used HUVEC cell line. Analyzing HED supernatants, GLUT-1 and MCP-1 were found in high concentrations. When applying ex vivo various concentrations of VEGF on HED there are a clear correlation of cell proliferation with the angiogenic factor concentrations, correlated moreover with decreased apoptosis, especially in the time range 3–24 hrs. When applying MMP-1 the effects are similar with the ones induced by VEGF in HED, while control cells display an inverse cellular behavior. When physiologically inhibiting MMP action with TIMP-1, there is a marked reduction of the cells proliferative capacity with an induction of early apoptosis mechanisms. Studying MCP-1 action, depending on the proliferative or regression tumor stage, its action is different. MCP-1 seems to be a factor that modulates positive the proliferative stage of the hemangioma. We can conclude that there is an intricate panel of pro-inflammatory factors displaying spatial and temporal action that converge towards the proliferative stages or to the regression one.

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Keywords: experimental model, hemangioma, inflammation.

SUN-206
Inhibition of MMP-9 gene expression and cancer cell proliferation by essential oils of *Ocimum sanctum*

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Matrix metalloproteinases (MMPs) released from inflammatory cells are involved in the development and progression of human cancers. Among the various MMPs, MMP-9 is found to be involved in metastasis of breast, colon and ovarian cancers. Natural products are effective in reducing inflammation and carcinogenesis. Essential oil from *Ocimum sanctum* was tested for its effect on inhibiting the proliferation of human breast cancer cells and reducing the expression of MMP-9 in human lymphocytes. Lymphocytes were treated with lipopolysaccharide to induce inflammation and then treated with essential oils. The expression of MMP-9 was analyzed using gelatin zymography and real-time reverse transcriptase PCR. Gelation zymography showed that MMP-9 expression was completely inhibited at 250 ug/ml of essential oil. A dose dependent decrease in the expression of MMP-9 was observed in real-time RT-PCR. The inhibitory effects of essential oils on the proliferation of breast cancer cells (MCF-7) were tested using the MTT assay and real-time PCR analysis. *Ocimum sanctum* essential oil (OSEO) inhibited proliferation (IC_{50} = 170 μg/ml) and migration (IC_{50} = 250 μg/ml) of MCF-7 cells in a dose-dependent manner. OSEO also induced apoptosis as evidenced by the increasing number of propidium iodide stained apoptotic nuclei. Flow cytometry analysis revealed that treatment with OSEO (50–500 μg/ml) increased the apoptotic cell population dose-dependently (by 16%> 84%) compared to the control. Gene expression analysis showed that OSEO upregulated the apoptotic genes p53 and Bid and elevated the ratio of Bax/Bcl-2. The results of our study indicate that OSEO has the ability to express both anti-inflammatory and anticancer activities.

Keywords: Anticancer, Essential oil, MMPs.

SUN-207
Innate immune response during NTM infections

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Background: As tuberculosis incidence declines in industrialized countries, nontuberculous mycobacteria (NTM) infections gained relevance. Human infection with NTM became relevant with AIDS pandemic, being currently recognized as a cause of pulmonary infection in humans. Despite this fact little is known about NTM pathogenesis. In the present work the role of innate immune response during NTM infection using THP-1 cells as a model of alveolar macrophages was evaluated.

Methods: *M. smegmatis* mc^2 155, 2 reference strains (*M. avium* ATCC25291; *M. fortuitum* ATCC6841) and 2 clinical isolates (*M. avium* 60/08; *M. fortuitum* 747/08) were used. Bacteria were grown until mid-exponential phase and stored at −80°C. Before each experiment an aliquot was thawed and diluted in RPMI with 10% HI- FCS in order to reach an $OD_{600\ nm}$ of 0.1. The inoculums were titrated by CFU enumeration on 7H10 medium supplemented with 10% OADC.

Briefly, 4×10^4 THP-1 cells were platted/well and incubated for 72 h with 100 nM PMA (37°C/5% CO_2) then fresh medium without PMA was added being the cells incubated for further 24 h. The cells were infected for 1 or 3 h for fast or slow growers, respectively. The intracellular persistence was evaluated by CFU enumeration at different time points from 1 to 24 h or 3–168 h for fast and slow growers, respectively.

Phagosome acidification was followed using confocal microscopy. The secretion of pro-inflammatory cytokines was assayed by ELISA, NO production using the Griess reagent and apoptosis was followed by flow cytometry and confocal microscopy. The ability of mycobacteria to persist at different pHs was evaluated using BACTEC-MGIT960.

Results: The mycobacteria experienced different fates within THP-1 macrophages. *M. smegmatis* and *M. fortuitum* ATCC6841 were cleared within 24 h, whereas 747/08 and the two *M. avium* strains were able to replicate. Despite this fact for the latest mycobacteria more than 50% of acidified phagosomes were present during the experience. Mycobacteria survival at acidic pHs (6.6; 5.4 and 4.6) was then evaluated. With the exception of *M. smegmatis* all strains grew at acidic pH showing that other factors than phagosome acidification were involved in mycobacteria killing.

Next, other components of the inflammatory response were evaluated. Measurable values of NO were present in supernatants of THP-1 infected for 3 days with 60/08 being this bacterium susceptible, to high concentrations of NO in vitro. Il-10 secretion was also assayed. For both fast growing NTM and *M. avium* ATCC25291 the production of IL-10 was not detectable. For 60/08 IL-10 production peaked at 3 days, decreasing afterwards until undetectable levels at 7 days.

Another factor being explored is apoptosis induction by NTM. Our preliminary results point to differential induction of apoptosis by different NTM.

Keywords: None.