Innate immunity in chronic infectious and inflammatory diseases

F. Haerynck, MD, PhD[1], F. De Baets, MD, PhD[1]

In the first part of this thesis, we analysed modifying genes encoding proteins involved in innate immune lung defence that influence pulmonary disease severity in cystic fibrosis patients. Firstly, we found that cystic fibrosis patients with *mannose binding lectin, ficolin 1* and *2* gene polymorphisms are at risk for earlier onset of chronic *Pseudomonas aeruginosa (Pa)* colonisation.[1] Secondly, we identified the involvement of toll-like receptor 1, 2 and 5 in the modulation of cystic fibrosis lung disease.[2]

In the second part of the thesis, we explored the innate immune response and genetic defects in patients with chronic inflammatory and infectious disease. We described at first a complete factor I deficiency caused by dysfunctional factor I in a patient with recurrent aseptic meningoencephalitis. Furthermore, we reported a novel mutation in the *IL-12receptorβ1* gene and we described c.1623_1624delGCinsTT mutation as the first founder effect on the *IL-12Rβ1* gene. A large survey on 141 patients with IL-12Rβ1deficiency reports a less favourable outcome, especially in patients with environmental mycobacteria.

In the scope of this journal, we will highlight the most important findings of the second part of this thesis.

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Introduction

The innate immune response is mediated by a limited number of pattern recognition receptors (PRRs) such as complement factors and toll-like receptors (TLRs) PRRs interact with specific components of micro-organisms, defined as pathogen specific associated molecular patterns (PAMPs), such as lipopolysaccharides (LPS). PRR-PAMP binding triggers intracellular signalling and cascades with the transcription of inflammatory cytokines, such as interleukin-12 (IL-12) and interferon-γ (IFN-γ), which activate innate immune cells.

Complement factors are the best known secreted pattern recognition receptors. The complement system provides protection against pathogens through antibody-dependent (classical complement pathway) and antibody-independent (alternative and lectin complement pathway) mechanisms. The complement system contributes to the homeostasis of inflammation.

Dysregulation of the innate immune response contributes to tissue damage, chronic inflammation, and autoimmune diseases. Defects in the innate immune response may predispose to increased susceptibility to infections.

Defects of innate immunity causing chronic inflammatory and infectious disease

Complement factor I deficiency and chronic inflammatory disease

Complement regulators control the activated complement system. Defects in this homeostasis result in tissue damage and autoimmune diseases with heterogeneity in clinical presentation. Complement factor I (FI), a serine protease, is an important regulator of the alternative complement pathway.

Two types of *complement factor I* gene mutations have been reported: i) type I complement factor I mutations resulting in low or absent serum level of FI and ii) type II complement factor I mutations with normal serum FI level but decreased activity of FI protein. To date, complete FI defects are only reported in patients with

[1]Department of Paediatric Pulmonology and Immunology, Ghent University Hospital, Ghent, Belgium.

Please send all correspondence to: F. Haerynck, MD, PhD, Ghent University Hospital, Department of Paediatric Pulmonology and Immunology, De Pintelaan 185, 9000 Ghent, Belgium, tel: + 32 9 332 64 39, fax: + 32 9 332 38 56, email: Filomeen.haerynck@ugent.be.

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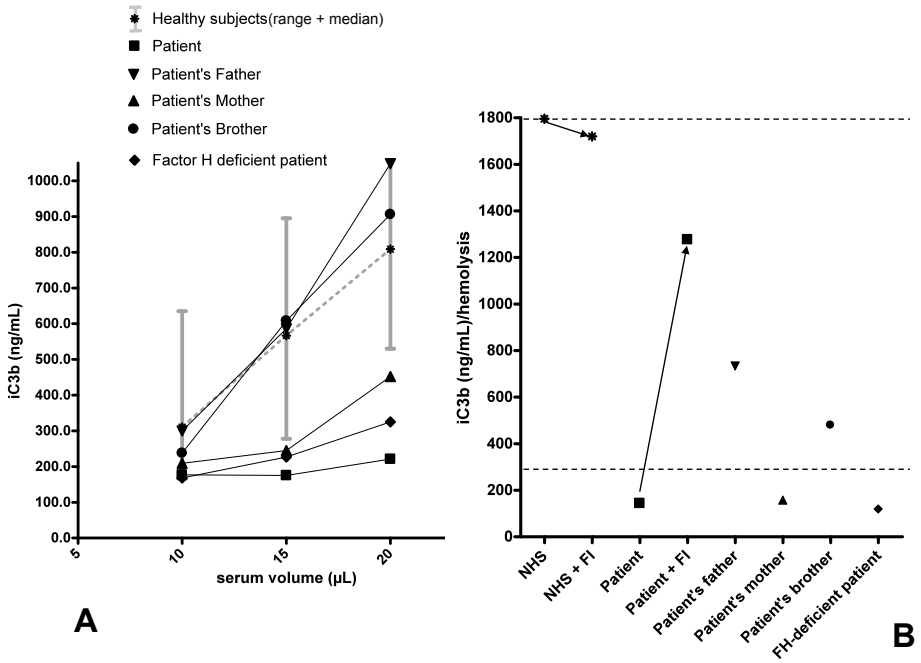

Figure 1. iC3b concentration in a haemolytic assay in serum from the patient, family members, 11 healthy controls and a patient with factor H deficiency.

Figure 1A. iC3b concentration (ng/mL) was quantified in the supernatant of the haemolytic assay, performed with 10, 15 and 20 µL of serum. The lowest concentration was observed in the patient. It was also decreased in the factor H-deficient patient, but to a lesser extent.

Figure 1B. iC3b concentration in the supernatant, reported to the haemolysis activity. The dashed lines represent the range of values obtained for 11 healthy subjects. NHS: pool of normal human serums. The arrows represent the effect of FI addition. The patient had a low iC3b concentration, similar to the FH-deficient patient, while both parents had a concentration similar to the healthy controls. The addition of purified FI to a final concentration of 3.5 mg/dL had a marginal effect on the NHS, while it significantly increased the concentration of iC3b in the supernatant of the patient.

a decreased or absent FI concentration. (type I FI deficiency).

Complete FI defects are mainly associated with severe bacterial infections. Partial FI defects are associated with atypical haemolytic uremic syndrome (aHUS).

In our study, we reported the diagnostic work-up of a patient with relapsing inflammatory mediated meningoencephalitis.[3]

Complement assays and measurement of FI activity were performed in the patient, family members, factor H-deficient patients, a patient with C3-nephritic factor and eleven healthy controls. The patient had absent alternative pathway activity with low levels of C3, factor B (FB) and normal serum level of FI. The very high ratio of FB/FBb (degradation product of FB) is compatible with overconsumption of FB suggestive for a regulatory defect in the alternative pathway. The patient's plasma FI did not degrade C3b with the lowest serum levels of inactivated C3b (iC3b). The addition of purified FI showed a normalisation of C3b degradation reflecting an isolated defect of FI function (*Figure 1*). Mutation analysis of the *complement factor I* gene revealed two heterozygous missense mutations (I322T and D506V). To our knowledge, this is the first description of a complete FI deficiency caused by a defect of FI activity. Thus far, all reported complete FI defects were associated with absent FI serum levels.[4]

Functional analysis of FI should be systemically performed in the work-up of a defect of complement activation. Recurrent aseptic meningoencephalitis is a rare clinical presentation of complete FI deficiency. Dysregulation of the alternative pathway needs to be explored in patients with central nervous involvement.

IL-12/IL-23-IFN-γ pathway defects and chronic infectious disease

Mendelian susceptibility to mycobacterial disease (MSMD) is a rare autosomal recessive disorder characterised by

Key messages for clinical practice

1. **A dysregulation of complement activation needs to be ruled out in patients with central nervous involvement. A normal factor I serum level does not exclude a complete factor I defect. Additional functional factor I analysis needs to be performed in the work-up of complement dysregulation.**

2. **IL-12Rβ1 deficient patients with disseminated *Mycobacterium avium* infections have the highest mortality compared to those with other infectious agents.**

3. **The first founder effect on the *IL12Rβ1* gene has been documented.**

predisposition to recurrent and/or severe infections caused by low virulent mycobacteria and salmonellae species. IL-12Rβ1 deficiency is the most common genetic aetiology for MSMD.

The genetic analysis of a young female with disseminated *Mycobacterium avium* revealed one known mutation in exon 14 (c.1623_1624delGCinsTT) and one novel mutation in exon 2 (c.65_68delCTGC) of the IL-*12β1Receptor-1 (IL12BR1) gene*, leading to a premature stop codon.[5] The nine-years old girl initially presented with persistent bilateral cervical adenopathies and two years later with chronic intestinal inflammation, multiple abdominal lymph nodes, and bone marrow involvement. She died due to cardiac insufficiency related to a Coxsackie B4 myocarditis.

The known c.1623_1624delGCinsTT mutation has been reported in seventeen patients from fifteen families from three different continents. We studied 34 polymorphic markers internal or proximal to the IL*12RB1* gene in five Argentinian and our Belgian patient with this mutation.[6] The detection of a common haplotype shared by all chromosomes carrying mutation c.1623_1624delGCinsTT confirms a mutational founder effect which occurred 475 years ago (95% CI: 175–1275), when Spaniards initiated the colonisation of the Americas. This mutation is the first founder effect described on the *IL-12RB1* gene. The clinical features of 141 IL-12Rβ1 deficient patients, including our patient, were described in a large survey.[7] Isolated environmental mycobacterium (EM) such as *M. avium* occurred in 6% of the IL-12Rβ1 deficient patients. The mortality rate was 32% (mean age at death was 7,5 years). Clinical outcome largely depends on the infectious agent, with the highest mortality in patients with EM (52%). This is concordant with the poor outcome in our studied patient. This survey reports a higher clinical penetrance, the rare recurrence of mycobacterial disease, the broader susceptibility to infections, and less favourable outcome than previously thought.

Conclusion

Through our research, we have gained more insight into the contribution of polymorphisms of *mannose binding lectin, ficolins* and *toll-like receptors* genes to lung disease diversity in cystic fibrosis patients.

In the second part of our research, we described a complete FI defect due to dysfunctional factor I. Functional analysis of FI should be systematically performed in the work-up of complement activation. Finally, we reported a novel mutation and a first founder effect mutation on the *IL12Rβ1* gene. In IL12Rβ1 patients, the clinical outcome largely depends on the infectious agent with the highest mortality in patients with environmental mycobacteria (*M. avium*).

References

1. Haerynck F, et al. Polymorphisms in the lectin pathway genes as a possible cause of early chronic Pseudomonas aeruginosa colonization in cystic fibrosis patients. Hum Immunol. 2012;73(11):1175-83.

2. Haerynck F, et al. Genetic variations in toll-like receptor pathway and lung function decline in Cystic fibrosis patients. Hum Immunol. 2013;74(12):1649-55.

3. Haerynck F, et al. Complete factor I deficiency due to dysfunctional factor I with recurrent aseptic meningoencephalitis. J Clin Immunol. 2013;33(8):1293-301.

4. Nilsson SC, et al. Complement factor I in health and disease. Mol Immunol. 2011;48(14):1611-20.

5. Haerynck F, et al. Disseminated Mycobacterium avium infection in a patient with a novel mutation in the interleukin-12 receptor-beta1 chain. J Pediatr. 2008;153(5):721-2.

6. Yancoski J, et al. A 475 years-old founder effectinvolving IL12RB1: a highly prevalent mutation conferring Mendelian Susceptibility to Mycobacterial Diseases in European descendants. Infect Genet Evol. 2009;9(4):574-80.

7. de Beaucoudrey L, et al. Revisiting human IL-12Rbeta1 deficiency: a survey of 141 patients from 30 countries. Medicine (Baltimore). 2010;89(6):381-402.