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Novel Mechanisms Underlying the Immediate and Transient Global Tolerization of Splenic Dendritic Cells after Vaccination with a Self-Antigen

Adam M. Farkas and Olivera J. Finn

Dendritic cells (DCs) are important orchestrators of the immune response, ensuring that immunity against pathogens is generated, whereas immunity against healthy tissues is prevented. Using the tumor Ag MUC1, we previously showed that i.v. immunization of MUC1 transgenic mice, but not wild-type, with a MUC1 peptide resulted in transient tolerization of all splenic DCs. These DCs did not upregulate costimulatory molecules and induced regulatory T cells rather than effector T cells. They were characterized by suppressed expression of a cohort of pancreatic enzymes not previously reported in DCs, which were upregulated in DCs presenting the same MUC1 peptide as a foreign Ag. In this article, we examined the self-antigen–tolerized DC phenotype, function, and mechanisms responsible for inducing or maintaining their tolerized state. Tolerized DCs share some characteristics with immature DCs, such as a less inflammatory cytokine/chemokine profile, deficient activation of NF-κB, and sustained expression of SOCS, suppressor of cytokine signaling; Tg, transgenic; Treg, regulatory T cell; WT, wild-type.

Dendritic cells (DC) can induce potent immunity by presenting foreign Ags derived from infectious agents or abnormal self-antigens derived from tumors (1). However, DCs can also maintain tolerance to self-antigen derived from healthy tissues, environmental agents, and commensal microbiota (2, 3). Ablation of DCs during homeostasis results in a breakdown of CD4+ T cell tolerance with ensuing fatal autoimmunity (4, 5). Multiple mechanisms assist DCs in maintaining peripheral tolerance to self-antigen, such as low expression of MHC class II (MHC II), costimulatory molecules CD40, CD80, and CD86, production of anti-inflammatory molecules such as IL-10, IDO, and retinoic acid (RA), exposure to low Ag dose, and expression of cell-surface molecules such as DEC-205 and CD103 that mark DCs that preferentially prime regulatory T cells (Tregs) (2, 3, 6–8).

Vaccines designed to induce immunity against tumors often use Ags that are closely related to self-antigens or are a combination of normal (self) and abnormal (foreign) epitopes, resulting in a suboptimal immune response (9). Immune responses to vaccines are controlled by many different mechanisms in the periphery. Better understanding of these mechanisms is important for designing vaccines that elicit the desired outcome: strong, protective immunity against pathogens and cancer, or tolerance of self-antigens to avoid autoimmunity.

We have been studying immune responses to cancer vaccines based on peptides derived from the human tumor Ag MUC1 that range from those closest to self (i.e., peptides with no sugar moieties) to those representing “abnormal self” (i.e., peptides decorated with tumor-specific, O-linked GalNAc adducts) (10, 11). We recently published that i.v. immunization of human MUC1 transgenic (MUC1.Tg) mice with an unglycosylated human MUC1 peptide (MUC1p) resulted in the transient tolerization (between 24 and 72 h) of the entire splenic DC population that failed to upregulate costimulatory molecules, lacked motility, and preferentially primed Foxp3+ Tregs. These vaccine-tolerized DCs also differed in their gene expression profile from vaccine-activated (immunogenic) DCs by suppressed expression of a group of “pancreatic” enzymes, specifically trypsin and carboxypeptidase B1 (CPB1) (12), which we proposed as predictive biomarkers of vaccine outcome. These results were consistent with the previously observed low level of vaccine-induced immunity against MUC1p in MUC1.Tg mice (10, 13, 14).

In this study, we elucidated several molecular and cellular pathways that characterize DCs tolerized by vaccination with MUC1p as a self-antigen. We show that, in contrast with endogenous splenic DCs recovered from vaccinated wild-type (WT) mice where MUC1p is a foreign Ag, splenic DCs from vaccinated MUC1.Tg mice where MUC1p is a self-antigen show inhibition of NF-κB whereas simultaneously increasing signaling through phospho-STAT3. As a result, they produce fewer inflammatory cytokines and chemokines. We also found that these DCs induce...
expression of aldehyde dehydrogenase 1/2 (Aldh1/2), the enzyme catalyzing the final step in the biosynthesis of immunosuppressive RA. Inhibition of Aldh before immunization of MUC1.Tg mice with MUC1p reverses DC tolerance as seen by increased expression of trypsin and CPB1 to levels observed in MUC1p vaccinated WT mice.

We also examined the functional consequence of the suppressed expression of “pancreatic” proteases in tolerized DCs. We show that increased expression by DCs of metallopeptidases, such as CBP1, is required for optimal proliferation of MUC1p-specific CD4+ T cells, suggesting a role for these enzymes in increasing efficiency of Ag processing and presentation. We found that trypsin expression, in contrast, is required for degradation of the extracellular matrix (ECM), facilitating DC motility. Suppressed expression of these enzymes in tolerized DCs contributes to their low T cell stimulatory capacity and low motility, resulting in low or no response to MUC1p when it is presented as a self-antigen.

Materials and Methods

Mice

C57BL/6 (WT) mice were purchased from The Jackson Laboratory. MUC1.Tg mice were originally obtained from Dr. Sandra Gendler (Mayo Clinic, Scottsdale, AZ) (15) and subsequently bred at the University of Pittsburgh. All colonies were housed under specific pathogen-free conditions. Experiments were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

Peptides

A 100-mer MUC1p represents 5 repeats of the 20-aa sequence HGVTSA-PDTRPAPGSTAPPA from the MUC1 VNTR region. It was synthesized as described previously by the University of Pittsburgh Genomics and Proteomics Core Laboratories (13).

DC culture and vaccines

Bone marrow–derived DCs (BMDCs) were generated according to a previously reported protocol (10). In brief, female C57BL/6 mice (Jackson) were sacrificed and their femurs and tibiae harvested. Marrow was flushed with RPMI 1640 and cells were passed through a 70-μm strainer and pelleted before RBC lysis using ACK buffer. Cells were resuspended in AIM-V (Life Technologies), counted, and plated at 1.25 × 10^6 cells/ml in AIM-V containing 20 ng/ml GM-CSF (Miltenyi Biotec). On days 3 and 5, half of the media was replaced with fresh AIM-V containing 20 ng/ml GM-CSF (Miltenyi Biotec). On days 3 and 5, of culture, DCs were harvested with 2 mM EDTA, counted, and (when indicated) loaded with 50 μg/ml MUC1 100-mer and matured overnight with 30 μg/ml polysinosine-polycytidylic acid and poly-l-lysine (poly-ICl; Hiltonol), a generous gift from Oncovir. On day 7, cells were harvested as described earlier. For immunizations, day 7 DCs were washed and resuspended in sterile PBS. Mice were immunized i.v. via the tail vein with 1 × 10^6 DCs.

DC protease inhibition

BMDCs were cultured overnight in the presence of 10 or 100 μM N-orthophenanthroline (Sigma) or 100 μM trypsin inhibitor from chicken OVA (Sigma), 30 μg/ml MUC1p 100-mer, and 10 ng/ml GM-CSF (Milenyi). The following day, MUC1p-specific TCR.Tg CD4+ T cells labeled with 5 μM CFSE (Invitrogen) were added to culture at a 5:1 CD4/DC ratio. After 5 d of coculture, cells were harvested and T cell proliferation determined by CFSE dilution on an LSR II cytometer (BD).

Aldh inhibition

Mice were given 100 mg/kg 4-diethylaminobenzaldehyde (DEAB) (Sigma) in 100 μl DMSO (i.p.) to inhibit Aldh1 activity as previously described (16). Immediately afterward, mice were immunized with unloaded DCs or MUC1p-loaded DCs as described earlier.

Quantitative RT-PCR

RNA was extracted from bead-isolated CD11c+ splenic DCs (Milenyi) using an RNeasy Mini kit (Qiagen) according to the manufacturer’s protocol. Reverse transcription was performed using Oligo(dT) primers and SuperScript III reverse transcriptase (Invitrogen). cDNA was amplified using the following primers: CCR2 (forward: 5’-CTCGAAAGCACAGAGAGGC-3’; reverse: 5’-CCACACCCCAATGGACTACA-3’), suppressor of cytokine signaling 1 (SOCS1; forward: 5’-CCAGCTGGCCTCACTAC-3’; reverse: 5’-GGCGAGGCGGTTCAAG-3’), Aldh1 (forward: 5’-CTTCGGCGGCGGTTCAAG-3’; reverse: 5’-GGCGAGGCGGTTCAAG-3’), or Rb X-p-STAT3 (Ser277) (Santa Cruz), and Rb X-p-p65 (93H1) (Cell Signaling).

Cytokine array

DCs were MACS (Miltenyi) isolated from spleens 24 h post-MUC1p immunization of WT and MUC1.Tg mice. DCs were then cultured overnight in the presence of 500 ng/ml LPS (Sigma) and GolgiPlug (BD). The following day, cells were lysed and total DC protein quantified for cytokine/chemokine expression using a Proteome Profiler Array (R&D Systems). Densitometry was conducted on blots using ImageJ (National Institutes of Health).

Gelatin degradation assay

DCs were MACS isolated (Miltenyi) from spleens 24 h post immunization of WT and MUC1.Tg mice. Cells (1 × 10^6) were immediately plated into eight-well chamber slides coated with FITC-gelatin using the QCM Gelatin Intradopedia Kit (Millipore). Images were captured on an Olympus Provis fluorescent microscope with a 40× objective. Areas of FITC-gelatin degradation were quantified at 4 and 24 h using binary thresholding in ImageJ (National Institutes of Health). Where indicated, DCs were cultured for indicated time points in the presence of 10 μM N-orthophenanthroline and/or 100 μg/ml trypsin inhibitor from chicken OVA (Sigma).

Results

Immunization with a self-antigen tolerizes DCs resulting in decreased chemotactic potential and a novel transcriptional signature

As we previously reported, splenic DCs recovered from MUC1.Tg mice immunized i.v. with MUC1p express lower levels of MHC II, CD40, and CD86, compared with DCs from WT mice immunized with the same peptide. They also preferentially prime naive CD4+ T cells into Tregs versus IFN-γ–producing effector T cells. In addition, there are fewer DCs 24 h postimmunization in the spleens of MUC1.Tg mice compared with WT (12). Low costimulatory molecule expression and priming of Tregs are hallmarks of immature DCs (iDCs) (2, 17, 18). We now show, using a cytokine blot array, that DCs recovered from MUC1p-immunized MUC1.Tg mice produce lower levels of proinflammatory cytokines (i.e., IL-1a, TNF-α, IL-6) relative to WT (Fig. 1A, 1B). These tolerized DCs also produced less chemokines such as MIP-1β, RANTES, and IL-17, compared with WT mice. In fact, DCs from MUC1p-immunized MUC1.Tg mice produce very low levels of IL-17, compared with WT mice (Fig. 2A), which may contribute to the functional impairment of these DCs (21, 22).

SOCS1/3 and histone deacetylase 11 (HDAC11) have been implicated in suppressing proinflammatory cytokine production and promoting IL-10 expression, respectively, in DCs (21, 22). Although SOCS1 expression was decreased by ∼50% in DCs recovered from MUC1p-vaccinated WT mice, as would be expected of a DC developing an immunostimulatory phenotype, its expression was unchanged in DCs from immunized MUC1.Tg mice (Fig. 2A). HDAC11 levels in immunized WT or MUC1.Tg mice

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were not significantly different (Fig. 2B). This suggested that these two transcription factors, which play a role in DCs that are actively immunosuppressive, do not have the same role in either inducing or maintaining the transient tolerized state of DCs from vaccinated MUC1.Tg mice. We also examined the transcription factor Zbtb46 (zDC) that has recently been identified as a conventional DC-lineage marker whose expression is inversely correlated with DC maturational status (5, 23, 24). Expression of zDC was ∼2.5-fold higher in tolerized DCs recovered from immunized MUC1.Tg mice compared with WT (Fig. 2C). Expression of zDC and CCR2 decreased after stimulation of DC with poly-ICLC ex vivo demonstrating that these DCs are not refractory to maturation (Fig. 2C, 2D).

Increase in phospho-STAT3 and decrease in NF-κB p65 in tolerized DCs

Activation of STAT3 characterizes DCs that are unable to prime efficient Th1 responses and is considered a negative regulator of DC function (25, 26). Splenic DCs isolated from MUC1p-immunized MUC1.Tg mice upregulated phospho-STAT3 24 h postimmunization (Fig. 3A). Conversely, NF-κB pathway activation that results in degradation of IκBα and phosphorylation of p65 is critical for DC phenotypic maturation and immunogenic function (27, 28). Tolerized DCs from immunized MUC1.Tg mice expressed less phospho-p65 (Fig. 3B) with a concurrent increase in total IκBα (Fig. 3C).

Aldh expression is induced in splenic DCs after immunization with self-antigen and is required for maintenance of the tolerized state

Aldh catalyzes the final biosynthetic step in RA production in multiple populations of DCs residing in the gut, skin, and lung (29). RA plays an important role in maintaining tolerance to oral and commensal-derived Ags (30). We found that splenic DCs from immunized MUC1.Tg mice expressed both Aldh1 and Aldh1/2 (Fig. 4A–C), whereas those recovered from immunized WT mice did not. This Aldh was biologically active as determined by its ability to oxidize aminoacetaldehyde to aminoaacetate (data not shown). Inhibition of Aldh1 activity before MUC1p vaccination of MUC1.Tg mice using the specific inhibitor DEAB led to reconstitution of normal expression of trypsin and CPB1 to levels observed in WT mice immunized with MUC1p as a foreign Ag (Fig. 4D). These data implicate Aldh activity and likely the subsequent production of RA as being required for self-antigen vaccine-induced tolerization of splenic DCs.

Pancreatic enzyme expression in DCs is associated with DC function

Expression of pancreatic proteases by splenic DCs is coordinately regulated such that immunization of WT mice with MUC1p results in a 10- to 40-fold increase in their expression by 24 h, whereas the same immunization in MUC1.Tg mice results in their profound suppression. We found that this expression profile is a predictive biomarker of DC immunogenicity and the ensuing T effector or Treg responses (Supplemental Fig. 2) (10, 12). We explored the potential function of these enzymes, focusing on CBP1 and trypsin as representatives of two main families of enzymes, metalloproteases (represented by CBP1) and serine proteases (represented by trypsin). We hypothesized that both might be involved in the processing and/or presentation of MHC II–restricted peptides derived from MUC1p, because a number of serine, aspartyl, and cysteine peptides, as well as asparaginyl endopeptidase, have been reported to be involved in this pathway (31). We loaded
BMDCs overnight with MUC1p in the presence of n-orthophenanthroline, an inhibitor of all metallopeptidases including CPB1, or a trypsin-specific inhibitor derived from chicken OVA. Neither inhibitor had an effect on DC viability as determined by Annexin V/propidium iodide staining (Supplemental Fig. 3). MUC1p-loaded DCs were then cocultured for 3 d with MUC1p-specific TCR.Tg CD4+ T cells (10) labeled with CFSE to quantify proliferation. Fig. 5A shows that inhibition of the metallopeptidase CPB1 inhibited CD4+ T cell proliferation, suggesting less efficient Ag processing and/or presentation. Inhibition of trypsin did not have any effect.

DCs in lymphoid and nonlymphoid tissues are known to remodel ECM proteins such as collagen, elastin, and fibronectin by using secreted or membrane-bound proteases (32). Because the optimal pH of both trypsin and CPB1 activity are within physiologic range (7.5, and 7.9, respectively) (33, 34), we cultured DCs and assayed the supernatants for secreted trypsin and CPB1 by Western blotting. Both proteins were secreted by 40 h with the majority of CPB1 being the 45-kDa proenzyme form. There were multiple trypsin bands detected with at least one of them corresponding to an active 23-kDa enzyme (Fig 6A). To determine whether there was a difference in the ability to degrade ECM between DCs recovered from MUC1p-immunized WT mice that upregulate these enzymes and DCs from MUC1p-immunized MUC1.Tg mice that do not, we plated both DC populations on FITC-gelatin–coated slides and examined them 4 h later. The regions of the slide devoid of FITC fluorescence correspond to the amount of gelatin degraded by the DCs. DCs from immunized WT mice were able to degrade more FITC-gelatin compared with those from immunized MUC1.Tg mice (Fig. 6B). When we then cultured DCs on FITC-gelatin in the presence of a specific trypsin inhibitor derived from chicken OVA, the ability of DCs from immunized WT mice to degrade the matrix was impeded (Fig. 6C).

**Discussion**

We previously showed that immunization with a self-antigen inhibits, within the first 24 h, the maturation and immunogenicity

**FIGURE 2.** SOCS1 and HDAC11 do not contribute to the tolerized state, whereas sustained expression of Zbtb46 and CCR2 maintains an iDC phenotype. WT and MUC1.Tg mice (n = 3/group) were immunized (i.v.) with DC:MUC1p (MUC1p; 1 $\times$ 10^6 cells) or unloaded DCs (Ctrl). One day later, isolated DCs were pooled for quantitative RT-PCR for SOCS1 (A) or HDAC11 (B). Bars represent mean ± SEM after normalization to respective control vaccinations and are representative of three independent experiments. (C and D) Mice were immunized with MUC1p as in (A); splenic DCs were isolated at 24 h and were left untreated (No Tx) or treated with 30 μg/ml poly-ICLC for 4 h before performing quantitative PCR for zDC (C) or CCR2 (D).

**FIGURE 3.** Deficient NF-κB activation and enhanced STAT3 signaling in tolerized DCs. WT and MUC1.Tg mice (n = 3/group) were immunized (i.v.) with DC:MUC1p (MUC1p; 1 $\times$ 10^6 cells) or unloaded DCs (Ctrl). Twenty-four hours later, isolated splenic DCs were pooled and whole-cell lysates were analyzed by Western blots for phospho-STAT3 (A), phospho-p65, (B) or total IkBa (C). β-Actin is shown as a loading control for each blot. Data are representative of two to three independent experiments for each molecule.
of all splenic DCs in the target lymphoid organ and not just the relatively few DCs that present the Ag (12). This rapid tolerization of all DCs is likely due to the increased frequency of pre-existing self-antigen–specific Tregs (12, 35). In this article, we report that early and transiently tolerized DCs have a less inflammatory cytokine/chemokine profile that results in impaired DC-mediated inflammation.

FIGURE 4. Production of Aldh by tolerized DCs. WT and MUC1.Tg mice (n = 3/group) were immunized (i.v.) with DC:MUC1p (MUC1p; 1 × 10^6 cells) or unloaded DCs (Ctrl). Twenty-four hours later, isolated DCs were pooled and quantitative RT-PCR (qRT-PCR) performed for Aldh1 (A). Concurrently, whole-cell lysates from recovered DCs were Western blotted for Aldh1/2 (B), and bands were quantified via densitometry (C). (D) MUC1.Tg mice were immunized as described earlier immediately after injection of the Aldh inhibitor DEAB (MUC1p+DEAB). Twenty-four hours later, isolated splenic DCs were pooled and qRT-PCR performed for Trpsin and CPB1. Bars represent mean ± SEM after normalization to respective control vaccinations (A, D). Data are representative of two (D) and three (A–C) independent experiments.

FIGURE 5. Activity of the metallopeptidase CPB1 in DCs is required for optimal proliferation of MUC1p-specific CD4+ T cells. (A) BMDCs were cultured overnight in the presence of 30 μg/ml MUC1p and 30 μg/ml poly-ICLC with or without protease inhibitors (no inhibition, 10 or 100 μM orthophenanthroline, and 100 μg/ml trypsin inhibitor, respectively). The following day, naive CFSE-labeled, MUC1p-specific CD4+ T cells were added at a 1:5 DC/T cell ratio and cultured for 3 d. Cells were then harvested and percent proliferation determined by CFSE dilution. Histograms are from one experiment and are representative of two independent experiments. (B) Quantification of pooled data from two independent experiments. Bars represent mean ± SEM with **p ≤ .001, and ***p ≤ .0001.
inflammation and chemotaxis. Specifically, tolerized DCs produced less CCL2, suggesting an inability to recruit additional CD11c+CCR2+ cells. DCs have been reported to rely on β-catenin, SOCS1/3, NF-κB, RAR/RXR, HDAC11, zDC, and STAT3 to induce and sustain various anti-inflammatory programs (2, 5, 21, 36–38). We found that activated DCs isolated from MUC1p-immunized WT mice downregulated SOCS1 as expected, but tolerized DCs from MUC1.Tg mice did not increase expression, suggesting that SOCS signaling was not involved in maintaining their tolerized state. Similarly, expression of HDAC11, known to inversely correlate with IL-10 transcription (22), did not decrease in MUC1.Tg DCs and there was no increase of IL-10 relative to WT DCs (Supplemental Fig. 4).

The transcription factor Zbtb46 (zDC), a repressor of DC maturation, was expressed ~2.5-fold higher in tolerized DCs. Importantly, the expression of zDC decreased when tolerized DCs were stimulated with a TLR3 agonist directly ex vivo, indicating that these cells maintain the potential for maturation when removed from in vivo suppressive signals, and are thus only transiently tolerized. This would allow the host to control a potentially autoimmune response against self-antigen whereas preserving the ability to prime a T cell response against foreign Ag.

DCs from immunized MUC1.Tg mice did not produce more IL-10 than those from WT (Supplemental Fig. 4), yet they demonstrated increased phosphorylation of STAT3, suggesting that a paracrine source of IL-10 was contributing to their tolerized state. We previously showed that both Tregs and IL-10 actively suppress expression of trypsin and CPB1 in splenic DCs and, by extension, contribute to tolerizing those DCs. Because MUC1.Tg mice have a higher frequency of MUC1p-specific Tregs (35), Tregs and paracrine IL-10 may act on DCs to induce STAT3 phosphorylation. IL-6 also signals through STAT3; however, tolerized DCs produce less IL-6 compared with immunogenic DCs and there is no increase in the IL-6 transcript in the spleens of WT or MUC1.Tg mice postimmunization (data not shown). The cellular source of this IL-10 remains to be identified. STAT3 can also directly inhibit NF-κB signaling, as well as the production of IL-6 and TNF-α, as observed in DCs recovered from immunized MUC1.Tg mice (39).

We show for the first time, to our knowledge, that self-antigen vaccine tolerized DCs express Aldh1/2, the enzymes responsible for oxidizing retinaldehyde to immunosuppressive RA. CD11c+ CD103+ cells from the gut have been shown to produce RA for the maintenance of tolerance to commensal flora, and RA plays a role in oral tolerance to food Ags (30). RA is also produced by CD11c+ CD103− in the lung and dermis (29), demonstrating that this molecule is not restricted to the gut and that CD103 may not be a marker of RA-producing DCs. Induction of Aldh or RA by DCs in a secondary lymphoid organ in response to vaccination with a self-antigen has not, to our knowledge, been demonstrated. Importantly, we identified a novel mechanistic link between Aldh activity and the relative immunogenicity of splenic DCs after immunization with self-antigen. In the absence of Aldh activity, DCs from MUC1p-immunized MUC1.Tg mice are not tolerized, as determined by levels of trypsin and CPB1 that resemble those observed after vaccination with a foreign rather than a self-antigen. Because enhanced expression of trypsin and CPB1 by splenic DCs is a biomarker of DCs that induces effective ensuing immunity (12), we conclude that Aldh, and likely subsequent production of RA, actively suppress the development of immunogenic DCs. These data show that Aldh production in the spleen is required for vaccine-induced tolerance in secondary lymphoid organs in addition to tolerance to oral and commensal Ags.

Our finding that metallopeptidases, such as CPB1, are necessary for DCs to prime an optimal Ag-specific CD4+ T cell response suggested that CPB1 might be involved in the generation of MHC II–restricted MUC1ps. The majority of endolysosomal peptidases involved in generating peptides to be loaded onto MHC II are cathepsins, consisting of cysteine, serine, and aspartyl peptidases. It is possible that the metallopeptidase inhibitor we used, n-orthophenanthroline, also affected other susceptible enzymes involved in the MHC II pathway. However, there are currently no known metallopeptidases reported to be involved in MHC II processing and presentation, and only two carboxypeptidases, suggesting that in this particular pathway, CPB1 can be implicated as playing a role directly or indirectly in increasing the efficiency of MHC II–restricted MUC1p presentation (40, 41).

Our previous data showed that DCs recovered from MUC1p-immunized MUC1.Tg mice were less motile ex vivo compared with those from WT. We wanted to determine whether this was due to a defect in the ability of DCs to migrate through the ECM. Because ECM degradation is dependent on extracellular protease expression, we examined DCs and found that they were able to secrete trypsin and CPB1, making them candidates for remodeling...
ECM proteins. Inhibition of trypsin decreased the ability of DCs recovered from immunized WT mice (immunogenic DCs) to degrade gelatin, suggesting that it is necessary for remodeling of the ECM. In vivo it is likely that suppressed expression of trypsin in tolerized DCs from immunized MUC1.Tg mice retards their ability to migrate to T cell areas within the spleen, as the majority of DCs are initially localized in the red pulp and marginal zone (42). We also observed that the 45-kDa proenzyme form of CPB1 was secreted into DC supernatant, whereas an active 23-kDa trypsin isoform was secreted. Because trypsin activates the CPB1 zymogen (43), it is likely that both enzymes are functional in the extracellular space. The active suppression of trypsin and CPB1 expression, and the resulting effects on DC gelatinolysis and T cell priming, also constitute part of the novel phenotype of tolerized DCs.

Together, our data demonstrate that vaccination with self-antigen has a rapid and global tolerizing effect on DCs within the draining lymphoid tissue. Once tolerized, these DCs possess some phenotypic hallmarks of iDCs as well as other novel features that preclude their immunogenicity, such as expression of Aldh1/2 and phospho-STAT3, and suppressed expression of trypsin and CPB1. Understanding of early events after immunization will ultimately allow for better rational design of vaccines for cancer and autoimmune diseases.

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Disclosures

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