Serogroup Y Meningococci (Y:14:P1.2,5) Are Rapidly Killed in an ex Vivo Whole Blood Model of Infection Whereas Serogroup B Meningococci (B:4:P1.7,16) Survive And Grow to Logarithmic Phase.

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Objective: To compare the survival and pathogenicity of an invasive and a carrier strain of *Neisseria meningitidis* in an *ex vivo* whole blood model of infection.

Design: Two strains of *N. meningitidis* (Y:14:P1.2,5 —carrier strain— and B:4:P1.7,16 —invasive strain—) were inoculated at low *cfu* (9009 +/-658 and 9863 +/-1580 per mL, respectively) in heparinized whole blood samples of healthy vaccinated (tetravalent polysaccharide vaccine) and non vaccinated donors. Remaining viable meningococci were determined at intervals up to 24 h after onset of experiments. In addition, leukocyte cell populations were studied using flow cytometry. Cytokine release was measured using ELISA.

Results: Serogroup Y meningococci were killed within 20 to 30 min after inoculation in whole blood independently of the status of the blood donor (e.g. vaccinated or not). Also independently of the donor, serogroup B meningococci reached logarithmic growth phase in whole blood samples usually within 4 hours after setup of the experiment. Accordingly, high amounts of cytokines (namely IL-1ß, IL-6, IL-10 and TNFα, but usually not IFNγ) were found to be released after incubation with serogroup B meningococci whereas only comparatively low amounts of IL-6 were released after incubation with serogroup Y meningococci. After incubation with serogroup B meningococci a considerable loss of neutrophils was observed. Neutrophils remained unchanged after incubation with serogroup Y meningococci.

Conclusions: The *ex vivo* whole blood model with low *cfu* seems to be highly useful to reflect the host-pathogen interactions as taking place *in vivo*. Results obtained using B meningococci indicate a pathogen mediated killing of neutrophils. However, host response to meningococci seems to be strain specific.

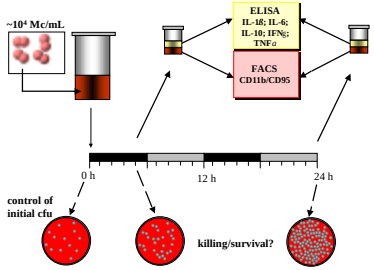

Fig. 1: Experimental outline.

Culture conditions for meningococci prior to addition into whole blood samples.

a.) subculture on Columbia agar (sheep blood agar) single colony **over night in RPMI1640*** extinction at λ=600 nm infection with ap. 10^4 meningococci/mL whole blood

b.) subculture on Columbia agar (sheep blood agar) harvesting and **re-suspension in RPMI1640*** extinction at λ=600 nm infection with ap. 10^4 meningococci/mL whole blood

c.) aliquots of both, a.) and b.) were blocked with antibodies specific for the respective serotype and serosubtype.

* no serum supplement

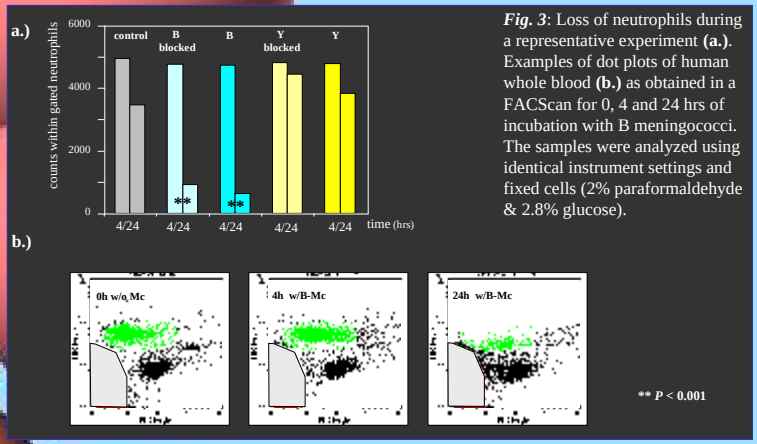

Fig. 3: Loss of neutrophils during a representative experiment **(a.)**. Examples of dot plots of human whole blood **(b.)** as obtained in a FACScan for 0, 4 and 24 hrs of incubation with B meningococci. The samples were analyzed using identical instrument settings and fixed cells (2% paraformaldehyde & 2.8% glucose).

** $P < 0.001$

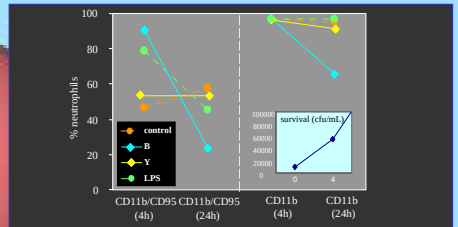

Fig. 4: CD11 and CD95 positive neutrophils during the 24 hrs incubation period. The survival of the meningococci is given for comparison. donor: male, vaccinated 1 year ago, 25 years of age

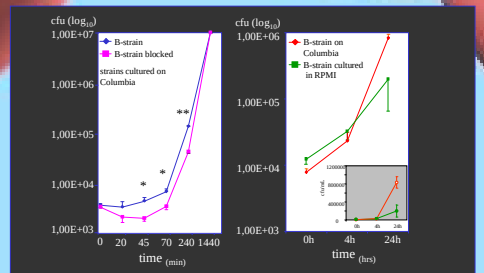

Fig. 2: Growth of B meningococci blocked with serotyp and subtype specific antibodies (left) or grown under different culture conditions (right) prior to be used in the whole blood assay. Y meningococci were killed within 20 min (not shown). * = $P<0.05$; ** = $P<0.001$

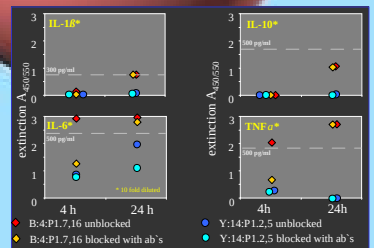

Fig. 5: Cytokine response in whole blood after incubation with blocked (serotype and subtype) or unblocked B meningococci. Data are given in pg/mL. donor: male, vaccinated 1 year ago, 25 years of age

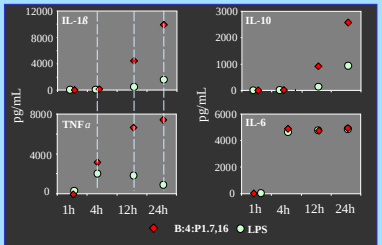

Fig. 6: Cytokine response pattern in whole blood after incubation with B meningococci or *E. coli* LPS as control. Data are given in pg/mL. donor: female, not vaccinated, 27 years of age