Single dose stromal cell transplants ameliorate cytopenia caused by depletion of FAPα-expressing cells

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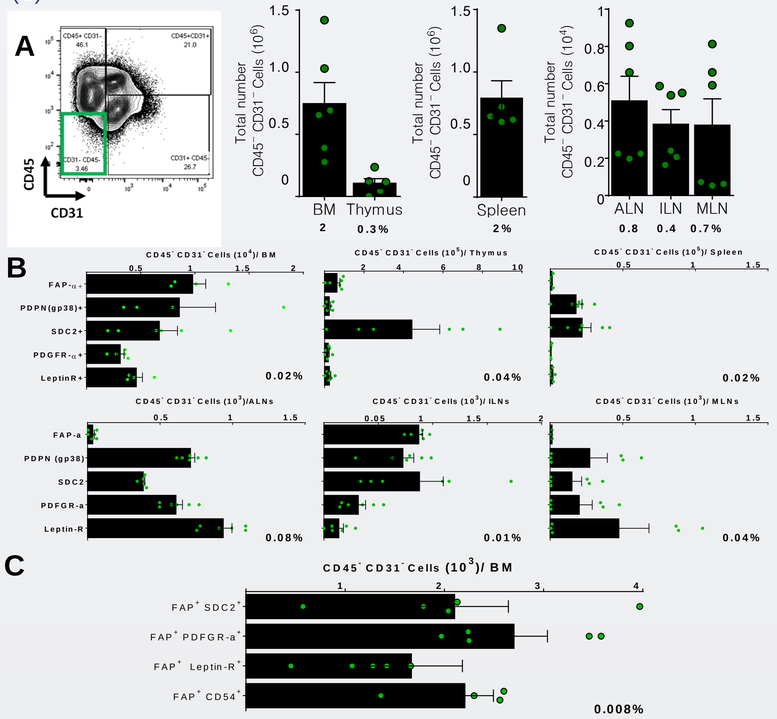
BACKGROUND

Mesenchymal stromal cell (MSC) therapies are in development for immune-mediated inflammatory diseases, including a recent Phase 3 clinical trial in patients with fistulising Crohns Disease. However, the endogenous roles of MSCs have not been fully elucidated. Fibroblast Activation Protein-α (FAP) is a marker of tissue resident stromal cells[2] and FAP$^+$ cells can be depleted in the FAP^{DM2} mice using Diphtheria Toxin (DTX) receptor-mediated conditional cell knockout (TRECK)[3]. We demonstrate that FAP$^+$ cell ablation induces cytopenic phenotypes and we present data that these cytopenias can be alleviated by a single low dose intravenous transplant of syngeneic marrow stromal cells .

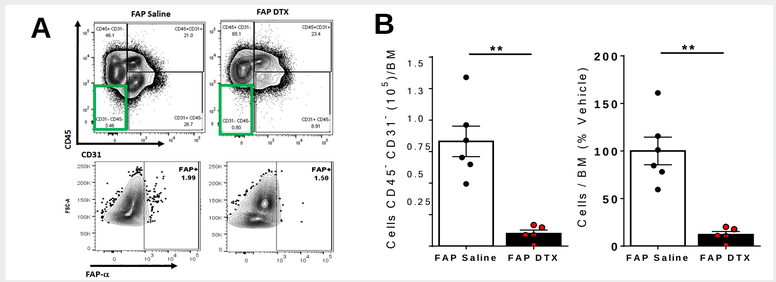
1. FAPα protein expressed by endogenous stromal cells

Bone marrow (BM), thymus and secondary lymphoid organs (SLOs) (spleen, axillary lymph node (ALN), inguinal (ILN) and mesenteric (MLN)) were digested to release mononuclear cells (MNCs) and labelled with fluorescently labelled antibodies (Ter119, CD45, CD31) to determine the number of stromal cells (Ter119$^-$, CD45$^-$ CD31$^-$) within each tissue (A). Detailed analysis of the stromal cell markers FAP-α, podoplanin (PDPN-gp38), SDC2, PDFGR-α and Leptin-R (B). FAPα$^+$ stromal cells co-localized with other stromal cells markers in very low percentage suggesting different distribution and localization within the BM (C).



2. Conditional ablation of FAPα$^+$ stromal cells

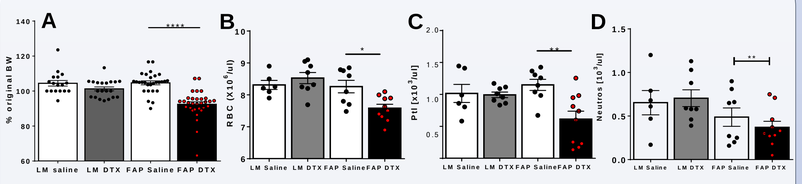
FAPα$^+$ stromal cells were ablated by administration of two doses of DTX in the FAP ^{DM2} strain [2,3].



Representative flow cytometry dot plots show FAPα$^+$ cell depletion the in CD45/Ter119$^-$ CD31$^-$ stromal cell population. (A) Bar graphs show the total number of cells ablated and the percentage of cell ablation calculated and referenced to the vehicle (saline) (B).

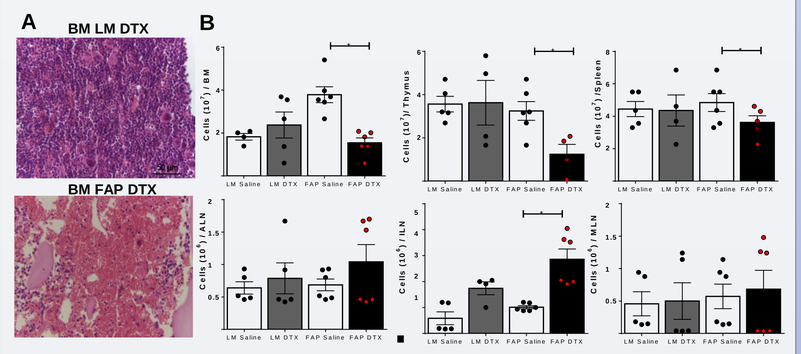
3. FAPα$^+$ cell ablation causes weight loss, anaemia and neutropenia

Body weight (A) is reduced in FAP^{DM2} mice at day 3 after second injection of DTX. FAPα$^+$ cell ablation causes anemia (B) - confirmed by the reduction of red blood cells (RBC). In addition, FAPα$^+$ cell ablation, cuases thrombocytopenia (C) and neutropenia (D) in peripheral blood (PB).

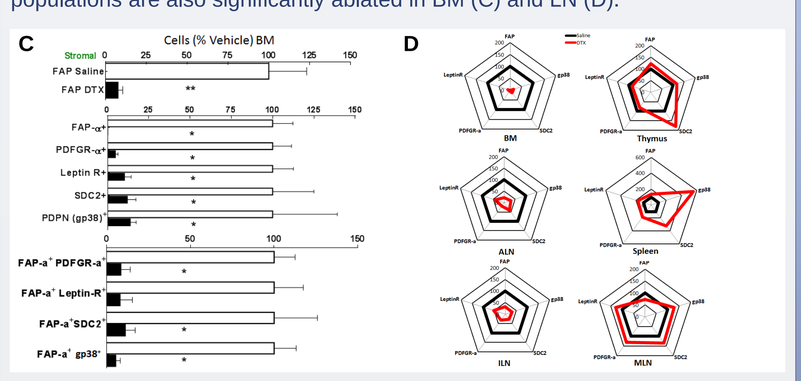


4. FAPα$^+$ cell ablation leads to alterations in lymphoid organ cellularity

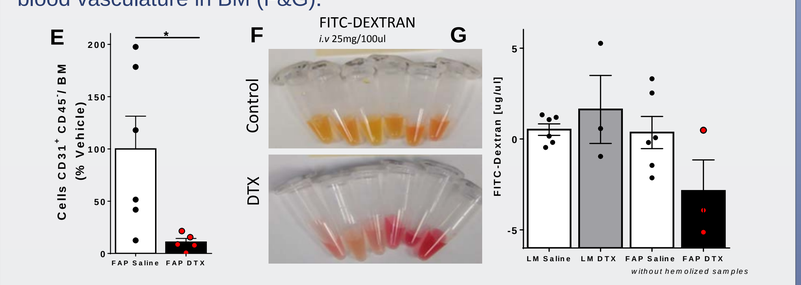
Hypo-cellularity observed by cytometric analysis was confirmed by H&E (A). FAPα$^+$ cell ablation caused marrow, thymus and spleen hypo-cellularity whereas SLOs (ILN, ALN and MLN) exhibit an increase in cellularity (B).



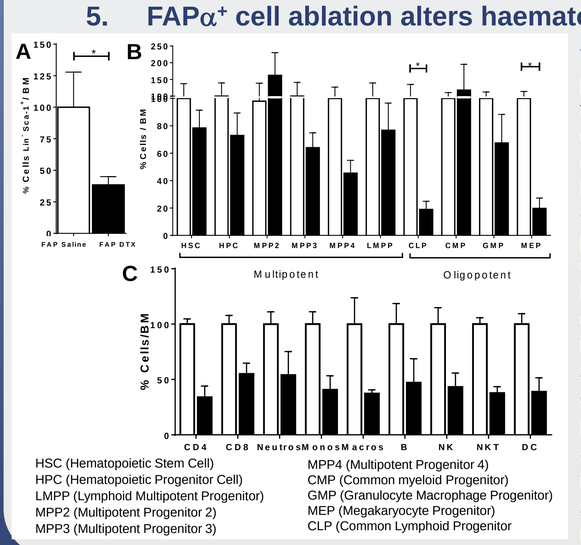
FAPα$^+$ cell ablation depletes FAPα$^+$ stromal cells - related stromal cell populations are also significantly ablated in BM (C) and LN (D).



Endothelial cells are also ablated (E) showing an increased permeability in blood vasculature in BM (F&G).



5. FAPα$^+$ cell ablation alters haematopoiesis



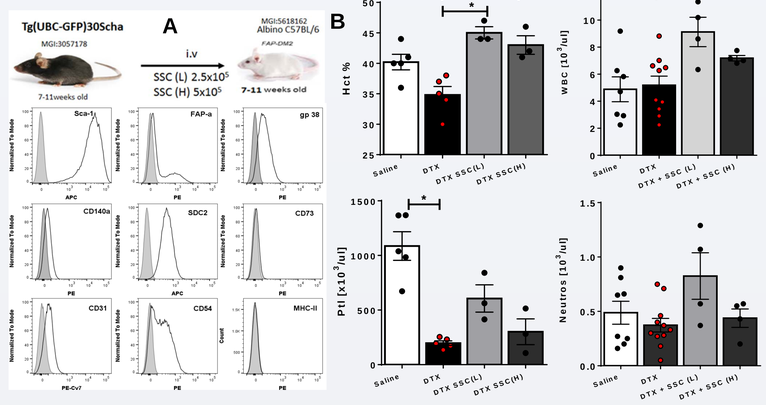
HSC (Hematopoietic Stem Cell)
HPC (Hematopoietic Progenitor Cell)
LMPP (Lymphoid Multipotent Progenitor)
MPP2 (Multipotent Progenitor 2)
MPP3 (Multipotent Progenitor 3)
MPP4 (Multipotent Progenitor 4)
CMP (Common myeloid Progenitor)
GMP (Granulocyte Macrophage Progenitor)
MEP (Megakaryocyte Progenitor)
CLP (Common Lymphoid Progenitor)

The blood forming process is altered from the progenitors (A) to the multipotent (HSC, HPC and LMPP) and oligopotent populations (MEP, CLP and GMP) (B). Mature immune cells (C) are also reduced in a preliminary study (n=3 per group).

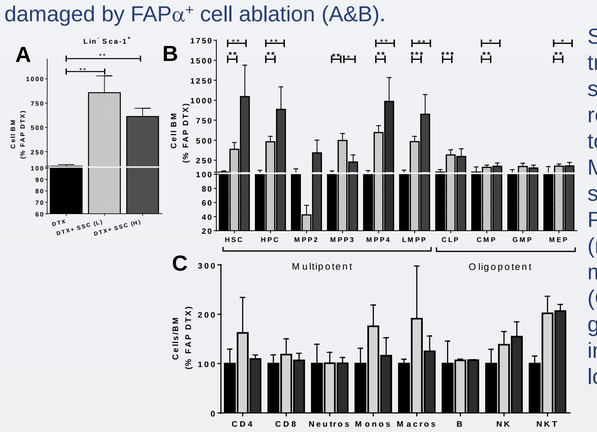
FAPα$^+$ cell ablation causes an increase in MPP2 progenitors suggesting a regenerative niche.

6. Intravenous (IV) transplant of single dose of stromal cells alleviates cytopenias caused by FAPα$^+$ cell ablation

A low dose (250,000 - SSC-L) of syngeneic marrow stromal cells (A) increases the blood volume (Hematocrit - Hct), ameliorates thrombocytopenia and neutropenia in PB (B).

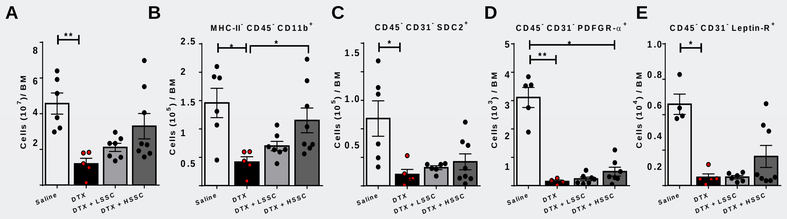


Stromal cell transplants increase the numbers of hematopoietic progenitors damaged by FAPα$^+$ cell ablation (A&B).



Stromal cell transplanted BM switches from regenerative state to homeostasis as MPP2/MEP axis shown (B). Preliminary data (n=3 per group) of mature subsets (C) indicates that granulocytes increases with the lower SSCs dose.

Stromal cell transplants improve BM cellularity (A). The cell subsets increased are a subset of dendritic cells (DCs) (B), and SDC2, PDFGR-α and Leptin-R stromal cell subsets (C,D &E).



Conclusions

We demonstrate that the DTX-mediated depletion of FAPα$^+$ cells - including rare FAPα$^+$ stromal cells causes cytopenia (thrombocytopenia and neutropenia), anemia and perturbs the numbers of endothelial cells and haematopoietic progenitors in the marrow. These phenotypes are not overly surprising given number of BM cells depleted by DTX in the FAP^{DM2} model is ~2x10^7.

What is notable was that the IV transplant of a single dose of 250,000 (L) to 500,000 (H) syngeneic BM derived stromal cells alleviated many of the cytopenia arising from FAPα$^+$ cell ablation.

Our data suggest that IV stromal cell therapy may correct immune-mediated pancytopenia in disease settings such as sepsis, GvHD and lupus.

STATISTICS & REFERENCES

Unless otherwise indicated, values and mean ± SEM were represented for unpaired Mann-Whitney test for 2 groups comparison and Kruskal-Wallis test, Dunn's multiple comparisons test for 3 or more (α=0.05). Statistical significance was expressed as follows: *,P<0.05; **,P<0.01; ***,P<0.001; ****,P<0.0001. Radar graphs (4D) contain sample average of n=6 per group. When values are not visible in a graph, mean ± SEM correspond to n=6 for FAP saline and FAP DTX and n=8 for FAP DTX+SSC(L) and (H) doses otherwise is specified in the section.

1.Panes J. et al. 2016 Sep 24;388(10051):1281-90. , 2.Roberts et al. J Exp Med, 2013. 210(6): p. 1137-51. , 3.Kraman et al. Science, 2010. 330(6005): p. 827-30.

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