**Title:** Identifying the mechanism of NF-kB activation by dense granule protein GRA15 “GRA15 (II)” of Toxoplasma gondii type II

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**Purpose:** Toxoplasma infection in humans manifests in a wide range of ways. Host’s immune status is critical, but even immunocompetent individuals can succumb to severe disease. For an infection to be effectively established, the parasite needs to modulate the host immune response. Specialized effector proteins from subcellular organelles known as rhoptries and dense granules enable Toxoplasma gondii to escape the host’s immunity. These effectors differentially interfere with the human immunity, and vary between T.gondii strains. Dense granules are key immune modulators. It is known that GRA15 activates the NF-κB pathway, but it is unclear whether GRA15 interacts directly with TNF receptor associated factor 6 (TRAF6) or acts indirectly through TRAF6 to cause NF-κB activation. How GRA15 activates the NF-kB pathway and which GRA15 regions are required for this activation remain unknown. The goal of this study is to elucidate the interference between the GRA15 and NF-kB pathway.

**Methods:** An in vitro study was performed and TRex- HEK 293 cells overexpressing GRA15 (II) was generated. Mass spectrometry was run on immunoprecipitates from whole cell lysates harvested 24 hours post induction with tetracycline. CRISPR/CAS9 technique was applied for gene editing on HEK 293 NF-kB reporter cell lines to generate the specific TRAFs knock out. Successful TRAFs knock out were verified by means of sequencing, western blotting and microscopy. Specific regions of GRA15 were engineered and expressed in a mammalian vector and subsequently transfected in the HEK 293 NF-kB reporter cell line to determine differences in activation**.**

**Results:** Co-immunoprecipitation identified host partners, upstream of the NF-κB adaptor proteins, as candidate host proteins interacting with GRA15. Infection with Toxoplasma strain pru (strain II) and pru GRA15k/o of specific engineered by CRISPR/Cas9 knocked out cell lines, established the crosstalk between GRA15 and NF-kB activation. Regions of GRA15 important for NF-κB activation were identified through structure-function mutant analysis.

**Conclusions:** Elucidating the interplay between the GRA15 and NF-κB activation provides a better understanding of the host - pathogen interactions, which may lead to the development of new targets to control the Toxoplasma infection.