Tube-Shunt Bleb Pathophysiology, the Cytokine Story

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Purpose: To define tube bleb-pathophysiology, outlining factors that may play a major role in the ultimate success or failure of the bleb. Methods that may be used to advance success of these blebs are discussed.

Materials and Methods: The study describes the importance of tube shunt bleb pathophysiology, including the role of cytokines, relating to bleb failure or success. Methods to influence these outcomes, are outlined.

Results: Understanding the various parameters involved with the pathophysiology of tube shunt blebs, especially intraocular pressure (IOP), and cytokine content of aqueous. The production of cytokines by tube shunt blebs, and the possible adverse results of this action on a second tube shunt in a different quadrant of the eye.

Conclusions: Tube shunts are conduits transporting aqueous from within the eye to the subconjunctival space. The ultimate end result is to create a drainage bleb over the tube plate. The formation of the bleb is controlled by multifactorial components, including age of the patient, racial background, presurgical IOP, and thereby aqueous cytokine content, and patient’s individual reaction to the presence of a foreign body beneath the conjunctiva. Prolonged IOP within the bleb results in cytokine production by the bleb lining. Occluding the tube of a failed implant can prevent damage to a new implant in a different eye quadrant.

Key Words: intraocular pressure, Bleb pathophysiology, tube shunts, cytokines

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Blebs formed by tube shunts, have a definitive pathophysiologic course, governed by different factors related to their development. Knowledge thereof can help in defining modalities available to procure a successfully functioning bleb.

Success, as well as failure of blebs, requires an understanding of the physiological mechanisms underlying these 2 parameters. The success of glaucoma filtration surgery, depends on the maintenance of a state consisting of a partial failure of normal wound healing. The wound healing process of glaucoma surgery is complicated by factors, both related to healing in general, as well as specific to glaucoma surgery. The factors involved include ethnicity of the patient, previous eye surgeries, and use of glaucoma medications. Aqueous content, namely cytokines, are additional important factors affecting wound healing in the eye.

MATERIALS AND METHODS

The ability to study the pathophysiology of blebs occurred to a large extent with the development and use of tube shunts. The blebs formed by these devices were large, and allowed for careful observation, and thereby assessment of their pathophysiology.

Historical Aspects of Tube Shunts

Multiple early studies using trans-limbal fistula maintaining devices, have been reported. The devices consisted of various substances, including gold, silk, and platinum. These devices were used where conventional fistula producing procedures had failed, but were mostly noneffective.

The Tube shunts in use today, had their origin in South Africa some 50 years ago. The history of large blebs began with an observation by a South African ophthalmologist, Dr Edward Epstein in 1958. Frustrated by the high incidence of bleb fibrosis in his full-thickness glaucoma filtration procedures, Epstein created a tube shunt out of polythene, (0.15 mm in diameter) to avoid major surgical trauma to the conjunctiva, which he assumed to be the root cause for this bleb fibrosis. His tube shunt was implanted with minimal trauma to the conjunctiva, and produced a subconjunctival bleb, which drained well for 3 months. Thereafter, fibrosis occurred at the subconjunctival draining site.

Histology revealed no fibrotic reaction around the inert subconjunctival body of the tube, but only at the aqueous drainage site. Epstein concluded that the aqueous, and thereby its constituents, were responsible for the bleb failure, and published his findings in a report “Fibrosing Response to Aqueous.” It’s relation to glaucoma. The main finding derived from Epstein’s tube shunt, was that small bore tubes, deliver aqueous to a very small and localized subconjunctival area. This results in the greater potential for any proinflammatory contents in the aqueous, (subsequently found to be cytokines), to cause bleb fibrosis, as was demonstrated by him.

Dr Anthony Molteno, some 9 years later, discussed Epstein’s findings with him, and decided that a device that creates a larger bleb, would spread and dilute the cytokines, thereby decreasing the possibility of bleb fibrosis, and additionally, increase the pressure-lowering effect of the larger bleb. Thus the original Molteno implant was devised.

RESULTS

Bleb Pathophysiology

This initial implant consisted of an acrylic trans-limbal tube, opening on to the upper surface of a thin, circular, curved episcleral plate 8.5 mm in diameter. This implant was placed with its trans-limbal tube occupying a corneo-scleral trephine opening, and its plate firmly sutured to the sclera (Figs. 1, 2).

The plate itself, in the absence of aqueous drainage, stimulates the formation of a thin episcleral covering. This episcleral covering will expand to form the definitive bleb
once aqueous is allowed to flow to the plate surface. During the succeeding few weeks, the bleb wall undergoes a series of physiological changes characterized by an inflammatory reaction, resulting at first in the thickening of the bleb wall, and subsequently a degenerative change in the bleb wall, giving rise to the final thinner-walled bleb. The bleb facilitates the passage of aqueous through the bleb wall into the surrounding tissue. A pressure gradient from the inside of the bleb to the surrounding tissues is required for this flow of aqueous. The effect of a large plate, increases the stretching force within the bleb wall, promoting early and effective distension of the bleb. Thus adequate drainage would occur despite the presence of a fibrous lining to the bleb wall. Molteno subsequently discovered that by increasing the plate size, the resulting larger bleb would increase the intraocular pressure (IOP)-lowering effect of the implant. This finding, led to the development of the long tube single and double plate implants, and eventually, the large single plate implant.\(^5\) The tissue reaction over the plate surface, allowed careful observation of the formed bleb. The nature of these blebs, resulted in the ability to carefully assess and describe, 3 distinct phases of bleb development. The changes that accompany bleb formation, are most clearly seen when a single plate implant is used to drain a case of severe and advanced glaucoma in a young adult. The changes were named according to the behavior of the IOP, during these phases of bleb formation.\(^1\)

The initial stage of the bleb, the hypotensive phase, occurs before the flow of aqueous onto the plate surface, and is due to the conjunctival reaction to the plate, thus named the “plate effect.” This phase lasts from 7 to 10 days. During this phase the integrity of the conjunctiva is disturbed setting off a tissue repair situation. The early tissue repair consists of the arrival of inflammatory cells mainly macrophages, lymphocytes, and fibroblasts. These cells, but predominantly the fibroblasts, produce cytokines. The main cytokine that is produced is TGF-$\beta_2$ (transforming growth factor-$\beta_2$). This cytokine is expressed in human Tenon fibroblasts and initiates the wound healing process by further stimulating fibroblast proliferation and collagen synthesis.\(^6\) The succeeding phase occurs after aqueous reaches the plate surface. Thus named the “aqueous effect.” This phase often results in the increase of IOP, and is then termed the “hypertensive phase.” The nature of the hypertensive phase is primarily governed by the patient’s tissue reaction to the presence and concentration of cytokines in the arriving aqueous, reaching its peak 5 to 6 weeks after the operation. This phase is followed by the “stable stage” of the bleb. This pattern of bleb development, will lack an isolated “plate effect” when aqueous is allowed to reach the surface of the plate immediately, as occurs in valved implants. The phases, however, tend to be different or absent in individual patients, and therefore understanding why this occurs is important.

**Factors Responsible for Bleb Development**

The nature of the bleb in general, depends on 3 very important factors. Most importantly, the inherent individual patient reaction to the surgical trauma inflicted by the presence of the tube plate, and the surgical procedure. This reaction in general, is more severe in black patients, but less severe in the very young and very old patient.\(^7\) The second important factor is the aqueous and its cytokine content. This in turn will be largely influenced by the prevailing IOP immediately before the release of aqueous onto the plate surface. The 3 factors combined, will have much to do with the success or failure of the bleb.

The cytokine content, as well as the IOP, are important variable components of bleb formation that can be modified. TGF-$\beta_2$ was the first, and most prolific cytokine discovered in aqueous from glaucomatous eyes.\(^8\)\(^-\)\(^10\) Subsequent studies, showed the presence of a multiplicity of additional cytokines in glaucomatous aqueous, as well as the direct relationship between IOP and cytokine levels.\(^11\) (Fig. 3).

The prevailing theory, whereby cytokines enter the aqueous, is the breakdown of the blood aqueous barrier as a result of increased IOP. Findings by Borisuth et al.,\(^12\) suggest an additional concept for the continued intraocular derivation of TGF-$\beta_2$. The hypothesis is that TGF-$\beta_2$ promotes the synthesis and deposition of extracellular matrix components in the trabecular meshwork, thereby decreasing the outflow of aqueous. This results in an increase in IOP, which stimulates the bleb lining to secrete TGF-$\beta_2$ as well as other cytokines. Clinical observation, as will be discussed, further supports this concept.

The hypertensive phase of the bleb does not occur in every patient, and is generally dependent primarily on the cytokine content of the aqueous. This in turn is predominately related to the IOP immediately before tube implantation. This has been demonstrated clearly in the greater frequency of the hypertensive phase seen in the valved tube.\(^13\) The valved tube is designed to carry the aqueous immediately to the surface of the plate, thus avoiding postoperative hypotony. The aqueous, if coming from an eye with high IOP, will generally contain high levels of...
pro-inflammatory cytokines, resulting in the greater incidence of the hypertensive phase, and the greater possibility of a more fibrotic bleb.\(^\text{13}\) This has been well illustrated by histologic evidence obtained from a dual chamber Molteno implant. This implant isolates the aqueous, containing high levels of cytokines, in the first receiving chamber. The resulting bleb develops a lining, which is somewhat thick. The resulting aqueous now slowly leaks into the main chamber of the implant, with a lowered cytokine content, resulting in a thin well-functioning bleb (Fig. 4). The reported use of aqueous suppressants early in the postoperative period of an Ahmed implant, resulted in a lower incidence of the hypertensive phase. This finding, suggested the reason to be lower cytokine levels in the aqueous, as discussed by the authors.\(^\text{14}\) Aqueous suppressants will also lower the IOP which in turn could additionally effect the frequency of the hypertensive phase.

**Cytokine Effect on Bleb Development**

Epstein and subsequently Molteno, both recognized the presence of “fibrosing” contents in aqueous. Molteno recognized that lowering the IOP for a minimum period of 3 weeks, before allowing aqueous to reach the plate surface, would result in a lower incidence of bleb fibrosis. The development of long tube implants, allowed Molteno to achieve this by delaying insertion of the tube into the anterior chamber for 6 weeks, his so called “two stage tube insertion.”\(^\text{15}\) The common method of decreasing cytokine effect today in nonvalved tubes, is by stenting the tube, and lowering the IOP with a draining slit and medications, before allowing aqueous onto the plate surface.\(^\text{16}\) Molteno, initially aware of the “fibrosing” effect of aqueous, also used a combination of drugs, consisting of steroids, nonsteroidal anti-inflammatories and colchicine, given in combination to reduce postoperative inflammation. This was given as soon as the aqueous reached the plate surface.\(^\text{17}\)

The variable factors involved in bleb success or failure can now be more succinctly classified as the patient’s inherent tissue reaction to the surgery, the cytokine content of the aqueous, and thereby the IOP both before, and after the surgical procedure.

The 2 variables associated with bleb formation that can be modulated, are the IOP and the cytokine content of the aqueous. The use of the antimetabolites Mitomycin C or 5-fluorouracil, to decrease the patient’s tissue reaction, has been shown to be generally noneffective in preventing tube bleb fibrosis.\(^\text{18}\)

**Clinical Importance of the Hypertensive Phase**

The hypertensive phase of bleb development in tube implants, allows for possible prognosis of bleb success or failure to be determined. The development of a hypertensive phase has been shown to result in a higher incidence of final bleb fibrosis.\(^\text{13}\) Lowering the pressure during the hypertensive phase is important, as this decreases the accumulation of cytokines, and thereby, may prevent bleb fibrosis and possible failure of the bleb.

The use of pressure-lowering medications is the common method used to treat the hypertensive phase. Aspirating aqueous from the bleb is an alternative method. Removing aqueous from the bleb, results in immediate lowering of the IOP, and can be attained by simply using a 30-G needle and tuberculin syringe (Fig. 5).

**FIGURE 3.** Depiction of intraocular pressure on levels of TGF-\(\beta 2\) in aqueous of cataract patients (low pressure); glaucoma patients (higher pressure) and aqueous from hypertensive phase (highest pressure). Figure 3 can be viewed in color online at www.glaucomajournal.com.

**FIGURE 4.** Histologic section of bleb in dual chamber Molteno implant. Illustrating thick capsule in first chamber receiving cytokine laden aqueous from eye. Bleb forms and pressure decreases, releasing aqueous with low cytokine levels, to second chamber, resulting in thin bleb capsule. Figure 4 can be viewed in color online at www.glaucomajournal.com.

**FIGURE 5.** Removal of aqueous with 30-G needle from bleb during hypertensive phase of bleb development. Figure 5 can be viewed in color online at www.glaucomajournal.com.
Removal of the aqueous, also decreases aqueous cytokine levels, thereby preventing further bleb lining inflammation.

The aspiration method of managing the hypertensive phase, requires patients to be followed at weekly intervals, wherein, repeat aqueous removal is done, until either the pressure is stabilized under 25 mm Hg, or the bleb has failed. Removing aqueous from the bleb, allows for measurement of cytokine levels. This was done in 15 patients post-Molteno implant insertion. The results in this group of patients were 7 successful blebs and 8 bleb failures. TGF-β2 levels were measured in aqueous removed from these patients. The averaged levels of TGF-β2 were low (<10,238 pg/mL), in the success group, and high (>22,733 pg/mL) in the failure group (Table 1).

The pattern of the levels of TGF-β2 seen in the success group was also different to the failure group. Consecutive weekly removal of aqueous in the success group, showed a trend of both lower levels of TGF-β2 and IOP, usually after 2 weeks. The failure group did not show a decline either in IOP or levels of TGF-β2, after 2 weeks, and bleb failure ultimately occurred. This finding, suggested that the bleb itself was producing cytokines. The bleb had thus become similar to the “Selye Rat Pouch,” wherein injection of air subcutaneously in a rat, produces an epithelial lined pouch, which under pressure will secrete cytokines.18 Lowering the IOP during the hypertensive phase either by the use of medication or by aspirating aqueous from the bleb to lower the IOP, results in increasing the function and longevity of the bleb.2019 The decrease in pressure also lessens the possibility of bleb wall thickening, resulting in either no hypertensive phase or a shortened one.

### Clinical Application of the “Selye Rat Pouch” Theory

This postulate, suggests a possible reason for a somewhat not uncommon clinical observation noted when placing a second tube shunt into an eye in the presence of the failed tube shunt. The second tube often appears to fail more rapidly than the second tube shunt into an eye in the presence of the failed tube (personal observation). This phenomenon also applies to valved tube shunts (confirmed by colleague who only uses valved tube shunts). This finding suggests the possibility that the failed implant has become a “Selye Rat Pouch” and is feeding high levels of TGF-β2 into the eye thus causing fibrosis to occur in the new tube shunt.

To test this theory, the following procedure was carried out in 4 patients with failed tube shunts who were undergoing the placement of a second tube shunt into the eye with the failed implant. Concurrent to inserting the second tube, the tubes of the failed implants were exteriorized and occluded with a liga-ture of 70 prolene and reinserted into the anterior chamber. The failed bleb collapsed in the postoperative period, thereby not feeding aqueous into the anterior chamber. Aqueous TGF-β2 levels were measured before the insertion of the second implants, and subsequently after establishment of the bleb over the new implant. The levels of TGF-β2 before inserting the second implant were high, averaging 22,733 pg/mL. Following the insertion of the second implant, TGF-β2 levels remained low 10,238 pg/mL, after long-term follow-up. These findings, are suggestive of the postulate, as previously stated, that the failing blebs themselves are responsible for producing cytokines, and thereby feeding the cytokines into the bleb of the new implant. This may ultimately result in the failure of the second implant. The low levels of TGF-β2 may also have occurred due to the pressure-lowering effect of the second tube, in conjunction with the absence of cytokines from the failed tied off tube. Removal of the tip of the tube from the anterior chamber, via a small limbal incision, and reinserting it after ligating the tip, proved to be a simple and easy procedure without any complications in any of the patients. This small series of cases suggested that the benefit of the procedure outweighed the possible complications that may have occurred, but did not.

### DISCUSSION

**Cytokines and Bleb Success or Failure**

Tube shunts produce large blebs, which in turn are capable of producing cytokines if subjected to the presence of elevated IOP, generally for an increased period of time. Prolonged observation suggests that pressures >25 mm Hg, may subsequently result in the production of cytokines from the eye as well as from the bleb itself. The cytokines being mediators of an inflammatory response within the walls of the bleb, will eventually cause bleb wall fibrosis, leading to an obstruction of aqueous flow through the bleb wall and thereby bleb failure. Normalization of IOP results in a decrease in the production of proinflammatory cytokines, leading to a gradual loss of fibroblasts, and the degeneration, fragmentation and disappearance of collagen fibrils in the inner bleb wall resulting in the stable stage of the bleb.19 Supporting evidence for these changes is seen, when further lowering of IOP is attained, when the ligature of a failed tubes is opened at a later date, following the insertion of a new tube (personal observation).

The use of tube shunts has highlighted the ability to observe the various parameters involved in bleb pathophysiology. This in turn depends on IOP both prior and subsequent to bleb formation. Thus, maintaining IOP below an arbitrary level of 25 mm Hg, as suggested by the long-term observations of Molteno, may result in the functional longevity of tube shunt blebs. Maintaining low pressure within the bleb itself, will prevent the bleb from reverting into a “Selye Pouch,” and thereby begin to secrete cytokines.

Tube-shunt blebs are uniquely able to both lower IOP, or to self-destruct, should the bleb lose its pressure-lowering potential. The blebs can therefore be defined as “living blebs” These blebs therefore require frequent follow up, to insure that IOP is maintained at a level, that is less likely to allow the bleb lining to secrete proinflammatory cytokines, resulting in bleb fibrosis and thereby failure.

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