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Challenging "privileged" stereotypes - leukemic blasts and the central nervous system

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The successful cure of over 90% of children with acute lymphoblastic leukaemia (ALL) is one of the most impressive achievements of the last 50 years in haematology. However, challenges remain. One particularly problematic area is how best to prevent and/or treat leukaemic relapse in the central nervous system (CNS). Early studies established that CNS-directed therapy is essential for cure. Accordingly, all modern ALL treatment protocols include large amounts of CNS-directed chemotherapy irrespective of the presence or absence of detectable CNS disease at presentation. Although reasonably effective, this therapy can cause significant acute and chronic neurotoxicity and relapsed disease can be highly refractory to currently available drugs. It is clear that identification of the molecular determinants of CNS involvement will aid understanding of leukaemia cell biology, allow prognostic models for CNS disease to be developed, and is an essential prerequisite for design of novel therapeutic approaches for CNS disease¹.

Development of CNS leukaemia involves both initial trafficking from the bone marrow to the CNS and survival within this compartment. The CNS is traditionally thought of as an immune-privileged site with specialised barriers to prevent unselected entry of immune cells². Several possible routes for entry of leukaemic cells across these barriers can be postulated (figure 1).

![Figure 1](image_url)

**Figure 1.** Schematic representation of potential trafficking routes of leukaemic blasts into the central nervous system. Potential sites of entry/exit are labelled A to E. There is experimental and clinical evidence for transit across the choroid plexus (A), meningeal vessels (B) and emissary veins (C). Leukaemic cells reside within the meninges and pass into the Virchow-Robin spaces surrounding the invaginating vessels. Although theoretically possible, there is currently little or no experimental support for transit across the blood-brain barrier (BBB) (D) or the dural lymphatics (E).
Importantly, histological analysis shows leukaemic cells reside within the leptomeninges, bathed in cerebrospinal fluid (CSF). Only rarely, and late in the disease course, is there any evidence of invasion into the brain parenchyma, via local breaches of the pial membrane within the leptomeninges. This effectively rules out the blood-brain barrier as the primary site of leukaemia entry, and use of in vitro BBB models to model leukaemic trafficking to the CNS should be avoided.

What about evidence for trafficking across the blood-CSF barrier (BCSFB)? Until now evidence had to be gleaned from static histology images showing leukaemic cells residing within the choroid plexus in xenograft models and also in the walls of the meningeal veins. Current knowledge of the mechanisms of BCSFB transit is extremely limited. In vivo modelling requiring sampling of CSF is technically difficult and often requires use of large animals such as pigs. Early models of the choroid plexus, using rat and porcine choroid plexus epithelial cells provided proof of principle that barrier properties such as tight junction formation could be replicated in vitro, but require large numbers of animals to harvest tissue and have limited applicability to human disease.

In this issue, März et al report a new in vitro model of the human Blood-CSF barrier and its use to investigate transit of B and T cell ALL into the CNS. The authors should be congratulated on developing this model, using human choroid plexus papilloma-derived cells. These cells have been extensively evaluated for the expression of choroid plexus specific proteins (i.e. tight junction proteins, specific transporter proteins), and morphological characteristics, indicating their similarity to choroid plexus epithelium. Use of this model should allow mechanistic insights into physiological and pathological cellular transit across this important barrier. Using transmission electron microscopy, the authors show evidence for transcellular migration of leukaemic cells across this barrier in vitro.

Interestingly, a recent publication from the Sipkins’ laboratory in Nature provides compelling evidence for an alternative and novel route of non-haematogenous entry for ALL cells to the leptomeninges. Using a xenograft model they provide histological evidence that leukaemic cell lines and primary cells, track along the outside of emissary vessels that bridge between the calvarial and vertebral bone marrow and the dural venous system. This system hijacks pathways used for neural progenitors early during neural development and the authors show data suggesting this is dependent upon α6 integrin receptor expression on ALL cells and laminin on the abluminal surface of the veins. It is noteworthy that intravital fluorescent microscopy was unable to visualise passage of cells across the leptomeningeal vessels in this model, although this could be explained by the imaging windows used or the experimental set-up. Interestingly, laminin, the ligand for α6 integrin, is also expressed on
the BCSFB and meninges, so use of both routes remains possible, and there might be important differences between mouse models (with very heavy calvarial bone marrow involvement) and human patients.

Both publications also investigate the role of the chemokine CXCL12 in trafficking to the CNS and show a modest increase in migration using *in vitro* modelling. It appears that a variety of chemokines provide attractant stimuli for leukemic cells to enter the CNS but that chemokine receptors on leukaemic cells do not orchestrate selective entry of cells to this compartment. Most notably there is no current experimental evidence for subclonal selection of “CNS-tropic” cells bearing specific receptors, suggesting the ability to enter the CNS is a universal property of leukaemic cells.

So, these recent publications bring us closer to understanding how cells physically enter the CNS, but that is of course only part of the story. Indeed, several lines of evidence support the hypothesis that trafficking to the CNS has already occurred at the time a patient presents with leukaemia. This suggests that the risk of CNS relapse is determined by the ability of blasts to adapt to the CNS niche and survive in this environment, rather than their ability to enter it in the first place. Successful adaptation permits cells to evade both systemic chemotherapy and normal immune surveillance. Thus, strategies to block leukaemic cell entry may be the equivalent of “locking the stable door after the horse has bolted”.

Of course, accurate *in vitro* models of the BCSFB and mechanisms of cellular trafficking into the CNS compartment are useful for fields outside ALL biology. CSF plays a central role in CNS homeostasis. Elucidating the mechanisms by which substances, microorganisms and cells cross this barrier is very important for understanding disorders of neurodevelopment, neurotoxicology and neuropharmacology, as well as many acute and chronic neurological disorders such as bacterial and viral meningitis, multiple sclerosis and solid tumour infiltration. Thus, the model presented by März *et al* provides a useful tool for researchers across a wide range of disciplines.

References