Light Microscopical Examination of Glomerular Basement Membrane in Systemic Lupus Erythematosus

J. V. KLAVINS, M.D., Ph.D.,*† Z. WESSELY, M.D.,† and P. PICKETT†

Departments of Pathology, Queens Hospital Center, Long Island Jewish-Hillside Medical Center Affiliation, Jamaica, NY 11432 and Duke University Medical Center, Durham, NC 27710

ABSTRACT

To obtain as much information as possible from light-microscopy about changes in the glomerular basement-membrane, a thin section microtome attachment is necessary in order to produce 1 to 3 micron sections.

The most informative staining method of paraffin embedded tissue is the Müller-Mowry-Masson trichrome (M-M-M) combination stain. The deposits, e.g., in systemic lupus erythematosus (SLE), stain red, the basement membrane light orange and the endothelial cell cytoplasm light pink. With this stain, as with the Müller-Mowry stain, normal glomeruli show an uninterrupted line of blue acid mucopolysaccharides along the basement membrane, where it is joined by foot processes of the epithelial cells. In contrast, in glomeruli from patients with SLE, focal loss of acid mucopolysaccharides is noted.

Introduction

Light microscopy, when utilizing special staining techniques and a thin section microtome attachment, can provide significant information about the glomerular basement membrane, e.g., in systemic lupus erythematosus (SLE).

During these studies, a new technique—the Müller-Mowry-Masson trichrome combination—was developed.

Materials and Methods

Fifty cases clinically classified as SLE were studied. The paraffin blocks were at first sectioned with the conventional microtome and approximately five to six micron thick sections were stained with hematoxylin and eosin (H&E). These sections included the entire tissue sample that was embedded in paraffin. Areas for more detailed studies were then selected from the preliminary H&E slides and were cut out from the paraffin blocks to provide a field approximately 5 x 5 mm. Such small blocks were necessary to obtain uniformly
thin sections on subsequent cutting with a thin section attachment to the microtome, according to the method by Churg and Grishman. These sections measured approximately 1 to 3 \( \mu \) in thickness.

**MÜLLER-MOWRY-MASSON TRICHROME COMBINATION STAIN (M-M-M)**

This was done by first utilizing steps 1 through 6 of the Müller-Mowry colloidal

**Figure 1.** Müller-Mowry-Masson trichrome stain \( \times \) 1380. Portion of glomerulus from patient with systemic lupus erythematosus. Red deposits can be differentiated from a light orange basement membrane, blue podocyte area and light pink cytoplasm of endothelial cells.

**Figure 2.** Müller-Mowry colloidal iron stain \( \times \) 800. Portion of normal glomerulus. Acid mucopolysaccharides are present uninterrupted along the entire basement membrane, where on electron-microscopic examination the foot processes of the epithelial cells join the basement membrane.
iron stain\(^7\) followed by a modification of the original Masson’s trichrome stain,\(^6\) namely:

(1) Staining with Ponceau acid fuchsin solution for 5 minutes; (2) Rinsing in 5 percent water; (3) Placing in 10 percent phosphomolybdic acid for 5 minutes; (4) Rinsing in acidified water; (5) Staining in light green solution for 5 minutes; (6) Rinsing in acidified water for 3 to 4 minutes; (7) Dehydrating in 95 percent alcohol, absolute alcohol and xylol and (8) Mounting.

**REAGENTS**

1. Ponceau acid fuchsin solution
   A. One g of Ponceau de xyledine and 1 g of acid fuchsin in 100 ml distilled water.
   B. Exactly 0.5 g of azophloxine G.A. in 100 ml of distilled water.

2. Working solution of Ponceau acid fuchsin
   A. Add 10 ml of solution A and 2 ml of solution B to 88 ml of solution C.

3. Light green solution
   A. One g of light green to 100 ml of 0.5 percent acetic acid solution.

4. Acidified water
   A. Exactly 0.5 g of glacial acetic acid to 100 ml of distilled water.

In addition, the following staining procedures were applied:

- Masson trichrome stain,\(^6\) Wilder’s reticulum stain,\(^5\) Müller-Mowry colloidal iron method,\(^7\) Periodic acid Schiff technique (PAS),\(^4\) Feulgen technique,\(^4\) and Periodic acid-methenamine-silver-Masson trichrome sequence (PA-MS-M).\(^1\)

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**Figure 3.** Müller-Mowry colloidal iron stain \(\times 1600\). Portion of glomerulus from patient with systemic lupus erythematosus. Focal absence of acid mucopolysaccharides over the glomerular basement membrane.
Results

Cross sections of a normal glomerular basement membrane were clearly demonstrated with all stains as a dense uninterrupted homogeneous line. They were best delineated by staining with methenamine silver.

When kidney containing "wireloops" were cut in five or six micron sections, a single thick membrane forming the glomerular loops was apparent with H&E and PAS stains. However, when the sections were cut with the thin section attachment to the microtome, a clear distinction could be made between the basement membrane and the deposits which, when using electron microscopy, appeared as more electron-dense osmiophilic material. The deposits appeared less intense than the basement membrane although they had the same color.

With Masson trichrome stain, the deposits appeared red while the basement membrane appeared light orange. When the PA-MS-M sequence was used, the deposits stained red and the basement membrane dark brown or black.

In a few instances, the deposits were also Feulgen positive. Utilizing the M-M-M sequence, the deposits were identified in all three predominant sites of localization in relationship to the glomerular basement membrane: subendothelially, subepithelially and within the basement membrane where the deposits appeared globular or cylindrical in shape. In addition, there was segmental interruption of the deposit by basement membrane-like material. Other changes consisted of thickening, fragmentation, splitting or focal loss of basement membrane.

Discussion

The anatomical manifestations of SLE, particularly those of the renal glomerulus, have been the subject of innumerable studies by light microscopy, electron microscopy and immunofluorescence. It has been established by electron microscopy and immunofluorescence that antigen-antibody complexes are deposited along the basement membrane of the glomerular capillary in a characteristic location, either between the basement membrane and the endothelium or between the basement membrane and the epithelium, within the basement membrane, within the mesangium or in any combination of these.

These deposits and their location were demonstrated using a thin section microtome attachment and the M-M-M staining sequence, which were developed in our laboratories. This technique provided the most useful information.

In our cases, as in those described earlier, the preferential location of the deposits occurred in the subendothelial and mesangial areas in active progressive disease. Clinically, inactive lesions were more prone to have subepithelial or mesangial deposits and basement membrane thickening.
The state of acid mucopolysaccharides (AMP) in kidney disease has not been investigated as widely as the other parameters. AMP was present between the basement membrane and the epithelium in glomeruli of normal control kidneys, and was stained with a M-M-M combination stain. There was spotty absence of AMP from the epithelial side of the basement membrane in some cases of SLE. Our light microscopic findings in the normal control kidneys reflected the electron-microscopic studies of Jones\textsuperscript{3} wherein a layer of colloidal iron positive material was described, covering the podocyte surface exposed to the urinary space. To the best of our knowledge, no comparable studies have been done in kidneys of patients afflicted with SLE. From our studies, it is not apparent whether the absence of histochromically demonstrable AMP indicates loss of foot processes or some other alteration in ultrastructurally visible sites of the epithelial cells.

References