Fully automated clinical-grade isolation of CD141 (BDCA-3)$^+$ cross-presenting dendritic cells for the generation of vaccines that stimulate antigen-specific CTL responses

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Introduction

It is established that CD8α$^+$ dendritic cells (DCs) in the mouse constitute a subpopulation that excels in cross-presentation. This cross-priming capacity of CD8α$^+$ DCs is essential for the initiation of effective cytotoxic T cell (CTL) responses against tumors. An equivalent subset of professional cross-priming DCs (XP-DCs) has recently been described in humans as lineage-negative, MHC class II^{high}, CD141 (BDCA3)$^+$, XCR-1$^+$, CLEC9A$^+$ DCs.[1–3]

Here we demonstrate that XP-DCs that were isolated using a fully automated clinical-grade process are able to stimulate T cells in an antigen (Ag)-specific manner. To this end, isolated XP-DCs were loaded with antigens and activated with TLR3 agonist poly (I:C). Antigen-loaded, mature XP-DCs were then cocultured with autologous, antigen-specific T cells.

Upon activation, XP-DCs up-regulated activation markers and produced a specific set of cytokines, including IFN-λ, a hallmark of XP-DCs[4]. Furthermore, Ag-loaded XP-DCs efficiently presented antigens and stimulated T cells in an Ag-specific manner, resulting in proliferation of antigen-specific T cells and secretion of effector cytokines. Taken together, we developed the first clinical-scale process for the manufacture of next-generation XP-DC–based vaccines capable of stimulating antigen-specific CTL responses. This is now going to be used in two clinical studies.

Figure 1

Results

1 Performance of the CliniMACS Prodigy® CD141 (BDCA-3) Enrichment System

XP-DCs were enriched from leukapheresis samples by a two-step procedure consisting of pre-depletion of monocytes, B cells, and T cells and the subsequent positive selection of XP-DCs on the CliniMACS Prodigy (fig. 1). As a result of the pre-depletion process, the numbers of monocytes, T cells, and B cells were reduced by more than 10,000-fold (fig. 2A). The mean purity of XP-DCs in the final fraction amounted to 65%, when starting from leukapheresis (LP) products with an average of 6.9×10^9 viable leukocytes and an XP-DC frequency of 0.08% (fig. 2B). The average XP-DC recovery was 46% (fig. 2C), which corresponded to a yield of 3.4×10^5 XP-DCs per 1×10^9 leukocytes in the LP sample (fig. 2D). The target cell fraction contained a residual 8% CD141^{low} pDCs and 10% CD141^{low} mDCs on average (not shown), resulting in a purity of CD141$^+$ cells of 83% on average (fig. 2B; n = 22).

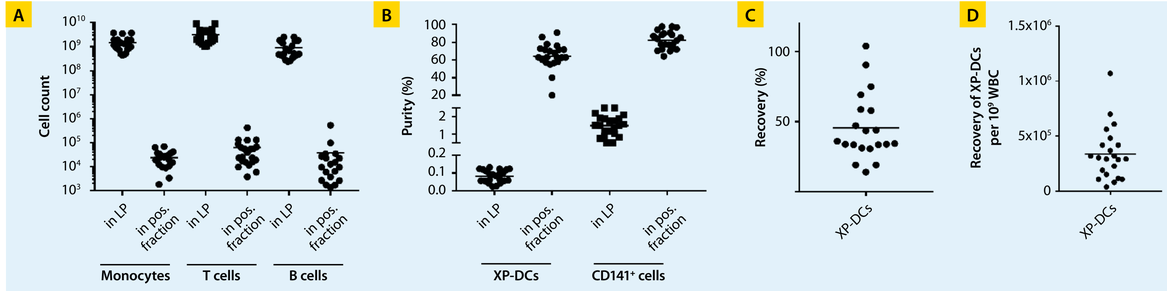

Figure 2

2 Activated XP-DCs produce a specific set of cytokines and show phenotypic characteristics of mature DCs

XP-DCs express high levels of TLR3 and respond to poly (I:C) stimulation by up-regulation of costimulatory molecules and production of large amounts of IFN-λ. To test their activation status upon TLR3 triggering, isolated XP-DCs were stimulated for 6 h or overnight in the presence or absence of poly (I:C) and analyzed by flow cytometry. Expression levels of some activation markers were already elevated in the absence of poly (I:C) while the levels of CCR7 and CD86 were additionally increased upon stimulation.

Overnight cultivation (17–19 h) further increased expression of all markers analyzed (fig. 3A; bar diagram; n = 3). In addition, cell culture supernatants were analyzed for cytokine secretion by ELISA or bead array (MACSPlex Cytokine 12 Kit, human). The stimulated cells secreted large amounts of IFN-λ, TNFα, and IL-6 with fast kinetics upon TLR3 triggering, whereas non-stimulated DCs failed to secrete cytokines. Substantial amounts of IFN-α could also be detected, presumably due to residual pDCs (fig. 3B).

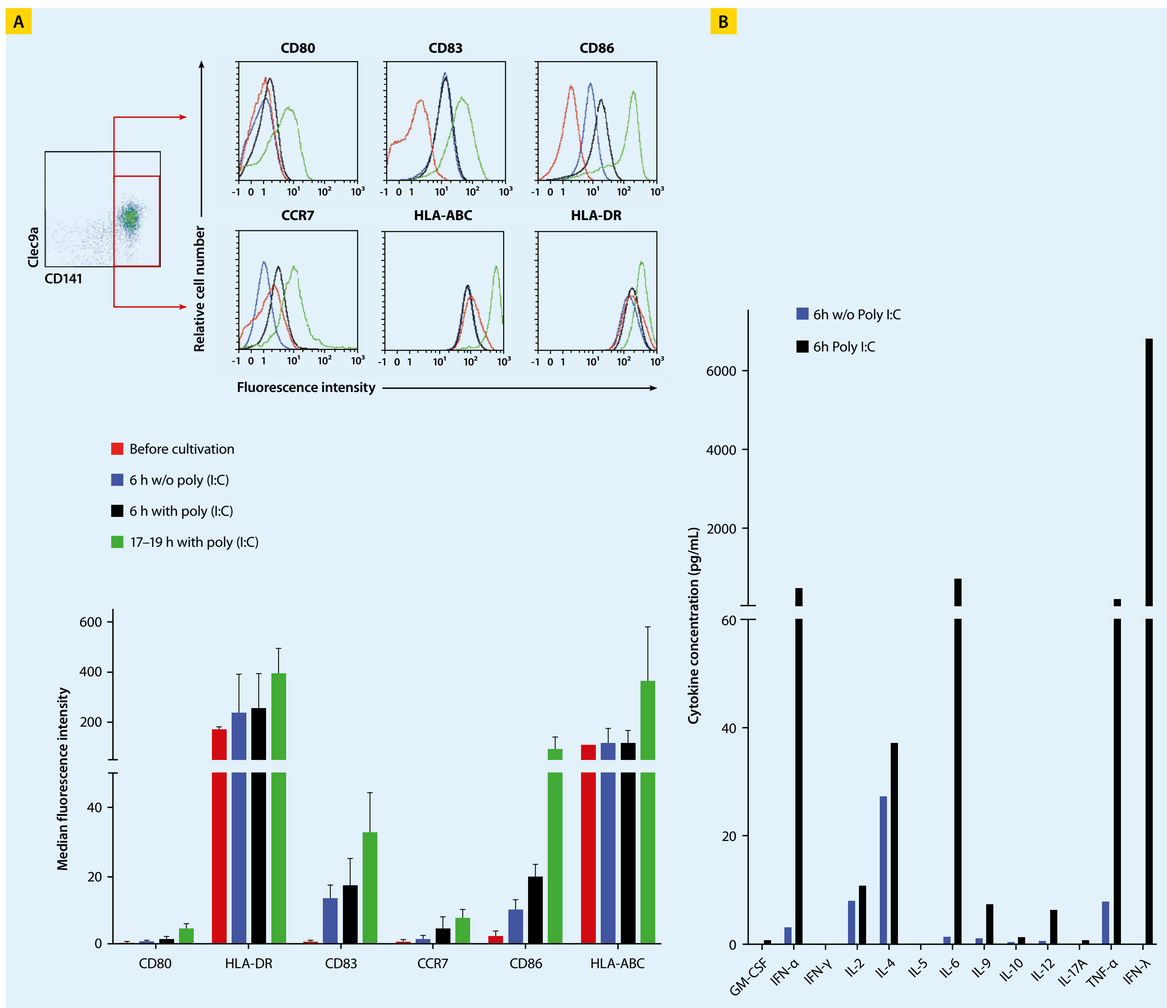

Figure 3

3 Peptide-loaded mature XP-DCs induce proliferation of antigen-specific T cells

The cross-presentation capability of activated CD141$^+$ DCs was demonstrated by their capacity to re-stimulate autologous Ag-specific CD8$^+$ T cells. To this end, XP-DCs were isolated from HLA-A2.1$^+$ CMV-seropositive donors. To analyze Ag-specific stimulation, enriched XP-DCs were then incubated with pp65- and IE-1-derived peptide pools (MACS® GMP PepTivator® HCMV pp65 and PepTivator CMV IE-1) and simultaneously activated with poly (I:C) for 6 h. Activated and antigen-loaded XP-DCs were then cocultured with CellTrace™ Dye–labeled autologous CD8$^+$ T cells. Non-loaded XP-DCs were used as control. After ten days T cell proliferation was assessed based on cell numbers and the reduction of CellTrace Dye staining intensity. Additionally, the specificity of T cell proliferation was analyzed by CMV pentamer staining; the analysis of T cell proliferation in the sample stimulated with non-loaded XP-DCs served as a control. T cells re-stimulated with XP-DCs loaded with pp65- and IE-1-derived peptide pools proliferated in an Ag-specific manner as shown by a 6-fold higher proliferation rate (fig. 4A) and reduced CellTrace Dye staining intensity (fig. 4B) compared to the sample with non-loaded XP-DCs. Furthermore, Ag specificity was shown by the increased frequency of pp65 peptide (NLVPMVATV)–specific and IE-1 peptide (VLAELVKQI)–specific CD8$^+$ T cells detected by pentamer staining after stimulation with antigen-loaded XP-DCs (fig. 4B).

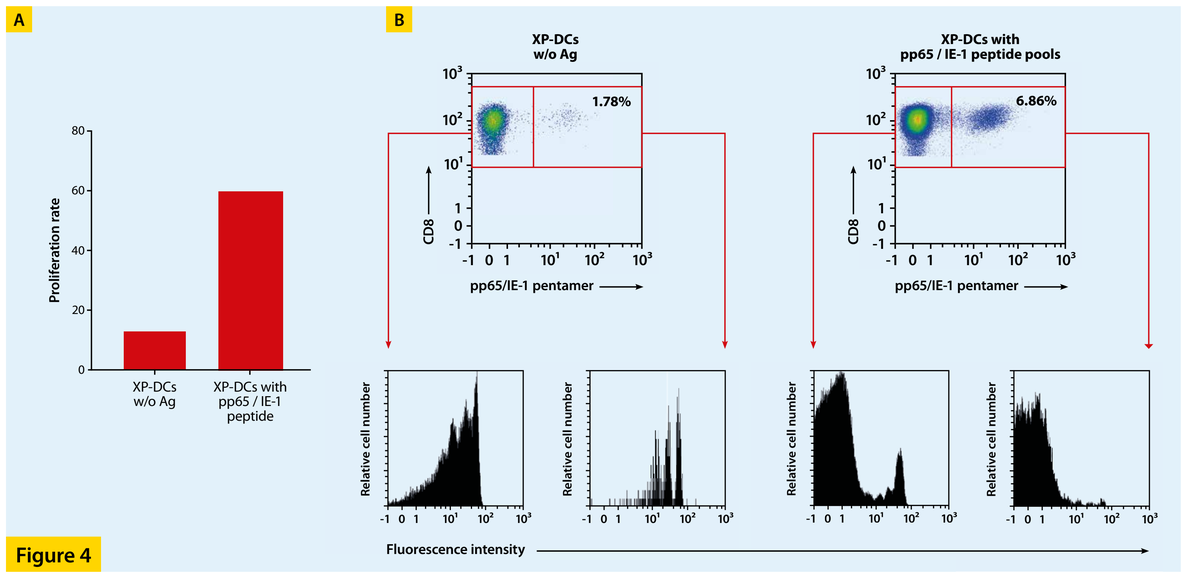

Figure 4

4 XP-DCs loaded with complete protein antigen are superior in inducing antigen-specific CTL responses

To show the antigen uptake and processing capacity of the XP-DCs, isolated cells from an HLA-A2.1$^+$ CMV-seropositive donor were loaded overnight with pp65 protein or pp65-derived peptide pool and stimulated for 3 h with poly (I:C). Activated, antigen-loaded XP-DCs were then cocultured with CellTrace Dye–labeled autologous PBMCs. After ten days cells were stained with pp65-specific pentamer and proliferation was determined by CellTrace Dye dilution and cell count. The Ag specificity of expanded T cells was further evaluated by re-stimulation with pp65- or MART-1–derived peptide pools and intracellular cytokine staining. T cells stimulated with pp65 protein–loaded XP-DCs showed a seven times higher frequency of CD8$^+$ T cells specific for the immunodominant pp65 peptide NLVPMVATV (fig. 5A) and also a higher proliferation rate (fig. 5B) compared to the sample with peptide pool–loaded XP-DCs. CD4$^+$ T cells showed only a negligible proliferation rate (fig. 5A). Expanded T cells re-stimulated with pp65-derived peptide pool produced IFN-γ and TNF-α, but no IL-4. In contrast, re-stimulation with the irrelevant MART-1–derived peptide pool did not induce cytokine production (fig. 5C). These data demonstrate the capacity of isolated XP-DCs to take up, process, and cross-present antigens, and efficiently restimulate antigen-specific CD8$^+$ memory T cells.

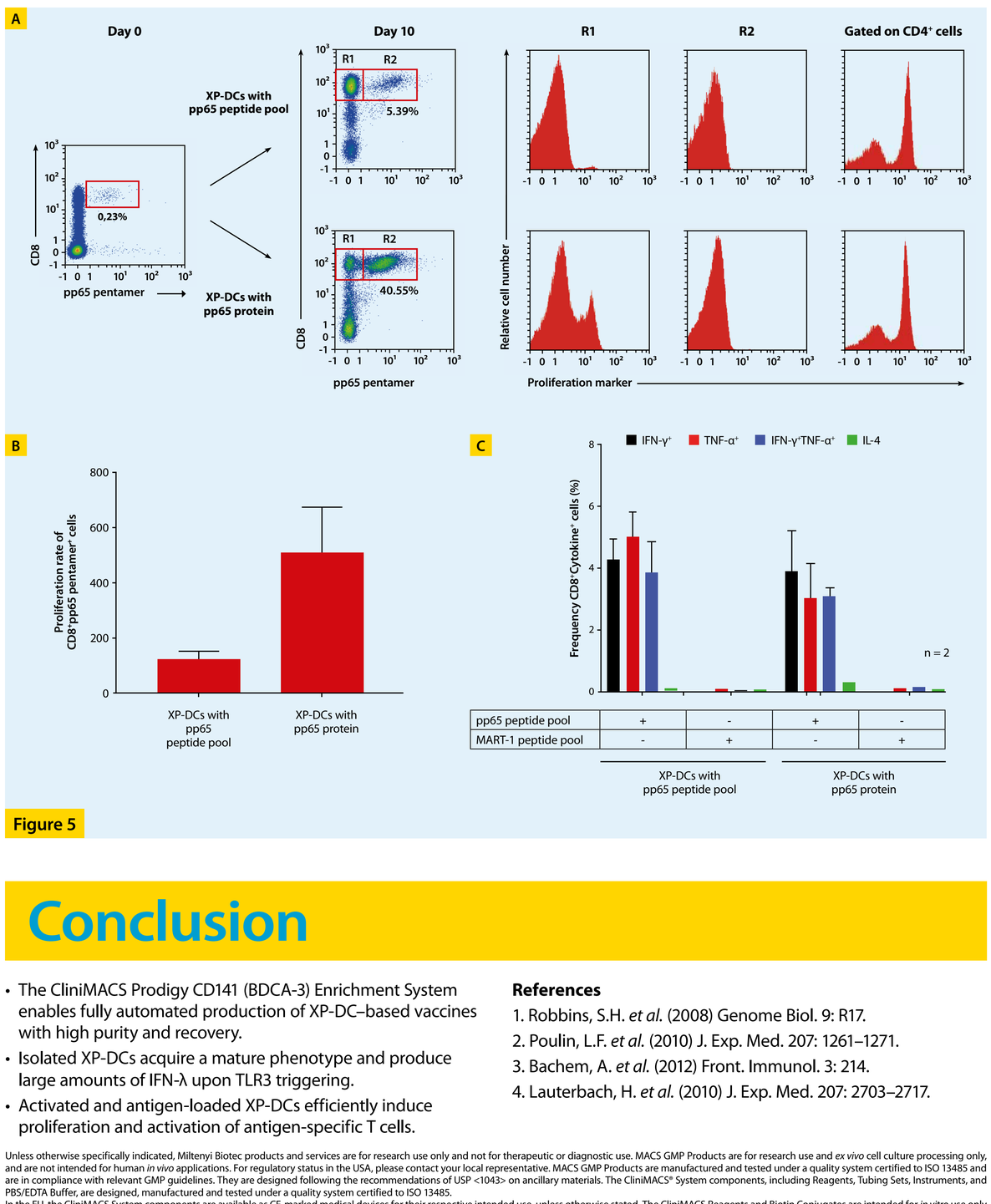

Figure 5

Conclusion

- The CliniMACS Prodigy CD141 (BDCA-3) Enrichment System enables fully automated production of XP-DC–based vaccines with high purity and recovery.
- Isolated XP-DCs acquire a mature phenotype and produce large amounts of IFN-λ upon TLR3 triggering.
- Activated and antigen-loaded XP-DCs efficiently induce proliferation and activation of antigen-specific T cells.

References

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