Transcriptional Effects of CpG-DNA Treatment on Bone Marrow Progenitor Niches.

Zoe Michael, MD[1-3]; Ekaterina Murzin, BS[2]; John Pulford, BS[2]; Alexandria Byskosh, MD[2-3]; James Lederer, PhD[2-3]
Division of Newborn Medicine, Boston Children's Hospital; [2] Department of Surgery, Brigham and Women's Hospital; [3]Harvard Medical School

Boston Children's Hospital
Where the world comes for answers

HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

BACKGROUND

With the escalating threat of antibiotic resistance, there is an urgent need for innovative therapeutic strategies to enhance immune function in immune compromised children and adults. In previous studies, we demonstrated that CpG-DNA preconditioning of mesenchymal stromal cells (MSCs) could amplify their therapeutic activity in a murine model of neutropenic sepsis. However, the biological and molecular effects on MSCs and hematopoietic stem cells (HSCs) in mice given therapeutic doses of CpG-DNA have not yet been explored. Herein, we present our findings on transcriptional responses in endogenous MSCs and HSCs after *in vivo* treatment with CpG-DNA, as well as the potential of using newly discovered mitochondrial CpG-DNA (mtCpG) sequences as future immunotherapeutic treatments in immune compromised sepsis.

CpG-DNA

- Short DNA sequences that enhance immune responses, investigated as adjuvants immunotherapies to boost responses against cancer.
- mtCpG: novel mitochondrial sequences

STUDY AIMS

Identify how *in vivo* administration of CpG or mtCpG affects:
1. BM progenitor cell milieu
2. HSC or MSC transcriptional profile

METHODS

In vivo studies

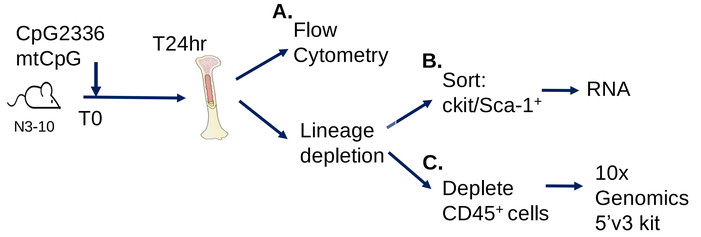

Fig 1. Systemic CpG-DNA uptake: Mice were given CpG2336 or mtCpG at 3 mg/Kg by subcutaneous injection. At 24hrs bone marrow was prepared for (A) flow cytometry to changes in bone marrow HSCs, MSCs cell subsets, (B) lineage depletion and sorting for RNA sequencing or (C) for single cell RNA sequencing.

Flow Cytometry

A.

HSC and Progenitor Populations Flow Panel	
Marker	**Color**
Lin- (CD3ε/Gr-1/CD11b/CD45R/Ter-119)	APC
CD34	PE
Sca-1	BV421
c-Kit	APC-Cy7
IL-7Ra	Alexa Fluor@488
CD16/32	PE-Cy7
Flk2(CD135)	PE-Cy5
MSC Flow Panel	
Marker	**Color**
Lin- (CD3ε/Gr-1/CD11b/CD45R/Ter-119)	APC
CD45	PE-Cy7
CD44	PE
Sca-1	BV421
CD29	APC-Cy7

B.

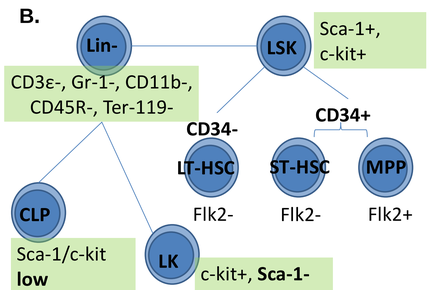

Fig 2. Bone marrow flow cytometry panels and gating schemes to identify HSCs, progenitor cells, and MSCs. (A) HSC, bone marrow progenitor, and MSC flow cytometry panels used to identify CpG-DNA effects on hematopoiesis (B) Flow chart illustrating our gating strategy to identify abundance changes in hematopoietic stem and progenitor cell populations.

RESULTS

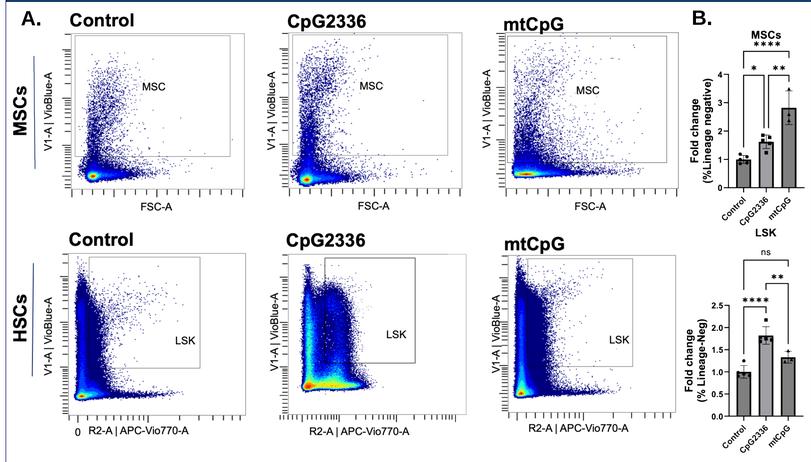

Fig 3. In vivo screen for CpG-DNA activity in mice. (A) FACS plots showing MSC and HSC number differences in equal subsampled stains from mice injected with CpG2336 or mtCpG-DNA33 sequences.(B) Statistical analysis sowing significant increase in bone marrow MSC and HSC in mice treated with CpG2336 or mtCpG. *p<0.05 **p<0.001 ***p<0.0001 One-way ANOVA

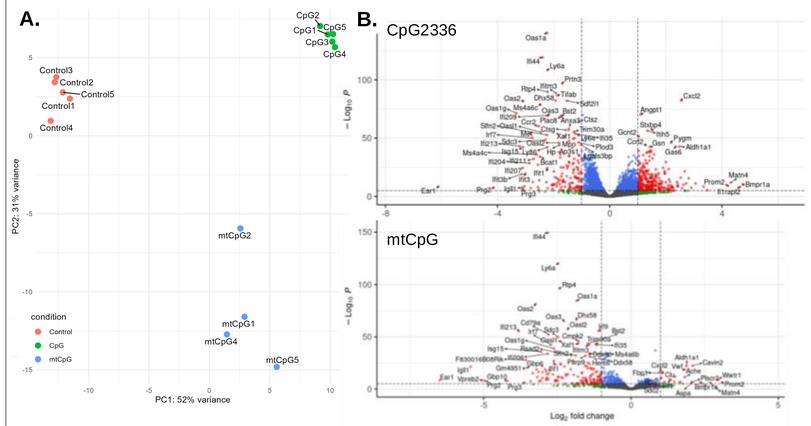

Fig 4. CpG2336 and mtCpg induced transcriptional modifications of HSCs. (A) Principal component analysis of bulk RNA sequencing of HSCs showing clustering of samples per treatment group (B) Volcano plots showing upregulated and downregulated genes in animals treated with either CpG2336 or mtCpG compared to controls.

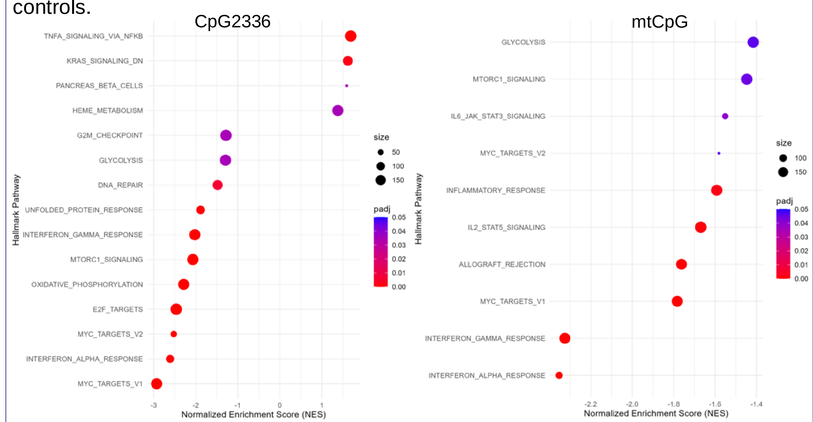

Fig 5. CpG2336 or mtCpG perturbed inflammatory, metabolic and mTORC1 signaling pathways in HSCs. Gene set enrichment analysis

RESULTS

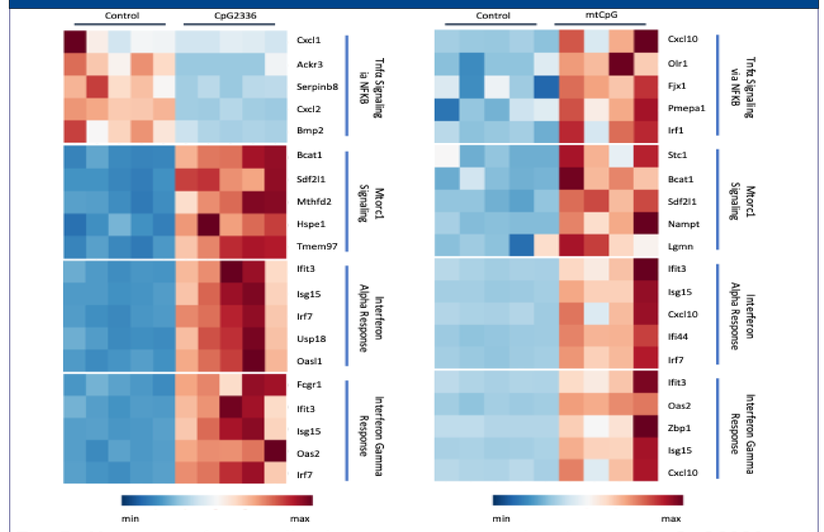

Fig 5. Heatmap of normalized counts per sample of control, CpG2336 and mtCpG groups. Genes present in leading edge of NES pathway from DESeq2 Control vs CpG or mtCpG comparison, filtered p < 0.05, sorted by log2FC, top 5.

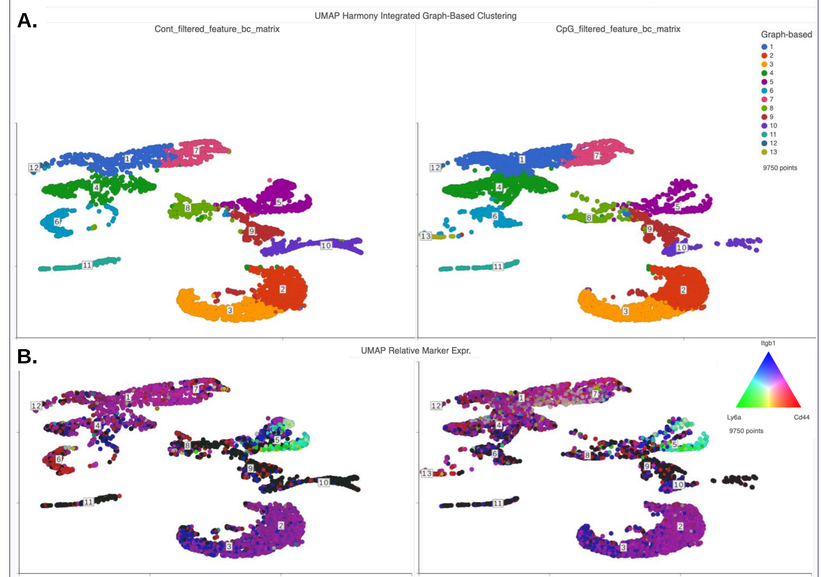

Fig 6. scRNA sequencing data analysis of non-hematopoietic bone marrow progenitor cells. UMAPs based on (A) cluster and (B) relative expression of known MSC markers.

CONCLUSIONS

Our findings underscore the immunomodulatory activities of CpG-DNA in HSCs and support the concept that CpG-DNA therapy has bone marrow regenerative activity in mice. Further investigations are warranted to elucidate the molecular mechanisms underlying the effects of CpG-DNA and mtCpG-DNA as potential therapeutics for infections and sepsis in immune compromised individuals.

ACKNOWLEDGMENTS

Support: NIH T32 HD 098061. NIH AI177803 JAL. BWH Stepping Strong Center for Trauma Innovation JAL and AB