OXFORD UNIVERSITY INNOVATION

Non-confidential information

CIRp-A programme (13645)

AP initiation inhibitor

Not for further distribution

CIRp-A
Summary

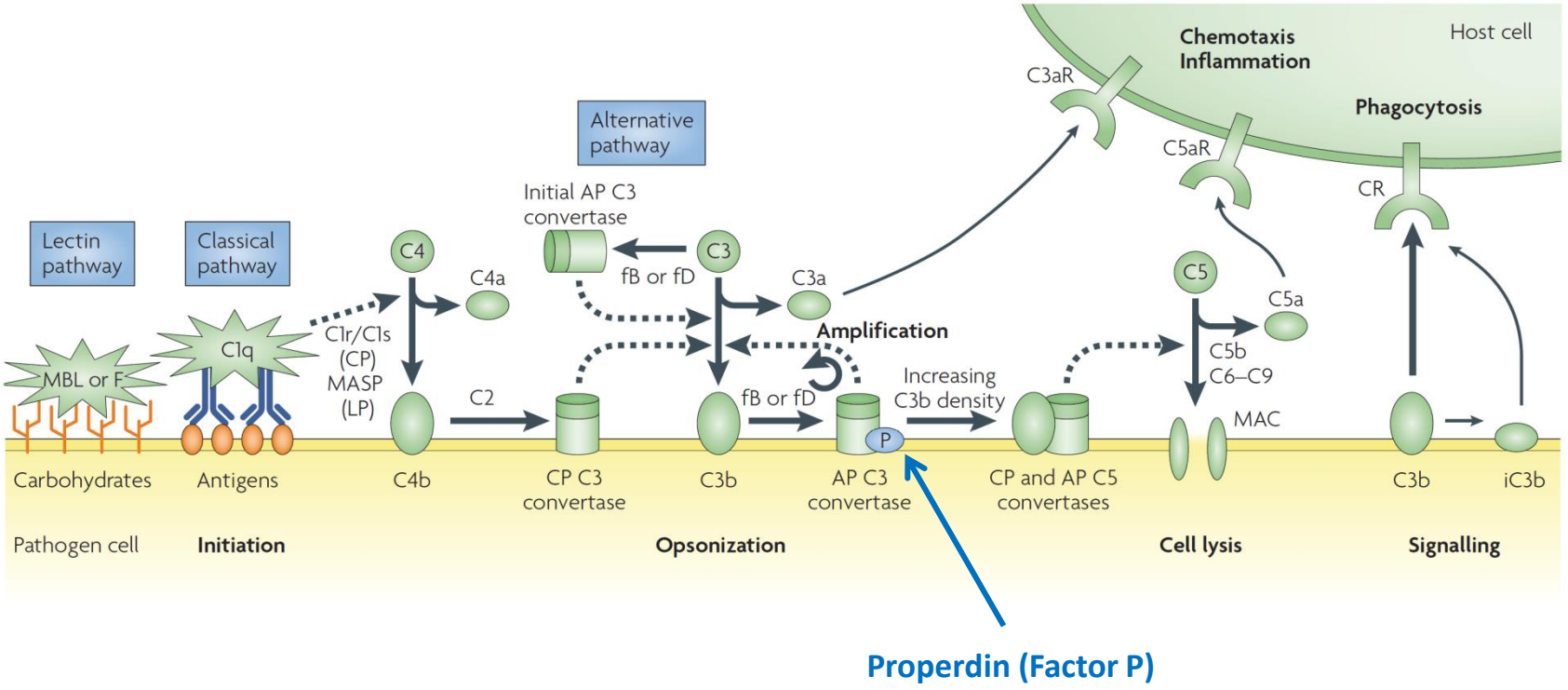
- Novel glycoprotein derived from tick saliva
- ~170 amino acids
- Four conserved Cys in two disulphide bridges
- Chemically and proteolytically stable
- Readily soluble

- Specifically inhibits initiation of the Alternative Pathway (AP)
- Specifically binds to properdin in human serum

- PCT patent application filed July 2018

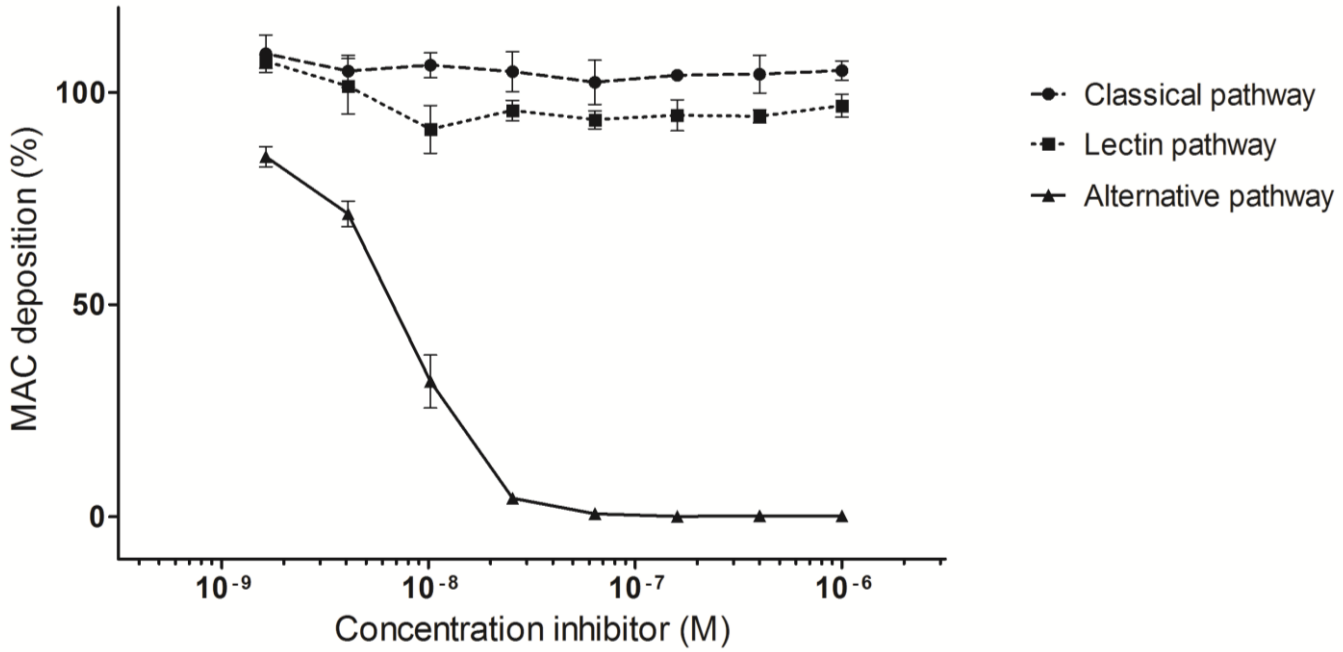
Overview of complement system

Classical, alternative and lectin pathways



CIRp-A
Specific AP initiation inhibitor

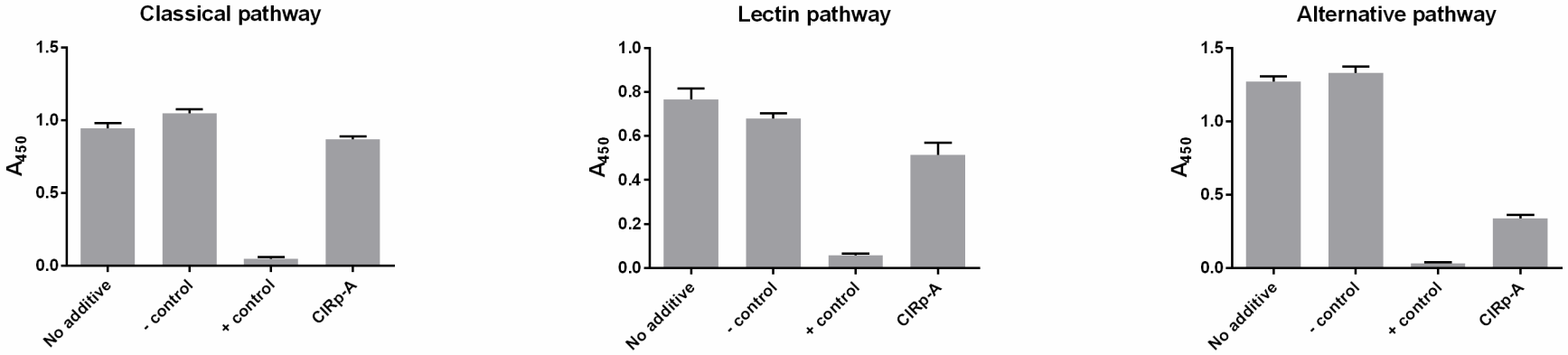
CIRp-A specifically inhibits initiation of the AP



The above figure shows the results of complement inhibition assays using CIRp-A purified from tick cells. A Wieslab assay (Eurodiagnostica, Sweden) was carried out with titrations of CIRp-A showing that CIRp-A inhibits the alternative pathway but not the classical and lectin pathways at the concentrations tested. Values were normalised for serum only (100% activity) and no serum (0% activity). N=3 and error bars represent s.e.m.

CIRp-A
Inhibition of C3a formation

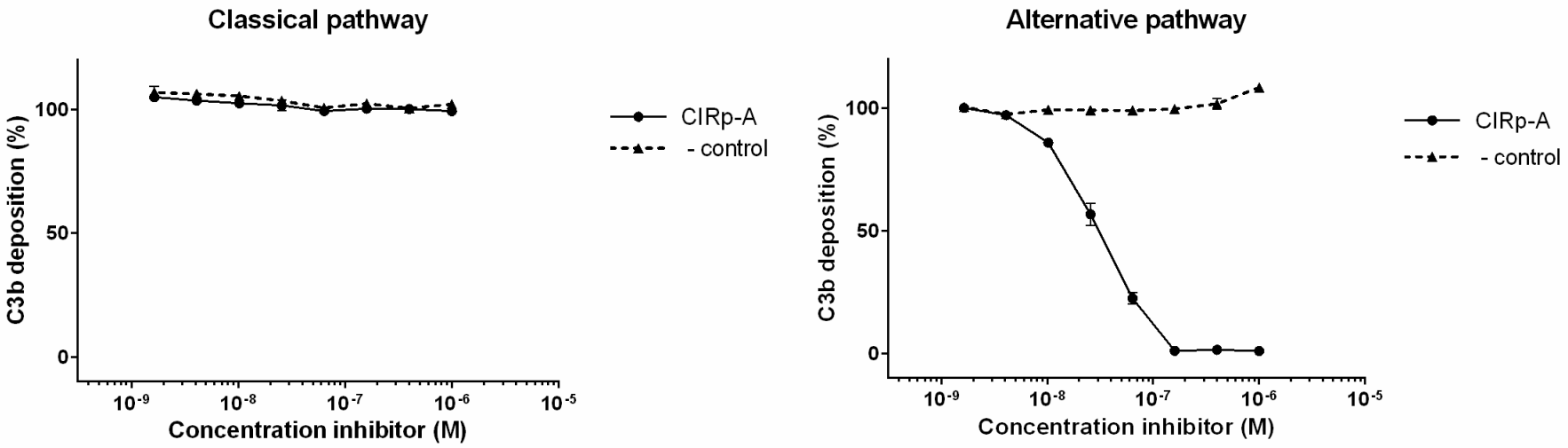
CIRp-A significantly inhibits C3a formation through the AP



The above figure shows C3a levels in the supernatants of the Wieslab assay at 1 µM inhibitor depicted in the previous slide. Levels were measured with a Microvue C3a kit (Quidel, USA). CIRp-A can be seen to significantly inhibit C3a formation through the alternative pathway - $p < 0.0001$ by unpaired two-tailed t test, with PBS as a reference. N=3 and error bars represent s.e.m.

CIRp-A
Inhibition of C3b deposition

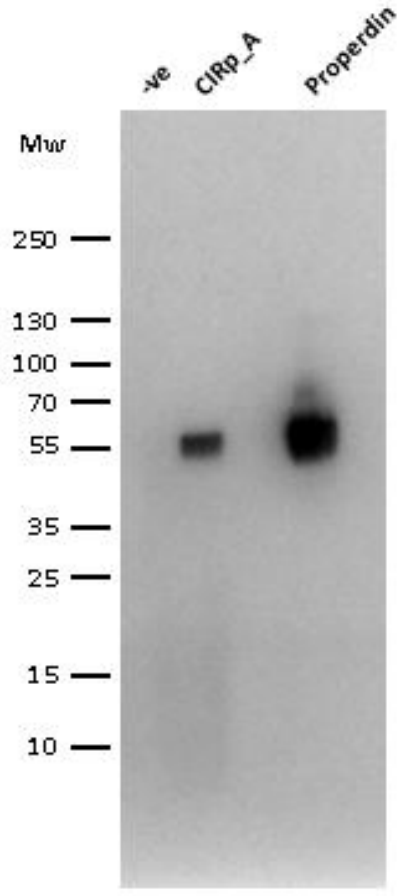
CIRp-A significantly inhibits C3b deposition through the AP



The figure shows C3b deposition in an ELISA based assay for Classical and Alternative pathway inhibition. 96 well plates were coated with IgM (classical pathway) or LPS (alternative pathway). Diluted normal human serum was added in the presence or absence of the CIRp-A. Plates were incubated for 1 hour at 37°C and C3b deposition was detected using an anti-C3 antibody coupled to HRP (Amsbio, UK). N = 3 and error bars indicate s.e.m. Values are normalised for no serum samples (0% activity) and no additive (100% activity).

CIRp-A
Specific binding to properdin

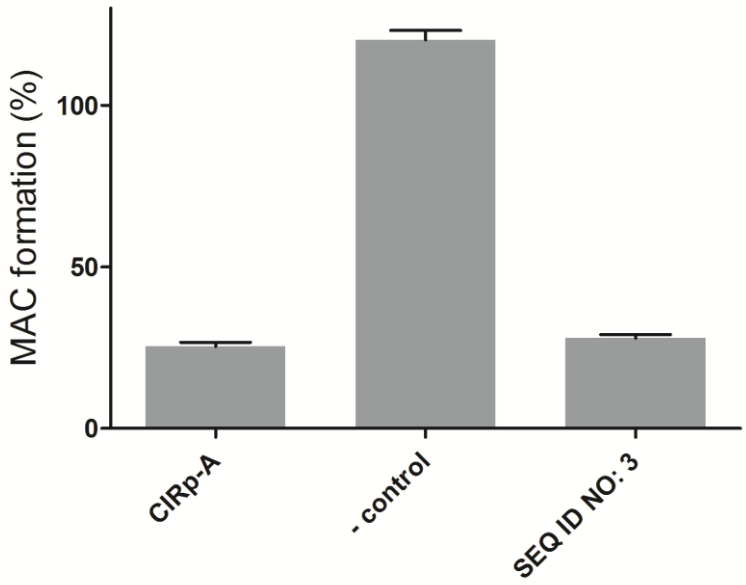
CIRp-A specifically binds to properdin in human serum



The figure shows the results of a western blot with a polyclonal antibody against properdin and shows that CIRp-A binds properdin, while empty beads (-ve control) do not bind properdin. CIRp-A was coupled to NHS-activated magnetic beads (Thermo Scientific, UK) following the manufacturer's instructions. The beads were incubated with normal human serum followed by three wash steps. Bound proteins were eluted by heating the beads in non-reducing SDS-loading buffer and resolved on a SDS-PAGE gel. Proteins were transferred to a PVDF membrane which was blotted for properdin using polyclonal anti-properdin antibodies (Comptech, USA). Pure properdin (Comptech, USA) was loaded as positive control.

CIRp-A
Activity of related sequences on the AP

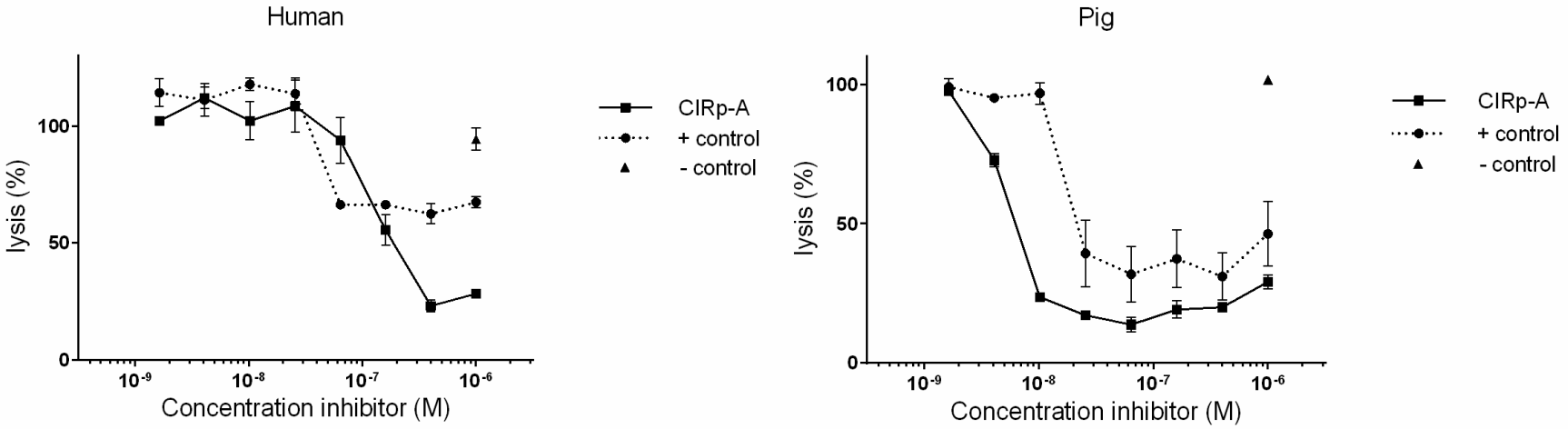
The patent application encompasses CIRp-A homologs



The figure shows the inhibition of the alternative pathway by CIRp-A and a homolog thereof with His tag (SEQ ID NO. 3), as determined by ELISA measuring MAC deposition. Supernatants of transfected cells were used. Only CIRp-A and the homolog show significant reduction in MAC formation. However, other experiments undertaken suggest that the other homologs may be active against pig serum. 96 well plates were coated with LPS and diluted normal human serum was added in the presence or absence of inhibitor. Plates were incubated for 1 hour at 37°C and MAC deposition was detected using an anti-C5b-9 monoclonal antibody (Abcam, UK). N = 3 and error bars indicate s.e.m. Values are normalised for no serum samples (0% activity) and no additive (100% activity).

CIRp-A
Inhibition of AP in pig serum

CIRp-A inhibits the AP in pig serum



The above figure shows that CIRp-A polypeptide potently inhibits serum from humans and pigs in an alternative pathway haemolytic assay using rabbit red blood cells. Final serum dilutions used were 1/8 for both. N = 3 and error bars indicate s.e.m. Values are normalised for no serum samples (0% lysis) and no additive (100% lysis).

CIRp-A programme
Further ongoing/planned work

Recently completed and ongoing

- Production of larger quantities of homologs/variants
- Further physicochemical characterisation of the above
- More detailed investigation of homologs/variants in a range of *in vitro* assays
  - Including further exploration of cross-species activity, especially rodents
- Partial localisation of the CIRp-A binding site within properdin

During 2019

- Crystal structure of properdin-CIRp-A complex to characterise in detail the binding sites in the two proteins

CIRp-A programme
Partnering objective

- To enter into a licence agreement in respect of the CIRp-A programme with an established industrial partner that:
  - demonstrates a strategic commitment to bring resultant pharmaceutical products to market as rapidly as possible;
  - has a clear vision for the diseases and indications for which it would seek to develop and market such products;
  - can set out specific plans for further research activities and for the subsequent pre-clinical and clinical development, registration and international marketing of resultant products for those indications; and
  - possesses and/or has access to all the necessary resources and capabilities.

CIRp-A programme
Envisaged transaction

- Worldwide exclusive licence to the patent application and associated intellectual property*
- Financial terms to include:
  - Up-front payment
  - Annual licence renewal fees / MARs
  - Milestone payments related to development progress, registration and market launch of resultant products (by indication and geography)
  - Royalty on net sales of resultant products
  - Sales-based milestone payments
- Associated research agreement with the Oxford group potentially available

* Subject to a provision that the inventors remain free to conduct further non-commercial research using the licensed intellectual property.

Contact details

Dr Robert V Fishleigh
bob.fishleigh@innovation.ox.ac.uk

www.innovation.ox.ac.uk

linkedin.com/company/oxford-university-innovation

twitter.com/OxUInnovation