Modelling CLL cell and T-cell migration in a dynamic circulating model of CLL

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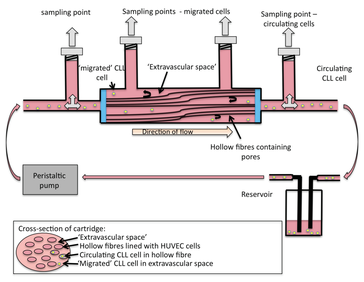
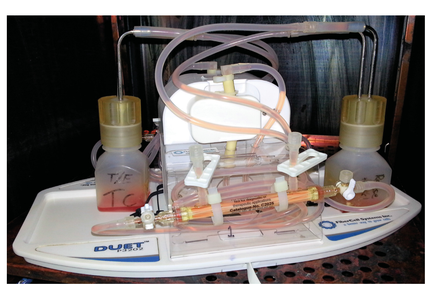
ABSTRACT

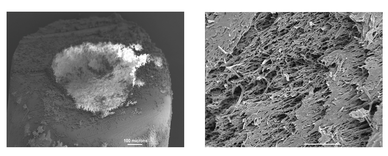
We have recently developed a novel circulating model of chronic lymphocytic leukaemia (CLL) that mimics the transient interactions that take place between circulating lymphocytes and vascular endothelium. Here we show that both normal and malignant lymphocytes actively underwent transendothelial migration. Furthermore, seeding of CXCL12-secreting MRC5 cells into the extravascular space (EVS) resulting in significantly enhanced CLL cell migration (P = 0.024) but no increase in T-cell migration. Both CLL cells and T-cells recovered from the EVS showed evidence of activation markers and entry into cell cycle as demonstrated by increased Ki-67 expression (P<0.0001). We subsequently established that CLL cell Ki-67 expression was dependent on the presence of T-cells, as depletion of the T-cells from the circulating compartment significantly inhibited Ki-67 induction (P = 0.003). It is worthy of note that as a proportion of all circulating peripheral blood mononuclear cells, $CD3^+$ T-cells migrated significantly more than $CD19^+$ CLL cells (P = 0.02). This seems likely to be promoted by CLL cell secretion of T-cell attracting chemokines CCL3 (298pg/ml ±197.5) and CCL4 (56pg/ml ±115.6) in our model. Interestingly, $CD38^+$ CLL samples showed elevated levels of CCL3 and CCL4 suggesting that increased T-cell recruitment may contribute to the inferior clinical outcomes seen in these patients. Taken together the data presented here highlight the requirement of T-cells in the pathology of CLL.

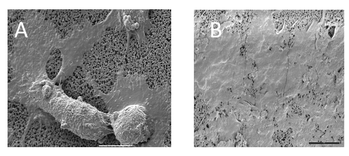
Novel circulating model system of CLL





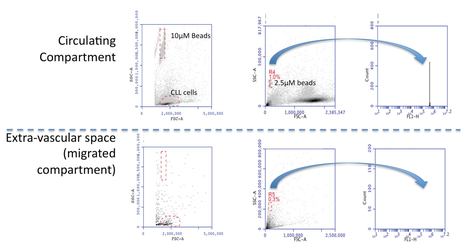
Cross-section of a hollow fibre in the cartridge showing CLL cells *in situ* and the network of pores through the fibres.

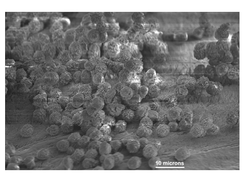




Endothelial cells on the inside of the hollow fibres after A: short and B: longer exposure to shear forces.

CLL cell migration from the circulating compartment to the extravascular space is an active process:

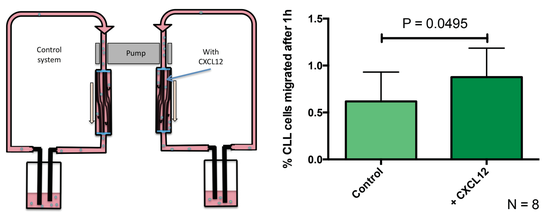




Migrated CLL cells on the outside of a hollow fibre

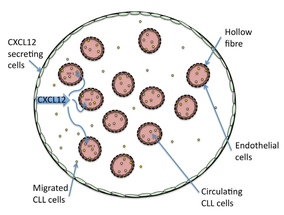
CXCL12 gradient increases migration

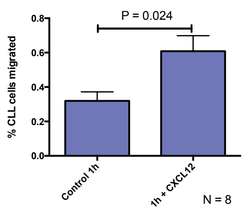
A transient CXCL12 gradient towards the extravascular space was generated by a single deposition of 100ng/ml CXCL12 into the extravascular space of one cartridge. In parallel, CLL cells isolated from the same patient at the same time were circulated in a separate cartridge without the CXCL12 gradient.



Increased CLL cell migration was seen in the presence of the CXCL12 gradient.

Seeding CXCL12-secreting MRC5 cells into the extravascular space produced a stable chemokine gradient towards the extravascular space.



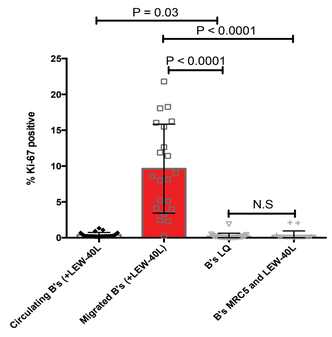


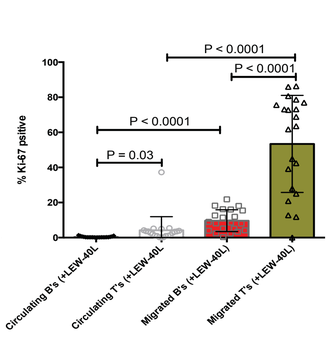
The constant, but lower, CXCL12 gradient (23pg/ml) created by secretion from MRC5 cells populating the extravasular space increased CLL cell migration.

Cells that migrate are actively in cell cycle

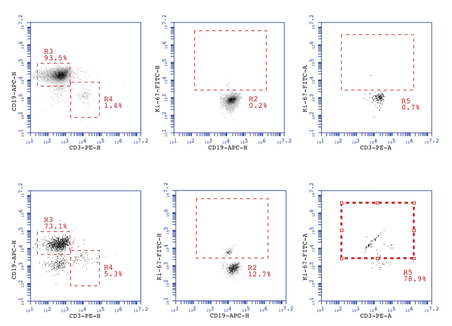
CLL cells in the peripheral blood are predominantly arrested in G_0. Ki-67 is expressed in all phases of the cell cycle except G_0. PBMCs from CLL patients were introduced into the circulating system and circulated for 48h before recovery from the circulating and extravascular compartments. CD19, CD3 and Ki-67 expression were measured by flow cytometry.

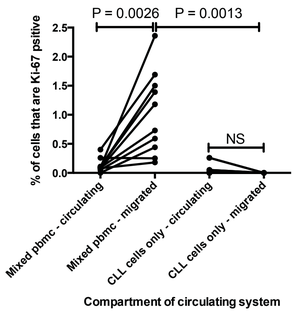
$CD19^+$ CLL cells in circulation, static mono-culture or static co-culture with CD40L-expressing fibroblasts had very low Ki-67 expression after 48h. In contrast, CLL cells that migrated out of circulation into the extravascular space showed significant increases in Ki-67 expression.





Identification of Ki-67 expression in $CD19^+$ CLL cells and $CD3^+$ T-cells.





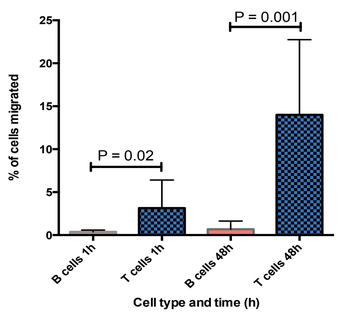
CLL B-cells require the presence of T-cells in order to enter cell cycle

Both CLL B-cell and T-cell migration increased over 48h. $CD3^+$ T-cells migrated significantly more than $CD19^+$ CLL cells (n=10).

Migrated $CD3^+$ T-cells showed remarkable increases in Ki-67 expression that were significantly greater than those seen in CLL cells.

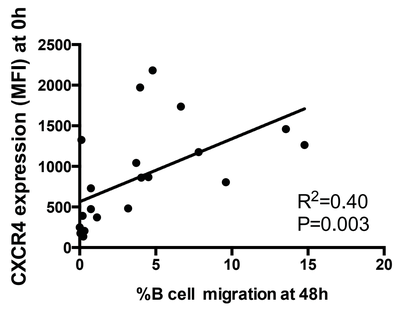
T-cells migrate more than CLL B-cells

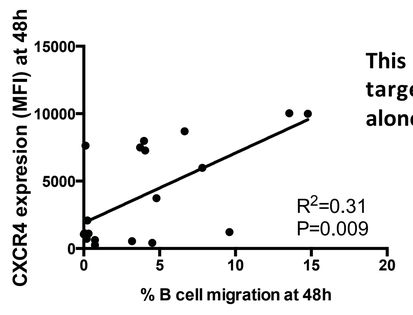
Unsorted PBMCs were introduced into the circulating system. Cells were recovered from the circulating and extravascular compartments. CLL B-cells were identified by CD19 expression and T-cells by CD3 expression using flow cytometry. The percentage of total CLL cells and T-cells that migrated was calculated as a proportion of circulating cells.



CXCR4 expression correlated with migration of CLL cells

CXCR4 expression is up-regulated on circulating CLL cells compared to static cultures. Basal expression of CXCR4 prior to circulation and expression after 48h of circulation correlated with the degree of CLL cell migration into the extravascular space.

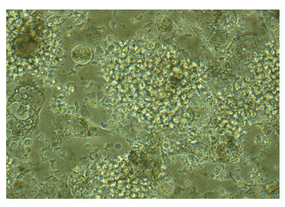




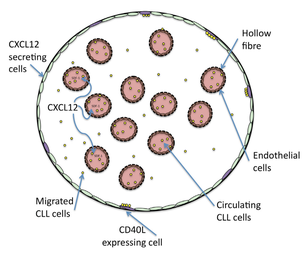
This makes CXCR4 a rational target for therapy either alone or in combination

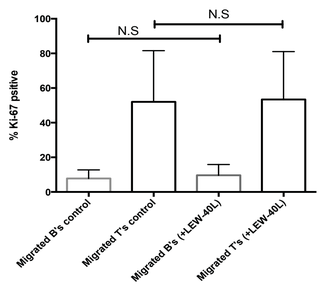
Additional CD40L signaling does not increase Ki-67 expression or CLL cell activation markers

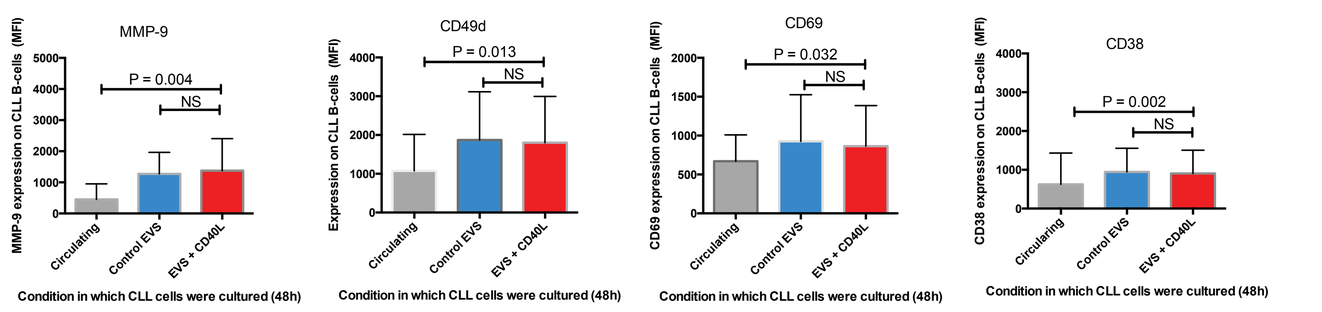
Co-culturing CLL cells *in vitro* with CD40L-expressing fibroblasts provides survival signals resulting in CLL cell activation and Ki-67 expression.



CLL cells co-cultured on CD40L expressing fibroblasts *in vitro.*



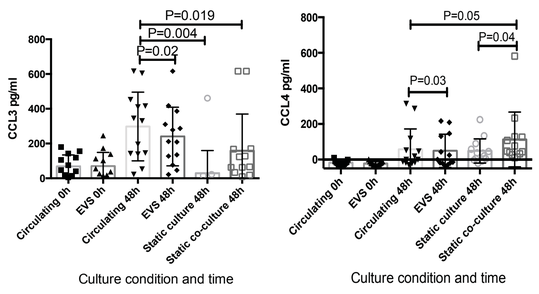


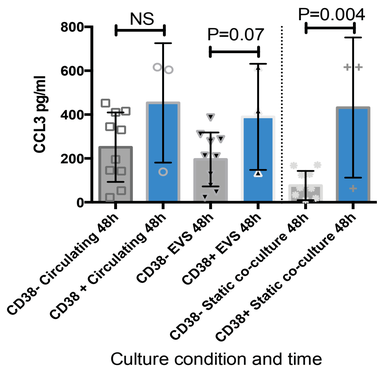


Addition of CD40L-expressing fibroblasts to the extravascular space of the circulating system did not result in any further increases in Ki-67 expression or expression of CLL cell activation markers MMP-9, CD49d, CD69 and CD38.

CLL cells secrete T-cell attracting chemokines

CLL cells and T-cells engage though specific ligand:receptor interactions (including CD40:CD40L). These interactions are facilitated by the release of chemokines.





In the circulating system CLL cells secreted the T-cell attracting chemokines CCL3 and CCL4. Furthermore, $CD38^+$ CLL cells secreted higher levels of both cytokines which is consistent with recruitment of supportive T-cells to the lymph nodes and the more aggressive nature of $CD38^+$ CLL.

Conclusions

We have added further sophistication to our circulating model by adding a CXCL12 chemokine gradient. Characterisation of the circulating and migrating cells under these conditions revealed that:

- **T-cells migrate at proportionally higher rates than CLL cells**
- **The presence of migrated T-cells in the EVS is critical for CLL cell entry into cell cycle**
- **CLL cell activation is not enhanced by additional CD40L signaling in the extravascular space**
- **CXCR4 expression correlates with migratory potential of CLL cells**