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Both apoptosis and complement membrane attack complex deposition are major features of murine acute graft-vs.-host disease

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Abstract

The parent-into-F1 mouse model (P→F1) of acute graft-vs.-host disease (GVHD) is a useful model of human acute GVHD because it allows the study of the T cell contribution to pathology without the complicating effects of conditioning regimens. To determine the similarity of this model to human GVHD, we assessed injury in organs typically involved in human acute GVHD (skin, liver) and less typically involved organs (spleen, kidney, lung). Mice were assessed histologically at early (2 weeks), intermediate (3 months) and late (6 month) time points. Based on the emerging roles of Fas ligand killing and complement deposition in allograft rejection, we correlated the amount of tissue specific TUNEL positive apoptosis and deposition of complement (C5b-9) with histopathologic changes. Our results indicate a striking similarity histologically between acute GVHD occurring in this model and in humans following bone marrow transplant. Moreover, C5b-9 deposition and apoptotic cell accumulation were found to parallel tissue injury in major organs of acute GVHD mice, although not all organs exhibited the same kinetic pattern. These results indicate a role for both adaptive immunity and innate immunity in this model of GVHD and support its use in modeling human acute GVHD in the nonmyeloablative setting.

Keywords: T lymphocytes; GVHD; Apoptosis; Fas; In vivo animal models

Introduction

Allogeneic bone marrow transplantation (BMT) is an important therapeutic option for a variety of hematological diseases. A major limitation to its use is the development of acute graft-vs.-host disease (GVHD) (see review, Reddy and Ferrara, 2003). Target organ damage in acute GVHD is mediated largely by donor T cells; however, conditioning regimens such as total body irradiation (TBI) or chemotherapy can also contribute to GVHD pathology. Both donor T cell activation and conditioning regimens promote secretion of the proinflammatory cytokines TNF-a and IL-1 which upregulate adhesion molecules, costimulatory molecules, and MHC molecules which in turn enhance donor T cell activation and promote GVHD (Chang and Lee, 1986; Pober et al., 1996; Reddy and Ferrara, 2003; Xun et al., 1994). In addition, TBI can directly damage the GI tract (Paris et al., 2001) allowing LPS entry into the systemic circulation (Teshima and Ferrara, 2002) further amplifying GVHD.

Conditioning regimens are required to prevent rejection of donor marrow and do so by rendering the host immunodeficient. Intensive conditioning regimens however

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are associated with increased GVHD risk (Clift et al., 1990; Hill and Ferrara, 2000). As a result, newer strategies such as nonmyeloablative conditioning have been devised in an effort to reduce GVHD toxicity. Typically, nonmyeloablative conditioning regimens use cytoreductive treatment that is either greatly reduced (Sykes et al., 1999) or eliminated altogether and replaced with agents that block host costimulatory molecules (Wekerle et al., 2000). Nevertheless, many difficulties remain in overcoming acute GVHD toxicity mediated by T cells in the absence of TBI.

A useful model of acute GVHD in the absence of conditioning regimens is the parent-into-F1 model (P→F1) of murine GVHD. In this model, acute GVHD is induced by the injection of fully allogeneic homozygous parental CD4+ and CD8+ T cells into unconditioned F1 mice (Rolink et al., 1983; Via and Shearer, 1988). Because the recipient F1 is tolerant to parental strain MHC, the F1 host is unable to reject the donor T cells and conditioning is not required. Thus, the model is highly analogous to human nonmyeloablative, noncytoreductive conditioning regimens that prevent recipient rejection of donor T cells e.g., costimulatory blockade. Following cell transfer, a strong donor anti-host cytotoxic T lymphocyte (CTL) response develops in the spleens of recipient F1 mice such that by 2 weeks after parental cell transfer, host splenic lymphocytes are largely eliminated by both perforin and FasL mechanisms (Kataoka et al., 2001; Kubota et al., 1983; Shustov et al., 1998; Via et al., 1987). The high mortality in this model of acute GVHD initially precluded long term (>1 month) studies. However, Hakim et al. (1991) demonstrated that mortality was associated with donor inocula constituted with lymph node derived donor cells. By contrast, donor inocula comprised of splenocytes resulted in the inadvertent transfer of stem cells permitting reconstitution of the host immune system, an eventual return of immunocompetence and markedly enhanced survival rates thus making long term studies of acute GVHD feasible.

It has been recently reported that early on (<1 month), liver histopathology in P→F1 acute GVHD mice strongly resembles human acute GVHD liver disease (Murai et al., 1999) supporting the suitability of this model for mimicking human disease. The purpose of our study was to determine whether longer term (<6 months) disease in this model results in organ pathology similar to human acute GVHD. We focused not only on organs typically involved in human acute GVHD (liver, skin, lung) but also on organs not typically involved directly (spleen and kidney). Because of the prominent role of Fas ligand (FasL) mediated killing in GVHD pathology (Baker et al., 1996; Bobe et al., 1997; Braun et al., 1996; Shustov et al., 1998), we sought to correlate the amount of tissue specific apoptosis with histopathologic changes. Moreover, we also quantitated deposition of complement C5b-9, the membrane attack complex (MAC) based on recent reports indicating a role for complement in acute and chronic rejection of solid organ allografts (Nakashima et al., 2002; Rahimi et al., 2004). Our results indicate a striking similarity between acute GVHD occurring in this murine model and in humans following BMT and a role for both adaptive immunity and innate immunity in this model of GVHD. Specifically, C5b-9 deposition and apoptotic cell accumulation were found to parallel tissue injury in major organs of both early and long term acute GVHD mice, although not all organs exhibited the same kinetic pattern.

Material and methods

Mice

C57BL/6J (B6) and B6D2F1 (BDF1) male mice 6–8 weeks old were purchased from Jackson Laboratory (Bar Harbor, ME).

Induction of GVHD

Single-cell suspensions of splenocytes were prepared from B6 donor mice in PBS, filtered through sterile nylon mesh, diluted to a concentration of 10^8 viable (trypan-blue excluding) cells/ml. Acute GVHD was induced by the injection of 50×10^6 B6 cells into the tail vein of F1 recipients. Controls consisted of uninjected age and sex matched BFD1 mice. Both controls and acute GVHD mice were assessed at 2 weeks, 3 months and 6 months (n = 3/group/time point).

Antibodies

Rabbit IgG anti-mouse/human C5b-9 neoantigens were purchased from Calbiochem (San Diego, CA). HRP-conjugated goat IgG anti-rabbit Ab was purchased from Jackson Immune Research Lab., Inc. (West Grove, PA).

Histopathology

Skin, liver, lung, kidney and spleen were harvested from euthanized mice. Half of each tissue fragment was fixed in 10% buffered formalin and the other half washed in cold phosphate buffered saline (PBS) 0.15 M, pH 7.4, and stored in cryotubes in liquid nitrogen until cryostat sectioning. The formalin-fixed tissue fragments were embedded in paraffin, cut into 4 μm thick sections and stained with hematoxylin and eosin. On selected slides, additional staining with Masson's trichrome, van Gieson's, and PAS was performed. All slides were examined under light microscopy and the photomicrographs obtained using a Zeiss Axioscop MC80 (Carl Zeiss, Goettingen, Germany). A qualitative/global assessment of glomerular cellularity was performed in a blinded fashion by a Pathologist (JCP).