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Expression of Chemokines in GVHD Target Organs Is Influenced by Conditioning and Genetic Factors and Amplified by GVHR

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ABSTRACT

Graft-versus-host disease (GVHD) is the most significant clinical problem that arises after allogeneic hematopoietic cell transplantation. Because chemokines induced by proinflammatory conditioning treatment may promote T-cell migration into GVHD target tissues, we addressed the influence of conditioning on chemokine expression in GVHD target organs. Our results showed that (1) conditioning leads to rapid and transient chemokine upregulation in GVHD target tissues before the time of GVHD-associated T-cell infiltration; (2) conditioning intensity and mouse strain influence chemokine expression in GVHD target organs; and (3) compared with syngeneic bone marrow transplantation, allogeneic bone marrow transplantation led to marked amplification of chemokine expression in GVHD target organs after myeloablative conditioning. This is also reflected by chemokine protein expression that is measured in the serum and colon. Intestines showed the greatest sensitivity to conditioning intensity, and chemokines affecting T-helper type 1 cells (eg, interferon γ-inducible protein 10 [CXCL10]) were most strongly expressed there after conditioning and during GVHD. However, severity of GVHD was not significantly different between recipients of CXCR3$^{+/+}$ or CXCR3$^{-/-}$ splenocytes, indicating that this chemokine pathway does not play a critical role. In summary, our data show that conditioning and recipient strain influence chemokine expression in GVHD target organs and that GVH alloreactivity markedly amplifies this expression, thus contributing to the inflammatory cascade associated with tissue GVHD.

KEY WORDS

Bone marrow transplantation • Graft-versus-host disease • Chemokine • CXCR3 • Knockout mouse

INTRODUCTION

Allogeneic bone marrow transplantation (BMT) is the only known curative treatment option for a number of malignant diseases, and tumor responses depend to a significant extent on an immunologically mediated graft-versus-leukemia (GVL) response [1,2]. However, in the clinical setting, GVL effects have frequently been linked to the development of GVHD [3]. This GVL reaction is primarily alloantigen driven but can occur in the absence of GVHD, as demonstrated in preclinical models [4,5] and in patients [3]. However, GVL effects are counterbalanced by GVHD, often leading to failure of improved relapse-free survival to translate into improved overall sur-

vival. Therefore, strategies are needed to inhibit GVHD without mitigating GVL effects.

The pathogenesis of GVHD is complex and influenced in part by the major or minor histocompatibility antigenic disparities between donor and recipient and the presence of host-derived antigen-presenting cells [6]. Other factors contributing to the development of GVHD include sequelae of conditioning therapy-induced toxicity. A large body of data has shown clearly that conditioning-induced tissue damage and cytokine secretion play a pivotal role in the development of GVHD [7-13]. This proinflammatory milieu is thought to critically influence the development of GVHD. Thus, administration of large numbers of fully major histocompatibility complex (MHC)-mismatched T cells on day 0 leads to the development of uniformly lethal GVHD in mice. However, administration of donor T cells after a delay of 5-8 weeks after BMT, when the inflammatory milieu created by the conditioning therapy has presumably subsided, does not induce GVHD [5,7]. When these nontolerant donor T cells are given to established mixed hematopoietic chimeras, conversion to full-donor chimerism ensues, demonstrating that donor T cells can mediate a GVH response (GVHR) that is confined to the lymphohematopoietic system (LGVHR) [7]. When donor lymphocyte infusions (DLIs) are given to established mixed chimeras, the LGVHR leads to powerful GVL effects without GVHD [5,14]. We have hypothesized that the absence of GVHD in this setting is related to the disappearance of the proinflammatory milieu in the GVHD target tissues over time after conditioning therapy [5,14].

Chemokines are predominantly small molecules (8-14 kd) that bind to a family of heterotrimeric G-protein–coupled receptors with a 7-transmembrane–spanning serpentine structure and play an important role in leukocyte trafficking [15]. Chemokines are involved in a variety of inflammatory and infectious conditions, including GVHD [16-21]. We hypothesized that conditioning-induced upregulation of chemokines and adhesion molecules in the epithelial GVHD target tissues plays a major role in converting LGVHR into GVHD. We sought to delineate the effect of myeloablative versus nonmyeloablative conditioning and other recipient factors on the expression of chemokines in response to conditioning and allogeneic BMT. Because T-helper types 1 and 2 (Th1 and Th2, respectively) have different roles in inducing GVHD in different target tissues and this specificity is somewhat strain dependent [22], we evaluated Th1- and Th2-attracting chemokines in 2 different strain combinations. Our data demonstrate that organ-specific chemokine expression patterns occur after conditioning, and this expression depends on conditioning intensity and genetic background.

METHODS

Animals

Female C57BL/6 (B6: $H2^b$) and BALB/c ($H2^d$) recipient mice were purchased from Frederick Cancer Research Facility (National Cancer Institute, Frederick, MD) and used after 8 weeks of age. $CXCR3^{-/-}$ mice were generated as described previously [23] and backcrossed 10 times to the B6 strain. All mice were housed in autoclaved micro-isolator environments, and all manipulations were performed in a laminar flow hood.

Nonmyeloablative Conditioning

Nonmyeloablative conditioning was performed as previously described [24]. Briefly, mice received depleting doses of anti-CD8 monoclonal antibody (mAb) 2.43 and anti-CD4 mAb GK1.5 intraperitoneally on day −5 and 200 mg/kg cyclophosphamide (Cytoxan, CTX, Pharmacia, Peapack, NJ) intraperitoneally on day −1. Purified mAbs were prepared at the National Cell Culture Center (Minneapolis, Minn). On day 0, the mice received 7 Gy thymic irradiation from a cobalt 60 source. Because our previous studies have shown that these high doses of CD4- and CD8-depleting antibodies deplete donor T cells that are given on the day of BMT, we could not compare the effects of allogeneic GVH-inducing inocula (versus syngeneic BMT) in recipients of this regimen, and we confined studies in the nonmyeloablative model to an analysis of the effect of conditioning alone on chemokine expression.

Lethal Conditioning and Induction of GVHD

Syngeneic or allogeneic control mice received lethal doses (8-9.75 Gy, depending on strain; dose rate, .8 Gy/min cesium 137 source; JL Shepherd Mark I Irradiator, San Fernando, CA) of total body irradiation (TBI) and were reconstituted within 4-8 hours with an intravenous inoculum (5×10^6 cells) of syngeneic (syngeneic control) or allogeneic (allogeneic controls) bone marrow cells (BMCs). For induction of GVHD, animals received donor splenocytes in addition to allogeneic BMCs. In BALB/c recipients, GVHD was induced by using 8-Gy TBI on day 0 followed by administration of 1×10^7 B6 BMCs and 13×10^6 B6 spleen cells. GVHD was induced in B6 recipients by using 9.75-Gy TBI followed by reconstitution with 1×10^7 BALB/c BMCs and 13×10^6 BALB/c spleen cells.

Assessment of GVHD

Animals from different groups were randomized in cages. Body weights were measured on the day of transplantation and then twice each week during the first month and once a week after that. Animals were