**Brief report**

**Peyer patches are not required for acute graft-versus-host disease after myeloablati ve 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 lymphotoxin-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 myeloablated 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 myeloablati ve conditioning and allogeneic BMT. (Blood. 2006; 107:410-412)**

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**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 nodule-type 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 lymphotoxin-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 myeloablative 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 TNF−/− mice were generated by LoxP/Ce 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 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.

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crypt-cell apoptosis, and hyperplasia in the colon; and vacuolation, cell apoptosis, and epithelial-cell sloughing in the small intestine; goblet-cell parameters in each organ (villous blunting, crypt-cell hyperplasia, crypt-scores were calculated based on the sum of individual scores for 3 to 5 logic changes ranked from 0 to 4 was used. Cumulative histopathology 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).

### Results and discussion

**Absence of PPs in TNFΔ/Δ mice**

TNFΔ/Δ mice completely lack PPs, recapitulating the previously reported phenotype of TNFR1 knockout. However, apart from the complete absence of PPs, TNFΔ/Δ mice shared characteristic phenotypic features with previously reported TNF−/− mice. In particular, both TNF−/− and TNFΔ/Δ mice develop a CP and all LNs, including mesenteric nodes (Figure 1A). In contrast, TNF−/− mice present with PPs, whereas the TNFΔ/Δ mice completely lack PPs. We therefore compared TNF−/− and TNFΔ/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]), TNFΔ/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, TNFΔ/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 TNFΔ/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+/+, TNFΔ/Δ, or TNF−/− mice received myeloablative total body irradiation (950 cGy) from a 137Cs source followed by intravenous infusion of $10^7$ bone marrow cells (BMCs) and splenocytes from BALB/c (H2a) donor mice. Some groups did not receive splenocytes to monitor non-GVHD-associated morbidity.Recipient mice were given amoxicillin ad lib in their drinking water for 2 weeks beginning 7 days before transplantation. Mice were monitored and weighed weekly. All moribund mice were humanely killed. Survival data were plotted by the Kaplan-Meier method and analyzed by the log-rank test.

### 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 histopathology 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).
Intestinal GVHD in mice with or without PPs following cytoreductive conditioning and allogeneic BMT

Histopathologic analysis of the liver and intestine from moribund animals revealed no differences in organ-specific GVHD-associated pathologic lesions in the gastrointestinal tract in WT, TNF^+/−, or TNF^−/− moribund animals 25 days after BMT (Figure 2A). 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 hematopoietic 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. 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

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