Brief report

Absence of donor T-cell–derived soluble TNF decreases graft-versus-host disease without impairing graft-versus-tumor activity

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Tumor necrosis factor (TNF) plays an important role in graft-versus-host disease (GVHD) and graft-versus-tumor (GVT) activity after allogeneic bone marrow transplantation (allo-BMT). TNF can be expressed in a membrane-bound form (memTNF) and as a soluble (solTNF) molecule after being cleaved by the TNF-α converting enzyme (TACE). To study the contribution of donor T-cell–derived memTNF versus solTNF in GVHD and GVT, we used mice containing a noncleavable allele in place of endogenous TNF (memTNF$^{\Delta/\Delta}$) as donors in murine BMT models. Recipients of memTNF T cells developed significantly less GVHD than recipients of wild-type (wt) T cells. In contrast, GVT activity mediated by memTNF T cells remained intact, and alloreactive memTNF T cells showed no defects in proliferation, activation, and cytotoxicity. These data suggest that suppressing the secretion of solTNF by donor T cells significantly decreases GVHD without impairing GVT activity. (Blood. 2007;110:783-786)

Introduction

Tumor necrosis factor (TNF) is a type I cytokine involved in many pathophysiological processes; it plays a critical role in the regulation of cell trafficking, inflammation, maintenance of lymphoid organ structure, and host defense against various pathogens.[1] TNF can be expressed as a transmembrane molecule (memTNF) on the surface of activated monocytes, macrophages, lymphocytes, and natural killer (NK) cells[2] and, when cleaved by the TNF-α converting enzyme (TACE), is secreted by the cell in its soluble form (solTNF).[3,4] Its important role in the development of graft-versus-host disease (GVHD) and graft-versus-tumor (GVT) activity has previously been demonstrated in murine bone marrow transplantation (BMT) models using anti-TNF antibodies,[5-8] TNF-deficient donors,[9] and TNF receptor–deficient recipients.[10] In our previous study, we showed that donor T-cell–derived TNF is required in the development of GVHD, especially in the thymus, small bowel, and large bowel target organs, as well as in GVT activity.[9] To distinguish between the roles of memTNF and solTNF in contributing to GVHD and GVT, we used memTNF$^{\Delta/\Delta}$ mice as the source of donor T cells in our BMT experiments. memTNF$^{\Delta/\Delta}$ mice have a combined deletion of wild-type (wt) TNF and a "knocked-in" allele of a mutated TNF resistant to TACE-mediated cleavage.[11]

Materials and methods

Cell lines, antibodies, and flow cytometry

P815 (H-2^d), a mastocytoma cell line of DBA/2 origin, A20 (H-2^d), a lymphoma cell line of BALB/c origin, and EL4 (H-2^b), a lymphoma cell line of C57BL/6 origin, were obtained from the ATCC (Manassas, VA). All the fluorochrome-labeled antimurine antibodies were obtained from Pharmingen (San Diego, CA). CFSE labeling of T cells and flow cytometric analysis were performed as previously described[12] using a LSRII cytometer (Becton Dickinson, San Jose, CA) and FlowJo (Treestar, Ashland, OR) for data analysis.

GVHD/GVT experiments

Female C57BL/6 (B6) (H-2^b), BALB/c (H-2^d), C3FeB6F1 (H-2$^{b/k}$), and B6D2F1 (H-2$^{b/d}$) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). MemTNF$^{\Delta/\Delta}$ mice were obtained from the DNAX Research Institute (Palo Alto, CA). The BMT procedure was performed as previously described.[12] Recipients were monitored for survival, weight loss, and clinical GVHD as previously described.[12] BMT protocols were approved by the Memorial Sloan-Kettering Cancer Center Institutional Animal Care and Use Committee. BMT recipients were killed for blinded histopathologic and flow cytometric analysis of GVHD target organs as previously described.[12]

Cytometric bead array

Serum concentrations of TNF and IFN-γ were determined by cytometric bead array (CBA) and analyzed with BD CBA Software (Becton Dickinson) according to the manufacturer's protocol.

Cytotoxicity assay

T cells were stimulated in vitro for 5 days with irradiated BALB/c splenocytes and cytotoxicity against ^{51}Cr-labeled target cells was determined as previously described.[12]

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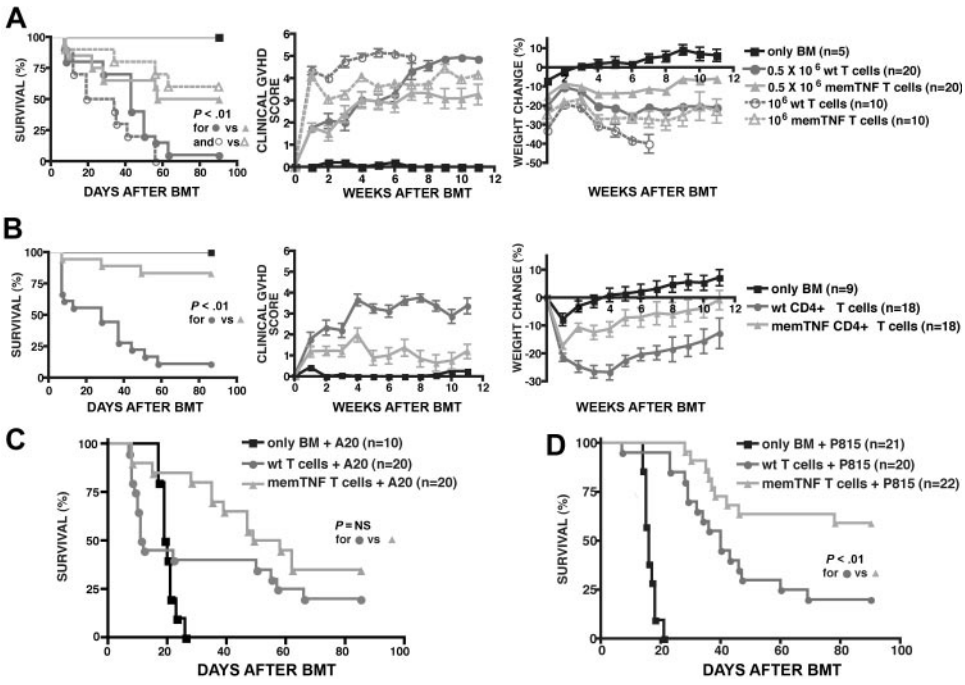

Figure 1. memTNF T cells induce less GVHD mortality and morbidity than wt T cells and have intact GVT activity. (A) Lethally irradiated (850 cGy) BALB/c mice received 5×10^6 TCD wt B6 BM cells alone (n = 5) or in combination with 0.5×10^6 (n = 20) or 1×10^6 (n = 10) wt or memTNF B6 T cells. Data represent 2 combined experiments. (B) Lethally irradiated (1300 cGy) C3FeB6F1 mice received 5×10^6 TCD wt B6 BM cells alone (n = 9) or in combination with 1×10^6 purified $CD4^+$ T cells from wt or memTNF B6 mice (n = 18). Data represent 2 combined experiments. (C) Lethally irradiated (850 cGy) BALB/c mice received 5×10^6 TCD wt B6 BM cells and 1×10^6 A20 lymphoma cells (n = 10) with or without the addition of 0.5×10^6 B6 wt or memTNF B6 T cells (n = 20). Survival was monitored daily and cause of death (tumor versus GVHD) was determined by necropsy and histopathology. Data represent 2 combined experiments. N/A indicates not analyzed due to tissue necrosis. (D) Lethally irradiated (1300 cGy) B6D2F1 mice received 5×10^6 TCD wt B6 BM cells and 1×10^3 P815 mastocytoma cells (n = 21) with or without the addition of 0.5×10^6 B6 wt (n = 20) or memTNF B6 T cells (n = 22). Survival was monitored daily and cause of death (tumor versus GVHD) was determined by necropsy and histopathology. Data represent 3 combined experiments.

Statistics

All values shown in graphs represent the mean ± SEM. Survival data were analyzed with the Mantel-Cox log-rank test. For all other analysis, nonparametric unpaired Mann-Whitney *U* test was used.

Results and discussion

To assess the contribution of donor T-cell–derived memTNF in the development of GVHD, we used a major histocompatibility complex (MHC)–mismatched murine BMT model in which BALB/c hosts received a transplant of 5×10^6 T-cell–depleted (TCD) wt B6 bone marrow (BM) cells and 0.5 or 1×10^6 T cells from wt or memTNF B6 mice. Recipients of memTNF T cells showed significantly improved survival and less morbidity compared with recipients of both doses of wt T cells (Figure 1A). The morbidity difference became evident at 5 weeks after BMT with a dose of 0.5×10^6 T cell, suggesting a late effect of memTNF donor T cells. We observed no difference in GVHD between wt or memTNF groups in a murine parent-into-F1 model (B6→C3FeB6F1) (data not shown). However, when we repeated the transplantation using 1×10^6 purified $CD4^+$ donor T cells, we found again that recipients of memTNF $CD4^+$ T cells showed less GVHD mortality and morbidity than recipients of wt $CD4^+$ T cells (Figure 1B). These data are in agreement with previous studies regarding the role of TNF (in particular donor T-cell–derived TNF) in GVHD.[5,7-9] Our data from 2 GVHD models suggest that solTNF released by donor T cells (especially $CD4^+$ T cells) is important for the development of GVHD.

To determine whether the presence of memTNF on T cells alone is sufficient to mediate GVT, we used a well-characterized GVHD/GVT model: B6→BALB/c with A20 lymphoma cells. We observed improved survival in recipients of memTNF T cells compared with recipients of TCD-BM, indicating that memTNF T cells were capable to exert GVT activity. Moreover, overall survival and deaths from lymphoma did not differ between recipients of memTNF versus wt T cells, suggesting that GVT activity was comparable (Figure 1C, Table 1). Interestingly, in a second GVHD/GVT model (B6→B6D2F1 with P815 mastocytoma cells), the mice receiving memTNF T cells showed significantly improved survival over the mice receiving wt T cells (Figure 1D, Table 1). Analysis of necropsy samples showed only tumor-related deaths with no GVHD-associated mortality (table in Figure 1D, Table 1). Our previous study demonstrated that TNF from donor T cells is required for GVT activity.[9] We conclude from the current experiments that donor T-cell solTNF is dispensable for GVT activity.

We then performed histopathological analysis of different GVHD organs and found that recipients of memTNF T cells developed significantly less damage to the liver, small bowel, and large bowel (Figure 2A). The role of TNF in intestinal[5,13,14] and hepatic[15] GVHD has previously been shown, and our data suggest that solTNF secreted by donor T cells is involved in intestinal and hepatic GVHD.

No difference in thymic GVHD (as defined by thymic cellularity and decrease in $CD4^+CD8^+$[16]) was observed (Figure 2B) in contrast from the decrease in thymic GVHD that we observed using $TNF^{-/-}$ mice as the source of donor T cells.[9] This suggests that the presence of memTNF alone may be sufficient to cause thymic GVHD.

To assess whether memTNF T cells differ in their capacity to undergo alloactivation, we analyzed splenic T cells from

Table 1. Cause of death for mice after lethal irradiation

Group	Tumor	GVHD	N/A
BALB-C			
only BM + A20	10/10	0/10	0/10
wt T cells + A20	4/20	11/20	1/20
memTNF T cells + A20	3/20	5/20	5/20
B6D2F1			
only BM + P815	21/21	0/21	
wt T cells + P815	16/20	0/20	
memTNF T cells + P815	9/22	0/22	

Descriptions as in Figure 1C,D.

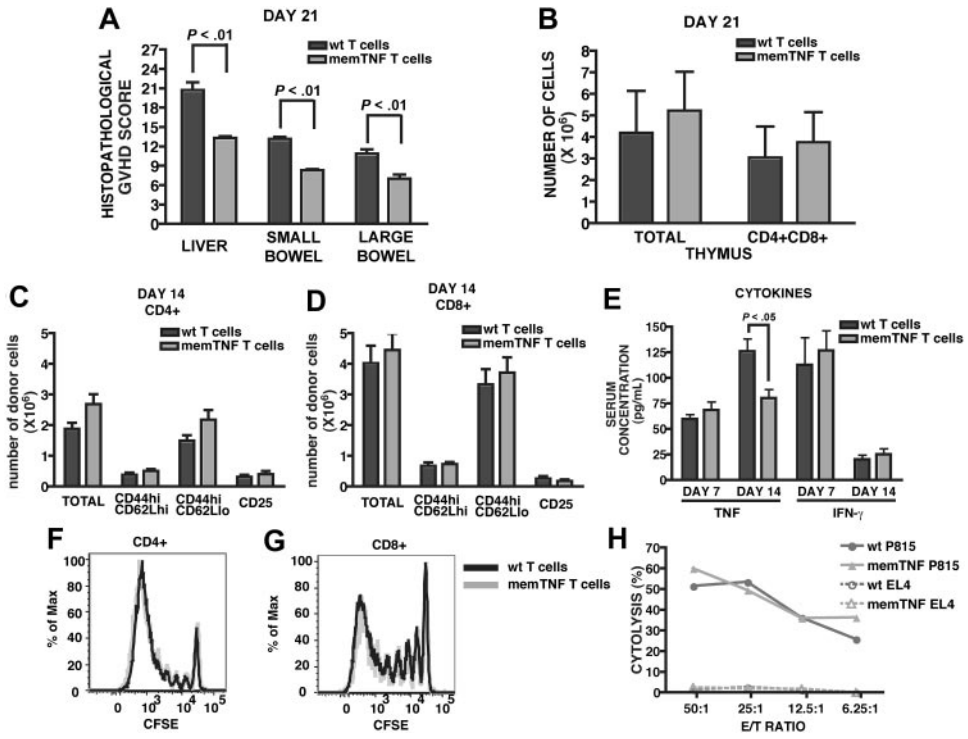

Figure 2. Alloreactive memTNF T cells cause less liver and gut damage than wt T cells and have intact proliferation, activation, and cytolytic activity. (A-E) Lethally irradiated (850 cGy) BALB/c mice received 5×10^6 TCD wt B6 BM cells in combination with 1×10^6 wt (black bar) or memTNF (gray bar) B6 T cells. (A) Liver, small bowel, and large bowel were harvested on day 21. Hematoxylin and eosin–stained slides were analyzed and scored for histopathological damage. Shown is the mean ± SEM for 7 mice per group. (B) Thymic cellularity (total cell counts and $CD4^+CD8^+$ thymocytes) was determined by flow cytometric analysis on day 21. Shown is the mean (± SEM) for 9 mice per group. (C-D) Recipient spleens were harvested at day 14 for flow cytometric analysis of CD44, CD62L, and CD25 expression on $CD4^+$ and $CD8^+$ T cells. Shown is the mean (± SEM) for 10 mice per group. (E) Serum cytokine levels were measured by CBA at days 7 and 14. Shown is the mean (± SEM) for 10 mice per group. (F-G) Lethally irradiated (850 cGy) BALB/c mice received 20×10^6 CFSE-labeled wt or memTNF B6 T cells. Recipient spleens were harvested after 72 hours for flow cytometric analysis. Data shown are from 1 representative mouse of 4 mice from 2 experiments. Histogram overlays for CFSE-labeled wt (black line) and memTNF (gray line) are shown for donor $CD4^+$ (G) and $CD8^+$ (H) T cells. (H) In vitro–activated splenocytes were used as effectors in a ^{51}Cr cytotoxicity assay. Targets were allogeneic P815 and third-party EL4.

recipients of wt versus memTNF T cells 14 and 21 days after BMT. We observed no difference in the number of splenic donor $CD4^+$ and $CD8^+$ cells and in the number of their subsets (Figure 2C,D; Figure S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). Analysis of serum samples showed lower levels of TNF in the recipients of memTNF T cells than in recipients of wt T cells (Figure 2E) on day 14 (but not onday 7), which confirms the late effect exerted by memTNF donor T cells shown in the morbidity graphs (Figure 1A). Host-derived TNF from irradiation-damaged gut may be exerting a confounding effect on differences in serum TNF levels early after transplantation, whereas the difference observed on day 14 indicates that TNF secretion by donor T cells contributes to the serum TNF levels during GVHD in addition to TNF secretion by monocytes and macrophages.[17] No difference was found in the IFN-γ serum levels between the 2 groups. To further assess alloactivation of memTNF T cells, we infused CFSE-labeled wt or memTNF B6 splenic T cells in BALB/c hosts and harvested spleens 72 hours after adoptive transfer. We found that the proliferation kinetics of $CD4^+$ and $CD8^+$ T cells were similar between wt and memTNF T cells (Figure 2F-G). Finally, cytotoxic activity of alloreactive memTNF T cells was intact, as measured in a ^{51}Cr release assay of in vitro–activated T cells against the P815 cell line (Figure 2H). Taken together, these results rule out any intrinsic defects of alloreactive memTNF T cells in activation, cytokine production (except TNF), proliferation, and cytotoxic activity.

In conclusion, our results show that eliminating donor T-cell–derived soluble TNF (especially from $CD4^+$ origin) causes a significant delayed decrease in GVHD morbidity and mortality after BMT without impairing the proliferation and activation of donor T cells. At the same time, the presence of membrane-bound TNF alone is sufficient to mediate GVT activity. Therefore, targeting the cleavage of membrane-bound TNF with a TACE inhibitor could be assessed as a new therapeutic strategy for attenuating gastrointestinal and hepatic GVHD without impacting GVT after allo-BMT.[18,19]

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Authorship

Contribution: C.B. designed and performed research, analyzed data, and wrote the paper; S.X.L. and O.M.S. performed research and wrote the paper; A.R.K.F., T.D.K., D.S., C.G.K., and A.C. performed research and analyzed data; C.L. analyzed data; O.A. designed and performed research; and M.R.M.v.d.B. designed research and wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References

1. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol. 2003;3:745-756.
2. Vassalli P. The pathophysiology of tumor necrosis factors. Annu Rev Immunol. 1992;10:411-452.
3. Black RA, Rauch CT, Kozlosky CJ, et al. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. Nature. 1997;385: 729-733.
4. Moss ML, Jin SL, Milla ME, et al. Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha. Nature. 1997; 385:733-736.
5. Hattori K, Hirano T, Miyajima H, et al. Differential effects of anti-Fas ligand and anti-tumor necrosis factor alpha antibodies on acute graft-versus-host disease pathologies. Blood. 1998;91:4051-4045.
6. Korngold R, Marini JC, de Baca ME, Murphy GF, Giles-Komar J. Role of tumor necrosis factor-alpha in graft-versus-host disease and graft-versus-leukemia responses. Biol Blood Marrow Transplant. 2003;9:292-303.
7. Tsukada N, Kobata T, Aizawa Y, Yagita H, Okumura K. Graft-versus-leukemia effect and graft-versus-host disease can be differentiated by cytotoxic mechanisms in a murine model of allogeneic bone marrow transplantation. Blood. 1999;93: 2738-2747.
8. Hill GR, Teshima T, Gerbitz A, et al. Differential roles of IL-1 and TNF-alpha on graft-versus-host disease and graft versus leukemia. J Clin Invest. 1999;104:459-467.
9. Schmaltz C, Alpdogan O, Muriglan SJ, et al. Donor T cell-derived TNF is required for graft-versus-host disease and graft-versus-tumor activity after bone marrow transplantation. Blood. 2003; 101:2440-2445.
10. Speiser DE, Bachmann MF, Frick TW, et al. TNF receptor p55 controls early acute graft-versus-host disease. J Immunol. 1997;158:5185-5190.
11. Ruuls SR, Hoek RM, Ngo VN, et al. Membrane-bound TNF supports secondary lymphoid organ structure but is subservient to secreted TNF in driving autoimmune inflammation. Immunity. 2001;15:533-543.
12. Terwey TH, Kim TD, Kochman AA, et al. CCR2 is required for CD8-induced graft-versus-host disease. Blood. 2005;106:3322-3330.
13. Piguet PF, Grau GE, Allet B, Vassalli P. Tumor necrosis factor/cachectin is an effector of skin and gut lesions of the acute phase of graft-vs.-host disease. J Exp Med. 1987;166:1280-1289.
14. Cooke KR, Hill GR, Crawford JM, et al. Tumor necrosis factor-alpha production to lipopolysaccharide stimulation by donor cells predicts the severity of experimental acute graft-versus-host disease. J Clin Invest. 1998;102:1882-1891.
15. El-Hayek JM, Rogers TE, Brown GR. The role of TNF in hepatic histopathological manifestations and hepatic CD8+ T cell alloresponses in murine MHC class I disparate GVHD. J Leukoc Biol. 2005;78:1001-1007.
16. Krenger W, Rossi S, Piali L, Hollander GA. Thymic atrophy in murine acute graft-versus-host disease is effected by impaired cell cycle progression of host pro-T and pre-T cells. Blood. 2000; 96:347-354.
17. Nestel FP, Price KS, Seemayer TA, Lapp WS. Macrophage priming and lipopolysaccharide-triggered release of tumor necrosis factor alpha during graft-versus-host disease. J Exp Med. 1992; 175:405-413.
18. Hattori K, Hirano T, Ushiyama C, et al. A metalloproteinase inhibitor prevents acute graft-versus-host disease in mice after bone marrow transplantation. Bone Marrow Transplant. 1999;23: 1283-1289.
19. Hattori K, Hirano T, Oshimi K, Yagita H, Okumura K. A metalloproteinase inhibitor prevents acute graft-versus-host disease while preserving the graft-versus-leukaemia effect of allogeneic bone marrow transplantation. Leuk Lymph. 2000;38:553-561.