The role of the complement system in a murine model of disseminated mucormycosis

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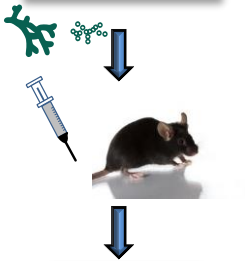
Background

Mucormycetes, a rather heterogeneous group of fungi, induce a life-threatening disease called mucormycosis. The prevalence of this disease, which shows high morbidity and mortality, increased within the last decade. Main risk factors for mucormycosis are immune deficiencies. An important link between innate and adaptive immunity is the complement system, which also provides several crucial functions in first-line defense against non-self-structures like fungi. To enlighten the responsibility of complement in the defense against mucormycosis, our objectives were, on the one hand, to compare the role of different parts of complement in a murine model of disseminated mucormycosis for different species and on the other hand, to study the relevance of complement for pathogenesis.

Aim 1:
To investigate the role of complement in disseminated mucormycosis

Experimental design

- *Lichtheimia corymbifera*
- *Lichtheimia ramosa*
- Mucor circinelloides
- *Rhizomucor pusillus*
- *Rhizopus arrhizus*
- *Rhizopus microsporus*



Survival clinical status

The survival and clinical status of immunocompetent, neutropenic, C3-deficient and C6-deficient mice, which were infected intravenously with $5x10^5$ spores of either *Lichtheimia corymbifera*, *Lichtheimia ramosa*, *Mucor circinelloides*, *Rhizomucor pusillus*, *Rhizopus arrhizus* or *Rhizopus microsporus* were monitored for 14 days.

Results

When intravenously challenged with *Mucor circinelloides*(Fig. 1C) or *Rhizopus arrhizus*(Fig. 1E), no significant difference between C3-deficient (ΔC3), C6-deficient (ΔC6), neutropenic (ΔNeu) and immunocompetent mice can be detected. Complement deficient animals presented a significantly aggravating course of infection when infected with either *Lichtheimia corymbifera*(Fig. 1A), *Lichtheimia ramosa*(Fig. 1B), *Rhizomucor pusillus*(Fig. 1D) or *Rhizopus microsporus*(Fig. 1F). A tendency towards higher lethality was demonstrated in complement deficient compared to ΔNeu mice infected with *L. corymbifera*(Fig. 1 A) or *R. pusillus*(Fig. 1D). ΔC3 mice exhibited higher mortality than ΔC6 mice when infected with *L. ramosa*(Fig. 1 B), whereas the opposite was the case in *R. pusillus*(Fig. 1D) infections.

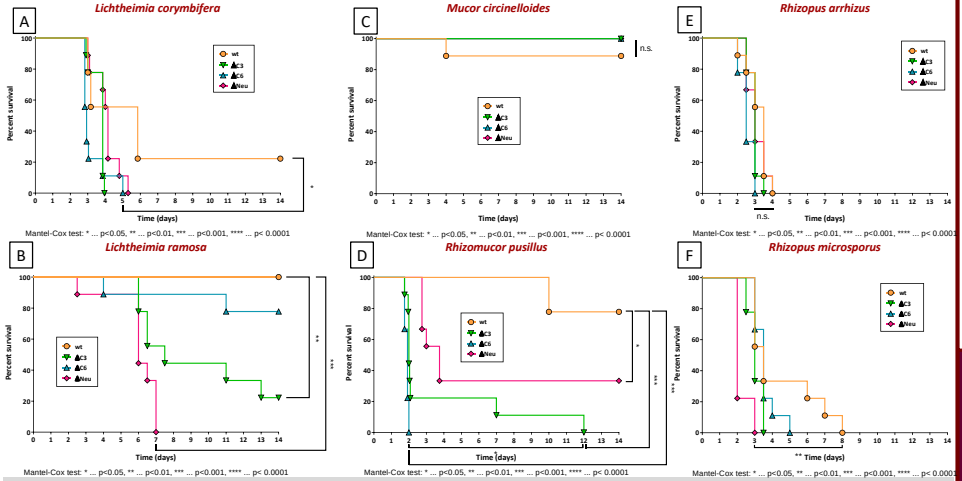


Fig. 1: Survival curves in a murine model of disseminated mucormycosis. Immunocompetent(wt), neutropenic(ΔNeu), C3-deficient(ΔC3) and C6-deficient(ΔC6) animals were intravenously infected with *Lichtheimia corymbifera*(A), *Lichtheimia ramosa*(B), *Mucor circinelloides*(C), *Rhizomucor pusillus*(D), *Rhizopus arrhizus*(E) or *Rhizopus microsporus*(F). The survival was monitored for 14 days. Stars(*) indicate the level of significance for a p-value resulting from a Mantel-Cox test analyzing pairs of survival curves.

Aim 2:
To check *in vitro* the complement opsonization on spores of selected mucormycete species

Experimental design

Spores of selected mucormycete species were opsonized with mouse serum and complement opsonization was detected using an anti-C3c antibody. Samples were measured using FACS Verse.

Results

Significantly higher levels of C3c were measured on spores of *L. corymbifera*, *L. ramosa* and *M. circinelloides* compared to *R. pusillus*, *R. arrhizus* and *R. microsporus* (Fig. 2). No significant difference in C3c deposition was seen between *R. pusillus*, *R. arrhizus* and *R. pusillus*.

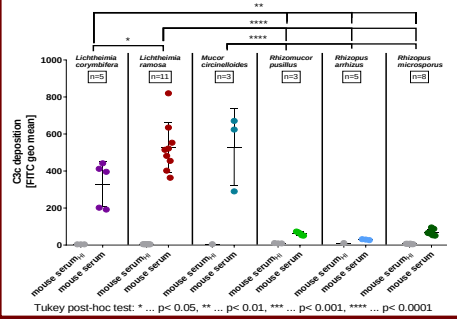


Fig. 2: C3c deposition on spores of selected mucormycete species. The deposition of C3c is detected using an anti-C3c antibody and shown in FITC geo mean. Stars(*) indicate an adjusted p-value, comparing pairs of groups using a Tukey post-hoc analysis after the one-way ANOVA reported significance.

Summary and Conclusion

- ✓ Complement plays a central role for the virulence of mucormycosis in mice
- ✓ Mortality of complement-deficient animals varies between mucormycete species
- ✓ Opsonization by the complement system inversely correlates with the mortality in murine model