Aqueous Humor Outflow Resistance (ISER Debate)

October 2002, Geneva, Switzerland

by Mark Johnson

It is a surprising fact that, despite many years of research, we still do not know how the majority of aqueous humor outflow resistance is generated, which implies that we do not understand the fundamental factors controlling intraocular pressure. Recently, two main schools of thought have emerged. One holds that extracellular material within the juxtacanalicular tissue (also known as the cribiform network) is responsible for the bulk of aqueous outflow resistance. The other holds that the cells within the aqueous outflow pathway, most likely cells of the inner wall of Schlemm's canal, are themselves directly responsible for aqueous outflow resistance.

In order to try to assess the merits of these two different ideas, as well as to stimulate discussion and exchange of ideas in this area, a debate was held at the recent International Congress of Eye Research in Geneva. The moderators of the debate were Mark Johnson (Northwestern University) and C. Ross Ethier (University of Toronto). Arguing for extracellular matrix were Douglas H. Johnson (Mayo Clinic; team captain), Paul L. Kaufman (University of Wisconsin, Madison), and Ernst R. Tamm (University of Erlangen). Arguing for the inner wall of Schlemm's canal were David L. Epstein (Duke University; team captain), Murray A. Johnstone (Swedish Medical Center, Seattle), and W. Daniel Stamer (University of Arizona).

It would be fair to say that no team won the debate outright. A common theme that emerged was that there are multiple ways in which the inner wall of Schlemm's canal can influence the extracellular material in the juxtacanalicular tissue, and *vice versa*. Below, we present in tabular form some of the main points that emerged from the debate, as well as our views on these points. The points in bold print are the ones that the moderators found most compelling.

Both teams agreed that neither cells nor matrix could be clearly ruled out in the generation of outflow resistance. Dr Epstein pointed out that, even if the funnelling hypothesis (whereby pores in the inner wall have negligible flow resistance on their own, but force a funnelling of the flow through the matrix surrounding the entrance to the pores and thereby increase the effective flow resistance of the matrix15) is accepted, these pores or openings pass through the inner wall cells. Thus, the inner wall cells would be important as 'modulators' of the flow resistance generated by the ECM upstream of these cells.

With regard to the case of paracellular flow versus flow through pores, there seemed to be less consensus on this issue.

Perhaps the most important questions asked came from the audience. For example, Dr Abe Clark (Alcon Laboratories) asked: where is the site of increased outflow resistance in the glaucomatous eye (not just in the normal eye)? Both sides agreed that this is a crucial question. Perhaps this will be the focus of the next OUTFLOW DEBATE!

We wish to thank the participants and audience for an extremely informative and engrossing debate.

For references see www.glaucom.com

Observation	ECM team	Inner Wall team	Moderators
No morphological study of the outflow pathway (including recent work in human using QFDE[1]) has demonstrated an extracellular matrix (in any species) that could generate the measured aqueous outflow resistance	ECM components are lost during tissue processing. There is a positive correlation between outflow facility and empty spaces in the JCT.[2] Biochemical assays of GAGs are consistent with generation of outflow resistance.[3]	**In some species (e.g. bovine) almost no ECM is seen; how then can matrix be responsible for significant flow resistance? In all species, structures seen in JCT using EM would generate negligible flow resistance.**[3]	Where is the resistance-causing matrix?
While increase in outflow facility caused by cytochalasin is associated with breaks in inner wall, facility returns to normal without repair of breaks; in fact, some regions of inner wall are devoid of cells and yet outflow resistance is near normal.[4]	If there are no cells and yet outflow resistance is near normal, then cells must play, at best, a minor role.	This is a non-physiological experiment (in organ culture); possible artifactual resistance. Different results have been found in live monkey experiments.	Physiological or not, if matrix can generate sufficient flow resistance in this situation, why not normally?
Inner wall has too many pores to have significant flow resistance.[5]	This explains why inner wall has highest hydraulic conductivity of any endothelium in the body.	Pores are artifacts that increase in number with length of fixation time and fixation volume.[6]	This is a major issue to be resolved.
Some inner wall pores have been shown to be artifacts[6,7]. If most or all of these pores are artifacts, then the inner wall is clearly the major site of flow resistance.	Without pores, the high hydraulic conductance of the inner wall and the passage of microspheres could not be explained.	Transport through intracellular pores is non-physiological. These pores are artifacts of tissue shrinkage, under tension, during fixation.	Pores do not pass through cyto-plasm as term "intracellular" suggests. Critical to determine how many pores are artifacts.
Large microspheres (e.g., 1 μm) pass relatively easily through outflow pathway; there must be many large pores and thus inner wall alone has negligible flow resistance.[8]	**One of the strongest arguments against paracellular pathway.**	Inner wall may deform, forming temporary pathways for microspheres to pass.	No evidence for such temporary pathways; possible experiment for cell culture study
Permeability of inner wall endothelium is the highest in the body. Endothelia with paracellular flow have much lower permeability than does inner wall endothelium.[9]	**Flow cannot be paracellular; flow must pass through pores (similar to flow through fenestra in other high permeability endothelia).**	This is a mathematical calculation, subject to errors in assumed values.	Possible errors in calculations are minimal and technique used to measure permeability is same as used in other endothelia.
Cationized ferritin binds to paracellular route while anionic ferritin does not; cationized ferritin decreases outflow facility while anionic ferritin does not.[10]	Ferritin binding to ECM might explain this result.	**This implicates the paracellular route in determining outflow resistance.**	This seems strong argument for ability of paracellular pathway to influence outflow resistance.
H7 greatly increases outflow facility. Careful morphological examination shows great expansion of the JCT region but no breaks in inner wall.[11]	If morphological effect is seen only in JCT (where matrix is), then this implicates matrix.	Maybe there were effects in the inner wall that were not detectable.	This argues against inner wall (alone).
Breaks in the inner wall lead to decreased outflow resistance.[12-14]	Breaks in inner wall lead to (i) loss of ECM (ii) decreased funneling and (iii) agents that cause inner wall disruption may also may alter the matrix	This demonstrates that inner wall is important either in directly generating outflow resistance or in modulating outflow resistance	Qualitatively consistent with predictions of funneling theory[15]
Micro-cannula pressure measurements show that most of the resistance is across the JCT and not across the inner wall endothelium[16].	This rules out the inner wall as being the primary site of flow resistance.	This observation was not addressed.	Experiments may have been flawed by large size of the cannula used and/or uncertainty about tip position.
MMPs (matrix metalloproteinases) alter outflow resistance[17].	These agents act on ECM, suggesting matrix in JCT has significant resistance.	These agents could also affect cell function through matrix-cell interactions.	Argument supports importance of JCT, although MMPs might also have effects on paracellular route.
JCT expands with increasing IOP (and becomes less densely packed) while Schlemm's canal cells undergo progressive deformation and reorientation.[18,19]	Some small resistance exists in inner wall. However, majority of resistance is still in JCT.	This indicates that there must be resistance in inner wall that then pulls on JCT and expands it.	Both teams have a valid arguments; not resolvable with existing data.
Outflow resistance increases with increasing IOP.[20]	Schlemm's canal collapse explains increasing resistance.	Since JCT is expanding, its resistance should drop; this is inconsistent with major resistance in ECM.	At low IOP, outflow resistance is relatively constant with increasing IOP.[20,21]