CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

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J Dent Res 91(11):1011-1018, 2012

Human Oral Mucosa and Gingiva: A Unique Reservoir for Mesenchymal Stem Cells

ABSTRACT

Mesenchymal stem cells (MSCs) represent a heterogeneous population of progenitor cells with self-renewal and multipotent differentiation potential. Aside from their regenerative role, extensive *in vitro* and *in vivo* studies have demonstrated that MSCs are capable of potent immunomodulatory effects on a variety of innate and adaptive immune cells. In this article, we will review recent experimental studies on the characterization of a unique population of MSCs derived from human oral mucosa and gingiva, especially their immunomodulatory and anti-inflammatory functions and their application in the treatment of several *in vivo* models of inflammatory diseases. The ease of isolation, accessible tissue source, and rapid *ex vivo* expansion, with maintenance of stable stem-cell-like phenotypes, render oral mucosa- and gingiva-derived MSCs a promising alternative cell source for MSC-based therapies.

KEY WORDS: gingival-derived mesenchymal stem cells, oral mucosa, multipotency, immunomodulation, inflammatory disease, regeneration.

INTRODUCTION

Mesenchymal stem cells (MSCs) represent a heterogeneous population of non-hematopoietic stem cells, which were first characterized from bone marrow (Luria *et al*., 1971) and subsequently identified from various adult tissues, including oral tissues (Gronthos *et al*., 2000; Miura *et al*., 2003; Zhang *et al*., 2009). Originally, because of their multipotent capabilities, MSCs were regarded as the major source of reparative progenitor cells in tissue engineering to replace damaged tissues (Hermann *et al*., 2006; Kuroda *et al*., 2010). However, such a paradigm has been challenged by recent findings that only a very small proportion of MSCs engrafted at the injured sites could differentiate into the types of resident cells essential for the replacement of damaged tissues (Prockop, 2009; Prockop and Oh, 2012); this evidence suggests that soluble mediators or direct interaction with host cells may contribute mainly to the therapeutic effects of transplanted MSCs (Roddy *et al*., 2011).

The trophic property of MSCs allows them to 'home' to the inflammatory site, where they become activated and produce an array of bioactive mediators with various biological functions (English and Mahon, 2011; Lee *et al*., 2011; Prockop and Oh, 2012). Accumulating evidence suggests that the immunomodulatory and anti-inflammatory functions of MSCs are flexible or plastic depending on their distinct tissue origins, the types of targeted immune cells, and specific pathophysiological settings (English and Mahon, 2011; Lee *et al*., 2011). Additionally, the lack of expression of MHC class II molecules and most of the classical co-stimulatory molecules may contribute to the low immunogenicity or immune privilege of MSCs (Salem and Thiemermann, 2010). In comparison with the well-studied MSCs derived from bone marrow (BMSC) and adipose tissues (ADSC), the immunomodulatory properties of MSCs derived from oral tissues remain largely unexplored. Herein, we focus on the characteristics of a unique population of MSCs derived from human gingiva and oral mucosa and their promising role as an easily accessible and feasible alternative source of MSCs in the treatment of several inflammation-related diseases.

GINGIVA AND ORAL MUCOSA: EXPRESSION OF STEM-CELL-RELATED GENES

The gingiva and oral mucosa share similarities to skin in histological structures and biological functions, specifically, oral defense and resistance to shear stress or friction (Stephens and Genever, 2007). However, the gingiva, in addition to its unique microenvironmental niche fueled by food residues,

DOI: 10.1177/0022034512461016

Received June 6, 2012; Last revision August 16, 2012; Accepted August 17, 2012

microbial flora, and saliva, has also been recognized for its sensitivity to inflammation, fibrosis response, and proneness to drug-induced overgrowth (Nakasone *et al*., 2009; Garlet, 2010). These biological properties suggest that MSCs derived from gingiva might possess some intrinsic properties distinct from those of oral mucosa-derived MSCs (Tang *et al*., 2011). Recent studies have shown that a population of clustered cells in the lamina propria layer of human gingiva displays positive signals for pluripotency-related markers, Oct-4, SSEA-4, and Stro-1 (Zhang *et al*., 2009; Tang *et al*., 2011), with some co-expressing Oct-4/SSEA-4 or Oct4/Stro-1 (Zhang *et al*., 2009). Additionally, the human oral mucosal/gingival lamina propria (OMLP) has been shown to harbor a population of cells positive for low-affinity neurotrophin (p75), a marker of neural stem cells, organized in cord-like structures that are also positively stained for Oct-4 and Sox2 (Marynka-Kalmani *et al*., 2010). These findings suggest that human oral mucosa and gingival tissues harbor progenitors or adult stem cells; however, the potential biological differences between these 2 related populations of oral MSCs remain to be determined.

CHARACTERIZATION OF MSCS FROM HUMAN ORAL MUCOSA AND GINGIVA

While progenitor cells isolated from the subepithelial layers of oral mucosa and gingival have been designated under different terms—*i.e*., gingiva-derived mesenchymal stem/stromal cells (GMSCs) (Zhang *et al*., 2009; Tang *et al*., 2011; Wang *et al*., 2011), gingival-tissue-derived stem cells (GT-MSCs) (Tomar *et al*., 2010), gingival multipotent progenitor cells (GMPCs) (Fournier *et al*., 2010), human oral mucosa stem cells (hOMSCs) (Marynka-Kalmani *et al*., 2010), and oral mucosa lamina propria progenitor cells (OMLP-PCs) (Davies *et al*., 2010)—they share similarities in MSC-associated properties.

Self-renewal

The self-renewal capabilities of human oral mucosa- and gingival propria-derived MSCs have been demonstrated by CFU-F assay (Zhang *et al*., 2009; Davies *et al*., 2010; Fournier *et al*., 2010; Marynka-Kalmani *et al*., 2010; Mitrano *et al*., 2010; Tomar *et al*., 2010; Tang *et al*., 2011; Wang *et al*., 2011). Importantly, human oral mucosa- and gingiva-derived MSCs invariably display a higher proliferation rate than do BMSCs (Zhang *et al*., 2009; Davies *et al*., 2010; Marynka-Kalmani *et al*., 2010; Tomar *et al*., 2010; Tang *et al*., 2011), which was likely attributed to the constitutive expression of human reverse telomerase transcriptase (hTERT) (Zhang *et al*., 2009; Davies *et al*., 2010). Moreover, the *in vivo* self-renewal capacity of gingiva-derived MSCs has been demonstrated by serial subcutaneous (s.c.) transplantation in immunocompromised mice (Zhang *et al*., 2009; Tang *et al*., 2011). These findings support that a population of MSCs with potent self-renewal and proliferative potentials can be readily isolated from human oral mucosa and gingival tissues and reliably expanded *ex vivo* for large-scale culture.

Multipotent Differentiation

Like BMSCs and ADSCs, human oral mucosa-/gingiva-derived MSCs can also differentiate into osteoblasts, adipocytes, and chondrocytes under specific *in vitro* differentiating conditions (Zhang *et al*., 2009; Davies *et al*., 2010; Fournier *et al*., 2010; Marynka-Kalmani *et al*., 2010; Mitrano *et al*., 2010; Tomar *et al*., 2010; Tang *et al*., 2011; Wang *et al*., 2011). In addition to these tri-lineage potentials, oral mucosa-/gingiva-derived MSCs are capable of differentiating into endodermal and ectodermal lineages, including various types of neural cells (Zhang *et al*., 2009; Davies *et al*., 2010; Marynka-Kalmani *et al*., 2010). As found *in vivo*, oral mucosa-/gingiva-derived MSCs embedded with carriers and subcutaneously transplanted into immunocompromised mice can generate connective tissue-like structures (Zhang *et al*., 2009; Tang *et al*., 2011), bone matrix (Fournier *et al*., 2010; Wang *et al*., 2011) and even 2 germ-layer-derived (teratoma-like) tissues (Marynka-Kalmani *et al*., 2010).

Expression of a Panel of MSC-associated Cell-surface Markers

Despite the lack of a specific cell-surface marker for adult MSCs of distinct tissue origins (Nombela-Arrieta *et al*., 2011), they invariably express a panel of mesenchymal cell markers such as CD73, CD90, CD105, and CD44 but are negative for endothelial and hematopoietic markers such as CD31, CD34, and CD45 (Dominici *et al*., 2006). Similarly, human oral mucosa- and gingiva-derived MSCs consistently express CD29, CD44, CD73, and CD90 (> 80%) and are negative for CD34 and CD45, but are positive for CD105, CD146, and Stro-1 in variable population subsets (Table).

Collectively, these fundamental biological properties conferred by human oral mucosa-/gingiva-derived progenitor cells fit the minimal criteria for human MSCs as proposed by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (Dominici *et al*., 2006). Lately, several studies have suggested the potential neural crest origin of this unique population of MSCs (Zhang *et al*., 2009; Davies *et al*., 2010; Marynka-Kalmani *et al*., 2010); however, like other heterogeneous populations of tissue-resident MSCs, the *in vivo* identity and physiological functions of oral mucosa- and gingiva-derived MSCs remain largely unclear.

IMMUNOMODULATORY AND ANTI-INFLAMMATORY PROPERTIES OF HUMAN ORAL MUCOSA-/GINGIVA-DERIVED MSCS

While the self-renewal and multipotent differentiation capabilities of human oral mucosa-/gingiva propria-derived MSCs have been well-characterized, their immunomodulatory and anti-inflammatory functions remain unexplored relative to BMSCs and ADSCs. Most recently, our group has performed serial *in vitro* and *in vivo* studies to investigate the immunomodulatory effects of human gingiva-derived MSCs (GMSCs) and their interplay with various types of innate and adaptive immune

Table. Cell Surface Marker Profiles of Human Gingiva/Mucosa-derived MSCs

CD29 (%)	CD44 (%)	CD73 (%)	CD90 (%)	CD105 (%)	CD106 (%)	CD13 (%)	CD146 (%)	CD166 (%)	SSEA-4 (%)	Stro-1 (%)	HLA-DR(%)	CD34 (%)	CD45 (%)	References
99.8		99.9	100	29.9			7.1		36.9	18.3			0.1	Zhang *et al.*, 2009
78.74	95.25	98.03	98.32	97.16								3.37	3.21	Tomar *et al.*, 2010
100	100	100	100	100			3-17			35	0	0	0	Fournier *et al.*, 2010
	99.4	98.98	99.52	96.1		99.48						0.95	0.85	Mitrano *et al.*, 2010
99.98			92.87	34.75						17.89		0.01	0.41	Wang *et al.*, 2011
82.4	90		76.4	92.5			93.3			75.6		0.3	0.5	Tang *et al.*, 2011
95.04		96.98	97.87	96.64	35.37		14.2	98.94		35.87	0	0	0	Marynka-Kalmani *et al.*, 2010

cells, as well as their potential clinical application in the treatment of several inflammation-related disease models in mice.

Effects of GMSCs on T-cells

GMSCs exhibit potent suppressive effects on the proliferation and activation of human peripheral blood mononuclear cells (PBMC) stimulated either by phytohemagglutinin (PHA) (Zhang *et al*., 2009) or allogenic lymphocytes in mixed lymphocyte reactions (MLRs) (Mitrano *et al*., 2010; Tang *et al*., 2011). GMSCs suppress PHA-stimulated T-lymphocyte proliferation and activation in a cell-cell contact-independent manner, apparently mediated *via* IDO (Zhang *et al*., 2009); whereas the inflammatory cytokine IFN-γ secreted by activated T-lymphocytes in the co-culture system serves as a feedback signal in the cross-talk between GMSCs and T-cells (Zhang *et al*., 2009) (Fig. 1). Davies *et al*. have recently reported that oral mucosa lamina-propria-derived progenitor cells induced inhibitory effects on activated T-lymphocytes independent of cell-cell contact, cell dose, or apoptosis, while IFN-γ or co-culture with T-lymphocytes also led to the up-regulation of IDO expression (Davies *et al*., 2012). Similar immunomodulatory mechanisms mediated by elevated IDO have also been reported for other types of oral MSCs, particularly human periodontal ligament stem cells (Wada *et al*., 2009). Additionally, findings from both *in vitro* and *in vivo* studies have indicated that GMSCs could significantly inhibit Th17 cells and simultaneously promote the expansion of $CD4^+CD25^+FoxP3^+$ regulatory T-cells (Tregs) (Zhang *et al*., 2009, 2010; Su *et al*., 2011; Tang *et al*., 2011). However, further studies are needed to elucidate the underlying mechanisms of interplay between gingiva-derived MSCs and specific types of T-helper cells.

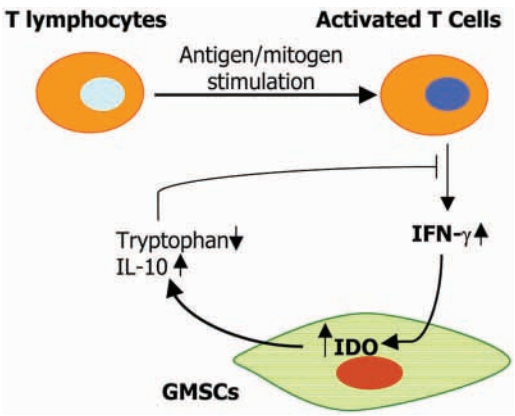

Figure 1. Potential interactions between activated T-lymphocytes and gingiva-derived MSCs. In response to antigen or mitogen stimulation, T-lymphocytes are activated and secrete the pro-inflammatory cytokine, interferon (IFN)-γ. Upon stimulation by IFN-γ, GMSCs express increased levels of IDO and IL-10, which subsequently dampen the pro-inflammatory function of activated T-cells. IDO, indoleamine 2, 3-dioxygenase.

Effects of GMSCs on Innate Immune Cells

The innate immune system is the first line of host defense, which consists of several types of innate immune cells (Galli *et al*., 2011). Similar to BMSCs (English and Mahon, 2011; Lee *et al*., 2011), GMSCs exhibit potent immunomodulatory effects on several types of innate immune cells, particularly dendritic cells (DCs), macrophages, and mast cells (Zhang *et al*., 2010; Su *et al*., 2011).

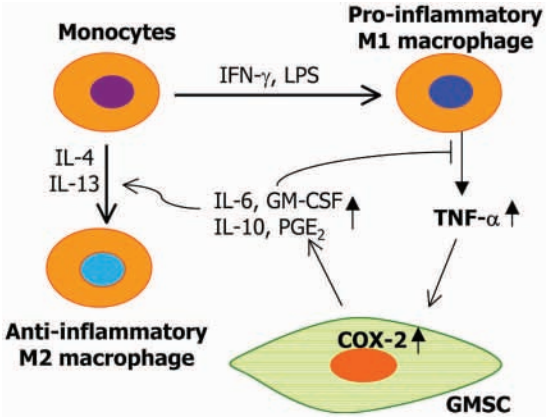

Figure 2. Potential interactions between macrophages and gingiva-derived MSCs. Activated by IFN-γ, TNF-α, or LPS, M1 macrophages produce TNF-α; which positively feeds back on MSCs to increase a variety of immunosuppressive or anti-inflammatory factors, some of which negatively regulate the M1 inflammatory responses. Other immunosuppressive factors produced by GMSCs promote the polarization of the M2 phenotype or the conversion of M1 to M2 macrophages. LPS, lipopolysaccharides; COX-2, cyclooxygenase-2; PGE2, prostaglandin E_2.

Dendritic Cells

Dendritic cells (DCs) can initiate and regulate effector T-cell activation and subsequently serve as major antigen-presenting cells that link the innate and adaptive immune responses (Galli *et al*., 2011). Previous studies have shown that MSCs possess profound capabilities to inhibit the maturation and activation of DCs under different settings (Spaggiari *et al*., 2009; Chiesa *et al*., 2011; Choi *et al*., 2012; Kapoor *et al*., 2012). Similarly, human GMSCs can significantly blunt the maturation and activation of DCs through the production of prostaglandin E_2 (PGE_2) (Su *et al*., 2011). This is in agreement with previous findings that MSC-derived PGE_2 plays a central role in BMSC-mediated inhibition of monocyte-derived DC maturation and functions (Spaggiari *et al*., 2009).

Macrophages

Macrophages constitute another essential cellular component of innate immune responses (Galli *et al*., 2011), which are generally categorized into M1 and M2 macrophages. Usually, M1 macrophages display pro-inflammatory properties, while M2 macrophages are considered to be anti-inflammatory because of their increased production of anti-inflammatory cytokines such as IL-10 and TGF-β (Laskin *et al*., 2011). Recent evidence has suggested an essential role of MSCs in modulating the phenotype and function of macrophages (Kim and Hematti, 2009; Nemeth *et al*., 2009; Bartosh *et al*., 2010; Maggini *et al*., 2010; Nakajima *et al*., 2012). Mice BMSCs have been shown to re-polarize macrophages from a pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype with enhanced interleukin-10 production (Nemeth *et al*., 2009), and co-culture with mouse BMSCs led to the conversion of activated macrophages to a regulatory-like profile (Maggini *et al*., 2010). In these studies, the secretion of PGE_2 by MSCs was critical in the MSC-mediated phenotype conversion of macrophages (Nemeth *et al*., 2009; Maggini *et al*., 2010). Similarly, co-culture with human BMSCs triggers acquisition of M2 phenotype characterized by up-regulated expression of IL-10, increased phagocytic ability, and a decreased expression of pro-inflammatory cytokines (Kim and Hematti, 2009). Human BMSCs could also promote the alternative activation of infiltrated rat macrophage when they were locally transplanted at the injured spinal cord site (Nakajima *et al*., 2012). Additionally, MSC-mediated polarization of M2 macrophages displays increased phagocytic and antimicrobial activities (Kim and Hematti, 2009; Nemeth *et al*., 2009; Maggini *et al*., 2010; Zhang *et al*., 2010), which may contribute to the emerging role of MSCs in host defense against infectious challenges (Auletta *et al*., 2012), as evidenced in a mouse model for sepsis (Nemeth *et al*., 2009; Krasnodembskaya *et al*., 2012) and zymozan-induced peritonitis (Bartosh *et al*., 2010; Choi *et al*., 2011). Likewise, GMSCs were shown to be capable of polarizing macrophages into the M2 phenotype *via* enhanced secretion of IL-6 and GM-CSF (Zhang *et al*., 2010) (Fig. 2). Given the unique anatomic location of oral mucosa and gingival MSCs in the oral cavity, a complex ecosystem that contains a diverse assemblage of micro-organisms with different pathogenic potentials, it would be conceivable to further investigate whether GMSCs are capable of antimicrobial activity as compared with BMSCs.

Mast Cells

Mast cells (MCs) are critical innate immune effector cells in allergic and inflammatory disorders (Sayed *et al*., 2008). To date, the immunomodulatory effect of MSCs on MCs is largely unknown. Most recently, it has been shown that mouse BMSCs and human GMSCs exhibit striking suppressive effects on specific functions of MCs *in vitro* and *in vivo* (Brown JM *et al*., 2011; Su *et al*., 2011). We found that human BMSCs and GMSCs suppressed *de novo* synthesis of the major pro-inflammtory cytokine, TNF-α, from activated human HMC-1 mast cells in a cell-cell contact-independent manner; however, it had no obvious inhibitory effects on their degranulation *in vitro* (Su *et al*., 2011). However, mouse BMSCs suppressed not only the production of pro-inflammatory cytokines by MCs, but also their degranulation, chemokinesis, and chemotaxis (Brown JM *et al*., 2011). Such discrepancies in MSC-mediated inhibitory effects on MCs may be due to the distinct cell contexts of both MSCs and MCs. However, in both studies, *in vivo* administration of BMSCs or GMSCs led to the suppression of MC degranulation in mouse skin and the peritoneal cavity (Brown JM *et al*., 2011; Su *et al*., 2011). The inhibitory effects of both human GMSCs and mouse BMSCs on MC functions were dependent on the COX2/PGE_2 pathway (Brown JM *et al*., 2011; Su *et al*., 2011), and were facilitated through the activation of EP4 receptors in mouse MCs (Brown JM *et al*., 2011). These findings suggest that the TNF-α/COX2/PGE_2 axis constitutes a negative feedback loop in the cross-talk between GMSCs and MCs (Su *et al*., 2011) (Fig. 3) and highlight the immunomodulatory functions of BMSCs and GMSCs on MCs and their potential application in cell-based therapy for MC-driven inflammatory diseases.

TREATMENT OF ANIMAL MODELS OF WOUND HEALING AND INFLAMMATORY DISEASES WITH HUMAN GMSCS

The compelling findings that human oral mucosa-/gingiva-derived MSCs possess potent immunomodulatory effects on several types of innate and adaptive immune cells prompted us to further explore their *in vivo* immunomodulatory functions and therapeutic effects in several inflammation-related disease models in mice.

Wound Healing

Wound healing is a complex process involving the participation of many types of immune and resident cells. Using a chemotherapy-induced oral mucositis (OM) mouse model, a compromised wound model in oral mucosa, we showed that systemic infusion of human GMSCs could mitigate the pathology of OM, as evidenced by reversal of body weight loss and restoration of the disrupted epithelial lining and proliferative basal cells (Zhang *et al*., 2011). In addition, Wang *et al*. found that local application of human GMSCs could significantly promote the repair of mandibular wounds and calvarial defects in rats (Wang *et al*., 2011). In a murine excisional full-thickness skin wound model, systemic infusion of human GMSCs significantly accelerated the repair process, as evidenced by rapid re-epithelialization and increased angiogenesis (Zhang *et al*., 2010). Compared with normal skin, increased numbers of infused MSCs were detected at the wound bed, where they were close to and interacted with resident macrophages, potentially contributing to their conversion to an anti-inflammatory M2 phenotype (Zhang *et al*., 2010). Meanwhile, systemic infusion of GMSCs significantly suppressed the local infiltration of inflammatory cells and pro-inflammatory cytokines such as TNF-α and IL-6, but simultaneously increased IL-10 (Zhang *et al*., 2010). These findings suggest that GMSCs enhance skin wound healing by promoting polarization of infiltrated monocytes or reprogramming resident macrophages into the M2 phenotype, thus preparing a special microenvironment for tissue repair and remodeling.

Dextran Sulfate Sodium (DSS)-induced Murine Colitis

The immunomodulatory and anti-inflammatory effects of GMSCs were also tested in a dextran sulfate sodium (DSS)-induced murine colitis model, in which Th1 and Th17 cells play an essential role (Brown JB *et al*., 2012). Systemic administration of GMSCs could reverse body weight loss, improve the overall colitis score, and restore normal intestinal architecture (Zhang *et al*., 2009). At the cellular level, GMSC treatment strikingly reduced the infiltration of $CD4^+IFN\gamma^+$ (Th1) and $CD4^+IL\text{-}17^+$ (Th17) cells at the colitic sites, and increased the recruitment of Tregs. At the molecular level, GMSCs remarkably suppressed pro-inflammatory cytokines such as IL-6, IL-17, and IFN-γ and increased IL-10 (Zhang *et al*., 2009). These findings suggest that GMSCs ameliorate inflammation-related tissue destruction caused by experimental acute colitis by suppressing the pro-inflammatory function of Th1 and Th17 cells and promoting the infiltration of Tregs.

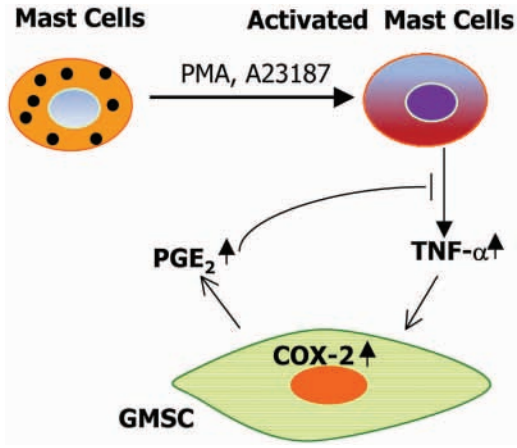

Figure 3. Potential interactions between activated mast cells and gingiva-derived MSCs. In response to PMA stimulation, activated mast cells synthesize and secrete the pro-inflammatory cytokine, TNF-α, which acts on GMSCs to induce increased levels of COX-2 and PGE-2. These factors negatively feed back and dampen the pro-inflammatory activity of activated mast cells. PMA, phorbol 12-myristate 13-acetate; COX-2, cyclooxygenase-2; PGE2, prostaglandin E_2.

Allergy-related Inflammatory Diseases

The pathological process of allergic contact dermatitis (ACD) or contact hypersensitivity (CHS) is comprised of multiple overlapping stages characterized by a dynamic and complex cellular network, including dendritic cells, $CD8^+$ T-cells, $CD4^+IFN\gamma^+$ (Th1), $CD4^+IL\text{-}17^+$ (Th17), mast cells, and Tregs, as well as their cytokines (Vocanson *et al*., 2009; Fonacier *et al*., 2010). Using a hapten (oxazolone)-induced murine CHS model, we showed that both prophylactic and therapeutic administration of GMSCs could mitigate clinical signs of CHS (Su *et al*., 2011). Following GMSC treatment, we observed a reduced infiltration of dendritic cells (DCs), $CD8^+$ T-cells, Th17, total and degranulated mast cells (MCs), a decreased level of a variety of inflammatory cytokines, and a reciprocal increased infiltration of Tregs and expression of IL-10 at regional lymph nodes and inflammatory areas. The underlying mechanism of GMSC-mediated attenuation of CHS involves the $COX2/PGE_2$ axis (Su *et al*., 2011). These findings suggest that GMSCs suppress CHS through targeting multiple types of innate and adaptive immune cells (Su *et al*., 2011), and the use of MSCs in cell-based therapy potentially contributes a novel modality for the treatment of allergic diseases.

Mouse Skin Allograft Model

Aside from our *in vivo* studies on cutaneous wound healing and inflammatory diseases, Tang *et al*. have recently reported that systemic infusion of GMSCs exhibited remarkable immune tolerance and promoted the survival of skin allografts, whereby the increased infiltration of Tregs may play a major role (Tang *et al*., 2011). These immunosuppressant capabilities in the graft *vs*. host disease model further extend the clinical spectrum based on the unique immunomodulatory functions conferred by GMSCs.

ROLE OF HUMAN ORAL MUCOSA-/GINGIVA-DERIVED MSCS IN TISSUE REGENERATION

Recently, accumulating evidence has challenged the previous paradigm that MSCs mediate tissue regeneration by virtue of their multipotent capabilities that enable them to replace damaged cells (Hermann *et al*., 2006; Kuroda *et al*., 2010). More studies have supported the new paradigm that MSCs promote tissue regeneration specifically through interaction with host/resident cells and production of a large array of trophic factors, capable of immunomodulatory and anti-inflammatory functions (Prockop, 2009; Roddy *et al*., 2011; Prockop and Oh, 2012). Despite the reported multipotent capabilities of oral mucosa- and gingiva-derived MSCs, both *in vitro* and *in vivo* (Zhang *et al*., 2009; Davies *et al*., 2010; Fournier *et al*., 2010; Marynka-Kalmani *et al*., 2010; Mitrano *et al*., 2010; Tomar *et al*., 2010; Tang *et al*., 2011; Wang *et al*., 2011), evidence supporting their direct role in tissue regeneration or replacement remains scanty. Using a chemotherapy-induced oral mucositis model, we demonstrated that only a very few GMSCs were found to 'home' to the injured sites and transdifferentiate into epithelial-like cells (Zhang *et al*., 2011). Mechanistically, the regenerative effects mediated by cultured GMSCs might be due to an increased expression of various chemokines and growth factors, as well as an increased resistance to oxidant stress-induced apoptosis (Zhang *et al*., 2011). In mouse models of skin wound and colitis, we showed that the mechanisms underlying GMSC-mediated acceleration of cutaneous and intestinal healing and regeneration may involve both pro-angiogenic and anti-inflammatory functions (Zhang *et al*., 2009, 2010). These findings further support that GMSCs, like other MSCs, may have promoted tissue regeneration *via* their trophic factors, not just their multipotent capabilities. Previous studies have implied that basal fibroblast growth factor (bFGF) can stimulate BMSCs to regenerate both bone and soft tissues, thus serving as an important growth factor for tissue regeneration (Sahoo *et al*., 2010; Tasso *et al*., 2012). However, its effect on GMSCs remains to be determined.

GMSCs *vs.* GINGIVAL FIBROBLASTS

Fibroblasts are the most abundant stromal cells in the connective tissue proper. It appears that fibroblasts share several common features with MSCs, including a spindle-like cell morphology, plastic adherence, and overlapping cell-surface-marker profile (Haniffa *et al*., 2009). Some studies have reported that fibroblasts derived from different tissue origins can exhibit multilineage differentiation potentials (Lysy *et al*., 2007; Sudo *et al*., 2007; Lorenz *et al*., 2008; Bouffi *et al*., 2011) and immunomodulatory functions (Haniffa *et al*., 2007; Cappellesso-Fleury *et al*., 2010; Bouffi *et al*., 2011; Pinchuk *et al*., 2011; Wada *et al*., 2011). Recent studies indicated that a population of MSC-like cells enriched from gingiva-derived fibroblasts grown on chitosan membranes expressed increased *Stro-1, Oct4, Nanog*, and *Sox-10* and enhanced chondrogenic differentiation (Hsu *et al*., 2012a,b). Mostafa *et al*. have reported that human gingival fibroblasts (HGFs) can be induced to differentiate into osteocytes *in vitro* (Mostafa *et al*., 2011). In addition, heterotopic gingival fibroblasts have been used as transplanted cells to facilitate tracheal epithelial regeneration (Kobayashi *et al*., 2007, 2010), periodontal tissue regeneration (Nakajima *et al*., 2008), and skin wound healing (Nishi *et al*., 2010). Moreover, HGFs display immunosuppressive effects on T-lymphocytes similar to those of periodontal ligament stem cells (Wada *et al*., 2009). These findings suggest that HGFs share similar properties with GMSCs. However, because of the lack of a specific marker and the unknown *in vivo* identity of MSCs, the exact relationship between MSCs and fibroblasts remains elusive. There has been some evidence that fibroblasts may represent a more differentiated subpopulation of MSCs, or, under certain conditions, may in fact be derived from MSCs (Haniffa *et al*., 2009; Aghajanova *et al*., 2010; Lee *et al*., 2010). Since the frequency of MSCs is very low *in vivo* as compared with the relative abundance of fibroblasts, further elucidation of the exact identity or relationship between these 2 populations of stromal cells would lead to the identification of an alternative source of stromal cells for cell-based tissue regeneration and therapy of immune- and inflammation-related diseases.

CONCLUDING REMARKS

The potent immunomodulatory and anti-inflammatory properties of human oral mucosa-/gingiva-derived MSCs position them as a promising cell source for MSC-based therapies for wound repair and a wide range of inflammation-related diseases. Further research on this unique population of MSCs will undoubtedly contribute to a deeper understanding of the mechanisms underlying their immunomodulatory and tissue-regenerative functions under different pathophysiological settings. Some topics to be addressed include: (1) What is the real identity or developmental origin of this population of cells? Are they identical to or different from MSCs isolated from other post-natal tissues? (2) Do GMSCs and gingival fibroblasts belong to the same hierarchical lineage of stromal cell? (3) Do these oral mucosa-/gingiva-derived MSCs with unique trophic properties exhibit distinct secretomes in response to specific stimuli? (4) Because of their specific anatomic location in the oral cavity, do these MSCs differ from BMSCs in terms of host defense immune response? Do these MSC-induced immunomodulatory effects contribute to the complexity of the oral mucosal immune network in mucosal wounds? Answers to these questions will substantially enhance our understanding of the biological properties of oral mucosa-/gingiva-derived MSCs and their important roles in tissue regeneration and cell-based therapy of immune- and/or inflammation-related diseases.

ACKNOWLEDGMENTS

This work was supported by a National Institutes of Health Research Grant (R01DE 019932) and an Oral and Maxillofacial Surgery Foundation (OMSF) Research Grant. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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