AMDCC Pilot Grant Report

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The effects of diabetes on inflammation are unclear. We have used models of type 1 diabetes mellitus (T1DM) to explore the specific role of hyperglycemia on white blood cell (WBC) biology. To do this, we have made mice diabetic using the AMDCC low dose streptozotocin (STZ) protocol and after 4 weeks treated some mice for an additional 4 weeks with inhibitors of the sodium glucose co-transporter 2 (SGLT2) to assess the specific effects of hyperglycemia.

This pilot and feasibility award funded two aims

Aim 1. To determine if hyperglycemia reduction leads to an alteration in the inflammatory state of circulating monocytes and neutrophils.

Results:
We have submitted files (some of which are still being analyzed) of gene expression data obtained by microarray and also real time PCR (RT-PCR) from isolated neutrophils and monocytes obtained by FACS of blood from these mice. The two data sets are not totally in concert.

Monocytes: RT-PCR showed increases in the follow genes, S100A8, S100A9. IL1-β was decreased. All these gene changes reverted towards control (some more than others) with SGLT2 inhibitor treatment and glucose reduction. Surprisingly, neither RAGE nor HMGB1 or adhesion molecular mRNA levels were altered.

Neutrophils: Changes in neutrophil gene expression were much more remarkable than those of circulating monocytes. RT-PCR showed increased TNFα, IL1-β, S100A8/9, HMGB1, RAGE, and CCR2. Many of these mRNA levels were reduced along with glucose reduction. Others – IL1-β, HMGB1, ICAM1 – were not. M-CSF was increased and increased even further with SGLT2 inhibitor.

T-cells – along with the FACS analysis we also obtained data for T-cells. Diabetic mice had increased Il1-β, IL6, S100A9, ICAM and M-CSF, but this was not responsive to glucose reduction. Only GM-CSF decreased with the SGLT1 inhibitor.

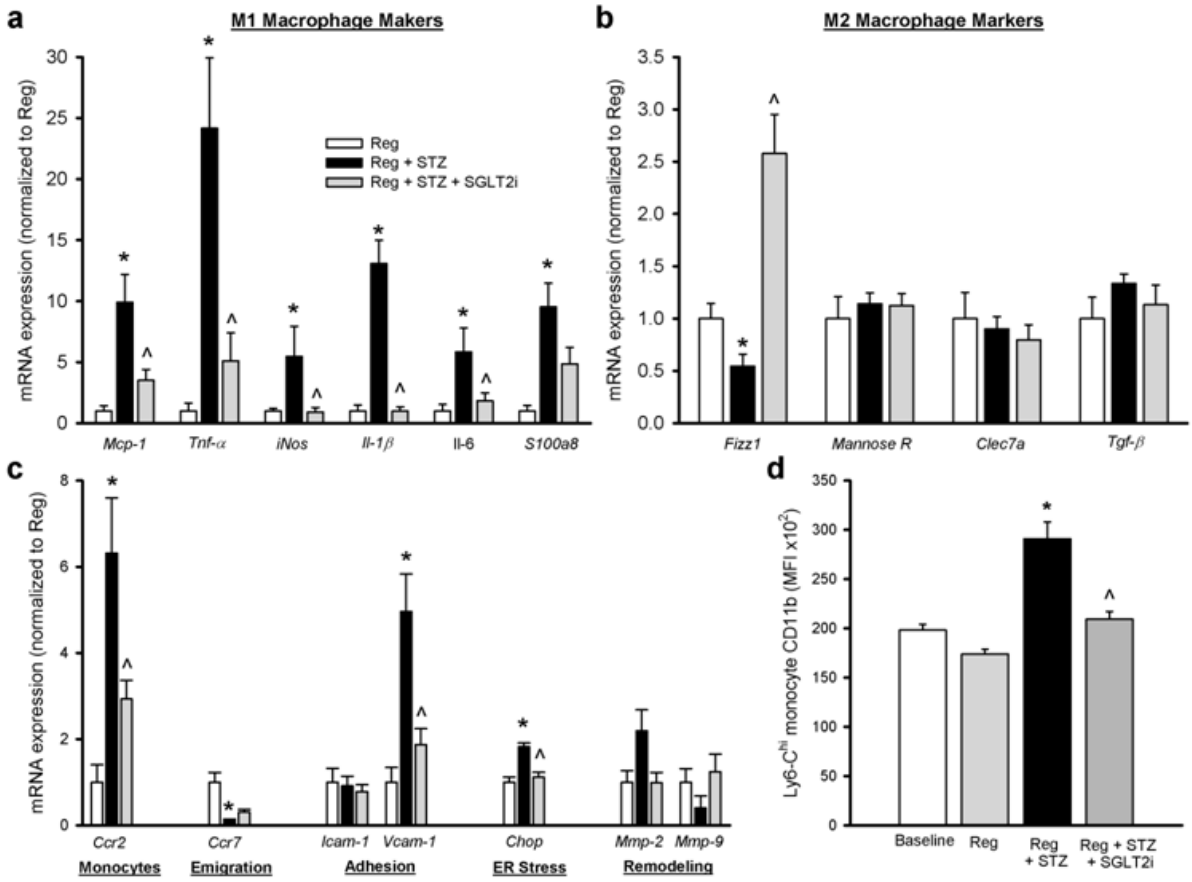
We also performed gene arrays on FACS isolated neutrophils and the file showing the genes most altered is attached. Of these genes the most remarkably changed gene is Egr1. Egr1 is marked increased with diabetes and reduced by SGLT2 inhibitor. This transcription factor is inflammatory and has been associated with insulin resistance in adipose tissue. It has been well studied in endothelial cells.

Conclusions: Diabetes leads to a number of inflammatory responses found in all three types of WBCs. We are unsure why the RT-PCR and array data are not identical and will follow this up by repeat analyses.

Aim 2. To determine whether glucose reduction in STZ-treated LDL receptor knockout mice with atherosclerosis will reduce the inflammatory profile of lesional CD68+ cells.

LDL receptor knockout mice were allowed to develop atherosclerosis by feeding a diet containing fat and cholesterol for 16 weeks. Thereafter, the mice were switched to chow, STZ diabetes created, and one group was then treated with a SGLT2 inhibitor. Lesion regression was assessed 6 weeks later. Lesional CD68+ cells were identified and RNA obtained by laser capture microdissection.

Results: Gene expression in lesional $CD68^{+}$ cells is shown in the figure. The three groups are non-diabetic mice with regression (Reg white bars), STZ-diabetic mice (Reg-STZ black bars) diabetic mice treated with SGLT2 inhibitor (Reg STZ-SGLT2, gray bars). Gene expression was quantified by qRT-PCR. mRNA expression of (**a**) M1 macrophage markers, (**b**) M2 macrophage markers and (**c**) other key genes as indicated. Expression was normalized to *Gapdh*. (**d**) Ly6-C^{hi} monocyte activation as measured by the cell surface abundance of CD11b expression via flow cytometry. All data are means ± SEM, n=9-11/group. **P*<0.05 vs. all groups and ^*P*<0.05 vs. Reg+STZ.



Conclusions: Diabetes was associated with a marked increase in inflammatory markers in arterial CD68+ cells that were obtained by LCM from lesions. MRNA levels of many of these genes reverted towards control regression values with glucose reduction. With the exception of *Fizz1,* genes indicative of alternative activation of macrophages (M2 markers) were no altered either by diabetes or glucose reduction. Thus, glucose regression in the setting of regression leads to less inflammatory arterial macrophages.