

Exploring existing human complement C4d, TCC & functional activity assays

Assessment of complement activation in laboratory animals

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INTRODUCTION

There is an increasing interest in analyzing the complement system of different species in biomedical research, especially in view of recent progress in complement assays. The animal models are used for evaluating efficacy of the corrective strategy, as it is possible to study systemic interactions and physiological functions. With the limited number of species-specific assays for assessment of complement activation and activity, we explored the possibility of using existing human complement ELISA assays for evaluation of complement activation in laboratory animals.

METHOD

Pooled or individual serum samples from eight different species; Rhesus monkey (*Macaca mulatta*), Cynomolgus monkey (*Macaca fascicularis*), mouse (CD-1), rat (Sprague Dawley), dog (Beagle), goat, minipig (Göttingen) and rabbit (New Zealand White), were analyzed in Wieslab® Complement System assays, i.e. Classical-, Alternative- and MBL Pathways (CP, AP and MP) and SVAR Complement C4d and Complement TCC assays (Svar Life Science AB, Sweden). All assays were performed as described in the "Instructions for use" manuals, using both activated (zymosan and heat-aggregated IgG) and non-activated serum. Commonly used complement inhibitors, i.e. 5mM/10mM MgEGTA, 10 µg/mL anti-human C1q monoclonal antibody (Svar Life Science AB), 30 mM D-mannose and 30 mM EDTA, were added to each pathway-specific buffer for the assessment of complement pathway specificity using previously determined dilution factor of the serum. For analysis of complement activation in rat, the detection antibody was replaced by an anti-rat TCC monoclonal antibody (Nordic BioSite) and an anti-rat C1q monoclonal antibody (ThermoFisher) was used for inhibition of the classical pathway.

“This study has shown how existing human complement assays can be used for evaluation of serum from different species and how serum from additional species could be analyzed with modification of detection conjugate antibody.”

CONCLUSION

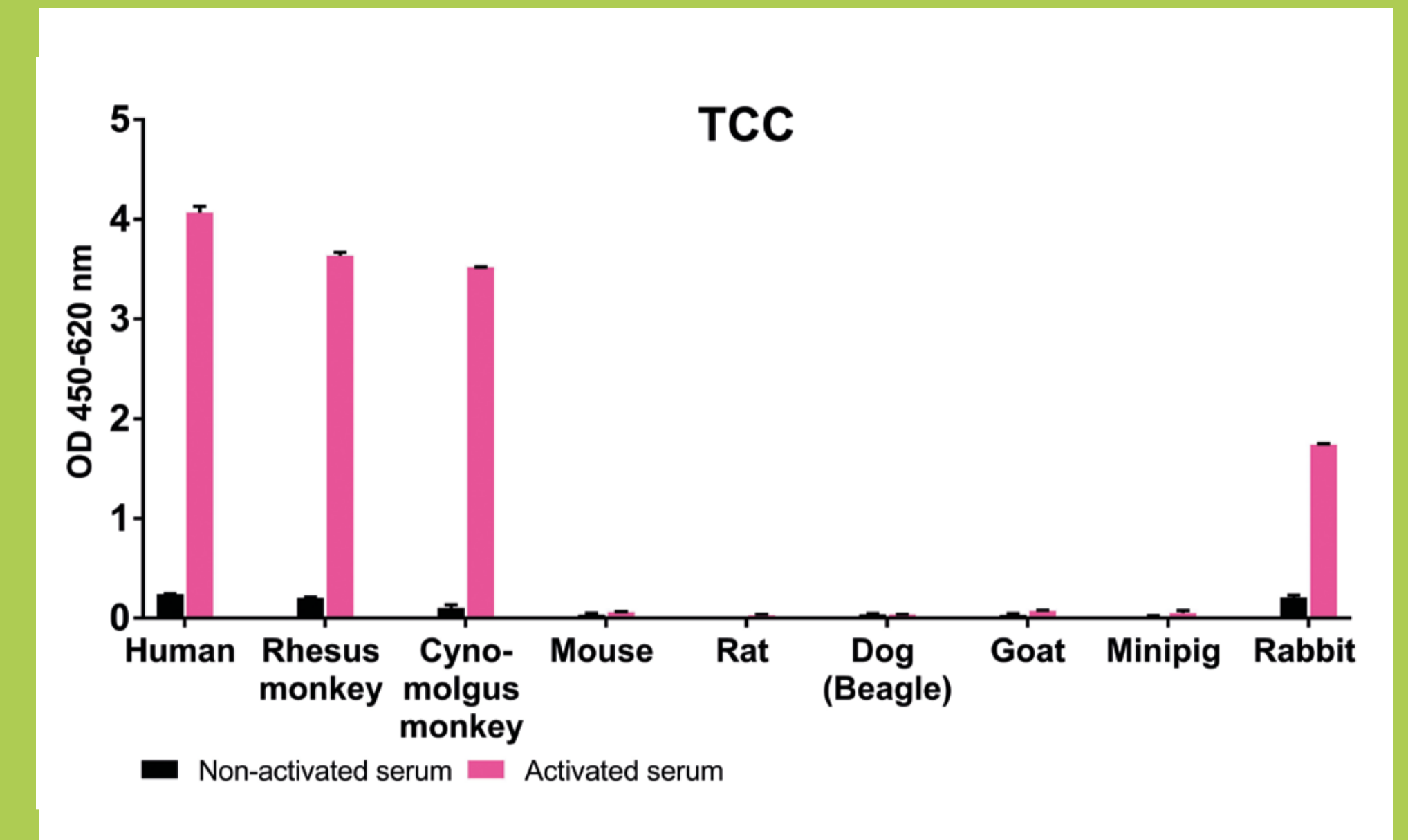
The human complement C4d assay (Svar Life Science AB) can be used for evaluating C4d in non-human primate serum. Soluble C5b-9 in non-human primate and rabbit serum can be analyzed with the human complement TCC assay (Svar Life Science AB).

Wieslab® complement system assays can be used for functional evaluation of all complement pathways in non-human primate-, minipig- and, after detection modifications, rat serum. Additionally, it is possible to detect functional complement activity via the AP and MP in goat serum and MP in rabbit.

This study has shown how existing human complement assays can be used for evaluation of serum from different species and how serum from additional species could be analyzed with modification of detection conjugate antibody.

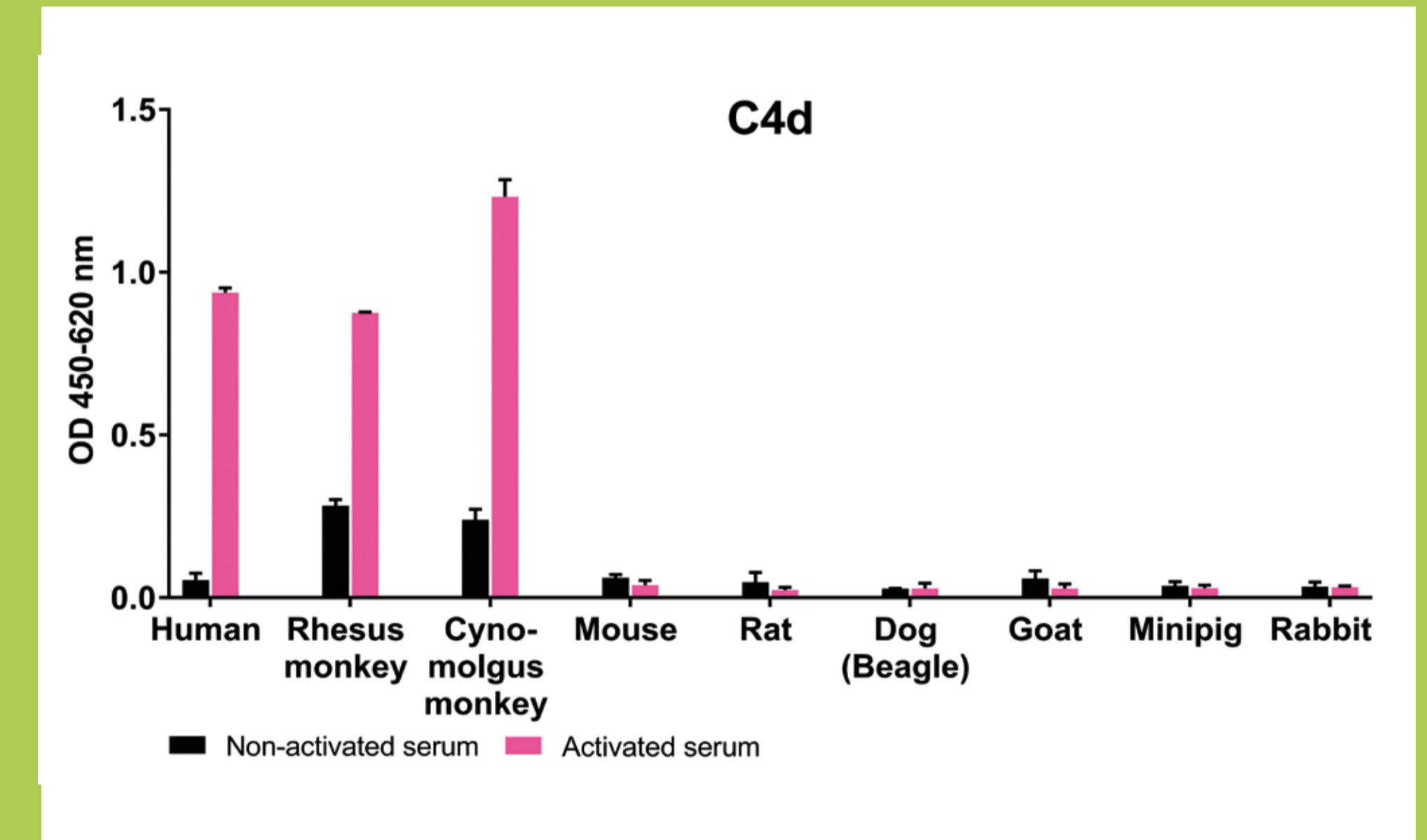
COMPLEMENT ACTIVITIES IN LABORATORY ANIMALS

ANALYSIS OF COMPLEMENT TCC IN DIFFERENT ANIMAL SERUM



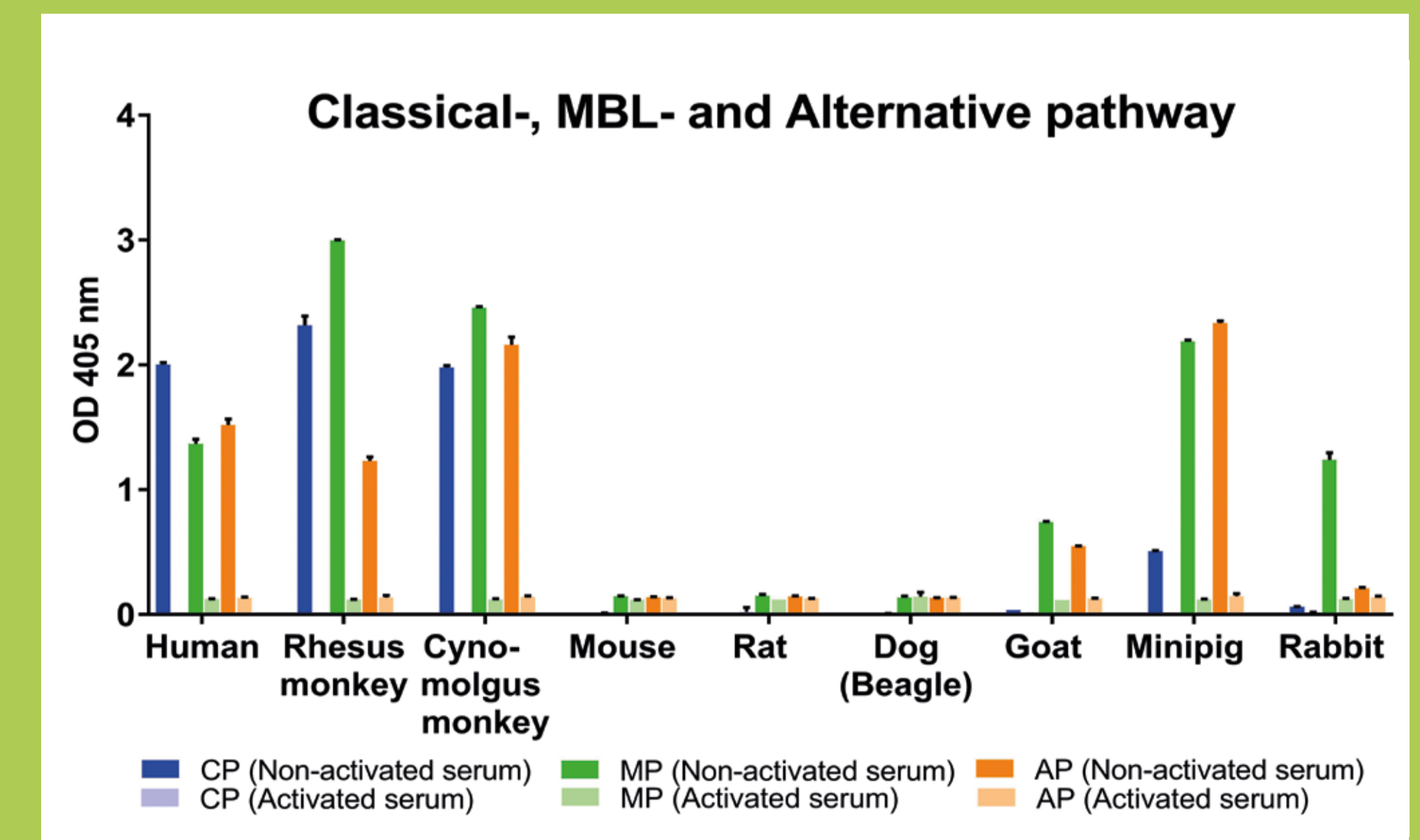
Serum samples from eight different species were analyzed in the human Complement TCC assay. Normal human serum was used as negative control and activated normal human serum was used as positive control. Serum from Rhesus monkey, Cynomolgus monkey and rabbit were diluted 1/200 in sample diluent while the remaining species were diluted 1/10 in sample diluent. Data are presented as mean ± SD of measured OD values at absorbance 450-620 nm.

ANALYSIS OF COMPLEMENT C4d IN DIFFERENT ANIMAL SERUM



Serum samples from eight different species were analyzed in the human Complement C4d assay. Normal human serum was used as negative control and activated normal human serum was used as positive control. Serum from all species were diluted 1/100 in sample diluent. Data are presented as mean ± SD of measured OD values at absorbance 450-620 nm.

ACTIVATION OF COMPLEMENT PATHWAYS IN DIFFERENT ANIMAL SERUM



Functional CP, MP, and AP activity in serum from eight different species. The activities of three complement pathways were assessed in Wieslab® Complement System assays. Normal human serum was used as positive control and activated normal human serum was used as negative control. Serum from Rhesus monkey and Cynomolgus monkey were diluted 1/101 in CP and MP and 1/18 in AP while the remaining species were diluted 1/5 in CP and MP and 1/3 in AP. Data are presented as mean ± SD of measured OD values at absorbance 405 nm.

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INHIBITION OF COMPLEMENT ACTIVATION FOR DEMONSTRATION OF PATHWAY SPECIFICITY

Specificity of functional CP, MP, and AP activity in serum from seven different species was assessed using selective inhibition of the complement activation. Each inhibitor was added to the pathway-specific diluents before diluting the serum (A-F).

The CP specificity, i.e. the dependency on C1q activation, was examined by adding 10 µg/mL anti-human C1q antibody. The addition of 5mM/10 mM MgEGTA inhibits the activation of both CP and MP while an addition of 30 mM EDTA inhibits the activation of all pathways. The MP activation was inhibited by addition of 30 mM D-Mannose.

Rat serum (G) was diluted in pathway-specific buffer with inhibitor and 10 µg/mL of an anti-rat C1q antibody was added for inhibition of CP and an HRP-conjugated anti-rat TCC monoclonal antibody was used as detection antibody with TMB as substrate. Activity was determined from duplicates and calculated as relative to the species-specific serum tested in original buffer.

