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Antigen Delivery to Plasmacytoid Dendritic Cells via BST2 Induces Protective T Cell-Mediated Immunity

Jakob Loschko,* Andreas Schlitzer,∗ Diana Dudziak,†‡ Ingo Drexler,§∗ Nadja Sandholzer,∥ Wolfgang Reindl,* and Anne B. Krug*

Plasmacytoid dendritic cells (PDCs) are capable of presenting Ags to T cells in a tolerogenic or immunogenic manner depending on the formulation of the Ag and the mode of stimulation. It has not been investigated whether effective adaptive immune responses useful for vaccination can be induced by Ab-mediated Ag targeting to PDCs in vivo. In this study, we show that Ag delivered to murine PDCs via bone marrow stromal cell Ag 2 (BST2)/CD317 in combination with TLR agonists as adjuvants is specifically presented by PDCs in vivo and elicits strong cellular and humoral immune responses. These include IFN-γ production by CD4+ T cells and high Ab titers with a broad range of IgG isotypes. In addition, BST2-mediated Ag delivery in the presence of polyinosinic-polycytidylic acid as adjuvant induces cytotoxic T lymphocytes that are functional in vivo. A single immunization with Ag-fused anti-BST2 Ab together with polyinosinic-polycytidylic acid as adjuvant is sufficient to trigger protective immunity against subsequent viral infection and tumor growth. We conclude that despite the potential tolerogenic properties of PDCs, Ag targeting to PDCs in combination with TLR agonists as adjuvants is an effective vaccination strategy.

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This approach has been tested extensively in the murine system to target Ags specifically to conventional DC (CDC) subpopulations. Ag delivery to CDCs with DEC205 (4–8) or CLEC9a/DNGLR-1 (9, 10) in the presence of adjuvant overcomes tolerance and induces effective CD4+ T cell and Ab responses as well as CD8+ T cell responses, as CD8α+ DCs are highly efficient in cross-presentation exogenous Ag. CD8α+ CD11b+ CDCs have also been targeted successfully leading to preferential expansion of CD4+ T cells and induction of humoral immune responses (6, 11–13). In the human system, Abs directed against DEC205, DC-SIGN, DCIR, and the mannose receptor were shown to be capable of targeting Ag to human DCs and to trigger CD4+ and CD8+ T cell responses in vitro (3, 11, 12).

Data on Ag targeting specifically to plasmacytoid DCs (PDCs) is rather limited to date. Sigle-H and bone marrow stromal cell Ag 2 (BST2) have been shown to be specifically expressed in murine PDCs (14, 15) and to be able to deliver Ags to PDCs for presentation (16, 17). The C-type lectin BDCA-2 expressed on human PDCs can mediate Ag presentation to T cells in vitro (18). PDCs, which are at the front line of antiviral immune responses because of their capacity to produce high amounts of type I IFN, differ considerably from CDCs with regard to Ag presentation. Whereas CDCs shut down the formation of new peptide–MHC class II (MHC II) complexes upon activation and stabilize high levels of peptide–MHC II complexes on the cell surface for a long time (19), activated PDCs have a high turnover of peptide–MHC II complexes. They may therefore be specialized to present Ags, which are available for MHC II loading for longer time periods (such as in viral infection), whereas they are rather poor at presenting soluble Ags (20, 21). However, presentation of exogenous Ags to CD4+ T cells in vitro can be achieved by Ag delivery to human PDCs via FcyRIIA (22) or DCIR, for example (23). PDCs have also been demonstrated to cross-present viral Ags derived from infected cells or directly from viral particles to CD8+ T cells in vitro (24–26), although it is still controversial if this also occurs in vivo (20). The data that are available to date show that Ag presentation by PDCs can induce both T cell-mediated tolerance (27–30) and immunity (18, 23–26). The outcome of Ag delivery to
PDCs in vivo with regard to the type of T cell response that is induced is therefore not predictable and is likely to depend on the route of Ag delivery and the mode of stimulation.

In the human system, PDCs constitute a major DC subpopulation in contrast to the equivalent of murine CD8α+ DCS, the CD141+ XCR1+ DCS, which have only a very low frequency (31–33). In addition, PDCs are directly activated by TLR7 and TLR9 ligands as adjuvant. Thus, PDCs may be attractive target cells for vaccination, if their tolerogenic properties can be reversed.

In this study, we investigate the efficacy of Ag targeting to PDCs (via the specific surface molecule BST2) for eliciting protective adaptive immune responses in vivo. We show that Ag delivery to murine PDCs via BST2 in the presence of CpG 1668 or polyinosinic-polycytidylic acid (polyI:C) as adjuvant is specific and efficient in inducing a Th1 response and high titers of class switched Abs including IgG1, IgG2b, and IgG2c. In addition, in combination with polyI:C as adjuvant, BST2-mediated Ag delivery to PDCs is potent in inducing CTLs that are functional in vivo. We show that a single immunization with a recombinant anti-BST2–Ag fusion protein together with polyI:C provides long-lasting protection against subsequent viral challenge and inhibits growth of an aggressive Ag-expressing tumor.

Materials and Methods

**Mice**

Specific pathogen-free, 6- to 8-wk-old, female C57BL/6 and C3H mice were purchased from Harlan Winkelmann (Borchen, Germany). OT-I and OT-II mice (C57BL/6) were bred under specific pathogen-free conditions according to the institute’s guidelines. Experiments were performed in accordance with German animal care and ethics legislation and were approved by the local government authorities.

**Generation of the recombinant anti-BST2–OVA and anti-BST2–pHEL Abs**

RNA was isolated from a hybridoma cell line that produces anti-BST2 Ab (rat IgG1) using TRIzol reagent (Invitrogen, Karlsruhe, Germany) (14). The sequences encoding for the V region of the L and H chain were amplified from cDNA using the 5′-RACE amplification system (Invitrogen) and cloned into a previously described vector encoding for a mouse IgG1 Ab whose C terminus is fused to full-length OVA (34). Because of a mutation in the Fc part, this recombinant Ab is unable to bind to Fc receptors. Where indicated, a peptide of hen egg lysozyme (pHEL) was used as Ag. The anti-BST2–pHEL Ab was generated by replacing the sequence encoding for OVA with the sequence encoding for pHEL (aa 48–62, DGSTDY-GILQINSR). The chimeric PDC-specific Abs were transiently expressed in HEK293T cells and purified from the supernatants using HitTrap Protein G columns (GE Healthcare, Munich, Germany). Each batch was tested for binding to PDCs by FACS analysis.

**Flow cytometry**

For FACS analysis, cells were stained using Abs against CD3, CD4, CD8, CD86, IFN-γ, IL-2, IL-4, anti-mouse IgG1 (BD Biosciences, Heidelberg, Germany), CD11c, CCR9 (eBioscience, San Diego, CA) and B220 (Southern Biotech, Birmingham, AL). 120G8 and 440c Abs were produced in Germany), CD11c, CCR9 (eBioscience, San Diego, CA) and B220 labels anti–IFN-γ, anti–IL-4, and anti–IL-2 Abs (BD Biosciences). Cells were fixed with 2% paraformaldehyde, (Sigma, St. Louis, MO) was added to exclude dead cells from the analysis.

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**Detection of OVA-specific Abs by ELISA**

ELISA for OVA-specific IgG Abs was performed according to the ELISA protocol provided by BD Biosciences. Briefly, Maxisorb ELISA plates (Nunc, Roskilde, Denmark) were coated with 5 μg/ml OVA (Hyglos, Regensburg, Germany). After washing and blocking, plates were incubated with 2-fold serial dilutions of collected sera. For detection, biotinylated anti-mouse IgG1, IgG2b, and IgG2c Abs (1 μg/ml; BD Biosciences) and streptavidin–HRP (1:3000; GE Healthcare) were used. ABTS was used as substrate (Roche, Basel, Switzerland), and the reaction was stopped by

1. Isolated OT-I and OT-II T cells were labeled with 5 μM CFSE, and 2 × 10^6 to 4 × 10^6 cells per mouse were transferred to C57BL/6 mice by i.v. injection. Recipient mice were immunized i.p. with 10 μg chimeric anti-BST2–OVA or anti-DEC205–OVA Abs 24 h later. T cell proliferation was analyzed in the spleen 4 d after immunization.

2. Mice were injected with 300 μg anti-Gr-1 Ab i.p. to deplete PDCs and were immunized 24 h later with anti-BST2–OVA/polyI:C. After 8 h, CD11c+ cells were enriched from splenocytes by MACS using CD11c microbeads (Miltenyi Biotech) and cocultured with CFSE-labeled OT-I T cells for 72 h (DC numbers as indicated, 10^6 T cells per well). T cell proliferation was measured by CFSE dilution.

3. Mice were injected i.p. with 20 μg CpG 1668 or 50 μg polyI:C. Anti-OVA Ab titers were determined in the serum on day 14 before the boost and on day 21, 1 wk after the boost. To detect OVA-specific CD4+ T cell responses, splenocytes were isolated 1 wk after the boost on day 21, and MACS-enriched CD4+ T cells were restimulated with OVA-pulsed CD11c+ DCs for 6 h in the presence of GolgiPlug and GolgiStop (BD Biosciences) according to the manufacturer’s protocol. To determine CD8+ T cell responses in vivo, killing assays were performed 1 wk after the boost on day 21. Briefly, splenocytes were pulsed with SINIFEKL peptide (500 nM) for 3 h and labeled with 0.1 μM CFSE or not pulsed and labeled with 1 μM CFSE. An equal number of cells (1 × 10^6) of each population was transferred to immunized mice, and specific killing was quantified 16 h later in spleens as described (35).

4. Mice were euthanized when tumor size exceeded 225 mm^2.

5. T cells were restimulated with OVA peptides Total splenocytes were incubated in vitro with 5 μg/ml SIINFEKL to restimulate CD8+ T cells or with a combination of 5 μg/ml OVA peptide (aa 323–339) and 5 μg/ml OVA peptide (aa 265–279) to restimulate CD4+ T cells for 6 h in the presence of GolgiPlug and GolgiStop (BD Biosciences) and then stained intracellularly for IFN-γ, IL-4, and IL-2. All peptides were purchased from GenScript (Piscataway, NJ).

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ELISA for OVA-specific IgG Abs was performed according to the ELISA protocol provided by BD Biosciences. Briefly, Maxisorb ELISA plates (Nunc, Roskilde, Denmark) were coated with 5 μg/ml OVA (Hyglos, Regensburg, Germany). After washing and blocking, plates were incubated with 2-fold serial dilutions of collected sera. For detection, biotinylated anti-mouse IgG1, IgG2b, and IgG2c Abs (1 μg/ml; BD Biosciences) and streptavidin–HRP (1:3000; GE Healthcare) were used. ABTS was used as substrate (Roche, Basel, Switzerland), and the reaction was stopped by...
Results

BST2 is a suitable target to deliver Ag specifically to PDCs in vivo

BST2 is highly expressed on murine PDCs (Supplemental Fig. 1B) (36, 37) and is therefore a promising target for delivering Ags to PDCs. We could show that purified recombinant anti-BST2 Ab fused with OVA as Ag (Supplemental Fig. 1A) binds specifically to PDCs whereas an isotype control Ab does not (Supplemental Fig. 1C). Depletion activity of the monoclonal rat anti-BST2 Ab is lost in the recombinant anti-BST2 Ab, which has a mutation in the Fc part (Supplemental Fig. 1D).

BST2 is also expressed in other cell types upon stimulation with type I IFN (37). We found that BST2 was clearly expressed on the majority of splenocytes 16 h after injection of polyIC, but only a very small shift of the BST2 staining signal was detected in splenocytes at 4 h after injection of polyIC (Supplemental Fig. 2, histograms on the right). However, we found that the recombinant anti-BST2–OVA Ab is bound exclusively to the surface of PDCs at 4 h and not detectable at later time points after injection even when coadministered with polyIC or CpG (Supplemental Fig. 2, anti-mlgG1 staining). Thus, binding of the injected anti-BST2 Ab precedes upregulation of BST2 expression by coadministered adjuvants. CDCs or other APCs are therefore not directly targeted by the anti-BST2 Ab in vivo even in the presence of adjuvants.

We next investigated whether Ag presentation on MHC II is specific for PDCs after Ag targeting to BST2 and simultaneous administration of adjuvants. For this purpose, C3H mice were immunized twice with an interval of 14 d with 20 μg anti-BST2–pHEL, without adjuvant or in combination with CpG 1668 or polyIC. Presence of pHEL/I-A$^k$ complexes on the surfaces of splenocytes was measured by FACS 16 h later. Even after two immunizations with anti-BST2–pHEL in the presence of CpG or polyIC as adjuvant, pHEL/I-A$^k$ complexes were detected exclusively on PDCs but not on CDCs, B cells, or remaining APCs in the spleen (Fig. 1A, 1B) and in lymph nodes (data not shown). pHEL/I-A$^k$ complexes were not detectable at later time points after immunization in any of the cell populations even in the presence of the adjuvant (Supplemental Fig. 3). Ag presentation was restricted to CCR9$^+$ PDCs and not detected in CCR9$^-$ PDCs in both spleen and lymph nodes (Fig. 1B and data not shown). The use of CpG 1668 and polyIC as adjuvant increased expression of the costimulatory molecule CD86 on PDCs. Thus, BST2 is a suitable target to deliver Ag specifically to PDCs for presentation on MHC II even in the presence of IFN-inducing adjuvants.

BST2-mediated Ag delivery to PDCs induces proliferation and expansion of adoptively transferred Ag-specific CD4$^+$ and CD8$^+$ T cells

To investigate whether Ag presentation is also followed by activation of Ag-specific T cells, we adaptively transferred CFSE-labeled OVA–TCR-transgenic CD8$^+$ OT-I and CD4$^+$ OT-II T cells into C57BL/6 mice. These recipients were then immunized i.p. with 10 μg anti-BST2–OVA with or without CpG 1668 or polyIC. Proliferation of OT-I and OT-II T cells was measured by CFSE dilution 4 d later. Isotype-OVA control Ab did not induce OT-I or OT-II cell proliferation (Supplemental Fig. 4A). Almost 100% of the CD8$^+$ OT-I T cells proliferated in response to anti-BST2–OVA with or without adjuvant (Fig. 2A, left panels). In the absence of adjuvants, OT-I T cells were expanded by immunization with anti-BST2–OVA ~7-fold compared with mice that had been injected with PBS alone. CpG 1668 slightly increased and polyIC strongly increased expansion of OT-I T cells (Fig. 2B). Immunization with anti-BST2–OVA also efficiently induced proliferation and expansion of CD4$^+$ OT-II T cells, which was further increased by CpG or polyIC (Fig. 2A, right panels, 2C). We therefore conclude that BST2-mediated Ag delivery to PDCs elicits priming of Ag-specific CD4$^+$ and CD8$^+$ T cells, which is significantly enhanced by coadministration of CpG or polyIC as adjuvant.

The ability of PDCs to cross-present Ags to CD8$^+$ T cells is controversial, and staining with the 25-D1.16 Ab was not sensitive enough to detect SHINFEKL–MHC class I (MHC I) complexes on the cell surface after DC targeting (data not shown). As shown in Fig. 3A, however, OT-I T cells proliferated after coculture with isolated PDCs treated with anti-BST2–OVA alone or together with CpG or polyIC in vitro thus providing evidence for direct cross-presentation of the Ag by PDCs. CD11c$^+$ cells isolated from splenocytes of mice that had been depleted of PDCs using the anti-Gr-1 Ab and immunized with anti-BST2–OVA/polyIC were unable to induce OT-I T cell proliferation demonstrating that PDCs were required for the cross-priming (Fig. 3B, 3C).

Targeting Ag to PDCs via BST2 in combination with adjuvant efficiently induces endogenous T cell and Ab responses

Successful vaccines induce expansion and acquisition of effector function of endogenous Ag-specific T and B cells in naive mice with a polyclonal T and B cell repertoire. To test if an immunization...
with Ag-carrying BST2 Abs induces long-lasting and protective immune responses, we immunized mice twice i.p. with anti-BST2–OVA in combination with CpG 1668 or polyI:C, as a positive control for the induction of a strong Ag-specific Th, CTL, and humoral immune response. The high efficiency and quality of this immunization strategy has been demonstrated previously in several studies (7, 8, 38). To determine anti-OVA Ab titers, we collected serum samples before the boost (day 14) and 1 wk after the boost (day 21). At this time point (day 21), mice were sacrificed and T cell responses were analyzed in the spleen. After in vitro restimulation of splenic CD4+ T cells with OVA-pulsed syngeneic splenocytes labeled with different concentrations of CFSE 6 d after the second immunization (day 20). Specific eradication of SIINFEKL-pulsed target cells was determined after 16 h. CTLs induced by immunization with anti-BST2–OVA/polyI:C were as efficient in killing target cells as CTLs induced by anti-DEC205–OVA/polyI:C. CpG 1668 (10 μg i.p.) was a weak adjuvant for anti-BST2–OVA immunization especially with respect to CTL induction (Fig. 4D). We conclude that in combination with polyI:C as adjuvant, Ag delivery to PDCs via BST2 is at least as efficient as Ag delivery to CD8α+ DCs via DEC205 in inducing strong Th1, CTL, and humoral immune responses.

To demonstrate the involvement of PDCs in the immune responses elicited by Ag delivery to BST2 in vivo, PDCs were depleted using the anti–Gr-1 Ab at the time of the first and second immunization with anti-BST2–OVA/polyI:C (depletion efficiency shown in Supplemental Fig. 1D). PDC depletion strongly reduced anti-OVA Ab titers (IgG1, IgG2b, IgG2c) by approximately 2 log (38). To determine anti-OVA Ab titers, we collected serum samples before the boost (day 14) and 1 wk after the boost (day 21). At this time point (day 21), mice were sacrificed and T cell responses were analyzed in the spleen. After in vitro restimulation of splenic CD4+ T cells with OVA-pulsed syngeneic splenocytes labeled with different concentrations of CFSE 6 d after the second immunization (day 20). Specific eradication of SIINFEKL-pulsed target cells was determined after 16 h. CTLs induced by immunization with anti-BST2–OVA/polyI:C were as efficient in killing target cells as CTLs induced by anti-DEC205–OVA/polyI:C. CpG 1668 (10 μg i.p.) was a weak adjuvant for anti-BST2–OVA immunization especially with respect to CTL induction (Fig. 4D). We conclude that in combination with polyI:C as adjuvant, Ag delivery to PDCs via BST2 is at least as efficient as Ag delivery to CD8α+ DCs via DEC205 in inducing strong Th1, CTL, and humoral immune responses.

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BST2-mediated Ag delivery in the presence of polyI:C elicits long-lasting protective immunity against viral infection

To investigate whether Ag targeting to PDCs via BST2 together with polyI:C as adjuvant can induce protective immune responses,
we chose the intranasal vaccinia virus infection model using VV-OVA. The prime boost protocol used in previous experiments was compared with a single i.p. injection of anti-BST2–OVA/polyI:C. To control for unspecific protective effects of the adjuvant, control groups received one or two injections of polyI:C alone. Mice were infected intranasally with $3 \times 10^4$ PFU VV-OVA 8 wk after the last immunization. We chose a sublethal dose of VV-OVA that allowed us to monitor loss and recovery of body weight for 14 d and subsequent analysis of T cell responses. Mice that had received one dose of anti-BST2–OVA/polyI:C lost only little weight and regained the weight they had before infection already on day 8 postinfection. In comparison, mice that had received one injection of polyI:C alone lost significantly more body weight and did not fully recover even after 14 d (Fig. 5A). Similar results were obtained when mice had received the prime and boost vaccination (Fig. 5B). The protective effect of the anti-BST–OVA/polyI:C immunization correlated with a significantly higher frequency of CD4+ T cells that produced IFN-γ and/or IL-2 in response to restimulation with OVA peptides 14 d after VV-OVA infection (Fig. 5C, 5D). In addition, a significantly higher percentage of CD8+ T cells producing IFN-γ in response to OVA peptide restimulation was detected after viral challenge in mice that had been immunized with anti-BST2–OVA/polyI:C compared with that in mice that had received only polyI:C (Fig. 5C, 5D). Our results show that systemic administration of a single dose of anti-BST2–OVA/polyI:C was sufficient to induce long-lasting protective antiviral T cell responses.

Targeting Ag to PDCs via BST2 combined with polyI:C induces a protective antitumor response

Efficient antitumor immunity requires strong Th1 and CTL responses. We therefore investigated if immunization with anti-BST2–OVA/polyI:C confers resistance against the growth of an OVA-expressing melanoma (B16-OVA). For this study, mice were injected i.p. with a single dose of anti-BST2–OVA in combination with polyI:C or with polyI:C alone. After 14 d, $1 \times 10^6$ B16-OVA cells were implanted s.c. Mice that had received only polyI:C developed rapidly growing tumors leading to sacrifice of the mice 18 d after injection of the tumor cells. In contrast to that, mice that had been immunized with anti-BST2–OVA in combination with polyI:C developed only very small and slow-growing tumors (Fig. 6A). The observed reduction in tumor growth correlated with increased survival of mice that had been injected with anti-BST2–OVA/polyI:C compared with that of mice that had received only polyI:C (Fig. 6B). BST2-mediated Ag delivery to PDCs together with polyI:C as adjuvant therefore protected mice from growth of a transplanted syngeneic Ag-expressing tumor.

Discussion

In this study, we show that Ag targeting to PDCs in conjunction with adjuvant is capable of inducing effective immune responses in vivo, which confer Ag-specific protection against viral infection and tumor growth. To target model Ags specifically to murine PDCs in vivo, we have chosen BST2/CD317 as target molecule, which is expressed at high levels on the surfaces of murine PDCs.
BST2 expression is specific for PDCs in naive mice but is upregulated on many cell types after stimulation with type I or type II IFNs, although expression is still significantly higher in PDCs after stimulation (37). We found that despite the pro-miscuous expression of BST2 induced by stimulation, MHC II presentation of Ag fused to anti-BST2 Ab was nevertheless restricted to PDCs even when a prime boost immunization protocol including a strong IFN-inducing adjuvant (CpG or polyI:C) was used. This preservation of specificity may be due to the fact that binding of the injected anti-BST2 Ab precedes upregulation of BST2 expression by the coadministered adjuvants. In addition, the higher expression levels of BST2 in PDCs or the more efficient internalization, trafficking, and loading onto MHC II in PDCs compared with other APCs could further contribute to the specificity of this Ag targeting strategy. It has been proposed that CCR9 expression distinguishes functionally different subpopulations of PDCs. CCR9+PDCs may develop into CDC-like cell type with higher Ag presentation capacity (29, 39, 40). However, after Ag delivery via BST2, we detected peptide–MHC II complexes only on CCR9+ but not CCR9− PDCs excluding a role for CCR9− PDCs in Ag presentation on MHC II after BST2-mediated Ag delivery.

Because direct detection of peptide–MHC I complexes on the cell surface after Ag targeting to BST2 was not successful due to low sensitivity of the available Ab, specificity of BST2 targeting for Ag presentation of MHC I by PDCs even when a prime boost immunization protocol including a strong IFN-inducing adjuvant (CpG or polyI:C) was used. This preservation of specificity may be due to the fact that binding of the injected anti-BST2 Ab precedes upregulation of BST2 expression by the coadministered adjuvants. In addition, the higher expression levels of BST2 in PDCs or the more efficient internalization, trafficking, and loading onto MHC II in PDCs compared with other APCs could further contribute to the specificity of this Ag targeting strategy. It has been proposed that CCR9 expression distinguishes functionally different subpopulations of PDCs. CCR9− PDCs may develop into CDC-like cell type with higher Ag presentation capacity (29, 39, 40). However, after Ag delivery via BST2, we detected peptide–MHC II complexes only on CCR9+ but not CCR9− PDCs excluding a role for CCR9− PDCs in Ag presentation on MHC II after BST2-mediated Ag delivery.

Because direct detection of peptide–MHC I complexes on the cell surface after Ag targeting to BST2 was not successful due to low sensitivity of the available Ab, specificity of BST2 targeting for Ag presentation of MHC I by PDCs was shown indirectly by the lack of OT-I T cell proliferation in response to CD11c+ cells isolated from spleens of PDC-depleted mice that had been immunized with anti-BST2–OVA/polyI:C. PDC-specific Ag presentation in vivo was therefore achieved in our study by targeting Ag to BST2 even in the presence of immunostimulatory adjuvant.

It has been shown that PDCs are rather poor at priming naive T cells in the absence of adequate stimulation but are capable of inducing T cell responses if they are activated by TLR agonists or viral infection (20). In our study, we demonstrate that transferred naive TCR-transgenic CD4+ and CD8+ T cells are sufficiently activated to proliferate after Ag targeting to PDCs via BST2 in the

FIGURE 5. Immunization with anti-BST2–OVA protects mice from weight loss in sublethal infection with OVA-encoding vaccinia virus. Mice were immunized either once (A, C) or twice (B, D) with anti-BST2–OVA/polyI:C or polyI:C alone. Eight weeks after the last immunization, mice were infected intranasally with 3 × 10^4 PFU VV-OVA. A and B display the loss of body weight compared with the weight before infection (mean ± SD, n = 6 mice per group). Asterisks indicate statistically significant differences between the two groups (*p < 0.05, Student t test). C and D show the frequency of OVA-specific IFN-γ+, IL-2−, and IFN-γ/IL-2+ CD4+ T cells and frequency of IFN-γ+ CD8+ T cells 14 d after viral challenge (mean ± SD, n = 6 mice per group). Asterisks indicate statistically significant differences between the two groups—polyI:C alone and anti–BST2-OVA/polyI:C (*p < 0.05, Student t test).

FIGURE 6. Anti-BST2–OVA mediated immunization confers resistance to tumor growth. A, Mice were immunized i.p. with anti-BST2–OVA/polyI:C or polyI:C alone. Two weeks later, mice were injected s.c. with 1 × 10^6 B16-OVA melanoma cells, and the growth of the tumor was monitored at the indicated time points (mean ± SD, n = 6 mice per group). B, Survival after tumor implantation is shown (n = 6 mice per group).
against VV-OVA infection as it has been shown for infection with wild-type virus (42), although the CD4+ T cell response appears to dominate the protective immune response in this model (8). BST2-mediated Ag delivery combined with polyLC as adjuvant also provided resistance against a rapidly growing tumor (B16 melanoma) expressing the specific Ag, which requires both efficient CD4+ and CD8+ T cell responses (4). Only treatment with anti-BST2–OVA/polyLC, but not polyLC alone, induced protective antiviral and antitumoral immune responses. We can therefore exclude that protection conferred by anti-BST2–OVA immunization was mediated by unspecific innate immune effects of the adjuvant polyLC itself.

To our knowledge, our study is the first to show that Ag targeting to PDCs in vivo combined with TLR agonists as adjuvant is an effective vaccination strategy that induces long-lasting protective cellular and humoral immune responses. This result was not expected, because PDCs have been reported to be tolerogenic even under immunostimulatory conditions. We therefore conclude that the route of Ag delivery and the mode of adjuvant stimulation greatly influences the type of immune response that is induced by PDCs in vivo. Thus, PDCs similar to CDC subpopulations can be harnessed to design effective vaccines. This strategy might be especially useful for vaccination in humans where PDCs constitute a major subpopulation of DCs.

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Disclosures

The authors have no financial conflicts of interest.

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