

decades. A wide array of available electroporators and electrodes available today require careful evaluation and optimization for different applications. The process can be demanding and involves the use of large number laboratory animals for preclinical testing. Objectives: We have adopted the use of a skin explant model to optimize the delivery of plasmid DNA using intradermal injections followed by electroporation.

Methods: Skin explants were obtained from mice, non-human primates collected post-mortem from marmosets euthanized in unrelated trials, or humans represented by aborted material after cosmetic operations. Skin explants were cultured in a humidified CO₂ incubator for up to 72 hours. The explants were injected intradermally with a DNA plasmid encoding a near-infrared fluorescent reporter protein (iRFP670). The explanted skin was immediately electroporated using either a 2-needle, multi-needle array, plate or fork-plate electrode utilizing varying pulse voltages (50V, 75V, 100V) and pulse polarities (+/-, +/+) (BEX Ltd, Japan). Expression of the DNA construct was monitored daily using a Spectrum CT device (Perkin Elmer) to quantify the fluorescence generated by iRFP670. At the end of the culturing period crawl-out cells emigrating from the tissue were collected, counted and evaluated for cell type and the efficacy of direct transfection/reporter expression using flow cytometry.

Results: Injection of the fluorescent reporter with subsequent electroporation resulted in observable expression indicated by up to 16-fold increase of baseline fluorescence intensity 3 days post immunization. Efficiency of DNA delivery/reporter expression was evaluated by the detected fluorescence and depended on the type of electrode, voltage and amount of DNA used. Fifteen micrograms or less resulted in fluorescence levels similar to the background regardless of the electrodes, parameters or species used. A dose of 30 µg DNA was sufficient to quantify the signal with good precision. As low as 300 ng of the reporter protein could be detected in the skin, and also *in vivo*, in injected animals, starting from 24 h post injection of 30 µg of the reporter gene. Using this amount, we observed the highest expression levels when electroporating with fork-plate electrode, followed by plate, multi-needle and 2-needle. Driving pulses of 70–70 volts were optimal for efficient expression and induced low levels of tissue damage. Too low (50V) voltage did not yield considerable expression. High voltage (>100V) supported similar expression levels to those after 75V electroporation, but resulted in more tissue trauma. Delivery of the driving pulses of the same (+/+) versus alternating (+/-) polarity demonstrated no enhancement of reporter expression due to electric field alterations. Methodology has been applied for optimization of delivery of immunotherapeutics based in plasmids encoding drug-resistant HIV-1 enzymes, for prevention of drug resistance in HIV-1/AIDS.

Conclusions: The skin explant model seems to be a promising alternative to animal preclinical and even human testing of delivery of gene therapeutics and genetic vaccines. Gene delivery can be significantly enhanced by optimized electroporation. Skin model allows such optimization. It is easy to work with, provides quick feedback of delivery efficiency when using a reporter gene, and considerably reduces the number of animals required for optimization of gene delivery. Study was in part supported by Russian Science Foundation 15-15-30039, and Thematic partnership of the Swedish institute grant 09272/2013.

Role of gut microbiota in microbial translocation: in HIV-infected patients

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Bacterial components, passed to the bloodstream from the gut as a result of microbial translocation, is known to induce immune hyperactivation causing progression of HIV infection. However, the pathogenesis of this process is not fully understood.

Hypothesis. Qualitative and quantitative gut microbiota abnormalities lead to intensification of microbial translocation.

Objective. To estimate the impact of intestinal flora abnormalities on concentration of serum markers of the microbial translocation in HIV infected patients, and compare these values with the clinical data.

Materials and methods. A cross-sectional study will be carried out in 120 ARV-naïve HIV-positive subjects at different stages of the disease.

Gas chromatography mass spectrometry will be used to evaluate microbiota of small and large intestine. To assess colon bacterial population stool cultures for potential pathogens also will be done. Microbial translocation will be analyzed through serum levels detection of endotoxin, 16s ribosomal DNA and soluble CD14.

The data obtained will be compared with the clinical and laboratory characteristics.

Expected Results. The findings extend the understanding of the gut microbiota's role in microbial translocation mechanisms in HIV-infected individuals. Furthermore, research forms basis for pathogenetic correction of some gastrointestinal symptoms and disease progression.

Co-infection of the human placenta and problem of the mother-to-child transmission of HIV A

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Background: The mother-to-child transmission of HIV in the absence of any interventions transmission rates range from 15–45%. This rate can be reduced to levels

below 5% with effective interventions. Placenta plays an important role in the prevention of the mother-to-child transmission (MTCT) of HIV during pregnancy. The immune parameters of the placenta of the HIV-infected woman during pregnancy have been linked to the mother-to-child transmission of HIV. The purpose of this study was to investigate the characteristics of the placentas and the expression of the CD14+ and CD68+ receptors in macrophages of the placenta of Russian HIV-infected women and to compare it with the expression of the immune receptors in placentas of women with co-infections and healthy women as controls.

Methods: The study prospectively investigated postpartum placentas obtained from deliveries at two different (general and specializing in HIV-complicated deliveries women) maternity wards in St.-Petersburg. Data on maternal age and delivery outcome were collected. The placentas were collected from three groups of patients: Group A — cases with children infected with HIV, Group B — cases with non-infected children born to HIV-infected mother and Group C — placentas from women without any infection.

In morphological analysis routine staining (hematoxylin and eosin) and microscope investigation were used. HIV-infection was confirmed immunohistochemically with use of p24 antibodies (Dako). The DNA-viruses of family *Herpesviridae* was detected immunohistochemically with use of antibodies against HSV (I and II) and CMV (Diagnostic BioSystems). Receptors expression was studied immunohistochemically with use of monoclonal antibodies CD14 (Novocastra) and CD68 (KP1 clone, Dako) and further morphometric analyses with the program Leica QWin Standard v2.8. Results: The study collected 11 placentas in Group A, 11 placentas in Group B and Group C had 16 placentas. The mean birth weight in Group A was 2965 (\pm SD=661) gm, in Group B 3056 (\pm 560) gm and in Group C (3536 \pm 306 gm). The average weight of placentas was lower in Groups A and B (434 \pm 48 gm and 445 \pm 55 gm, respectively) compared to Group C (566 \pm 59 gm).

Placental infection was detected in 91% (n=10) of placentas Group A, 64% (n=7) of Group B. In Group A the majority of placental inflammation (73%; n=8) represented inflammatory changes (chorioamnionitis, placental membrane inflammation), including 46% (n=5) combined bacterial and viral changes, and 18% (n=2) had isolated viral inflammatory changes — HIV and DNA-virus (one with HSV-1, two with CMV, and one with combined HSV-1 + CMV). In Group B the majority of placentas had HIV changes — 55% (n=6) and the smaller proportion — 18% (n=2) had combination of viral and bacterial infection associated changes. The presence of the bacterial and viral inflammatory changes was statistically associated with MTCT ($p<0,05$). The chro-

nic insufficiency of placenta was detected in Group A in 45,5%, in Group B in 36% (n=4). HIV RNA effects were detected in villous chorion of all placentas in Group A and Group B with positive p24 antigen. Expression of CD14+ in cytoplasm of chorion villi cells and endotheliocytes was the highest in Group A (14,14 \pm 1,11%), followed by Group B (10,04 \pm 1,37%), when compared with control Group C (3,21 \pm 0,43%, $p<0,05$ for both comparisons). Similarly, the expression of CD68+ was the highest in Group A (13,07 \pm 0,83%), followed by Group B (7,21 \pm 0,89%) when compared to the control Group C (2,02 \pm 0,60%, $p<0,05$ for both comparisons).

Conclusion: In our study there was a significant prevalence of bacterial and combined bacterial and viral inflammatory changes in the placentas of women with MTCT of HIV compared to the placentas of the women without MTCT. Further studies of the role of the placenta may help to better understand the mechanisms of the vertical transmission of the HIV. The presence of viral infections (HSV and CMV) and HIV was accompanied by the significant increase of CD14+ and CD68+ macrophages in the placenta of Russian women at time of delivery. Further studies of the role of the immune factors of the placenta may help to better understand the mechanisms of the transmission of the HIV viruses to the infants of the infected women.

The epidemic of comorbid and advanced stages of HIV infection in the Northwest of Russia

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Background: The numbers of registered HIV cases and advanced forms of HIV infection and the rate of deaths caused by the infection are increasing in Russia. In 2015, high HIV prevalence (more than 0,5% of the total population) was reported in 26 regions of Russia where 41,5% of population reside. The aggravation of epidemic situation is associated with not only increasing HIV prevalence and HIV-caused death rate but also with increasing HIV expansion from high-risk groups to the general population.

Objective: To assess the dynamics of the development of HIV epidemic, including the comorbid forms of HIV infection, in the Northwest of Russia from the time of detecting the first HIV cases up to the present, in particular in the recent years.

Tasks: To study HIV epidemic in the Northwest Federal Region; to determine the main causes of the increasing HIV incidence, the development of comorbid forms of HIV infection, and the increasing HIV-associated mortality; and to pinpoint the specific features of diagnostics, course and severity of HIV infection at different times before the onset and in the course of antiretroviral therapy.