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Abstract

Feline chronic gingivostomatitis (FCGS) is a painful, immune-mediated, oral mucosal inflammatory disease in cats. The etiology of FCGS remains unclear, with evidence pointing potentially toward a viral cause. Full-mouth tooth extraction is the current standard of care, and cats that are non-responsive to extraction therapy may need lifelong medical management and, in some cases, euthanasia. Adipose-derived mesenchymal stromal cells (adMSCs) have been demonstrated to have advantages in the treatment and potentially the cure of non-responsive FCGS in cats. Therefore, adMSCs have attracted a series of ongoing clinical trials in the past decade. AdMSC therapy immediately after full-mouth tooth extraction was not explored, and we postulate that it may benefit the overall success rate of FCGS therapy. Here, we aim to summarize the current knowledge and impact of adMSCs for the therapeutic management of FCGS and to suggest a novel modified approach to further increase the efficacy of FCGS treatment in cats.

Keywords: Gingivostomatitis; inflammation; immune modulation; stem cell

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Background

Scientific and public interest in stem cell therapy for chronic inflammatory diseases has been on a steep trajectory in recent years. Several reports exist on using stem cells to treat chronic inflammatory diseases, with the promise of a cure or reduction of clinical signs and a chance for an improved quality of life. However, many questions remain to be answered regarding its safety and efficacy before definitive recommendations can be made.

Mesenchymal stromal cells (MSCs) are multipotent cells with the potential to differentiate into three well-known cell lineages: chondrocytes, osteoblasts and adipocytes.[1] In addition, MSCs are known for their self-renewal capacity, immune evasiveness and potential for immune modulation.[2] MSCs may be harvested from various tissues, including, but not limited to, bone marrow, placenta, amniotic membranes, gingiva and adipose tissue.[3] Adipose tissue has been established as a reliable and abundant source of MSCs.[4] Adipose-derived MSCs (adMSCs) can be obtained with minimal invasiveness and have high frequency and expansion potential.[3,4] The presence of MSCs in tissues may range from 0.001% in the bone marrow to 2% in the stromal vascular fraction of adipose tissue.[5] Alternative tissue sources of MSCs are the placenta, umbilical cord and skin dermis.[6,7]

Feline chronic gingivostomatitis (FCGS) is a painful, immune-mediated, chronic, oral mucosal inflammatory disease in cats with potential viral etiopathogenesis, as demonstrated in a recent metagenomic and transcriptomic study (Figure 1).[8] FCGS cats have a dysregulated cellular immune response with a predominance of cytotoxic T cells systemically and in affected tissues.[9]

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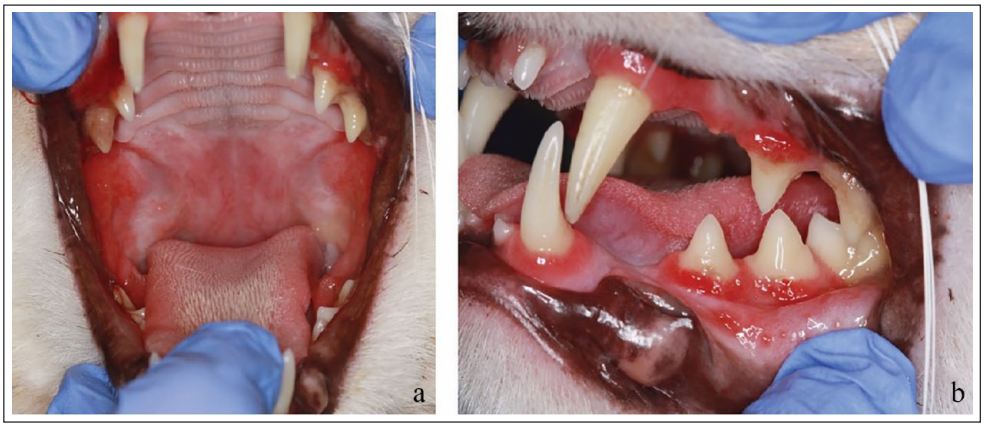

Figure 1 Clinical images from the (a) rostral view and (b) left lateral view from a cat affected by feline chronic gingivostomatitis before full-mouth tooth extractions. Note the inflammation lateral to the palatoglossal folds and the gingiva that are the typical phenotype of the disease

A comprehensive review of FCGS, by Soltero-Rivera et al, has been published as a companion article in *JFMS*.[10] In the context of the present review, cats affected by FCGS that are not responsive to tooth extraction (refractory FCGS) presented a unique opportunity to explore the efficacy and safety of the immunomodulatory potential of MSCs in clinical settings.

The safety and efficacy of adMSCs in treating non-responsive FCGS have been established in clinical trials over the past decade.[11–13] Recently, a study was conducted in eight cats using placenta-derived MSCs to treat refractory FCGS.[14] In both adMSCs and placenta-derived MSCs treatments of FCGS, improvement and, in some cases, complete cure of clinical signs have been reported.[11–14] Yet, a small percentage of patients with refractory FCGS do not respond to adMSC therapy.[11–13] While the immunomodulatory efficacy of adMSCs relative to other MSC sources remains to be explored, adMSCs' ease of collection, processing, abundance and possibility for autologous and allogeneic applications are apparent.

This review summarizes the current knowledge of adMSC therapy for FCGS, its biological and immunomodulatory basis, manufacturing, adverse events and future directions.

Biological and immunological properties of MSCs

MSC biology

MSCs are undifferentiated cells that reside in multiple tissues. A prevalent biological feature of MSCs is their ability to differentiate into various tissues (trilineage differentiation), such as bone, cartilage and adipose tissue, in vitro.[15] Morphologically, MSCs have a spindle-shaped phenotype in standard cell-culture conditions (Figure 2).[16] Feline MSCs express CD44, CD90, CD105 and major histocompatibility complex I (MHC-I) on the cell surface, markers almost replicative of human MSCs.[18] These markers, however, are not specific and can be expressed by other cell types,[7] precluding their utility for the purification of MSCs.

Other defining characteristics of MSCs include clonal expansion and theoretically almost unlimited proliferation potential.[15] Nevertheless, studies on human bone marrow-derived MSCs show that the clonal complexity of MSCs declines with increased passaging.[19] Specifically, at early passages, many cells give rise to multiple unique clones, but with an increase in passage number, the amount of clones declines.[19] The relevance of the latter is that with passaging, MSC populations change due to the dominance of some clones and the disappearance of others, which may be challenging to predict statistically. It also remains to be determined whether particular clones are linked to specific functions of MSCs. Some clones may have a stronger immunomodulatory capacity or specific lineage differentiation qualities than others. In addition, the proliferation potential of MSCs is not unlimited.[20] For example, via assessment of β-galactosidase activity, Lee et al determined that the proportion of senescence in feline adMSC cultures increases with passage number.[20] Further, the ability of adMSCs to differentiate along osteogenic lineages was diminished after the fifth passage.[20] In concert, these studies indicate that more work needs to be done to determine the ultimate passage number for a specific utility of MSCs.

The source of MSCs and the cell-culture conditions play an important role in the biology of the cells. For instance, from work performed on equine MSCs, we know that bone marrow-derived MSCs had more limited proliferation potential compared to adMSCs or umbilical cord MSCs.[21] A similar cell source difference was detected by Li et al in a comparison of human adMSCs and bone marrow-derived MSCs.[22] Cell-culture media composition was also shown to play an important role in MSC biology.[23–25]

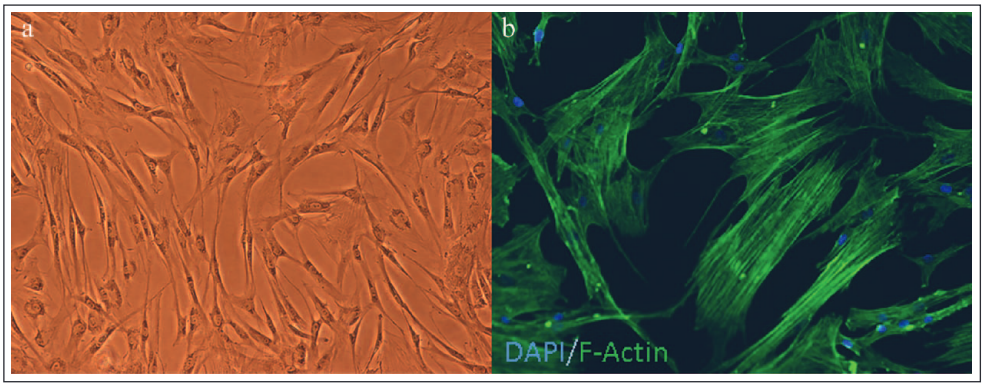

Figure 2 Morphology of mesenchymal stromal cells (MSCs) in culture conditions. (a) Cellular morphology of normal feline MSCs in standard cell culture (10× objective). (b) Fluorescent feline MSCs using 4 ,6-diamidino-2-phenylindole (DAPI) nuclear staining (blue) and F-actin staining (green). F-actin provides cellular structure and results in a fibroblastic-like shape (40× objective). With permission from Mary Ann Liebert, Inc. Arzi et al. Feline foamy virus adversely affects feline mesenchymal stem cell culture and expansion: implications for animal model development. *Stem Cells Dev* 2015; 24: 814–823[17]

Specifically, the addition of fetal bovine serum (FBS) is traditionally considered an influential factor in facilitating culture flask adhesion and proliferation.[26] However, FBS composition is not entirely known and may vary greatly between batches.[27] FBS substitutes have been explored for ethical and safety reasons, but no consensus was reached on what formula is identical to FBS.[27] Furthermore, studies comparing the effects of FBS alternatives on MSC growth and differentiation reported conflicting results.[28,29] In essence, a high degree of protocol variability to MSCs' isolation, growth, expansion and preservation is the most likely reason for controversy and disagreements between scientific reports and clinical outcomes of MSC therapy. Added to the latter, donor and cell-source complexity and heterogeneity make the comparison between studies difficult to between studies nearly impossible. Hence, it is likely that before more uniform guidelines are applied, MSC therapies may remain under-regulated and unapproved by the US Food and Drug Administration (FDA).

MSCs and immunomodulation

One of the first studies documenting the immunomodulatory properties of MSCs was performed on baboons receiving skin allografts.[30] Specifically, the authors of this study reported that lymphocyte proliferation was significantly reduced in co-culture with MSCs, despite mitogen supplementation.[30] Furthermore, skin allografts had prolonged survival in MSC-treated animals.[30] Later studies, summarized elsewhere, identified multiple mechanisms of MSC immunomodulation.[31] In short, these mechanisms include direct contact with immune cells and paracrine effects through the secretion of cytokines, growth factors and other mediators.[31]

Specifically in cats, it was shown that primarily upon MSC stimulation with inflammatory cytokines, such as interferon-gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), they secrete factors that directly reduce inflammation and interact with cells of the immune system to alter T-cell and B-cell proliferation, function and circulation (Figure 3).[32,33] Even more specific in vivo studies focusing on MSC mechanisms of function in FCGS cats revealed a significant decrease in the quantity of circulating cytotoxic T cells, normalization of CD4/CD8 lymphocyte ratios, a decrease in circulating neutrophils and, most importantly, remission of FCGS in 65.5% of patients.[12]

Measuring the secretion of soluble factors by MSCs is a promising tool for understanding the immunoregulation mechanisms.[18,33] Dozens of soluble factors can be secreted by MSCs to regulate the host immune system and relieve inflammation caused by the inflammatory responses of chronic disease.[34] Specifically, it was determined that secretion of anti-inflammatory cytokine prostaglandin E2 (PGE2) by IFN-γ-pretreated adMSCs induces macrophage and T-cell polarization towards tolerogenic and anti-inflammatory phenotypes.[35] IFN-γ stimulation is especially relevant in FCGS cats, because this pro-inflammatory cytokine is upregulated at the systemic level in these patients.[12] Consequently, adMSC treatment reduces IFN-γ levels in patients with FCGS.[12]

The induction of cell-cycle arrest in mitogen-activated lymphocytes and its associated decrease in feline peripheral blood mononuclear cell (PBMC) proliferation are some additional mechanisms in feline adMSCs' immune modulation.[36] Explicitly, this effect is achieved by direct interaction between adMSCs and T cells via adhesion molecules (such as ICAM-1/LFA) and PGE2 secretion.[36] Cell-cycle analysis revealed inhibited proliferation of T-cell lymphocytes with a cell-cycle arrest in growth phases G0 and G1 of mitosis.[36]

Importantly, feline adMSCs also alter the phenotypical and functional characteristics of cytotoxic T cells (CD8+ T cells) and upregulate the expression of effector compounds such as granzyme B, interleukin-2 (IL-2) and the

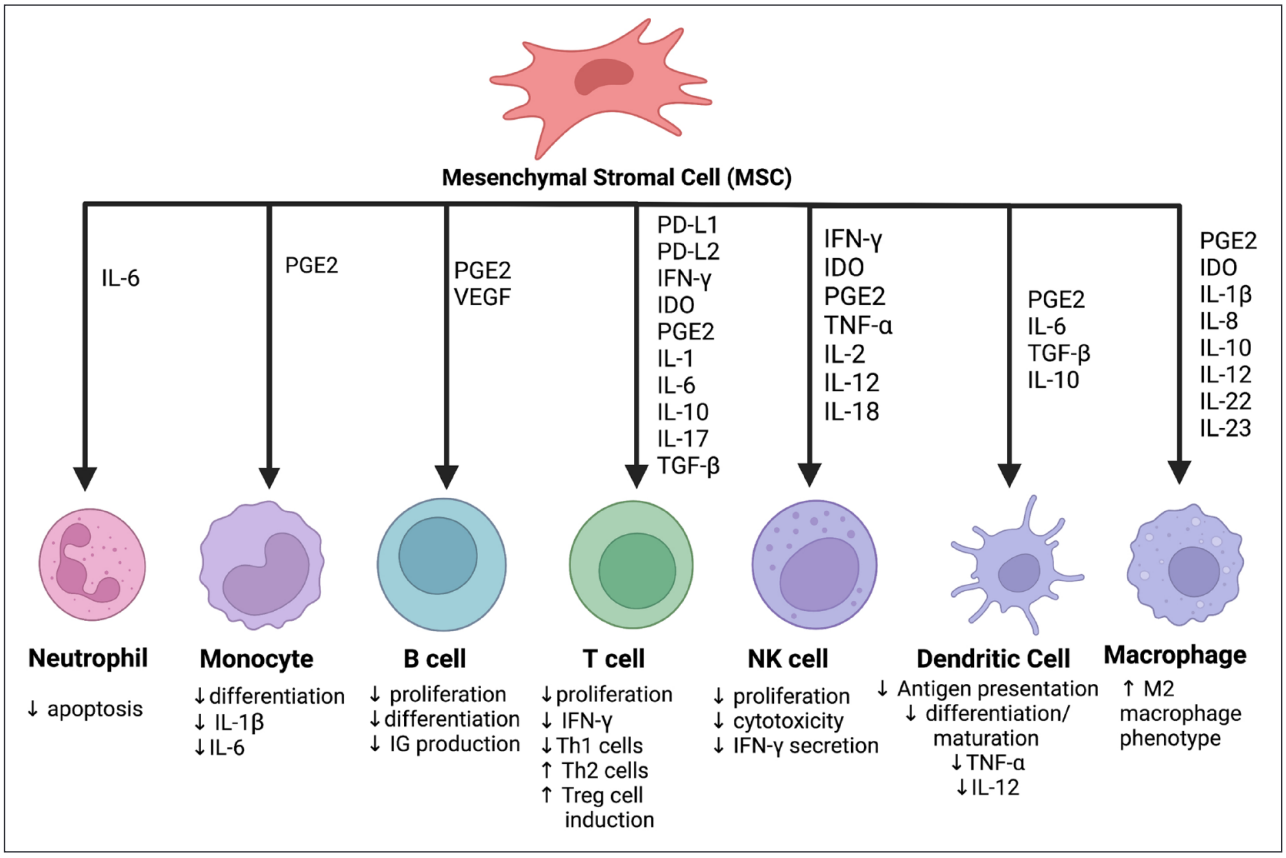

Figure 3 Immunomodulation by mesenchymal stromal cells (MSCs). Feline MSCs secrete paracrine factors and have a direct effect on humoral and cellular host immune players. These secretory factors decrease the inflammatory response and pose as an immunoregulatory mechanism to fight inflammatory diseases. Created on BioRender. MSC mesenchymal stromal cell; PGE2 prostaglandin E2; VEGF vascular endothelial growth factor; PD-L1 programed death ligand 1; PD-L2 programmed death ligand 2; IFN-γ interferon-gamma; IDO indoleamine 2,3-dioxygenase; TGF transforming growth factor; TNF-α tumor necrosis factor-alpha; IG immunoglobulin; Th1 T helper 1; Th2 T helper 2; Treg T regulatory; M2 Th2 macrophage

killer cell lectin-like receptor subfamily G1 (KLRG-1) in these cells.[37] Even more specifically, when MSCs are co-cultured with T cells, they reduce CD8 and CD62L expression and upregulate CD57 and CD25.[37] Cumulatively, this phenotype switch means that cytotoxic T cells assume terminally differentiated, effector phenotypes and manifest an enhanced potency to fight off viral intruders. The latter interpretation of mechanistic studies indirectly agrees with the calicivirus etiology recently determined as a potential cause of FCGS.[8]

To conclude, adMSCs have potent immunomodulatory properties resulting from direct contact with and paracrine influences on patients' leukocytes. In the case of FCGS, this effect is beneficial for the clinical improvement or resolution of the disease with a potentially viral etiology. However, as noted in people, caution should be exercised in applying MSCs to the treatments of other immune-mediated conditions, such as multiple sclerosis, Crohn's disease and systemic sclerosis, which may not benefit, or even suffer from, the observed changes in lymphocyte behavior, polarization and proliferation.[7]

MSCs and viral infections

Susceptibility of MSCs to viral infection

MSCs are not immune to viral infection, but are generally considered more resistant than terminally differentiated cells.[38] Viruses cannot exist without the host cell and need to enter the cell and take over its metabolic machinery.[39] To enter the cells, viruses usually use receptor binding, upon which internalization of the virus occurs.[39] Over millions of years, viruses evolved to perfect this task, and so the variety of receptors they utilize for cell entry varies greatly among viruses.[39] Human MSCs have been widely explored for viral susceptibility due to concerns for their efficacy upon viral infection in virus-infected recipients after MSC infusion.[40] Indeed, it was determined that viral infection of human bone-marrow-derived MSCs with cytomegalovirus can render them dysfunctional

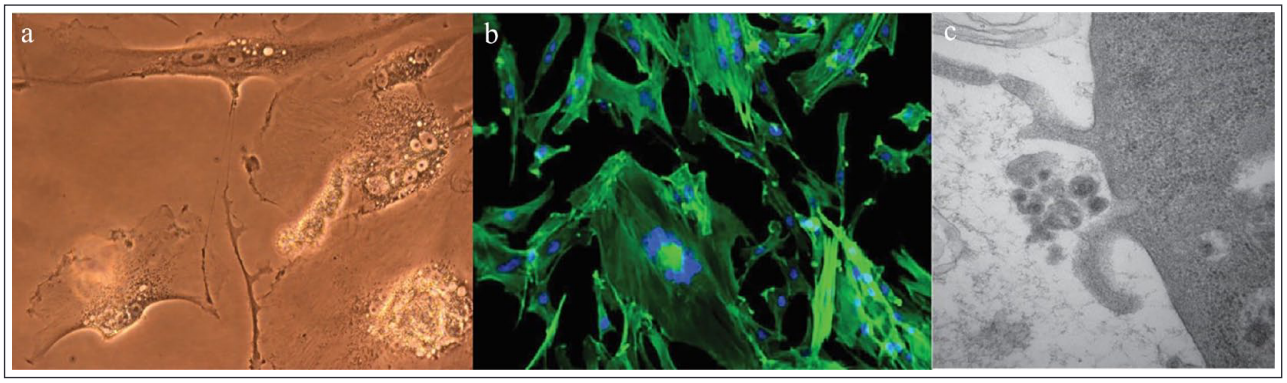

Figure 4 Syncytial cell formation of mesenchymal stromal cells (MSCs) caused by viral infection. (a) Transmission electron microscope (TEM) image of giant, multinucleated feline MSCs in cell culture infected with feline foamy virus (40× objective). (b) Fluorescent syncytial feline MSCs using 4′,6-diamidino-2-phenylindole nuclear staining (blue) and F-actin staining (green). Multinucleations are common in virus-infected adipose-derived mesenchymal stromal cells (adMSCs) (40× objective). (c) TEM photograph depicting a feline foamy virus particle gaining entry to the MSC membrane through tethering and endocytotic mechanisms (500 nm scale). With permission from Mary Ann Liebert, Inc. Arzi et al. Feline foamy virus adversely affects feline mesenchymal stem cell culture and expansion: implications for animal model development. *Stem Cells Dev* 2015; 24: 814–823[17]

in terms of differentiation and immune modulation.[41] Furthermore, MSCs were also shown to serve as parvovirus carriers and were able to transmit the virus to hematopoietic cells in vitro.[42] Thus, very stringent criteria for MSC screening and knowledge of the potential effects of MSC viral infections should be established.

Specifically to cats and FCGS, it was found that viral recrudescence (active virion production by previously quiescent cells) can occur upon in vitro culture of feline adMSCs.[17] Our group detected feline foamy virus (FFV) in 55% of adMSC cell lines obtained from client-owned cats (Figure 4).[17] Viral infection of the cell lines became apparent in passages 3–5 and manifested in a slow replication capacity in the adMSCs, formation of syncytial cells (multinucleated cells composed of many fused cells) and their early senescence.[17] The latter meant that the adMSCs were unusable for autologous applications, because sufficient healthy cells could not be generated for infusions. Interestingly, the marker phenotype of the virus-infected MSCs was identical to virus-free controls. The lack of marker change underscores the limited power of MSC surface-marker screening as the sole method for quality control.[17] The serological evidence of FFV antibodies in the serum of the affected cats was determined to be a possible screening tool for excluding unsuitable donors, but it is yet to be commercialized. Lastly, the antiretroviral agent tenofovir had limited efficacy in abrogating negative viral effects.[17]

Despite the strong evidence of MSC susceptibility to a variety of viral infections, multiple reports also documented the beneficial effects of MSCs in combating viral infections.[38] Moreover, MSC resistance to viral infections has also been reported.[38] In brief, the antiviral activity of MSCs was linked to the activation and expression of IFN-stimulated genes (ISGs). The products of these genes were shown to interfere with viral replication on multiple levels, including transmembrane migration, RNA transcription, protein translation and virion assembly, among others.[38,43,44] Another antiviral mechanism is indoleamine 2,3-dioxygenase (IDO) production.[45] This mechanism was shown to be very effective in combating cytomegalovirus and human herpes simplex infections by human bone-marrow-derived MSCs.[45] IDO is a key modulator of tryptophan metabolism and is a well-known pathway in immune tolerance and immunomodulation.[45] It remains to be studied whether similar mechanisms are in place in feline species.

To that end, Teshima et al investigated the potential antiviral effects of feline adMSCs on feline calici- and herpes viruses.[46] They found out that soluble factors present in the media where adMSCs are grown can prevent viral replication but not viral entry in the feline kidney cell line.[46] Although more studies are needed to identify the exact mechanism of this antiviral property of adMSCs, the clinical data support its correctness and relevance, especially in light of metagenomic sequencing by Fried et al, where calicivirus was consistently found in cats with refractory FCGS not resolving upon teeth extractions.[8] In summary, databased evidence of MSCs' antiviral properties supports the carrying out of further studies and promises interesting results.

Challenges with feline stromal cell production and use

The fate of MSCs after systemic administration

The fate of MSCs after systemic administration remains an active area of research in regenerative medicine. It is known that MSCs follow similar steps of vascular adherence and transmigration to lymphocytes.[47] First, MSCs are

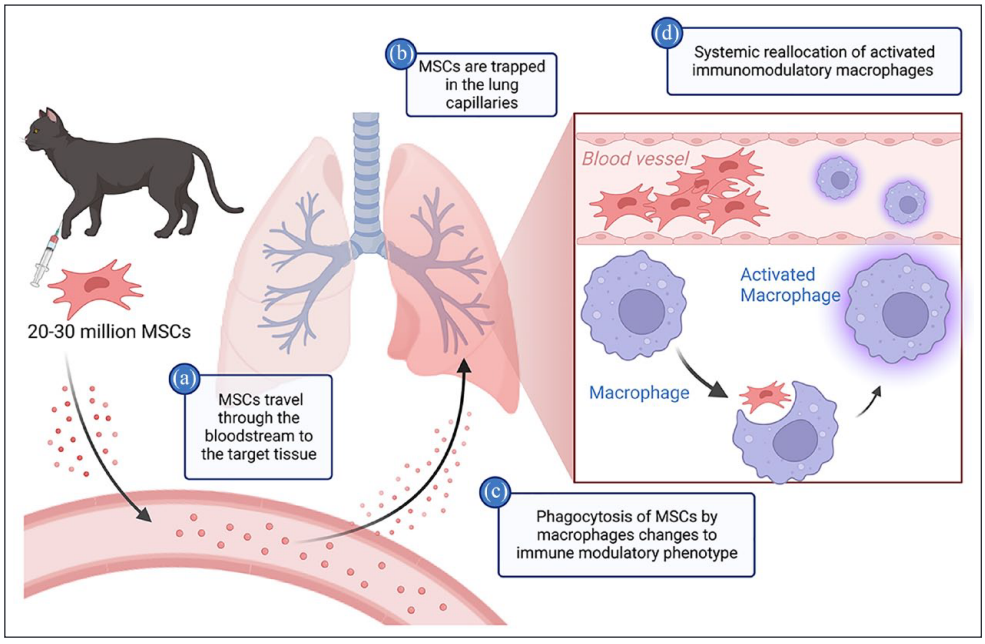

Figure 5 Mesenchymal stromal cell (MSC) homing mechanism. An overview describing the homing mechanism and systemic fate of adipose-derived mesenchymal stem cells (adMSCs) after intravenous (IV) administration. (a) 20–30 million MSCs are injected into the bloodstream of the recipient. (b) MSCs travel from the bloodstream to the target tissue, but immediately get trapped in the lung capillaries due to their enormous size (15–30 µm). (c) Inactivated macrophages phagocytose the overly populated MSCs in the capillary system of the lungs, and adversely affect macrophages to become activated and switch phenotypes. (d) Once activated, macrophages are induced to M2-macrophage phenotypes and secrete factors that decrease the inflammatory response. Created on BioRender

tethering to endothelial cells through the CD44 receptor on the MSCs and P-selectin and VCAM-1/VLA-4 receptors on endothelial cells.[47,48] Next, the MSCs are activated by G protein-coupled chemokine receptors CXCR4[49] and CXCR7[50] in response to ligands such as stromal cell-derived factor-1 (SDF-1).[51,52] Then, MSC arrest is facilitated by VLA-4 integrin in response to SDF-1 and binds to VCAM-1 receptors on endothelial cells.[53] After arrest, the MSCs transmigrate through the endothelial cell layer and basal lamina by secreting matrix metalloproteinases MMP2, MMP9 and MT1-MMP, which break down the basement membrane.[53,54] Finally, the MSCs migrate to the injury site through the interstitium, following the chemotactic gradient of cytokines (IL-8, insulin-like growth factor 1, TNF-α) released in response to inflammation and tissue damage.[55,56] Our group investigated feline adMSC homing by labeling them with technetium (Tc)-hexamethylpropyleneamineoxime (HMPAO).[11] Immediately after the injection, most of the ^{99m}Tc-HMPAO adMSCs were observed in the lungs, but a low radioactive signal was detected in the oral cavity in FCGS, but not healthy control cats.[11] These findings suggest that adMSCs can 'home' to the site of inflammation, probably following the chemotactic signals described above (Figure 5).

Circling back to feline studies, there is initial evidence of a slightly higher initial efficacy of autologous than allogeneic MSCs, albeit not significant.[11,12] The allogeneic study also reported that the positive effect of allogeneic MSCs took longer to materialize and was less effective in more severe cases of FCGS.[11] Given that FCGS is a systemic disease characterized by increased systemic IFN-γ concentration, allogeneic MSCs may be identified as non-self and eliminated by natural killer (NK) cells upon upregulation of MHC-I induced by IFN-γ. The latter hypothesis agrees with the findings by Spaggiari et al showing that MSCs treated with IFN-γ are detected and eliminated by NK cells.[57] In summary, more research is needed to better understand the fate of MSCs upon systemic administration, as well as the potential ways to manipulate it.

Adverse events associated with systemic administration of MSCs

The adverse events associated with the administration of MSCs may be divided into acute and chronic. A transient transfusion-like reaction is the most common acute adverse effect.[58] The most common transfusion-like response includes lethargy and/or a temporary increased respiratory rate within minutes to hours of adMSC administration. However, more severe presentations, such as vomiting and diarrhea, may occur in rare cases.[58] These adverse events were observed upon autologous and allogeneic adMSC administration to feline patients with refractory FCGS.

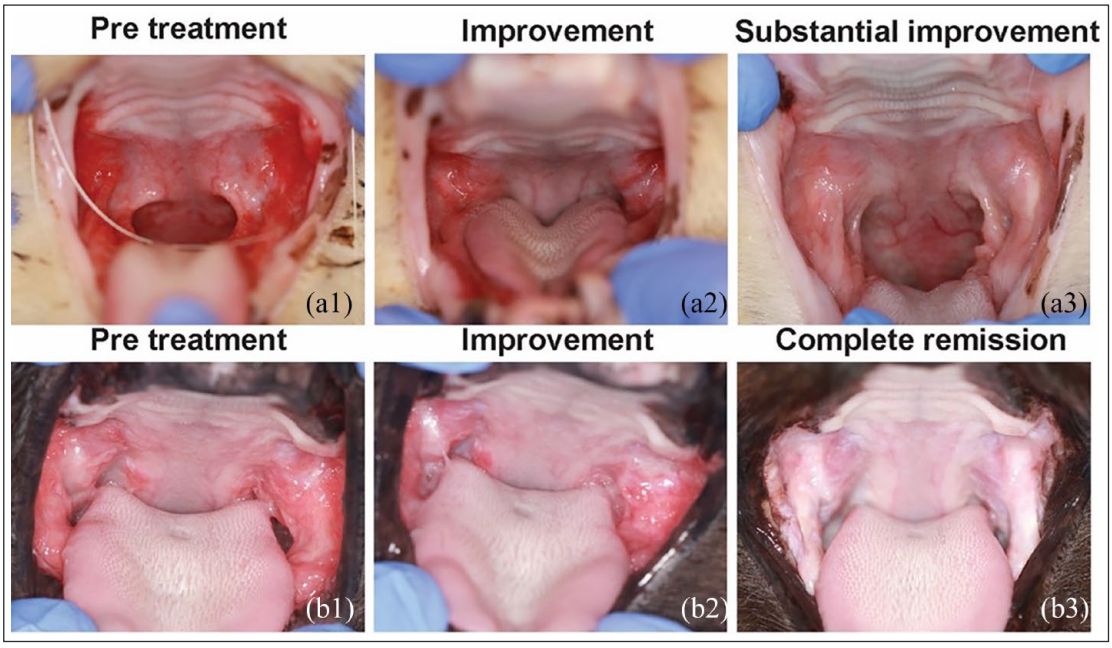

Figure 6 Positive clinical response to mesenchymal stromal cell (MSC) therapy in cats with feline chronic gingivostomatitis (FCGS). Clinical images from two cats that underwent a clinical trial using allogeneic adipose-derived mesenchymal stem cells (adMSCs) for the treatment of non-responsive FCGS. Representative pre treatment images for two different cats (a1,b1) are characterized by proliferative and ulcerative inflammation at the caudal oral cavity. For two cats, a clinical response was observed within 3 months (a2), with substantial improvement at 6 months (a3). In two cats, a delayed response was observed and resulted in improvement at 6 months (b2) and complete resolution in 18 months (b3). With permission from John Wiley & Sons, Inc. Arzi et al. Therapeutic efficacy of fresh, allogeneic mesenchymal stem cells for severe refractory feline chronic gingivostomatitis. *Stem Cells Transl Med* 2017; 6: 1710–1722[11]

The potential chronic adverse event of MSC therapy is tumorigenesis.[59] The latter is reasonable given the MSC proliferation qualities and the ability to differentiate into various lineages. Indeed, a prolonged culture of human bone-marrow-derived MSCs resulted in chromosomal aberrations in several studies.[60,61] Similar examples exist in studies performed on murine bone-marrow-derived MSCs.[62] Data on tumor progression after the administration of MSCs also exist, it being supposedly facilitated by angiogenic factor secretion by MSCs.[45] With that said, 9 years of follow-up of 38 patients with FCGS that received two systemic doses of adMSCs reported no evidence of tumor development.[58]

Manufacturing of MSCs

As mentioned before, many factors can influence the biology and thus the clinical behavior of MSCs. At the authors' laboratory, MSCs are grown and passaged no more than twice before cryopreservation. Furthermore, the banked MSCs are regularly monitored for quality by screening for undesired pathogens, and validated for consistent surface marker expression with flow cytometry. These processes are labor-intensive and require meticulous precision and supervision. The process of MSC culture and preservation is complex and, at this juncture, considered more than minimal manipulation by the FDA.[63]

In the clinical trials performed at University of California, Davis in cats with FCGS, fresh (not frozen) cells were administered.[11,12] In a multicenter clinical trial involving more academic and private veterinary clinics, cells were manufactured at University of California, Davis and shipped to distant locations. Before this strategy was approved, our group verified that shipping did not alter the properties of feline adMSCs, using the same vigorous quality control methods mentioned above.[13] Other studies utilizing feline adMSCs may have different quality checkpoints. Moreover, some studies chose not to culture the MSCs before administration, but instead used thawed, previously cryopreserved stocks of cells.[14] Hence, outcomes of studies, even when performed on the same disease entity, are difficult to compare due to manufacturing detail differences.

Although the FDA publishes stringent guidelines for human applications, there is no regulatory mechanism to ensure that these guidelines are observed by the manufacturers offering various cell products. The production of MSCs for clinical application must comply with good manufacturing practice for clinical intervention and application.[64] Before such practices and regulations are in place in veterinary medicine, it will be difficult to compare the clinical outcomes of studies and draw conclusions on the efficacy of various sources of MSCs.

MSCs vs stromal vascular fraction

As stated previously, feline adMSCs may be isolated from subcutaneous or intra-abdominal adipose tissue. After fat removal, a mixture of endothelial cells, lymphocytes, macrophages, pericytes and MSCs remains. This mixture is known as the stromal vascular fraction (SVF).[65] In some instances, adipose-derived SVF (adSVF) is used as a first step for obtaining high cellular yield for subsequent culture expansion of adMSCs.[66] In other instances, SVF is offered as a sole therapy. Traditionally, adSVF is obtained by the enzymatic digestion of fat.[67] Mechanical separation of SVF is an alternative method, and has been shown to be cheaper and faster, but it has the disadvantage of containing a higher frequency of blood mononuclear cells and fewer progenitor cells.[68] To summarize, SVF is a mixture of multiple cell types, but less than 0.1% of nucleated cells in adipose SVF are MSCs.[69] A study comparing SVF to MSC-enriched cells for treating hypertrophic scars in humans reported higher efficacy in the MSC-enriched group.[70] At this juncture, there are no reports on clinical trials using SVF for treating inflammatory diseases such as FCGS.

Future directions: exploring the potential early intervention of stem cell therapy

Intravenous administration of MSCs has been conducted in previous clinical trials to test their immunomodulatory effects and improvement of FCGS.[12,13,71] Two clinical trials have targeted cats unresponsive to tooth extractions for over 6 months postoperatively.[11,12] A substantial improvement or cure was achieved in 57% of cats with FCGS treated with allogeneic MSCs[11] (Figure 6) and in 71% of cats treated with autologous MSCs.[12] A subsequent clinical trial evaluating the efficacy of adMSCs in cats with FCGS before teeth extraction reported a very limited clinical response and no evidence of immune modulation.[71] These clinical trials suggest that extractions are still essential for resolving clinical signs; however, the waiting period of 6 months was arbitrarily selected primarily to ensure no clinical response to extractions. Thus, it is plausible that administering adMSCs immediately after teeth extraction therapy may improve clinical outcomes and speed up the clinical improvement of patients with FCGS (ie, early intervention).

It is expected that once the teeth are extracted, the inflammatory burden from periodontitis will subside, and there will be a substantial reduction in the following: (1) chronic antigenic stimulation and (2) T-cell and B-cell proliferation. On top of that, pro-angiogenic factors accompanied by marked anti-fibrotic activities reported to be some of the critical mechanisms in MSCs therapy[72] will facilitate faster healing of the extraction sites. This new approach is yet to be explored in clinical trial settings.

Conclusions

In this review, we have summarized the biological and immunomodulatory functions of MSCs with a particular emphasis on feline adMSCs and FCGS as an example of immune-mediated disease. We also reviewed some shortcomings, limitations and potential risks associated with MSC therapies and manufacturing processes. Although some ground-breaking advancements were made possible by incorporating MSCs therapy, many questions and unresolved mysteries remain. However, focusing on a single disease entity in a single species with the application of adipose-tissue-sourced MSCs minimizes the number of significant variables. It allows us to take a cleaner and closer look at the process as a whole and come up with a new and exciting direction for treating immune-mediated diseases such as FCGS in cats.

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval This work did not involve the use of animals and therefore ethical approval was not specifically required for publication in *JFMS*.

Informed consent This work did not involve the use of animals (including cadavers) and therefore informed consent was not required. No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

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