Therapeutic Potential for Blockade of the CD40 Ligand, gp39

JANET E. BUHLMANN and RANDOLPH J. NOELLE

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INTRODUCTION

The ligand for CD40 (gp39, TBAM, CD40L) is an integral membrane protein that is transiently expressed on the surface of activated CD4+ helper T cells. Initial studies on the function of this molecule showed that its interaction with CD40 (prominently expressed on B cells) played an essential role in the development of humoral immune responses. Evidence in support of this conclusion came from studies using a blocking antibody to gp39 which prevented primary and secondary humoral immune responses to T-dependent antigens (1, 2). Later studies showed that this ligand-receptor pair was also involved in the development of germinal centers and memory B cells (3, 4). Additional genetic evidence for the importance of gp39 and CD40 was provided when a genetic defect in gp39 was found to be the cause of the human immunodeficiency, hyper-IgM syndrome (HIM) (5-8). In this disease, the gp39 gene contains mutations which prevent the expression of the protein or the binding of gp39 to its ligand CD40. These afflicted individuals have significant to complete reductions in TD humoral immune responses. Genetic models in the mouse which have disrupted either the CD40 (9, 10) gene or the gp39 (11, 12) gene have supported the importance of this ligand–receptor pair in humoral immunity.

Although the distribution of CD40 was originally thought to be restricted to B cells, dendritic cells, and basal epithelial cells (13), recent studies have shown that CD40 is functionally expressed on a variety of cell types, including macrophages (14), Langerhan cells (15), endothelial cells (16, 17), and thymic epithelial cells (18). Since CD40 is expressed on such a wide variety of cell types involved in antigen presentation and inflammation, it is possible that gp39 effector functions may impinge on other functions of the immune response than those restricted to the humoral arm. For example, it has been shown that expression of the costimulatory molecules, B7.1 and B7.2, on dendritic cells and B cells is CD40 dependent (19, 20), suggesting that the ability of these cells to provide co-stimulatory function may be dependent on CD40 triggering. In fact, it appears that B cells are not capable of maturing to functional APC without a CD40 signal in vivo (21). Therefore, T cell priming may be indirectly influenced by interfering with gp39 function. Antigen presentation is not the only function that may be regulated by CD40, as it has been shown that engagement of CD40 by gp39 on macrophages can elicit nitric oxide (NO) generation (22) and IL12 production (23), suggesting that the pathology of some inflammatory responses may be driven by CD40-mediated activation of macrophages. Furthermore, the fact that the expression of adhesion molecules on endothelial cells is also regulated by CD40 signaling (16, 17) indicates that gp39–CD40 interactions may be involved with the extravasation and accumulation of activated T cells at the site of inflammation.

Given this broad range of effector functions for gp39, its role in antibody and T cell-mediated autoimmune disease, graft-vs-host disease (GVHD), and graft rejection has been investigated.
GRAFT-VERSUS-HOST DISEASE

GVHD occurs when donor T cells recognize minor and/or major histocompatibility antigens that are expressed by the host that are different from those expressed on the donor lymphocytes (as reviewed in Ref. 24). Both acute and chronic forms of this disease are observed in mouse and in man and are a major complication in allogeneic bone marrow transplantation. The main mediators of aGVHD are cytotoxic T cells (CTL), although other cells such as NK cells also have an important role in this form of the disease. In addition, it is believed that cytokine production plays an important role in the induction and activation of cells that cause aGVHD, along with playing a role in organ damage (as reviewed in Ref. 25). In contrast to aGVHD, cGVHD is caused mainly by the polyclonal activation of host B cells and the production of self-reactive antibodies, which lead to destruction of tissues expressing reactive epitopes. T cells are very important to the development of either form of GVHD and it has been found that depletion of T cells before transplantation greatly decreases the risk of GVHD. One drawback to this solution is that, in the case of bone marrow transplants, depletion of T cells leads to decreased levels of successful marrow engraftment. Current treatments for GVHD focus on trying to silence the reactive cells with immunosuppressive drugs or monoclonal antibodies directed against cytokines or cytokine receptors.

Recent work published by Durie and co-workers showed that a brief prophylactic treatment with anti-gp39 blocked the onset of both aGVHD and cGVHD (26). At the time of GVHD induction, mice were treated with either anti-gp39 or control hamster Ig and development of disease was followed. In the aGVHD model, the high-titered anti-allogeneic CTL activity seen in control animals was not detectable in anti-gp39-treated animals. In the cGVHD model, anti-gp39 treatment prevented the generation of splenomegaly, spontaneous antibody production in vitro, and anti-DNA antibodies, all hallmarks of cGVHD. Even though the treatment with anti-gp39 was brief, the administration of mab produced long-lived protection against the development of this disease. Supportive evidence for a central role of gp39 in GVHD comes from more recent studies that have shown that donor T cells derived from gp39 knockout mice were unable to cause aGVHD (unpublished data). Although the mechanism of how anti-gp39 prevents the development of GVHD is not completely resolved, we propose that in the absence of gp39 function, the host is rendered incapable of upregulating essential costimulatory molecules (e.g., B7.1 and B7.2) that are necessary to trigger alloreactive, donor T cells to initiate GVHD.

It is possible, if not likely, that anti-gp39 interferes with GVHD development at multiple levels of cellular interaction. As stated earlier, cytokine production plays an important role in organ damage and in activating cells that cause destruction associated with GVHD (25). The disruption of gp39–CD40 interactions may alter the cytokines that are produced during aGVHD and modify the course of disease. One scenario in which this may occur is via the blockade of CD40-dependent IL12 production by macrophages. One could speculate that blocking IL12 production would limit Th1 differentiation (27–29) and skew the differentiation of T cells to a Th2 phenotype. Fortunately, even in the face of primed, alloreactive Th2 activity, anti-gp39 would prove effective in blocking their capacity to polyclonally activate B cells to produce Ig. Therefore, anti-gp39 may skew inflammatory responses toward humoral immune responses, then impose a blockade in antibody production. Even if some inflammatory, alloreactive T cells emerge in the presence of anti-gp39, one may predict that their effector functions may be impeded by the mab. For example, some of the pathology and the immunosuppression that develops as a result of aGVHD has been shown to be associated with the production of nitric oxide by macrophages (30). Since gp39–CD40 interactions have been shown to play a role in the generation of nitric oxide, blockade of gp39 function on primed, inflammatory T cells may block development or progression of disease. Because anti-gp39 blocks both the afferent and efferent arms of the immune response, blockade of this ligand–receptor pair should prove to be an effective target for treatment of GVHD in humans. Since massive T cell depletion is not required or observed with anti-gp39 therapy, anti-gp39 should have minimal impact on the success of marrow engraftment in allogeneic bone marrow transplantation.

AMPLIFICATION OF DONOR-SPECIFIC TRANSFUSION TOLERANCE (DST) BY ANTI-gp39 TO INDUCE LONG-TERM TRANSPLANTATION TOLERANCE

The use of donor cell transfusions to potentiate tolerance to subsequent allografts has been noted by retrospective clinical studies and basic studies in murine models of transplantation tolerance (31, 32). Transfused B cells have been reported to be effective at inducing tolerance to a limited scope of antigens (33, 34) but have not been shown to induce tolerance to allogeneic MHC antigens. We know from in vitro studies that resting B cells are extremely poor APC because of the low levels