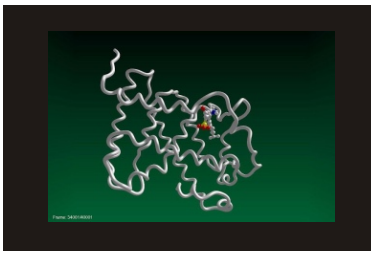

Bacterial Capnine Blocks Transcription of Human Antimicrobial Peptides

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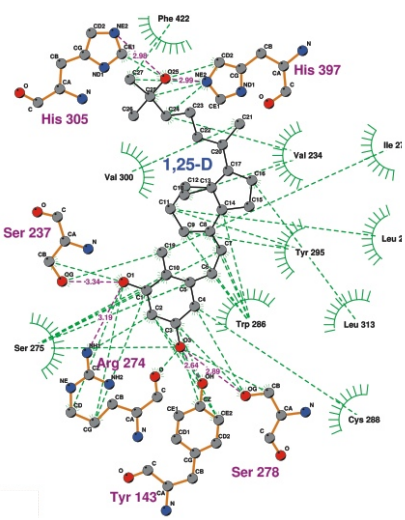
Video was created using the GROMACS Molecular Dynamics software on a small computing cluster assembled from PCs based on Core-2-duo CPU technologies. Capnine can be seen to maintain the stable configuration in the ligand binding pocket of the human VDR. Video available at http://autoimmunityresearch.org/models/capnine-vdrh.ram

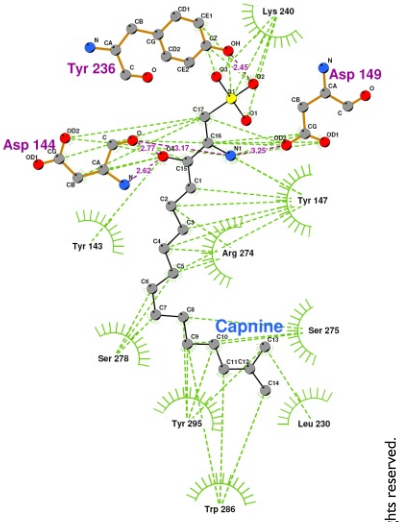
The US CDC believes that 65% of all infections in developed countries may be caused by pathogens in biofilms. Electron Microscopy has shown that these bacterial communities can evade phagocytosis, and persist in the cytoplasm of monocytes, macrophages, lymphocytes and neutrophils. Three decades ago, Wirostko, et al, found such intraphagocytic communities in Crohn's disease, Juvenile Rheumatoid Arthritis and Sarcoidosis [1]. However, the mechanism(s) by which such persistent bacteria could evade the immune system have remained elusive. Recently, 16S RNA from species of gliding bacteria never thought to be able to survive in-vivo, have been found in surgically removed biofilms [2]. This study set out to identify whether the genomes of these gliding bacteria might yield insight into mechanisms by which such persistent pathogens could evade phagocytosis.

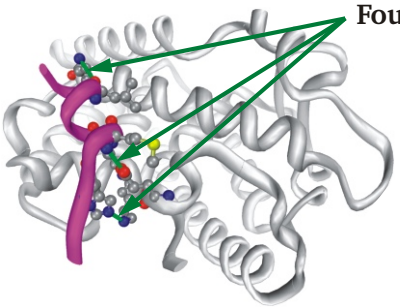
METHODS: A single Type 1 Nuclear Receptor, the VDR (commonly known as the 'Vitamin D Receptor'), is responsible for transcription of LL-37, the human Cathelicidin antimicrobial peptide, as well as the beta Defensin anti-microbial peptides defB2/defB4 [3]. Disabling transcription by the VDR would allow a pathogen to persist inside phagocytes without threat from these anti-microbial peptides. Static molecular modeling (primarily using AutoDock) was used to screen a number of proteins and peptides known to be produced by the genomes of the gliding bacteria.

RESULTS: A candidate bacterial sulfonolipid, Capnine, was identified to have a nanomolar Ki for the ligand binding pocket (LBP) of the VDR. Molecular Dynamics simulation of the human VDR in complex with Capnine confirmed that this substance is indeed stable in the VDR LBP, and that its action is that of a strong transcriptional antagonist.

CONCLUSION: Medical Metagenomics has demonstrated the ability to deliver important results in silico, potentially underpinning an infectious pathogenesis for idiopathicnic illness [4,5].







Four hydrogen bonds

Analysis of the rat VDR and DRIP205 model (PDB:1RK3) show that the activation of the VDR is only partly due to the AF-2 domain. There are hbonds from GLU416 to DRIP MET629/LEU630, but there are also hbonds from LYS260 to HIS627, and LYS242 to LEU633.

References:

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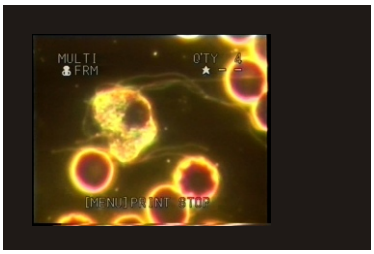
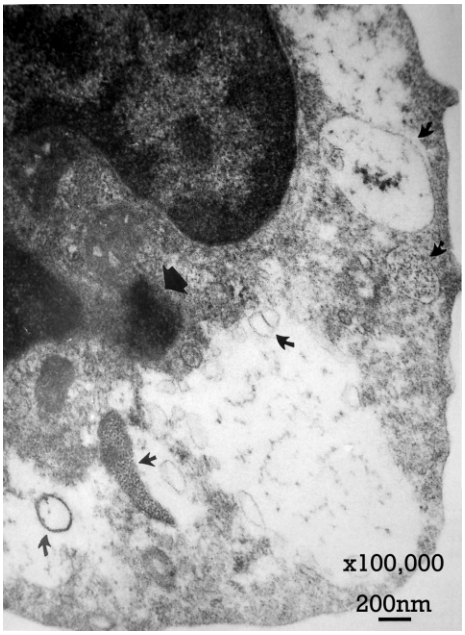


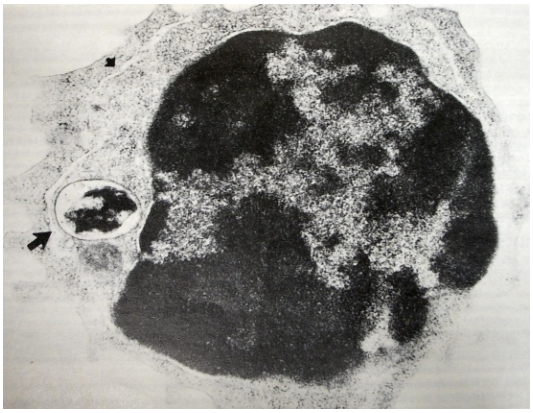
Metagenomic 'Biofilm' Communities, ex-vivo/in-vivo

Pinprick blood from Th1 patients. Blood left to stagnate 6-36 hours. Picture shows L-forms in biofilms leaving cells. Sources include: Dr Andrew Wright, UK. Video available at http://www.autoimmunityresearch.org/lax2006.ram

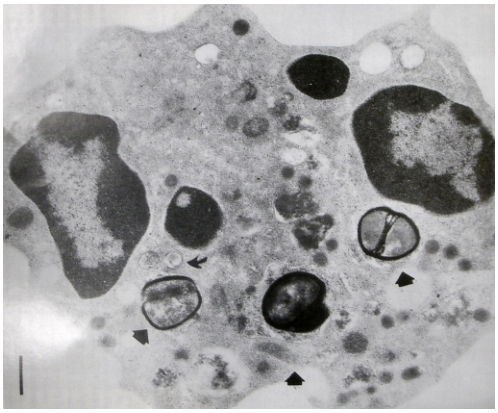
Wirostko E, et al. [1989 Columbia University TEM]



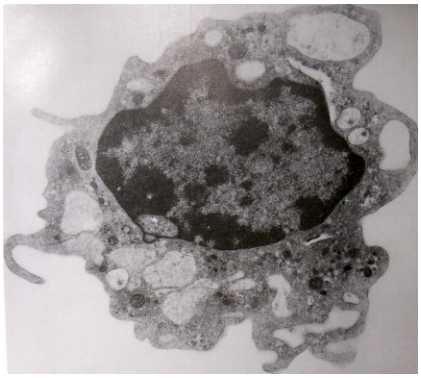
Sarcoidosis MLO parasitized vitreous monocyte. Uranyl acetate-lead citrate stain. Innumerable pleomorphic variably staining tubulo-spherical-fliamentous bacterial bodies lie within a severely lysed cytoskeleton (small arrows). The upper nuclear pole has a normal appearance, whereas the lower pole is abnormal (large arrow). (Bar = 0.2 micron)



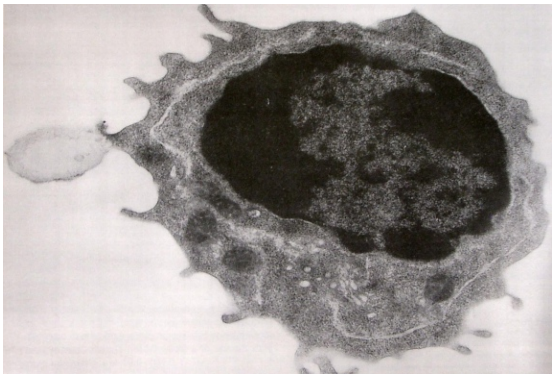
JRA MLO parasitized vitreous lymphocyte. Uranyl acetate-lead citrate stain (x34,722). Undulating variably staining tubules (small arrow) and a prominent 0.7 um ovoid body (large arrow) are detectable within the cytoplasm. The latter has a well defined outer membrane, abundant periplasm, and a plasma membrane surrounding amorphous electron dense material. The nucleus has an irregular contour with focally indistinct areas.



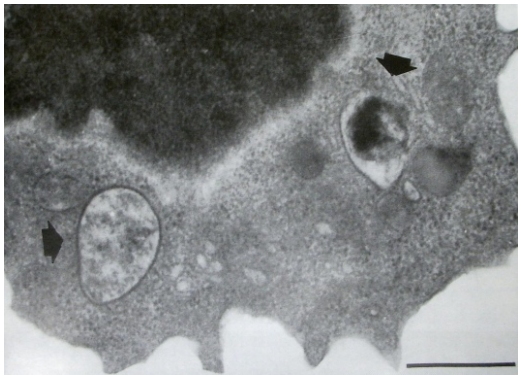
Sarcoidosis MLO parasitized vitreous polymorphonuclear leucocyte. Uranyl acetate-lead citrate stain. Three cocci with thick homogenous cell walls, a distinct periplasm, internal tubulo-filamentous structures, and varying degrees of septal formation, lie within the cytoskeleton (large arrows). Numerous intracytoskeletal pleomorphic variably staining cell wall deficient bacterial forms are also present (small arrow). There is a paucity of normal granules. The nuclear lobes display multifocally frayed contours and perinuclear 'halos' (Bar = 0.5 microns)



Crohn's MLO parasitized vitreous monocyte. The cytoplasm of the monocyte is replaced by numerous pleomorphic variably staining trilaminar membrane-bound bodies. These bodies are attached to the nucleus by a 0.005-0.01 um filaments that extend into the cytoplasm from several "moth eaten" appearing foci in the peripheral chromatin. The nuclear contour is convoluted and peripheral chromatin is clumped. Uranyl acetate-lead citrate stain (x21,485)



Crohn's MLO parasitized vitreous lymphocyte. The lymphocyte cytoplasm is replaced by trilaminar membrane-bound pleomorphic variably staining 0.01-0.7 um tubules and sperules. These arise from 0.005 to 0.01 um diameter branching filaments that are also detectable along the nuclear envelope. The envelope has a convoluted and 'hairy' appearance, and the peripheral chromatin is clumped. An MLO "bag-like" body protrudes from the lymphocyte cell surface. Uranyl acetate - lead citrate stain (x25,915)



Sarcoidosis MLO parasitized vitreous lymphocyte. Uranyl acetate-lead citrate stain. Two bacterial bodies with a varied internal appearance lie within the cytoskeleton (arrows). Numerous smaller intracytoskeletal tubulo-spherical shaped structures are also present. The nucleus has a prominent scalloped frayed contour and a 'halo-like' perinuclear space (Bar = 0.5 micron)