

Mediadors entre l'activació glial i l'increment de la permeabilitat vascular en la retinopatia diabètica: ús de la proteòmica en retines humanes.

Simó-Servat O¹, Hernández C¹, Sundstrom J², García-Ramírez M¹, Mesa J¹, Gardner TW³, Simó R¹.

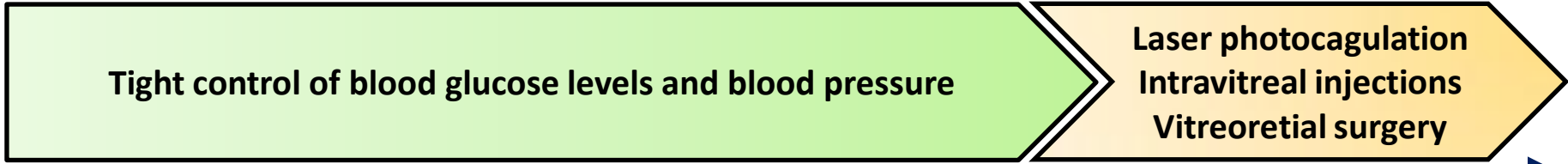
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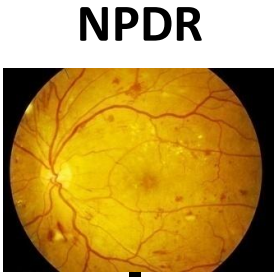


*Up to 30 % of diabetic patients present some degree of DR
10% with advanced disease (sight-threatening DR)*

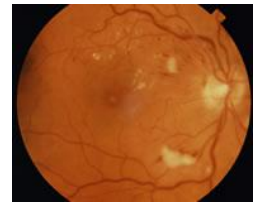


Neurodegeneration

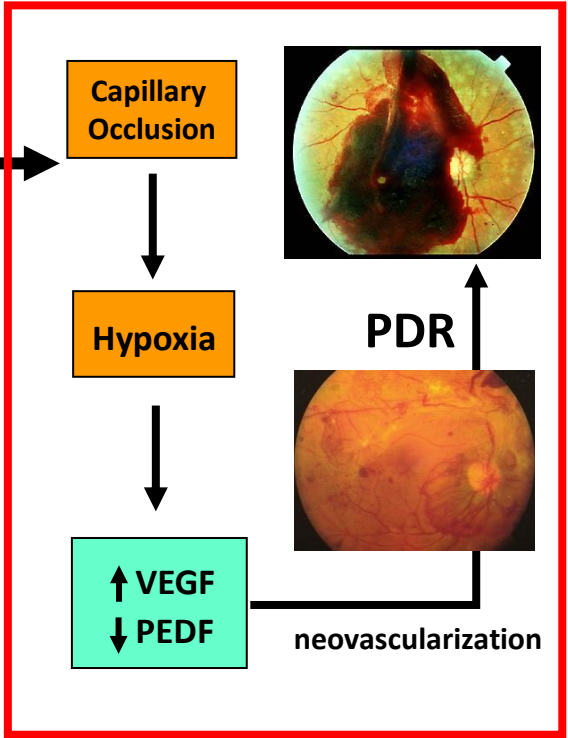
Antedates and participates in microcirculatory abnormalities



VEGF
Proinflammatory Cytokines

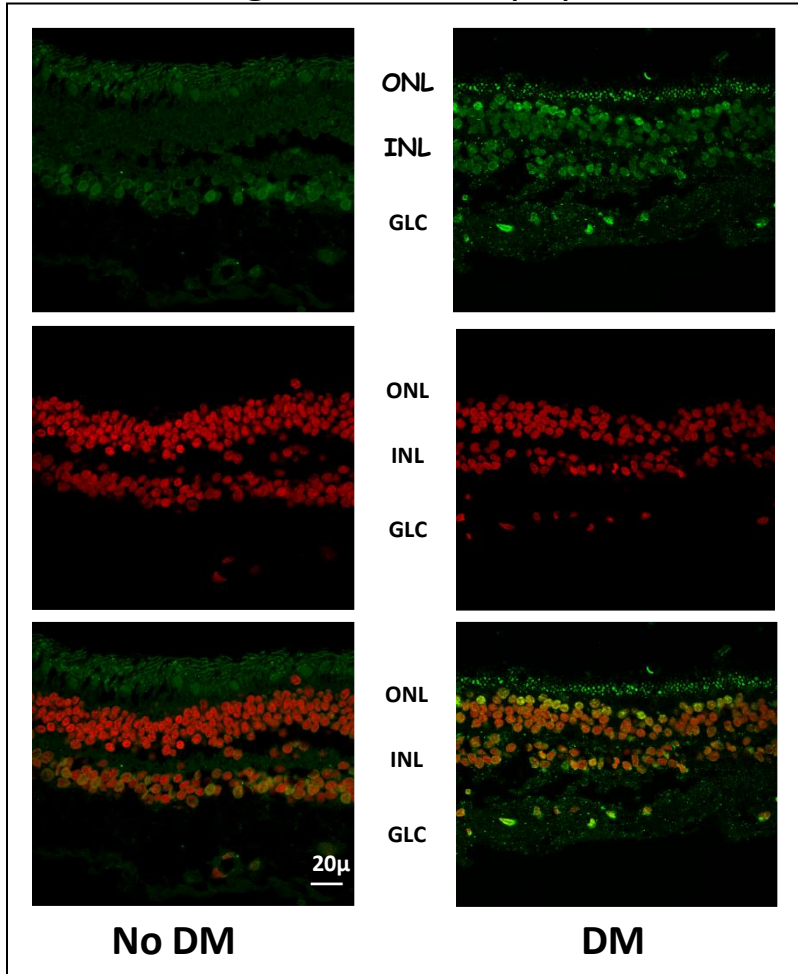


BRB breakdown

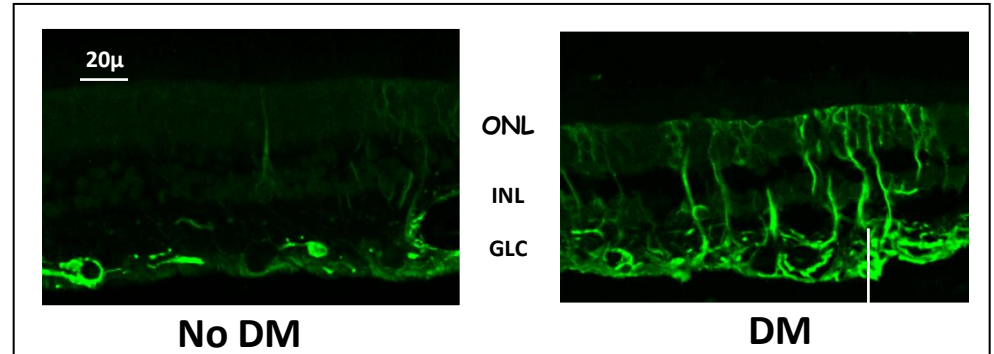


Neurodegeneration in retinas from diabetic donors

Ganglionic cells apoptosis



Glial activation (GA)

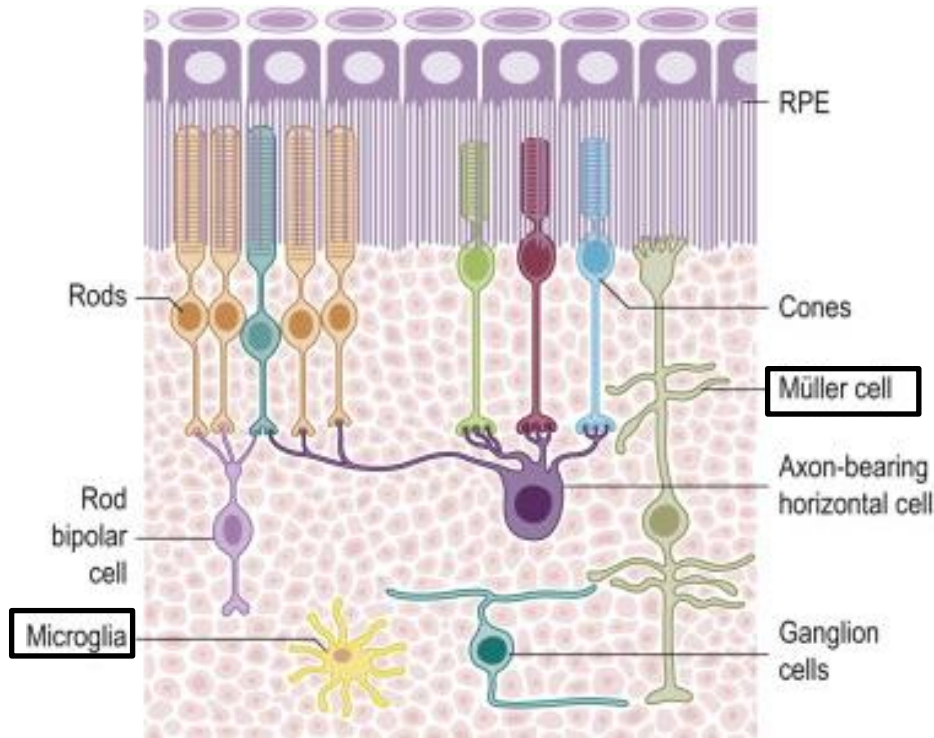


Absence of microvascular abnormalities

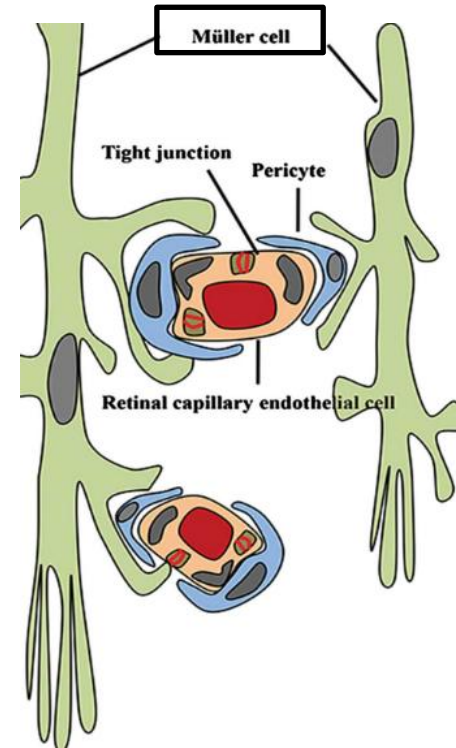
*Diabetes Care 2007; Diabetes Care 2008
Mol Vis 2008; Diabetologia 2009*

**Neurodegeneration is an early event
in the pathogenesis of DR**

Glial activation



Blood Retinal Barrier



Müller cells produce factors capable of modulating blood flow, vascular permeability, and cell survival



The effect of glial activation on early microvascular impairment remains to be elucidated

OBJECTIVE

To identify potential mediators of vascular leakage associated with glial activation by means of a proteomic analysis approach.

MATERIAL AND METHODS

Human Retinas:

Retinal samples were obtained from 5 non-diabetic donors, and 10 type 2 diabetic donors without (n=5; group A) or with (n=5; group B) reactive gliosis.

Diabetic donors did not presented microcirculatory abnormalities in the ophthalmoscopic examinations performed during the two years before death.

Proteomic Analysis:

Retinal lysates from each group were pooled and run on an SDS-PAGE gel



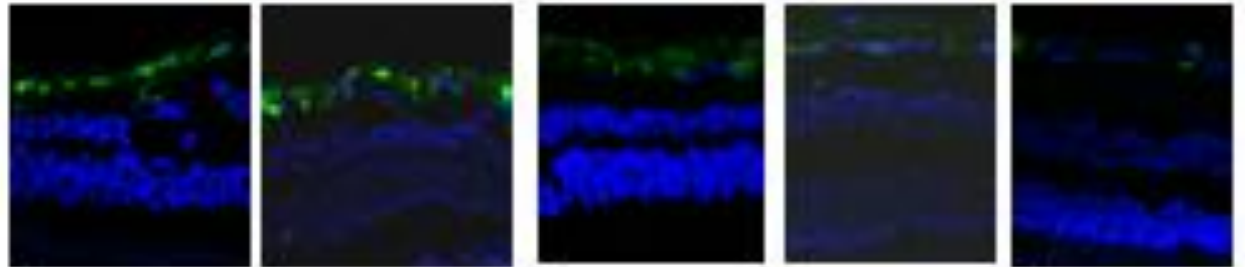
Bands were excised and analyzed sequentially by
Label-free Liquid Chromatography-Mass Spectrometry



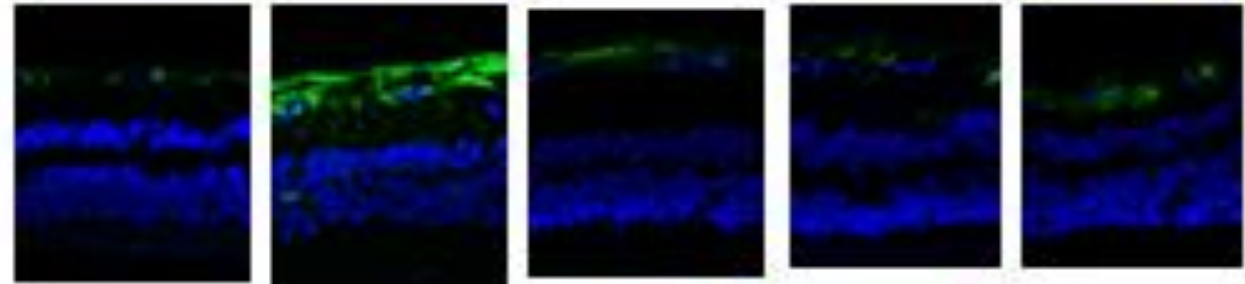
Data analysis (software: Scaffold)

RESULTS

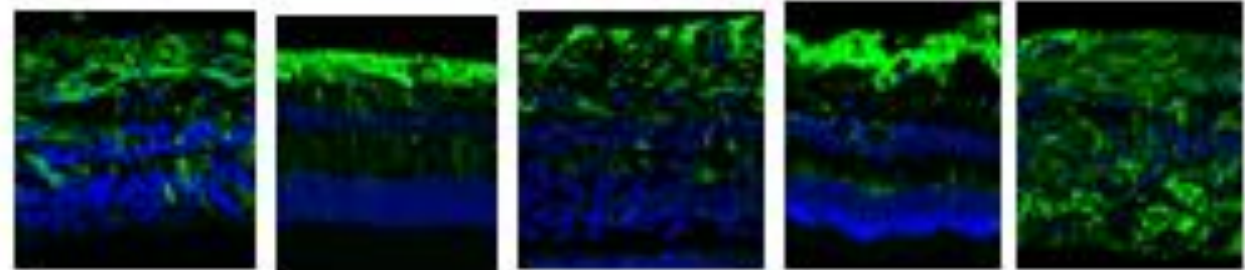
**Non-diabetic donors
(C)**



**Diabetic donors
without glial activation
(D)**

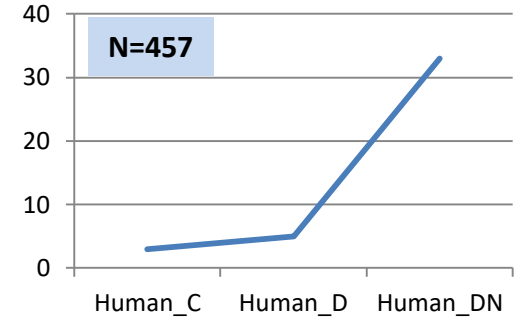
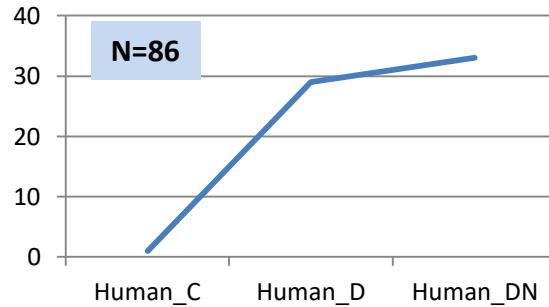
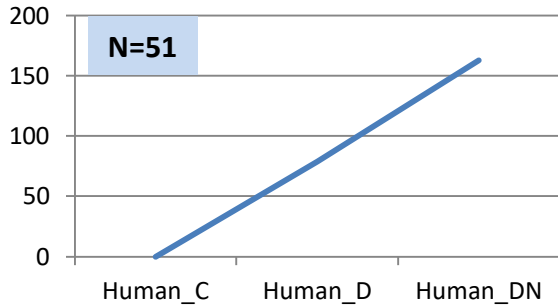


**Diabetic donors
with glial activation
(GA)**

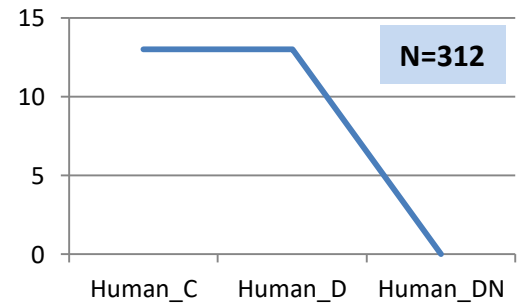
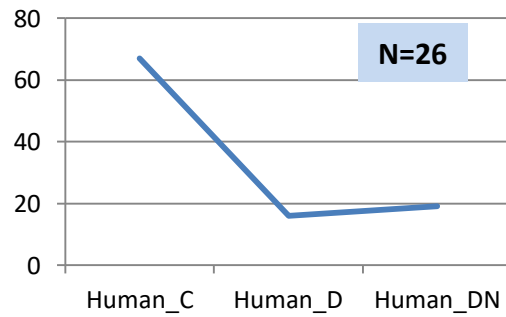
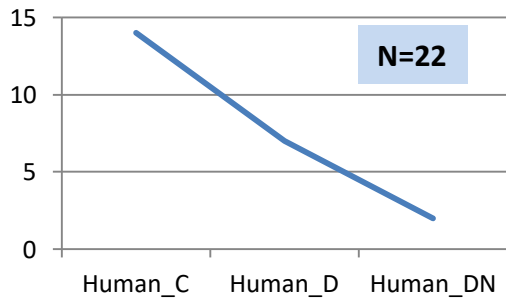


RESULTS

UP-REGULATED

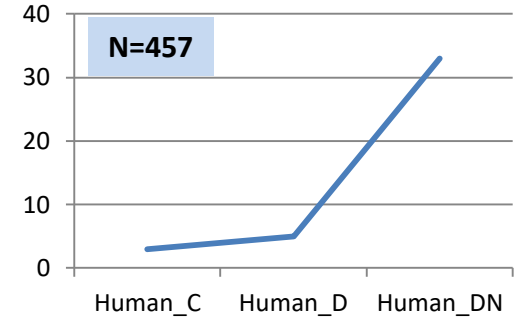
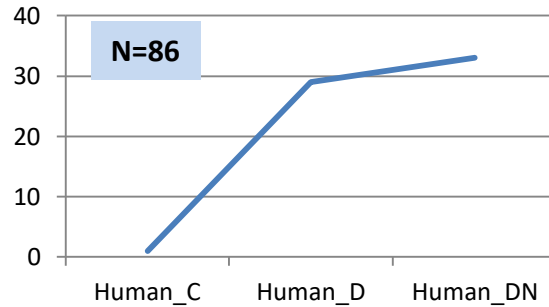
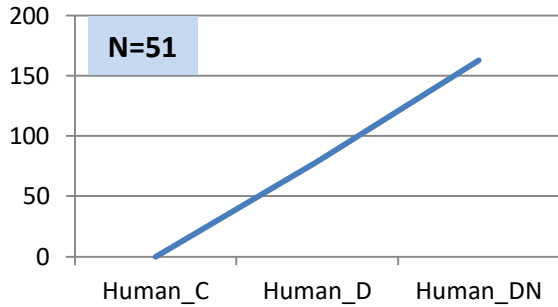


DOWN-REGULATED

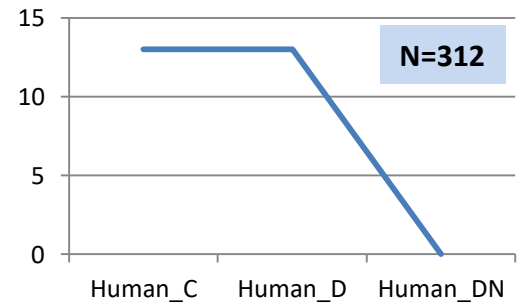
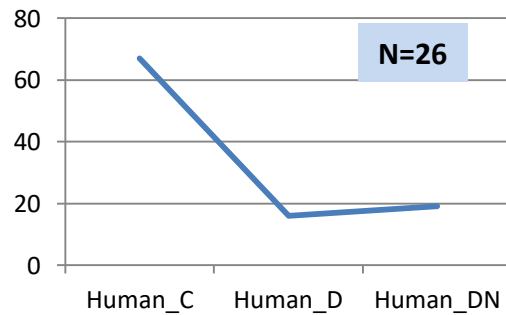
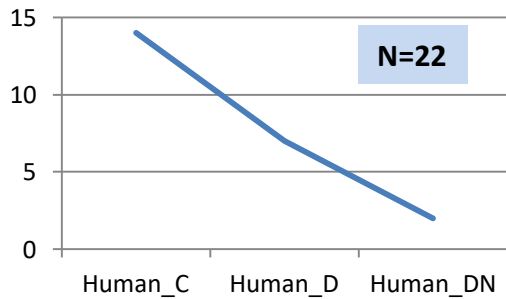


RESULTS

UP-REGULATED



DOWN-REGULATED

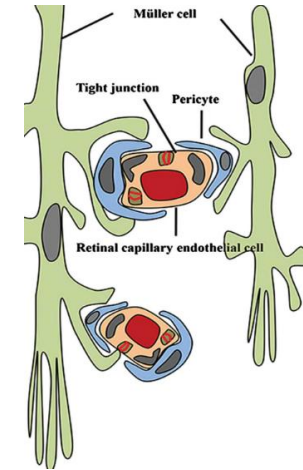
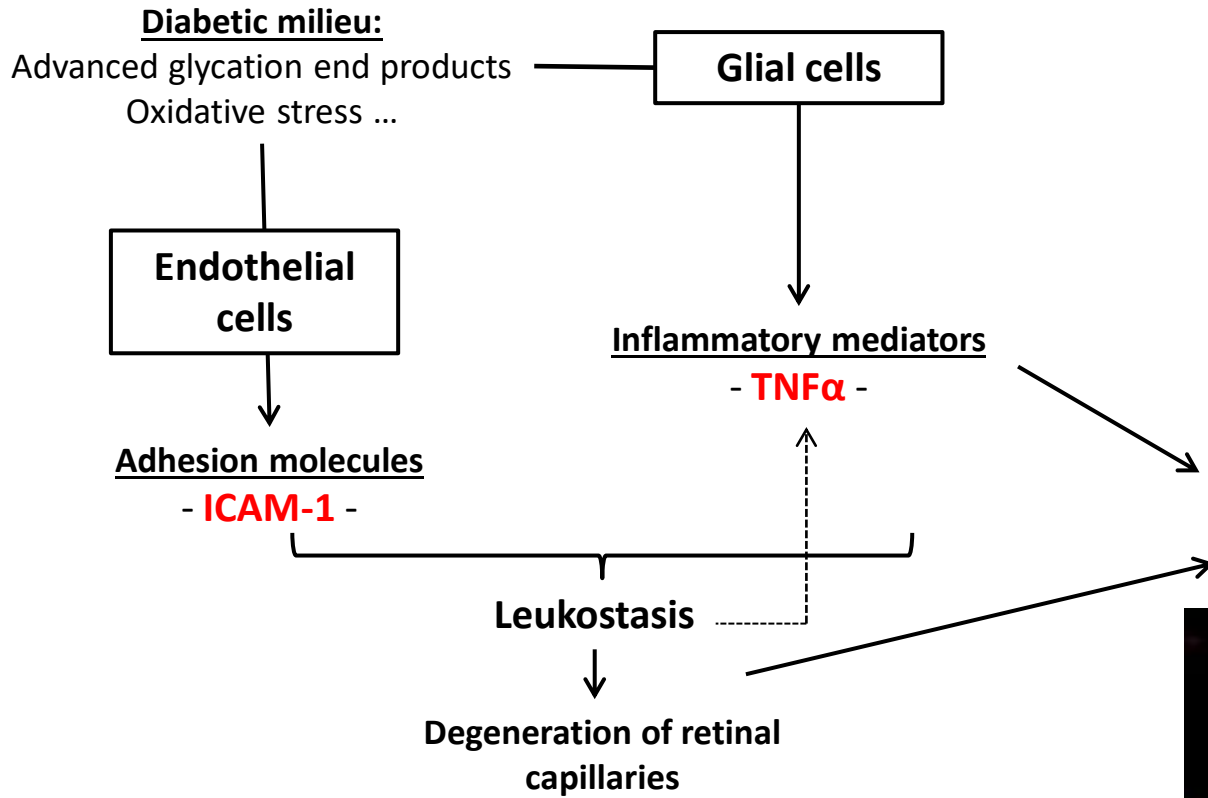


RESULTS

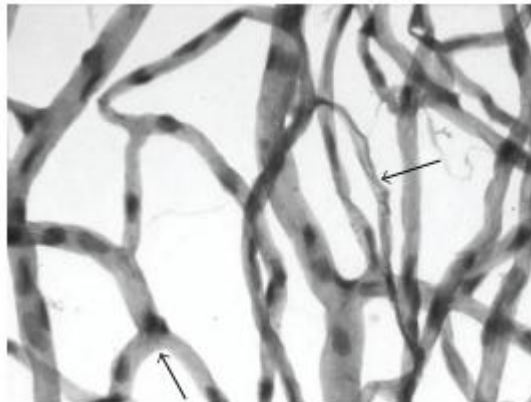
Early microvascular impairment and inflammation

	<u>Controls</u> <u>(C)</u>	<u>Diabetes without</u> <u>glial activation</u> <u>(D)</u>	<u>Diabetes with</u> <u>glial activation</u> <u>(GA)</u>	
GFAP	123	240	528	
Albumin	83	89	231	
TNF Receptor	7	23	46	
ICAM-1	5	22	94	
Complement	C4-B	22	30	75
	Complement factor H	8	2	0
Leukotriene biosynthesis	Leukotriene A-4 hydrolase	39	37	72
	Coactosin-like protein 1	6	3	15
	Prostaglandin reductase 1	10	18	2

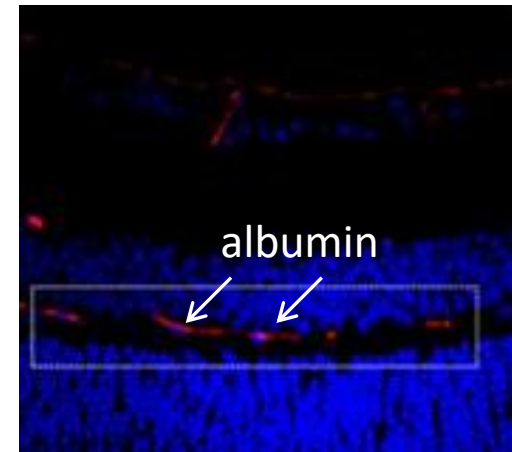
RESULTS:
TNF Receptor and ICAM-2



Increased permeability of blood retinal barrier



Kern TS. Exp Diab Res. 2007



Hernández et al. Diabetes 2016.

RESULTS:

Complement activation

CLASSIC PATHWAY (Ag-Ab)

C1
C4
C2

LECTIN PATHWAY (carbohydrates, lectins)

MBP, MASP1, MASP2

C4

ALTERNATIVE PATHWAY (pathogens)

C3
B
D

← - Complement factor H ↓

C3 convertase

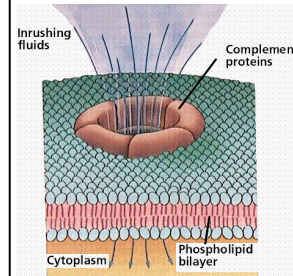
C4a
C3a
C5a

Inflammatory mediators
Phagocyte recruitment

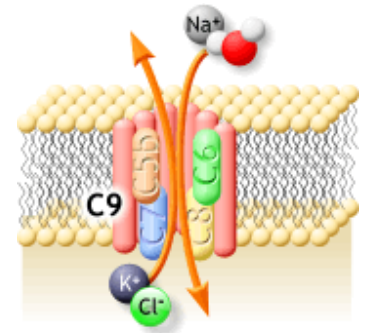
C3
C3b

Opsonization

C5b
C6
C7
C8
C9

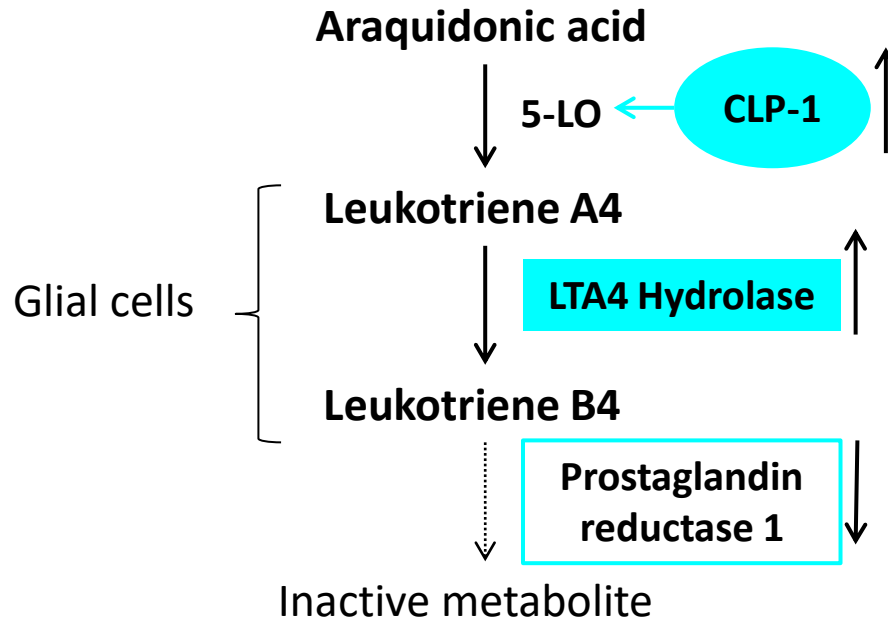


Membrane attack complex (MAC)
cell lysis



Proteomic analysis of human vitreous fluid by fluorescence-based difference gel electrophoresis (DIGE): a new strategy for identifying potential candidates in the pathogenesis of proliferative diabetic retinopathy. *Diabetologia* 2007;50:1294-1303

RESULTS:
Increased leukotriene synthesis

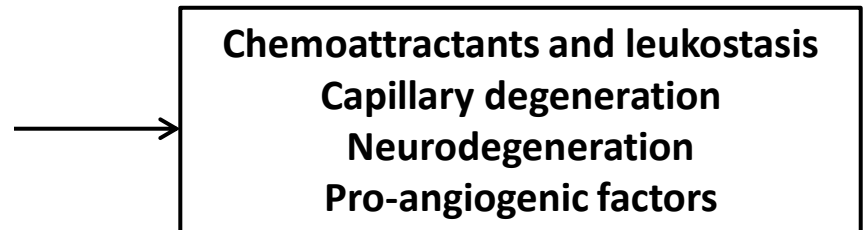


Review

Role of leukotrienes in diabetic retinopathy

Tapan Behl^{a,*}, Ishneet Kaur^b, Anita Kotwani^a

Prostaglandins and Other Lipid Mediators, 2016

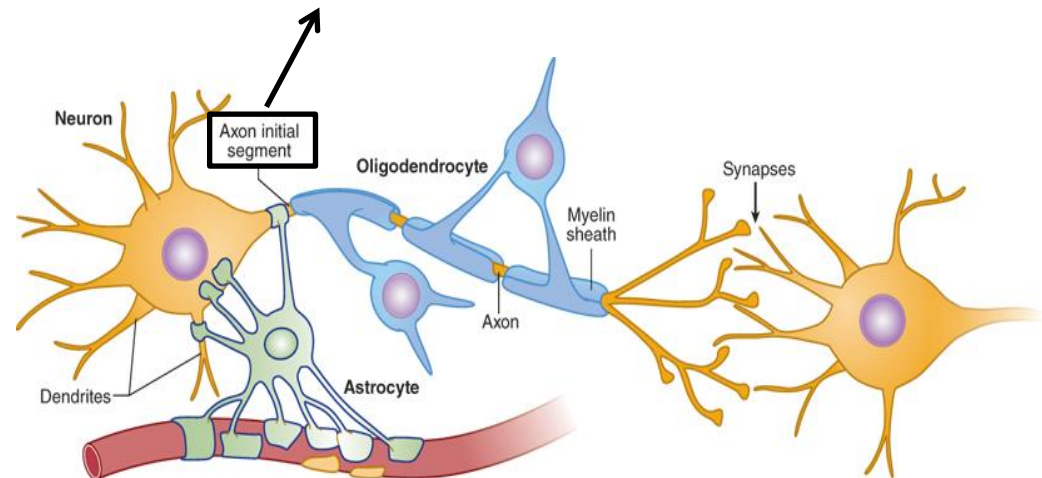
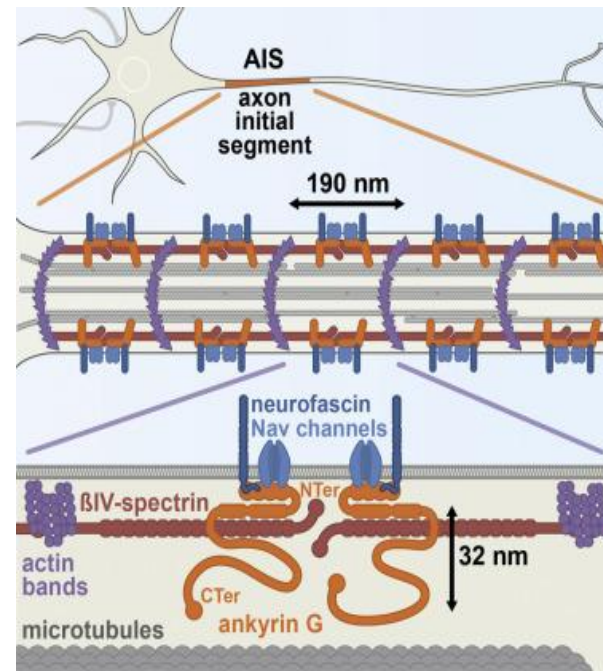


RESULTS

Axon initial segment proteins

$$C = D > DN$$

Ankyrin	Isoform Br21 of Ankyrin 1
	Ankyrin-2
	Ankyrin-3
	Isoform 4 of Ankyrin repeat and FYVE domain-containing protein 1
Spectrin	Isoform 2 of Spectrin alpha chain
	Spectrin beta chain, non erythrocytic 1
Neural adhesion molecule	Neural cell adhesion molecule L1
	Neural cell adhesion molecule 1
Neurofascin	
Dynein	Cytoplasmic dynein 1 heavy chain 1
	Cytoplasmic dynein 2 heavy chain 1
	Cytoplasmic dynein 1 light intermediate chain
	Dynein light chain 2
Dynactin	Dynactin subunit 2
	Isoform 6 of dynactin subunit 1



Source: Bertram G. Katzung, Anthony J. Trevor: Basic & Clinical Pharmacology, 13th Ed.
www.accesspharmacy.com
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Glial activation induced by diabetes lead to downregulation of axonal initial segment proteins

CONCLUSIONS

- **Proteomics is a useful tool to identify potential candidates implicated in the pathogenesis of diabetic retinopathy.**
- **Glial activation plays a relevant role in generating inflammatory mediators that can participate in vascular leakage.**
- **Structural proteins of the axon initial segment are downregulated in retinas from diabetic patients with glial activation.**

- This approach could open up new therapeutic strategies for early stages of diabetic retinopathy -



Moltes gràcies!