Phase I-II Trial of a Monoclonal Anti-Tumor Necrosis Factor α Antibody for the Treatment of Refractory Severe Acute Graft-Versus-Host Disease

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In a multicenter pilot study, 19 patients with severe acute graft-versus-host disease (aGVHD) refractory to conventional therapy and serotherapy with a monoclonal anti–interleukin-2 receptor antibody were treated by in vivo infusion of a monoclonal anti-tumor necrosis factor α (TNFα) antibody (B-C7). Ten patients were grafted from a genotypically identical sibling, five from an HLA-mismatched family member, and four from an HLA-matched unrelated donor. Before B-C7 treatment, 15 patients had grade IV and four had grade III GVHD. In all cases, patients received cyclosporine/methotrexate as aGVHD prophylaxis. Patients were administered increasing doses of antibody (from 0.1 to 0.4 mg/kg). The antibody was infused in bolus daily for 4 days and then every other day twice (6 doses). No side effects were observed during treatment regardless of the dose level used. Changes in peripheral blood cell counts occurred in 8 of the 19 patients and appeared to be unrelated to B-C7. No truly complete response was observed; eight patients achieved a very good partial response (42.6%) and six a partial response (31.5%). The treatment was ineffective in five patients (26.4%). When present, the response occurred early (<3 days). In the 14 responding patients, gut lesions responded best (100%), followed by skin (85%) and liver (35.7%) lesions. In 9 of 11 evaluable patients (81%), GVHD recurred when treatment was discontinued in a median delay of 3 days (range, 2 to 120 days). All except one died from aGVHD. Two patients did not experience GVHD recurrence and are still alive 13 and 18 months post-bone marrow transplantation. This pilot study shows that a monoclonal anti-TNFα antibody may be of benefit to some patients with severe refractory aGVHD, but is ineffective to prevent GVHD recurrence in the majority of cases.

ACUTE GRAFT-VERSUS-HOST disease (aGVHD) has an ambivalent activity. When moderate, it has a beneficial effect in terms of survival without relapse (graft-versus-leukemia effect), but when severe, it becomes deleterious. In a setting of HLA-identical sibling marrow transplantation, data from the International Bone Marrow Transplant Registry (IBMTR) shows that 45% of the patients presented grade ≥ II aGVHD. Among them, 48% died from GVHD-related complications. In a situation other than HLA-identical sibling marrow transplantation (mismatched bone marrow transplantation [BMT], BMT from matched unrelated donor), when the graft had not been T-depleted, the incidence of aGVHD was 75% to 90%, 60% of which were grade III and IV aGVHD.[1] Survival after an episode of aGVHD depends on whether complete or partial response to corticotherapy is achieved. In moderate forms (grade ≤ II) a 50% survival rate was obtained, but in grade III aGVHD, it did not exceed 29%, and grade IV GVHD was lethal in most cases. Thus, the incidence of mortality in severe forms of aGVHD is extremely high, ranging from 50% to 90% of the cases, depending on the published series.[2-5]

Experimental animal models have highlighted the primary role played by T lymphocytes in the genesis of aGVHD.[6,7] Later, the role of interleukin-2 (IL-2) as a recruiting and proliferating agent of GVHD effector cells was established. Based on these pathophysiologic concepts, techniques of prevention of aGVHD have been developed using total or selective T-cell depletion of the graft. Also, trials of in vivo therapy of aGVHD with the use of monoclonal anti-T cell or anti–IL-2 receptor antibodies (MoAbs) have been made in an attempt to control the effector cell population.[8-11] Yet, although T lymphocytes and IL-2 play a major role, other effector cells or cytokines play an important role in GVHD.[7]

Among effector cytokines, tumor necrosis factor α (TNFα), produced mainly by monocytes/macrophages and by T lymphocytes, is regarded as a major effector cytokine in GVHD.[12-16] TNFα, in combination with interferon γ (IFN γ), potentiates the mechanisms of allogeneic recognition.[17] TNFα also upregulates intercellular adhesion molecule-1 (ICAM-1) expression on target cells, thus enhancing their susceptibility to cytotoxic cells.[18]

In an experimental murine model, TNFα infusion mimics most GVHD manifestations[13]: fever, cachexia, and cell necrosis. In this model, a polyclonal or monoclonal anti-TNFα antibody administered as prophylactic therapy prevents all the manifestations of aGVHD without affecting T-lymphocyte functions.[16,19] In humans, it has been suggested that an increase in serum TNFα level frequently preceded the occurrence of aGVHD and that GVHD severity was correlated with TNFα level.[20] In our experience, patients with aGVHD who did not respond to an anti–IL-2 receptor MoAb (B-B10) had significantly higher pretreatment levels of circulating TNFα as compared with responders ($P = .03$).[21]

All these results suggest that therapy directed against TNFα in severe aGVHD in humans could be highly useful.

An anti-TNF MoAb capable of blocking cytotoxicity in vitro was developed in our laboratory. We report the results

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Submitted July 15, 1991; accepted February 11, 1992.

Supported in part by a grant from the Association pour la Recherche sur le Cancer (ARC).

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0006-4971/92/7912-0011$3.00/0

of a phase I-II multicenter pilot study, including 19 patients and assessing the potential clinical efficacy of this MoAb in the treatment of severe forms of refractory aGVHD.

PATIENTS AND METHODS

Monoclonal Anti-TNFα Antibody (clone B-C7)

B-C7 MoAb (J. Wijdenes, Besançon Regional Blood Transfusion Center) is a murine IgG1 κ MoAb with a Ka value of 1.8×10^9 mol/L obtained by immunizing mice (Balb/c X 63/Ag 8653) with human recombinant TNFα. After adaptation of the B-C7 cell line to serum-free medium, B-C7 MoAb was produced in a bioreactor. The MoAb is able to block the cytotoxic activity of natural and recombinant human TNFα on the murine cell line L929 with a specific biologic activity of 10 ng.

Patients

From November 1989 to January 1991, 19 patients aged 4 months to 43 years (median, 21 years) entered this pilot study. Their clinical characteristics are summarized in Table 1. Ten patients received a marrow transplant from HLA-matched related donors (MRD), five from partially matched related donors (PMRD; one pheno-identical, one with one incompatible locus, and two with two incompatible loci), and four from HLA-matched unrelated donors (MUD). BMT conditioning regimens were variable (fractionated total body irradiation [12 to 14 Gy], associated with single drug chemotherapy [4 patients] or multiple drug chemotherapy [4 patients]) or exclusive multiple drug chemotherapy (busulfan associated with another chemotherapeutic agent [eight patients]). Two patients (unique patient nos. [UPN] 1 and 19) were transplanted for the second time after the first BMT. One patient with SCID was not conditioned before transplantation. All patients were nursed under strict protective isolation using laminar air-flow or closed plastic bubble systems. They received a total nonselective gut decontamination using nonabsorbable antibiotics.

As aGVHD prophylaxis, three patients received a T-lymphocyte–depleted donor marrow without postgraft immunosuppressive therapy, and 16 patients received cyclosporine, either alone (n = 3) or associated with methotrexate (n = 9) and with an anti–IL-2 receptor antibody (n = 4).

The criteria we adopted for diagnosis and grading of aGVHD are those established at Seattle.[7] aGVHD was histologically confirmed by skin biopsy. Due to the clinical and/or hematologic status of the patients, gut and liver biopsies were seldom performed. The intervals between BMT and the onset of GVHD and from refractory GVHD to B-C7 treatment were 27 ± 21 days and 40.7 ± 36.6 days, respectively. Three patients initially presented grade I GVHD, five grade II, five grade III, and six grade IV (4 of 6 had a clinical picture of toxic epidermal necrolysis). Eighteen patients were administered corticosteroids before B-C7, either at standard dose (2 mg/kg), intermediate dose (5 mg/kg), or high dose (10 mg/kg) combined with monoclonal anti–IL-2 receptor antibodies (12 patients) or antithymocyte globulin (6 patients). Eighteen patients received B-C7 either as second-line (n = 5), third-line (n = 5), or fourth-line therapy (n = 8). In one infant with SCID (UPN 7) who presented acute transfusional GVHD before BMT, B-C7 was initiated as first-line therapy.

Before B-C7 treatment was initiated, four patients had grade III and 15 had grade IV refractory GVHD (Table 1).

During B-C7 administration, all patients were administered corticosteroids at standard or intermediate dose and all except one received cyclosporine (Table 1).

Before B-C7 treatment, 13 patients did not exhibit any infection, but six patients did (three had cytomegalovirus [CMV] infections, one had aspergillosis, one had sepsis, and one had interstitial pneumonitis).

At the initiation of B-C7 treatment, hematopoietic recovery was complete in six patients, partial in 10, two had poor graft function, and one had pancytopenia justifying a second marrow transplant 1 month later.

B-C7 Treatment Protocol

As part of this phase I-II protocol and with the goal of assessing the feasibility, tolerance, and kinetics of this MoAb, the patients included in this pilot study were administered increasing doses of MoAb in vivo: 0.1 mg/kg (n = 4), 0.2 mg/kg (n = 9), 0.3 mg/kg (n = 2), 0.4 mg/kg (n = 4) (Fig 1). The MoAb was diluted in 100 or 150 mL of saline and was infused for 15 to 30 minutes every day for 4 days and then every second day for a further 4 days (6 doses). To avoid GVHD recurrence when B-C7 was discontinued, eight patients received high-dose corticosteroids followed by rapid dose reduction over 4 days. Three patients received anti-T MoAbs (CD25 ± CD5, 10 mg/day for 10 days).

The sera of patients treated in Besançon were collected before each B-C7 infusion for a study of B-C7 pharmacokinetics. Their sera were also collected before and after treatment to determine the levels of TNFα,[22] soluble IL-2 receptor (sIL-2R), and soluble CD8 (sCD8).

The protocol received the approval of the Committee of Ethics of the University of Besançon (France). Patients or their legal guardians gave their written informed consent.

GVHD response to B-C7 treatment was assessed upon completion of B-C7 treatment and fell into four groups. The complete response (CR) patients were those in whom GVHD lesions totally resolved in all organs involved. The very good partial response (VGPR) patients were those in whom greater than 50% of GVHD lesions disappeared in all of the organs initially involved. In particular, improvement of gastrointestinal symptoms was assessed by the number of stools per day (bloody or not), the volume of diarrhea, and the disappearance of abdominal pain. Partial response (PR) was considered to be a reduction of lesions in at least one organ involved and no response (NR) to be an unchanged status or progressive evolution of aGVHD.

Measurement of BC-C7 Serum Levels

Circulating BC-7 was measured by serial dilutions in a double sandwich enzyme-linked immunosorbent assay (ELISA) with a sensitivity of 10 ng/mL. Rabbit antimouse Ig was coated overnight in carbonate buffer. Control and patient sera were incubated at different dilutions for 1 hour, and underwent a further 1 hour of incubation with biotinylated goat antimouse Ig, and were finally incubated for 1 hour with streptavidin peroxidase. O-Phenylenediamine was used as a substrate and optical density (OD) was measured after 30 minutes at 405 nm.

Laboratory Assays

All assays were performed on frozen (−20°C) aliquoted samples. Consecutive evaluable samples were assayed for sCD8, sIL-2R, and TNFα. sCD8 and sIL-2R levels were measured with two-site sandwich enzyme immunoassays (Cell Free test kits; T Cell Science, Cambridge, MA). In our laboratory, control values were 346 ± 116 U/mL and 473 ± 180 U/mL for sCD8 and sIL-2R levels, respectively. TNFα levels were determined with an immunoradiometric assay (IRA-Medgenix, Brussels, Belgium). Polypropylene tubes coated with MoAbs directed against distinct epitopes of TNFα (polyclonal system) were incubated with a mixture of

Table 1. Clinical and Treatment Characteristics of Patients Treated With an Anti-TNFα Antibody for Severe aGVHD

UPN	Age/ Sex	Diagnosis	HLA Matching	GVHD Prophylaxis	Organ Involvement (grading)	B-C7 Dose (mg/kg)	Medications Administered With B-C7	Response	Sites of Response	Side Effects	aGVHD Recurrence	Time to Recurrence (d)	Treatment After B-C7	Outcome
1	1/F	SAA	PMRD (3/6)	TCD + CSP	S4 + G4 (IV)	0.1	MP + CSP	VGPR	S1 + G1 (I)	No	Yes	12	MP (SD) anti IL2R antibody	Dead day 74 post-BMT aGVHD multiorgan failure
2	30/M	CML	MRD	CSP + MTX	S2 + G4 + L4 (IV)	0.1	MP + CSP	PR	S1 + G2 + L4 (III)	No	Yes	2	MP (HD)	Dead day 135 post-BMT aGVHD IP
3	35/F	CML	MUD	CSP + MTX	S3 + G4 + L1 (IV)	0.1	MP + CSP	PR	S1 + G2 + L1 (II)	No	Yes	120	MP (HD)	Dead day 175 post-BMT liver failure (aGVHD)
4	25/M	CML	MRD	CSP + MTX	S1 + G3 + L2 (III)	0.1	MP + CSP	PR	S0 + G2 + L2 (II)	No	Yes	3	MP (HD)	Dead day 202 post-BMT aGVHD IP
5	6/M	AML	PMRD (6/6, MLR+)	CSP + MTX anti–IL-2R	S4 + L3 (IV)	0.2	MP + CSP	PR	S3 + L1 (II)	No	No	—	MP (ID)	Alive and well 15 mo+
6	6/M	SAA	PMRD (5/6)	CSP + MTX anti–IL-2R	S4 + G3 + L3 (IV)	0.2	MP + CSP	NR	—	No	—	—	ATG	Dead day 47 post-BMT aGVHD cerebral hemorrage
7	4 mo/F	SCID	PMRD (3/6)	TCD	S4 + G2 + L4 (IV)	0.2	MP + CSP	VGPR	S2 + G0 + L1 (II)	No	Yes	3	MP (HD)	Dead day 155 post-BMT ARDS no aGVHD
8	39/F	Hodgkin	MRD	TCD	S3 + G4 (IV)	0.2	MP + CSP	VGPR	S1 + G1 (I)	No	NE	—	MP (HD)	Dead day 94 post-BMT suicide
9	4/F	AUL	MRD	CSP + MTX	S4 + G2 (IV)	0.2	MP + CSP	VGPR	S1 + G1 (I)	Septic shock	NE	—	MP (SD)	Dead day 79 post-BMT septic choc (candida)
10	6 mo/F	ADA deficiency	PMRD (4/6)	TCD	S3 + G4 (IV)	0.2	MP	NR	—	No	—	—	MP (ID)	Dead day 30 post-BMT aGVHD hemorragic alveolitis
11	5/M	ALL	MUD	CSP + MTX anti–IL-2R	S4 + G2 (IV)	0.2	MP + CSP	NR	—	No	—	—	ATG	Dead day 185 post-BMT Chronic GVHD multiorgan failure
12	2/M	AMML	MRD	CSP + MTX	S2 + G3 (III)	0.2	MP + CSP	VGPR	S1 + G1 (I)	Pancreatic seizure	Yes	3	MP (ID) CD5 + CD25 ATG	Dead day 143 post-BMT IP no aGVHD
13	27/M	ALL	MRD	CSP + MTX	S2 + G3 + L2 (III)	0.2	MP + CSP	PR	S1 + G0 + L2 (II)	No	Yes	2	MP (ID)	Dead day 50 post-BMT aGVHD toxoplasmosis
14	32/M	CML	MUD	CSP + MTX	S2 + G3 + L3 (IV)	0.3	MP + CSP	PR	S1 + G1 + L3 (III)	No	Yes	3	Anti–IL-2R antibody MP (ID)	Dead day 115 post-BMT aGVHD fungal infection
15	17/M	Blackfan Diamond	MUD	CSP + MTX anti–IL-2R	S2 + G4 + L3 (IV)	0.3	MP + CSP	VGPR	S1 + G1 + L1 (II)	No	NE	—	MP (SD)	Dead day 100 post-BMT aGVHD IP
16	21/M	AML	MRD	CSP + MTX	G1 + L3 (III)	0.4	MP + CSP	VGPR	G0 +L1 (I)	No	No	—	MP (SD)	Alive and well 15 mo+
17	26/M	ALL	MRD	CSP	S3 + G4 + L1 (IV)	0.4	MP + CSP	VGPR	S1 + G0 + L0 (I)	No	Yes	27	MP (HD)	Alive and well mild chronic GVHD 6 mo+
18	43/F	AML	MRD	CSP + MTX	S2 + G4 + L2 (IV)	0.4	MP + CSP	NR	—	No	—	—	MP (HD) ATG	Dead day 121 aGVHD multiorgan failure
19	35/F	CML	MRD	CSP	S2 + G4 + L3 (IV)	0.4	MP + CSP	NR	—	No	—	—	MP (HD)	Dead day 68 aGVHD ARDS

Abbreviations: SAA, severe aplasic anemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AUL, acute undifferentiated leukemia; AMML, acute myelomonocytic leukemia; ADA, adenosine deaminase; CML, chronic myeloid leukemia; SCID, severe combined immune deficiency; CSP, cyclosporin; MTX, methotrexate; TCD, T-cell depletion; S, skin; G, gut; L, liver; NE, not evaluable; HDM, high-dose methylprednisolone; ATG, antithymoglobulins; ARDS, acute respiratory distress syndrome; MP, methylprednisolone; HD, high dose; ID, intermediate dose; SD, standard dose.

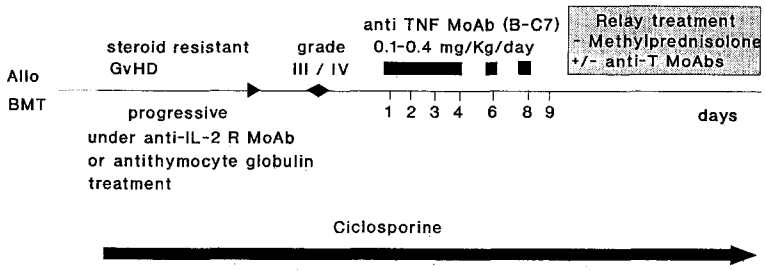

Fig 1. Anti-TNFα MoAb protocol for the treatment of severe aGVHD.

^{125}I-labeled anti-TNFα MoAb and the sample to be tested. After decantation, the bound fraction was counted in a gamma counter. Control values for serum TNFα levels were less than 15 pg/mL.

RESULTS

B-C7 MoAb Tolerance

The infusion of B-C7 antibody was very well tolerated over the 8 days of treatment. No clinical signs of intolerance (chills, hypotension) were observed regardless of the dose level used. In 11 patients, no significant change in white blood cell (WBC), absolute neutrophil count (ANC), monocyte, lymphocyte, or platelet counts was detected during B-C7 administration. In eight patients, changes in blood cell counts occurred during B-C7 treatment (Table 2). These variations of peripheral blood cell counts appeared to be random and are probably attributable to direct or indirect effects of severe GVHD. Of the eight patients presenting documented infection or interstitial pneumonitis (IP) before B-C7 treatment, no deleterious effects of B-C7 on the evolution of infection were observed during the treatment. When the treatment was completed, one patient (UPN 9) presented a septic shock due to candida. The clinical symptoms of this candida septicemia, which occurred during the course of B-C7 treatment, might have been masked by the anti-TNFα MoAb. Another patient (UPN 12) presented acute pancreatitis, as well as neurologic deterioration with a seizure, both of which appeared unrelated to B-C7 treatment and resolved completely with no sequelae.

Response to B-C7 Treatment

B-C7 treatment was initiated in a median delay of 25 days (range, 5 to 116 days) after the onset of aGVHD. The response was clinically assessed at the end of B-C7 treatment. Responses are given in Tables 1 and 3. Eight patients (42.1%) achieved a VGPR (6 grade IV, 2 grade III). Six patients (31.5%) achieved a PR (4 grade IV, 2 grade III) and five patients (26.3%) did not respond (5 grade IV). Skin and gut lesions responded best (11 of 18 [61%] and 13 of 18 [72%], respectively), followed by liver lesions (5 of 13 [38.5%]). Among the 14 responding patients (VGPR + PR), 14 of 14 (100%) gut lesions, 12 of 14 (85.7%) skin lesions, and 5 of 14 (35.7%) liver lesions, respectively, responded to B-C7 administration. The clinical response was particularly clear in gut lesions, because clinical manifestations disappeared in six patients (43%) and eight patients (57%) achieved a greater than 50% response.

In all the gut responders (10 adults, 4 children) the decreasing in stooling was clinically obvious: 1,726 ± 869 mL diarrhea before B-C7 treatment and 566 ± 324 mL diarrhea after treatment was completed. In five patients with bloody diarrhea, three patients changed to nonbloody diarrhea.

Skin improvement was also satisfactory, although no truly complete response was observed. Liver improvement was mediocre (8 failures), although two patients achieved a complete response. Response delays for skin and gut were very short ($\leq$3 days).

Clinical responses were seldom histologically documented.

Nevertheless, when skin (n = 6), rectum, or liver biopsies (n = 3) were performed, they consistently showed a staging decrease in histologic GVHD manifestations. There was no significant correlation between B-C7 dose levels and GVHD response (p = .8, Fischer's exact test). The small sample size did not make it possible to establish a response curve. In terms of GVHD response, there was no difference whether patients received standard (2 mg/kg, n = 7) or higher doses (>2 mg/kg, n = 11) of prednisolone during B-C7 treatment.

Table 2. Leucocyte and Platelet Modifications During B-C7 Administration in Eight Informative Patients

UPN	B-C7 Dose (mg/kg)	WBC		ANC		Lymphocytes		Monocytes		Platelets	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
2	0.1	2,500	1,500	2,000	1,275	75	85	3,100	110	40	20
3	0.1	5,400	3,600	4,644	3,132	54	180	640	252	80	50
5	0.2	2,600	4,200	1,946	1,780	178	1,210	182	812	130	83
10	0.2	11,000	15,500	10,160	13,125	330	875	220	875	26	45
11	0.2	3,410	1,330	2,100	254	688	574	605	470	41	26
13	0.2	500	600	100	420	400	180	50	0	20	20
17	0.4	14,600	16,300	12,550	14,507	438	326	1,022	863	70	80
18	0.4	4,100	600	2,500	224	1,066	70	328	280	40	20

In the other 11 patients, no change in blood cell counts was detected the day of B-C7 MoAb completion.

Table 3. Response to B-C7 Treatment Categorized by Individual Organs

Outcome	Organ		
	Skin	Gut	Liver
Evaluable	18	18	13
CR	—	6 (33)	2 (15.5)
VGPR	5 (28)	5 (28)	2 (15.5)
PR	6 (33)	2 (11)	1 (7.5)
NR	7 (39)	5 (28)	8 (61.5)

Percentages in parentheses.

Abbreviations: CR, complete response; VGPR, very good partial response (≥50%); PR, partial response (<50%); NR, no response (stable, progression).

B-C7 Pharmacokinetics

Pharmacokinetic analysis showed that peak serum B-C7 levels were dose-dependent and ranged from 1.2 to 13.4 μg/mL (Fig 2).

Alloreactivity Markers

TNFα. TNFα levels were measured in 10 patients immediately before B-C7 treatment (control values, <15 pg/mL). Five had high levels (118 ± 71 pg/mL) and five had levels less than 15 pg/mL. There was no correlation between the initial TNFα level and the response to B-C7 treatment. A follow-up of serum TNFα levels was performed on five patients during and after B-C7 treatment. Although the number of patients was too low for statistical analysis, the posttreatment TNFα level was higher in the PR/NR group (94 ± 53 pg/mL) as compared with the VGPR group (<20 pg/mL).

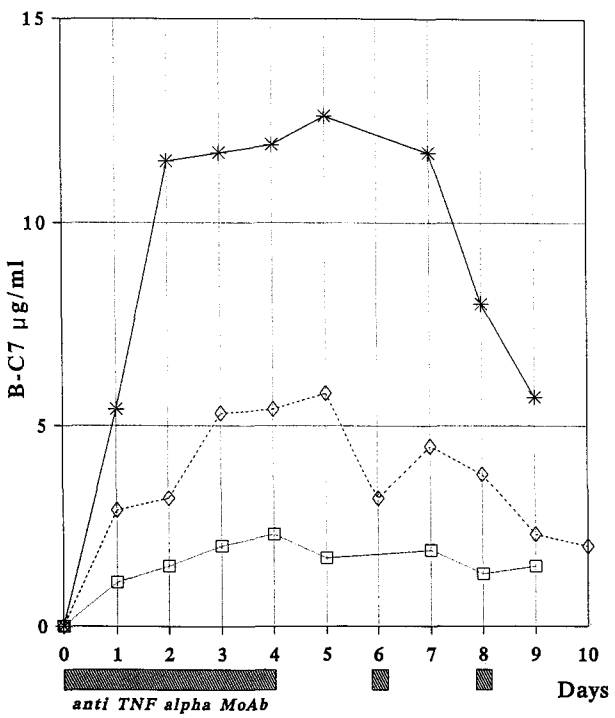

Fig 2. Evolution of serum B-C7 levels according to the infused dose of B-C7 (0.1, 0.2, 0.4 mg/kg). Serum B-C7 was measured in a double sandwich ELISA with a sensitivity of 10 ng/mL (described in Patients and Methods).

sIL-2R and sCD8. Before B-C7 treatment in the eight evaluable patients, sIL-2R and sCD8 levels were, respectively, 2,270 U/mL (range, 200 to 5,000 U/mL) and 294 U/mL (range, 160 to 410 U/mL). In patients evaluated posttreatment, sIL-2R levels were 2,300 U/mL (range, 200 to 5,000 U/mL) and sCD8 levels were 423 U/mL (range, 110 to 1,200 U/mL).

GVHD Recurrence

When B-C7 treatment was completed, all responding patients received additional immunosuppressive therapy (Table 1). Among the 14 responders, only 11 were evaluable for GVHD recurrence. Three patients died immediately after treatment was discontinued (one suicide, one septic shock, one interstitial pneumonitis). In two patients (14%), GVHD did not relapse; both are alive 15 and 13 months post-BMT. In nine patients, GVHD recurred in a median delay of 3 days after B-C7 treatment was discontinued (range, 2 to 120 days). Except for a female patient whose GVHD recurrence involved only liver (UPN 3), all patients presented multiorgan involvement at the time of recurrence.

Although salvage treatments were administered at the time of relapse, only one of the relapsing patients survived and is currently alive on day +180, with moderate chronic GVHD.

Survival

Among the 19 patients included in this study, three are alive (15.8%) at 6, 13, and 15 months post-BMT. Two do not exhibit chronic GVHD and one presents moderate chronic GVHD. Sixteen patients died (84.2%). The causes of death (Table 4) are mainly due to GVHD recurrence.

DISCUSSION

In murine models, Piguet[16] showed the protective role of a polyclonal or monoclonal anti-TNFα antibody against aGVHD-induced endothelial lesions, but not against those induced by chronic GVHD. These antibodies also enhanced engraftment and prevented cachexy. These combined effects lead to a clearly reduced mortality (70% animals surviving in the group treated with anti-TNFα

Table 4. Primary Causes of Death Among Patients Treated With Anti-TNFα (B-C7) for Severe aGVHD

Causes of Death		
aGVHD	5	56%
aGVHD + IP	3	
Chronic GVHD	1	
Acute respiratory distress syndrome	2	
Interstitial pneumonitis	1	
Suicide	1	
Septic choc	1	
Toxoplasmosis	1	
Fungal infection	1	
Total	16	

antibody versus 10% in the control group). High serum TNFα levels are reported to be associated with severe aGVHD.[20] However, this association is not true in each individual patient undergoing allogeneic BMT. Indeed, severe aGVHD can be observed with low serum TNFα levels.[21]

B-C7 was well tolerated at all dose levels. Changes in blood cell counts were observed in eight patients, but appeared to be unrelated to the B-C7 administration. In one patient (UPN 9), a septic shock was observed when B-C7 was discontinued. The occurrence of infectious complications on discontinuation of a treatment with monoclonal anti-TNFα antibody administered in a context of infection is a potential risk, given the role played by TNFα in the mechanisms of antibacterial resistance.[22] Nevertheless, the target-specificity of an anti-TNFα MoAb suggests that such a therapy, with respect to infectious complications, could be less dangerous than immunosuppressive agents with a broader action such as ATG. The maximal tolerated dose of this anti-TNFα MoAb is unknown. Doses greater than 1 mg/kg have been used in the treatment of septic shock with serum TNFα concentrations in excess of 1,400 pg/mL without any clinical intolerance.[23] In our study, the dose escalation was interrupted at 0.4 mg/kg/d because patients did not appear to get any additional benefit, at this dose level, in terms of GVHD response.

With a 72.8% response rate, our pilot study shows that anti-TNFα may be of benefit to some patients with severe aGVHD after BMT from HLA-matched or mismatched donors. B-C7 has an influence on all the target organs of GVHD, with a decreasing efficacy on the following organ involvements: gut (72% response, among which 33% were complete responses and 39% partial responses), skin (61% response), and liver (38.5% response). Regression occurs particularly early in the case of gut disorders. The reasons why the most dramatic responses were observed in gut lesions are presently unknown. In our phase II trial involving an anti–IL-2R MoAb,[9] the best responses were also observed in skin and gut lesions.

In our series, only 5 of 10 evaluable patients had high levels of TNFα just before B-C7 treatment. Serum TNFα levels less than 10 pg/mL do not exclude a possible GVH response to B-C7 treatment. Low serum TNFα levels may correspond to a tissue cell fixation of TNFα. In accordance, serum TNFα levels in a GVH murine model were never detectable, also suggesting that local versus systemic production of TNFα is predominant in most cases of GVH.[16]

The major problem encountered in MoAb serotherapy is GVHD recurrence when the treatment is discontinued.[9,24] In our study, 9 of 11 (81%) evaluable patients relapsed. The rate of relapse is higher than that observed with MoAbs directed against the presumed effector cells of aGVHD.[25] One can assume that a monoclonal anti-TNFα antibody has an influence only on a GVHD effector cytokine, and not on an effector cell.

Several alternatives exist to reduce the incidence of GVHD recurrence. An anti–T-cell MoAb (like anti-CD2 or anti-CD25) could be associated with an anti-TNFα MoAb and this type of serotherapy could be maintained for several weeks discontinuously. Preliminary data suggest that a 1- to 2-month maintenance treatment with anti–IL-2R MoAb (B-B10) is well tolerated and might be effective in preventing GVHD relapse (unpublished data). When anti-TNFα is discontinued, MoAbs conjugated with a toxin (ricin-conjugated CD25 or CD5) could be adopted to obtain a better target effect, because this type of conjugated MoAb is less dependent on the host's natural effectors as compared with unconjugated MoAbs.[8] High doses of corticosteroids, which also prove to be an effective anti-TNFα agent, could be associated with or follow B-C7 treatment.[25]

Progress in the treatment of aGVHD will clearly depend on our understanding of the cytokine network and its role in allospecific immune organ cytotoxicity.[26]

The rapidly occurring responses observed in severe potentially lethal aGVHD lesions and the high relapse rate observed after B-C7 treatment both suggest that anti-TNFα serotherapy could be used as an "emergency" medicine in severe GVH situations ideally associated with effective therapy directed against the allo-reactive cellular aGVHD component. B-C7 MoAb could also be used earlier in the therapeutic strategy of aGVHD, as second-line therapy, as soon as steroid resistance is established.

In summary, the possible efficacy of an anti-TNFα MoAb has been shown in severe refractory aGVHD. A major problem remains yet to be solved as to how to achieve long-term control of steroid-resistant GVHD. Associating an anti-TNFα MoAb with effective innovative therapy directed against the allo-reactive cellular GVHR component could be very promising.

ACKNOWLEDGMENT

We especially thank the physicians and nurses of the different BMT units who cared for the patients. We are grateful to the Biotechnology laboratory staff of the CRTS of Besançon and Biotest Company for providing an anti-TNFα MoAb for clinical use. We also thank Dany Trestchenkoff for her secretarial assistance and Jacqueline Kerveillant for her assistance in translating and revising the manuscript.

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Phase I-II trial of a monoclonal anti-tumor necrosis factor alpha antibody for the treatment of refractory severe acute graft-versus-host disease

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.