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Mannose-binding lectin: The Dr. Jekyll and Mr. Hyde of the innate immune system.

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CHAPTER 6

Summary and general discussion

Submitted in part



SUMMARY AND GENERAL DISCUSSION

The aim of the thesis was to obtain insight in the immunological aspects of transplantation and diabetes, with the emphasis on mannose binding lectin (MBL) as a crucial part of the innate immune system. The results of this thesis underscore the Jekyll-and-Hyde character of MBL as it is shown that MBL deficiency is detrimental in liver transplantation patients whereas it could be beneficial in type 1 diabetes. In order to fully appreciate the genetic, functional and serological impact of MBL under discrete pathological conditions, novel assays were required and designed. As opposed to the vast majority of groups studying MBL in relation to disease, presently it was opted to expand the focus of the current research by tallying not only MBL genetics and MBL serum levels but also MBL function. By doing so, a discrepancy was identified between MBL serum levels and functional MBL activity under specific circumstances, underlining the necessity to study all dimensions of MBL when correlating the lectin pathway (LP) to disease. Furthermore, being capable to study MBL in transplantation and diabetes resulted in ability to link specific parts of innate and adaptive immunity.

Mannose binding lectin, as a key player of natural immunity, is capable of binding common carbohydrate structures of a diversity of microorganisms (including bacteria, viruses and fungi) and facilitates elimination of these pathogens (1). In the general population there is a wide variety in MBL serum concentrations as well as functional MBL activity (2). This great diversity in concentration and function has been shown to be determined by single nucleotide polymorphisms in exon 1 of the *mb12* gene and its promoter(3; 4). Currently, it has well been acknowledged that an impaired MBL function significantly increases the risk for infections in individuals who largely depend on their innate immune system for anti-microbial defense, like immune-compromised patients and young children (5-9). It is therefore intriguing that the frequency of variant alleles, resulting in low MBL production, is above 40% depending on ethnicity. This percentage is even higher when promoter SNPs are taken into account (3). The high frequency of variant genotypes resulting in low levels of high molecular MBL weight and decreased MBL function indicate that in particular cases impaired MBL function and low serum concentrations may be beneficial. This has been suggested to be the case for mycobacterial infections, inflammatory bowel disease like ulcerative colitis, and transplantation (1; 10; 11).

Molecular and immunological characteristics of MBL

Chapter 2 is build up of three parts and shows the close interaction between the different complement pathways. The first part of this chapter discusses the functional characterization of the lectin pathway in human serum. Here, a novel functional as-

say is described that enables quantification of autologous complement activation via the LP in full human serum up to the formation of the membrane attack complex. This assay offers novel possibilities for both patient diagnostics and research.

The second part of this chapter is continuation of the observation shown in the previous section, that normal human serum contains IgG, IgA and IgM antibodies against mannan. The high frequency of MBL deficiency suggests that MBL-mediated innate immunity can be compensated by alternative defense strategies. To examine this hypothesis, complement activation by MBL-binding ligands was studied. The results show that the prototypic MBL ligand mannan can induce complement activation via both the LP and the classical pathway (CP). Furthermore, antibody binding to mannan restored complement activation in MBL-deficient serum in a C1q-dependent manner. Cooperation between the CP and the LP was also observed for complement activation by the protein 60 from *Listeria monocytogenes*. It is concluded that antibody-mediated CP activation can compensate for impaired target opsonisation via the LP in MBL-deficient individuals.

The last part of this chapter describes binding of MBL to polymeric serum IgA as a novel mechanism for activation of the lectin pathway. Furthermore, it is shown that MBL binding to IgA results in complement activation and propose that this leads to a synergistic action of MBL and IgA in antimicrobial defense, especially in the mucosal immune system.

It is concluded that the LP of the complement system as a part of the innate immunity is interwoven with the adaptive immune system, a characteristic that previously was only allocated to the CP within the complement system.

MBL and type 1 diabetes

Type 1 (insulin-dependent) diabetes mellitus (T1D) is an autoimmune disease characterized by the specific destruction of beta cells in the pancreas. The role of the adaptive immune system in the autoimmune process leading to type 1 diabetes is well established (12). Presently the interest for the innate immune system in the immunopathogenesis of T1D is mounting (13-15). Having previously shown that MBL is able to meticulously link the adaptive and innate immune system, it is speculated that MBL could be involved in the pathogenesis of type 1 diabetes. It could be hypothesized that low MBL serum levels could result in an impaired clearance and inactivation of pathogens responsible for beta cell destruction. Contrarily, insulinitis resulting in tissue damage could activate the lectin pathway via MBL, facilitating additional beta cell damage and a more fulminant insulinitis. **Chapter 3** describes MBL in relationship to type 1 diabetes at clinical presentation. In this study new-onset juvenile type 1 diabetic patients are investigated in comparison with their non-diabetic siblings and healthy unrelated control subjects. The polymorphisms of MBL exon

1 and promoter were determined and serum MBL concentration and MBL-complex activity were measured. Initially, the genetic MBL constitution did not account for disease predisposition, since the genetic epidemiology did not show pronounced differences between patient and unrelated control subjects. However, genetic stratification based upon MBL polymorphisms in both the coding exon 1 and promoter region, proved to be essential to reveal T1D associated differential MBL functionality. MBL serum concentration as well as MBL complex activity was significantly higher in new onset diabetic patients compared to their siblings matched for high producing MBL genotypes. The increase in MBL complex activity in high MBL producing patients could only partially be explained by high MBL production as demonstrated by an increased MBL complex activity/MBL concentration ratio. It is concluded that MBL serum concentration and complex activity are increased in early onset diabetic patients upon manifestation independently of genetic predisposition to high MBL production, indicating a possible role in the immunopathogenesis of type 1 diabetes. The study described in this chapter is the first to show an association between MBL and the onset of T1D. Although MBL is a major recognition molecule of the lectin pathway, it should be appreciated that various other molecules are involved, including MBL-associated serine protease and fluid phase complement inhibitors like C1 esterase inhibitor. In order to be able to fully comprehend the involvement of the lectin pathway in the onset of T1D, further extension of research is required and should include all relevant players. However, with our present knowledge it can be hypothesized that high MBL levels and MBL activity could facilitate an immune response by inflicting or maintaining damage during the insulinitis phase of T1D. In accordance with the danger model of the immune system (16), the presence of damage is essential for an (auto)immune response

MBL and liver transplantation

Infection is the primary cause of death after liver transplantation. As transplant patients require immune suppressive drugs in order to insure graft survival, they rely to a great extent on their innate immunity to counteract infections. Studying MBL as a major component of the innate immune system, the effect of MBL gene polymorphisms on the susceptibility to infection after liver transplantation was evaluated in **chapter 4**. Furthermore, although it is generally assumed that MBL is hepatically produced, it was sought to establish the role of the liver in production of serum MBL. Patients undergoing orthotopic liver transplantation were investigated. MBL promoter and exon 1 polymorphisms were determined in both patients and in liver donors. MBL serum concentration was monitored shortly before and during one year after transplantation. MBL deficiency was evaluated in association to the occurrence of clinically significant infections during this period. This study shows that

transplantation of MBL-wildtype recipients with donor livers carrying MBL-variant alleles resulted in a rapid and pronounced decrease of serum MBL levels. This serum conversion was associated with the disappearance of high molecular weight MBL. No indication for extrahepatic production of serum MBL could be obtained. The presence of MBL variant alleles in the MBL gene of the donor liver, but not in the recipient, was associated with a strongly increased incidence of clinically significant infections following transplantation in a gene-dose-dependent way. It was concluded that serum MBL is produced by the liver under strong genetic control, and established that the liver is the primary producer of serum MBL. Following liver transplantation, the MBL genotype of the donor liver is a major risk determinant for life-threatening infections.

The ability to unambiguously identify a group of patients severely prone to infection post transplantation is of significant clinical value. In an era of donor shortage, donor selection based upon MBL genotype is inconceivable. However, this study suggests that patients receiving an 'MBL-variant' liver could benefit from MBL replacement therapy similar to that presently being studied in phase I/II and III studies (17; 18). Furthermore, prophylactic approaches including intensified clinical follow-up, preemptive antimicrobial therapy, and prolonged selective digestive decontamination could be considered dependent on the MBL genotype of the liver donor. Moreover, administering intravenous immunoglobulins (IVIg) should be considered. By administering a diverse repertoire of immunoglobulins that possess the same wide spectrum of antibacterial, antiviral and antifungal specificities as MBL, IVIg could compensate MBL deficiency via the CP. As clearly shown in chapter 2, complement activation by prototypic MBL ligands can be achieved in MBL-deficient serum via the antibody-mediated activation of the classical pathway, thus compensating MBL deficiency.

Having shown the direct implications of MBL deficiency in liver transplantation, randomized clinical intervention studies should be set up in transplant patients receiving an MBL variant liver in order to diminish the increased susceptibility to life-threatening infections.

Adaptive immunity in pancreatic islet transplantation

As shown and discussed in chapter 3, type 1 diabetes is an autoimmune disease in which both adaptive and innate immune processes are involved, ultimately leading to beta cell destruction. An attractive novel therapy for type 1 diabetes is pancreatic islet transplantation. In order to successfully apply islet transplantation as a treatment for diabetes, two distinct tribulations have to be resolved. Primary, the recurrence of islet autoimmunity, a process theoretically equivalent to the pathogenesis of T1D (chapter 3), should be prevented. It is conceivable that MBL is also involved

in this process as implied in chapter 3, however this topic remains subject to future investigation. Secondly, to assure pancreatic islet graft survival, it is fundamental to prevent alloimmunity resulting in allograft rejection. In order to optimise the current islet transplantation, it is essential to study the reaction of T-cells to islets. **Chapter 5** describes the effect of HLA incompatibility and immunogenicity of human pancreatic islet preparations and peripheral blood mononuclear cells (PBMC) of healthy blood donors. The aim of this study was to examine whether the degree of MHC incompatibility between PBMC and donor islet cells is related to the degree of proliferative T-cell responses. The experiments included co-culturing of PBMC with human islet-cell preparations with a composition similar to that of islet grafts used in clinical transplantation trials [i.e. mixed islet lymphocyte reaction (MILR)]. PBMCs isolated from immunologically uncompromised healthy blood donors were used to exclusively study alloreactivity independently of recurrence of autoreactivity. Prominent T-cell responses were observed in the vast majority of cases of complete HLA class II-mismatches. Intermediate T-cell responsiveness was observed in single HLA class II mismatches, whereas HLA-matches islet preparations did not induce a T-cell response.

These results identify the potential immunogenicity of islet preparations transplanted between HLA-DR incompatible subjects regardless of an autoimmune background of the recipient. These findings underscore the difficulty of islet allotransplantation, but provide leads to reduce the extent of alloreactivity in clinical islet transplantation.

CONCLUDING REMARKS

The interplay between innate and adaptive immunity is complex and multifaceted as chapters 2 and 3 of this thesis show. The beneficial effect of high MBL serum levels in liver transplant patients stand in vast contrast to the potential harmful effect of elevated serum MBL levels in diabetic patients emphasizing the pluripotency of mannose binding lectin. This paradox is reflected in chapters 3 and 4. Since the first report of the clinical implication of MBL deficiency somewhat four decades ago (19), our knowledge of the lectin pathway has expanded tremendously. Strikingly however, clinical implementation of our current knowledge occurs tediously lethargic. Presently, MBL replacement therapy is being studied in phase I/II and III studies (17; 18). However several other therapeutic interventions can be put forward to compensate for MBL deficiency in immunocompromised patients, including intensified clinical follow-up and preemptive antimicrobial therapy. Intravenous and subcutaneous

immunoglobulin administration might also be an alternative therapy to counterweigh a malfunctioning lectin pathway (20; 21).

Finally, pancreatic islet transplantation as a potential therapy for diabetes currently remains an immunological pitfall. Major hurdles include the occurrence of alloimmunity and the recurrence of autoimmunity. Further complicating immunological disentanglement is the fact that both innate and adaptive immunity appear to be involved. Presently islet graft rejection can only be counteracted by pharmacological immune suppression. The substitution of insulin replacement therapy by immune suppressive drugs is a debatable issue, which should be evaluated conscientiously by physicians and patients, taking both short and long term perils into account. Currently, diabetic patients appear to be stuck between a rock and a hard place. Therefore studies aimed to identify immunological aspects of islet transplantation are of eminent importance in order to optimize immune suppressive therapy and to reduce morbidity and mortality.

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