Antigen-presenting cell–derived complement modulates graft-versus-host disease

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Acute graft-versus-host disease (GvHD) is a serious complication of allogeneic hematopoietic cell transplantation (allo-HCT) that results from donor allogeneic T cell attack on host tissues. Based on previous work implicating immune cell–derived C3a and C5a as regulators of T cell immunity, we examined the effects of locally produced C3a and C5a on murine T cell–mediated GvHD. We found that total body irradiation, a conditioning regimen required to permit engraftment of allo-HCT, caused upregulation and activation of alternative pathway complement components by recipient APCs. Allo-HCT with decay accelerating factor–null ($Daf1^{-/-}$) host BM and $Daf1^{-/-}$ donor lymphocytes led to exacerbated GvHD outcome and resulted in splenic and organ-infiltrating T cell expansion. T cells deficient in C3a receptor (C3aR) and/or C5a receptor (C5aR) responded weakly in allogeneic hosts and exhibited limited ability to induce GvHD. Using a clinically relevant treatment strategy, we showed that pharmacological C5aR blockade reduced GvHD morbidity. Our data mechanistically link APC-derived complement to T cell–mediated GvHD and support complement inhibition as a therapeutic strategy for GvHD in humans.

Introduction

Graft-versus-host disease (GvHD) — a potentially lethal complication of allogeneic hematopoietic cell transplantation (allo-HCT), which is performed to treat malignancies or nonmalignant conditions — is initiated by cognate interactions between donor T cells and host, MHC-disparate, or minor antigen–disparate (mH-disparate) DCs (1–4). Total body irradiation (TBI), a requirement in allo-HCT, activates recipient DCs through incompletely deciphered mechanisms (4–6) and facilitates alloreactive T cell activation. Subsequent costimulatory and TCR-initiated signaling pathways orchestrate pathogenic alloresponses (7, 8), but emerging evidence indicates that innate mechanisms influence the strength of GvHD-inducing T cell immunity (9, 10).

Previous work by our laboratories showed that DCs and T cells synthesize and secrete complement proteins; that these cells express C3a receptor (C3aR), C5a receptor (C5aR), and complement regulators on their surfaces; and that locally produced C3a and C5a influences the strength/phenotype of T cell responses (11–13). These findings raise the possibility that C3a and/or C5a modulate T cell–induced GvHD and that targeting C3aR/C5aR signaling could be therapeutically efficacious.

Results and Discussion

We examined the effect of TBI used for allo-HCT on the synthesis of complement components by recipient APCs. Compared with nonirradiated controls, DCs and macrophages isolated 3–24 hours after TBI produced up to 40-fold more mRNA for C3, C5, factor B, and factor D (Figure 1, A and B). Immunoblots performed on DC lysates confirmed that TBI upregulated C3 protein (Figure 1C). Irradiation lowered DC expression of decay accelerating fac-

tor (DAF, also referred to as CD55; Figure 1D), a surface-expressed complement regulator that prevents amplification of the complement cascade at the C3 convertase step (14). The lowered DAF expression lifted restraint on activation of the DC-derived complement proteins; we detected more C5a in serum-free culture supernatants obtained from DCs of irradiated mice (Figure 1E). To test for functional effects, we performed in vitro mixed lymphocyte reactions using splenic DCs isolated before and after TBI (Figure 1, F and G). T cells responded most strongly to allogeneic DCs obtained from irradiated mice, but responses were prevented in cultures containing C3/C5-deficient DCs. Splenic macrophages do not induce allogeneic T cell expansion (15). In vivo T cell allo-reactivity was also reduced in irradiated BALB/c recipients reconstituted with C3/C5-deficient BM, in which C3/C5 deficiency was restricted to the host hematopoietic compartment (Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI61019DS1). Thus, C3/C5 produced by host hematopoietic cells controlled allogeneic T cell reactivity upon allo-HCT.

To test for effects on GvHD, we used DAF-deficient ($Daf1^{-/-}$) mice, in which restraint on local C3a/C5a production is lifted (13). We transplanted B6 $Daf1^{-/-}$ mice with MHC-disparate $Daf1^{-/-}$ BALB/c BM plus 2 different doses of $Daf1^{-/-}$ BALB/c splenocytes (Figure 2, A–C). We observed worse clinical (Figure 2, A and B) and histological GvHD scores in $Daf1^{-/-}$ recipients (large bowel, WT, 0.25 ± 0.1; $Daf1^{-/-}$, 2.8 ± 0.2; $P < 0.05$; $n = 4$ per group; data not shown). Flow cytometry of spleens of $Daf1^{-/-}$ recipients showed approximately 5-fold more IFN-γ^+ T cells (Figure 2C).

To examine the role of recipient DAF in protecting against GvHD, we transplanted WT or $Daf1^{-/-}$ B6 mice with BALB/c BM and spleen cells. We found that $Daf1^{-/-}$ recipients had reduced survival (Figure 2D) and worsened clinical scores (Supplemental Figure 2A) versus WT recipients. We also observed enhanced proliferation/expansion of naive and in vitro–primed T cells 5 days after transfer into irradiated $Daf1^{-/-}$ allogeneic hosts (Sup-
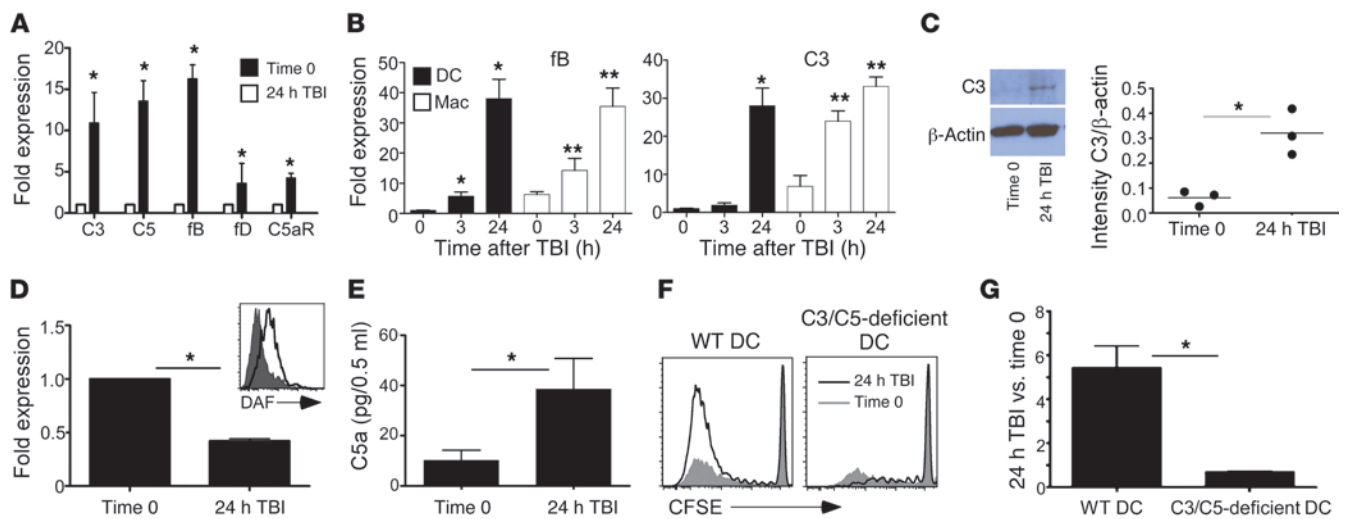
Figure 1
TBI causes DC complement upregulation and complement-dependent augmentation of T cell reactivity. (**A**) Quantitative PCR for complement components performed on splenic DC mRNA purified from B6 mice before (time 0) or 24 hours after TBI. fB, factor B; fD, factor D. (**B**) Kinetics of gene expression in splenic DCs or macrophages obtained from untreated or TBI-treated mice. Experiments were performed in triplicate. (**C**) Representative immunoblot for C3 (and β-actin control) performed on lysates of splenic DCs isolated before or 24 hours after TBI. Quantitative densitometry ($n = 3$) normalized to β-actin is also shown. (**D**) Quantitative PCR for DAF and expression of DAF assessed by flow cytometry (inset) on splenic DCs isolated from untreated (solid line) or TBI-treated (24 hours; filled) B6 mice. Each experiment in **A**–**D** was repeated twice with similar results. (**E**) C5a ELISA, performed on supernatants of unstimulated splenic DCs obtained from untreated or TBI-treated (24 hours) B6 mice, pooled from 4 experiments. (**F**) Representative CSFE dilution plots of B6 T cells mixed with allogeneic $H\text{-}2^d$ WT or C3/C5-deficient splenic DCs obtained from untreated or TBI-treated mice. (**G**) Quantification of proliferation (>4 divisions), calculated as the ratio of response to DCs from irradiated mice to that from nonirradiated mice, and expressed as a percentage. Data are representative of 2 different experiments. *$P < 0.05$.

plemental Figure 3, A and B). Thus, recipient DAF deficiency augmented T cell alloreactivity during priming and reactivation. Host DAF deficiency also worsened GvHD in a mH-disparate combination (C3H.SW→B6; Supplemental Figure 3C).

To distinguish the contribution of host hematopoietic cell–expressed DAF on GvHD, we generated BM chimeric mice reconstituted with congenic $Daf1^{-/-}$ or WT BM to use as recipients. Control experiments showed equal reconstitution of donor-derived hematopoietic cells in blood 14 days posttransplant (WT, 81%; $Daf1^{-/-}$, 84%; P = NS; n = 4 per group), which indicates that DAF deficiency did not affect engraftment. At 8 weeks after reconstitution, chimeric mice were irradiated and injected with BALB/c BM and splenocytes. We observed worse GvHD in $Daf1^{-/-}$ BM chimeric mice (Figure 2E), which suggests that DAF deficiency on host hematopoietic cells was responsible for the increased alloreactivity observed in $Daf1^{-/-}$ animals.

To assess the contribution of donor cell DAF in protecting against GvHD, we transplanted BALB/c recipients with B6 WT or $Daf1^{-/-}$ BM/spleen cells. Recipients injected with $Daf1^{-/-}$ cells developed exacerbated weight loss and shorter survival (Figure 2F) and contained greater numbers of skin-infiltrating donor T cells (Figure 2G) with increased staining for C3d in the skin at sites of cell infiltration (Supplemental Figure 2, B and C) 21 days posttransplant. The same result was observed with the B6→C3H.SW, mH-disparate combination (WT, 87% of baseline weight at 21 days posttransplant; $Daf1^{-/-}$, 73%; $P < 0.05$; n = 5 per group; data not shown).

Since irradiation triggered DC complement production (Figure 1), activation of which is enhanced by DAF deficiency (11, 13, 16), we postulated that the increased C3a/C5a would exacerbate GvHD

in part via C3aR/C5aR signaling on responder T cells. To test this hypothesis, we reconstituted irradiated BALB/c mice with B6 BM plus WT, $C5ar1^{-/-}$, $C3ar1^{-/-}$, or $C3ar1^{-/-}C5ar1^{-/-}$ T cells (Figure 3, A and B). The clinical/pathological expression of GvHD was diminished in BALB/c recipients of B6 $C3ar1^{-/-}C5ar1^{-/-}$ T cells (large bowel scores, WT, 6.0 ± 2.2; $C3ar1^{-/-}C5ar1^{-/-}$, 2.6 ± 1.5; $P < 0.05$; n = 8 per group pooled from 2 experiments). We observed partial protection from GvHD in recipients of either $C5ar1^{-/-}$ (Figure 3A) or $C3ar1^{-/-}$ T cells (Supplemental Figure 4), indicative of overlapping, but not fully redundant, effects.

To further assess the role of T cell–expressed C3aR/C5aR, we used a competition strategy. We reconstituted irradiated BALB/c recipients with B6 allogeneic BM plus equal numbers of B6 CD45.1⁺ WT, B6 CD45.2⁺ $C3ar1^{-/-}C5ar1^{-/-}$, and B6 Thy1.1 $Daf1^{-/-}$ T cells. After 5 weeks, we compared the expansion/infiltration of each congenic B6 T cell population in the same recipient animal (Figure 3C and Supplemental Figure 5). We observed fewer $C3ar1^{-/-}C5ar1^{-/-}$ than WT T cells, and more $Daf1^{-/-}$ T cells, in recipient skin and large bowel (Figure 3, C and D). Together, these data indicate that T cell expression of DAF, C3aR, and C5aR regulate in vivo T cell alloreactivity during GvHD.

To develop a treatment strategy, we examined the effect of C5aR blockade on GvHD. Continuous 30-day infusion of a specific cyclic-peptide C5aR antagonist (C5aR-A) by osmotic pump (17) starting 7 or 14 days posttransplant (after initiation of GvHD-induced weight loss) improved the clinical manifestations of GvHD in BALB/c recipients of B6 cells (Figure 3E). In the mH-disparate B6→C3H.SW combination, C5aR-A administration prevented the exacerbated disease induced by $Daf1^{-/-}$ compared

Figure 2
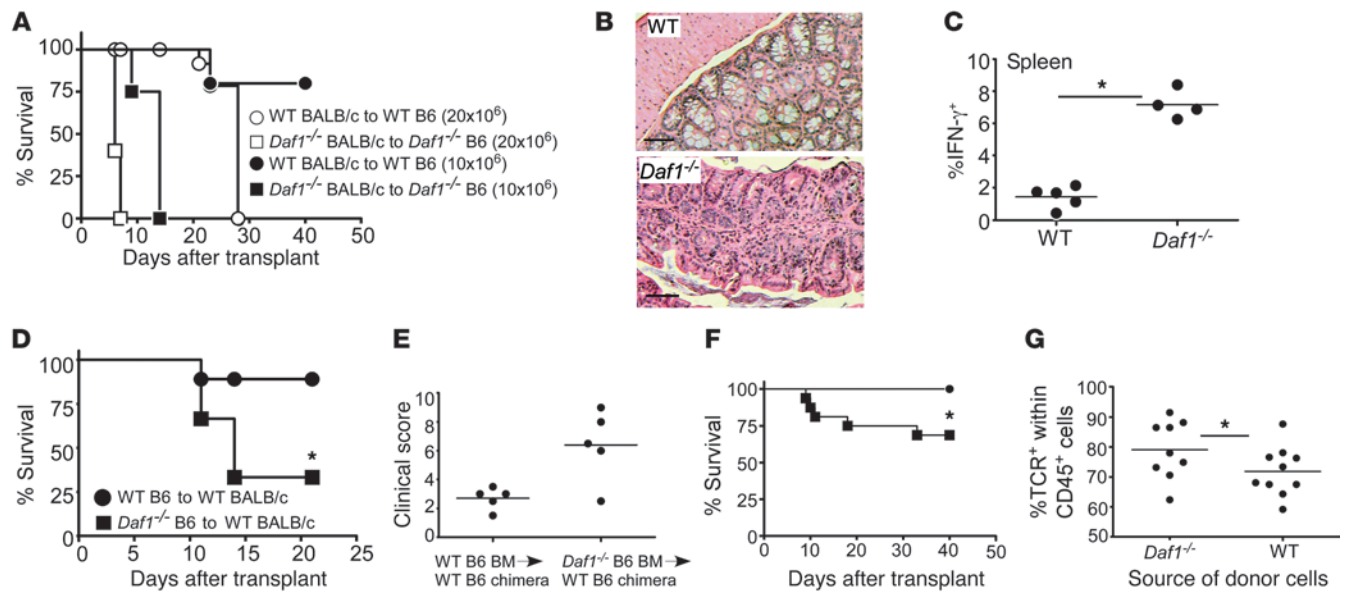DAF deficiency exacerbates GvHD. (**A–C**) GvHD in WT B6 recipients of WT BALB/c transplants or *Daf1⁻/⁻* B6 recipients of *Daf1⁻/⁻* BALB/c transplants. (**A**) Survival curves for 10×10^6 or 20×10^6 doses of donor spleen cells. $n = 5$ per group. (**B**) Representative H&E-stained bowel tissue (8 days posttransplant) from a separate experiment (5×10^6 donor spleen cells). Scale bars: 50 μm. (**C**) IFN-γ–producing spleen cells stimulated ex vivo with αCD3/CD28 (day 8 posttransplant). (**D**) Survival in WT or *Daf1⁻/⁻* B6 recipients transplanted with WT BALB/c BM plus 1×10^6 BALB/c spleen cells. $n = 5$ per group, repeated with the same results. (**E**) Clinical GvHD scores in B6 chimeras of allogeneic BALB/c BM transplants. Shown are pooled data from 2 separate experiments ($n = 10$ per group). (**F** and **G**) WT BALB/c mice received B6 WT or *Daf1⁻/⁻* T cell–depleted BM plus 0.5×10^6 B6 WT or *Daf1⁻/⁻* splenocytes. (**F**) Survival ($n = 15$ per group, pooled from 3 experiments). (**G**) Percent skin-infiltrating T cells at 5 weeks, pooled from 2 experiments. *$P < 0.05$.

with WT donor cells (Figure 3F), which indicates that the effects of DAF deficiency are dependent in part on C5a. Whether short-term C5aR blockade prevents late GvHD and/or induces immune tolerance requires further study.

Our results indicated that APC-expressed DAF, through controlling local C3a/C5a production, regulated T cell–dependent GvHD. We showed that TBI-induced APC activation was dependent on APC-produced C3/C5 (Figure 1), providing a molecular explanation for observations by others that irradiation-induced, gut-derived LPS modulates APC maturation (18, 19). LPS induces complement production by DCs (20), complement amplifies TLR signals (21, 22), and T cells respond weakly to allogeneic, LPS-stimulated DCs from *C3⁻/⁻* mice (13, 16, 20). Thus, while gut-derived LPS may contribute to DC maturation after TBI, our data suggest that the effects are dependent upon immune cell–derived complement activation. Because previous work showed that radio-resistant DCs drive GvHD (1, 3, 4) and that host macrophages can be protective (15), our findings strongly implicate DCs as the primary, pathogenic source of complement following TBI.

While serum complement could contribute to the observed effects on alloreactive T cells, several of our observations support the conclusion that the mechanisms involve immune cell–derived complement: (a) DAF deficiency on donor T cells augmented T cell expansion, while (b) *C3ar1⁻/⁻C5ar1⁻/⁻* T cells expanded minimally in the same host, and (c) in vivo T cell alloresponses were modulated by BM cell–derived C3/C5 and DAF. Finally, as we showed that C5aR blockade treated murine GvHD (Figure 3), our data support the need for studies to assess the efficacy of analogous strategies in humans.

Methods

Figure 3

T cell–expressed C3aR and C5aR regulate in vivo T cell expansion and GvHD. (**A**) Weight change of BALB/c recipients of B6 BM plus 1.5×10^5 WT, $C5ar1^{-/-}$, or $C3ar1^{-/-}C5ar1^{-/-}$ T cells in 2 experiments. $n = 10–17$ per group per experiment. *$P < 0.05$ versus WT, #$P < 0.05$ versus $C3ar1^{-/-}C5ar1^{-/-}$. (**B**) H&E-stained slides of large bowel (week 5) in recipients of WT or $C3ar1^{-/-}C5ar1^{-/-}$ T cells. Scale bars: 50 μm. (**C** and **D**) Representative flow cytometry (**C**) and quantitation (**D**) of skin- and large bowel–infiltrating T cells in a competition experiment. Irradiated BALB/c mice were injected with B6 BM (CD45.1$^+$) plus 5×10^4 purified T cells from B6 WT (CD45.1$^+$CD45.2$^+$Thy1.2$^+$), $Daf1^{-/-}$ (CD45.1$^-$CD45.2$^+$Thy1.1$^+$), and $C3ar1^{-/-}C5ar1^{-/-}$ (CD45.1$^-$CD45.2$^+$Thy1.2$^+$) mice. Assays were performed 5 weeks after transplantation (see Supplemental Figure 5 for gating) to determine normalized percentage of cells infiltrating the same skin and large bowel. $n = 9$, pooled from 2 independent experiments. (**E**) Weights of BALB/c recipients of B6 BM and B6 0.5×10^6 spleen cells with or without C5aR-A (1 mg/kg/d) delivered beginning 7 or 14 days posttransplant by osmotic pump ($n = 10–15$ per group). Clinical scores for experiment 1 at day 35 were as follows: control, 5.5 ± 0.2; C5aR-A, 3.8 ± 0.2 ($P < 0.05$). (**F**) Weights of C3H.SW recipients of mH-disparate B6 BM and 5×10^6 spleen cells with or without C5aR-A (initiated on day 0). $n = 11–14$, pooled from 2 experiments. *$P < 0.05$.

Real-time PCR. Splenic CD11c$^+$ DCs or F4/80^{hi} macrophages were isolated by flow sorting. RNA isolation, cDNA synthesis, reverse transcription, and real-time RT-PCR were performed as described previously (13) using TaqMan (Applied Biosystems) primers. PCR products were normalized to the control gene (Mrp132) and expressed as fold increase compared with unstimulated cells using the $\Delta\Delta$Ct method.

Statistics. Data are presented as mean ± SEM. Recipient survival was compared using log-rank survival statistics. Immunology assay results were compared using 2-tailed Student's t test. In scatter-plot graphs, symbols denote individual mice or assays, and horizontal bars represent means. A P value less than 0.05 was considered significant.

Study approval. All mice were housed in the Mount Sinai School of Medicine Center for Comparative Medicine and Surgery in accordance with guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International. The study protocols described herein were reviewed and approved by the Institutional Animal Care and Use Committee at Mount Sinai School of Medicine, New York, New York, USA.

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