Neuroimmune Alterations in Diabetes: Implications to Molecular Pathomechanisms of Complications

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1 Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Bonow & Gheorghiade, 2004).

The number of patients with DM is steadily increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity (Bonow & Gheorghiade, 2004; Wild et al., 2004; Shaw et al., 2010). It was estimated that there were 171 million people suffering from diabetes mellitus in 2000 and in 2030 the number of diabetes-affected people will increase to 439 million (Bonow & Gheorghiade, 2004; Wild et al., 2004; Shaw et al., 2010). The expected rise in the number of diabetic patients will lead to an enormous increase in the socioeconomic burden.

DM is characterized by multiple vascular complications. Endothelial and vascular smooth muscle cell dysfunction inflammation and hypercoagulability are the key factors in diabetic arteriopathy (Huysman & Mathieu, 2009). Beside the severe microvascular complications, diabetic nephropathy, retinopathy or neuropathy, macrovascular complications including diabetic cardiomyopathy are frequent among diabetic patients (Marshall & Flyvbjerg, 2006; Huysman & Mathieu, 2009; Keymel et al., 2011). DM leads to premature and accelerated atherosclerosis with an increased risk of cardiovascular and cerebrovascular events (Wingard et al., 1993; Grundy et al., 1999; Fox et al., 2004; Booth et al., 2006). Thus, coronary artery disease (Devine et al., 1981; Cuocolo et al., 2009), myocardial ischemia (Ambepityia et al., 1990), infarction (Weitzman et al., 1982; Kapur & De Palma, 2007) and ischemic stroke (Oppenheimer et al., 1985; Olsson et al., 1990; Jorgensen et al., 1994; Laing et al., 2003; Idris et al., 2006) commonly occurs and represent the ultimate cause of death in patients with DM (Weitzman et al., 1982; Kapur & De Palma, 2007; Oppenheimer et al., 1985; Olsson et al., 1990; Jorgensen et al., 1994; Laing et al., 2003).

Research efforts aim to elucidate pathophysiological mechanisms contributing to the disease process, progression and complications. However, the complexity of molecular events and pathways involved in etiopathomechanisms of DM and responsible for diabetic complications creates big obstacles in understanding the real consequence of DM and related complications. This highly limits the development of the efficient measures on early diagnosis, prevention, and treatment of DM and its complications.

In the present chapter we summarize the results of our studies of neuroimmune state of patients with long-term diabetes and also provide brief overview of the related data obtained by other research groups. These findings suggest that DM is characterized by neuroimmune dysfunction, which underlies the development and progression of diabetic complications and is responsible for high frequency of cerebrovascular and cardiovascular pathologies in DM patients, as well as for association of diabetic stroke with high severity and mortality, and poor clinical outcome.

2 Alterations in the Immune Response in DM

Patients with DM have infections more often than those without DM. The course of the infections is also more complicated in this patient group. One of the possible causes of this increased prevalence of infections is defects in immunity. Both type 1 and type 2 DM (DM1 and DM2, respectively), two main forms of DM, are characterized by the immune system disturbances mainly related to the innate immunity (Moutschen et al., 1992; Geerlings & Hoepelman, 1999).
DM1, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas Langerhans islet. Markers of the immune destruction, the β-cell including autoantibodies to Langerhans islet cells, insulin, glutamic acid decarboxylase (GAD65), and tyrosine phosphatase-like protein (islet antigen-2 and islet antigen-2b) are detected in the blood of DM1 patients. In addition, DM1 is often accompanied by other autoimmune disorders including Graves’ disease, Hashimoto's thyroiditis, Addison disease, pernicious anemia, and diffuse toxic goiter. Onset of DM1 is often accompanied by insulitis, an inflammation of the islets of Langerhans islets of the pancreas (Kukreja et al., 2002). Interestingly, that at the same time in DM1 patients the reduced primary antibody response to T-cell dependent antigens as well as the T-cell response to primary protein antigens was shown (Eibl et al., 2002).

It is well known, that early disability and mortality of DM2 patients are mainly conditioned by macrovascular complications (atherosclerosis, coronary artery disease, acute myocardial infarction, stroke, etc), provoking the development of inflammatory response (Huysman & Mathieu, 2009; Bariş et al., 2009). On the other hand, hyperactivation of the immune response triggered by oxidative stress or other factors, results in the dysfunction of the insulin producing β-cells and to insulin resistance (Kaneto et al., 2005; Solinas et al., 2007), thus promoting DM2. There are probably many different causes of DM2. Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur. It is proposed that immune mediated acute phase reactions that are part of the innate immune response are involved in DM2 pathogenesis. This suggestion is supported by a large number of experimental data showing elevated levels of serum amyloid A, C-reactive protein, and cytokines in the blood of DM2 patients (Crook et al., 1993; Pickup & Crook, 1998; Crook, 2004; Donath & Shoelson, 2011). Regarding cytokines, in early stage of DM2 the increase in interleukin (IL)-1 and tumor necrosis factor-α (TNF-α) was observed (Ozer et al., 2003), whereas in long-term DM2 the decreases production of IL-1β and IL-2 by monocytes and T lymphocytes was detected (Geerlings & Hoepelman, 1999). Furthermore, it was shown that poorly controlled diabetics have reduced lymphocyte proliferation in response to different stimuli (Geerlings & Hoepelman, 1999). Long-term DM2 is also characterized by a decreased chemotaxis of polymorphonuclear lymphocytes and monocytes (Delamaire et al., 1997), impaired monocyte adhesion to vascular endothelium (Jialal et al., 2002), and reduced phagocytic activity (Lecube et al., 2012).

In long-term DM accompanied with diabetic complications the presence of antibodies against membrane phospholipids, vascular endothelium (Kluz & Adamiec, 2003), C3d opsonins and membrane attack complex (MAC) and development of the inflammatory reactions in capillary wells (Rosoklija et al., 2000) were detected. In addition, it was shown that CD40–CD40L interactions promote pancreatic and adipose tissue, as well as vascular inflammation in DM2 (Seijkens et al., 2013). The role of the immune response abnormalities in the development of diabetic complications is unclear. Here the main question is whether the immune system disturbances are primary pathogenic factors for DM complications or not.

In our own studies we investigated the functional state of the major mediators of the immune response, immune complexes and the complement cascade, in patients with DM1 and DM2 (Hovsepyan et al., 2002; Hovsepyan et al., 2002; Hovsepyan et al., 2004; Ovsepyan et al., 2004; Hovsepyan et al, 2006; Arakelova et al., 2011). In total, 86 patients with DM1, 110 patients with DM2, and 96 healthy subjects have been involved in the studies. Duration of the illness was 8-10 years; all patients have DM-specific complications, micro- and macroangiopathies.

Formation of immune complexes (IC) is a normal physiological reaction of organism to foreign or autoantigen. IC may interact with both humoral and cellular components of the immune recognition system, activate the complement cascade, and thus affect the immune response on multiple levels (Schifferli
et al., 1986; Ng et al., 1988; Moulds et al., 2009). In healthy conditions IC are easily eliminated from circulation through complement deposition, followed by their opsonization, phagocytosis, and further processing by proteases (Schifferli et al., 1986; Ng et al., 1988; Hebert, 1991; Thornton, 1994). In pathologic conditions inappropriate clearance or deposition of IC result in increased levels of IC in circulation. Circulating IC (CIC) may deposit in endothelial of vascular structures provoking prolonged inflammatory response by permanent activation of the complement cascade through the classical pathway, generation of cytotoxic agents and tissue damage (Theofilopoulos, 1980; McDougal & McDuffie, 1985; Konstantinova, 1996; Shmagel & Chershnev, 2009; Burut et al., 2010). Deposition of CIC is a prominent feature of many diseases characterized by altered immune response and development of inflammatory reactions (Shmagel & Chershnev, 2009; Burut et al., 2010; Theofilopoulos & Dixon, 1980), and plays a decisive role in atherogenesis (Burut et al., 2010). The most aggressive sub-population of CIC is so called “pathogenic” CIC, which may originate in the excess of either antibody or antigen. Pathogenic CIC are smaller in size than classic CIC, are hardly recognized by phagocytes and removed from circulation (Konstantinova, 1996; Cavallo & Granholm, 1990; Monsalvo et al., 2011).

We evaluated the total levels of CIC and the levels of their pathogenic sub-population in the blood of patients with DM1 and DM2 in comparison to healthy controls (Hovsepyan et al.; 2002a; Hovsepyan et al., 2002b; Ovsepyan et al., 2004) using earlier described procedures for isolation of CIC and spectrophotometric determination of their levels (Digeon et al., 1977; Tarnacka et al., 2002). According to the obtained results in both groups of patients the increased levels of both total CIC and pathogenic CIC were detected. Thus the average levels of total CIC and pathogenic CIC in the blood of DM1 patients were 1.87and 2 times, respectively, significantly higher than in controls ($p<0.05$). In case of DM2 patients the average blood levels of total CIC and their pathogenic sub-population were, respectively, 1.67and 4 times significantly higher than in controls ($p<0.05$). A positive correlation between the blood levels of total and pathogenic CIC in DM1 ($r = 0.9, p< 0.05$) and DM2 ($r = 0.85, p<0.05$) was detected indicating that elevation in the total blood CIC levels in DM mainly occurs due to increase in pathogenic species of CIC in their general population.

Further we evaluated the protein composition of total CIC isolated from the blood of DM1 and DM2 patients by sodium dodecyl sulphate (SDS)-gel electrophoresis. According to the obtained results (Figure 1) high level of specificity for protein composition of CIC was detected, when comparing DM1 and DM2 with each other or with other diseased conditions characterized by the increased blood levels of CIC and their pathogenic subpopulation. Here for comparison we include data related to schizophrenia (Hakobyan et al., 2004; Hakobyan et al., 2001), strokes (Boiadzhian et al., 2007; Arakelian et al., 2003), and familial Mediterranean fever (complicated and not complicated with renal amyloidosis) (Mkrtchyan et al., 2002). In each case different distribution of the CIC proteins by molecular weights was detected.

The complement system is major effector of the immune response, which acts on the interface of innate and adaptive immunity and consists of more than 40 soluble proteins (mostly serum glycoproteins), cell surface receptors and regulators. Many of the complement soluble proteins are proenzymes (serine proteases), producing and circulating in inactive forms (zymogens), but activating when cleaved into two peptides, which represent immunoregulatory molecules and inflammatory mediators. Mostly all tissues and organs, including brain, are able to produce complement proteins, but liver is their main source. Being the first line of defense against infections and initiating a variety of cellular and humoral
Figure 1: Composition of CIC in different diseased conditions. Data related to healthy subjects were subtracted from the patients data by computer simulation. Electrophoresis was performed in non-reduced conditions.

reactions and intermolecular interactions, complement represents a cytotoxic host defense system. Complement mediates a variety of effector functions and is a key component and trigger of many immunoregulatory mechanisms. It is a complex cascade involving proteolytic cleavage of its components, soluble proteins, often activated by cell receptors. This cascade ultimately results in induction of the antibody responses, inflammation, phagocyte chemotaxis, and opsonization of immune complexes, foreign pathogens, transformed, apoptotic and necrotic cells or cell debris, facilitating their recognition, clearance, and lysis. Complement exhibits three activation pathways - classical, alternative, and lectin, initiated via separate mechanisms and result in formation of opsonins, anaphylatoxins and chemotaxins, and a single terminal pathway that results in a formation of MAC and subsequent cell lysis (Figure 2) (Sim & Laich, 2000; Cole & Morgan, 2003; Nauta et al., 2004). Changes in the functional activity of the complement cascade contribute to the pathology of many human diseases (Sakamoto et al., 1998; Volanksi & Frank, 1998; Mollnes et al., 2002). The alterations in the complement cascade have been considered as indicator of the implication of inflammatory component in disease etiology, pathogenesis and/or progression (Sakamoto et al., 1998; Volanksi & Frank, 1998; Mollnes et al., 2002).

In our study we assessed functional activity of the complement cascade in DM by determining total hemolytic activities of its classical pathway and hemolytic activities of its individual components, C1, C2, C3, and C4 proteins, in the blood serum of DM1 and DM2 patients and healthy controls (Hovsepyan et al., 2004; Hovsepyan et al., 2006). In addition, in DM2-affected subjects total hemolytic activity of the
Complement activation pathways, and the levels of MAC, final product of the complement activation, were determined (Hovsepyan et al., 2006; Arakelova et al., 2011). C1, C2 and C4 are main components of the classical pathway, and C3 is the initial point for the alternative pathway and a converge point of all three complement activation pathways, starting up for the terminal pathway leading to MAC formation (Figure 2) (Sakamoto et al., 1998; Volankis & Frank, 1998; Mollnes et al., 2002). Hemolytic activities were measured by application of the earlier developed methods (Doods & Sim, 1997; Morgan, 2000; Watford et al., 2000).

According to the obtained results presented in Table 1, mean values of the total hemolytic activity of the complement classical pathway (TC), as well as of the activities of its C1, C3 and C4 components in DM1 and DM2 patients were significantly higher than in healthy controls ($p<0.05$). The detected changes were significantly more pronounced in DM2 patients, compared to DM1 ($p<0.05$). Mean value of the C2 component activity in DM2 patients was also significantly higher than in healthy controls ($p<0.05$), whereas no significant difference in this parameter between DM1 patients and healthy controls was observed ($p<0.05$). In addition, in case of DM2 patients, as compared to healthy subjects, a significantly increased mean level of the total hemolytic activity of the complement alternative pathway (TA) was detected ($p<0.05$).
t activation pathways. This terminal pathway is launched by splitting of the complement protein into two active fragments and is finally leading to formation of MAC (Fig 2). Insertion of MAC into plasmatic membrane results in its perforation and cell lysis. MAC may also trigger apoptosis, initiate production of inflammatory mediators, cytokines, prostaglandins, thromboxanes, leukotrienes, and active forms of oxygen as well as expression of adhesion molecules (Sim & Laich, 2000; Cole & Morgan, 2003; Nauta et al., 2004; Sakamoto et al., 1998; Volankis & Frank, 1998; Mollnes et al., 2002).

According to the obtained results, in patients with DM2 the mean level of MAC was 1.6 times significantly higher than in healthy controls (mean±SD: 11.1±3.0 µg/ml vs. 6.9±1.7µg/ml, respectively; p<0.0007) indicating hyperactivation of the terminal complement cascade. This data is in consistence with the results of earlier reported studies, which demonstrated accumulation of the complement activation intermediate and terminal products, C3-derived opsonins and MAC in endoneurial and retinal microvessels of DM2 patients (Rosoklija et al., 2000; Gerl et al., 2002).

Correlation between the rate of the carbohydrate metabolism compensation and the incidence of micro- and macroangiopathies in DM2 reflects a causal relationship between hyperglycemia and the risk for development of chronic diabetic complications. It was shown that, the risk for development of vascular pathology in DM2 significantly increases even when a slight increase (1%) in glycated hemoglobin level is observed (UKPDS Group, 1998). In this regard, it is interesting that deposition of MAC on the membranes of endothelial cells, apart from cytotoxic effects, also results in the release of the growth factors, which stimulate cell proliferation and under a long-term influence can induce hypertrophy and thrombogenicity of the vessel wall (Sim & Laich, 2000; Cole & Morgan, 2003; Nauta et al., 2004; Sakamoto et al., 1998; Volankis & Frank, 1998; Mollnes et al., 2002). Normally, the MAC production, even in the conditions of the complement system hyperactivation, is controlled by the regulatory protein CD59 capable to inhibit a formation of MAC (Acosta et al., 2004; Huang et al., 2005). However, the experimental data demonstrate that, the glycated CD59 loses its ability to inhibit formation of MAC (Acosta et al., 2000; Qin et al., 2004; Cheng & Gao, 2005). This fact represents particular interest in the light of our

<table>
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<tr>
<th>Study group</th>
<th>Hemolytic activity, % of lysed cells*, mean±SD</th>
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<tr>
<td></td>
<td>TC</td>
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<tr>
<td>DM1 patients</td>
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<tr>
<td>(n=86)</td>
<td>56.5±9.0&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Healthy controls</td>
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<tr>
<td>(n=96)</td>
<td>52.1±7.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>DM2 patients</td>
<td></td>
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<tr>
<td>(n=110)</td>
<td>79.2±12.0&lt;sup&gt;c&lt;/sup&gt;</td>
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* as a target cells, sheep erythrocytes sensitized with rabbit anti-sheep erythrocyte antibody (TC, C1, C2, C3 and C4) and rabbit erythrocytes (TA) were used.<sup>a</sup>-p<0.05, <sup>b</sup>-p<0.01, <sup>c</sup>-p<0.005, <sup>d</sup>-p<0.05, <sup>e</sup>-p<0.002, <sup>f</sup>-p<0.05, <sup<g>-p<0.005, <sup<h>-p<0.05, <sup>i>-p<0.05, <sup>j>-p<0.05, <sup>k>-p<0.05, <sup<l>-p<0.05, <sup<m>-p<0.05, <sup<n>-p<0.05, <sup<o>-p<0.0004, <sup<p>-p<0.05, <sup<q>-p<0.00015, <sup<r>-p<0.05.

We also determined the levels of MAC in the blood of DM2 patients and healthy controls by the enzyme-linked immunosorbent assay (ELISA) (Arakelova, 2011) using earlier developed method (Morgan, 1988). As it was mentioned before, the terminal cascade of the complement activation is similar for all three complement activation pathways. This terminal pathway is launched by splitting of the C3 complement protein into two active fragments and is finally leading to formation of MAC (C5b-9), the terminal product of the complement system activation (Figure 2). Insertion of MAC into plasmatic membrane results in its perforation and cell lysis. MAC may also trigger apoptosis, initiate production of inflammatory mediators, cytokines, prostaglandins, thromboxanes, leukotrienes, and active forms of oxygen as well as expression of adhesion molecules (Sim & Laich, 2000; Cole & Morgan, 2003; Nauta et al., 2004; Sakamoto et al., 1998; Volankis & Frank, 1998; Mollnes et al., 2002).
findings, since, as it is well known, the enhanced smooth muscle cell and retinal endothelial cell proliferation and, as a result, the hypertrophy and thrombogenicity of vascular wall lead to disruption of vascular permeability and development of angiopathies (Huysman & Mathieu, 2009; McMillan, 1997).

Besides that, it was also demonstrated that the DM2 is characterized by intensifications of apoptotic activity in neuronal and vessel cells that stimulates development of retinopathies (Barber et al., 2011; Park et al., 2003). In this regard, taking into consideration our own data, it can be proposed that the products of proteolytic activation of the complement C3 and C4 proteins (C3b, C4b, iC3b and C3dg), which are known triggers of apoptosis (Sim & Laich, 2000; Cole & Morgan, 2003; Nauta et al., 2004; Sakamoto et al., 1998; Volankis & Frank, 1998; Mollnes et al., 2002), can contribute to this pathologic process.

Thus, based on the results of our investigations we concluded that one of the mechanisms of DM2-associated hyperglycemia-induced complications may include glycosylation and subsequent inactivation of the inhibitor of MAC formation.

In summary, it is obvious that long-term DM accompanied with diabetic complications is characterized by hyperactivation of the complement cascade that may be induced by the complex of hormonal, metabolic and genetic alterations observed in DM. On the other hand, the accumulation of complement activation products may in turn provoke further progressing of DM and development of diabetic complications by damaging vascular walls and affecting platelet-vascular and humoral components of hemostasis. This will change the antigenic and functional characteristics of vessels, alter permeability and resistance of their walls, induce development of the immunopathological reactions and finally lead to the narrowing of vessel lumen and reduction of the inner vessel surface, development of edema and endothelial dystrophy. Hyperproduction of chemotactic agents, opsonins, anaphylatoxins and MAC as a result of hyperactivation of the complement system, along with other factors, may, to a large extent, force the progressing of these pathogenic changes.

3 Neuronal Changes in DM

In addition to abnormalities in the immune response DM1 and DM2 are also accompanied by neuropsychological alterations (Ryan et al., 1984; Skenazy et al., 1984; Perlmutter et al., 1984; Ryan et al., 1985; Mooradian, 1988; Perlmutter et al., 1989). The neurologic manifestations most frequently described in association with DM are involved changes of the peripheral nerves and nerve roots and are often accompanied by delays in integrative and sensory transduction processes (Cracco et al., 1980; Harkins et al., 1985; Khardori et al., 1986). On the other hand, it was shown that in absence of acute vascular deficiency DM related structural changes in the central neuronal system (CN S) are not prominent (de Jong, 1977). However, both myelopathy and encephalopathy may be part of the diabetic process (de Jong, 1977; Troisi et al., 1999). DM has a differential effect on different subpopulations of myenteric neurons. DM-related alterations in the enteric nervous system include changes in the inhibitory and excitatory enteric neurons, including loss of inhibitory neurons in early DM enteric neuropathy. The functional consequences of these neuronal changes result in altered gastric emptying, diarrhea or constipation. DM can also affect gastrointestinal motility through alterations in extrinsic neuronal control. Recent research on the neuro–immune interactions demonstrates inflammation-associated neurodegeneration which can lead to motility related problems in DM (Chandrasekharan & Srinivasan, 2007).

According to brain imaging studies DM2 has increased incidence of small vessel disease including white matter lesions and lacunae infarcts (Vermeer et al., 2002) and increased risk of temporal lobe atro-
Reduction of hippocampus and amygdala volumes in DM2 patients compared to non diabetics was shown (Korf et al., 2006; den Heijer et al., 2003) as well as association of DM2 with degeneration of ganglions, demyelization and axon loss (de Jong, 1977; Moscou & Pereant, 2010).

Patients with DM1 have increased activity of hypothalamic-pituitary axis (Asfeldt, 1972; Mooradian, 1997), increased growth hormone secretion in response to exercise and stimulation of dopamine and thyrotropin releasing hormone (Mooradian, 1997; Merimee et al., 1978; Ceda et al., 1982).

In our investigations we determined the activity of a marker enzyme for adrenergic neurons, dopamine β-monooxygenase (DBM; EC 1.14.17.1), and a modulator of β-adrenergic receptor activity, in patients with DM1, DM2, and healthy subjects (Hovsepyan et al., 2002). The enzyme is catalyzing formation of noradrenaline from dopamine, and its activity in the blood reflects central and peripheral adrenergic activity (Beliaev et al., 2009). Study subjects represent the same patients and healthy controls as described in the previous section. Blood activity of DBM was measured according to earlier described spectrophotometric assay using tyramine as a substrate (Nagatsu & Udenfriend, 1972). According to the obtained results the specific activity of DBM in the blood of DM1 patients was in average 5.7 times significantly lower than in healthy controls ($p<0.00000001$). The same applies to DM2 patients; in this case the specific activity of DBM in patients group was in average 9.5 times significantly lower than in healthy controls ($p<0.00000001$). The obtained results are presented in Figure 3 and suggest that long-term DM1 and DM2 are associated with deficient adrenergic activity.

![Figure 3: Specific activity of DBM (SD=±20%) in the blood of patients with DM1 (n=86), DM2 (n=110) and healthy controls (n=96). Control vs. DM1 - $p<0.00000001$; Control vs. DM2 –$p<0.00000001$; DM1 versus DM2 –$p<0.0005$.](image)

Animal studies also revealed a number of neuro-humoral and related biochemical alterations in DM (Mooradian, 1988; Srinivasan & Ramarao, 2007; Islam& Loots, 2009). Experimental DM lowers...
glycogen levels in CNS and promotes glycogen consumption (Srinivasan & Ramarao, 2007; Islam & Loots, 2009). In addition, there were detected changes in brain amino acid patterns as well as in levels of monoamines and their metabolites (Crandall et al., 1981; Crandall & Fernstrom, 1983; Glanville & Anderson, 1986; Chu et al., 1986; Kwok & Juorio, 1986; Bitaret et al., 1986). Furthermore, the increased levels of dopamine in hippocampus and decreased levels of serotonin in hypothalamus and brain stem were observed in diabetic animals (Mooradian, 1997). Although the changes in the blood brain barrier (BBB) are readily demonstrable in animal models of DM in human subjects the changes have not been consistently demonstrable (Horani & Mooradian, 2003). To determine if clinical long-term DM is associated with disruption of the blood–brain barrier (BBB), we measured blood serum levels of antibodies to neuron-specific enolase (NSE; EC4.2.1.11), a peripheral marker of BBB disruption (Selakovic et al., 2005); in the same cohorts of DM1 and DM2 patients using a chemiluminescent Western blot (Hovsepyan et al., 2004). There was detected a significant increase in antibodies to NSE in both DM1 and DM2 subjects compared to controls. This study suggests that DM in humans may be associated with alterations in the BBB integrity that allow the emergence of antibodies against neuronal antigens.

Thus, DM in humans as well as in experimental animals leads to various structural and functional disturbances in nervous system resulting in its dysfunction and BBB damage.

The oxidative stress and production of free radicals are the main causes leading to the development of insulin resistance, β-cell dysfunction, decrease in glucose tolerance, and development of DM. The oxidative stress plays an important role in the pathophysiology of DM and is the common factor in the development of diabetic complications, micro- and macroangiopathies (Wright et al., 2006) and DM-associated neuronal changes (Voukali et al., 2011). We performed a comparative analysis of the functional activity of antioxidant system and intensity of lipid peroxidation process in the blood serum of acute ischemic stroke patients complicated and none-complicated with DM2 (on the first day of stroke onset), DM2 patients, and healthy controls (Tsakanova et al., 2010; Tsakanova et al., 2011). In particular, we determined total activity of non-enzymatic water-soluble low-molecular-weight antioxidants by photochemiluminescent analysis, ferrooxidase activity of ceruloplasmin and the content of lipid hydroperoxides by spectrophotometric assays (Tsakanova et al., 2010; Tsakanova et al., 2011). All patients with DM2 have micro- and macroangiopathies. Duration of the illness was 9-12 years. The obtained results suggest that stroke complicated with DM2 is characterized by significantly higher intensity of the lipid peroxidation process as compared to stroke none-complicated with DM2 (p<0.05), that, probably, is one of the determining factors responsible for more severe clinical course of strokes in patients with DM compared to those who are not suffering from DM. Our study also demonstrated that mechanisms of the compensatory response to oxidative stress on the level of antioxidants in stroke patients complicated with DM2 differ from those detected in stroke none-complicated with DM2. On the base of the obtained results we suggested that metabolic, molecular, and cellular level alterations typical for long-term DM2 impair compensatory mechanisms protecting the body from oxidative.

On the other hand, it is known that diabetic angiopathies of cerebral arteries lead to microcirculation disturbances and contribute to the development of cerebral ischemia. DM is a risk factor for ischemic stroke, and the prevalence of stroke, its severity and frequency of deaths among patients with DM is much higher (Bonow & Gheorghiade, 2004; Tuomilehto et al., 1996). In our own study we investigated the relationship between post-ischemic inflammatory response and the state of BBB in acute stroke progression in patients with acute ischemic stroke complicated and none-complicated with DM2 by measuring the levels of proinflammatory and chemotactic cytokines (IL-1β, IL-6, TNF-α and monocyte chemotactant protein-1 (MCP-1), chemokine (C-X-C motif) ligand 1 (CXCL1), respectively), brain-specific
proteins (NSE and S100b) and antibodies to these proteins in the blood serum of the study subjects on different time points of stroke onset using ELISA and Western blot (Boyajyan et al., 2007; Boyajyan et al., 2008). Patients with long-term DM2 and healthy controls were also involved in this study. All patients with DM2 have micro- and macroangiopathies. Duration of the illness was 9-12 years. According to the obtained results a significant increase of the levels of all the analyzed cytokines in both groups of the ischemic stroke patients was detected on days 1-5 of stroke onset, with the maximum level on day 1. Notably, the levels of cytokines in DM2-complicated patients were significantly higher than in those none-complicated with DM2 (p<0.05). In all groups of patients the presence of antibodies specific to NSE and S100b was detected with highest concentration in case of ischemic stroke patients complicated with DM2. According to the obtained results, significantly increased levels of NSE and S100b were detected on days 1-7 of stroke onset with the maximum level on day 3. As in case of cytokines, here also the levels of NSE and S100b in DM2-complicated patients were significantly higher than in those none-complicated with DM2 (p<0.05). In addition, a positive correlation between the levels of analyzed cytokines, on the one hand, and the levels of NSE and S100b, on the other hand, in ischemic stroke patients, both complicated and none-complicated with DM2, was detected suggesting about the relationship between the intensity of postischemic inflammatory response and BBB disruption.

Therefore, the results of our study suggest that in ischemic stroke complicated with DM2 the systemic inflammatory reactions are more intense than in case of stroke none-complicated with DM2. That may be a result of the initial BBB destruction detected by us in long-term DM2 (Hovsepyan et al., 2004), since our data presented above indicated the presence of positive correlation between the intensity of postischemic inflammatory response and BBB disruption (Boyajyan et al., 2007; Boyajyan et al., 2008). The alteration in BBB integrity may, on the one hand, promote the influx of immunomodulators to the brain and enhance the inflammatory response in the damaged areas of the brain, and, on the other hand, promote a migration of immunocompetent cells and inflammatory mediators from the brain to the peripheral circulation through the brain endothelium triggering the development of the inflammatory reactions on the systemic level.

References


Ozer, G., Teker, Z., Cetiner, S., Yilmaz, M., Topaloglu, A.K., Onenli-Mungan, N. & Yuksel, B. (2003). Serum IL-1, IL-2, TNFalpha and INFgamma levels of patients with type 1 diabetes mellitus and their siblings. J Pediatr Endocrinol Metab, 16(2), 203-10.


