for the first immunisation Freund’s complete adjuvant was used while for the second to fifth immunisation we use Freund’s incomplete adjuvant, in the sixth week only the antigen was used. At week 0, 3 and 6, rabbits were bled for the evaluation of the immune response by the indirect ELISA. The final bleeding was performed and the serum obtained was stored at -20°C until use. For the purification of the antibodies we use a protein A purification kit and the integrity of the immunoglobulins was verified by electrophoresis, the titration of the obtained polyclonal antibody was performed by indirect ELISA. We evaluated the antibody by Western blot. Dot blot and inhibition of hemagglutination using as antigen the ATCC 14018 of G. vaginalis. To corroborate that the antibody inhibited erythrocyte lysis, hemagglutination inhibition assays were performed. The western blot showed that this antibody recognised a band of approximately 56 kDa that matched with the molecular weight reported for the VLY, whereas the Dot blot showed that the antibody recognises the VLY of ATCC 14018 of G. vaginalis. To corroborate that the antibody inhibited erythrocyte lysis, hemagglutination inhibition assays were performed and we showed that the use of this antibody decreased the cell lysis in around 80%.

Conclusion We produce a polyclonal antibody against the VLY of G. vaginalis capable of inhibiting the erythrocyte lysis. This antibody will be useful in investigating the role of VLY in the pathogenesis of G. vaginalis during the development of BV.

Introductions: Treponema pallidum is the causative agent of venereal syphilis, a human-specific sexually transmitted infection characterised by multi-stage disease and diverse clinical manifestations. T. pallidum undergoes rapid hematogenous dissemination, accessing distant organ sites and penetrating tissue, placental, and blood-brain barriers. Tp0751 is an adhesion that interacts with the host vasculature and mediates bacterial adherence to endothelial cells under shear flow conditions. This study explores Tp0751-mediated adhesion to the vascular endothelium.

Methods Tp0751, expressed in a non-infectious model spirochete, was assayed for a gain-of-function adherence phenotype using attachment assays. Interaction specificity was probed with competitive inhibition studies using synthetic peptides of Tp0751 host-binding regions. Affinity chromatography coupled with mass spectrometry was used to identify endothelial receptors for Tp0751. Membrane receptors isolated from human umbilical vein endothelial cells (HUVECs) were incubated with Tp0751-affinity columns and interacting proteins were identified with mass spectrometry.

Results Here we demonstrate that Bb-Tp0751 adheres to HUVECs under stationary conditions. The laminin receptor (LamR) was identified as an endothelial receptor for Tp0751. LamR is a brain endothelial receptor for other neurotropic invasive pathogens, including Neisseria meningitidis. Current investigations will validate the Tp0751-LamR interaction and characterise the functional outcomes of Tp0751 adhesion to endothelial cells.

Conclusion These investigations reveal the mechanics of T. pallidum attachment to endothelial cells, the fundamental step in the process of T. pallidum vascular dissemination. A complete understanding of this process will provide opportunities to prevent T. pallidum attachment to the host vasculature to facilitate syphilis vaccine development.

Abstracts

P1.29 ATTACHMENT OF THE SYTPLIS SPIROCHETE, TREPONEMA PALLIDUM, TO THE VASCULAR ENDOTHELIUM

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Introduction: Treponema pallidum is the causative agent of venereal syphilis, a human-specific sexually transmitted infection characterised by multi-stage disease and diverse clinical manifestations. T. pallidum undergoes rapid hematogenous dissemination, accessing distant organ sites and penetrating tissue, placental, and blood-brain barriers. Tp0751 is an adhesion that interacts with the host vasculature and mediates bacterial adherence to endothelial cells under shear flow conditions. This study explores Tp0751-mediated adhesion to the vascular endothelium.

Methods: Tp0751, expressed in a non-infectious model spirochete [Borrelia burgdorferi (Bb-Tp0751)], was assayed for a gain-of-function adherence phenotype using attachment assays. Interaction specificity was probed with competitive inhibition studies using synthetic peptides of Tp0751 host-binding regions. Affinity chromatography coupled with mass spectrometry was used to identify endothelial receptors for Tp0751. Membrane receptors isolated from human umbilical vein endothelial cells (HUVECs) were incubated with Tp0751-affinity columns and interacting proteins were identified with mass spectrometry.

Results: Here we demonstrate that Bb-Tp0751 adheres to HUVECs under stationary conditions. The laminin receptor (LamR) was identified as an endothelial receptor for Tp0751. LamR is a brain endothelial receptor for other neurotropic invasive pathogens, including Neisseria meningitidis. Current investigations will validate the Tp0751-LamR interaction and characterise the functional outcomes of Tp0751 adhesion to endothelial cells.

Conclusion: These investigations reveal the mechanics of T. pallidum attachment to endothelial cells, the fundamental step in the process of T. pallidum vascular dissemination. A complete understanding of this process will provide opportunities to prevent T. pallidum attachment to the host vasculature to facilitate syphilis vaccine development.