CHAPTER 50

The role of mesenchymal stromal cells in the treatment of ulcerative colitis and Crohn's disease

Céline Gregoire[a], Chantal Lechanteur[b], Alexandra Briquet[b], Etienne Baudoux[b], Olivier Giet[b], Olivier Delloye[b], Frédéric Baron[a], Edouard Louis[c], and Yves Beguin[a,b,]*

[a] Department of Hematology, Le Centre Hospitalier Universitaire of Liège and Unit of Hematology, University of Liège, Liège, Belgium
[b] Laboratory of Cell and Gene Therapy (LTCG), Le Centre Hospitalier Universitaire of Liège and University of Liège, Liège, Belgium
[c] Department of Gastro-Enterology, Le Centre Hospitalier Universitaire of Liège and University of Liège, Liège, Belgium

50.1 Inflammatory bowel diseases

Inflammatory bowel diseases (IBDs) are chronic relapsing diseases associated with debilitating symptoms and leading to progressive intestinal tissue damage and disability (Figure 50.1).

Crohn's disease (CD) can affect any part of the gastrointestinal tract, and lesions are often segmental and transmural. The disease was named after gastroenterologist Dr Burrill Bernard Crohn, who, in 1932, together with two other colleagues at Mount Sinai Hospital in New York, described a series of patients with inflammation of the terminal ileum of the small intestine, the area most commonly affected by the illness. Active CD is characterized by focal inflammation with granulomas and formation of fistula tracts or abscesses, and chronic lesions include fibrosis and stricture of the bowel (Figure 50.2).

In ulcerative colitis (UC), mucosal inflammation is continuous and limited to the mucosa and superficial submucosa. Lesions can affect the rectum and to a variable extent the colon, without granulomas or fistulas. The age of onset of IBD is most frequently in late adolescence or early adulthood, and the disease is equally

* Corresponding author: University of Liège, Department of Hematology, Le Centre Hospitalier Universitaire Sart Tilman, 4000 Liège, Belgium. Email: yves.beguin@chu.ulg.ac.be.
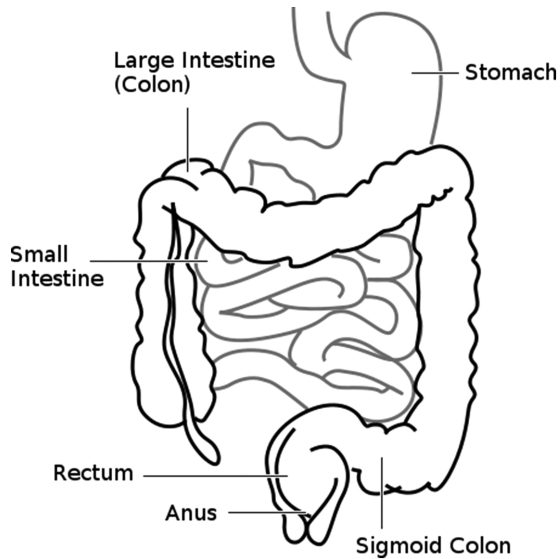
Figure 50.1 Diagram of the human intestine. Drawn by Duncan Lock and released into the Public Domain. *Source:* http://commons.wikimedia.org/wiki/File:Intestine-diagram.svg#mediaviewer/File:Intestine-diagram.svg.

Figure 50.3 Erythema nodosum is an inflammatory condition characterized by inflammation of the fat cells under the skin, resulting in tender red nodules or lumps. It can be caused by a variety of conditions and typically resolves spontaneously within 3–6 weeks. The commonest distribution of erythema nodosum is on the shins – the anterior aspect of the lower legs. *Source:* 1905, William Wood and Company.

distributed between the sexes. The incidence and prevalence are stable in high-incidence areas (northern Europe and North America), and rising in low-incidence areas (southern Europe, Asia, and most developing countries) [1,2].

Symptoms of CD are heterogeneous, but commonly include chronic diarrhea, abdominal pain, weight loss, anorexia, and/or fever. Rectal bleeding occurs commonly in UC, while perianal fissures, fistulas, or abscesses can occur in CD. Extraintestinal features can be related to extraintestinal inflammation, such as joint inflammation (spondyloarthritis, peripheral arthritis),

Figure 50.2 A portion of resected jejunum showing the typical cobblestone appearance of CD affecting the jejunum. *Source:* http://commons.wikimedia.org/wiki/File:Crohn_Jejunum.PNG#mediaviewer/File:Crohn_Jejunum.PNG.

cutaneous manifestations – erythema nodosum (Figure 50.3), pyoderma gangrenosum (Figure 50.4) – ocular inflammation (uveitis, episcleritis, sclero-conjunctivitis), and primary sclerosing cholangitis, or are consequences of malabsorption (cholelithiasis, nephrolithiasis, and osteoporosis) [3].

The diagnosis is based on clinical evaluation and a combination of endoscopic, histological, radiological (computed tomography scan, magnetic resonance imaging (MRI)), and/or biochemical (C-reactive protein (CRP), full blood count, and fecal calprotectin) investigations. The histologic diagnosis is based on the presence of discontinuous chronic inflammation, focal crypt irregularity, and granulomas in CD [4].

50.2 Pathogenesis of inflammatory bowel diseases

The etiology of IBD is multifactorial, polygenic [5], and environmental [6] and includes an abnormal systemic and mucosal

Figure 50.4 Pyoderma gangrenosum affecting the lower leg of a person with CD. This is a condition that causes tissue to become necrotic, resulting in deep ulcers that usually occur on the legs. Ulcers usually initially look like small bug bites or papules and then progress to larger ulcers. While the ulcers rarely lead to death, they can cause pain and scarring. Although the etiology is not well understood, the disease is thought to be due to immune system dysfunction, and particularly abnormal functioning of neutrophils. The commonest diseases associated with pyoderma gangrenosum are the IBDs (CD and UC), followed by the arthritides such as rheumatoid arthritis, followed by malignant hematological disorders. (See color plate section.)
Source: http://commons.wikimedia.org/wiki/File:Crohnie_Pyoderma_gangrenosum.jpg#file.

of inflammation [14–18]. In a later phase, the importance of the Th17 response increases. Th17 cells are induced by IL-6 and transforming growth factor (TGF)-β, and secrete IL-17 and IL-22. IL-23, produced by DCs, interacts with Th17 cells and increases the secretion of IL-6 and IL-17 by T cells, thus enhancing the inflammatory response [19–24]. This overactive immune response can lead to the development of mucosal lesions. Defective apoptosis also promotes autoimmunity, by a reduced autoantibody clearance. Interestingly, almost all drugs with clinical efficacy, including infliximab [25], are able to induce apoptosis of immune cells *in vitro*, while etanercept, an anti-TNF-fusion protein that does not induce apoptosis of immune cells [26], is not efficacious in the treatment of CD [27]. Nevertheless, certolizumab pegol, another anti-TNF agent, is unable to induce cell apoptosis [28], and yet is effective for inducing and maintaining remission in active CD [29,30]. Eventually, chronic inflammation induces intestinal fibroblasts to proliferate and produce a greater amount of extracellular matrix, resulting in local intestinal fibrosis [31].

Endoscopic appearances are shown in Figure 50.5.

In UC, inflammation is mainly driven by IL-5 and IL-13, while production of INF-γ is normal. The disease is qualified as "Th2-like" given the normal production of IL-4, the most defining Th2 cytokine [11]. A Th17 response may also play a role, but its importance is probably lower than in CD.

immune response against intraluminal antigens, favored by microbial factors [7] and alteration of the mucosal barrier [8]. A key role in the pathogenesis of IBD seems to be played by enhanced proliferation and defective apoptosis of immune cells, attributed to an imbalance of the antiapoptotic protein BCL-2 and the pro-apoptotic protein BAX [9,10]), as well as by proinflammatory cytokines arising from T helper cells (Th)1 and Th17 CD4 T cell differentiation in CD, and Th2 T cell differentiation in UC [11–13]. The loss of balance between proliferation and apoptosis is responsible for the presence of an abnormal population of T cells with extended lifespan, which remains in the mucosal compartment and secretes proinflammatory cytokines. The result is an activation of other inflammatory cells, including macrophages and B cells, and a recruitment of circulating leukocytes into the gut through interactions between homing receptors (e.g., $\alpha_4\beta_7$ integrin) and addressins on vascular endothelium, such as the mucosal vascular addressin cell adhesion molecule (MAdCAM)-1 or the vascular cell adhesion molecule (VCAM)-1.

In CD the initial phase of the inflammation seems to be predominantly driven by a Th1 response. Interleukin (IL)-12, a proinflammatory cytokine produced by dendritic cells (DCs), promotes the conversion of T cells into Th1 cells, secreting interferon (INF)-γ and tumor necrosis factor (TNF)-α, two major actors in the induction and propagation

Normal ileum

(A)

Figure 50.5 (A) Normal ileum: the mucosa is homogeneously pink and the villi are regular and well developed. (B) Crohn's ileitis: diffuse inflammation characterized by erythema and mucosal hemorrhage. (C) Crohn's ileitis: presence of erythema and ulcers; red dots indicate mucosal hemorrhages. (D) Normal colon: colonic folds are present and regular. The vascular pattern is also readily visible. (E, F) Crohn's colitis: presence of diffuse inflammation with erythema and superficial ulcers. (G) Healed colon. (See color plate section.)

Crohn ileitis
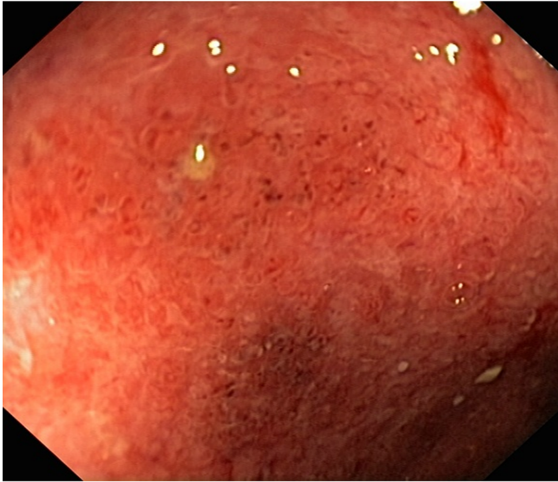
Crohn ileitis

(B)

(C)

Normal colon

Crohn colitis

(D)

(E)

Crohn colitis

Healed colon

(F)
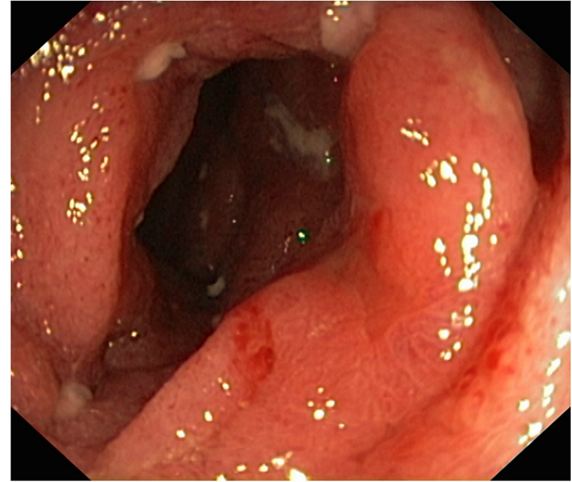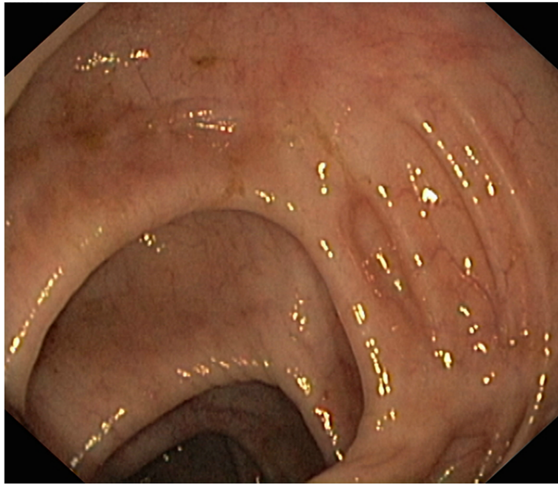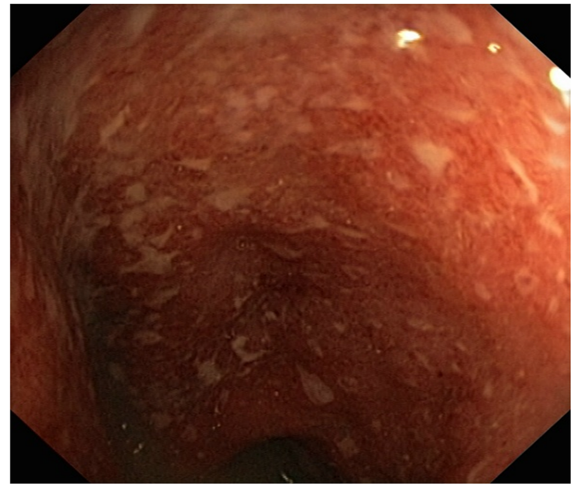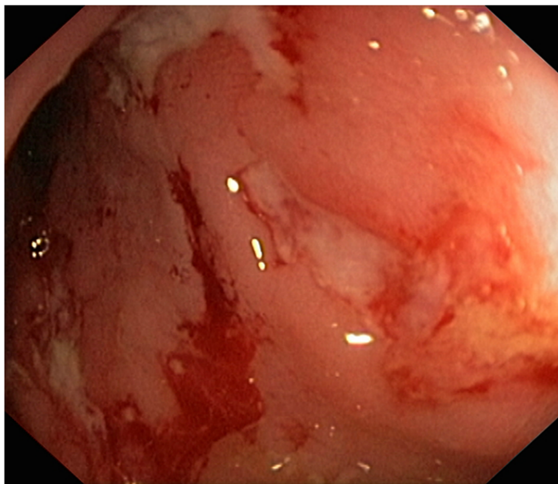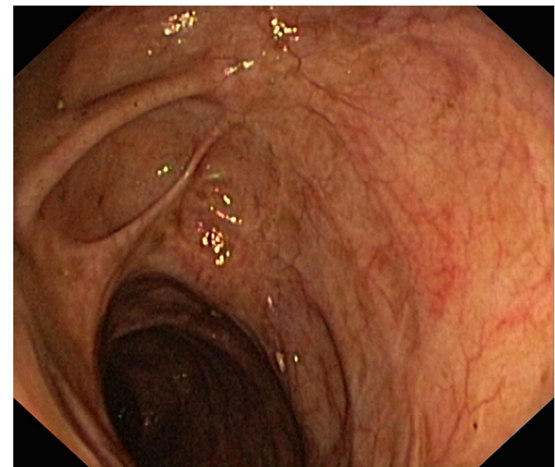
(G)

Figure 50.5 (*Continued*)

Table 50.1 The CDAI is a research tool used to quantify the symptoms of patients with CD.

Clinical or laboratory variable	Weighting factor
Number of liquid or soft stools each day for 7 days	×2
Abdominal pain (graded from 0 to 3 on severity) each day for 7 days	×5
General wellbeing, subjectively assessed from 0 (well) to 4 (terrible) each day for 7 days	×7
Presence of complications[a]	×20
Taking Lomotil or opiates for diarrhea	×30
Presence of an abdominal mass (0 as none, 2 as questionable, 5 as definite)	×10
Hematocrit of <0.47 in men and <0.42 in women	×6
Percentage deviation from standard weight	×1

Source: Best et al. [32].
Remission of CD is defined as CDAI below 150. Severe disease is defined as a value of greater than 450.
[a] One point each is added for each set of complications: (a) the presence of joint pains (arthralgia) or frank arthritis; (b) inflammation of the iris or uveitis; (c) the presence of erythema nodosum, pyoderma gangrenosum, or aphthous ulcers; (d) the presence anal fissures, fistulae, or abscesses; (e) the presence of other fistulae; (f). Fever during the previous week.

50.3 Treatment of Crohn's disease

The choice of therapy is based on anatomic location, severity, and behavior of disease. Severity can be assessed by the CD activity index (CDAI), a score based on daily symptoms and repercussions of CD such as hematocrit, weight, and other factors (Table 50.1).

CD can be classified as mild or mild–moderate (CDAI between 150 and 220), moderate or moderate–severe (CDAI between 220 and 450), or severe or severe/fulminant (CDAI >450). There is a current trend to use CRP levels in conjunction with the CDAI to evaluate severity of the disease [4,33]. Disease remission is defined in the majority of clinical trials as a CDAI of less than 150 [34]. Response is often defined by a variation of CDAI of 100 points or more, although a variation of 70 points or more has been used in some studies. The perineal disease activity index (PDAI) is sometimes used if perineal lesions are predominant. Endoscopic evaluation can be standardized using the CD endoscopic index of severity (CDEIS) or a simplified variant, the simple endoscopic score for CD (SESCD) [35].

50.3.1 Current treatment options for Crohn's disease

Current treatment strategies include oral 5-aminosalicylates (sulfasalazine, mesalamine), antibiotics (metronidazole, ciprofloxacin), topically or systemically acting steroids (controlled ileal release budesonide or prednisone), and immunomodulator therapy, including azothioprine, 6-mercaptopurine, methotrexate, calcineurin inhibitors (cyclosporine, tacrolimus) and anti-TNF antibodies (infliximab, adalimumab, certolizumab pegol). Nutritional therapy (elemental or polymeric diets) can be considered as an adjunct treatment. Mildly to moderately active localized ileocecal disease can often be managed with budesonide, while severe active or extensive disease generally requires systemic corticosteroids with or without an immunomodulator. Anti-TNF therapy, used alone or in adjunction to an immunomodulator, is recommended in moderate to severe or extensive relapsing disease or in previously steroid-refractory, -dependent, or -intolerant patients. All currently available anti-TNF therapies appear to have similar efficacy.

If there is loss of response to anti-TNF therapy, increasing doses or frequency of administration can be tried before switching to another anti-TNF agent. Surgery should be considered in localized disease. Treatment of relapse is essentially based upon previously successful therapies, but other factors such as patient preference, time to relapse, concurrent therapy, and adherence to therapy are taken into account. The choice of maintenance therapy is essentially based upon the course and extent of disease, and the effectiveness and tolerance of previously used treatments. Some patients do not need maintenance. Azathioprine is generally the first choice, followed by methotrexate and anti-TNF therapy [36].

Perianal and fistulizing disease can be treated by surgery, including surgical drainage, fistulotomy and setons, advancement flaps, antibiotics, or an immunomodulator (including anti-TNF agents) [33]. A seton or seton stitch is a procedure used to aid the healing of fistulas. The procedure involves running a surgical-grade cord through the fistula tract so that the cord creates a loop that joins up outside the fistula. The cord provides a path that allows the fistula to drain continuously while it is healing, rather than allowing the exterior of the wound to close over. Keeping the fistula tract open can help keep from trapping pus or other infectious material in the wound. The procedure was mentioned by Hippocrates in 400 BC. Flap surgery is a technique in plastic and reconstructive surgery where any type of tissue is lifted from a donor site and moved to a recipient site with an intact blood supply. Advancement flaps are used when the patient is in overall good health and the defect is free of tumor and obvious infection.
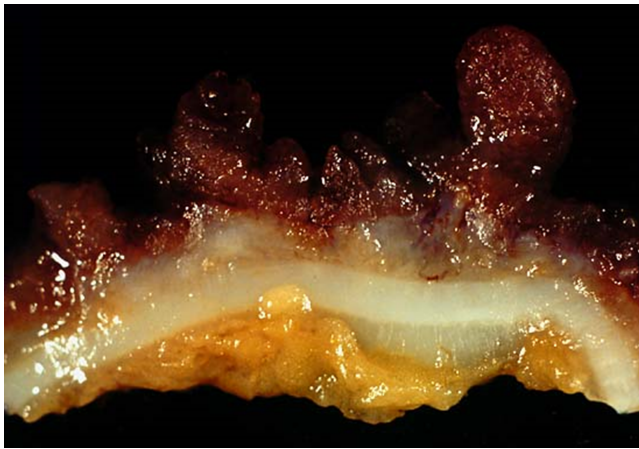
Figure 50.6 Chronic UC. The image shows a view of a longitudinal section through the colon wall. This demonstrates not only the angry red mucosa but also the tendency for the inflamed tissue to throw itself up into inflammatory pseudopolyps. (See color plate section.) *Source:* http://commons.wikimedia.org/wiki/File:Chronic_Ulcerative_Colitis_1.jpg#mediaviewer/File:Chronic_Ulcerative_Colitis_1.jpg.

Whereas 80–90% of the patients can be satisfactorily treated with these approaches, some patients are refractory, intolerant, or lose response to them. In this setting, few options are left apart from debilitating surgery with a permanent stoma or experimental treatments. Images of surgical resections are shown in Figures 50.6 and 50.7.

50.3.2 Potential treatment options

New therapies that have demonstrated some efficacy are inhibiting leucocyte migration, targeting inflammatory cytokines, enhancing immune regulatory mechanisms, and/or promoting tissue repair.

Figure 50.7 Colonic pseudopolyps of a patient with chronic severe intractable UC. Colectomy specimen. (See color plate section.) *Source:* http://commons.wikimedia.org/wiki/File:Chronic_Ulcerative_Colitis_1.jpg#mediaviewer/File:Chronic_Ulcerative_Colitis_1.jpg.

50.3.2.1 Drugs targeting leucocyte homing

Alpha-4 integrins are members of the cell-surface adhesion family expressed by several cells, including leucocytes, and binding several ligands, including VCAM-1 expressed on endothelial cells, and extra-cellular matrix proteins such as fibronectin. These interactions lead to various cellular processes, such as activation, proliferation, and migration of α_4-integrin-expressing cells toward sites of inflammation, including areas of the gut in CD [37]. Natalizumab, a humanized monoclonal antibody against the α_4 subunit of both $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins, has demonstrated therapeutic benefit in patients with moderate to severe active CD. In the ENACT-1 randomized clinical trial (RCT) evaluating natalizumab versus placebo as induction therapy in 905 patients with active CD, similar rates of response (decrease in CDAI of $\geq$70 points) and remission (CDAI <150) were observed at week 10 (respectively 56% versus 49%, $P = 0.05$, and 37% versus 30%, $P = 0.12$). In a second part of the trial (ENACT-2) evaluating natalizumab versus placebo as maintenance therapy in 339 patients who had a response to natalizumab in the first trial, a significantly higher rate of sustained response (61% versus 28%, $P < 0.001$) and remission (44% versus 26%, $P = 0.003$) was observed at week 36 [38]. In ENCORE, an RCT evaluating the efficacy of natalizumab versus placebo as induction therapy in 509 patients with moderately to severely active CD and active inflammation (CRP >2.87 mg/L), significantly higher rates of response (decrease in CDAI of $\geq$70 points) at week 8 and remission (CDAI <150) were observed (respectively 48% versus 32%, $P < 0.001$, and 26% versus 16%, $P = 0.002$) [39]. In a meta-analysis of five trials comparing natalizumab with placebo, the relative risk (RR) of not achieving remission was reduced with natalizumab (0.88) [40]. In the USA, monotherapy with natalizumab is indicated for inducing and maintaining clinical response and remission in adult patients with moderately to severely active CD and evidence of inflammation who have had an inadequate response or who are unable to tolerate conventional CD therapies and anti-TNF agents [33]. The most dangerous adverse effect is progressive multifocal leukoencephalopathy caused by the JC virus.

Vedolizumab, a humanized anti-$\alpha_4\beta_7$ integrin monoclonal antibody, has also been approved by the US Food and Drug Administration and the European Medicine Agency for use in patients with moderate to severe CD. In the induction phase comparing intravenous vedolizumab therapy (300 mg) versus placebo in 368 adults with active CD, a higher rate of clinical remission (CDAI $\leq$150) was observed (14.5% versus 6.8%, $P = 0.02$), while CDAI-100 response was similar (31.4% versus 25.7%, $P = 0.23$). In the maintenance phase comparing intravenous vedolizumab therapy every 8 or 4 weeks versus placebo in 461 patients who had had a response to vedolizumab, there was a significantly higher rate of clinical remission (39.0% and 36.4% for the two vedolizumab groups, respectively, versus 21.6% for the placebo group; $P < 0.001$ and $P = 0.004$), but also a higher rate of serious adverse events (24.4% versus 15.3%) and infections (44.1% versus 40.2%) [41].

Chemokine receptor 9 (CCR9) is a highly specific receptor expressed predominately by T cells. Its overexpression leads to the migration of T cells to the small and large intestines. In PROTECT-1, an RCT with 436 subjects suffering from moderate to severe CD (CDAI between 250 and 450 and CRP >7.5 mg/L), a CCR9 antagonist (CCX282-b/Traficet-EN) given 250 mg twice daily for 36 weeks demonstrated efficacy in maintaining remission in patients identified as responders (decrease in CDAI of ≥70 points) after the induction phase (47% of subjects on CCX282-B in remission at 36 weeks versus 31% on placebo; $P = 0.01$). CCX282-B was also superior to placebo in normalizing CRP at week 36 (19% versus 9%; $P = 0.04$), and decreasing the number of subjects requiring corticosteroid rescue therapy (11% versus 21%, $P = 0.04$). Serious adverse events occurred in similar rates in the two groups [42].

Other molecules targeting T cells, but not interfering with homing, have been studied with less success. Visilizumab, a humanized monoclonal antibody directed at the cell surface marker CD3 expressed only by activated T cells, inducing selective apoptosis of these activated T cells, has shown some efficacy in an uncontrolled trial [43] but was associated with an increased rate of adverse events, including cytokine release syndrome and liver toxicity [44].

Cytokine release syndrome is a common immediate complication occurring with the use of anti-T cell antibody infusions such as antithymocyte globulin (ATG), OKT3 (muromonab-CD3), and TGN1412, and also with the CD-20 antibody rituximab against B cells. Severe cases are known as cytokine storms. The antibodies bind to the T cell receptor (TCR), activating the T cells before they are destroyed. The cytokines released by the activated T cells produce a type of systemic inflammatory response similar to that found in severe infections and characterized by hypotension, pyrexia, and rigors. The patient feels very unwell, as if in a high fever – indeed, the cytokine release syndrome *is* effectively a type of noninfective fever. Deaths due to cytokine release syndrome with OKT3 have been reported, and it can cause life-threatening pulmonary edema if the patient is fluid overloaded. However, if treated appropriately it is usually not dangerous, just extremely unpleasant for the patient.

Abatacept, a CTLA-Ig (cytotoxic T-lymphocyte-associated protein) that blocks the co-activation of T cells by competing with CD28 for CD80/CD86, and which is used in the treatment of rheumatoid arthritis, was not found to be effective in CD [45].

50.3.2.2 Drugs targeting the proinflammatory cascade

The proinflammatory cytokines IL-12 and IL-23 are involved in Th1 and Th17 responses respectively, and both are implicated in the pathogenesis of CD [46]. Ustekinumab, a human monoclonal antibody blocking the p40 common subunit of IL-12 and IL-23, was evaluated in a phase II RCT with 526 adults suffering from moderate-to-severe CD that was resistant to TNF antagonists. In the induction phase, clinical response at 6 weeks was significantly higher in a subgroup of patients receiving 6 mg/kg of ustekinumab intravenously (39.7% versus 23.5% in the placebo group,

$P = 0.005$), while there was no significant difference in clinical remission. In the maintenance phase of the trial, comparing 90 mg of ustekinumab subcutaneously versus placebo in 145 patients who had a response to ustekinumab at 6 weeks, significantly increased rates of clinical response (69.4% versus 42.5%, $P < 0.001$) and remission (41.7% versus 27.4%, $P = 0.03$) at 22 weeks [47]. Phase III trials are currently underway to evaluate the safety and efficacy of ustekinumab (www.clinicaltrials.gov; NCT01369355, NCT01369342, NCT01369329).

The proinflammatory cytokine IL-6 has been implicated in the pathogenesis of colitis in various animal models and in patients with CD [48,49]. In a small pilot study with 36 patients suffering from active CD, a biweekly 8 mg/kg infusion of tocilizumab, an anti-IL-6 receptor humanized monoclonal antibody used in rheumatoid arthritis, showed promising results with 8/10 (80%) patients experiencing a clinical response compared with 4/13 (31%) patients in the placebo group ($P = 0.019$), although only two patients achieved remission. The incidence of adverse events was similar in the two groups [50].

Thalidomide has anti-inflammatory effects, by decreasing the production of proinflammatory cytokines, including TNF-α and IL-12. It appears to be effective in inducing remission in refractory pediatric CD, as shown in a randomized controlled trial with 56 children with active CD despite immunosuppressive treatment (prolonged by an open-label extension of the study, where nonresponders to placebo received thalidomide). Overall, 32/49 (65.3%) patients treated with thalidomide achieved a response (reduction in pediatric CDAI by ≥75%) and 31/49 (63.3%) achieved clinical remission [51].

Since IL-17 and IFN-γ have been identified as major actors in the pathogenesis of CD, molecules inhibiting these agents have been developed. However, anti-IL-17 antibodies (secukinumab [52], brodalumab [53]) and anti-IFN-γ antibodies (fontolizumab [54]) all failed to demonstrate efficacy. Despite encouraging preliminary results, attempts to administer anti-inflammatory cytokines, such as IL-10 [55,56] and IL-11 [57,58], were also disappointing, as no significant improvement was observed.

50.3.2.3 Other treatments

Some other treatments have been studied with encouraging preliminary results, including human growth hormone [59], linoleic acid [60], low-dose naltrexone [61], helminths [62,63], or extracorporeal photopheresis [64]. Larger studies are needed to confirm these results. Other agents have so far failed to demonstrate efficacy including granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) [65,66], probiotics [67–70], and prebiotics [71]. If intestinal failure occurs, home parenteral nutrition and intestinal transplantation can be discussed [72]. Interestingly, several cases of CD recurrence after intestinal transplantation have been reported [73–75], which may indicate that the immune system plays a more important role than the mucosal barrier in the pathogenesis of CD.

50.3.2.4 Bone marrow transplantation and stem cell therapy

The first reported success of autologous hematopoietic stem cell transplantation (HSCT) in CD was published in 1993 in a patient with non-Hodgkin's lymphoma [76]. Since then, at least 19 cases of autologous or allogeneic HSCTs for malignancies in CD patients have been reported, with remission of CD achieved in most cases, even after discontinuation of any immunosuppressive therapy [77]. Given these results, HSCT trials in patients with CD refractory to conventional therapy were initiated. In 1995, the Autoimmune Diseases Working Party of the European Group for Blood and Marrow Transplantation recommended the use of autologous rather than allogeneic HSCT because of the lower risk of severe toxicity [78]. The largest study is a phase I–II trial on 24 patients with severe CD (CDAI >250 and/or Crohn Severity Index >16) despite anti-TNF therapy, who underwent autologous HSCT after mobilization of their peripheral blood hematopoietic stem cells (HSCs) with cyclophosphamide and G-CSF, *ex vivo* HSC enrichment by CD34$^+$ selection, and non-marrow ablative preparation with cyclophosphamide and equine or rabbit ATG.

All patients went into remission (CDAI <150). After 5 years, the percentage of clinical relapse-free survival was 19% and the percentage of patients in remission, steroid-free, or medication-free was at least 70%, 80%, and 60% respectively. Infectious complications were frequent, including early bacteremia and/or central venous line infection (six patients) and late infections (10 cases, including seven severe infections, in the first year after transplantation) [79]. A randomized phase III study was initiated, but recruitment was halted prematurely for safety reasons (http://clinicaltrials.gov/ct2/show/NCT00297193). Preliminary results on 48 patients after 1 year follow-up showed decreases in mean CDAI from 324 to 161, CRP from 16.6 to 6.5 mg/L, with six patients achieving a normal CDAI and three patients allowed to stop immune-suppressive medications. However, high rates of adverse events were observed (100 serious adverse events, among which 42 infective episodes requiring or prolonging hospitalization and eight patients with temporary flare of CD activity or a need for surgery) [80].

50.4 Treatment of ulcerative colitis

The choice of therapy is based on the activity, distribution, and pattern of disease. Disease activity can be evaluated by Truelove and Witts' criteria (number of bloody stools/day, pulse, temperature, hemoglobin, and erythrocyte sedimentation rate or CRP) or the Mayo score, and is classified as mild, moderate, or severe. Remission is defined as complete resolution of symptoms and endoscopic mucosal healing. Response is defined as clinical and endoscopic improvement (generally a decrease in the activity index of >30%, associated with a decrease in the rectal bleeding and endoscopic scores in the Mayo score) [2].

50.4.1 Current treatment options for ulcerative colitis

Current treatment strategies include oral 5-aminosalicylates (sulfasalazine, mesalamine), antibiotics (metronidazole, ciprofloxacin), topically or systemically acting steroids (prednisone, beclomethasone dipropionate), and immunomodulatory therapy, including azathioprine, 6-mercaptopurine, calcineurin inhibitors (cyclosporine, tacrolimus), and anti-TNF antibodies (infliximab, adalimumab, golimumab). Surgery (procolectomy with ileo-anal anastomosis) is an option to treat acute severe colitis or to manage refractory disease (see Figures 50.5 and 50.7) [81].

50.4.2 Potential new treatment options

The most promising therapies for UC are essentially acting through an inhibition of leukocyte migration or inflammatory cytokines. Several anti-integrin antibodies have been studied in UC, including vedolizumab [82], which has been recently approved by the US Food and Drug Administration, and etrolizumab, a humanized monoclonal antibody targeting the β_7 subunit of $\alpha_4\beta_7$ and $\alpha_E\beta_7$) [83,84] and PF-00547,659, a monoclonal antibody to MAdCAM [85], which have been found effective in preliminary trials.

Alicaforsen, an inhibitor of intracellular adhesion molecule-1 [86–88], and tofactinib, a Janus kinase-3 inhibitor [89], have also shown promising results in UC. The roles of methotrexate [90–94], probiotics, and fecal microbiota transplantation [95,96] in the treatment of UC are still debated, and randomized controlled trials are needed to evaluate their effectiveness. Despite encouraging preliminary results, anti-IL-2 receptor (daclizumab [97,98], basiliximab [99–101]) and anti-CD3 (visilizumab [102–104]) monoclonal antibodies have failed to demonstrate efficacy in larger trials. Rituximab (an anti-CD20 antibody against B cells) is ineffective and can even mediate exacerbation of UC [105–107].

50.5 Properties of mesenchymal stromal cells

Mesenchymal stromal cells (MSCs) are multipotent progenitors within the bone marrow and all other vascularized organs that are capable of differentiating into various cells and tissues, such as chondrocytes, osteoblasts, and adipocytes *in vitro* [108]. MSCs can be isolated after *ex vivo* culture of the plastic-adherent mononuclear cell fraction. After *ex vivo* expansion human MSCs have a fibroblast-like morphology, and are uniformly positive for CD29, CD44, CD71, CD73, CD90, CD105, CD106, CD120a, CD124, and CD166 but do not express common hematopoietic markers such as CD14, CD45, or CD34. No marker specific for MSCs has been found yet. The International Society for Cellular Therapy has proposed three minimal criteria to define MSCs: (1) adhesion to plastic in standard culture conditions, (2) expression of CD105, CD73, and CD90, and lack of expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and human leukocyte antigen (HLA)-DR surface

molecules, and (3) *in vitro* differentiation into osteoblasts, adipocytes, and chondroblasts [109]. MSCs can be isolated from bone marrow and many other tissues, including umbilical cord [110], umbilical cord blood [111], placenta [112], adipose tissue [113], gingival tissue [114], skin [115], lung [116], liver [117], heart, and spleen [118]. They are pericytes residing in the endothelium in the connective tissues of most organs [119].

50.5.1 Immunomodulation

MSCs also exert powerful immunomodulatory effects, including inhibition of proliferation and function of T cells, B cells, and natural killer (NK) cells. These properties have been demonstrated *in vitro* with both murine and human cells, and *in vivo* in murine models of experimentally induced colitis. Dextran sodium sulfate (DSS) and trinitrobenzene sulfonate (TNBS) are commonly used to induce colitis in mice, by initiating epithelial cell lesions and altering the epithelial barrier integrity. Both acute DSS- and TNBS-induced colitis are characterized by a Th1–Th17-mediated acute inflammation with increased TNF-α, IL-6, IL-17 and increased IL-12, IL-17, IFN-γ respectively. However, while chronic TNBS-induced colitis is associated with an enhanced Th1/Th17 intensity of response, chronic DSS-induced colitis switches to a Th2-biased (IL-4, IL-10) profile. Similarly, histological DSS-colitis features are "UC-like" (epithelial disruption, focal lesions, superficial inflammation), while histological TNBS-colitis features are "CD-like" (transmural inflammation and edema) [120].

The first observed immunomodulatory effect of MSCs was their ability to inhibit T cell proliferation *in vitro* [121–123]. This phenomenon was observed independently of whether the T cells were CD4^{+} or CD8^{+}, naive or memory T cells [124], stimulated with allogeneic cells or nonspecific mitogens [123], and regardless of their functional state or the type of TCR expressed [125]. The immunosuppressive activity of MSCs is independent of the major histocompatibility complex (MHC), as inhibition is similar using third-party MHC-unmatched MSCs or MSCs that are autologous to the responder or stimulating lymphocytes [121]. MSC-inhibited T cells do not undergo apoptosis [123], but are blocked in the G_0/G_1 phase of the cell cycle in a state of anergy by inhibition of cyclin D2 expression [126]. Whether *in vitro* addition of IL-2 can [122] or cannot [127] reverse this inhibition is controversial. However, a recent study found that the systemic infusion of murine BM-MSCs induced T cell apoptosis via the FAS ligand/FAS pathway, and that FASL^{−/−} bone-marrow-derived MSCs (BM-MSCs) could not ameliorate DSS-induced colitis in mice [128]. Interestingly, it has been demonstrated that FAS-mediated apoptosis is lower in the T cells of people with CD than in control T cells [129]. However, another study demonstrated that *in vitro* MSCs prevented activation-induced cell death through downregulation of Fas receptor and Fas ligand on TCR-activated T cells [127]. It is important to note that the effect of MSCs on T cells seems dependent on the MSC/T cell ratio: a high MSC/T cell ratio exerts strong inhibitory effects, while low MSC/T cell ratios enhance T cell proliferation [130].

Other studies suggest that an important part of MSC-mediated immunomodulation results from the recruitment of immune-regulatory T cells (Tregs), which can be either CD4^{+} CD25^{+} FoxP3^{+} or CD8^{+} from both naive and memory T cells [131–133]. *In vivo*, MSC-mediated Treg expansion has been observed in several immune-mediated diseases, including experimental autoimmune encephalomyelitis [134], experimental arthritis [135], and type 1 diabetes mellitus [136]. Mechanisms of this T-reg expansion appear multiple and include secretion of soluble factors by MSCs, such as prostaglandin E_2 (PGE$_2$) and TGF-β1 [137–139] or HLA-G [140,141], interaction between MSCs and chemokine ligand 1 and chemokine receptor 8 on T cells [142], and induction of immature DC [143] and monocytes [144] to a regulatory profile. MSC-mediated T cell modulation was observed in several DSS- and TNBS-induced colitis mouse models, treated with intravenous or intraperitoneal injection of human adipose-derived MSCs (ASCs) [145], umbilical-cord-derived MSCs (UCMSCs) [146,147], or BM-MSCs [148]. In these studies, MSCs effectively treated colitis with both a clinical response and histological improvement, showing decreased infiltration of inflammatory cells in the lamina propria. The investigators mostly observed a decreased number of IFN-γ-producing Th1 cells and higher numbers of CD4^{+} CD25^{+} Foxp3^{+} Tregs in mesenteric lymph nodes and the lower colon and systemic levels of Th1 and Th17 proinflammatory cytokines, including TNF-α, IFN-γ, IL6, IL-12, IL-17, and IL-23, as well as higher colonic and systemic levels of the anti-inflammatory/regulatory cytokine IL-10. In a murine model of TNBS-induced colitis, MSCs co-localized with CD11b^{+} cells in the spleen, and these cells were essential to the expansion of Tregs in draining mesenteric lymph nodes [149]. In another murine model of TNBS-induced colitis, intravenous injection of murine BM-MSCs was followed not only by downregulation of both Th1 and Th17 responses and activation of regulatory T cells, but also by a significant upregulation of Th2 activities (IL-4, IL-10, GATA-3) [150].

The effect of MSCs on B cells remains controversial, and may depend on environmental signals. According to most authors, MSCs inhibit B cell activation, proliferation, chemotaxis, and immunoglobulin G secretion, both in murine [126,151,152] and human [153] studies. In these studies, B cells were enriched from the spleen or purified from peripheral blood and were exposed to various stimuli, including allogeneic cells, mitogenic agents and lipopolysaccharide. Inhibition of B cell function was mediated by soluble factors in transwell experiments. In another study, B-cell co-stimulatory molecule expression and cytokine production seemed unaffected by human MSCs [153]. However, in a study of highly purified B cells, MSCs promoted proliferation and differentiation into immunoglobulin-secreting cells of naive B cells isolated from healthy donors and total B cells from children with the autoimmune disease systemic lupus erythematosus (SLE), stimulated with an agonist of toll-like receptor (TLR) 9 in the absence of B cell receptor triggering [154].

The relationship between MSCs and NK cells is complex and also not yet fully understood. MSCs inhibit the IL-2- or

IL-15-driven proliferation of human resting NK cells and their production of IFN-γ, TNF-α, and IL-10 [155,156], but their effects on the cytotoxic activity of NK cells is controversial. Some authors report have reported MSC-mediated decreased NK cell cytotoxicity [140,156,157], while others have not observed this effect [158,159]. This discrepancy might depend on MSC/NK ratios or on whether NK cells were freshly isolated or not [155].

MSCs also interact with antigen-presenting cells, including DCs. MSCs inhibit differentiation of DC precursors, such as monocytes or CD34$^+$ hematopoietic progenitors into mature DCs, by blocking them in the G_0/G_1 phase of the cell cycle, through a downregulation of cyclin D2 and p27^{Kip1} expression [160]. DCs cultured with MSCs have impaired antigen-presenting [161] and migration [162] functions and show increased secretion of IL-10 and decreased production of IL-12 [163]. These effects have been confirmed *in vivo* in murine models [164]. MSCs also induce DC precursors (CD34$^+$ hematopoietic progenitors) [165] and mature DCs [166] to differentiate into a regulatory DC population through the Notch signaling pathway.

MSCs may also modulate the functions of macrophages, for example, through increased IL-10 secretion [167] or induction into a regulatory-like profile [144]. *In vitro* addition of increasing numbers of monocytes or DCs into co-cultures of human ASCs and activated CD4$^+$ cells progressively inhibited T cell proliferation and IFN-γ production [145]. In murine models of DSS- and TNBS-induced colitis, the intraperitoneal injection of murine bone marrow macrophages co-cultured with ASCs was effective in treating both colitis and sepsis. *In vitro* these macrophages showed a regulatory phenotype that was different from activated macrophages and characterized by high arginase activity and increased expression of IL-10 and inducible nitric oxide synthetase (iNOS) upon restimulation with lipopolysaccharide, while secreting low levels of the inflammatory cytokines TNF-α and IL-12. They showed potent immunosuppressive activity on splenocytes that was significantly reversed by the iNOS inhibitor L-NG-nitroarginine methyl ester and IL-10-blocking antibodies [168].

Thus the mechanisms of MSC-mediated immunomodulation are not yet fully elucidated and probably vary among species [169]. They include direct cell-to-cell contact through the expression of adhesion molecules and secretion of soluble factors. Multiple factors are involved, including PGE$_2$ [156,167,170], indoleamine 2,3-dioxygenase [114,156,171], nitric oxide [172,173], HLA-G [140,174], TGF-β and hepatocyte growth factor [123], IL-1 receptor antagonist [175], TNF-stimulated gene 6 protein [176], and IL-10, which, although probably not secreted directly by MSCs, also participates in the immunosuppressive effect of MSCs, as IL-10 blockade partially reversed the colitis and sepsis in the murine model [145]. In the DSS murine models of DSS- or TNBS-induced colitis, PGE$_2$ produced by nucleotide-binding oligomerization domain-containing protein-2-activated human UCMSCs increased production of IL-10 and Tregs and was required to reduce the severity of colitis [177].

Transcriptional repression of the proinflammatory cytokine early T cell activation factor 1 (osteopontin) by the autoimmune regulator (AIRE) has also been implicated in MSC immunomodulation as it was required for the efficacy of BM-MSC administration in a model of chronic colitis by transfer of CD4$^+$ CD45RB^{hi} T cells in Rag$^{-/-}$ mice. However, AIRE did not control MSC suppression of T cell proliferation *in vitro* [178].

The immunomodulatory effects of MSCs depend on the inflammatory environment and in some circumstances MSCs can act as immune-stimulating cells. TLRs have an important role in inducing MSCs into an immunosuppressive or immunostimulating pathway, as different TLR agonists influence MSC proliferation, differentiation, migration, and immunomodulatory functions in different ways [179–181]. Many inflammatory cytokines promote MSC-mediated immunosuppression, including IFN-γ, TNF-α, and IL-1β [173]. Pretreatment of human BM-MSCs with IFN-γ increased expression of MHC class II molecules, indoleamine 2,3-dioxygenase, and iNOS and significantly inhibited peripheral blood mononuclear cell (PBMC) proliferation at lower PBMC : MSC ratios. In murine models of DSS- and TNBS-induced colitis, intraperitoneal administration of INF-γ-pretreated MSCs was more effective in treating colitis, in reducing serum amyloid A levels, in reducing colonic inflammatory cytokines (TNF-α, IL-6, IFN-γ, and IL-17A), and in increasing migration into inflamed intestine [182]. Similarly, IL-1β-primed human UCMSCs significantly attenuated the development of DSS-induced murine colitis, increased the number of peritoneal M2 macrophages and splenic or mesenteric lymph node Tregs, and enabled migration more efficiently into the spleen, mesenteric lymph nodes, and colon, which was related to upregulation of CXCR4 expression. IL-1β stimulation elevated COX-2, IL-6, and IL-8 mRNA expression in MSCs [183].

50.5.2 Immune tolerance

Human MSCs are immunoprivileged cells because they express low levels of HLA class I molecules and do not express HLA class II molecules nor the co-stimulatory molecules CD80, CD86, and CD40 under normal circumstances [184,185]. However, expression of HLA class I antigens, even at a low level, may be responsible for vulnerability of MSCs to activated NK-cell lysis [186]. Moreover, a recent study observed the capacity of murine MSCs to present extracellular antigen through their MHC class I molecules by cross-presentation and to induce an effective CD8$^+$ T cell immune response [187]. In addition, it has been demonstrated *in vivo* that, in the presence of a narrow window of IFN-γ, MSCs upregulate expression of MHC class I and II molecules and are able to act as antigen-presenting cells and stimulate CD4$^+$ cell proliferation [188–190]. This immunogenicity has also been observed *in vivo* in animal models in which donor-derived MSCs, unlike autologous MSCs, could promote bone marrow [191] or skin graft [192] rejection. Systemic or local administration of allogeneic MSCs in rats resulted in the development of anti-donor T cell and antibody responses, which could

promote a rapid clearance of MSCs and a limitation of their long-term benefits [193,194].

50.5.3 Tissue regeneration

MSCs can differentiate *in vitro* to cells of the mesenchymal lineage. The relative importance of this property for the efficacy of MSCs in experimental colitis and IBD is still being investigated.

50.5.4 Homing

MSCs are able to selectively home to sites of tissue injury and inflammation, including intestine, kidney, lung, liver, thymus and skin [195] (and see Chapters 22–24). In murine models of colitis, MSCs have demonstrated their ability to migrate selectively into inflamed zones of the intestine, mesenteric lymph nodes, and spleen, whether they were murine or human, bone marrow, adipose, or umbilical cord derived, and whether administered intravenously or intraperitoneally [145,147–149,196]. MSCs have been found in the lamina propria [196], the muscular layer, and the submucosa of the inflamed intestine [197]. However, homing capacities may be altered in MSCs that have been cryopreserved. In a murine model of TNBS-induced colitis, intraperitoneally but not intravenously injected cryopreserved MSCs were found in the inflamed colon [197]. MSC migration is directed by a multitude of signals depending on the MCS source, type or environment, and growth factors and cytokines involved [198,199]. In order to better understand the selective migration of MSCs into the inflamed intestine, Gonzalez-Rey *et al.* [145] studied the expression of various chemokine receptors involved in cell recruitment to inflammatory sites and demonstrated that human ASCs expressed on their surface the chemokine receptors CCR1, CCR2, CCR4, CCR5, CCR7, CCR9, CXCR1, and CXCR5 and that most of these receptors were functional since human ASCs migrated in response to chemokines such as CCL5, CCL22, CCL19, CCL25, CXCL8, and CXCL13. Moreover, human ASCs expressed a panel of adhesion molecules involved in tissue transmigration, including CD54, CD49e, CD44, CD29, CD105, CD106, and CD166. Activation and targeting may improve delivery of MSCs into mesenteric lymph nodes and colon, and hence their efficacy in murine colitis, as demonstrated with IFN-γ- [182] or IL-1β-primed MSCs [183] and VCAM antibody-coated MSCs (AbVCAM-1-MSCs) [200]. IFN-γ upregulates expression of the chemokine receptor CXCR7, lectins LGALS3BP and LGALS9 and ADAM15, a matrix metalloproteinase involved in epithelial adhesion [182], while IL-1β upregulates CXCR4 expression [183]. How long MSCs do stay into the inflamed intestine is unknown, but it seems that they remain there for at least 2 weeks [147].

50.5.5 Differentiation and stimulation of tissue repair

In addition to their capacity to differentiate into chondrocytes, osteoblasts and adipocytes, MSCs can also differentiate into other cells of the mesenchymal lineage, such as myoblasts [201]

or cardiomyocytes [202]. Moreover, UCMSCs [203] and BM-MSCs have shown an ability to differentiate *in vitro* into cells of ectodermal lineage, such as neuroglial cells [204,205], and of endodermal lineage, such as renal tubular epithelial cells [206] and hepatocytes [207–209], although this a controversial area; and while MSCs may take on the morphological and even the cell surface markers of, for example, neurons, there is little, if any, evidence that they function as neurons. This not to say that they are not beneficial in the treatment of diseases involving damaged neurons, but they most likely mediate this effect by secretion of paracrine factors and by modulation of the inflammatory reaction.

As indicated earlier, in response to inflammation or tissue injury, MSCs are capable of engrafting in many tissues [210], albeit transiently, and have demonstrated their efficacy in promoting tissue repair in myocardial infarction [211], kidney diseases [212,213], liver diseases [214], lung diseases [215] and injury [216], and neurological disorders [217]. In DSS-induced colitis in rats, intravenous injection of rat MSCs enabled significant healing of epithelial injuries, and the MSCs extracted from the lamina mucosa had upregulated their expression of α-smooth muscle actin, suggesting a reprogramming to myogenic lineage differentiation, possibly involved in intestinal repair. In TNBS-induced colitis in rats, topical implantation of rat BM-MSCs healed mucosal injuries. MSCs engrafted into the submucosal layers, but only a small proportion was positive for α-smooth muscle actin and desmin, indicating differentiation into myofibroblasts. A larger proportion of MSCs surrounding the lesion area expressed vascular endothelial growth factor and TGF-β1, two growth factors known to play important roles in gastrointestinal wound healing [218]. In a DSS-induced colitis model in rats, intravenous injection of allogeneic MSCs resulted in clinical improvement with markedly reduced epithelial injury and, significantly, restoration of expression of claudin-2, -12 and -15, proteins constituting tight junctions that play a major role in the paracellular permeability of the epithelial barrier [219].

Again, as indicated earlier, it is thought that the tissue repair properties of MSCs are mainly due to their ability to stimulate survival and recovery of local tissues, rather than to transdifferentiation ability [220]. MSCs have demonstrated their ability to stimulate angiogenesis [221] and to inhibit apoptosis and fibrosis in injured tissues [222].

50.6 Mesenchymal stromal cell administration in inflammatory bowel diseases

Two different applications of MSCs in IBDs have been evaluated in human trials: local injection of MSCs to treat fistulizing CD and intravenous injection of MSCs to treat luminal CD or UC. MSC administration has been studied in CD more than it has in UC.

50.6.1 Mesenchymal stromal cell administration for fistulizing Crohn's disease

Management of fistulas in patients with CD is an extremely challenging problem, because many such fistulas do not respond to available treatment, despite recent improvements using anti-TNF agents and improvements in surgical treatments. Endoanal advancement flap is the standard surgical technique, but anal incontinence and recurrence are not uncommon. Several trials have evaluated the efficacy of local injections of ASCs or BM-MSCs.

50.6.2 Autologous mesenchymal stromal cell administration for fistulizing Crohn's disease

50.6.2.1 Adipose-tissue-derived mesenchymal stromal cells

In a phase I study García-Olmo *et al.* treated four patients with one or more refractory, complex Crohn's fistulas with a single intrafistular injection of MSCs. The fistulas were enterocutaneous, suprasphincteric, and/or rectovaginal fistulas, were unresponsive to medical treatment, and had been unsuccessfully treated by surgery at least twice. The injection consisted of $3–30 \times 10^6$ autologous ASCs from passages 1 to 3. Six of eight (75%) fistulas had healed 8 weeks after treatment, and the other two (25%) were incompletely closed with decreased output flow. No relationship was found between the number of injected MSCs and the success of the treatment. No adverse effects were observed within 12–22 months follow-up, and no aberrant differentiation was seen in biopsies [223].

In a phase IIb study the same group showed that ASCs were effective in healing 69% of complex perianal fistulas in patients with moderate to severe CD who were intolerant or resistant to the anti-TNF agent infliximab [224]. Forty-nine patients with complex perianal fistulas were included. Thirty-five of the fistulas were of cryptoglandular origin and 14 were associated with CD. Cryptoglandular anal fistulas arise from inflammation of the proctodeal glands of the intersphincteric space. The mucus secretions of the anal glands empty into the anal crypts, thereby lubricating the anus. Anal glands are present in the subepithelium and the internal sphincter, with a large number also deeply sited within the intersphincteric space. Infection of these intersphincteric glands is thought to give rise to an intersphincteric abscess if the draining duct is blocked by the resulting infectious debris. The abscess may resolve by spontaneous drainage into the anal canal or it may progress to an acute anorectal abscess that, in most cases, subsequently develops into a perianal fistula. This type of fistula is not associated with CD.

Patients were randomly assigned to the administration of an intrafistular injection of fibrin glue or fibrin glue containing 20 million ASCs. If fistula healing had not occurred by 8 weeks, a second double dose of ASCs was administered. Fistula healing was observed in 17/24 patients receiving ASCs (71%); 11 patients healed after the first injection and six patients healed after a second injection compared with 4/25 (16%) in the control group (RR 4.43, $P < 0.001$). A similar proportion was observed in the CD patients, with healing of the fistula in five out of seven patients receiving ASCs compared with one out of seven patients in the control group (RR 5.00, $P = 0.10$). Improvement of quality of life, assessed by a quality-of-life questionnaire, was significantly greater in the ASC group compared with the control group, even in patients whose fistula did not heal [225]. After an average long-term follow-up of approximately 40 months, 7 out of 12 patients treated with ASCs remained free of recurrence compared with two out of three in the control group. Perianal sepsis occurred more frequently in the control group than in the ASC group ($P = 0.04$). No adverse effects related to ASC administration were noted, confirming their excellent safety and tolerability profile, and no patient suffered from anal incontinence [226].

Another group performed a dose-escalation phase I study, in which 10 patients with a perianal fistula associated with CD were treated with an intrafistular injection of autologous ASCs from passages 3 and 4 at a dose of either 1×10^7 (group 1), 2×10^7 (group 2), or 4×10^7 ASCs/mL (group 3). The doses used were selected with regard to the size of the fistula. Eight weeks after injection, complete healing occurred in two out of four patients in group 2 and one out of three patients in group 3, while partial closure with no output drainage was observed in all other patients. After follow-up of 8 months the three patients whose fistula had been completely healed were free of recurrence and no adverse effects were observed [227]. The same group subsequently led a phase II trial studying the effects of the intrafistular injection of ASCs from passages 3 and 4 in 43 patients with perianal fistulas associated with CD. The first injection again contained a number of ASCs that was proportional to the size of the fistula: 3×10^7 ASCs per 1 cm length of fistula when the fistular diameter was less than 1 cm, and 6×10^7 ASCs per 1 cm length of fistula when the diameter was between 1 and 2 cm. The average number of ASCs was 15.8×10^7 cells per fistula. Patients without complete fistula closure at 8 weeks received a second injection of 1.5 times more cells than the first injection. The fistula tract was filled with a mixture of ASCs and fibrin glue. Complete fistula healing was observed in 27/42 patients (64.3%) by 8 weeks after the final ASC injection and was sustained after 1 year in 23/26 patients (88%). Six other patients showed incomplete closure of the fistula, of which five patients achieved closure of more than 50% of the fistula tract with a decrease in drainage of more than 50%. Five other patients discontinued the study before week 8. No relation was found between the outcome of the treatment and fistula type, length, or diameter or duration of CD. No adverse events related to ASCs were observed [228].

50.6.2.2 Bone marrow-derived mesenchymal stromal cells

The safety and efficacy of serial intrafistular injections of autologous BM-MSCs in the treatment of fistulizing CD was studied by

Ciccocioppo *et al.* in 10 patients refractory to all previous medical treatments including anti-TNF agents or unsuccessfully treated by surgery. They administered intra-fistular injections of $15–30 \times 10^6$ MSC from passage 3 every 4 weeks until improvement was obtained or when autologous MSCs were no longer available Patients received between two and five injections. Sustained complete closure of the fistula tract occurred in 7/10 patients and incomplete closure in 3/10 patients as assessed by surgical exploration and MRI. MRI provided images of regenerative tissue without fibrotic tissue. All patients had a significant reduction of their CDAI scores: pre- and posttreatment median values were 294 ± 49 and 99 ± 32 at 12 months after the last procedure ($P < 0.001$). Their median PDAI scores pre- and posttreatment were 13.0 ± 2.2 and 4.5 ± 2.4 at 12 months after the last procedure ($P < 0.001$). Patients who achieved remission had CDAI scores ≤150 and PDAI scores ≤8, usually after the second administration of ASCs. Lower endoscopic examination performed at the end of the follow-up period demonstrated rectal mucosal healing in seven out of seven patients. In addition, they observed a significant increase in the percentage of circulating CD4$^+$ CD25^{bright} FoxP3$^+$ Tregs as soon as after the second injection of ASCs which was stable at 12 months ($P < 0.01$). Additionally, mucosal FoxP3$^+$ Tregs were present in the inflamed areas at 12 months ($P < 0.0001$). There were no adverse effects during the procedure nor during the 12-month follow-up period [229].

50.6.3 Allogeneic mesenchymal stromal cell administration for fistulizing Crohn's disease

García-Olmo and co-workers also explored the use of expanded allogeneic ASCs for the treatment of complex perianal fistulas in patients with nonactive luminal CD (CDAI ≤200) in a single-arm multicenter phase I/IIa clinical trial. Twenty-four patients received an intrafistular treatment with 20×10^6 allogeneic ASCs followed by a second administration of 40×10^6 ASCs if fistula closure was incomplete after 12 weeks. After 12 weeks, 12/20 patients (60.0%) had an improvement of at least one of their fistulas and complete closure occurred in 8/21 (38.1%) on clinical examination. After 24 weeks, these values were respectively 9/13 patients (69.2 %) and 9/16 patients (56.3%). Based on a combination of clinical and MRI assessment, fistula closure was seen in 6/24 patients (30%) at 24 weeks. A statistically significant decrease in the MRI score of severity was observed at week 12. PDAI significantly decreased by more than 37% compared with baseline ($P < 0.01$). No effect was observed on the CDAI scores. Luminal relapse was observed in 0/24 patients (0.0%) at week 12 and in 5/24 patients (20.8%) at week 24. After a 6-month follow-up period the safety profile was acceptable, with a low number of serious adverse events: anal abscess in three patients, pyrexia in one patient, and uterine leiomyoma in one patient [230].

50.6.4 Autologous mesenchymal stromal cell administration for luminal inflammatory bowel diseases

Intravenous injections of autologous BM-MSCs have been tested in a phase I trial. Nine adult patients with moderate to severe CD with a CDAI score between 220 and 450 and who were refractory to conventional standard treatment, including corticosteroids, immunosuppressive agents, or anti-TNF therapy, were treated with two intravenous injection of $1–2 \times 10^6$ cells/kg body weight 7 days apart. Three patients had a clinical response (defined as a decrease in CDAI score of ≥70 points) but none achieved remission (CDAI <150), and four patients experienced worsening of their disease, requiring surgery in three cases or rescue medication in one case within 14 weeks after BM-MSC administration. Endoscopic improvement using the SESCD was seen in two patients at week 6. Biopsies of inflamed mucosa showed a trend to lower CD4$^+$ T cell and higher CD4$^+$ CD127$^+$ Treg numbers [231]. CD127 is the receptor for IL-7.

50.6.5 Allogeneic mesenchymal stromal cell administration for luminal inflammatory bowel diseases

Intravenous injection of allogeneic BM-MSCs has also been evaluated with more success in a phase II pilot study. Nine patients with refractory moderate-to-severe CD with a CDAI score ≥220 and previously unsuccessful treatment with corticosteroids and immunomodulatory agents were randomized to receive either a low (2×10^6 BM-MSCs/kg) or a high (8×10^6 BM-MSCs/kg) dose of MSCs by intravenous infusion. After 28 days all patients had a decrease in their CDAI score, with a mean decrease of 105 ($P = 0.004$), and three patients had a clinical response (reduction in CDAI ≥100), one of whom entered clinical remission (CDAI <150). Patients reported an increased quality of life, as attested by a significant increase in mean Inflammatory Bowel Disease Questionnaire (IBDQ) score by day 28 ($P = 0.008$). No infusion reaction was observed, although five patients experienced mild to moderate adverse events [232].

The Nanjing group, who studied the use of MSCs in the autoimmune disease SLE, reported their experience of allogeneic MSC transplantation in seven patients with IBD, among whom four had CD and three had UC. They administered one intravenous infusion of BM-MSCs from healthy family members or UCMSCs at a dose of 1×10^6/kg body weight. Patients continued their treatment with corticosteroids and/or immunosuppressive agents after the transplant. After 3 months they observed a significant reduction in disease activity (CDAI or Clinical Activity Index) in all patients, with remission achieved in five out of seven patients (two out of four with CD and three out of three with UC) and endoscopic improvement with a decrease in Endoscopic Index of Severity score or in Endoscopic Activity Index in three out of seven patients, two out of four with CD and one out of three with UC. Remission lasted for more than 2 years in two patients, while two other patients relapsed at 6 and

7 months. One CD patient had a significant reduction in fistula size and drainage. Histological analysis of biopsy specimens showed a reduction in the extent of the inflamed area and in the lymphocytic infiltration in the mucosa propria. One patient had a significant reduction in fistula size and drainage. No serious adverse events were reported after a mean follow-up of 19 months (range 6–32 months) [233].

50.7 The future of mesenchymal stromal cell treatment in inflammatory bowel diseases

50.7.1 Ongoing protocols

50.7.1.1 Mesenchymal stromal cell treatment for fistulizing Crohn's disease

50.7.1.1.1 The Navarra study (NCT01157650)

This phase I–II study is evaluating viability, safety, and tolerance of locally implanted autologous ASCs in 15 patients with non-active CD (CDAI ≤200) and one or more enterocutaneous, rectovaginal or complex perianal fistulas. Patients should not have perianal abscesses or rectal and/or anal stenosis, and should not have received anti-TNF therapy or have received tacrolimus or cyclosporine within 8 weeks and 4 weeks before MSC therapy respectively. Secondary outcomes include an evaluation of efficacy (fistula healing, quality of life, presence of systemic CD, relapse) and a biological characterization of the MSCs used in terms of their phenotype, immune suppressive capacities, and cytokine production. This study is ongoing, but is no longer recruiting participants (Figure 50.8).

50.7.1.1.2 The Leiden study (NCT01144962)

This dose-escalation phase I–II study aims to determine the safety and efficacy of intrafistular injection of allogeneic BM-MSCs for the induction of response of active fistulizing CD. The fistulas are peri-anal fistulas refractory to conventional medical treatment. Patients are randomized into one of four groups, receiving either placebo or MSCs at doses of 10 million, 30 million, or 90 million. Exclusion criteria include acute peri-anal infection, rectovaginal or complex peri-anal fistulas with more than two internal openings, and anti-TNF therapy less than 8 weeks prior to enrolment. Secondary outcomes at 12 weeks are improvement of clinical and endoscopic scores, quality of life details, and serum CRP. Safety is evaluated after 12 and 24 weeks. This study is ongoing, but is no longer recruiting participants.

50.7.1.1.3 The Anterogen studies (NCT01314092, NCT01623453)

In a phase II trial, patients with complex perianal fistulas are randomized to receive autologous ASCs at either low (1×10^9/mL) or high (2×10^9/mL) dose, followed by a second double-dose injection if complete closure is not achieved. Primary outcome is complete closure of the fistula at week 8. Safety is assessed by the reporting of all adverse events. Subsequently, an open follow-up clinical trial will evaluate the number of patients with sustained complete closure and the number of adverse events 6 months after the final dose injection.

50.7.1.1.4 The TiGenix study (NCT01541579)

This phase III randomized double-blind placebo-controlled multicenter study is evaluating the efficacy of allogeneic ASCs for the treatment of complex anal fistulas in patients with CD. Patients with nonactive or mildly active luminal CD (CDAI ≤220) and two or less complex perianal fistulas are randomized to receive either an intrafistular injection of 120×10^6 allogeneic ASCs or placebo. Patients with abscesses, rectovaginal fistulas, surgically treated fistulas, and ongoing corticosteroid treatment or corticosteroid within the preceding 4 weeks are excluded from
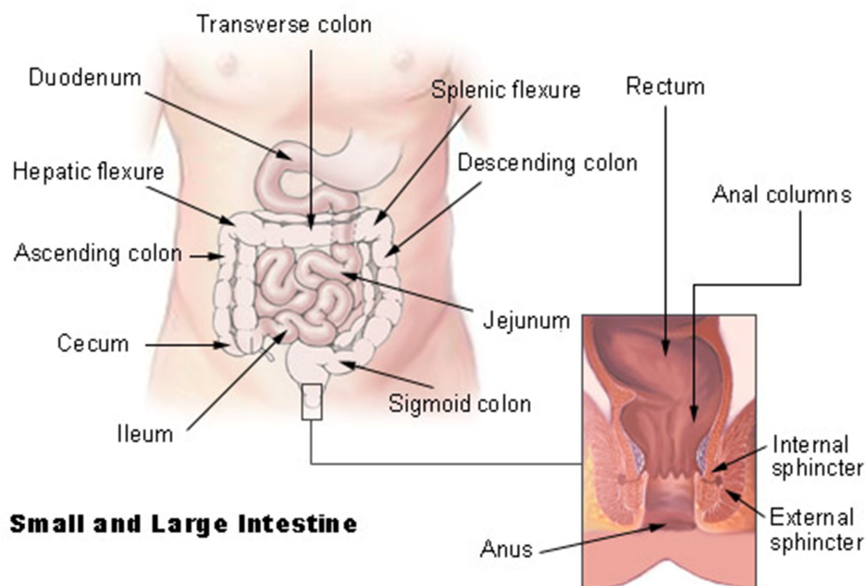
Figure 50.8 The small and large intestine with an insert of the perianal anatomy, an area where perianal fistulas occur in persons with CD. *Source:* http://commons.wikimedia.org/wiki/File:Illu_intestine.jpg#mediaviewer/File:Illu_intestine.jpg.

the study. Primary outcomes include remission of perianal fistulizing CD at week 24 by clinical and MRI assessment. Other outcomes are efficacy, including response, time to response, and time to remission, and assessment using the PDAI and IBDQ criteria, as well as safety at weeks 24 and 52. This study is currently recruiting participants.

50.7.1.1.5 The Royan study (NCT01874015)
This study is a prospective, randomized, phase I trial in which patients with refractory CD, a CDAI score >220, and perineal fistulas are randomized to receive four monthly intrafistular injections of autologous BM-MSCs with or without fibroblasts. Outcomes are fistula closure and CDAI score at month 4. This study is currently recruiting participants.

50.7.1.2 Mesenchymal stromal cell administration for luminal inflammatory bowel diseases
50.7.1.2.1 The Liège study (NCT01540292)
This prospective open-label phase I–II pilot trial was initiated in 2013 to explore the safety and efficacy of allogeneic BM-MSC infusions in CD refractory or intolerant to conventional therapies. Twenty patients with active refractory CD defined by a CDAI >220 despite conventional treatment, including mesalazine, corticosteroids, purine analogues, methotrexate, infliximab, and adalimumab, are treated with two successive injections of $1.5–2.0 \times 10^6$ allogeneic BM-MSCs/kg at the beginning of the trial and 4 weeks later. Conventional treatments of CD, such as mesalazine, methylprednisolone, budesonide, azathioprine, 6-mercaptopurine, or methotrexate, are allowed, while anti-TNF antibodies are forbidden, and antibiotics are allowed only for the treatment of a concurrent infection. The primary endpoint is a clinical response defined by a 100-point decrease in the CDAI score at week 8. Secondary endpoints include clinical response, remission defined by a CDAI score <150, CDAI levels, CRP levels, and fecal calprotectin levels at weeks 2, 4, 8, and 12. Immune modulation is being investigated by monitoring of nucleated cell counts, leucocyte subpopulations, Treg numbers, immunoglobulin levels, Vβ repertoire of T lymphocytes, and the quantification of TRECS in T lymphocytes. TRECS are TCR excision circles, which are small circles of DNA created in T cells during their passage through the thymus as they rearrange their TCR genes. This is a measure of thymic function. Safety is being assessed by recording side effects, including infections.

50.7.1.2.2 The Mesoblast international studies (NCT01233960 and NCT00543374)
In a first phase III study, patients with moderate-to-severe CD (CDAI scores between 250 and 450) who are intolerant to, or who have previously failed, therapy with at least one corticosteroid course and at least one immunosuppressive agent course and an anti-TNF treatment are randomized into three groups to receive a placebo or an intravenous injection of allogeneic MSCs at low $(6 \times 10^6$ MSCs) or high (1.2×10^9) doses on four occasions over 2 weeks. The primary outcome is disease remission (CDAI score

≤150) at day 28, and secondary outcomes are disease improvement (reduction in CDAI score of ≥100 points), improvement in quality of life (using the IBDQ), and reduction in number of draining fistulas. In a second phase III study, patients who successfully achieved clinical benefit in the first study will be enrolled and followed for duration and reinduction of clinical benefit using the CDAI questionnaire and improvement in quality of life using the IBDQ after 6 months.

50.7.1.2.3 The Qingdao study (NCT01221428)
This phase I–II study aims to determine the safety and efficacy of UCMSCs in refractory UC. First, 2×10^7 MSCs are injected intravenously followed 1 week later by 1×10^7 MSCs injected into the mesenteric artery. Outcomes evaluated after 3 months are improvement in endoscopy findings and histology and amelioration of clinical symptoms.

50.7.1.2.4 The La Paz study (NCT01914887)
In this phase I–II study, patients with left-sided colitis and moderate activity as assessed using a modified Truelove–Witts score between 11 and 21, who did not respond to 4 weeks of treatment with oral and/or topical 5-aminosalicylates, receive multiple endoscopic injections of allogeneic ASCs into the affected colonic submucosa at a total dose of 6×10^7 MSCs. Safety and efficacy assessed by the modified Truelove–Witts score, IBDQ, Mayo endoscopic index, CRP, and fecal calprotectin are evaluated at weeks 0, 4, 8, and 12. The study is currently recruiting.

50.7.1.2.5 The Washington study (NCT02150551)
In this phase I trial, pediatric patients with IBDs will be randomized into three dose groups and will receive eight weekly infusions of allogeneic BM-MSCs. Safety and efficacy will be assessed using the pediatric CDAI or Pediatric Ulcerative Colitis Activity Index questionnaires, quality of life using the Impact III IBD questionnaire, laboratory tests, including CRP, fecal calprotectin, and proinflammatory cytokines, as well as endoscopic healing evaluated at day 24 and day 77.

50.8 Issues to be resolved

50.8.1 Source of mesenchymal stromal cells
Most of the published human trials in people with CD have used ASCs. Only Ciccocioppo *et al.* used BM-MSCs [229]. ASCs have the advantage of being easily accessible in large numbers. MSC treatment requires a large number of cells, and, although MSCs have great proliferation potential, extended *ex vivo* expansion can alter their properties, resulting in reduced differentiation potential and senescence from the sixth passage onward [234].

Alternatively, expanded ASCs could be superior to nonexpanded ASCs, known as the stromal vascular fraction, for the treatment of enterocutaneous fistula in CD, as shown by García-Olmo *et al.* in eight patients: three of four fistulas healed in the ASC group compared to one of four in the stromal vascular

fraction group [235]. We lack information on differences on immunophenotype, differentiation potential, transcriptome, and proteome between BM-MSCs and ASCs. *In vitro* studies suggest that ASCs might be superior to BM-MSCs in suppressing immune responses [236,237]. In practice, both types of cells have demonstrated some efficacy, but formal studies comparing the efficacy of BM-MSCs and ASCs will be needed to resolve this question.

50.8.2 Autologous versus allogeneic mesenchymal stromal cells

The use of autologous MSCs has raised a debate on whether MSCs are affected by, or may contribute to, CD. MSCs from patients with other autoimmune diseases, such as SLE, have lower proliferation capacities [238], and the results in human studies on SLE were disappointing when autologous MSCs were used. However, several *in vitro* studies have demonstrated that MSCs from patients with CD have similar growth potential and T cell suppression properties to MSCs from healthy donors [231,239]. Moreover, both autologous and allogeneic MSCs seem promising in human trials, with the exception of a trial using autologous MSCs in SLE.

50.8.3 Dosage and modalities of administration

Because of the extent and dissemination of luminal lesions in CD, systemic administration seems required. In mice, intravenous and intraperitoneal routes of administration have been used with equal success, apart from a few studies in which MSCs did not appear to home to inflamed intestine [196,218]. In humans, intravenous infusion of cells is simple, minimally invasive, routinely performed, and safe. However, little is known about the optimal amount of MSCs and the proportion of MSCs that will reach an inflamed intestine. Duijvestein *et al.* used $1–2 \times 10^6$ autologous MSCs/kg body without much success [231], while Onken *et al.* observed no significant difference between low (2×10^6 MSCs/kg) and high (8×10^6 MSCs/kg) [232]. Administration of MSCs via selective mesenteric artery cannulation has been successfully performed in one patient, without any complication [240]. This route of administration may increase the number of MSCs reaching the inflamed organ, but its superiority over the intravenous administration is yet to be demonstrated.

Local injection of MSCs has been successfully used in several studies to treat CD fistulas. In studies showing efficacy of intrafistular injections, $10–60 \times 10^6$ MSCs were injected into each fistulous tract, and these injections were sometimes repeated. Ongoing trials are testing higher systemic doses: the Mesoblast international studies are testing the injection of 6×10^8 or 1.2×10^9 cells four times over a 2-week period. Several local dose-escalation trials are also currently ongoing.

50.8.4 Concomitant use of other drugs

MSCs are used concomitantly with immunosuppressive drugs, and, as they have common targets, it is important to know if the drugs can affect MSC function. MSCs exposed to physiological concentrations of azathioprine, methotrexate, 6-mercaptopurine, and anti-TNF-α antibodies had normal survival and inhibitory capacities on PBMC proliferation when tested *in vitro*. An additive effect could even exist with 6-mercaptopurine and anti-TNF-α antibodies [241].

50.9 Safety

So far there have been no reports of any serious adverse events or ectopic tissue growth in clinical trials using MSC-based therapy for CD, nor for graft-versus-host disease [242–244], solid-organ transplantation [245], cartilage disorders [246], or in many completed clinical trials for a variety of applications [247,248]. In some studies there was a mild and transient fever shortly after the time of administration, and this association was found to be statistically significant in a meta-analysis [248]. More experience is needed in order to confirm the long-term safety of MSCs.

The immunosuppressive properties of MSCs could theoretically increase susceptibility to infections and cancers. However, no infections or malignant diseases have been reported as serious adverse events in MSC-treated CD patients, and there is no evidence of any increased risk in other applications [248]. In the context of solid organ transplantation with MSC-based immunosuppression, one group has even observed a decreased infection risk compared with standard immunosuppressive drugs [245]. Another reason for concern for an increased risk of malignant disease is the need to expand MSCs *in vitro* in order to obtain a sufficient number of cells for MSC-based therapy. While murine MSCs have shown a potential for the development of chromosomal aberrations and tumor generation after long-term *in vitro* culture [249,250], human MSCs have not shown any of these risks so far [251–253]. Moreover, IBDs are associated with a higher risk of developing colorectal cancer. In a murine model of colitis-associated tumorigenesis induced by azoxymethane and DSS, MSC-treated animals showed a significant reduction in tumor number and tumor load compared with control mice, while tumor size remained comparable. This could be linked to a decreased expression of proinflammatory cytokines and downregulation of STAT3 phosphorylation [254].

Finally, the effects of MSCs on preexisting cancers have also been studied. In animal studies some authors found that MSCs could promote tumor growth [255,256], while others observed a tumor-suppressive activity of MSCs [257–259]. This dual effect could be explained by a context-dependent role of MSCs in regulating tumor growth [260,261]. In a small human trial of 25 patients, co-transplantation of MSCs and HSCs was associated with a higher relapse rate compared with control patients (60% versus 20%). However, this observation has not been confirmed by other studies [262].

50.10 Conclusions

MSCs represent a promising therapy for IBD, especially for CD. They probably exert their effects through a combination of

immunomodulation, myogenic differentiation, and the promotion of epithelial repair. Both local injection of MSCs for fistulizing CD and intravenous injection of MSCs for luminal IBD have shown interesting results in several human trials. Unlike current surgical strategies, MSC administration is a minimally invasive procedure, which does not injure the anal sphincter and even promotes a nonfibrotic reparative process of fistulas, with no cases of incontinence reported after MSC treatment. MSCs seem superior to conventional drugs in term of side effects, such as opportunist infections. There are still many questions to answer concerning the optimal source of MSCs, as well as dosage and routes of administration.

The efficacy of MSCs compared with conventional treatments still needs to be demonstrated in randomized controlled trials, several studies of which are ongoing. They will also likely help us in the understanding and use of MSCs to treat IBDs.

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