Bone Marrow Stromal Cell Antigen 2 Is a Specific Marker of Type I IFN-Producing Cells in the Naive Mouse, but a Promiscuous Cell Surface Antigen following IFN Stimulation

Amanda L. Blasius, Emanuele Giurisato, Marina Cellar, Robert D. Schreiber, Andrey S. Shaw and Marco Colonna

*J Immunol* 2006; 177:3260-3265;
doi: 10.4049/jimmunol.177.5.3260
http://www.jimmunol.org/content/177/5/3260

---

**References**

This article cites 32 articles, 18 of which you can access for free at:
http://www.jimmunol.org/content/177/5/3260.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2006 by The American Association of Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Bone Marrow Stromal Cell Antigen 2 Is a Specific Marker of Type I IFN-Producing Cells in the Naive Mouse, but a Promiscuous Cell Surface Antigen following IFN Stimulation

Amanda L. Blasius, Emanuele Giurisato, Marina Cella, Robert D. Schreiber, Andrey S. Shaw, and Marco Colonna

Type I IFN-producing cells (IPC), also called plasmacytoid dendritic cells (DC), are early responders to viral infections (1, 2). IPC detect viruses through TLRs 7 and 9 (3) and produce large amounts of type I IFN, i.e., IFN-α and IFN-β and proinflammatory chemokines. Through secretion of type I IFN, IPC direct both the innate and adaptive immune response by promoting NK cell and CD8+ T cell cytotoxicity, enhancing DC maturation, presenting Ag to T cells and inducing their Th1 polarization, and inducing B cell differentiation into Ab-secreting plasma cells (1, 2). IPC develop in the bone marrow and then circulate through the blood, eventually migrating into lymphoid and nonlymphoid organs (1, 2), with increased recruitment sites into sites of inflammation (4, 5). Although IPC have significant effects on other cells, their numbers in blood and tissues are very small. This along with their complex surface phenotype makes them extremely difficult to identify. Specifically, mouse IPC are negative for CD11c+CD11b-CD25-CD44-CD11b+Ly-6C+CD62L+ (6–9). Identification of cell surface markers specifically expressed by IPC allows for more accurate and simpler means of studying them. Furthermore, determination of functions of IFN-specific Ags may lend insight as to the specialized role of IPC within the immune response.

A number of IPC-specific receptors have been described recently. Blood DC Ag-2 is expressed exclusively on human IPC and functions in Ag uptake (10). Siglec-H is expressed preferentially by mouse IPC in both naïve and virally stimulated mice and also may function in Ag uptake (11–13). Ly-49Q is expressed in peripheral mouse IPC and in some strains is specific for IPC under resting conditions (14–16). Interestingly, all of these markers are species specific and can only be found in either humans or mice. Two additional Abs have been described to be specific for IPC in the mouse, 120G8 (17) and plasmacytoid DC Ag-1 (PDCA1), although the Ags bound by these Abs have not been described previously.

In this study, we have identified a new series of Abs specific for mouse IPC in naïve mice. We have further determined that the Ag bound by these Abs is bone marrow stromal cell Ag 2 (BST2) (18–21). Surprisingly, BST2 is also recognized by the Abs PDCA1 and 120G8. BST2 is predominantly expressed by IPC in the naïve mouse, but is up-regulated on numerous cell types following stimulation that triggers an IFN response. BST2 cycles between cell surface and intracellular compartments and may function to regulate trafficking of secreted cytokines.

Materials and Methods

Mice

The 129/SVJ mice were obtained from The Jackson Laboratory; B6 and 129SvEv mice were obtained from Taconic Farms. Mice lacking both IFN-α receptor (IFNAR)1 and IFN-γ receptor (IFNGR)1 were described previously and originally obtained from M. Aguet (Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland) (22). For influenza A infection, mice were injected i.v. with 5 × 10⁶ PFU WSN strain, and BST2 expression was assessed at day 3 postinfection on splenocytes. For depletion, mice were injected with 500 μg of mAb 927 or control rat IgG at 0 and 24 h, and splenocytes were analyzed by flow cytometry at 48 h.

Cell culture

Bone marrow-derived IPCs and conventional DCs were prepared by culturing bone marrow cells with fms-like tyrosine kinase 3 ligand (FLT3L) and GM-CSF, respectively, as previously described (23). Conventional DC
were identified as CD11c<sup>+</sup> cells within the GM-CSF cultures. IPC were identified as B220<sup>+</sup> CD11b<sup>+</sup> cells within the FLT3L cultures.

For stimulation of DC, cells were incubated for 24 h with 10 ng/ml IFN-γ (PeproTech), 100 U/ml IFN-α (PBL Biomedical Laboratories), 100 U/ml IFN-β (PBL Biomedical Laboratories), 10 μg/ml LPS (Sigma-Aldrich), 6 μg/ml CpG oligodeoxynucleotide 2216, 10 ng/ml TNF-α (PeproTech), 20 ng/ml IL-6 (PeproTech), and 10 ng/ml IL-12 (PeproTech). Alternatively, DC were incubated with irradiated J558L cells expressing CD40L (23).

For determination of IPC production of IFN-α, freshly isolated splenic or bone marrow-derived IPC were sorted and cultured for 16 h on plates coated with mAb 927 or isotype control with or without 10 μg/ml CpG 2216. IFN-α released into cell culture supernatants was measured by ELISA (PBL Biomedical Laboratories).

**Generation of Abs to mouse IPC**

Wistar/CRL rats were immunized s.c. in the hind footpads with 10<sup>7</sup> bone marrow-derived IPC with either 100 μg of CpG oligodeoxynucleotide 1826 or 1 mg of heat-killed *Mycobacteria tuberculosis* (Difco Laboratories) as adjuvant at days 0, 4, and 7. On day 8, popliteal lymph nodes were fused with SP2/0 myeloma cells. Hybridoma supernatants were selected that stained bone marrow-derived IPCs and fractions of primary CD11c<sup>+</sup> spleen cells. We further identified hybridoma supernatants that stained only CD11c<sup>+</sup>Ly-6C<sup>+</sup>CD11b<sup>+</sup> splenocytes. mab 927 is rat IgG2b isotype.

**Flow cytometric analysis**

Spleen, lymph node, and thymus were mechanically disrupted and digested with collagenase D (Sigma-Aldrich). Blood, bone marrow, and spleen were treated with ammonium chloride to lyse RBC. Cells were stained with hybridoma supernatants for Abs against BST2, which were detected with anti-rat IgG or anti-rat IgG2b conjugated to FITC, PE, or biotin. Alternatively, mAb 120G8 (AbCys) or PDCA1 (Miltenyi Biotec) were used. Following staining with secondary Abs against rat IgGs, unoccupied binding sites were blocked with rat IgG. Subsequently, staining was performed with the following Abs: Ly-6C, CD11b, CD8α, B220, CD11c, CD3ε, DX5, CD19, CD138 (all obtained from BD Biosciences), and Ly-49Q (Medical and Biological Laboratories). Siglec-H was detected with biotin-or FITC-conjugated mAb 440c (anti-Siglec-H, rat IgG2b isotype; Hycult Biotechnology (11, 12), and in some with cases mAb 257 or mAb 551, which were detected with anti-rat IgG2a (BD Biosciences) or anti-rat IgGl (BD Biosciences), respectively. Biotinylated Abs were detected with streptavidin-allophycocyanin ( Molecular Probes). In some experiments, cells were stained with rat IgG2b isotype control Ab (eBioscience) instead of anti-BST2 Abs to determine background. Live cells were gated on by forward light scatter, side light scatter, and propidium iodide negativity. Sorting was performed on a Mo Flo cell sorter (DakoCytomation) or a FACSVantage SE with FACSDIVA option (BD Biosciences).

**Expression cloning**

IPC were purified from FLT3L bone marrow cultures by B220 magnetic cell sorting (MACS; Miltenyi Biotec). RNA was extracted by the Triazol method (Invitrogen Life Technologies) and used to prepare a customized cDNA library in Express-1 (Open Biosystems). The IPC cDNA library was transiently transfected with Lipofectamine 2000 (Invitrogen Life Technologies) into 293 cells and cells binding mAbs 927, or 129c1 were sorted by flow cytometry. DNA was extracted from the sorted cells, transformed into bacteria, and DNA was prepped from bacterial plates. The harvested DNA was again transfected into 293 cells, and the procedure was repeated three more times. The enriched library was divided into pools, and positive pools were identified by transfection into 293 cells, followed by analysis by flow cytometry. Positive pools were subdivided until a single positive clone was identified and sequenced. All IPC-specific Abs with unknown Ags were tested for binding to this clone.

**Confocal microscopy**

To detect the cellular localization of BST2 protein, J558L or bone marrow-derived IPC cells were placed onto poly(L-lysine)-coated glass slides for 30 min at 37°C. After fixation for 15 min with 4% paraformaldehyde, cells were permeabilized for 10 min with 0.01% saponin in blocking solution (2% BSA in PBS) and incubated for 1 h with mAb 927. After washing, cells were incubated for 1 h with FITC-conjugated goat anti-rat Ig (1:400; BD Biosciences) and with Cy3-labeled cholera toxin (CTx) B subunit (1: 300). Alternatively, slides were incubated with anti-golgin 97 (Invitrogen Life Technologies), which was detected with Cy3-labeled anti-mouse IgG (Jackson ImmunoResearch Laboratories). Slides were then washed several times with PBS and covered with coverslips in a mounting medium of antifade (Molecular Probes). Confocal microscopy was performed with a Zeiss LSM 510 laser-scanning confocal microscope.

**Results**

**BST2 is a specific marker of IPC under naive conditions**

To generate Abs to murine IPC, rats were immunized with bone marrow-derived IPC. Thirteen Abs were isolated that specifically recognized CD11c<sup>+</sup>B220<sup>+</sup>Ly-6c<sup>+</sup>CD11b<sup>+</sup> cells within the spleen, represented by staining with one Ab, mAb 927 (Fig. 1A). One of these Abs, mAb 440c, has been reported previously and was shown to recognize Siglec-H, a highly specific marker of IPC (11, 12). Eight other Abs (mAbs 257, 385, 551, 1021, 1121, 1313, 1331, and 1165) additionally recognized Siglec-H (data not shown). The remaining four Abs, mAbs 927, 129c1, 646, and 404, were shown. The remaining four Abs, mAbs 927, 129c1, 646, and 404, were shown. The remaining four Abs, mAbs 927, 129c1, 646, and 404, were shown. The remaining four Abs, mAbs 927, 129c1, 646, and 404, were shown. The remaining four Abs, mAbs 927, 129c1, 646, and 404, were shown. The remaining four Abs, mAbs 927, 129c1, 646, and 404, were shown. The remaining four Abs, mAbs 927, 129c1, 646, and 404, were shown. The remaining four Abs, mAbs 927, 129c1, 646, and 404, were shown. The remaining four Abs, mAbs 927, 129c1, 646, and 404, were shown. The remaining four Abs, mAbs 927, 129c1, 646, and 404, were shown. The remaining four Abs, mAbs 927, 129c1, 646, and 404, were shown. The remaining four Abs, mAbs 927, 129c1, 646, and 404, were shown. The remaining four Abs, mAbs 927, 129c1, 646, and 404, were shown. The remaining four Abs, mAbs 927, 129c1, 646, and 404, were shown.
all stained an identical cell population as the Siglec-H Abs in all lymphoid organs (Fig. 1, A and B, and data not shown).

To identify the Ag(s) bound by the IPC-specific Abs, we screened a cDNA library generated from bone marrow-derived IPC by expression cloning for cDNAs encoding Ags bound by mAbs 927 and 129c1. We identified a single cDNA clone that mediated binding to both of these Abs and to two additional Abs, mAbs 404 and 656 (Fig. 1C and data not shown). Upon sequencing, we found that the Ag recognized was BST2. We went on to test whether two IPC-specific Abs for which the Ag specificity is unknown, 120G8 and PDCA1, recognized either of the IPC-specific Ags Siglec-H or BST2. We found that both 120G8 and PDCA1 bound to cells expressing BST2, but not cells expressing Siglec-H (Fig. 1D).

The BST2 transcript cloned out of the cDNA library was shorter than the cDNA reported in the National Center for Biotechnology Information nucleotide database (accession no. BC056638), lacking the upstream methionine. The cDNA began at a second methionine 13 aa downstream the first methionine. We cloned a full-length BST2 cDNA containing the upstream methionine from IPC and expressed it in 293 cells. These transfectants had only minimal cell surface expression of BST2, as detected by mAb 927 (data not shown). Interestingly, the downstream methionine is conserved in human BST2, and it may be that this is the major start site for BST2 expressed on the cell surface. Expression from the upstream methionine may lead to instability or altered localization.

**BST2 is a promiscuous marker for cellular activation**

BST2 was originally identified in the human as an Ag preferentially expressed in terminally differentiated B cells, Ab-secreting plasma cell lines, and myeloma cells (18, 20). Independently, it was reported to be expressed by bone marrow stromal cell lines that could promote growth of a murine pre-B cell line (19). Recently, BST2 was designated CD317 and found to be promiscuously expressed in all stages of B cell differentiation as well as in T cells, NK cells, DC, monocytes, CD34+ progenitors, and non-hemopoietic cells in humans (21). BST2 has previously been cloned from the mouse (20), although characterization and expression pattern of the mouse protein had not yet been addressed.

As BST2 expression on human cell lines was widespread, we examined BST2 expression on murine cell lines. We found expression on a wide variety of cell lines generated from diverse cell sources, including T cell lines (EL4, RMAS, Yac), mast cell lines (P815), B cell lines (Baf3, A20, J558L), fibroblast cell lines (3T12), and a pluripotent embryoary carcinoma cell line (P19) (Fig. 2A). This result was unexpected, as in the naive mouse spleen, BST2 was preferentially expressed by IPC. We further investigated the expression of BST2 on mouse splenocytes following viral infection. In influenza-challenged mice, BST2 expression on IPC was unchanged, but DC and other myeloid cells began to express BST2 (Fig. 2B). Furthermore, BST2 expression was found on T cells, B cells, NKT cells, and some NK cells (Fig. 2B). Further analysis indicated that within the DC subset, both CD8+ and CD8− DC up-regulated BST2 (Fig. 2C). Similar results were seen in splenocytes stimulated in vitro or in vivo with a variety of stimuli, including CpG, LPS, murine CMV, poly(E,C), and imiquimod (data not shown). Although 120G8 had been reported to be expressed on activated B cells and DC (17), expression had not been reported previously on activated T cells and NK cells. 

BST2 in the human had originally been described to be highly expressed on plasma cell lines (20). We further investigated this specialized B cell subset in the mouse and found that BST2 was expressed on CD138+ plasma cells in naive mice at low levels (Fig. 2D). This expression was below that seen on IPC, and did not interfere with specific recognition of IPC by flow cytometry. However, following viral stimulation, BST2 was highly up-regulated on plasma cells, with expression levels similar to that of IPC (Fig. 2D). We conclude that BST2 is predominantly a marker for IPC, but also for plasma cells and other cell types following stimulation. This has implications for many previous studies using mAbs 120G8 and PDCA1 for specific identification of IPC following viral challenge or in other models of immune system activation.

**BST2 is induced by both type I and type II IFN**

The promoter of BST2 contains a number of elements that would suggest transcriptional regulation by IFNs and IL-6 through transcription factors, including STAT1 and STAT3 (20, 24). There are additional elements for binding by AP-2 and GATA1 (20, 24).
further examine what stimuli could induce BST2 expression, we incubated murine bone marrow-derived DCs with various stimuli and cytokines (Fig. 3A). Expression was induced upon treatment with the TLR ligands LPS and CpG and also with the cytokines IFN-α, IFN-β, and IFN-γ. Because the promoter for human BST2 contained IL-6-responsive elements (20), we investigated whether IL-6 could induce expression. On the contrary, treatment with IL-6 did not induce expression of BST2 in bone marrow-derived DC (Fig. 3A) or the A20 cell line (data not shown). Furthermore, BST2 expression was not induced by treatment with IL-12, TNF-α, or CD40L.

As the TLR stimuli LPS and CpG are able to induce secretion of type I IFNs through the TLR4/Toll/IL-1R domain-containing adaptor-inducing IFN-β pathway and the TLR9/MyD88 pathway, respectively (25), we investigated whether TLR ligand-induced BST2 expression on DC was dependent on IFNs. Bone marrow-derived DC from mice doubly deficient in the IFNAR1 and IFNGR1 were unable to up-regulate BST2 in response to LPS and CpG (Fig. 3B). We therefore conclude that BST2 is up-regulated in response to all IFNs and stimuli that cause an IFN response.

As IPC are specialized to produce type I IFNs, expression of BST2 on naive IPC may be attributed to autocrine secretion of type I IFNs. Although it has been reported that 120G8 continued to bind to IPC derived from IFNAR-deficient mice (17), it was previously unknown that up-regulation could occur in response to IFN-γ exposure. We further compared mice deficient in both IFNAR and IFNGR. IPC continued to express high levels of BST2 even in the absence of IFN responsiveness (Fig. 3C). We conclude that IFNs can induce cell surface expression of BST2 by most cell types. However, BST2 expression on resting and activated IPC is independent of IFNs.

Abs to BST2 deplete IPC and plasma cells

Depletion of IPC during viral infection or tumor challenge would allow identification of the precise function of IPC in these immune responses. Upon treating mice in vivo with our four different Abs to BST2, we found that all Abs gave at least a partial depletion of IPC, but only mAb 927 efficiently depleted IPC upward of 95% (Fig. 4). In addition, treatment with mAb 927 depleted IPC as defined by the markers CD11c<sup>+</sup> Ly-6C<sup>−</sup> B220<sup>−</sup> (data not shown). As we found BST2 to be expressed on plasma cells, we additionally examined how treatment with mAb 927 affected numbers of CD138<sup>−</sup> CD19<sup>−</sup> cells. We found that mAb 927 treatment in vivo also significantly reduced plasma cells, even in naive animals that only express low levels of BST2 (Fig. 4). In light of this finding, previous studies using 120G8 or PDCA1 for specific depletion of IPC may need to be re-examined to determine whether depletion of plasma cells may have contributed to any observed phenotype.

**FIGURE 3.** BST2 is induced by IFNs. A, Mouse bone marrow-derived DC were cultured with the indicated stimuli, and CD11c<sup>+</sup> cells were analyzed for expression of BST2 with mAb 927. Gray shaded indicates medium only control stimulation, and black line indicates stimulated cells. B, Mouse bone marrow-derived DC from mice deficient in both IFNGR and IFNAR were treated as above. Gray shaded indicates medium only control stimulation, and black line indicates stimulated cells. C, BST2 expression was evaluated on freshly isolated splenic Siglec-H<sup>−</sup>CD11c<sup>+</sup> IPC from wild-type control mice (gray shaded) or mice deficient in both IFNAR and IFNGR (black line).

**FIGURE 4.** IPC and plasma cells are depleted by mAb 927. Mice were treated with mAb 927 or control Ab. Percentage of IPC was determined as CD11c<sup>+</sup>CD138<sup>−</sup> cells. Results are representative of at least three experiments.

**BST2 is localized to the cell surface and intracellular compartments**

Human BST2 amino acid sequence shows similarity to BAP31, a molecule that is important in regulating export or import of receptors to or from the cell surface. BAP31 has been shown to associate with and regulate intracellular traffic of cell surface molecules such as CD11b (26) and IgD (27, 28). Moreover, rat BST2 has been shown to have both a cell surface and an intracellular juxtanuclear location and to reach apical plasma membranes of polarized rat cells via cholesterol-rich lipid microdomains (29). These observations suggest that BST2 may be important in sorting molecules within the Golgi apparatus for targeting to the cell surface, possibly regulating cytokine secretion in IPC.

To address this hypothesis, we localized BST2 within mouse IPC and mouse myeloma cells J558L by confocal microscopy. Cells were counterstained with CTb X, a marker of sphingolipid- and cholesterol-rich lipid microdomains on plasma, Golgi, and endosomal membranes. We determined that BST2 is located on the cell surface and in an intracellular compartment with the morphological characteristics of the Golgi apparatus (Fig. 5, A and B). In both locations, BST2 and CTb largely overlapped, consistent with BST2 presence in lipid rafts. Localization of BST2 to the Golgi was further confirmed by costaining with Ab to golgin (Fig. 5, C and D).

To determine whether BST2 may have a role in secretion by IPC, we investigated whether these Abs to BST2 could alter secretion of IFN-α in IPC. We found that treatment with mAb 927 could abrogate IFN-α secretion by IPC in response to CpG (Fig. 5E), further suggesting a role for regulation of secretion. This inhibition was most efficient with plate-bound mAb 927 as opposed to soluble Ab (data not shown), indicating that immobilization or
extensive cross-linking is required. Taken together, these data suggest that BST2 may be an important molecule in promoting secretion in IPC, sorting proteins between the Golgi apparatus and the plasma membrane.

**Discussion**

We have identified new Abs specific for naive mouse IPC. These Abs recognize the Ag BST2, which we also show is the Ag recognized by the previously described Abs 120G8 (17) and PDCA1. Identification of these and other Abs specifically recognizing mouse IPC has been an important step forward for the field of IPC biology. These Abs allow IPC to be identified with better purity and allow IPC to be depleted so that their contribution to the immune response can be assessed. From the consolidated work of multiple groups, only three Ags, Siglec-H, Ly-49Q, and now BST2, have been identified as mouse IPC-specific Ags. It will be interesting to see whether any further Ags highly specific for IPC will be identified. Although IPC appear to be important first responders and regulators of the immune response, their precise role is still unclear. Most likely, Ags that are specifically expressed by IPC will be responsible for producing some of the specific functions that make IPC unique.

The function of BST2 remains unknown. However, several observations suggest that BST2 may be important in sorting membrane and secreted proteins in the Golgi apparatus, the trans-Golgi vesicles, and the cell membrane. First, the predominant expression of BST2 in IPC, which secrete high levels of type I IFN, and also plasma cells, which secrete Igs, indicates that BST2 could be important in sorting secreted proteins. Moreover, BST2 is not only on the cell surface of IPC and myeloma cells, but also in an intracellular compartment that corresponds to the Golgi apparatus (and possibly the trans-Golgi network), both sorting sites for proteins en route to the cell membrane or internalized from cell membranes into the endosomes. Similar localization and internalization have been observed previously for rat BST2 in cell lines (29). A function in sorting transmembrane and secreted proteins is further indicated by BST2 sequence and topology. BST2 shows sequence homology with BAP31, a chaperone involved in intracellular transport of cell surface proteins, and contains sequence motifs, such as an N terminus tyrosine-based motif (Y-x-Y-x-x-x-P-M, Y, tyrosine; P, proline; M, methionine; x, any amino acid) and KKxx (K, lysine; x, any amino acid) that may function as transport signals (30). Finally, BST2 presents an unusual topology with an N terminus transmembrane domain and a C terminus GPI anchor that locates BST2 in glycosphingolipids- and cholesterol-rich lipid microdomains (29). Thus, BST2 may mediate protein sorting via lipid rafts. The constitutive high level of BST2 expression in IPC may be the basis for the ability of these cells to secrete high amounts of IFN. BST2 has additionally been proposed to be important in development of B cells (19) and signaling through NF-κB and MAPK pathways (31). Ultimately, the function of BST2 in IPC and other cells would be best determined by generation of knockout mice.

Although cell surface expression of BST2 is predominantly restricted to IPC in the naive mouse, this Ag is promiscuously expressed on many cell types following viral or other IFN-inducing stimulation. Consistent with this observation, BST2 was found to be more abundant in Th1 T cells, which produce IFN-γ, than Th2 T cells (32). Although the expression on most cells remains low following stimulation, plasma cells and IPC cannot be distinguished on the basis of BST2 expression alone and many other cell types may be difficult to separate. Therefore, caution should be taken in the interpretations of experiments when using anti-BST2 Abs to identify IPC in models of infection and perhaps tumor challenge and autoimmunity. Because during stimulation both Ly-49Q and BST2 can be up-regulated on other cell types, Siglec-H may be the best marker of IPC under activating conditions. Careful analysis of the cell populations depleted with these Abs needs to be done in studies attempting to assess the role of IPC in these models. Depletion of IPC during tumor challenge poses an additional problem, as many tumor cell lines express BST2. However, we have been unable to deplete IPC using any of nine independent Abs to Siglec-H (our unpublished observations). Therefore, anti-BST2 Abs remain the best choice for depleting IPC.

**Acknowledgments**

We thank Daved Fremont, Susan Gilfillan, and Tom Brett for helpful suggestions; Rachel Presti for reading the manuscript; William Eades and Jacqueline Hughes in the Siteman Cancer Center High Speed Sorter Core Facility; and Amy Boyet for cell sorting experiments.
Disclosures
The authors have no financial conflict of interest.

References