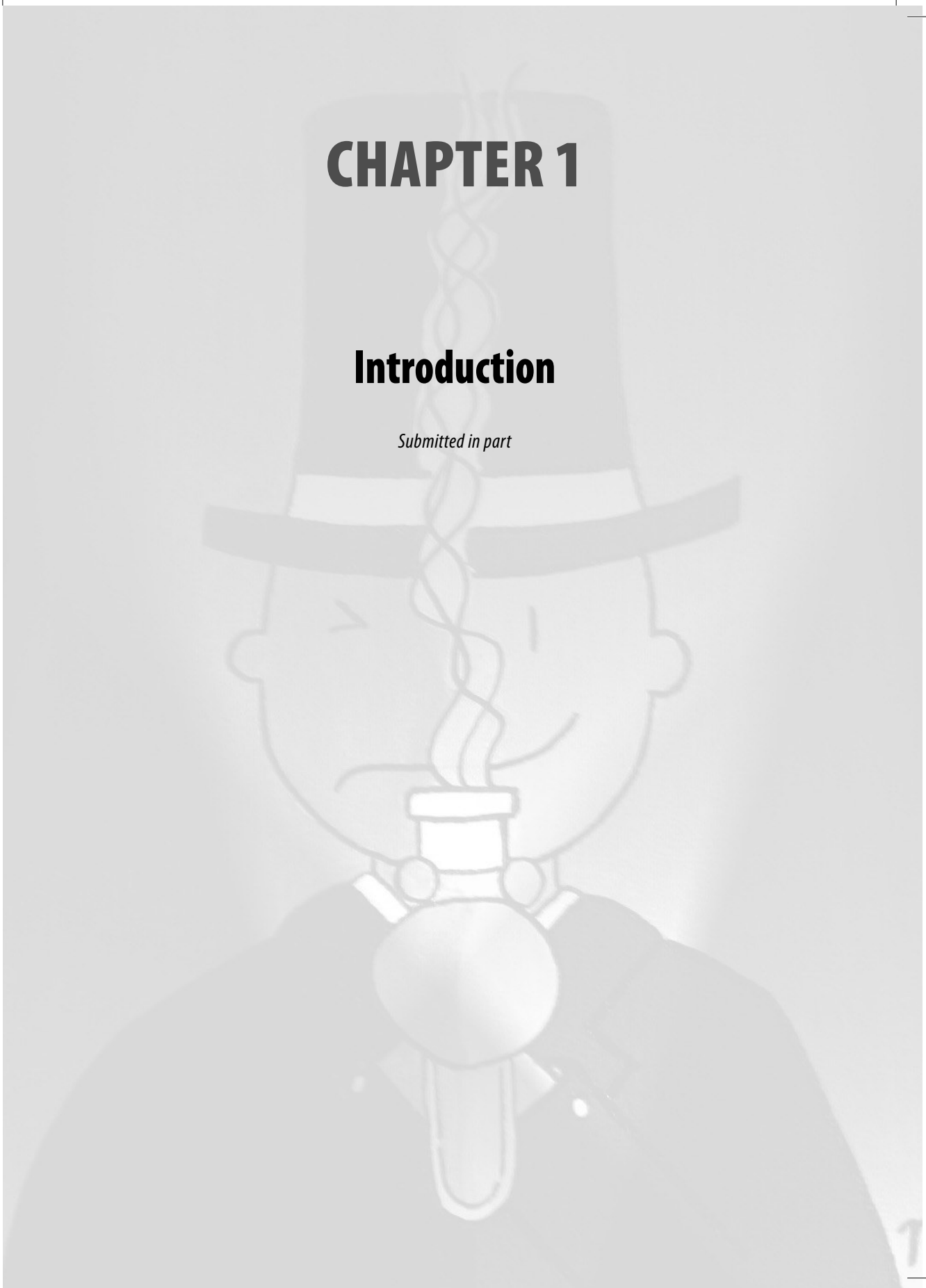
Mannose-binding lectin: The Dr. Jekyll and Mr. Hyde of the innate immune system.

Bouwman, L.H.

CHAPTER 1

Introduction

Submitted in part



1. INTRODUCTION

The term immunity is derived from the Latin word *immunitas*, which referred to the exemption from various civic duties and legal prosecution offered to Roman senators during their tenures in office. Since Roman times immunity had been taken to mean freedom from infections, which connoted the ability to resist infection. The immune system is a complex assemblage of cells and molecules, which allow the body to function in a habitat crowded with pathogens.

The immune system can be divided in natural or innate immunity and acquired, adaptive or specific immunity. This general and crude division is based on the ability of the adaptive immune system to increase defense mechanisms in magnitude and specificity following exposure to infectious agents, enabling a more rigorous immune response after a second infection. The innate immunity differs from adaptive immunity as it recognizes a restricted array of structures on a broad range of microorganisms, the so-called pathogen-associated molecular patterns. Repeated contact with pathogens always results in a constant innate immune reaction, which is not amplified.

The ability to vastly counteract a great variety of pathogenic microorganisms is of eminent importance for homeostasis. However, profligate functioning of the immune system is disadvantageous under specific circumstances. Two situations in which excessive immunological response is unwanted are autoimmunity and transplantation. The inability of the immune system to distinguish self-tissue and self-proteins from non-self pathogens (i.e. to maintain of self-tolerance) can result in autoimmune disease causing tissue and organ damage. Traditionally, it is assumed that the recognition of self-determinants in autoimmune diseases is confined to the adaptive immune system, ignoring the role of the innate immune system in auto-immunity. More recently it has been argued that, in order to initiate an (auto)immune response, additional signals generated by the innate immune system (i.e. danger signals) are required (1). Evidence is growing that (auto) antigen recognition by the innate immune system, in a certain context, could lead to autoimmunity, either by priming or promoting aggressive immune responses. In transplantation, disproportionate immune responses can cause both acute and chronic graft rejection as the adaptive immune system may consider the graft hazardous.

The present thesis underscores the current concept of collaboration between the innate and adaptive immune system by showing close interactions between both immune systems. This thesis shows that several constituents of the complement system, a core component of innate immunity, are highly allied with the adaptive immune system. Furthermore the involvement of innate immunity in type 1 diabetes, generally accepted as an adaptive immune system-mediated autoimmune disease is addressed.

In the following paragraphs, a general overview will be given of a specific component of the innate immune system, the complement system. The most recently discovered pathway of the complement system, the lectin pathway and particularly its activating molecule mannose binding lectin (MBL) will be discussed in greater detail. After having outlined the current view on association between MBL and various diseases and disease complications, the role of MBL in transplantation will be addressed. Subsequently, the role of the adaptive immune system will be discussed in pancreatic islet transplantation. Finally, the aim and content of the studies in this thesis will be presented.

2. THE COMPLEMENT SYSTEM

The complement system consists of a variety of functionally linked proteins, including complement factors classified as C1 to C9, which act in conjunction and result in many effects in humoral immunity and inflammation. The five principal biological functions of the complement system are: 1) complement-mediated cytolysis; 2) opsonisation of foreign organisms; 3) activation of inflammation; 4) clearance of self and non-self debris; and 5) amplification of adaptive immunity.

The complement system consists of three pathways, the classical, the alternative and the lectin pathway. All pathways are activated differently, however they converge in a shared terminal pathway and result in the same basic effects. The classical pathway is activated when the first classical pathway component C1, binds to the fragment crystalline (Fc) portion of an immune complex. Activation of the alternative pathway occurs when complement factor C3b binds to various activating surfaces, like microbial cell walls. Finally the lectin pathway, a recently discovered complement activation pathway, is triggered when MBL binds to common carbohydrate structures of a variety of microorganisms (including bacteria, viruses and fungi) (2-6).

Central in the complement system is the complement factor C3. All activated pathways result in generation of a C3 convertase, facilitating the proteolytic cleavage of complement factor C3 into C3a and C3b. Subsequent binding of C3b to C3 convertase enzymes allow the formation of C5 convertase, enabling proteolytic cleavage of the complement factor C5. Upon C5 cleavage, all pathways converge and enter the terminal pathway of complement activation, eventually resulting in the formation of a so-called membrane attack complex (MAC), a lipid-soluble pore structure which causes osmotic lysis of cells (figure 1). Cleavage products C3a and C5a are potent chemotactic products amplifying inflammation. C3b facilitates both opsonisation of foreign organisms and clearance of immune complexes.

As the MBL forms a major topic of the present thesis, the lectin pathway will be discussed in greater detail in the next section.

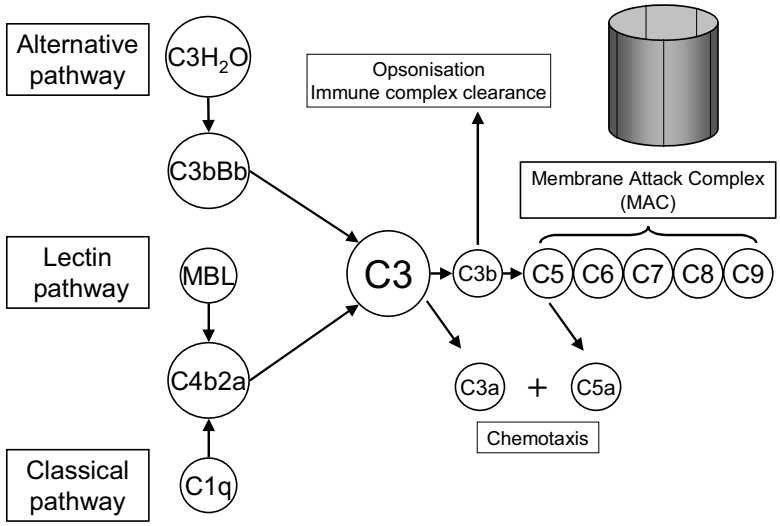


Figure 1: A bird's eye view of the complement system.

3. MANNOSE BINDING LECTIN

THE PLURIPOTENT MOLECULE OF THE INNATE IMMUNE SYSTEM

Mannose binding lectin, also referred to as mannan binding lectin or mannan binding protein, is believed to be a central player in the innate immune response. The first case of an association of MBL deficiency and infectious disease dates back to 1968. A small girl, suffering from severe dermatitis, consisting diarrhea and recurrent bacterial infections indifferent to antibiotic and steroid therapy, was reported. Hematological examination revealed a defect in the phagocytosis of yeast particles from *Saccharomyces cerevisiae*, rice starch and *Staphylococcus aureus* in polymorphonuclear leukocytes. This defect was serum-dependent. Infusion of fresh plasma corrected the phagocytic deficiency. As the same phagocytic defect was observed in several direct relatives of the patient, it was concluded that this condition had a genetic origin (7).

In order to fully appreciate the implication of MBL in clinical settings, biological characteristics of MBL will be discussed prior to focusing on the association of MBL with various diseases.

3a. MBL Characteristics

Mannose binding lectin is a C-type serum lectin and is presumed to be produced by the liver. MBL is build up out of 96 kDa structural units, which in turn are composed of three identical 32 kDa primary subunits. The subunits consist of an N-terminal cross-linking region, a collagen-like domain and a C-terminal carbohydrate-recognition (CRD) domain (8). Circulating MBL is comprised of higher-order oligomeric structures, which include dimers, trimers, tetramers, pentamers and hexamers of the structural homotrimeric unit. The oligomeric configuration of the structural units allows the MBL molecule to have multiple CRDs facilitating multivalent ligand binding (figure 2). Each CRD of MBL is structurally identical and is able to bind a wide range of oligosaccharides including N-acetylglucosamine, mannose, N-acetylmannosamine and fucose (5). Although the various sugars are bound with different affinities, the cluster-like array of multiple binding sites allows activation of the complement activation to be most effective. MBL is considered to play its major role in innate defense against pathogens, involving recognition of arrays of MBL-binding carbohydrates on microbial surfaces. However, more recent studies have shown that MBL is also involved in the recognition of self-targets, such as apoptotic and necrotic cells.

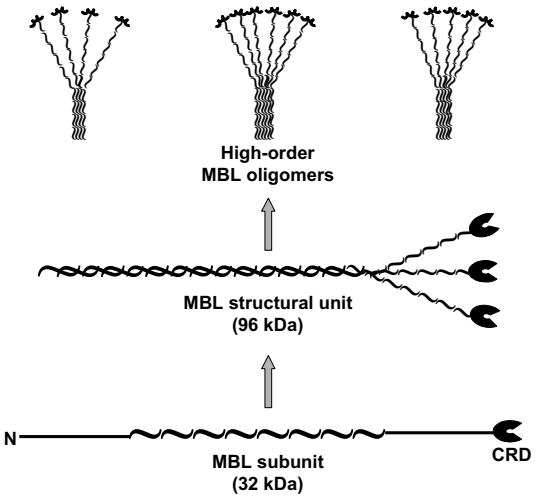


Figure 2: Mannose binding lectin (MBL) structural build-up.
MBL is composed of 32 kDa subunits. The primary subunits combine to form a trimeric MBL structural unit, which in term forms high-order oligomers. (figure modified from D.P. Eisen and R.M. Minchinton (22)).

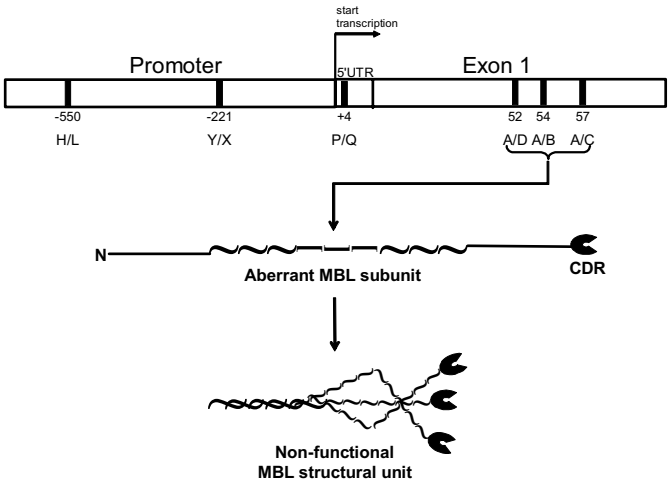


Figure 3: Location of single nucleotide polymorphisms located in the promoter and exon 1 of the *mbl-2* gene.
Promoter SNPs are located at positions -550 (H/L variant), and -221 (X/Y variant), both G to C nucleotide substitutions. Another SNP is located in the 5'-untranslated portion of the MBL gene, at position +4 (P/Q variant). Exon 1 SNPs are located at codon 52 (Arg→Cys; allele 'D'), codon 54 (Gly→Asp, allele 'B') and codon 57 (Gly→Glu, allele 'C').
The SNPs of exon 1 result in aberrant formation of MBL subunits, which are unable to form functional MBL structural units and high-order oligomers.

In plasma, MBL is associated with MBL-associated serine proteases (MASP) (9). Currently, three MASPs have been identified, MASP-1, MASP-2 and MASP-3 (10-12). Although the function of MASP-1 and MASP-3 remains subject to debate, there is a general consensus of the role of MASP-2, which is responsible for cleavage of C4 and C2, generation of the C3 convertase C4b2a and subsequent complement activation.

Exon 1 of the *mbl-2* gene, which is located at chromosome 10, contains three known single nucleotide polymorphisms (SNPs) at codons 52 (CGT to TGT; Arg → Cys), referred to as allele 'D', codon 54 (GGC to GAC; Gly → Asp, allele 'B') and codon 57 (GGA to GAA; Gly → Glu, allele 'C') (8). All SNPs of exon 1 result in altered collagenous regions and as a consequence, interfere with the formation of high-order oligomers. This impairment of polymerization causes low serum levels of high molecular weight MBL and impaired MBL function. Dependent on ethnicity, the allele frequency of variant alleles B, C and D, commonly referred to as O-alleles, may be above 40% (wildtype = A/A) (13). In addition to the three SNPs in exon 1, there are several other polymorphic sites located in the MBL promoter region, including SNPs located at positions -550 (H/L variant), and -221 (X/Y variant), both G to C nucleotide substitutions. Furthermore a polymorphic site is located at position +4 of the 5'-untranslated portion of the *mbl-2* gene (P/Q variant, C→T) (14-16) (figure 3). The common allele A of exon 1 is associated with the following haplotypes: HYPA,

LYPA, LYQA and LXPA with high, high-intermediate, intermediate and low promoter activity (17). Although there is great variety of MBL levels between the different haplotypes, it has been advocated in order to ease interpretation, to only show the most significant promoter allele in position –221 (X/Y), which is only found in normal A haplotype background (YA or XA) exhibiting high and low promoter activity and serum MBL levels (14). The structural alleles carry the following haplotypes: LYPB, LYQC and HYPD.

3b. MBL and associated diseases

MBL has been studied in a great diversity of diseases. Both decreased and elevated serum levels of MBL and different SNPs of the *mbl2* gene and its promoter have been associated with a variety of diseases, indicating the Jekyll-and-Hyde character of MBL. In order to structure the discussion of this double-edged sword phenomenon, involvement of MBL in different diseases will be discussed according to the aetiology.

MBL and infectious diseases

When the adaptive immune response is either immature or compromised, the innate immune system constitutes the principle defense against infection. A logical consequence of impaired MBL function would be an enlarged susceptibility to infectious disease. The phenomenon of an increased incidence of infectious disease in MBL-deficient patients has been shown in pediatric patients and in immune compromised patients. However it also has been shown that adult patients with recurrent infectious disease are more likely to have insufficient serum MBL levels.

MBL and bacterial infections

Pediatric patients are still in the developmental stage of the adaptive immune system and rely to a great extent on their innate immune system to counteract infectious pathogens. In support of the theory that MBL has an important protective role in early childhood is a British study amongst 266 pediatric patients (mean age 3.5 years) suffering from meningococcal disease (18). Showing a clinical association between MBL variant alleles and meningococcal disease, the authors of this study suggested that genetic variants of MBL gene might account for a third of all meningococcal disease cases.

Patients undergoing myeloablative bone marrow transplantation or cytotoxic chemotherapy are severely immune-compromised. MBL deficiency has been shown to be associated with severe bacterial infections after chemotherapy and major infections following allogeneic hemopoietic stem cell transplantation (19-21).

In immune-competent Caucasians, it has been suggested that homozygotes for MBL exon 1 codon variants could have an increased risk of invasive pneumococcal

disease (22; 23). Considering post-operative infections a surgical complication, low MBL levels are associated with significantly increased infection rates (24).

In vast contrast to the protective properties of MBL against extracellular bacterial infections is the observation that mycobacterial infections (*Mycobacterium tuberculosis and M. leprae*) occur more frequently in patients with increased serum MBL levels. Complement-mediated enhanced phagocytosis as a result of opsonization has been suggested to facilitate these intracellular infections (25).

MBL and virus infections

MBL has been studied in relation to various viruses. Persistent hepatitis B virus infection has been reported to be associated with the variant alleles located at codons 52 and 54 of the MBL gene, responsible for low MBL serum levels (26; 27). Furthermore it has been suggested that high MBL serum levels are associated with increased survival rates among Japanese patients with hepatitis B (28). MBL in hepatitis C has been studied to lesser extent and appears to be somewhat contradictive (22; 29-31) .

The role of MBL in HIV infection and progression has been a conflict of debate over the last years. The envelope protein gp120 of the HIV-1 virus is highly glycosylated with N-linked carbohydrates, enabling MBL to bind (32; 33). Although it is conceivable that MBL-mediated complement activation could facilitate the immune response directed against HIV infection, reports on the effects of MBL on HIV infection and progression are contradictory.

Although common pediatric virus infection, including RSV and EBV, lack association with MBL (34-36), it has been shown that MBL is able to neutralize the influenza A virus and inhibit the spread of this virus (37; 38).

MBL and autoimmunity

It is generally assumed that the recognition of self-determinants is confined to the adaptive immune system, neglecting the role of the innate immune system in autoimmunity. However, evidence is growing that the innate immune system could lead to autoimmunity, either by priming or promoting aggressive immune responses (39; 40). Low MBL serum levels and genetic polymorphisms associated with impaired MBL function have been shown to be associated with different autoimmune diseases including celiac disease and systemic lupus erythematosis (41; 42). A major current pathophysiological concept of autoimmunity is impaired apoptotic cell clearance. MBL has been shown to facilitate the clearance of apoptotic cells (43; 44). A result of cells going into apoptosis is alteration of membrane carbohydrates leading to increased expression of fucose and *N*-acetyl-glucosamine (45; 46). Redistribution or clustering of glycoproteins has been suggested to enable MBL to bind to these carbohydrates expressed on apoptotic cells, thereby facilitating clearance (47; 48).

When studying the association between MBL and Rheumatoid arthritis, it has been shown that MBL is able to bind to rheumatoid factor (RF) complexes and as a consequence could assist RF clearance by the reticuloendothelial system (49; 50). Although several other studies have not yet been able to reproduce these findings (51), the observations that MBL insufficiency is associated with both elevated IgM RF and increased joint erosions, inflammation and early disease onset are in support of the MBL RF clearance theory (52-56).

MBL and transplantation

Tissue damage and impaired organ function as a result of ischemia/reperfusion (I/R) injury still remain enormous predicaments in solid organ transplantation. The hypoxic state to which an organ is subjected during organ harvesting, transport and implantation, result in activation of various immunological events (57-60). The complement system plays an important role in mediating tissue injury after oxidative stress. Activation and deposition of complement on the vascular endothelium following oxidative stress has been shown (61-63) and more interestingly, tissue injury after I/R is significantly reduced by complement inhibition (64; 65). Complement activation via the lectin pathway has been shown following oxidative stress, indicating that inhibition of MBL could be a novel approach in reducing ischemia/reperfusion damage (66;

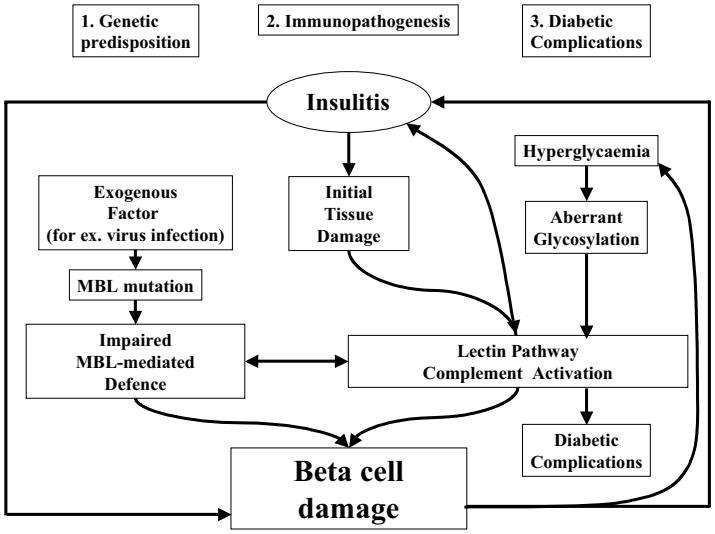


Figure 4: Theoretical association between MBL and type 1 diabetes.
The genetic predisposition (1) of low MBL serum levels could result in an impaired clearance and inactivation of pathogens responsible for beta cell destruction. Contrarily, high MBL serum levels during insulitis could facilitating further beta cell damage and fulminant insulitis (2). Finally, aberrantly glycosylated proteins could result in MBL mediated complement activation and result in subsequent tissue damage (i.e. diabetic complications) (3).

67). In support of the involvement of MBL in I/R injury is the fact that MBL-depositions were observed early after transplantation of ischemically injured kidneys (68). Furthermore, it has been suggested that high MBL levels are associated with a more severe form of rejection leading to graft loss in kidney transplantation (69).

MBL and diabetes

A major source of mortality and morbidity in diabetes is caused by microvascular complications, as a substantial portion of diabetic patients develop diabetic nephropathy and retinopathy. MBL has been associated with diabetic microvasular complications. Several studies have shown the association between an increased risk of developing renal failure and high MBL producing genotypes in diabetic patients (70-72). The involvement of MBL in the pathogenesis of diabetic nephropathy now appears to be appreciated, however the exact immunological process remains to be studied (figure 4). Controversially, it has been suggested that high MBL serum levels may predict a decreased likelihood of myocardial infarction in diabetic patients (73).

4. ADAPTIVE IMMUNITY IN ISLET TRANSPLANTATION

Type 1 (Insulin Dependent) Diabetes Mellitus (T1D) is an autoimmune disease characterized by the specific destruction of beta cells in the pancreas resulting in the loss insulin production. The aetiology of T1D is multifactorial, consisting of genetic predisposition and environmental factors possibly including a variety of viruses and dietary components (74-78). Currently insulin substitution is the most common therapy for patients with T1D. A potential novel therapy for diabetes is transplantation of insulin-producing beta-cells of isolated pancreatic islets. Two major immunological hurdles have to be tackled in order to obtain successful islet transplantation. As the autoimmune response of T1D potentially can destroy the transplanted islets, the recurrence of T-cell autoreactivity against islet determinants, needs to be prevented. In addition, induction of alloimmunity to donor antigens, has to be inhibited (79-81). The attractive and recently successful therapy of transplanting pancreatic islets in T1D is overshadowed by the need for permanent immune suppression. Without the administration of these non-specific and potentially harmful immunosuppressive drugs, graft failure seems inevitable. Islet transplantation is thus limited to diabetic patients already receiving immune suppression for a previous organ transplant, or to patients with severe hypoglycemia unawareness or uncontrollable hyperglycemia. The introduction of a new glucocorticoïd-free immunosuppressive regime, the so-called Edmonton protocol, has improved the outcome of islet transplantation considerably (82). This protocol includes sirolimus, tacrolimus and dacluzimab. All

these immunosuppressive drugs share the same basic quality that they all inhibit T-cell stimulation and proliferation, identifying once again T-cells as key-players in this rejection process (83).

Prediction and prevention of ongoing beta-cell destruction after islet transplantation, resulting in long-term graft survival is of utmost importance. In order to be able to optimise the current islet transplantation, it is essential to study the reaction of T-cells to islets.

5. SCOPE OF THE THESIS

The scope of the current thesis is to obtain insight in immunological aspects of transplantation and diabetes. As previously stated, the present thesis underscores the current concept collaboration between the innate and adaptive immune system by showing close interactions between both immune systems. Mannose binding lectin as a major recognition molecule of the lectin pathway and as a key protein of the immune system was studied in relation to its functional characteristics. Appreciating the Jekyll-and-Hyde character of MBL and the fact that MBL serum levels and functionality are under strict genetic control, MBL was studied under distinct pathological conditions. **Chapter 2** describes molecular and biological aspects of mannose binding lectin and the interaction of MBL with the adaptive immune system. The first part focuses on functional characterizion of the lectin pathway of complement. The second part discusses the possible compensation of MBL deficiency by antibodies and the classical complement pathway as an example of interaction between adaptive and innate immunity. Furthermore, it describes the functional consequences of genetic MBL polymorphisms for the activation of the lectin pathway in serum of healthy individuals. In the last part of this chapter, lectin pathway complement activation via human IgA is described, presenting a novel linkage between innate and adaptive immunity. **Chapter 3** focuses on the involvement of MBL in autoimmunity, by studying juvenile type 1 diabetic patients at disease onset. Prior to starting the study, it was hypothesized that MBL could be associated with type 1 diabetes in three different manners, as shown in figure 4. The genetic predisposition of low MBL serum levels could result in an impaired clearance and inactivation of pathogens responsible for beta cell destruction. Contrarily, high MBL serum levels could contribute to complement activation and inflammation via the recognition of injured tissue, facilitating further beta cell damage and aggravating insulitis progression. Finally, aberrantly glycosylated proteins, as a consequence of the diabetic state, could result in MBL-mediated complement activation resulting in subsequent tissue damage in eyes, kidneys and in various vascular structures (i.e. diabetic complications).

The last theory has been shown in several different studies during the completion of this thesis (70-72; 84). **Chapter 4** addresses the role of the liver in production of serum MBL and to evaluates the effect of MBL variant alleles on the susceptibility to infection after liver transplantation.

Having studied the role of innate immunity in new onset diabetic patients and transplantation, **chapter 5** focuses on the effect of the adaptive immune system on islet transplantation, a novel treatment of type 1 diabetes. The aspects of HLA incompatibility in human pancreatic islet transplantation and the subsequent adaptive allogeneic immune response are discussed. Finally, the results and conclusions of all studies will be summarized and discussed in **chapter 6**.

REFERENCES

1. Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol 1994;12:991-1045.
2. Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ, Turner MW. Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. Infect Immun 2000;68:688-693.
3. Neth O, Jack DL, Johnson M, Klein NJ, Turner MW. Enhancement of complement activation and opsonophagocytosis by complexes of mannose-binding lectin with mannose-binding lectin-associated serine protease after binding to Staphylococcus aureus. J Immunol 2002;169:4430-4436.
4. Townsend R, Read RC, Turner MW, Klein NJ, Jack DL. Differential recognition of obligate anaerobic bacteria by human mannose-binding lectin. Clin Exp Immunol 2001;124:223-228.
5. Turner MW. Mannose-binding lectin: the pluripotent molecule of the innate immune system. Immunol Today 1996;17:532-540.
6. van Emmerik LC, Kuijper EJ, Fijen CA, Dankert J, Thiel S. Binding of mannan-binding protein to various bacterial pathogens of meningitis. Clin Exp Immunol 1994;97:411-416.
7. Miller ME, Seals J, Kaye R, Levitsky LC. A familial, plasma associated defect of phagocytosis. Lancet 1968;60-63.
8. Turner MW, Hamvas RM. Mannose-binding lectin: structure, function, genetics and disease associations. Rev Immunogenet 2000;2:305-322.
9. Thiel S, Vorup-Jensen T, Stover CM, Schwaeble W, Laursen SB, Poulsen K, Willis AC, Eggleton P, Hansen S, Holmskov U, Reid KB, Jensenius JC. A second serine protease associated with mannan-binding lectin that activates complement. Nature 1997;386:506-510.
10. Zundel S, Cseh S, Lacroix M, Dahl MR, Matsushita M, Andrieu JP, Schwaeble WJ, Jensenius JC, Fujita T, Arlaud GJ, Thielens NM. Characterization of recombinant mannan-binding lectin-associated serine protease (MASP)-3 suggests an activation mechanism different from that of MASP-1 and MASP-2. J Immunol 2004;172:4342-4350.
11. Presanis JS, Hajela K, Ambrus G, Gal P, Sim RB. Differential substrate and inhibitor profiles for human MASP-1 and MASP-2. Mol Immunol 2004;40:921-929.
12. Dahl MR, Thiel S, Matsushita M, Fujita T, Willis AC, Christensen T, Vorup-Jensen T, Jensenius JC. MASP-3 and its association with distinct complexes of the mannan-binding lectin complement activation pathway. Immunity 2001;15:127-135.
13. Minchinton RM, Dean MM, Clark TR, Heatley S, Mullighan CG. Analysis of the relationship between mannose-binding lectin (MBL) genotype, MBL levels and function in an Australian blood donor population. Scand J Immunol 2002;56:630-641.
14. Garred P, Larsen F, Madsen HO, Koch C. Mannose-binding lectin deficiency--revisited. Mol Immunol 2003 Sep; 40 (2-4):73 -84 2003;40:73-84.
15. Madsen HO, Garred P, Thiel S, Kurtzhals JA, Lamm LU, Ryder LP, Svejgaard A. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. J Immunol 1995;155:3013-3020.
16. Roos A, Garred P, Wildenberg ME, Lynch NJ, Munoz JR, Zuiverloon TC, Bouwman LH, Schlagwein N, Fallaux van den Houten FC, Faber-Krol MC, Madsen HO, Schwaeble WJ, Matsushita M, Fujita T, Daha MR. Antibody-mediated activation of the classical pathway of complement may compensate for mannose-binding lectin deficiency. Eur J Immunol 2004;34:2589-2598.
17. Madsen HO, Satz ML, Hogh B, Svejgaard A, Garred P. Different molecular events result in low protein levels of mannan-binding lectin in populations from southeast Africa and South America. J Immunol 1998;161:3169-3175.
18. Hibberd ML, Sumiya M, Summerfield JA, Booy R, Levin M. Association of variants of the gene for mannose-binding lectin with susceptibility to meningococcal disease. Meningococcal Research Group. Lancet 1999;353:1049-1053.
19. Peterslund NA, Koch C, Jensenius JC, Thiel S. Association between deficiency of mannose-binding lectin and severe infections after chemotherapy. Lancet 2001;358:637-638.
20. Neth O, Hann I, Turner MW, Klein NJ. Deficiency of mannose-binding lectin and burden of infection in children with malignancy: a prospective study. Lancet 2001;358:614-618.

21. Mullighan CG, Heatley S, Doherty K, Szabo F, Grigg A, Hughes TP, Schwarer AP, Szer J, Tait BD, Bik TL, Bardy PG. Mannose-binding lectin gene polymorphisms are associated with major infection following allogeneic hemopoietic stem cell transplantation. Blood 2002;99:3524-3529.
22. Eisen DP, Minchinton RM. Impact of mannose-binding lectin on susceptibility to infectious diseases. Clin Infect Dis 2003 Dec 1;37 (11):1496 -505 2003;37:1496-1505.
23. Roy S, Knox K, Segal S, Griffiths D, Moore CE, Welsh KI, Smarason A, Day NP, McPheat WL, Crook DW, Hill AV. MBL genotype and risk of invasive pneumococcal disease: a case-control study. Lancet 2002 May 4;359 (9317):1569 -73 2002;359:1569-1573.
24. Siassi M, Hohenberger W, Riese J. Mannan-binding lectin (MBL) serum levels and post-operative infections. Biochem Soc Trans 2003;31:774-775.
25. Garred P, Harboe M, Oettinger T, Koch C, Svejgaard A. Dual role of mannan-binding protein in infections: another case of heterosis? Eur J Immunogenet 1994;21:125-131.
26. Yuen MF, Lau CS, Lau YL, Wong WM, Cheng CC, Lai CL. Mannose binding lectin gene mutations are associated with progression of liver disease in chronic hepatitis B infection. Hepatology 1999;29:1248-1251.
27. Thomas HC, Foster GR, Sumiya M, McIntosh D, Jack DL, Turner MW, Summerfield JA. Mutation of gene of mannose-binding protein associated with chronic hepatitis B viral infection. Lancet 1996;348:1417-1419.
28. Hakozaki Y, Yoshiba M, Sekiyama K, Seike E, Iwamoto J, Mitani K, Mine M, Morizane T, Ohtani K, Suzuki Y, Wakamiya N. Mannose-binding lectin and the prognosis of fulminant hepatic failure caused by HBV infection. Liver 2002;22:29-34.
29. Sasaki K, Tsutsumi A, Wakamiya N, Ohtani K, Suzuki Y, Watanabe Y, Nakayama N, Koike T. Mannose-binding lectin polymorphisms in patients with hepatitis C virus infection. Scand J Gastroenterol 2000;35:960-965.
30. Matsushita M, Hijikata M, Ohta Y, Iwata K, Matsumoto M, Nakao K, Kanai K, Yoshida N, Baba K, Mishiro S. Hepatitis C virus infection and mutations of mannose-binding lectin gene MBL. Arch Virol 1998;143:645-651.
31. Matsushita M, Hijikata M, Matsushita M, Ohta Y, Mishiro S. Association of mannose-binding lectin gene haplotype LXPA and LYPB with interferon-resistant hepatitis C virus infection in Japanese patients. J Hepatol 1998;29:695-700.
32. Ezekowitz RA, Kuhlman M, Groopman JE, Byrn RA. A human serum mannose-binding protein inhibits in vitro infection by the human immunodeficiency virus. J Exp Med 1989;169:185-196.
33. Saifuddin M, Hart ML, Gewurz H, Zhang Y, Spear GT. Interaction of mannose-binding lectin with primary isolates of human immunodeficiency virus type 1. J Gen Virol 2000;81:949-955.
34. Kristensen IA, Thiel S, Steffensen R, Madhi S, Sorour G, Olsen J. Mannan-binding lectin and RSV lower respiratory tract infection leading to hospitalization in children: a case-control study from Soweto, South Africa. Scand J Immunol 2004;60:184-188.
35. Nielsen HE, Siersma V, Andersen S, Gahrn-Hansen B, Mordhorst CH, Norgaard-Pedersen B, Roder B, Sorensen TL, Temme R, Vestergaard BF. Respiratory syncytial virus infection--risk factors for hospital admission: a case-control study. Acta Paediatr 2003;92:1314-1321.
36. Homoe P, Madsen HO, Sandvej K, Koch A, Garred P. Lack of association between mannose-binding lectin, acute otitis media and early Epstein-Barr virus infection among children in Greenland. Scand J Infect Dis 1999;31:363-366.
37. Kase T, Suzuki Y, Kawai T, Sakamoto T, Ohtani K, Eda S, Maeda A, Okuno Y, Kurimura T, Wakamiya N. Human mannan-binding lectin inhibits the infection of influenza A virus without complement. Immunology 1999;97:385-392.
38. Thielens NM, Tacnet-Delorme P, Arlaud GJ. Interaction of C1q and mannan-binding lectin with viruses. Immunobiology 2002;205:563-574.
39. Carroll M. Innate immunity in the etiopathology of autoimmunity. Nat Immunol 2001;2:1089-1090.
40. Ezekowitz RA. Role of the mannose-binding lectin in innate immunity. J Infect Dis 2003;187 Suppl 2:S335-S339.

41. Boniotto M, Braida L, Baldas V, Not T, Ventura A, Vatta S, Radillo O, Tedesco F, Percopo S, Montico M, Amoroso A, Crovella S. Evidence of a correlation between mannose binding lectin and celiac disease: a model for other autoimmune diseases. J Mol Med 2005.
42. Garred P, Voss A, Madsen HO, Junker P. Association of mannose-binding lectin gene variation with disease severity and infections in a population-based cohort of systemic lupus erythematosus patients. Genes Immun 2001 Dec; 2(8):442 -50 2001;2:442-450.
43. Nauta AJ, Castellano G, Xu W, Woltman AM, Borrias MC, Daha MR, van Kooten C, Roos A. Opsonization with C1q and mannose-binding lectin targets apoptotic cells to dendritic cells. J Immunol 2004;173:3044-3050.
44. Roos A, Xu W, Castellano G, Nauta AJ, Garred P, Daha MR, van Kooten C. Mini-review: A pivotal role for innate immunity in the clearance of apoptotic cells. Eur J Immunol 2004;34:921-929.
45. Russell L, Waring P, Beaver JP. Increased cell surface exposure of fucose residues is a late event in apoptosis. Biochem Biophys Res Commun 1998;250:449-453.
46. Duvall E, Wyllie AH, Morris RG. Macrophage recognition of cells undergoing programmed cell death (apoptosis). Immunology 1985;56:351-358.
47. Ogden CA, deCathelineau A, Hoffmann PR, Bratton D, Ghebrehiwet B, Fadok VA, Henson PM. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. J Exp Med 2001;194:781-795.
48. Nauta AJ, Raaschou-Jensen N, Roos A, Daha MR, Madsen HO, Borrias-Essers MC, Ryder LP, Koch C, Garred P. Mannose-binding lectin engagement with late apoptotic and necrotic cells. Eur J Immunol 2003;33:2853-2863.
49. Sato R, Matsushita M, Miyata M, Sato Y, Kasukawa R, Fujita T. Substances reactive with mannose-binding protein (MBP) in sera of patients with rheumatoid arthritis. Fukushima J Med Sci 1997;43:99-111.
50. Day JF, Thornburg RW, Thorpe SR, Baynes JW. Carbohydrate-mediated clearance of antibody . antigen complexes from the circulation. The role of high mannose oligosaccharides in the hepatic uptake of IgM . antigen complexes. J Biol Chem 1980;255:2360-2365.
51. Saevarsdottir S, Vikingsdottir T, Valdimarsson H. The potential role of mannan-binding lectin in the clearance of self-components including immune complexes. Scand J Immunol 2004;60:23-29.
52. Saevarsdottir S, Vikingsdottir T, Vikingsson A, Manfredsdottir V, Geirsson AJ, Valdimarsson H. Low mannose binding lectin predicts poor prognosis in patients with early rheumatoid arthritis. A prospective study. J Rheumatol 2001;28:728-734.
53. Ip WK, Lau YL, Chan SY, Mok CC, Chan D, Tong KK, Lau CS. Mannose-binding lectin and rheumatoid arthritis in southern Chinese. Arthritis Rheum 2000;43:1679-1687.
54. Graudal NA, Homann C, Madsen HO, Svejgaard A, Jurik AG, Graudal HK, Garred P. Mannan binding lectin in rheumatoid arthritis. A longitudinal study. J Rheumatol 1998;25:629-635.
55. Garred P, Madsen HO, Marquart H, Hansen TM, Sorensen SF, Petersen J, Volck B, Svejgaard A, Graudal NA, Rudd PM, Dwek RA, Sim RB, Andersen V. Two edged role of mannose binding lectin in rheumatoid arthritis: a cross sectional study. J Rheumatol 2000;27:26-34.
56. Jacobsen S, Madsen HO, Klarlund M, Jensen T, Skjodt H, Jensen KE, Svejgaard A, Garred P. The influence of mannose binding lectin polymorphisms on disease outcome in early polyarthritis. TIRA Group. J Rheumatol 2001;28:935-942.
57. Leff JA, Repine JE. Blood cells and ischemia-reperfusion injury. Blood Cells 1990;16:183-191.
58. Keith F. Oxygen free radicals in cardiac transplantation. J Card Surg 1993;8:245-248.
59. Sheron N, Eddleston A. Preservation-reperfusion injury, primary graft non-function and tumour necrosis factor. J Hepatol 1992;16:262-265.
60. Rudiger HA, Graf R, Clavien PA. Liver ischemia: apoptosis as a central mechanism of injury. J Invest Surg 2003;16:149-159.
61. Weisman HF, Bartow T, Leppo MK, Marsh HC, Jr., Carson GR, Concino MF, Boyle MP, Roux KH, Weisfeldt ML, Fearon DT. Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. Science 1990;249:146-151.

62. Collard CD, Vakeva A, Bukusoglu C, Zund G, Sperati CJ, Colgan SP, Stahl GL. Reoxygenation of hypoxic human umbilical vein endothelial cells activates the classic complement pathway. Circulation 1997;96:326-333.
63. Collard CD, Agah A, Stahl GL. Complement activation following reoxygenation of hypoxic human endothelial cells: role of intracellular reactive oxygen species, NF-kappaB and new protein synthesis. Immunopharmacology 1998;39:39-50.
64. Collard CD, Agah A, Reenstra W, Buras J, Stahl GL. Endothelial nuclear factor-kappaB translocation and vascular cell adhesion molecule-1 induction by complement: inhibition with anti-human C5 therapy or cGMP analogues. Arterioscler Thromb Vasc Biol 1999;19:2623-2629.
65. Buerke M, Murohara T, Lefer AM. Cardioprotective effects of a C1 esterase inhibitor in myocardial ischemia and reperfusion. Circulation 1995;91:393-402.
66. Collard CD, Vakeva A, Morrissey MA, Agah A, Rollins SA, Reenstra WR, Buras JA, Meri S, Stahl GL. Complement activation after oxidative stress: role of the lectin complement pathway. Am J Pathol 2000;156:1549-1556.
67. Collard CD, Montalto MC, Reenstra WR, Buras JA, Stahl GL. Endothelial oxidative stress activates the lectin complement pathway: role of cytokeratin 1. Am J Pathol 2001;159:1045-1054.
68. de Vries B, Walter SJ, Peutz-Kootstra CJ, Wolfs TG, van Heurn LW, Buurman WA. The mannose-binding lectin-pathway is involved in complement activation in the course of renal ischemia-reperfusion injury. Am J Pathol 2004;165:1677-1688.
69. Berger SP, Roos A, Mallat MJ, Fujita T, de Fijter JW, Daha MR. Association between mannose-binding lectin levels and graft survival in kidney transplantation. Am J Transplant 2005;5:1361-1366.
70. Hovind P, Hansen TK, Tarnow L, Thiel S, Steffensen R, Flyvbjerg A, Parving HH. Mannose-binding lectin as a predictor of microalbuminuria in type 1 diabetes: an inception cohort study. Diabetes 2005;54:1523-1527.
71. Saraheimo M, Forsblom C, Hansen TK, Teppo AM, Fagerudd J, Pettersson-Fernholm K, Thiel S, Tarnow L, Ebeling P, Flyvbjerg A, Groop PH. Increased levels of mannan-binding lectin in type 1 diabetic patients with incipient and overt nephropathy. Diabetologia 2005;48:198-202.
72. Hansen TK, Tarnow L, Thiel S, Steffensen R, Stehouwer CD, Schalkwijk CG, Parving HH, Flyvbjerg A. Association between mannose-binding lectin and vascular complications in type 1 diabetes. Diabetes 2004;53:1570-1576.
73. Saevarsdottir S, Oskarsson OO, Aspelund T, Eiriksdottir G, Vikingsdottir T, Gudnason V, Valdimarsson H. Mannan binding lectin as an adjunct to risk assessment for myocardial infarction in individuals with enhanced risk. J Exp Med 2005;201:117-125.
74. Virtanen SM, Knip M. Nutritional risk predictors of beta cell autoimmunity and type 1 diabetes at a young age. Am J Clin Nutr 2003;78:1053-1067.
75. Vreugdenhil GR, Geluk A, Ottenhoff TH, Melchers WJ, Roep BO, Galama JM. Molecular mimicry in diabetes mellitus: the homologous domain in coxsackie B virus protein 2C and islet autoantigen GAD65 is highly conserved in the coxsackie B-like enteroviruses and binds to the diabetes associated HLA-DR3 molecule. Diabetologia 1998;41:40-46.
76. Hiemstra HS, Schloot NC, van Veelen PA, Willemen SJ, Franken KL, van Rood JJ, de Vries RR, Chaudhuri A, Behan PO, Drijfhout JW, Roep BO. Cytomegalovirus in autoimmunity: T cell crossreactivity to viral antigen and autoantigen glutamic acid decarboxylase. Proc Natl Acad Sci U S A 2001;98:3988-3991.
77. Roep BO, Hiemstra HS, Schloot NC, de Vries RR, Chaudhuri A, Behan PO, Drijfhout JW. Molecular mimicry in type 1 diabetes: immune cross-reactivity between islet autoantigen and human cytomegalovirus but not Coxsackie virus. Ann N Y Acad Sci 2002;958:163-165.
78. Roep BO. Molecular mimicry in autoimmune neurological disease after viral infection. Curr Med Chem 2003;10:1939-1943.
79. Roep BO, Stobbe I, Duinkerken G, van Rood JJ, Lernmark A, Keymeulen B, Pipeleers D, Claas FH, de Vries RR. Auto- and alloimmune reactivity to human islet allografts transplanted into type 1 diabetic patients. Diabetes 1999;48:484-490.

80. Keymeulen B, Ling Z, Gorus FK, Delvaux G, Bouwens L, Grupping A, Hendrieckx C, Pipeleers-Marichal M, Van Schravendijk C, Salmela K, Pipeleers DG. Implantation of standardized beta-cell grafts in a liver segment of IDDM patients: graft and recipients characteristics in two cases of insulin-independence under maintenance immunosuppression for prior kidney graft. Diabetologia 1998;41:452-459.
81. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 2000;343:230-238.
82. Shapiro AM, Nanji SA, Lakey JR. Clinical islet transplant: current and future directions towards tolerance. Immunol Rev 2003 Dec; 196 :219 -36 2003;196:219-236.
83. Roep BO. The role of T-cells in the pathogenesis of Type 1 diabetes: from cause to cure. Diabetologia 2003;46:305-321.
84. Hansen TK, Thiel S, Knudsen ST, Gravholt CH, Christiansen JS, Mogensen CE, Poulsen PL. Elevated levels of mannan-binding lectin in patients with type 1 diabetes. J Clin Endocrinol Metab 2003 Oct; 88 (10):4857 -61 2003;88:4857-4861.