Autologous Hematopoietic Stem Cell Transplantation in Patients With Refractory Crohn's Disease

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Background & Aims: **Crohn's disease (CD) is an immunologically mediated inflammatory disease of the gastrointestinal tract. Due to a high morbidity and/or an increase in mortality in refractory cases, a new treatment approach is needed. In theory, maximum immune ablation by autologous hematopoietic stem cell transplantation (HSCT) can induce a remission.** ***Methods:*** **We conducted a phase 1 HSCT study in 12 patients with refractory CD. Candidates were younger than 60 years of age with a Crohn's Disease Activity Index (CDAI) of 250–400 despite conventional therapies including infliximab. Peripheral blood stem cells were mobilized with cyclophosphamide and granulocyte colony-stimulating factor and $CD34^+$ enriched. The immune ablative (conditioning) regimen consisted of 200 mg/kg cyclophosphamide and 90 mg/kg equine antithymocyte globulin.** ***Results:*** **The procedure was well tolerated with anticipated cytopenias, neutropenic fever, and disease-related fever, diarrhea, anorexia, nausea, and vomiting. The median days for neutrophil and platelet engraftment were 9.5 (range, 8–11) and 9 (range, 9–18), respectively. The initial median CDAI was 291 (range, 250–358). Symptoms and CDAI improved before hospital discharge, whereas radiographic and colonoscopy findings improved gradually over months to years following HSCT. Eleven of 12 patients entered a sustained remission defined by a CDAI ≤150. After a median follow-up of 18.5 months (range, 7–37 months), only one patient has developed a recurrence of active CD, which occurred 15 months after HSCT.** ***Conclusions:*** **Autologous HSCT may be performed safely and has a marked salutary effect on CD activity. A randomized study will be needed to confirm the efficacy of this therapy.**

Crohn's disease (CD) is a chronic illness that is immunologically mediated and of unknown etiology but probably induced by an exposure to xenogeneic antigen(s) leading to an excessive Th1-mediated chronic inflammation of the gastrointestinal tract. The disease has a variable course from a mild, intermittently active illness requiring only symptomatic therapy to a fulminant illness requiring potent and potentially toxic immunosuppressive therapy, surgery, or both. CD responds to anti-inflammatory agents, antibiotics, and immunosuppressive drugs such as mesalamine,[1] metronidazole,[2] quinolone antibiotics, corticosteroids, 6-mercaptopurine,[3,4] azathioprine,[5] methotrexate,[6] cyclosporine,[7] tacrolimus,[8] and intravenous pulse cyclophosphamide.[9] A tumor necrosis factor α inhibitor, infliximab, has also shown consistent benefit.[10] Unfortunately, none of these treatments are curative. In addition, many patients with severe disease will have a recurrence after some response or will not respond. This leads to a significant morbidity from disease and treatment complications. Although the reported mortality from CD[11–15] does not seem to be excessively high, a subgroup analysis in severe refractory cases has not been performed. Also, risk factors for mortality have not been established. Therefore, the mortality in severe cases is unknown but is believed to be higher than reported. In addition, patients with severe disease experience an inability to eat, frequent nausea, vomiting, diarrhea, malnutrition, growth retardation in children, fistulae, abdominal pain, multiple surgeries, extraintestinal symptoms, iatrogenic addiction to narcotics, and toxicities of therapy. In these therapy-refractory cases, a new treatment approach is needed.

Autologous hematopoietic stem cell transplantation (HSCT) is an extension of immune modulation/suppression by maximizing immune suppression to the point of immune ablation. In theory, the transplant-conditioning

Abbreviations used in this paper: CCSI, Craig Crohn's Severity Index; HSC, hematopoietic stem cell; HSCT, hematopoietic stem cell transplantation; TPN, total parenteral nutrition.

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regimen ablates aberrant disease-causing immune cells, while hematopoietic stem cells (HSCs) regenerate a new and antigen-naive immune system similar to the normal ontogeny of the immune system during fetal development.

Twelve patients who had highly active disease despite standard therapies underwent autologous T cell–depleted ($CD34^+$ cell–enriched) HSCT. We previously reported an early experience on the first 2 subjects[16] and now report on the 12 subjects who have completed a phase 1 portion of the study.

Materials and Methods

Study Design

This is a pilot study designed to investigate the safety and efficacy of HSCT utilizing high-dose cyclophosphamide, equine antithymocyte globulin, and autologous T cell–depleted ($CD34^+$ cell–enriched) HSCT in patients with chronic active CD refractory to conventional therapies including infliximab. Primary end points were treatment-related toxicity and engraftment. Secondary end points were an HSC mobilization and disease response.

Patient Selection

Patients and/or parents read and signed an informed consent form for study IDE 7846, which was approved by our institutional review board and the US Food and Drug Administration. Candidates for HSCT had to have clinical and histologic evidence of CD, be younger than 60 years of age, and have failed treatment with corticosteroids, mesalamine, metronidazole, azathioprine (or 6-mercaptopurine), and a monoclonal antibody to tumor necrosis factor α receptor (infliximab). Failure was defined as a Crohn's Disease Activity Index (CDAI) of 250–400 despite those therapies. Exclusion criteria included active ischemic heart disease or congestive heart failure, left ventricular ejection fraction $<40\%$, forced expiratory volume in 1 second/forced vital capacity $<50\%$, diffusing capacity for carbon monoxide $<50\%$, serum bilirubin level >2.0 mg/dL, serum transaminase levels >2 times the upper limit of normal, serum creatinine level >2.0 mg/dL, platelet count $<100{,}000/\mu L$, absolute neutrophil count $<1500/\mu L$, active infection, toxic megacolon, or intestinal perforation. All of the patients had undergone an upper endoscopy, a colonoscopy, and small bowel radiography within 6 weeks before transplantation.

HSC Procurement

Peripheral blood stem cells were mobilized with cyclophosphamide 2 g/m^2 and granulocyte colony-stimulating factor 10 μg/kg/day beginning 72 hours following completion of cyclophosphamide administration. A leukapheresis was initiated when the white blood cell count rebounded to $>1000/\mu L$ and continued daily until an enriched target $CD34^+$ cell count (2.0×10^6/kg) was achieved. T-cell depletion was performed by enrichment of $CD34^+$ cells using Isolex 300i magnetic immunoselection (Baxter, Chicago, IL). The HSC graft was cryopreserved until the date of transplantation (reinfusion).

Conditioning Regimen

The conditioning regimen consisted of cyclophosphamide 50 mg/kg/day on days -5, -4, -3, and -2 (total, 200 mg/kg) and equine antithymocyte globulin 30 mg/kg/day on days -4, -3, and -2 (total, 90 mg/kg). Mesna was administered along with the cyclophosphamide to prevent hemorrhagic cystitis, and 1.0 g/day methylprednisolone was administered before each dose of equine antithymocyte globulin to prevent an infusion reaction and serum sickness. HSCs were infused intravenously on day 0, 48 hours following the last dose of cyclophosphamide. Granulocyte colony-stimulating factor (5 μg/kg/day) was started on day 0 and continued until the absolute neutrophil count reached $500/\mu L$.

Supportive Care

Patients were treated on a high efficiency particulate air (HEPA)-filtered medical floor. A low microbial diet, oral ciprofloxacin 500 mg twice daily, fluconazole 400 mg once daily, metronidazole 500 mg 3 times daily, valacyclovir 500 mg 3 times daily, and aerosolized pentamidine 300 mg were started on admission. Ciprofloxacin was discontinued and intravenous piperacillin/tazobactam 3.375 g every 4 hours was started when the neutrophil count decreased to $<500/\mu L$. Metronidazole and piperacillin/tazobactam were stopped on neutrophil recovery. Valacyclovir (500 mg twice daily) and fluconazole (400 mg once daily) were continued for 12 and 6 months post-HSCT, respectively. Trimethoprim/sulfamethoxazole double-strength (160 mg/800 mg) 3 times weekly was started on hematopoietic engraftment and continued for 6 months post-HSCT. Hemoglobin levels and platelet counts were maintained above 8 g/dL and $20{,}000/\mu L$, respectively, with leukoreduced, irradiated, and cytomegalovirus-safe blood transfusions. Total parenteral nutrition (TPN) was used during transplantation in patients who were malnourished, had already been on home TPN, or were unable to maintain adequate oral intake due to CD. All immunosuppressive and disease-modifying agents were discontinued upon the stem cell mobilization except systemic corticosteroids, which were tapered over 2–6 months.

Assessment of Outcomes

Neutrophil engraftment was defined as the first day of 3 consecutive days with an absolute neutrophil count $\geq 500/\mu L$. Platelet engraftment was defined as the first day of 3 consecutive days with a platelet count $\geq 20{,}000/\mu L$ without a transfusion.

Each patient was asked to return to this center at 6 and 12 months following HSCT and then yearly thereafter. Evaluation at those dates included the following: history and physical examination, review of systems, medication use, history of hospitalizations, infections, CDAI,[17] Craig Crohn's Severity

Table 1. CCSI

Feature	Score
Diarrhea	
3–10 times per day	1
>10 times per day	2
Pain	
Intermittent cramping	1
Steady, mild to moderate	2
Steady, severe	3
Long-term opiate use for pain	2
Well-being	
Fair	1
Poor	2
Terrible	3
Corticosteroid use	2
Immunosuppressive use	2
Mesalamine or antibiotic use	1
Total parenteral nutrition use	3
Enteropathic arthritis/arthralgias	1
Hepatobiliary complication	2
Perianal fistula or abscess	2
Enteroentero fistula	2
Enterovaginal fistula	2
Enterovesical fistula	2
Enterocutaneous fistula	2
Perianal fissure, anal pain	1
Vulvar inflammation	1
Intestinal obstruction	2
Abdominal mass	2
Erythema nodosum	1
Pyoderma gangrenosa	2
Aphthous stomatitis	1
Iritis	2
Fever >1 week	2
Weight loss	
10% of usual	1
20% of usual	2
Hematocrit	
1–5 less than normal	1
5 or more less than normal	2
Serum albumin level (*g/dL*)	
2.5–3.5	1
<2.5	2
C-reactive protein abnormality	1
Erythrocyte sedimentation rate >20 mm/h	1
Colonoscopic abnormality	
Mild	1
Moderate	2
Severe	3
Small bowel radiographic inflammation	
Mild	1
Moderate	2
Severe	3
Upper gastrointestinal endoscopy abnormality	
Mild	1
Moderate	2
Severe	3

Index (CCSI)[18,19] (Table 1), erythrocyte sedimentation rate, C-reactive protein level, hemoglobin level, serum albumin level, weight/body mass index, colonoscopy with biopsy, small bowel radiography, and upper gastrointestinal endoscopy if indicated by symptoms or presence of pre-HSCT upper gastrointestinal CD. Results on the Inflammatory Bowel Disease Questionnaire[20,21] were also assessed. Clinical remission was defined as a CDAI ≤150.

Results

Patient Demographics and Pre-HSCT Disease Manifestations

Twelve white patients (6 female and 6 male patients) with a median age of 27 years (range, 15–38 years) were enrolled. Five patients had significant pre-HSCT complications from standard immunosuppressive therapies. Three patients had either anaphylaxis or an anaphylactoid reaction from infliximab, while 6-mercaptopurine caused pancreatitis in 1 patient and prolonged cytopenias due to bone marrow suppression in another patient. The median duration of disease was 10 years (range, 1.5–20 years). By protocol eligibility, all patients had a CDAI ≥250. The median CDAI was 291 (range, 250–358). The median CCSI was 27.5 (range, 20–33). All patients failed multiple immunosuppressive therapies, and most had failed nutritional interventions and surgeries. The pre-HSCT endoscopies, small bowel radiographs, and weight/body mass index are described in Table 2.

HSC Mobilization, Engraftment, Transfusion Requirement, and Discharge Day

Disease improvement was observed in most patients following the HSC mobilization (Table 3). No mobilization-related disease flares or infections were observed. The median number of leukapheresis was 2 (range, 1–4). The median preselection and postselection $CD34^+$ and $CD3^+$ cell counts were as follows: $CD34^+$, 7.69×10^6/kg (range, 3.05–17.5 $\times 10^6$/kg) preselection and 5.64×10^6/kg (range, 1.73–9.93 $\times 10^6$/kg) postselection; $CD3^+$, 1.51×10^8/kg (range, 0.6–5.33 $\times 10^6$/kg) preselection and 0.59×10^4/kg (range, 0.3–3.09 $\times 10^6$/kg) postselection. The median days of neutrophil and platelet engraftment were 9.5 (range, 8–11) and 9 (range, 9–18), respectively. The median numbers of packed red blood cells and single donor platelet transfusions were 4 (range, 2–8) and 2.5 (range, 1–14), respectively. The median day of hospital discharge after the HSC reinfusion (transplant) was 11 (range, 10–17). Engraftment was prompt and complete, and no one developed late cytopenias.

Toxicity

Besides fever that was either neutropenic or disease related, the procedure was well tolerated without a documented infection. One patient developed hemate-

Table 2. Demographics of Patients and Disease Manifestations Before HSCT

Patient	Age/sex	Disease duration (*y*)	CDAI	CCSI	Disease manifestations at the time of transplantation	Prior medical therapies	Prior surgeries	Narcotic use	Route of nutritional support	Weight (*kg*)/body mass index (kg/m^2)	Karnofsky performance scale	CMV	Small bowel radiograph	Endoscopies
1	21/F	10	337	32	Diarrhea 25 times per day Abdominal/anal pain Anal-vulvar fistulae Fever Oral ulcers	Mesalamine, AB, CS, 6-MP, MTX, infliximab	Sigmoid colon resection Small bowel resection Hemorrhoidectomy	CI	TPN, TF	58.1/22.7	40	−	Ulcers and fixed separation of terminal ileum	Pancolitis Inflammation in terminal ileum
2	16/M	7	293	33	Diarrhea 3 times per day Abdominal/anal pain Abdominal mass Anal fistulae Intestinal obstruction Arthralgias Oral ulcers Growth retardation (Tanner stage 2)	Mesalamine, AB, CS, 6-MP, AZA, MTX, infliximab	None	Oral	TPN, TF	55.4/18.2	50	+	Multiple skip lesions (irregular mucosa with nodularity and stricture) in small intestine	Severe colitis Transverse colon stricture
3	38/F	20	289	30	Diarrhea 20 times per day Abdominal/anal pain Anal fissures Abdominal mass Intestinal obstruction Fever Arthralgias	Mesalamine, AB, CS, 6-MP, MTX, infliximab, budesonide	Sigmoid colon resection Ileocecal resection	Oral	Oral	52/19.7	70	−	2 segments of strictures in small intestine Enteroentero fistula	Ulcers in ileotransverse colostomy anastomosis Stricture with inflammation in colon Severe anal stricture and inflammation
4	27/F	12	312	23	Diarrhea 20 times per day (bloody) Abdominal/anal pain Perianal fistulae Abdominal mass Arthritis	Mesalamine, olsalazine, AB, CS, 6-MP, AZA, infliximab, antimycobacterial agents, interleukin 11	Seton for perianal fistula	Oral	Oral	38.2/15.3	50	−	Irregular mucosa in large bowel with wall thickening, diffuse cobblestoning, and strictures	Deep ulcers and pseudopolypoid disease in anal/rectosigmoid Sigmoid stricture

(Continued on following page)

Table 2 (continued). Demographics of Patients and Disease Manifestations Before HSCT

Patient	Age/sex	Disease duration (*y*)	CDAI	CCSI	Disease manifestations at the time of transplantation	Prior medical therapies	Prior surgeries	Narcotic use	Route of nutritional support	Weight (*kg*)/body mass index (kg/m^2)	Karnofsky performance scale	CMV	Small bowel radiograph	Endoscopies
5	35/M	12	329	31	Diarrhea 10 times per day (bloody) Abdominal/anal pain Abdominal mass Perianal fistulae Intestinal obstruction Fever	Mesalamine, AB, CS, 6-MP, AZA, MTX, infliximab	Seton for perianal fistula Surgical drainage of perianal fistula	Oral	Oral	77/24	50	−	Ulcers in terminal ileum	Diffuse friable mucosa Deep serpiginous ulcers in colon sparing rectum
6	27/F	6	304	20	Diarrhea 15 times per day Abdominal/anal pain Abdominal mass Rectovaginal fistula Nausea/vomiting Fever	Mesalamine, AB, CS, 6-MP, AZA, etanercept, infliximab, 6-thioguanine, budesonide	Ileocolic resection Stricturoplasty Multiple procedures for perirectal fistulae Small bowel resection and ileostomy for SBO Multiple surgeries for rectovaginal fistulae	Oral	TPN	59.1/19.5	40	−	No lesion	Anal ulcers Duodenal ulcers
7	25/M	13	282	22	Diarrhea 7 times per day Abdominal/anal pain Perianal fistulae Severe proctitis Anal abscess Fever Anal stricture Arthralgias	Sulfasalazine, AB, CS, 6-MP, MTX, infliximab	Ileostomy and bowel resection for stricture (twice) Bowel resection for SBO and sigmoid fistula Diverting colostomy	Oral	TPN	63/19.4	50	+	Terminal ileum stricture	Proctitis with fistula Cecal ulcers
8	15/M	8	250	29	Diarrhea 5 times per day Abdominal pain Pancreatitis from duodenal lesion Arthritis Iritis Oral ulcers Growth retardation (Tanner stage 2)	Mesalamine, sulfasalazine, AB, CS, 6-MP, infliximab	Partial jejunectomy Resection of terminal ileum	None	TPN, TF	38.2/14.5	40	−	Nodular and thickened mucosa in terminal ileum	Ulcers in stomach, duodenum, anus, and ileum

9	31/F	10	278	26	Diarrhea (unable to evaluate due to ileostomy) Abdominal/anal pain Anal fissure, fistulae, and abscess Fever Pyoderma gangrenosum on ileostomy opening	Mesalamine, AB, CS, AZA, infliximab	Resection of sigmoid colon and ileum Ileostomy Multiple procedures for preanal fistula	Oral	TPN	59.1/20.2	30	−	No lesion except presence of ileostomy	Diffuse erythema and atrophy of colonic mucosa Anal stricture
10	37/M	6	288	22	Diarrhea 10 times per day (bloody) Abdominal/anal pain Anal fistulae Anal stricture Fever	Mesalamine, AB, CS, 6-MP, infliximab	None	None	Oral	81.8/23.1	70	+	Normal	Severe ulcers, cobblestoning, friability in rectum and anus
11	16/M	1.5	274	24	Abdominal pain Abdominal mass Nausea Enterocutaneous fistula Weight loss Arthralgias	Mesalamine, AB, CS, 6-MP, infliximab	None	Oral	Oral	48/17.1	60	−	Mucosal thickening in distal ileum, proximal to the stenosis Stenosis in ileum with prestenotic dilatation Ileoileal fistula	Inflammation in terminal ileum Erythema in cecum
12	27/M	14	358	29	Diarrhea 15 times per day (bloody) Frank rectal bleeding Fever Abdominal pain Colon perforation Oral ulcers	Mesalamine, CS, 6-MP, MTX, infliximab, budesonide, tacrolimus	Cecal resection due to stricture Ileostomy and subtotal colectomy for colon perforation	Oral	Oral	66/22.0	40	−	No lesion except presence of ileo rectal anastomosis	Deep ulceration and stricture in remaining colon Anal stricture

CMV, anti-cytomegalovirus immunoglobulin G antibody; AB, antibiotics (metronidazole and ciprofloxacin); CS, systemic corticosteroids; 6-MP, 6-mercaptopurine; MTX, methotrexate; CI, continuous hydromorphone infusion; TF, tube feeding; AZA, azathioprine; SBO, small bowel obstruction.

Table 3. Mobilization, Engraftment, Transfusion Requirement, and Discharge Day

Patient	L	Pre-CD34	Post-CD34 (infused)	Pre-CD3	Post-CD3 (infused)	White blood cell engraftment (day)	Platelet engraftment (day)	PRBC	PLT	DC
1	3	14.4	8.33	1.1	3.09	10	9	4	5	12
2	1	5.86	4.4	0.6	0.49	8	9	4	2	10
3	4	3.05	1.73	0.76	0.30	11	18	4	5	14
4	2	8.91	6.81	2.5	1.69	10	10	6	2	11
5	1	4.44	4.83	1.03	0.5	9	9	4	3	11
6	2	11.4	9.93	2.3	0.46	8	9	5	14	17
7	1	12.1	6.29	1.36	0.66	10	10	8	11	10
8	2	6.31	4.65	1.44	0.56	9	9	2	1	10
9	1	11.0	5.18	3.4	0.6	8	9	4	2	10
10	1	17.5	8.35	3.24	0.88	8	9	4	5	10
11	2	6.47	4.05	1.57	0.57	10	14	4	2	13
12	2	6.42	6.09	5.33	0.84	10	8	6	1	15

L, number of leukapheresis; Pre-CD34, $CD34^+$ cell count before $CD34^+$ enrichment (10^6/kg); Post-CD34, $CD34^+$ cell count after $CD34^+$ enrichment (10^6/kg); Pre-CD3, $CD3^+$ cell count before $CD34^+$ enrichment (10^8/kg); Post-CD3, $CD3^+$ cell count after $CD34^+$ enrichment (10^4/kg); PRBC, number of packed red blood cell transfusions; PLT, number of single-donor platelet transfusions; DC, day of discharge after infusion of HSCs.

mesis secondary to Mallory–Weiss syndrome followed by transient hypotension without compromised organ function that responded to intravenous fluids. No early or late bacterial, fungal, or viral infections were documented. Two patients with CD-related fever before HSCT developed prolonged fever (7 and 14 days, respectively) that resolved following neutrophil engraftment. Both patients improved without consequence and remained culture negative. After discharge from the hospital, 3 patients developed complications that were unrelated to HSCT. One patient with an indwelling central venous catheter placed 1 year before HSCT for home patient-controlled analgesia with hydromorphone and narcotic dependency developed a line-related bacteremia 15 months post-HSCT that was complicated by acute renal failure requiring temporary hemodialysis that reversed without long-term consequence. One patient needed resection of a chronically narrowed small bowel segment 5 months post-HSCT. The resected segment demonstrated narrowing secondary to scarring. One patient developed mild diarrhea and abdominal pain following an upper respiratory tract infection 8 weeks post-HSCT. Symptoms lasted 3 weeks and improved with temporary cessation of oral intake and TPN. The etiology was attributed to viral gastroenteritis. Five of 12 patients were on TPN during their HSCT. Three of 12 patients were positive for cytomegalovirus immunoglobulin G, which indicates pre-HSCT exposure. No cytomegalovirus reactivation, infection, or disease occurred after HSCT.

Survival

At a median follow-up of 18.5 months (range, 7–37 months), 11 of 12 patients were alive. One patient died 37 months after HSCT due to an accidental cause. Postmortem examination showed no evidence of CD.

Disease Response

The CDAI had a rapid and dramatic post-HSCT improvement (Figure 1*A* and Table 4). In general, diarrhea and abdominal pain stopped before hospital discharge. All patients discontinued all immunosuppressive therapies post-HSCT. Lassitude and Karnofsky performance scale have improved in all patients. All patients have regained normal appetite and oral intake. One patient developed a recurrence of abdominal pain and diarrhea at 15 months post-HSCT after achieving a remission at 6 months post-HSCT. The patient needed to resume prednisone and methotrexate for recurrent disease at 21 months that was confirmed by colonoscopy with biopsy. Interestingly, smoking has been reported to exacerbate CD and this patient continued smoking at least 1 pack per day after HSCT. Another patient also developed mild diarrhea after an episode of what appeared to be *Clostridium difficile* colitis 14 months post-HSCT. The patient was treated with oral vancomycin but also received budesonide for 4 weeks followed by leflunomide for 8 weeks with complete disappearance of her diarrhea. No colonoscopy was performed and it is unknown whether symptoms were CD related or not, but she has been asymptomatic for 3 months since then without medications. After a median follow-up of 18.5 months (range, 7–37 months) following HSCT, 11 of 12 patients have remained in a remission (CDAI $\leq$150) off all immunosuppressive drugs. Changes of small bowel radiographs and colonoscopies showed an unequivocal and continuing improvement. Although most patients

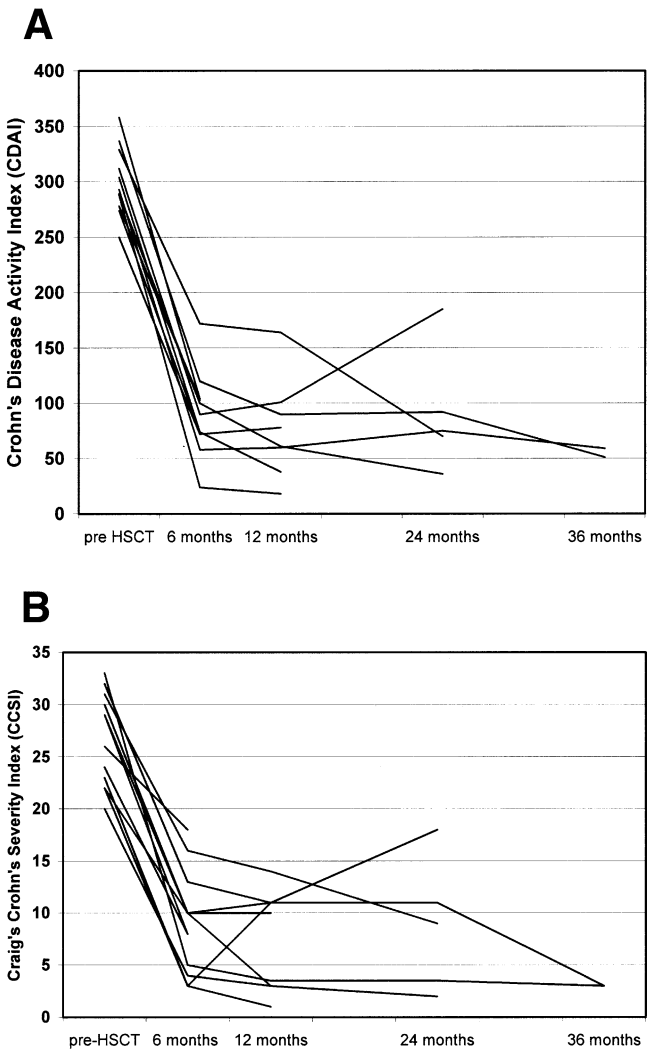

Figure 1. Clinical and laboratory results pre-HSCT and post-HSCT. (*A*) CDAI. (*B*) CCSI.

are improving gradually, they still have remaining minor ulcerations on colonoscopy. The Inflammatory Bowel Disease Questionnaire indicated marked improvement in all patents except one who developed a recurrence. For those patients who improved, all returned to a productive life, including full-time work or school. CCSI has correlated with CDAI (Figure 1*B*). Erythrocyte sedimentation rate and hemoglobin level have improved as follows: average pre-HSCT and 12 months post-HSCT erythrocyte sedimentation rates were 33 mm/h (range, 1–70 mm/h) and 22.2 mm/h (range, 6–46 mm/h), respectively, and average pre-HSCT and 12 months post-HSCT hemoglobin levels were 10.9 g/dL (range, 7.7–14.9 g/dL) and 12.1 g/dL (range, 10.3–14.6 g/dL), respectively. C-reactive protein level did not parallel with improvement of CDAI; average pre-HSCT and 12 months post-HSCT C-reactive protein levels were 2.9 mg/L (range, 0.1–10.8 mg/L) and 2.9 mg/L (range, 0.2–10.8 mg/L), respectively. Findings on colonoscopy for patient 5 pre-HSCT and 1 year post-HSCT are shown in Figure 2.

Discussion

The etiology of CD is unknown. No intestinal self-antigen (initiating or spread epitope) that is pathogenic has been identified. On the other hand, several animal gene knockout models suggest that inflammatory bowel disease may be a result of immune dysregulation between Th1 and Th2 cytokines. Deficiency of multiple Th2 cytokines may cause colitis in animal models. Interleukin 10–deficient mice develop acute and chronic colitis.[22] Interleukin 2–deficient,[23] double mutant interleukin 2– and interleukin 4– deficient, and transforming growth factor β–deficient[24] mice also develop colitis. When raised in a germ-free environment, these gene knockout mice remain free of disease. Therefore, animal models demonstrate the need for both cytokine imbalance and gut bacterial flora as disease triggers.

The molecular defect(s) causing CD have not been characterized but probably involve a cellular interaction with bacterial pathogens. Dendritic cells function at an interface between innate immunity and adaptive T- and B-cell immunity. Dendritic cells express molecules that enable them to detect structures not found in self-tissues but present on bacteria and other pathogens. These "pattern recognition receptors" include a Toll-like receptor family. Toll-like receptor transmembrane signaling receptors bind a wide variety of ligands, including proteins (flagellin), modified bacterial lipids, lipopolysaccharides, and nucleic acids (DNA and double-stranded RNA). NOD (nucleotide-binding oligomerization domain) proteins are Toll-like receptor proteins involved in programmed cell death and innate immune responses.[25] NOD2 is restricted to monocytes and Paneth cells and activates nuclear factor κB in response to bacterial products. A premature truncation of the *N0D2* gene confers disease susceptibility in a subset of patients with CD.[26] The truncated *N0D2* by itself is not sufficient to cause CD, and two thirds of patients with CD do not have a mutated *N0D2* gene.[27] Therefore, the clinical presentation of CD probably requires a number of genetic and pathogenic factors that dysregulate the immune response, perhaps by bacterial activation of dendritic cells, which initiate a persistent T cell–mediated Th1 inflammatory response.

Although the CDAI has been commonly used to monitor CD severity, it has several important drawbacks: (1) it favors predominantly colonic disease (severity of diarrhea), while diseases that primarily affect the upper gas-

Table 4. Outcome

Patient	Follow-up post-HSCT (*mo*)	CDAI	CCSI	Disease manifestations	Current therapy	Narcotic use	Route of nutritional support	Weight (*kg*)/body mass index (kg/m^2)	Karnofsky performance scale	Small bowel radiograph	Colonoscopy	Remote complications
1	37	51	3	No diarrhea Occasional abdominal pain	—	Methadone tapering	Oral	58.2/22.7	100	Normal	Slight ulcerations in ileocecal anastomosis Mild rectal stricture	Line-related bacteremia and acute renal failure at 15 months, fully recovered
2	36	59	3	Asymptomatic Grew 4 inches over 2 years (Tanner stage 4)	—	—	Oral	61.4/18.8	100	Normal	Equivocal inflammation in cecum	—
3	27	36	2	Asymptomatic	—	—	Oral	45.5/17.2	100	Normal	Single erosion in colon Slight erythema in terminal ileum	Persistent stenosis of small bowel resected at 5 months
4	25	185	18	Diarrhea with occasional blood 10 times per day Daily abdominal pain Persistent sigmoid strictures	Methotrexate, prednisone	Oral	Oral	38/15.3	70	Normal	Sigmoid stricture with ulceration Anal inflammation	—
5	24	70	9	Soft stool 3 times per day Occasional abdominal pain Perianal drain without fistula	—	—	Oral	75/23.8	90	No fistulous connection and cobblestoning in terminal ileum	Normal	—
6	20	38	3	Asymptomatic	—	—	Oral	59/19.5	100	Normal	Rare mild aphthous ulcers in coloileal anastomosis and terminal ileum	Recurrence of diarrhea lasted for 6 weeks at 14 months
7	17	81	11	No diarrhea Perianal disease with proctitis and fistulae	—	—	Oral	79/25.4	90	Refused	Severe anal stenosis Proctitis Normal colon	—

Patient	Follow-up post-HSCT (*mo*)	CDAI	CCSI	Disease manifestations	Current therapy	Narcotic use	Route of nutritional support	Weight (*kg*)/body mass index (kg/m^2)	Karnofsky performance scale	Small bowel radiograph	Colonoscopy	Remote complications
8	16	78	10	Occasional abdominal pain and diarrhea Grew 5 inches over 1 year (Tanner stage 4)	—	—	Oral, TPN	46.4/17.9	80	Lesser mucosal thickening and nodularity in ileum	Normal both upper gastrointestinal and colon	Recurrence of diarrhea and abdominal pain at 2 months, improved with nothing by mouth and TPN
9	14	103	18	Improving abdominal/anal pain Anal stricture no change Healed pyoderma Anal fistulae Perianal ulcers	—	Methadone tapering	Oral	59.1/24.6	60	Normal	Scattered ulcerations in colon Inflammation in distal sigmoid and anus	—
10	14	18	1	Asymptomatic	—	—	Oral	89.1/25.2	100	Normal	Pseudopolyp in sigmoid colon due to scarring and healed fistula	—
11	7	74	8	Asymptomatic	—	—	Oral	56.7/19.6	90	Persistent stricture in terminal ileum	Normal	—
12	7	104	8	Asymptomatic	—	Oral	Oral	70.8/23.7	90	Not performed	Mild anal stricture Solitary small ulcer in rectum	—

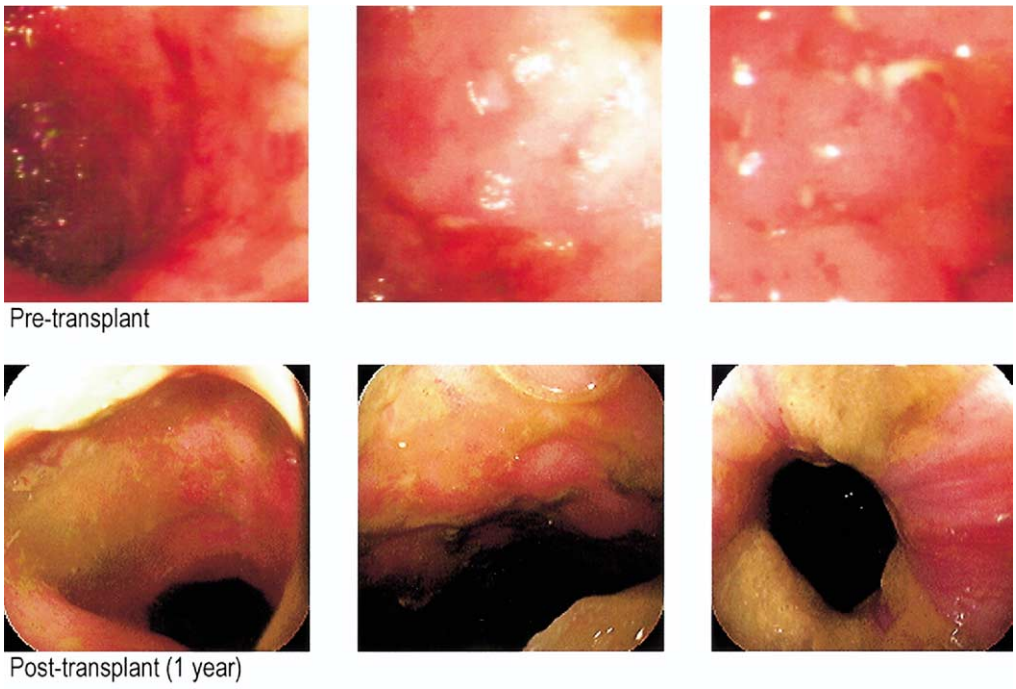

Figure 2. Colonoscopic findings pre-HSCT (*upper panels*) and post-HSCT (*lower panels*).

trointestinal tract and small bowel will be scored less severely; (2) it relies heavily on the subjective state of the patient (pain and general well-being); (3) it gives equal weight to anal fissures and fistulae; (4) the presence of an abdominal mass and/or anemia is given an unnecessarily high score; (5) many other important objective variables are not used, such as endoscopic findings, C-reactive protein level, erythrocyte sedimentation rate, and albumin level; and (6) current and prior therapies are not considered in evaluating disease severity. Therefore, we have created a new comprehensive grading scale, CCSI, which takes into account the above factors. It eliminates the above drawbacks and is being validated against the CDAI in this study.

Anecdotal case reports from HSCT recipients who had coexistent CD in addition to a malignancy suggested that HSCT could be an effective therapy providing a durable long-term remission.[28–31] In our study, most of the patients, despite their clinical remission, are still showing slowly improving but nonsymptomatic histologic and/or radiographic evidence of CD. The significance of these findings is unclear. In our study, only 1 patient developed a documented clinical recurrence 15 months post-HSCT, but all other patients have maintained a clinical and drug-free remission.

The ultimate treatment effect of autologous HSCT comes from the immunosuppressive/cytoreductive effects of the conditioning regimen. Autologous HSCs are infused only to shorten the post-HSCT neutropenic interval. Unlike allogeneic HSCT, which replaces a recipient's immune and hematologic system with a genetically non–disease-prone donor, autologous HSCT is not expected to alter a genetic tendency to develop CD. Therefore, in autologous HSCT, the risk of disease recurrence may be higher than after allogeneic HSCT. However, the morbidity and mortality of allogeneic HSCT, including the risk of graft-versus-host disease, mandated an initial clinical study with autologous HSCT. Following autologous HSCT, achievement of a treatment-free remission for more than 3 years without any active therapy raises the possibility that autologous HSCT may have reset the patient's own immune system to its predisease status.

Regimen-related toxicity from the procedure was fever that was either from anticipated neutropenia or disease related. Hematopoietic engraftment was prompt and durable. HSCs were easily mobilized with a conventional mobilization regimen. These results suggest that this therapy may be given safely. The safety data in this study were also consistent with our data in other autoimmune diseases.[32] All of the patients with CD undergoing HSCT were completely disabled and their quality of life was grim, and 5 patients had experienced significant and sometimes life-threatening complications from their therapies before HSCT. Considering the increased morbidity and mortality in these patients, the risk-benefit ratio of HSCT seems to be justified.

Although longer follow-up is needed, further investigation of autologous HSCT for CD appears warranted. A randomized trial will be necessary to confirm these results.

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