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Transient decrease in human peripheral blood myeloid dendritic cells following influenza vaccination correlates with induction of serum antibody

James J. Kobie^{1,*}, John J. Treanor¹, and Christopher T. Ritchlin²

¹Infectious Diseases Division, University of Rochester Medical Center, Rochester NY 14642

²Division of Allergy, Immunology, and Rheumatology, University of Rochester Medical Center, Rochester NY 14642

Abstract

Dendritic cells (DC) are critical inducers of the adaptive immune response. Extensive characterization of tissue-resident and monocyte-derived DC has revealed diverse stimulatory and regulatory actions, although the role of peripheral blood dendritic cells (PBDC) in maintaining homeostasis remains unclear. Examination of various myeloid (CD11c+CD303–) and plasmacytoid (CD11c–CD303+) DC populations in the peripheral blood of seasonal trivalent inactivated influenza vaccine recipients revealed a transient decrease in the frequency of CD11c+CD1c– myeloid DC subsets 5–10 days following vaccination, including both CD141+ and CD141– myeloid DC subsets of this population. These populations rebounded by 1 month, while plasmacytoid DC remained stable. The magnitude of the decrease in the CD141+ myeloid DC subset at d5–7 significantly correlated with the induction of influenza specific serum antibodies measured at 1 month following vaccination. These results demonstrate a mobilization of peripheral blood myeloid DC following vaccination and indicate these cells are potential biomarkers of immune response.

Keywords

dendritic cell; vaccine; influenza; human; blood; antibody

Introduction

Dendritic cells (DC) are sensors of microorganisms and induce the adaptive immune response to maintain immune homeostasis (1, 2). These cells exhibit substantial phenotypic and functional heterogeneity that is associated with tissue localization and unique functional niches (3). The peripheral blood dendritic cell (PBDC) compartment, although quantitatively minute (<1% of total peripheral blood mononuclear cells), provides ready assessment of these cell populations, but the impact of specific subsets on the adaptive immune response is poorly understood. The PBDC are primarily segregated as myeloid or plasmacytoid

*corresponding author, James J. Kobie, 601 Elmwood Avenue, Box 689, University of Rochester, Rochester, NY 14642, james_kobie@urmc.rochester.edu, phone: 585-275-6632.

lineages. Myeloid DC (mDC), with high endocytic and phagocytic activity and expression of MHC II and co-stimulatory molecules (e.g. CD40 and CD80/CD86) are preferentially suited for antigen uptake and presentation for the induction of T and B cell response. mDC defined in-part by their expression of CD11c, have been further delineated into finer subsets including the major population (mDC1) which expresses CD1b/c+ (BDCA1), an MHC class I-like molecule which is able to present lipid antigens to T cells; and a minor population (mDC2) which expresses CD141+ (thrombomodulin/BDCA3) (4–7). Plasmacytoid DC (pDC) are the primary source of type I interferon, produced in response to Toll Like Receptor (TLR) 7 and TLR9 engagement, and this is the primary mechanism by which they are thought to contribute to anti-viral activities and autoimmunity (8, 9). Additionally, regulatory activities of DC have been described that are mediated through indoleamine 2,3-dioxygenase (IDO) (2, 10), inducible costimulator ligand (ICOS-L) (11), and programmed death receptor 1-ligand (PD-L1) (12), highlighting the potential functional diversity of the PBDC compartment. Thus, while phenotypic and functional diversity of the human PBDC compartment has been well described, we lack understanding of their role in maintaining homeostasis, their response to pathogens, tissue migration patterns, and interactions with tissue-resident dendritic cells.

The induction of serum antibody is a primary mechanism for vaccine mediated-protection, however resolution of the *in vivo* DC response to vaccination and its relationship to antibody responses in humans is limited. The objective of this study was to assess phenotypic and functional alterations in PBDC compartment at baseline and following influenza vaccination.

Methods and Materials

Subjects and Sampling

We enrolled 84 healthy subjects at the University of Rochester Medical Center from 2006 to 2010, all of who were administered Fluzone (Sanofi Pasteur) intramuscular seasonal inactivated trivalent influenza vaccine (TIV) as standard-of-care. All subjects provided signed written informed consent. All procedures and methods were approved by the Research Subjects Review Board at the University of Rochester. Peripheral blood was obtained from subjects at one time point prior to receiving TIV. Based on subject willingness, availability, and logistical constraints, a subset of subjects (n=6) provided three additional samples following 2009–2010 TIV immunization; one obtained on day five to day seven post-vaccination, another obtained day eight to day ten post-vaccination, and a final sample collected 1 month post-vaccination. PBMC and serum were isolated and cryopreserved as previously described (13). Briefly, PBMC were isolated within two hours of sampling using CPT tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Tubes were immediately inverted 8 to 10 times and processed according to manufacturer's instructions. Peripheral blood mononuclear cells (PBMCs) were cryopreserved and stored in liquid nitrogen. Serum was collected, aliquotted and stored at –80°C. All sample processing was performed in a blinded manner.

Flow Cytometry

PBMC samples were stained and analyzed by flow cytometry on a BD LSRII (BD Biosciences, San Jose, CA) using FlowJo analysis software (Treestar, Ashland, OR) as previously described (14).

The following monoclonal antibodies were used in this study: CD1c-PE (AD5-8F7, Miltenyi Biotec, Auburn, CA), CD3-PE-Cy5.5 (S4.1, Invitrogen, Carlsbad, CA), CD4-APC-Alexa Fluor 750 (RPA-T4, eBioscience, San Diego, CA), CD4-Qdot655 (S3.5, Invitrogen), CD11c-PE-Cy7 (3.9, Biolegend, San Diego, CA), CD14-Alexa Fluor 700 (M5E2, BD Biosciences, San Jose, CA), CD14-Qdot800 (TüK4, Invitrogen), CD16-PerCp-Cy5.5 (3G8, BD Biosciences), CD16-PE-TexasRed (3G8, Invitrogen), CD19-PerCp-Cy5.5 (SJ25C1, BD Biosciences), CD34-PerCp-Cy5.5 (8G12, BD Biosciences), CD40-APC-H7 (5C3, BD Biosciences), CD86-Pacific Blue (IT2.2, Biolegend), CD141-biotin (AD5-14H12, Miltenyi Biotec), CD303-APC (AC144, Miltenyi Biotec), HLA-DR-Qdot605 (Tü36, Invitrogen). Streptavidin-Pacific Orange and Streptavidin-Qdot585 (Molecular Probes/Invitrogen, Carlsbad, CA) were used as secondary staining reagent for CD141-biotin. 7-Amino-Actinomycin D (7-AAD) (BD Biosciences) or Live/Dead Aqua (Invitrogen) was included in the antibody cocktails as a vital dye to exclude dead cells. All dendritic cell subsets were identified as live, lineage negative, CD14 negative (to exclude monocytes), CD4 positive.

FITC-dextran uptake was determined by incubating cells with FITC-dextran in duplicate plates at 4 °C and 37 °C, respectively. Briefly, 50 µl of PBMC (1×10^6 cells) in 1% BSA/HBSS were added to triplicate wells on each of the two 96-well V-bottom plates before adding 4 µl of FITC-dextran (molecular weight = 40,000; Invitrogen) at 12.5 mg/ml for a final concentration of FITC-dextran of 1 mg/ml. The FITC-dextran solution was vortexed for 30 s and sonicated for an additional 30 s immediately before use. One plate was incubated at 37 °C and the second was incubated at 4 °C (to determine baseline FITC-dextran uptake level) for 30 min. Both plates were gently tapped every 5 to 10 min to ensure adequate mixing. Following FITC-dextran incubation, 200 µl of 1% BSA/HBSS was added into each well and the plates were spun at $400 \times g$ at 4 °C for 6 min, decanted supernatant, washed one more time with 250 µl of 1% BSA/HBSS, and followed by cell surface marker staining (see above).

A minimum of 3 million events was collected from each sample. Gating was performed in a blinded manner and gates set based on fluorescence minus one (FMO) controls for anti-CD1c, CD4, CD14, and CD141 antibody staining.

Hemagglutination Inhibition Assay

A standard hemagglutination inhibition assay was performed in a blinded manner from samples obtained at baseline and at 1 month following vaccination as previously described (15). Briefly, serial two-fold serum dilutions were assayed individually against influenza H1N1, H3N2, and B using strains contained in the 2009–2010 TIV vaccine or an appropriately matched closely related strain.

Statistical Analysis

Repeated Measures ANOVA with Tukey's Multiple Comparison Test was used to determine significance unless otherwise noted. Correlative analysis of 2009–2010 HAI average change for each subject was determined as: $((H1\ 1mo/H1\ baseline) + (H3\ 1mo/H3\ baseline) + (B\ 1mo/B\ baseline))/3$ (13). Spearman two-tailed correlation co-efficient was used to measure the correlation of two variables. Statistical analyses were performed using Prism 5.0 software (GraphPad Software, La Jolla, CA, USA).

Results

Homeostatic characteristics of the PBDC compartment

Baseline peripheral blood samples from 84 healthy subjects were obtained prior to receiving seasonal trivalent inactivated influenza vaccine (TIV). Plasmacytoid dendritic cells (CD303+CD11c–) and three CD11c+ mDC subsets; mDC1 (CD303–CD11c+CD1c+CD141–), mDC2 (CD303–CD11c+CD1c–CD141+), and CD303–CD11c+CD1c–CD141– which are henceforth referred to as mDCX were examined (Figure 1A). Consistent with previous findings, the pDC population was significantly ($p<0.05$) more frequent (0.275%), compared with mDC1 (0.047%), mDCX (0.028%), and mDC2 (0.004%). And the mDC1 were significantly ($p<0.05$) more frequent than the mDC2 subset (Figure 1B).

Twenty subjects were analyzed in-depth for relative expression of HLA-DR, CD40, CD86, and endocytic ability amongst the DC subsets, as an indicator of co-stimulatory and antigen-uptake potential. The mDC1 cells exhibited the highest HLA-DR expression, which was significant ($p<0.05$) compared to all subsets including the CD14+ monocytes (Figure 1C). mDC1 also exhibited the lowest CD40 expression (Figure 1D) compared to the other DC subsets, and substantial endocytic activity, as determined by internalization of FITC-labeled dextran particles (Figure 1E). The mDC2 subset exhibited substantial HLA-DR expression although significantly lower ($p<0.05$) than mDC1. mDC2 exhibited substantial CD40 expression and endocytic activity that was higher although not significant as compared to mDC1.

The mDCX subset showed modest HLA-DR expression and endocytic ability, accompanied by the highest CD40 expression which was significant ($p<0.05$) compared to CD14, mDC1, and pDC. The pDC subset exhibited the lowest HLA-DR expression and endocytic ability, although modest CD40 expression was observed. None of the PBDC subsets exhibited substantial CD86 expression (not shown).

Influence of vaccination on the PBDC compartment

Having demonstrated the steady-state characteristics of PBDC, we next focused on the response of the PBDC compartment to an immunological trigger, specifically the seasonal influenza vaccine. Although, thought to be a relatively minor immunological event, increases in peripheral blood antigen-specific T cell and B cell responses are apparent during the first 10 days following TIV (13, 16, 17). Hence we sought to determine if variation in the PBDC compartment was also evident during this time. A longitudinal assessment of the frequency of PBDC subsets following TIV revealed a decrease in myeloid DC subsets

(mDC1, mDC2, mDCX) 5–10 days post vaccination; this was most apparent and reached significance at days 8–10 for the mDC2 and mDCX subsets (Figure 2A–C). The mDC2 subset, the least frequent of the myeloid DC subsets had the greatest decrease ($\approx 40\%$) from baseline. The myeloid DC subsets returned to baseline within 1 month. The pDC frequency did not change significantly following vaccination (Figure 2D), neither did the CD14+ monocytes (not shown).

To assess the relationship of the PBDC compartment and the induction of the humoral response, influenza-specific serum antibodies were measured by hemagglutination inhibition (HAI) assay. The induction of serum influenza HAI antibody, a presumptive measure of vaccine-mediated protection, was assessed at 1 month post vaccination for vaccine-matched H1, H3, and B viruses. The change in mDC1, mDC2, and mDCX frequency at days 5–7 from baseline inversely correlated with the change in HAI antibody from baseline to 1 month post vaccination (Figure 2E), and reached significance for mDC2 ($r = -0.886$, $p = 0.0167$) indicating that the magnitude of decrease in blood myeloid DC subsets shortly after TIV, correlated with the magnitude of increase in the serum HAI antibody.

Discussion

We can now identify multiple PBDC subsets with distinguishing markers by flow cytometry. These tools have allowed investigators to define phenotypic and functionally distinct subsets, but the role of these populations in the maintenance of homeostasis and response to vaccines remain to be determined. Our results provide a cross-sectional analysis of the different subsets at baseline and show a decrease in the myeloid DC compartment following TIV.

To our knowledge, this is the first report to describe decreased blood myeloid DC subsets following vaccination in humans, however transient decreases in blood myeloid DCs have been observed in other conditions: 1) during acute myocardial infarction, where it is suggested that they are being recruited into the vessel wall and accumulating in atherosclerotic plaques (18–22), 2) in burn patients where it is associated with the incidence of sepsis (23), and 3) during acute viral infections including CMV (24) and pandemic H1N1 influenza (25). Additionally, following injection of the TLR7/8 agonist R-848 or the TLR9 agonist CpG-ODN in rhesus macaques, a decline in mDC were observed (26).

In response to vaccination, mDC subsets may be reduced in the blood as a consequence of migration to the injection site and draining lymph node, but the fate of these mDC remains to be determined. To that end, the origin, kinetics, and trafficking of PBDC is poorly understood, although comprehensive transcriptional profiling of tissue-resident and blood DC subsets such as that recently described by Lundberg et al. is providing informative resolution of the relationships among DC subsets (27). Whether the decline in PBDCs following vaccination is critical to develop a protective response is unknown, so it is critical to build on our pilot study with larger cohorts with a variety of vaccines. Certainly, detailed transcriptional analysis of the PBDC subsets before and after vaccination will determine if our findings are a generalizable feature of the immune response and if vaccination alters the DC transcriptome, particularly signatures of survival and migratory potential.

The mDC2 subset, which appeared most responsive to vaccination, has been previously shown to have the increased ability to cross-present antigen, produce IL-12p70 cytokine which promotes Th1 and cytotoxic T cell responses, and also express TLR3 compared with other mDC subsets suggesting increased pro-inflammatory potential and similarity to mouse CD8 α + DC (4, 28, 29). Although the TLR ligands present in influenza vaccine are uncharacterized, it has been demonstrated that influenza vaccine induces a Poly I:C (TLR3 ligand)-like transcriptional profile characterized by up-regulation of PKR (protein kinase R), RIG-I (retinoic acid-inducible gene I), and 2,5-OAS (oligoadenylate synthetase) mRNA (30), suggesting TLR3 responsiveness may be a possible mechanism for the increased sensitivity of mDC2 to influenza vaccination we observed compared to the other myeloid DC subsets. Recent studies also showed a central role for DC IL-1R signaling in promoting CD8+ T cell responses to live influenza infection, but the importance of this pathway in response to vaccination has not been studied (31). Future studies examining the mechanism of mDC2 responsiveness to influenza vaccine, and the mDC2 contribution to the induction of protective adaptive immune responses are warranted.

We speculate that the mDCX subset, which is 5-fold more abundant than mDC2, overlaps with the previously described CD16 (Fc γ RIII) positive and 6-sulfo LacNAc positive (slan) DC subset, which consistent with our results is CD1c negative, has higher CD40 expression than CD1c+ mDC (mDC1) and CD141+ mDC (mDC2), and lower HLA-DR expression than CD1c+ mDC (32, 33). Recently, this CD16+ human dendritic cell subset has been described as having increased ability to bind IgG immune complexes as compared to other blood dendritic cell and monocyte populations, and this is mediated through the expression of the low-affinity Fc γ receptor CD16 (34). Our observation of decreased mDCX 8–10 days following influenza vaccination corresponds to the peak of influenza-specific serum antibodies (13) and may in part be associated with this population responding to influenza-specific IgG immune complexes. More detailed functional and phenotypic characterization of the mDCX subset to determine its relationship with other CD1c negative dendritic cell subsets will be valuable, however are outside the scope of the current study.

It remains to be determined if the correlation of the acute decrease in blood mDC shortly after vaccination with the increase in influenza specific antibody over one month is the result of a direct interaction of the mDC subsets in the antigen-specific B cell response or a indirect measure of the overall immune response to vaccination. Follow-up studies to evaluate the interactions of blood mDC populations and B cells *in vitro* and complementary *in vivo* animal models may provide further insight into this relationship.

In conclusion this study has demonstrated the heterogeneity of the PBDC compartment and its responsiveness to influenza vaccination. Further studies of the PBDC population may provide insights into the mechanisms that promote effective adaptive immune responses and inform strategies for future vaccine development.

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Figure 1. Homeostatic frequency and phenotype of peripheral blood dendritic cell subsets
 PBMC were isolated from healthy subjects prior to receiving seasonal influenza vaccine and analyzed by flow cytometry. **(A)** Gating strategy to identify PBDC subsets, initial plot is gated on CD3-7AAD-Lin- cells. **(B)** Frequency of PBDC subsets (n=84). Expression of HLA-DR **(C)** and CD40 **(D)** and endocytic activity **(E)** (n=20). Each symbol represents an individual subject. Solid line indicates mean. A solid line between subsets indicates significant difference (p<0.05). Significance determined by Repeated Measures ANOVA with Tukey Multiple Comparisons Test.

Figure 2. PBDC dynamics following seasonal influenza vaccination

PBMC and serum were isolated from healthy subjects prior to and following immunization with trivalent inactivated seasonal influenza vaccine. The frequency of mDC1 (A), mDC2 (B), mDCX (C), and pDC (D) was determined by flow cytometry ($n=6$). Solid line indicates mean. A solid line between subsets indicates significant difference ($p < 0.05$). Significance determined by Repeated Measures ANOVA with Tukey Multiple Comparisons Test. Influenza specific serum antibodies (H1, H3, and B) were measured by hemagglutination inhibition assay at baseline and 1 month following TIV and correlation with change in PBDC subsets from baseline to day 5–7 determined by Spearman two-tailed test (E). Each symbol represents an individual subject.