

The Effect of Hyperglycemia in CD4 T Cell Proliferation & Activation

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Introduction

The major driving force in the pathogenesis of prediabetes and Type 2 Diabetes is thought to be chronic and low grade systemic inflammation. Immune cells are key players in the induction of this chronic inflammation; however, the type of immune cells and mechanisms underlying the rapidly progressing pathogenesis of this disease are unclear. Therefore, we examined whether hyperglycemia alters CD4 T cell activation, differentiation and survival.

Dendritic cells and T cells were isolated from human peripheral blood and cultured with varying amounts of glucose. Using flow cytometry we found dendritic cells primed in hyperglycemic conditions induced CD4 T cells to exhibit a more activated phenotype capable of mobilization, as evident by upregulation of the adhesion molecule CD11a. Additionally, these activated CD4 T cells had a lower degree of proliferation and decreased expression of the apoptotic protein caspase-3 compared to CD4 T cells primed by dendritic cells stimulated with a physiological concentration of glucose. We conclude that hyperglycemia drives CD4 T cells towards an activated immunophenotype, which could be assessed via flow cytometry in the clinic to further stratify patients with prediabetes and Type 2 Diabetes and potentially predict those patients at highest risk for progression of the disease.

Results

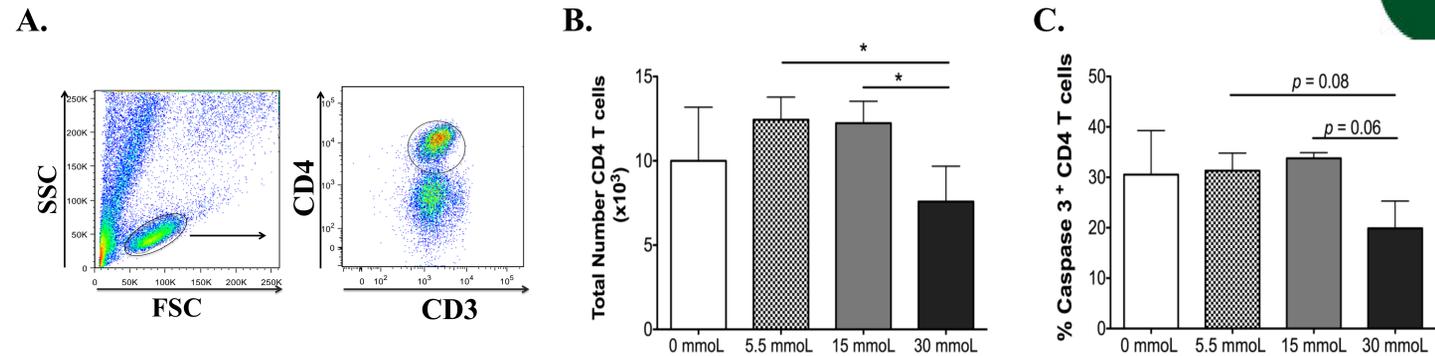
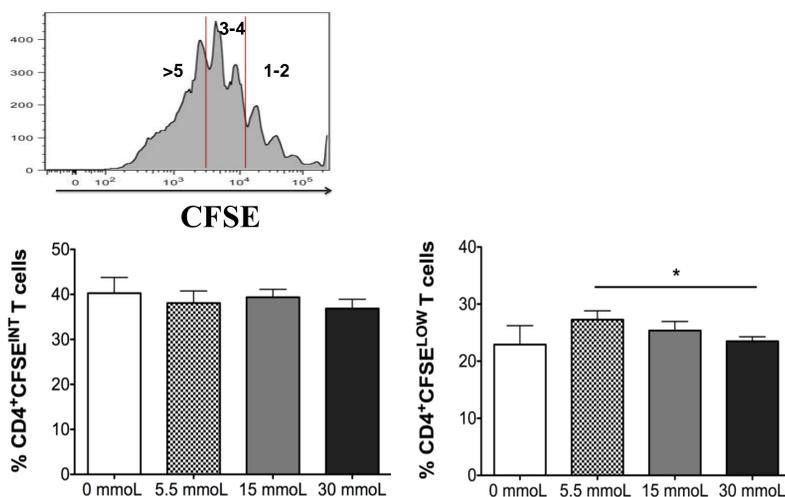


Figure 2. Total number of CD4 T cells and percentage of Caspase 3+ CD4 T cells recovered from co-culture with dendritic cells primed in various concentrations of glucose. Peripheral blood derived CD3+ CD4 T cells were co-cultured with autologous dendritic cells primed in varying concentrations of glucose. After 7 days in culture, the total CD3+CD4+ T cells (CD4 T cells) per well were enumerated via flow cytometry. Gating Scheme (A), bar graph (B). Percentage of Caspase 3+ (apoptotic) CD4 T cells were enumerated via flow cytometry (C). *p ≤ 0.05

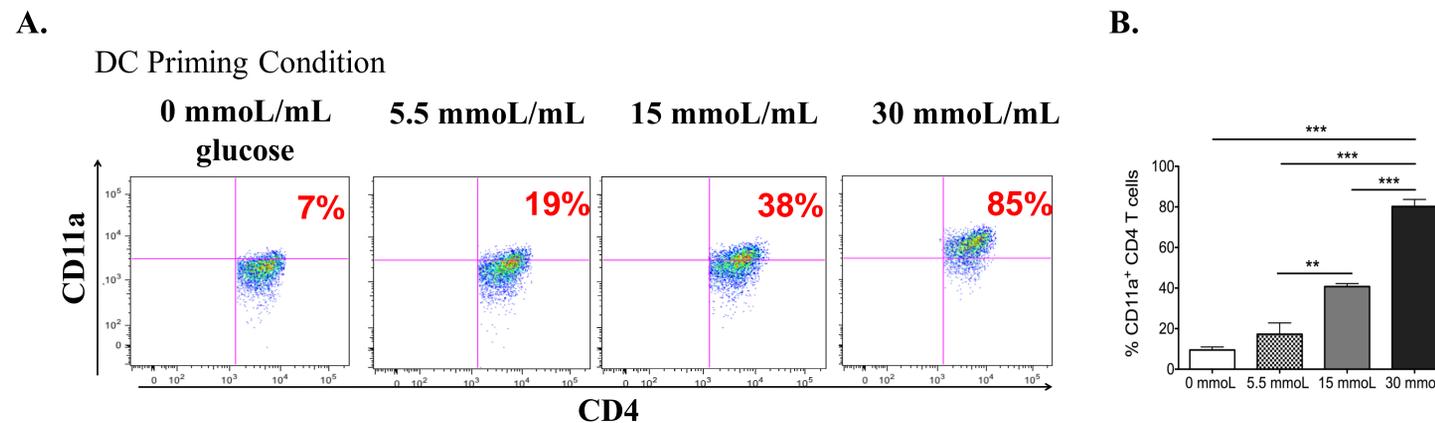


Figure 3. Human CD4 T cells increase their CD11a expression when cultured with DCs primed in increasing concentrations of glucose. Peripheral blood derived human CD4 T cells were co-cultured with autologous dendritic cells primed in varying concentrations of glucose. After 7 days in culture, effector status of CD3+ CD4+ T cells was determined by analysis of CD11a expression using flow cytometry. (A) Representative dot plots of this expression and (B) bar graphs are shown. **p ≤ 0.01; ***p ≤ 0.0001

Conclusion

Hyperglycemic (diabetic) culture conditions negatively affected the proliferative capacity of CD4 T cells, evident not only by a decrease in total number of cells recovered after co-culture, but also by the total number of cell divisions CD4 T cells underwent when primed with DCs exposed to hyperglycemic culture conditions.

There is a dramatic glucose dose-dependent increase in CD11a expression on CD4 T cells in co-culture with DCs.

Taken together these results suggest CD4 T cells are part of a mechanism that may be contributing to the systemic inflammatory process occurring in pre-diabetes and Type 2 Diabetes.

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Figure 1. CD4 T cell proliferation after co-culture with dendritic cells primed in increasing concentrations of glucose. Peripheral blood derived CFSE-labeled CD3+ CD4 T cells were co-cultured with autologous dendritic cells primed in varying concentrations of glucose. Percentage of CFSE high (left), intermediate (middle) and low (right) CD4 T cells was assessed using flow cytometry. CFSE high was defined as 1-2 cellular divisions, CFSE intermediate was defined as 3-4 divisions and CFSE low was defined as >5 cell divisions. *p ≤ 0.05