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Biology of Pathogenicity (Theoretical Review)

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Author's contribution

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Research Article

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ABSTRACT

Phenomenon of pathogenicity is the polyfunctional biological potency of germs that are realized by factors (determinants) of pathogenicity [1].
Bacterial pathogenicity is an ability of bacteria to induce and develop infectious diseases in multi-cellular organisms (human, animals and plants).
Virulence is a degree of pathogenicity measured by the *in vivo* (*LD*50) and *in vitro* (*ID*50) tests (highly virulent, weakly virulent and non-virulent strains).
Pathogenic factors (determinants) are the bio-molecules produced by pathogen and are responsible for interaction with the host tissue cells.
"Pathogenicity Islands" are the bacterial genome mobile elements that carry genes encoding the pathogenicity factors production.

Keywords: Pathogenicity; virulence; "pathogenicity islands"; adhesions; invasions; toxins; receptors.

1. INTRODUCTION

The first attempt to consider bacterial pathogenicity as a peculiar biological phenomenon had been made in the Russian academic issue "Biomolecular Bases of Bacterial Pathogenicity" at the end of the 1970s by Dr. Yu. Ezepchuk [2]. The principle point of analysis was the poly-functional character of pathogenicity phenomenon. It was demonstrated that some biological functions realized by a pathogen through bio-molecules

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production, lie in the basis of pathogenicity phenomenon. The biological active molecules named “Pathogenicity Factors” (PF) are grouped in accordance with their functional features. The expression of virulence is regulated by combination of PF-s produced by pathogenic strain and conditioned by the microenvironment in the host- bacteria interactions.

The epithet “Pathogenicity Island” (PAI) appeared in the 1980s in a special publication related to genetic study of bacterial pathogenesis. The concept of PAI was founded by Dr. Jorg Hacker and coauthors [3] who investigated genetic basis of virulence of UPEC strains. It was shown that PAI are mobile elements of bacterial genome carrying the genes that code the pathogenicity determinant production. The elements play a pivotal role in virulent function of bacterial pathogens of humans, animals and plants. PAI apparently have been acquired during the speciation of pathogens from their nonpathogenic ancestors. The acquisition of PAI demonstrates the appearance of the new biological function that causes the interaction of a pathogen with eukaryotic host cells and the effect in a new ecological niche.

Comparative genomics, transcriptomics, and proteomics have become the modern tools discovering the PF-s in bacterial pathogens [4].

There are three approaches in consideration of pathogenicity phenomenon: biological, medical and applied aspects.

Biology of pathogenicity is based on the study of evolution, speciation of pathogens, ecology, genetic determination and encoding of biosynthesis of macromolecules possessing the function of pathogenicity factors (PF). The mechanism of interactions of PF and the host target-cells are also an important part in the biological process of pathogenicity.

The medical aspect of bacterial pathogenicity indicates a realization of pathogenic potency in the host organism. It is a problem of infectious disease, diagnostic, prophylactic and treatment of illness induced by the pathogen.

In the applied area, the knowledge of PF, their structure and functions may have important practical implication such as providing delivery systems for vaccination, tools for cell biology, and tools for the development of new strategies for therapy of bacterial infections.

2. “PATHOGENICITY ISLANDS” ARE THE BACTERIAL GENOME MOBILE ELEMENTS

According to the concept founded by Dr. Jorg Hacker and coauthors [3], pathogenicity factors are predominantly encoded by or associated with mobile genetic elements such as phages, plasmids, insertion elements, or transposons, and a large number of such determinants are located with PAI (Pathogenicity Islands). These elements play a pivotal role in virulence of bacterial pathogens, and are also essential for virulence in pathogens of animal and plants.

More frequently, PAI are included in bacterial genome and occupy relatively large genomic regions. They are in range of 10 to 200 *kb*.

PAI are present in the genome of bacterial virulent forms, but are absent from the genomes of non-virulent representative of the same species. PAI may carry one or more virulence

genes. However genomic elements with characteristics similar to PAI but lacking virulence genes are referred to as “genomic or metabolic islands”.

PAI often differ from the core genome in their base composition that is expressed as a percentage of guanine and cytosine (*G+C*). The average *G+C* content of bacterial *DNA* can range from 25% to 75%. Usually the horizontally acquired PAI has the base composition of the donor species.

PAI are frequently located adjacent to *tRNA* genes that may serve as anchor points for insertion of foreign *DNA* acquired by horizontal gene transfer. After acquisition by horizontal gene transfer, a *DNA* fragment that contains the genes can insert into recipient genome by recombination between the *tRNA* genes.

PAI are frequently associated with mobile genetic elements. They can be flanked by direct repeats (DR) that are presented as *DNA* sequences of 16 to 20 base pairs, up to 130 *bp*. DR might have served as recognition sites for the integration of bacteriophages and for enzymes involved in excision of mobile genetic elements, thus contributing to the instability of a PAI flanked by a DR. Some PAI contain genes that are similar to integrase and resolvase genes of transposons. These mobile genetic elements can change their location within the chromosome, but transposons can also jump from a chromosomal location into a plasmid and vice versa. Insertion sequence elements presented in PAI can also result in the mobilization of large portions of *DNA.* Integrated plasmids, conjugated transposons, bacteriophages or parts of these elements can also be observed in PAI. Pathogenicity factors encoded by PAI are lost with a frequency that is higher than the normal rate of mutations. The genetic mechanism that allows the distribution of PAI by horizontal gene transfer, determines their intrinsic genetic instability. Several characteristic elements such as integrases, transposases, and insertion elements have been identified that contribute to mobilization as well as instability of PAI.

Modern knowledge about the features of PAI gives evidence that the genomes of prokaryotes have a highly diverse mosaic structure. Besides a core genome, which mostly demonstrates homogeneous *G+C* content and codon usage, there exists a flexible gene pool that is formed by mobile genetic elements.

3. ECOLOGICAL ASPECT OF BACTERIAL PATHOGENICITY

The use of molecular biology in the experimental study of infectious disease agent's pathogenicity brought us closer to understanding the biological nature of the phenomenon, based on the interaction of prokaryotic and eukaryotic organisms. Based on the definition of ecology* as a science, an infectious disease can be considered a special model of an ecosystem, in which a living organism is a pathogen, and its natural environment is multi-cellular tissues of humans, animals or plants. In this habitat the microbe is able to perform its vital functions and interact with the tissue cells of its host's body. Death of the host's body as an outcome of infectious disease is a type of the ecological-like disaster, causing collapse of this agent's natural environment, followed by death of the majority of pathogen population.

In the very distant past, our planet was inhabited by microbial society that consisted of saprophytes (non-infectious microorganisms) whose natural environment was water and soil. The development of pathogenic germs that were able to induce infectious diseases was connected with the appearance of multi-cellular organisms. The potential ecological space

represented by human and animal tissues provided pathogens with definite advantages in comparison to natural reservoirs of water and soil. For example, the warm-blooded environment protected microbes from volatile characteristics of the wild, such as changes in temperature. Under stable temperatures, organisms could reproduce and increase the number of individuals that possessed newly developed biological capabilities. Heredity of these characteristics was regulated through natural selection that preserved the genetic structures of the best adapted individuals, and eventually resulted in origination of new species. It leads to the appearance of a new group of paratrophic microorganisms capable of inhabiting various plants and animals.

The appearance of saprophyte individuals that could interact not only with hard inert surfaces but also with living cells of multi-cellular tissues can be viewed as the first step in the establishment of a new ecological space. In these bacteria, specific macromolecules appeared and morphological structures composed of them emerged as a result of new function of the cell walls. Eventually, they transformed into functional active organelles with an adhesive role. These new morphological structures are called *fimbriae* or *common pili*. Lately, other types of adhesions were discovered [5,6].

The adhesive property of bacteria turned out to be very important as the organisms took possession of the new ecological niche. Among the adhesive macromolecule variations that had resulted from various gene mutations, some showed high affinity to the process of physic-chemical binding with eukaryotic cells. In contrast to the simple electrostatic contact with hard surfaces that was typical for many saprophytes, the ligand-receptor interaction of bacterial adhesions and the cell surface demonstrated a high degree of specificity. After adherence to a cell surface, microorganisms begin to reproduce intensely and increase their population while colonizing parts of the host tissue. The capability to extend microbial population in a more complex multi-cellular environment, as opposed to soil and water, meant that it was a new property allowing new germ species to broaden their habitat.

Microbial cells that possessed such adhesive properties in relation to animal or plant tissues became commensals and potential pathogens. Natural selection completed formation of the new species and contributed to the promotion of the specificity of adhesive function. For example, certain pathogenic bacteria infect only certain species of animals, e.g. *N. gonorrhoeae* infections are limited to humans; Enteropathogenic *E. coli* K-88 infections are limited to pigs; *E. coli* CFA I and CFA II infect humans; *E. coli* K-99 strain infects calves.; Group A streptococcal infections occur only in humans [6,7].

Another morphological structure of bacterial cell, the capsule, also played an important role in helping microbes to conquer multi-cellular environment. The capsule is located on the surface of a bacterial cell and has a gelatinous consistence usually reinforced by chains or threads of linear polymers. When separated from a cell, a capsule can easily transform into hydrophilic gel.

In saprophyte organisms that inhabited water and soil, the capsule was primarily a protective device against mechanical damage of the bacterial cell, and a barrier to poisons that could penetrate it. The role of the capsule for bacteria that colonized tissues in humans, animals, and plants was amplified since in the new environment it acquired a new function protecting the pathogen from destructive biological mechanisms of the host organism. The mammals were armed with an immune system and phagocytosis as the first powerful line of defense. To resist compliment activation and absorption by phagocytes the capsule of microbial cells needed certain chemical traits [2].

A good example of the new biological properties of the capsule can be found in some pathogenic bacteria of the *Bacillus* genus. One of these species, *Bacillus anthracis*, is the agent that causes anthrax in human and animals. The critical development in its evolution was the mutation of a gene coding the glutamic acid biosynthesis and the assembly of its linear polymers in its capsule. The bacterial cells that had a capsule consisting of *D*-isomers glutamic acid became invulnerable to host phagocytes. Indeed, it is known that peptides that consist of *D*-amino acids isomers are biologically resistant to the action of proteolytic degradation. The saprophyte members of this family such as *B. subtilis* and *B. megatherium* also are able to produce capsule polypeptides but they were built of *L*- and *D*-glutamic acids isomers. In the process of colonization of the new ecological niche, a few individuals with the *D*-isomer mutant gene were selected from an array of saprophytes bacilli through natural selection. These genetic changes contributed to the growth of microbial population residing in multi-cellular media and, finally, helped to form a new species named *Bacillus anthracis*. However, in order to become a real pathogen, the new microorganism had to acquire an offensive weapon - the toxin [2].

Many pathogens, however, possess additional structural or biochemical features which allow them to resist the main lines of host internal defense against them, i.e., the phagocytic and immune responses of the host [7]. These bacterial substances had different molecular and chemical structure. This group of bio-molecules consisted of peptides and proteins such as staphylococcal protein A and streptococcal protein M, lipopolysacharides (*LPS*) produced by gram-negative bacteria, glycoproteins and other mixed polymers. Some pathogens such as *Streptococcus pyogenes*, *Staphylococcus aureus* and *Treponema pallidum* use fibronectin-binding proteins to provide an antigenic disguise if they clotted fibrin on the cell surface to avoid host defenses. Pathogenic mycobacteria have a waxy cell wall that resists attack or digestion by most tissue bactericides. An intact lipopolysaccharides (*LPS*) of Gram-negative pathogens may protect the cells from complement-mediated lysis or the action of lysozyme . An original function of S-layer proteins as a defense against antibacterial peptides has been demonstrated by some authors [8].

Despite different mechanisms of resistance to phagocytosis, these substances played the same functional role. They protected microbial germs from the host phagocyte and immune system.

Another property that turned out to be helpful in conquering multi-cellular media was the ability to penetrate intercellular space or invasive capacity. Only a small number of pathogens acquired this ability. To that effect, bacteria employed the enzymes that saprophytes used to degrade organic remains in water and soil. Using enzymes such as hyaluronidase, lecithinase, proteases and some glycopeptidases, pathogen that colonized tissue surface were able to split intercellular concreted compounds and invade the tissue. These enzymes have other functions related to bacterial nutrition or metabolism, but may aid in invasion either directly or indirectly. The invasive function is another property of the pathogenicity complex, in addition to selective adhesion to host eukaryotic cells and anti-phagocyte capacity [6].

We can assume that as a result of the first step of evolution all three functions played a very important role in the process of colonization of the new ecological niche by microbial pathogens, and guaranteed its necessary life in the host organism. Their biological action was devoid of aggressive features and did not induce any damage to human or animal multi-cellular systems. Thus, during this first stage of bacterial colonization of the new environment, the interaction between the pathogen and the tissue cells of the host organism

can be characterized as a kind of a symbiotic balance. Typical examples are the oral microflora, the intestinal flora, and the urogenital flora in the human host organism.

Formation of the pathogenic complex was further complicated after the damaging function developed in the bacterial cells, which possessed the above mentioned properties. The aggressive activity was directed to induce various types of dysfunction in the host tissue cells. Development of damaging function was realized in two ways: bio-synthesis of endo- and exo-toxins.

Endotoxons are constituents of the outer membrane of the bacterial cell wall. The biological activity of endotoxin is associated with lipopolysaccaride (*LPS*). *LPS* participate in a number of outer membrane functions that are essential for bacterial growth and survival in host-pathogen interaction. The above mentioned feature is a basic argument for the hypothesis that the damaging factor had been developed in Gram-negative bacteria on the early steps of evolutionary process of pathogenic function.

Toxicity of the cell unbound endotoxins is associated with the lipid component (*Lipid A*), and antigenic activity is associated with the polysaccharide components. *LPS* activates complement by the alternative pathway and may be a part of pathology of Gram-negative bacterial infections induced by *E. coli, Salmonella, Pseudomonas, Neisseria, Haemophilus* and other pathogens. Regardless of the bacterial source, all endtoxins produce the same range of biological effects in the host organism. They cause a wide spectrum of non-specific pathophysiological reactions and are responsible for fever, changes in white blood cell counts, disseminated intravascular coagulation, hypotension, shock and lethality. *Lipid A* is known to react at the surfaces of macrophages causing them to release tumor necrosis factor (*TNF-α*) and probably other cytokines. The polysaccharide part of *LPS* may supply a bacterium with its specific ligands for adhesion which is essential for colonization as the first stage of any infection. In contrast to the protein exotoxins, endotoxins do not act enzymatically and they are less potent and less specific in their action. Blood and lymphoid cells as well as immune system cells and compliment system, are targets that undergo endotoxin action [7].

Another family of aggressive factors is represented by protein exotoxins that were able to damage or to destroy the vitally important physiological systems in the organ tissue cells. Production of protein toxins is specific to a particular pathogenic bacteria species, although it consists of both populations of virulent and non-virulent strains. Virulent strains of pathogen are able to produce the toxin while non-virulent are not. Both Gram-positive and Gram-negative bacteria produce soluble protein toxins. Most exotoxins possess an enzymatic activity that can be realized in contact with the host tissue target cells. Bacterial protein exotoxins are different in their molecular structure; some of them are represented by simple polypeptide molecules and others have a complicated subunit structure.

The bacterial cell produces toxin molecules that are not toxic for the prokaryotic organism, but their damaging actions are aimed at eukaryotic cells. There is no direct connection between production of these toxin macromolecules and viability of pathogenic bacteria. Facts show that the capacity of exotoxin synthesis is caused by a biological stimulus to an increasing number of the microbial population.

We cannot rule out the possibility that the development of the major determinant of pathogenicity resulted from functional mimicry that pathogen borrowed from the host cell environment. Close contacts between two types of cells, prokaryotic (microorganism) and

eukaryotic, lead to communicative events at the molecular level and had dramatic consequences for both the pathogen and the multi-cellular host environment. Apparently, under the action of the outside genetic information, new elements of non-chromosome heredity appeared in the bacterial genome. These elements usually were not integrated in microbial chromosome but they contained some genes that regulated production and assembly of original protein macromolecules. In spite of limited information it was shown that the genes responsible for biosyntheses of some bacterial exo-toxins are located on plasmids or in lysogenic bacteriophages such as *B. anthracis* toxin, *Ps. aeruginosa* toxin, *Cl. botulinum* toxin, *Cl. tetani* toxin, and *Cor. diphtheria* toxin.

These so-called "chimera" proteins [9] had both hormone-like as well as enzyme-like properties and played an important role in the development of the pathogenic complex. Their precursors were molecules secreted by germ cells into the liquid media. Under the exposure of limited proteolysis, the precursor polypeptide chain was cleaving and eventually developed into a bi-functional molecular structure. The hormone-like component of the macromolecule was able to recognize specific membrane receptor on the sensitive tissue cell and bind to the cell surface. After the ligand-receptor binding, the enzymatic active components of the bi-functional structure were subjected to endocytosis or pore-forming mechanism and targeted one of the vital systems of the host cell. Microbial protein toxins with this type of "chimera" structure were highly damaging to eukaryotic cells.

Molecular model of the "chimera" toxin complex is often represented by the formula *A* + *B* (?) where the subunit *B* is the peptide (or peptides) responsible for the membrane receptor binding; and the subunit "*A*" is the peptide that is able to penetrate the cell and damage the intracellular target. Membrane receptors for many bacterial toxins are the same lipid-containing components, for example, gangliosides, that are used by hormone molecules.

Certain protein toxins have broad cytotoxic activity and cause both very specific as well as nonspecific damage of tissue cells. Pore-forming mechanism of action underlies cytotoxicity of some exotoxins, such as hemolysins and leukocidins. A family of staphylococcal and streptococcal exotoxins such as enterotoxins, *TSST*, pyrogenic toxins and others were named as superantigens [10]. These simple proteins possess the distinct domain structure and are able to elicit massive activation of the immune system and to inhibit antibody response simultaneously. They stimulate *T* cell proliferation by interaction with *Class II MHC* molecules on *APC*s and specific *Vß* chains of the *T* cell receptor. Production of *IL-1, TNF*, and other lymphokines is a very important result of this interaction.

The study of the genetic bases of bacterial pathogenicity showed that the development of pathogenic potency had been synchronized with the appearance of the distinct genetic elements into the bacterial genome of pathogenic organism. These genomic islands are acquired by horizontal gene transfer, and they encode genes which contribute to production of pathogenic determinants. “Pathogenicity islands” (PAI) are usually absent from non-pathogenic organisms of the same species. PAI are found in pathogens that undergo gene transfer by plasmid, phage or conjugative transposon. They may be incorporated in the bacterial chromosome or may be a part of a plasmid [11].

PAI apparently have been acquired during the specification of pathogens from their non-pathogenic or environmental ancestors. The acquisition of PAI can be considered an ancient evolutionary event that led to the appearance of phenomenon based on the interaction of prokaryotic and eukaryotic organisms on a timescale of millions of years. We can assume

that the feature of the multi-cellular environment surrounding a bacterial cell could result in the development of new mechanisms of horizontal gene transfer.

Given the current understanding of bio-molecules produced by various causative microorganisms, it can be concluded that the development of the pathogenicity in some non-pathogenic forms and the colonization of a new ecological niche developed in parallel. The laws of Nature dictate that the supreme goal of all biological species, including pathogens, is unrestrained growth of the population. This means in turn that microbial mass in a tissue colonized by a pathogen can grow logarithmically with a corresponding growth in the amount of the tissue-damaging substance. The damaging action frequently results in degradation and eventual death of the multi-cellular host organism, also killing the majority of the colonizing bacteria. Interaction between pathogen and its human or animal environment ends in ecological-like catastrophe.

Bacterial species are saved from disappearance by a few individual cells that escape the host and infect other human or animal subjects. Some germs are also able to survive in air, water, or soil using the capabilities inherited from their saprophyte precursors.

Is it possible to prevent catastrophic destruction of an organism invaded by pathogens? Modern civilization has acquired an arsenal of epidemiological and medical tools for prevention and treatment of infectious diseases. However, as we are constantly reminded by sporadic outbreaks, the causative microorganisms can not be eradicated for as long as there are carriers they can inhabit.

**Ecology is the study of the interaction of organisms with their physical environment and each with other.*

COMPETING INTERESTS

Author has declared that no competing interests exist.

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