MINIREVIEW

Permeability of Basement Membranes to Macromolecules (43782B)

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Basement membranes are extracellular structures that underlie most epithelia and surround the cells of nerve and muscle. They are generally stronger than the cells they support and so provide a firm anchoring site for cells, lending physical strength to the tissue and functioning to mediate attachment between cells and fibrillar connective tissue. Basement membranes also play important roles in development and wound healing, and differentiation of cells is modified by the basement membrane substrate on which they lie. Finally, basement membranes can act to separate compartments within the body by acting as barriers to the movement of cells and macromolecules. All of these functions are described in detail in a number of recent reviews (1–9). The function of basement membranes as barriers to macromolecules has been studied extensively, because it is thought that loss of this function is a part of the progression of several diseases, particularly diabetes mellitus (10–23). However, no consensus concerning a basic understanding of this function of basement membranes has developed. Thus, this review will concentrate on the function of basement membranes in retarding movement of macromolecules between compartments in the body, and—because extensive reviews of older literature already exist—will describe mostly work that has been published in the past 5 years.

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0037-9727/94/2071–0013$10.50/0

Basement Membranes as Barriers to Macromolecules

The most common method used for studying the permeability of basement membranes has been the examination of electron-dense tracer distribution in tissue. A tracer, such as ferritin, is injected into the blood, and tissues subsequently are isolated and fixed for electron microscopy. The presence of the electron-dense tracer on one side of a basement membrane and not on the other indicates that the basement membrane is impermeable to the tracer.

Several workers have used this method to demonstrate the ability of the basement membrane in the renal glomerulus (GBM) to restrict the passage of serum proteins into the urine (to be discussed further below), but little recent work in other tissues has been done. Work with ferritin in the epidermis has demonstrated that native (anionic) ferritin was less concentrated in the space between the epithelial cells and the basement membrane than was cationic ferritin (24), suggesting that this basement membrane does provide some restriction to diffusion of macromolecules based on charge. Alternatively, this could also mean that fixed, negative charges within this space lead to a reduction in the concentration of anionic ferritin. The latter explanation was put forth to explain a similar difference that was seen between concentrations of native and cationic ferritin in the space between cells of the macula densa and its basement membrane (25); that is, native ferritin was seen in lower concentration than cationic ferritin in this space, and this difference could be due to fixed anionic charges in the space, rather than to a selective permeability of the basement membrane.

Permeation of a molecule as large as ferritin (~450 kDa) suggests that epidermal and renal tubular basement membranes would not restrict the diffusion of

interstitial macromolecules such as immunoglobulins. For a tissue such as skin, this probably means that the concentrations of most macromolecules in the space between the cells and basement membrane are about the same as those in the interstitium of the dermis. For the macula densa of the nephron, the situation is not as clear: because this spot on the side of the thick ascending limb is apparently permeable to water, it is probable that a constant flow of water normally occurs from the cells of the macula densa into the interstitium (26). Would this flow of water oppose the influx of macromolecules from the interstitium? Experiments to answer this question have not been done for the macula densa, but we discuss below evidence that such a situation may indeed exist in the renal proximal tubule.

Besides the use of electron-dense tracers to probe the permeability of basement membranes, efforts also have been made to study the properties of basement membranes *in vitro*. Robinson and colleagues have developed a method for isolating basement membranes and layering them onto filters for direct study of permeability (27, 28), and this method has been adapted in other laboratories as well (29, 30). In addition, basement membranes of renal tubules have been studied individually by the isolated, perfused tubule method (31–33). All of these studies suggest that basement membranes *in vitro* show some restriction for the permeation of macromolecules, but that this restriction is incomplete for all but very large molecules.

In studies of the GBM, interpretation of the results of tracer studies and *in vitro* studies conflict. Because the GBM has been studied so extensively, we will now consider it in detail.

Studies on the GBM

In the renal glomerulus, the basement membrane is the only continuous barrier that separates blood from the fluid in Bowman's space (Fig. 1) (34, 35). Because the fluid in Bowman's space is essentially devoid of the proteins found in blood plasma, a barrier must exist in the glomerular capillary that allows water and relatively small solutes to pass, but that restricts the passage of larger molecules. From early studies with the electron microscope, some workers hypothesized that the epithelial cells surrounding the capillary constituted the filtration barrier that prevents the passage of proteins (36). This idea was challenged by the work of Farquhar and coworkers, who used histochemistry and electron-dense tracers to provide extensive evidence that the GBM is the primary barrier that prevents plasma proteins from entering the urine (reviewed in 37 and 38).

Recent studies with electron-dense tracers have confirmed the picture of the basement membrane as the primary barrier to protein loss into the urine. Fujigaki *et al.* (39) used drip-fixation followed by immunogold labeling to identify the location of endogenous serum albumin, transferrin, IgG, and IgM (respectively, ~69, 90, 180, and 900 kDa) in the wall of the glomerular capillary in the rat kidney. All but IgM were found within the basement membrane, but all showed restricted penetration of the GBM, with most of the labeling on the blood side and little near the podocytes. When the kidneys were perfusion fixed, almost no labeling for these proteins was seen, suggesting that the proteins were not bound in place, but were indeed serum proteins that had partially penetrated the GBM.

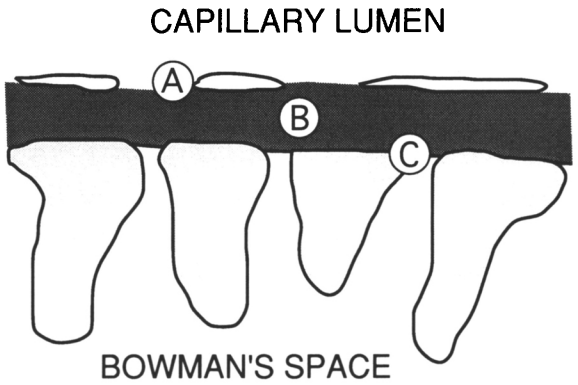

Figure 1. Schematic of glomerular filtration barrier. Structures that possibly restrict the passage of serum protein from the capillary lumen into Bowman's space include: A, the charged surface of the endothelial cells; B, the glomerular basement membrane (GBM); and C, the charged surface of the foot processes and slit diaphragms of the podocytes. The GBM almost certainly does not contain prominent laminae rarae (34, 35) as was once thought.

Russo & Bendayan (40) saw a similar distribution of serum albumin across the basement membrane of normal human glomeruli. In contrast, glomeruli from nephrotic patients showed no restriction of albumin within the GBM, but instead showed roughly even staining across its width, from endothelium to the podocytes. Desjardins & Bendayan (41) studied rat kidneys during development, and saw even distribution of endogenous serum albumin across the GBM during early development, but this distribution changed to one in which the albumin was restricted toward the subendothelial region as the animals matured.

These three studies seem to confirm the important role of the basement membrane of the glomerulus in restricting the entry of serum proteins into the urine. Work with glomerular matrix[2] *in vitro,* however, has

[2] Work *in vitro* with GBM invariably involves isolating glomeruli and obtaining basement membrane by sonication or treatment with detergent. Thus, the preparations contain both GBM and mesangial matrix. We will refer to isolated GBM as glomerular matrix, which more properly describes the material used for these studies.

not confirmed that the basement membrane possesses the ability to block the passage of serum albumin to the extent seen in the glomerulus *in vivo*. Robinson and Walton (42) layered glomerular matrix from pig kidney onto filters and found that the concentration of albumin in the filtrate was 3%–17% of the concentration in the filtrand. Similar restriction was found by Daniels *et al.* with rat glomeruli (30), while Bertolatus and Klinzman measured lower values of about 1% using dog glomeruli (29). These three studies were done with different amounts of glomerular matrix and cannot be easily compared directly. Nonetheless, it is significant that all showed albumin levels in the filtrate that were considerably higher than glomerular filtrate *in vivo*, which contains albumin at a concentration about 1000 times lower than in plasma (43, 44). The glomerular barrier *in vivo* has only one layer of basement membrane, rather than the stacks of glomerular basement membrane used in these studies, so the differences between filtrate concentration of albumin *in vivo* and that seen with isolated glomerular matrix are clearly significant.

Thus, the data *in vitro* show some conflict with those *in vivo*, and this disagreement is also seen in recent studies and reviews of the glomerular filtration barrier. Myers and Guasch (45) reviewed studies of glomerular permselectivity and concluded that the filtration barrier consists of a proximal electrostatic barrier, probably the glycocalyx of the endothelial fenestrae, and a distal size-selective barrier, probably the slit diaphragms of the podocytes. In support of the idea that the glomerular cells are most important, Comper *et al.* (46) measured the anionic charge concentration in isolated glomerular matrix and concluded that the basement membrane could not be a significant barrier for anionic macromolecules. Daniels and coworkers (47, 48) maintained that the basement membrane can provide some selectivity, but that the crucial barrier in the glomerulus is provided by the podocytes. In contrast, Remuzzi and Remuzzi (49) reviewed studies of glomerular permselectivity and concluded that no details about the structural location of the glomerular size-barrier can be stated with certainty. Hostetter and Rosenburg (50) reviewed the effects of hemodynamic alterations on glomerular permeability and similarly concluded that the changes could be due to effects on the cells, the basement membrane, or both.

The difficulty in distinguishing the relative roles of glomerular cells and the GBM in restricting the movement of protein into the glomerular filtrate is illustrated by trying to explain the results of van den Born *et al.* (51), who injected rats with a monoclonal antibody against a glomerular basement membrane heparan sulfate. The antibody was an IgM and was shown not to penetrate past the subendothelial region of the basement membrane. The injections induced in the animals an acute proteinuria that was marked by a decrease in the normal selectivity of the glomerular barrier between albumin and IgG. The albuminuria was greatest 2 hr after injection and gradually declined over several days.

Because this antibody was specific for a major glycosaminoglycan in the glomerular basement membrane, one might hypothesize that the cause of the subsequent proteinuria was masking of anionic charge in the basement membrane by the antibody. This would have allowed a higher-than-normal concentration of serum albumin to enter the basement membrane. Because heparan sulfate is bound by other molecules of the basement membrane, its masking by the IgM also could have led to a rearrangement of the noncovalent structure of the basement membrane, thereby altering the effective pore size of this filter.

Alternatively, these data could be explained by actions on the glomerular cells. Directly, the IgM could have bound to heparan sulfate on the endothelial cells (some inconsistent staining of the surfaces of these cells was seen [51]), and thereby could have disrupted the proximal charge barrier (45). Indirectly, the IgM binding could have clogged the basement membrane sufficiently that the rate of glomerular filtration was reduced. In response to this reduction, podocytes may have been somehow induced to increase the permeability of the slit diaphragms, and the proteinuria could have resulted from this change.

Thus, distinguishing between effects on the GBM or the glomerular cells is difficult. Neither of the above hypotheses is strongly supported, and we have not even considered the possible effects of hemodynamic changes that may have been induced by antibody binding. *In vitro* studies of the components of the glomerular barrier may help to define the relative roles of cells and basement membrane, but eventually the integration of these components into the complete glomerular barrier must be understood if we are to be able to understand the loss of barrier function in glomerular disease.

Studies of the Renal Tubular Basement Membrane (TBM)

The permeability of the renal tubular basement membrane (TBM) has been studied more directly than that of any other basement membrane. By isolating and perfusing individual renal tubules and then removing the tubule cells with osmotic shock or detergent, Welling and coworkers studied the permeability properties of single basement membranes (31, 32). They found these structures to be very permeable to water, but permeability to macromolecules was assessed indirectly. Recently, a similar study looked more directly at albumin permeability in the basement membrane of the renal proximal tubule and concluded that

this basement membrane can be a barrier that allows oncotically driven water flow (33). This study also raises the possibility that the basement membrane could be a useful barrier, even though it possesses a finite permeability to serum albumin, as we will now describe.

The rate of fluid absorption in the proximal tubule is affected by the peritubular concentration of protein (52), and this effect has never been explained. One hypothesis suggested that the tubular basement membrane could act as a barrier between a protein-containing interstitial space and a protein-free intercellular space (see Fig. 2), and that this oncotic force then could affect the function of the tubule. However, when this basement membrane failed to block the movement of radiolabeled albumin (53), it appeared that the intercellular space could therefore not be protein-free, and thus the hypothesis was considered to be untenable.

An alternative hypothesis (Fig. 2) suggests that sites of albumin permeability could also be sites for solvent drag of albumin across the basement membrane. In this scheme, fluid transported into the intercellular space would then move across the basement membrane, and some of this flow would drag albumin with it. The consequent reduction in albumin concentration in the intercellular space would then provide a force for water flow across the portions of the membrane that are not permeable to albumin. This system would work only if the albumin permeability was limited relative to that of water, and it appears that this indeed is the case for the basement membrane of the proximal tubule (33).

Thus, restriction of permeability of proteins in a basement membrane may be important, even if the restriction to permeation is not absolute. This situation exists in the proximal tubule, because transport by the epithelium results in a constant flow of fluid across the basement membrane, and restriction of macromolecules in this dynamic system can then lead to redistribution of physical forces within the tissue. Such a situation may exist in other tissues, but none has been described.

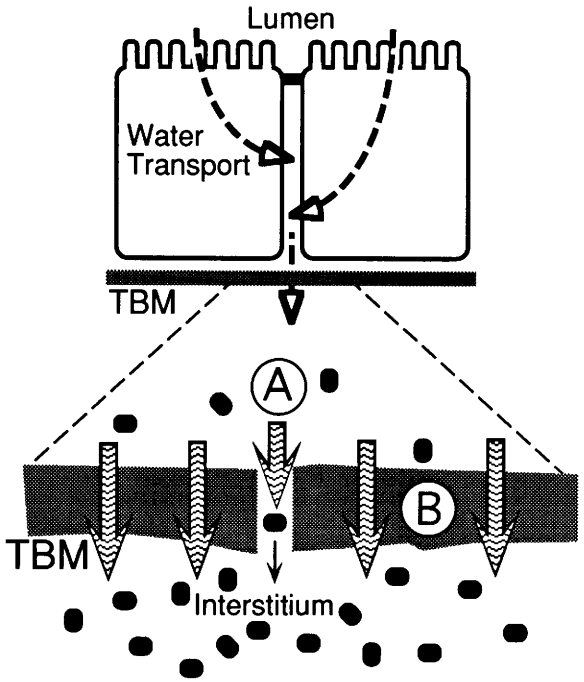

Figure 2. Possible function of basement membrane (TBM) of renal proximal tubule in aiding transport of fluid across the epithelium. The presence of colloid around the tubule is known to enhance the rate of water transport, and this effect of peritubular colloid could be mediated by oncotic action across the TBM (54). Lower portion of figure shows blow-up of TBM with hypothesis for how water flow across the TBM could lead to reduction of colloid (black ovals) concentration in the intercellular space by solvent drag (A), with the resulting oncotic gradient driving transport of fluid across other portions of the TBM (B). Permeability of TBM to albumin suggests that this process is feasible, and could result in substantial reduction in the hydrostatic pressure that develops in the intercellular space during fluid transport (33). This is an example of how basement membranes could have a significant function in restricting the movement of macromolecules without being a perfectly tight barrier.

Problems and Promise with *in Vitro* Studies of Basement Membrane

Work with isolated basement membrane may be the only way to answer questions about the actual permeability properties of these barriers, but some significant hurdles need to be overcome before *in vitro* studies can become more widely utilized. We must learn how close our *in vitro* basement membranes are to those *in vivo*, with regard to composition and physical properties. We need to learn more about the diversity of basement membranes in different tissues. And we need to know the contribution of attached cells to basement membrane permeability.

Basement membranes *in vitro* may contain 50 or more different proteins (2), yet we presently know the identity of only a few of these (4, 5, 7, 9). Some work has been done on the diversity of composition among basement membranes in different tissues (55–57), but most of this work has used immunostaining and so can give only qualitative information on composition. Quantitative results have been variable; even for type IV collagen, a principal component of all basement membranes, estimates of its content vary from 50%–80% of dry weight (3, 58).

The effects of isolation on basement membrane composition are essentially unknown. All known basement membrane proteins appear to be present in *in vitro* preparations of basement membrane (30, 55), but whether the content of these is the same as *in vivo* is a difficult question to answer. Isolation of basement membrane must use strong detergents or physical disruption to separate the basement membrane from cells. Some basement membrane proteins could be re-

moved during this process, but if they are some of the proteins that are not yet identified, then there is no way to know that they are missing. As more components of basement membrane are identified, the correlation between content of basement membranes *in vivo* observed by immunostaining and the content determined *in vitro* will continue to be assessed.

The effects of isolation on the physical properties, such as permeability, of basement membrane are also unknown. Basement membrane that is isolated using detergent becomes sticky as the detergent is removed (30). This stickiness could be due simply to the exposure on the surface of the basement membrane of sites that were covered previously by cells, but also it could indicate that some of the noncovalent interactions within the basement membranes have been broken, thereby changing the physical properties. When detergent-isolated basement membranes are further extracted with urea or sodium dodecylsulfate, their permeability properties are altered (59). Thus, it is possible that isolation of basement membrane alters permeability. Further work needs to be done in this area to test different methods of isolation of basement membrane to see possible effects on permeability.

Related work in this area can also be done using a model for basement membrane, Matrigel, which is an extract of the Engelbreth-Holm-Swarm tumor (60, 61). Matrigel contains the same major components as basement membrane, and forms a gel at 37°C that shows basement membrane-like properties (60). The hydraulic conductivity of Matrigel has been measured (62), and we recently have reported its ability to restrict the passage of serum albumin (63). This material, or other basement membrane-like matrices (64), may be useful in exploring the effects of compositional changes on the physical characteristics, including permeability properties, of basement membranes.

Conclusions

Basement membranes are important structures within many tissues, and for at least some tissues, the ability of the basement membranes to restrict the passage of macromolecules is important for function. We have concentrated on the renal glomerular and tubular basement membranes because they have been studied more than others, but the basement membranes of capillaries may be important in many different organs (65). In the renal glomerulus, the GBM certainly plays an important role in restricting the passage of serum proteins into the forming urine; however, some data are conflicting, and the relative roles of cells and the GBM are not yet defined. In the renal proximal tubule, the TBM may play a part in the process of epithelial transport of fluid, and the example that we have given shows the importance of considering the dynamics of the *in vivo* situation in assessing the role of the basement membrane. Describing the permeability properties of basement membrane will require more work *in vitro*, and investigators need to be aware of the possible artifacts induced by isolation procedures.

The author is an Established Investigator of the American Heart Association. Research work mentioned here was funded by National Institutes of Health Grant DK39023 and by grants from the American Diabetes Association Indiana Affiliate and the Juvenile Diabetes Foundation International. The author thanks Judy Boyd-White, George Tanner, Jeff Siemon, and Andrew Evan for helpful critique of the manuscript.

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