O063
Oral Session
Basic science: pathogenesis and epidemiology of Gram-positive bacteria

PATHOGENESIS OF NECROTIZING SOFT TISSUE INFECTIONS: ANALYSES AT THE LOCAL TISSUE SITE IN PATIENT BIOPSIES AND IN A NOVEL 3D SKIN MODEL

N. Siemens[1], L. Johansson[1], B. Chakrakodi[1], INFECT Study Group[1], M. Svensson[1], **A. Norrby-Teglund**[1]

[1]Center for Infectious Medicine, Karolinska Institutet, Stockholm, Sweden

Objectives. This study is part of an EU-funded project called INFECT that aims to elucidate the complex host and pathogen signatures that dictate the outcome of necrotizing soft tissue infection (NSTI). Here we study host-pathogen interaction at the local tissue site of NSTI through analyses of infected patient tissue biopsies as well as in a novel 3D skin tissue model.

Methods. Snap-frozen tissue biopsies (n=26) collected from NSTI patients enrolled in the INFECT project were included. The tissue model of human skin was developed by use of primary skin fibroblasts together with the skin keratinocyte cell line N/TERT-1. Briefly, upon culture the fibroblasts form a supportive scaffold (stroma matrix) on which the keratinocytes are seeded and stratified. Once the stratified epithelial layer formed, the models were infected with *Streptococcus pyogenes* strains (one *emm*1 and two *emm*3 strains) isolated from NSTI patients. Analyses of infected tissue were achieved through immunostainings and microscopy analyses of host factors in cryosectioned tissue models and biopsies. Also gene expression analyses of host responses were done by qPCR.

Results. Hematoxylin/eosin staining of tissue models revealed that the fibroblasts and keratinocytes formed a multicellular tissue specific organization with the fibroblast forming structures resembling dermis whereas the keratinocytes formed structures resembling epidermis and stratum corneum. To confirm that the skin model produces structural framework proteins associated with skin and epithelial barriers, sections of the model were analysed for the presence of Claudin, Fibronectin, Cytokeratin 10 and Cytokeratin 16, using confocal microscopy analyses. The data confirms that the established skin tissue model closely resembles normal human skin tissue both with respect to morphology as well as production of structural proteins associated with specific areas of the human skin. NSTI isolates caused efficient and highly disseminated infections in the skin models. Significant increases of Cytokeratin 10 and Claudin were evident both at protein and mRNA levels after 8-24h post-infection with all 3 strains as compared to uninfected models ($p<0.05$). Similarly, a robust inflammatory response was seen with increased expression of the IL-6 and IL-8 genes. Analyses of *S. pyogenes* infected patient tissue showed similar patterns in terms of bacterial dissemination and in inflammatory responses.
Conclusion. *S. pyogenes* NSTI isolates were capable of causing infections through the epithelial layer of the skin tissue model, which resulted in a disseminating infection of the dermis layer. The infections are associated with significant alterations of host responses, including hyper-inflammatory responses, which are evident in both the tissue model as well as infected patient tissue. Our findings show that the artificial skin tissue is an elegant novel model system that allows in-depth studies of host-pathogen interactions in human tissue.