Letter to the Editor

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Increased PD-L1 surface expression on peripheral blood granulocytes and monocytes after vaccination with SARS-CoV2 mRNA or vector vaccine

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To the Editor,

The immune response is accompanied by the activation of a highly complex network of immune activator and inhibitor pathways. Immune defense reactions coexist with reactions maintaining self-tolerance and the balance between these processes is crucial. Immune checkpoints play an important role in controlling this network. Inhibitory immune checkpoint molecules minimize collateral tissue damage and are essential for the prevention of autoimmune diseases [1]. One important inhibitory checkpoint receptor is programmed cell death protein 1 (PD-1, CD279) which is typically found on T cells and also on other cells like mature B-cells [2]. Its ligands, programmed death-ligand 1 (PD-L1) and PD-L2, are regularly expressed on antigen presenting cells like dendritic cells and macrophages; upregulation of PD-L1 is observed after activation of monocytes and granulocytes [3, 4].

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Since the onset of the SARS coronavirus 2 (SARS-CoV-2) pandemic, several effective vaccines such as nucleic acid vaccines or vector vaccines have been developed and applied worldwide [5]. These vaccines cause the synthesis of viral proteins within the cells of the vaccinated person, mimicking a viral infection. Numerous vaccination studies have been conducted recently, also with vaccination regimens combining mRNA and vector vaccines.

We were interested to know whether vaccine-induced activation of the immune system also causes measurable regulating effects, for instance an increase in PD-L1 expression. As part of a large ongoing vaccination study [6], we therefore compared the PD-L1 expression on peripheral blood monocytes and granulocytes of healthy vaccinated probands and of a not vaccinated control group. In parallel, interferon gamma (IFN-γ) release assays were conducted to determine T cell induction.

62 samples from healthy volunteers after SARS-CoV-2 vaccination were analyzed. The control group of healthy non-immunized probands comprised only 12 individuals since the variance of leucocyte PD-L1 expression within this group was very low. Written informed consent prior to participation and Ethics Committee approval from the Medical University of Innsbruck was obtained. Three different vaccine combinations involving the vaccines AZD1222 (AZ, Astra Zeneca) and BNT162b2 (BNT, BioNTech-Pfizer) were administered. Samples from each of the vaccinated individuals were collected on day 2 after the second vaccination. PD-L1 surface expression was determined by flow cytometry as recently described [7]. Ten days following the second vaccination SARS-CoV-2-specific antibody and T cell response were measured as described previously [6]. SARS-CoV-2-specific interferon-γ release from blood cells was determined by QuantiFERON (QFN) SARS-CoV-2 RUO IGRA (Qiagen) according to the manufacturer's instructions. Briefly, samples were loaded onto QFN tubes coated with specific CD4 (Ag1) and CD4+CD8

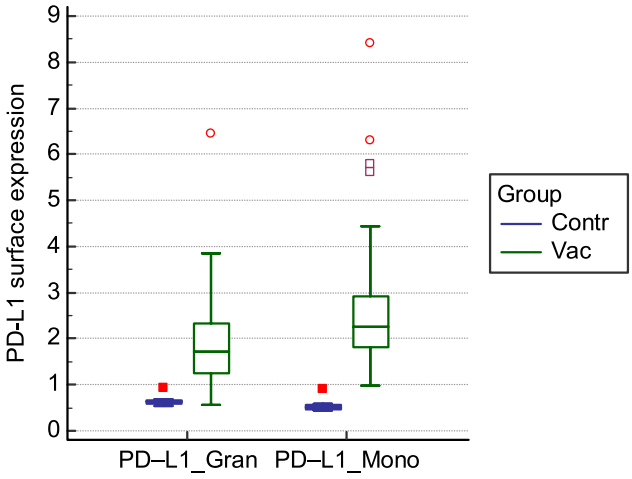

Figure 1: PD-L1 delta-MFI surface expression on granulocytes (Gran) and monocytes (Mono) of healthy controls (left boxplots, n=12) and SARS-CoV-2 vaccinated individuals (right boxplots, n=62). MFI was determined by flow cytometry using an anti-human PD-L1 Ab or isotype control. The differences between the MFIs of the PD-L1 and the isotype control signal were calculated and plotted. Horizontal lines indicate median with IQR. As positive control a peripheral blood sample after stimulation with AB0 incompatible erythrocytes was used (delta MFI Gran 0.69, delta MFI Mono 2.22). The stimulation was confirmed by qPCR as elevated PD-L1 mRNA expression. A Mann–Whitney U test was performed for statistical analysis using MedCalc 19.6.1 software. Delta-MFI on granulocytes and monocytes from vaccinated individuals significantly differ from healthy controls ($p<0.01$).

(Ag2) SARS-CoV-2 peptide pools from spike antigen (S1 S2 RDB). QFN tubes including a negative and a positive control (Mitogen) served as controls. After 24 h incubation at 37 °C, supernatants were harvested and stored at −80 °C until use. Interferon-γ release was measured by QFN human IFN-γ ELISA Kit (Qiagen) according to the manufacturer's instructions and the stimulation index (quotient of stimulated vs. unstimulated IFN-γ concentration) was calculated.

Our results demonstrate a statistically significant ($p<0.01$) increase in PD-L1 expression both on peripheral granulocytes and monocytes of the vaccinated individuals (Figure 1; median delta mean fluorescence intensity [MFI] 1.71 and 2.26, respectively) in comparison to the control group of healthy non-vaccinated individuals (median delta MFI 0.65 and 0.51). Using different vaccine combinations had no effect on the results (Supplementary Figure 1). We observed a moderate inverse correlation between PD-L1 expression and mitogen-induced T-cellular IFN-γ release (Figure 2), but we could not find a direct correlation between PD-L1 surface expression and SARS-CoV-2 antibody levels or a SARS-CoV-2-specific T cell response (Supplementary Figures 2 and 3).

The present study shows that the PD-L1 expression of peripheral granulocytes and monocytes of vaccinated individuals is significantly higher than the expression found in non-vaccinated individuals. Furthermore, PD-L1 expression correlates inversely with mitogen-induced T-cell stimulability in the IFN-γ release assay. Interestingly, stimulability with SARS-CoV-2-specific S-antigen in the interferon-γ release assay was not associated with PD-L1 surface expression on granulocytes and monocytes. A possible explanation for this might be that T-cell stimulation by proteins or mitogens occurs by different pathways. While antigens can only activate B/T cells via specific binding receptors (true immune response), mitogens cause unspecific stimulation. In an acute situation like a vaccination, it would be biologically meaningful that the antigen specific reactions are less influenced by the dampening effect of PD-L1 while non-specific reactions with the tendency to escalate are more sensitive to inhibition.

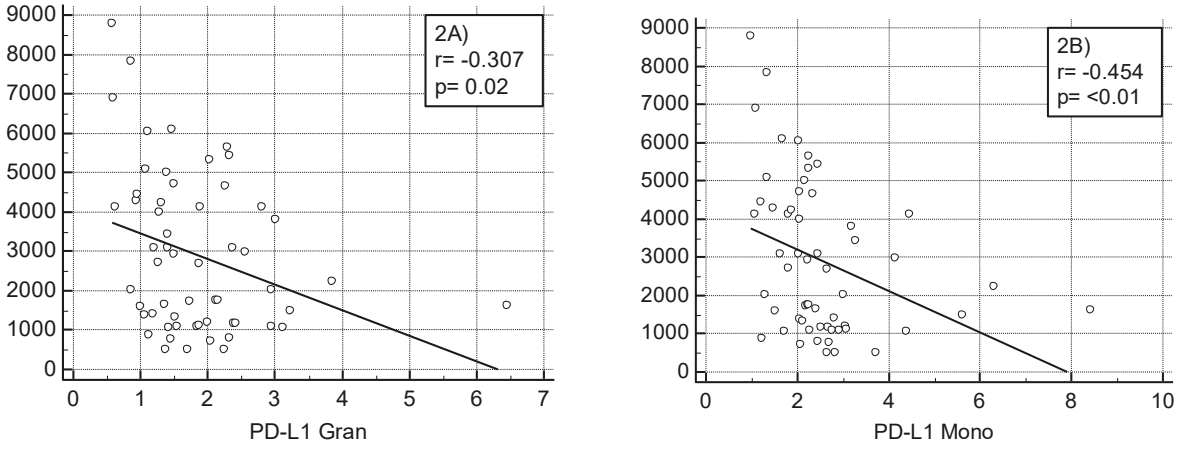

Figure 2: Regression/correlation analysis between mitogen-induced interferon (IFN)-γ represented by stimulation index and PD-L1 delta-MFI surface expression on (A) granulocytes and (B) monocytes of all SARS-CoV2 vaccinated individuals (n=57). Correlation coefficients were calculated using the Spearman rank method. The slightly different number of subjects between the figures is due to the lack of isolated results for certain subjects.

The significance of PD-L1 expression following immunization is currently unknown. Upregulation of PD-L1 might merely reflect the physiological immune regulation, but on the other hand it could also influence the outcome of vaccinations or the incidence and type of side effects. Indeed, a recently published case report suggests that PD-L1 upregulation after vaccination may correlate with some vaccination side-effects [8], a finding which deserves further investigation. It seems plausible that PD-L1 is upregulated after a strong vaccine-related activation because an activated immune system needs to be regulated to avoid autoimmune collateral damage. This raises the question whether a high PD-L1 expression has an effect on the success of vaccination. Vaccinations may have a non-specific immunosuppressive effect lasting for a certain period of time (which remains to be determined). If this holds true, it would be worth-while to consider whether an adjustment of vaccination intervals or a modulation of the checkpoint system could possibly improve the vaccination response. This might be of particular importance with regard to the currently recommended short vaccination intervals for SARS-CoV-2 booster vaccinations.

Our present studies focused on the investigation of PD-L1. In the future, also other immune checkpoint molecules should be included in vaccination studies such as cytotoxic T lymphocyte antigen-4 (CTLA-4) which has been shown to play an essential role in the anti-tumor immune response [9].

Furthermore, the available data do not allow to differentiate between PD-L1 overexpression as a sign of a general neutrophil activation or as a result of specific receptor upregulation. To investigate this, granulocyte activation markers such as elastase should be included in further studies.

To our knowledge these are the first results to date in the context of PD-L1 surface expression and vaccination. In summary, we believe that immune checkpoints such as PD-L1 as important regulators of the immune system should also be considered in future vaccination studies to further elucidate their impact on vaccination outcome and side effects.

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Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The study was approved by the Institutional Ethics Committee.

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