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IMMUNOBIOLOGY OF DIPHTHERIA. RECENT APPROACHES FOR THE PREVENTION, DIAGNOSIS, AND TREATMENT OF DISEASE

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Diphtheria is a highly contagious life-threatening disease caused by the toxigenic strains of *Corynebacterium diphtheria*, which are transformed by a bacteriophage carrying the toxin gene. Diphtheria causative agent and its major virulence factor diphtheria toxin are well studied, but outbreaks of disease still occur worldwide. Rapid development of new methods in immunology and molecular biology is currently leading to improvement of prophylaxis, diagnosis and treatment of diphtheria. This review highlights the microbiological, epidemiological and immunological aspects of diphtheria infection, role of diphtheria toxin and others virulence factors in diphtheria pathogenesis and role of humoral anti-toxic immunity in the protection against disease. Perspectives in development of new diagnostic tests, anti-diphtheria vaccines, immunobiological preparations and antidotes for prevention of diphtheria infection, and other anti-diphteria means was also discussed.

Key words: diphtheria, diphtheria toxin, immunity, diagnostic tests, vaccines, antidotes, recombinant proteins.

Diphtheria is an acute infectious disease caused by the bacterium *Corynebacterium diphtheriae* (also known as Klebs-Löffler bacillus) [1]. Typically, diphtheria has respiratory or cutaneous localization. Respiratory diphtheria has various forms, usually restricted to upper respiratory tract: nasal, pharyngeal, tonsillar and laryngeal. As rule, main symptoms of respiratory diphtheria are sore throat, low fever, and an adherent membrane at the site of bacterial colonization [2]. Milder forms of diphtheria are often restricted to the skin [3].

Long time diphtheria was considered as well-controlled vaccine-preventable disease because it has largely been eradicated in all industrialized countries presumably through broad vaccination [4–6]. However, a diphtheria epidemic at the former Soviet Union territory at 1990s has again attracted the attention to incomplete understanding of the epidemiology, microbiology and especially immunobiology of this infection [7–12].

Today cases of diphtheria are still occur in Ukraine, Russia, and Latvia and also it is endemic in India, Indonesia, Nepal, Angola and Brazil, but only sporadic cases are reported in developed countries [11, 13–16]. However, the majority of the adult populations in Europe, Australia and the United States have no immune protection against this infection [13, 17]. This issue draws renewed attention to the immunology of this infection, because lowered immunity levels within population can cause outbreaks of diphtheria.

Bacterial pathogenesis

Biological properties of *C. diphtheriae*. *C. diphtheriae* is an aerobic nonmotile, rod-shaped gram-positive bacillus, which can form metachromatic granules at the ends of the rod. Bacterial cells form irregular V-shaped aggregates resembling Chinese letters [1]. Species *C. diphtheriae* has three biotypes: *gravis*, *intermedius*, and *mitis*, which are differ by the colony morphology and growth characteristics [18]. Genomic sequence of *C. diphtheriae* has been recently characterized [19], but molecular basis for differences in *C. diphtheriae* biotypes is not well defined and requires further investigation [20, 21]. The most severe diseases are often associated with the *gravis* biotype, but

every strain has ability to produce toxin. Consequently, all isolated strains of *C. diphtheriae* should be tested in the laboratory for toxigenicity [22].

Besides *C. diphtheriae* there are two other species *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis*, which can optionally produce diphtheria toxin and hence can cause respiratory illness resembling classical diphtheria [23–25]. It is worth noting that in recent years some severe infections caused by *C. ulcerans* have been recorded [26, 27]. Other well-known human pathogen *C. pseudodiphtheriticum* causes respiratory disease and is associated with high mortality in compromised hosts [28], however it is little known about the virulence factors and pathogenesis of such infections. Some other species of *Corynebacterium* are part of human normal flora, which able to find niches in every suitable anatomic location of the host [29].

Most the clinical manifestationsof diphtheria result from the action of an exotoxin produced by pathogen. Consequently, diphtheria toxin (DT) produced by toxigenic strains of *C. diphtheriae* is considered as the main pathogenic factor of infection. Toxigenicity of *C. diphtheriae* is controlled by bacteriophage conversion [30–32]. Thus toxin production occurs only when the bacterium is infected by lysogenic corynephage carrying the *tox* gene encoding DT.

Production of DT by toxinogenic *C. diphtheriae* is strictly repressed by high concentrations of iron ions from the extracellular environment. The main source of the iron at site of bacterial colonization is the red blood cells, which appear from the bleed via damaged mucous epithelium [33]. The chromosomally encoded diphtheria toxin repressor DtxR depends of iron ions as a co-repressor factor [34]. The iron bound form of DtxR binds to DNA sequence and prevents initiation of transcription of bacteriophagal *tox* gene. Hence, DtxR can be considered as iron-dependent transcriptional repressor [35]. In bacterial cell, DtxR regulates several chromosomally encoded genes, which encode products involved in iron utilization and acquisition [36, 37]. Some mutant forms of DtxR or iron-regulated promoters could constitutively repress the expression of diphtheria toxin gene, and phenotypically nontoxigenic strains may represent a potential reservoir for the emergence of toxigenic strains [38–40].

Strains of *C. diphtheriae* that do not produce diphtheria toxin are still frequently considered as non-virulent. Nevertheless, the association of nontoxigenic strains with localized disease is well known. There is an option that nontoxigenic strains may be responsible for pharyngitis and should be treated [22, 41, 42]. However, additional studies are still required to obtain complete information about the pathogenicity or co-pathogenicity of nontoxigenic *C. diphtheriae* associated with cases of infection in the respiratory tract [43].

The introduction of a toxigenic strain of *C. diphtheriae* into a community may initiate an outbreak of diphtheria by bacterial spreading or by transfer of the bacteriophage to nontoxigenic strains carried in the respiratory tracts of susceptible human subjects. Both toxigenic and nontoxigenic strains of *C. diphtheriae* could be isolated during outbreak of infection, but the epidemiological role of nontoxigenic strains is under the question [44, 45].

Only toxigenic strains can cause respiratory diphtheria, conversely nontoxigenic strains can live in the organism without any clinical manifestation or cause some other pathologic states.

Recently, it was shown that nontoxigenic strains are associated with cases of invasive infection, particularly with endocarditis [46–48]. These microorganisms also can be associated with other invasive diseases, such as septic arthritis and osteomyelitis [49], or catheter-related infection [50].

The systemic diseases caused by *C. diphtheriae* often related to invasive clones. Invasive diseases add new aspects to the infectious processes caused by *C. diphtheriae*. Entry of *C. diphtheriae* by invasive processes can be caused by percutaneous trauma, skin and throat colonization. Unlike classical diphtheria, invasive disease caused by *C. diphtheriae* affects both vaccinated and non-vaccinated persons, and mostly induced by nontoxigenic isolates.

The patterns of adherence to HEp-2 cells (epidermoid carcinoma tissue from the larynx) of *C. diphtheriae* strains can be used to predict their invasive character [48, 51]. Invasive microorganisms yielded simultaneous expression of localized adherence-like and aggregative-like adherence patterns to HEp-2 cells. Microbial adhesive properties may contribute to the spread and outcome of invasive processes.

Diphtheria toxin. Since the discovery of diphtheria toxin by Roux and Yersin in 1888 [52] it became one of the most extensively studied bacterial toxins. The minimal lethal dose of diphtheria toxin for humans and animals is below 0.1 mkg per kg of body weight [53]. The delivery of a single molecule of diph-

theria toxin to the cell is sufficient to kill a eukaryotic cell [54].

Diphtheria toxin is an A-B type toxin consisting of two fragments: A (active) and B (binding) (Fig. 1). This protein consists of three domains: catalytic C-domain, transmembrane T-domain and receptor-binding R-domain. C-domain encompasses the fragment A (SubA — subunit A), T-domain and R-domain together constitute the fragment B (SubB — subunit B) [55].

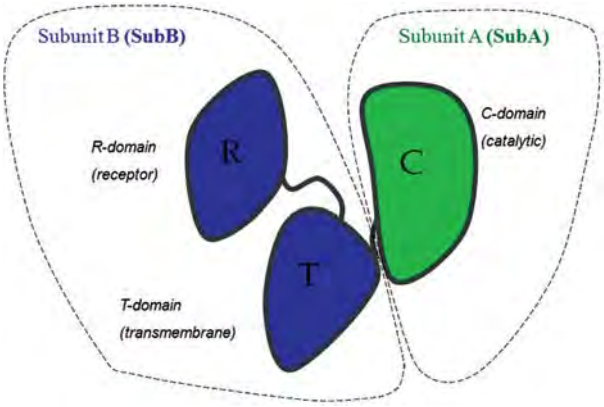

Fig. 1. **Schematic structure of diphtheria toxin (DT)**

Receptor for DT (Fig. 2) is well characterized [56, 57]. Membrane-anchored precursor of heparin-binding epidermal growth factor-like growth factor (pro-HB-EGF) binds with toxin with high affinity (Kd of approximately 10^{-8} –10^{-9} M) [58]. DT is only one known natural ligand for pro-HB-EGF, which causes its internalization.

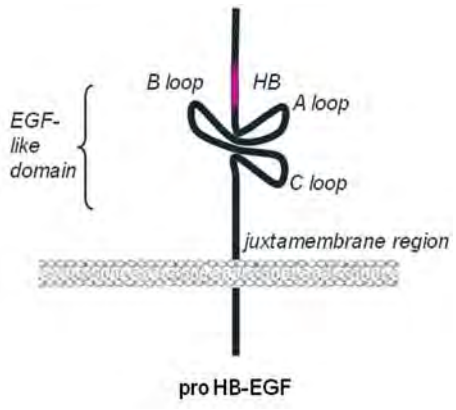

Fig. 2. **Schematic structure of the DT receptor — heparin-binding EGF-like growth factor (HB-EGF) exposed on the cell surface in the form of profactor. (The Subunit B of DT binds to the EGF-like domain of HB-EGF)**

HB-EGF is a member of the EGF family growth factors, which has high affinity for heparin and heparan sulfates [59]. Pro-HB-EGF is synthesized as type I transmembrane protein, which after processing by metalloproteases like ADAMs turns into soluble form (sHB-EGF). sHB-EGF acts as ligand for the EGF receptors of I and IV type, thus it is considered as a potent mitogen and chemoattractant for different cell types, including malignant cells.

The first step in intoxication of eukaryotic cells by diphtheria toxin is binding of the toxin to a specific cellular receptor pro-HB-EGF. The fragment B of DT is responsible for interaction with receptor on the cell surface and translocation of the fragment A across endosomal membrane into the cell cytosole. Two domains of fragment B have different functions. R-domain mediates binding of DT to its surface receptor, which promotes endocytosis of the toxin-receptor complex. T-domain facilitates C-domain translocation across lipid bilayer.

Upon endosome formation, endosomal low pH induces conformational changes that result in diphtheria toxin T-domain interaction with the endosomal membrane [60].

Thereafter T-domain mediates translocation of C-domain into the cell cytosol. Mechanism of C-domain translocation remains unclear, but obvious that it depends on conformational switching of T-domain and its affinity to proteins in molten globule state.

After C-domain translocation across endosomal membrane, it restores the ability to inactivate eukaryotic translation elongation factor 2 (eEF2). Subunit A possesses ADP-rybosyl transferase activity and specifically inactivates eEF2. Accumulation of large number of inactivated eEF2 leads to inhibition of cellular protein biosynthesis and cell death [61].

The target of ADP-dribosylation by diphtheria toxin is unusual amino acid residue in eEF-2-diphthamide (a posttranslationally modified histidine). The diphthamide residue is unique to EF-2 from eukaryotes and Archea [62]. Synthesis of diphthamide is a complex process that requires the participation of several different proteins [63]. It has been suggested that ADP ribosylation of diphthamide in EF-2 may occur as a regulatory event in normal cellular physiology, but evidence for the precise physiological role of diphthamide in eukaryotes remains unknown [64]. Mutant cell lines that cannot produce diphthamide remain viable and are resistant to diphtheria toxin [65].

There are several important questions regarding DT functions remain to be investigated, like mechanism of translocation of the A fragment across endosomal membranes, mechanism of rodent toxin-resistance, the physiologic role of diphthamide residue of EF-2 in eukaryotic cells, as well as mechanism of immune recognition and protection mediated by toxin-specific antibodies etc.

Diphtheria toxin is responsible for the local cell damage at site of bacterial colonization as well as for distant toxic effect on peripheral nervous system, kidneys and heart. Apparently DT also helps bacteria to evade immune defense mechanisms and to escape from phagocytosis. Thus, DT and probably other surface structures of *C. diphtheriae* toxigenic strains show apoptogenic effect on mice peritoneal macrophages *in vitro*[66]. Small amounts of toxin can impair protein synthesis in both polymorphonuclear leukocytes and mononuclear cells from humans and guinea pigs [67]. DT could penetrate into phagocytes and B-cells specific to DT and kill these cells even if they derived from toxin-resistant animals [68]. This observation confirms that DT is potentially able to inhibit self-directed antibody response and phagocytosis and in this way escape from host defense mechanisms.

Additional bacterial virulence factors. Despite that the role of DT in bacterial virulence is well established, there are little known about other virulence factors of *C. diphtheriae*. These factors could be crucial for colonization of the host and recognition of corresponding host receptors since colonization is an essential step in pathogenesis. However, host cell receptors and invasion-associated proteins of the pathogen remains unknown.

On the HEp-2 cell system was shown distinct patterns of bacterium adherence: an aggregative, a localized and a diffuse [48, 51], which confirm an existence of several adhesion factors and different receptors on the host cell surface. Some bacterial adhesion factors have been recently characterized on the molecular level. Certain *C. diphtheriae* strains able to express three types of pili (SpaA, SpaB and SpaC) on its surface [69, 70], which are sufficient for adhesion to pharynx cells. There some additional proteins besides pili proteins involved in adhesion to larynx, pharynx and lung epithelial cells. For instance, *C. diphtheriae* invasion-associated protein (DIP1281) involved in cell surface organization, adhesion and internalization in epithelial cells [71]. In addition, the disruption of the *C. diphtheriae* DIP1621 gene leads to decreased adherence to epithelial cells [72]. DIP0733 (67-72p) may be directly implicated in bacterial invasion and apoptosis of epithelial cells in the early stages of diphtheria and *C. diphtheriae* invasive infection [73]. Non-fimbrial surface protein 67-72p also involved in adhesion to human erythrocytes. Iron supply has effect on binding properties of the microorganisms to erythrocytes as well as HEp-2 cells [74].

Number of studies suggesting the multifactorial mechanism of adhesion [75]. In addition, biofilm formation and fibrin deposition may contribute to the persistence of *C. diphtheriae* at the infected site [50].

Intracellular survivelance strategy. *C. diphtheriae* generally considered an extracellular colonizer. However, some strains of *C. diphtheriae* possess the ability to enter into cells and to survive within cultured cells [51, 76]. *C. diphtheriae* strains can adhere to epithelial cells and erythrocytes and has ability to survive within these cells. Probably, *C. diphtheriae* strains might use epithelial cells as an environmental niche supplying protection against antibodies and macrophages [71]. Some *C. diphtheriae* strains even without the *tox* gene exhibit strategies to survive within macrophages and to exert apoptosis and necrosis in human phagocytic cells [77]. Invasion of these cells is an active process; tetracycline-treated *C. diphtheriae* was still able to attach to host cells, but lost its ability to invade the cytoplasm [78]. As rule, the interaction between bacteria and macrophage determines the outcome of most infectious diseases.

The ability of diphtheria infectious agent to cause macrophage apoptosis is one of the mechanisms of realization of its pathogenic properties determined by the effect of diphtheria exotoxin, as well as its surface structures and pathogenicity enzymes. The presence of the *tox* gene influences the susceptibility of *C. diphtheriae* to human macrophages and the outcome of non-opsonic phagocytosis [77]. Analyses of molecular mechanisms of non-opsonic phagocytosis should lead to new approaches for the prevention of diphtheria and systemic *C. diphtheriae* infections. Homologous *C. diphtheriae* tox^+ and tox^- strains can survive within U-937 human macrophages but viable intracellular bacteria can be detected after 24 hr only for the tox^- strain.

Pseudomembrane formation and coagulase-like activity. The characteristic feature of disease is a pseudomembrane formation that usually covers the posterior pharynx and

tonsils, which may also extend to the larynx and lower respiratory tract [2, 79]. Pseudomembrane of respiratory diphtheria is composed of fibrin matrix with incorporation of bacteria, necrotic epithelial and inflammatory cells, which adheres tightly to the underlying tissue [2]. The severity of the disease usually related to the extent of the local infection, although the potential role of these pseudomembranes in the maintenance of viable *C. diphtheriae* is still uncharacterized.

Although fibrin pseudomembrane is a characteristic feature of diphtheria, there is little known about the fibrinogen-binding properties and fibrin clot formation activity of *C. diphtheriae* strains and the role of the DT in these processes. The production of fibrinous exudates may play an important role in determining of the pseudomembrane formation. Diphtheria toxin generally considered as the major factor responsible for local cellular destruction and production of fibrinous exudates, suggesting that the presence of bacteriophages carrying the diphtheria toxin gene (*tox*) is essential for pseudomembrane formation. Recently reported property of *C. diphtheriae* iscoagulase-like activity. The capacity to bind to fibrinogen and to convert fibrinogen to fibrin may play a role in pseudomembrane formation and act as virulence determinants for both nontoxigenic and toxigenic strains [43].

Consequently, production of DT is important for the epithelial cell damage and production of fibrinous exudates, while coagulase-like activity of *C. diphtheriae* may be important forfibrin polymerization. That is why we could consider DT as molecular instrument used by *C. diphtheriae* at the site of colonization for the partial epithelial cell damage in order to get small portion of fibrinogen from the blood for the fibrin formation. After short bleeding, the production of DT is inhibiting by iron ions from incoming hemoglobin. DT also can get to the blood circulation via damaged epithelia and thus cause severe systemic toxic effects. Diphtheria toxin exerts its effects on distant tissues and organs, especially the heart (causing myocarditis), and the peripheral and cranial nerves (causing weakness progressing to paralysis), if absorbed from the site of infection.

Assessment of anti-diphtheria protection

Serologic methods of diphtheria diagnosis based on the detection of diphtheria toxin or on increased level of antitoxic antibodies. Therefore, measurement of antitoxin level in diphtheria patients could provide important clinical information about course of infection.

In addition, determination of anti-toxin antibodies is essential for characterization of the immune status of population, and evaluation of the immunogenicity of diphtheria vaccines in clinical trials, as well as for monitoring long-term immunity and thus provides recommendations for vaccination policy. Data obtained from serological studies serve as an important guide in choosing of local strategy of vaccination. Detecting the existence of a cohort of susceptible subjects can predict the risks for disease outbreaks. Therefore, it is of critical importance to have methods for assessment of anti-diphtheria immunity that are accurate, reproducible, specific, and sensitive.

Most symptoms of diphtheria are resulted from the diphtheria toxin action; therefore, protection against disease depends on antibody level against the toxin (antitoxin). The assessment of the anti-diphtheria protection in healthy population is common for a surveillance system within any National Program of Immunization. According to the Order № 545 of Ministry of Health of Ukraine from 24.11.2003 «About Ukrainian population immunity against diphtheria and tetanus», clinicists also need «to provide annual studies of population immunity to diphtheria and tetanus among healthy population (1.2.)»

Antitoxic antibodies probably play a main role in the immunity against diphtheria. Serum titers of antitoxin usually are expressed in International Units per milliliter (IU/ml) according to the diphtheria antitoxin standard. The cut-off of protective serum level of antitoxin is 0.01 IU per ml. (but it also depends on the method of titer determination). As believed, the powerful anti-toxin immunity (>1.0 IU/ml) can completely protects the body from infection caused by toxigenic strains. Although, the very little is known about protection associated with nontoxigenic strains.

Classical serological tests tend to underestimate low concentrations of diphtheria antibody. That is why antitoxin level under 0.1 IU per ml could not be defined precisely in many laboratories where hemagglutination test is used for this purposes. In clinical interpretation of results, antibody titers classified into one of the following categories: insufficient protection (<0.1 IU/ml), satisfactory protection (0.1–1.0 IU/ml) and high levels of protection (>1.0 IU/ml) [44]. However, with more reliable techniques it is possible to define an

additional categories like short-term protection (0.01–0.1IU/ml) and no protection (<0.01 IU/ml).

Numerous *in vivo* and *in vitro* tests for the measuring of diphtheria antitoxin levels in serum have been standardized and implemented for laboratory practice. Among the *in vivo* protocols are the Schick test in humans and the classical toxin neutralization (TN) assay in rabbits or guinea pigs. There is also the *in vitro* toxin neutralization test in microcell culture plates using highly sensitive Vero (green monkey renal epithelium) cell line [80]. Several *in vitro* serologic techniques for diphtheria antitoxin determination are described [81].

Toxin neutralization tests. DT is a toxic agent that can kill eukaryotic cells and can cause systemic reaction in sensitive organisms. Protective antibodies can block specific binding of DT to cell receptor and therefore to protect the cell and the body from toxic action of DT. Existing toxin neutralization tests exploit *in vivo* or *in vivo* models oftoxicity for measuring the level of antitoxin by dose-depended neutralization effect.

DT can cause *in vivo* inflammatory response when injected in small doses intracutaneously into the skin of humans or sensitive animals. The ability of specific antibodies to prevent this reaction can be utilized to determine the activity of antitoxin. Thus, the *in vivo* neutralization tests show the functional capacity of antibody to neutralize toxin in live organisms.

One of the first methods to estimate immunity against diphtheria in humans was intradermal Schick test with active diphtheria toxin [82]. Bŭla Schick designed this test in 1913 as an approach to determine susceptibility to diphtheria in children. This method allows controlling the population immunity against diphtheria. At that point, immunization was available for those who had never been naturally immunized by exposing to live diphtheria bacilli. This test eventually led to the eradication of the childhood disease, made Bŭla Schick world famous [83].

The Schick test involves injecting a very small amounts (0.1 ml of diluted 1/50 MLD for the guinea pigs) of the toxin into the skin of the forearm and evaluating the reaction at the injection site after 48 hours [84]. The result of positive test manifested in inflammatory reaction indicates susceptibility to diphtheria, whereas result of negative test without any reaction indicates immunity (antibody neutralizes toxin). A control injection with inactivated toxin had to be performed to exclude allergic reactions to toxin. Schick test results usually correlate well with serum antitoxin levels. The average antitoxin level up to 0.1–0.3 IU/mL is corresponded to the negative Schick test reaction when antibodies can completely neutralize injected toxin. However this test is no longer used in healthcare due to safety requirements, painful effect when results are positive, time-consuming, need for two visits, occurrence of pseudo negative reaction, etc. Currently, passive hemagglutination reaction with red blood cells is commonly used for this purpose.

There are several biological tests on sensitive animals are also available to quantify the level of antitoxin. The *in vivo* neutralization tests can be performed on rabbits (Jensen, 1933) or guinea-pigs (Glenny & Llewellyn-Jones, 1931). Different dilutions of serum mixed with fixed amounts of diphtheria toxin can be injected into the depilated skin of the animal, and the antitoxin concentration could be estimated based on the presence or absence of an inflammatory reaction. The *in vivo* toxin neutralization test using guinea pigs or rabbits is referred as the «gold standard» method for determining protective levels of antitoxin in serum. The toxin neutralization assay has been recognized as an accurate and sensitive test able to detect antitoxin levels as low as 0.001 IU/mL [85]. However, this test requires large numbers of animals, relatively large volumes of serum as well as specialized facilities and personnel trained to work with animals. Therefore, this test is highly expensive and time-consuming, thus it is not convenient for practical use in serological diagnosis or epidemiological monitoring.

Consequently, *in vitro* methods as alternative to *in vivo* approaches can reduce time, costs and improve animal welfare. *In vitro* tests with cultured cells (neutralization test on microcell culture) have been developed as “humane” alternatives to the *in vivo* test for detection of diphtheria antitoxin [86]. The ability of diphtheria toxin to cause cell death in cultured mammalian cells used to determine diphtheria toxin or antitoxin amounts. Thisneutralization test based on the observation that the presence of antitoxin in serum samples can promote survival of sensitive mammalian cells in culture treated with DT in dose-dependent manner.

Vero cells [87] commonly used in neutralization tests *in vitro* are most sensitive to diphtheria toxin cells since they have largest numbers of receptors on their surface [88].

Results of *in vitro* neutralization test read as a change in color of the medium (from red to yellow) in the cell-culture plate wells after 3–4 days of incubation. Only alive cells can change the medium color due to the metabolic formation of acid, which changes the pH of medium. Treated with DT cells retain their ability to grow when serum samples contains antitoxin in sufficient amount [80].

The *in vitro* neutralization test in microcell culture is highly sensitive (minimum detectable level is 0.005 IU/mL) and provides comparable results to *in vivo* neutralization test on guinea pig and rabbit skin [89–91]. Thus, the Vero cell toxin neutralization assay is recommended by World Health Organization and European Pharmacopeia as *in vitro* alternative method for guinea pig assay for potency testing of vaccines [92, 93].

Alternatively, cell culture test without native DT use was proposed. In these test native DT replaced with recombinant fusion protein consisting of B-subunit of DT and enhanced green fluorescent protein (EGFP-SubB) (Fig. 3). This protein was able to bind to DT receptor on Vero cells surface, but had no toxicity due to absence of C-domain [94]. This method based on the ability of anti-toxin antibodies to block the binding of fluorescently-labeled recombinant B subunit of DT to the cell surface receptor of Vero cells [95]. We called this method Vero-cell based toxin-binding inhibition test (Vero-ToBI). Proposed *in vitro* method for quantitative evaluation of protective antibodies in sera is significantly rapid than existing tests and not require native DT.

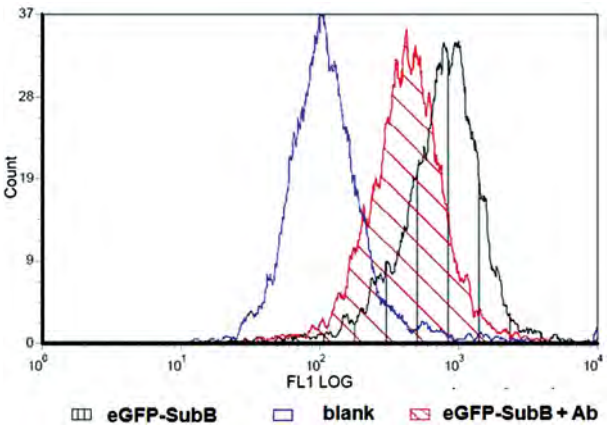

Fig. 3. **The Vero-cell based toxin-binding inhibition test. This method is based on the ability of anti-toxin antibodies to block the binding of fluorescently-labeled recombinant B subunit of DT to the cell surface receptor. This process could be measured by flow-cytometry**

Most important practical issues with all cell-culture tests are the relatively high complexity and time-consuming of the procedure, skilled staff and special laboratory equipment requirements. Therefore, a number of diagnostic laboratories may prefer to use more simplified format of diagnostic kits like serological assays.

Serological tests. For diagnostic and monitoring purposes, serological test can offer significant advantages in terms of cost, speed, ease of use and adaptability to automation. There are a number of serological methods for the estimation of antitoxin level available, like the passive hemagglutination assay [96] and the latex agglutination test [97], toxoid or toxin based ELISA, the double-antigen ELISA [98] and the double-antigen dissociation-enhanced lanthanide fluorescence immunoassay [99], as well as the toxin binding inhibition assay [100] and multiplex assay [101] etc.

Currently, passive hemagglutination reaction (PHA) with red blood cells is still the most frequently used method in many laboratories for the detection of anti-toxin antibodies. The PHA test use coated with diphtheria toxoid sheep red blood cells for agglutination by diphtheria antitoxin [96, 102, 103]. Overall it is relatively simple and inexpensive method, but there is poor correlation of PHA with contemporary toxin neutralization tests, which considered as standard reference methods. In addition, PHA test tends to underestimate low concentrations of antitoxin [104], lacks sensitivity and obviously needs improvement or replacement [81, 105]. Therefore, new assays for the detection of diphtheria antitoxin levels in the population extremely desirable.

An enzyme-linked immunosorbent assay can make the good alternative to PHA for the detection of anti-toxin antibodies. The indirect ELISA which is the simplest variant of this assay involves the estimation of antitoxin bounded to diphtheria toxin (or toxoid) adsorbed on ELISA plates [106]. The almost exact correlations between both the Toxoid-ELISA and the Toxin–ELISA were indicated [81]. Indirect ELISA tests in addition have the ability to measure class-specific antibodies such as IgG, IgM or IgA.

Results of the ELISA have high reliability and reproducibility. When the antibody titer is >0.1 IU/mL results of ELISA have good correlation with results of the neutralization tests in guinea-pigs [107] or in tissue culture[108], however there is poorer correlation with results of the neutralization test when the antibody titer is lower 0.1 IU/mL.

Better correlation can be achieved with modified ELISA tests [98, 100, 107] like the toxin binding inhibition test (ToBI-test) and the double-antigen ELISA, however potential drawback of these tests is inability to measure class-specific antibodies.

In the double antigen format of serologic tests one arm of the antibody binds to antigen immobilized on the plate and the other arm binds to labeled antigen providing enzymatic (DAE — double-antigen ELISA) [98] or fluorescent signal (DELFIA — dissociation-enhanced lanthanide fluorescence immunoassay) [99]. These assays showed a good correlation with established toxin neutralization assays. In addition, the double antigen system is indifferent for origin of serum permitting the use of calibration standard serum of animal origin to measure antitoxin levels in humans in international units (IU/ml). The detection limit for DELFIA format with Eu^{3+}-labeled toxoids corresponded to 0.0003 IU/ml. This fast with a high capacity assays can be alternative to above mentioned methods in serological surveillance studies [99].

High sensitivity and specificity together with the highest correlation with the reference test has ToBI–ELISA. The toxin binding inhibition test (ToBI-test) based on inhibition of the binding of toxin to an antitoxin-coated immunoassay ELISA plate by free antitoxic antibodies[100]. Consequently, the ToBI-test resembles classical sandwich-ELISA combined with competition assay. Antitoxin titers as low as 0.002 IU/ml were detectable by the ToBI-test, it is far below the level considered to be protective for human [100]. The ToBI-test shows good correlation (r = 0.91–0.93) with the *in vitro* neutralization test on Vero cells [100]. From samples with a titer below 0.1 IU/ ml, as estimated by the reference test, 96% were correctly identified by the ToBI–ELISA [81]. Antibody affinity thought to be a key factor to influence the resulting relative antibody titer in ToBI-test [109].

Apparently, determination of antibody titer against the whole toxin molecule cannot provide information about the precise contents of protective antibodies. Protective properties are inherent mainly to the antibodies against B-subunit of the toxin, because only these antibodies can inhibit the toxin binding to the receptor. Antitoxic antibodies to A-subunit of DT often predominate over antibodies to B-subunit in children with diphtheria (as opposed to carriers and vaccinated children) (Fig. 4) [110]. Therefore, recombinant A- and B-subunits of DT [111] was proposed to use in ELISA and flow chromatography test-systems for diphtheria diagnosis and for monitoring of vaccine efficiency. Differential assessment of antibodies to distinct DT fragments with recombinant analogues of A and B subunits can be used for a rough evaluation of protective anti-diphtheria antibodies. In addition, the information regarding level of antibodies to A-fragment of DT provides indirect information about the contact of the person with DT during naturally occurred immunization process.

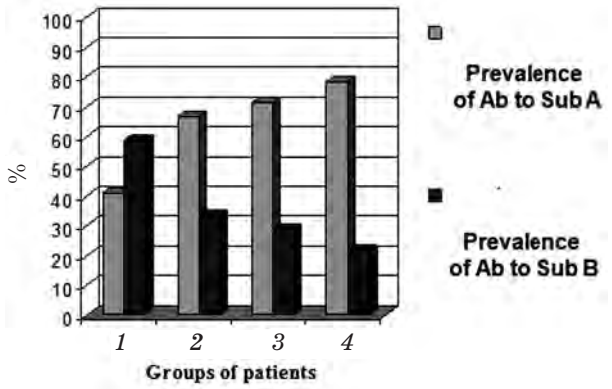

Fig. 4. **The specificity of serum antibodies to separated subunits of diphtheria toxin.**
1 — patients with diphtheria;
2 — carriers of toxigenic strains of *C. diphtheriae*;
3 — carriers of non-toxigenic strains of *C. diphtheriae*;
4 — healthy volunters immunized with DTP-vaccine

Recombinant soluble form of DT receptor sHB-EGF was proposed to use instead capture antibodies in sandwich ELISA for functionally active DT detection. Affinity constant for interaction of recombinant sHB-EGF with DT was similar to the affinity of natural sHB-EGF with DT. The developed sandwich ELISA allowed detection DT with sensitivity up to 1.9 ng/ml [112]. Another test-system for the detection of protective antibodies against DT was based on the competitive ligand-receptor enzyme immunoassay. Recombinant DT receptor sHB-EGF as the bottom layer (bait) and the enzyme-labeled toxin B-subunit as the second layer (prey) allowed identifying anti-toxin antibodies with protective properties (able to prevent the toxin-receptor interaction) (Fig. 5).

Developed methods for evaluation of protective anti-diphtheria immunity can be applied in clinics for monitoring the effectiveness of vaccination within the healthy population, as well as in search for new means of anti-diphtheria immunotherapy and immunoprophylaxis. (Fig. 6)

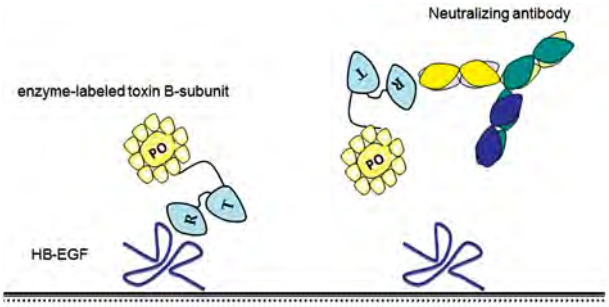

Fig. 5. **The competitive ligand-receptor enzyme immunoassay for the detection of protective antibodies against DT.** Application of recombinant DT receptor (HB-EGF) as the bottom layer (bait) and the enzyme-labeled toxin B-subunit as the second layer (prey) allows to identify anti-toxin antibodies with protective properties able to prevent the toxin-receptor interaction

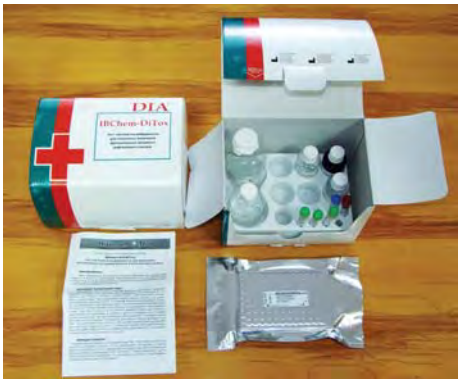

Fig. 6. **Kit for the detection of protective antibodies against DT based on the competitive ligand-receptor enzyme immunoassay**

Immunity to diphtheria

Diphtheria toxin produced by *C. diphtheriae* during the disease or the carrier state has ability to induce production of naturally acquired antibodies against the toxin (antitoxin). Artificial immunity to diphtheria can be stimulated with diphtheria toxoid immunization. Antitoxin can pass through the placenta providing passive immunity to the infant during the first months of life. Patients can acquire passive immunity to diphtheria by injection of equine antitoxin in course of the disease therapy.

As supposed, the primary role in the protection against diphtheria belongs to the antibodies of IgG class, but protection potential of IgA and IgM antibodies is remains underestimated. As mentioned earlier, antibodies to B-fragment of DT are more protective than antibodies to A-fragment.

Recovery from diphtheria is also associated with activity of phagocytes at site of infection. However, there is little known about cell-mediated immune responses to toxin or toxoid and other antigenic substances of *C. diphtheriae*.

Passive immunity to diphtheria. Passive immunity to diphtheria can occur naturally when maternal antibodies are transferred to the fetus through the placenta. Thus, most infants have protective antitoxin level acquired passively from their mothers [113]. However, the half-life of passively acquired antitoxin by newborns is about 30 days [114], thus level of these antibodies significantly decreases between 6 and 12 months. Mothers and their infants have highest diphtheria antitoxin titers (above 0.1 IU/ml) in areas with normal circulation of toxigenic *C. diphtheriae* in population [115].

High titers of maternal antibodies can interfere with serologic response of infants to diphtheria vaccination. The modifying effect of passively-acquired maternal antibodies in young infants is strongest under the age of 4 weeks [116]. High titers of passively transferred antibodies may temporarily interfere with active immunization of infants [117, 118]. Maternal transferredantibodies may suppress responses to the first or second vaccination [119]. Thus in the countries where circulation of toxigenic *C. diphtheriae* is common the early immunization of infant is not so effective due to the presence of high level of maternal antitoxin. At the other hand, early immunization of these infants can deplete their passive immunity due to the absorbance of maternal antibodies by injected toxoid.

Equine diphtheria antitoxin and other antidotes. Passive immunity to diphtheria can be also induced artificially, when high levels of horse antibodies (DAT — diphtheria antitoxin) specific for toxin are transferred to non-immune individuals in order to prevent or cure disease [120]. DAT neutralizes circulating toxin and can prevent progression of the disease. However, DAT could not directly kill live microorganism colonizing mucous epithelia. Therefore, additional treatment with antibiotics required.

This antitoxin was first used in 1890s for prevention of the disease [121–123]; however, modern application of DAT involves only the diphtheria treatment, but not its prophylaxis [124]. Patients with diphtheria suspected have to be given antitoxin and antibiotics in adequate dosage and placed into isolation. The treatment with DAT has serious drawbacks resulted in serum sickness, an immune complex disease, thus a better treatment for diphtheria patients remains desirable [125].

Ways to reduce immunogenicity and allergenicity of therapeutic equine antibodies are to decrease their molecular mass, to modify their Fc fragment and/or to humanize them. Therefore, monoclonal human antibodies, humanized rabbit or mouse antibodies, recombinant single chain fragment variable (scFv) antibodies with molecular mass of 25–35 kDa and camel nanobodies with molecular mass of 15 kDa are now considered as perspective toxin-neutralizing agents [126].

Antibody gene cloning coupled with phage display technique seems to be a relatively fresh solution for the issue of developing better therapeutic means. Generated antitoxic murine and human scFv antibodies have high affinity constants to B-subunit of DT (up to 10^9 M^{-1}) and could neutralize toxin binding to its receptor [127, 128]. Therefore, recombinant scFv antibodies against DT can be utilized for developing new therapeutic reagents.

Another concept of diphtheria treatment is based on preventing diphtheria toxin binding to its cellular receptor pro-HB-EGF by the soluble form of HB-EGF. In order to minimize its side effects sHB-EGF lacking grows-factor activity proposed [129].

Natural acquired active immunity to diphtheria occurs when a person is exposed to a live pathogen, and develops a primary immune response, which leads to immunological memory. When toxigenic *C. diphtheriae* commonly circulated in population natural immunity to diphtheria can be acquired with unapparent infection. Subsequently immunity rise rapidly in early childhood reflecting increasing exposure to diphtheria microorganisms. In the pre-vaccination era diphtheria was primarily an infection of children. At some developing countries at the age of 10–15 years almost all individuals had natural acquired immunity to diphtheria [130–132]. This pattern was observed in Europe and the United States in pre-vaccination era [133, 134] and in developing countries until nowadays.

Residual coetaneous diphtheria is considered as an ongoing source of natural immunity, but insufficient data are available regarding the current prevalence of skin infections [135, 136]. Furthermore, exposure to live *C. diphtheriae* can determine natural boosting of diphtheria immunity after vaccination. The low level of antitoxin among adults in developed countries may result from reduced exposure to live toxigenic microorganisms and thus reduced opportunity to acquire naturally immunity [15] (Schou et al., 1987; Simonsen et al., 1987; Simonsen, 1989). That is why the schoolchildren in developed countries sometime have low titers of antitoxin.. Thus, many authors emphasize the urgency of general revaccination against diphtheria of schoolchildren and adults [137–142].

Vaccines. Invention of toxoid in 1923 by Gaston Ramon provided safe and effective means for vaccination [143]. Formaldehyde treatment of DT eliminates its enzymatic activity and ability to bind to cell receptor, but retains its antigenic properties. In addition, formaldehyde treatment enhances immunogenicity of toxoid while preserving its structural integrity and ability to induce highly active toxin-neutralizing antibodies [144, 145]. Such treatment converts toxic DT to harmless toxoid, which is widely used for immunization against diphtheria. Diphtheria toxoid is still the basis of current anti-diphtheria vaccines. Diphtheria toxoid in vaccines presented most commonly alone or in combination with tetanus toxoid (TD or Td) and whole cell pertussis (DTwP) or acellular pertussis (DTaP) formulations. Addition of aluminum salts as adjuvant increases immunogenicity of this vaccine preparations. DTwP was licensed in 1949 and DTaP — in 1981. Other combined vaccine with diphtheria toxoid may include combination of DTP with poliomyelitis virus vaccine (tetra-component formulation) or with vaccines against *Haemophilus influenzae* type B and hepatitis B (penta-component formulation).

The dosage diphtheria toxoid is measured in flocculation (Lf) units. The Lf unit is used to control quality of produced diphtheria toxoid and to confirm antigenic purity and content of toxoid prior to use in vaccine formulations [146]. It can also be used for determination antigen content in the final products. The 1st International Reference Reagent (IRR) of Diphtheria Toxoid for Flocculation Test (DIFT) was established by the WHO in 1988. This reagent is essential for the standardization of assays used to calculate Lf units of toxoids.

Vaccines for children under 7 years of age usually contain 7.5–25 Lf of toxoid per dose while vaccines for schoolchildren and adults contain 2–3 Lf per dose [44]. Diphtheria vaccine for adults is typically prepared without pertussis component (Td).

Non-toxic mutants of diphtheria toxin are considered as possible alternatives to the formaldehyde treated toxoid. These mutants called CRMs (cross-reactive materials) are naturally nontoxic and do not require chemical inactivation. Most promising antigen among

them is CRM197, which is an enzymatically inactive and nontoxic form of diphtheria toxin that contains a single amino acid substitution (G52E) in the enzymatic A subunit [147]. Currently CRM197 is used as the carrier protein in several licensed polysaccharide-protein conjugate vaccines, for example pneumococcal conjugate vaccine (Prevnar, Synflorix etc.). However, it is considered as antigen for immunization against diphtheria [148]. In addition, new vaccines based on CRM197 and directed against diphtheria are also being developed [149, 150].

Instead of diphtheria toxoid and CRM197 for immunization against diphtheria non-toxic recombinant subunits of diphtheria toxin can be considered as potential antigens to elicit immune response to distinct parts of DT molecule. Preliminary immunization with DT B-subunit was able to protect sensitive to DT animals from toxin action [95]. Recombinant B subunit had great potential to elicit protective immune response in immunized organisms, which allow considering this antigen as prospective component for future vaccine development.

The current diphtheria vaccines are delivered by parenteral route. They can induce high level of antitoxin, mainly IgG, which prevent systemic spread of the toxin. IgG antibodies also may exert a local protective effect, probably through transudation at the mucosal surfaces at site of bacterial colonization. However, IgA antibodies play more important role in the protection of mucosal surfaces of the body from mucosal-associated pathogens like *C. diphtheriae*. Mucosal vaccines can induce an immune response that more closely resembles natural immunity. In animal models of immunization, the nasal route of toxoid or CRM197 administration appears to have the advantage of inducing IgA mucosal response, making it highly attractive for the delivery of vaccines [151, 152]. However, the immunity to diphtheria in human subjects usually estimated in IU by the formation of protective serum IgG or IgM levels of immunoglobulin, but there are no accepted criteria for evaluation of toxin-neutralizing activity of secretory IgA response. This circumstance significantly slows down the progress in intranasal vaccine approval.

Vaccination schedules. The World Health Organization introduced Expanded Programme on Immunization (EPI) with the aim to make vaccination available to all children throughout the World [153, 154]. The WHO recommendation for primary immunization of infants includes administration of three doses of DTP vaccine at the age of 6, 10 and 14 weeks (WHO, 2006) [44]. Generally accepted, that after three DTP vaccinations almost all children can achieve antibody levels higher than 0.01 IU/mL [44, 155].

However, there is no universal schedule for immunization against diphtheria appropriate for each country. The choice of a right schedule depends on the epidemiological pattern of diphtheria in defined territory. In developing countries where the reservoir of *C. diphtheriae* remains large and natural immunity plays significant role in protection against the disease, the first priority of WHO is to ensure 90% coverage of infants with the primary series of three doses of DPT vaccine. In developed countries, primary immunization usually consists of 3 doses of DPT vaccine given at intervals of one month from 2nd or 3rd months of age, and boosted by a fourth dose given in the second year of life or later [44]. According to the Ukrainian schedule of immunization primary series of DPT vaccine was given at 3rd, 4th, and 5th months of age, and a booster dose was administered at 18 months of age [Order of the Ministry of Health of Ukraine № 595 from 16.09.2011 «On the procedure of vaccination in Ukraine and quality control and circulation of medical immunobiological preparations»].

Unfortunately, in developed countries antitoxin serum concentration in infants shows a dramatic decline after the primary series of vaccinations. For example, infants vaccinated against diphtheria at the ages of 3, 5 and 12 months according the Swedish vaccination schedule results incessary for long-term protection (titers 0.1 IU/ml) [156]. The decline of the antibody titers indicates a necessity of further studies to establish the duration of protection.

In countries with high rates of infant vaccination, the cases of diphtheria during childhood significantly declined. On the other hand, this leads to disappearing of toxigenic strains of *C. diphtheriae* circulating in population, which results in declining of antibody levels with age. Populations with high rates of infant vaccination can acquire susceptibility to outbreaks of diphtheria among adults, because their post-vaccination immunity decreased without permanent contact with toxigenic strains. Therefore, WHO recommend for industrialized countries to include additional boosters of DTP vaccine to the primary series of infancy immunization in order to compensate the loss of naturally acquired boosting. Various national immunization schedules propose two booster doses: one during the second year of life and a second before school entry. In addition, people living in non-endemic areas

may require additional boosters every 10 years period to retain all-time protection. Typically, a booster dose administered any time stimulates strong antitoxin production with mean levels above 1.0 IU/mL [44, 142, 157].

The Ukrainian schedule of immunization offers quite a lot of booster doses: one at 18 months of age and a second at six years of age, then at 14, 18, 23, 28 years of age and additional boosters at about 10-year intervals to maintain life-long protection [Order of the Ministry of Health of Ukraine № 595 from 16.09.2011]. Serological monitoring of antitoxin titers in population can be helpful tool for improving current immunization schedule.

Epidemiology. Economic and cultural changes including improved sanitation and hygiene may change the epidemiologic patterns of diphtheria [158]. Today diphtheria evolves from children's disease into disease affecting predominantly adults, with severe respiratory forms of infection. Outbreaks of diphtheria can reemerge until population-wide immunity will be restored by naturally acquired immunization or by broad vaccination of adults [15].

Current vaccines are very effective in preventing from severe forms of infection and infection-caused death, but they are not so effective against mild diseases or asymptomatic carrier states. Their effectiveness in protection from infection is estimated only at 70–90%. Diphtheria outbreaks still can occur among highly vaccinated populations [159, 160].

It is assumed that there is no exactly defined level of antitoxin that gives complete protection from infection, and same antitoxin titers may give diverse protection in different subjects. Thus, an antibody concentration from 0.01 to 0.1 IU/ml may be considered as basic protection, whereas a higher titer of antitoxin may be needed for complete protection. Asymptomatic diphtheria carriers show high antitoxin titers [161].

Human cases or carriers are the reservoir for this infection. In general, total immunization resulted in considerable reduction of diphtheria incidences. It also results in some changes in the immune profile of various age groups following reduction of circulation of toxigenic strains. It is believed, that circulating toxigenic strains can provide opportunities for natural boosting and maintenance of immunity to infection. Adults become more susceptible to diphtheria due to reduced opportunities to keep high immunity through subclinical infections. Protective antibody levels decreases with age, thus in some developed countries, less than 50% of adults could be immune to diphtheria. The age groups with the lowest level of diphtheria antibodies are 20–40 year old or older [15, 44]. A large pool of susceptible persons creates an epidemic potential. In some countries, old persons are still immune to diphtheria, and this is probably due to natural immunity.

Numerous studies have indicated that immunization against diphtheria toxin does not protect from the challenge of non-toxigenic *C. diphtheriae* strains. In highly immunized populations, toxigenic strains virtually disappear, although non-toxigenic strains may continue to circulate [42]. Among them the emergence of invasive non-toxigenic clones of *C. diphtheriae* [41, 162] has been described, but role of this infection as a potential source for respiratory diphtheria remains unclear.

Lessons from the diphtheria epidemic in the Former Soviet Union. Diphtheria was well controlled in the Soviet Union due to well-established childhood vaccination program initiated in the late 1950s. The huge recurrence of diphtheria at the former Soviet Union Countries was the first large-scale diphtheria epidemic in developed countries in vaccination era. Diphtheria incidence started to increase in those countries in the early 1980s, reached its first peak in 1983 to 1985 and its second peak in 1994 to 1995. Epidemic factors were a large population of susceptible adults and children due to decreased immunization coverage, terrible socioeconomic conditions and returning of the military forces from Afghanistan where diphtheria was endemic[163–165].

Due to the reappearance of the epidemic diphtheria in the Newly Independent States in 1990, the European Laboratory Working Group on Diphtheria was established in 1993 [166]. In 2006, diphtheria surveillance network has been expanded and become officially recognized by the European Commission as a dedicated surveillance network, called DIP-NET, covering 25 European countries as members. One of the main goals of this network is the evaluation and standardization of laboratory methods for diagnosis of diphtheria, especially due to the fact that diphtheria has become a rare disease in the majority of the participating countries [167].

Conclusions and Future Directions

Diphtheria was a major cause of childhood mortality in the pre-vaccination era. Routine childhood vaccination virtually eliminated diphtheria in most of industrialized countries. But outbreaks of diphtheria still occur in non-immunized and immunocompromised groups

even in developed countries. Today it is clear that high immunization coverage, prompt diagnostics and rapid identification of close contacts are principal things in controll of diphtheria outbreaks. Nevertheless, deeper understanding of the molecular mechanisms of bacterial pathogenesis is still required for efficient struggle nith the complete combating disease.

Diphtheria represents a unique model for the study of the host-microbe interaction due to phage-encoding mechanism of DT production. Most symptoms of diphtheria are resulted from the diphtheria toxin, which is a product of phage genome. Therefore, immunity against disease is antitoxin-mediated. It is believed that potent humoral immune response to DT can provide the full protection of the body against disease. Peculiarities of humoral immune response also determine current form of diphtheria infection and carrier state. However it is still unclear how the antibodies to extracellular secreted protein can help to eliminate bacterial cells. At the other hand it is apparent that antitoxic immunity could not provide protection against nontoxigenic strains, which could represent the dormant source of pathogen for the possible outbreaks of the disease.

The major characterized virulence factor of *C. diphtheriae* diphtheria toxin helps bacteria to invade the host, cause disease and evade host defense mechanisms. Years of study of the structure and function of DT have made its one of the best characterized bacterial protein toxins. But the identification of other virulence factors are still needed for complete understanding of the full picture of bacterial pathogenesis, including bacterial adhesion to the cells and spreading through the body by invasive process, biofilm formation and fibrin polymerization, intracellular viability and deal host defense mechanisms. Several experimental systems are available to clarify the mechanisms underlying *C. diphtheriae* infections: *in vivo* tests on rabbits and guinea pigs and *in vitro* tests on sensitive cell lines. Other opportunities can provide genomic information and post-genomic comparative analysis of different isolates with different pathogenic potential.

There are many important questions have been raised by recent epidemic of diphtheria in the Newly Independent States of the former Soviet Union and current outbreaks of the disease. First, this epidemic emphasized the necessities for new rapid diagnostic kits and new vaccination surveillance system for prevention of the disease and reducing the risk of the disease among children and adults in future.

Determination of anti-toxin antibodies during vaccination is essential step for the characterization of the immune status of population and monitoring long-term immunity. Such information could provide advanced recommendations for vaccination policy and can predict the spread of future diphtheria epidemics. Accurate determination of anti-diphtheria toxin antibodies is essential to establish susceptible cohorts and to obtain reliable information on the immune status of a given person in population. Therefore, it is of critical importance to develop new serological methods for this purpose that will be fast and specific.

The further understanding of the host immune response to *C. diphtheriae* will suggest novel strategies for treatment and prevention of diphtheria, along with infections caused by nontoxigenic *C. diphtheriae* strains.

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ІМУНОБІОЛОГІЯ ДИФТЕРІЇ. НОВІ ПІДХОДИ ДО ПРОФІЛАКТИКИ, ДІАГНОСТИКИ ТА ЛІКУВАННЯ ЗАХВОРЮВАННЯ

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Дифтерія є висококонтагіозним і небезпечним для життя бактеріальним токсинопосередкованим захворюванням, яке спричинюється токсигенними штамами *Corynebacterium diphtheria*, трансформованими бактеріофагом, який несе ген токсину. Збудник дифтерії та його основний фактор вірулентності — дифтерійний токсин досить добре вивчені, проте спалахи цього захворювання ще й досі виникають в усьому світі. На цей час бурхливий розвиток нових методів у галузі імунології та молекулярної біології сприяє удосконаленню профілактики, діагностики та лікування дифтерії.

В огляді висвітлено мікробіологічні, епідеміологічні, а також імунологічні аспекти дифтерійної інфекції, роль дифтерійного токсину та інших факторів вірулентності в патогенезі захворювання, роль гуморального антитоксичного імунітету в протидифтерійному захисті, а також перспективи розроблення нових діагностичних тестів, протидифтерійних вакцин, імунобіологічних препаратів та антидотів для боротьби з дифтерійною інфекцією.

Ключові слова: дифтерія, дифтерійний токсин, імунітет, діагностичні тести, вакцини, антидоти, рекомбінантні протеїни.

ИММУНОБИОЛОГИЯ ДИФТЕРИИ. НОВЫЕ ПОДХОДЫ К ПРОФИЛАКТИКЕ, ДИАГНОСТИКЕ И ЛЕЧЕНИЮ ЗАБОЛЕВАНИЯ

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Дифтерия является высококонтагиозным и опасным для жизни бактериальным токсинопосредованным заболеванием, которое вызывается токсигенными штаммами *Corynebacterium diphtheria*, трансформированными бактериофагом, несущим ген токсина. Возбудитель дифтерии и его основной фактор вирулентности — дифтерийный токсин достаточно хорошо изучены, однако вспышки этого заболевания до сих пор возникают по всему миру. В настоящее время бурное развитие новых методов в области иммунологии и молекулярной биологии способствует совершенствованию профилактики, диагностики и лечения дифтерии.

В обзоре освещены микробиологические, эпидемиологические, а также иммунологические аспекты дифтерийной инфекции, роль дифтерийного токсина и других факторов вирулентности в патогенезе заболевания, роль гуморального антитоксического иммунитета в противодифтерийной защите, а также перспективы разработки новых диагностических тестов, противодифтерийных вакцин, иммунобиологических препаратов и антидотов для борьбы с дифтерийной инфекцией.

Ключевые слова: дифтерия, дифтерийный токсин, иммунитет, диагностические тесты, вакцины, антидоты, рекомбинантные протеины.