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REVIEW ARTICLES

Yersinia pestis FACTORS ENSURING CIRCULATION AND PERSISTENCE OF THE PLAGUE PATHOGEN IN ECOSYSTEMS OF NATURAL FOCI. COMMUNICATION 1

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For constant circulation in natural foci, the plague pathogen must penetrate into the host organism, counteract the protective bactericidal systems of the rodent, and reproduce to ensure bacteriemia, essential for further transmission of the infection by fleas to a new host. Each of these stages in the cyclic existence of *Yersinia pestis* is supported by numerous factors of the plague pathogen, which may exert an influence jointly or each individually, participating in various stages of the infectious process or transmission. However, only in aggregate do these factors ensure persistence of the plague pathogen in natural foci, no matter how significant or insignificant their individual effect might be. This review examines not only the biomolecules, organelles, and systems of the bacterium ensuring the implementation of the pathogenic properties but also other factors essential for the vital activity of *Y. pestis* cells, as well as the relationship of individual pathogenicity factors and the expression of various "housekeeping" genes to the virulence of the plague pathogen. The first communication examines questions associated with the adaptational plasticity of *Y. pestis,* proposes a classification of factors of the plague pathogen ensuring its persistence in nature, and also discusses the factors that ensure survival of *Y. pestis* in the host organism. In compiling the review, not only widely known publications, but also studies published in relatively inaccessible sources, especially to English-speaking specialists, were utilized.

The main processes of the vital activity of any organisms are growth and reproduction, which require sources of energy and plastic materials. Bacteria, which arose during the evolution of the organic world, are characterized by varied types of nutrition and are correspondingly capable of inhabiting various ecological niches — abiotic (soil, water, air) and biotic (protists, plants, animals, and humans). Autotrophic microorganisms can reproduce in an inorganic medium. Heterotrophs require ready-made organic compounds for their vital activity and are arbitrarily subdivided into saprophytes, which feed on organic substances that are outside of living organisms, and parasites, which satisfy their requirements at the expense of nutrients of "other organisms that they parasitize" [122]. Actually, bacteria "can lead a truly saprophytic existence, can be parasites, or can enter into different symbiotic relationships with fauna and flora" [87].

The possible pathways of evolution of parasites and the formation of parasitism have been discussed in detail in a whole series of publications [17, 27, 58, 61, 87, 96, 113, 154, 181, 202, 214]. It is now generally recognized that a change in the ecological niche of a heterotrophic bacterial cell, with transition from saprophitism to parasitism, in which the host organism is utilized as a food source and a dwelling place, is accompanied by the appearance of new biological properties, which facilitate adaptation of the pathogen to the changed conditions of existence.

In the first place, the availability of a whole series of organic compounds leads to a loss of the enzyme systems necessary for their synthesis and to the formation of systems ensuring effective absorption of ready-made organic substances from the host organism; in the second place, antagonistic interactions of the co-members of the parasitocoenosis suggests the appearance of specialized mechanisms and systems in pathogenic bacteria, ensuring: a) their penetration into the macroorganism, and b) reproduction in it up to the levels and persistence for the periods necessary for effective transmission to a new host; in the third place, mechanisms are formed to provide for a route of transmission to a new host characteristic of the given parasite, which ultimately also ensures a cyclic existence of pathogenic bacteria [26, 27, 51, 54, 61, 96, 154, 181, 186, 202, 214].

The classic model in the study of the interrelationships of bacterial pathogens with the host organism and the vector is the plague pathogen — *Y. pestis* [8, 9, 72, 81, 82, 150, 154, 156, 179, 231]. Plague has been the cause of several pandemics and has led to the death of millions of people, devastation of cities and villages, and the collapse of governments and ancient civilizations. At the present time the circulation of *Y. pestis* has been detected in populations of more than 200 species of wild rodents inhabiting natural plague foci on all the continents except for Australia, and transmission of plague is provided for by a minimum of 80 species of fleas. Plague epizootics, during which the pathogen spreads through new territories, alternate with a decrease in epizootic activity. When natural foci of infection are investigated during periods between epizootics, no antibodies to *Y. pestis* are detected in the animals, and the plague microbe is not detected by bacteriological and biological methods. Morbidity in humans is noted, as a rule, when epizootics become acute and is a consequence of bites of blocked fleas, direct contact with infected animal tissues, the consumption of insufficiently cooked meat products as food, or the inhalation of aerosolized respiratory excreta of animals with the pneumonic form of infection [12, 47, 50, 52, 72, 109, 124, 159, 231, 232].

Plague still remains a serious problem for international public health today. Recent epidemic outbreaks of illness in India [209], the isolation of "natural" strains with multiple antibiotic resistances [188], and an increase in the resistance of fleas to insecticides [163] are evidence of the probability that the epidemiological situation for plague will get worse in the near future.

ADAPTATIONAL PLASTICITY OF *Y. pestis*

The persistence and circulation of *Y. pestis* in the ecosystems of natural plague foci are ensured by the high adaptational plasticity of the pathogen [51, 124], manifested at a minimum of three levels:

1) macroevolutionary – irreversible changes of the genome;

2) microevolutionary or population – partially reversible changes in the genome (within the framework of a constant genotype with respect to the storable information);

3) phasic – regulation of the expression of individual genes or whole groups of genes.

Macroevolutionary variability reflects the fact that the plague microbe has diverged from the enteropathogenic yersiniae. To ensure constant circulation in natural foci, the plague pathogen must have acquired in the course of evolution factors that permitted it to penetrate into the host organism, to counteract the protective bactericidal systems of the rodent, and to reproduce to ensure bacteriemia, necessary for further transmission by blocked fleas to a new host. An acquaintance with a description of the possible "ecological trigger factors for speciation" of *Y. pestis* can be gained in the publication by V. V. Suntsov and N. I. Suntsova [121].

On the basis of a study of the population-genetic structure of five "housekeeping" genes, as well as a gene involved in the determination of lipopolysaccharide (LPS) biosynthesis of *Y. pestis* and other representatives of the genus *Yersinia (Y. pseudotuberculosis* and *Y. enterocolitica),* M. Achtman *et al.* [138] arrived at the conclusion that *"Y. pestis* is a clone that evolved from *Y. pseudotuberculosis* 15,000-20,000 years ago, not long before the first known plague pandemic."

The question of the correlation of the already established phenotypic peculiarities, as well as putative differences of the organization of the *Y. pestis* and *Y. pseudotuberculosis* genomes, has been examined most fully in the publications of R. Brubaker [154]. The main "acquisitions" of the plague microbe were the plasmids pPst and pFra (see section 1), as well as constitutive expression of the *hmsT* gene – a component of the *hms* operon, providing for the formation of a block in the flea's gizzard (see section 5). "Losses" are more numerous: loss of the ability to synthesize the adhesin YadA and urease (frameshifts), aspartase, invasins Inv and Ail (insertions of IS elements), certain amino acids, enzymes, and loss of motility (unestablished mechanisms of mutations).

The plague pathogen also lost the ability to synthesize the O-side chains of LPS. According to the data of M. Skurnik *et al.* [255], the most probable ancestor of *Y. pestis* was *Y. pseudotuberculosis* of the serovar O: 1b, the nucleotide sequence of the O-antigen gene cluster of which is 98.9% homologous to the similar cryptic gene cluster

of the plague microbe. Out of 17 "biosynthetic" genes detected in *Y. pseudotuberculosis,* 5 are inactivated in the genome on account of insertions or deletions.

Computer analysis of the complete genome of the strain *Y. pestis* CO92 [228] showed the presence of 149 pseudogenes. The retention of the detected pseudogenes (with the exception of those in IS elements and phages) with the nucleotide sequence of the *Y. pseudotuberculosis* genome known by the time of the analysis (more than 60%) showed that more than 90% of the gene-analogs of the pseudotuberculosis pathogen do not carry mutations.

Microevolutionary variability reflects the intraspecies diversity of the strains of the plague microbe. This diversity is manifested in the existence of intraspecies populations, ensuring geographic separation of plague foci with pronounced ecological differences of the conditions of existence of the microbe organism in them and characterized by differences in certain properties of bacteria from different foci. Thus, the strains isolated in different foci differ in degree of virulence for different species of wild and laboratory animals [1,12,55,60,72,76,108,109,158,231], plasmid composition [15,127,130,184,231], nutrient requirements and enzymatic activity [12,109,115,173,231]. Since phenotypic variability arose "evolutionarily not as a mechanism of generation of diversity and further divergence but as a method of stabilization of the species," it has been proposed that it be called "phenotype metastability" [22]. Various reversible intragenomic rearrangements, including displacements of IS elements, integration of plasmids and bacterial phages into the chromosome, frameshift mutations of a regulatory gene, *etc*., have been designated as mechanisms of phenotype metastability.

It has been suggested that the strains of *Y. pestis* be subdivided into three biovars on the basis of their different ability for nitrate reduction, ammonia oxidation, and fermentation of glycerol: Antiqua, Medievalis, and Orientalis [173]. A determination of the polymorphism of the macrorestriction SpeI profiles using pulse electrophoresis has shown that strains of *Y. pestis* from different natural foci did not differ in size of the chromosomes; however, each of the three biovars was characterized by its own restriction profile [220]. A study of the restriction fragment length polymorphism of the EcoRI fragments containing the insertion element IS*100* also confirmed the fact that these biovars represent different branches of the phylogenetic tree [130, 138].

An investigation of 70 strains of *Y. pestis* using the probe 16S-23S rRNA revealed the presence of 16 ribotypes. The ribotypes B and O, detected in 65.7% of the investigated strains, predominated. Strains of the Orientalis biovar were represented by ribotypes from A to G, Antiqua from F to O, and Medievalis strains were assigned to the ribotypes O and P. The strains isolated in Africa were distinguished by the greatest ribotype diversity. Heterogeneity was least pronounced in isolates from Asia, and strains from South and North America were represented by only one ribotype [196].

The repetitive sequence $(CAAA)_N$ has been identified in the *Y. pestis* genome. This region of tandem repeats – VNTR (variable-number tandem repeat) – was possessed by 9 alleles (from 3 to 13 repeats) in 35 *Y. pestis* strains investigated, isolated in various natural foci. Each of the three classic *Y. pestis* biovars possessed different identifying alleles, with the exception of the allele H, detected in a number of representatives both of the Antiqua biovar and of Orientalis. Strains from the Antiqua biovar were distinguished by the greatest diversity – 4 alleles were detected in 5 strains. In representatives of the Orientalis and Medievalis biovars, 5 alleles (in 21 strains) and 3 alleles (in 8 strains) were detected respectively. It was established that the VNTR $(CAAA)_N$ localized in direct proximity to the transcription promoters, flanking the open reading frames, and is capable of influencing their activity [139]. Further analysis of the *Y. pestis* genome permitted the detection of a whole series of segments of the VNTR. An investigation of the variety of alleles of 42 loci of the VNTR on the chromosome of 24 strains of *Y. pestis* showed that this method of multiple locus genotyping – MLVA (multiple-locus VNTR analysis) – permits not only grouping of the strains in biovars and differentiation of individual isolates but also a determination of their phylogenetic relationship [208].

On the basis of 16 different phenotypic traits, a conference of specialists of antiplague institutions of the Soviet Union, held in Saratov in 1985, recommended that all the variants of the plague pathogen isolated on the territory of the USSR and Mongolia be classified into the following subspecies: *Y. pestis* subsp. *pestis* (the major one), *Y. pestis* subsp. *altaica, Y. pestis* subsp. *caucasica, Y. pestis* subsp. *hissarica,* and *Y. pestis* subsp. *ulegeica* [12] (Table 1). We should emphasize that this classification is national and is not included in the international nomenclature of bacteria. A study o the restriction fragment length polymorphism of EcoRI, containing the insertion element IS*100*, on the whole confirmed the justification of such a intraspecies differentiation of the strains; however, according to the data o IS-typing, the subspecies *altaica* and *ulegeica* were combined, and the major subspecies was additionally subdivided into three groups of strains, isolated from different rodents [43]. However, on the basis of the results of the comparison of the chromosomal IS profiles, A. A. Filippov [130] suggests that all the strains of the plague pathogen be divided into four groups: *Y. pestis* subsp. *caucasica*, as well as the biovars Antiqua, Medievalis,

Table 1

Taxonomic Characteristics of Strains, Characterizing Different Subspecies of the Plague Microbe [12] and their Correspondence to Biovars [173]

***Yersinia pestis* subspecies**	**Fermentation of**				**Nitrate reduction**	**Pesticin I production**	**Susceptibility to pesticin I**	**Fibrinolytic activity**	**Coagulase activity**	**Dependency upon nutrition factors**				**Virulence for guinea pigs**	**Region of circulation**	**Hosts**	**Biovar**
	rhamnose	**melibiose**	**arabinose**	**glycerol**						**leucine**	**methionine**	**arginine**	**thiamine**				
pestis	−	–	+	±	±	+	–	+	+	±	+	–	–	+	Central Africa, central and northern Asia, China (Manchuria), Mongolia	Rodents of genus: *Marmota, Citellus, Meriones, Rattus, etc*.	Antiqua, Medievalis, Orientalis
altaica	+	+	–	+	–	+	+	+	+	+	–	+	–	–	Mountain Altai	*Ochotona pricei*	Medievalis
caucasica	+	+	+	+	+	–	+	–	–	±	+	+	+	–	Transcaucasian highland, Mountain Dagestan	*Microtus arvalis*	Antiqua
hissarica	+	+	–	+	–	+	±	+	+	+	+	–	–	–	Hissarian Ridge	*Microtus carruthersi*	Medievalis
ulegeica	+	+	+	+	–	+	+/–	+	+	–	–	–	–	–	Northeast Mongolia, Gobi Desert	*Ochotona pricei*	Medievalis

Note. +) presence of trait; –) absence of trait; ±) trait is not present in all strains; +/–) sensitive to pesticin of strains of the subspecies *pestis* and *altaica* and insensitive to pesticin of strains of their own subspecies.

and Orientalis.

On the basis of data on the epidemic activity of natural foci of the former USSR and the peculiarities of *Y. pestis* strains isolated in them, A. M. Kokushkin [72] isolated two main variants of glycerin-positive plague bacteria:

—highly virulent for guinea pigs, rhamnose-negative strains, isolated in foci with varied epidemic activity *(Y. pestis* subsp. *pestis);*

—weakly virulent and avirulent for guinea pigs, rhamnose-positive strains, which are the cause of rare illnesses, not accompanied by anthroponose distribution among humans (*Y. pestis* subsp. *altaica, Y. pestis* subsp. *caucasica, Y. pestis* subsp. *hissarica, Y. pestis* subsp. *ulegeica*).

The rhamnose-positive strains of *Y. pestis* subspp. *altaica, caucasica, hissarica,* and *ulegeica,* which circulate in populations of voles of various species and in Pallas's pikas, are distinguished: 1) according to plasmid profiles [15, 127, 130, 184]; 2) according to DNA fingerprint pictures, obtained in IS-typing [19, 43, 130]; 3) according to the production of pesticin and sensitivity to pesticin; 4) according to fibrinolytic/plasmocoagulase activities; and 5) according to dependence on growth factors [12, 53]. However, the ability to ferment rhamnose, as a rule, is accompanied by fermentation of melibiose, and, which is the most interesting, by selective virulence. The rhamnose-positive strains of *Y. pestis* are virulent for white mice and individual species of wild rodents but weakly virulent for guinea pigs [12, 50]. In strains of the plague pathogen isolated from voles *Microtus brandti* and their fleas, Chinese researchers have established the absence of a 32-40 kDa outer membrane protein that is present "in all the known ecotypes of *Y. pestis* virulent for guinea pigs and humans" [274].

We should mention that most of the phenotypic traits used for intraspecies differentiation of the plague pathogen are not absolute. Each of them may be entirely absent, and also may be expressed to the highest degree [55]. Thus, the virulence of vole strains for guinea pigs ranges from complete avirulence to death of 50% of the experimental animals at infecting doses from 10 to 10^9 CFU. In an investigation of more than forty strains isolated in different years in the Transcaucasian mountain focus, "higher virulence for guinea pigs was noted for strains obtained in the Leninakan mesofocus, in comparison with strains isolated in the Zanzegur-Karabakh mesofocus" [60]. Other researchers have also noted high virulence for guinea pigs of individual strains of *Y. pestis* isolated from common voles on the Armenian highlands [1], in the Dagestan mountain focus [108], and in Mongolia [82].

The ability for microevolutionary variability of the plague microbe in the course of the adaptation to a definite species of carrier has been shown in model experiments on infection of rodents by strains of *Y. pestis* isolated from different carriers. In the case of repeated subcutaneous passage (10-15 passages) through an uncharacteristic host, in a number of cases strains acquired some of the phenotypic traits typical of the *Y. pestis* variant characteristic of the given carrier [66, 70,114]. However, the studies of other researchers cite data on an inability of "foreign" strains to adapt to a new carrier and become established in its populations [3, 55].

In an investigation of 187 strains of *Y. pestis* isolated in Madagascar from 1939 to 1996, it was established that all the cultures isolated prior to 1982 belonged to the classic ribotype B. In 1982, 1983, and 1994 strains of new ribotypes were isolated for the first time — R, Q, and T, respectively. The new variants of *Y. pestis* also differed in restriction fragment profiles of the chromosome (NotI) and plasmids (EcoRV). Each of the new ribotypes had its own region of distribution, which had a tendency to expand. Judging by the clinical data, strains of the ribotype R, in comparison with the classic ribotype B, caused a more severe illness in humans, with a higher level of mortality [197].

In 1990, in two cantons in the south of China, one culture each of *Y. pestis* with cryptic 5919-bp plasmid pYC were isolated. By 1999, 230 strains of the plague pathogen with the plasmid pYC were already detected, and the indicated cultures were already circulating in twenty cantons that had previously been characterized by the isolation of strains lacking this plasmid [174].

Still another manifestation of microevolutionary variability is the circulation in natural foci of so-called atypical strains, which differ in a number of traits from the variant of the plague pathogen characteristic of the given focus. Under natural conditions, variability leading to the appearance of atypical strains of *Y pestis* is correlated with the phases of the epizootic process [73,116,123,124]. It is interesting that the frequency of appearance of variable forms of microbes differs in different natural plague foci. The lowest was the variability in the Volga-Ural steppe focus (1.58%) and the Trans-Ural steppe focus (3.3%). In the Gissar and Central Asian desert foci, this index was 6.59 and 6.55 percent, respectively. In the Volga-Ural sandy focus, no atypical forms were detected. In the Trans-Ural steppe focus, atypical cultures were detected in all phases of the epizootic process with an approximately equal frequency. In the Volga-Ural steppe and Gissar foci, such strains were detected only in the acute epizootic phase. Materials on the Central Asian desert focus provide evidence of the absence of differences in the frequency of findings in the phase of the beginning of the epizootic and in the first acute disseminated epizootic. In the phase

of quieting down, the percent of changed cultures increase [116].

In the Ural-Emba focus the largest number of changed forms (6.5% of the total number of strains isolated) was detected in the acute epizootic phase: 24.4% of the number of changed strains had differences in biochemical activity, 17.6% had differences in damage by bacteriophages, 13.5% exhibited a change in the requirement of growth factors, 14.27% showed a predominance of cells with impaired pigment adsorption in the isolated cell culture, 16.75% had reduced virulence, 9.93% did not produce the capsule antigen, 7.14% were calcium-independent, 2.36% were non-pesticinogenic, and 0.4% were phage-resistant variants. The lowest frequency of variability was observed in the case of a virtually continuous course of the epizootic [63]. According to other data [32], in most of the foci strains with reduced virulence constituted from 3-7% to 16-48% of the total number of cultures isolated, avirulent strains constituted 3-10%, those lacking the autonomous plasmid pFra came to 0.2-1.2%, and calcium-independent variants were 0.2-8.4%.

In a series of experiments on preliminarily immunized guinea pigs, albino rats, and wild rodents *(Rombomis opimus, Citellus pigmeus)* it was shown that after their infection with the virulent strain of the plague pathogen, auxotrophic mutants (4.2×10^{-3}-5.3×10^{-5}), variants with sharply reduced virulence, mutants defective for pigment adsorption, and clones differing in level of capsule antigen production are detected in high frequencies in populations of wild rodents. Subsequent experiments with phagocytic cells of immune animals *in vitro* suggested that precisely the mutagenic activity of the phagocytic cells with respect to *Y. pestis* is the leading mechanism in the variability of the plague pathogen in nature [143].

The phasic variability is manifested in differences in phenotypic traits of the same strain under different conditions of its growth and reflects different phases of the cyclic lifestyle of the plague pathogen. The existence in nature of *Y. pestis* populations in the form of two parts: hostal (in the body of a warm-blooded host) and vector (in the body of a flea), which differ in antigenic composition, is generally recognized [12]. However, a number of researchers believe that the scheme of circulation of *Y. pestis* in nature — rodent→flea→rodent — is definitely a simplification of the actual biocoenotic relationships. Thus, the possibility of transmission of the plague microbe along the chain rodent→flea→rodent→soil→plant→rodent is considered; this assumes the presence of a minimum of at least two phenotypic phasic variants of *Y. pestis:* soil and plant (see section 6.2).

Phasic variability is determined by the global regulation of genes. The expression of individual or whole groups of *Y. pestis* genes depends on a number of the following factors: temperature [9, 53, 93, 136, 154, 156, 162, 168, 169, 231, 245, 248, 252], pH [9, 148, 167, 231], the concentration of organic and inorganic components in the medium [154, 168,169,227, 231], the concentration of bacterial cells [203], *etc*., which presupposes the possibility of "turnon" and "turnoff" of individual genes at different stages of the infection process or interaction with the body of the vector.

CLASSIFICATION OF FACTORS OF THE PLAGUE PATHOGEN ENSURING ITS PERSISTENCE IN NATURE

In the classification of factors of the plague pathogen responsible for its persistence in nature, presented in Table 2, an attempt was undertaken to systematize both the already established variants of "strategy and tactical methods" ensuring a cyclic existence of *Y. pestis* in natural foci of infection and the proposed variants. This classification is based on three principles:

1. All bacterial factors are considered from the anthropocentric standpoint.
2. Individual bacterial factors, considering pleiotropy of action and polyfunctionality of biomolecules, can be represented simultaneously in several taxonomic groups.
3. Certain strategical approaches are in turn considered as tactical methods necessary for implementation of strategic approaches of a higher level.

FACTORS ENSURING SURVIVAL IN THE HOST ORGANISM

1. Pathogenicity Factors

As a result of their vital activity, pathogenic bacteria induce pathological, physiological and anatomical changes in the host organism. In a number of cases these changes are not manifested clinically, but in a significant portion of the infected animals symptoms of disease are noted, and some individuals even die. Specialized biomolecules, mechanisms, and systems of the microorganism that promote the pathogenesis of infection, the action

Table 2

Classification of *Y. pestis* Factors Ensuring Its Persistence in Nature

Factors ensuring survival of *Y. pestis* in the host organism	Factors ensuring transfer of *Y. pestis*
1. Pathogenicity factors 2. Factors responsible for reversible transition into partially attenuated forms, which induce a chronic course of infection 3. Factors responsible for antibiotic resistance 4. Factors responsible for nutrient requirements	5. Factors ensuring flea-borne transfer 6. Factors ensuring non-flea-borne mechanisms of transfer

of which ultimately also determines the clinical picture of the disease, are customarily classified as pathogenicity factors [21, 59, 181, 186].

When disease-causing microbes were discovered, discussions began on what terminology should be used to describe the properties of microorganisms determining their ability to cause disease. By the end of the 1930s, most researchers had achieved a consensus on the meaning of the terms "pathogenicity" and "virulence" [120], although it should recognized that in our time also, individual specialists who believe it possible to determine virulence *in vitro* are also encountered. The study o disease-causing bacteria has shown that the factors determining their pathogenicity and virulence may be: antilysozyme activity, resistance to the action of complement, O-antigens, capsules, resistance to phagocytosis, toxins, *etc*. To support the infectious process, several pathogenicity factors may function both individually and in combination. The "turnoff" of any of these factors in turn may either not influence the virulence of the microbe or lead to its attenuation [5, 9, 54, 92, 155, 156, 176, 186]. On the model of the plague microbe the principle of polydetermination of virulence was postulated in the 1950s as a result of the cycle of investigations by T. Burrows [155, 156]. However, up to this time there is no unanimity on the names of these bacterial factors. In studies in medical and veterinary microbiology the following terms may be encountered: "determinants of virulence," "effectors of virulence," "determinants of pathogenicity," "pathogenicity factors," "pathogenicity elements," "aggressive factors," "markers of bacterial persistence," *etc*. We should mention that in the publications devoted to the plague pathogen, until recently "determinants of virulence" predominated. The popularity of this term can be explained by the following factors. The importance of the study of the molecular mechanisms of pathogenesis and immunogenesis of plague is emphasized in one form or another by all the authors in the introductory sections of their works devoted to microbiology, biochemistry, or immunogenicity of the plague microbe, but, unfortunately, in the overwhelming majority of cases, the bulk of the review literature is a conglomerate of information describing primarily individual plasmids of *Y. pestis* and some of the pathogenicity factors and antigens encoded by them and/or chromosomal factors and antigens (Table 3). Actually, in the course of evolution, in the *Y. pestis* genome [228], just as in the genomes of other pathogenic bacteria [22], dynamic structures were formed that provide for reversible functional changes in the genome, determining the regulation of the expression of a whole series of genes. In this formulation of the question, actually it is correct to speak of "determinants" or "virulence genes." However, in contrast to viral infections, in bacterial diseases of animals the genome of the microbe does not interact directly with the host's body; instead products encoded by it interact with the host. The course and outcome of the illness is not determined by the site of localization of the "virulence genes" and their expression; therefore, it would be more correct to speak of "virulence factors," subdivided under this concept not only into biomolecules, organelles, and systems of the bacterium responsible for implementing the pathogenic properties but also various factors and primarily the immune status of the macroorganism, essential for the implementation of these properties of the microbe. As is well known, virulence is only the degree of pathogenicity of a concrete strain with respect to animals of a definite species (or even a definite population) under standard conditions of natural or artificial infection. Virulence depends on numerous conditions: the immune status of the host, the conditions of maintenance and nutrition of the animal, on the mode of infection (subcutaneous, cutaneous, intracerebral, *etc*.) and even the time of infection [99]; therefore, any biomolecules, organelles, and systems of the bacterium, damage to which leads to a decrease in the viability even of nonpathogenic microbes, should be classified as bacterial "virulence factors. A pathogenic microorganism includes a whole series of genes determining a complex of properties essential for the initiation and development of

Table 3

Distribution of Certain Genes on Plasmids and the Chromosome of *Y. pestis* (compiled on the basis of the data of [154, 168, 169, 231, Sanger Centre for Genome Research: http://www.sanger.ac.uk/Projects/y. *pestis*])

Genes	Localization	Encoded trait
caf1	Plasmid pFra	Capsule antigen FI
ymt	Plasmid pFra	"Murine" toxin
Genes of the Yop virulon	Plasmid pCad	Yop virulon — the only system permitting extracellular yersiniae to counteract the nonspecific immune response on account of an impairment of phagocytic and signaling activity of macrophages and induction of apoptosis of phagocytic cells
pla	Plasmid pPst	Plasminogen activator
pst and *pim*	Plasmid pPst	Bacteriocin — pesticin (Pst) and immunity to it, ensuring stable inheritance of the plasmid pPst
mob	Plasmid pPst	Provision for mobilization of the plasmid pPst for conjugal transfer
psaABE	Chromosome	pH 6 antigens
"Housekeeping" genes	Chromosome	Provision for vital functions

the infection process. Part of these genes code for classical pathogenicity factors, such as adhesins, capsules, toxins, *etc*. But full-valued functioning of any bacterial cell requires the expression of so-called "housekeeping" genes – genes of vitally important functions. They include genes providing for the work of the transport systems that carry out the import of nutrients, such as trace elements, vitamins, sugars, *etc*., the ability for *de novo* synthesis biomolecules (including purines and aromatic amino acids) necessary for construction of the chromosome and other cell organelles, and mechanisms responsible for respiration, cell division, and repair [44, 115, 175,201,206,269]. Full-valued expression of the "housekeeping" genes can play the deciding role in the adaptation of a pathogen to the host organism and therefore may be essential for survival and manifestation of virulence. As was correctly noted by L. N. Klassovskii and V. S. Petrov [69], "any loss by bacteria of the ability to synthesize" or obtain "a compound necessary for cell development, which is absent in the host organism or present in an insufficient amount, should or in any case may lead to a loss in virulence." In V. M. Stepanov's opinion [115], factors of nutrition can affect the virulence of *Y. pestis* "in two ways: on the one hand, they provide conditions for the synthesis or appearance of the determinants of virulence, and on the other, by limiting the reproduction of cells possessing all the determinants or promoting their reproduction, they determine the possibility of manifestation of virulence of a pathogen."

Attempts to classify the properties determining differences of pathogenic and non-disease-causing bacteria have been undertaken repeatedly. Back in 1964, I. N. Morgunov [94] noted that "until a comparatively recent time, the virulence of microbes was explained by the presence of their invasiveness, aggressiveness, and infectiousness. They reflect the ability to penetrate into an organism, to suppress nonspecific protective mechanisms, and to develop (reproduce) in it. But these attempts explain essentially nothing." The rapid development of the methodology of molecular biology provided the possibility of determining the complete nucleotide sequence of the genome of individual microorganisms; the appearance of PCR technology and biochips, redevelopment of new methods of cell immunology, the wide use of physical methods of studying the structural and functional organization of individual biomolecules, *etc*. have made it possible to reevaluate bacterial pathogenicity factors. The experimental data obtained using modem approaches lay at the basis of the modem classifications of bacterial factors providing for the pathogenicity of microorganisms [21, 100, 186], their ability for persistence in the host organism [26, 27, 97], or various forms of adaptation of pathogenic bacteria to an ecological niche [51]. Taking these classifications into consideration, the pathogenicity of *Y. pestis* can be subdivided into a minimum of eight groups (Table 4).

Table 4 Pathogenicity Factors of the Plague

Pathogen

1.1. Spreading factors that promote generalization of infection: Pla, YopM, neuraminidase, facultative intracellular parasitization, and resistance to the bactericidal action of serum
1.2. Adhesive activity: pH6, Pla, and FI
1.3. Factors preventing initiation of the host's immune response and/or preventing opsonization
1.3.1. Shielding of LPS: Its own capsule or formation of a capsule-like layer around the cell from the host's biomolecules
1.3.2. Antigenic mimicry
1.3.3. Formation of L-forms
1.3.4. Factors promoting inactivation of cellular elements or humoral factors of the immune system: FI, Caf1A, Ymt, V, Yops, Pla, pH6, neuraminidase, and yersiniabactin
1.4. Protection of bacteria from capture by intact host phagocytes: Capsule, adhesins, Yops, neuraminidase, pH6, FI
1.5. Intracellular parasitization
1.5.1. Persistence within macrophages: Catalase, superoxide dismutase, peroxidase, FI, Ymt, adenylate cyclase, pH6, proteins of S-layer, R-form of LPS
1.5.2. Penetration into cells incapable of phagocytosis
1.6. Resistance to the bactericidal action of serum LPS
1.7. Antigenic variability: Capsule, V-antigen
1.8. Switching of an immune response to a "false" target: FI, Pla, pH6
1.9. Factors determining the development of infectious toxic shock: LPS, Ymt

Note. Caf1 (FI, F1) — capsular antigen fraction I; fraction I, the major component of the capsule formed at 37°C; Caf1A — the "usher" protein of capsular antigen fraction I assembly; pH6 (PsaA) — an antigen that forms adhesion pili of the plague pathogen (pH six antigen) at the temperature 37°C and pH values ≤ 6; Pla — plasminogen activator, which possesses proteolytic properties and determines the fibrinolytic (37°C) and plasmocoagulase (28°C) activities of the plague pathogen (plasminogen activator Pla protease); V (LcrV) — V-antigen (low-calcium response V antigen) — a polyfunctional regulatory and effector protein, formed at 37°C under conditions of the absence of calcium or nucleotides in the nutrient medium; Ymt (Tox, FII) — "murine" toxin (*Yersinia* murine toxin, "fraction II"), maximum production of which is noted at 28°C; Yops — outer membrane proteins (*Yersinia* outer membrane proteins), formed at 37°C under conditions of the absence of calcium ions or nucleotides in the nutrient medium.

1.1. Factors for Spreading that Promote Generalization of Infection

Earlier the term "invasiveness" was used to denote the ability of microbes to penetrate and spread in the host's tissues and organs, overcoming the protective mechanisms of the macroorganism. The ability to penetrate into nonphagocytizing cells, for example, into epithelial cells, was considered as a particular case of invasiveness and was more often called "penetration" [92,99]. However, recently most microbiologists have subsumed precisely the ability to penetrate into epithelial and endothelial cells, providing specialized bacterial invasins to enteropathogenic representatives of the genus *Yersinia* (Inv), *Escherichia coli* (Inv), *Listeria monocytogenes* (InlA), *Shigella flexneri* (IpaB-D), and *Salmonella* Typhimurium (SipB-D) under invasiveness [186]; therefore, a word combination — the

ability to promote generalization of infection — will be used in the context of this publication to denote the ability to penetrate and spread in the host's tissues and organs.

The protease Pla, which determines the ability of the plague microbe to lyse fibrin clots that hinder its spread, is customarily considered as the major factor of *Y. pestis* promoting generalization of the infection process after transfer by blocked fleas or subcutaneous infection [150, 152, 154, 257, 263]. In pathological anatomical investigations, "a complete or almost complete absence of fibrin" is noted in the organs of animals and people who have died of plague. This also suggested that plasminogen activator "is not only involved in local fibrinolysis in foci of infection but also plays the role of a "spreading factor" [52].

The YopM protein disrupts the interaction of thrombin with thrombocytes and hinders their aggregation, essential for the formation of a blood clot; it inhibits platelet activation, which leads to a cessation of the formation of inflammation mediators and prevents the development of a local inflammatory reaction [215, 238, 258].

The neuraminidase detected in *Y. pestis* is a generally recognized spreading factor [33, 92].

Facultative intracellular parasitization (see section 1.5) and resistance to the bacterial action of serum (see section 1.6) promote the spread of *Y. pestis* bacteria with the flow of lymph and blood.

1.2. Adhesive Activity

The adhesion pili of *Y. pestis* [35, 36] are formed by the pH6 antigen [219] and are responsible for the agglutination of erythrocytes [148]. These pili are capable of binding fibronectin, mucin, and ganglioside [37]. Isolated in pure form from *E. coli* cells containing cloned *psa* genes, the pH6 antigen bound to gangliotetraosylceramide (GM1A), gangliotriosylceramide (GM2A), and lactosylceramide (LS), and also exhibited the ability to attach to hydroxylated galactosylceramidase. The pH6 antigen, present on the surface of undamaged *E. coli* cells, possessed the same properties as the purified antigen, with the exception of binding to nonhydroxylated galactosylceramide. The observed binding profile indicated that the presence of β_1-bound galactosyl residues in glycophospholipids is the necessary minimum for binding of the pH6 antigen [229].

The high-molecular-weight capsule antigen possessed hemagglutinating activity on account of the ability to bind specifically to D-galactosamine-HCl and glucuronic acid [111]; it formed a weak bond to fibrinogen and fibrin [56].

An analysis of the complete genome of *Y. pestis* revealed the presence of eight more systems organized similarly to the operons *psa* and *caf1*, each of which is potentially capable of promoting biogenesis of the pili adhesins [228].

Transfer of the *pla* locus, which codes for the production of plasminogen activator, to *E. coli* cells imparted adhesive activity to the latter with respect to a number of eukaryotic cells [207] on account of the ability of the plasminogen activator to bind to the extracellular matrix [212, 213].

The S-layer protein in a concentration of 8-16 μg/ml agglutinated rabbit erythrocytes; this agglutination was inhibited by 0.1 M sugars — rhamnose (by a factor of 4) and dulcitol (by a factor of 8). It did not react with human, sheep, and guinea pig erythrocytes. The S^+ cells had an index of specific binding to fibrinogen 3-4 times higher than the S^- cells [57].

Until recently it was customarily considered that the plague microbe has lost the ability to synthesize adhesin/invasin Ail on account of the insert IS*285* [154]; however, in the genome of the virulent strain CO92, this gene is undamaged [228].

1.3. Factors Preventing Initiation of the Host's Immune Response and/or Preventing Opsonization

It is customarily considered that the peptidoglycan of the bacterial cell wall should be shielded or "blocked," so as not to induce the host's immune response. This is associated with the fact that the peptidoglycan is an immunologic target, since it is absent in eukaryotes. We should mention that in gram-negative bacteria the peptidoglycan is "covered" by the outer membrane, the upper layer of which contains a complex amphipathic biopolymer, LPS, which, like the peptidoglycan, possesses a pronounced ability to induce an alternative pathway of complement activation [44, 59].

For this reason, the pathogenic bacterium can be retained in the host organism only after implementing the protection of LPS and/or peptidoglycan on account of supplementary shielding structures, or by the production of specialized factors directed against the protective mechanisms of the organism, or by "antigenic mimicry." Finally, a simple loss of these components of the cell wall by the bacterium during the formation of L-forms is possible [26].

1.3.1. Shielding of LPS

Cells of *Y. pestis* at 37°C are capable of forming a capsule [245, 252], formed at pH values from 7.0 to 8.0 by the capsule antigen FI [9, 169]. In investigations utilizing scanning electron microscopy T. Chen and S. Elberg [165] showed that the capsule antigen forms a granular layer on the surface of the bacteria, which gradually diffuses into the surrounding medium. Combined use of the immunoferritin method and electron microscopy permitted A. G. Zolotarev *et al.* [64] to confirm these data. On the electron micrographs, the capsule is represented in the form of tears in the fibrillar material (diameter of individual fibrils about 3 nm) [68] or the intracellular fibrillar matrix [74, 165]. However, in most cases the *Y. pestis* capsule was determined as an amorphous substance, and only at sufficient magnification could elements of a honeycomb structure and individual "fimbria-like strands" with a length of the order of 0.25-15 nm, diverging in different directions from the surface of the bacterium, be seen on the photographs [74, 111]. It is interesting that the capsules of certain enterotoxigenic strains of *E. coli* or strains capable of causing septicemia were formed by the protein antigen CS31A, which represents fimbria 2 nm in diameter [191], while the structural subunit of these pili — ClpG — is phylogenetically related to the structural subunit of the capsular antigen Caf1 [147].

Plasmocoagulating ability may be a factor that to some degree shields the cell wall of the bacterium through the formation of a fibrin clot around the microbial cell [48, 52, 178].

The pH6 antigen (antigen I, antigen 4, PsaA, or adhesion pili) binds the 500 kDa apolipoprotein B100 from nonimmune blood serum of humans and animals [166] and reacts with the Fc subunits of human immunoglobulins of subclasses G1, G2, and G3 [137, 272], which may lead to the formation of a capsule-like layer, covering the surface of the bacterium. Transfer of the operon *psa*, which codes for its formation, to *E. coli* cells gave them the ability to form a capsule consisting of the pH6 antigen [9, 133]. According to the data of immunoelectron microscopy, the cells of the plague pathogen, grown at 37°C and pH values of the medium around 6.0, were covered with a capsule-like polymer structure, similar to the capsule formed by the capsule antigen FI [9, 167]; the antigen FI was present in such capsules only in trace amounts in this case [9, 10].

An analysis of the capsule-forming ability at 37°C and pH values from 7.0 to 8.0 *in vitro* for sixteen natural FI^- and $F1^{\pm}$ isolates of *Y. pestis* showed that the populations of all these strains contained from 10 to 97 percent of cells with capsules distinctly visible under a light microscope. According to the data of immunoelectron microscopy, the substance of such atypical capsules did not include the FI and pH6 antigens, but in a number of cases S-layer proteins were one of the major components. These data were confirmed by electrophoresis of the total proteins of the atypical capsules in polyacrylamide gel with 0.1% SDS and immunoblotting using polyclonal monospecific rabbit antibodies against the FI and pH6 antigens and S-layer proteins. The "proteinograms" of the proteins of the atypical capsules of various strains of the plague microbe, grown under the same conditions, differed appreciably from one another. Most of the major and minor proteins contained in the atypical capsules have not been identified [9, 10].

An ability of the S-layer proteins to protect the bacterium from the action of complement has been established [57].

1.3.2. Antigenic Mimicry

Antigenic similarity of *Y. pestis* cells to erythrocytes [23,42,62] and tissues of the human heart [42], guinea pig spleen and liver [62], has been detected. The 45 kDa and evidently the 14 kDa proteins of *Y. pestis,* encoded by the plasmid pCad, are cross-reacting antigens, common to the plague pathogen and human liver and spleen cells [34]. In the tissues of rodents with different natural resistance to plague, differing spectra of mimicry antigens with *Y. pestis* have been detected in a number of immune reactions [18].

1.3.3. Formation of L-forms

The possibility of isolation of *Y. pestis* in the L-form from rodents in natural plague foci [65], and also transition to the L-form in the course of long-term laboratory storage [79] has been demonstrated.

1.3.4. Factors Capable of Inactivating Cellular Elements or Humoral Factors of the Immune System

The capsular or envelope antigen "fraction I" exhausts the complement system on account of selective activity of the C'2 and C'4 components of the complement system of human blood serum and thus prevents the

complement-mediated opsonization of the bacteria [265]. In the case of its prolonged action on macrophages (for 36 h), a pronounced cytopathic effect was detected [117], possibly on account of the ability to form pores permeable to water in the double-layered phospholipid membranes of the macrophages [240]. The capsule antigen is capable of inhibiting the activity of the receptor apparatus of guinea pig helper T cells, presumably by exerting an immunoparalytic effect [29, 30]. It was established that in the bodies of mice after parenteral administration of derivatives of the attenuated *S. typhimurium* strain SL3261, which carry recombinant plasmids with the complete *caf1* operon, which cannot be stably inherited *in vitro*, there is a selection of cells that retain and express the genes responsible for production of FI. Only recombinant FI^+ cells could be inoculated out of the liver and spleen of animals that died on the first to seventh days after infection [192, 260].

The state of functional activity of peritoneal leukocytes of various species of gerbils, subjected to the action of FI, was correlated with the sensitivity of these animals to infection by plague [67].

V. P. Zav'yalov *et al.* [271] detected a high affinity of the molecular usher Caf1A, which is a component of the secretory apparatus of the structural subunits of the capsule antigen FI - Caf1 and provides for attachment on the cell surface of the capsule formed from them, not only to the structural subunit of the capsule antigen but also to human interleukin 1β. On the basis of these experimental data it was suggested that FI may compete with interleukins 1α, 1β, and 1γ for binding to receptors on the lymphoid cells, preventing the development of an adequate immune response. In turn, the molecular usher Caf1A, in their opinion, may adsorb interleukins, preventing contact of the latter with macrophages. On the model of cells of the continuous mouse fibroblast line NIH 3T3 it was shown that a dimer of the *Y. pestis* capsule protein Caf1 competes with interleukin rHuIL-1β for common receptors on the immunocompetent cells [2].

Ymt has a lethal effect on mice and rats, but not on other hosts [150]; it inhibits the oxidation-reduction processes in heart and liver mitochondria of sensitive animals [156], functioning as an adrenergic antagonist [149]; it leads to a decrease in the efficiency of phosphorylation of high-molecular-weight proteins in peritoneal leukocytes of white mice [14]; it possesses phospholipase [93, 104, 177, 246], phosphatase, autokinase, phosphodiesterase, deaminase, and NAD-glycohydrase activities [93]; it exhibits cytokine-inducing activity [31]; its superantigenic properties are manifested at low concentrations (10^{-6} g/ml) — under conditions of a sharp decrease in the synthesis at the temperature 37°C [93].

The V-antigen inhibits chemotaxis of neutrophils [264], suppresses the synthesis of γ-interferon and tumor necrosis factor α — cytokines necessary for nonspecific activation of professional phagocytes and the formation of productive granulomas [223], on account of a stimulation of the production of interleukin 10 — a repressor of the cytokines indicated above [225].

The outer membrane proteins (Yops) are encoded by the plasmid pCad, the presence of which in the cell is essential for the manifestation of virulence of the plague pathogen [71,234]. At the present time the complex of traits encoded by the plasmid pCad is considered as the Yop virulon— the only system permitting extracellular bacteria to disarm the cells involved in the host's immune response, disrupt their binding, and induce their apoptosis by injection of bacterial effector proteins. This system consists of the Yops proteins and the apparatus of their type III secretion, called Ysc. The Ysc apparatus consists of 25 proteins, including secretin. Most of the Yops proteins can be divided into two groups according to their functions. Some of them are intracellular effectors (YopE, YopH, YpkA/YopO, YopP/YopJ, YopM, YopT), whereas others (YopB, YopD, LcrV) form the translocation apparatus (injectosome), which is formed on the surface on the bacterium to deliver the effectors into eukaryotic cells through the plasma membrane. The secretion of Yops proteins is triggered by contact with eukaryotic cells and is controlled by proteins of the virulon, including YopN, TyeA, and LcrG, which cover the bacterial secretory channel, presumably in the form of a trapdoor. Precise functioning of the system also requires chaperones, called Syc proteins, which are present in the bacterial cytosol. Transcription of the genes is controlled by the temperature and activity of the secretion apparatus [168, 169].

It is believed that YopM is capable of influencing the inflammatory process, by decreasing the amount of thrombin and preventing platelet aggregation [215,238,258]. YpkA/YopO possesses Ser/Thr phosphokinase activity [189]. YopH and YopE inhibit phagocytosis [242, 243]. YopH dephosphorylates cellular structures of the host on account of the tyrosine phosphatase activity [194], while YopE possesses cytotoxicity on account of the ability to depolymerize the network of actin microfilaments of the phagocytic cell [244]. It has been suggested that the antiphagocytic activity of YopE is associated with its lectin properties, namely, with the ability to bind specifically to N-acetylglucosamine, thereby preventing interaction of lysozyme with the oligosaccharide residue on the *Y. pestis* surface and lysis of the bacterial cell wall [182].

The plasminogen activator is responsible for the fibrinolytic activity of *Y. pestis;* it is capable of hydrolyzing the C3 component of complement [257] and such cytokines as tumor necrosis factor *α,* interferon, *γ*, interleukin-8, monocyte chemotaxis protein 1 [251]; it has been suggested that it has Ig-protease activity [248]. It has also been established that Pla provides for post-translational degradation of Yops proteins [256]. This function of plasminogen activator, in the opinion of V. Kutyrev *et al.* [211], may be responsible for the ability of *Y. pestis* to cause acute illness by cleaning out necrotic damages to tissues of the macroorganism, removing Yops proteins that have penetrated not into eukaryotic cells but into the intercellular space, where they may be recognized as foreign proteins. This hypothesis is supported by data on the fact that pathological morphological changes in the organs of mice infected with the pseudotuberculosis pathogen, phylogenetically related to the plague microbe, but not producing the Pla protease, was manifested in the form of abscesses surrounded by professional phagocytes. The *Y. pestis*, on the contrary, induce the formation of necrotic nodules, entirely devoid of traces of inflammation [224, 261]. There is a well-known hypothesis that precisely the acquisition of the plasmid pPst, which codes for the production of plasminogen activator, became the basic stage of evolution of *Y. pseudotuberculosis* into *Y. pestis* [257]. O. Sodeinde *et al.* [257] also noted negligible signs of inflammation at the site of introduction of the wild-type strain *Y. pestis* KIM10 in comparison with pronounced infiltration by neutrophils in response to infection with its isogenic *pla* mutant. However, we should mention that transfer of derivatives of the plasmid pPst with an intact *pla* gene to *Y. pseudotuberculosis* cells led to a 6.4-31.3 fold decrease in virulence [211], while the "wild-type" strain *Y. pestis* CO92 and its $pPst^-$ variant induced an equally pronounced inflammatory reaction [263].

The pH6 antigen possesses cytotoxicity with respect to peritoneal [148] and alveolar macrophages [118], leads to a decrease in the number of antibody-forming cells, inhibits the reaction of mitogen-dependent blast transformation of spleen cells and the reaction of a mixed culture of lymphocytes, and suppresses growth of cells in a system of interleukin-2-dependent proliferation of T-blasts [40].

Neuraminidase possesses pronounced cytotoxicity with respect to peritoneal macrophages of mice and guinea pigs [92].

It has been suggested that the siderophore yersiniabactin can regulate the activity of cells of the host's immune system [144, 180].

1.4. Protection of Bacteria from Capture by Intact Host Phagocytes

Pathogenic bacteria use four different mechanisms for protection from capture by professional phagocytes of the host. Three of them — motility, capsule formation, and adhesion to surfaces in places relatively inaccessible to the host's leukocytes — are directed toward avoiding contact with phagocytic cells. The fourth, on the contrary, is offensive. It consists of the production of cytotoxins that produce rapid death of the host phagocytes [153]. In view of the absence of motility, the plague pathogen utilizes only three of the indicated approaches.

The capsule formed from FI protects *Y. pestis* cells from capture by intact phagocytes of the macroorganism [156, 162, 171, 205]. Recombinant *E. coli* cells, capable of forming the capsule of *Y. pestis,* were resistant to phagocytosis by mouse peritoneal macrophages [187].

Factors providing for the adhesive activity of *Y. pestis* were discussed in section 1.2.

When a bacterium comes in contact with phagocytic cells, the virulon Yop is "turned on," which leads to translocation of the effector proteins Yops into the cytoplasm of the eukaryotic cells and death of the latter [168,169]. Cytotoxic activity has also been detected for neuraminidase [92], and the antigens pH6 [118, 148] and FI [240].

A more detailed acquaintance with the antiphagocytic properties of pathogenic yersiniae can be obtained in the review publication by L. M. Kukleva *et al.* [78].

1.5. Intracellular Parasitization

An intracellular localization prevents contact of microbes with cellular elements and humoral factors of the host's immune system [222].

1.5.1. Survival Within Macrophages

Y. pestis cells are capable of surviving and reproducing in monocytes of sensitive animals [162]. Various enzymes may be responsible for inactivation of components of the oxygen-dependent bactericidal system [151]. Thus, antigen 5, responsible for catalase activity [150, 152, 156] exceeding that of a whole series of other bacteria [157],

has been detected in the plague pathogen. It has been shown that the high catalase activity of *Y. pestis* may be correlated with virulence [239], but there are also data contradicting this observation [45, 157]. The superoxide dismutase activity of *Y. pestis* was 2-10 times as high at the host's temperature (37°C) than at 28°C [53, 136]. Peroxidase of the plague microbe has also been detected and characterized [89]. The recent publication of E. Garcia *et al.* [190] described the cloning and sequencing of the *katY* gene, as well as the isolation, purification, and study of the physicochemical properties of the product of this gene — KatY (antigen 5), which possesses significant homology to the catalase-peroxidases of other enterobacteria.

Other factors are also responsible for ensuring the intracellular survival of the plague pathogen. Thus, highly purified preparations of FI inhibit the completion of phagocytosis of plague bacteria by macrophages [77,107]. The same inhibiting effect is exhibited by preparations of FI isolated from *Y. pestis* cells and from the recombinant strain *E. coli* HB101pFS2, in the phagocytosis both of zymosan and of the vaccine strain EV [117]. Preparations of purified FI antigen in a wide range of doses (from 10 to 100 μ/ml) significantly inhibited the chemiluminescence of peritoneal mouse macrophages in the phagocytosis of various particles: latex, zymosan, or formalin-killed staphylococci. The more active the macrophages were, the more strongly their oxygen metabolism was suppressed. This antigen exerted the same effect on human neutrophils and monocytes [39, 119]. It was also shown that the FI antigen intensified the oxidative burst of peritoneal leukocytes of mice, but suppressed the activity of guinea pig leukocytes [14].

Ymt suppressed the bactericidal oxidative burst of peritoneal leukocytes of mice [14].

The extracellular form of adenylate cyclase of the plague pathogen was involved in changing the nature of the metabolic oxidative burst at the first stages of interaction of the phagocyte and bacteria [92].

The pH6 antigen induced a disruption of the digestive function of macrophages [36].

The presence of a protein forming an S-layer on the surface of the cells was correlated with resistance of the bacteria to the digestive activity of phagocytic cells [57].

YopE can compete with lysozyme for binding to the receptor (N-acetylglucosamine) on the surface of *Y. pestis* cells, thereby preventing digestion of the bacteria within phagolysosomes [182].

It is known that the reproduction of *Y. pestis* cells inside macrophages is a necessary step in plague pathogenesis [162]. The virulence of the plague microbe is correlated not with resistance to capture by phagocytes [205] but with the ability to survive and reproduce in phagolysosomes of phagocytic cells on account of a suppression of their antibacterial functions [29, 30, 75, 77, 134, 164]. Indirect evidence of obligate intracellular parasitization by the plague pathogen is also provided by the putative mechanism of resistance of FI variants of *Y. pestis* to a number of antibiotics *in vivo* [142], discussed in section 3.2. In histological investigations of tissues of the bodies of animals and humans who died of plague, *Y. pestis* cells found inside the host phagocytes are detected [88, 171]. It has also been shown that the LPS of *Y. pestis* is a weak inducer of interieuktn-6 and tumor necrosis factor α [235], which is also characteristic of other intracellular parasites [160,273]. It has been established that *Y. pestis* surpasses *Y. pseudotuberculosis* and *Y. enterocolitica* in resistance to the action of bactericidal cationic peptides, which are among the components of the bactericidal system of phagocytic cells of vertebrate animals. Restoration of resistance was correlated with an increase in the degree of reduction of LPS. The total absence of negatively charged polysaccharide O-side chains in the structure of *Y. pestis* was accompanied by minimal ability of the bacteria to adsorb cationic peptides [146].

Recently some researchers, under the impression of success in the study of the type III secretion system encoded by the plasmid pCad, which is responsible for aggression of extracellular *Y. pestis* with respect to macrophages, have been inclined, if not to entirely deny the significance of the intracellular stage of the plague pathogen, then to delimit it only to the initial stage of the infection process in the case of the bubonic form of plague [168, 169]; here they ignore the investigations cited above, conducted on a model of *Y. pestis,* based on an electron microscopic study of the pathological morphology of experimental pseudotuberculosis [253] and intestinal yersiniosis [198,199,216]. In our opinion, the plague microbe is capable both of extra- and of intracellular existence. Selection of the concrete "scenario" depends on the current conditions that have developed in the interactions of the parasite and host. Reproduction of the portion of the pathogen population that was under the most favorable conditions occurs at the maximum rate, and a redistribution of the bacteria between cells and the intercellular medium of the host may occur in the course of the infection process.

1.5.2. Penetration into Cells Incapable of Phagocytosis

For a long time it was believed that the plague pathogen is noninvasive for epithelial cells, but recently C.

Cowan *et al.* [170] have established that the presence of the plasmid pPst, which determines the formation of plasminogen activator, in *Y. pestis* cells provides the bacteria with high invasiveness with respect to HeLa cells. In the opinion of the authors of the publication, when *Y. pestis* enters the body of a warm-blooded host by respiratory or alimentary routes, precisely this ability permits it to penetrate into cells of the mucous membranes of the digestive tract and respiratory tract, to reproduce in them, and then to induce a generalized infection. This hypothesis is indirectly supported by the decrease in virulence of $pPst^-$ variants of the plague pathogen in comparison with the initial strains in the case of alimentary infection of mice [72] or respiratory infection of guinea pigs [9, 248].

At the temperature 37°C, the invasive activity of *Y. pestis* cells was sharply reduced, which, in the opinion of C. Cowan *et al.* [170], may be associated with formation of a capsule consisting of Caf1 subunits at this temperature.

K. Lähteenmäki *et al.* [213] have shown that transfer of the *pla* gene to *E. coli* XL1 cells gave the bacteria the ability to invade human epithelial cells ECV304. The invasive activity of Pla was unchanged by substitutions of amino acid residues S99 and D206, essential for the manifestation of proteolytic activity.

The presence of an intact gene of the invasin *ail* and three other genes, which code for Ail-like proteins (YPO1850, YPO2190, YPO2506), has been demonstrated in the genome of the virulent strain *Y. pestis* CO92 [228].

1.6. Resistance to the Bactericidal Action of Serum

In the overwhelming majority of virulent and avirulent strains of the plague microbe, LPS is present in the R-form [12] and determines resistance to the bactericidal action of serum, i.e., to lysis of the bacterial cells by the complement system, and, just as in other pathogenic bacteria [233], "regardless of whether this bactericidal property depends on normal or immune antibodies" [54].

According to the data of N. A. Gvozdenko *et al.* [41], strains of *Y. pestis* subsp. *caucasica,* in contrast to the major subspecies, prove entirely sensitive to the action of "normal" human serum. The introduction of iron preparations (hemoglobin or ferritin) into the serum reduce the bactericidal effect.

1.7. Antigenic Variability

One of the most labile properties of the plague pathogen is synthesis of the capsule antigen FI. This trait is directly dependent on the nature of the epizootic process: as the epizootic dies down, there is an increase in the number of cultures with reduced ability to synthesize the capsular antigen, until it is entirely lost. This is correlated with the increase in the number of serum positive animals with respect to FI [106, 110, 123]. The wide "immunization" of sensitive animals in the course of the plague epizootic leads to the formation of long-term bacteria carrying among them, which can give rise an acute infection process long after [102,123]. Table 5 presents summary data on the isolation of such strains in various natural foci of plague in the CIS.

As is well known, the natural variability of pathogens of infectious diseases that circulate in nature and primarily the interrelationship of the variation of antigenic structure of pathogens and their ability to overcome the collective population immunity of the hosts lies at the basis of the self-regulation of parasitic systems [16]. Even a cursory analysis of the literature data shows that variability with respect to one definite antigen makes a difference for the development of the epidemic process in different carriers and, correspondingly, in different natural foci.

Thus, immunization of great gerbils with FI^+ strains of *Y. pestis* created permanent immunity to their infection by FI^+ bacteria and weak immunity to infection by FI^- bacteria. Immunization with FI^- variants increase the resistance of the great gerbils to infection by FI^- strains without affecting their sensitivity to FI^+ strains [76]. When intact great gerbils were infected with FI^+ strains, the animals died of generalized infection on the fifth to ninth days, and FI^+ cultures were isolated. Death in periods later than one month or sacrifice of the animals at the same times, as a rule, led to the detection of FI^- forms of the plague pathogen in abscesses that formed at cites of injection of the initial FI^+ culture [105]. The isolation of FI^+ or FI^- bacteria alternated depending on the phase of the epizootic and was correlated primarily with fluctuations of the percent of zero positive great gerbils with respect to FI in the population [106].

In experiments on the infection with virulent nonisogeneic strains of FI^+ or FI^- of *Y. pestis* of Altai marmots surviving after preliminary infection with the same strains, it was shown that "capsuleless microbes provided practically no protection from death as a result of infection with the capsule strain, whereas capsule microbes provided weak protection. Both capsule and capsuleless microbes possessed pronounced immunogenic properties with respect to the capsuleless strain ..." [28]. We should mention that there is no information in the literature available to us on

Table 5

Natural Plague Foci of the CIS in Which FI^- Strains of *Y. pestis* have been Isolated (compiled on the basis of original certificates of strains from the Russian collection of pathogenic bacteria "Microbe" and literature sources available to us; epidemic significance of foci are indicated according to the data of A. M. Kokushkin [72])

Name of region	Sources of isolation	Epidemic significance of foci
Caucasus	Burrow fleas of *Ceraiophyltus resquorum,* fleas of the common vole	Low
Foci of the Northern Caspian	Lesser suslik, greater suslik, fleas	High
Central Asian desert	Great gerbil, midday gerbil, fleas, steppe polecat	Medium
Central Asian mountains and Siberia	No data on isolation	Medium

the isolation of FI^- strains from marmots and their ectoparasites.

Site-directed mutagenesis of the *caf1* operon of *Y. pestis* in combination with complex use of a set of immunochemical, biochemical, and biophysical methods, light and electron microscopy, has established that the formation of an atypical capsule, differing from the "wild-type" organelle in antigenicity, protectiveness, hydrophobicity, and electrokinetic potential [4, 6, 7, 9, 11] and similar to the atypical capsules of natural FI^- and $FI^\pm$ isolates of *Y. pestis,* occurs in cells defective for synthesis of the chaperone Caf1M (see section 1.3.1). Strains of *Y. pestis* with atypical capsules possessed selective advantages in mice preliminarily immunized with "wild-type" strains or with the "classic" capsular antigen. Protein preparations of the atypical capsule possessed weak protective activity with respect to homologous strains of the plague pathogen. In a pathomorphological investigation of the internal organs of intact and immune mice that died as a result of infection with strains of the plague pathogen with an atypical capsule, morphological signs of acute infection were detected, corresponding to those in the case of infection with the original "wild-type" strain [9, 11, 91].

Until recently it was customarily considered that the antigenic variability of *Y. pestis* is associated only with a different degree of expression of immunogenic factors (primarily the FI antigen), but not with variability of the actual antigenic epitopes of the protective proteins. However, it has recently been shown that in "vole" strains of *Y. pestis* the V-antigen is represented by the variant characteristic of *Y. enterocolitica* O:3 serovar [268]. Both serovars of the V-antigen detected in the plague pathogen possessed pronounced protective activity with respect to strains with the homologous variant of the V-antigen but did not ensure protection of the animals in the case of infection with *Y. pestis* strains of a different serovar [193, 241].

1.8. Switching of an Immune Response to a "False" Target

To explain the death of individual immunized animals possessing high titers of antibodies against FI, G. Andrews *et al.* [141 have suggested that the FI antigen secreted by the bacteria into the surrounding medium binds significant amounts of immunoglobulins, thereby preventing opsonization of the bacteria. In their opinion, a "scaling off" from the capsule fragments of antibodies already attached to them is also possible.

It has been established that Pla provides for posttranslational degradation of Yops [256]. The fragments of Yops "scaled off" from the bacteria may bind a large portion of the antibodies, preventing their interaction directly with *Y. pestis* cells.

Experiments on active immunization of laboratory animals with preparations of the pH6 antigen provide evidence of the absence of its protective properties in the presence of a pronounced ability to induce antibody formation [38, 118,132]. Probably the absence of protectiveness is associated with peculiarities of the pH regulation of this antigen. The formation of PsaA by *Y. pestis* cells inside phagolysosomes of the macrophages or in the center of necrotically degenerated tissues of abscesses [36, 218, 219] makes $PsaA^+$ bacterial cells inaccessible to contact

with antibodies and immunocompetent cells. Correspondingly, the induction of an immune response is like a "blank shot" switching of the immune system toward an unachievable goal exhausting its resources.

1.9. Factors Determining the Development of Infectious Toxic Shock

It is generally recognized that "the basis of plague pathogenesis is the action of the lipopolysaccharide of the pathogen on polymorphonuclear leukocytes. In the case of activation by endotoxin, followed by degranulation of neutrophils, a number of biologically active substances are released, which trigger the system of the postaggression oscillatory reaction, its disharmonization, and the development" of infectious toxic shock, while "plague pathogenesis itself in any form is essentially a stage of development" of infectious toxic shock [46]. In *Y. pestis* cells, in addition to the endotoxin the protein exotoxin Ymt, which possesses selective toxicity with respect to mice and rats (*Muridae*) but not guinea pigs, is formed [150,231]. However, in the opinion of V. I. Tynyanova *et al.* [125], "plague bacteria have one toxin, which belongs to the class of complex toxins and represents a glycolipoprotein complex. It contains a minimum of two functional units": Ymt and LPS. The formation of the toxic complex occurs under the action of a low molecular weight glycolipid "with pronounced polarity" present in the host organism, and non-lethal doses of these toxins, simultaneously injected into mice or guinea pigs, led to death of the animals.

2. FACTORS ENSURING REVERSIBLE TRANSITION TO PARTIALLY ATTENUATED FORMS, WHICH CAUSE A CHRONIC COURSE OF THE INFECTION

The adaptational variability of bacteria is most pronounced at the population level. One of the main characteristics of natural populations of microorganisms is their heterogeneity and dynamic variability. A population existing in a changing environment should react to it, neutralize its influences, and thereby ensure survival for its constituent elements. The degree of heterogeneity of a population of microorganisms, the rate and range of variations in its structure depend on the species of microbe, its isolation from other populations, and the pressure of selection factors [16]. As was noted above, "turnoff" of any of the pathogenicity factors or a disruption of the expression of the "housekeeping" genes either may not influence the virulence of the microbe or may lead to its attenuation [5, 9, 54, 92, 155, 156, 176,186,226]. If such attenuation is reversible and is accompanied by a change in the antigenic composition of the bacterium (see section 1.7), by a transition of *Y. pestis* cells and to the L-form (see section 1.3) or into unculturable forms (see sections 6.2.3), then the infection may have a protracted course in a persistent form, turning into a generalized illness in the long-term periods, promoting further transmission of the plague pathogen to new hosts.

Thus, the overwhelming majority of publications describe a sharp decrease in the virulence of FI^- strains of *Y. pestis* with respect to guinea pigs [28, 81, 82, 84, 90, 110, 123, 155, 156, 262] and rats [85, 267]. In the southern Balkhash region in 1963-1964, T. P. Kudinova [76] isolated and characterized FI^- strains of *Y. pestis* avirulent for white mice, rats, and guinea pigs, weakly virulent for midday gerbils, red-tailed jirds, and crested gerbils, but virulent for great gerbils. In 1964-1965, N. K. Kunitsa [79] tested their virulence for mice and guinea pigs and established that "they were highly virulent and caused death of the animals in doses from 10 cfu and higher at period from 60-90 days with isolation of the bacterial form of plague," while retaining the FI^- phenotype. During the course of a subsequent 30-year storage, the strains indicated above were converted to the L-form, which caused the development of a persistent infection, leading to death of mice after periods from 8 days to 291 days.

What factors of the plague pathogen might determine the reversible transition to partially attenuated forms, which cause a chronic course of infection? As is well known, a population of bacteria undergoes changes caused by the action of external and internal factors, which influence the rate of population variability. The internal factors include the frequency of formation of mutations, as well as the growth rate and viability of cells of the mutants and of the parent strain under concrete conditions [24]. In addition to the usual relatively rare and random mutations, "a significant fraction of the hereditary variability is caused by basic genetic structures - jumping genes": transposons, IS elements, and certain bacteriophages and plasmids capable of independent replication and of integration into the chromosome [131]. The mobile genetic elements are capable of being incorporated into various genes or close-lying regions and being precisely excised from them, participating actively in negative and positive regulation of the virulence of pathogenic bacteria on account of the formation of terminator and promoter sequences, leading to turnon, turnoff, or a change in expression of the target genes, and also on account of the insertion mutations induced by these genes and secondary rearrangements of the deletion type [221].

2.1. Intragenomic Rearrangements Due to IS Elements or Bacteriophages

It has been established that 3.7% of the genome of the plague pathogen is accounted for by IS elements [228]: IS*100* [130, 185, 234], IS*285* [126, 130, 185], and an IS*200*-like element (IS*1541*) [254]; the number of their copies and their localization in the chromosomes and plasmids of different strains vary [20, 234, 254]. In the chromosome of the strain CO92, 66 complete or partial copies of IS*1541*, 44 of IS*100*, 21 of IS*285*, and 9 of IS*1661* have been isolated [229]. It has been shown that these IS elements are capable of inducing mutations of calcium independence [185, 234]. In pFra plasmids from "wild-type" strains of *Y. pestis* 231 and 358, as well as the vaccine strain EV, resident copies of IS*285* were localized in direct proximity to the operon of capsule formation (281 bp from the stop codon of the *caf1R* gene) [19]. IS-mediated rearrangements of the *caf1* operon, leading to the phenotype $FI^{\pm}$, have been detected [20]. It was established that the $FI^{\pm}$ strain M-493 carries a deletion in ninth HindIII fragment of the pFra plasmid, with a length of about 2 kb, including a unique BamHI restriction site, situated in the *caf1M* gene [130,236]. This deletion "starts" at the end of IS*285* and covers the *caf1R* and *caf1M* genes [128, 130]. Questions associated with the structure of the IS elements of pathogenic yersiniae, their distribution, localization in the genomes, as well as their involvement in the regulation of virulence, and the formation of larger potentially mobile structures – transposons and pathogenicity islands – are discussed in detail in the review article by A. A. Filippov [129].

Virulent and temperate phages specific for *Y. pestis* have been isolated from the plague microbe, from the bodies of rodent plague carriers, from fleas, from laboratory animals infected with plague, and from people who have recovered from plague [135, 232]. Their influence on the variability of the plague pathogen has been shown, and it has been suggested that they play a role in the self-regulating system of the plague enzootic [86, 98].

2.2. Reversible Integration of Plasmids with the Chromosome

The ability of *Y. pestis* to cause an acute lethal illness sharply distinguishes this microbe from the enteropathogenic yersiniae related to it – *Y. pseudotuberculosis* and *Y. enterocolitica,* which are the cause of subacute or chronic processes, which, as a rule, end in recovery. Evidently primarily these differences are determined by the fact that the plague pathogen acquired two additional plasmids in the course of evolution (pPst and pFra) and also lost the ability to produce a number of metabolic enzymes, the adhesin YadA, the invasin Inv, *etc*. [152, 154, 228]. Strains both lacking individual plasmids and carrying supplementary replicons or plasmids with a changed molecular mass have been isolated from various natural foci, together with typical strains of the plague pathogen, which carry the plasmids pPst, pCad, and pFra [15, 72, 127, 130, 184]. The possibility of reversible integration of the plasmid pFra into various portions of the *Y. pestis* chromosome has been shown. In the opinion of A. A. Filippov [130], IS elements are involved in the cointegration of the replicons of *Y. pestis*. In a number of cases, integration of plasmids may be accompanied by a cessation of expression of the genes situated on these plasmids and by a decrease in the virulence of the bacteria [72, 103, 187, 231, 236, 262]. In natural foci of the northern Caspian region, up to 10 percent of strains in which one or several plasmids are integrated into the chromosome are isolated; in a number of cases this was accompanied by a loss of expression of the genes localized on them. These data served as a basis for suggesting that "the mechanism of integration of intrinsic plasmids into the chromosome and their emergence into an autonomous state can serve as a regulator of the activity of epizootic manifestations" [72]. In the opinion of A. Friedlander *et al*. [187], the decrease in virulence of FI^{-} strains carrying the plasmid pFra in an integrated state in a number of cases may be associated with the fact that this integration has an influence on the expression of other pathogenicity factors. An analysis of clones from lyophilized cultures and cultures stored on Hottinger's agar showed that in 10-30 percent of the cases clones that had lost the plasmid pFra appeared; an increase in the molecular mass of this plasmid was detected in 8-25 percent of the clones, whereas 8-10 percent of the clones had a replicon with molecular mass greater than that of the "wild-type" pFra plasmid in addition to the three plasmids. Passage of a heterogenous agar culture through the body of a mouse led to elimination of the atypical clones from the population of isolates of the plague microbe [103].

3. FACTORS PROVIDING FOR ANTIBIOTIC RESISTANCE

Several cases of isolation of antibiotic-resistant strains of *Y. pestis* in natural foci of Mongolia, Madagascar, and South Vietnam have been described [12, 188, 266]. The rarity of isolation of such variants of the plague pathogen in natural foci is explained by I. V. Domaradaskii [50] by two factors. In the first place, plague in humans is relatively rarely encountered at present, and as a rule, has an acute course. In second place, plague is classified

as a "blood" infection, in which infection of a person occurs primarily by a flea-borne pathway, reducing the probability of contact of the plague pathogen with the natural reservoir of R-plasmids.

3.1. Conjugative R-plasmids

A natural isolate of *Y pestis* 17/95, carrying the conjugative plasmid pIP1202, determining resistance to eight antibacterials recommended for therapy and emergency prophylaxis of plague, was first isolated in 1995 in Madagascar from a 16-year-old boy with the bubonic form of the disease [188]. In discussing this case, D. T. Dennis and J. M. Hughes [172] raise a whole series of questions.

Is this finding a single case or does the observed phenomenon of multiple drug resistance have a definite distribution in natural populations of the plague pathogen? In most diagnostic laboratories, according to their data, the sensitivity of freshly isolated strains of *Y. pestis* to antibacterials usually is not determined, which may be the cause of the absence of other reports on the isolation of multidrugresistant strains of the plague pathogen. Did transfer of the conjugative plasmid to *Y pestis* cells from a multidrugresistant organism in the patients intestinal tract occur during the course of the illness? Is a determination of drug resistance being conducted on strains of *Y. pestis* isolated from fleas and rodents? Do multidrugresistant forms of enterobacteria circulate in rat populations on Madagascar? Rats are everywhere; consequently, the high probability of acquisition of the R-plasmid by *Y. pestis* cells in the population of rats inhabiting environs contaminated by multidrugresistant human fecal microflora is high. Are there any selective advantages of the *Y. pestis* strain with multiple drug resistance in natural plague foci?

The elucidation of the mechanisms of the origin and evolution of the observed multidrugresistant, as well as the probability of spread of such strains in nature, in the accurate opinion of D. T. Dennis and J. M. Hughes [172], determine the epidemiological significance of the "Madagascar finding."

3.2. Absence of FI Production

E. D. Samokhodkina *et al.* [249, 250] have shown that treatment of experimental plague caused by non-capsulated strains of *Y. pestis* proved ineffective with the use of tetracycline, β-lactam antibiotics, and quinolones in moderate therapeutic doses, which have a pronounced therapeutic effect with respect to the infection caused by "wild-type" strains. Analogous antibiotic resistance has also been observed *in vitro* in a culture of macrophages but not on nutrient media. No explanation of this phenomenon is presented in the indicated publications.

It has been shown that *Y. pestis* cells are capable of surviving and reproducing in the phagolysosomes of macrophages [162]. At the same time, it is known that the antibiotics indicated above are incapable of penetrating into phagocytes in the active form [247]. More likely the capsule of *Y. pestis* may influence the level of penetration of these antibiotics through the plasma membrane of macrophages.

The antigen FI, which forms the capsule of *Y. pestis,* is capable of being incorporated into bilayer phospholipid membranes, forming pores in them that are permeable to water. In the opinion of C. Rodrigues *et al.* [240], this lies at the basis of cytopathic action of *Y. pestis* on phagocytic cells. It is also known that after treatment with channel-forming toxins, cell membranes become permeable to a whole series of molecules [206]. Considering all that has been stated above, we can assume a priori that *in vivo* the "water" pores induced by FI may be the main cause of penetration of antibiotics into the macrophage and its phagolysosomes and thereby ensure sensitivity of the "wild-type" *Y. pestis* cells to antibiotics [142].

4. FACTORS RESPONSIBLE FOR NUTRIENT REQUIREMENTS

Growth and reproduction of microorganisms requires nutrient substances — sources of energy and plastic materials. Depending on their role in metabo1ic processes, nutrients are customarily subdivided into hydrogen donors, hydrogen acceptors, carbon sources, nitrogen sources, and minerals. Some microbes, having received these nutrient components, are capable of independently synthesizing all the organic constituent elements of the bacterial cell. Bacteria that lack some biosynthetic processes, however, must receive the end products of these processes — "growth factors" necessary for the synthesis of more complex organic compounds — from outside as off-the-shelf components. The growth factors include vitamins, amino acids, purine and pyrimidine bases, *etc*. The absence or deficiency of a growth factor in the medium inhibits the growth and reproduction of the microorganisms. If, however, microorganisms are capable of synthesizing the end products but can receive them from the surrounding medium, as a rule, they utilize exogenous growth factors, and endogenous synthesis is repressed [195].

T. Lucier and R. Brubaker [220] have noted that the size of the chromosome of the generally recognized obligate parasite *Y. pestis,* which consists of 4208.4 kb, constitutes 89.5% of the size of the genome of *E. coli,* which possesses substantially greater metabolic activity, and only 71.3% of that of *Pseudomonas aeruginosa* — a saprophyte capable of existing in various ecological niches. We should mention that according to the sequence data, the chromosome of the strain *Y. pestis* CO92 consists of ~ 4653.7 kb, and that of the three resident plasmids — 96.2, 70.3, and 9.6 kb [228].

Let us take up in more detail some peculiarities of the nutrition of the plague pathogen. We should take special note of the fact that systems that provide for the nutrient requirements of microorganisms must be differentiated from the intrinsic pathogenicity factors. We should mention that T. Burrows [156] has already isolated factors of nutrition into a separate group of virulence factors of *Y. pestis.* The metabolism of the plague microbe is discussed in detail in the review publications [50, 52, 152, 154].

4.1. Cytotoxins That Provide Access of Extracellular Bacteria to the Nutrient-Rich Cytoplasm of Destroyed Eukaryotic Cells

This method of providing for nutrient requirements is implemented primarily through the action of the effector proteins Yops [153]. Cytotoxic activity has also been detected for neuraminidase [92] and the antigens pH6 [118, 148] and FI [240].

The plague microbe possesses hemolytic properties and lyses the erythrocytes of certain species of animals (horse, rabbit, and guinea pig) [109]. Hemolytic activity provides the *Y. pestis* bacteria with an additional source of iron in the form of hemoglobin from the destroyed erythrocytes. It appears at temperatures of 37°C and higher but not at 28°C and is determined by the *pla* gene of the plasmid pPst [25].

4.2. Systems Providing for the Accumulation and Uptake of Iron and Manganese by *Y. pestis* Cells

Until recently, in a whole series of publications [72, 82, 101, 112,181, 200], products of the chromosomal locus denoted as HPI (high-pathogenicity island) have been classified as "necessary" pathogenicity factors of the plague microbe, just as, moreover, for other representatives of the genus *Yersinia* as well. A total loss of this "island" leads to a significant decrease in the virulence of HPI^- mutants with respect to mice [204]. The expression of genes localized in it is manifested in the Pgm^+ phenotype, responsible for satisfaction of the nutrient requirements of *Y. pestis* cells for iron on account of two different mechanisms [231].

The first of them is determined by a siderophore — "yersiniabactin." In our opinion, its recognition as one of the major pathogenicity factors is erroneous and was based on the fact that mutations on the *irp2*, *psn*, and *ybtE* genes, leading to a disruption of the biosynthesis or transport of yersiniabactin, are accompanied by a loss of virulence [231]. However, iron is just as necessary a nutrient component for all pro- and eukaryotic cells, and at physiological pH values, "iron salts (Fe^{3+}) form an almost insoluble ferric hydroxide $Fe(OH)_3$" [112]. To assimilate the negligible portion of the iron that is still present in the dissolved state, both in the mammalian organism and in microorganisms, special iron-binding proteins — siderophores — are synthesized. They are not unique for pathogenic bacteria, since they have been found in virtually all the investigative representatives of the prokaryote kingdom [112, 186]. We should mention that siderophore activity has been detected only for "highly pathogenic" yersiniae, capable of causing the death of mice infected with low infecting doses: *Y. pestis, Y. pseudotuberculosis,* and *Y. enterocolitica* of biotype IB (the "New World" strains), but has not been determined in low-pathogenic biovars II—V of *Y. enterocolitica* ("Old World" strains) [16]. However, HPI have also been detected in other representatives of the family *Enterobacteriaceae*, often HPI^+ strains of *E. coli* are isolated from the excretions of healthy humans, and they constitute about 20 percent of the isolates from the external environment [237]. In our opinion, the production of siderophores cannot be considered as a pathogenicity factor, but the genes coding for their formation should be classified as "housekeeping" genes. An analogous viewpoint is confirmed by A. Rakin *et al.* [237]. They note that although HPI contains a cluster of genes associated with "high lethality" for mice of "highly pathogenic" strains of yersiniae containing this locus, not one determinant directly associated with pathogenicity has been detected on the "island." The genes situated on HPI, on the other hand, are more likely responsible for the reproduction of the pathogens in the macroorganism than for direct damage to the eukaryotic cells, i.e., they are the factor ensuring adaptation of the bacteria to the body of the warm-blooded host.

The second mechanism is determined by a cluster of *hms* genes and provides for adsorption of hemin on the surface of the microbial cell. We should emphasize that in other microorganisms (with the exception of *Y.*

pseudotuberculosis), structural analogs of none of the four proteins encoded by this locus have as yet been detected [231], whereas functionally analogous iron-binding proteins have also been observed in other pathogenic bacteria [186]. It has been shown that the selective absence of expression only of the genes responsible for the trait of pigment formation and localized in this "island" does not lead to a decrease in the virulence of Hms^- mutants of the plague pathogen [80, 82, 83, 210, 217].

It is interesting that the recently discovered transport system "necessary for complete virulence," providing for the passage of iron and manganese into *Y. pestis* cells [145], is still unique; the HmuTUV system, necessary for the use of hemin, hemin-albumin, myoglobin, but not hemoglobin, hemoglobin-haptoglobin, or heme-hemopexin as iron sources, does not play a vital role in the development of infection in mice [259].

An analysis of the genome of the strain *Y. pestis* CO92 revealed several more iron uptake systems: three partially functional operons of siderophore biosynthesis, five systems of siderophore uptake, and one siderophore-independent heme uptake system [228].

4.3. Pesticin Provides Selective Advantages to *Y. pestis* Cells in Competition with Other Pathogenic Yersiniae Possessing Similar Mechanisms of Uptake of Inorganic Iron and Hemin

Y. pestis produces bacteriocin — a pesticin that hydrolyzes the peptidoglycan of sensitive bacteria [183]. The spectrum of its activity is limited to several atypical strains of *E. coli,* serovars IA and IB of *Y. pseudotuberculosis,* serovar O:8 of *Y. enterocolitica,* and non-pesticinogenic but Pgm^+ variants of *Y. pestis* [152]. The relationship between sensitivity to pesticin and the Pgm^+ phenotype is explained by the fact that the surface receptor for pesticin Psn is simultaneously a receptor for the iron-yersiniabactin complex, i.e., a component of the system of siderophore-dependent iron uptake [231]. Evidently pesticin provides selective advantages to *Y. pestis* cells in competition with other pathogenic representatives of the genus *Yersinia,* possessing analogous HPI [237] and, correspondingly, similar mechanisms of uptake of inorganic iron and hemin [230].

4.4. Ability to Stop Endogenous Synthesis of a Number of Amino Acids and Significantly Increase Their Uptake from the Culture Medium at 37°C

The factors responsible for the nutrient requirements of *Y. pestis* in the body of a warm-blooded host, in addition to iron uptake, should include the ability of the plague microbe to stop endogenous synthesis of a number of amino acids (valine, isoleucine, cysteine) at 37°C [13,49] and significantly increase the uptake of amino acids from the culture medium [95]. This should substantially reduce its energy expenditures, since it has been shown on a model of *E. coli* that the synthesis of one molecule of histidine requires 41 molecules of ATP, whereas transport of the ready-made amino acid into the cell takes only 1-2 molecules of ATP [140]. The ability to synthesize aromatic amino acids should also be assigned to this category of factors, since they are not synthesized in mammalian cells [101]. It is interesting that *aroA* mutants of the plague pathogen, avirulent for guinea pigs, retained virulence for mice at the level of the parent strain, although the lifetimes of the animals that died were increased by approximately two days [226].

In concluding the section devoted to factors of *Y. pestis* ensuring survival in the host organism, we should cite R. Brubaker [154]. He notes that from the time of discovery of the plague pathogen by A. Yersin in 1894 [270] up to the 1980s, this acute infectious disease was usually considered as a consequence of continuous and uncompromising conflict between the host and parasite. However, in his opinion, the results of the latest investigations provide evidence that the symptoms of the disease to a large degree reflect the use of "stealth" and "deception" tactics, which prompt the host to ignore the lethal danger of the developing generalized infection. In this context, the term "stealth" means the ability of yersiniae that have come in contact with eukaryotic cells for direct transfer of cytotoxins to the host cytoplasm, using the system of type III protein secretion, which allows these proteins to avoid interacting with the immune system. The word "deception" means the ability of *Y. pestis* to suppress the inflammatory reaction by inhibiting the production of the cytokines that initiate it. We should mention that in a consideration of *Y. pestis* factors responsible for survival in the host organism, R. Brubaker divides them into only two groups of virulence factors: essential for dissemination and essential for vegetative growth in organs.

You can learn about the most interesting (in our opinion) attempts to reproduce possible scenarios of the pathogenic process in play on the basis of the modern concepts of *Y. pestis* in the review publications [78,154, 231].

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