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Cutting Edge: Paradoxical Roles of BST2/Tetherin in Promoting Type I IFN Response and Viral Infection

Melissa Swiecki,* Yaming Wang,* Susan Gilfillan,* Deborah J. Lenschow,†‡ and Marco Colonna*

Bone marrow stromal Ag 2 (BST2) is a transmembrane protein that prevents virus release from infected cells. It was also reported that BST2 inhibits type I IFN production by plasmacytoid dendritic cells. To determine BST2 impact on antiviral responses in vivo, we generated BST2−/− mice. Following infection with a murine retrovirus, BST2−/− mice had slightly elevated viral loads; however, infection with other enveloped viruses revealed unexpected roles of BST2. BST2−/− mice showed reduced type I IFN production by plasmacytoid dendritic cells. Moreover, BST2−/− mice had lower viral titers in lungs following intranasal infection with vesicular stomatitis virus expressing OVA and influenza B and increased numbers of virus-specific CD8 T cells in the lungs, suggesting that BST2 may facilitate entry and/or replication of enveloped viruses and modulate priming of CD8 T cells. These findings suggest complex roles of BST2 beyond retroviral control in vivo, possibly reflecting the involvement of BST2 in endocytosis and intracellular trafficking of viruses, viral nucleic acids, and Ags. The Journal of Immunology, 2012, 188: 2488–2492.

Bone marrow stromal Ag 2 (BST2), also known as tetherin, CD317, and HM1.24, is a type II membrane protein that was first identified on plasma cells and stromal cells (1, 2). More recently, it has been shown that BST2 is expressed by plasmacytoid dendritic cells (pDC) and is induced on most cell types following exposure to type I IFN (IFN-I) and type II IFN (3). BST2 is a rather unique protein with atypical membrane topology (4). It consists of an N-terminal transmembrane domain, extracellular coiled–coiled domain, and a C-terminal GPI anchor. BST2 is found on the cell surface, with the GPI anchor inserted in cholesterol-rich lipid microdomains, and also in the trans-Golgi network following internalization via clathrin-dependent endocytosis (5, 6). These localizations and the biased expression of BST2 in plasma cells, which secrete IgGs, and pDC, which produce IFN-I, have suggested that it might be involved in the trafficking of secreted proteins.

Cross-linking BST2 on mouse pDC with a specific Ab reduces the IFN-α response to CpGA, suggesting that it may function as a modulator of IFN-I production (3). Furthermore, it was recently reported that human BST2 interacts with a receptor expressed on pDC called Ig-like transcript 7. Ig-like transcript 7 triggers an FcRγ-dependent signaling pathway that leads to the reduction of IFN-I and proinflammatory cytokine secretion by pDC in response to TLR9 and TLR7 ligands (7). In addition to the role of BST2 in regulating the secretion of IFN-I, there is a growing literature illustrating its function in limiting viral spread and infection. BST2 has been shown to be an essential molecule that inhibits the egress, release, and spread of retroviruses such as HIV and SIV as well as other enveloped viruses (8–15); specifically, BST2 tethers budding viruses at the cell surface by having the transmembrane domain inserted in the cell membrane and the GPI anchor inserted into the virions during the process of budding. However, the role of BST2 in vivo has remained unclear.

In this study, we investigated the impact of BST2 on viral infections in vivo using newly generated BST2−/− mice. Following infection with Moloney murine leukemia virus (MMLV), we observed an expected modest increase in retroviral infection in BST2−/− mice. However, infection with other enveloped viruses revealed unexpected roles of BST2 in viral infections. BST2−/− pDC and mice secreted moderately less IFN-I than their wild-type (WT) counterparts in response to enveloped viruses in vitro and in vivo. Furthermore, BST2−/− mice had lower viral titers in the lung following intranasal infection with vesicular stomatitis virus expressing OVA (VSV-OVA) or influenza B (FluB) at early time points. Finally, we observed that BST2−/− mice had increased numbers of virus-specific CD8 T cells in the lungs after VSV-OVA infection. These findings suggest complex roles of BST2 in viral infections in vivo, which involve IFN-I production, replication of enveloped viruses, and induction of virus-specific CD8 T cells.
Materials and Methods

Mice and infections

All animal studies were approved by the Washington University Animal Studies Committee. BST2−/− mice were generated with 129/SvJ ES cells and maintained on a 129/SvJ background. WT and BST2−/− 129/SvJ mice were bred in-house and used between 6 and 12 wk of age. Salivary gland stocks of murine CMV (MCMV) Smith strain (2 × 105 PFU) were administered i.p. Mice were infected with different doses of VSV-OVA i.v. or intranasally (i.n.), specified in text and/or figure legends. FluB/Yamagata strain/88 (2 × 105 PFU) was administered i.n. HSV-1 KOS strain was injected i.v. at 1 × 105 PFU/mouse. Supernatant from MMLV-infected cells was injected i.p.

Viral titers

VSV-OVA, MCMV, and FluB titers were determined as previously described (16, 17). MMLV titers were determined by quantitative PCR (qPCR).

Cell culture and preparation

Primary cells were grown in complete RPMI: RPMI 1640 medium with 10% FCS, 1% glutamax, 1% nonessential amino acids, 1% sodium pyruvate and 1% kanamycin sulfate (Invitrogen Life Technologies). Single-cell suspensions were prepared as described previously (16, 18). Whole blood was collected by cardiac puncture, and bone marrow cells were harvested from tibias and femurs. For in vitro stimulations and infections, primary cells were cultured with viruses or TLR ligands, and supernatants were analyzed by ELISA.

Abs, flow cytometry, and cytokine analysis

The following reagents were from BD Biosciences, ebioscience, or Bio-Legend: fluorochrome-labeled anti-Siglec-H (551), anti-B220 (RA3-6B2), anti-CD11c (HL3), anti-CD8α (53-6.7), anti-CD4 (GK1.5), anti-CD19 (1D3), and streptavidin. Anti-BST2 (clone 927, rat IgG2b) Ab (3) was purified from ascites and biotinylated using the Fluororeporter Minibiotin kit (Invitrogen). Virus-specific CD8+ T cells in mice infected with VSV-OVA were detected with H-2Kb OVA257–264 peptide tetramers (Beckman Coulter). Dead cells were excluded with propidium iodide. All flow cytometry was conducted on a dual laser FACSCalibur flow cytometer (BD Biosciences) and analyzed with FlowJo software (Tree Star). B220+Siglec-H+ pDC were sorted from the bone marrow of WT and BST2−/− mice infected with VSV-OVA or FluB/Yamagata strain/88 (2 × 105 PFU) were analyzed with FlowJo software (Tree Star). B220+Siglec-H+ pDC were sorted from the bone marrow of WT and BST2−/− mice and stimulated with viruses or CpGA (Fig. 1B). We next evaluated IFN-I responses by pDC in vivo. Mice were infected i.p. or i.v. with MCMV (Fig. 1C) or VSV-OVA (Fig. 1D), respectively and serum IFN-α was measured. We have previously reported that MCMV and VSV-OVA infections induce IFN-α production by pDC in vivo at early time points p.i. (16). Infection with either virus resulted in modest reductions of serum IFN-α in BST2−/− mice compared with WT mice. Taken together, these data suggest that BST2−/− mice have reduced IFN-I responses by pDC and perhaps other cell types to viruses, which might promote viral replication in vivo.

Statistics analysis

The statistical significance of differences in mean values was analyzed with unpaired, two-tailed Student t test. A value of p < 0.05 was considered statistically significant.

Results and Discussion

Generation and characterization of BST2−/− mice

To investigate the impact of BST2 on antiviral responses in vivo, we generated mice lacking BST2 on a pure 129/SvJ background. The targeting construct contained a neomycin cassette replacing exons 1–4. BST2 expression or lack thereof was confirmed in WT and BST2−/− mice by DNA blot, PCR, and Ab staining before and after systemic infection with HSV-1 (Supplemental Fig. 1A, 1B; data not shown). BST2−/− mice are viable and resemble their WT counterparts in terms of size and weight. Furthermore, BST2−/− mice have no apparent defects in lymphocyte development in spleen, bone marrow, or thymus (Supplemental Fig. 1C; data not shown). These data indicate that BST2−/− mice can be used to study the functions of this molecule in vivo.

BST2 deficiency reduces IFN-I secretion by pDC

It has been reported that BST2 inhibits pDC responses to TLR9/7 ligands in vitro (3, 7). Therefore, we hypothesized that BST2−/− pDC would have more robust IFN-I responses to TLR ligands than pDC from WT mice. To test whether BST2 expression altered IFN-I secretion by pDC, we sorted B220+Siglec-H+ pDC from the bone marrow of WT and BST2−/− mice and stimulated them with viruses or synthetic TLR9/7 ligands then measured IFN-α in supernatants. To our surprise, BST2−/− pDC secreted less IFN-1 than WT pDC under most conditions (Fig. 1A). Similar findings were obtained when total splenocytes were incubated with viruses or CpGA (Fig. 1B). We next evaluated IFN-I responses by pDC in vivo. Mice were infected i.p. or i.v. with MCMV (Fig. 1C) or VSV-OVA (Fig. 1D), respectively and serum IFN-α was measured. We have previously reported that MCMV and VSV-OVA infections induce IFN-α production by pDC in vivo at early time points p.i. (16). Infection with either virus resulted in modest reductions of serum IFN-α in BST2−/− mice compared with WT mice. Taken together, these data suggest that BST2−/− mice have reduced IFN-I responses by pDC and perhaps other cell types to viruses, which might promote viral replication in vivo.
burden in various organs by plaque assay. Viral titers in WT and BST2−/− mice infected with MCMV i.p. were similar in spleens and salivary glands on days 3 and 14 p.i., respectively (Fig. 2B). It has been reported that BST2 inhibits the release of VSV from infected cells (19, 20), so we next infected mice i.v. with VSV-OVA. Infection of WT and BST2−/− mice with different doses of VSV-OVA revealed that both groups of mice were able to control viral replication in spleens and liver to a similar degree (Fig. 2C; data not shown). These data suggest that during certain systemic infections, BST2 deficiency does not strongly impact viral replication or spread.

We next asked whether a local infection would be controlled in the absence of BST2. To this end, we infected mice i.n. with VSV-OVA and measured viral titers in the lungs at different time points p.i. Interestingly, BST2−/− mice had reduced viral titers at 24 h p.i. compared with WT mice; however, on day 3 p.i., both groups of mice had similar viral loads, and on day 5 p.i., viral burden in WT and BST2−/− mice was below detection limit as determined by plaque assay (Fig. 3A; data not shown). Consistent with viral titers, evaluation of IFN-I in the lung tissue of VSV-infected mice revealed that BST2−/− mice also had significantly lower levels of IFN-α compared with their WT counterparts (Fig. 3B).

The difference in viral titers and IFN-I levels we observed in the lungs after VSV-OVA infection compelled us to look at another respiratory virus infection with FluB. It has been shown that BST2 does not restrict influenza infection but does prevent the release of influenza virus-like particles into supernatants from infected cells (21). On day 3 p.i., the lungs of FluB-infected BST2−/− mice contained less virus than WT...
mice; however, on day 6 p.i., both groups had comparable levels of virus (Fig. 3C; data not shown). Taken together, these data suggest that BST2 may promote the entry or early replication of some viruses in vivo during local infections, at least in the lung.

**BST2<sup>−/−</sup> mice have increased virus-specific CD8 T cells in the lung after i.n. VSV-OVA infection**

We next examined virus-specific CD8 T cell responses in WT and BST2<sup>−/−</sup> mice. Frequencies of MHC class I tetramer<sup>+</sup> CD8 T cells were determined at different time points p.i. by flow cytometry. Analysis of mice infected i.n. with VSV-OVA indicated that BST2<sup>−/−</sup> mice had higher numbers of OVA-specific CD8 T cells in the lungs on day 15 p.i. but not at day 7 p.i. (Fig. 4A). Thus, even though BST2<sup>−/−</sup> mice produced less IFN-I and had lower viral burden than WT mice early on and cleared the virus around the same time as WT mice, they appeared to have an increase in the lung, BST2<sup>−/−</sup> mice had increased numbers of virus-specific CD8 T cells at later time points. In contrast, after i.v. infection with VSV-OVA, the spleens of WT and BST2<sup>−/−</sup> mice contained similar numbers of CD8 T cells and OVA-specific CD8 T cells on days 7 and 14 p.i. (Fig. 4B; data not shown). When higher doses of VSV-OVA were administered i.v., we also observed similar frequencies and numbers of virus-specific CD8 T cells on days 7 and 14 p.i. (Fig. 4B; data not shown). When higher doses of VSV-OVA were administered i.v., we also observed similar frequencies and numbers of virus-specific CD8 T cells in spleens of WT and BST2<sup>−/−</sup> mice (data not shown).

In summary, we have generated BST2<sup>−/−</sup> mice to study the impact of BST2 on antiviral responses in vivo. Our data indicate that BST2 has a modest impact in limiting MMLV infection in mice. A recent study corroborates our findings, showing that BST2 moderately inhibits MMLV replication in vivo, unless BST2 expression is upregulated by IFN-I (22). Taken together, these findings suggest that BST2 inhibits MMLV egress and that MMLV, in contrast to HIV and SIV (8, 9, 15, 23), is inefficient at antagonizing BST2, such that MMLV spreading is slightly higher in BST2<sup>−/−</sup> mice than WT mice. The impact of BST2 deficiency on MLV spreading is more evident when mice are infected with MLV strains that induce stronger IFN-I responses and subsequent expression of BST2 (22).

The most surprising finding in our study is that the influence of BST2 on antiviral responses is more complex than previously anticipated. Although earlier studies have shown that BST2 inhibits pDC secretion of IFN-I and proinflammatory cytokines, we found that pDC and splenocytes from BST2<sup>−/−</sup> mice have reduced IFN-I secretion in response to a purified TLR9 ligand and viruses known to engage TLR9 in pDC. In addition, BST2<sup>−/−</sup> mice produced less IFN-I than their WT counterparts in vivo after systemic viral infections that have been shown to induce IFN-I secretion by pDC through TLR9/7 (16). These results suggest that BST2 promotes pDC secretion of IFN-I, whereas the modulatory effects of BST2 previously reported may be the result of an artificial cross-linking by Abs.

Because it has been shown that BST2 restricts viral release and we observed lower levels of IFN-I in BST2<sup>−/−</sup> mice, we expected to see increased viral burden in mice lacking BST2. However, our data indicate that in the lung, replication of VSV-OVA and FluB was initially reduced in BST2<sup>−/−</sup> mice compared with WT mice. These data imply that BST2<sup>−/−</sup> mice might have defects in viral entry. Indeed, a recent study has shown that BST2 enhances the entry of human CMV by a reverse-tethering mechanism (24). Thus, certain viruses such as human CMV and perhaps VSV and FluB may exploit BST2 to gain entry into BST2-expressing cells. The fact that we did not observe increased viral burden or spreading during systemic infections in BST2<sup>−/−</sup> mice suggests that 1) the viruses used in these studies may have mechanisms to antagonize BST2 functions and 2) that BST2 may play a predominant role in the early phase of infection when viral replication is confined to a mucosal barrier and becomes less important when the virus has spread beyond the initial site of infection.

Finally, we observed that BST2<sup>−/−</sup> mice had increased numbers of virus-specific CD8 T cells in the lungs following i.n. infection with VSV-OVA, even though the virus was cleared around the same time as WT mice (day 5). These findings suggest that BST2 may inhibit presentation of viral Ags and subsequent CD8 T cell responses. By promoting viral entry in APCs, BST2 may accelerate their death, reducing their ability to migrate into the draining lymph nodes and present viral Ags to CD8 T cells. BST2 may also preferentially direct newly synthesized viral proteins toward intracellular viral factories rather than endolysosomal compartments where viral peptides are processed and loaded onto MHC. Thus, the lack of BST2 may result in sustained antigenic stimulation, even in the absence of obvious viral replication.

BST2 is localized in clathrin-rich lipid rafts as well as in the trans-Golgi network following internalization (4–6). VSV and influenza can gain entry into cells via clathrin-mediated endocytosis (25). IFN-I production by pDC is also dependent on the localization of TLR9/7 and viral nucleic acids in specialized endosomal compartments. Priming of CD8 T cells depends on efficient Ag processing and MHC class I loading in specialized intracellular compartments. A recent study found that targeting Ag to cells via BST2 resulted in protective T cell-mediated immunity suggesting a role for BST2 in Ag presentation (26). Thus, we envision that perturbation of lipid rafts and the trans-Golgi network in the absence of BST2 may affect endocytosis and vesicular trafficking of viruses or viral nucleic acids and Ag presentation machinery.

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**Disclosures**

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