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SCIENCE DIRECT
Experimental and Molecular Pathology 80 (2006) 283–288
Experimental and Molecular Pathology
www.elsevier.com/locate/yexmp

Complement activation in diabetic ketoacidosis brains

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Received 5 December 2005
Available online 21 February 2006

Abstract

The metabolic crisis of diabetic ketoacidosis (DKA) and its treatment can result in the life-threatening complication of clinical brain edema. However, there is limited information available regarding either the pathophysiology or histology of this acute complication. It has been reported that DKA and its treatment are associated with a systemic inflammatory response involving the activation of the complement cascade with increases of SC5b-9 serum level. We studied the brains of two patients, both of whom died as the result of DKA-related brain edema, for the presence of C5b-9, C1q and the expression of the CD59. Apoptosis was also evaluated by the TUNEL method. All regions of the brain demonstrated varying degrees of C5b-9 deposits on neurons, oligodendrocytes and blood vessels. C5b-9 was co-localized with C1q, suggesting the activation of classical pathway. No expression of CD59 was found on neurons, oligodendrocytes or blood vessels in DKA brain, but this complement inhibitor was present on these cells in the normal brain. Rarely, C5b-9 was co-localized with apoptotic neurons and OLG. Our data demonstrate that the metabolic crisis of DKA results in a loss of CD59 expression and assembly of C5b-9 on neurons and oligodendrocytes, suggesting that complement activation and C5b-9 may play a role in the pathophysiology of the brain edema of DKA.

Keywords: Complement activation; C5b-9; CD59; Cerebral edema; Diabetic ketoacidosis; Type 1 diabetes mellitus

Introduction

Activation of the complement system is part of the innate immune response in the brain (Gasque et al., 2000). A role for complement in the development of inflammatory autoimmune diseases, as well as tissue injury and repair, in disorders affecting the central nervous system (CNS) is well established (reviewed by Rus and Niculescu, 2001a). Deposition of complement activation products and C5b-9 has been demonstrated in multiple sclerosis (MS) (Lucchinetti et al., 2000), Alzheimer disease (Tenner, 2001), ischemia/reperfusion injury (D'Ambrosio et al., 2001), Huntington (Singhrao et al., 1999) and Prion disease (Kovacs et al., 2004). The pathophysiology of the complement system can be mediated either directly, by the activation products and the assembly of the C5b-9 complement complex (Esser, 1991), or indirectly, by the components of the complement cascade inducing other factors such as cytokines (Blatteis et al., 2004) and growth factors (Halperin et al., 1993; Rus et al., 1996b). The lytic C5b-9 forms a transmembrane channel which can cause cell death, while assembly of sublytic C5b-9 activates multiple signaling pathways (Carney et al., 1990; Niculescu et al., 1997), induces cell cycle (Rus et al., 1996a) and protects from apoptosis (Soane et al., 2001; Rus et al., 2001b). Unrelated to disruption of the blood–brain barrier (BBB), neurons have been reported to display C5b-9 immunoreactivity in the penumbra of cerebral contusions (Bellander et al., 2001, 2004) and formation of C5b-9 in the cerebral cortex of the rat has cytotoxic effects (Xiong et al., 2003). Oligodendrocytes (OLG), the myelinating cell of the CNS, are also the target of the complement activation (Rus et al., 1996a, 1997). These cells are also sensitive to increased oxidative stress (Ernst et al., 2004) that occurs during both diabetic ketoacidosis and its treatment (Jain et al., 2002; Lee et al., 2002).

Diabetic ketoacidosis and to a greater extent its treatment are associated with a systemic inflammatory response (SIR) (Dalton et al., 2003; Hoffman et al., 2003a,b). The SIR in DKA has recently been reported to include an increase in the plasma concentrations of several bioactive complement peptides and SC5b-9 (Jerath et al., 2005). Neuroradiologic studies

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0014-4800/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexmp.2005.12.007

suggest that a vasogenic component is likely to be involved in the subclinical brain edema that occurs both prior to (Durr et al., 1992; Hoffman et al., 1988) and during the treatment of DKA (Durr et al., 1992; Glaser et al., 2004; Hoffman et al., 1988; Krane et al., 1985). Due to the infrequent occurrence of this life-threatening event (Edge et al., 2001), there is minimal information available regarding the histology of the brain (Dillon et al., 1936) and no immunohistochemical studies on complement activation in the brain of patients with clinical brain edema associated with DKA.

In this study, we investigated the C5b-9 deposition in conjunction with that of C1q and CD59 in the brains of two patients with type 1 diabetes mellitus (T1DM) who died of brain edema during the treatment of severe DKA. We found that C5b-9 and C1q were deposited on neurons, OLG and blood vessels in the absence of CD59 expression, suggesting that C5b-9 might play a role in the pathophysiology of the brain edema of DKA.

Materials and methods

Case 1

An adolescent female had a 4-year history of poorly controlled T1DM, which resulted in recurrent hospitalizations for DKA. The admission was preceded by a 12-h history of abdominal pain and several episodes of emesis. There was no history of fever or enteritis. On physical examination, the patient was orientated but drowsy. Her height was 163 cm; weight was 68.5 kg; blood pressure was 140/70 mm Hg; pulse was 140/min; respirations were 30/min; and temperature was 98.5°F. The mucous membranes were dry, the abdomen was diffusely tender and bowel sounds were hypoactive. There was no evidence of infection. The Tanner stage of puberty was B3 and P3. Admission laboratory tests consisted of a pH 7.10; pCO_2 15 mm Hg; pO_2 106 mm Hg; glucose 810 mg/dl; Na 132 meq/l; K 5.7 meq/l; Cl 93 meq/l; HCO_3 5 meq/l; and BUN 30 mg/dl. Treatment was in a Pediatric Intensive Care Unit, and correction of the hyperglycemia and metabolic acidosis was unremarkable. Twelve hours following the initiation of treatment, the child developed a mild headache. Seven hours later, she developed sudden onset of labored respirations and within 20 min had a cardiorespiratory arrest. An emergency CT scan of the head showed sulcal effacement and cerebral and pontine edema with evidence of herniation. Efforts at resuscitation were unsuccessful, and she was pronounced dead 1.5 h after the cardiorespiratory arrest.

Case 2

An adolescent female had an 8-year history of poorly controlled T1DM, which had resulted in recurrent hospitalizations for DKA. The admission was preceded by an 18-h history of capillary blood glucoses of over 300 mg/dl; ketonuria; a 4-h history of headache; and several episodes of emesis. There was no history of fever or enteritis. On physical examination the patient was slightly confused and lethargic. Her height was 154 cm; weight was 45 kg; blood pressure was 135/68 mm Hg; pulse was 132/min; respiration was 26/min; and temperature was 97°F. Diffuse abdominal tenderness and decreased bowel sounds were present. The Tanner stage of puberty was B5 and P4. There was no evidence of infection. Admission laboratory tests consisted of: pH 7.16; pCO_2 17 mm Hg; pO_2 100 mm Hg; blood glucose 581 mg/dl; Na 130 meq/l; K 4.8 meq/l; Cl 89 meq/l; HCO_3 6 meq/l; and BUN 28 mg/dl. Treatment was in a Pediatric Intensive Care Unit, and correction of the hyperglycemia metabolic acidosis was unremarkable. Ten hours following initiation of treatment, she became unresponsive and was treated with mannitol and had hyperventilation and placed on mechanical ventilation. An emergency CT scan of the head showed diffuse cerebral edema and decreased intercaudate diameter. She was pronounced dead approximately ten hours after the cardiorespiratory arrest.

Control brain tissue arrays were obtained from Pro Sci Incorporated (Poway, CA) and Chemicon (Temecula, CA).

Immunohistochemistry

The paraffin sections were processed as described previously (Niculescu et al., 2004). After xylene deparaffinization and epitope retrieval using a Target Retrieval Solution (DAKO, Carpanteria, CA), sections were treated with 3% H_2O_2 to remove endogenous peroxidase. Sections incubated for 30 min with normal goat serum then with the monoclonal antibody against C5b-9 (Quidel, San Diego, CA) or C1q (Quidel) for 1 h at room temperature (RT) in a humid chamber. In separate experiments, consecutive sections were incubated with mouse monoclonal anti-CD59 (clone MEM-43, Serotec, Oxford, UK). The sections were washed 3 times for 3 min at RT with PBS and then incubated for 1 h at RT with HRP-conjugated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA). After washing, the specific deposits were developed using NovaRed (Vector Laboratories, Burlingame, CA). The nuclei were counter-stained with Mayer's hematoxylin (Sigma Chemical Co, St. Louis). Controls for the specificity of each immunohistochemical reaction were performed by replacing the primary antibody with PBS, mouse or rabbit IgG. Immunostaining was independently evaluated by two investigators.

Analysis of apoptosis

The "ApopTag" Peroxidase in Situ Apoptosis Detection Kit (Intergen Co., Purchase, NY) was used as described (Niculescu et al., 2004). This kit detects DNA strand breaks by measuring terminal deoxynucleotidyl transferase (TdT)-dependent incorporation of dUTP. The sections were incubated for 1 h at 37°C with TdT enzyme, washed, and incubated with the anti-digoxigenin peroxidase-conjugated antibody. The reaction was developed using ImmunoPure metal-enhanced diaminobenzidine tetrachloride (DAB) substrate kit (Pierce, Rockford, IL). Apoptosis was defined as TUNEL positive cells with nuclear fragmentation demonstrated by either karyorhexis or pyknosis.

Results

Histopathology

On gross examination, the brains of both patients were swollen. There was flattening of gyri, narrowing of sulci and uncal and tonsillar herniation. Coronal sections of both brains showed expansion of the white matter with compression of the lateral ventricles. On microscopic examination, there was diffuse pallor of the white matter and neuronal ischemia in the hippocampus of both DKA brains. Only a small percentage of cortical neurons showed ischemic changes (data not shown). No cellular reaction was evident in the cortex or white matter in either case nor was there evidence of cerebral infarction or thrombosis of cerebral vessels in either case. Case 2 showed the features of a non-perfused brain with diffuse pericellular vacuolation in the cortex and white matter.

Immunohistochemical localization of C5b-9 and C1q in DKA brains

C5b-9 was localized by indirect immunoperoxidase in various areas of the brain. Hippocampal neurons exhibit extensive C5b-9-specific deposits (Figs. 1A and C). Similar deposits were found on midbrain, substantia nigra and Purkinje neurons (Figs. 1D–F). C5b-9 deposits co-localized with C1q (Fig. 1D, insert), suggesting that activation of complement occurs through the classical pathway. Controls of the immuno-peroxidase reaction using isotype IgG (Fig. 1B) show no specific deposits in the same area as in Fig. 1A. C5b-9 deposits