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Nerve growth factor plays a role in the neurotherapeutic effect of a CD45$^+$ pan-hematopoietic subpopulation derived from human umbilical cord blood in a traumatic brain injury model

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Abstract

Background aims. Human umbilical cord blood (HUCB) is an important source of stem cells for therapy of hematopoietic disorders and is a potential therapy for various neurological disorders, including traumatic brain injury (TBI). The expression of nerve growth factor (NGF) and its receptors TrkA, p75^{NTR} and α9β1 integrin on an HUCB CD45$^+$ pan-hematopoietic subpopulation was investigated in the context of its neurotherapeutic potential after TBI. *Methods.* NGF and its receptors were detected on CD45$^+$ cells by reverse transcriptase polymerase chain reaction, flow cytometry analysis and confocal microscopy. CD45$^+$ cells were stimulated by TBI brain extracts, and NGF levels were measured by enzyme-linked immunosorbent assay. TBI mice were divided into six groups for xenogeneic intravenous transplantation, 1 day post-trauma, with 1×10^6 CD45$^+$ cells untreated or treated with the anti-NGF neutralizing antibody K252a, a TrkA antagonist; VLO5, an α9β1 disintegrin; or negative (vehicle) and positive (NGF) controls. *Results.* The HUCB CD45$^+$ subpopulation constitutively expresses NGF and its receptors, mainly TrkA and p75^{NTR} and minor levels of α9β1. *In vitro* experiments provided evidence that trauma-related mediators from brain extracts of TBI mice induced release of NGF from HUCB CD45$^+$ cell cultures. HUCB CD45$^+$ cells induced a neurotherapeutic effect in TBI mice, abrogated by cell treatment with either anti-NGF antibody or K252a, but not VLO5. *Conclusions.* These findings strengthen the role of NGF and its TrkA receptor in the HUCB CD45$^+$ subpopulation's neurotherapeutic effect. The presence of neurotrophin receptors in the HUCB CD45$^+$ pan-hematopoietic subpopulation may explain the neuroprotective effect of cord blood in therapy of a variety of neurological disorders.

Key Words: *CD45$^+$ pan-hematopoietic subpopulation, cord blood, nerve growth factor, neuroinflammation, neurotherapeutic effect, TBI, TrkA receptor*

Introduction

Human umbilical cord blood (HUCB) represents an abundant source of pluripotent stem and progenitor cells with high engraftment rates when used for hematopoietic stem cell population replacement and shows low rates of graft-versus-host disease compared with bone marrow (BM) [1]. Approximately 730 000 HUCB units have been donated and stored to date in cord blood banks worldwide, and about 35 000 HUCB transplants have been performed [2] for the treatment of various hematological and oncological disorders [3]. The unfractionated HUCB mononuclear cells (MNC) population is highly heterogeneous [4], including hematopoietic stem/progenitor cells (HSC), myeloid and lymphoid cells [5], as well as small populations of embryonic progenitors [6] and mesenchymal stromal cells [7] containing a subpopulation with the ability to transdifferentiate into neuronal progenitors [8]. Therefore, the spectrum of diseases for which HUCB is effective has been expanded to nonhematopoietic diseases or immune modulations [9]. Clinical trials most commonly focused on therapy of various neurological disorders, indicating safety and partial efficacy [10] and proposing their potential use for cell therapy in traumatic brain injury (TBI).

TBI is a major burden to hospitals in terms of intensive care unit utilization and hospitalizations [11]. Recent estimates indicate that up to 3 million

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individuals in the United States suffer a TBI annually [12], and the World Health Organization states that TBI will be a major health problem and the main reason for disability and mortality in 2020 [13]. Current guidelines for the management of TBI are primarily supportive, with an emphasis on preventive measures to maintain cerebral homoeostasis and reduce and/or prevent secondary brain damage [11,14]. Because the therapeutic drug options remain limited, the pace of TBI preclinical research has accelerated with hope that HUCB therapy will provide a possible therapeutic modality [11]. Since the first proof of concept in preclinical study, indicating that intravenous (IV) administration of HUCB MNC reduced neurological deficit in the rats after TBI [15], additional studies have confirmed this experimental therapeutic approach [16]. In a recent study [17], we demonstrated that intracerebroventricular (ICV) or IV administration of HUCB MNC, 1 and/or 8 days post-trauma, corrected neurobehavioral deficits in a moderate TBI mouse model, suggesting that this therapy could be commenced at any time after the traumatic event. Moreover, we found that the HUCB MNC-derived pan-hematopoietic $CD45^+$, but not $CD45^-$, subpopulation improved the neurobehavioral deficits upon IV transplantation, an effect decreased by pretreatment of the cells with anti-human CD45 antibody. In addition, HUCB $CD45^+$ cells were detected at the site of brain injury 1.5 h after transplantation, and the therapeutic effect was in direct correlation to a reduction in the brain lesion volume [17].

From a mechanistic point of view, it is well established that the secondary brain damage that develops after TBI involves neuroinflammatory processes of different pro- and anti-inflammatory cytokines, as well as neurotrophic factors [18]. Neurotrophins are neurotrophic growth factors secreted from neuronal, glial and hematopoietic cells and are responsible for survival, differentiation and neuroprotection of neuronal networks across the entire brain. More specifically, neurotrophins regulate synaptic plasticity, protect neurons from oxidative stress and apoptosis and can stimulate neurogenesis [19]. The neurotrophin family members include nerve growth factor (NGF), brain-derived neurotrophic factor, neurotrophin (NT)-3, and NT-4/5, which are classified together on the basis of their structural similarity to NGF, the prototype of neurotrophins [19]. Neurotrophins exert their neurotherapeutic effects through three main classes of transmembrane receptors, which include the Trk family of tyrosine kinase receptors, the p75 neurotrophin receptor ($p75^{NTR}$) and the integrin $\alpha9\beta1$. NGF specifically binds to TrkA, brain-derived neurotrophic factor, NT-4/5 to TrkB, and NT-3 to TrkC, and each of these neurotrophins indiscriminately binds either $p75^{NTR}$ receptor and/or integrin $\alpha9\beta1$ [19]. Neurotrophins levels are up-regulated in the brain upon TBI injury [20], and their important role in neural repair and regeneration has been well established [21]. However, among the various neurotrophins, NGF has been the most investigated in TBI both preclinically and clinically. NGF exerts trophic (survival) and tropic (axonal growth) effects on both developing and mature neurons and angiogenic effects on endothelial cells and regulates hematopoietic cells [19]. NGF expression was found to positively correlate with severity and outcome of TBI in children, and its up-regulation after traumatic injury was associated with better neurological outcome [22]. In experimental TBI models, regional brain changes in the expression of neurotrophins and their receptors were observed [23]. For example, NGF was up-regulated after cortical trauma [24] or injection of NGF lentivirus fused-gene attenuated cholinergic memory deficits and rescued cognitive function [25,26], and intranasal delivery of NGF attenuated TBI-induced brain edema [27]. Moreover, transplantation of embryonic neuronal progenitors or BM cells after experimental TBI clearly demonstrated NGF-dependent neurotherapeutic effects, measured by improved cognitive and neuromotor functions [28–30]. Also, HUCB MNC-derived subpopulations have profound neurogenic potential by anti-inflammation, enhanced neurogenesis [31,32], antioxidant activity and release of neurotrophins, such as NGF [33]. Altogether, these studies demonstrate the potential neurotherapeutic effect of NGF and call for evaluation of its role in HUCB $CD45^+$ subpopulation–induced beneficial effects in TBI model.

There is insufficient information on the HUCB $CD45^+$ subpopulation from the perspective of neurotrophic factors (neurotrophins) such as NGF and its receptors' expression, which are relevant for TBI neurotherapy. It is therefore hypothesized that part of the beneficial effects of the HUCB pan-hematopoietic $CD45^+$ subpopulation seen on xenogeneic transplantation in TBI is due to expression of NGF and its receptors that, by autocrine and/or paracrine mechanisms, confers neurotherapeutic effects.

Methods

HUCB collection and separation of the $CD45^+$ subpopulation

HUCB collection was performed as previously described [34] according to a protocol (0336-10-HMO) approved by Hadassah-Hebrew University Medical Center ethical committee. The product was transported to the laboratory and used fresh within 24 h. Thirty-five women gave consent to participate in the study. The $CD45^+$ subpopulation was separated according to a previously reported protocol [34].