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The Cells of Innate Systems in Tick-Borne Encephalitis

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1. Introduction

The immune system belongs to the protective basic system of an organism which is capable of identifying and destroying pathogens. There are two forms of the immune response of an organism: innate and adaptive ones. The innate response occurs within minutes of infection, providing the first wave of defense against the invading pathogen. The concept of innate or nonspecific immunity includes many factors of protection of an organism: biomechanical barriers, physiological, physical, chemical and biochemical factors, etc. The phagocyte (neutrophils, monocytes/macrophages, basophil cells, eosinophils and corpulent cells), into epithelium subpopulations of lymphocyte Tγδ-cells, killer cells - natural (NK-cells), lymphokine activity killer cells (LAK-cells) and so-called Pit-cells - a subpopulation of NK-cells with phenotype CD56/CD16 belong to cellular elements of innate immunity (Litvitskij & Sinelnikov, 2009). Functional properties of these cells are so diverse, that their inferiority as the consequence or the reason of pathological process, in due course inevitably forms the system defeat of an organism accompanied by immune insufficiency. Following virus infection, the host cell deploys the rapid response to limit virus replication in both the infected cell and in neighboring ones.

The family of Flaviviridae includes three genius: Flavivirus, Pestivirus and Hepacivirus. The tick-borne encephalitis (TBE) serocomplex comprises viruses belonging to the genus *Flavivirus* of the family *Flaviviridae* and includes TBE virus (TBEV), Powassan (POWV), Omsk hemorrhagic fever virus (OHFV), and Kyasanur Forest disease virus (KFDV). These viruses cause encephalitis, meningitis, and/or hemorrhagic fevers and represent a serious public health threat due to high morbidity and mortality rates following the infection. The TBEV species include 3 subtypes, Far Eastern (previously RSSE), Siberian (previously West-Siberian), and Western European (previously Central European encephalitis [CEE]) viruses. Recently, taxonomic improvements were proposed, and TBEV were divided into 4 types: Western, Eastern, Turkish sheep, and Louping ill (Grard et al., 2007). Eleven thousand TBE

cases annually occur in Russia, but only ≈150 cases are registered in Primorsky District, Russia (Leonova et al., 2006). The Far Eastern subtype is considered to be the most pathogenic for humans, with a mortality rate of >20%. The Western European subtype is less virulent and lethal (Leonova et al., 2006). In addition to the tick-borne viruses, the flaviviruses include mosquito-borne dengue virus (DEN, serotypes 1 through 4), yellow fever virus (YFV), Japanese encephalitis virus (JEV), and West Nile virus (WNV).

All flaviviruses are virions uniform in shape; spheroidal; enveloped; 40-60 nm in diameter. An isometric nucleocapsid (composed of a single protein designated C) contains the positive-stranded RNA genome of about 11 000 nt, which has of single open reading frame (ORF) flanked by non-coding regions at its 5′- and 3′- noncoding ends regions. Following translation, the single polyprotein is cleaved by viral and cellular proteases into three structural proteins, C (capsid), prM/M (membrane and its precursor), and E (envelope), and seven nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Murray et al., 2007). The C protein binds viral RNA to form the spherical nucleocapsid, which is enclosed within a host-derived lipid bilayer studded with viral prM and E proteins.

The transmission of flaviviruses to humans basically occurs following the bite of infected insects – tick, mosquito and so on. When the skin tissue is damaged during the sting of insects, the soluble mediators and cells contribute to the response. Soluble mediators released by injured cells activate polymorphonuclear neutrophils and other types of cells that accumulate at the site of injury. Concurrent with neutrophil accumulation, other cells migrate from both the epidermis [Langerhans cells (LC), dendritic cells (DC) and γδ T cells] and blood (granulocytes, monocytes, natural killer cells, and CD4/CD8 T cells). Following their recruitment, these cells play important roles in the early signaling that activates and orchestrates the immune response. It's establish that upon inoculation of dengue virus into the skin, initial infection and replication occurs in dendritic cells (DCs) localized in the skin and neutrophils (Wu et al., 2000). The macrophages and dendritic cells (DCs) are critical early responders in host defense against West Nile virus infections infection, not just because of their role in orchestrating the immune response, but also because of their importance as sites of early peripheral viral replication (Schneider et al., 2010). The research also showed that cutaneous West Nile virus (WNV) infection leads to a decrease in LC density in the epidermis with a concomitant increase in LC concentration in draining lymph nodes. DCs are thought to transport virus to nearby lymph nodes. Virus replication at this site leads to viremia and spread to peripheral organs including the central nervous system (CNS). The primary cellular targets of infection in the CNS are neurons. However, the mechanisms by which the virus enters and damages the CNS are not defined. Although critical for controlling virus infection in the CNS, the host immune response has been implicated in contributing to neuropathology (Chen et al., 2002). Recently, genetic analysis of TBE patients in Lithuania suggested that a deletion within the chemokine receptor CCR5 is a host genetic factor associated with severe TBE (Rios et al., 2006). Similarly, a mutation in the CCR5 gene was shown to correlate with severe disease in West Nile virus infections (Cologna & Rico-Hesse, 2003).

2. The role of neutrophils (polymorphonuclear cells) in Tick-Borne Encephalitis

The immune function of neutrophils (polymorphonuclear cells, PMNs) at infectious diseases, mainly, associates with phagocytoses and production of cytokines, including nitric

oxide and oxygen radicals. The process of output neutrophils from blood in tissue occurs within the first minutes after infection for some stages: a stage of primary adhesion and rolling of leukocyte on endothelium surface; activation and a stop of cells on the certain site of endothelium; the adhesion to a surface and diapedesis of leukocyte with movement in loci of inflammation. The rolling and adhesion are necessary stages for transformation of neutrophils and monocytes in more mature cells and in this process the selecting receptors and integrins take part. The two kinds of selectin receptors: L (CD 62L) and E (CD 62E) and the 5 kinds of integrins to which concern three of heterodimers, consisting from the general β-chain (CD 18) and three α- chain (CD 11a, CD 11b, CD 11c) are identified (Holzer et al., 2010).

Leukocyte chemoattractants induce a series of metabolic changes including activation of trimeric G-proteins followed by enhancing intracellular calcium levels, lipid remodeling, and protein kinase activations. These events culminate in fusion of granule membranes with phagosomes or with the plasma membrane (Smolen & Boxer, 2001). Leukocyte chemoattractants stimulate signaling pathways that are evolved in Rho GTPases, including Rac-dependent NADPH oxidase activation, Rac- and Rho-dependent phospholipase D activity, and Racand CDC-42 regulate p21-activated protein kinases (Bokoch, 1996). The studies by Bokoch have illuminated the role of the GTPases especially the necessary requirement for Rac-dependent and NADPH oxidase activation.

Recruitment and activation of neutrophil is among the principal defensise mechanisms of innate immunity. However, neutrophil-derived proteinases and reactive oxygen species, which are required for the elimination of microorganisms, are also capable of inflicting tissue damage (Klebanoff, 1992). Myeloperoxidase (MPO), a heme protein abundantly expressed in the azurophilic granules, plays a central role in these events. MPO catalyzes the formation of hypochlorous acid, a potent oxidant that has been implicated in killing infects and tissue destruction through induction of necrosis and apoptosis. MPO, through formation of secondary oxidants and nitration of protein tyrosine residues, could modulate intercellular signaling in the vasculature and affect the activation state of neutrophils.

Neutrophil trafficking into inflamed tissues is intimately linked to prolonged survival. Mature neutrophils have the shortest half-life (7 hours) among leukocytes and die rapidly via apoptosis (Kebir & Filep, 2010). This constitutively expressed cell death program renders neutrophils unresponsive to proinflammatory stimuli and promotes their removal from inflamed areas by scavenger macrophages with minimal damage to the surrounding tissue, thereby facilitating the resolution of inflammation (Kennedy & DeLeo, 2009; Fox et al., 2010). Neutrophil survival is contingent on rescue from apoptosis bysignals, such as lipopolysaccharide or proinflammatory cytokines, from the inflammatory microenvironment.

Upon contact with their bacterial targets, neutrophils fire a barrage of antimicrobial peptides, sometimes releasing structures called neutrophil-associated extracellular traps, or NETs. In releasing their NETs, they commit suicide—unleashing their entire antibacterial arsenal via a specialized form of programmed cell death called NETosis (Hakkim et al. 2010). It is established, that NET release is a marker of disease progression in Feline leukemia virus infection in domestic cats (Wardini et al., 2010).

Neutrophils are key in innate immunity, but their role in viral pathogenesis is incompletely understood. In Flavivirus infection the protective role of neutrophils was researched in Japanese encephalitis, West Nile and Denge viruses infections. After entry into the host,

these viruses generate a rapid inflammatory response, including peripheral neutrophil leucocytosis and infiltration of neutrophils into extraneural tissue (Khanna et al., 1994). In Japanese encephalitis virus (JEV) the inflammation results in an increased level of cytokines such as macrophage-derived chemotactic factor (MDF), tumour necrosis factor alpha (TNF-α) and interleukin 8 (IL-8) in the serum and cerebrospinal fluid (CSF) (Winter et al., 2004). JEV-stimulated neutrophils released reactive oxygen metabolites with maximum activity between days 7 and 9 (Chen & Wang, 2002). The increased levels of inflammatory mediators appear to play a protective role or to initiate an irreversible immune response leading to cell death. In West Nile virus (WNV) disease the neutrophils are rapidly recruited to the loci of infection and support efficient replication of virus. Thus the expression of neutrophil attracting chemokines, Cxcl1 and Cxcl2, was rapidly and dramatically elevated in macrophages. Mice depleted of neutrophils after West Nile virus inoculation developed higher viremia and experienced earlier death, compared with the control group, which suggest a protective role for this cells. These data suggest that neutrophils have a biphasic response to WNV infection, serving as a reservoir for replication and dissemination in early infection and later contributing to viral clearance (Bai et al., 2010).

Defensins product of neutrophils are antimicrobial peptides important to innate host defense. In addition to their direct antimicrobial effect, defensins modulate immune responses. Increasing evidence indicates that defensins exhibit complex functions by positively or negatively modulating infections of both enveloped and non-enveloped viruses (Ding et al., 2009). The effects of defensins on viral infections appear to be specific to the defensin, virus and target cell. Regulation of viral infection by defensins is achieved by multiple mechanisms. In virus infection: defensins bactericidal/permeability-increasing proteins – DEF1A and DEF4A are neutrophil-associated defensins with antiviral activity (Hartshorn et al., 2006; Hazrati et al., 2006; Furci et al., 2007). Dengue is a pantropic public health problem. It is established that in dengue shock syndrome (DSS) at children plasma concentrations of several canonical proteins associated with neutrophil degranulation (bactericidal/permeability-increasing protein, elastase 2, and defensin 1 alpha). Elevated levels of neutrophil-associated transcripts were independent of the neutrophil count and also of the genotype of the infecting virus, as genome-length sequences of dengue virus serotype 1 and serotype 2 sampled from DSS patients were phylogenetically indistinguishable from those sampled from uncomplicated dengue patients. Collectively, these data suggest a hitherto unrecognized association between neutrophil activation, pathogenesis, and the development of DSS and point to future strategies for guiding prognosis of diseases (Hoang et al., 2010).

We study the morphofunctional characteristic of neutrophils in TBEV infections. The TBEV strain Primorye-73 isolated from the brain of a person who died from TBE and these was the virulent for newborn white mice. TBEV was propagated in pig embryo kidney (PEK) cell line was grown at 37 ^{0}C in Eagle's minimal essential medium (MEM) that was supplemented with 8% fetal calf serum and L-glutamine and 100 U of penicillin/ml, and 10 µg of streptomycin/ml. The specific-pathogen free male mice (6 to 8 weeks old) peritoneal neutrophils were elicited by intraperitoneal injection of 5 mL of peptone water with meat extract (10%). Neutrophils 18 h after injection were collected by washing the peritoneal cavity of mouse once with 3 ml of cold minimal essential medium 199 (Sigma) containing preservative-free heparin (5 IU/ml). The cells were isolated by 3%dextran sedimentation and were placed in glass vials (2X10^6 /ml) in 2 mL medium for the following neutrophils

adherence for 45 min at 37 ^{0}C in 95% air ± 5% CO^2. Non-adherent cells were removed by washings and the cell monolayer were kept in tissue culture medium 199 containing 10% heat-inactivated fetal bovine serum (FBS) and 1000 U/mL penicillin and 1 mg/mL streptomycin (Invitrogen). For infection of neutrophils we used the virus containing culture liquid of PEK cells (2 units of the titer of cytopathogenic virus action on 50% of the cell culture; TCA50; multiplicity of infection [MOI]=1). The time of the contact of the neutrophils with TBEV was 15, 30, 45, and 60 min, 2, 3, 5, 6, 8, 24 and 48 h.

The cells were washed twice in PBS containing 1% FBS and stained for 30 min at -4 ^{0}C with antibodies for antigen of TBEV and then were fixed in gas of formalin for 15 min. After several washes, the bound antibodies were detected with Alexafluore 546 conjugated with IgG1 of mice (Molecular Probes) After incubation, slides were washed several times, and stored at -4 ^{0}C. The cells were examined by a LSM510META multiphoton confocal laser scanning microscope (Carl Zeiss, Germany). Fluorescence from the Alexafluore-546 dye was excited by 633 nm light and collected after passing through an HQ660LP emission filter. The lasers were programmed to scan over successive focal planes (0.25–0.5 μm intervals) at 50 lines per second. Lasersharp software was used to control the confocal system and to reconstruct individual focal planes into three-dimensional renderings.

The activity of enzymes ATPase, 5′ nucleotidase, succinate and lactate dehydrogenases and myeloperoxidase were determinated in infected neutrophils by F.G. Hayhoe and D. Quaglino's (1983) methods with our updating (Plekhova et al., 2007). The absorption of the product of cytochemistry reaction was measured by using a Multiscan Titertek Plus spectrophotometer (Flow lab, Finland) at lengths of waves corresponding to each reaction. Samples containing the reagent without cells and uninfected cells were used as controls.

The results of experiments were calculated using the equation: $T = [(N_0 - N_k)/N_k]*100\%$, where T is the index of stimulation of the phagocytes, N_k is the average absorption of the cytochemistry reaction product of uninfected cells, N_0 is the average absorption of this product of infected cells.

For determination of apoptosis neutrophils the method of staining by Hoechst 333258 (Sigma, USA) was used. Preparations investigated in system LSM510META, at excitation of 405 nm counted up 100 cells, and from them, on presence of a specific luminescence, defined a share of the apoptoses changed cells which expressed in the form of an index apoptoses (IA).

After 15 min of the contact of TBEV with the neutrophils, specific fluorescence of the cytoplasm was detected using the indirect fluorescent antibody method (Fig. 1). The fluorescence was mainly of diffuse character, and its intensity reached 42,6±5,6 standard units and the individual antigen-positive cells exhibited a localized fluorescence. The maximum intensity was determined in 4 h post infection made 234,56±15,7 standard units and then remained constant within 8 h of observation. After the first day of post infection the cells degraded.

The plasma membrane of cells is known to transform spatially during chemotaxis, and this directly depends on activities of its ectoenzymes 5′ nucleotidase and ATPase. In order to differentiate activated and resting cells, the intracellular contents of these enzymes were determined [Plekhova et al., 2007]. We found that an increase of the number of antigen positive cells was associated with a decrease of the activity of its enzymes (Fig. 2a). The parameters were significantly different from of the control ones in 2 hours post infection with TBEV. The values of stimulation index have made for 5′ nucleotidase 12.3±1.2 and for ATPase 11.4±1.1 %.

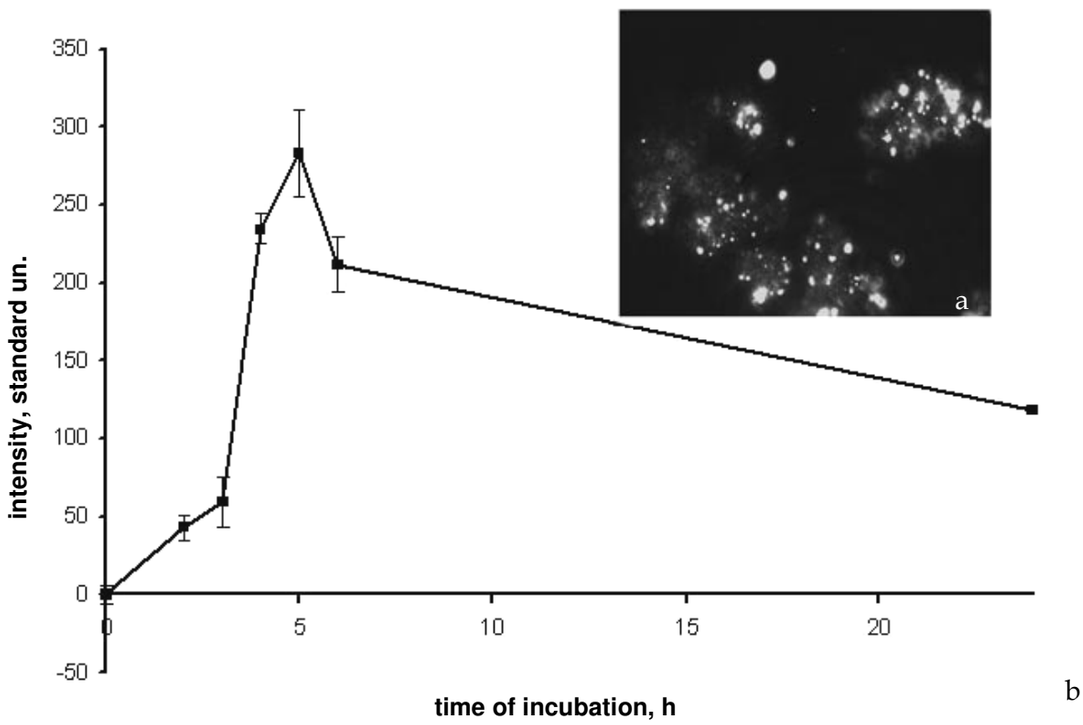

Fig. 1. Micrograph shows the neutrophils staining on TBEV by methods of indirect fluorescent antibody and analyzed by confocal laser scanning microscope, 5 hours post infection, original magnification, X 120 (a), intensity of fluorescence antigen-positive cells (b)

In terms of biochemical characteristics, phagocytes do not differ significantly from other cells. Nevertheless, the characteristic feature of their metabolism is the ability to generate various oxygen radicals under the influence of different factors. This phenomenon is called "respiratory burst". The formation of these short-lived, but reactive oxygen metabolites, which include superoxide anion, hydrogen peroxide, hydroxyl radical and others, is catalyzed in leukocytes by the membrane-associated enzyme, NADPH oxidase. This multi-component enzyme consists of both membrane-bound components (Nox2, p22 ^{phox}) and cytosolic regulatory proteins (p40 ^{phox} , p47 ^{phox} , p67 ^{phox} and Rac2 GTPase) (Klebanoff, 2005). Besides the pathway when the oxygen molecule accepts electrons, reactive oxygen species in phagocytes can be formed due to the hydrogen transfer from a substrate being oxidized (hydrogen donor) to another substrate (hydrogen acceptor). This reaction is catalyzed by dehydrogenases, participating in hydrogen transfer to substrates is specific for both the hydrogen donor and the hydrogen acceptor. The succinate dehydrogenases (SDH) is a flavoproteid dehydrogenase and belongs to the succinate oxidase enzyme complex that forms the membrane respiratory chain. The flavin group of this enzyme contains four iron atoms and is bound covalently to the protein. The SDH activity depends on the SH groups of the enzyme (Hayhoe & Quaglino, 1980). Investigation of the intracellular content of SDH in neutrophils infected with TBEV demonstrated a significant increase of its activity (18.2±1.6%, Fig. 2b) in 5 h post infection. The maximal value of the stimulation index for this enzyme was observed in 8 h post infection (27.95±2.4%).

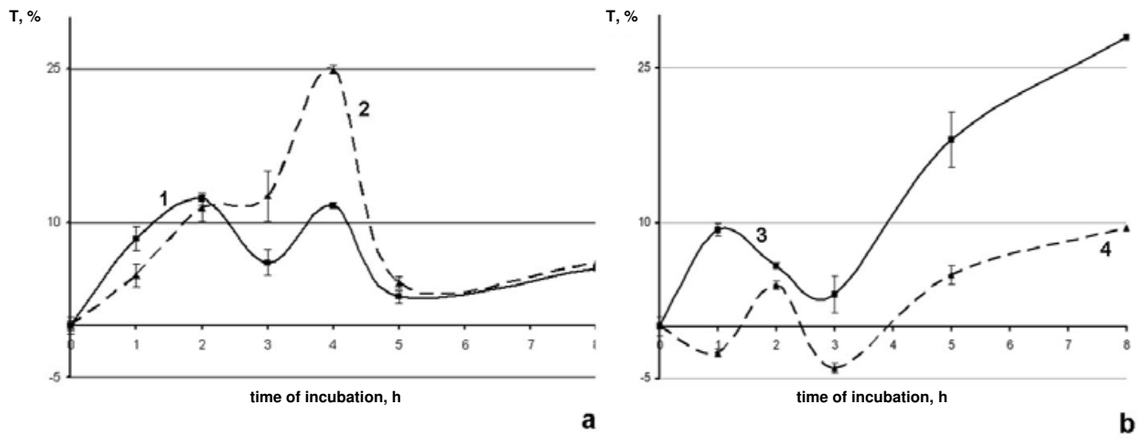

Fig. 2. The enzyme activity in infected TBEV neutrophils: a) 5′ nucleotidase (1) and ATPase (2); b) succinate dehydrogenases (3) and lactate dehydrogenases (4)

The lactate dehydrogenases (LDH) is a coenzyme dependent dehydrogenase and catalyzes the transfer of a reduced equivalent (hydrogen) from lactate to NAD+ or from NADPH to pyruvate. Usually, NAD+ is the coenzyme of LDH, but NADPH also can function as the coenzyme. LDH acts at the last step of hydrolysis that occurs under anaerobic conditions and results in the reduction of pyruvate yielding lactate and NAD+. Most of the enzyme in the cell is weakly bound to the cell structure and localized in the cytoplasm, a smaller part being attached firmly to mitochondrial membranes (Loida et al., 1982). Histochemical analysis can result in the enzyme diffusion into the incubation media. The spectrophotometric analysis used in the present work provided more precise determination of LDH activity in the cells. We determined a slight change in the activity of its enzyme in the neutrophils infected by TBEV that did not differ significantly from the value obtained for the intact cells during the experiment. Thus, in contrast to LDH, a significant increase in the intracellular content of SDH in neutrophils was observed at the later period of TBEV infection.

Among the antimicrobial systems formed in the phagosome is one consisting of myeloperoxidase (MPO), released into the phagosome during the degranulation process, hydrogen peroxide (H_2O_2), formed by the respiratory burst and a halide, particularly chloride. The initial product of the MPO-H_2O_2-chloride system is hypochlorous acid, and subsequent formation of chlorine, chloramines, hydroxyl radicals, singlet oxygen, and ozone has been proposed. These same toxic agents can be released to the outside of the cell, where they may attack normal tissue and thus contribute to the pathogenesis of the disease. The MPO-H_2O_2-halide system is strongly toxic to human immunodeficiency virus type 1, as measured by the inability of the virus to replicate in the lymphocyte cell line CEM (Klebanoff & Coombs, 1991). Investigation of the intracellular content of MPO in neutrophils infected by TBEV demonstrated a significant decrease of its activity (Fig. 3a). The minimal value of the stimulation index for this enzyme was observed in 3 h post infection (-34.4±2.8%). These data specified in TBEV infection cells the deficits of activity of protective system at the formation of oxygen radicals in them.

A variety of cationic peptides and proteins present in leukocyte granules has been implicated in the microbicidal activity of phagocytes. These include defensins, seprocidins,

bactericidal/permeability-increasing protein, lysozyme, cathelicidins, phospholipase A_2, and lactoferrin. Defensins are antimicrobial peptides important to innate host defense. In addition to their direct antimicrobial effect, defensins modulate immune responses. Defensins are classified into two subfamilies, α and β, which differ in their three disulfide bond paring. Neutrophil α-defensins are mainly synthesized as prepropeptides in promyelocytes, neutrophil precursor cells in the bone marrow, and the mature peptide is stored in primary granules of neutrophils (Ganz, 2003). In studies of enveloped viruses, defensins α have been shown to have a potent direct inhibitory effect on herpes simplex viruses, a moderate direct effect on vesicular stomatitis virus, and little effect on cytomegalovirus (Daher et al., 1986). We researched the activity of cationic peptides in neutrophils infected by TBEV and found that intracellular included its enzymes decreased in reply to infection by TBEV. It specified on exocytose by infected TBEV cells of cationic peptides, which can influence on neutrophils in intracellular environment. The inhibition of its cell function can partially suppress of organism immune response. Recent evidence indicates that defensins modulate viral infection through multiple mechanisms (Ding et al., 2009).

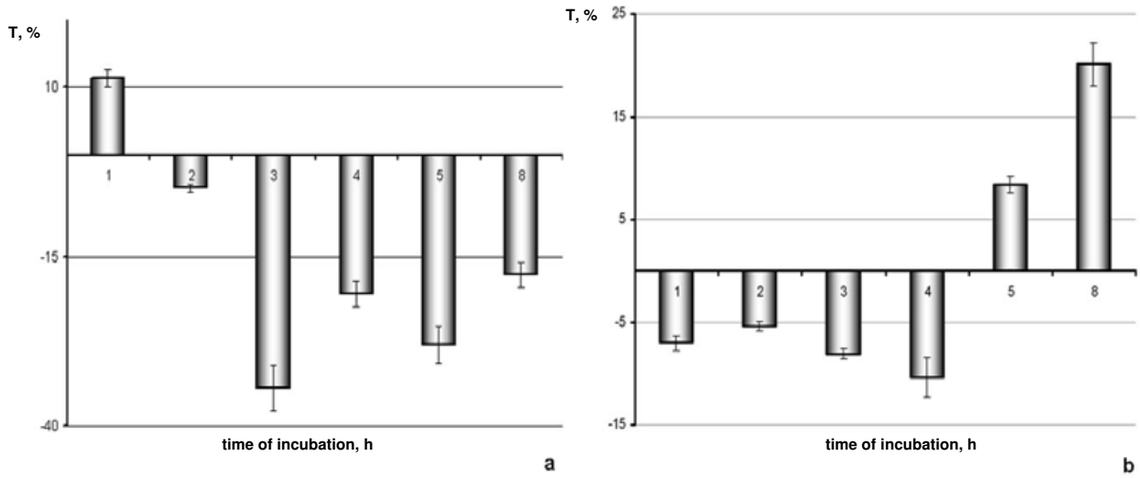

Fig. 3. The activity of myeloperoxidase (a) and cationic peptides (b) in infected TBEV neutrophils

Apoptosis, a programmed cell death that occurs to conserve toxic neutrophil contents, is one of such mechanisms. The benefits of apoptotic neutrophils are twofold as they also stimulate macrophages into a proresolution phenotype, reducing the inappropriate inflammatory response further (Fox et al., 2010). The increase of apoptosis these cells has been reported in chronically Simian Immunodeficiency Virus infected patients, and shown that degradation of neutrophils function is the consequence of upregulation apoptoses (Elbim et al., 2008). Morphologically the apoptosis of neutrophils is characterized the reduction of cytoplasm granules quantity, a rounding of nucleus and the condensation of chromatin, that is accompanied by depression of cells functions, especially their antiinfect abilities (Kennedy & DeLeo, 2009).

We researched the degradation of neutrophils infected by TBEV. Early display of the programmed destruction of neutrophils is sharply outlined of nucleus in the form of homogeneous substances with a uniform green luminescence (fig. 4a). At this stage

apoptosis can be stopped by the action of inhibitors and it is designated as preapoptosis (Wilkie et al., 2007). The intermediate stage of apoptosis destructions of cell is accompanied by reduction of the sizes of nucleus and at the final stage it breaks up to discrete fragments the quantity of which are 3-5 and more (fig. 4b). Hoechst 33342 is used by us contacts with damaged sites of DNA cells at the final stage of apoptosis. We established the increase of quantity of apoptosis changed neutrophils, infected by TBEV (fig. 4c). This quantity accrued with the increase of time post infection, and reached the maximal value in 8 hours - 48±7.3 %. The decrease of apoptosis index in 18-24 hours of incubation was connected with degradation of cells.

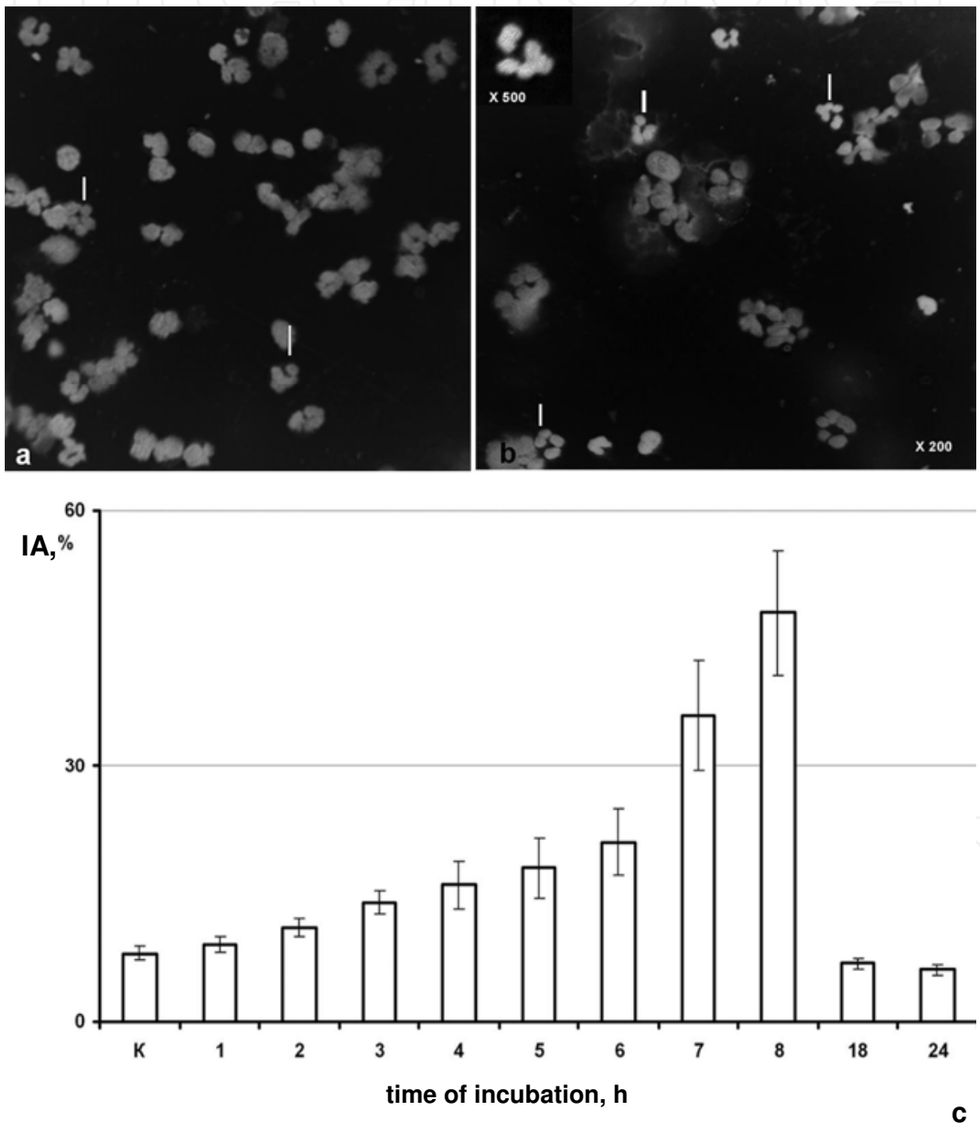

Fig. 4. The apoptosis of neutrophils infected by TBEV. a) preapoptosis (arrows) of cells – 2 hours post infection; b) apoptosis (arrows) of cells – 8 hours post infection; c) the quantity of apoptotic cells, AI – apoptotic index. The method of staining by Hoechst 333258, slides investigated in system LSM510META, at excitation of 405 nm counted up to 100 cells

Recently, a new microbicidal mechanism named NETosis was described in human, bovine and fish neutrophils, (Brinkmann et al., 2004; Palic et al., 2007). In this mechanism, neutrophils die after releasing neutrophil extracellular traps (NETs), threads composed of DNA, histones and granular proteins that not only ensnare microorganisms but also provide a high local concentration of antimicrobial molecules (Brinkmann et al., 2004; Lippolis et al., 2006). The release of NETs is dependent on reactive oxygen species (ROS) production and can be stimulated by bacteria, fungi, protozoa and some soluble molecules (e.g. interleukin-8, lipopolysaccharide, b-glucan and phorbol esters) (Brinkmann et al., 2004). NET formation requires a specialized form of cell death that is distinct from apoptosis and which is dependent on NADPH oxidase activity. It is show that a late phase of Nox2-mediated ROS production is sensitive to DNase treatment and suggest a role for myeloperoxidase-derived ROS in NET-dependent extracellular killing. In TBEV virus infection we have found in neutrophils the increase of activity enzymes which take part in reproduction of reactive oxygen species 8 h post infection (fig. 2a). The formation of NETs was determined in 6 h post infection (fig. 5). Their quantity was the small nearby 5 per 100 cells, but with the increase of time incubation it has made 8-10 per 100 cells. In our opinion the formation of NETs really occurs to participation of ROS which was produced at TBEV infection and the protective MPO-H_2O_2-chloride system of cells does not work (fig. 3).

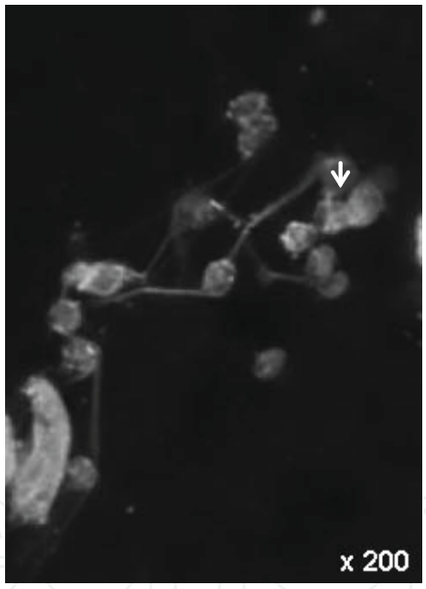

Fig. 5. Micrograph show the neutrophils staining on TBEV (arrow) and DAPI fluorescence shows NET analyzed by confocal laser scanning microscope 6 hours post infection. Cells were fixed with paraformaldehyde and stained with DAPI (5 ng ml^{-1})

Thus in TBEV infection the neutrophils activity of plasmalemma enzymes and degidrogenese increased. It is known, that neutrophils belong to cells with mainly aerobic type of an exchange (Walmsley et al., 2008). In these cells SDG has the important metabolic value as the increase of its activity does not give accumulation of lactate and the full oxidation of glucose occurs through a cycle of threecarbon acids with production of a plenty of energy (Arruda & Barja-Fidalgo, 2009). In this case the increase of an energy potential of a cell is the display of adaptive reaction neutrophils on TBEV infection. In its turn, the

increase of LDG activity is connected with changes of glycolyses intensity the biological value of which consists in formation of phosphoric connections rich with energy that testifies an intensification of power supply neutrophils due to inclusion anaerobic glycolys (Koolman & Rohm, 1998). This way of oxidation can be regarded as compensated the mechanism of adaptive reaction neutrophils on TBEV infection. Thus, the moderate increase of SDG activity presence evidences about direction to anaerobic ways of production energy – the activity of glycolys in TBEV infected neutrophils testify the relative stability of a homeostasis of cells. Finally it brings to killing of neutrophils mainly by apoptoses as it is known, that necroses is accompanied with acid bioenergy hypoxia of cells at full inhibition of enzymes activity of a respiratory circuit (Arruda & Barja-Fidalgo, 2009).

3. The monocytes/macrophages in Tick-Borne Encephalitis

The existence of different populations of monocytes in the blood of a man and mammalian homologues of these populations is established (Geissmann et al., 2003). In addition to the classical monocytes, which are strongly positive for the CD14 cell surface molecule ($CD14^{++}$ $CD16^{-}$ monocytes), a population of monocytes was discovered, which coexpresses CD16 and low levels of CD14 antigens. These cells have some characteristic patterns of cell surface molecules when compared with the classical monocytes, and this includes high HLA-DR, epidermal growth factor module-containing mucinlike receptor 2 (EMR2), Ig-like transcript 4 (ILT-4), CD43, and CD45RA expression and expression of MDC8 on a fraction of $CD14^{+}$ $CD16^{+}$ monocytes (Ziegler-Heitbrock, 2007). The $CD14^{+}$ $CD16^{+}$ monocytes and their mammalian homologues are potent producers of TNF but show no-to-low IL-10 production supports the concept that they are proinflammatory cells. Also, these cells and their derived cells show a higher antigen-presenting capacity. The process of differentiation monocytes in macrophages accompanies the rises of adhesion receptors CD11c/CD18, and an activation of cells – the parity of receptors CD11a/CD18 (Ammon et al., 2000). The basis functions of monocytes and tissue macrophages are phagocytosis and intracellular killing of microorganisms. In addition, macrophages are capable of extracellular killing of infected or altered self target cells. In response to such a stimulus, monocytes adhere to activated endothelial cells on the blood vessel wall and extravasate into the adjacent tissue. While residing in the tissue, they differentiate to monocyte-derived macrophages. One of the most immediate responses of monocytes to a variety of activating stimuli is the production of the potent oxygen free radical, superoxide anion. Furthermore, macrophages contribute to tissue repair and act as antigen presenting cells, which are required for the induction of specific immune responses.

The applicability of monocytes/macrophages in virus infection is determined by their participation in the production of antibodies and interferon, cooperation with lymphocytes and maintenance of more perfect antibodies presentation for stimulation of B-lymphocytes. These cells can accept direct participation in elimination of infect agents by phagocytosis of the cells-targets infected by a virus and their fragments or adsorbing directly on the plasmalemma a virus with its subsequent ingesting. At present anti-virus activity of monocytes/macrophages subdivide on direct and mediated (Baskin, 1997). Both intrinsic and extrinsic antiviral mechanisms of macrophages have been described. Intrinsic antiviral activity is defined as the ability of macrophages to restrict virus replication in macrophages per se and thus to serve as nonpermissive targets for virus replication. Extrinsic antiviral activity is defined as the ability of macrophages to influence extracellular virus and to

interfere with virus replication in surrounding permissive cells. Also it is marked, that during the development of some virus infections the activated macrophage gets an ability to distinguish infected and intact cells (Silva et al., 2007). Thus, the value of monocytes/macrophages in virus infections is defined by their functional condition. On the one hand, infected by virus monocytes as host cells at their maturation in macrophages can be a source of this virus in an organism. On the other hand, for viruses, inactivated by macrophages, these cells are the biological barrier interfering these distribution from the primary loci inflammation. It protects the high-sensitivity cells of the central nervous system and parenchymal bodies from the further infection. It is especially value able to studying influences on monocytes/macrophages at the first hour and day of the organism infection by virus. It is necessary to consider, that the concrete mechanisms of activation of these cells in various virus infections are not identical and are not clearly defined yet.

After contact with virus, the macrophages were considered as activated macrophages. In the resident macrophages, the activity of most lysosomal enzymes is in a latent state, being associated with glycolipid membrane complexes. As in resting primary human monocytes the enzyme complex nicotinamide adenine dinucleotide phosphate oxidase (NADPH) for the production of highly reactive oxygen species is unassembled and its components are located in the cytosol and the membrane. After activation, the cytosolic components translocate to the membrane and associate with the membrane components, and the newly formed enzyme complex actively catalyzes the production of superoxide anion. The membrane components include the cytochrome b558, consisting of gp91phox and p22phox (Babior, 1999). Cytosolic components include p47phox, p67phox, and Rac1 – Rho family member of small G-proteins (Abo et al., 1991). Also in contrast to resident macrophages the activated macrophages have a larger size, higher content of protein and RNA and exhibit a high enzymatic activity. While carrying out phagocytosis, pinocytosis, and exocytosis, macrophages constantly consume (interiorize) the plasma membrane, which is compensated by the constant synthesis of its components (Ballinger et al., 2010). The degree of macrophage stimulation can be estimated by the activity of their membrane ectoenzymes (ATPase, 5' nucleotidase, leucine aminopeptidase, D amino acid oxidase, serine esterase, alkaline phosphodiesterase) (Nathan & Shiloh, 2000). Numerous primary lysosomes of macrophages contain acid hydrolases (β-D-glucuronidase, N-acetyl-β-glucosaminidase, β-glycerophosphatase, α mannosidase, acid phosphatase, α naphthylesterase, esterase, RNase, arylsulfatase, lipase, β- galactosidase, naphthylamidase, cathepsins B, D, and G, and elastase) and antibacterial enzymes (lysozyme, neutral proteases, and B_{12} binding protein) (Cathcart et al., 2004). Secondary lysosomes (phagolysosomes) are formed by the fusion of the primary lysosomes with pinocytic and phagocytic vacuoles. Many lysosomal enzymes demonstrate combined and cumulative action (Williams & Burgo, 2009).

We researched the macrophages infected by TBEV. The patterns of peritoneal exudates were obtained by washing the peritoneal cavity of specific-pathogen free male mice (6 to 8 weeks old) once with 3 ml of cold minimal essential medium (RPMI) containing preservative-free heparin (5 IU/ml). Cell suspensions were placed in a glass vials ($4X10^6$ /ml) for 60 min at 37 ^{0}C in 95 % air ± 5% CO^2 to allow macrophages adherence. Non-adherent cells were removed by washings and the cell monolayer was kept in tissue culture medium RPMI containing 5% heat-inactivated fetal bovine serum (Sigma) for 72 h before infection. Thus we received the completely functionally mature population of cells.

The high-virulent for newborn white mice Primorye-73 TBEV strain was isolated from the brain of a person who died from tick borne encephalitis and two avirulent strains 202 and

69, isolated from blood of patient's disease of TBE subclinical form were used in this study. The monolayer of cells was washed once in serum-free RPMI, followed by suspension in 0.2 mL of virus (73, 69 and 202) giving the multiplicity of infection 0.3 plaque-forming units on cells (PFU/cell). For adsorbing the viruses were left with cells for 60 min at 37 ^{0}C. After 1 h at 37 ^{0}C the non-adsorbed viral particles were removed by serial washing of serum-free RPMI and monolayer of cells in medium RPMI containing 5 % heat-inactivated fetal bovine serum was incubated within 4 days. The supernatant medium was collected and frozen at – 70 ° at 1, 5, 9, 24, 48, 72 and 96 h post infection time periods. The virus titers were determined by plaque assay on porcine kidney stable (PS) cell monolayers by A.T. De Madrid's method (1969). The tenfold dilutions of virus were placed in 24-well tissue culture plates to which a suspension of PS cells was added (0.5 – 1.5 x 10 ^{5} cells per well). After an incubation for 4 h, the suspension was overlaid with carboxymethylcellulose (1.5% in L-15 medium). After incubating at 37 ° and 0.5% CO 2 for 5 – 6 days, the infected plates were washed with saline, and cell monolayers were stained with naphthalene black. Infectivity was expressed as PFU/ml.

The cells were stained with indicated antibodies to viral proteins conjugated secondary antibodies with followed by Alexafluore 546 (Molecular Probes). In brief, the monolayer of cells was fixed in gas of formalin for 15 min. Cells were then washed with PBS, labeled with either polyclonal mice anti–TBEV antibodies. After several washes, the bound antibodies were detected with rabbit anti-mouse antibodies directly labeled with Alexafluore. After incubation, the slides were washed several times, and stored at -4 ^{0}C until viral antigen was assessed. Caveolin-1 (CAV 1) were visualized by staining of anti-Caveolin-1 antibody produced in rabbit IgG fraction of antiserum (Molecular Probes) with Alexafluore 488 conjugated with IgG1 of mice. The revealing activity of inducible nitric oxide synthase (iNOS) was revealed by staining of monoclonal anti-iNOS antibody produced in mouse IgG fraction of antiserum (1:50; Sigma) with Alexafluore 586 conjugated with IgG1 of mice.

The slides were examined by a LSM510META multiphoton confocal laser scanning microscope (Carl Zeiss, Germany). Alexa-488 immunostain was excited using 488 nm light from a Krypton-Argon laser and the emitted light passed through an HQ515/30 filter. Fluorescence from the Alexa-546 dye was excited by 633 nm light and collected after passing through an HQ660LP emission filter. The lasers were programmed to scan over successive focal planes (0.25–0.5 μm intervals) at 50 lines per second. Lasersharp software was used to control the confocal system and to reconstruct individual focal planes into three-dimensional renderings.

For NO metabolite assays (nitrite ions) the infected monolayer of cells and the culture liquid were frozen and stored at –20°C. The suspension of destroyed cells was treated with 100 μl of Griess reagent (Schulz et al., 1999). The activity of enzymes ATPase, 5′ nucleotidase, succinate and lactate dehydrogenases and myeloperoxidase were determined in infected neutrophils by methods of F.G. Hayhoe and D. Quaglino (1983) with our updating (Plekhova et al., 2007). The absorption of the product of cytochemistry reaction was measured by using a Multiscan Titertek Plus spectrophotometer (Flow lab, Finland) at lengths of waves corresponding to each reaction. Samples containing the reagent without cells and uninfected cells were used as controls.

The results of experiments were calculated using the equation: $T = [(N_0 - N_k)/N_k]*100\%$, where T is the index of stimulation of the phagocytes, N_k is the average absorption of the cytochemistry reaction product of uninfected cells, N_0 is the average absorption of this product of infected cells.

Electron Microscopy. The monolayer of macrophages was prefixed in 1 % glutaraldehyde in 0,1 mol/L cacodylate buffer for 18 hours at room temperature, then it was postfixed for an hour in 1 % OsO4 in the same buffer, dehydrated in a graded series of ethanols and embedded in epon-araldite medium. Thin sections unstained or stained with lead citrate were examined in Jeol 100S electron microscope. The control patterns consisted of macrophages without TBEV. To elucidate the role of nitric oxide (NO) in TBEV infected macrophages, the ultrastructural localization of the enzyme NO synthase (NOS), based on the cytochemical NADPH-diaphorase staining was studied in a cell. For the localization of NADPH-diaphorase activity a special tetrazolium salt (BSPT, Sigma) was applied (Faber-Zuschratter, 1994).

After 60 min of the contact of, specific fluorescence of the cytoplasm was detected using the indirect fluorescent antibody method. The fluorescence of TBEV in the macrophages was mainly of diffuse character, and 20 ± 0.9% of the antigen-positive cells exhibited a localized lumpy fluorescence (Fig. 6b,c). After 4 and 5 h of incubation, the number of such cells increased to 35±1.4 and 87±5.3%, respectively, and then remained constant within 24 h of observation. After 4 days of observation, the number of the antigen-positive macrophages was minimal, constituting 17.5 ± 0.8%.

The adhesion and reproduction of TBEV in the macrophages was confirmed by the titration method (Fig. 6d). After 60 min of incubation, the original virus titer decreased by 2.0 log units. After 24 h, this value increased in the cell monolayer by 1.0 log, decreasing to the end of the experiment (4 days). In the supernatant, the virus titer increased to 2.0 log on the second day. These data indicate the adsorption and subsequent reproduction of TBEV in the primary macrophage culture, and then its liberation from the cells 2 days after the infection.

Microscopic investigation of the preparations stained 60 min after the contact with TBEV revealed mainly activated macrophages with large nucleus occupying 1/3 of the cell area and numerous vacuoles on the periphery of the phagocyte cytoplasm. The intact cells were lengthened forms with 2 pseudopodials (Fig. 7a). After infected by TBEV the macrophages were stimulated and accepted the approximated form (fig. 7b). In infected by virulent strains TBEV, 15.0 ± 0.8% of cells with chemotactic activity were detected (fig. 7c). These cells acquired lengthened forms due to the formation of two pseudopodias on the opposite sides, and the number of such cells significantly increased in 7 h after the contact of the virus with macrophages, constituting 25.0 ± 2.1%. Then contacting phagocytes were observed, and 18 h later, symplast-like conglomerates composed (Fig. 7d), where the maximal specific fluorescence of the viral antigen was detected. In this period, macrophages with destroyed nucleus were detected (10.0 ± 1.3%), as well as apoptotic cells (5.0 ± 0.46%) with 2-3 nuclear fragments and a reduced cytoplasm area. After 24 h of incubation, the intensive vacuolization of the peripheral part of the phagocytes was observed, this indicating the beginning of degradation of the cell culture Further, the number of degrading cells increased, and at the end of the observation (the fourth day) all cells were destroyed.

To reveal the activated cells, investigators use the test for intracellular 5′ nucleotidase or adenosine 5′ phosphatase (EC.1.3.5), the enzyme bound to the external side of the plasma membrane through the glycosylphosphatidyl residue. We determined a slight change in the activity of 5′ nucleotidase in the macrophages infected by virulent strains TBEV that did not differ significantly from the value obtained for the intact macrophages during the experiment (fig. 8a). Whereas in cells infected by avirulent strains TBEV the parameters of enzyme activity increased it due to the activation of cells.

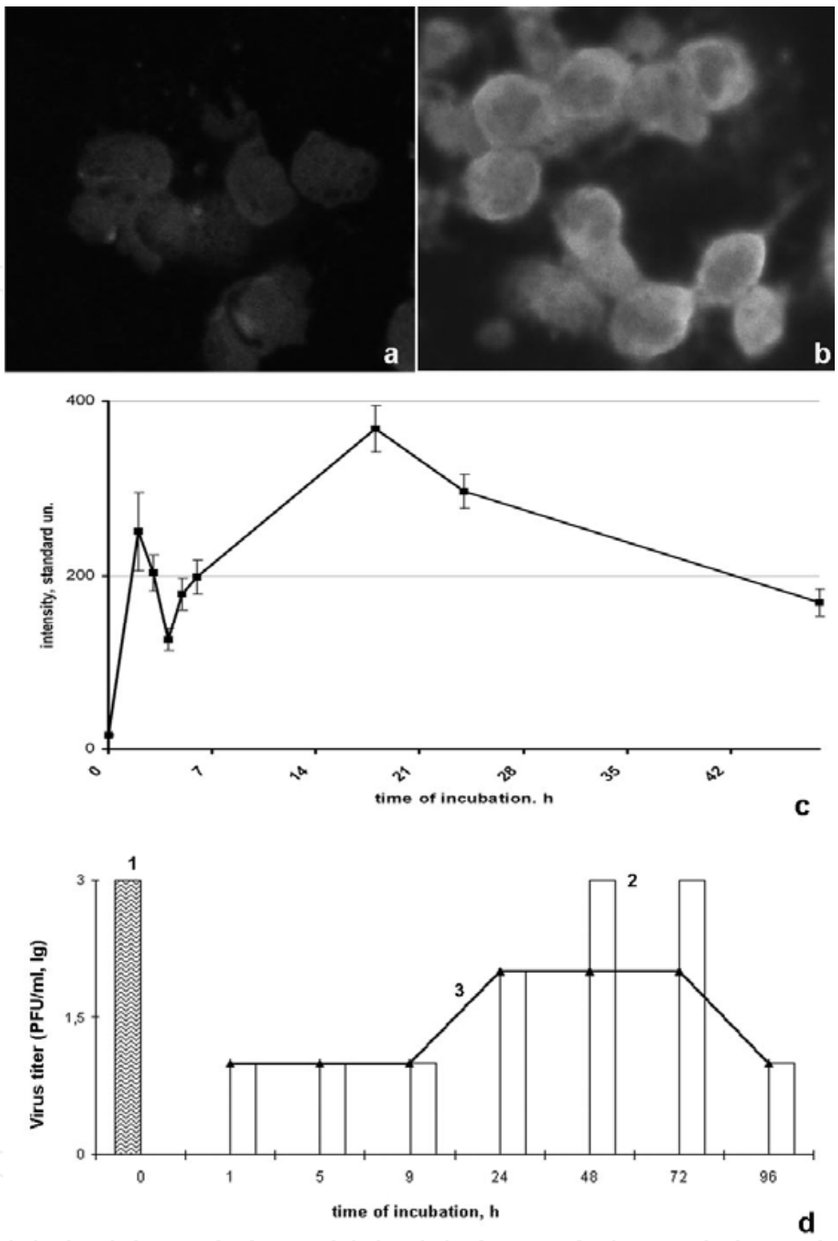

Fig. 6. Accumulation and visualization of TBEV antigen in macrophages. Uninfected macrophages (a) or cells (b) infected with virus were stained for presence of TBEV proteins. After infection, cells were fixed to spot slides and stained for TBEV proteins, followed by Alexafluor 546–conjugated antibodies. The bar represents 10 mkm. Data points show the mean of fluorescent intensity of antigen-positive cells (c); d) The growth of the high-virulence TBEV strain (73) in cultured mouse macrophages. The viral titers were quite stable after 2-day infection. In the case of the low-virulence strain (69 and 202), the virus was detected only at 18 h after infection; at later time point intervals, the virus titer was under the detection limit. These results are representative of 3 experiments of similar design. reproduction of TBEV in macrophages: 1) quantity of introduced TBEV; 2) TBEV in culture liquid; 3) TBEV in cells

Adenosine triphosphatase (ATPase, EC 3.6.1.3) of cell membranes is activated by potassium and sodium ions. This enzyme catalyzes hydrolysis of the high energy phosphate bonds, and the changes in its activity reflect the activation of cell metabolism. Investigation of the ATPase activity in avirulent strains of TBEV infected macrophages revealed a significant change in the intracellular content of this enzyme (fig. 8b). The ATPase activity decreased by 12.2 ± 1.5% compared to the control value 0.5 h post infection for the cells infected by strain 69 of TBEV, remaining constant for 8 h of incubation. Then the activity decreased until the end of the experiment (the third day). Thus, the data on the ATPase activity indicate pronounced stimulation of the macrophages infected by avirulent strains of TBEV.

The pathway when the oxygen molecule accepts electrons, reactive oxygen species in macrophages can be formed due to the hydrogen transfer from a substrate being oxidized (hydrogen donor) to another substrate (hydrogen acceptor). This reaction is catalyzed by dehydrogenases, besides, cytochrome oxidases can also be involved in the oxidation, acting as the additional substrates of the respiratory chain. To evaluate the activity of oxygen metabolism of the resident macrophages infected with TBEV, the intracellular content of SDH, LDH, and cytochrome oxidase was determined and the total activity of the respiratory chain enzymes was evaluated in the NBT test. Among the known methods for determination of the oxygen dependent activity of phagocytes, the cytochemical test for reduction of nitroblue tetrazolium (NBT test) is the simplest and the most reproducible method. The NBT test allows evaluation of the total activity of NADPH dependent enzymes of the respiratory chain. The activated macrophages consume tetrazolium and their enzymes convert it into insoluble diformazan. The formation of NBT into diformazan is attributed mainly to the powerful redox agent superoxide anion (Babior, 1999). Significant increase in the index in NBT test (91.6 ± 6.5%) was observed in macrophages after 15 min post infection (Fig. 9a). During 3 h of the experiment, the index decreased to 17.3 ± 0.9%, and then increased again, reaching its maximal value after 6 h of incubation (130 ± 11.6%). This value exceeded slightly the value obtained for macrophages treated with the bacterium *Staphylococcus aureus* as the stimulating agent (129.8 ± 7.8%). The parameters in NBT test of cells infected by avirulent strains 69 and 202 of TBEV statistically authentically did not differ from those of high virulent strain infected macrophages.

Dehydrogenases catalyzing hydrogen transfer to substrates are specific for both the hydrogen donor and the hydrogen acceptor. The investigation of the intracellular content of SDH in macrophages infected by avirulent strains 69 and 202 of TBEV demonstrated a significant increase in the enzyme content compared to the intact cells (Fig. 9b). As seen from the figure, 30 min after the contact of the cells with the strains 69 and 202, the stimulation index for SDH was 22.5 ± 1.9% and 28.5 ± 2.1% accordingly. Then the index decreased to the level of intact cells, and again increased during the observation time. The activity of LDH in macrophages infected by high-virulence strain 73 of TBEV decreased significantly (-13.4 ± 1.6%, Fig. 8c) after 30 min post infection. The maximal value of the stimulation index for this enzyme was observed after 2 h post infection in cell infected by avirulance strain 202 (11.8 ± 0.7%). Thus, in contrast to SDH, a significant decrease in the intracellular content of LDH in the macrophages was observed.

The level of oxygen radicals in the cells is regulated by the highly specific antioxidant enzyme superoxide dismutase (SOD, EC 1.15.1.11) with molecular weight of 31 kD (Allen et al., 1997). The scavenging of the oxygen radicals by SOD proceeds in the presence of $O_2^{\cdot}$ at pH > 4.8 (Webb et al., 2001). It is known that in an inflammation area, 80% of H_2O_2 is generated by phagocytes due to this reaction. The activity of SOD can be evaluated as the

amount of enzyme required for 50% inhibition of the superoxide anion formation in the presence of xanthine oxidase and NBT. An increase in SOD activity in macrophages infected by high virulent strain of TBEV was detected after 1 h of incubation (41.6 ± 3.4%, Fig. 9d). During the observation, the activity remained constant, exceeding significantly the value for the intact macrophages. The SOD activity was maximal after 72 h of incubation and constituted 26.4 ± 1.8%. In macrophages infected by avirulent strains 69 and 202 the SOD activity slightly differed from parameters for high virulent strain of TBEV infected cell.

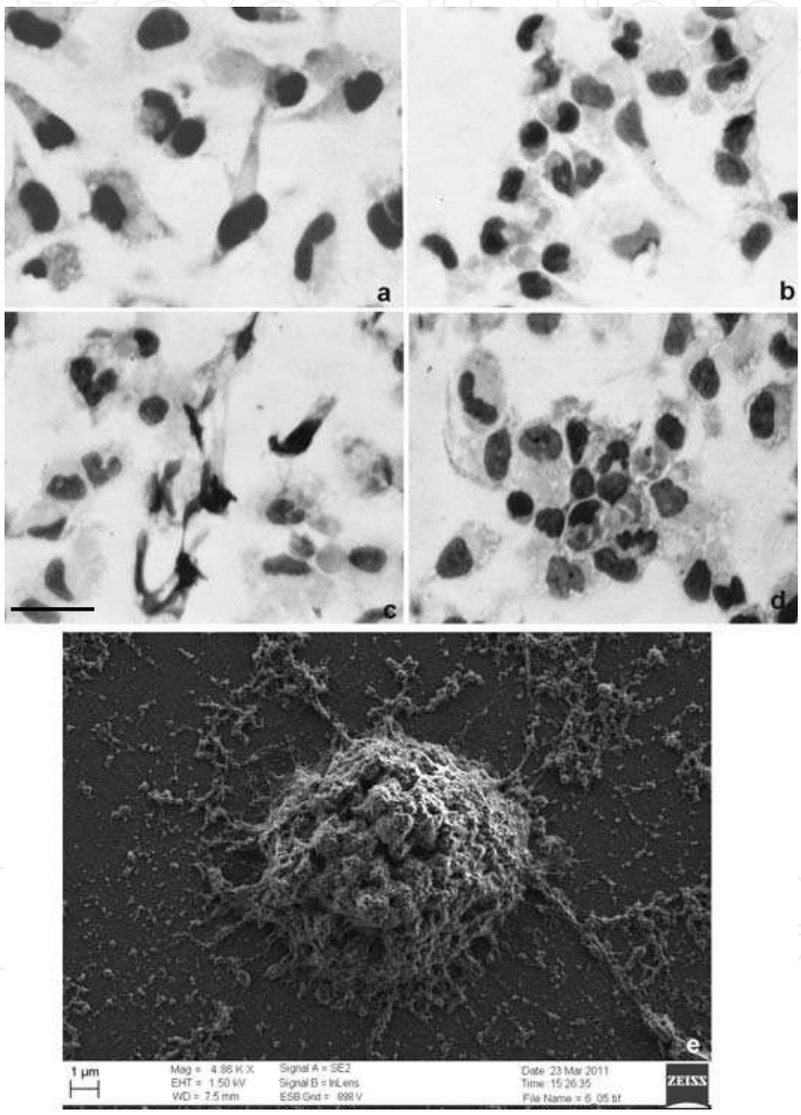

Fig. 7. Cytological investigation of infected by TBEV: a) intact cells; b) the stimulated cells in infected avirulant strain of TBEV; c) the chemotactic cells in infected virulant strain of TBEV (7 h); c) symplast (24 h); d) apoptotic macrophages with fragmentation of the nucleus. The cells were stained by method of Nocht–Maksimov (bar represents 8 mkm). e) micrograph show the stimulated macrophages infected by avirulent strain TBEV (69) with numerous long pseudopodia, scanning electronic microscopy

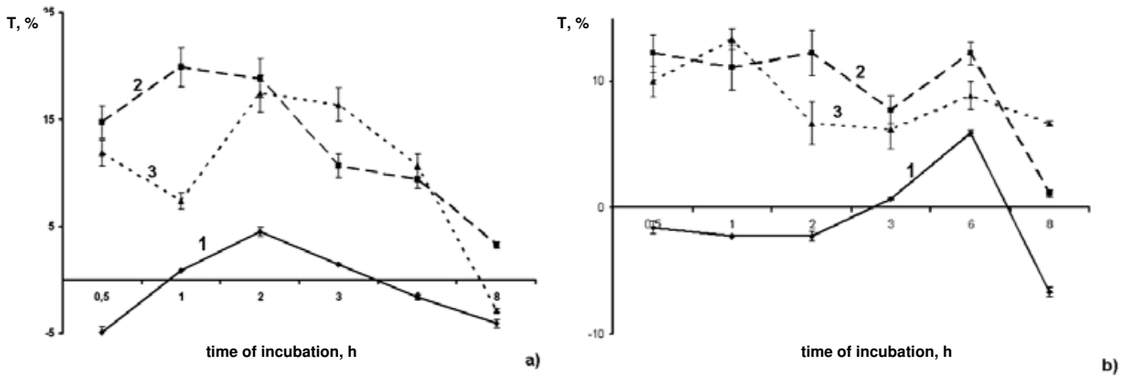

Fig. 8. The enzyme activity in infected by high-virulence strain 73 (1), avirulence strain 69 (2) and 202 (3) of TBEV macrophages: a) 5′ nucleotidase and b) ATPase

Thus, the research of metabolic activity of TBEV infected macrophages established the stimulation of cellular oxygen metabolism; the increase of NADPH-oxidase complex activity, as well as the mitochondrial enzymes lactate dehydrogenase, succinate dehydrogenase, and cytochrome oxidase. A wave-like change of these enzymes activity in the macrophages reflected the reaction of the cells in the first period (within 3 h) on virus penetration and in the second period (from 5 to 48 h) on the synthesis of the viral components and exit of virus into the extracellular space. The activity of these enzymes in cells depended on virulent property of TBEV strains which were infected macrophages. In infected cells high virulent strain 73 of TBEV the activity of enzymes was less than in macrophages infected by avirulens strains.

Nitric oxide (NO) is a free radical gaseous molecule that is produced in cell from molecular oxygen and a guanidino nitrogen from l-arginine, which is converted to citrulline l (Nathan & Shiloh, 1992). In activated macrophages this is exerted through the induction of expression of the inducible isoform of nitric oxide synthase (iNOS) gene, which encodes this enzyme. In recent years, much interest has been paid to NO as an important mediator of vital physiological functions, including host defense against virus (Kreil & Eibl, 1996). Unlike neutrophils, macrophages generally lack sub-stantial MPO activity, but have the capacity to induce a highly active inducible isoform of nitric oxide synthase (iNOS) with following activation by bacterial lipopolysaccharide and inflammatory cytokines. It is determinated that the stimulating influence of flavivirus on NO production activity of monocytes/macrophages, and in phagocytes NO-mediated inhibit action on replication these viruses was established (Chen & Wang, 2002; Chaturvedi et al., 2006). On model of the resident mouse macrophages infected by Denge virus, it is revealed, that for high-grade anti-virus action of phagocytes is necessary activity of oxygen depending enzymes system too. In this case at one-stage production of active oxygen metabolites and NO there is a formation of peroxynitrites which strengthens cytotoxicity of these cells concerning a virus. Thus, studying NO dependence activity of macrophages in infected by flaviviruses represents the certain interest and in this connection, the purpose of our research was the definition of production NO in resident macrophages in their infection by TBEV.

Cytochrome oxidase and SDH are the main components of the normal aerobic oxidative system of the tissue cells that are also known as the succinate dehydrogenase complex, where SDH is the first component and cytochrome oxidase is the second. Cytochromes are

subdivided into three groups according to their chemical structure and spectrum: cytochromes a, b, and c. Oxidized cytochrome oxidase is reduced by cytochrome c catalyzing the transfer of four electrons to the oxygen molecule. Thus, cytochrome oxidase is a representative of the third group of oxidases. The cytochrome oxidase activity in the macrophages reflects the level of oxidative metabolism (Koolman & Rohm 1998). This enzyme contains cytogemmin with which molecule of NO communicates. In this case, the peroxynitrites of super oxygen anion with NO are formed at interaction - the powerful oxidizer capable of inhibiting activity of mitochondrial enzymes cell. The definition of this enzyme activity allows indirectly to estimating the ability of cells to produce NO on nitrite reductase ways.

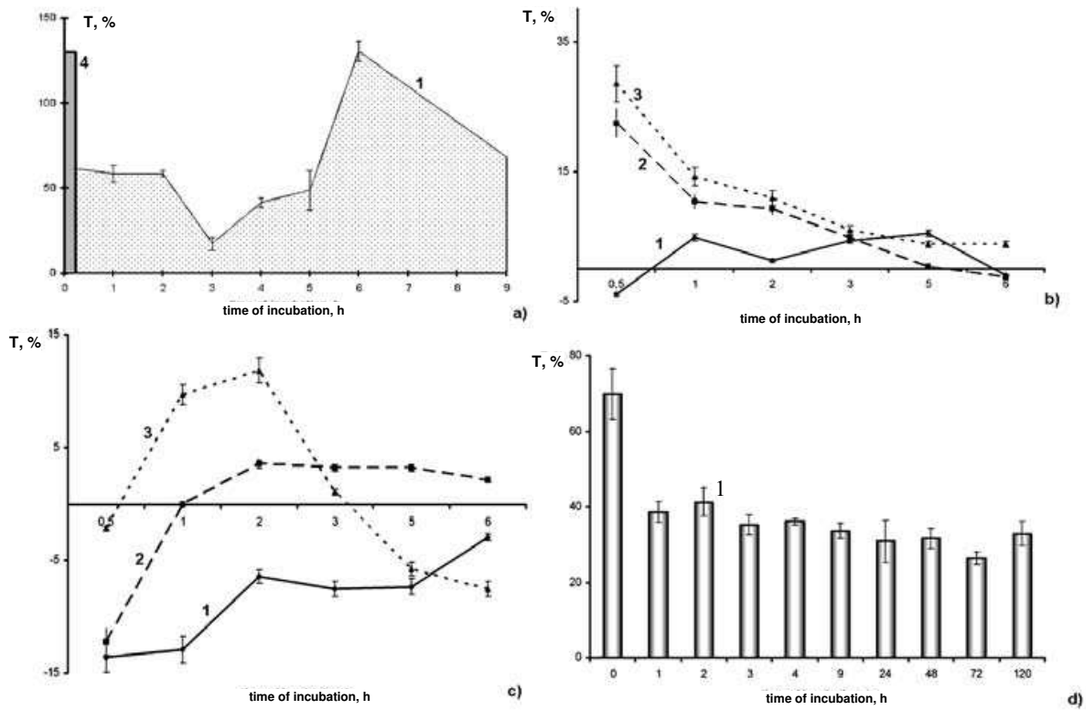

Fig. 9. The enzyme activity of oxygen dependent system in infected by high-virulence strain 73 (1), avirulence strain 69 (2) and 202 (3) of TBEV macrophages: a) NBT test; b) succinate dehydrogenases; c) lactate dehydrogenases; d) superoxide dismutase. 4 – NBT test, macrophages after 45 min of incubation with S. aureus

It was shown that the activity of cytochrome oxidase in the macrophages increased after 30 min post infection (10.6 ± 0.9%, Fig. 10b). A decrease in the cytochrome oxidase activity was observed during the period from 1 to 4 h post infection. After 6 h, the activity increased and reached 27.6 ± 1.8%, and then decreased again. It is necessary to emphasize the opposite direction in the changes of the activities of cytochrome oxidase and SDH in the macrophages infected with TBEV (the increase in the activity of one enzyme while decreasing the activity of the second).

At studying activity iNOS by methods of indirect immunofluorescent in the macrophages infected TBEV (fig. 10a), the greatest quantity of cells with a activity of this enzymes is marked after 1 h of incubation and has made 65±6,5%. Then in 5 h it decreased to a level of

intact cells - 5±0,6% and in 7 h it increased up to 25±1,6%, going down to the end of experiment time. It is necessary to note the difference in the direction of iNOS activity changes and cytochrome oxidase in the macrophages infected by TBEV. It was shown in the increase of percentage of cells with positive reaction on iNOS whereas at this time the activity of cytochrome oxidase decreased, and the parameters of the NO metabolites quality in cells remained at a high level (fig. 10b).

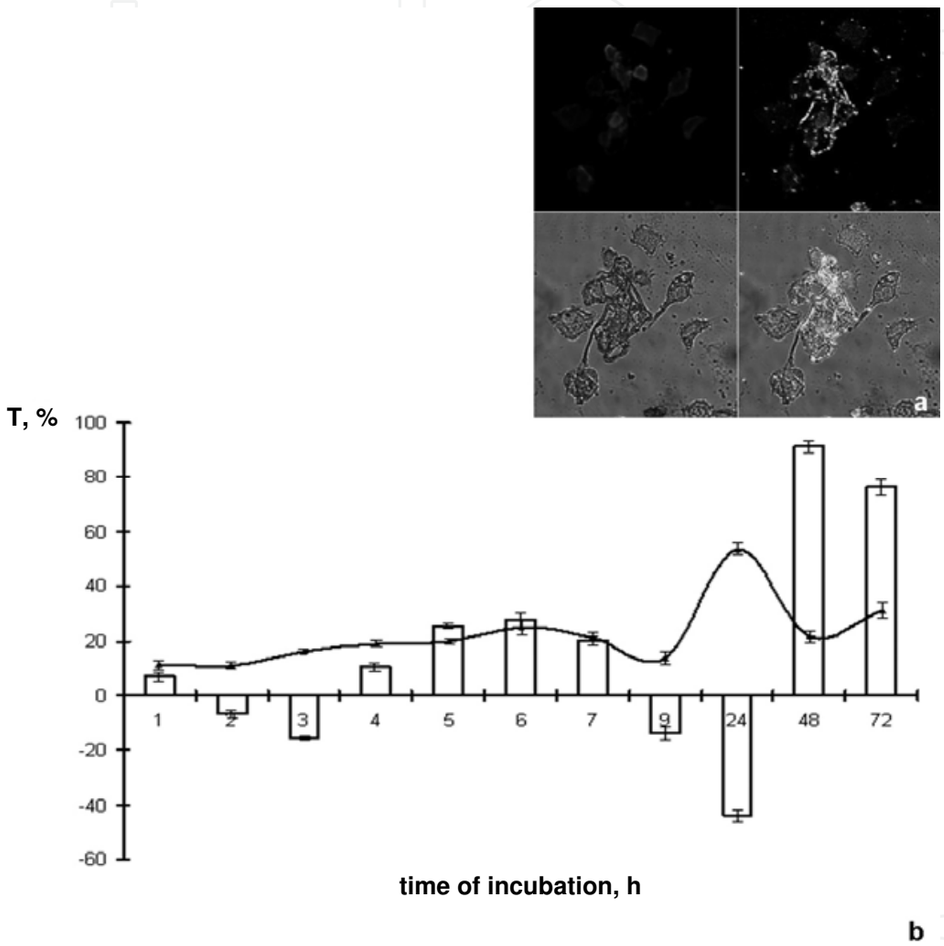

Fig. 10. a) the localization of iNOS in the infected TBEV macrophages, methods of indirect immunofluorescent, X 200. b) the quality of NO metabolites in cells (curve); the activity of cytochrome oxidase in the macrophages (columns). T - an index of stimulation

The ultrastructural research showed, that localization of NADPH diaphorase in the TBEV infected macrophages, it was mainly observed in intracellular vesicles (fig. 11a). BSPT-formazan, the product of the osmiophilic reaction, was found to be attached to intocellular membranes, predominantly the endoplasmic reticulum. Quantitative studies of TBEV infected macrophages revealed that the quantity of cells with similar granules was about 25 % in 10 min after inoculation and 15 % in 2 h and remained in these limits up to the end of experiment time. In parallel preparations which incubated in the environment without the addition of NADPH, the osmiophilic granules with positive reaction on NADPH diaphorase it was not revealed. It is necessary also to note the presence of NADPH diaphorase positive

reaction at 7 % of intact cells which have been not infected with TBEV. These data confirm the known fact of enzymes of monocytes/macrophages of activation in the response on adhesion (Reutov, 2002).

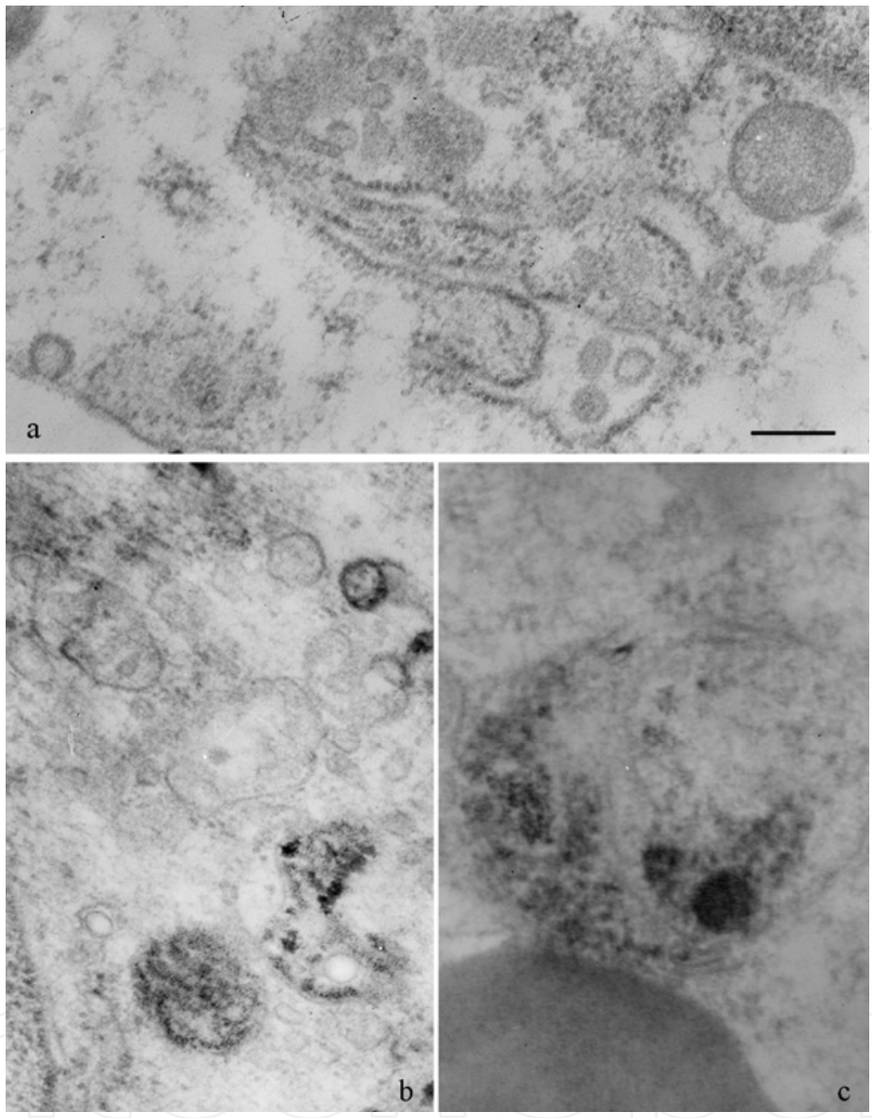

Fig. 11. The localization of TBEV in macrophages cytoplasm (a), the osmiophilic granules with positive reaction on NADPH diaphorase in the TBEV infected macrophages (b, c). Bar represents 100 nm

Now the monocytes/macrophages at virus infections are considered as the basic significance defined by their ability at stimulation to produce of reactivity metabolites oxygen and nitric oxide. These metabolites may influence on the synthesis of γ-interferon and tumor necrosis factor. In the researche by R. Kreil et al. (1996) it has been shown, that the macrophages from TBEV-infected mice, but not from control mice, spontaneously produced NO upon culture in vitro. In contrast to the inhibitory effect of NO on replication of several poxviruses and herpes simplex virus, high levels of NO production did not

display an inhibitory influence on TBEV replication in vitro. And finally, in vivo administration of a competitive inhibitor of NO production, aminoguanidine, to TBEV-infected mice significantly increased their mean survival time. Thus the antiviral activity of NO in vitro may be confined to certain viruses, whereas others remain unaffected. Also, other researchers at infection of populations of monocytes/macrophages by the viruses of the Western Nile, Denge and Japanese encephalitis revealed the increase of NO metabolic production by cells in reply to their infection (Chaturvedi et al., 2006). Alongside with the increase in vitro a level of NO metabolic, produced by monocytes of the blood of the patients infected by virus Dehge, in the same cells it has been established the expression of iNOS (Chen et al., 2002) and it is proved, that activation of oxygen depended enzymes system is necessary for high-grade anti-virus action of macrophages.

With the help of different research methods we established the stimulation of NO production activity of TBEV infected macrophages. On the background of active production cells of NO metabolites during all observable time the activity of NADPH diaphorase and iNOS was determined. And the parameters of these enzymes activity in TBEV infected phagocytes were not always combined with the dynamics of activity of heme including enzymes of mitochondria – cytochrome oxidase. In our opinion, during this moment the generation of NO in cells can be carried out on nitrite reductase ways. It was shown the data the increase of cytochrome oxidase activity in TBEV infected macrophages. At the same time at the initial stage of infection the cells produce the NO metabolites in nitric oxide synthase way of the participation as the catalyst of this enzyme.

The activity of TNF α cytokine and nitric oxide production in TBEV infected macrophages depended on strains of virus. The avirulent strain considerably enhanced the production of TNF α cytokine and nitric oxide by phagocytes during an early period of infection (Fig. 12). On the contrary, the cells infected by highly virulent strain TBEV generated the small quantity of these components. Thus, the early increase in the activity of the cell enzymes indicates the activation of the macrophages, and the subsequent increase in their activity corresponds to the enhanced synthetic activity of the macrophages.

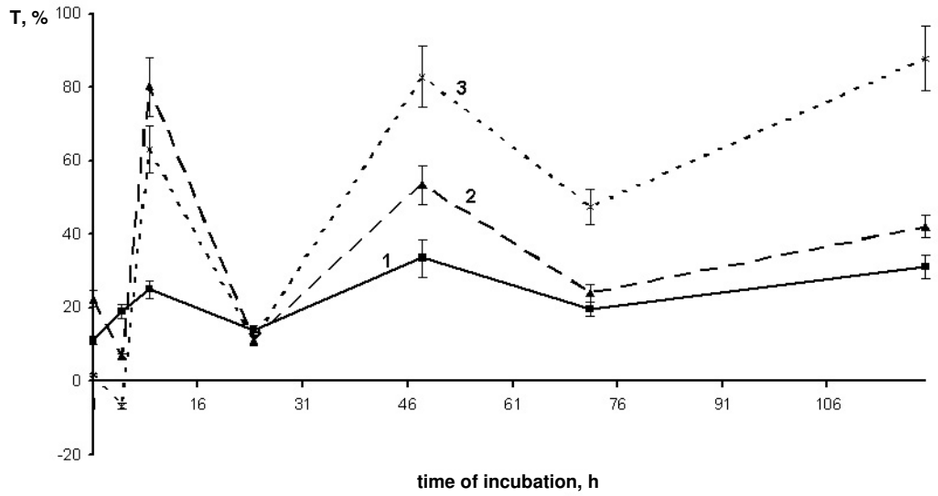

Fig. 12. The quality of NO metabolites in infected by high-virulence strain 73 (1), avirulence strain 69 (2) and 202 (3) of TBEV macrophages

4. Conclusion

So, for understanding the mechanisms of immunity reactions of an organism and their role in pathology of TBE infections it is necessary to estimate the role of phagocytoses, interferon, antibodies and local factors of immunity where the key cellular elements are neutrophils and monocytes/macrophages. As the data cited above specify, the functional expressiveness of these cells in TBE infections is various. So, alongside with that neutrophils and macrophages can carry out positive anti-viruses effect by absorption, neutralization and elimination of viruses and the cells infected by them, thus, becoming more active and producing in foci inflammation cytokines. As well, these cells can possess the negative influence during in the primary immune response in reproduction of ingesting viruses and them dissemination in various peripheral bodies. In that case, the given phagocytes act as the so-called "Troian horse", mediating a new formation of the foci inflammation. Thus, both depression of functional activity cells, and the display of undesirable consequences of similar change, namely, that they can destroy healthy cells in the place of an inflammation, excessively producing reactive radicals of oxygen and NO, arise. These metabolites are capable besides anti-virus to initiate actions process of apoptosis cells and to increase the permeability of capillaries. Thus, neutrophils and monocytes/macrophages in TBEV infection of an organism does not always prove as a protective barrier. At the same time, the activation of these cells at a reproduction in them of a virus allows them to carry out the antigen representing function for stimulation B-and T-lymphocytes and activation of cytotoxic T lymphocytes for destruction of the infected cells at the development of the specific immune response.

It was established by us that in TBEV infection of neutrophils the activity of plasmalemma enzymes and degidrogenese increased. The increase of succinate dehydrogenases activity is the display of adaptive reaction of neutrophils on TBEV infection. In its turn, the increase of LDG activity is connected with the changes of glycolyses intensity which consists of the formation of phosphoric connections rich with energy that testifies the an intensification of power supply neutrophils due to the inclusion of anaerobic glycolys (Koolman & Rohm, 1998). This way of oxidation can be regarded as a compensating mechanism of adaptive reaction neutrophils in TBEV infection. Finally it brings to killing neutrophils mainly by apoptoses as it is known, that necroses is accompanied by acid bioenergy hypoxia of cells at full inhibition of enzymes activity of a respiratory circuit.

We have demonstrated that TBEV is able to adhere from the virus containing liquid to the surface of the neutrophils and resident macrophages, penetrate into the cells, and reproduce in these cells. Similar data on selective adsorption to monocytes/macrophages were obtained while studying the interaction of these cells with TBEV (Ahantarig et al., 2009). Morphological examination of the cell culture infected by virulent strains TBEV revealed the features of the cytopathic action of this virus on the macrophages in contrast with the cells infected by avirulent strains. The virulent strains infected cells exhibited a granular cytoplasm, enhanced eosinophilicity of the cytoplasm, which characterizes the increase of the RNA and protein content, and karyopyknosis in some cells. Apoptotic neutrophils and macrophages were also observed.

It is established by us that the activation of oxygen metabolism in the cells depended from virulence strains of TBEV. Under physiological conditions, in contrast to other cells of the organism, superoxide anion is not produced in phagocytes or produced in small amounts. NADPH oxidases and oxidases of D-amino acids on the internal surface of the membranes

also virtually do not function. After the infection of cells by TBEV, the activity of the primary NADPH oxidase complex (plasma membranes) increased according to the results of the NBT test. The activities of the mitochondrial enzymes of the third level (LDH, SDH, and cytochrome oxidase) also increased. These data indicate the stimulating effect of TBEV on the neutrophils and macrophages. It is known that redox molecules, active metabolites of oxygen, take part in the transmission of external signals arising on the plasma membrane. Further intracellular transfer of the signal can also be connected with the activation of the redox molecules of certain cytoplasm elements and transcriptional factors (Kliubin & Gamalei, 1998). Our results have revealed an instant reaction of the phagocytes in response to the infection by TBEV, since within the first 15-30 min of incubation a sharp increase of the activity of the enzymes involved in the generation of superoxide radicals was detected. In our opinion, the observed recurrence in the dynamics of the activity of the investigated enzymes in the neutrophils and macrophages infected by TBEV reflect the cell reaction to the penetration of the virus into the cell in the first period (within 3 h), and the replication and emission of the virus particles in extracellular space in the second period (18-48 h). The subsequent (after 48 h) decrease in the activity of the indicated enzymes of oxygen metabolism can be accounted by the beginning of degradation of the cell culture under the influence of TBEV.

It is necessary to mention the enzymes defending the macrophages from the excess of peroxide products. Although the cell culture infected with TBEV exhibited characteristic features of cell degradation in the late period of the observation (1-4 days), the activity of SOD (the enzyme scavenging excessive amounts of reactive oxygen species) Thus, complex evaluation of enzymatic changes in macrophages is a highly sensitive method of the indication of the virus reproductive activity in the cell cytoplasm allowing differentiation of the types of its cytopathogenic action.

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6. References

Abo, A., Pick, E., Hall, A. Totty, N., Teahan, C.G., Segal, A.W. 1991. Activation of the NADPH oxidase involves the small GTP-binding protein p21rac1, *Nature*, Vol. 353, pp. 668-670, 0028-0836.

Ahantarig, A., Růzek, D., Vancová, M., Janowitz, A., St'astná, H., Tesarová, M., Grubhoffer, L. 2009. Tick-borne encephalitis virus infection of cultured mouse macrophages, *Intervirology*, Vol. 52, N 5, pp. 283-290, 0300-5526.

Allen, R.G., Keogh, B.P., Tresini, M., Gerhard, G.S., Volker, C., Pignolo, R.J., Horton, J., Cristofalo, V.J. 1997. Development and age-associated differences in electron transport potential and consequences for oxidant generation, *J. Biol. Chem.*, Vol. 272, N 40, Oct 3, pp. 24805-24812, 1864-6158.

Ammon, C., Meyer, S.P., Schwarzfischer, L., Krause, S.W., Andreesen, R., Kreutz, M. 2000. Comparative analysis of integrin expression on monocyte-derived macrophages and monocyte-derived dendritic cells, *Immunol.*, Vol. 100, N 3, pp. 364-389, 0019-2805.

Arruda, M.A., Barja-Fidalgo, C. 2009. NADPH oxidase activity: In the crossroad of neutrophil life and death, *Front. Biosci*., Vol.14, pp. 4546-4556, 1945-0494.

Babior, B. M. 1999. NADPH Oxidase: An Update, *Blood*, Vol. 93, N 5, pp. 1464-1476, 0268-960X.

Ballinger, M.N., Welliver, T., Straight, S., Peters-Golden, M., Swanson, J.A. 2010. Transient increase in cyclic AMP localized to macrophage phagosomes, *PLoS One*, Vol. 5, N 11, Nov 11, pp. e13962, 1932-6203.

Baskin, H.S. 1997. Herpes simplex virus type 2 synergizes with interferon-γ in the induction of nitric oxide production in mouse macrophages through autocrin secretion of tumour necrosis factor α, *Gen. Virol*., Vol. 78, pp. 195-203, 0022-1317.

Bokoch, G.M. 1996. Chemoattractant signaling and leukocyte activation, *Blood*, Vol. 86, pp. 1649-1660, 0268-960X.

Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D. S., Weinrauch, Y., Zychlinsky, A. 2004. Neutrophil extracellular traps kill bacteria, *Science*, Vol. 303, pp. 1532–1535, 0193-4511.

Cathcart, M.K. 2004. Regulation of superoxide anion production by NADPH oxidase in monocytes/macrophages: contributions to atherosclerosis, *Arterioscler. Thromb. Vasc. Biol.* Vol. 24, N 1, pp. 23-28, 1079-5642.

Chen, C.-J., Raung, S.-L., Kuo, M.-D., Wang, Y.-M. 2002. Suppression of Japanese encephalitis virus infection by non-steroidal anti-inflammatory drugs, *J. Gen.Virol*., Vol. 83, pp. 1897-1905, 0022-1317.

Chen, Y.-C., Wang, S.-Y. 2002. Activation of terminally differentiated human monocytes/macrophages by Dengue virus: productive infection, hierarchical production of innate cytokines and chemokines, and the synergistic effect of lipopolysaccharide, *J. Virol*., Vol. 76, pp. 9877–9887, 1816-4900.

Chaturvedi, U. C., Mathur, A., Tandon, P., Natu, S. M., Rajvanshi, S. & Tandon, H. O. 1979. Variable effect on peripheral blood leucocytes during JE virus infection of man, *Clin. Exp. Immunol*., Vol. 38, pp. 492-498, 0009-9104.

Chaturvedi, U.C., Nagar, R., Shrivastava, R. 2006. Macrophage & dengue virus: Friend or foe? *Indian J. Med. Res*., Vol. 124, pp. 23-40, 0022-4286.

Cologna, R., Rico-Hesse, R. 2003. American genotype structures decrease dengue virus output from human monocytes and dendritic cells, *J. Virol*., Vol. 77, pp. 3929-3938, 1816-4900.

De Madrid, A.T., Porterfield, J.S. 1969. A simple microculture method for the study of group B arboviruses, *Bull. WHO*, Vol. 40, pp. 113–121, 0042-9686.

Daher, K.A., Selsted, M.E., Lehrer R.I. 1986. Direct inactivation of viruses by human granulocyte defensins, *J. Virol*., Vol. 60, pp. 1068–1074, 1816-4900.

Dale, D.C., Boxer, L., Liles, W.C. 2008. The phagocytes: neutrophils and monocytes, *Blood,* Vol. 112, N 4, Aug., pp. 15935-15945, 0268-960X.

Ding, J., Chou, Y.-Y., Chang, T.L. 2009. Defensins in viral infections, *J. Innate Immun*., Vol. 1, pp. 413–420, 1662-811X.

Elbim, C., Monceaux, V., Mueller, Y.M., Lewis, M.G., François, S., Diop, O., Akarid, K., Hurtrel, B., Gougerot-Pocidalo, M.A., Lévy, Y., Katsikis, P.D., Estaquier, J. 2008. Early divergence in neutrophil apoptosis between pathogenic and nonpathogenic simian immunodeficiency virus infections of nonhuman primates, *J. Immunol*., Vol. 181. , pp. 8613-8623, 1754-1441.

Faber-Zuschratter, H. 1994. Ultrastructural distribution of NADPH-diaphorase in cortical synapses, *Neuroreport*, Vol. 27, N 5, pp. 2029-2032, 0959-4965.

Fox, S., Leitch, A.E., Duffin, R., Haslett, C., Rossi, A.G. 2010. Neutrophil apoptosis: relevance to the innate immune response and inflammatory disease, *J. Innate. Immun*., Vol. 2, pp. 216–227, 1662-811X.

Furci, L., Sironi, F., Tolazzi, M., Vassena, L., Lusso, P. 2007. Alphadefensins block the early steps of HIV-1 infection: interference with the binding of gp120 to CD4, *Blood*, Vol. 109, pp. 2928–2935, 0268-960X.

Ganz, T. 2003. Defensins: antimicrobial peptides of innate immunity, *Nat. Rev. Immunol*., Vol. 3, pp. 710–720, 1474-1733.

Geissmann, F., Jung, S., Littman, D. R. 2003. Blood monocytes consist of two principal subsets with distinct migratory properties, *Immunity*, Vol. 19, pp. 71–82, 1074-7613.

Grard, G., Moureau, G., Charrel, R.N., Lemasson, J.J., Gonzalez, J.P., Gallian, P. 2007. Genetic characterization of tickborne fl aviviruses: new insights into evolution, pathogenetic determinants and taxonomy, *Virology*, Vol. 361, pp. 80–92, 0042-6822.

Hartshorn, K. L., White, M. R., Tecle, T., Holmskov, U., Crouch, E. C. 2006. Innate defense against influenza A virus: activity of human neutrophil defensins and interactions of defensins with surfactant protein D. *J. Immunol*., Vol. 176, pp. 6962–6972, 1754-1441.

Hakkim, A., Fürnrohr, B.G., Amann, K., Laube, B., Abu Abed, U., Brinkmann, V., Herrmann, M., Voll, R.E., Zychlinskya, A. 2010. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis, *PNAS*, Vol. 107, N 21, May 25, pp. 9813–9818, 0027-8424.

Hazrati, E., Galen, B., Lu, W., Wang, W., Ouyang, Y., Keller, M. J., Lehrer, R. I., Herold. B.C. 2006. Human alpha- and beta-defensins block multiple steps in herpes simplex virus infection, *J. Immunol*., Vol. 177, pp. 8658–8666, 1754-1441.

Hoang, L.T., Lynn, D.J., Henn, M., Birren. B.W., Lennon, N.J., Le, P.T., Duong, K.T., Nguyen, T.T., Mai, L.N., Farrar, J.J., Hibberd, M.L., Simmons, C.P. 2010. The early whole-blood transcriptional signature of dengue virus and features associated with progression to dengue shock syndrome in Vietnamese children and young adults, *J. Virol*., Vol. 84, N 24, Dec., pp. 12982-12994, 1816-4900.

Kebir, E.l.D., Filep, J.G. 2010. Role of neutrophil apoptosis in the resolution of inflammation, *Scientific. World Journal*, Vol. 1, N 10, Sep., pp. 1731-1748, 1537-744X.

Kennedy, A.D., DeLeo, F.R. 2009. Neutrophil apoptosis and the resolution of infection. *Immunol. Res*., Vol. 43, N 1-3, pp. 25-61, 0257-277X.

Khanna, N., Mathur, A., Chaturvedi, U.C. 1994. Regulation of vascular permeability by macrophage-derived chemotactic factor produced in Japanese encephalitis, *Immunol. Cell. Biol*., Vol. 72, N 3, Jun., pp. 200-204, 0818-9641.

Klebanoff, S.J., Coombs, R.W. 1991. Viricidal effect of Lactobacillus acidophilus on human immunodeficiency virus type 1: possible role in heterosexual transmission, *J. Exp. Med*., Vol. 174, pp. 289-292, 0022-099X.

Klebanoff, S.J. 1992. Oxygen metabolites from phagocytes. In: Gallin J.I., ed. Inflammation. New York: Raven Press.

Klebanoff, S.J. 2005. Myeloperoxidase: friend and foe, *J. Leukocyte biology*, Vol. 77, pp. 598-625, 0741-5400.

Kliubin, I.V., Gamalei, I.A. 1997. NADPH oxidase a specialized enzyme complex for the formation of active oxygen metabolites, *Tsitologiia,* Vol. 39, N 4-5, pp. 320-340, 0564-3783.

Koolman, J., Rohm, K.-H. 1998. Taschenatlas der Biochemie. New York: Georg Thieme Verlag Stuttgart.

Kreil, T.R., Eibl, M.M. 1996. Nitric oxide and viral infection: NO antiviral activity against a flavivirus in vitro, and evidence for contribution to pathogenesis in experimental infection in vivo, *Virology,* Vol. 219, N 1, May 1, pp. 304-306, 0042-6822.

Leonova, G.N., Pavlenko, E.V., Krylova, N.V. 2006. Prophylactic of the tickborne encephalitis with vaccines [in Russian]. Vladivostok: Dal'nauka.

Lippolis, J.D., Reinhardt, T.A., Goff, J.P., Horst, R.L. 2006. Neutrophil extracellular trap formation by bovine neutrophils is not inhibited by milk. *Vet. Immunol. Immunopathol*., Vol. 113, pp. 248–255, 0165-2427.

Litvitskij, P.F., Sinelnikov, T.G. 2009. The innate immunity: mechanisms of realization and pathological syndromes, *Ques. Mod. Pediatrics*. Vol. 8, № 1, 2, 3, 4.

Loida, Z., Gossrau, R., Schiebler, T. 1982. Enzymes Histochemistry. A laboratory Manual, Springer-Verlag, Berlin, Heidelberg, New York, 272 p.

Murray, C.L., Jones, C.T., Rice, C.M. 2008. Architects of assembly: roles of Flaviviridae non-structural proteins in virion morphogenesis, Nat. Rev. Microbiol., Vol. 6, pp. 699–708,

Nathan, C., Shiloh, M. 2000. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. *Proc. Natl. Acad. Sci. USA*., Vol. 97, N 16, pp. 8841-8848, 0027-8424.

Palic, D., Ostojic, J., Andreasenc, C. B. & Roth, J. A. 2007. Fish cast NETs: neutrophil extracellular traps are released from fish neutrophils. *Dev. Comp. Immunol*., Vol. 31, pp. 805–816, 0145-305X.

Plekhova, N.G. , Somova, L.M., Drobot, E.I., Krylova, N.V., Leonova G.N. 2007. Changes in the metabolic activity of macrophages under the influence of Tick Borne Encephalitis virus, *Biochemistry (Moscow),* Vol. 72, N 2, pp. 199-207, 0006-2979.

Reutov, V.P. 2002. Nitric oxide cycle in mammals and the cyclicity principle, *Biochemistry (Mosc*), Vol. 67, N 3, Mar., pp. 293-311, 0006-2979.

Rios, M., Zhang, M. J., Grinev, A., Srinivasan, K., Daniel, S., Wood, O., Hewlett, I.K., Dayton, A.I. 2006. Monocytes-macrophages are a potential target in human infection with West Nile virus through blood transfusion *Transfusion*, Vol. 46, pp. 659-662, 0041-1132.

Schneider, B.S., Soong, L., Coffey, L.L., Stevenson, H.L., McGee, C.E., Higgs, S. 2010. Aedes aegypti saliva alters leukocyte recruitment and cytokine signaling by antigen-presenting cells during West Nile virus infection, *PLoS One*, Vol. 22, N 5(7), Jul., pp. e11704, 1932-6203.

Schulz, K., Kerber, S., Kelm, M. 1999. Reevaluation of the Griess method for determining NO/NO2- in aqueous and protein-containing samples, *Nitric Oxide*, Vol. 3, N 3), Jun., pp. 225-234, 1089-8603.

Silva, M.C., Guerrero-Plata, A., Gilfoy, F.D., Garofalo, R.P., Mason, P.W. 2007. Differential activation of human monocyte-derived and plasmacytoid dendritic cells by West nile virus generated in different host cells, *J. Virology*, Vol. 81, N 24, Dec., pp. 13640–13648, 0022-538X.

Smolen, J.E., Boxer, L.A. 2001. *Function of neutrophils.* (In: Beutler, E., Lichtman, M.A., Coller, B.S., Kipps, T.J., Seligsohn, U., eds. Williams Hematology. 6th ed.), NY: McGraw Hill pp. 761-784, ISBN

Walmsley, S.R., McGovern, N.N., Whyte, M.K., Chilvers, E.R. 2008. The HIF/VHL pathway: from oxygen sensing to innate immunity. *Am. J. Respir. Cell. Mol. Biol*., Vol. 38, N 3, Mar., pp. 251-255, 1044-1549.

Wardini, A.B., Guimarães-Costa, A.B., Nascimento, M.T., Nadaes, N.R., Danelli, M.G., Mazur, C., Benjamim, C.F., Saraiva, E.M., Pinto-da-Silva, L.H. 2010. Characterization of neutrophil extracellular traps in cats naturally infected with feline leukemia virus, *J. Gen. Virol*., Vol. 91, N Pt 1, Jan., pp. 259-264, 0022-1317.

Webb, J.L, Harvey, M.W., Holden, D.W., Evans, T.J. 2001. Macrophage nitric oxide synthase associates with cortical actin but is not recruited to phagosomes, *Infect. Immun.* , Vol. 69, N 10, Oct, pp. 6391-400, 0019-9567.

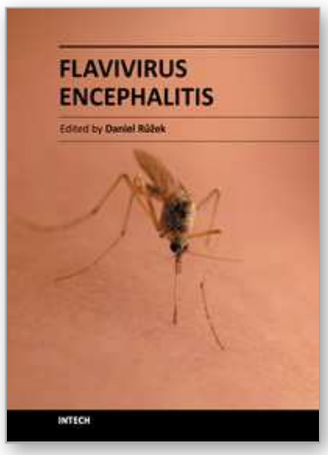
Wilkie, R.P., Vissers, M.C., Dragunow, M., Hampton, M.B. 2007. A functional NADPH oxidase prevents caspase involvement in the clearance of phagocytic neutrophils, *Infect. Immun*., Vol. 75, N 7, Jul., pp. 3256-3263, 0019-9567.

Williams, K.C., Burdo, T.H. 2009. HIV and SIV infection: the role of cellular restriction and immune responses in viral replication and pathogenesis, *APMIS*, Vol. 117, N 5-6, May, pp. 400-412, 0903-4641.

Winter, P.M., Dung, N.M., Loan, H.T., Kneen, R., Wills, B., Thu le, T., House, D., White, N.J., Farrar, J.J., Hart, C.A., Solomon, T. 2004. Proinflammatory cytokines and chemokines in humans with Japanese encephalitis, *J. Infect. Dis*., Vol. 190, N 9, Nov. 1, pp. 1618-1626, 0022-1899.

Wu, S.J., Grouard-Vogel, G., Sun, W., Mascola, J.R., Brachtel, E., Putvatana, R., Louder, M.K., Filgueira, L., Marovich, M.A., Wong, H.K., Blauvelt, A., Murphy, G.S., Robb, M.L., Innes, B.L., Birx, D.L., Hayes, C.G., Frankel, S,S. 2000. Human skin Langerhans cells are targets of dengue virus infection, *Nat Med.,* Vol. 6, N 7, Jul., pp. 816-820, 1078-8956.

Ziegler-Heitbrock, L. 2007. The CD14+ CD16+ blood monocytes: their role in infection and inflammation, *J. Leukoc. Biol*., Vol. 81, N 3. pp. 584-592, 0741-5400.



Flavivirus Encephalitis

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Encephalitis is an inflammation of the brain tissue associated with clinical evidence of brain dysfunction. The disease is of high public health importance worldwide due to its high morbidity and mortality. Flaviviruses, such as tick-borne encephalitis virus, Japanese encephalitis virus, Murray Valley encephalitis virus, or St. Louis encephalitis virus, represent important causative agents of encephalitis in humans in various parts of the world. The book Flavivirus Encephalitis provides the most recent information about selected aspects associated with encephalitic flaviviruses. The book contains chapters that cover a wide spectrum of subjects including flavivirus biology, virus-host interactions, role of vectors in disease epidemiology, neurological dengue, and West Nile encephalitis. Special attention is paid to tick-borne encephalitis and Japanese encephalitis viruses. The book uniquely combines up-to-date reviews with cutting-edge original research data, and provides a condensed source of information for clinicians, virologists, pathologists, immunologists, as well as for students of medicine or life sciences.

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