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SHORT REPORT

Immune profiling of responses to influenza vaccination in patients with myeloproliferative neoplasms

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Abstract

Myeloproliferative neoplasms (MPNs) are associated with immune dysregulation and increased susceptibility to infection, emphasizing the importance of vaccination for patients. This pilot study evaluated immune responses to influenza vaccination in MPN patients compared with healthy donors using mass cytometry and serology. We observed diminished CXCR5+ B-cell, CXCR3+ T-cell, activated CD127+ memory T-cell subsets, and a trend toward lower hemagglutinin inhibition titer in MPN patients. These results indicate that patients with MPN exhibit distinct responses to influenza vaccination suggestive of impaired migration to lymphoid organs and T-cell maturation which may impact the development of protective immunity.

KEYWORDS
immunology, myeloproliferative disease, vaccines

Myeloproliferative neoplasms (MPNs) are a clonal cell hematopoietic malignancy characterized by aberrant JAK-STAT signaling, and significant immune dysregulation resulting in treatment complications and infection susceptibility [1]. While infection prevention through vaccination is recommended by the CDC for all cancer patients, including those with MPNs, some studies have indicated lower seroconversion rates after influenza vaccination in this population [2]. The purpose of this pilot study is to assess the feasibility of conducting deep immune profiling by mass cytometry together with serological assessments of samples from healthy donors and patients with MPN after influenza vaccination, gain preliminary insights into the immune system changes in MPNs and identify immunological correlates of protection to the influenza vaccine response.

We conducted a retrospective analysis of peripheral blood samples collected 1–6 months after the administration of the annual influenza vaccine from healthy donors and patients with MPNs including polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF) (Figure 1A, Table S1). Hemagglutinin inhibition (HAI) titers were determined and immune cell profiling by mass cytometry was performed after labeling with a 41-antibody panel (Table S2). Data were

Petra Bachanová and Joan How authors contributed equally to this study

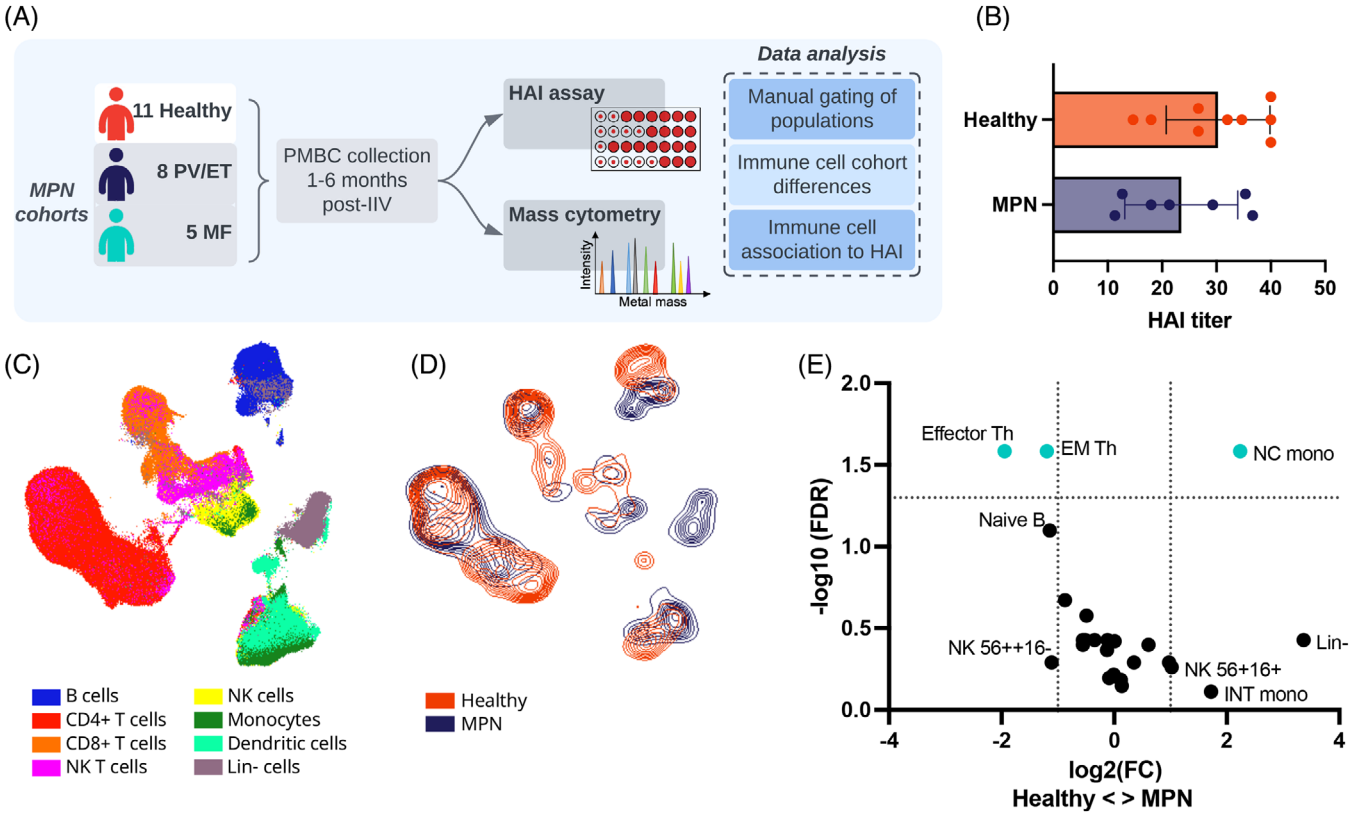

FIGURE 1 **Workflow and major immune differences between MPN patients and Healthy controls.** (A) Study design and experimental and data analysis workflow. (B) HAI titers in cohorts. Median titer for MPN is 21.3 and for Healthy 32.0. C–D) UMAP dimensionality reduction coloured (C) by major gated populations and (D) by cell density in cohorts. (E) Differentially abundant immune cell populations in cohorts. Those with multiple comparison-adjusted *p*-value < 0.05 and absolute log2 foldchange > 1 are shown in green. Those above the foldchange threshold are annotated regardless of their *p*-value.

normalized with Fluidigm bead standards, debarcoded, corrected for spillover [3], and arcsinh transformed. Gating (Figure S1) and dimensionality reduction by UMAP (Uniform Manifold Approximation and Projection [4]) were performed in Omiq (for all cohorts see Figure S2) and cell subsets were interrogated for abundance differences between Healthy and MPN cohorts using nonparametric Mann–Whitney Test with Holm–Sidak multiple comparison correction or ANOVA with sphericity correction for the comparisons of the three cohorts: Healthy, PV/ET, and MF (Figures S3 and S4). Clustering analysis (Figure S5) informed manual interrogation of specific markers across B-, T-, and innate cell lineages. We linked the quantified HAI titers with the gated immune populations using linear regression with elastic net regularization to determine cellular correlates of serological protection. All visualization was performed in Prism.

Our analysis revealed a trend toward decreased HAI titers in MPNs as compared with Healthy (Figure 1B) that did not reach statistical significance, likely due to the limited number and heterogeneity of MPN samples assayed. This trend toward reduced HAI titer appears to be driven by individual donor responses, rather than through a time-dependent waning of antibody following vaccination (Figure S6). However, substantial differences in immune cell distribution were observed between Healthy and MPNs as depicted on the UMAPs (Figure 1C, D) which were found statistically significant as quantified in Figure 1E. MPNs had diminished proportions of effector and effector memory helper T cells, along with decreased maturation and migration marker expression across T-cell subsets, namely CD127, CCR2, and CXCR3 (Figure 2A). CD127 is expressed by antigen-specific cytotoxic T cells [5] and marks T-cell differentiation in both influenza and RSV infections [6]. CCR2+ helper T cells have been previously ascribed immune regulatory functions in the lung [7] and CXCR3 expression is known to promote migration of activated T cells [8]. Diminished expression of CXCR3 has previously been described in MPNs [9] and we note that in our data this was true for all T-cell subsets. Additionally, we observed diminished frequencies of CXCR5+ B-cell subsets (Figure 2B), which has not been reported previously for MPN, though has been observed in other inflammatory disorders such as rheumatoid arthritis and systemic lupus erythematosus [10]. Altered chemokine receptor expression may impact B-cell trafficking to secondary lymphoid organs, impeding productive B–T cell interactions that facilitate maturation and survival of memory and effector T-cell subsets [11]. We hypothesize that impaired postvaccination T-cell responses, consistent with previous data by Alimam et al. [12], may be linked to reduced chemokine receptor expression within B cells. Similarly, impaired T-cell and B-cell responses may contribute to the trend toward reduced HAI titers in MPN patients, a novel observation that requires further investigation within a larger cohort.

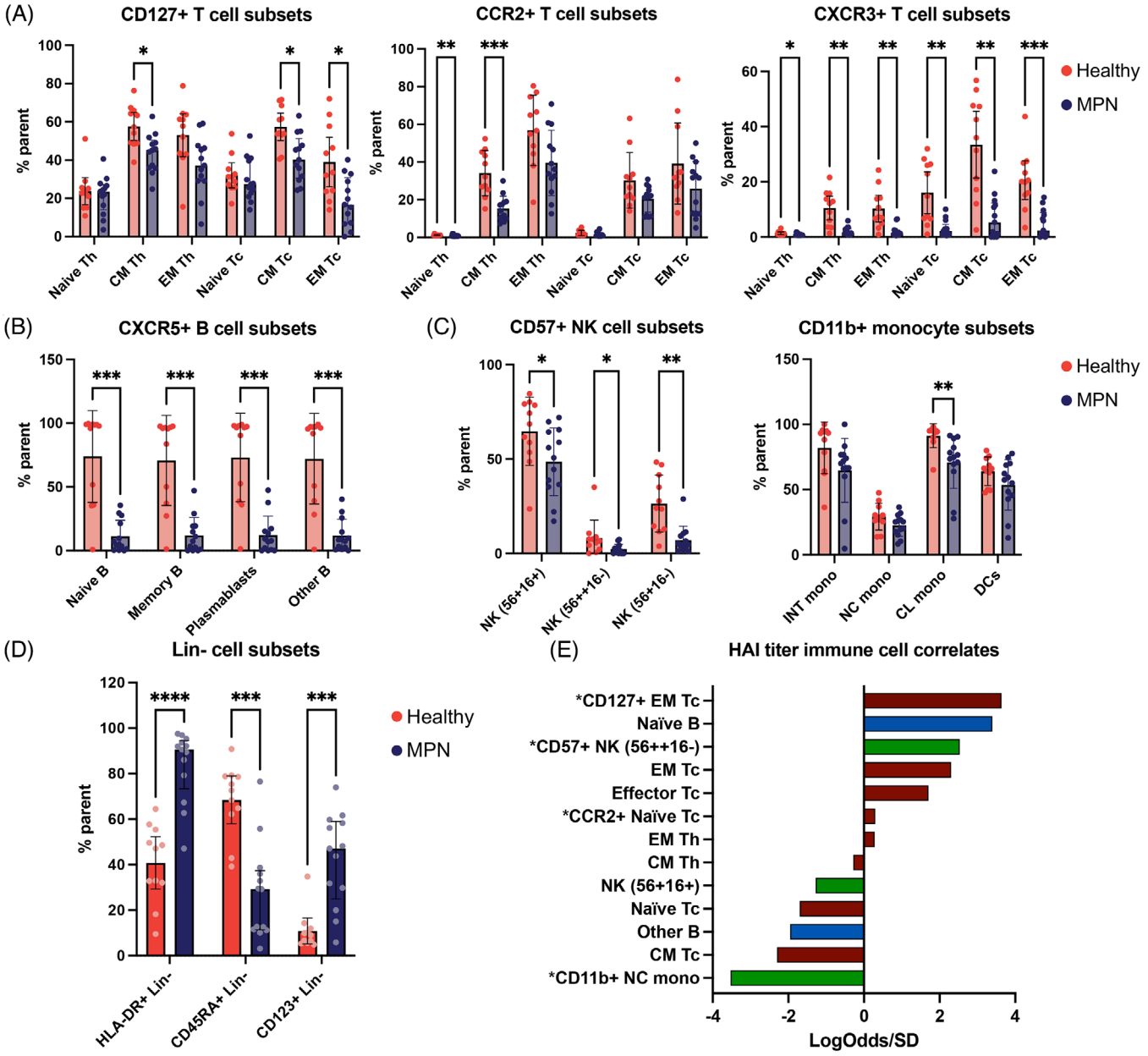

FIGURE 2 **Specific immune cell differences in cohorts and correlates of immune protection**. (A) Quantification of T cell subset frequencies gated for activation and migration markers CD127, CCR2 and CXCR3, in MPN patients and Healthy controls. (B) Quantification of B cell subset frequencies gated for a migration marker, CXCR5. (C) Quantification NK cells gated for a maturation marker, CD57, and monocyte subsets and dendritic cells gated for an activation marker, CD11b. (D) Characterisation of Lin-populations in MPN and Healthy, gated for immune markers: HLA-DR, CD45RA and CD123. Adjusted *p*-values: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, $^{****}p \leq 0.0001$. (E) Correlates of immune protection, as measured by HAI titers. Waterfall plot depicts linear regression coefficients for gated immune populations, selected by elastic net regularisation. Colour scheme refers to B, T or innate parent cell population with B cell gates in blue, T cell gates in red and innate cell gates in green. * denote populations gated with functional markers.

MPN patients exhibited increased frequencies of nonclassical monocytes, which induce potent inflammatory responses that underpin chronic inflammation in MPN [13]. CD11b+ classical monocytes, a population reported to reduce inflammation [14], were decreased in MPNs and CD57+ mature NK cell subsets were also diminished in MPNs (Figure 2C). Of note, MPNs overall had increased frequencies of Lin− cells, which were significantly elevated in MF patients (see Supporting information S3 for PV/ET and MFs plotted separately). Specifically, MPNs were more abundant in HLA-DR+ CD123+ Lin− cells, and less abundant in CD45RA+ Lin− cells, which likely constitute Innate Lymphoid cells (Figure 2D). It remains to be determined how these altered proportions of immune subsets impact the amplitude and diversity of antigen-specific serological and cellular responses post-vaccination in MPN.

Previous work has not explored the association between immune cell composition and profiles with serological response to influenza

vaccination in MPN patients. Therefore, we utilized a subset of samples to identify cellular immune correlates to serological response as measured by HAI titer (Figure 2E). Reduced HAI titers in healthy and MPN cohorts were associated with higher abundances of CD11b+ nonclassical monocytes, naïve, and central memory cytotoxic T cells. Conversely, increased HAI titers were associated with CD127+ EM cytotoxic T cells, CD57+ NK (CD56++ CD16−) cells and EM helper T cells. Interestingly, these cell subsets associated with higher HAI titers were diminished in MPN populations, which may indicate cellular deficiencies in MPN patients affecting serologic responses. Naïve B cell as well as Effector cytotoxic T cells, which were similarly distributed between MPN and Healthy cohorts, were also linked with higher HAI titers. Identification of cellular correlates to HAI serologic response in MPN patients is an important component in understanding their elevated risk for infection and more severe outcomes. Probing the relationships between cellular and serologic responses may also provide insights into immune dysregulation in MPN, which we will explore in future studies in a larger set of longitudinal samples.

Significant differences in postvaccination immune profiles between MPN and healthy controls are indicative of an attenuated immune response and diminished seroconversion following vaccination. MPN patients display increased innate cell subsets and decreased B- and T-cell subsets with altered immune marker expression, as well as differing cellular correlates of serological protection. Multiple factors contribute to the immune system impairment in MPN patients, including direct impact of the mutated clone on hematopoietic cell function, chronic inflammation, and treatment effects. The specific influence of these factors on diminished T- and B-cell responses detected among samples from patients with MPN remains unclear.

Vaccination is a component of routine care of patients with hematologic malignancies and has been shown to reduce the risk of infection-related morbidity and mortality [13]. Seroconversion after influenza vaccination is generally reduced in hematologic malignancy patients compared with healthy populations [2], though this has not been specifically reported in MPN patients. Interestingly, the rate of seroconversion after SARS-CoV-2 vaccination was not found statistically different between MPN patients and healthy controls, despite a trend toward reduced anti-Spike antibody responses in MF patients [15]. Importantly, treatment with immunomodulatory drugs including Ruxolitinib, hydroxyurea, and IFN-α likely contribute to baseline differences as well as vaccine responses observed in MPN patients as compared with healthy controls. Ruxolitinib has been reported to reduce NK, DC, and CD4+ T-cell function, and reduce both antibody and IFN-γ production following SARS-COV-2 vaccination in MPN patients receiving Ruxolitinib [16]. It has been demonstrated that IFN-α, hydroxyurea, and Ruxolitinib treatment may impact the abundance of immune subsets in a study of influenza vaccination response [12]. Therefore, the impact of immunomodulatory therapies is an important area of research and clinical consideration for vaccination of patients with MPN. Although the COVID-19 pandemic focused attention on immunization strategies against SARS-CoV-2, our results indicate that vaccine responsiveness in MPN patients should also be studied for other infectious agents. This would allow for assessing the need for enhanced vaccination protocols such as additional boosters and further infection control measures for the most at-risk MPN patients, guiding clinical practice.

The limitations of this pilot study include its retrospective timeline along with a limited sample number and heterogeneity. Additionally, the MPN patient group is notably older than healthy controls, which can influence vaccine response. MPN samples were from a patient sample repository, while healthy donor samples were from a commercial resource. Samples were collected previously over a 3-year period and pre-vaccination samples were not available for either group. Only a subset of samples was available for hemagglutinin inhibition assay. Comparison of MPN patients on Hydroxyurea or Jakafi regimens was not possible due to MPN subtype bias to specific treatment (Table S1). Future studies will focus on confirming our results in a larger cohort and a further investigation of immune dysregulation in MPN subtypes as well as within-subtype relationship to HAI titers. Our end goal is to further investigate the key immune hallmarks that define protective immunity in MPN patients receiving vaccines to protect against influenza, COVID-19, pneumococcus, varicella zoster, and other infectious diseases.

AUTHOR CONTRIBUTIONS

Sonia Mukherjee, Maia Pavlovic, and Jennifer Lombardi performed research and collected data; Petra Bachanová, Richard Dzeng, and Patrick M. Reeves performed statistical analyses; Petra Bachanová, Richard Dzeng, Joan How, Gabriela Hobbs, and Patrick M. Reeves performed research and collected data, analyzed and interpreted data, and wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

G.H.: Consulting fees from Abbvie, BMS, Moprhosys, CTI, Cogent, Protagonist, Pfizer, Novartis; research support from Incyte; Stock ownership, Regeneron. P.R.: Consulting fees from Merck and PDTx, speaking honoraria from Standard Biotools, member of ImmunoScape scientific advisory board.

DATA AVAILABILITY STATEMENT

Data that support the findings of this study, including cytometry, serological analyses, and de-identified metadata are available https://doi.org/10.7910/DVN/ZLZCOM. Please send inquiries and additional requests to pmreeves@mgb.org

ETHICS APPROVAL

The tissue bank used in this study has been approved by Dana Farber Harvard Cancer Center (DFHCC) Institutional review board.

CLINICAL TRIAL REGISTRATION

The authors have confirmed clinical trial registration is not needed for this submission.

PATIENT CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study through protocols approved by the Institutional Review Board.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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