



Arthur H Neufeld

AMD – a disease of the ageing RPE

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in Vienna

THE next challenge in age-related macular degeneration (AMD) research is to synthesise the vast body of pre-clinical and clinical research knowledge to improve and extend our understanding of the disease. Three main areas providing future therapeutic opportunities include genetics, drug delivery and a deeper understanding of the molecular and cellular biology underlying the pathophysiology of AMD.

At this year's Winter EURETINA Conference, Prof Arthur H Neufeld, of Northwestern University School of Medicine, Chicago addressed the third topic – understanding the molecular and cellular biology of AMD – and reported to attendees on the evolving picture of how AMD might occur.

In considering the disease of AMD Prof Neufeld admitted that one question that had always bothered him is, “how can an individual be born with a genetic mutation for which the phenotype doesn't show up for 60, 70 or 80 years?”

For most of our evolutionary history, AMD was never a problem and, according to Prof Neufeld, it is increasingly evident that growing old is at the core of the matter. In Prof Neufeld's own words the answer to his rhetorical question is remarkably simple – “being old is different than being young”.

To convince the conference of this relatively simple thesis, Prof Neufeld presented data to show that three key events related to ageing are likely to affect the normal physiology of the retinal pigment epithelium (RPE). These changes included age-related damage to the mitochondria, age-related iron overload and an age-related increase in excretory activity.

A review of some of the basic physiology of the retina shows that the RPE cells are phagocytic, “every morning they take a bite out of a photoreceptor cell, put it into a phagosome and digest the material which is then released from the cell and washed away by the blood vessels,” explained Prof Neufeld.

The question that had exercised Prof Neufeld and his research group, directed by Dr Ai Ling Wang, for a number of years was, “what age-related changes in the RPE would make this process of normal physiology susceptible to AMD?” According to Prof Neufeld a broad range of experiments on mitochondrial DNA damage, iron overload and excretory activity now show that such events may be sufficient to create the substrate for AMD pathology.

Mitochondrial damage

Measurements of mitochondrial DNA (mtDNA) damage over the life span of an

animal show that such damage accumulates over time. Analysis of the eyes in an old mouse versus a young animal show that the drusen contains increased levels of markers for mtDNA damage. This new finding suggests that the increase in mtDNA damage adds additional work to an ageing retina and, when combined with other factors, a tipping point will be reached in older animals whereby the “system” begins to stutter.

The iron factor

One of these “other factors” is an age-related iron overload in which the RPE of older animals becomes loaded up with iron that eventually creates functional consequences. Phagocytosis experiments performed by Prof Neufeld's group in tissue culture experiments show cells loaded with iron have a markedly reduced level of phagocytosis. In iron-treated cells, iron overload decreases one of the key players in the phagosome – a protein known as FAK (focal adhesion kinase). Extending the findings to animals shows that older animals have less FAK and, less phosphorylated FAK. Consequently, given that FAK phosphorylation is required for internalisation of the phagosome, phagocytosis now runs into a serious problem in older animals.

Release of waste products

Knowing that the RPE cells, with time, accumulate high levels of “junk”, Prof Neufeld turned his attention to understanding how the cells get rid of such waste materials from the cell. It is well established that phagosomes come into the cell, lysosomes empty into the phagosome and form an endosome and the exosomes contained in the endosomes are released and washed away by the blood circulation. In fact you can find exosomes in abundance in the blood, urine and other waste materials of the body. They are ubiquitous tiny vesicles released from every cell, including RPE cells. The exosomes are involved in the release of intracellular proteins into extracellular space and these released intracellular proteins are also found in drusen, representing a significant link.

Prof Neufeld and his research team looked at markers for this exosome activity, including CD63, LAMP2 and CD81. In tissue culture systems when the mtDNA of ARPE19 is damaged together with feeding them outer segments, large increases in several of the exosome markers have been observed. In essence, when the cells are under stress, exosome activity is increased. In vivo in humans with AMD and in older animals, the drusen is loaded with exosome markers and the exosome markers appear to be

clinging to Bruch's membrane. Guilt by association was the minimum suspicion. It soon came as no surprise that looking at the proteomics of drusen and comparing the results with what is excreted by exosomes, there appeared to be an almost fingerprint match. In other words, exosomes may be a major source of the proteins found in drusen.

When considered together it can be seen that there is a lot going on in the RPE cells: there is increased demand for phagocytosis of damaged mtDNA, there is impairment in the phagosome biology itself due to iron overload and there is increased excretory activity. In short, RPE cells in the ageing retina represent extremely busy places. Such an abundance of activity in old cells would obviously increase the stress to the point where such cells might seek help by calling in the cavalry and it is exactly such calls that were detected by the research group when they examined chemokine signalling.

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CCL2 (chemokine [C-C motif] ligand 2) is a chemokine that recruits leukocytes to a “stressed” tissue and it appears to be up-regulated in older animals. Microarray analysis identified several other pathways that are up-regulated in the RPE cells, including complement cascades. This cry for help from the RPE is answered by leukocytes, which begin to invade the RPE in an effort to assist clearing up the growing accumulation of cellular debris.

Microarray studies looking at young versus old animals have shown an increase in the activity of genes associated with the complement pathway in the old animals. Immuno-histological examination of the tissue of younger animals shows that complement is laid down in a thin line along Bruch's membrane however, in the older animals the pattern is discontinuous and clumpy; ageing appears to associate “with a rearrangement of how complement is being laid down on Bruch's membrane,” according to Prof Neufeld.

As mice don't have a macula or drusen, interpretation can be difficult. However, despite the absence of a macula and human drusen in the mouse model, Prof Neufeld argues that there is “mouse drusen” and that their research has shown abundant proteins to be associated with such deposited material.

All of these events lead to an increase in inflammatory and immunological activity and, according to Prof Neufeld, this is likely to be a consequence of normal age-related changes in the RPE. Pulling all these various strands of evidence together paints a credible explanation of what is occurring behind the scenes.

In the young animals, explained Prof Neufeld, the lysosomes empty into the phagosome to produce an endosome and the material is released by the exosomes, which is delivered to the blood to be washed away and excreted. However, in the older animal things are very different: phagocytosis is slowed down, iron has affected the internalisation of the phagosome, mitochondria are being damaged, all of which results in a lot of accumulating junk to be cleared.

Although the volume is processed through the “system” some of it literally “sticks” and what sticks grows into drusen. At that point inflammation becomes important, leukocytes invade the RPE and the seeds of AMD are sewn.

“If you don't have the correct CFH (complement factor H [allele]), if you don't have the correct complement system, if you cannot control the activity of these cells, you will go on to develop AMD – everything before that is normal,” explained Prof Neufeld. “It is normal to have the chemokines call for help, it is normal for the leukocytes to invade the retina; however, it is the lack of the control of the otherwise normal ageing activity that makes the difference in AMD pathology.”

Prof Neufeld's hypothesis to explain the events that lead to AMD are that “the ageing RPE/choroid provides the substrate for the development of the disease; normal ageing of the RPE/choroid results in increased immunological/inflammatory activity and AMD is caused by a loss of control of the immunological/inflammatory pathways during the normal ageing process.”

If such a picture turns out to be validated among other research groups, then significant advances may be expected to meet the challenge of obtaining a more detailed understanding of the molecular and cellular biology of AMD in the years ahead.

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