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A novel subpopulation of peripheral blood mononuclear cells presents in major burn patients

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ABSTRACT

Hypertrophic scars (HTS) are generally believed to result from proliferation and activation of resident connective tissue fibroblasts after burns. To demonstrate a potential role of blood-borne cells, the peripheral blood mononuclear cells (PBMCs) and the effect of PBMCs on dermal fibroblast behavior was investigated.

Flow cytometry was used to analyze the surface and intracellular protein expression of PBMCs and fibroblasts. Transwell migration assay, enzyme-linked immunosorbent assay and real-time reverse transcription polymerase chain reaction was performed to assess fibroblast functions.

We identified a novel subpopulation of PBMCs in burn patients in vivo that appears at an early stage following major thermal injuries, which primarily express procollagen 1, leukocyte specific protein 1, CD204, toll-like receptor 4 and stromal cell-derived factor 1 (SDF-1) receptor CXCR4. In vitro, the conditioned media from burn patient PBMCs up-regulated the expression of fibrotic growth factors and extracellular matrix molecules, down-regulated antifibrotic factor decorin, enhanced cell chemotaxis and promoted cell differentiation into contractile myofibroblasts in dermal fibroblasts.

After thermal injury, this novel subpopulation of PBMCs is systemically triggered and attracted to the wounds under SDF-1/CXCR4 signaling where they appear to modulate the functions of resident connective tissue cells and thus contribute to the development of HTS.

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Abbreviations: αSMA, alpha smooth muscle actin; P-PBMC, culture media from peripheral blood mononuclear cells of burn patients; COL-1, type 1 collagen; CTGF, connective tissue growth factor; CXCR4, CXC chemokine receptor 4; DCN, decorin; ECM, extracellular matrix; FN, fibronectin; HTS, hypertrophic scar(s); LPS, lipopolysaccharide; LSP-1, leukocyte specific protein 1; C-PBMC, culture media from peripheral blood mononuclear cells of normal individuals; PBMCs, peripheral blood mononuclear cells; SDF-1, stromal cell-derived factor 1; TBSA, total body surface area; TGF-β, transforming growth factor β; Th2, type 2 T helper cell; TLR4, Toll-like receptor 4.

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1. Introduction

Hypertrophic scars (HTS) in humans that follow deep thermal injuries and severe traumas are a dermal fibroproliferative disorder where abnormal extracellular matrix (ECM) accumulation and cellular activity result in raised, red, itchy, firm and symptomatic scar tissue [1,2]. Although the molecular and cellular events that lead to HTS have been extensively studied, the pathogenesis of this condition is still not well understood. HTS are generally believed to result from proliferation and activation of resident connective tissue fibroblasts and myofibroblasts [3] which may migrate from adjacent uninjured cutaneous tissue, originate from resident pericytes and mesenchymal stem cells [4], or are derived from blood-borne fibroblast-like cells (fibrocytes) in the circulation [5–9].

The idea that matrix-producing cells could be derived from peripheral blood mononuclear cells (PBMCs) was suggested by Metchnikov and others [10,11]. Circulating fibrocytes identified in 1994 in the context of wound repair were described previously as a subpopulation of PBMCs and are unique bone marrow-derived mesenchymal progenitor cells that are implicated in the pathogenesis of fibrotic diseases in diverse organs including liver, lungs, skin and kidneys [12]. They express markers of leukocytes, hematopoietic progenitor cells and fibroblasts as well as a number of other markers including chemokine receptors and adhesion molecules, but do not normally express CD14 [13].

Stromal cell-derived factor 1 (SDF-1) is a small cytokine belonging to the chemokine family, which is also called CXCL12, and is often induced by pro-inflammatory stimuli such as lipopolysaccharide (LPS), tumor necrosis factor (TNF), or interleukin-1 (IL-1). It was originally identified as a bone marrow SDF [14] from stromal cells which includes immune cells, pericytes, endothelial cells, inflammatory cells and fibroblasts. SDF-1 has been found to be a potent chemoattractant for lymphocytes and monocytes in vitro and subsequently in vivo [15] and functions by binding to its receptor, CXCR4 [16]. Emerging experimental evidence of wound healing research indicates that circulating fibrocytes mobilized from the bone marrow may contribute significantly to wound healing and hypertrophic scarring [17,18]. Fibrocytes are believed to migrate into inflamed tissue, such as the wounded dermis the SDF-1/CXCR4 signaling axis. It has been shown that circulating fibrocytes can rapidly enter the sites of injuries, not only producing ECM molecules, but also regulating the functions of the surrounding cells. These cells secrete inflammatory cytokines, growth factors and chemokines, present antigens, stimulate angiogenesis, contribute to wound contraction, and synthesize collagen and fibronectin (FN) [19].

Of the PBMCs, macrophages also appear to be of fundamental importance in the development of post-burn immune dysfunction because they are the major producers of pro-inflammatory mediators and the productive capacity for these mediators is markedly enhanced following thermal injury. Studies suggest that γ/δ T-cells and alterations in cAMP-dependent processes in part mediate the expression of macrophage hyperactivity post-burn [20].

Thus, we hypothesize that a special subset of blood-borne cells originated in responding to injuries may recruit from circulation into wound sites through chemokine pathways in the early stages following the burns, where they interact with resident cells and lead to the development of dermal fibrosis.

2. Materials and methods

2.1. Patients

Blood samples were collected within 2 weeks following burns from four major burn patients with ≥50% total body surface area (TBSA) and six normal individuals. Normal fibroblasts were cultured from normal skin of a burn patient with 25% TBSA. Detailed demographic information of patients and control individuals is listed in Table 1. The patients were treated at the Firefighter's Burn Treatment Unit and Outpatient Burn Clinic at the University of Alberta Hospital. The Health Research Ethics Board of the University of Alberta

Table 1 – Demographic information of burn patients and normal individuals.

	Age (year)	Gender (M/F)	Diagnosis	TBSA (%)	Collection time (day post injury)	Sample	HTS
P1	29	M	Burn	90	1	PBMCs	Yes
P2	41	M	Burn	70	3	PBMCs	Yes
P3	21	M	Burn	60	4, 9	PBMCs	Yes
P4	34	M	Burn	50	11	PBMCs	Yes
Mean ± SE	31.3 ± 3.7 years	4M		67.5 ± 7.4%	5.4 ± 1.6 days		
C1	46	M	NA	NA	NA	PBMCs	No
C5	41	F	NA	NA	NA	PBMCs	No
C2	21	M	NA	NA	NA	PBMCs	No
C3	26	M	NA	NA	NA	PBMCs	No
C4	28	M	NA	NA	NA	PBMCs	No
C5	34	M	NA	NA	NA	PBMCs	No
Mean ± SE	32.7 ± 3.9 years	5M/1F					
P5	39	M	Burn	25	NA	Fibroblasts	Yes

P, patient; C, control; M, male; F, female; HTS, hypertrophic scar; PBMCs, peripheral blood mononuclear cells; NA, not applicable.