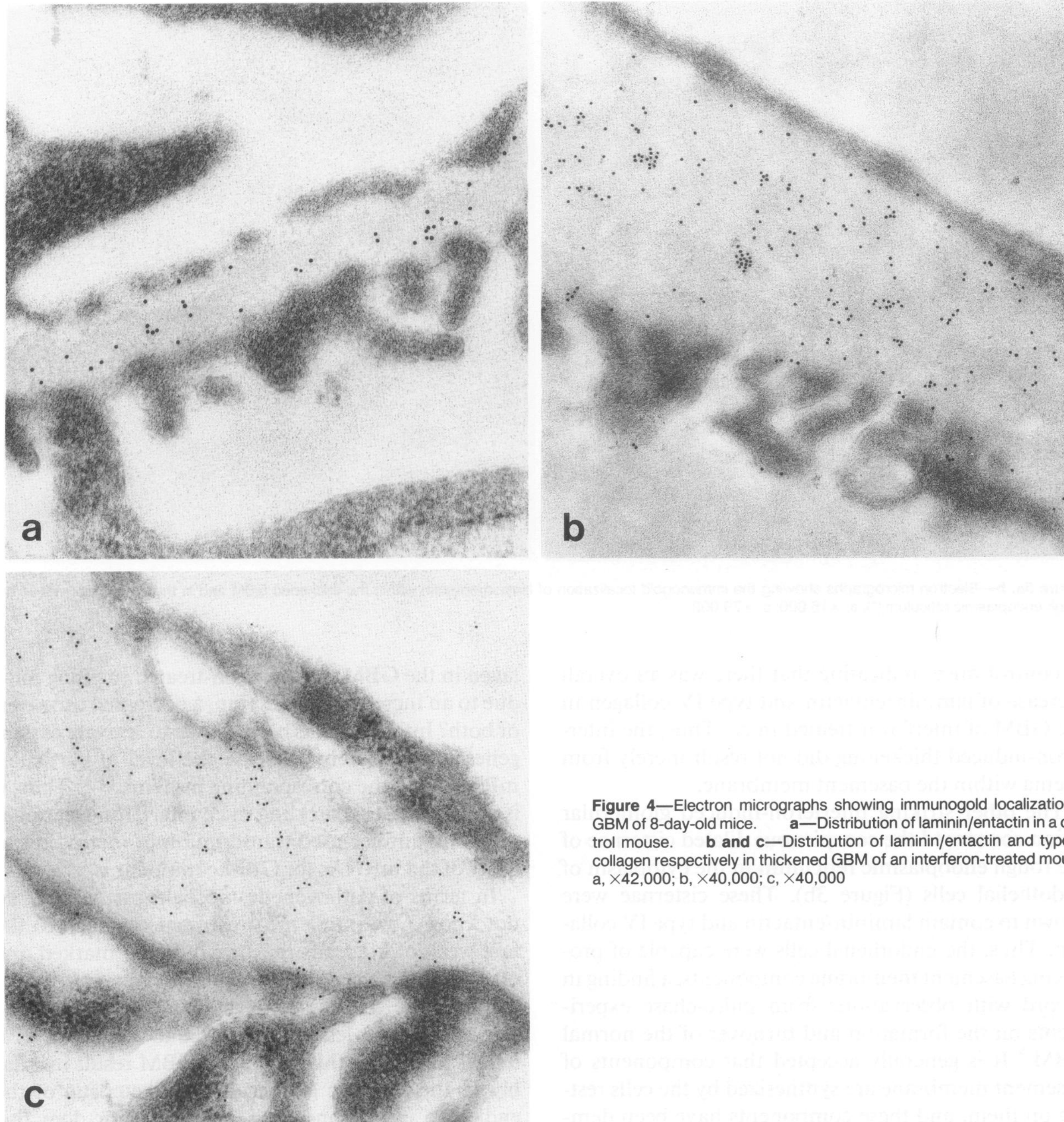

Figure 4—Electron micrographs showing immunogold localization in GBM of 8-day-old mice. a—Distribution of laminin/entactin in a control mouse. b and c—Distribution of laminin/entactin and type IV collagen respectively in thickened GBM of an interferon-treated mouse. a, ×42,000; b, ×40,000; c, ×40,000

Discussion

We have investigated the GBM lesion in 8-day-old interferon-treated mice (Figure 1) using antibodies to the normal basement membrane components: laminin/entactin and type IV collagen. The purpose of this study was twofold: 1) to determine whether the markedly thickened GBM of interferon-treated mice contains the same components as the GBM of normal mice and if so, to determine their density relative to the normal GBM, and 2) to determine, if possible, whether glomerular endothelial, mesangial, or epithelial cells contribute to the genesis of these lesions.

In interferon-treated mice, the thickened areas of GBM were stained by immunofluorescence with an intensity equal to that of the GBM in control mice (Figure 2). These results suggested that the thickened GBM of interferon-treated mice consisted of an accumulation of normal basement membrane components. Immunogold studies confirmed that the interferon-induced areas of GBM thickening contained normal basement membrane components and that these were distributed over the full thickness of the GBM (Figure 4b, c). The number of gold particles per square micron in the thickened GBM of interferon-treated mice was roughly the same as that in the GBM

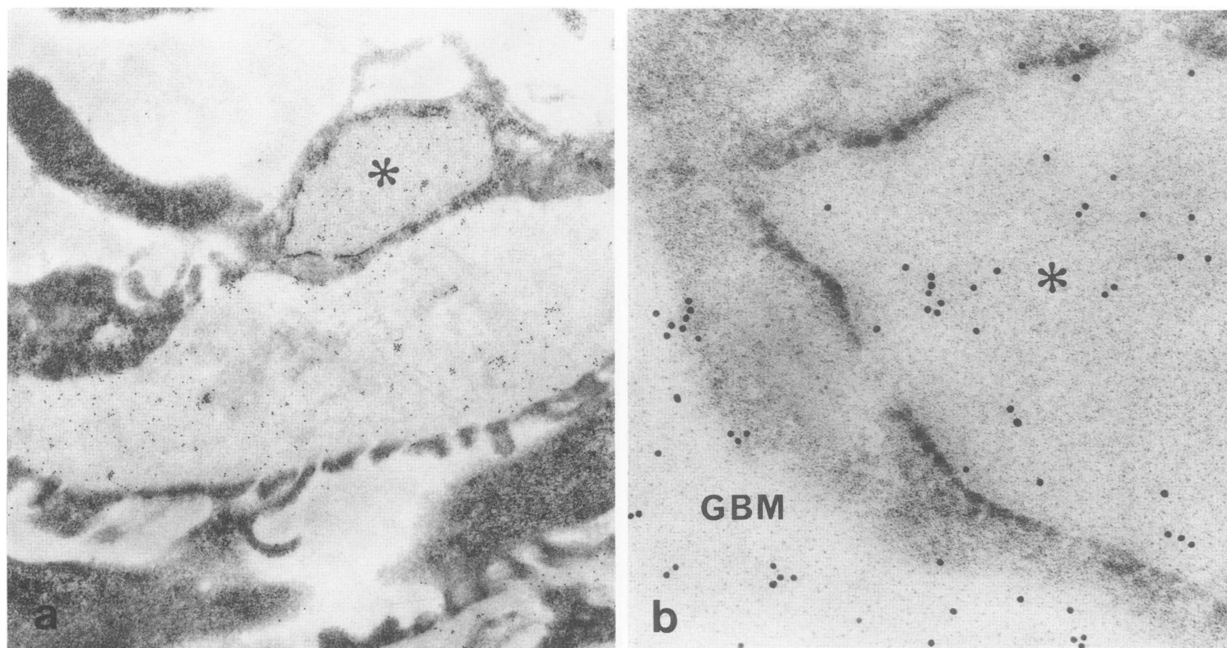

Figure 5a, b—Electron micrographs showing the immunogold localization of laminin/entactin within the thickened GBM and in the dilated cisterna of the rough endoplasmic reticulum (*). a, ×16,000; b, ×79,000

of control mice, indicating that there was an overall increase of laminin/entactin and type IV collagen in the GBM of interferon-treated mice. Thus, the interferon-induced thickening did not result merely from edema within the basement membrane.

The other striking interferon-induced glomerular lesion concerned the conspicuous dilated cisternae of the rough endoplasmic reticulum in the cytoplasm of endothelial cells (Figure 3b). These cisternae were shown to contain laminin/entactin and type IV collagen. Thus, the endothelial cells were capable of producing basement membrane components, a finding in accord with observations from pulse-chase experiments on the formation and turnover of the normal GBM.[9] It is generally accepted that components of basement membrane are synthesized by the cells resting on them, and these components have been demonstrated in the rough endoplasmic reticulum of rat endodermal cells,[10,11] of endothelial cells of developing tissues,[12,13] and of cultured neuroblastoma cells.[14] In our experiments the epithelial cells showed no abnormalities, and within the GBM the lamina densa and lamina rara externa were not altered. Our observations that interferon administration induced an accumulation of GBM components within dilated regions of the rough endoplasmic reticulum of endothelial cells suggests therefore that the interferon-induced thickening of the endothelial aspect of the GBM is related in some manner to endothelial cell function.

Several questions remain to be answered. Is the marked increase of laminin/entactin and type IV collagen in the GBM of interferon-treated suckling mice due to an increased production, a decreased turnover, or both? Interferon has been shown to activate certain genes,[15] and thereby increase the level of particular mRNAs and the corresponding proteins.[16–18] Thus, it is possible that in suckling mice interferon treatment results in an increased transcription or increased stability of the mRNAs for GBM components.

In terms of pathogenetic mechanisms, we still do not know how interferon treatment confined to the first week of life leads inexorably to the marked and often progressive glomerulonephritis observed in the adult mouse. Likewise we do not know how these early glomerular lesions are related to the ultimate renal lesions. Does this thickened GBM result inevitably in the sclerotic glomerulus characteristic of the end stage interferon-induced lesion,[2–4] or does this thickened GBM trap "circulating immune complexes" that then contribute to the progression of glomerular lesions?

References

1. Gresser I, Tovey MG, Maury C, Chouroulinkov I: Lethality of interferon preparations for newborn mice. Nature 1975, 258:76–78
2. Gresser I, Maury C, Tovey M, Morel-Maroger L, Pontillon F: Progressive glomerulonephritis in mice treated with interferon preparations at birth. Nature 1976, 263: 420–422
3. Gresser I, Aguet M, Morel-Maroger L, Woodrow D, Puvion-Dutilleul F, Guillon J-C, Maury C: Electropho-

retically pure mouse interferon inhibits growth, induces liver and kidney lesions, and kills suckling mice. Am J Pathol 1981, 102:396–402

4. Gresser I: Can interferon induce disease, Interferon 4. Edited by I Gresser. New York, Academic Press, 1982, pp 95–127
5. Morel-Maroger L, Sloper JC, Vinter J, Woodrow D, Gresser I: An ultrastructural study of the development of nephritis in mice treated with interferon in the neonatal period. Lab Invest 1978, 39:513–522
6. Gresser I, Morel-Maroger L, Châtelet F, Maury C, Tovey MG, Bandu M-T, Buywid J, Delauche M: Delay in growth and the development of nephritis in rats treated with interferon preparations in the neonatal period. Am J Pathol 1979, 95:329–346
7. Tovey MG, Begon-Lours J, Gresser I: A method for the large scale production of potent interferon preparations (38196). Proc Soc Exp Biol Med 1974, 146:809–815
8. Semoff S, Hogan BL, Hopkins CR: Localization of fibronectin, lamin-entactin, and entactin in Reichert's membrane by immunoelectron microscopy. EMBO J 1982, 1:1171–1175
9. Nayyar RP, Volini FI, Borke JL: The role of visceral epithelial, endothelial and mesangial cells in the formation and turnover of glomerular basement membrane, The Glomerular Basement Membrane. 1st Int. Symp. Renal Physiol., Basel 1980, 3:212–219
10. Laurie GW, Leblond CP, Martin GR: Intracellular localization of basement membrane precursors in endodermal cells of the rat parietal yolk sac. II. Immunostaining for type IV collagen and its precursors. J Histochem Cytochem 1982, 30:983–990
11. Laurie GW, Leblond CP, Martin GR, Silver MH: Intracellular localization of basement membrane precursors in endodermal cells of the rat parietal yolk sac. III. Immunostaining for laminin and its precursors. J Histochem Cytochem 1982, 30:991–998
12. Abrahamson DR: Origin of the glomerular basement membrane visualized after in vivo labeling of laminin in newborn rat kidneys. J Cell Biol 1985, 100:1988–2000
13. Laurie GW, Leblond CP, Cournil I, Martin GR: Immunohistochemical evidence for the intracellular formation of basement membrane collagen (type IV) in developing tissues. J Histochem Cytochem 1980, 28:1267–1274
14. Liesi P: Laminin in cultured mouse C1300 neuroblastoma cells: Immunocytochemical localization by pre- and postembedding electron microscope procedures. J Histochem Cytochem 1983, 31:755–764
15. Gribaudo G, Toniato E, Engel DA, Lengyel P: Interferons as gene activators. Characteristics of an interferon-activatable enhancer. J Biol Chem 1987, 262:11878–11883
16. Farrell PJ, Broeze RJ, Lengyel P: Accumulation of an mRNA and protein in interferon-treated Ehrlich ascites tumour cells. Nature, 1979, 279:523–525
17. Shulman L, Revel M: Interferon-dependent induction of mRNA activity for (2′-5′)oligo-isoadenylate synthetase. Nature 1980, 288:98–100
18. Colonno RJ: Accumulation of newly synthesized mRNAs in response to human fibroblast (β) interferon. Proc Natl Acad Sci USA 1981, 78:4763–4766

Acknowledgment

The authors thank Mrs. G. Miller, Mrs. P. Porter, and Mr. R. Fletcher for technical assistance.