

ABSTRACT

The alternative pathway (AP) of the complement system can be activated excessively in several inflammatory diseases, particularly when there is a defect in regulatory components of the complement system. For instance, defects of complement regulatory protein Factor H are associated with atypical hemolytic uremic syndrome (aHUS), whereas deficiency in glycosylphosphatidylinositol anchored proteins, including complement regulators decay accelerating factor (CD55) and CD59, leads to paroxysmal nocturnal hemoglobinuria (PNH), which causes severe prothrombotic pathologies. Understanding molecular mechanisms involved in complement activity are essential for developing new treatments. Properdin, the positive regulator of complement, is essential for complement amplification by stabilizing enzymatic convertases, and can also initiate complement activation. It exists as head-

P_n) accumulate in purified properdin preparations, due to prolonged storage and freeze/thaw cycles, and these P_n are artificially highly active (greater than P_4) and should be removed before use in research studies. There is no commercially available assay to measure properdin function in biological samples and clinical and research laboratories have been limited to using cumbersome functional assays that require fresh red blood cells. Here we have developed and characterized monoclonal antibodies (MoAbs) against properdin and standardized a functional enzyme linked immunosorbent assay (ELISA) that allows to determine the function of properdin *in vitro*, in an ELISA format. The data indicate that the functional ELISA can detect differences in how quickly and effectively the different properdin forms (P_2 - P_4 and P_n) activate the AP and can also detect properdin function in normal human serum. We have also developed a highly sensitive sandwich ELISA for measuring properdin concentration, using MoAbs pairs, with a lower properdin detection limit than commercially available kits (20 pg/ml). Finally, when tested in *in vitro* models of aHUS and PNH, which measure complement-mediated lysis due to defects in complement regulation, the inhibitory MoAbs had lower IC_{50} values than all other complement inhibitors tested, including Soliris (FDA approved for use to treat patients with these diseases). Further studies aimed at determining the therapeutic value of inhibiting properdin in human inflammatory diseases are warranted.

DISSERTATION COMMITTEE

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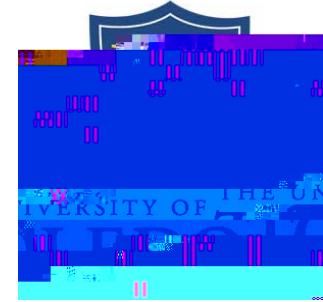
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THESIS
PRESENTATION

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**Anti-properdin monoclonal
antibodies: Relevance in
detecting functional differences
between properdin polymers and
in inhibiting properdin function**

**M.S. in Biomedical
Sciences**

PUBLICATIONS

N. Galwankar, H.N. Emch, C. Cortes, J.M. Thurman, J.D. Lambris, D. Ricklin, V.P. Ferreira. Inhibition of properdin is more effective than other inhibitors of the complement system at preventing cell lysis in the *in vitro* models of atypical hemolytic uremic syndrome and paroxysmal nocturnal hemoglobinuria. (In preparation).

N. Galwankar, V.P. Ferreira. Development of an assay that allows detection of the function of properdin. (In preparation).

N. Galwankar, H.N. Emch, A.C. Lad, C. Cortes, V.P. Ferreira. Inhibition of properdin is more effective than other inhibitors of the complement system at preventing cell lysis in an *in vitro* model of atypical hemolytic uremic syndrome. 104th annual meeting of the American Association of Immunologists (AAI), May 2017, Washington, DC, USA. Abstract published in the *Journal of Immunology* 198 (1 supplement) 222.11.