# 2001 Middlemore Lecture

## The Ins and Outs of Uveitis

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1

## The Ins and Outs of Uveitis

- Setting the scene
- Defining the problems
- Going "Back to Basics"
  - Why does the eye get inflamed?
  - Immune privilege
  - Programmed cell death (apoptosis) - why don't the cells die?
  - What keeps cells in the eye or stops them from getting out?
- What we have found

2

## Uveitis

- Accounts for approximately 10% of causes of blindness worldwide
- In USA 2.3 million suffer with uveitis
- Disease of the working population and has a huge economic impact
- Blindness from this disease in USA
  - Annual cost $242.6 million
  - Diabetes annual cost $242.7 million

BUT

uveitis is 250 times less common than diabetic retinopathy

3

## Uveitis - problems

1. We do not know what causes it in nearly all of our patients
2. We know little about the underlying disease mechanism(s) involved
3. We have no satisfactory treatment for sight-threatening disease

4

## Defining the issues

Where is the evidence?

- Scientific evidence
- Experience
- Experiment
- Anecdote

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"Can't concentrate, wake up early, no interest, low mood, suicidal...."
"I'm sorry to hear that Doctor"

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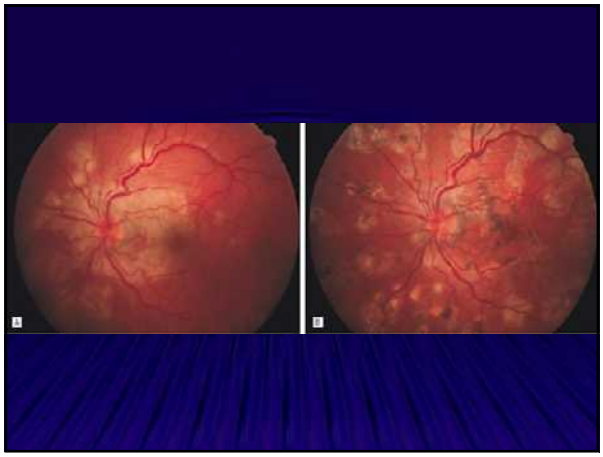

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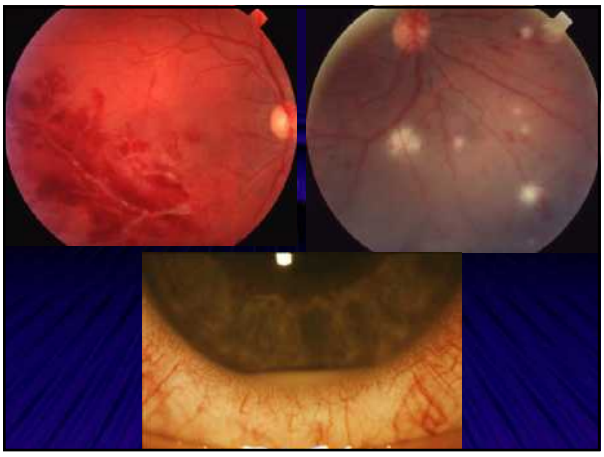

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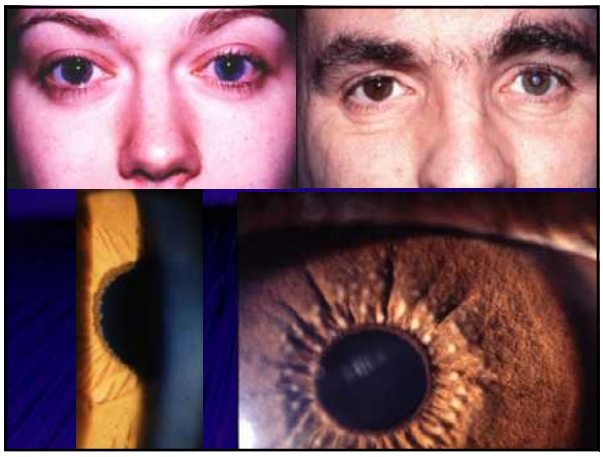

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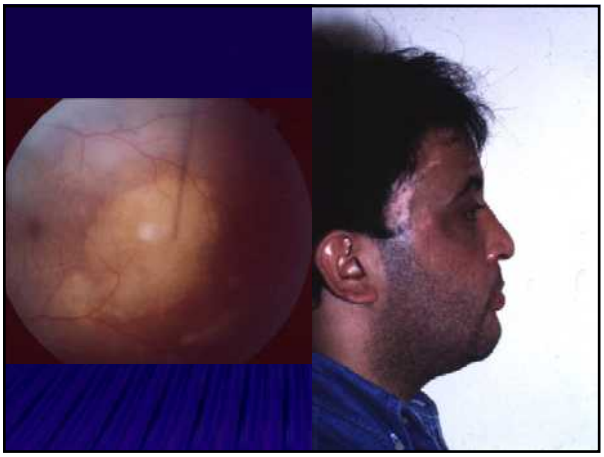

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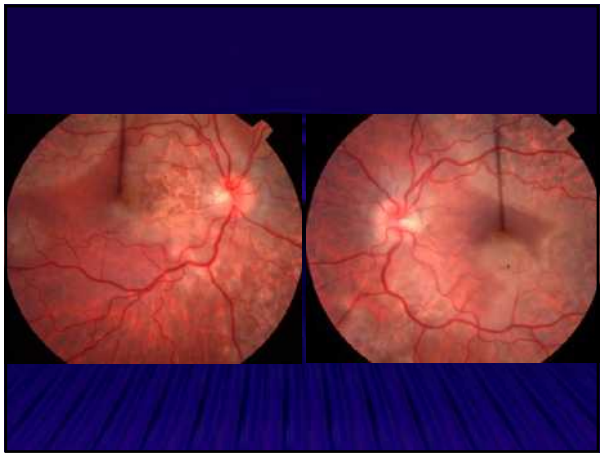


12

**Why, in uveitis, do leucocytes accumulate in the eye?**

13

## Immune Privilege

Immune responses in sites of immune privilege do not cause significant tissue damage and resolve rapidly

Sites of immune privilege

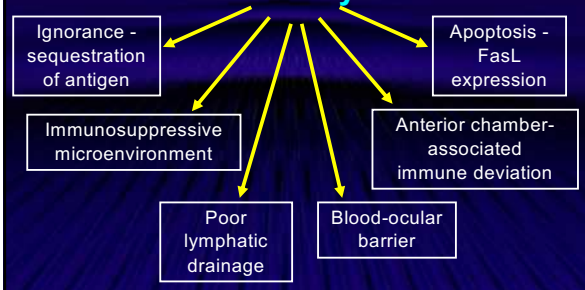
Eye
Brain
Testis
Placenta

14

- Eye an immune privileged site
- Protects the visual axis from disruption secondary to immunogenic inflammation
- In uveitis, the presence of substantial numbers of inflammatory cells within the eye appears to contradict the immune privilege status of the tissue

15

## Mechanisms of Immune Privilege in the Eye




16

## Fas/FasL and Immune Privilege

- Fas (CD95, Apo-1)
  - A receptor protein belonging to the TNF-receptor family
  - Expressed on most cell types
- Fas Ligand (FasL) (CD95 Ligand)
  - Type II membrane protein expressed predominantly on NK cells and activated T-cells
  - Shares significant homology with members of the TNF family

17

## Fas/FasL and Immune Privilege

- FasL expression by ocular structures also has an important role in immune privilege
- Fas+ inflammatory cells are brought into contact with FasL on ocular tissue leading to apoptosis (programmed cell death)

18

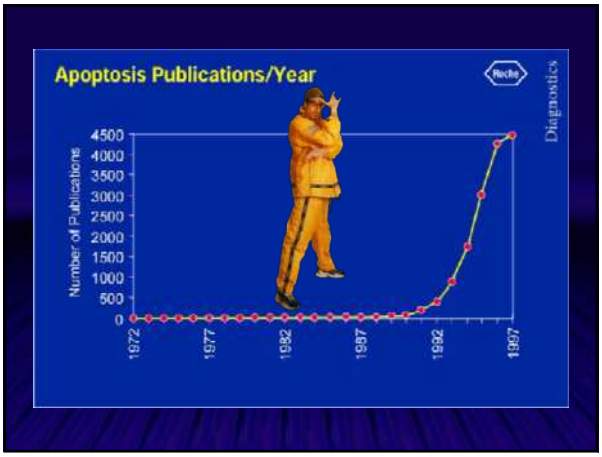
## Apoptosis

- An active, controlled form of cell death
- The most common physiological form of cell death
- A process that does not lead to inflammation

## Necrosis

- A passive, uncontrolled form of cell death that leads to inflammation

19

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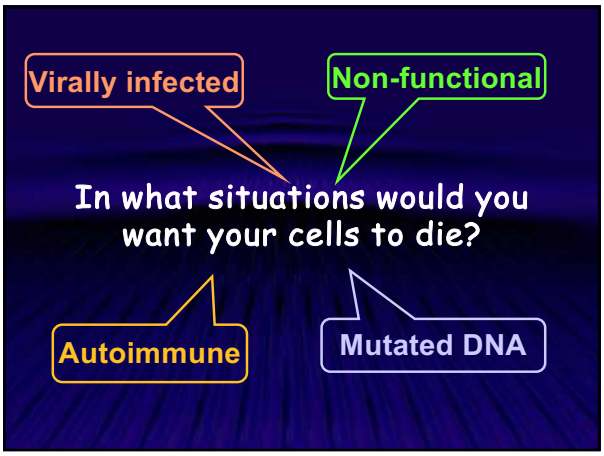



21

**Destroy the dangerous**

**Scrap the surplus**

**Whack the worthless**

22

## Pathways of apoptosis

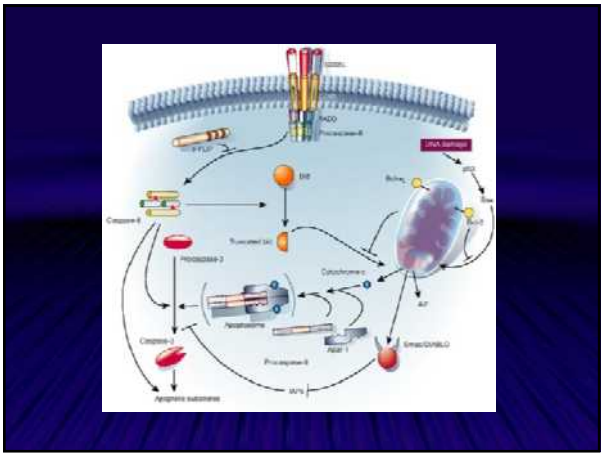
- Active
  - Fas/FasL
- Passive
  - Inadequate levels of stimulatory cytokines e.g. cytokine deprivation

23

## The Apoptotic Cascade

- FasL binds to receptor Fas
- Receptors containing “death domains” oligomerise
- Cytoplasmic factors (containing “death domains”) associate with receptor
- Allow receptors to associate with caspases and downstream signalling molecules
- The major proteases involved in the apoptotic pathway are caspases (protease which cleaves at aspartic acid residues)
- Present as precursor proteins in cytosol, cleaved in response to apoptotic signals

24



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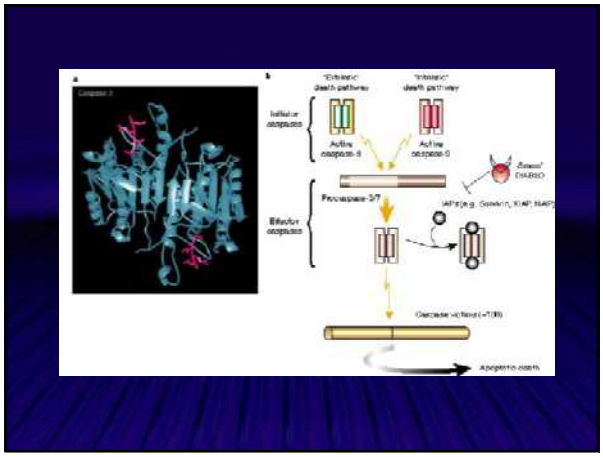

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## Four phases of apoptosis

1. Initiation (Trigger)
2. Signal integration (Decision)
3. Execution (Demolition)
4. Clearance (Disposal)

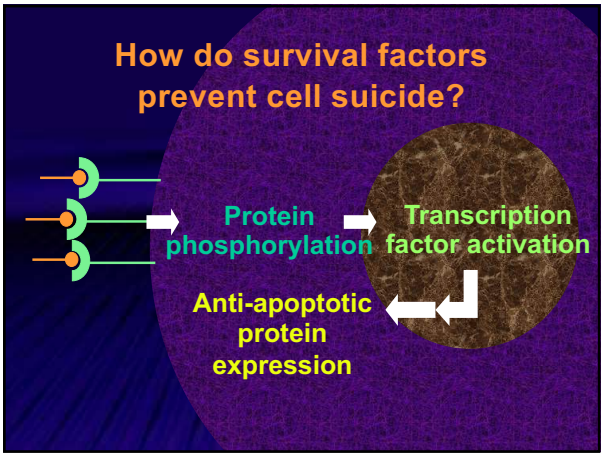
27

## Cells need continuous signals to survive

- All mammalian cells are programmed to self-destruct…

  …unless they receive survival signals:
- Soluble factors e.g. cytokines
- Adhesion to matrix
- Cell-cell interactions

28

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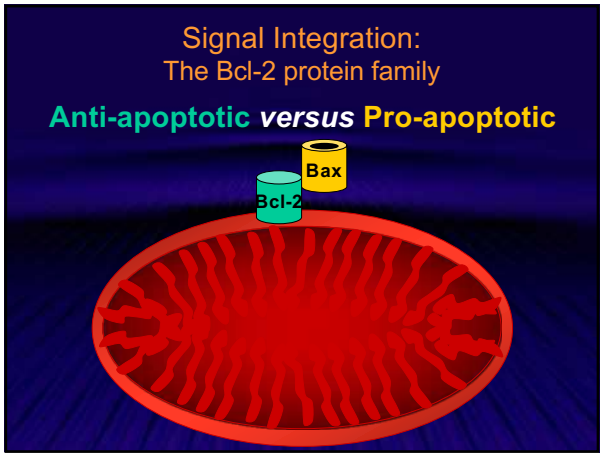


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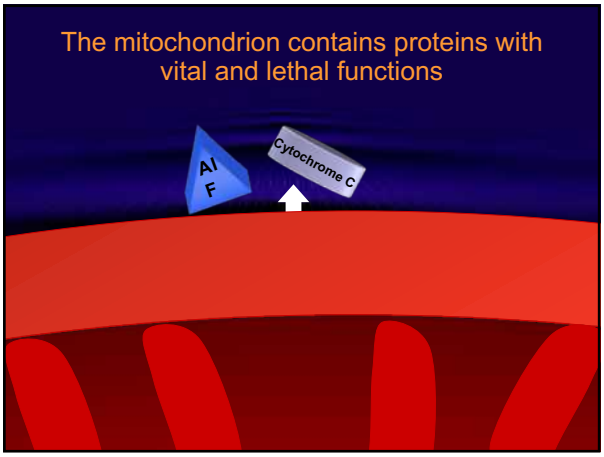


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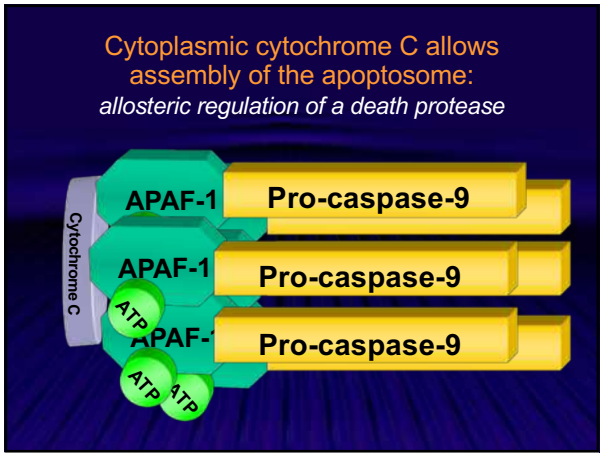


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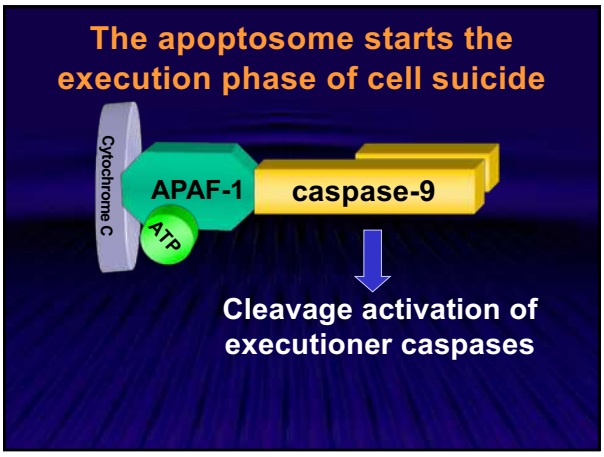


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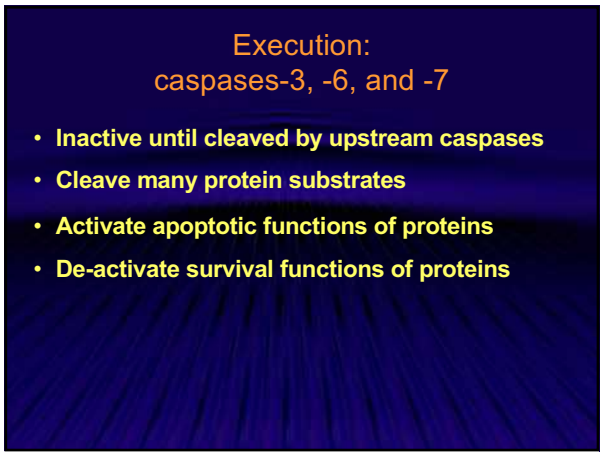


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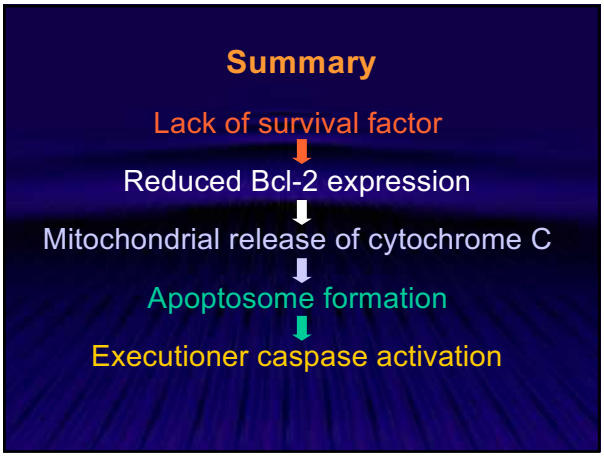


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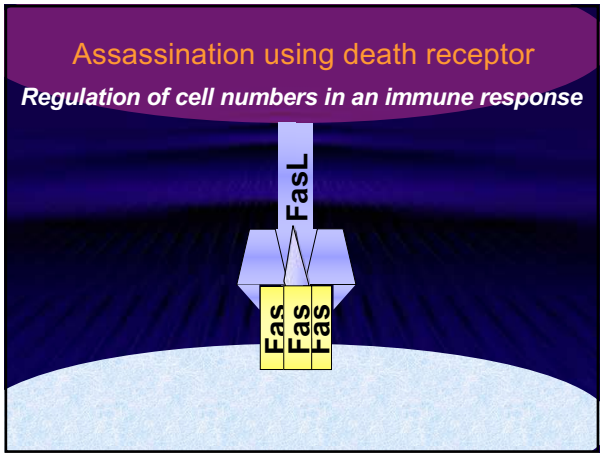


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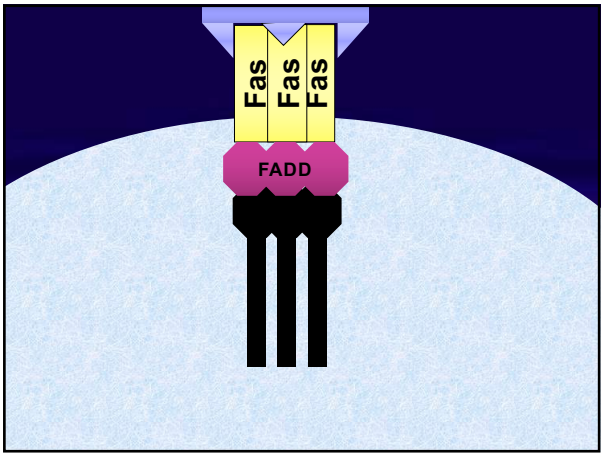


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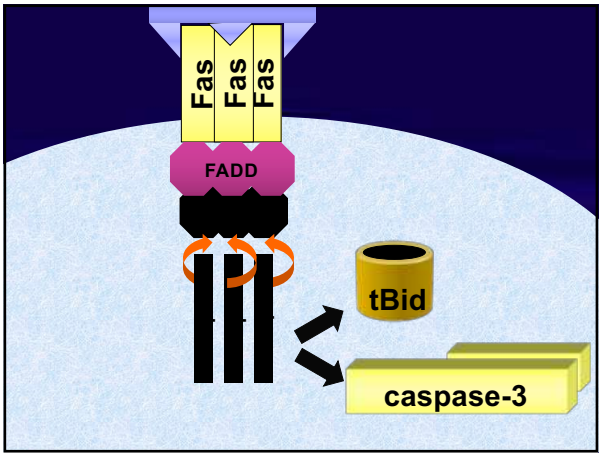


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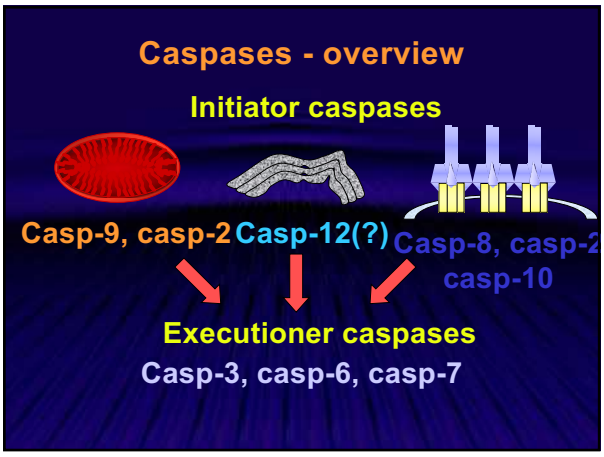


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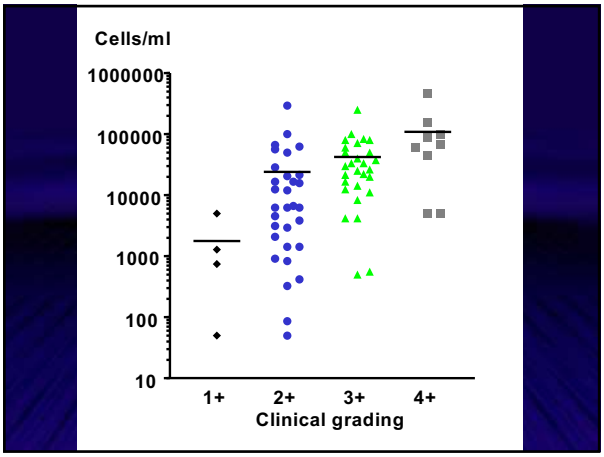


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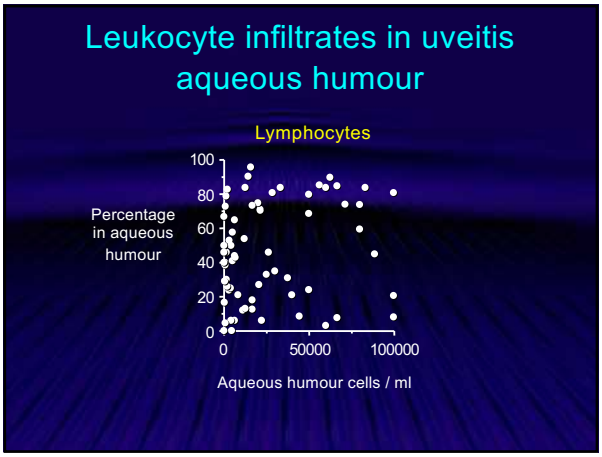
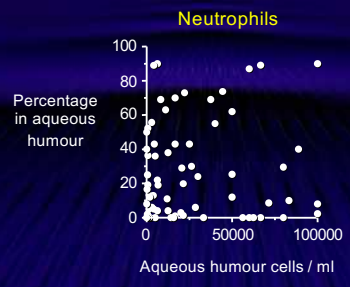



42

## Leukocyte infiltrates in uveitis aqueous humour




43

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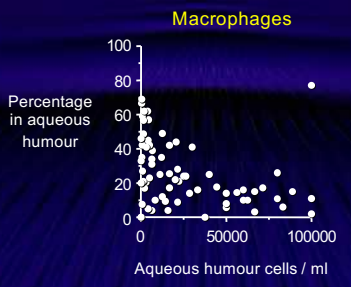


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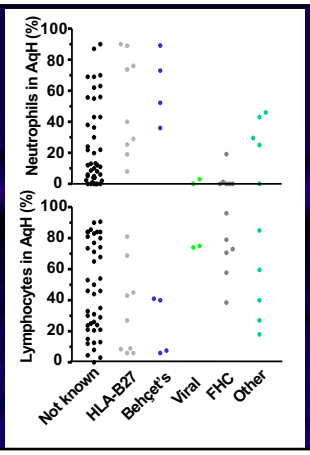
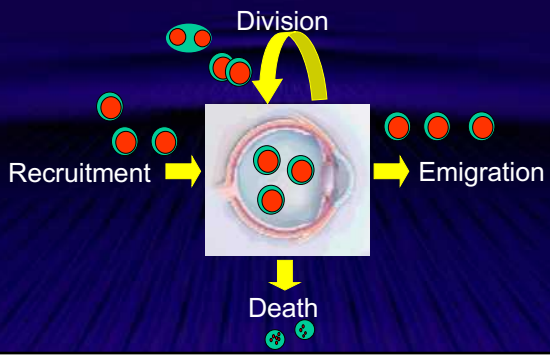


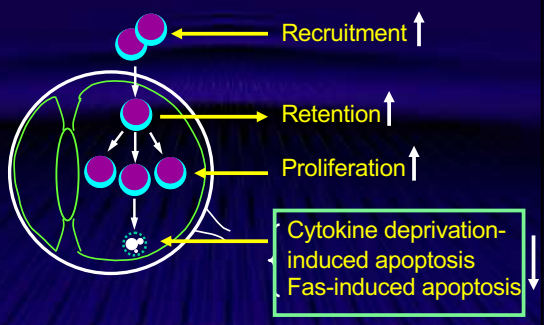

45

## Dynamics of a cellular infiltrate




46

## Possible mechanisms leading to inflammation in the eye




47

## Hypothesis

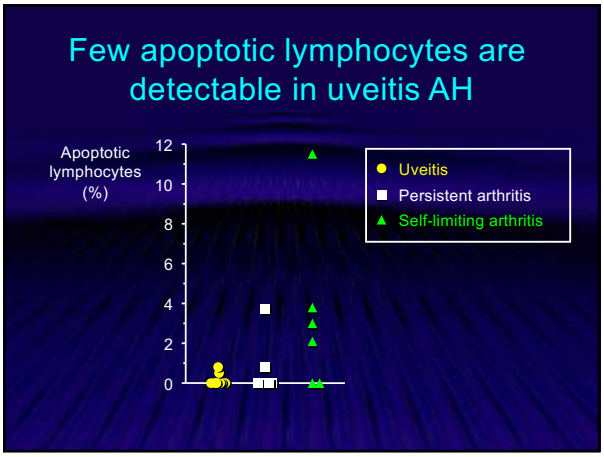
Inhibition of leukocyte apoptosis by the ocular microenvironment contributes to the accumulation of leukocytes in uveitis

## Predictions

1. Few apoptotic cells will be present in uveitis aqueous humour
2. Uveitis, but not control, AH will inhibit Fas-induced and/or cytokine deprivation-induced apoptosis

48

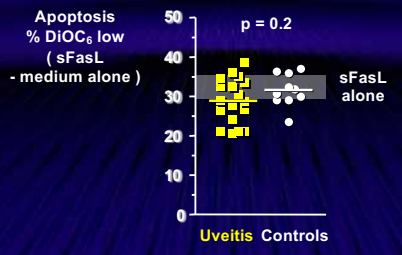
## Few apoptotic lymphocytes are detectable in uveitis AH




49

## Fas-induced T cell apoptosis

**Uveitis aqueous humour does not inhibit Fas-induced apoptosis of CD4⁺ T cells**

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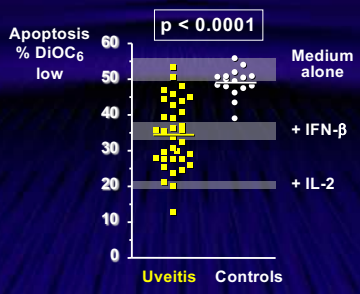
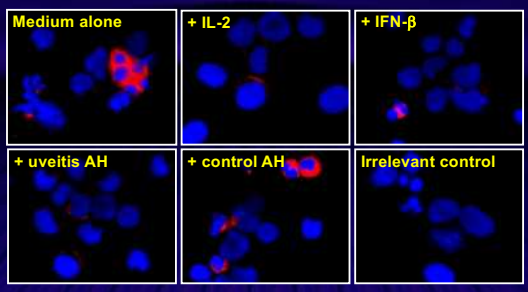
## Cytokine deprivation-induced T cell apoptosis

**Uveitis, but not control, aqueous humour inhibits cytokine deprivation-induced apoptosis of CD4⁺ T cells**



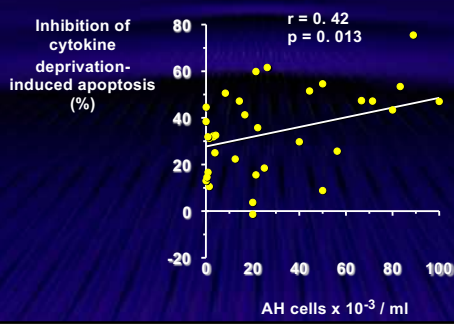

51

## Caspase-3 activation and nuclear fragmentation are also inhibited by uveitis AH




52

## Apoptosis inhibition correlates with cell number in the inflammatory infiltrate

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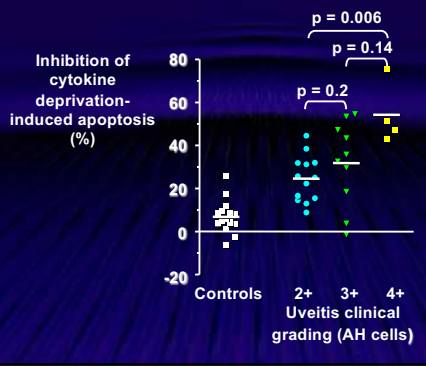
## Apoptosis inhibition correlates with clinical grading

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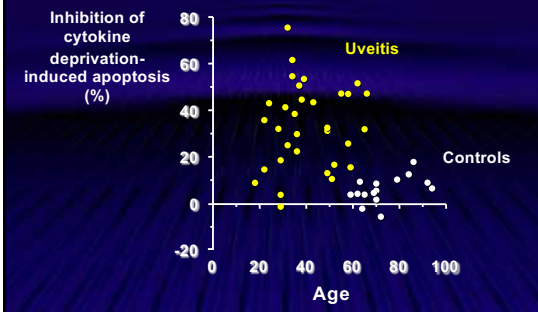
## Apoptosis inhibition does not correlate with age

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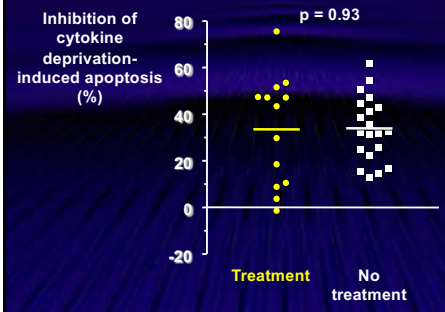
## Treatment for uveitis does not affect inhibition of apoptosis




56

## Conclusions

- Few apoptotic leukocytes are present in uveitis aqueous humour
- Fas-induced apoptosis is not affected by the ocular microenvironment
- Uveitis, but not control, AH inhibits cytokine deprivation-induced T cell apoptosis
- Inhibition of apoptosis correlates with the number of cells in the AH and clinical disease score

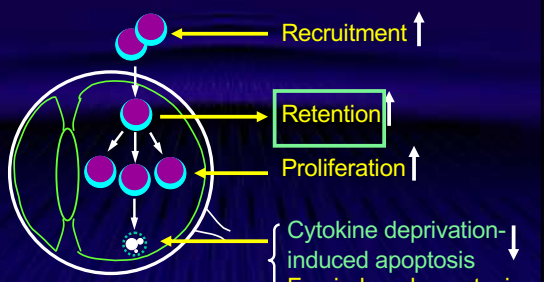
57

## Conclusions

- Immune privilege in the eye is compromised in uveitis
- Inhibition of cytokine deprivation-induced apoptosis contributes to the accumulation of leukocytes in uveitis

58

## Possible mechanisms leading to inflammation in the eye




59

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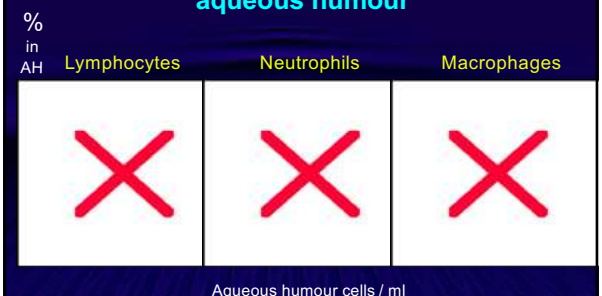



60

## How are these patterns established?

Probably a chemokine-driven process...

……...the same as anywhere else

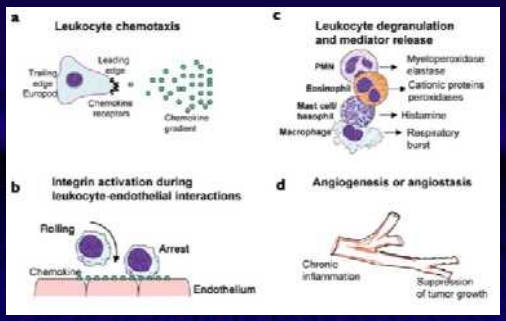
61

## Chemokines

Chemotactic cytokines involved in
- cell recruitment and retention
- haemopoiesis
- angiogenesis
- metastasis

Functional subgroups
inflammatory (inducible)
homeostatic (constitutive)

62




63

## Retention Hypothesis

Once within the tissue, chemokine receptor ligation on leucocytes will activate integrins and thus actively retain cells within the eye

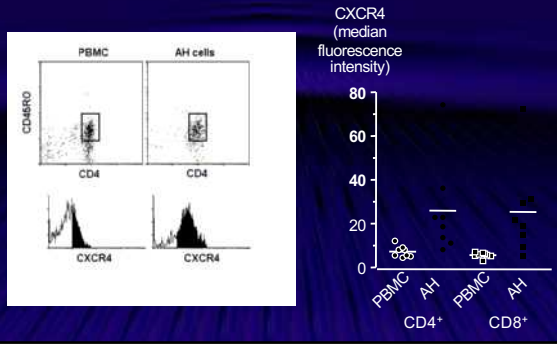
64

## Does retention play a role in the accumulation of leucocytes in uveitis?

- Screen AH leucocytes for the expression of chemokine receptors
- Can AH modulate their expression?

65

## Increased expression of CXCR4 on AqH CD45RO⁺ lymphocytes



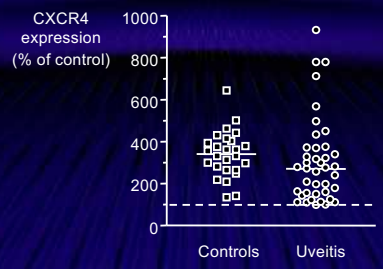

66

## Does retention play a role in the accumulation of leucocytes in uveitis?

- CXCR4 expression is elevated on AH lymphocytes
- Can AH modulate their expression?

67

## AH up-regulates CXCR4 expression on CD4⁺ T cells *in vitro*



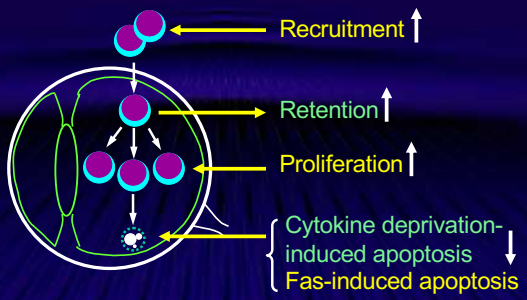

68

## Does retention play a role in the accumulation of leucocytes in uveitis?

- CXCR4 expression is elevated on AH lymphocytes
- AH increases CXCR4 expression

69

## Possible mechanisms leading to inflammation in the eye

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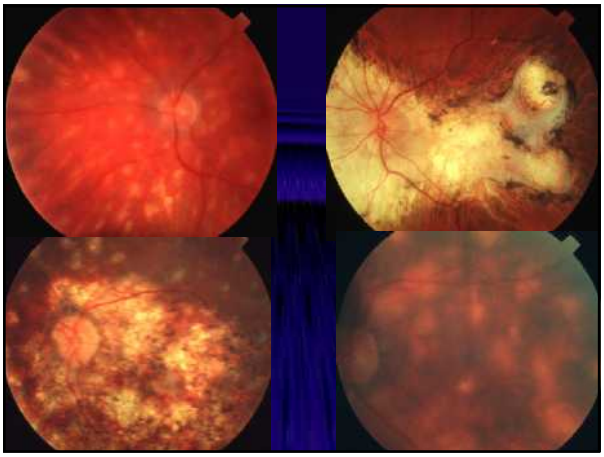

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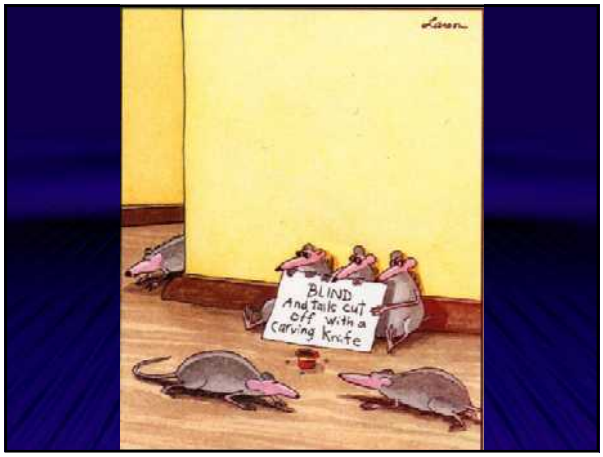


74

## Acknowledgements

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Jenny Hudson
Kate Martin

75