

# Time-Saving Dendritic Cell Generation No Medium Change - Less Reagents With HumanKine® GM-CSF and HumanKine IL4

## INTRODUCTION

Dendritic cells (DCs), originally identified by Steinman and his colleagues<sup>1</sup>, represent the pacemakers of the immune response. DCs are derived from bone marrow progenitors and circulate in the blood as immature precursors prior to migration into peripheral tissues. These cells are crucial to the presentation of peptides and proteins to T and B lymphocytes<sup>2</sup>. Within different tissues, DCs differentiate, and become active in the taking up and processing of antigens, and their subsequent presentation on the cell surface linked to major histocompatibility (MHC) molecules. The peptide binding proteins are of two types, MHC class I and II, which interact with and stimulate cytotoxic T lymphocytes and T helper cells, respectively. DCs are receiving increasing scientific and clinical interest due to their key role in anti-cancer host responses and potential use as biological adjuvants in tumor vaccines, as well as their involvement in the immunobiology of tolerance and autoimmunity<sup>3</sup>.

An important advance in DC biology, within the past few years, has been the ability to propagate, in vitro, large numbers of DCs, using defined cytokines. The current standard protocol requires the addition of recombinant GM-CSF and IL-4 to complete RPMI 1640 at nominal concentrations of 50 ng/mL (this media is often referred to as G4 DC). The standard protocol requires medium replacement on day 3 and day 5. Scientists at HumanZyme have developed an efficient human-cell based technology, HumaXpress®, for the scalable production of authentic human cytokines. Recombinant HumanKine GM-CSF and HumanKine IL-4 produced using the HumaXpress system show substantially higher stability in culture medium and have higher potency.

We determine, in the following report, that G4 DC using HumanKine GM-CSF and IL-4 at 5ng/mL and **without** medium replacement (HZ G4 DC) is similar to G4 DC made using industry standard E. coli expressed cytokines in a standard protocol (50 ng/mL, **with** medium replacement at day 3 and day 5) (ED G4 DC) for the differentiation of human monocyte-derived DCs in terms of the expression of surface markers, production of cytokines, antigen uptake, and antigen-presenting capacity.

## METHODS

Purified human peripheral blood monocytes were cultured in either G4 DC medium (as specified below) at  $5 \times 10^5$ /mL in humidified air containing 5% CO<sub>2</sub> at 37°C for a total of 7 days. HZ G4 DC was used at 5 ng/mL without medium replacement whereas EC G4 DC was used routinely (50 ng/mL with 50% medium replacement on day 3 and day 5). On day 6, LPS was added to half of the wells to induce DC maturation while the other half of the wells were used as sham treatment. At the end of the culture (7 days), the supernatants were harvested for cytokine measurement, while the resulting DCs were analyzed for the surface markers by flow cytometry, the antigen uptake by phagocytosis of FITC-Dextran, and the antigen-presenting capacity by allogeneic MLR.

## RESULTS

### DC expression of surface markers

Compared to DCs generated in a routine protocol (EC G4 DC at 50 ng/mL with 50% replacement on day 3 and day 5), DCs generated in the presence of HZ G4 DC at 5 ng/mL **without** medium replacement exhibited similar levels of costimulatory molecules CD80, CD83, and CD86, and slightly higher levels of MHC (HLA-ABC and HLA-DR) molecules (the red line histograms of the top and bottom panels of Figure 1). Upon LPS-stimulated maturation, DCs

differentiated in the presence of HZ G4 DC showed similar upregulation of costimulatory and MHC surface molecules as DCs generated in a routine (standard) protocol (Green line histograms of Figure 1). The higher levels of HLA-ABC and HLA-DR before and after LPS-induced maturation on DCs differentiated in the presence of HZ G4 DC were better illustrated by Figure 2. Thus, DCs differentiated in the presence of HZ G4 DC at 5 ng/mL without medium replacement are similar to or even better than DCs differentiated in the presence of EC G4 DC in a standard protocol in terms

of surface expression of costimulatory and MHC molecules.

### DC cytokine production

The profile of DC generation of selected cytokines and chemokines was measured by Pierce Cytokine Array. The data indicate that DCs generated in the presence of HZ G4 DC (5 ng/mL without medium replacement) showed a similar profile of cytokines and chemokines as DCs generated in the presence of EC G4 DC (50 ng/mL with medium replacement) before and after maturation (Figure 3).

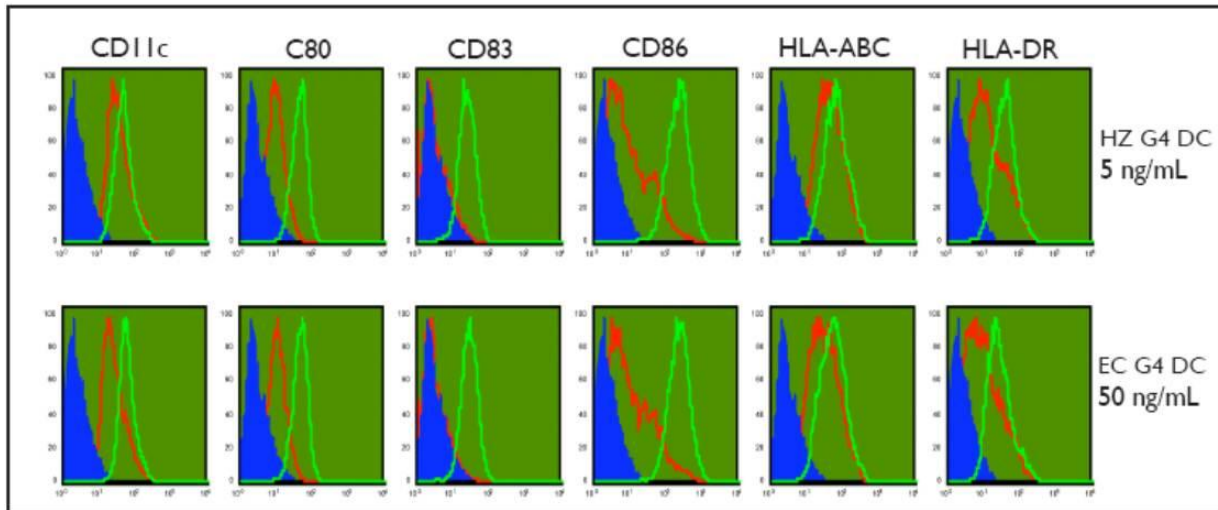


Figure 1. Overlay histograms of the expression of various surface markers by DCs generated and matured under different conditions. Filled Blue = isotype-matched control; Open Red = 24 h w/o LPS treatment; Open Green = 24 h LPS treatment.

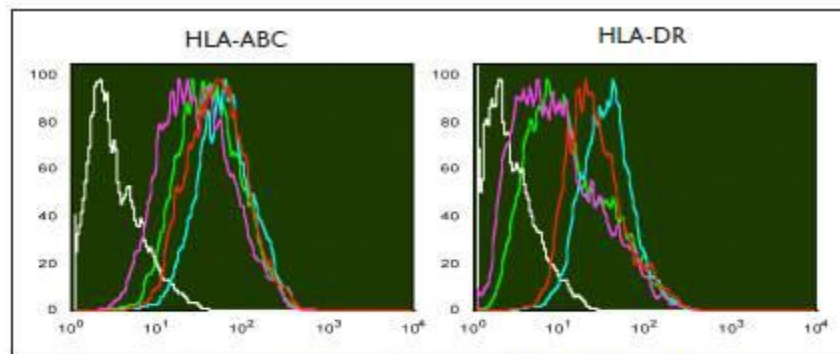


Figure 2. Re-plot of the expression of HLA-ABC and HLA-DR by DCs of Figure 1. White = isotype-matched control; Green = HZ G4 DC Without LPS treatment; Blue = HZ G4 DC with LPS treatment; Pink = EC G4 DC without LPS treatment; Red = EC with LPS treatment.

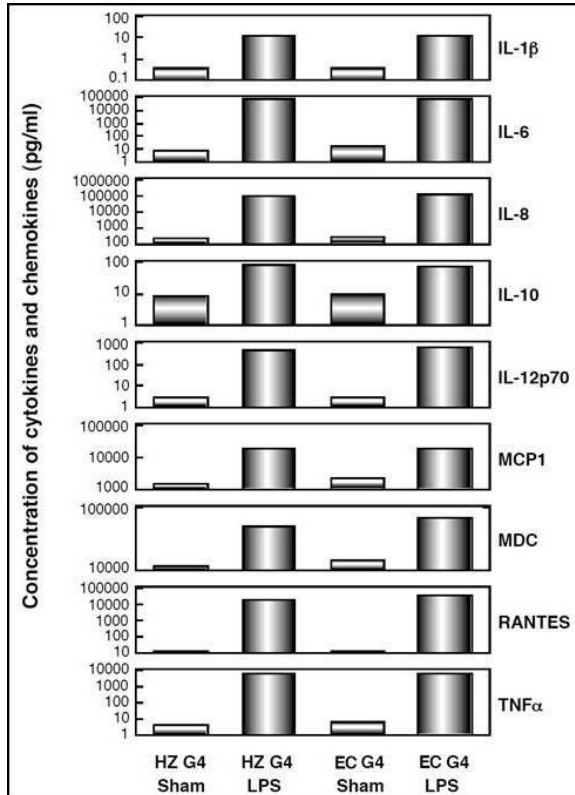


Figure 3. Production of selected cytokines and chemokines by DCs generated and matured under different conditions. (Scale: Cytokine and Chemokine concentration pg/mL.)

### DC antigen uptake capacity

DCs generated under different conditions were incubated with FITC-Dextran at 0.2 mg/mL at either 4°C (on ice) or 37°C for 1 h and the phagocytosis of FITC-Dextran was measured by flow cytometry. DCs generated in HZ G4 DC showed a similar capacity to engulf FITC-Dextran as DCs generated in EC G4 DC (Red histogram of Figure 4), suggesting a similar antigen uptake capacity. Upon LPS stimulated maturation for 24 h, DCs generated in the presence of HZ G4 DC decreased their antigen uptake capacity as did DCs generated in the presence of EC G4 DC, suggesting they also responded similarly to LPS-induced maturation.

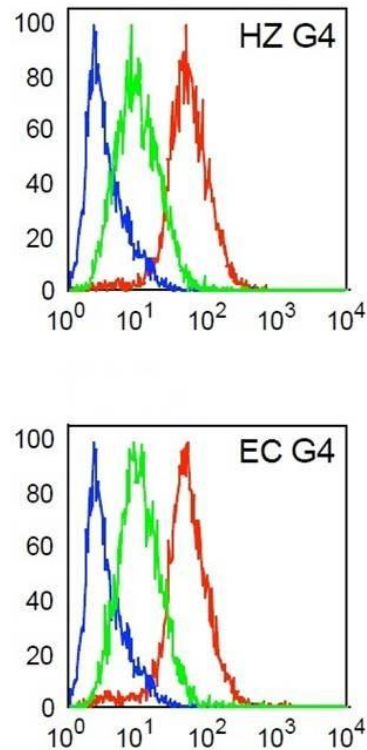


Figure 4. Phagocytosis of FITC-Dextran by DCs generated and matured under different conditions. Blue: FITC-Dextran uptake by DCs on ice for 1 hr.; Red: FITC-Dextran uptake by sham-treated DCs in an incubator for 1 hr.; Green: FITC-Dextran uptake by LPS-treated DCs in an incubator for 1 hr.

### DC antigen-presenting capacity

DCs differentiated in the presence of HZ G4 DC or EC G4 DC before or after LPS maturation were cultured in triplicate with allogeneic human peripheral blood T cells at various ratios for 5 days. The cultures were pulsed with 3H-TdR (0.5 uCi/well) for the last 18h before cell harvest. The proliferation of T lymphocytes was measured by beta scintillation counting. As shown by Figure 5, DCs differentiated in the presence of either HZ G4 DC or EC G4 DC showed similar low capacities to stimulate the proliferation of allogeneic T cells, in particular when DC:T ratio was low. After LPS-induced maturation, DCs differentiated under both conditions increased their capacity to stimulate the proliferation of allogeneic T cells. DCs generated in the presence of HZ G4 DC seemed to be even better than DCs generated in the presence of EC G4 DC in this regard (Figure 5).

Therefore, DCs differentiated in the presence of HZ G4 DC had similar or better antigen-presenting capacity than DCs differentiated in the presence of EC G4 DC.

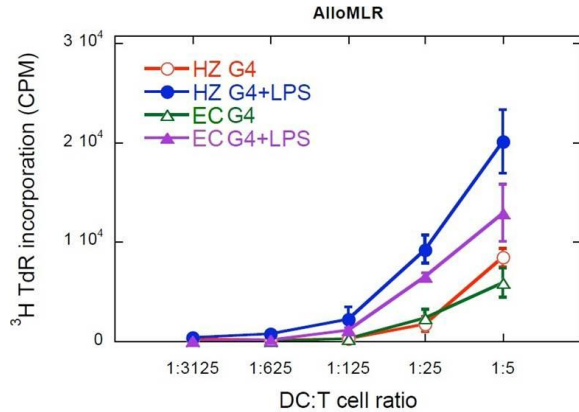


Figure 5. Allogenic MLR of DCs generated and matured under various conditions. Red: HZ G4 DC; Blue: HZ G4 DC and LPS maturation; Green: EC G4 DC; Purple: EC G4 DC with LPS maturation.

## CONCLUSION

In comparison with DCs generated in a standard protocol (EC G4 at 50 ng/mL with 50% medium replacement on day 3 and day 5), DCs generated in HZ G4 (5 ng/mL without medium replacement) demonstrated similar levels of surface expression of costimulatory & MHC molecules, a similar profile of cytokines and chemokines, a similarly high capacity

to engulf antigens, and a similarly low capacity for antigen-presentation. Upon maturation by LPS for 24 h, HZ G4 DCs, similar to EC G4 DCs, upregulated the expression of costimulatory & MHC molecules, elevated the production of many cytokines and chemokines, downregulated their antigen uptake capacities, and upregulated their antigen-presenting capacities. HZ G4 DCs showed slightly higher surface expression of MHC molecules as well as higher capacity to stimulate T cell proliferation in an allogeneic MLR setting. Overall, the usage of low concentration of HZ G4 DC without medium replacement is equal to or better than using E. coli-derived G4 (EC G4 DC) in a standard protocol (higher concentrations and with medium replacement).

In addition to G4 Media, HumanZyme has successfully produced an expanding range of tag-free cytokines, including difficult-to-express protein members of the TGFβ superfamily, using the HumaXpress technology. HumanZyme's authentic cytokines can be used as highly preferred reagents for cancer, inflammation, stem cell research, and antibody development.

## References

1. Steinman R and Cohn Z. J 1971. Exp Med. 137:1142-1162
2. Sathaporn S and Eremin O. 2001. JR Coll Surg 46:9-20