Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol14Issue2abst020P.pdf

Adrenomedullin signalling via CLR plays a role in cell-cell communication in microvascular endothelium.

Joshua Liu[1], Matthew Skidmore[1*], Lydia Wharton[1], Christelle Langlet[2], Dhan Limbu[1], Karolis Mikalauskas[1], Tejumola E Akerele[1], Margaret C.P. Rees[3], Patrick Robberecht[2] and Leonid L. Nikitenko[1*] (l.nikitenko@hull.ac.uk) [1]University of Hull, United Kingdom; [2]Université Libre de Bruxelles, Belgium; [3] University of Oxford, United Kingdom. [*]Presenting author.

Adrenomedullin (AM) and calcitonin-gene related peptide (CGRP) are potent vasoactive and angiogenic peptides that play important roles in microvascular blood and lymphatic vessels. Deficiency in AM signalling has been associated with predisposition to cardiovascular disease and lymphoedema **(1, 2)**. AM signals via G-protein coupled receptor (GPCR) calcitonin receptor-like receptor (CLR), which forms heterodimers with receptor activity modifying proteins (RAMPs) 2 or 3 to generate pharmacologically distinct subtypes of AM receptors - AM_1 and AM_2 respectively **(3, 4)**. However, the translational research on AM receptors has been limited due to the lack of inhibitors for CLR/RAMP2 and CLR/RAMP3 heterodimers, when compared to available antagonists for CLR/RAMP1 complex (CGRP receptor). The aim of this study was to develop monoclonal antibody against human CLR for targeting AM receptors endogenously expressed in endothelial cells (EC).

The genetic immunization technology **(5)** was used to generate mouse monoclonal antibodies against human CLR in its native (cell-surface expressed CLR/RAMP heterodimer) form. Next, one of the six obtained clones was extensively characterised using immunofluorescence and MTS assays utilising cultured *in vitro* primary human microvascular blood and lymphatic EC **(2, 6)**. When compared to control immunoglobulins, anti-CLR antibody bound cell surface-expressed receptor, significantly inhibited both endogenously produced and exogenously supplemented (10μM) AM-induced proliferation/survival in blood (1±0.1 v 0.52±0.04; 1.3±0.16 v 0.76±0.0.06; n=3; $p<0.01$) and lymphatic (1±0.24 v 0.54±0.12; 1.04±0.23 v 0.59±0.11; n=3; $p<0.01$) EC.

In summary, anti-human CLR antibody acted as a potent inhibitor for AM receptors endogenously expressed in microvascular blood and lymphatic EC, suggesting the potential for its use in further functional and imaging studies, and possibly targeted therapy. Furthermore, these data confirmed the key role for CLR in mediating AM effects in EC and demonstrated that endogenously produced AM is essential for autocrine signalling and cell-cell communication in microvascular blood and lymphatic endothelium. The development of an antibody targeting CLR expressed in the human endothelium is a significant step towards addressing currently unmet need for the development of specific inhibitors for AM_1 and AM_2 receptor subtypes. The work in progress is aimed at dissecting which CLR/RAMP heterodimer is targeted by this antibody.

(1) Ehret GB *et al*. (2011) *Nature* 478: 103-9.
(2) Nikitenko *et al*. (2013) *Journal Invest Dermatology* 133(7): 1768-76.
(3) McLatchie *et al*. (1998) *Nature* 393: 333-339.
(4) Poyner *et al*. (2002) *Pharmacol Rev* 54(2): 233-46.
(5) Langlet *et al*. (2004) *Peptides* 25(12): 2079-86.
(6) Nikitenko *et al*. (2006) *Journal of Cell Science* 119(Pt 5): 910-22.