bjh short report

Altered immune response to the annual influenza A vaccine in patients with myeloproliferative neoplasms

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Received 15 July 2020; accepted for publication 17 August 2020

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Summary

The seasonal influenza A vaccine is recommended for patients with myeloproliferative neoplasms (MPNs). We hypothesised that immune deregulation associated with MPNs may affect the immune response gained following vaccinations when compared to healthy controls. Using deep immunophenotyping with high-dimensional single-cell analysis and mass cytometry we could demonstrate an altered immune response in MPN patients following vaccination. We found that prior to vaccination, MPN patients had reduced numbers of naive CD4 T cells. Furthermore, at 3-weeks and 3-months post-vaccination there was evidence of both delayed and impaired B- and T-memory cells responses. Thus, although, the immune systems of MPN patients can 'recognise' the Influenza A vaccine, the response appears inferior compared to healthy controls.

Keywords: myeloproliferative disorder, vaccines, influenza A, flu.

From a global perspective, seasonal influenza viruses are associated with both significant morbidity and mortality. Patients with haematological diseases, such as myeloproliferative neoplasms (MPNs), are at higher risk of developing serious complications following influenza virus infection.[1] MPN patients demonstrate both a pro-inflammatory state and associated immune dysfunction, indeed it is speculated that MPNs may evolve and progress due to inherent defects in 'tumour' immune surveillance.[2] This is evidenced by dysregulation of several pivotal immune and inflammatory genes in addition to impaired regulatory T cell, CD4 and natural killer cell function, all potentially contributing to altered vaccination responses.[3,4] Moreover, the complexity of immunogenic responses in MPN patients may be further altered by cytoreductive agents and immunosuppressive therapies such as JAK inhibitors. MPN patients are frequently recommended for annual influenza vaccination in most national vaccination guidelines.[5] However, limited data exists concerning the true efficacy of influenza vaccine approaches in patients with MPNs, immunological responses ranging from 5 to 75% have been reported in patients with underlying diverse haematological conditions.[6] Effectiveness of the influenza A

vaccine is commonly extrapolated from immunogenicity data in the general population with limited understanding of the true efficacy in these disease states.[7] Many proposed strategies for such patients exist, including that proposed by de Lavallade *et al*., who studied vaccine responses in patients with Chronic Myeloid Leukaemia, with administration of two doses of influenza vaccine to illicit optimal vaccine response.[8] However, no universaly accepted guidance on vaccination response and assessment in such patients is available.

We hypothesise that MPN patients may display an altered immune response to the recommended seasonal influenza A vaccination when compared with healthy donors (HD). During October 2016, patients with a diagnosis of Essential Thrombocythaemia (ET), Polycythaemia Vera (PV) or Myelofibrosis (MF) were enrolled in a study to assess immune responses to the recommended annual influenza A vaccine within our institution. Written informed consent was obtained in accordance with the Declaration of Helsinki and in accordance with approval from the local ethical review committee. In line with Department of Health Guidelines, inactivated influenza A vaccine (Split virion, inactivated) was administered by intramuscular injection. A total of 19 patients were enrolled in addition to 6 HD, clinical characteristics of these subjects are outlined in Table I. Samples were collected pre-vaccination and at approximately 3-weeks and 3-months post-vaccination. Peripheral blood mononuclear cells were isolated (PBMCs) via the Ficoll-hypaque density based technique and immediately stored in −80°C freezers for subsequent analysis.

Deep immunophenotyping was performed using high-dimensional single-cell analysis with Mass Cytometry (CyTOF). PBMCs were stained with 35 metal-tagged antibodies and analysed using CyTOF (Table SI). Data were normalised with Fluidigm EQ™ Four Element Calibration Beads using the CyTOF® 6.7 system control software, and gated via Cytobank[9] (see Figure S1 for gating details). Analysis was performed using the ImmunoCluster package in R (https://github.com/kordastilab/ImmunoCluster). Dimensionality reduction of data were carried out using Uniform Manifold Approximation and Projection (UMAP), and the FlowSOM algorithm was applied to the scaled and transformed (arcsinh (cofactor 5)) data for clustering. Mean expression of all markers measured for each cluster was used for cell type identification (Figure S2 and Table SII). Nonparametric Pairwise Wilcoxon Rank Sum Tests were used to identify significant changes in cell cluster abundance between groups.

Pre-vaccination we note significantly less naïve CD4 T-cells ($P = 0{\cdot}01$), and activated CD4 T-cells ($P = 0{\cdot}02$) in MPN patients compared to HDs (Figure S3 and Table SIII). At 3 weeks post-vaccination, MPN patients demonstrated less memory cell clusters, including central memory (CM) CD4 ($P = 6{\cdot}93 \times 10^3$) and CM CD8 ($P = 5{\cdot}11 \times 10^3$), memory B ($P = 0{\cdot}03$, $P = 0{\cdot}01$, and $P = 0{\cdot}05$) and resting memory B-cells ($P = 0{\cdot}05$), compared to HDs (Fig 1A). When compared to HDs at this time point, we also noted a significantly

Table I. Characteristics of patients and healthy donors enrolled onto the study who received the annual influenza A vaccine (Split virion, inactivated).

	MPN patients			Healthy donors
Male/Female	6/13			2/4
Median age (years)	50 (range 30–78)			41·5 (range 25–59)
Median disease duration (years)	PV	ET	MF	NA
	5 (1–41)	3 (0·5–5)	13·5 (3–22)	
*Available sample time points for analysis				
Pre-vaccine	11			2
3 weeks post-vaccination	15			4
3 months post-vaccination	18			6
Treatment	PV	ET	MF**	
Hydroxycarbamide	1	3	0	NA
Pegylated interferon	3	3	0	NA
Ruxoltinib	1	0	1	NA
Momelotinib	0	0	1	NA
No treatment	1	2	2	NA
Molecular status				NA
JAK2 V617F mutated	7	3	2	NA
CALR mutated	–	2	2	NA
'Triple Negative'	–	2	–	NA
Unknown	–	1	–	NA

PV, polycythaemia vera; ET, Essential thrombocythaemia; MF, myelofibrosis (**includes patients with post ET MF); NA, not applicable; CALR, calreticulin; JAK2, Janus Kinase 2.

*19 patients and 6 healthy donors were enrolled into study, however, at time of analysis viable cells were not available for all patients at all the pre and post vaccination time points.

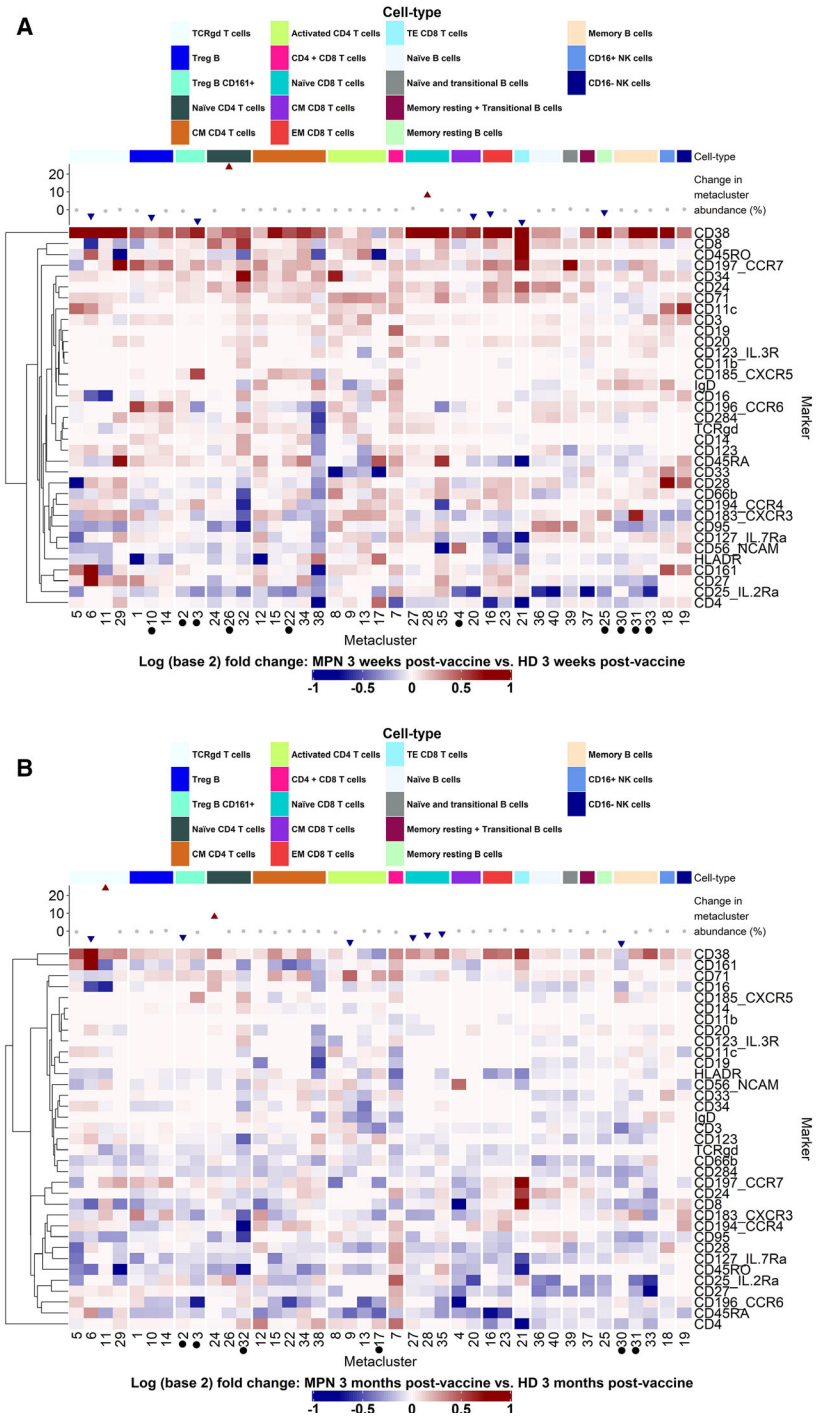

Fig 1. Cell cluster marker expression and abundance changes. (A) MPN patients vs HD 3 weeks post-vaccination. (B) MPN patients vs HD 3 months postvaccination. Change in marker expression between time points measured (log2 fold change in marker expression (log2 (MPN marker expression/HD marker expression)). Change in magnitude of immune cell cluster abundance (mean change in percentage of cell abundance, red arrow = ≥1·0, and blue arrow = ≤1·0). •cell clusters with significant changes in abundance between MPN patients and HDs. Cell cluster abundance split by MPN patients' molecular status and healthy donors. MPN, myeloproliferative neoplasms; HD, healthy donor. [Colour figure can be viewed at wileyonlinelibrary.com]

lower subset of Tregs known as Treg B-cells[10] ($P = 0{\cdot}01$), including CD161+ Treg B subpopulations ($P = 9{\cdot}32 \times 10^3$ and $P = 3{\cdot}73 \times 10^3$, respectively) in MPN patients (Fig 1A), which are a highly suppressive subpopulation of Tregs.[11] Additionally, three weeks post-vaccination MPN patients had a significantly higher number of naïve CD4 T-cells compared to HDs ($P = 6{\cdot}93 \times 10^3$) (Fig 1A and Table SIV), which may suggest a delayed immune response. By 3 months post-vaccination significant reductions in memory B cells ($P = 0{\cdot}04$ and $P = 0{\cdot}01$) and CD161+ Treg B-cells ($P = 0{\cdot}01$ and $P = 0{\cdot}01$) were still evident in MPN patients (Fig 1B and Table SV). Although this was to a lesser extent, it had not reverted to the pre-vaccination state. Compared to the HDs, reductions in naïve CD4 T-cells ($P = 0{\cdot}03$) from pre-vaccination in MPN patients could also be observed at 3 months post-vaccination, paralleled with an increase in activated CD4 T-cells ($P = 0{\cdot}03$; Fig 1B and Table SV). In our cohort, we did not observe significant effect of disease

subtype, molecular status or cytoreductive therapy on the vaccination responses. Comparisons between disease subtypes, molecular status and treatment effects, where available, are summarised in the supplementary information.

Efficacy of the annual influenza vaccine in oncology patients has been previously investigated, with reports of adequate protection evidenced by low rates of influenza illness rather than delineation of specific immunological responses.[12] However, patients with clonal haematological disorders, especially in receipt of cytoreductive therapy, display inadequate humoral responses compared to healthy individuals.[13] Vaccination should result in memory B- and T-cell formation which facilitates adaptive immune responses to the pathogen if challenged later and can take approximately 2 weeks for a so-called 'healthy' immune system.[12] At 3 weeks post-vaccination, MPN patients displayed significantly lower B and T memory cells compared to HD. Additionally, a reduction of 'Treg B' and CD161+ populations (highly suppressive) were also identified in the MPN patients which may indicate that the HDs immune system was returning to homeostasis post immune response to the vaccine. MPN patients did show a significantly increased number of naïve CD4 T-cells which may denote a delayed response to the vaccine, and therefore we could have missed the 'peak' response from these patients based on our choice of analysis time points. At 3 months post-vaccination the differential responses between MPN patients and HD responses remains the same, albeit to a lesser extent.

Limitations of our study include a small and heterogeneous cohort, in addition to the missing analytical time points due to absence of viable cells. Nonetheless, using novel approaches, we demonstrate an inferior immunological response to the inactivated influenza A vaccine in MPN patients compared to HDs. Whether this immune response is sufficient for robust clinical protection from influenza remains unclear. This study is timely in view of the current coronavirus pandemic, where Fattizzo *et al.* noted patients with myeloid neoplasms, including MPNs were at higher risk of contracting COVID-19, presenting with atypical features and displaying higher mortality.[14] Therefore, as the scientific community searches for a COVID-19 vaccine, it is important to recognise the potential limitations of vaccinations in patients with MPNs and a requirement for improved strategies to address this issue.

In summary, our data supports routine influenza A immunisation in accordance with national recommendations; however, additional studies are mandated to evaluate both the effectiveness of the vaccine responses and 'memory' in a larger cohort of MPN patients to determine if alternative strategies for vaccination are required.

Acknowledgements

The authors gratefully thank all the patients and healthy volunteers who participated in this study. We would also like to acknowledge all the work of MPN Voice.

Conflict of interest

C.H.: Funded research: Novartis; Speaker fees: Novartis, Jannsen, CTI, Celgene, Medscape; Advisory Board: Incyte, CTI, Sierra Oncology, Novartis, Celgene, Roche, AOP pharma, Geron, Astra Zenica. S.K.: Celgene and Novartis research grant; Alexion speaker honorarium. D.M.: Speaker fees and advisory boards Novartis, Celgene and Jazz pharmaceuticals.

Supporting Information

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

Table SI. Mass cytometry panel.

Table SII. Cluster cell type identification.

Table SIII. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: Prevaccine: MPN vs HD.

Table SIV. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 weeks postvaccine: MPN vs HD.

Table SV. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 months postvaccine: MPN vs HD.

Table SVI. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: prevaccine: JAK2 vs CALR and TN.

Table SVII. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 weeks postvaccine: JAK2 vs CALR and TN.

Table SVIII. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 months postvaccine: JAK2 vs CALR and TN.

Table SIX. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: Prevaccine: CALR vs JAK2.

Table SX. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 months postvaccine: CALR vs JAK2.

Table SXI. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 months postvaccine: JAK2 vs TN.

Table SXII. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 months postvaccine: CALR vs TN.

Table SXIII. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 weeks postvaccine: HC vs IFN.

Table SXIV. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 weeks postvaccine: HC vs Rux.

Table SXV. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 weeks postvaccine: IFN vs Rux.

Table SXVI. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 months postvaccine: HC vs Rux.

Table SXVII. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 months postvaccine: HC vs IFN.

Table SXVIII. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 months postvaccine: IFN vs Rux.

Table SXIX. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: Prevaccine: ET vs MF.

Table SXX. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: Prevaccine: MF vs PV.

Table SXXI. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 weeks postvaccine: ET vs MF.

Table SXXII. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 weeks postvaccine: MF vs PV.

Table SXXIII. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 months postvaccine: ET vs MF.

Table SXXIV. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 months postvaccine: ET vs PV.

Table SXXV. Nonparametric Pairwise Wilcoxon Rank Sum Test comparing cell cluster proportion: 3 months postvaccine: MF vs PV.

Fig S1. Gating strategy used to remove beads, dead cells and doublets and select for CD45+ live cells.

Fig S2. Heatmap showing mean expression of all markers measured for all samples combined for each FlowSOM cluster (1–40).

Fig S3. Cell cluster marker expression and abundance changes.

Fig S4. Cell cluster abundance split by MPN patients molecular subtype and healthy donors.

Fig S5. Cell cluster abundance split by MPN patients Treatment and healthy donors.

Fig S6. Cell cluster abundance split by MPN patients disease subtype and healthy donors.

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