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REVIEW

Advances in mesenchymal stromal cell therapy in the management of Crohn's disease

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ABSTRACT

Introduction: The aim of therapy in Crohn's disease (CD) is induction and maintenance of remission, promotion of mucosal healing and restoration of quality of life. Even the best treatment regimes, including combinations of biologics and immunomodulators lack durable efficacy and have well documented side effects. Accordingly, there is an unmet need for novel therapies. Mesenchymal stromal cells (MSCs) are a subset of non-hematopoietic stem cells that home to sites of inflammation where they exert potent immunomodulatory effects and contribute to tissue repair. Their utility is being explored in several inflammatory and immune mediated disorders including CD, where they have demonstrated favourable safety, feasibility and efficacy profiles.

Areas covered: This review highlights current knowledge on MSC therapy and critically evaluates their safety, efficacy and potential mechanisms of action in CD.

Expert commentary: Building on positive early phase clinical trials and a recent phase 3 trial in perianal CD, there is considerable optimism for the possibility of MSCs changing the treatment landscape in complicated CD. Although important questions remain unanswered, including the safety and durability of MSC therapy, optimal adjunctive therapies and their sourcing and manufacturing, it is anticipated that MSCs are likely to enter mainstream treatment algorithms in the near future.

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IBD; Inflammatory bowel disease; Crohn's disease; refractory Crohn's disease; perinanal fistula; luminal Crohn's disease; mesenchymal stem cells; mesenchymal stromal cells; MSC; autologous MSC; allogenic MSC

1. Introduction

1.1. Background to Crohn's disease (CD)

CD is a chronic inflammatory disorder that can affect any part of the gastrointestinal tract, although most commonly involves the small bowel, large bowel and perineum. Most patients initially present with significant mucosal inflammation which is often present throughout the thickness of the gut wall. Unfortunately, over time, disease behavior changes and many patients progress to penetrating complications including fistula, sinus and abscess formation [1]. There is no cure for CD and although sustained clinical remission remains the goal of treatment, many patients continue to experience persistent symptoms, frequent relapses, disease progression and significant complications. Medical management comprises nonspecific anti-inflammatory agents such as corticosteroids and immunomodulators, as well as biological therapies targeting specific immune molecules or cells.

While treatment with anti-tumor necrosis factor alpha (anti-TNFα) is considered a game-changing therapy for CD [2–4], approximately one-third of patients do not respond to treatment and an additional one-third subsequently lose response or become intolerant [5,6]. Even powerful combinations of immunomodulators and anti-TNFα, administered for over 1 year, induce mucosal healing in fewer than 50% of CD patients [3]. Although newer agents, including vedolizumab, targeting the gut-homing integrin α4β7 expressed by circulating leukocytes, and ustekinumab, targeting the p40 subunit common to both cytokines interleukin-12 (IL12) and IL23, are efficacious in CD, most patients experience disease relapse even when they have been preselected as drug responders [7,8]. Thus, there is a clear unmet need for identifying new therapeutic approaches. Moreover, the current paradigm of targeting individual cytokines fails to acknowledge the likelihood that inflammation in different individuals may be driven by different immune pathways. Similarly, the human immune system is complex and has built in redundancy and compensatory mechanisms, such that targeting only one immune pathway with selective cytokine blockade may well result in activation of alternative immune pathways that emerge and continue to drive disease. For example, it was recently shown that inflammatory bowel disease (IBD) patients with poor clinical responses to anti-TNFα therapy have increased expression of the novel cytokine oncostatin M and the transcriptional network linked to its activation [9].

Another limitation of current therapeutic strategies is the focus on targeting inflammation, but not on coordinating tissue remodeling and repair. Regenerative medicine with cell therapy approaches, including the application of mesenchymal stromal cells (MSCs), attempts to bridge this gap and offers an alternative paradigm to suppress inflammation while simultaneously promoting tissue restitution.

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This approach might be especially beneficial in CD, where complex inflammatory pathways promote progressive tissue damage and organ failure despite the use of expensive biological therapies.

1.2. Etiology of CD

The pathophysiology and etiology of CD are not fully understood but dysregulation of mucosal immunity, ostensibly to intraluminal microbial antigens, is considered a critical event. Key immune changes in the gut of CD patients include excessive accumulation of mucosal $CD4^+$ helper T cells producing effector cytokines, such as TNFα and interferon-γ (Th1 cells) and IL17 and IL22 (Th17 cells) [10]. Inappropriate activation of these T-cell lineages drives excessive recruitment and stimulation of mononuclear phagocytes (MPs), such as $CD14^+$ inflammatory monocytes (especially for Th1 cells), as well as neutrophils (triggered by Th17-derived cytokines), which in turn orchestrate tissue injury [11]. It is important to recognize that MPs are not merely involved in responding to T-cell-derived signals. They are also involved proximal to T-cell activation, through bacterial recognition, processing, and presentation of microbial antigens to infiltrating T cells. In CD, dendritic cells (DCs), which are professional antigen-presenting cells and sample luminal antigens, upregulate molecules involved in bacterial sensing, such as toll-like receptors [12] and prime T-cell responses. Mucosal MPs are a key source of cytokines involved in driving activation of T cells and shaping their differentiation toward the harmful effector lineages. Th1 differentiation/activation is triggered by MPs-derived IL12, IL15, and IL18, and Th17 differentiation/activation and maintenance is promoted by IL1β, IL6, and IL23 produced by tissue MPs [10].

A population of innate lymphocytes termed innate lymphoid cells (ILCs) also contribute to inflammation [13]. ILCs closely resemble effector $CD4^+$ T-cell lineages but are activated independently of recombined antigen-specific receptors. ILCs are expanded in CD [14] and have been shown to play an indispensable role in preclinical models of chronic intestinal inflammation [13,15]. In addition to expansion of pro-inflammatory lymphocytes, in CD there is also contraction of immunomodulatory immune mechanisms. For instance, the number of $FOXP3^+$ regulatory T cells (Tregs), which produce anti-inflammatory cytokines such as IL10 and TGF-β, is significantly diminished in CD patients compared to inflammatory control patients [16]. Accordingly, the imbalance between pro-inflammatory and counterregulatory mechanisms is likely to be central to the progression and maintenance of gut inflammation in CD.

In addition to environmental triggers such as luminal microbes, host genetics also play a significant role in conferring increased susceptibility to CD. Indeed, CD concordance among monozygotic twins is about 50%, and having an affected first-degree relative increases the risk for developing CD by 5–35-fold [17]. More than 200 genetic loci have been associated with altered IBD risk, the majority of which are shared between CD and ulcerative colitis (UC), and are located at loci enriched for immune genes [18]. Key pathways implicated by genetic studies include bacterial sensing (*NOD2*), autophagy (*IRGM*), selected inflammatory pathways, such as the IL23/IL17 axis (*IL12B, IL23R, STAT3, TYK2, TNFSF15*), and immune cell trafficking (*ITGA4, ITGAL, ICAM1*). Efforts at identifying causal variants by fine mapping have confirmed involvement of many of these loci, most notably *NOD2* and *IL23R* [19].

2. Mesenchymal stromal cells

2.1. Background

It was almost five decades ago when Friedenstein and colleagues isolated MSCs (also known as mesenchymal stem cells, multipotent stromal cells, marrow stromal cells, and colony-forming unit-fibroblastic cells) from the bone marrow (BM) of mice (Figure 1) [20].

As well as BM, MSCs can be easily isolated and expanded *in vitro* from adipose tissue, skeletal muscle, skin, liver, dental pulp, placenta and umbilical cord blood. Although cultures of MSCs have a uniform appearance of spindle-like fibroblast cells (Figure 2), they are heterogeneous, and younger passages have higher rates of plasticity and proliferation compared with higher passages [21].

Like hematopoietic stem cells (HSC), only a small percentage of infused MSCs (often <1%) reach the target tissue [22]. A large fraction becomes entrapped in the lungs or in precapillary vascular beds. Ongoing genetic and chemical engineering approaches are attempting to enhance the tissue-homing capacity of MSCs with a view to augmenting their tissue specific therapeutic efficacy [23]. MSCs were initially considered for therapy based on their multilineage differentiation capacity (Figure 1). However, it is now appreciated that these cells modulate inflammation and possess antiapoptotic and proangiogenic properties, which makes them ideally suited for application in diseases caused by chronic inflammation and associated tissue injury.

2.2. Immunomodulatory properties

While MSCs represent only a minority of the cells in the BM (0.001–0.01%), they constitute the niche in which HSC self-renew and differentiate. Apart from their ability to repair bone, cartilage and other stromal compartments [24], MSCs exhibit potent immunomodulatory effects through a variety of mechanism (Figure 3). They can influence the phenotype of multiple immune cell populations, including adaptive and innate lymphocyte populations and MPs, which comprise monocytes, macrophages and DCs.

Unlike many of their immune cell counterparts, and especially MPs, an important property of MSCs is their lack of major histocompatibility complexes (MHC) class II or co-stimulatory molecule expression and poor antigen-presenting properties. This means they have very low immunogenicity and are poor primers of adaptive immune responses. This is especially useful for the deployment of allogeneic MSCs, which would otherwise trigger a proliferative response from allogeneic lymphocytes, and thus abrogates the need for donor–recipient matching. Despite this 'immunopriviledged' property, recent data indicate that allogeneic MSCs may elicit detectable humoral and cellular immune responses [25–27]. In a recent phase 3 clinical trial in fistulizing CD, serological testing performed in a subgroup of patients receiving allogeneic adipose-derived MSCs or placebo

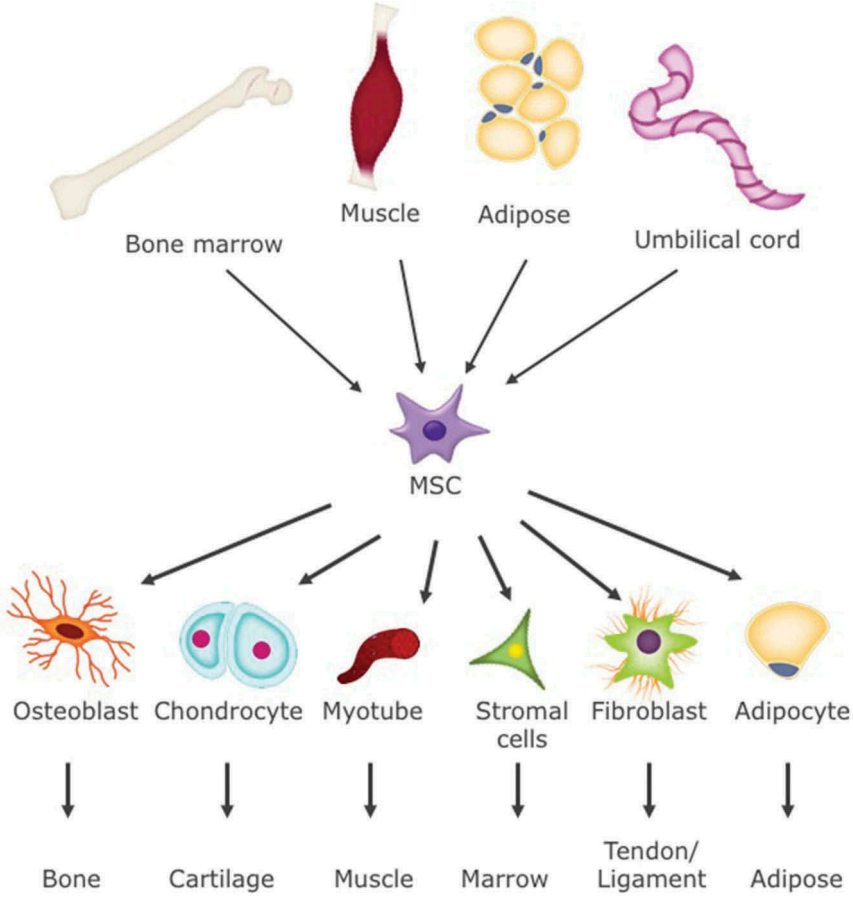

Figure 1. Sources of MSC and their differentiation capacity. MSCs are present in all tissues but have mainly been isolated from bone marrow, muscle, adipose and umbilical cord tissue. They are capable of differentiating into several cell types including osteoblasts, chondrocytes, myotubes, stromal cells, fibroblasts and adipocytes rendering them valuable in promoting tissue repair.

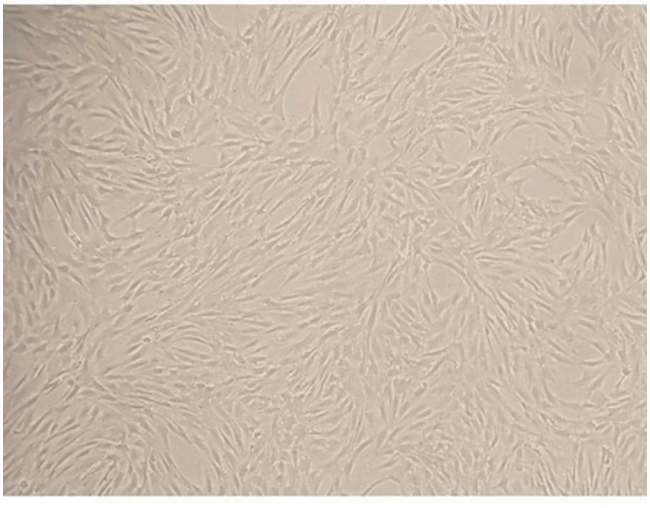

Figure 2. Morphology of human adipose derived MSC. MSCs firmly adhere to the plastic surface and exhibit a spindle shape appearance.

demonstrated that 36% of MSC-treated patients mounted donor-specific anti-HLA class I antibodies as compared to 0% of placebo-treated patients. However, there were no adverse events associated with antibody formation and no impact on clinical response to MSCs [28].

MSCs are not constitutively immunosuppressive but rather acquire this ability during exposure to the inflammatory microenvironment, which then activates them to produce growth factors, promote tissue regeneration and adopt immunosuppressive functions. Pro-inflammatory cytokines such as IFNγ, TNFα and IL1β are key players in this 'licensing step' [29,30]. MSCs pretreated with IFNγ demonstrated enhanced inhibition of peripheral blood mononuclear cells and T-lymphocyte proliferation *in vitro* compared with resting MSCs, and exhibit superior immunosuppressive function and improved migration to inflamed gut in preclinical models of intestinal inflammation [31]. However, one theoretical problem associated with IFNγ licensing is the tendency of this cytokine to increase expression of MHC I and co-stimulatory molecules and potentially increase the risk of immunogenicity.

More recent data has shown that IL17 can also license MSCs. Whole-genome transcriptional profiling of MSCs treated with IFNγ or IL17A confirmed that IFNγ induced expression of MHC, co-stimulatory molecules and transcripts encoding proteins involved in antigen presentation, which could potentially enhance their immunogenicity [32]. On the other hand, IL17A

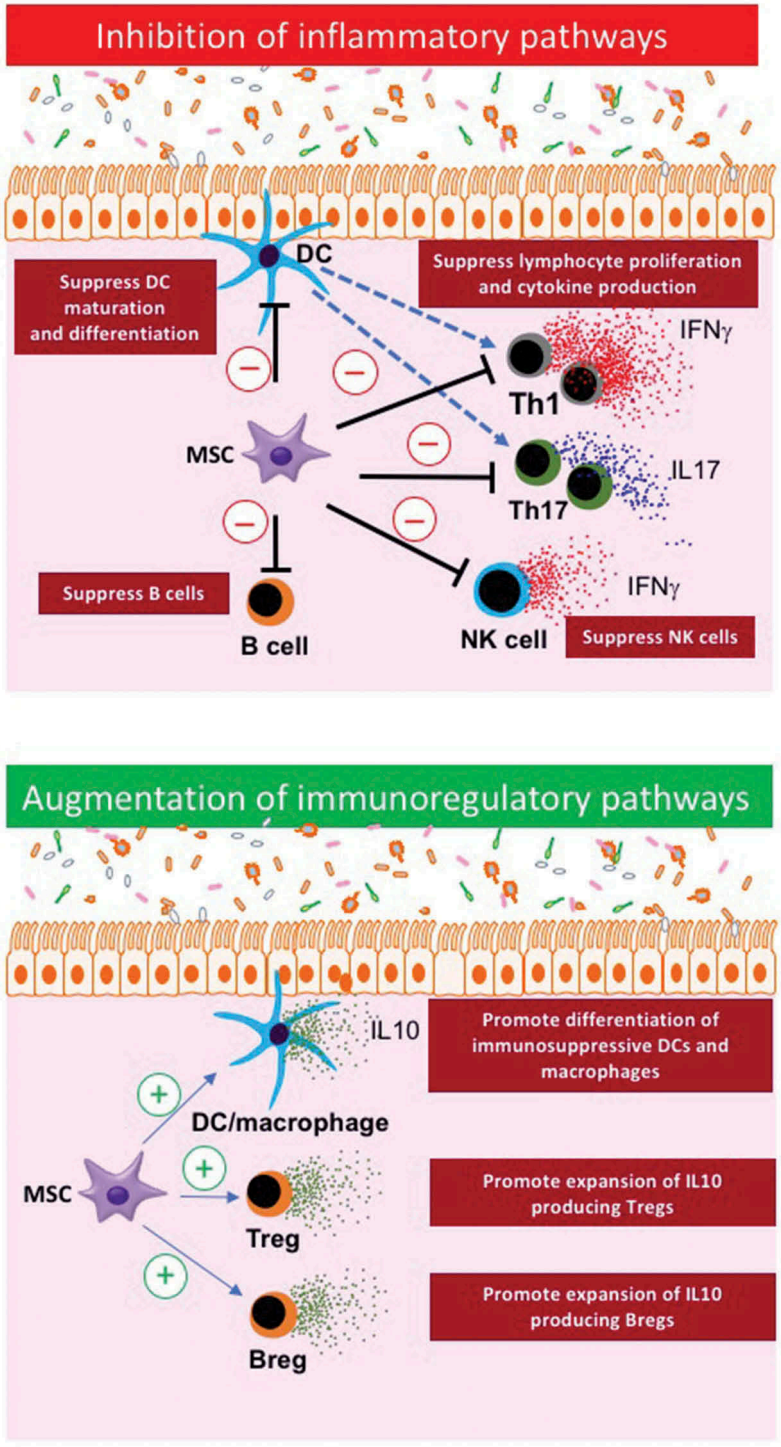

Figure 3. Mechanisms of MSC immunomodulation. MSCs exert a wide variety of immunomodulatory properties which contribute to their therapeutic effect. This includes inhibition of inflammatory pathways (top image) via suppression of DC cells, B cells, NK cells and lymphocyte proliferationresulting in reduced production of pro-inflammatory cytokines including IFN and IL-17. MSC are also able to augment immunoregulatory pathways that have a protective function (bottom image) including promoting differentiation of the regulatory phenotype of DCs and macrophages, and promoting expansion of the anti-inflammatory cytokine IL-10 producing Tregs and Bregs.

activation of MSCs failed to induce transcripts involved in these pathways, and instead triggered induction of transcripts encoding molecules involved in chemotaxis (e.g. *CCL2, CXCL6, CCL8*), and molecules that might permit MSCs to invade inflamed tissues by degrading extracellular matrix networks (e.g. *MMP13, MMP1*). Notably, IL17A-licensed MSCs exhibit increased suppression of Th1 cells and enhanced potentiation of Tregs [33].

As discussed above, effector T-cell lineages including Th1 and Th17 cells, play a central role in orchestrating the pro-inflammatory response in inflamed tissue in CD. There are multiple studies demonstrating that MSCs can suppress IFNγ

production by Th1 T cells, however, the role of MSCs in suppressing Th17 cells is more controversial. One study reported that MSCs can suppress IL17 production by T cells, which was cell contact independent and could be reversed by inhibiting indoleamine 2,3-dioxygenase (IDO) [34]. Although MSC-mediated suppression of Th17 cells results have been corroborated by other groups [35], other studies have observed a reciprocal expansion of Th17 cells after exposure to MSC, while confirming the potent suppression of Th1 cells [36]. Irrespective of their effects on cytokine production, it is well documented that MSCs inhibit T-cell proliferation [37,38], and induce apoptosis [39], which would ofcourse serve to downregulate effector T-cell responses.

The antiproliferative effects of MSCs are at least partly mediated by cyclin D2 which arrest the cell cycle in the G0/G1 phase [37,40]. Importantly, T-cell lines isolated from the intestine of CD patients exhibit reduced proliferative responses, augmented apoptosis, and impaired production of IFNγ, IL17, IL21 and TNFα when cocultured with MSCs [41]. These inhibitory effects were dependent on IDO and cell contact. Although the immunosuppressive effects of MSCs on T cells has received much research attention, it is important to remember that MSCs also modulate other lymphocyte populations, including B cells (inhibiting activation, proliferation and IgG secretion) [42] and natural killer (NK) cells [43].

MSCs also impact on the phenotype and activation of cells of the MP system. MPs play a key role in priming and activating adaptive immunity, as well as responding to T-cell signals to drive inflammation. MSCs suppress the differentiation and maturation of DCs from monocytes or HSC [44,45]. DCs suppressed by MSC fail to upregulate co-stimulatory molecules such as CD80, CD86 and CD40, and fail to produce key cytokines involved in T-cell polarization, such as IL12, which is critical for Th1 development [45,46].

In addition to suppressing pro-inflammatory immune pathways, MSCs also promote the expansion and immunosuppressive action of immune cells involved in countering excessive inflammation. Tregs are the best known immunomodulatory lymphocytes, and play a key role in suppressing gut inflammation. MSCs promote the generation of Tregs, increase their suppressive action, and in models of autoimmunity MSCs act synergistically to promote disease amelioration [47]. *In vivo* administration of MSCs is associated with expansion of $FoxP3^+$ Tregs and reduced severity of colitis [48,49].

More recent work suggests that a new regulatory subset of IL10 producing $CD5^+$ B cells (Bregs) may also contribute to suppression of exaggerated immune responses in experimental colitis [50], and *in vivo* administration of MSCs has been shown to promote the expansion of Bregs in different disease settings [51,52].

Myeloid cells also participate in the anti-inflammatory processes. MSCs promote the differentiation of IL10 producing immunosuppressive macrophages, which potently suppress T-cell activation [53]. Notably, adoptive transfer of MSC-conditioned regulatory macrophages attenuates preclinical models of IBD.

Although the molecular mechanisms responsible for mediating the immunosuppressive effect of MSC have yet to be resolved, there is experimental support for IDO as an important contributor to this process. IDO depletes the microenvironment of tryptophan, resulting in cell proliferation arrest [54]. Besides IDO, other soluble factors implicated in the delivery of MSC immunosuppression include prostaglandin E2 [55], HLAG5 [56], transforming growth factor beta-1 and leukocyte inhibitory factor [57].

2.3. Tissue repair properties

As well as their anti-inflammatory effects, MSCs have been shown to accelerate tissue repair. While they probably differentiate into the wound, they mainly achieve healing by promoting increased epithelialization, formation of granulation tissue and neovascularization [58,59]. The exact mechanisms are not well understood but one pathway is via the secretion of paracrine growth factors including vascular endothelial growth factor (VEGF)-alpha, keratinocyte growth factor, insulin-like growth factor and angiopoietin-1 which facilitate the recruitment of macrophages and fibroblasts to the site of inflammation. This enhances angiogenesis and collagen production and reduces scar formation at the wound site [60]. In a mouse model, local injection of MSCs into inflamed gut mucosa was more effective in preventing the development of penetrating ulcers than intravenous injection of MSC. Locally injected MSCs were observed to be more efficiently recruited to sites of colonic inflammation and stimulated angiogenesis in a VEGF-dependent manner [61].

2.4. Therapeutic application of MSCs in immune-mediated disorders

The capacity for MSCs to regulate inflammation and promote tissue restitution renders them an attractive therapeutic tool for immune-mediated disease. Therapeutic application of MSCs has perhaps gained most traction in graft-versus-host disease (GvHD), a feared complication of BM transplantation that results in immune-mediated damage from donor immune cells. This manifests with inflammation in the gut, liver and skin. The first case report suggesting a benefit was in 2004 when MSCs were administered in a patient with severe treatment-resistant grade IV acute GvHD, who then went into complete remission for at least a year following treatment with MSCs [62]. Since then, their efficacy has been suggested in several early clinical trials, including a breakthrough phase 2 multicenter study where 30 out of 55 patients with steroid-resistant grade II–IV acute GvHD entered remission following MSC therapy [63]. These results have been largely corroborated in other early phase trials [64–66]. The key phase 3 clinical trials (NCT00366145 and NC00562497) using the commercial MSC product (Prochymal®, Osiris Therapeutics, Albert Einstein Drive, Columbia, MD) have yet to be published.

Encouraging results of MSC therapy have also been reported in other inflammatory disease settings, including acute respiratory distress syndrome [67], multiple sclerosis [68] and renal transplantation [69], consistent with the conceptually attractive notion that MSCs may also be effective in chronic gut inflammation.

Table 1. Comparison of allogenic and autologous mesenchymal stromal cells.

	Allogenic	Autologous
Source	Healthy donor	Patient
Sourcing	Sourced commercially – therefore can be used by any center	Generation of MSCs dependent on center expertise
Consistency	Commercial production fosters consistency	Consistency varies between patients
Time to administration	Immediate – 'off-the-shelf'	Following extraction and *in vitro* expansion (days–weeks)
Number of doses per specimen	Up to 10,000. Provisions for many patients.	More limited especially in elderly or thin patients. Provision for one patient.
Immunogenicity	Minimal	Nil
Cost	More cost effective	Less cost effective
Efficacy	Equivalent	Equivalent

MSCs: mesenchymal stromal cells.

2.5. Autologous versus allogeneic MSCs

Currently, both autologous and allogeneic MSCs are under investigation (Table 1). The quality of autologous MSC from CD patients was initially questioned given earlier findings that autologous MSC derived from patients with systemic lupus erythematosus (SLE) showed lower proliferation rates and immunosuppressive capacity compared to healthy controls [70]. However, this does not appear to be the case with MSCs from CD patients [71,72]. Even though the tolerability and safety of autologous MSC therapy has not been challenged, their clinical application has been hampered by the lack of standardization in terms of sourcing and manufacturing as well as the time needed to expand cells. This has been overcome using allogenic MSCs, which can be mass produced and readily available at the point-of-care. For example, Prochymal® has already been approved in the USA and Canada. MSCs are derived from BM of healthy donors and expanded *ex vivo* with one donor being able to provide up to 10,000 doses [73].

3. MSC therapy in CD

3.1. Fistulizing disease

Fistulae commonly complicate CD. In a population-based cohort study from Olmsted County (USA), the cumulative incidence of fistula development was 33% after 10 years and 50% after 20 years, with perianal fistulae accounting for 54%, entero-enteric 24%, and rectovaginal 9% [74]. The goal of treatment is to achieve complete fistula closure without compromising anal sphincter function. However, this is challenging and many patients experience high rates of recurrence even with the combination of maximal pharmacotherapy (antibiotics and biologics) and surgical drainage. Failure to respond leaves proctectomy or diversion ileostomy as the last resort, which significantly impairs quality of life [75]. The pathophysiology of CD fistulae is complex, however, dysregulated adaptive and innate immune responses play a prominent role. Large numbers of macrophages, which are the chief source of TNF in the gut, line the fistula tract and are surrounded by pockets of T cells and B cells in deeper layers of the lamina propria [76]. The phenotype of T cells shows significant accumulation of IL17A and IFNγ producing $CD161^+$ memory $CD4^+$ T cells [77]. Given the robust activation of host immunity and significant degree of tissue injury in CD fistulae, coupled to striking accumulation of immune cell types and pathways that have been shown to be amenable to suppression by MSCs, there has been intense interest in exploring the therapeutic potential of these cells in this disease setting. There have been several clinical trials investigating the utility of locally injected MSC into perianal fistulae (Table 2). The safety and therapeutic potential of MSCs in treating perianal CD was first demonstrated in 2005 when autologous adipose-derived MSC was injected into nine perianal fistulae from four patients. After 8 weeks, complete healing was observed in six fistulae [78]. The same group later performed the first randomized controlled trial of expanded autologous adipose-derived MSC in perianal fistulae, which included both CD and cryptoglandular fistulae [79]. In this study, all patients received adjunctive surgical therapy with tract curettage and closure of the internal opening of the fistula with a stitch. Control patients were then treated by sealing of the tract with fibrin glue. In MSC-treated patients, prior to tract closure with fibrin glue, 20 million MSCs were superficially injected (≤2 mm) into the fistula tract wall. A second dose of 40 million MSCs was administered if no closure was observed at 8 weeks. The primary end point was fistula closure 8 weeks after the last treatment administered. Fistula tract healing was observed in 71% of patients treated with MSC and fibrin glue as compared to 16% of patients treated with fibrin glue alone, with similar results observed in patients with CD or cryptoglandular fistulae. In patients receiving MSCs, closure was observed in 46% of patients after a single treatment and in a further 25% after a second rescue treatment. In patients treated with fibrin glue alone, healing occurred in 8% after a single treatment and a further 8% after a second treatment.

An alternative approach is to adjust the MSC dose according to the length of the fistula tract. In an open-label study, 43 patients received adipose-derived autologous MSCs with fibrin glue, at a dose that was proportional to the length of the fistula tract. Complete closure was achieved in 64% of patients at 8 weeks (82% in the per protocol analysis), and 88% of this group had sustained closure at 1 year with no MSC-related adverse events [82].

Impressive early phase results have also been reported using allogeneic MSCs, including statistically significant improvements in radiological closure of fistulae, defined as the absence of collections >2 cm in three axes on magnetic

Table 2. Completed clinical trials for local MSC injections in fistulizing perianal Crohn's disease.

Study	Phase	*N* receiving treatment	Source	Regimen and dose (/10^6 cells)	Follow-up	Response (*n*)	Treatment-related AE
Garcia-Olmo et al. [78]	1	4 (8 fistulae)	Autologous Adipose derived	1 dose: 3–30 cells	8 W	Clinical closure: ×6 (complete) ×2 (partial)	Nil
Garcia-Olmo et al. [79]	2	14	Autologous Adipose derived	1 ± 2nd dose: Fibrin glue or fibrin glue plus 20 cells ± 40 cells or fibrin glue at week 8 if incomplete closure	12 M	Clinical closure: 5/7 in MSC + fibrin group 1/7 in fibrin group	Nil
Ciccocioppo et al. [49]	1	10	Autologous BM derived	4 doses (weeks 0, 4, 8, and 12): 20 cells	12 M	Clinical closure: ×7 (complete) ×3 (partial) ×7 rectal mucosal healing	Nil
Cho et al. [80]	1	10	Autologous Adipose derived	1 dose: 10, 20, or 40 cells/ml (proportional to size of fistula tract)	6 M	Clinical closure: ×3 (complete)	Nil
De La Portilla et al. [81]	2	24	Allogenic Adipose derived	1 dose ± 2nd dose: 20 million cells ± 40 million cells at week 12 if incomplete closure	24 W	Clinical and radiological closure: ×9 reduction in no. draining fistula ×7 (complete) (6/7 clinical and radiological closure)	×3 anal abscess ×1 pyrexia ×1 uterine leiomyoma
Lee et al. [82]	2	43	Autologous Adipose derived	1 dose ± 2nd dose 30–60 cells/cm, with fibrin glue ± 1.5 times more cells at week 8 if incomplete closure	12 M	Clinical closure: ×27 (complete) at week 8 ×23 (complete) at 12 months ×6 (partial) (5/6 > 50% closure)	Nil
Molendijk et al. [83]	2	21	Allogenic BM derived	1 dose: 10, 30, or 90 cells or placebo	24 W	Clinical and radiological closure: ×7 (complete) (×2 in placebo)	Nil
Panes et al. [28]	3	212	Allogenic Adipose derived	1 dose: 120 cells or placebo	24 W	Clinical and radiological closure: 53/107 (complete)	18/103 MSC group 30/103 placebo Mainly anal abscess and proctalgia

AE: adverse events; BM: bone marrow; M: months, N: number; W: weeks; MSC: mesenchymal stromal cell.

resonance imaging (MRI) [84]. An open-label study of autologous MSCs injected into fistula tracts confirmed radiological healing using axial T1-weighted MRI with the emergence of regenerative tissue replacing the fistula track in the absence of fibrosis – an invaluable consideration since fibrotic repair can compromise anal sphincter integrity and continence [49]. Interestingly, this study also showed expansion of Tregs in the rectal mucosa following of MSC treatment. Furthermore, MSCs cocultured with lamina propria T cells resulted in significantly increased production of the anti-inflammatory cytokine IL10.

Crucially, there is now a phase 3 study evaluating the efficacy of MSC versus placebo in 212 patients with treatment refractory, perianal fistulizing CD [28]. Patients were randomized to allogenic adipose-derived MSC local injections (Cx601, 120 million cells injected directly into the tract) or placebo (saline injected directly into the tract). The primary end point was combined clinical and radiological remission at 24 weeks. In the intention-to-treat analysis, significantly more patients treated with intralesional MSC achieved the primary end point in comparison with patients treated with intralesional saline (50% vs. 34%, absolute difference 15.2%, 97.5% confidence interval (CI) 0.2–30.3; $p = 0.024$). All patients received adjunctive surgical management including fistula curettage, drainage, and internal orifice closure, performed as part of the protocol 2 weeks prior to the study. This may account for the high remission rate observed. In terms of safety outcomes, proctalgia (five in the treatment group vs. nine in placebo) and anal abscesses (six vs. nine) were the most commonly reported events but their presence in both groups suggests the preparation procedure may account for these events, rather than the actual cell therapy.

The only study that looked at long-term outcomes beyond 1 year was a retrospective analysis of patients that had initially received autologous local injections of MSCs and fibrin glue in perianal fistulae. From the Crohn's cohort, five out of seven had complete closure at 24 weeks [79] and from these, two out of five had sustained closure at 3 years [85]. There were no new safety issues identified suggesting a favorable safety and tolerability profile, although the small number of patients in the study limits interpretation of the longer-term efficacy and safety data.

There is a paucity of data on the role of MSCs in fistulae at other anatomical locations. Rectovaginal fistulae are particularly detrimental to quality of life, but there is no clear consensus on their management, probably due to a lack of large-scale clinical trials. Surgical treatment is challenging with unsatisfactory response rates and high complication rates. A recent systematic review reported that anti-TNFα therapy led to a complete response in 41%, partial response in 21.8% and no response in 37.9% [86]. This low response rate has been partly attributed to a poorly vascularized rectovaginal septum [87] which renders local therapy with MSC even more attractive in this group. However, there is hardly any MSC data from this cohort even though the first ever case of a patient treated with MSCs was in a 33-year old with a refractory rectovaginal fistula who achieved complete healing after 3 months, despite flares of intervening perianal disease [88].

A small proof-of-concept study investigated the impact of autologous MSC on enterocutaneous fistulae, reported complete closure (defined as complete epithelialization of the external closure) at 8 weeks and 1 year in three out of four patients, consistent with a potential signal of efficacy [89].

3.2. Luminal disease

Despite advances in biological therapy, the number of patients requiring surgical resection for the stenosing and uncontrolled inflammatory complications of CD has not declined significantly. Moreover, following a surgical resection many patients will require a second operation [90]. The potential for systemically infused MSCs to attenuate a dysregulated inflammatory response and repair damaged tissue has exciting implications for reducing the need for surgery. Furthermore, there is still an important unmet need to develop multifaceted anti-inflammatory therapies for patients with inflammatory luminal disease, since biological therapies frequently fail in this setting [3].

One of the first studies to demonstrate the safety and feasibility of MSC in luminal disease was an early phase 1 trial in the Netherlands, where nine patients with refractory CD received two infusions of autologous BM-derived MSC (days 0 and 7, with a dose of 1–2 million cells/kg body weight). At 6 weeks, endoscopic improvement was reported in two patients, clinical improvement in three, while three patients required surgery due to worsening disease [72]. This study also demonstrated a trend toward fewer infiltrating $CD4^+$ T cells, increased numbers of Tregs, and lower cytokine levels (TNFα, IL1β, IL10, and IL6) in colonic biopsies at week 6, which is in line with earlier *in vitro* work and experimental colitis studies in mice [48]. The phenotype of MSCs from the CD patients was reported to be comparable to MSCs from healthy donors, which is also consistent with previous studies [71].

Another study confirming the safety and feasibility of autologous MSC therapy administered a single dose of either 2, 5, or 10 million cells/kg in 12 refractory CD patients. Clinical response as defined by a decrease in the Crohn's disease activity index (CDAI) of more than 100 points, was observed in five patients, and worsening disease in five others at the 9-week follow-up [91].

Prochymal® was used in a study by Onken et al. in 2006 where nine patients with active refractory CD (CDAI ≥220 and C-reactive protein ≥5 mg/L) were randomized to two doses a week apart, of either 2 or 8 million cells/kg. A reduction in CDAI score at day 28 was experienced by all patients (mean reduction of 105 points, $p = 0.004$), with three patients achieving the primary end point of a CDAI score reduction ≥100 points. The mean reduction in CDAI was greater in the higher-dose group although this did not reach statistical significance. All infusions were well tolerated, with no treatment-related adverse events [92]. Lazebnik et al. went a step further to demonstrate that as well as reducing CDAI scores, MSC therapy enabled the majority of the 10 steroid refractory CD patients to discontinue or reduce their corticosteroid burden [93].

Promising results were also seen in 15 CD patients with moderate-to-severe active disease who were refractory to anti-

TNFα therapy. Study patients received weekly infusions of BM-derived allogenic MSC at 2 million cells/kg for 4 weeks [94]. At 6 weeks, a clinical response (decrease in CDAI >100 points) was observed in 12 patients (80%), clinical remission (CDAI <150) in 8 patients (53%), and endoscopic improvement in 7 patients (47%). Treatments were well tolerated but a serious adverse event related to the diagnosis of a malignant sigmoid dysplastic lesion observed in one patient at the 6-week endoscopy. However, this patient had previously documented low-grade dysplasia at other sites and had been recommended to undergo colectomy for cancer prophylaxis. The investigators suggested that the malignancy was probably present before the endoscopy at 6 weeks but potentially missed due to active colitis, however, the possibility of MSCs contributing to progression of dysplasia to cancer should not be excluded. There is currently no evidence that MSC therapy contributes to neoplastic development, and this is supported by findings from a systematic review that followed patients up to 5 years, and reported no significant difference in incidences of malignancy between 103 MSC-treated IBD patients (56 UC and 47 CD) and 208 matched controls [95]. However, since not all these patients were assessed by repeat endoscopy at follow-up, the presence of dysplastic lesions cannot be excluded. Although there have been no suggestions of increased risk of malignancy from the numerous MSC trials in patients with GvHD, there remains a need for long-term prospectively collected safety data in MSC-treated patients to confidently address this line of enquiry.

While clinical outcomes for MSC therapy in luminal Crohn's are modest at best, it is worth noting that the small patient numbers, the heterogeneous study designs, sourcing, and dosing regimens of trials, as well as absence of phase 3 data, render meaningful conclusions premature (Table 3). There is an ongoing phase 3 randomized, double-blind, placebo controlled, multicenter study which is investigating the safety and efficacy of Prochymal® in treatment refractory moderate-to-severe CD. Patients are randomized to receive four infusions over 2 weeks of 600 million cells, 1200 million cells, or placebo with an expected completion date of 2018. The primary end point is remission at day 28 [96].

4. Conclusion

MSCs are an attractive therapeutic strategy in CD owing to their capacity for active participation in tissue regeneration at sites of inflammation and ability to mediate potent anti-inflammatory effects. Their low immunogenicity permits the use of reliable and reproducible sources of allogenic preparations, which appear to be efficacious and safe. Although MSC therapy in CD is still in its infancy, preliminary findings from early phase clinical studies, and a phase 3 trial, place MSCs as viable therapeutic options in perianal fistulizing CD. The possibility of using a minimally invasive procedure to induce repair and regeneration of damaged tissue and potentially reduce the need for surgical intervention has favorable implications for patient outcomes, quality of life as well as cost-effectiveness. However, there are hurdles that still need to be addressed before MSC therapy can be widely adopted in clinical practice. Future work needs to be directed at determining the optimal source of MSC, treatment dose, timing and frequency of administration, long-term safety as well as the mechanisms underlying *in vivo* immunomodulatory properties.

5. Expert opinion

Mucosal healing is considered an important treatment goal in CD since it predicts sustained clinical remission and resection-free survival [97,98]. Therefore, the ideal CD therapy should attenuate inflammation and promote tissue restitution and remodeling without incurring loss of tissue function. Unfortunately, in many patients, current therapies including combinations of immunomodulators and biological drugs fall short of this desirable treatment target.

The ability of MSCs to exhibit potent immunosuppressive effects and promote tissue repair has favorable implications on clinical, endoscopic, and quality of life markers. Clinical trial data including a phase 3 study, show positive results [28], especially in the context of perianal fistulizing CD where the safety, feasibility and short-term efficacy are very encouraging [49,78,79,82,84,85]. A meta-analysis of all perianal MSC CD studies reported that 61.3% (95% CI 35.6–84.6) of patients had fistula closure after local MSC administration, albeit with considerable heterogeneity

Table 3. Completed clinical trials for systemic infusion of MSCs in active luminal Crohn's disease.

Study	Phase	*N* receiving treatment	Source	Regimen and dose (10^6 cells/kg)	Follow-up	Response (*n*)	Treatment-related AE
Onken et al. [92]	1	9	Allogeneic BM derived	2 Doses (days 0 and 7): either 2 or 8 cells/kg	4 W	×3 Clinical response (reduction in CDAI >100)	Nil
Duijvestein et al. [72]	1	9	Autologous BM derived	2 Doses (days 0 and 7): 1–2 cells/kg	6 W	×3 Clinical response (reduction in CDAI >70) ×2 Endoscopic improvement	×3 Headache ×1 Transfusion reaction
Lazebnik et al. [93]	1	11	Allogeneic BM derived	1 Dose: 1.5–2 cells/kg	4–8 M	×9 Clinical response Reduction in steroid dose (number N/A)	Mild transfusion reaction (number N/A)
Forbes et al. [94]	2	16	Allogeneic BM derived	4 Doses (weeks 0, 1, 2, and 3): 2 cells/kg	6 W	×12 Clinical response (reduction CDAI >100) ×8 Clinical remission (CDAI <150) ×7 Endoscopic improvement	In one patient: ×1 Low-grade dysplasia ×1 Colonic adenocarcinoma
Dhere et al. [91]	1	12	Autologous BM derived	1 Dose: either 2, 5, or 10 cells/kg	9 W	×5 Clinical response (reduction CDAI >100)	(Unlikely to be treatment related:) ×1 severe Crohn's colitis flare and appendicitis ×1 *C. diff* colitis

AE: adverse events; BM: bone marrow; *C. diff*: *Clostridium difficile*; M: months, *N*: number; N/A: not available, W: weeks.

(I^2 = 68.9%) [99]. Some of this heterogeneity can be explained by differences in clinical protocols, source of MSCs, end points used and the type of adjunctive surgical and medical therapy administered in addition to the MSCs.

Although these outcomes herald the emergence of a new treatment modality in the armamentarium of physicians and surgeons treating CD, important questions remain unanswered. These include the optimal dosing regime, frequency of administration and in fistulizing disease, where to inject these cells and the optimum adjunctive surgical therapy. There are also outstanding questions about the long-term safety of MSCs and the durability of response. Moreover, while it is clear that fistulizing perianal CD will be at the vanguard of MSC deployment, their effectiveness for other fistulae including rectovaginal and enterocutaneous fistulae remains to be seen. Similarly, harnessing MSCs for isolated luminal disease and maybe even in UC remains an intriguing possibility and the results of large-scale controlled trials are eagerly awaited. Additional questions include identifying where to place MSC therapy in current treatment algorithms and developing strategies to identify patients most likely to benefit from treatment. Given the need for cytokine-mediated licensing of MSCs to elicit their immunosuppressive and tissue repair actions, it is conceivable that patients with significant inflammatory burden and/or tissue damage may benefit the most. Alternatively, identifying patients with host immune responses most likely to support effective *in vivo* MSC licensing, such as patients with pronounced Th17 responses, is an interesting concept to consider in a personalized medicine approach.

Another pertinent issue relates to uncertainty of the fate of MSCs following systemic administration both in terms of survival as well as where they home to. While evidence from preclinical trials suggest that MSCs home to areas of inflammation regardless of their origin or route of administration [100–102], less is known about their survival and persistence in tissues. MSCs have been found in inflamed colonic tissue 15 days after injection in mice [103]. However, the proportion of cells that reach the site of inflammation in human studies has not been evaluated.

In summary, emerging data in this rapidly evolving field strongly supports the likelihood that MSCs will soon find a place in the treatment algorithm in perianal fistulizing CD. This may well pave the way for use in other IBD disease settings including the pregnant and pediatric cohort.

6. Five-year view

The clinical impact of MSC on CD outcomes is currently being evaluated in multiple clinical trials. Indeed, there are currently 13 active clinical trials registered with the ClinicalTrials.gov database, investigating the safety and efficacy of MSC in CD. If the early promise of existing clinical trial experience is replicated, it is tempting to speculate that MSC therapy may well become established as part of the treatment algorithm in the management of perianal fistulizing disease, and potentially in other CD settings including luminal disease. More work is needed to determine optimal dosing schedules, best adjunctive surgical practice, requirement for concomitant medical therapy, methods for maximizing MSC survival and engraftment (e.g. the need for licensing with cytokines), and development of biomarker strategies such that these therapies can be tailored to patient populations most likely to benefit.

Key issues

- MSCs are non-hematopoietic multipotent cells present in most tissues, that are capable of differentiating into osteoblasts, chondrocytes and adipocytes.
- In addition to multilineage differentiation and participation in the hematopoietic niche, MSCs can home to sites of inflammation where they exert potent immunomodulatory effects.
- This includes inhibition of DCs, NK cells and B and Th1 and TH17 proliferation as well as promotion of regulatory T cell differentiation.
- MSCs do not express MHC class II or co-stimulatory molecules permitting allogenic MSCs to be used safely and conveniently.
- Successful preclinical studies using MSCs in models of autoimmunity, inflammation and tissue damage have prompted a sharp rise in the number of clinical trials utilizing MSC in treatment of immune mediated diseases including CD.
- MSC therapy has been administered either locally (for external fistulae) or systemically (for luminal disease) with good short term safety and tolerability.
- The most encouraging therapeutic benefits have emerged from locally administered MSCs into perianal fistulae.
- Preliminary findings for systemic therapy in luminal disease shows some benefit in terms of signals of efficacy but there is no data from phase 3 studies to substantiate this.
- There is an unmet need to broaden the evaluation of MSC therapy to include internal fistulae, other external fistuale, the pediatric population, pregnancy and early CD.
- Enthusiasm is this field needs to be driven by more phase 3 trials.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Cosnes J, Gower-Rousseau C, Seksik P, et al. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology. 2011;140:1785–1794.

•• **An elegant review on the epidemiology, disease progression, and mortality associated with IBD.**

2. Colombel JF, Sandborn WJ, Rutgeerts P, et al. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. Gastroenterology. 2007;132:52–65.
3. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med. 2010;362:1383–1395.
• **The SONIC trial, a key phase 3 multicenter study that showed combination of infliximab and azathioprine was superior to monotherapy in achieving steroid-free clinical remission and mucosal healing in CD.**
4. Rutgeerts P, Van Assche G, Sandborn WJ, et al. Adalimumab induces and maintains mucosal healing in patients with Crohn's disease: data from the EXTEND trial. Gastroenterology. 2012;142:1102–11.e2.
5. Akobeng AK, Zachos M. Tumor necrosis factor-alpha antibody for induction of remission in Crohn's disease. The Cochrane Library; Cochrane Database Syst Rev. 2004;(1):CD003574.
6. Cottone M, Criscuoli V. Infliximab to treat Crohn's disease: an update. Clin Exp Gastroenterol. 2011;4:227–238.
7. Sandborn WJ, Feagan BG, Rutgeerts P, et al. Vedolizumab as induction and maintenance therapy for Crohn's disease. N Engl J Med. 2013;369:711–721.
8. Feagan BG, Sandborn WJ, Gasink C, et al. Ustekinumab as induction and maintenance therapy for Crohn's disease. N Engl J Med. 2016;375:1946–1960.
• **A key phase 3 trial demonstrating efficacy of the newly licensed biologic, ustekinumab, in CD.**
9. West NR, Hegazy AN, Owens BMJ, et al. Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor-neutralizing therapy in patients with inflammatory bowel disease. Nat Med. 2017;23:579–589.
10. Shale M, Schiering C, Powrie F. CD4+ T-cell subsets in intestinal inflammation. Immunol Rev. 2013;252:164–182.
• **A thorough review exploring advances in understanding the role of CD4$^+$ T cells in intestinal immunity.**
11. Chao K, Zhang S, Yao J, et al. Imbalances of CD4(+) T-cell subgroups in Crohn's disease and their relationship with disease activity and prognosis. J Gastroenterol Hepatol. 2014;29:1808–1814.
12. Hart AL, Al-Hassi HO, Rigby RJ, et al. Characteristics of intestinal dendritic cells in inflammatory bowel diseases. Gastroenterology. 2005;129:50–65.
13. Goldberg R, Prescott N, Lord GM, et al. The unusual suspects–innate lymphoid cells as novel therapeutic targets in IBD. Nat Rev Gastroenterol Hepatol. 2015;12:271.
14. Powell N, Lo JW, Biancheri P, et al. Interleukin 6 increases production of cytokines by colonic innate lymphoid cells in mice and patients with chronic intestinal inflammation. Gastroenterology. 2015;149(456–67):e15.
15. Powell N, Walker AW, Stolarczyk E, et al. The transcription factor T-bet regulates intestinal inflammation mediated by interleukin-7 receptor+ innate lymphoid cells. Immunity. 2012;37:674–684.
16. Maul J, Loddenkemper C, Mundt P, et al. Peripheral and intestinal regulatory CD4+ CD25(high) T cells in inflammatory bowel disease. Gastroenterology. 2005;128:1868–1878.
17. Russell R, Satsangi J. IBD: a family affair. Best Pract Res Clin Gastroenterol. 2004;18:525–539.
18. De Lange KM, Moutsianas L, Lee JC, et al. Genome-wide association study implicates immune activation of multiple integrin genes in inflammatory bowel disease. Nat Genet. 2017;49:256.
19. Huang H, Fang M, Jostins L, et al. Fine-mapping inflammatory bowel disease loci to single-variant resolution. Nature. 2017;547:173–178.
20. Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet. 1970;3:393–403.
•• **The nominal study in 1970 where MSCs were first described.**
21. Prockop DJ. Repair of tissues by adult stem/progenitor cells (MSCs): controversies, myths, and changing paradigms. Mol Ther. 2009;17:939–946.
22. Phinney DG, Prockop DJ. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair – current views. Stem Cells. 2007;25:2896–2902.
23. Karp JM, Leng Teo GS. Mesenchymal stem cell homing: the devil is in the details. Cell Stem Cell. 2009;4:206–216.
24. Watson L, Elliman SJ, Coleman CM. From isolation to implantation: a concise review of mesenchymal stem cell therapy in bone fracture repair. Stem Cell Res Ther. 2014;5:51.
25. Le Blanc K, Tammik C, Rosendahl K, et al. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol. 2003;31:890–896.
26. Nauta AJ, Westerhuis G, Kruisselbrink AB, et al. Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting. Blood. 2006;108:2114–2120.
27. Zangi L, Margalit R, Reich-Zeliger S, et al. Direct imaging of immune rejection and memory induction by allogeneic mesenchymal stromal cells. Stem Cells. 2009;27:2865–2874.
28. Panes J, Garcia-Olmo D, Van Assche G, et al. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial. Lancet. 2016;388:1281–1290.
•• **The first and only phase 3 study of MSC in fistulizing CD.**
29. Krampera M, Cosmi L, Angeli R, et al. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. Stem Cells. 2006;24:386–398.
30. Ren G, Zhang L, Zhao X, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell. 2008;2:141–150.
31. Duijvestein M, Wildenberg ME, Welling MM, et al. Pretreatment with interferon-gamma enhances the therapeutic activity of mesenchymal stromal cells in animal models of colitis. Stem Cells. 2011;29:1549–1558.
•• **An important murine study showing stimulation of MSCs with IFN-γ enhances their capacity to inhibit Th1 inflammatory responses, and attenuate mucosal damage in experimental colitis.**
32. Sivanathan KN, Rojas-Canales D, Grey ST, et al. Transcriptome profiling of IL-17A preactivated mesenchymal stem cells: a comparative study to unmodified and IFN-γ modified mesenchymal stem cells. Stem Cells Int. 2017;2017:1025820. doi: 10.1155/2017/1025820.
33. Sivanathan KN, Rojas-Canales DM, Hope CM, et al. Interleukin-17A-induced human mesenchymal stem cells are superior modulators of immunological function. Stem Cells. 2015;33:2850–2863.
34. Milosavljevic N, Gazdic M, Simovic Markovic B, et al. Mesenchymal stem cells attenuate liver fibrosis by suppressing Th17 cells. Transplant International; 2017;Aug 14. doi: 10.1111/tri.13023. [Epub ahead of print].
35. Liu Q, Zheng H, Chen X, et al. Human mesenchymal stromal cells enhance the immunomodulatory function of CD8(+)CD28(-) regulatory T cells. Cell Mol Immunol. 2015;12:708–718.
36. Darlington PJ, Boivin MN, Renoux C, et al. Reciprocal Th1 and Th17 regulation by mesenchymal stem cells: implication for multiple sclerosis. Ann Neurol. 2010;68:540–545.
37. Di Nicola M, Carlo-Stella C, Magni M, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood. 2002;99:3838–3843.
38. Krampera M, Glennie S, Dyson J, et al. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood. 2003;101:3722–3729.
39. Plumas J, Chaperot L, Richard MJ, et al. Mesenchymal stem cells induce apoptosis of activated T cells. Leukemia. 2005;19:1597–1604.
40. Glennie S, Soeiro I, Dyson PJ, et al. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood. 2005;105:2821–2827.

41. Ciccocioppo R, Cangemi GC, Kruzliak P, et al. Ex vivo immunosuppressive effects of mesenchymal stem cells on Crohn's disease mucosal T cells are largely dependent on indoleamine 2, 3-dioxygenase activity and cell-cell contact. Stem Cell Res Ther. 2015;6:137.
42. Corcione A, Benvenuto F, Ferretti E, et al. Human mesenchymal stem cells modulate B-cell functions. Blood. 2006;107:367–372.
43. Spaggiari GM, Capobianco A, Becchetti S, et al. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood. 2006;107:1484–1490.
44. Zhang W, Ge W, Li C, et al. Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells. Stem Cells Dev. 2004;13:263–271.
45. Spaggiari GM, Abdelrazik H, Becchetti F, et al. MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: central role of MSC-derived prostaglandin E 2. Blood. 2009;113:6576–6583.
46. Zhang B, Liu R, Shi D, et al. Mesenchymal stem cells induce mature dendritic cells into a novel Jagged-2–dependent regulatory dendritic cell population. Blood. 2009;113:46–57.
47. Burr SP, Dazzi F, Garden OA. Mesenchymal stromal cells and regulatory T cells: the Yin and Yang of peripheral tolerance? Immunol Cell Biol. 2013;91:12–18.
48. Gonzalez MA, Gonzalez-Rey E, Rico L, et al. Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. Gastroenterology. 2009;136:978–989.
49. Ciccocioppo R, Bernardo ME, Sgarella A, et al. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. Gut. 2011;60:788–798.
•• **A feasibility, safety, and efficacy study of MSC intrafistular injections in 12 patients, with an investigation of potential mechanisms of action, including the positive effect on Tregs.**
50. Chao K, Zhang S, Qiu Y, et al. Human umbilical cord-derived mesenchymal stem cells protect against experimental colitis via CD5(+) B regulatory cells. Stem Cell Res Ther. 2016;7:109.
51. Guo Y, Chan KH, Lai WH, et al. Human mesenchymal stem cells upregulate CD1dCD5(+) regulatory B cells in experimental autoimmune encephalomyelitis. Neuroimmunomodulation. 2013;20:294–303.
52. Peng Y, Chen X, Liu Q, et al. Mesenchymal stromal cells infusions improve refractory chronic graft versus host disease through an increase of CD5+ regulatory B cells producing interleukin 10. Leukemia. 2015;29:636–646.
53. Anderson P, Souza-Moreira L, Morell M, et al. Adipose-derived mesenchymal stromal cells induce immunomodulatory macrophages which protect from experimental colitis and sepsis. Gut. 2013;62:1131–1141.
54. Meisel R, Zibert A, Laryea M, et al. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood. 2004;103:4619–4621.
55. Nemeth K, Leelahavanichkul A, Yuen PS, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med. 2009;15:42–49.
56. Selmani Z, Naji A, Zidi I, et al. Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4 +CD25highFOXP3+ regulatory T cells. Stem Cells. 2008;26:212–222.
57. Najar M, Raicevic G, Boufker HI, et al. Adipose-tissue-derived and Wharton's jelly-derived mesenchymal stromal cells suppress lymphocyte responses by secreting leukemia inhibitory factor. Tissue Eng Part A. 2010;16:3537–3546.
58. Nuschke A. Activity of mesenchymal stem cells in therapies for chronic skin wound healing. Organogenesis. 2014;10:29–37.
59. Otero-Vinas M, Falanga V. Mesenchymal stem cells in chronic wounds: the spectrum from basic to advanced therapy. Adv Wound Care (New Rochelle). 2016;5:149–163.
• **An interesting review summarizing how MSCs enhance wound closure, increase tensile strength within the wound, promote angiogenesis and reduce inflammation.**
60. Hocking AM, Gibran NS. Mesenchymal stem cells: paracrine signaling and differentiation during cutaneous wound repair. Exp Cell Res. 2010;316:2213–2219.
61. Manieri NA, Mack MR, Himmelrich MD, et al. Mucosally transplanted mesenchymal stem cells stimulate intestinal healing by promoting angiogenesis. J Clin Invest. 2015;125:3606.
62. Le Blanc K, Rasmusson I, Sundberg B, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet. 2004;363:1439–1441.
63. Le Blanc K, Frassoni F, Ball L, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. The Lancet. 2008;371:1579–1586.
64. Introna M, Lucchini G, Dander E, et al. Treatment of graft versus host disease with mesenchymal stromal cells: a phase I study on 40 adult and pediatric patients. Biol Blood Marrow Transplant. 2014;20:375–381.
65. Sanchez-Guijo F, Caballero-Velazquez T, Lopez-Villar O, et al. Sequential third-party mesenchymal stromal cell therapy for refractory acute graft-versus-host disease. Biol Blood Marrow Transplant. 2014;20:1580–1585.
66. Te Boome LC, Mansilla C, Van Der Wagen LE, et al. Biomarker profiling of steroid-resistant acute GVHD in patients after infusion of mesenchymal stromal cells. Leukemia. 2015;29:1839–1846.
67. Wilson JG, Liu KD, Zhuo H, et al. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. Lancet Respir Med. 2015;3:24–32.
68. Llufriu S, Sepúlveda M, Blanco Y, et al. Randomized placebo-controlled phase II trial of autologous mesenchymal stem cells in multiple sclerosis. PLoS One. 2014;9:e113936.
69. Tan J, Wu W, Xu X, et al. Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial. JAMA. 2012;307:1169–1177.
70. Sun LY, Zhang HY, Feng XB, et al. Abnormality of bone marrow-derived mesenchymal stem cells in patients with systemic lupus erythematosus. Lupus. 2007;16:121–128.
71. Bernardo ME, Avanzini MA, Ciccocioppo R, et al. Phenotypical/functional characterization of in vitro-expanded mesenchymal stromal cells from patients with Crohn's disease. Cytotherapy. 2009;11:825–836.
• **An interesting study demonstrating that immunological markers, genetic stability, and proliferation potential of MSC from CD patients do not differ significantly to MSC from healthy donors.**
72. Duijvestein M, Vos ACW, Roelofs H, et al. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. Gut. 2010;59:1662–1669.
73. Rattue P (May 2012). "Prochymal - first stem cell drug approved." Available from: http://www.medicalnewstoday.com/articles/245704.php.
74. Schwartz DA, Loftus EV, Tremaine WJ, et al. The natural history of fistulizing Crohn's disease in Olmsted County, Minnesota. Gastroenterology. 2002;122:875–880.
75. Gecse K, Khanna R, Stoker J, et al. Fistulizing Crohn's disease: diagnosis and management. United European Gastroenterol J. 2013;1:206–213.
76. Bataille F, Klebl F, Rummele P, et al. Morphological characterisation of Crohn's disease fistulae. Gut. 2004;53:1314–1321.
77. Maggi L, Capone M, Giudici F, et al. CD4+ CD161+ T lymphocytes infiltrate Crohn's disease-associated perianal fistulas and are reduced by anti-TNF-α local therapy. Int Arch Allergy Immunol. 2013;161:81–86.
78. Garcia-Olmo D, Garcia-Arranz M, Herreros D, et al. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. Dis Colon Rectum. 2005;48:1416–1423.
79. Garcia-Olmo D, Herreros D, Pascual I, et al. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: a phase II clinical trial. Dis Colon Rectum. 2009;52:79–86.

80. Cho YB, Lee WY, Park KJ, et al. Autologous adipose tissue-derived stem cells for the treatment of Crohn's fistula: a phase I clinical study. Cell Transplant. 2013;22:279–285.
81. De La Portilla F, Alba F, García-Olmo D, et al. Expanded allogeneic adipose-derived stem cells (eASCs) for the treatment of complex perianal fistula in Crohn's disease: results from a multicenter phase I/IIa clinical trial. Int J Colorectal Dis. 2013;28:313–323.
82. Lee WY, Park KJ, Cho YB, et al. Autologous adipose tissue-derived stem cells treatment demonstrated favorable and sustainable therapeutic effect for Crohn's fistula. Stem Cells. 2013;31:2575–2581.
83. Molendijk I, Bonsing BA, Roelofs H, et al. Allogeneic bone marrow-derived mesenchymal stromal cells promote healing of refractory perianal fistulas in patients with Crohn's disease. Gastroenterology. 2015;149:918–27.e6.
84. De La Portilla F, Alba F, Garcia-Olmo D, et al. Expanded allogeneic adipose-derived stem cells (eASCs) for the treatment of complex perianal fistula in Crohn's disease: results from a multicenter phase I/IIa clinical trial. Int J Colorectal Dis. 2013;28:313–323.
85. Guadalajara H, Herreros D, De-La-Quintana P, et al. Long-term follow-up of patients undergoing adipose-derived adult stem cell administration to treat complex perianal fistulas. Int J Colorectal Dis. 2012;27:595–600.
86. Kaimakliotis P, Simillis C, Harbord M, et al. A systematic review assessing medical treatment for rectovaginal and enterovesical fistulae in Crohn's disease. J Clin Gastroenterol. 2016;50:714–721.
87. Sands BE, Blank MA, Patel K, et al. Long-term treatment of rectovaginal fistulas in Crohn's disease: response to infliximab in the ACCENT II Study. Clin Gastroenterol Hepatol. 2004;2:912–920.
88. García-Olmo D, García-Arranz M, García LG, et al. Autologous stem cell transplantation for treatment of rectovaginal fistula in perianal Crohn's disease: a new cell-based therapy. Int J Colorectal Dis. 2003;18:451–454.
•• **The first and fascinating case report that demonstrated the therapeutic potential of MSC in CD.**
89. Garcia-Olmo D, Herreros D, Pascual M, et al. Treatment of enterocutaneous fistula in Crohn's disease with adipose-derived stem cells: a comparison of protocols with and without cell expansion. Int J Colorectal Dis. 2009;24:27–30.
90. Frolkis AD, Lipton DS, Fiest KM, et al. Cumulative incidence of second intestinal resection in Crohn's disease: a systematic review and meta-analysis of population-based studies. Am J Gastroenterol. 2014;109:1739–1748.
91. Dhere T, Copland I, Garcia M, et al. The safety of autologous and metabolically fit bone marrow mesenchymal stromal cells in medically refractory Crohn's disease - a phase 1 trial with three doses. Aliment Pharmacol Ther. 2016;44:471–481.
92. Onken JGD, Hanson J, Pandak M, et al. (2006). Successful outpatient treatment of refractory Crohn's disease using adult mesenchymal stem cells. American College of Gastroenterology Conference NV, Las vegas.
93. Lazebnik LB, Konopliannikov AG, Kniazev OV, et al. [Use of allogeneic mesenchymal stem cells in the treatment of intestinal inflammatory diseases]. Ter Arkh. 2010;82:38–43.
94. Forbes GM, Sturm MJ, Leong RW, et al. A phase 2 study of allogeneic mesenchymal stromal cells for luminal Crohn's disease refractory to biologic therapy. Clin Gastroenterol Hepatol. 2014;12:64–71.
•• **The largest MSC trial for luminal disease to date (*n* = 16) which shows optimistic remission and response rates.**
95. Kniazev OV, Parfenov AI, Konoplyannikov A, et al. [Safety of mesenchymal stromal cell therapy for inflammatory bowel diseases: results of a 5-year follow-up]. Ter Arkh. 2015;87:39–44.
96. Raina, P. e. a. (2007). Evaluation of Prochymal® adult human stem cells for treatment-resistant moderate-to-severe CD. (ClinicalTrials.gov identifier: NCT00482092).
97. Frøslie KF, Jahnsen J, Moum BA, et al. Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort. Gastroenterology.2007;133:412–422.
98. Schnitzler F, Fidder H, Ferrante M, et al. Mucosal healing predicts long-term outcome of maintenance therapy with infliximab in Crohn's disease. Inflamm Bowel Dis. 2009;15:1295–1301.
99. Dave M, Mehta K, Luther J, et al. Mesenchymal stem cell therapy for inflammatory bowel disease: a systematic review and meta-analysis. Inflamm Bowel Dis. 2015;21:2696–2707.
•• **The only meta-analysis that describes the efficacy and safety of MSC therapy in CD and UC (12 studies included), showing a good therapeutic potential and low adverse event profile.**
100. Tanaka F, Tominaga K, Ochi M, et al. Exogenous administration of mesenchymal stem cells ameliorates dextran sulfate sodium-induced colitis via anti-inflammatory action in damaged tissue in rats. Life Sci. 2008;83:771–779.
101. He XW, He XS, Lian L, et al. Systemic infusion of bone marrow-derived mesenchymal stem cells for treatment of experimental colitis in mice. Dig Dis Sci. 2012;57:3136–3144.
102. Bruck F, Belle L, Lechanteur C, et al. Impact of bone marrow-derived mesenchymal stromal cells on experimental xenogeneic graft-versus-host disease. Cytotherapy. 2013;15:267–279.
103. Liang L, Dong C, Chen X, et al. Human umbilical cord mesenchymal stem cells ameliorate mice trinitrobenzene sulfonic acid (TNBS)-induced colitis. Cell Transplant. 2011;20:1395–1408.