Brief report
Peyer patches are not required for acute graft-versus-host disease after myeloablative conditioning and murine allogeneic bone marrow transplantation
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Graft-versus-host disease (GVHD) is a multistep disease process following allogeneic bone marrow transplantation (BMT). It has been postulated that the induction of acute GVHD requires the presence of Peyer patches (PPs). A new tumor necrosis factor (TNF)–deficient strain has been developed that totally lacks PPs and displays the defects characteristic of TNF ablation but not lympho toxin-associated defects characterized by lack of both PPs and lymph nodes. To determine the necessity of PPs in acute lethal GVHD induction, we transplanted full major histocompatibility complex (MHC)–mismatched grafts into myelol ated TNF knockout recipients. No differences in the survival or GVHD-associated histopathologic lesions were observed between the recipients. We conclude that neither PPs nor host TNF-α is required for the development of acute lethal GVHD in mice that undergo myelolative conditioning and allogeneic BMT. (Blood. 2006; 107:410-412) © 2006 by The American Society of Hematology

Introduction

Gut-associated lymphoid tissue (GALT) is found throughout the intestine. It consists of the lamina propria of the submucosa, gut cryptopatches, intraepithelial lymphocytes (IELs), and the nodulectype tissues similar to a lymph node (LN) in function called Peyer patches (PPs). Development of PPs, cecal patches (CPs), and LNs depends on the expression of certain members of the tumor necrosis factor (TNF) ligand and receptor superfamilies. LNs, CPs, and PPs are absent in lympho toxin-deficient mice. However, the presence of PPs varies between different independently generated TNF and TNF receptor 1 (TNFR1) knockout strains, ranging from nonexistent to virtually normal.

The availability of TNF knockout strains of mice with or without PPs allowed us to address the role of both TNF and PPs in resistance to graft-versus-host disease (GVHD) caused by major histocompatibility (MHC)–mismatched hematopoietic grafts. Previous reports have shown that although TNF-α is produced by diverse types of activated cells, only donor-derived TNF is important in the induction of acute lethal GVHD as well as leukocyte movement in autoimmune disease. PPs are reported to be important for the homing and priming of T-cell effector cells in intestinal GVHD and have been reported to be essential for GVHD induction in a murine model that does not use conditioning of the host prior to adoptive transfer of the allogeneic donor cells. However, as a clinical entity, acute GVHD is encountered primarily in patients who receive a conditioning regimen in preparation for the hematopoietic-cell transplant.

We report here that the absence of TNF and PPs still allowed for acute GVHD induction in models in which myelolative conditioning was applied before bone marrow transplantation (BMT).

Study design

Mice

BALB/c were purchased from the Animal Production Area (National Cancer Institute [NCI] at Frederick, Frederick, MD). C57BL/6 TNFα−/− mice were generated by LoxP/Cre technology. Details of the targeting strategy and animal phenotype are reported elsewhere. C57BL/6 TNF−/− mice were provided by Dr M. Marino (Memorial Sloan-Kettering Cancer Center, New York, NY). Normal littermates or C57BL/6 mice purchased from the Animal Production Area were the source of C57BL/6 TNF−/− mice. Mice were 2 to 4 months of age at the initiation of experiments. Experimental groups were balanced for age and sex of mice. Animals were maintained under specific-pathogen conditions at both NCI-Frederick and the University of Nevada, Reno, facilities. Animal care was provided in accordance with the procedures outlined by the NIH. Animal studies were performed at each of the 2 animal facilities according to approved protocols and in accordance with the Animal Care and Use Committees of each institution.
Results and discussion

Absence of PPs in TNFα/Δ mice

TNFα/Δ mice completely lack PPs, recapitulating the previously reported phenotype of TNFR1 knockout. However, apart from the complete absence of PPs, TNFα/Δ mice shared characteristic phenotypic features with previously reported TNF−/− mice. In particular, both TNF−/− and TNFα/Δ mice develop a CP and all LNs, including mesenteric nodes (Figure 1A). In contrast, TNF−/− mice present with PPs, whereas the TNFα/Δ mice completely lack PPs. We therefore compared TNF−/− and TNFα/Δ mice in an experimental GVHD model in which myeloablative conditioning was applied.

The absence of PPs does not confer protection from acute lethal GVHD using models with cytoreductive conditioning and hematopoietic-cell rescue. Using normal littermates (wild-type [WT]), TNFα/Δ, and TNF−/− mice as recipients of fully allogeneic BM- and splenic-cell grafts allowed us to directly test the necessity of PPs in GVHD induction. As demonstrated, TNFα/Δ, TNF−/−, and WT recipients showed similar kinetics of mortality (Figure 1B) and weight loss (Figure 1C) from acute GVHD following allogeneic BMT. In addition, no statistical differences in GVHD mortality between TNFα/Δ and WT recipients were observed when lower doses of spleen cells were used to induce GVHD (Figure 1D).

Induction of GVHD

To induce acute GVHD associated with allogeneic BMT, recipient C57BL/6 (H2b) TNF−/−, TNFα/Δ, or TNF−/− mice received myeloablative total body irradiation (950 cGy) from a 137Cs source followed by intravenous infusion of 107 bone marrow cells (BMcs) and splenocytes from BALB/c (H2b) donor mice. Some groups did not receive splenocytes to monitor non-GVHD-associated changes. Representative photomicrographs are depicted. While arrows designate areas of inflammation. Black arrows designate sloughing of epithelium. (B) No difference in GVHD-associated histologic changes observed in the small intestine, large intestine, and liver of TNFα/Δ or WT recipients of allogeneic BM grafts in combination with 3 × 107 spleen cells (SCs) (n = 3/group). Some groups received BM only to control for non-GVHD-associated changes. Representative results of 2 independent experiments are shown for survival kinetics. No significant differences were observed between experimental groups for either dose of spleen cells.

Histology

Formalin-fixed, paraffin-embedded tissue sections were stained with hematoxylin and eosin and evaluated and graded in coded fashion by a veterinary pathologist as previously described. A semiquantitative scale for histopathologic changes ranked from 0 to 4 was used. Cumulative histopathologic scores were calculated based on the sum of individual scores for 3 to 5 parameters in each organ (villous blunting, crypt-cell hyperplasia, crypt-cell apoptosis, and epithelial-cell sloughing in the small intestine; goblet-cell depletion, inflammation, sloughing of epithelial cells into the lumen, crypt-cell apoptosis, and hyperplasia in the colon; and vacuolation, necrosis, and inflammation in the liver). Comparison of cumulative histopathologic scores were analyzed the Kruskal-Wallis and Dunn multiple comparison tests (P < .05). Images were visualized using an Olympus Vanox AHBS3 microscope with an Olympus SPPlan Apo 20×/0.70 numeric aperture objective (Olympus, Woodbury, NY). A Diagnostic Spot RT color digital camera using Spot software version 4.0.2 was used to acquire the images (Diagnostic Instruments, Sterling Heights, MI).

Figure 1. Acute GVHD induced after allogeneic BMT using extensive conditioning in mice with or without PPs. (A) TNFα/Δ and TNF−/− mice develop mesenteric LNs and cecal patches (CPs), TNF−/− mice develop PPs, but TNFα/Δ mice do not. Not all PPs formed in the TNF−/− mice are visible. Fresh mouse organs, from representative adult naive animals, were photographed using Nikon Coolpix 2500 digital camera set to macro mode. (B) TNFα/Δ, TNF−/−, or WT mice were used as recipients of allogeneic BM grafts in combination with 3 × 107 spleen cells (SCs) as a source of T cells (n = 9-11 mice/group/experiment). Some groups received BM only to control for non-GVHD-associated changes (n = 3-8 mice/group/experiment). Combined results of 2 independent experiments are shown. No significant differences were observed in mice that received BM and spleen cells. (C) Kinetics of weight loss in one of the 2 independent experiments represented in panel B. (D) TNFα/Δ or WT mice were used as recipients of allogeneic BM grafts in combination with 2.5 or 1.25 × 107 spleen cells (SCs) as a source of T cells (n = 5-8 mice/group). Combined results of 2 independent experiments are shown for surviver kinetics. No significant differences were observed between experimental groups for either dose of spleen cells.

Figure 2. No difference in intestinal GVHD in TNF−/− deficient hosts using extensive conditioning and allogeneic BMT at either early or late time points. (A) Photomicrograph of colonic tissue sections taken from mice with or without PPs. Representative photomicrographs are depicted. While arrows designate areas of inflammation. Black arrows designate sloughing of epithelium. (B) No difference in GVHD-associated histologic changes observed in the small intestine, large intestine, and liver of TNFα/Δ or WT recipients of allogeneic BM grafts in combination with 3 × 107 spleen cells (SCs) (n = 3/group). Some groups received BM only to control for non-GVHD-associated changes. Mice were assessed 6 days after transplantation. Tissues were assessed for histologic changes in 3 to 4 parameters as described in Study design and the sums of these scores are represented for each tissue.
Intestinal GVHD in mice with or without PPs following cytoreductive conditioning and allogeneic BMT

Histopathologic analysis of the liver and intestine from moribund allogeneic BM and spleen cells (Figure 2B). As summarized in Figure 2B, TNF$^{+/+}$ and WT recipients were also evaluated at day 6 after BMT for histopathologic lesions associated with GVHD. GVHD-associated changes were evident, particularly in the colon and small intestines. However, there were no significant differences in the cumulative histopathologic scores in TNF$^{+/+}$ and WT recipient mice of allogeneic BM and spleen cells (Figure 2B). Specifically, no differences in the frequency or grade of crypt hyperplasia and apoptosis were observed in the small and large intestines of TNF$^{+/+}$, TNF$^{-/-}$, and WT recipients nor were there differences in goblet-cell depletion or the presence of sloughed epithelial cells in the crypt lumen at day 6 or day 28 after BMT (Figure 2A-B). Similar frequency and grade of subacute inflammation and hepatocellular vacuolation were observed in the livers of all recipient types (Figure 2B and data not shown).

Therefore, the data demonstrate that in a BMT model using myeloablative conditioning and hematopoietic-cell rescue, neither host-derived TNF nor PPs were required for induction or progression of acute GVHD. These results are in contrast with a recent report that PPs are required for the induction of acute GVHD.12 That report used administration of anti-interleukin 7 receptor (anti-IL7R) antibodies to prevent PP formation that may have altered other tissues in the recipient that could affect GVHD progression. Importantly, the study used an adoptive lymphocyte transfer model that involved no or only myelosuppressive conditioning of the recipient and lacked hematopoietic rescue. The use of myeloablative conditioning and allogeneic BM reconstitution is more reflective of clinical BMT regimens and most likely results in the release of inflammatory cytokines that can alter the requirements for GVHD induction and pathobiology. We and others have recently found that the presence or absence of cytoreductive conditioning of the host also results in markedly contrasting effects on the role of the chemokine receptor, CCR5, in GVHD pathobiology.19,20 Wysocki and colleagues have shown that expression of proinflammatory chemokines important in the trafficking of alloreactive T cells into target tissues was dependent on the use of a myeloablative conditioning regimen.20 Therefore, these data demonstrate that the use of myeloablative conditioning has a marked impact on acute GVHD pathobiology and that PPs are not necessary for its induction or progression when such conditioning is applied.

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References


