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Concurrent Allorecognition Has a Limited Impact on Posttransplant Vaccination

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Transplantation of allogeneic hematopoietic stem cells with or without immunocompetent lymphocytes has proved a successful strategy in the treatment of hematological malignancies. We have recently shown that this approach can also cure mouse prostate cancer, provided that it is combined with tumor-specific vaccination. Whether the response to alloantigens acts by providing helper function to enhance vaccine-specific responses or in other ways impinges on vaccine immunogenicity remains to be clarified, and this question is of clinical relevance. In this study, we have addressed this issue by comparing the immunogenicity of dendritic cells pulsed with a peptide derived from a tumor/viral model Ag in recipients of donor cells either syngeneic to the host or differing for either Y-encoded or multiple minor H antigens. We report that vaccination elicits comparable proliferation and differentiation of peptide-specific CD8+ T cells despite concurrent expansion and differentiation of minor H antigen-specific IFN-γ effector T cells. Depletion of alloreactive CD4+ T cells reduced alloreactivity but not vaccine-induced CD8+ T cell priming, suggesting that alloresponses do not provide helper functions in peripheral lymphoid tissues. Vaccine-mediated T cell priming was also preserved in the case of multiple minor H antigen disparities, prone to graft-versus-host disease. Thus, in the context of nonmyeloablative allotransplantation aimed at restoring an effective tumor-specific T cell repertoire, minor H antigen-specific T cells do not interfere with vaccine-induced T cell priming, supporting the notion that posttransplant vaccination is a valuable strategy to boost tumor and pathogen-specific protective immunity. The Journal of Immunology, 2011, 186: 1361–1368.

Allogeneic hematopoietic stem cell transplantation (HSCT) combined with a donor lymphocyte infusion (DLI) is widely used in treatment of hematological malignancies and is a possible therapeutic option for nonhematological malignancies. The potency of the strategy is based on the notion that the infusion of a fresh repertoire of naive T cells recognizing tumor-associated Ags in a nontolerogenic environment accounts for eradication of residual malignancy and/or durable immunologic control. In addition to bona fide tumor-associated Ags, broadly expressed minor histocompatibility (H) antigens have also been shown to play a significant role in the graft-versus-tumor (GVT) response (1, 2). These Ags are peptides presented in conjunction with HLA class I and II molecules and are derived from endogenous proteins encoded by polymorphic genes inherited independently from HLA (3). The first set of minor H antigens identified were those encoded by genes on the Y chromosome (4–6); sex mismatch was readily identified as a risk factor for chronic rejection of liver allografts (7), and more recently, it has been reported that male patients receiving female donor hematopoietic allografts experience both a higher severity of graft-versus-host disease (GVHD) and more effective GVT responses than recipients of sex-matched allografts, presumably because of recognition of Y-chromosome–encoded minor H antigens by the female donor T cells (8). The GVT effects are often accompanied, even in an HLA-identical sibling, by deleterious alloreactivity against normal host tissue, manifesting as GVHD (9–11).

Hematopoietic stem cell transplant recipients are also uniquely predisposed to develop chronic illness, possibly because of pre-transplant conditioning and the prolonged lymphopenic state, because of a variety of common and opportunistic viruses (e.g., herpes, CMV, and EBV) (12). Patients acquire viral infections from the donor (donor-transmitted infections), from reactivation of endogenous latent virus, or from the community. In the effort to improve patient immunoreactivity, limiting opportunistic infections and, in the case of neoplastic patients, favoring GVT effects without boosting graft-versus-host responses, posttransplant vaccination has been proposed (13, 14).

Although vaccination against tumor-associated Ags was proven effective in the autologous setting, there are only limited data on the use of vaccination after allogeneic transplantation (15–18). Because activation of host-specific alloreactive minor H antigen-specific donor T cells is observed shortly after transplantation, it might be conducive to concurrent vaccine-induced T cell priming. This might augment vaccine responses via an adjuvant effect or alternatively might be immunosuppressive. For instance, interference between responding cells, described as immunodominance, has been found in the responses to many different Ags (19), including viruses and also among minor H antigens in the context of multiple minor H antigen disparities (20, 21). The initial precursor frequency of naïve epitope-specific CD8+ T cells that can be engaged in the
response and the relative abundance of cognate peptide and major H complex and other more poorly understood factors contribute to determining the magnitude of the response against both viral and nonviral epitopes (22–24). Also, the extent of naive T cell recruitment and subsequent clonal expansion contributes to both the size and diversity of a T cell response (25). Alloantigen cross-presentation, as well as alloreactive intermolecular help/suppression, might also impact on vaccine immunogenicity. Thus, addressing whether post-transplant vaccination is influenced by concurrent alloreactivity is of relevance for optimizing vaccine formulation or administration.

In this study, we investigated whether minor H antigen-specific, alloreactive T cell responses could perform a helper function or be detrimental to posttransplant vaccine-mediated T cell priming. We choose this particular setting as we have recently reported that tumor-specific vaccination promotes optimal tumor-specific T cell priming and disease-free survival in mice undergoing nonmyeloablative allogeneic transplantation (26). This previous study mainly focused on alloresponse to Y-encoded H Ags and provided the proof of principle that minor H antigen- and tumor-specific T cells cooperate for prostate cancer eradication but did not determine whether alloreactive T cell responses would influence immunogenicity if peptides were presented on allogeneic rather than syngeneic dendritic cells (DC). In this study, we have addressed this important issue and compared the ability of peptide-pulsed DC to induce CD8+ effector cells in the presence of allosresponses evoked by HY or multiple minor H antigen disparities, in the presence or absence of graft-versus-host responses/disease. We choose the immunodominant peptide from the SV40 large T Ag (Tag-IV), as this is commonly used as representative of both virus- and tumor-associated Ags. We first analyzed the potency of syngeneic or allogeneic peptide-pulsed DC to determine the impact of the simultaneous presentation by DC of minor H antigen and of nominal Ag. Then, we analyzed vaccine- and minor H antigen-specific T cell responses in allogeneic HSCT plus DLI recipients. We report that soon after HSCT/DLI infusion and vaccination both minor H antigen- and vaccine-specific T cells undergo simultaneous, but independent rapid clonal expansion in peripheral lymphoid organs increase in frequencies and numbers and differentiate into IFN-γ effector T cells. Although concomitant alloresponses do not provide helper function for Tag-IV-specific CD8+ expansion, they are not detrimental to vaccine-induced T cell immunity, supporting the idea that post-HSCT/DLI vaccination is a valuable strategy to boost Ag-specific T cell immunity, also in the context of multiple minor H antigen disparities.

Materials and Methods

Mice, cell lines and reagents

BALB.B and C57Bl/6 (B6) mice, either CD45.2<sup>+</sup>, CD45.1<sup>+</sup>, or F<sub>1</sub> heterozygous CD45.1<sup>10.2</sup> following use of appropriate congenic strains, were housed and bred in the San Raffaele-specific pathogen-free animal facility in accordance with European Union guidelines and with the approval of the Institutional Ethical Committee (IACUC number 388). Rag2-deficient Thy1.1<sup>+</sup> MataHari and Rag2-deficient Thy1.1<sup>+</sup> Marilyn mice were housed at the animal facility of the Imperial College London under the specific pathogen-free conditions. CD8<sup>+</sup> T cells from MataHari mice express a transgenic TCR specific for the HY Uty peptide WMHHNMDLI (27, 28), whereas CD4<sup>+</sup> T cells of Marilyn origin express a transgenic TCR specific for the HY Dby peptide NAGFNSNRANSS (29, 30). Unless specified, all chemical reagents were from Sigma-Aldrich, and mAbs were from BD Pharmingen (San Diego, CA).

DC vaccination

Bone marrow-derived DC (BMDC) were obtained and matured by LPS stimulation as previously described (31), before being pulsed with 2 μM Tag-IV peptide (VYDFLKC; Research Genetics, Huntsville, AL), 2 μM mSTEAP186–193 (RSYRYKLL), or 2 μM HY Dby (NAGFNSNRANSS) or Uty (WMHHNMDLI) peptides and subsequently injected s.c. into the mice (4 × 10<sup>6</sup> DC/mouse).

HSCT and DLI

B6 or BALB.B mice were sublethally irradiated (600 rad) and transplanted (i.v.) next day with 1 × 10<sup>7</sup> viable bone marrow cells of B6 or BALB.B origin (sex and strain combination are specified for each individual experiment). Two weeks later, they also received a DLI consisting of 6 × 10<sup>7</sup> splenocytes. In some cases for preparing donor lymphocytes, female B6 mice were presensitized against minor H antigens, HY, by i.v. injection of 5 × 10<sup>6</sup> BM cells from syngeneic male B6 mice (21). The day after the DLI, mice were vaccinated as described above.

CFSE dilution assay

To trace donor cell proliferation in vivo, the splenocytes of HY-primed B6 female mice were labeled with CFSE, according to the protocol described by Lyons-Parish et al. (32). In brief, after extensively washing with PBS, the cells (2 × 10<sup>7</sup> cells/ml in PBS) were incubated with 2 μM fluorescent dye CFSE (Molecular Probes) for 8 min at room temperature and resuspended. Unbound CFSE was quenched by adding extra FBS (at a final concentration of 50%). After washing with PBS, the CFSE-labeled cells were injected i.v. into male recipients (6 × 10<sup>7</sup>/mouse) and then analyzed by flow cytometry.

Flow cytometry analyses

Cells were stained with the appropriate fluorochrome-labeled mAb, or they were cultured ex vivo in the absence (Nil) or in the presence of 2 μM Tag-IV, Uty, or Dby peptides for 4 h, of which, the last 2 h were in the presence of brefeldin A. Then, cells were surface stained, fixed, and analyzed for intracellular cytokine staining as described previously (26). Unless otherwise indicated, cytokine production in the absence of stimulation (Nil) was considered as background release and subtracted from values obtained by the specific peptides.

Statistical analyses

Statistical analyses were performed using the χ<sup>2</sup> test or the two-tailed Student t test. Whenever differences were found statistically significant, p values were reported in the figures (*p < 0.05; **p < 0.005; and ***p < 0.001).

Results

Syngeneic and allogeneic peptide-pulsed DCs elicit comparable peptide-specific CD8<sup>+</sup> T cell priming

Tumor-specific vaccination by peptide-pulsed DC following allogeneic hematopoietic cell transplantation is critical for prolonging disease-free survival (26) and optimizing tumor-specific T cell priming (R. Hess Michelini, T. Manzo, M. Freschi, V. Basso, M. Rocchi, E. Simpson, M. Bellone, and A. Mondino, submitted for publication). Whether the immunogenicity of DC-based vaccines is influenced by concomitant alloreactive T cell responses remained to be determined. We initially investigated whether the immunogenicity of BMDC pulsed with a nominal Ag (SV40 large T Ag-derived immunodominant Tag-IV peptide) might benefit or suffer from the simultaneous presentation of minor H antigens. To test this hypothesis, we used female B6 mice vaccinated with Tag-IV-pulsed DC either sex-matched (syngeneic) or sex-mismatched (allogeneic, naturally presenting Y-encoded Ags). Male C57BL/6 (B6) mice were similarly vaccinated for use as controls. Mice were sacrificed 1 wk later, and Tag-IV-specific splenic T cells were quantified by flow cytometry. Although Tag-IV-specific CD8<sup>+</sup> T cells were undetectable in naive mice and in mice vaccinated with unpulsed DC (data not shown and Ref. 33), a sizeable frequency of Tag-IV-specific CD8<sup>+</sup> CD44<sup>high</sup>IFN-γ<sup>+</sup> was detectable in both male and female recipients, respectively, lacking and harboring T cell precursors for HY-specific T cell effectors (Fig. 1A). Tag-IV-specific IFN-γ responses were quantified in between five and nine individual mice in each group: we observed that male mice vaccinated with either
male or female DC, or female mice vaccinated with either female (syngeneic) or male (allogeneic) DC, revealed comparable frequencies of Tag-IV–specific T cells (Fig. 1B). This was despite the male DC expressing both MHC class I- and MHC class II-restricted HY Ags (30). These results suggest that simultaneous presentation of Tag-IV and HY Ags does not influence vaccine immunogenicity.

**DC-based vaccination preserves immunogenicity in the context of allotransplantation**

We next investigated whether vaccine immunogenicity might differ in the context of syngeneic or allogeneic HSCT and DLI (a schematic representation of the experimental scheme is depicted in Fig. 2A). B6 male hosts (CD45.2+) were sublethally irradiated (600 rad) and the next day were transplanted with 1 × 10^7 bone marrow cells from F1 congenic B6 mice (CD45.1+CD45.2+). Sublethal irradiation was used to obtain mixed chimeraism at the time of DLI infusion such that both male and female APC would be operational. Two weeks after HSCT, ~50% of bone marrow-derived cells were of donor origin (data not shown and Ref. 26). Mice then received a DLI of 6 × 10^6 BM cells from F1 congenic B6 females (CD45.1+CD45.2+). Two weeks later, mice received a CFSE-labeled DLI (CD45.1+, 6 × 10^6/mouse) from either female donors either unmanipulated (fDLI) (B, C) or presensitized against male Ag (fpDLI) (D, E). Mice were then vaccinated with Tag-IV–pulsed female DC. A week later, mice were sacrificed, and splenocytes were ex vivo analyzed by flow cytometry for IFN-γ intracellular secretion following exposure to HY peptides HY/Dby, HY/Uty, or no peptide (control, Nil, depicted for fpDLI) stimulation. A, Schematic representation of experimental setting. B–E, Representative dot plots depict the percentage of CFSE^low/IFN-γ^ cells gated on CD8+ T cells. B, The percentage of CD44+/IFN-γ^ cells within the splenic CD8+ T cell population of individual mice of different four groups. p Values did not reach statistical significance (fDC/mDC in female: p = 0.06; mDC/fDC in male: p = 0.27; fDC in female/mDC in male: p = 0.45; mDC in female/mDC in male: p = 0.308).

II (H2Aβ/Dby)-restricted peptides are known (34). Soon after infusion into male hosts, a significant percentage of CD4+ and CD8+ T cells (up to 20 and 47%, respectively) within both mDLI and fDLI populations revealed a lower CFSE content (Fig. 2B, 2C). This donor sex-independent expansion was also found in tumor-bearing TRAMP mice (26) and most likely reflects the ability of the cells to proliferate in the lymphopenic environment (35). Indeed, it was similarly observed upon cell infusion in female mice preconditioned by total body irradiation, reported to favor donor cell engraftment (36), and not in nonpreconditioned control ones (data not shown). Despite the expansion of T cells from both male and female DLI donors, HY Ag-specific (H2Aβ/Dby and H2Dβ/Uty) T cells remained below the level of detection, because of central deletion in males and their extremely low frequency in females (Fig. 2G), as expected (34).
additional set of mice received a DLI from female donors presensitized to male Ags (fpDLI) to increase the frequency of HY-specific CD4+ and CD8+ T cells (21) and to facilitate their detection following infusion in male mice (26) (Fig. 2D, 2E). When an fpDLI was given to male recipients, both CD4+ and CD8+ T cells were enriched for cells of donor origin (data not shown and Fig. 2F, respectively) and for fast-dividing cells identified by a CFSE low content (60–80% of CFSE+ cells), which likely reflected HY-specific T cells reactivation in vivo (Fig. 2D, 2E). In line with this interpretation, CD4+ and CD8+CFSElow T cell populations were found to contain significant proportions of HY Dby- and Uty-specific T cells capable of IFN-γ secretion upon ex vivo stimulation (Fig. 2G). As expected, CFSElow cells were not enriched for when fpDLI were infused in female hosts (data not shown and Ref. 26), in which IFN-γ–producing cells remained below the level of detection (Fig. 2G). Thus, HY-specific T cells contained within the DLI of minor H antigens mismatched donors are able to recognize endogenously expressed recipient minor H antigens and undergo rapid T cell expansion and differentiation into IFN-γ–secreting cells.

We next enumerated Tag-IV–specific T cells in the presence or absence of mismatch of minor H antigens between a DLI donor and recipient. As in the case of HY-specific effectors, Tag-IV–specific CD8+ T cells were also found within the cell populations with a CFSE low content in recipients of fpDLI, fDLI (Fig. 3A), and mDLI (data not shown). Although the frequency of Tag-IV IFN-γ–secreting cells was significantly lower in recipients of fpDLI when compared with fDLI (Fig. 3B), absolute numbers of Tag-IV–responding CD8 T cells between groups were slightly higher in fpDLI than in fDLI recipients (98,296 ± 72,285, 45,424 ± 28,488, and 42,065 ± 25,500, respectively, and 54,343 ± 21,748 when fpDLI were infused in female hosts) (Fig. 3C). This was explained by a higher engraftment of donor-derived CD8+ T cells in fpDLI recipients when compared with fDLI and mDLI recipients (Fig. 2F). Splenocytes of mDLI, fDLI, and fpDLI recipients were also stimulated in vitro with the Tag-IV peptide and tested for cytotoxicity. Following the in vitro culture, Tag-IV–specific CD8+ IFN-γ–secreting T cells were found to be enriched regardless of the origin of the DLI (data not shown) and to exert comparable cytotoxicity against Tag-IV–pulsed target cells (Fig. 3D). Thus, posttransplant vaccination of mDLI, fDLI, and fpDLI male recipients induces comparable Tag-IV–specific IFN-γ–secreting T cells, capable of cytotoxic activity.

Tag-IV–directed CD8+ T cell responses are of a high-affinity nature, because the large SV40 T Ag is a virus-derived foreign Ag. To investigate whether alloreactivity could either promote or suppress T cell responses against self-Ags against which only low-affinity T cells would be available, HSCT-transplanted male mice received either an mDLI or an fpDLI and a vaccine composed of male or female DC, respectively, pulsed with a peptide derived from the six-transmembrane epithelial Ag of the prostate (STEAP), found in human and prostate cancer, and previously used in vaccination strategies (37, 38). Results depicted in Fig. 3E and 3F indicate that albeit very low, possibly because of the nature of the Ag, vaccination–elicited STEAP-specific IFN-γ–producing CD8+ T cells were induced regardless of HY disparity. These data also support the notion that vaccination against both a foreign and a self-Ag elicits CD8+ T cell priming in the presence (fDLI and fpDLI) or in the absence (mDLI) of alloreactive T cell responses.

We next addressed possible helper function by HY/Dby-specific CD4+ T cells. Copulsing female-derived DC with the Dby and Tag-IV peptides did not improve either the frequency (Fig. 4A) or the total number (data not shown) of Tag-IV–specific effectors, over those induced by Tag-IV–pulsed DC. Likewise, the almost complete removal of donor CD4+ T cells from an fpDLI (a possible source for intermolecular help) abrogated responses to Dby (Fig. 4B, 4E) and resulted in reduced responses to Uty (Fig. 4C, 4F) without, however, diminishing and rather improving the response to Tag-IV-DC (Fig. 4D, 4G). Taken together, these data indicate that DC pulsed with MHC class I peptides derived from either a tumor/virus foreign model Ag (Tag-IV) or a self-Ag (STEAP) prime CD8+ effector T cells regardless of donor/host disparities in HY minor H antigens.

Potential minor H antigen-specific T cell responses did not contribute to vaccine-mediated T cell priming

To further address the impact of minor H antigen-specific T cell responses on DC-induced T cell priming, we took advantage of MataHari and Marilyn transgenic T cells expressing TCR specific...
male and female host mice, despite the fact that the former and not the latter contained substantial numbers of alloreactive CD4+ and CD8+ T cells. Taken together, these results demonstrate that the provision of stronger minor H antigen-restricted CD4+ (Marylin) or CD8+ (MataHari) T cell help neither contributes to nor interferes with DC-mediated CD8+ T cell priming, supporting the notion that minor H antigen- and vaccine-specific T cells can be independently primed in peripheral lymphoid organs.

Vaccine-mediated T cell priming is preserved in the presence of multiple minor H antigen mismatch disparities and signs of systemic GVHD

We next investigated whether vaccine immunogenicity was preserved also in the presence of multiple minor H antigen disparities, a situation more clinically relevant. BALB.B and B6 mouse strains are matched for major H Ags, but they express different allelic variants of many minor H antigens including H7, H13, and H4 (39). B6 male mice were conditioned by total body irradiation (600 rad) and transplanted with hematopoietic precursors and DLI from female BALB.B mice (Ly9.1+), presensitized against recipient B6 minor H antigens, including HY, by an i.v. injection of 5 × 10^6 male B6 cells (fpDLI BALB.B). The day after DLI, the recipient B6 males were vaccinated with female BALB.B Tag-IV-pulsed DC. A DLI from presensitized BALB.B donors gave a strong immunodominant H7-specific CD8+ T cell response. This indeed dominated the H13- and the HY Uty-specific responses that were undetectable in this transplant setting (Fig. 6A–C) and that were instead enumerable in recipients of hematopoietic precursors and fpDLI of B6 origin (Fig. 6B). This was not the case for Y-encoded class II-restricted IFN-γ-producing CD4+ T cell responses specific for the Dby peptide that were found in mice transplanted with hematopoietic precursors and fpDLI of both BALB.B or B6 origin (Fig. 6C). Likewise, B6 male recipients of fpDLI from either B6 or BALB.B donors supported a comparable expansion of CD8+, IFN-γ+ Tag-IV-specific T cells, which was not statistically different in several independent experiments (Fig. 6D). Also, in this case, Tag-IV–specific CD8+ T cells were endowed with cytotoxic activity (data not shown) and proved efficacious against established prostate adenocarcinomas (R. Hess Michelini, T. Manzo, M. Freschi, V. Basso, M. Rocchi, E. Simpson, M. Bellone, and A. Mondino, submitted for publication).

The combination of BALB.B into B6 DLI transplantation evokes signs of clinical GVHD, the major clinical complication of allotransplantation, in tumor-bearing mice (R. Hess Michelini, T. Manzo, M. Freschi, V. Basso, M. Rocchi, E. Simpson, M. Bellone, and A. Mondino, submitted for publication) but not in tumor-free mice analyzed in this study. This is possibly due to the absence of the immunodominant H60 minor H antigen (20, 40) and/or a predisposing inflammatory condition. To determine whether clinical GVHD might impact on vaccine immunogenicity, we performed the reciprocal transplantation of fB6 HSCT and DLI into mBALB.B and then vaccinated mice with Tag-IV–pulsed fB6 DC. One week after vaccination, H60-specific CD8+ T cells dominated the minor H antigen-specific T cell response, as expected (20), and Tag-specific IFN-γ+ effector T cells were enumerable (Fig. 6E). By 2 wk from vaccination, both H60 and Tag-specific CD8+ T cell effectors remained detectable, albeit in lower frequency. At later times, all the mice developed clinical GVHD (weight loss, tremor, and conjunctivitis) and died within 1 mo of lymphocyte infusion (data not shown). Thus, transplant settings prone to GVHD do not impede DC-induced CD8+ T cell priming but might exert some immunosuppressive function and interfere with long-term persistence of the cells, as previously reported in myeloablative allo-transplantation (18).
FIGURE 5. Potent minor H antigen-specific T cell responses do not contribute nor are detrimental to vaccine-mediated T cell priming. Female or male B6 mice (CD45.2+) were subjected to nonmyeloablative conditioning (600 rad) and transplantation of female B6 bone marrow cells (CD45.2+), as described in Fig. 2. Two weeks later, mice received a DLI composed of 58 × 10^6 splenocytes from congenic male donors (CD45.1+) mixed with 10^6 Thy 1.1 Marilyn CD4 T cells, 10^5 Thy1.1 MataHari CD8 T cells, or both. Next day, the mice were vaccinated with Tag-IV–pulsed DC. One week later, splenocytes from individual mice of each group were analyzed ex vivo by flow cytometry after staining with Thy1.1 mAb (A, B) and for IFN-γ intracellular secretion following HY/Dby (C–E), HY/Uty (F, G), and Tag-IV (H, I) peptide restimulation. Unpulsed splenocytes (Nil) were analyzed as control. C–E, HY/Dby-specific responses (triangles) are depicted following electronic gating on Thy1.1“CD47“/Vb6” cells. C, F, and G, HY/Uty-specific responses are depicted following electronic gating on Thy1.1“CD87“/Vb8.3” cells. H and I, Percentages (H) and total numbers (I) of Tag-IV–specific IFN-γ-producing cells are depicted following electronic gating on CD8“CD45.1“ cells (i.e., DLI cells). *p < 0.05.

To further analyze this possibility in nonmyeloablative settings, we measured long-term persistence of DC-induced T cells in B6→B6 and BALB.B→B6 settings (the latter with possible subclinical GVHD). B6 recipients of B6mDLI, B6fpDLI, or BALB.B fpDLI were analyzed 6 wk after vaccination for the presence of Tag-IV–specific IFN-γ“ cells by ex vivo intracellular staining. IFN-γ“ cells could be retrieved in recipients of B6mDLI and B6fpDLI but not BALB.B fpDLI (Fig. 6F).

Taken together, these results demonstrate that although DC-mediated CD8 T cell priming is preserved in the presence of concomitant activation of alloreactive T cells specific for ubiquitously expressed multiple minor H antigens, long-term persistence of the cells might be hindered in mice with large numbers, or particularly strong, for example, H60 minor H antigen disparities.

Discussion

Active vaccination of allogeneic HSCT recipients provides a safe strategy to overcome suboptimal immunocompetence of transplanted patients and has the potential to boost the GVT response without exacerbating GVH responses. Although vaccine-induced immunity is becoming advisable in a number of clinical conditions (41–43) whether alloreactive T cell responses, frequently observed in the context of allotransplantation, might interfere with vaccine immunogenicity remained to be determined. Although our previous study provided the proof of principle that minor H antigen- and tumor-specific T cells could team up for prostate cancer rejection and highlighted the importance of postransplant tumor-specific vaccination (26), it focused whether the alloresponse could perform a helper function and enhance vaccine-induced responses or instead impinge on vaccine immunogenicity. We found that Tag-IV–pulsed DC induced comparable CD8“ T cell priming in female mice when female or male DC presented the peptide or when Tag-IV was presented together with the Dby peptide, eliciting HY-specific T cell help. These data suggest that the concomitant presentation of natural or exogenously provided Y-encoded minor H antigen-specific epitopes to allogeneic T cells does not promote or interfere with T cell priming against a nominal Ag. This is of importance because donor-derived DC, most likely preferred to host-derived DC as APC, are likely to cross-present host-restricted minor H antigens (44).

In this study, we have investigated this issue by 1) comparing the efficacy of DC-based vaccine presenting a nominal Ag alone or together with peptides from the Y-encoded minor H antigens, HY and 2) by enumerating minor H antigen-specific and vaccine-specific T cell responses following allotransplantation and asking whether the alloresponse could perform a helper function and enhance vaccine-induced responses or instead impinge on vaccine immunogenicity. We also compared the ability of donor cells from unmanipulated B6 male mice (mDLI), from fDLI mice, or from HY-primed B6 female mice (fpDLI) (21) transplanted into male recipients to respond to female DC pulsed with the Tag-IV peptide. We found comparable frequencies of Tag-IV–reactive donor cells, regardless of the presence of graded HY-specific T cell responses (absent in male into male settings, below the level of detection in fDLI recipients, detectable by flow cytometry in fpDLI recipients). Furthermore, depletion of alloreactive CD4“ T cells reduced alloreactivity but not vaccine-induced CD8“ T cell priming, supporting the notion that alloresponses do not perform helper functions in this experimental setting. Similar results were observed in the case of multiple minor H antigen disparities, which evoked more potent...
polyclonal alloresponses. When donor DLI cells were infused into multiple minor H antigen-disparate recipients (fpBALB.B into B6 and B6 into BALB.B), DC pulsed with the Tag-IV peptide initially induced the generation of CD8+ T cells to an extent comparable to those found in fully syngeneic settings, also despite clinical signs of GVHD. Nevertheless, we found that alloresponses concurrent to vaccination limit the long-term persistence of vaccine-induced CD8+ T cells. Thus, it is possible that alloreactivity and GVHD exert different effects, depending on the nature of the Ag and/or the responding T cells (naive and memory). We speculate that the use of donor-matched DC and appropriate patient boosting might overcome these hurdles.

Several vaccination strategies, including autologous as well as heterologous DC, recombinant proteins or peptides in adjuvant, DNA-mediated in vivo transfection, or use of exosomes, are currently being considered for clinical practice. Although we have evaluated only DLI donor-matched DC as a possible strategy of vaccination, the results predict that immunogenicity should be preserved with other vaccination strategies, but it would be important to extend this study to define optimal strategies to boost pathogen- or tumor-specific immunity following transplantation. In addition, it will be critical to evaluate the impact of alloreactive T cell immunity on long-lasting Ag/vaccine-mediated T cell memory. Experiments are currently ongoing to address these unsolved issues.
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The authors have no financial conflicts of interest.

References