An Animal Component-Free, Serum-Free Culture Method for Generation of Human Dendritic Cells

Nooshin Tabatabaei-Zavareh[1], Tim A. Le Fevre[1], Stephen J. Szilvassy[1], Terry E. Thomas[1], Allen C. Eaves[1,2], and Albertus W. Wognum[1]
[1] STEMCELL Technologies Inc., Vancouver, BC, Canada [2] Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Introduction

Dendritic cells (DCs) are potent antigen-presenting cells that have a central role in T cell immunity. Immature DCs in peripheral tissues capture antigens which triggers their maturation in the presence of inflammatory cytokines and other signals. Mature DCs present pathogen-derived antigens to T cells thereby inducing T cell activation. Mature DCs also produce cytokines such as interleukin-12 (IL-12) which aid in activating pro-inflammatory T cell subsets (T helper type 1 (Th1)) as well as cytotoxic T cells. DCs are rare in peripheral blood (PB) and not readily accessible from tissues, however large numbers of both immature and mature DCs can be generated *in vitro* from more prevalent precursors such as PB monocytes.

The use of human or animal-derived media components is undesirable in the development of cell culture procedures that may be used for clinical applications. Described here is the use of an animal component-free (ACF), serum-free medium, ImmunoCult™-ACF DC, for *in vitro* generation of DCs from monocytes. ImmunoCult™-ACF DC contains recombinant proteins and synthetic components and does not contain serum or other human or animal-derived components.

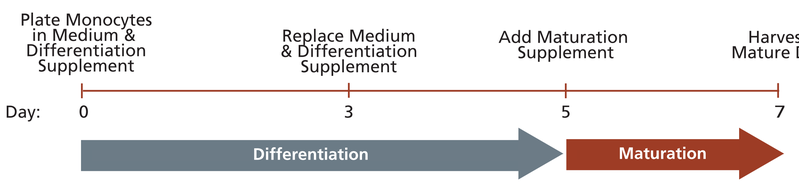
ImmunoCult™-ACF DC medium and differentiation supplement supported differentiation of PB monocytes into immature $CD14^{-}CD83^{-/lo}$ DCs. A maturation supplement containing pro-inflammatory cytokines and mediators then enabled maturation of immature DCs into fully mature $CD14^{-}CD83^{+}$ DCs with a yield of 45 ± 25% (mean ± SD, n=39). The cultured mature DCs produced high levels of IL-12p70 (361 ± 81 pg/ml, mean ± SEM, n=27 in cultures initiated with 10^6 monocytes/mL). In MLR assays, mature DCs induced the proliferation of allogeneic T cells and also promoted the proliferation of autologous $CD8^{+}$ T cells when loaded with antigenic viral peptides (a peptide pool derived from CMV, EBV and FLU viruses). In conclusion, functional DCs can be generated efficiently by differentiation of monocytes in a completely animal and human component-free medium. This medium and culture method will enable research into the development of DC-based cellular therapies.

Methods

Culture Protocol

$CD14^{+}$ monocytes were isolated from peripheral blood mononuclear cells using the EasySep™ Human Monocyte Isolation Kit. The isolated monocytes (day 0) were plated out at $1x10^6$ cells/mL in ImmunoCult™-ACF DC medium supplemented with Differentiation Supplement (containing animal component-free GM-CSF and IL-4). On day 3, medium was replaced with fresh medium and Differentiation Supplement and cells were cultured for an additional 2 days to promote differentiation of monocytes into $CD14^{-}CD83^{-/lo}$ immature DCs. On day 5, Maturation Supplement (containing pro-inflammatory cytokines and mediators) was added to the cultures and mature DCs were harvested after 2 days (day 7, Figure 1).

FIGURE 1: Protocol Diagram

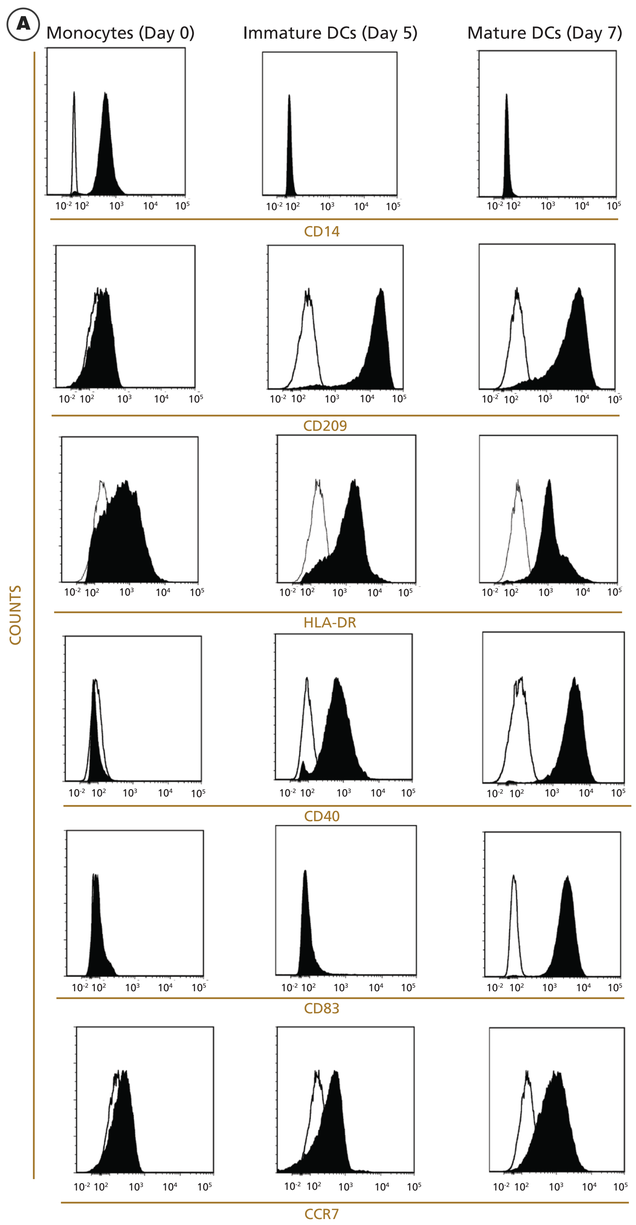


Assessment of Cultured DCs

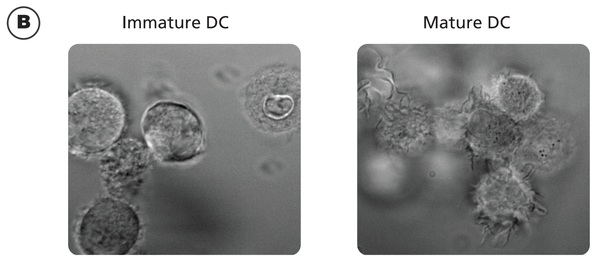
Mature DCs were counted and analysed for the expression of cell surface markers by flow cytometry (Figures 2 and 3). Cell yields were calculated relative to the number of monocytes plated on day 0. IL-12p70 production was measured by ELISA on supernatants harvested on day 7 (Figure 4). T cell activation was measured in mixed-leukocyte reaction (MLR) co-cultures of allogeneic T cells with immature and mature DCs, respectively (Figure 5). Antigen-specific activation of autologous $CD8^{+}$ T cells was measured using DCs loaded with HLA Class I CMV, EBV and Flu virus peptide pool. DC:T co-cultures were performed in ImmunoCult™-XF T cell expansion medium using CFSE-labelled T cells. T cell proliferation was assessed after 5 - 7 days of co-culture by measuring the decrease of CFSE fluorescence by flow cytometry. In all flow cytometry analyses dead cells and debris were excluded by light scatter profile and 7-AAD staining.

Results

FIGURE 2: Phenotype of immature and mature DCs generated in ImmunoCult™-ACF DC

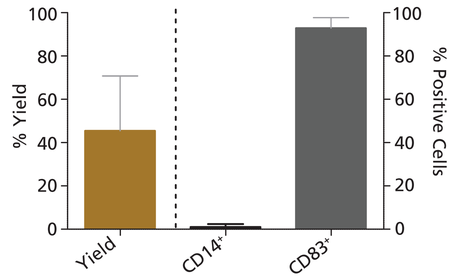


Monocytes (day 0), immature DCs (day 5) and mature DCs (day 7) were stained with fluorochrome-conjugated antibodies against CD14, CD209, HLA-DR, CD40, CD83 and CCR7. Isotype control antibody staining is shown as empty histograms. Cell debris and dead cells were gated out using scatter profile and 7-AAD staining. Mature DCs are $CD14^{-}$ similar to immature DCs but upregulate CD40, CD83 and CCR7 upon maturation. CD209 expression is higher in immature DCs compared to mature DCs which is consistent with their antigen uptake role.



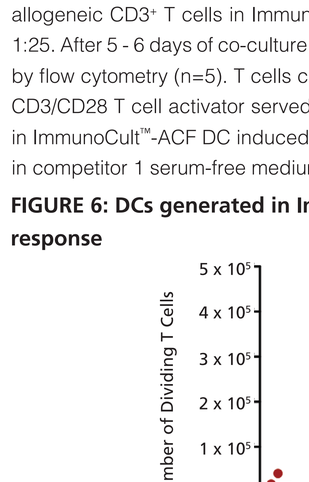
Bright field images of live immature and mature DCs at 630X magnification.

FIGURE 3: Mature $CD14^{-}CD83^{+}$ DCs are obtained by differentiation and maturation of monocytes in ImmunoCult™-ACF DC medium and supplements



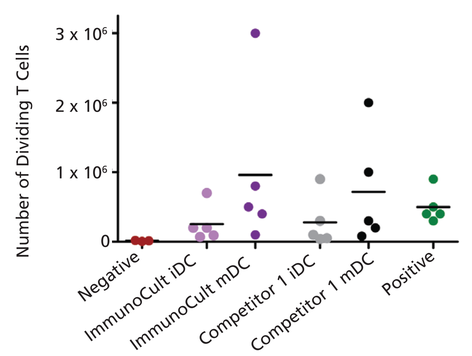
Starting with $1x10^6$ monocytes per mL, the yield of viable mature DCs after 7 days of culture was 45 ± 25% (Mean ± SD, n=39) with only 1 ± 1% of cells still expressing the monocyte marker CD14 and 93 ± 5% expressing the mature DC marker CD83.

FIGURE 4: Mature DCs cultured in ImmunoCult™-ACF DC produce the pro-Inflammatory cytokine IL-12



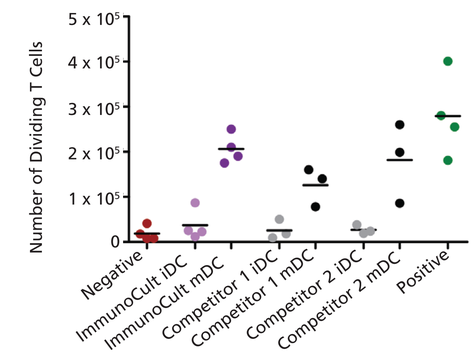
Immature DCs (day 5) were cultured in ImmunoCult™-ACF DC medium supplemented with Maturation Supplement for 2 days, or cultured without Maturation Supplement for the same time (shown as immature DCs). Supernatant was collected and assayed for the production of IL-12p70 using ELISA. IL-12p70 concentrations in supernatants of immature and mature DCs were 5 ± 2 and 361 ± 81 pg/ml, respectively (mean ± SEM of 27 independent experiments).

FIGURE 5: Mature DCs generated in ImmunoCult™-ACF DC promote allogeneic T cell proliferation in MLR assays



Immature and mature DCs (iDC and mDC) generated in ImmunoCult™-ACF or in a serum-free medium of another supplier (competitor 1) were co-cultured with $1x10^5$ CFSE-labelled allogeneic $CD3^{+}$ T cells in ImmunoCult™-XF T cell expansion medium at a DC:T cell ratio of 1:25. After 5 - 6 days of co-culture, the number of dividing $CD3^{+}CFSE^{lo}$ T cells was determined by flow cytometry (n=5). T cells cultured without DCs either without or with the ImmunoCult™ CD3/CD28 T cell activator served as negative and positive controls. Mature DCs generated in ImmunoCult™-ACF DC induced proliferation of allogeneic T cells similar to DCs generated in competitor 1 serum-free medium.

FIGURE 6: DCs generated in ImmunoCult-ACF DC induce antigen-specific T Cell response



Immature DCs generated in ImmunoCult™-ACF or in serum-free media of two other suppliers (competitors 1 and 2) were loaded with HLA Class I peptide pool from CMV, EBV and Flu viruses (CEF peptide pool) and stimulated for 2 days using Maturation Supplement. Competitor 2 mDCs were matured using supplier's Maturation Supplement. Immature DCs (iDCs) left unstimulated on day 7 were used as a control. Peptide-loaded mature DCs and iDCs were co-cultured with $1x10^5$ CFSE-labelled autologous $CD8^{+}$ T cells at DC:T cell ratios of either 1:4 or 1:10 in ImmunoCult™-XF T cell expansion medium. After 5 - 7 days of co-culture, the number of dividing T cells ($CD3^{+}CFSE^{lo}$) was measured. Mature DCs in ImmunoCult™-ACF DCs stimulate antigen-specific T cell proliferation similar to DCs generated in either competitor media.

Conclusions

- ImmunoCult™-ACF DC medium and supplements support the differentiation and maturation of monocytes into DCs in the absense of serum or other animal- or human derived components
- Mature DCs generated in ImmunoCult™-ACF DC lack CD14 expression and are $CD209^{+}$, $CD40^{hi}$, $CD83^{hi}$ and $CCR7^{hi}$
- Mature DCs generated in ImmunoCult™-ACF DC are functional as indicated by their ability to:
  - Produce the pro-inflammatory cytokine IL-12p70
  - Induce allogeneic T cell proliferation in MLR assays
  - Stimulate antigen-specific T cell responses to a CEF peptide pool

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