Pathogenesis of ocular fungal Infections

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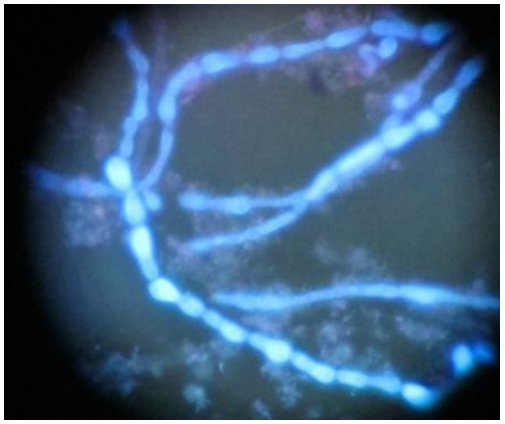
Introduction

- Fungal infections of the eye are rare

  (exception: keratitis)
- High morbidity and in some case with mortality.
- The incidence is higher in a tropical country.



Issues with fungal infections of the eye. :

- Difficulty in recognition
- In diagnosing fungal infections of the eye.
- effective anti-fungal agents for treatment is limited.

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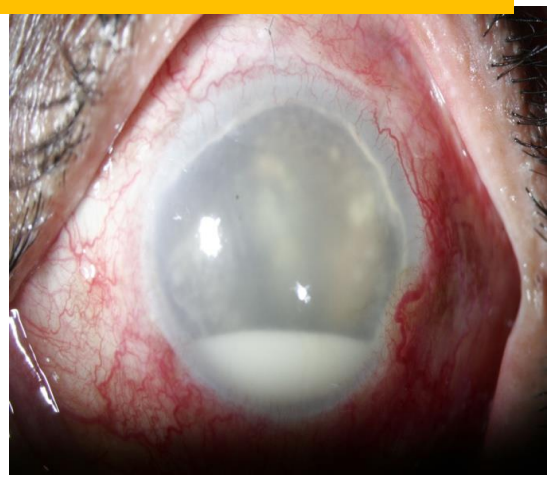
Fungal infections of the eye.

blepharitis,
conjunctivitis,
adnexal
lacrimal gland infections.



The more serious infections with increased morbidity :

- *corneal ulcers*
- *endophthalmitis*
- *uveitis.*
- *Orbital*

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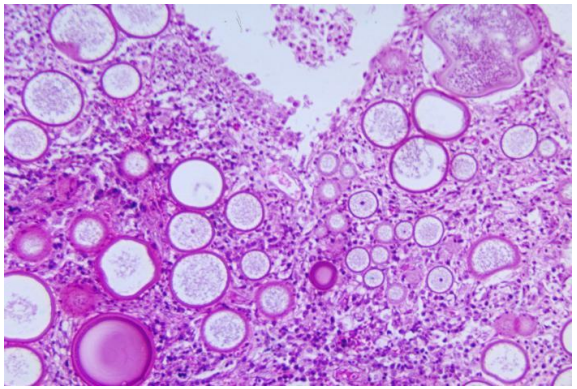
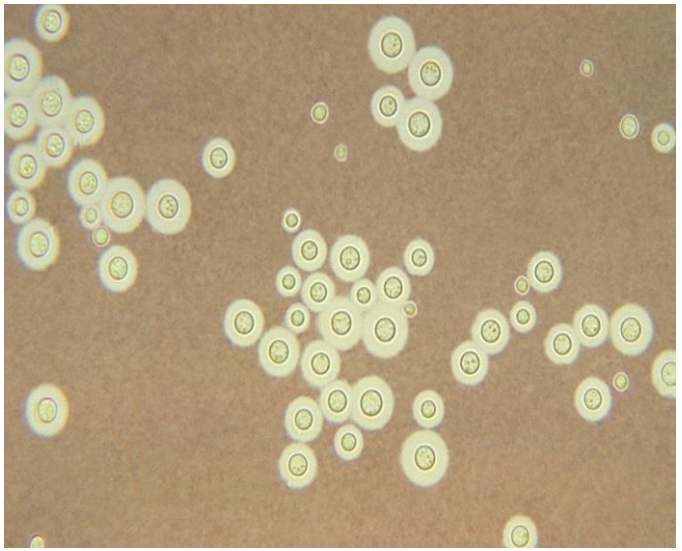


- **blepharitis,**
- *Cryptococcus neoformans* after trauma
- *Rhinosporidium seeberi*
- *Candida* spp: antibiotics or immunosuppressive drugs

- **The prevalence of fungal conjunctivitis is low,**

secondary to inflammation of the cornea, lacrimal sac and tear ducts

- *Rhinosporidium* .
- *Candida* spp., *Aspergillus* spp.
- *Sporotrichum* spp., *Blastomyces* spp.,
- *Coccidioides* spp.,
- *Malassezia* spp. and dermatophytes



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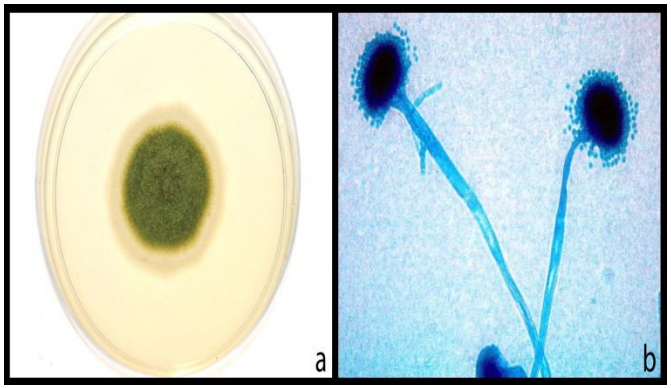
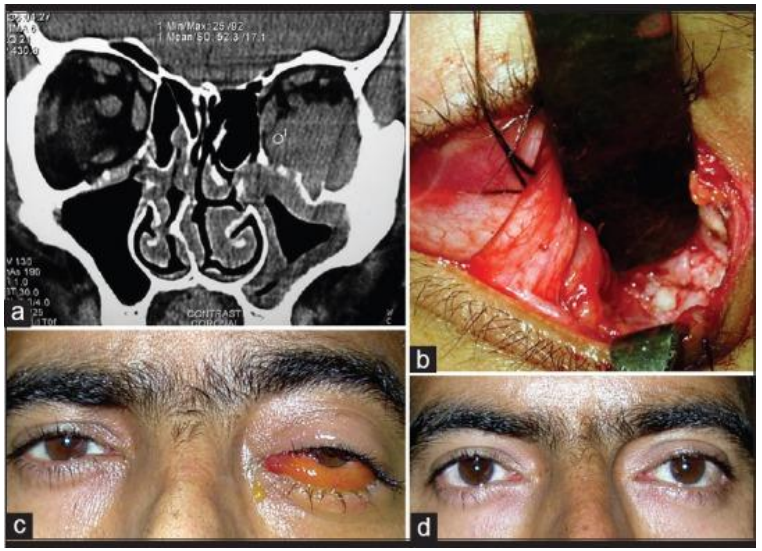
Fungal infections of the Obit

- Orbital fungal infections are vision- threatening
- also associated with high mortality.
- Imperative to diagnose fungal disease at the earliest and initiate appropriate therapy.
- The most common orbital fungal infections are :
  mucormycosis and aspergillosis.





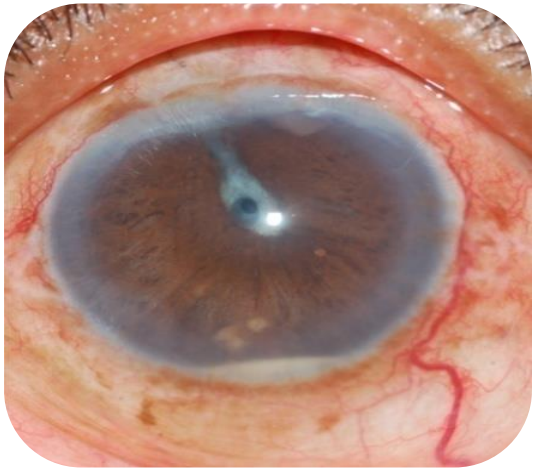
Fungal endophthalmitis

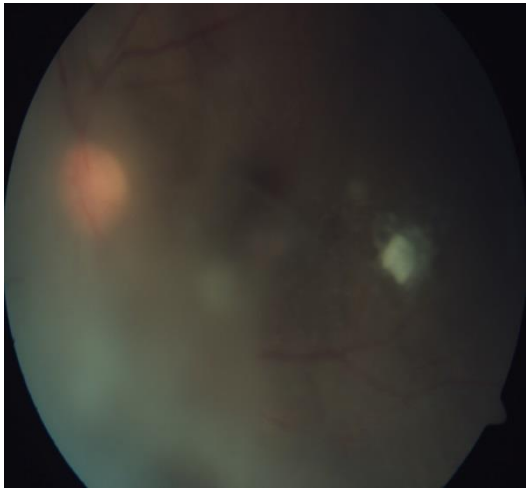
- Exogenous :

  intraocular surgery, keratitis, trauma

- Endogenous:

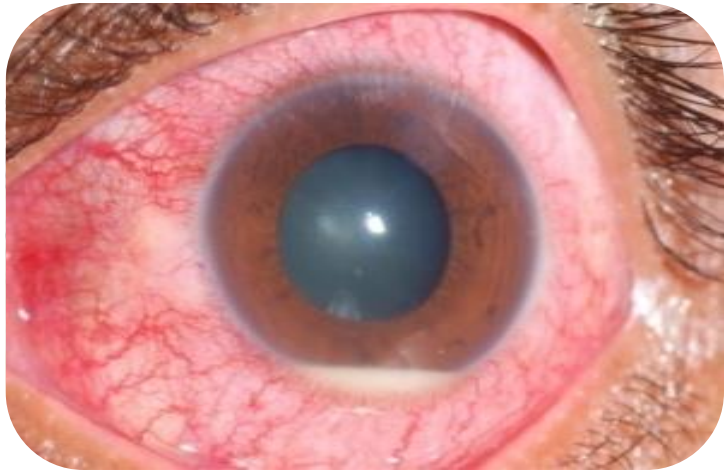
  associated with systemic fungal diseases

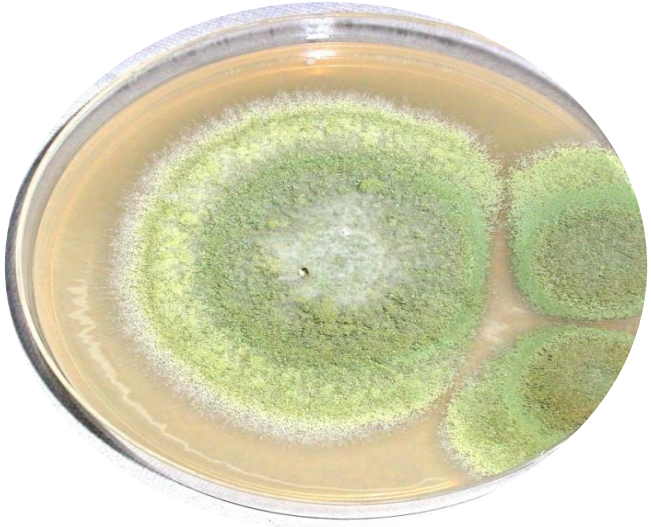


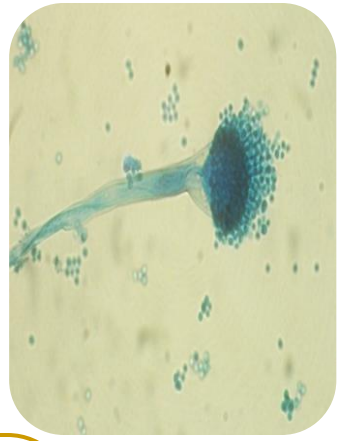


Fungal endophthalmitis

Clinical example: confirmation of the culture.







Aspergillus Flavus
Isolated both from Sputum and anterior chamber.

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Incidence of fungal endophthalmitis

- Incidence varies with geographical regions:
- Higher in tropical vs temperate *Gupta A, 2008; Lalwani GA,2008*

- Endogenous endophthalmitis accounts for 2–15%
- Endogenous endophthalmitis *:*

  50% Binder; 68.7% Leibovitch; 62% Schiedler

- *Post-operative Fungal endophthalmitis accounts for 5–10%*
- Postoperative endophthalmitis :, *21.8% Anand AR*
- Aravind Data from 2010 to 2014 *: 39(8.9 %)*

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Causative organisms

Post-operative endophthalmitis	Traumatic endophthalmitis	Endogenous endophthalmitis
Aspergillus flavus	*Aspergillus fumigates*	*Aspergillus flavus* (40%)
Aspergillus fumigatus	*Aspergillus niger*	*Candida albicans* (40%), *Candida tropicalis* (10%)
Aspergillus terreus	Curvularia	*Aspergillus fumigates* (10%),
Fusarium	Dematiaceous fungi	Penicillium (10%).
hyaline fungus	*Aspergillus terreus,* hyaline fungus Lasodiplopia	

Onset of an Outbreak of *Bipolaris hawaiiensis* Fungal Endophthalmitis after Intravitreal Injections of Triamcinolone

Kent W. Small, MD,[1] Candy K. Chan, MD,[1] Rosemary Silva-Garcia, MD,[1] Thomas J. Walsh, MD[2,3,4]

Purpose: To report a series of cases with fungal endophthalmitis occurring after intravitreal injection of triamcinolone derived from a single lot prepared by a compounding pharmacy.

Research

Original Investigation | CLINICAL SCIENCES

An Outbreak of Fungal Endophthalmitis After Intravitreal Injection of Compounded Combined Bevacizumab and Triamcinolone

Alan T. Sheyman, MD; Ben Z. Cohen, MD; Alan H. Friedman, MD; Jessica M. Ackert, MD

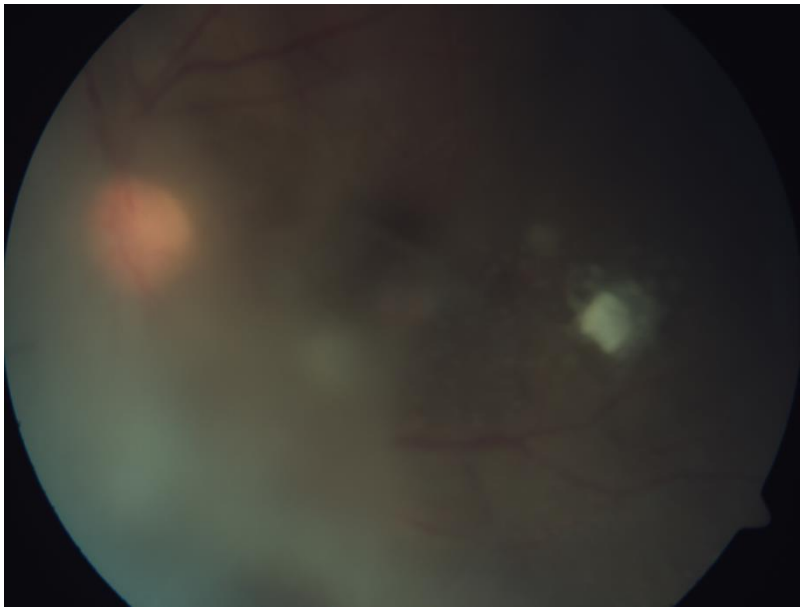
IMPORTANCE Our experience may be useful to other practitioners using compounded intravitreal agents, those suspecting infectious outbreaks, and those managing fungal

- intravitreal triamcinolone injection /anti VGEF
- obtained from the same compounding pharmacy.
- Long duration of onset
- Poor outcome ,
- Vitreous tap inadequate/direct vitreous biopsy preferred for laboratory tests
- fungal infection was confirmed in 57% (8/14)

Risk factors: Endogenous endophthalmitis

- debilitating disease
- intravenous drug use,
- chemotherapy,
- corticosteroids therapy,
- alcoholism, diabetes.

presenting sign of a systemic fungal infection.

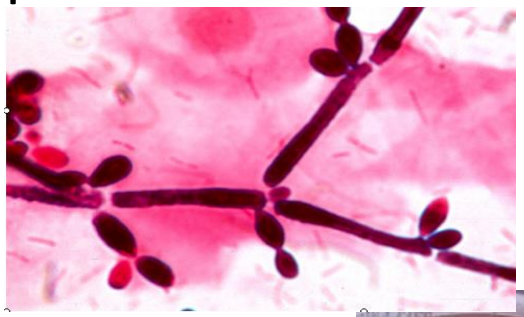


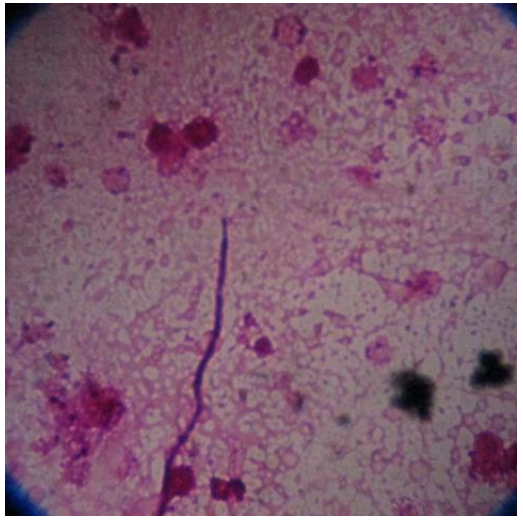
Candida endogenous endophthalmitis

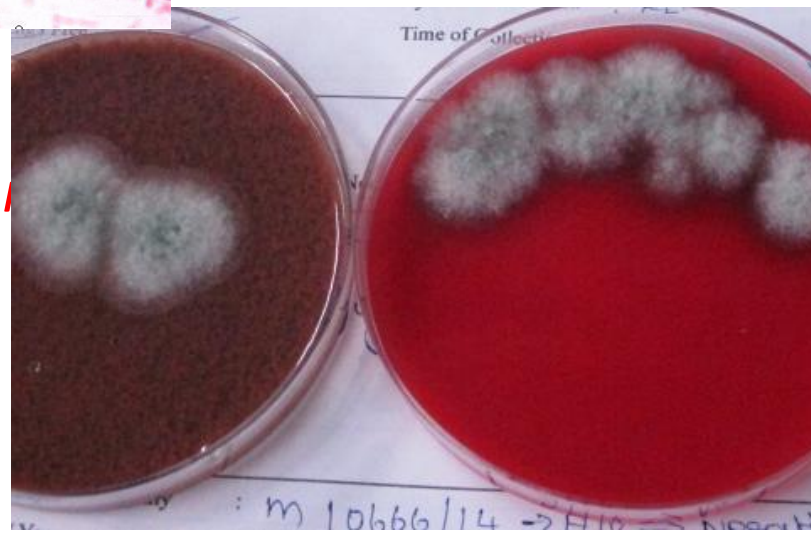
endophthalmitis within 10 days to 25 days of contaminated dextrose infusion
Chakrabarti 2008 ;Narang 2001

Challenges in fungal endophthalmitis

- Identification of risk factors
- Delay in presentation
- Clinical presentation
- Diagnosis
- Management







- *Rare*
- *Suspect fungal aetiology at presentatio*
- *Accurate diagnosis*
- *Laboratory confirmation*
- *Prompt therapy with antifungals*

Fungal corneal ulcers

- Fungal ulcers of the cornea are a major cause of blindness .
- *Fusarium* and *Aspergillus:*
- *Topical anti-fungal eye drops: Natamycin, Voriconazole.*
- *But nearly 50% of ulcers fail treatment.*

Microbiology data from Aravind Eye Hospital: 2013 to 2017

Particulars	2013	2014	2015	2016	2017	Total (%)
corneal ulcer cases	2785	2988	2285	1982	950	10990
Culture Negative	1058 (49.2%)	1214 (51.2%)	933 (48.5%)	707 (43.4%)	355 (45.4%)	4267 (48.2%)
Culture Positive	1092 (50.8%)	1156 (48.8%)	989 (51.5%)	922 (56.6%)	427 (54.6%)	4586 (51.8%)
Bacteria	355 (32.5%)	409 (35.4%)	332 (33.6%)	242 (26.2%)	124 (29.0%)	1462 (31.9%)
Fungus	673 (61.6%)	657 (56.8%)	617 (62.4%)	648 (70.3%)	284 (66.5%)	2879 (62.8%)

	2013	2014	2015	2016	2013-2016	Total %
Fungus						
Fusarium sp	310	265	253	266	**1094**	**39.8**
Aspergillus flavus	133	119	135	155	**542**	**19.7**
Other hyaline spp	125	90	104	102	**421**	**15.3**
Other Aspergillus sp	48	42	36	36	**162**	**5.9**
Unidentified fungus	139	150	128	93	**510**	**18.5**
Candida sp	4	5	8	4	**21**	**0.8**

Trend in fungal keratitis in the world.

- Multistate outbreak of *Fusarium* keratitis associated with use of a contact lens solution

  Khor WB et al 2006 , Chang DC, et al, 2006

- ***Trend in fungal keratitis in USA 2001 to 2007***
- *Fusarium*-related fungal keratitis cases among CLWs returned to baseline levels after the removal of ReNu with MoistureLoc from the market
- However, number of **non-Fusarium, culture-positive fungal keratitis** cases among both CLWs and non-CLWs had actually increased .

  Gower et al 2010

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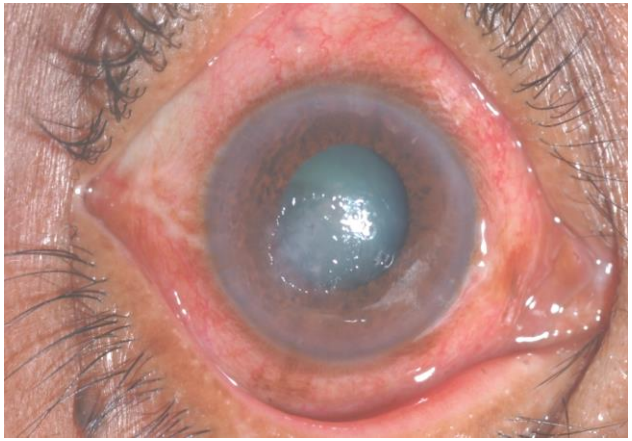
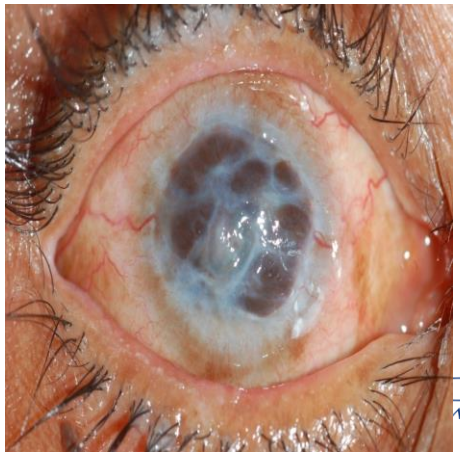
Fungal corneal ulcers

- The outcome of fungal corneal ulcers is due to a *combination of host factors and fungal virulent factors.*
- As we understand the exact pathogenicity we might be able to have a more customized treatment options
- *“ personalized medicine”*





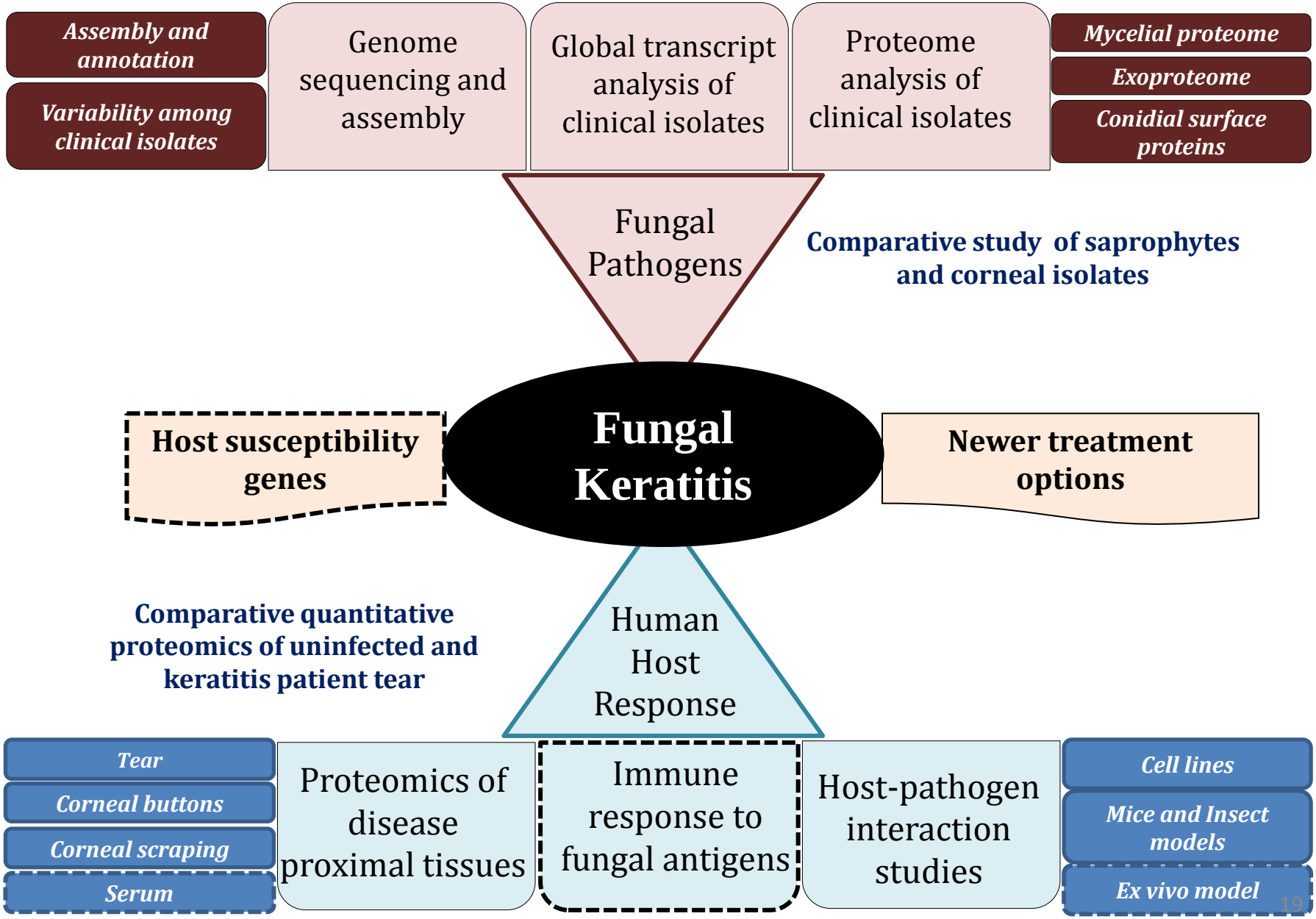
Pathogenesis Fungal corneal ulcers

- To understand the pathogenesis underlying fungal keratitis:

  Comprehensive approach to examine the *fungal virulence factors* and the *host response* to the infection was undertaken.

- *Aim to identify biomarkers that can be used for diagnosis/ prognosis / treatment.*

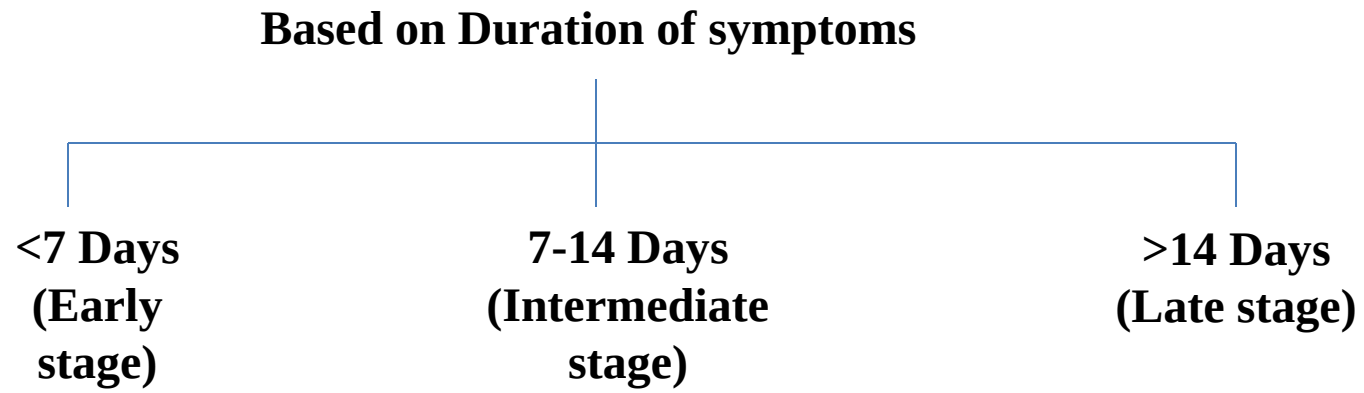
Thematic Focus



Tear collection and processing

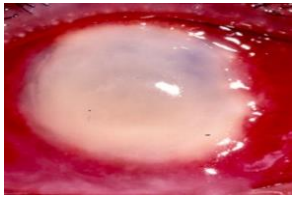
1. Tear from healthy individuals and fungal keratitis patients were collected (60-80µl).
2. Tear is collected using 10 µl-capillary tubes
3. Tear samples are Centrifuge to remove cellular debris.
4. Frozen in liquid nitrogen until analysis.

Categorization of keratitis tear samples

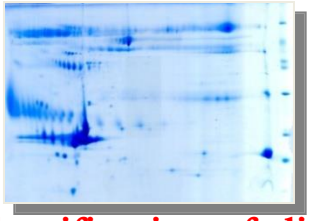


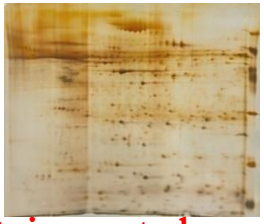
Experimental Approach

Tear/Cornea Collection from Healthy/Cadaver Controls & Fungal keratitis patients



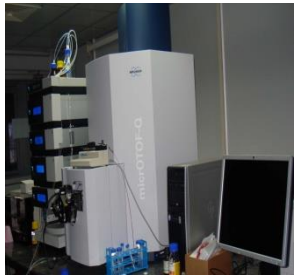
Separation Of Proteins by 1D/2D PAGE



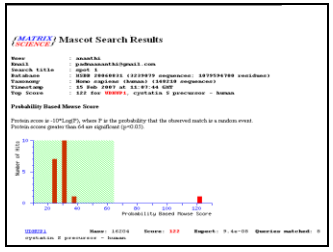


Identification of differentially expressed protein spots by

MALDI



LC-MS/MS



Mascot

	Database/ Accession no.	Mowse score[a]	Mr/ p*I* 2-D[b]	Mr/ p*I* data base[c]	Peptides Matched[d]	Sequence Coverage (%)[e]	Function[f]
	MSDB / UDHUP1	495 (36)	16/4.8	16.2/ 4.95	R.IIPGGIYDADLNDEWVQR.A R.ALHFAISEYNK.AK.ATEDEYYRRPLQ VLR.A K.SQPNLDTCAFHEQPELQK.K K.KQLCSFEIYEVPWEDR.M K.QLCSFEIYEVPWEDR.M	55	Cysteine protease inhibitor

Expression of Innate and Adaptive Immune mediators in Human Corneal Tissue Infected With Aspergillus or Fusarium

Rajapandian et al JID 2011:

- Gene expression studies of patients with corneal ulcers

  Corneal scrapping / Post transplant corneas

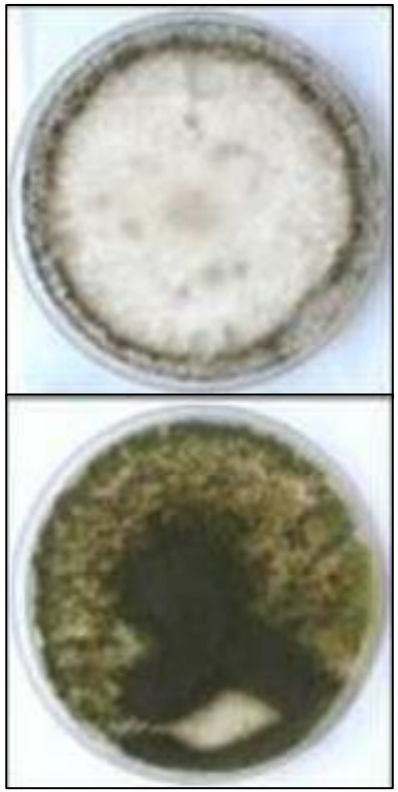
Expression of Dectin-1, Toll-like receptor 2 (TLR2), TLR4, TLR9, and NOD-like receptor protein were looked for .

- There is a common innate and adaptive immune response to these filamentous fungi, which includes the generation of T-helper 1 and T-helper 17 cell.
- Targeting mediators along with antifungal therapy, could restrict excessive cellular infiltration into infected corneas and minimize host- mediated tissue damage

A. flavus corneal isolates are more virulent: Animal models

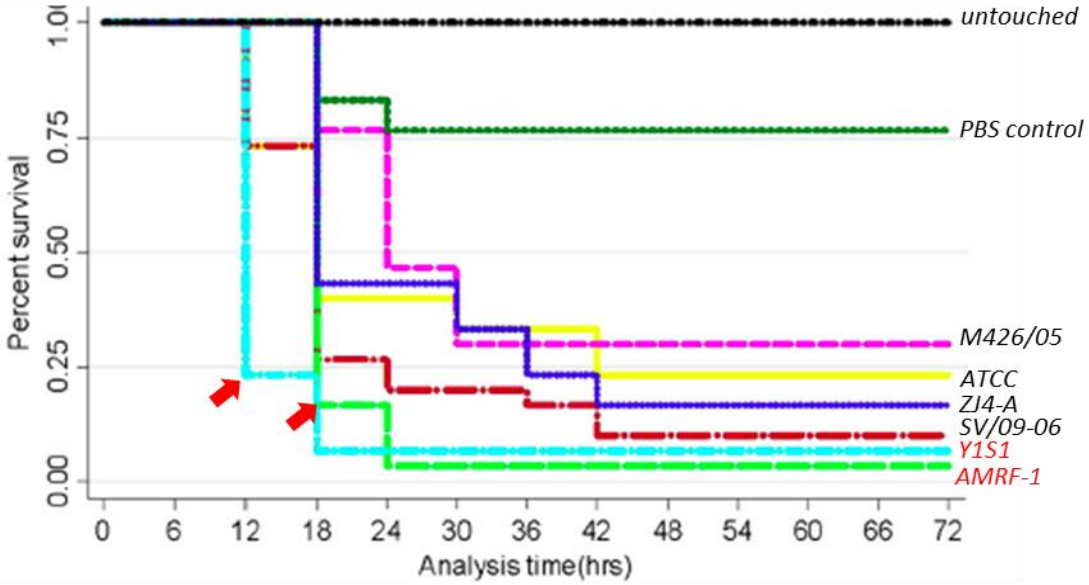
Corneal isolates produce more melanin

Saprophyte



Corneal isolate

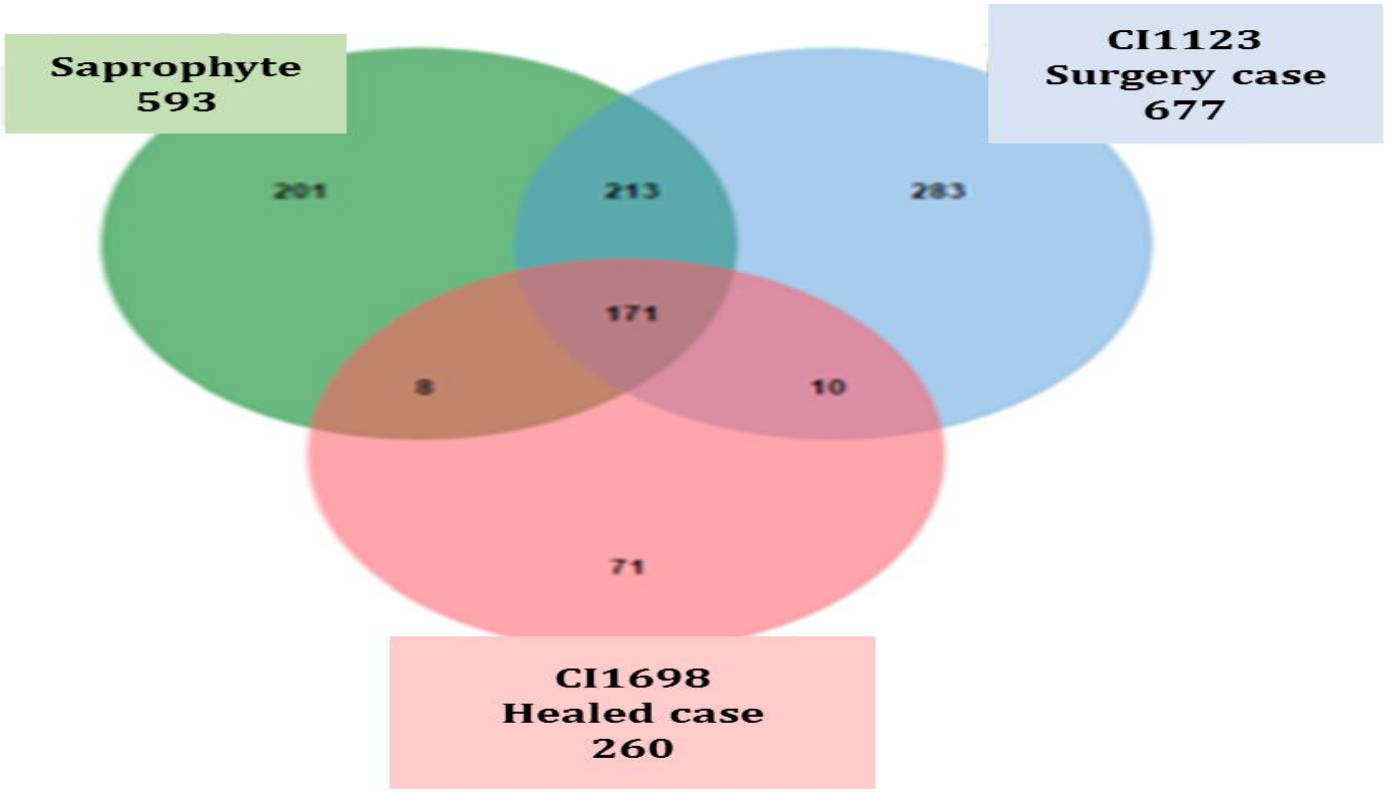
Corneal isolates in general are more virulent in Galleria larvae



Kaplan-Meier Survival Estimates

Selvam, R.M., et al., (2015). *Exoproteome of Aspergillus flavus corneal isolates and saprophytes: Identification of proteoforms of an oversecreted alkaline protease*. **J Proteomics** *115*, 23-35

Exoproteome differ between A. flavus strains
Clinical isolates secrete more proteins: Through non-classical pathways



Proteome depends on life style

Selvam, R.M., et al., (2015). **J Proteomics** *115*, 23-35
Selvam, R.M., et al., (2015). **Data in Brief** *2*, 42-47

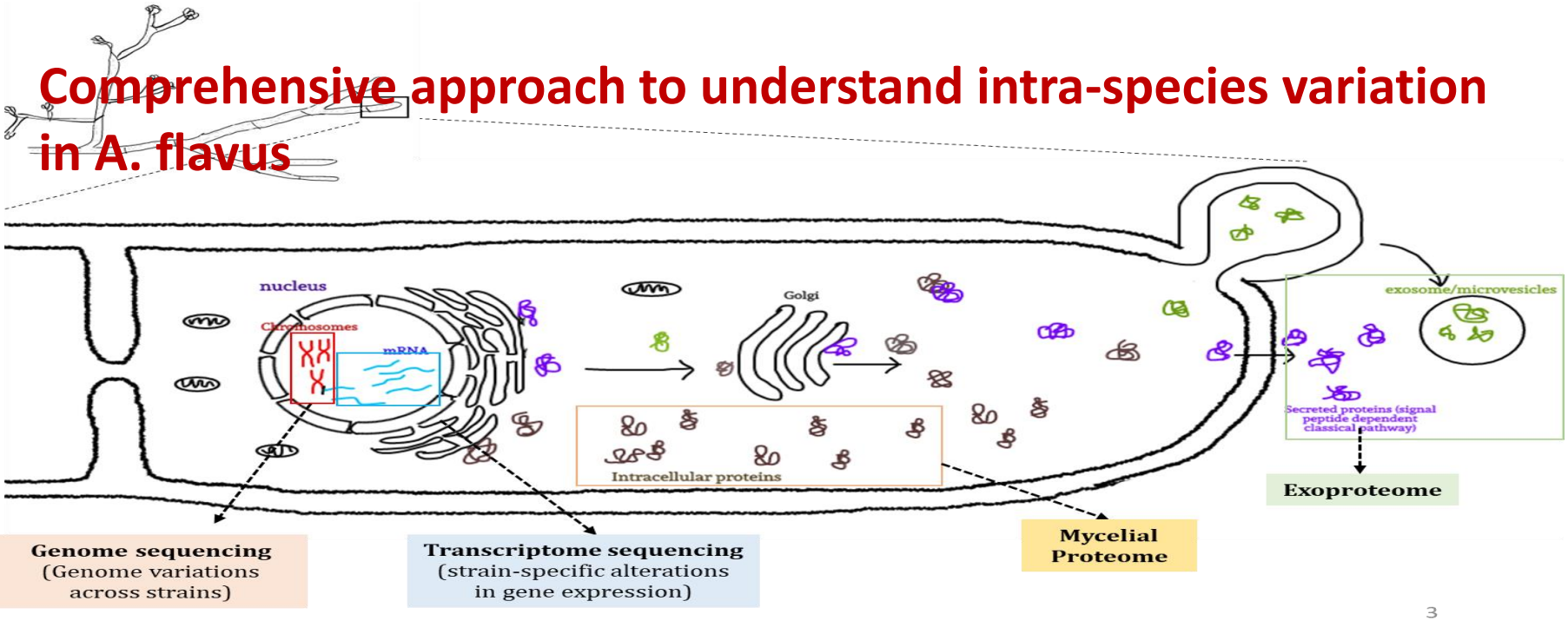
Selvam, R.M., et al., (2015). *Exoproteome of Aspergillus flavus corneal isolates and saprophytes: Identification of proteoforms of an oversecreted alkaline protease*. **J Proteomics** *115*, 23-35

- High resolution two-dimensional electrophoresis and mass spectrometry were used to generate A. flavus exoproteome reference map as well as to profile most of the exoproteins.
- Nearly 50% of the exoproteins possess catalytic activity
- ***One of these, an alkaline serine protease (Alp1) is present in high abundance as well as multiple proteoforms.***
- Many proteins in the A. flavus exoproteome have been shown to be virulence factors in other pathogens indicating the probable role for these proteins in the corneal infection as well.
- Thus, this study provides a clue to the early strategies employed by the pathogen to establish an infection in an immunocompetent host.

Tear Proteomics to understand host response

- Multiple pathways are activated in response to fungal infection
- Complement system is a major mediator of inflammatory response
- An intricate balance exists between pro- and anti-inflammatory factors

Comprehensive approach to understand intra-species variation in A. flavus



Pathogenesis of fungal corneal ulcers

Jeyalakshmi, K., et al., (2017).

- In-depth identification and analysis of tear proteins revealed that *A. flavus* infection activates multiple pathways representing the host response, namely the

  complement and coagulation pathways along with the recruitment of neutrophils.

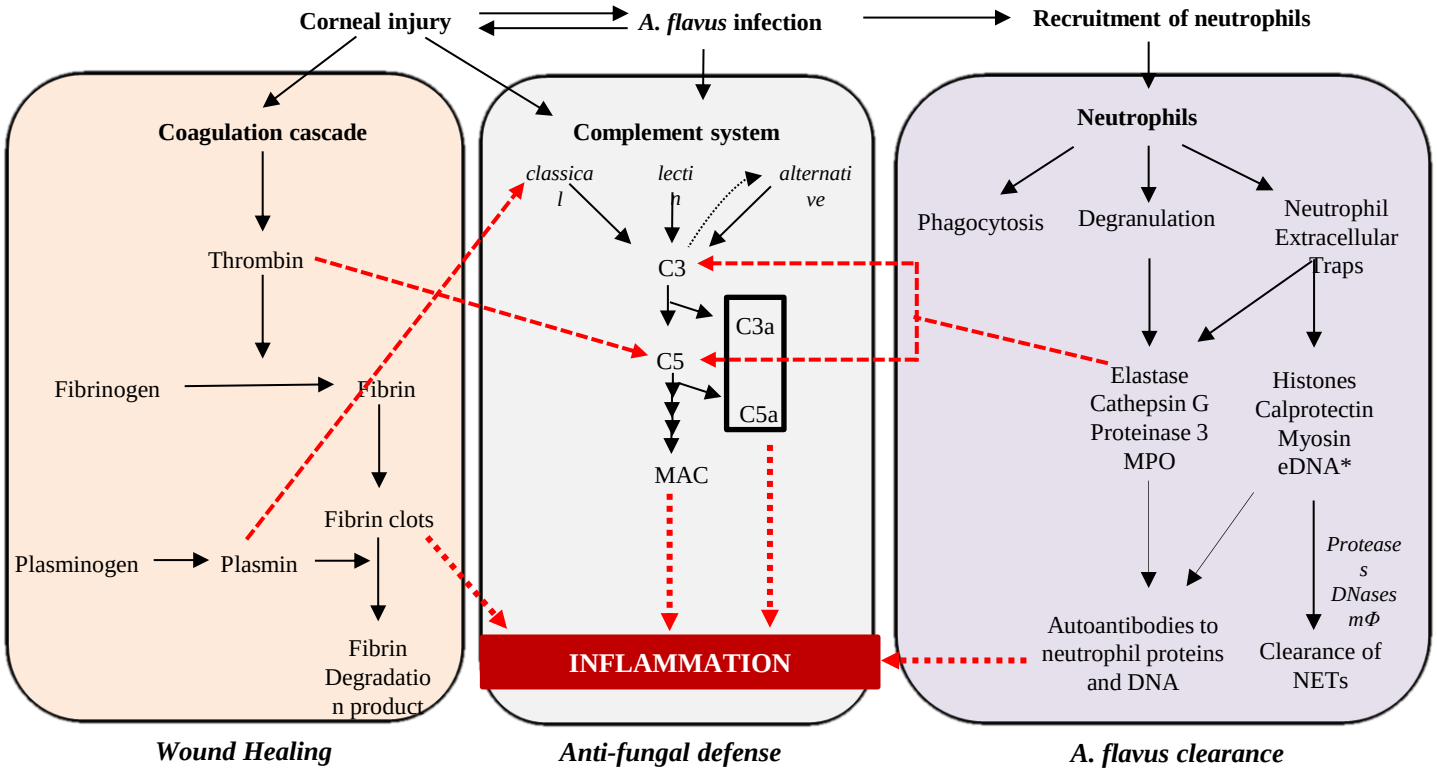
- All these pathways are activated only in patients and are favourable to the host in resolving the fungal infection.

Pathogenesis of fungal corneal ulcers

Jeyalakshmi, K., et al., (2017).

- However, the presence of a number of pro- and anti-inflammatory proteins in tear suggest that there is an intricate balance between these two groups of proteins and the outcome of the ulcer is dependent on which of these factors dominate.
- Further, studies with infected corneal tissue from keratitis patients also provide a direct characterization of the host response to pathogenic fungi in infected human tissues at early and later stages of disease

Fungal infection alters multiple pathways: Wound healing, inflammation and host defense



Jeyalakshmi, K., et al., (2017). *Aspergillus flavus* induced alterations in tear protein profile reveal pathogen-induced host response to fungal infection. J Proteomics.

Conclusion

- These findings will allow us to identify

  : potential targets for immune intervention

Targeting immune mediators along with antifungal therapy, could restrict excessive cellular infiltration into infected corneas and minimize host- mediated tissue damage.

- The outcome of the study is expected to allow the development of better diagnostic methods in combination with effective treatment strategies

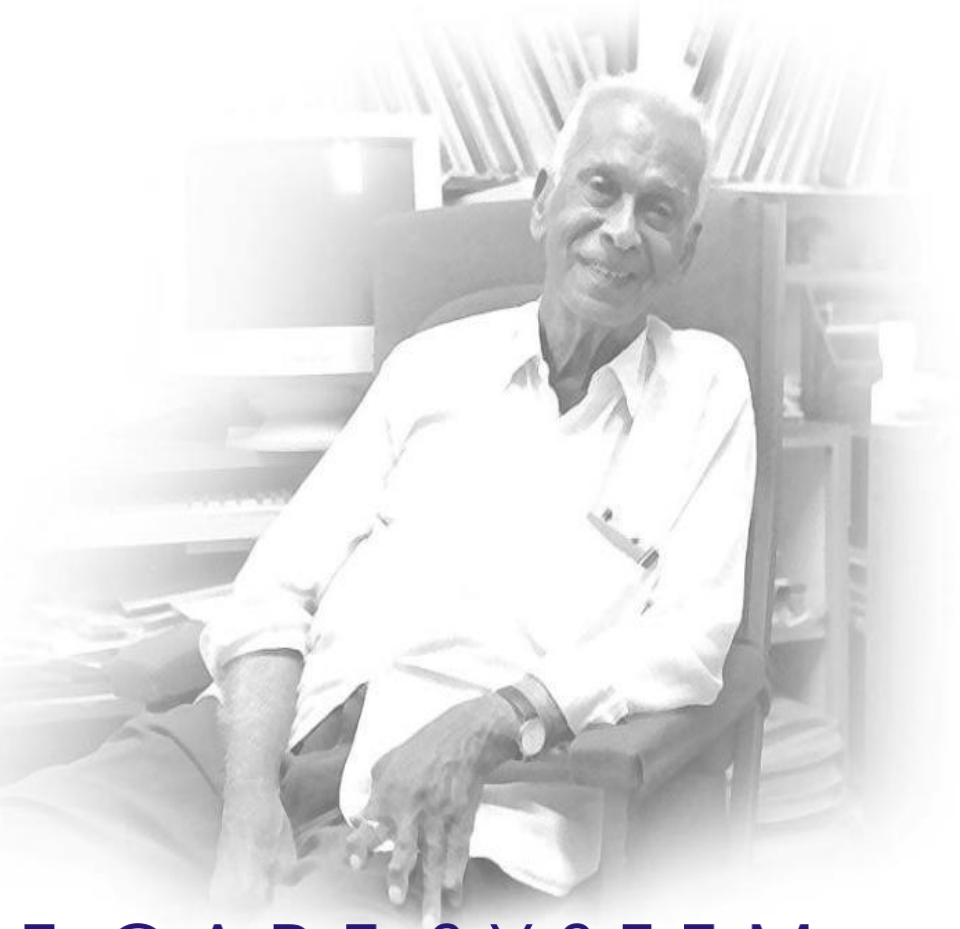
Future work: **Pathogenic *Aspergillus*: Interaction with innate immune cells**

1. Analyzing the differential interactions of two *Aspergillus*, *A. fumigatus* and *A. flavus,* with their host in the context of their specific pathologies(*invasive vs superficial*)
2. Understanding the phagocytic responses toward *Aspergillus* morphotypes (dormant and germinating conidia)
3. Identifying and characterizing the fungal cell surface components which activate or repress the host immune response
4. Studying in depth the immunogenic function of the core cell wall fungal polysaccharides – uptake by phagocytes, surface receptor identification and degradation
5. Recognizing the components of the phagolysosome involved in the intracellular recognition of the fungus

“Intelligence & capabilities are not enough.

There must be the joy of doing something beautiful..”

- Dr. V



Thank You!

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