Chapter

Remodeling of Phenotype $CD16^+CD11b^+$ Neutrophilic Granulocytes in Acute Viral and Acute Bacterial Infections

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Abstract

Neutrophilic granulocytes (NGs) are very important cells of innate immunity that can very quickly realize antibacterial and antiviral defense. Until the present time, the phenomenon of different levels of presentations of membrane receptors CD16 and CD11b NG in normal and pathological conditions wasn't studied. We had studied the population of $CD16^+CD11b^+$NG in two groups of patients with acute viral and acute bacterial infections in the models of acute bacterial tonsillitis (ABT) and acute viral tonsillitis-EBV infection (AEBVI), having the same clinical symptoms in early stages of the disease. Comparative analysis of the redistribution of equipment intensity of CD16 and CD11b has detected three subpopulations of $CD16^+CD11b^+$NG population—$CD16^{bright}CD11b^{bright}$, $CD16^{bright}CD11b^{dim}$, and $CD16^{dim}CD11b^{bright}$—in normal and pathological conditions. It was found that subpopulation $CD16^{bright}CD11b^{dim}$NG dominates in healthy individuals; subpopulation $CD16^{bright}CD11b^{bright}$NG dominates in patients with acute viral infection; subpopulation $CD16^{dim}CD11b^{bright}$NG dominates in patients with acute bacterial infections. We had demonstrated that the study of $CD16^+CD11b^+$NG subpopulations allows in early stage of diseases to diagnose acute viral and acute bacterial infections. Our studies have demonstrated the positive effects of eukaryotic DNA sodium salt on the negatively altered phenotype subpopulation $CD16^+CD11b^+$NG, in particular, through the remodeling of the expression of CD11b on NG membrane.

Keywords: neutrophilic granulocytes, subset, phenotype, receptors, acute viral and bacterial infections, eukaryotic DNA sodium salt

1. Introduction

Neutrophil granulocytes (NGs) are the most mobile and numerous populations of innate immunity cell, which reacts lightly to any aggression, which also carries out powerful anti-infectious protection.

The surprising universality and multifunctionality of this cell, once again, underline the existence of heterogeneity within the NG population, that is,

the presence of subpopulations with different immunological roles. The use of monoclonal antibodies made it possible to confirm the existence of NG subpopulations using phenotypic characteristics. In 1998, the first nomenclature of human neutrophils antigens (HNAs) was created on the basis of membrane-expressed glycoprotein groups: HNA-1 (FcγRIIIb, CD16), HNA-2 (CD177), HNA-3 (CTL2), HNA-4 (CD11b/CD18, Mac-1, CR3), and HNA-5 (CD11a/CD18) [1]. The concept of heterogeneity of NG was discussed by scientists for more than 20 years and was confirmed with the accumulation of evidence on the presence of subsets of NG with various functions both in healthy subjects and in various diseases. Various methods have been used to detect the subpopulations of NG, such as cell maturity, functional activity, and localization, including receptors or markers of the cell surface.

2. Neutrophil granulocyte receptors

Cell populations and subpopulations of NG show a high degree of plasticity and functional heterogeneity depending on the characteristics of the course of physiological or pathological scenarios of the immune response, which, first of all, is due to potent receptor equipment. The membrane complex of NG expresses adhesion molecules, receptors for different ligands: cytokines, immunoglobulins, other cell membrane molecules, etc. NGs are capable to express MHC-1, selectins (CD62L), selectin receptors (CD162 (PSGL-1)), integrins (CD18 (β2-integrin), CD11a (LFA-1), CD11b (CR3), CD11c (CR4), CD11d), integrin receptors (ICAM receptors for β2-integrins - ICAM-1 (CD50), ICAM-3 (CD54). NG expresses receptors for chemoattractants (PFPR and FPLR for fMLP), receptors for chemokines (CXCR1, CXC2, CCR1), FcR receptors (CD16 (FcγRIII), CD32 (FcγRII), CD64 (FcγRI), CD89 (FcαRI), FcεR), receptors for complement components (CR1 (CD35), CR3 (CD11b), CR4 (CD11c), C5aR, C3aR, C5L2), receptor for LPS and endotoxins (CD14), cell adhesion receptor (CD15). NG receptors are involved in binding bacteria, in angiogenesis and apoptosis (CD17), in cell proliferation and differentiation (CD24), in PAMP recognition (TLR 1, 2, 4-10; NOD - receptors). In addition, on NG membrane there is a costimulatory receptor for B- lymphocytes (CD28), apoptosis activation/induction receptor (CD95), IL-2 receptor (CD25), which is NG activation marker; there are also molecules that determine the ability of NG to be APC (CD40, CD80, CD86, MHC II). NGs have multiple receptors for cytokines (IL-8, TNFα, IL-1, IL-2, IL-15, IL-17, IFNα, IFNγ, G-CSF, GM-CSF, etc.), hormones, neuropeptides, histamine, and kinases. The recently revealed expression of TCR-like (TCRL, TCRαβ) receptors on NG membrane, present throughout the life of a person and decreasing in old age, opens up new, previously unknown immune mechanisms for the functioning of NG [2] (**Figure 1**).

NGs are equipped with receptors that recognize endogenous molecules of "danger" alarms or danger-associated molecular patterns (DAMPs)—extracellular ATP, fragments of the extracellular matrix, heat shock proteins, nucleic acids (DNA and RNA fragments of its own cells), nuclear protein HMGB-1, and others—through which activation of the cell takes place and its inclusion in the inflammation reaction [3]. It has been established that the initiation of apoptosis of NG in clinically healthy individuals is under the influence of TNFα, sTRAIL, and IL-4 ligand [4]. Recently, new ways of activating the NG signal via ITAM/Syk-CARD9 have been described in the interaction of β-glycans with dectin-1, which triggers the synthesis of the cytokine IL-23 inducing the formation of Th17 cells [5]. NG receptor pool is located on intracellular membrane of secretory vesicles, gelatinase and specific granules, these receptors are translocated to surface membrane of NG only under the action of inducing stimuli [6]. Thus, the membrane expression of NG not only reflects the

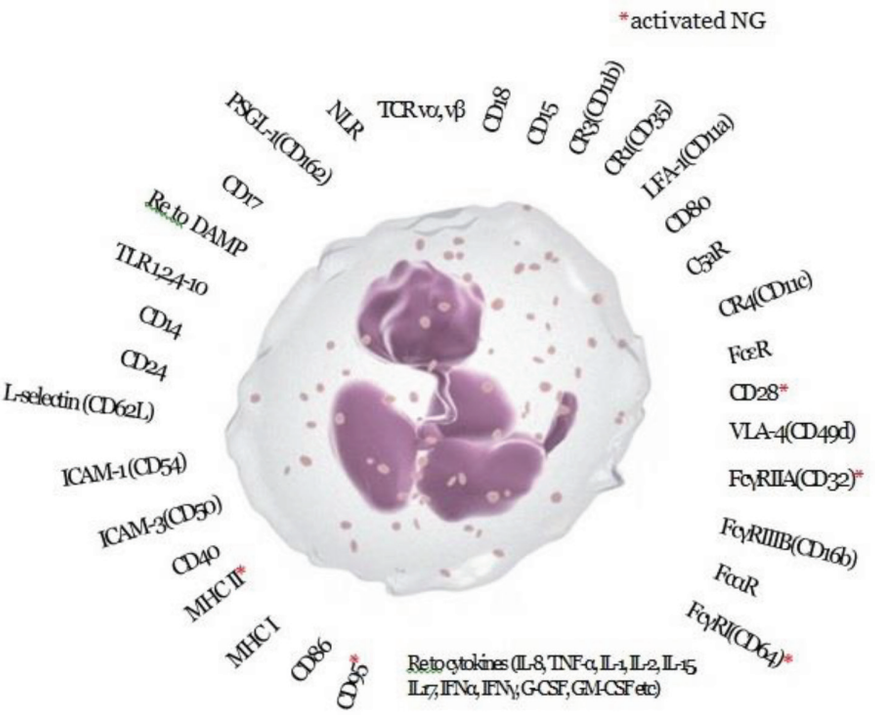

Figure 1.
Surface membrane receptors of neutrophilic granulocytes.

processes occurring during the life cycle of the cell but also allows us to evaluate the functional priming by reorganizing the surface cytoplasmic membrane of NG.

3. Phenotypic profile and functional features of neutrophil granulocytes

The study of the subpopulations of NG presents a new approach to the determination of functional activity of NG, allowing to assess the adequacy of the inclusion of NG in the implementation of the immune response, as well as to diagnose and predict the outcome of the disease. It is known that various phenotypic profiles and the level of equipment with surface receptors are associated with morphological features and determine the functional potential of NG-cytokine production, transendothelial migration, intracellular and extracellular killing, and formation of NET [7–9]. The existence of a sufficiently large number of NG subpopulations with different possibilities is demonstrated. NGs that receive complex cytokine influences not only acquire new features but also undergo different stages of activation and differentiation while expressing MHCII antigens, CD80, CD86, ICAM-1, and LFA-1 [7, 10, 11]. It has been shown that inducing cytokine stimuli differentiates NG in a unique hybrid subpopulation with dual phenotypic and functional properties characteristic of both NG and dendritic cells (DC) involved in innate and adaptive immune responses [12]. We have identified in our earlier works the following subpopulations of NG: regulatory; suppressor; pro-inflammatory, initiating inflammatory reaction; inflammatory with a positive microbicidal potential (antibacterial, antiviral, antifungal); inflammatory with negative cytotoxic potential, "aggressive"; anti-inflammatory, regulating inflammation regression; antineoplastic, TAN1; and pro-tumor, TAN2 and hybrid [13]. Phagocytic and microbicidal function and virucidal activity of NG are directly dependent on phenotypic features: the number and density of such expressed receptors as CD11b/CD18, CD10, CD15, CD16, CD32, CD64, CD35, etc. [6]. Expression on NG membrane of CD32 and CD16 is important

in the realization of phagocytic function and antibody-dependent cellular cytotoxicity (ADCC), which is associated with CD11b-/CD18-dependent enhancement of adhesion, degranulation, and killing [14]. CD64, CD32, and CD16 are triggering molecules that induce immune phagocytosis and killing processes [15].

The variants of remodeling of NG phenotype simultaneously expressing functionally significant receptors CD64, CD32, CD11b, and CD16 in patients with infectious and inflammatory diseases, including newborns of different gestational ages [15], patients with neoplastic processes [10, 16], women of reproductive age with genital and extragenital infectious-inflammatory diseases [17] have great diagnostic and prognostic significance. When we study the variability of the simultaneous presentation of NG receptors CD64, CD32, CD16, and CD11b on the membrane, it was established that in healthy adults and children of different ages in the peripheral blood, there is one major subpopulation of $CD64^{-}CD32^{+}CD16^{+}CD11b^{+}$ and five minor subpopulations of NG, $CD64^{-}CD32^{+}CD16^{+}CD11b^{-}$, $CD64^{-}CD32^{-}CD16^{+}CD11b^{+}$, $CD64^{+}CD32^{+}CD16^{+}CD11b^{+}$, $CD64^{+}CD32^{+}CD16^{+}CD11b^{-}$, and $CD64^{+}CD32^{-}CD16^{+}CD11b^{+}$, with different equipment and density of studied receptors. We detected a significant increase in NG subpopulation with $CD64^{+}CD32^{+}CD16^{+}CD11b^{+}$ phenotype with a high expression density of CD11b and CD16 in newborns with infectious and inflammatory diseases of bacterial etiology (congenital pneumonia, neonatal sepsis) (**Figure 2**).

The observed increase in this subpopulation of NG in the peripheral blood is directly related to the severity of the infectious-inflammatory process: the more clinically severe the disease, the greater the number of NG with this phenotype $CD64^{+}CD32^{+}CD16^{+}CD11b^{+}$ is in circulation [18]. In fertile age women with genital and extragenital infectious and inflammatory diseases planning pregnancy, the phenotypic variability of NG—the appearance of a subpopulation of $CD16^{+}CD32^{+}CD11b$—was also revealed, which indicates a persistent violation of their receptor function and the need for its adequate correction consisting in restoring the phenotypic composition of NG. Thus, the provision of pre-gravity training with the inclusion of immunotherapy has a positive clinical and immunological effect, which consists in normalizing the receptor function of NG, which

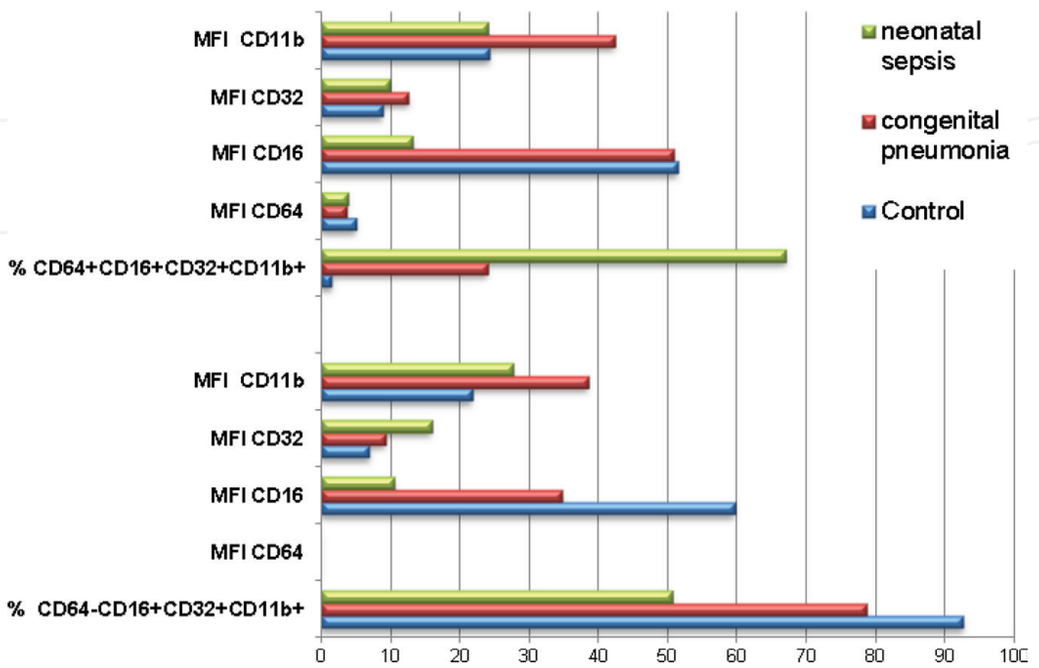

Figure 2.
Phenotypic profiles of $CD64^{+}CD32^{+}CD16^{+}CD11b^{+}$ NG in children with congenital pneumonia and neonatal sepsis.

correlates with an increase in the percentage of women who become pregnant [17]. The obtained data allow us to develop criteria for monitoring the course of associated viral infections and bacterial pro-inflammatory diseases, to diagnose and/or predict the aggravation of their severity, and to optimize immunotherapy methods aimed at correcting NG dysfunction. Multiple increases in the subpopulation of $CD64^-CD32^-CD16^+CD11b^+$ NG have been shown in children with repeated acute respiratory viral infections associated with herpesviral mono- or mixed infection. There was a significant replicative activity of herpesviruses such as HSV I/HSV II, CMV, EBV, and HHV VI [18, 19]. In addition, authors of this article put together all information into the table for the period from 2010 to 2016, about NG subpopulation phenotype according to their studies (**Table 1**).

Specific NG subpopulation composition and also adequate level of corresponding surface membrane marker expression density is important for the proper NG function. Thus, Pillay et al. found several subpopulations of NG with different phenotypes, which differed in the number and density of equipment with receptors: mature NG with the phenotype $CD16^{high}CD62L^{high}$, immature NG with the phenotype $CD16^{low}CD62L^{high}$, suppressive NG with the phenotype $CD16^{high}CD62L^{low}$, and NG precursors with the phenotype $CD16^{low}CD62L^{low}$ [11]. Circulating NG subpopulation with the phenotype $CD16^{low}CD62L^{high}$ was observed in children with respiratory syncytial viral infection, as well as in viral and bacterial coinfection

Group	Subpopulation	Functions/diagnostic significance
Healthy adults and children	$CD64^-CD32^+CD16^+CD11b^+$ $CD16^{bright}CD11b^{dim}$ $CD62L^{bright}CD63^{dim}$ $CD62L^{dim}CD63^{dim}$ (1:1)	-Anti-inflammatory and antitumor effect
		-Major subpopulations in healthy individuals -Full implementation of ADCC, microbicidal activity
Purulent-septic diseases in children and adults	$CD64^+CD32^+CD16^+CD11b^+$ $CD62L^{dim}CD63^{mid}$ $CD62L^{dim}CD63^{dim}$	-Marker of severity of bacterial infection process
		- Minor subpopulation in healthy individuals - Activated NG in vivo by bacterial antigens (significant increase in circulation)
Acute bacterial infection in adults	$CD16^{dim}CD11b^{bright}$	-Marker of acute process of the bacterial infection
		- Major subpopulation (significant increase in circulation)
Respiratory and herpes infections in children	$CD64^-CD16^+CD32^-CD11b^+$	-Prognostic sign of adverse course of viral infection
		- Minor subpopulation in healthy individuals -Significant increase at viral respiratory and herpetic infection - Depression of NG phagocytic and microbicidal activity
Acute EBV infection in adults	$CD16^{bright}CD11b^{bright}$	- Marker of severity of viral infection process - Prognostic sign of concomitant bacterial infection
		- Major subpopulation (significant increase in circulation) - High level of ADCC reaction and ROS-dependent inhibition of T-cell proliferation

Table 1.
Neutrophilic granulocyte subpopulation phenotypes and their function and diagnostic significance (Nesterova I.V. et al., 2010–2016).

[20, 21]. It is shown that the subpopulation of immature NG did not possess the ability to protect against microorganisms. Activated mature NG with immunosuppressive properties was found in patients with HIV infection [22]. Suppressive NG can cause paralysis of the immune system, as a result of which anti-infective protection is disrupted, which facilitates the occurrence of bacterial complications and the emergence of viral and bacterial coinfection [20, 23]. The appearance of $CD16^{high}CD62L^{low}$ NG significantly increases with bacterial infection or viral and bacterial coinfection, and at the same time, in the lower respiratory tract, in lungs this subpopulation is practically not detected in patients with viral infection [11, 20]. Neutrophilic subpopulation characterized by the phenotype $CD16^{low}CD62L^{low}$ was observed in children with severe viral respiratory infection without bacterial coinfection and in patients with bacterial sepsis [20, 21]. Using flow cytometry in combination with a visual evaluation of cells, it was shown that a large number of myelocytes and metamyelocytes are included in this subpopulation, so NG with the phenotype $CD16^{low}CD62L^{low}$ was called a subpopulation of NG precursors. A sequential increase in the number of NG precursors was statistically significant ($p < 0.001$) and did not depend on the presence of bacterial coinfection [20]. It was suggested that the NG precursors originate from a heterogeneous family of granulocyte myeloid-derived suppressor cells (G-MDSCs), which include granulocyte cells with the property of immune inhibition [20, 24]. Significant differences in the number of markers of activation and degranulation of CD11b, CD54, CD63, and CD66b in the above four subpopulations (mature NG, immature NG, suppressor NG, and NG "precursors") in viral infections and in bacterial coinfections in newborns with severe viral infection, practically, are not revealed. It was noted that the activation and degranulation of suppressor NG revealed a high level of expression of CD11b and CD63, whereas in NG "precursors," the highest level of expression of CD63 and CD66b and a low level of expression of CD11b and CD54 were observed [20]. Interestingly that NG number in the bloodstream equipped with CD62L on the surface membrane is larger than CD62L NG obtained from bronchoalveolar lavage, which is presumably associated with the loss of this receptor during migration. Pillay et al. [11, 24] discovered the existence of a new subpopulation $CD11c^{bright}CD62L^{dim}CD16^{bright}CD11b^{bright}$ NG—mature hypersegmented human NG with immunosuppressive activity. This subpopulation was able to suppress the proliferation of T cells through the release of active forms of the oxygenate and showed high expression of CD11b. Earlier studies by Woodfin A. and co-authors demonstrated that suppressive NGs—mature cells with hypersegmented nucleus, expressing high levels of ICAM-1—have the ability to reverse transendothelial migration (TEM) [25]. Later, Cortjens and his colleagues in 2017 [20] showed that in severe respiratory viral infection in infants, the expression of the activation marker CD11b was significantly increased in the suppressor subpopulation of NG. These suppressor subpopulations of NG, which appeared in viral and bacterial coinfections in newborns, also had the highest expression of CD63 molecules on surface membranes, which indicated active degranulation of NG.

4. Neutrophil granulocytes in infectious diseases

Defective functioning NGs (deficiency of NG amount; violation of phagocytic function; deficiency of myeloperoxidase, defensins, lactoferrin, glucose-6-phosphate dehydrogenase, NADPH oxidase, etc.; defects in the formation of NET) do not provide adequate antimicrobial protection, which leads to the development of atypically occurring infectious and inflammatory diseases, sepsis, acute hematogenous osteomyelitis, recurrent purulent infections, chronic bacterial infections,

etc. Adequate response of NG in contact with various aggressive pathogens (viruses, bacteria, fungi) can develop in different ways. At the same time, the lack of functional activity of NG is a risk factor for the development of many pathological conditions. Changes in both quantitative and functional characteristics of different subsets of NG are recorded in pathological conditions, that is, NG phenotype is transformed due to a multivariate change in the expression of various receptors. In this case, the defectiveness of microbicidal and regulatory functions of NG leads to a violation of antigen elimination and, as a result, to the aggravation of the course of acute or chronic bacterial, viral, and fungal infections [13]. At present, various dysfunctions of NG are described, which can proceed according to different scenarios in patients with infectious diseases with atypical current: (1) hypofunction and NG deficiency in recurrent and persistent-relapsing purulent processes and chronic infectious diseases, viral and bacterial etiology, not amenable to standard treatment; (2) blockade of functional activity of NG, manifested by the development of an inadequate response up to the state of non-response in chronic sluggish infectious and inflammatory processes with a protracted course of exacerbations in socially significant infections and sepsis; and (3) hyperfunctioning of NG (e.g., extracellular production of oxygen radicals in a high concentration), which can lead to suppression of the T cells and other members of the immune system and damage different organs and tissues in chronic immune-dependent diseases or septic shock [26–28]. Atypically occurring infectious and inflammatory diseases against the background of immune system disorders, and, in particular, against the background of NG dysfunction, lead to increased morbidity, partial and sometimes complete loss of ability to work, and high lethality in sepsis, both in adult subjects and in children especially in the neonatal period [29, 30].

Neonatal NG characterized qualitative and quantitative deficit compared to adult NG. Neonatal sepsis is a global problem because it has the most severe consequences and is characterized by high mortality. This occurs against the background of impairments in the functioning of the immune system and defective NG, which contributes to the rapid dissimilation of the infection and, as a result, to the death of the newborn [31]. Thus, three important violations of NG that contribute to the emergence of severe neonatal sepsis and septic shock are described: neutropenia, decreased plasticity, and delayed apoptosis [32]. In the case of sepsis or the syndrome of a systemic inflammatory reaction, a large number of immature forms of NG appear in circulation. NGs are characterized by a decrease in phagocytic functional activity, a decreased production of ROS, a defective expression level of CD14 receptors, and a violation of the migratory ability. Immature NGs are characterized by a high basal level of intracellular TNF-α/IL-10 ratio, which confirms their pro-inflammatory phenotype. They have a longer life cycle, are resistant to spontaneous apoptosis, and can mature ex vivo [33]. Patients with sepsis (a more severe inflammatory reaction) have a more pronounced decrease in some receptors, in particular TREM-1, which has a key role in amplifying the production of inflammatory cytokines than patients suffering from a noninfectious systemic inflammatory reaction syndrome [34].

NGs affect the adaptive immune response in viral infection [13, 22] through antigen presentation, translocation of pathogenic viruses to the lymph nodes, suppressor modulation of the T-cell response, and expression of Toll-like receptors recognizing the herpesvirus DNA (TLR-9) [35–37]. NGs are important elements of antiviral immunity, realizing their capabilities through the process of phagocytosis, the formation of active forms of oxygen (ROS), the formation of NET, and the ability to synthesize and secrete cytokines, defensins, and interferons [18, 38–40]. Recent studies have shown that on the one hand, NG can perform antiviral protection and on the other hand, many viruses, in particular herpesviruses, can

negatively affect the function of the NG, transform their phenotype, and influence the formation of populations/subpopulations with different functional properties [22]. Herpes viruses block NG antiviral activity, increase NG apoptosis, which leads to neutropenia. Damage to the NG by herpesviruses disrupts their functioning and leads in combination with other factors to disruption of adaptation reactions [13, 22, 36, 41, 42]. In recent years, it has been shown that in chronic herpesviral infection, there are numerous subpopulations of the NG, characterized by different phenotypes with different receptor equipment, possessing different functional properties: the ability to restructure chromatin, express cytokine genes and secrete cytokines, realize the activity of the granular apparatus, produce active oxygen species, and form NET and cytotoxicity.

4.1 $CD16^{+}CD11b^{+}$ phenotype of neutrophilic granulocytes in acute viral and acute bacterial infections

The first reports of the heterogeneity of CD16 expression (FcγIII) on NG membrane (induces oxidative burst and phagocytosis) appeared more than 25 years ago [25, 43, 44], but only recently the mechanisms and functional consequences of this heterogeneity have been studied. In particular we have identified different $CD16^{+}CD11b^{+}$ NG phenotypes with individual characteristics in patients with acute viral (acute viral Epstein-Barr (EBV) infection) and acute bacterial infectious-inflammatory diseases (acute bacterial tonsillitis) [45]. Summarizing the obtained data, it should be noted that in healthy subjects, $CD16^{bright}CD11b^{dim}$NG subpopulation was major. The NG of this subpopulation in trace amounts was detected with acute viral infection and was completely absent in acute bacterial infection. In acute viral infection, the number of NGs of a highly equipped subpopulation—$CD16^{bright}CD11b^{bright}$NG—significantly increased, whereas in acute bacterial infection, there was a significantly lower increase in the number of NGs of this subpopulation. At the same time, in healthy individuals the subpopulation of $CD16^{bright}CD11b^{bright}$NG was minor. In the case of acute bacterial infection, the $CD16^{dim}CD11b^{bright}$ NG subpopulation, which was absent in conditionally healthy individuals, became dominant, and in the case of acute viral infection, it appeared in an insignificant amount. Subpopulation $CD16^{dim}CD11b^{dim}$ was detected only in healthy individuals (**Figure 3**).

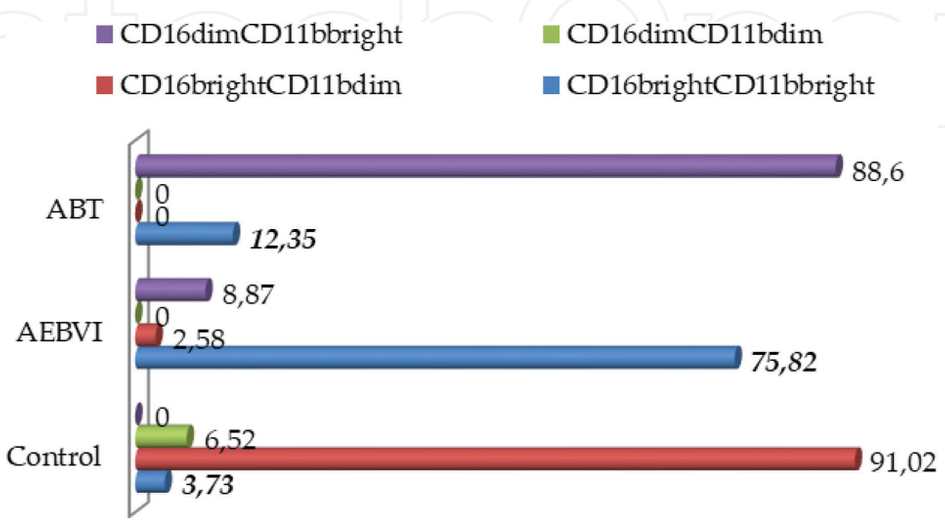

Figure 3.
Phenotypic profiles of $CD16^{+}CD11b^{+}$NG in acute viral (AEBVI) and acute bacterial (ABT) infections.

Apparently, this is a reserve nonactivated pool of circulating NG, since it is known that in the resting state, the NG is insignificantly equipped with membranes CD16 and CD11b. It should be noted that there are certain difficulties and differences in interpreting the data concerning the reasons for the low equipment of the NG CD16 receptor. Thus, early works of Elghetany M. T. (2002) [6] states that, in inflammation, the expression level of CD16 decreases and explain this phenomenon by shading this receptor; then Elghetany and Lacombe note that surface antigen expression of granulocytes depends on age, sex, race, and the presence of stress [46]. Pillay et al. [24] noted the appearance of a "paradoxical" NG population of low-membrane CD16-CD16^{dim} NG in the experiment with the introduction of LPS in vivo. The authors linked this phenomenon to the release of immature forms of granulocytes in the blood, reinforcing this conclusion with morphological studies: CD16^{dim}NG demonstrates the morphology of "young" band nuclear NG [47]. Thus, it is possible that the appearance of the prevalent major population of CD16^{dim}CD11b^{bright} in the acute bacterial infection of the pharyngeal lymphoid ring in patients in a state of moderate severity or with a severe condition is associated with the release of immature forms of the NG into circulation, which is a stereotype response of the NG in severe bacterial infection. The predominant subpopulation of CD16^{bright}CD11b^{bright}NG in patients with acute viral infection has a high cytotoxic antiviral potential due to the high level of CD16 and CD11b expression. According to Kushner and Cheung [48], the detectable enhanced expression of CD16 on NG in a viral infection may be due to the greater functional significance of cytotoxic NGs expressing FcγRIII (CD16) for the implementation of ADCC associated with CD11b-dependent increase in adhesion and degranulation [14].

The clinical picture of many infectious diseases of viral or bacterial etiology in the early stages of the disease can proceed according to a similar scenario. In this case, when verifying the diagnosis of an acute infectious process of viral or bacterial etiology, there are often certain difficulties that prevent timely proper selection of etiotropic therapy. Conducting an express analysis that allows us to specify dominant NG subpopulation—CD16^{bright}CD11b^{bright} or CD16^{dim}CD11b^{bright} can contribute to the differential diagnosis of acute viral and acute bacterial processes of the lymphogenous ring, which will allow timely optimization of etiotropic therapy. On the other hand, it is possible that the evaluation of these subpopulations of NG can be used for early differential diagnosis of various acute viral and acute bacterial processes of other localizations; however, this requires further study.

Thus, CD16^{bright}CD11b^{dim} NG subpopulations prevail in healthy subjects from 80 and up to 99.9%. In acute viral infection of the lymphogenous ring—infectious mononucleosis associated with EBV—the predominance of the subpopulation CD16^{bright}CD11b^{bright}NG is detected in an amount of 40% or more. In acute bacterial infection of the lymphatic pharynx, a subpopulation CD16^{dim}CD11b^{bright} NG predominates—from 40% and higher. The observed phenomenon of different dynamics of presentation of CD16 and CD11b membrane receptors in the CD16⁺CD11b⁺ NG population in healthy individuals and acute inflammation in the region of the lymphopharynx ring reflects the differentiated response of NG to acute viral and acute bacterial infections.

4.2 Evaluation of the effects of the sodium salt of eukaryotic DNA and ODN2395 on CD16⁺CD11b⁺NG phenotype in patients with acute viral and bacterial infection

The use of drugs to improve the accuracy of exposure to target cells and selectively trigger the type of effector reaction is currently considered topical;

in particular, the use of exogenous oligomers of RNA and DNA, in the process of metabolism of which nucleotides and deoxynucleotides are formed, is promising [49]. Nucleic acid preparations of immunomodulating action of various natures are widely used in practical medicine. Pharmacopoeial preparations are known: sodium nucleate (RNA derived from yeast) [50], sodium deoxyribonucleate (sodium salt of native DNA isolated from sturgeon fish milt) [51], and placentex-integro (DNA from trout milt) [52]. Now, the team of authors [49] showed that the substance of sodium deoxyribonucleate mainly contains short and medium DNA chains ending in the CpG motif. Recognition of CpG motifs by the immune system occurs through their interaction with the Toll-like receptor 9 (TLR9), which acts on the cells as "alarm," activating innate and acquired immunity and many times enhancing the body's response even to low-immunogenic antigens [53] with effects of increased proliferation, maturation, and secretion of a number of biologically active molecules—cytokines, costimulatory molecules, molecules of the main histocompatibility complex, etc. [51, 54]. Inflammation is directly related to neutrophilic granulocytes (NGs), which express almost all known TLRs [55], which explains their crucial role in the regulation of phagocytic cells. It should be noted that activation of TLR-4 induces e production of pro-inflammatory cytokines and chemokines (IL1β, IL8, TNFα), TLR-2 activation induces production of chemokine MCP-1, and synchronous activation of TLR-4 and TLR-9 is accompanied by a pronounced respiratory burst a change in the expression of NG adhesion molecules [56, 57]. In the current literature, there are data on the cooperation of TLR9 with the functionally significant receptor of phagocytes—CD11b in the process of recognition of pathogens, even at a low level of exposure to such pathogens [58]. CD11b is also known to both positively and negatively regulate TLR9-mediated mechanisms: control TLR9-triggered NK cell cytotoxicity and macrophage inflammatory responses [59, 60]. On the other hand, it was found that bacterial DNA enhances expression of CD11b genes, while TLR9 expression in NG does not change under the influence of bacterial DNA [61].

Comparative evaluation of the effect of in vitro sodium deoxyribonucleate and the TLR9 agonist (ODN2395) on $CD16^{+}CD11b^{+}$subpopulation composition of NG in both healthy individuals and infectious diseases was of interest [62].

In particular, it was shown that when the peripheral blood of conventionally healthy volunteers with sodium deoxyribonucleate is incubated in vitro, the density of surface-localized CD11b and CD16 receptors is increased, which is expressed by a significant increase in the content of $CD16^{br}CD11b^{br}$NG. The effect of the TLR9 agonist (ODN2395) on this subpopulation in patients with AEBVI allowed us to identify a tendency to decrease its relative content, the effects of the TLR9 agonist and sodium deoxyribonucleate did not affect the relative content of $CD16^{br}CD11b^{br}$NH in patients with ABT (**Figures 4–6**).

The assessment of the content of the $CD16^{br}CD11b^{br}$NG subpopulation in the incubation of the peripheral blood of patients with acute viral and acute bacterial processes made it possible to reveal a significant immunomodulating effect only in the experiment with sodium deoxyribonucleate. When the blood of patients with AEBVI was incubated, a significant decrease in the initially high relative content of $CD16^{br}CD11b^{br}$NG was found.

In acute bacterial infection (ABT), there was an increase in the percentage of $CD16^{br}CD11b^{dim}$NG and a decrease in the initially predominant subpopulation of $CD16^{dim}CD11b^{dim}$NG (as in incubation with sodium deoxyribonucleate and with the TLR9 agonist), whereas in acute EBV infection, an increase in $CD16^{br}CD11b^{dim}$NG was observed under the influence of sodium deoxyribonucleate in vitro (**Figure 4**).

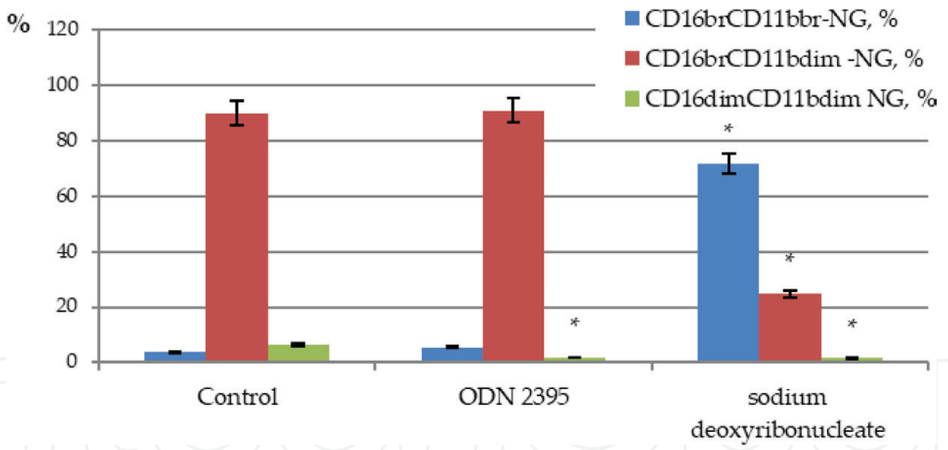

Figure 4.
Comparative analysis of the effect of the TLR9 agonist and sodium deoxyribonucleate in vitro on the subpopulation of CD16⁺CD11b⁺NG of healthy volunteers.

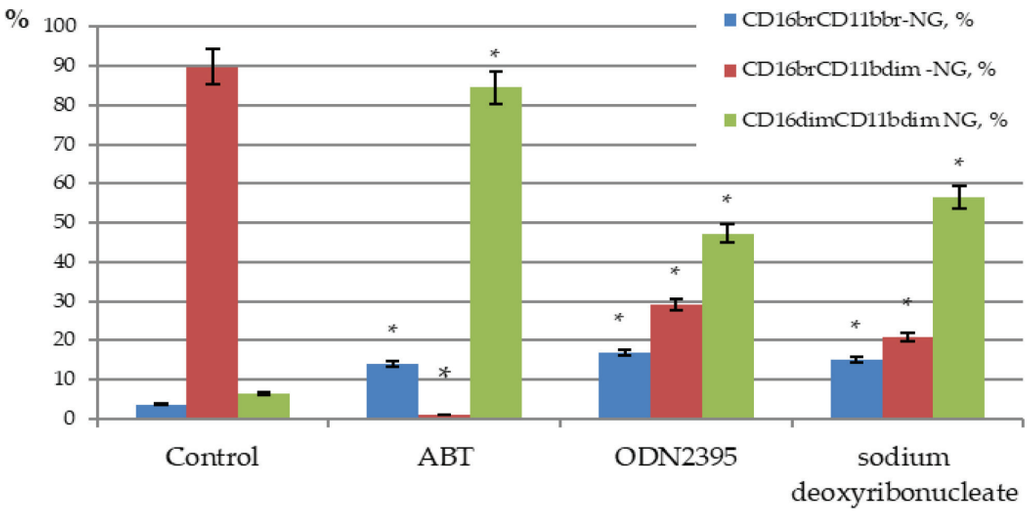

Figure 5.
Comparative analysis of the effect of the TLR9 agonist and sodium deoxyribonucleate in vitro on the subpopulation composition of CD16⁺CD11b⁺NG in patients with ABT.

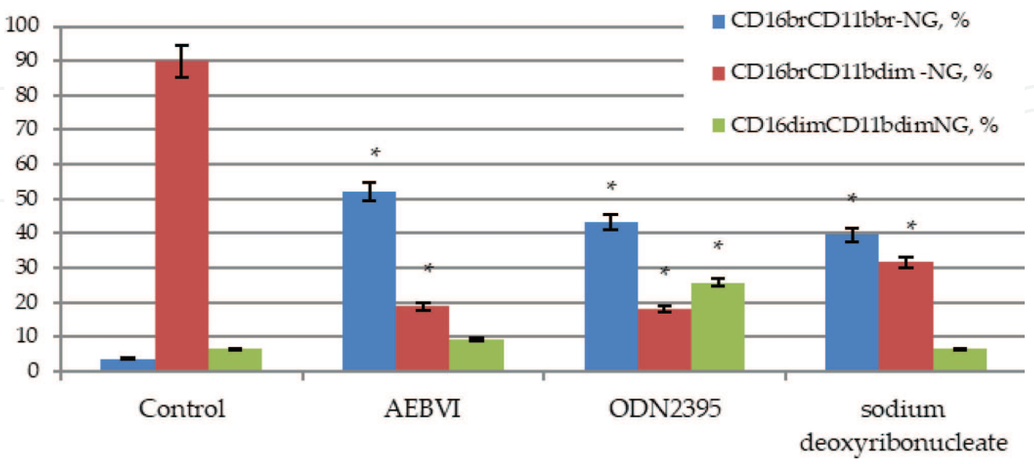

Figure 6.
Comparative analysis of the effect of the TLR9 agonist and sodium deoxyribonucleate in vitro on the subpopulation of CD16⁺CD11b⁺NG in patients with acute EBV infection.

It is important to note that the redistribution of NG subpopulation composition occurring under the action of both agonist and, especially, sodium deoxyribonucleate has a modulating nature, which suggests the involvement of Toll-like type 9 receptors in the regulation of functional NG activity in infectious processes.

5. Conclusion

The classical view of the NG, as short-lived finally differentiated cells, which carry out only phagocytosis, killing, and elimination of extracellular pathogens, is convincingly refuted by numerous recent studies. New scientific facts obtained during the last 10–15 years have demonstrated that NGs possess certain regulatory influences of activating, modulating, and suppressive nature, practically on all cells, both innate and adaptive immunity. The development of new diagnostic technologies allowed us to expand and deepen our understanding of the role of the NG in immune homeostasis and to evaluate the dynamic interrelation of the functional potential of the cell with gene expression and phenotypic polarization of the NG in response to inducing signals of intra- and extracellular environment. It is important to note that, to date, not all NG subpopulations have been identified.

Today it is well known that the population of $CD16^{+}CD11b^{+}$NG plays an important role in the reactions of phagocytosis and ADCC in infectious processes of various natures. It is also known that CD11b and CD16 NG are the most important triggers inducing the cascade of activation and regulatory processes of the NG. The resting unactivated NGs express the low levels of CD11b and CD16 membrane molecules. After activation additional translocation of intracellular CD16 and CD11b molecules to the NG membrane takes place [6, 36, 63]. Our studies showed that the subpopulation $CD16^{bright}CD11b^{dim}$NG prevailed in healthy people, and the NG with the phenotype $CD16^{bright}CD11b^{bright}$ was absent in healthy volunteers but appeared and dominated in patients with acute EBV infection. It has been established that $CD16^{bright}CD11b^{dim}$NG subpopulation predominates in healthy individuals, subpopulation $CD16^{bright}CD11b^{bright}$NG prevails in patients with acute viral infection, and $CD16^{dim}CD11b^{bright}$NG subpopulation dominates in patients with acute bacterial infection. Identified by us in acute bacterial infection (acute bacterial tonsillitis), emergence of the prevalent population of $CD16^{dim}CD11b^{bright}$ NG indicated, in our opinion, the release into circulation of immature forms of NG in a bacterial attack. At the same time, $CD16^{bright}CD11b^{bright}$ NG subpopulation predominated in patients with acute viral infection (acute EBV infection). We hypothesized that on the one hand, the appearance of $CD16^{bright}CD11b^{bright}$ NG with high cytotoxicity (high levels of CD16 expression) and with a suppressive effect on T-cell proliferation (high levels of CD11b molecules) is necessary for the implementation of antiviral activity of the NG in their fight against EBV infection. $CD16^{bright}CD11b^{bright}$NG should have high antiviral activity. On the other hand, their suppressor properties (high levels of CD11b expression) may lead to various complications in the form of secondary bacterial infections. Thus, in severe acute EBV infection, we revealed the transformation of the NG phenotype and the appearance of a new subpopulation of $CD16^{bright}CD11b^{bright}$NG with high cytotoxicity and suppressive effects. Further studies are needed to determine the functional significance of the $CD16^{bright}CD11b^{bright}$ NG subpopulation for both EBV infection and other herpesvirus infections. In addition, early diagnosis of the etiological factors that cause an acute infectious process of a viral or bacterial nature is extremely important for the appointment of early etiopathogenetic therapy. The results of the present study demonstrate that the determination of various subpopulations of the NG in the early stages of an acute infectious process can contribute to the early differentiation of an acute viral process in which the $CD16^{bright}CD11b^{bright}$ NG subpopulation dominates and the acute bacterial process dominated by the $CD16^{dim}CD11b^{bright}$ NG subpopulation. On the other hand, it is extremely important to search for new substances that have immunomodulatory effects on the "negatively transformed" phenotype of the NG with the possibility of positive remodeling, which can prevent the attachment of serious complications, both in viral and bacterial infections [63].

Our studies of the eukaryotic DNA sodium salt effect on the expression of functionally significant CD16 and CD11b NG receptors in healthy individuals and in infectious diseases of viral and bacterial etiology have demonstrated the potential for transformation of the negatively altered phenotype of the NG, in particular, by remodeling the expression of CD11b on the NG membrane [64]. The obtained data open certain prospects for the development of new therapeutic strategies that allow correcting the negatively transformed phenotype of various subpopulations of defective functioning NG in severe infectious and inflammatory processes, both viral and bacterial etiologies.

Conflict of interest

Authors declare that there is no conflict of interest.

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References

[1] Bux J. Nomenclature of granulocyte antigens. Transfusion. 1999;**39**:662-663

[2] Fuchs T, Püellmann K, Scharfenstein O, Eichner R, Stobe E, Becker A, et al. The neutrophil recombinatorial TCR-like immune receptor is expressed across the entire human life span but repertoire diversity declines in old age. Biochemical and Biophysical Research Communications. 2012;**419**(2):309-315

[3] Matzinger P. Friendly and dangerous signals: Is the tissue control? Nature Immunology. 2007;**8**:11-13

[4] Cassatella MA. On the production of TNF-related apoptosis inducing ligand (TRAIL/Apo-2L) by human neutrophils. Journal of Leukocyte Biology. 2006;**79**:1140-1149

[5] Kiseleva EP. New ideas about anti-infectious immunity. Infection and Immunity. 2011;**1**(1):9-14

[6] Elghetany MT. Surface antigen changes during normal neutrophilic development: A critical review. Blood Cells, Molecules & Diseases. 2002;**28**(2):260-274

[7] Nesterova IV, Kovaleva SV, Evglevsky AA, Chudilova GA, Lomtididze LV, Fomicheva EV. Remodeling of chromatin structure and change of the phenotype of neutrophilic granulocytes under the influence of G-CSF in patients with colorectal cancer. Modern Problems of Science and Education. 2014;**3**:1-8. http://science-education.ru/ru/article/view?id=13006

[8] Nesterova IV, Kolesnikova NV, Kleshchenko EI, Tarakanov VA, Smerchinskaya TV, Sapun OI, et al. Different variants of defects in the functioning of neutrophilic granulocytes in congenital pneumonia in newborns. Russian Immunological Journal. 2012;**6**(2):170-176

[9] Beyrau M, Bodkin JV, Nourshargh S. Neutrophil heterogeneity in health and disease: A revitalized avenue in inflammation and immunity. Open Biology. 2012;**2**(11):120-134

[10] Nesterova IV, Kovaleva SV, Chudilova GA, Lomatidze LV, Yevlevsky AA. The dual role of neutrophilic granulocytes in the implementation of antitumor protection. Immunology. 2012;**33**(5):281-288

[11] Pillay J, Kamp VM, van Hoffen E, Visser T, Tak T, Lammers JW, et al. Subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1. The Journal of Clinical Investigation. 2012;**122**(1):327-336

[12] Matsushima H, Geng S, Lu R, Okamoto T, Yao Y, Mayuzumi N, et al. Neutrophil differentiation into a unique hybrid population exhibiting dual phenotype and functionality of neutrophils and dendritic cells. Blood. 2013;**121**(10):1677-1689

[13] Nesterova IV, Kolesnikova NV, Chudilova GA, Lomatidze LV, Kovaleva SV, Yevlevsky AA. Neutrophilic granulocytes: A new look at the "old players" on the immunological field. Immunology. 2015;**35**(4):257-265

[14] Metelitsa LS, Gillies SD, Super M, Shimada H, Reynolds CP, Seeger RC. Antidisialogangliosid/granulocyte macrophage—colony-stimulating factor fusion protein facilitates neutrophil antibody-dependent cellular cytotoxicity and depends on FcγRII (CD32) and Mac-1 (CD11b/CD18) for enhanced effector cell adhesion and azurophil granule exocytosis. Blood. 2002;**99**(11):4166-4173

[15] Nesterova IV, Kolesnikova NV, Kleshchenko EI, Chudilova GA, Lomtididze LV, Smerchinskaya TV, et al. Variants of the transformation of the phenotype of neutrophilic granulocytes CD64+CD32+CD11b+ in newborns with various infectious and inflammatory diseases. Cytokines and Inflammation. 2011;**10**(4):61-65

[16] Nesterova IV, Kovaleva SV, Chudilova GA, Kokov EA, Lomtatidze LV, Storozhuk SV, et al. Peculiarities of the phenotype of neutrophilic granulocytes in neoplastic processes. Russian Immunological Journal. 2010;**4**(4(13)):374-380

[17] Kolesnikova NV, Kovaleva SV, Nesterova IV, Chudilova GA, Lomtatidze LV. Correction of violations of receptor function of neutrophilic granulocytes at the stage of pregravid preparation. Russian Immunological Journal. 2014;**8**(3(17)):697-699

[18] Nesterova IV, Chudilova GA, Lomatidze LV, Kovaleva SV, Sapun OI, Kleshchenko EI, et al. Remodeling of the phenotype of CD64-CD16+CD32+CD11b+ and CD64+CD16+CD32+CD11b+ neutrophilic granulocyte subspecies in congenital pneumonia in deeply premature neonates. Russian Journal of Immunology. 2014;**8**(17):1, 48-53

[19] Nesterova IV, Kovaleva SV, Kolesnikova NV, Kleshchenko EI, Shinkareva ON, Chudilova GA, et al. Optimization of interferon-and immunotherapy in immunocompromised children with associated viral infections. In: Allergy, Asthma & Immunophysiology: From basic science to clinical management. Medimond. International Proceedings. 2013. pp. 101-104

[20] Cortjens B, Ingelse SA, Calis JC, Valar AP, Koendetman L, Bem RA, et al. Neutrophil subset responses in infants with severe viral respiratory infection. Clinical Immunology. 2017;**176**:100-106

[21] Lukens MV, van de Pol AC, Coenjaerts FE, Jansen NJ, Kamp VM, Kimpen JL, et al. A systemic neutrophil response precedes robust CD8(+) T-cell activation during natural respiratory syncytial virus infection in infants. Journal of Virology. 2010;**84**(5):2374-2383

[22] Scapini P, Cassatella MA. Social networking of human neutrophils within the immune system. Blood. 2014;**124**(5):710-719

[23] Leliefeld PH, Wessels CM, Leenen LP, Koenderman L, Pillay J. The role of neutrophils in immune dysfunction during severe inflammation. Critical Care. 2016;**20**:73

[24] Pillay J, Tak T, Kamp VM, Koenderman L. Immune suppression by neutrophils and granulocytic myeloid-derived suppressor cells: Similarities and differences. Cellular and Molecular Life Sciences. 2013;**70**(20):3813-3827

[25] Woodfin AL, Voisin MB, Beyrau M, Colom B, Caille D, Diapouli FM, et al. The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo. Nature Immunology. 2011;**12**(8):761-769

[26] De Oliveira-Junior EB, Bustamante J, Newburger PE, Condino-Neto A. The human NADPH oxidase: Primary and secondary defects impairing the respiratory burst function and the microbicidal ability of phagocytes. Scandinavian Journal of Immunology. 2011;**73**(5):420-427

[27] Klebanoff SJ, Kettle AJ, Rosen H, Winterbourn CC, Nauseef WM. Myeloperoxidase: A front-line defender against phagocytosed

microorganisms. Journal of Leukocyte Biology. 2013;**93**(2):185-198

[28] Winterbourn CC, Kettle AJ. Redox reactions and microbial killing in the neutrophil phagosome. Antioxidants & Redox Signaling. 2013;**18**(6):642-660

[29] Markova TP. Often Ill Children. The Look of the Immunologist. Moscow: Torus Press; 2014. 192 p

[30] Khaitov RM, Ignatieva GA, Sidorovich IG. Immunology. Norm and Pathology. 3rd ed. Moscow: Medicine; 2010. 752p

[31] Wynn JL, Levy O. Role of innate host defenses in susceptibility to early-onset neonatal sepsis. Clinics in Perinatology. 2010;**37**(2):307-337

[32] Maródi L. Innate cellular immune responses in newborns. Clinical Immunology. 2006;**118**(2-3):137-144

[33] Drifte G, Dunn-Siegrist I, Tissières P, Pugin J. Innate immune functions of immature neutrophils in patients with sepsis and severe systemic inflammatory response syndrome. Critical Care Medicine. 2013;**41**(3):820-832

[34] Oku R, Oda S, Nakada TA, Sadahiro T, Nakamura M, Hirayama Y, et al. Differential pattern of cell-surface and soluble TREM-1 between sepsis and SIRS. Cytokine. 2013;**61**(1):112-117

[35] Gusakova NV, Novikova IA. Functional activity of neutrophils in chronic recurrent herpetic infection. Medical Immunology. 2013;**15**(2):169-176

[36] Rusinova TV, Chudilova GA, Kolesnikova NV. Comparative evaluation of immunotropic effects in vitro of derinata and synthetic agonist TLR9 on the receptor function of neutrophilic granulocytes and monocytes in normal and in infectious process. Kuban Scientific Medical Journal. 2016;**5**(160):94-97

[37] Pillay J, den Braber I, Vrisekoop N, Kwast LM, de Boer RJ, Borghans JA, et al. In vivo labeling with 2H2O reveals a human neutrophil lifespan of 5.4 days. Blood. 2010;**116**(4):625-627

[38] Zlotnikova MV, Novikova IA. Functional activity of neutrophils and peroxidation of lipids in severe form of herpetic infection. Problems of Health and Ecology. 2011;**27**(1):70-76

[39] Didkovskii NA, Malashenkova IK, Tanasova AN, Shepetkova IN, Zuikov IA. Herpesvirus infection: The clinical significance and principles of therapy. BC. 2004;**12**(7):459-464

[40] Nagoev BS, Kambachokova ZA. Functional-metabolic activity of neutrophilic granulocytes in patients with recurrent herpetic infection. Journal of Infectology. 2011;**3**(3):38-41

[41] Novikova IA, Romanova OA. Features of the production of cytokines in recurrent herpetic infection. Medical Immunology. 2013;**15**(6):571-576

[42] Drescher B, Bai F. Neutrophil in viral infections, friend or foe? Virus Research. 2013;**171**:1:1-1:7

[43] Krause PJ, Malech HL, Kristie J, Kosciol CM, Herson VC, Eisenfeld L, et al. Polymorphonuclear leukocyte heterogeneity in neonates and adults. Blood. 1986;**68**:200-204

[44] Spiekermann K, Roesler J, Elsner J, Lohmann-Matthes ML, Welte K, Malech H, et al. Identification of the antigen recognized by the monoclonal antibody 31D8. Experimental Hematology. 1996;**24**:453-458

[45] Nesterova IV, Chudilova GA, Lomtatidze LV, Kovaleva SV,

Kolesnikova NV, Avdeeva MG, et al. Remodelling of the phenotype $CD16^{+}CD11b^{+}$ neutrophilic granulocytes granulocytes in acute Epstein-Barr viral infections. Allergy, asthma, COPD, immunophysiology & norehabilitology: Innovative technologies. In: Filodiritto International Proceedings; April; Bologna, Italy. 2017. pp. 181-187

[46] Elghetany MT, Lacombe F. Physiologic variations in granulocytic surface antigen expression: Impact of age, gender, pregnancy, race, and stress. Journal of Leukocyte Biology. 2004;**75**:157-162

[47] Pillay J, Ramakers BP, Kamp VM, Loi ALT, Lam SW, Falco H, et al. Functional heterogeneity and differential priming of circulating neutrophils in human experimental endotoxemia. Journal of Leukocyte Biology. 2010;**88**(1):211-220

[48] Kushner BH, Cheung NK. Absolute requirement of CD11/CD18 adhesion molecules, FcRII and the phosphatidylinositollinked FcRIII for monoclonal antibody-mediated neutrophil antihuman tumor cytotoxicity. Blood. 1992;**79**(6):1484-1490

[49] Filatov OY, Kashaeva OV, Bugrimov DY, Klimovich AA. Morphophysiological principles of immunological action of eukaryotic DNA. Russian Immunological Journal. 2013;**7**(16):4

[50] Rykova EY, Laktionov PP, Vlasov VV. Activating influence of DNA on the immune system. Advances of Modern Biology. 2001;**121**(2):160-171

[51] Besednova NN, Zaporozhets TS. The action of deoxyribonucleic acid prokaryotes on the humoral and cellular factors of congenital and adaptive immunity of vertebrates. Pacific Medical Journal. 2009;**3**:8-12

[52] Shutikova AN, Zaporozhets TS, Serebryakova MF, Epstein LM, Korneeva NA. The effect of DNAC on immunity indices in elderly people. Journal of Microbiology, Epidemiology and Immunobiology. 2006;**3**:68-71

[53] Goldfarb Y, Levi B, Sorski L, Frenkel D, Ben-Eliyahu S. CpG-C immunotherapeutic efficacy is jeopardized by ongoing exposure to stress: Potential implications for clinical use. Brain, Behavior, and Immunity. 2011;**25**:67-76

[54] Takeda K, Sh A. Toll-like receptors in innate immunity. International Immunology. 2005;**17**:1:1-1:14

[55] Berezhnaya NM, Sepiashvili RI. Physiology of TOLL-like receptors—Regulators of congenital and acquired immunity. Journal of Physiology. 2011;**57**(5):26-29

[56] Hyang LT, Paredes CG, Papoutsakis ET, Miller WM. Gene expression analysis illuminates the transcriptional programs underlying the functional activity of ex vivo-expanded granulocytes. Physiological Genomics. 2007;**31**(10):114-125

[57] Yoshimura A, Ohishi HM, Aki D, Hanada T. Regulation of TLR signaling and inflammation by SOCS family proteins. Journal of Leukocyte Biology. 2004;**5**(3):422-427

[58] Figueiredo MM. Expression of Toll–like receptors 2 and 9 in cells of dog jejunum and colon naturally infected with leishmania infantum. BMC Immunology. 2013;**14**(22):1-12. http://www.biomedcentral.com/1471-2172/14/22

[59] Bai Y. Integrin CD11b negatively regulates TLR9–triggered dendritic cell cross–priming by upregulating microRNA–146a. The Journal of Immunology. 2012;**188**(11):5293-5302

[60] Han C. Integrin CD11b negatively regulates TLR–triggered inflammatory responses by activating Syk and promoting degradation of MyD88 and TRIF via Cbl–b. Nature Immunology. 2010;**11**:734-742

[61] Itagaki K, Adibnia Y, Sun S. Bacterial DNA induces pulmonary damage via TLR9 through cross–talk with neutrophils. Shock. 2011;**36**(6):548-552

[62] Rusinova TV, Kolesnikova NV, Chudilova GA, et al. Comparative evaluation of the immunotropic effects of TLR9 agonists and the sodium salt of vertebrate DNA in normal and infectious processes. Russian Immunological Journal. 2016;**10/2**(19/1):77-79

[63] Nesterova IV, Chudilova GA, Lomtatidze LV, Kovaleva SV, Kolesnikova NV, Avdeeva MG, et al. Differentiation of variants of subpopulations of the transformed CD16+CD11b+ phenotype of neutrophilic granulocytes in acute viral and acute bacterial infections. Immunology. 2016;**37**(4):199-204

[64] Tamassia N, Cassatella MA, Bazzoni F. Fast and accurate quantitative analysis of cytokine gene expression in human neutrophils. Methods in Molecular Biology. 2014;**1124**:451-467