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The role of C1q in (auto) immunity

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Chapter 8

Summary and discussion

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In this thesis we report on the production of C1q by immune cells and non-immune cells. Deficiency of C1q as a consequence of a genetic mutation is strongly associated with the development of Systemic Lupus Erythematosus (SLE). In chapter 2 we describe a not previously reported genetic mutation in one of the C1q genes. The patient is currently a teenager and has suffered from infectious problems but did so far not develop SLE. When patients are diagnosed with C1q deficiency due to a genetic mutation the risk to develop SLE is high, but there is high degree of variation between the patients in clinical manifestations. In chapter 3, we used questionnaires to get an overview of C1q deficient patients worldwide, revealing that once the C1q deficient patients reach adulthood that then the chance of fatal infections is reduced. This overview highlighted the importance of a personal approach for therapy, especially in young children.

A mutation in of the genes of C1q can also lead to a non-functional structure of C1q, Low Molecular Weight-C1q (LMW-C1q). In chapter 4 we described a C1q deficient patient who has low levels of LMW-C1q. This patient demonstrates a severe form of SLE and neuropsychiatric SLE (NPSLE). Our data indicate that the classical pathway activity is not required for NP involvement in SLE, but the absence of C1q and the biological consequences may have a role in the pathogenesis of NPSLE.

When we investigated other NPSLE patients we see many different clinical manifestations. To investigate if complement activation and components play a role in NPSLE we performed data analyses and serum analyses. The NPSLE patients have a high degree of complement activation and the levels of anti-C1q and C1q circulating immune complexes are increased compared to healthy controls. The association NPSLE and the levels of anti-C1q, C3/AP50 and C4 are probably due to the disease activity and the presence of anti-phospholipid antibodies (discussed in chapter 5).

In the second part of this thesis we described the production of C1q by different cells. We demonstrate that mast cells, which are originating from the same myeloid progenitor cells as the already known C1q producing cells; macrophages and dendritic cells, are able to produce functional active C1q (chapter 6). We were able to detect C1q secreted from chondrocytes. This was surprising as these cells are originating from mesenchymal stem cells, which is different from haematopoietic stem cells. The main role of chondrocytes is to produce cartilage and maintain the homeostasis of the cartilage (chapter 7).

Overall, all these studies demonstrate the involvement of C1q in disease and the production of C1q by immune cells and non-immune cells.

The role of C1q in disease

In our institute we have identified a C1q deficient patient with a previously unknown mutation in the C1qB chain of C1q resulting in a complete C1q deficiency. Several studies have already shown the association between the absences of C1q in disease like SLE [1-3]. However, this patient did demonstrate several infections but no signs of autoimmunity. Currently, he is receiving prophylactic antibiotics to protect the patient from bacterial infections. If and when patients with a C1q deficiency will develop lupus is unpredictable and it is therefore important to keep him under close control to detect any signs of autoimmunity as early as possible.

Because C1q deficiency is often reported in literature as case reports describing only the initial presentation and no follow-up, we investigated the clinical manifestations of C1q deficient patients around the world. Our data shows that even during follow up the clinical presentation and severity of symptoms in persons that are deficient for C1q is very divers. Even though this case series comprised 45 individuals (comprising the majority of cases known to date) there is no clear algorithm to describe how to manage C1q deficiency clinically. Also differences in clinical presentation are seen within families with the same mutation in one of the C1q genes. Indicating, that other factors like environmental factors or epi-(genetic) changes can influence the different outcome of clinical presentations [4, 5]. From this study we can conclude that the manifestation of the disease in C1q deficient patients is unfortunately not predictable. Especially in young children, where the risk of developing a fatal infectious disease is high. In future studies C1q deficient patients should be monitored regularly from a young age if possible. Together with the familial history and clinical manifestations of the C1q deficient patient, the clinician can decide which treatment will be applied, like FFP or HSCT.

Patients with established SLE have a wide diversity in clinical presentations. Patients can e.g. demonstrate cutaneous lupus or glomerulonephritis, but can also demonstrate symptoms involving the nervous system resulting in neuropsychiatric SLE (NPSLE). In the literature neuropsychiatric involvement in patients with a deficiency in the early components of the classical pathway is only described for patients with a C1q deficiency. We have had the opportunity to investigate in detail a C1q deficient patient demonstrating NPSLE. With this study together with literature research we suggest that the classical pathway activity is not required for NP involvement in SLE but the absence of C1q and subsequently some of its biological functions may have a role in the pathogenesis of NP-SLE.

Typical phenomena in SLE are B-cell hyper activation, production of autoantibodies and formation of immune complexes [6, 7]. Since immune complexes activate complement a state of secondary complement deficiency can develop. The role

of C1q and other complement components in NPSLE is not exactly known. Due to the complexity of the disease no specific biomarkers are known for NPSLE. As anti-C1q antibodies are very common in SLE patients we measured the presence of anti-C1q antibodies and also the presence of C1q circulating immune complexes in serum of a large cohort of NPSLE patients. As previous studies have reported, the levels of anti-C1q in NPSLE patients is higher compared to healthy controls. These high levels of anti-C1q antibodies correlated with the SLE activity. Unfortunately no association of the presence anti-C1q antibodies and NSPLE patients compared with SLE patients is found, indicating that anti-C1q is not a useful biomarker that can be used to identify NPSLE patients. Furthermore, in NPSLE patients complement activation is taking place, which is seen as decreased levels of C1q and C3 but this is probably the result of the presence of autoantibodies that form immune complexes.

C1q production by immune and non-immune cells

The production and secretion of complement factors by immune cells is of importance in case of local infection or local processes such as clearance of immune complexes or apoptotic cells. In this thesis we demonstrate that mast cells are able to produce and secrete functionally active C1q. This could be of importance in maintaining the homeostasis of tissues were mast cells are abundant, like in the synovial tissue of joints affected by arthritis [8, 9]. In infections mast cells can be of importance due to degranulation and/or the release of IL-8, which is an important chemokine that attracts other immune cells. When mast cells are stimulated with LPS or via FcεRI triggering the mast cells release IL-8 and also C1q. The release of C1q can have a role by activating the complement system resulting in attracting more immune cells due to the cleavage products C3a and C5a [10, 11]. Another role that could be of importance in the release of C1q by mast cells is the clearance of apoptotic cells, macrophage polarization or stimulating cytokine production [12-14].

Previous studies have described a role for C1q in different inflammatory tissues [15, 16]. Like in RA patients the haemolytic activity is up-regulated in synovial fluid as a consequence of complement activation and due to genetic variations in and around the C1q genes, the levels of C1q are increased in RA patients [15, 17]. C1q is predominantly acting in the clearance of apoptotic cells, macrophage polarization or stimulating cytokine production [13, 14, 18]. Several studies demonstrated that C1q is able to bind to cartilage fragments of which could be of importance in the pathogenesis of joint disease. Interference of C1q with the cartilage oligomeric matrix protein (COMP), decorin and biglycan will result in an inhibition of complement activation [19, 20]. On the other site, aggrecan, fibromodulin and osteoadherin are

also able to bind to C1q and enhance the activation of the classical pathway [21-23]. C1q produced by mast cells can play a role in the complement activation in synovial tissue. When mast cells are activated and secrete C1q, the activation of complement can be either dampened or enhanced by binding of certain cartilage fragments. More interestingly is that not only immune cells in the joint are able to produce C1q [24]. In our study we demonstrate that chondrocytes have also the capability to produce C1q. This is quite remarkable, because chondrocytes are originating from mesenchymal stem cells compared to dendritic cells, macrophages and mast cells, which are from haematopoietic origin. The production of C1q by chondrocytes was already described in 1996 [25], but the exact role and the regulation of secretion was not described. We show in our study that non-stimulated chondrocytes are able to produce C1q and that the production is increased after stimulation of pro-inflammatory cytokines including IL-1ß. Also the proteases C1r and C1s had the same mRNA expression pattern as the C1q genes. Compared to the expression of collagen the C1q genes and C1r and C1s expression pattern were opposed. Why chondrocytes produce C1q should be further investigated, due to the high molecular weight of C1q (460 kD) it is unable to diffuse through the cartilage of which the maximum 65 kD is [25].

Perspectives

The involvement of the complement system in inflammation has been studied for many years. Especially the role of complement on cell development, attraction and differentiation is well studied by different research groups [26-28]. Remarkably, the clinical relevance and physiological importance is not yet well understood. Ricklin et al reviewed in 2010 the importance of complement in the maintenance of the homeostasis [29]. For example in normal pregnancy, C1q plays a key role in trophoblast invasion, spiral artery remodelling and the normal placentation [30]. The sources of C1q in the maternal tract are trophoblasts and decidual endothelial cells (DECs) [24]. In mouse studies it is shown that C1q -/- mice were unable to clear apoptotic trophoblasts resulting in an accumulation of apoptotic trophoblasts and an abnormal placentation. These mice showed features of preeclampsia like hypertension, albuminuria, endotheliosis, less placenta vascular endothelial growth (VEGF), an increase of soluble VEGF receptor 1 (sFlt-1) and oxidative stress. Furthermore, decreased blood flow, increased fetal death, diminished litter size, abnormal invasion of trophoblasts and increased levels of STAT-8 (inhibitor trophoblast migration) [31]. Unfortunately, this is all based on mouse studies and

no literature is known about pregnancy in women with C1q deficiency. In our questionnaires no problems with pregnancy were described. However, since half the cohort was male and different age groups were present, such an effect could easily have been missed. Apparently C1q is of major importance in pregnancy and therefor it would be of importance and interesting to know if there are problems with pregnancy in C1q deficient women.

Another important role for C1q is the role in synaptic pruning [32]. During developmental stage the neural network is growing continuously leading to excessive synaptic formation, which needs to be under control to maintain a proper functioning of the central nervous system (CNS). The role of C1q in this stage is to contribute to the elimination of synapses during developmental stages of the CNS. This process is also well studied in mice. C1q -/- mice have aberrant synaptic connectivity and show forms of epilepsy, which is probably a result of excessive excitatory synapses [33, 34]. Also this phenomenon is not observed in our questionnaires. The clinicians treating C1q deficient patients describe no epilepsy or other CNS abnormalities. However, in the literature NPSLE symptoms are described in patients with a C1q deficiency in previous studies[3, 35-38]. For future research it would be interesting to find out if there is an association with C1q deficiency and the development of NPSLE. Due to absence of C1q in the brain during the developmental stage, the synaptic connectivity could also be aberrant as in C1q -/- mice leading to NPSLE events during aging.

A standard treatment for C1q deficiency is not available. Most of the patients receive immunosuppressive drugs or are receiving fresh frozen plasma (FFP) on a regularly basis [39, 40]. Another therapy, which is more radical, is hematopoietic stem cell transplantation (HSCT). Already 2 patients are treated successfully after HSCT. Following HSCT, the levels of C1q in serum are in the normal range and they are cured from SLE [41, 42]. Because there are people with C1q deficiency that live a normal life without any infections or symptoms of autoimmunity the question is raised if those people should also undergo a HSCT, because the chance to develop SLE is almost 100%. If the patient is feeling healthy and does not have signs of autoimmunity it may not be ethical to perform HSCT, because it will be 'cutting in a healthy body'. Also one patient, who underwent HSCT to normalize the C1q levels in the serum, died of multiorgan failure and an intracerebral hemorrage. For the clinicians treating C1q deficiency patients it should be important to check the patient on a regular basis and to determine if the patient is vulnerable to infections in his surroundings. An infection will trigger the immune system, which can also be a trigger the development of SLE. An extra consideration the clinicians should make is to vaccinate the patient with additional vaccinations like, Pneumococcus,

Meningococcus, Hepatitis B and the seasonal influenza vaccinations next to the regular childhood vaccines such as measles, diphtheria, tetanus and poliomyelitis. As described before the role of complement in the human body is diverse. The complement system and especially C1q can be involved in many processes. C1q can be involved in the induction of angiogenesis and tissue repair. In mouse studies C1q is believed to be a unique player in the angiogenesis and thereby be a potential therapeutic target in wound healing of the skin [43]. In this study they demonstrate that C1q will bind to the endothelial cells by their globular head and without activating the complement system will induce an angiogenic phenotype in endothelial cells. The suggested source of C1q is probably plasma, but by analysing our data we can consider that the mast cells in the skin are a source of C1q production as the mast cells in the skin are the most abundant C1q positive cells. Vascular endothelial growth factor (VEGF) plays a major role angiogenesis and mast cells are an important source of VEGF. This together will suggest that C1q producing mast cells have a potential role in inducing an angiogenic phenotype in endothelial cells and thereby promote vascularization.

Angiogenesis is a phenomenon that also takes plays in the bone repair [44-46]. For example, chondrocytes are able to produce VEGF and mice lacking the VEGF gene in chondrocytes have an impaired embryonic bone development, reducing angiogenesis and reduced removal of terminally differentiated hypertrophic chondrocytes [47-49]. In the mouse studies they suggested gC1qR on the endothelial cells as potential receptor of C1q inducing the angiogenic phenotype of the endothelial cells. In a study in 2012 it is also demonstrated that chondrocytes are positive for gC1qR [50]. This could indicate that C1q produced by chondrocytes can regulate a feedback loop to chondrocytes by binding to gC1qR and subsequently induce VEGF resulting in the survival of the chondrocytes. When C1q is produced by chondrocytes C1r and C1s are also produced. These proteases are able to degrade collagen, which is an important matrix molecule [51, 52]. This is also an indication that C1q and other complement components could be of importance in the survival of chondrocytes. C1q, C1r and C1s can bind matrix molecules surrounding the chondrocytes and subsequently degrade the collagen to maintain the lacunae.

In diseased joints as in rheumatoid arthritis (RA) and in osteoarthritis (OA) higher levels of complement components are detected in the synovial fluid. As described by Wang et al more deposition of MAC is found on the cartilage of osteoarthritic joints [16]. Together with our findings and with the findings of Bradley et al, who described that chondrocytes are positive for C1q [25], we can suggest that C1q produced by chondrocytes can play a role in the pathogenesis of OA and maybe

also in RA. However, the major source of C1q is possibly C1q from the circulation that deposits on the cartilage due to damage by pro-inflammatory cytokines. So the additional value of C1q producing chondrocytes should be investigated.

Conclusion

In this thesis the combination of epidemiological research of C1q deficient patients and NPSLE patients in combination with cell biology of C1q producing cells is described. This combination provides more insight into how C1q producing cells can play a role in autoimmune diseases like SLE, NPSLE, RA and OA.

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