



**Universidad
de Guanajuato**

Interaction of the parasite *Trichomonas vaginalis* with human neutrophil extracellular traps

Eva Edilia Avila

Universidad de Guanajuato, México

- **Yordan Jhovani Romero Contreras**
- **Valeria Janeth Elías Soria**
- **María Guadalupe Ramírez**
- **Mayra Cecilia Rodríguez Solís**



Universidad
de Guanajuato

Trichomonas vaginalis and trichomoniasis

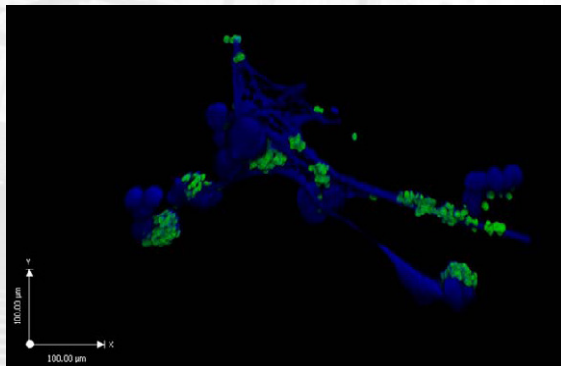
- ❖ ***Trichomonas vaginalis* is a flagellated protozoan that contain lipoglycan in the cell surface.**
- ❖ **This parasite causes the most common non-viral sexually transmitted infection in human.**
- ❖ **Trichomoniasis is associated with others disorders:**
 - Premature births
 - Higher risk for getting and transmitting HIV
 - Higher risk of contracting Human Papilloma Virus
 - Bacterial vaginosis
 - Infertility



Functions of neutrophils in infections

Universidad
de Guanajuato

- ❖ Neutrophils are the most abundant white blood cells and the first to arrive to an infection site.
- ❖ Neutrophils contribute to the excessive inflammatory process that occur during trichomoniasis.
- ❖ The microbicidal effect of neutrophils may occur by endocytosis, degranulation and the formation of extracellular traps (NETs).



Maren von Köckritz-Blickwede & Victor Nizet, 2009



Universidad
de Guanajuato

The role of neutrophils in the response to *T. vaginalis*

T. vaginalis induces IL-8 secretion by neutrophils and this cytokine activates neutrophil degranulation. Altstaed & col., 1996; Sook Ryu & col., 2003; Hee Nam & col., 2011.

Neutrophils activated by *T. vaginalis* express myeloperoxidase and produce nitric oxide and superoxide anion. Ouk-song & col., 2013.

Lysates of *T. vaginalis* delay neutrophil apoptosis; but live trophozoites induced neutrophil apoptosis, this induction is depending of reactive oxygen species. Kang & col., 2006; Ouk-song & col., 2007; Ouk-song & col., 2010.

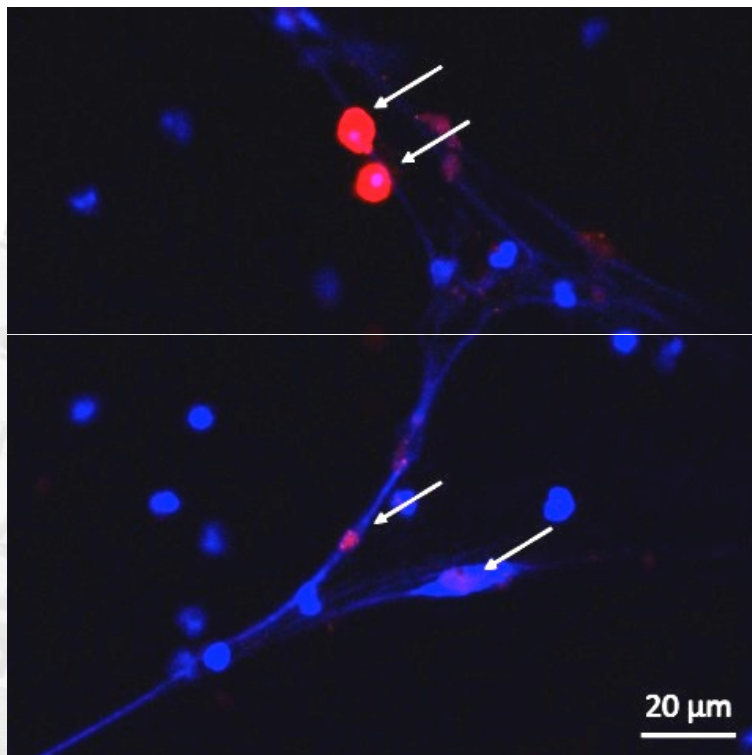
Groups of neutrophils surrounding individual *T. vaginalis* are able to fragment trophozoites and to phagocytose the pieces. Rein & col., 1980.

T. vaginalis infection activates neutrophils through toll-like receptor- 4. Zarifarrd & col., 2004.

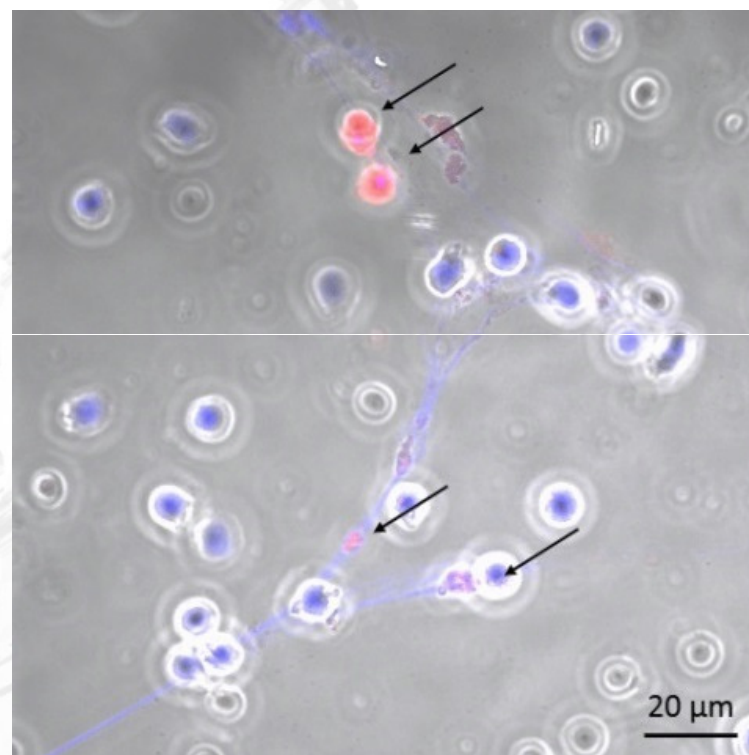


Universidad
de Guanajuato

T. vaginalis induces the formation of neutrophil extracellular traps



Hoechst 33342 + PKH26



Merge with phase contrast

Ratio: 1 trophozoite for every 5 neutrophils(1:5)

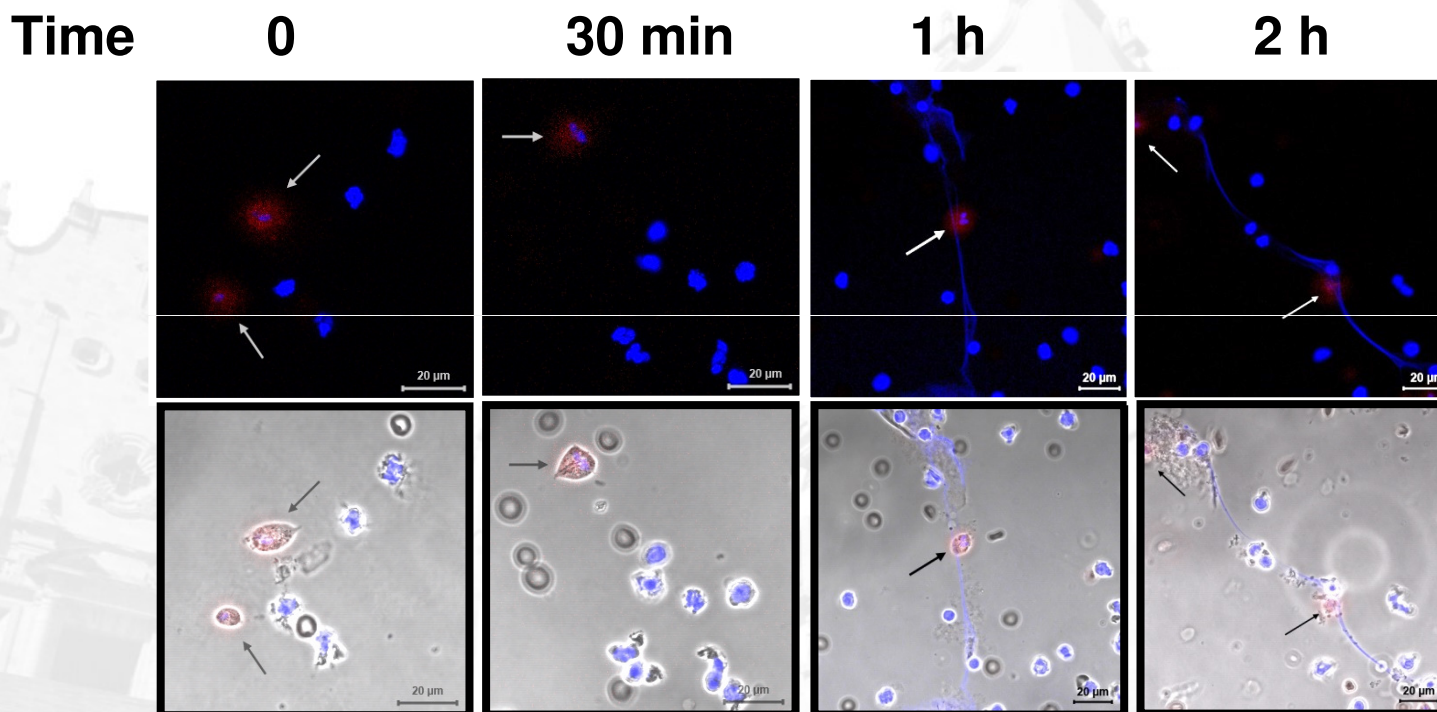
Yordan Jhovani Romero Contreras

www.ugto.mx



Universidad
de Guanajuato

Kinetics of neutrophil extracellular traps induction by *T. vaginalis*



DNA staining with HOECHST 33342, *T. vaginalis* pre-staining with PKH26.
Bar, 20 μm .

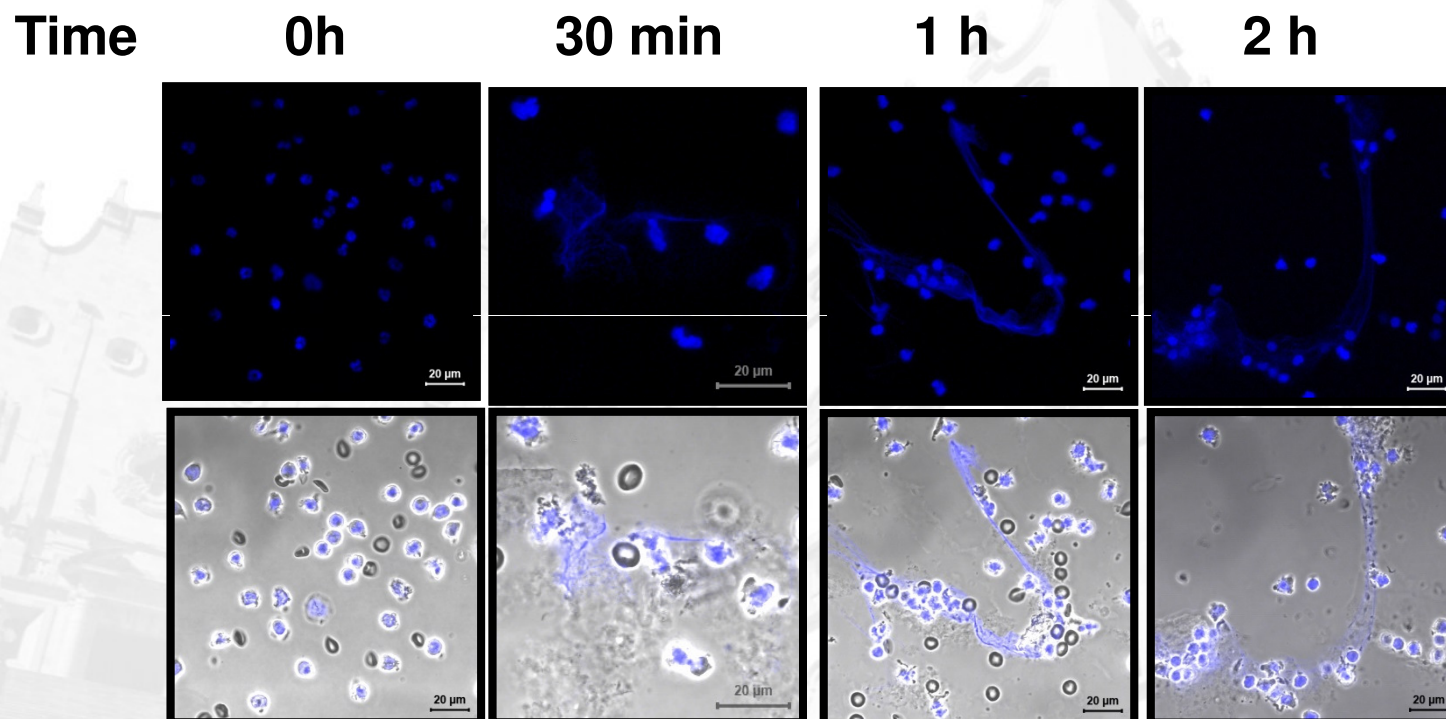
Yordan Jhovani Romero Contreras
Valeria Janeth Elías Soria

www.ugto.mx



Universidad
de Guanajuato

Lipoglycan from *T. vaginalis* surface induces neutrophil extracellular traps



T. vaginalis Lipoglycan at 10 ng/ml, DNA staining with HOECHST 33342. Bar, 20 μ m.

Yordan Jhovani Romero Contreras
Valeria Janeth Elías Soria

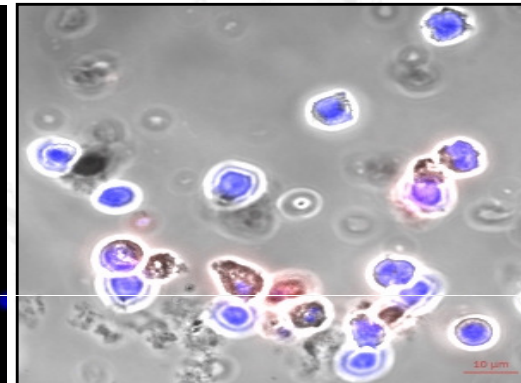
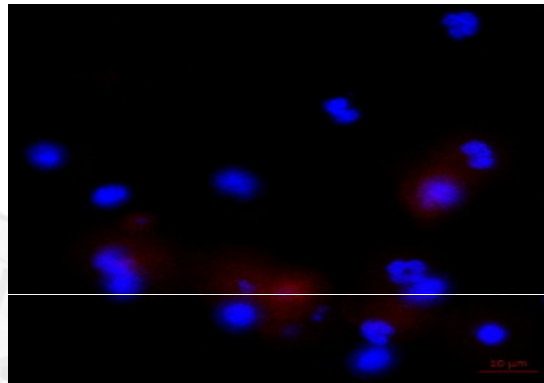
www.ugto.mx



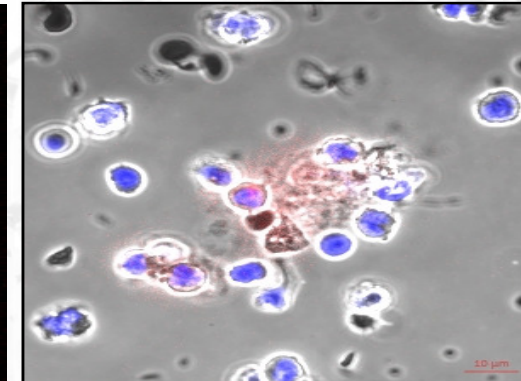
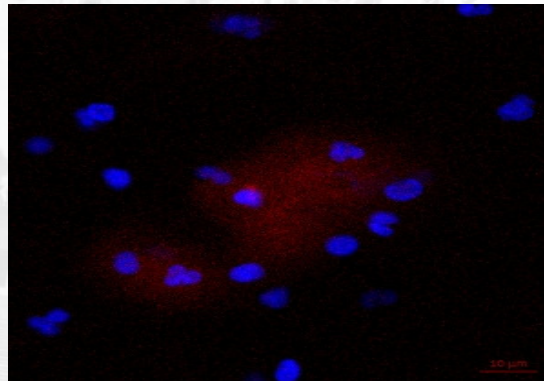
Universidad
de Guanajuato

Inhibition of NET formation by antibodies to TLR-4 and TLR-2

Neutrophils
+ α -TLR-4
+
T. vaginalis



Neutrophils
+ α -TLR-2
+
T. vaginalis



Merge of dyes

Merge with phase contrast

María Guadalupe Ramírez

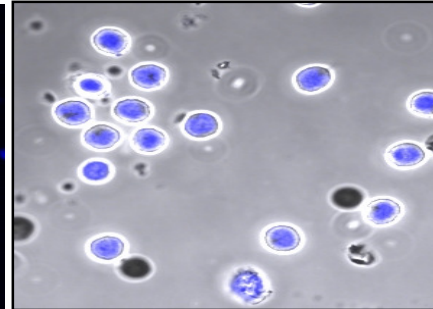
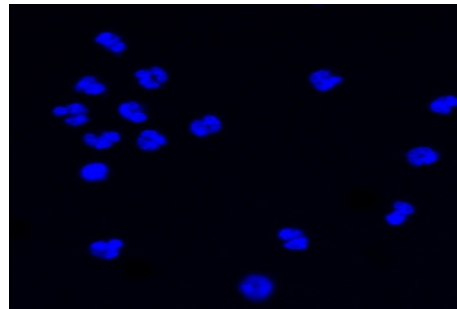
www.ugto.mx



Induction of neutrophil extracellular traps by *T. vaginalis* in presence of normal IgG

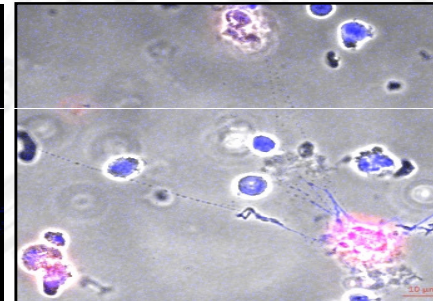
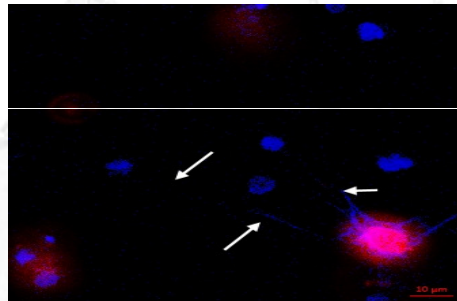
Universidad
de Guanajuato

Neutrophils



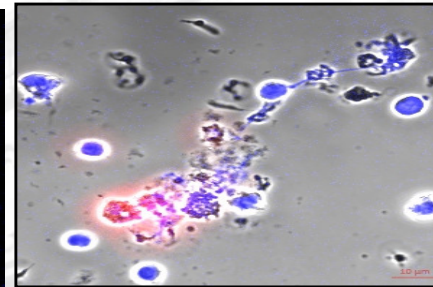
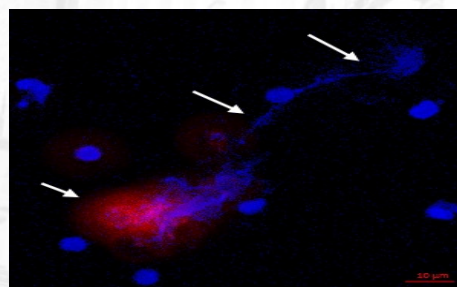
Negative
control

Neutrophils
+ *T. vaginalis*



Positive
control

Neutrophils
+ IgG +
T. vaginalis



10 µg/ml
rat IgG

Merge of dyes

Merge with phase contrast

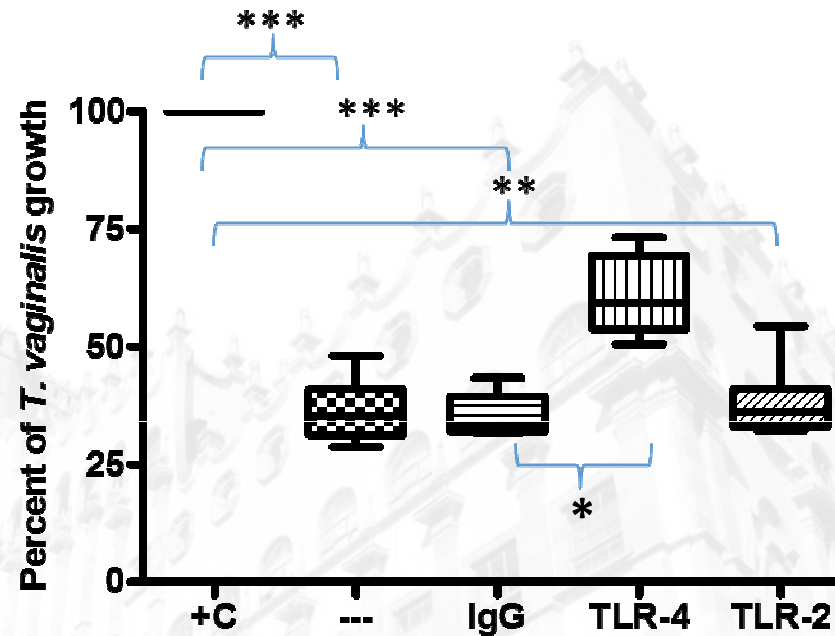
María Guadalupe Ramírez

www.ugto.mx



Universidad
de Guanajuato

T. vaginalis growth after interaction with human neutrophils and NETs



Neutrophils	-	+	+	+	+
IgG	-	-	+	-	-
α -TLR-4	-	-	-	+	-
α -TLR-2	-	-	-	-	+
<i>T. vaginalis</i>	+	+	+	+	+

Kruskal-Wallis statistic analysis, four independent experiments performed by duplicate. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$



Universidad
de Guanajuato

CONCLUSIONS

- ***Trichomonas vaginalis* and its surface lipoglycan induce the formation of neutrophil extracellular traps.**
- **Neutrophil extracellular traps sequester *T. vaginalis* and reduce parasite growth.**
- **Blocking TLR-2 or TLR-4 receptors inhibit NET formation.**
- **When TLR-2 is blocked, neutrophils reduce *T. vaginalis* growth similarly to NETs,. However, when TLR-4 is inhibited, neutrophils are less trichomonocidal.**



Thank you

Universidad
de Guanajuato

