Blood DC preparations generated using automated CliniMACS Prodigy® CD1c/CD304 enrichment and activation system efficiently activate CD8+ antigen-specific T cells

Caroline Angerer[1], Carola Schöggl[1], Katja Petry[1], Gerty Schreibelt[2], Jeanette M. Pots[2], I. Jolanda M. De Vries[2], Andrzej Dzionek[1] and Mareke Brüning[1]
[1]Miltenyi Biotec GmbH, Bergisch Gladbach, Germany | [2]Radboud University Medical Center, Department of Tumor Immunology, Nijmegen, The Netherlands

Introduction

The innate and adaptive immune functions of plasmacytoid and myeloid dendritic cells (pDCs and mDCs) make them an attractive tool for anti-cancer therapy. Clinical efficacy of vaccination with activated pDCs or mDCs loaded with tumor-derived peptides could recently be demonstrated in phase I/II studies in melanoma patients. The treatment resulted in successful induction of anti-tumor immune responses and improved overall survival[1,2]. In addition, data from the mouse system[3,4] suggest that IFN-α–producing pDCs are capable of trans-activating mDCs, thereby enhancing the cross-presentation capacity of mDCs resulting in an improved tumor response. A combination of both natural blood DC (BDC) subsets therefore provides a promising vaccination approach in cancer immune therapy.

Workflow for the BDC enrichment and activation system on the CliniMACS Prodigy®
The preparation of BDC vaccines consisting of antigen-loaded CD304 (BDCA-4)+ pDCs and CD1c (BDCA-1)+ mDCs requires a separation system, which enables the pre-depletion of monocytes and B cells and the subsequent enrichment and overnight culture of BDCs. Using the CliniMACS Prodigy®, Tubing Set 310, and the LP-BDC Pre-Depletion process, monocytes and B cells are depleted efficiently in the first step, while CD1c (BDCA-1)+ mDCs are labeled with CliniMACS® CD1c (BDCA-1)-Biotin. In the second step, utilizing the LP-BDC Enrichment + Culture process and Tubing Set 510, BDCs are enriched by means of CliniMACS Anti-Biotin and CD304 (BDCA-4) Reagents and subsequently cultured overnight, activated, and loaded with tumor-related antigen (fig. 1).

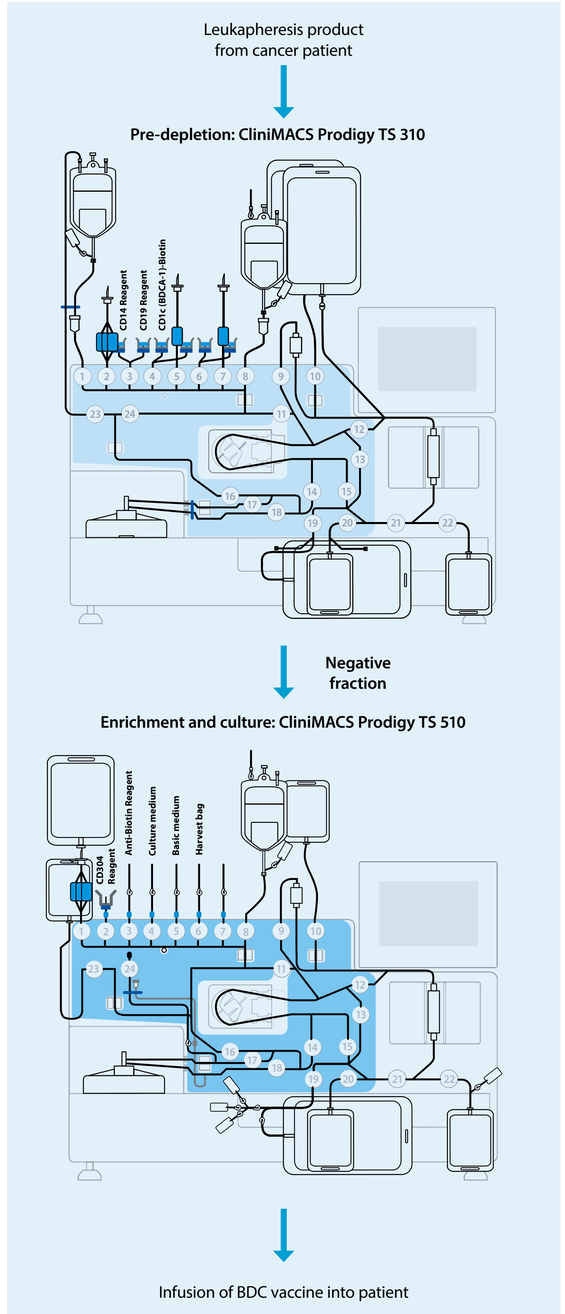


Figure 1

Results

1 Performance of the automated BDC enrichment and culture process

Using the CliniMACS Prodigy CD1c/CD304 System, BDCs were routinely enriched to a purity of 81% with a recovery of 89% (fig. 2A). Isolated BDCs were cultured overnight in the presence of GM-CSF and IL-3 and activated with TLR ligands. Activated BDCs were generated with recoveries of 53% on average, as calculated from the number of DCs present in the starting material. The viability of BDCs was evaluated by flow cytometry based on scatter signals and PI fluorescence and amounted to 83% (fig. 2A). DC subsets were analyzed by an 8-color staining procedure: pDCs and mDCs were determined within viable PI-CD45+CD14-CD20- cells based on BDCA-2 and CD123 expression and FcεR1 and CD123 expression, respectively (fig. 2B). Monocyte and B cell depletion was verified according to analysis of CD14 and CD20 staining.

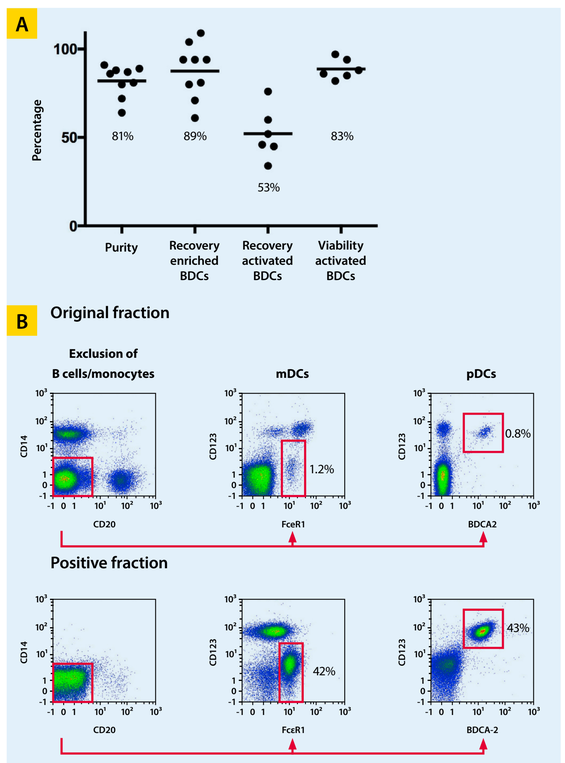


Figure 2

2 Activated BDCs show phenotypic characteristics of mature DCs

Isolated BDCs were cultured overnight in the presence of TLR agonists for activation. Upon culture mDCs and pDCs were distinguished from each other by use of the myeloid marker CD11c. The mDCs showed a high expression level of CD11c, whereas pDCs were characterized by low expression levels for CD11c. For exclusion of residual B cells, T cells, monocytes, and granulocytes a cocktail of fluorochrome-conjugated antibodies against lineage markers was used. According to flow cytometry analysis after overnight stimulation, BDCs acquired phenotypic characteristics of mature DCs. Expression of costimulatory receptors CD80 and CD86 as well as the activation marker CD83 was up-regulated (fig. 3). CCR7, which is required for migration of DCs to draining lymph nodes, was also significantly up-regulated (fig. 3). MFI denotes the mean fluorescence intensity. Isotype controls are indicated in black, specific antibodies in blue (BDC culture w/o stimulus) and red (BDC culture + TLR agonist). Importantly, BDCs maintained their activated phenotype after freezing and thawing and showed high viability of 87% on average (not shown).

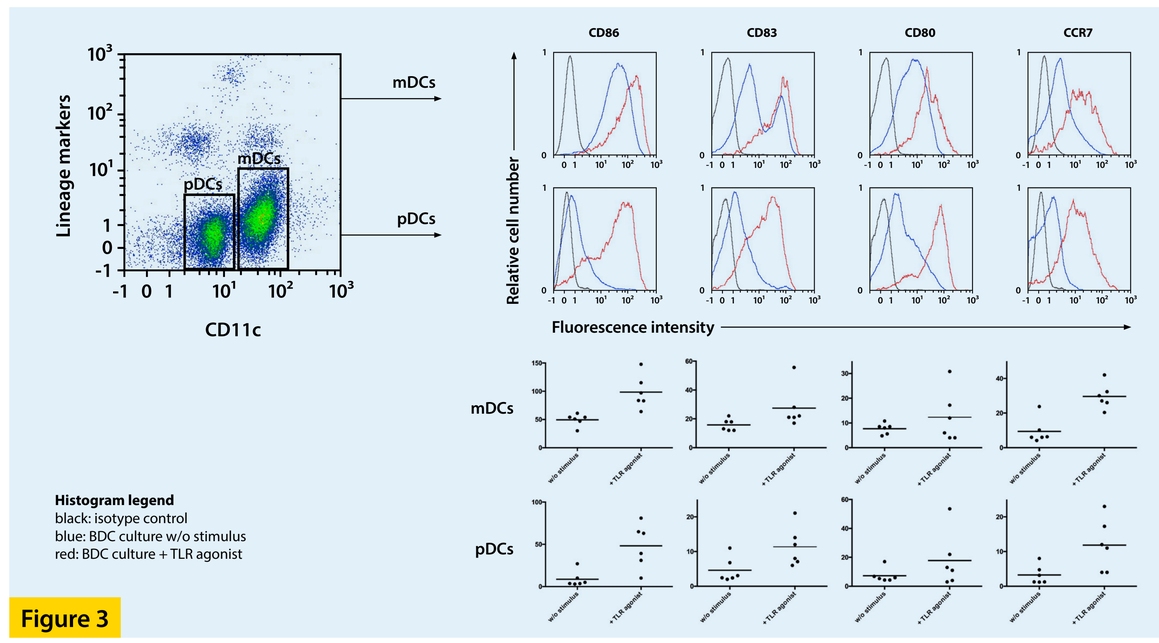


Figure 3

3 Peptide-loaded mature BDCs induce activation of antigen-specific T cells

The ability of BDCs to activate T cells was demonstrated by the restimulation of antigen-specific, autologous pan T cells originated from HLA-A2.1+ CMV–seropositive donors. To this end, isolated BDCs were cultured in the CliniMACS Prodigy overnight in the presence of IL-3, GM-CSF, and a pp65-derived peptide pool (MACS® GMP PepTivator® HCMV pp65, Miltenyi Biotec). Subsequently, antigen-loaded BDCs were cocultured with autologous T cells for an additional 6 hours. Production of IFN-γ and TNF-α in re-stimulated T cells was analyzed by intracellular staining (fig. 4). pp65-loaded BDCs induced strong secretion of IFN-γ and TNF-α in cocultured CD8+ T cells in an antigen-dependent manner, as BDCs loaded with irrelevant antigen or cultured without antigen did not induce cytokine production.

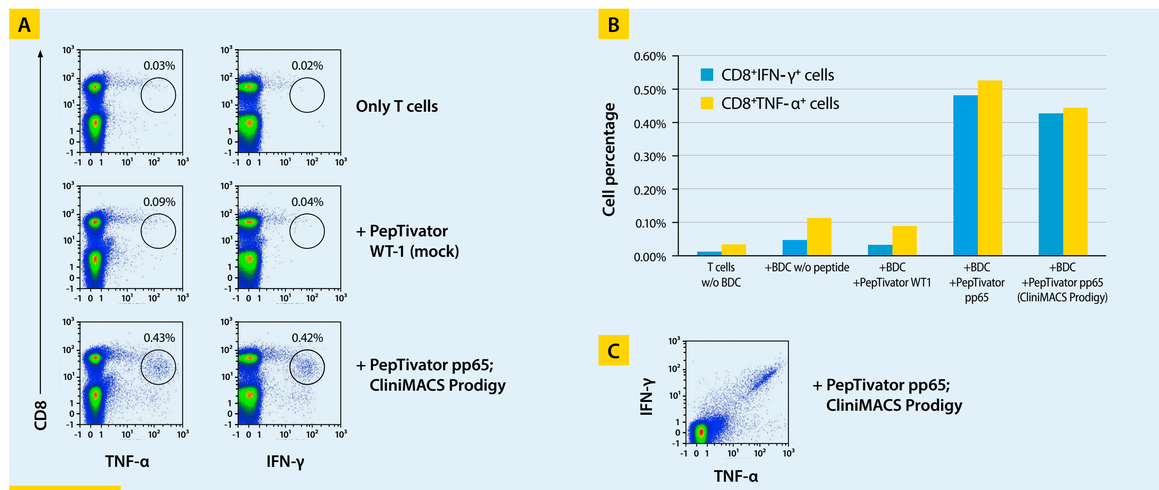


Figure 4

4 Peptide-loaded BDCs are strong inducers of T cell proliferation

The T cell activation capacity of BDCs was additionally evaluated in a T cell proliferation assay. For this purpose, autologous T cells from HLA-A2.1+ CMV–seropositive donors were labeled with CellTrace™ Violet and cocultured with activated BDCs loaded with PepTivator HCMV pp65. After 6 and 9 days of stimulation cell numbers and the CellTrace Violet staining intensity of the CD8+ T cells were analyzed (fig. 5). BDCs induced strong proliferation of CD8+ T cells as indicated by the increasing cell counts and the reduction of CellTrace Violet staining. Antigen specificity of proliferating T cells was confirmed by CMV pp65 tetramer (NLVPMVATV) staining (fig. 5C). As expected after 9 days of coculture, all pp65 tetramer–positive T cells showed reduced CellTrace Violet staining. These data demonstrate the functional capacity of BDCs to efficiently re-stimulate CD8+ memory T cells and additionally confirms the antigen specificity of the stimulation.

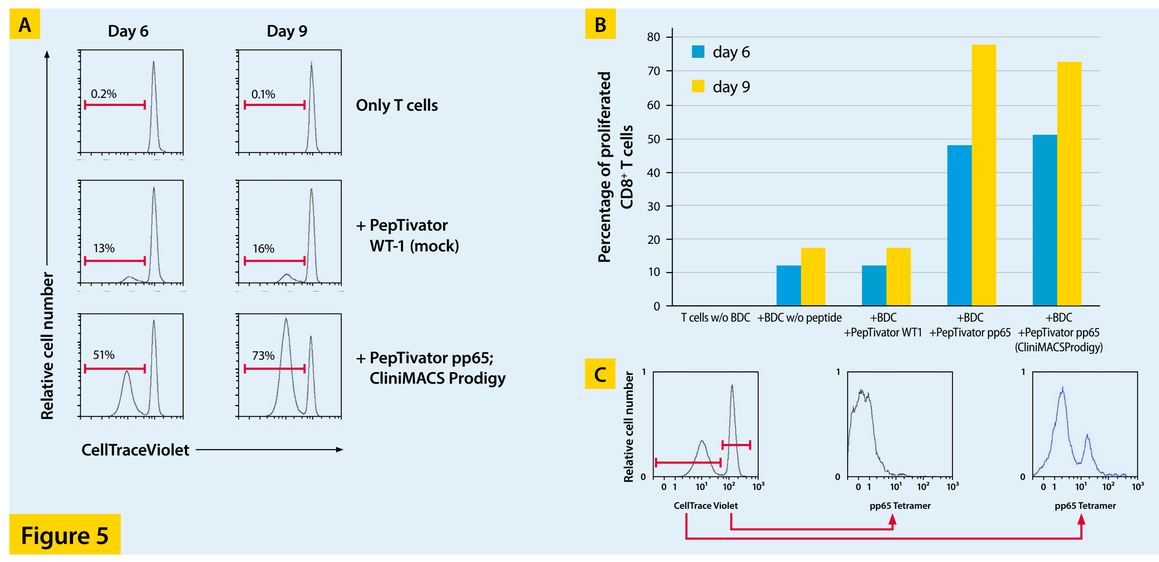


Figure 5

Conclusion and outlook

- The CliniMACS Prodigy CD1c/CD304 enrichment and activation system enables fully automated production of BDC-based vaccines with high purities and recoveries.
- BDCs prepared using the CliniMACS Prodigy enrichment and activation system show characteristics of mature DCs in terms of surface marker expression and functional capacity to induce activation and proliferation of antigen-specific CD8+ T cells.

Reference
1. Tel, J. *et al.* (2013) Cancer Res. 73: 1063–1075.
2. Schreibelt, G. *et al.* (2015) Clin. Cancer Res. 10: Epub ahead of print, Dec. 28. DOI: 10.1158/1078-0432.CCR-15-2205.
3. Cantisani, R. *et al.* (2011) Hum. Immunol. 72: 1018–1021.
4. Lou, Y. *et al.* (2007) J. Immunol. 178: 1534–1541.

The CliniMACS® System components, including Reagents, Tubing Sets, Instruments, and PBS/EDTA Buffer, are manufactured and controlled under an ISO 13485–certified quality system. In the EU, the CliniMACS System components are available as CE-marked medical devices. In the US, the CliniMACS CD34 Reagent System, including the CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Sets TS and LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved; all other products of the CliniMACS Product Line are available for use only under an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE). CliniMACS MicroBeads are for research use only and not for human therapeutic or diagnostic use. Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. CliniMACS, CliniMACS Prodigy, MACS, and PepTivator are registered trademarks or trademarks of Miltenyi Biotec GmbH. All other trademarks mentioned in this document are the property of their respective owners and are used for identification purposes only. Copyright © 2016 Miltenyi Biotec GmbH. All rights reserved.