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Review

Complement activation and diabetic vascular complications

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Abstract

Diabetes mellitus is a major and increasing health problem worldwide. One of the most serious consequences of diabetes is the development of diabetic angiopathy, which includes cardiovascular disease, neuropathy, retinopathy and nephropathy. Diabetic nephropathy alone affects 15–25% of patients with type 1 diabetes and 30–40% of patients with type 2 diabetes and is the single-most important cause of end-stage renal failure in the Western World. Existing research has demonstrated the involvement of glycation factors, growth factors/cytokines, hemodynamic factors and intracellular changes in the pathogenesis of diabetic kidney disease. An emerging amount of recent data suggests that the complement system, especially the MBL pathway, plays an important role in the pathogenesis of diabetic vascular complications. Although the numerous therapeutic interventions available today may delay the development and progression of diabetes vascular complications, there is an ongoing need for new therapeutic strategies. In this article the evidence for a connection between the complement system and vascular dysfunction will be reviewed, with a special focus on the relation to diabetic kidney disease. Several ways of specifically manipulating the complement system already exist. However, whether or not these drugs provide new targets for intervention on diabetic vascular complications is still unknown.

Keywords: MBL; Mannose-binding lectin; Diabetic nephropathy; Type 1 diabetes; Lectin complement pathway; Diabetes mellitus

Contents

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1. Introduction

Diabetes mellitus is a major health problem and the disease is responsible for substantial personal and economical costs. The most serious complications to diabetes are micro- and macrovascular complications that may lead to cardiovascular disease (CVD), neuropathy, retinopathy and nephropathy. Diabetic kidney disease affects about 15–25% of all patients with type 1 diabetes [1,2] and about 30–40% of all patients with type 2 diabetes [2]. Although an increasing number of therapeutic interventions have been developed for the treatment of diabetic kidney disease [3], renal dysfunction in diabetic patients is still the single most important cause of end-stage renal failure (ESRF) in the Western World. Studies have shown that markers of chronic low-grade inflammation correlates with markers of endothelial dysfunction in diabetic patients without clinical signs of macrovascular disease [4], suggesting that diabetic angiopathy may have an inflammatory pathogenesis. This is in line with the inflammatory component in atherosclerosis [5] as well as data showing that diabetic patients have increased levels of acute-phase proteins [6–8]. Furthermore, C-reactive protein (CRP) well within the normal range, is a predictor of CVD in patients with angina pectoris [9] and among healthy men [10]. It is still uncertain whether increased acute-phase proteins per se may lead to diabetes [11].

Recent data have shown that type 1 diabetic patients with nephropathy have significantly higher circulating levels of mannose-binding lectin (MBL), a member of the complement system, than normoalbuminuric patients [12]. These data thus support the idea of an involvement of the immune system in the development of diabetic complications. Family clustering of nephropathy [13] and the association between CVD and nephropathy in families with diabetic members [14] suggests a common genetic predisposition. However, this idea was not supported by data from a study preformed in patients with type 2 diabetes, where a history of previous CVD had no association with increased risk of development of increased urinary albumin excretion [15].

2. The complement system

The complement system is activated in a cascade manner through three different pathways, i.e. the classical, the alternative and the MBL complement pathway, respectively. These three pathways merge and stimulate the formation of a C3 convertase (Fig. 1). The various enzymes, which are all serine proteases, of the three systems are synthesized in the liver as inactive zymogens. Activation occurs when the components are cleaved into smaller pro-inflammatory and larger proteolytic fragments, which subsequently activates the next step. Initiation of the classical complement pathway relies on the adaptive immune system and occurs by the interaction of antigen–antibody complexes with the C1q portion of complement component C1 (Fig. 1). C1q activates the two C1r and the two C1s components of C1. Subsequently the activated C1s cleave C4 and C2 leading to formation of the classical pathway C3 convertase (C4bC2a), cleaving C3 into C3a and C3b (Fig. 1).

The alternative complement pathway is activated in an antibody-independent manner on microbial surfaces, biomaterials and by tissue type plasminogen activator leading to the formation of the alternative pathway C3 convertase, C3bBb (Fig. 1). The same convertase is also formed when factor D cleaves factor B bound to the C3b generated by the classical pathway (see above) or the MBL pathway (see below). In this way the alternative pathway C3 convertase accelerates the classical and the MBL pathway [16].

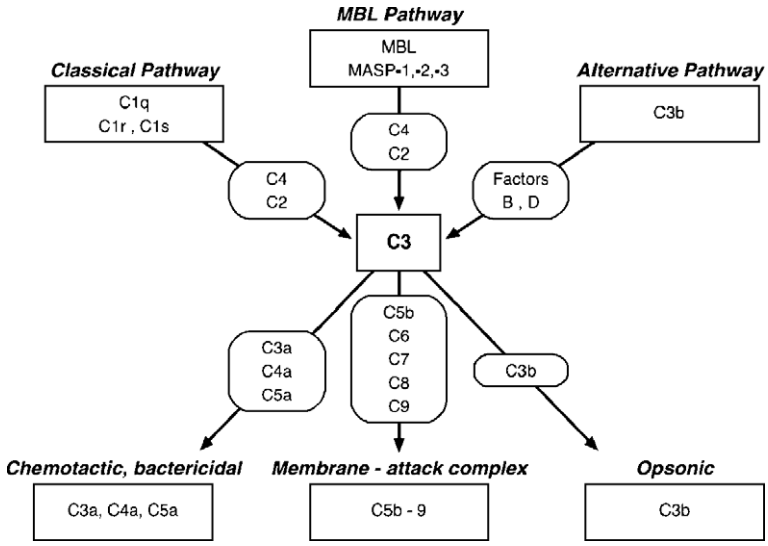

Fig. 1. Schematic depiction of the complement system. The complement cascade is activated by either of the three pathways: the classical, the MBL, or the alternative pathway. Activation leads to formation of the membrane–attack complex as well as formation of opsonic, chemotactic and bactericidal factors. For further information, please see text.

The MBL pathway is initiated when MBL binds to carbohydrate structures (mannose, *N*-acetyl-D-glucosamine, *N*-acetyl-mannosamine, fucose or glucose) found in a repetitive pattern on microbial surfaces which increases the overall binding affinity [17]. This interaction activates MBL-associated serine proteases (MASPs) of which three are known: MASP-1, MASP-2, and MASP-3. When MASP-2 is activated it may cleave C4 and C2 leading to formation of the classical complement pathway C3 convertase described above [18]. From the level of C3 cleavage into C3a and C3b the complement system runs by a common trail. A number of C3b molecules bind to either of the C3 convertases (C4b2a or C3bBb), forming a C5 convertase, which cleaves C5 into C5a and C5b. In succession C6-9 binds to C5b and the membrane–attack complex (MAC or C5b–9) is formed inserting a pore in the pathogen (Fig. 1). Many leucocytes carry receptors for C3b, the so-called CR1, and in this way C3b covalently bound to the pathogen serves as an opsonin [19,20] and clearing of immune complexes etc. from the blood (Fig. 1). Other effects of complement activation are mediated by C5a, C3a and C4a, which serve as chemokines or, as sometimes referred to, as anaphylatoxins [16] (Fig. 1). C3a by itself may further serve as a bactericidal peptide [21]. Interestingly, despite obvious beneficial effects of the complement system, advantages following blockade of activation have been reported in some pathophysiological conditions [22–29].

3. The complement system in vascular disease

Many studies have focused on the complement system in relation to vascular diseases. Both preclinical (in vitro and animal models) and clinical data suggest a potential role of the system in macro- and microvascular disease, including diabetic angiopathy. These results and data obtained from research in myocardial ischemia/reperfusion injury studies will be presented below.

3.1. Preclinical data

3.1.1. The kidney

The association between complement and renal diseases has been shown in various studies. Depending on the genetic background C1q-null mice develop autoimmune renal disease [30,31]. To examine the potential relationship between the complement system and diabetic nephropathy, the degree of renal deposition of component C3 has been examined in different animal models of diabetes. In immunohistological

studies deposition of C3 (named β1C in this paper) was demonstrated in glomeruli and the walls of glomerular capillaries of diabetic KK mice (a model of type 2 diabetes) [32]. Further, a more intense C3 staining was demonstrated in the renal mesangium of alloxan-induced diabetic rats (a model of type 1 diabetes mellitus), when compared with age-matched control [33,34]. Interestingly, the complement deposition appeared before structural renal changes were demonstrated [33]. In streptozotocin (STZ)-diabetic rats (a model of type 1 diabetes) demonstrating positive glomerular C3 staining, glomerular and tubular changes, transplantation of pancreatic islets resulted in complete normalization of C3 staining, improved tubular morphology and a decreased amount of mesangial material [35,36]. These data were confirmed in uni-nephrectomized, diabetic rats with a partial reduction in renal C3 staining following islet-transplantation [37]. Furthermore, transplantations of kidneys from STZ-diabetic rats into non-diabetic rats and vice versa revealed similar results [38]. Prior to transplantation increased mesangial volume and positive C3 staining were observed in kidneys from diabetic animals. Following transplantation into non-diabetic rats, approximately half of the kidneys had unchanged morphology, while the rest had a significantly decreased mesangial volume and a clear reduction in/or no C3 staining. The non-transplanted kidney of the healthy animals remained generally normal while the non-transplanted kidney in the diabetic animals had worsened morphology and increased or unchanged C3 staining.

When antibodies raised against the C9 portion of the MAC were used for immunohistochemistry on human kidney tissue from type 1 diabetic patients with varying degrees of renal dysfunction, MACs were localized to the glomerular basement membrane, the tubular basement membrane and the Bowman's capsular membrane [39]. Further, the MAC deposition correlated with the degree of mesangial expansion [39]. Finally, in healthy subjects the differences in deposition of MACs were shown to correlate with the age of the person [39]. It has to be noted, however, that in this rather small study, the tissue used as control ($n=3$) was obtained post-mortem and the diabetic kidney tissue ($n=12$) was obtained from biopsies for clinical evaluation or prior to transplantation.

It has been shown that inhibition of complement component C5 by K-76COONa in Otsuka Long-Evans Tokushima Fatty (OLETF) rats (a model of type 2 diabetes), resulted in a reduction both in urinary protein excretion and mesangial expansion [40] (Table 1). In the same study immunohistochemical examination for C3 revealed a more intense staining in the placebo-treated diabetic animals than in the diabetic group of animals treated with K-76COONa. These data thus indicate that blockade of the complement system at C5-level inhibits further complement activation as a result of Ig deposition.

Recently it was demonstrated that MBL-null mice showed less renal damage after kidney ischemia/reperfusion along with diminished deposition of C3 [41].

3.1.2. The heart

In an in vitro model of myocardial ischemia/reperfusion (I/R) injury, i.e. human umbilical vein endothelial cells (HUVECs) subjected to hypoxia and subsequent reoxygenation, significantly increased C3 deposition was observed when compared with normoxic HUVECs [28,29,42]. Addition of $MgCl_2$/EGTA to human serum (inhibiting the classical and the MBL pathway, but allowing the alternative pathway to be active), as well as the use of human serum depleted of C2, attenuated the C3 deposition to the same level as normoxic controls [28]. In contrast the use of factor B depleted human serum did not attenuate the increased C3 deposition due to I/R, indicating

Table 1
Specific inhibitors of the complement system

Inhibitor	Target site	References
C1 INH	C1r, C1s, MASP-1, MASP-2	[22,44–46,76]
mMBL-Ab	MBL	[23,29]
Anti C3 (Compstatin)	C3	[77]
sCR1 (TP10)	C3b, C4b	[26,78]
mC5-Ab (Pexilizumab)	C5	[24,40,64]

C1 INH, C1 inhibitor; *mMBL-Ab*, monoclonal inhibitory MBL antibody; Compstatin inhibits C3 cleavage; *sCR1*, soluble complement receptor 1; *mC5-Ab*, monoclonal inhibitory C5 antibody. The soluble CR1, the C1 inhibitor and the monoclonal anti C5 antibodies are currently under clinical evaluation. For more detailed information please read text and the publications indicated in the reference column.

that the Ca^{2+}-dependent classical and/or the Ca^{2+}-dependent MBL pathway initiates the complement system after I/R [28] (Fig. 1). Human serum with an admixture of either D-mannose, GlcNAc, MBL-inhibitory monoclonal antibodies (Table 1) or human serum deficient of MBL inhibited iC3b deposition. Accordingly, these data suggest that the MBL pathway is the C2-dependent pathway dissected previously [29]. This is in line with the co-localization of MBL and C3 after I/R [29] and the protection against myocardial I/R injury observed in MBL-null mice [43].

Several publications have demonstrated the involvement of the complement system in myocardial I/R injury in vivo. Administration of a recombinant soluble form of CR1 (which inhibits C3b and C4 (Table 1)) to rats prior to I/R, showed a significant cardioprotective effect [26]. Also administration of C1 esterase inhibitor, *C1 INH* (Table 1), significantly attenuated myocardial I/R injury [22,44–46]. Immunohistochemistry has demonstrated MBL and C3 deposition following myocardial I/R, but not by ischemia alone [29] and blockade of the MBL pathway by monoclonal anti rat MBL antibodies (Table 1) significantly decreased infarct size after I/R [23].

3.2. Clinical data

3.2.1. The kidney

In a group of patients with type 1 diabetes, approximately a fourth had subnormal serum C4 concentration without correlation to the amount C3 fragments present (i.e. a measure of complement activation) [47,48]. Further, a significant correlation in serum C4 levels between the diabetic probands and their monozygotic twins was demonstrated, even in individuals without diabetes. These observations led to the hypothesis, that low C4 level could be a risk factor for the development of type 1 diabetes. Further, it was shown that type 1 diabetics with microangiopathy had significantly lower serum levels of C4, when compared to those without microangiopathy and that C4 correlated to markers of complement activation [49]. A study has reported data in agreement with these results [48]. This correlation was not consistent in a later study which proposed low C4 levels rather to be a manifestation of type 1 diabetes more than a pathogenic factor of the disease [50]. However, it is very interesting that about the same proportion of patients with type 1 diabetes has low C4 levels and develops diabetic kidney disease. Evaluation of allotypes in diabetics with or without microangiopathy has not revealed conclusive results [51–54].

Deposition of MBL, MASP-1, C3b/C3c and C5b-9 has been reported in patients with IgA nephropathy as well as in patients with Henoch–Schonlein purpura nephritis, suggesting that the MBL pathway is activated in the course of these diseases [55,56].

The MBL pathway has come in focus as a potential pathogenic factor in diabetic nephropathy, as normoalbuminuric type 1 diabetics have higher levels of serum MBL than non-diabetic controls, with a stepwise increase in circulating MBL-levels with increasing levels of urinary albumin excretion (UAE) within the normal range [57]. Of interest no correlation between MBL and CRP was seen. In a Caucasian population the median MBL level is 800–1000 μg/L, one third of the population have a MBL level lower than 500 μg/L and in about one tenth it is below 100 μg/L [58,59]. The serum level of MBL is largely determined by the genetic composition [60] and of importance the within-subject variation is small [61]. Thus the correlation between MBL-level and UAE in type 1 diabetes is most likely a result of a genetic predisposition. Data from a recent study showed that a significantly larger proportion of patients with a high MBL-genotype had diabetic nephropathy compared with the group with low MBL-genotypes (OR = 1.52) [12]. Furthermore, in patients with a high MBL genotype the serum MBL levels were significantly higher in nephropathic patients than in patients without nephropathy [12]. The elevated serum MBL levels in type 1 diabetic patients with diabetic nephropathy were confirmed in another recent study, which also showed that elevated serum MBL-levels are seen even in microalbuminuric type 1 diabetic patients [62]. Further, no significant correlations were found between MBL levels and CRP, interleukin-6, glomerular filtration rate, duration of diabetes or urinary albumin excretion. Finally, in a recent 18-year follow-up study in type 1 diabetic patients, the association between serum MBL-levels and development of microalbuminuria was evaluated [63]. In patients with type 1 diabetes and MBL-levels below the median of 1597 μg/L the risk of developing micro- or macroalbuminuria was 26%, while the patients with MBL-levels above the median had a risk of 41% of developing

micro- or macroalbuminuria over an average follow-up period of 18 years [63]. In conclusion, these data show that diabetic kidney disease is associated with a high MBL expression genotype and circulating MBL-levels, and that a high MBL geno- and phenotype is associated with an increased risk of developing diabetic kidney disease.

3.2.2. The heart

Pexelizumab, an inhibitor of C5 (Table 1), has recently been demonstrated to significantly reduce mortality following acute myocardial infarction treated with percutaneous intervention [64] and to reduce the incidence of death or myocardial infarction in patients after coronary artery bypass graft surgery with or without valve surgery [24]. Interestingly, in the study reported above dealing with type 1 diabetics with nephropathy, patients with CVD had significant higher levels of MBL than those without [12]. These data support the hypothesis of a possible role of MBL and the complement system in the pathogenesis of diabetic nephropathy and CVD.

4. On the mechanisms of the complement system in diabetic vascular complications

As presented in this review an increasing amount of evidence indicates that the complement system may play a role in the development of diabetic vascular lesions. As will be appreciated from this review most recent data point at the MBL pathway as the initiating pathway of complement activation in patients with diabetes mellitus, with a close link to the development of diabetic kidney disease. Extensive research through the last decades has shown that glycation factors, several growth factors/cytokines, hemodynamic factors and intracellular changes are all involved in the development of diabetic kidney disease. A comprehensive review has recently been published on this topic [65] and a detailed description of these pathways is beyond the scope of the present article. At present the knowledge about a possible interaction between the complement system and these various pathways is sparse. The mechanism responsible for a potential activation of the complement system in patients with diabetes is still unknown. However, inactivation of the complement regulatory proteins by glycation as well as reactive oxygen species (ROS)-mediated cell alternations may be possible.

Homologous cells are protected against attack by the complement system due to membrane bound complement regulatory proteins, e.g., the decay-accelerating factor (DAF) that inactivates the C3 and C5 convertases and CD59 which prevents the formation of MAC [16]. In the blood C1 inhibitor deactivates activated C1, while factor I and H degrade C4b and C3b [16]. It has been suggested that inactivation of complement regulatory proteins by glycation could explain the attack on autologous tissue by the complement system. In the course of glycation of proteins, intermediate Amadori products are formed before the stabile advanced glycation end products (AGEs) are finally produced. Glycation of CD59 have been shown to attenuate its MAC-inhibitory effects in vitro most likely due to formation of Amadori products rather than AGEs [66]. Furthermore treatment of HUVECs with 50 mM ribose prior to exposure of MAC-forming components was demonstrated to cause an increased release of growth factors from the cells. This occurred even without lysis of the cells by formation of pores [66]. Examination of autopsy tissue from diabetic patients or patients with severe glomerulosclerosis revealed no normal CD59 in the areas of the arterial tunica media where MAC-deposition was most pronounced [67]. In the same samples the degree of deposition of both MAC and the form of AGE designated carboxymethyllysin (CML) were reported as severe and prominent. In all groups examined, including the controls, CML was found in areas positive for MAC [67]. A significant correlation between the volume fractions of the arterial tunica media positive for MAC and positive for CML was only found in the groups of diabetic patient with severe and moderate glomerulosclerosis [67]. The volume fractions of the arterial media positive for MAC and CML were greatest in group with diabetes and severe glomerulosclerosis although not when the MAC deposition was compared with diabetics with moderate glomerulosclerosis [67]. Further, the same study demonstrated that AGE-modified bovine serum albumin did not activate complement in vitro [67]. In a recent study MACs and glycated CD59 were reported to be localized in the kidneys, especially in the glomerular capillaries, and in nerves, especially in vasa nervorum, of about 60% of the diabetic

patients studied and in none of the non-diabetic controls. MAC and glycated CD59 were co-localized in the tissues examined [68]. In addition, the authors demonstrated a glycation-mediated decrease in CD59 activity on red blood cells from diabetics when compared with non-diabetic controls [68].

As indicated above a potential activation of the complement system in patients with diabetes may be through intracellular generation of ROS. Hyperglycemia has been shown to induce intracellular formation of ROS due to mitochondrial production [69]. In a model of myocardial I/R injury, increased intracellular production of ROS was reported [42]. By addition of cell permeable ROS scavengers, the I/R induced complement activation was attenuated [42]. Interaction between AGEs and the receptor for AGE (RAGE) results in ROS generation [70]. Accordingly, it may be hypothesized that hyperglycemia in diabetic patients may activate the complement system through mitochondrial production of ROS or by augmented RAGE activation.

5. Perspective

Tight metabolic and antihypertensive control are cornerstones in the treatment of micro- and macrovascular complications in patients with type 1 or type 2 diabetes. Although the numerous therapeutic interventions available today may postpone the development and progression of diabetic vascular complications, there is an ongoing need for the development of new therapeutic strategies. As reviewed in this article an emerging amount of evidence is in support of the complement system playing a role in the pathogenesis of diabetic vascular complications. Accordingly, such data imply that the complement system may be a potential new target for the development of drugs with an impact on the development of diabetic vascular dysfunction. However, much still remains to be clarified before the exact role of the complement system in diabetic vascular dysfunction is fully elucidated. Future research in the area should include in vitro experiments, with vascular cells in culture, examining the potential interaction between components of the complement system, glycation factors and growth factors/cytokines. Genetic manipulated animal models (e.g. MBL or C3 knockout mice) offer opportunities for specific evaluation of the role of the different components of the complement system in the pathogenesis of diabetic complications. Administration of monoclonal antibodies represents another tool by which manipulation at various sites in the complement cascade is possible and may be evaluated (Table 1). Administration of the soluble complement receptor (*sCR1*), a modified C1 inhibitor (*C1 INH*) and C5 inhibitory antibodies is already being evaluated in clinical trials (Table 1) and might be used in the future as a treatment of diabetic complications. However, whether or not manipulation of the complement system will be a way to protect patients from vascular complication is still unknown, as are the long-term effects of such a treatment. It is well-known that low levels of MBL are associated with an increased susceptibility to a broad range of infections [71–78] and potential adverse effects of manipulation of the MBL pathway may hinder the use of such agents in the treatment of diabetic complications.

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