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REVIEW ARTICLE

Endosymbiosis and its significance in dermatology

K. Kubiak,[1] H. Sielawa,[1] W. Chen,[2,*] E. Dzika[1]

[1]Department of Medical Biology, Faculty of Health Sciences, Warmia and Mazury University, Olsztyn, Poland
[2]Department of Dermatology and Allergy, Technische Universität München, Munich, Germany
*Correspondence: W. Chen. E-mail: wenchieh65.chen@gmail.com

Abstract

Proposed at the beginning of the twentieth century to explain the origin of eukaryotic organelles from prokaryotes, endosymbiosis is now medically defined by various interaction patterns between microorganisms and their residing hosts, best exemplified by the bacterial endosymbiont *Wolbachia* identified in arthropods and filarial nematodes, which can influence normal development, reproduction, survival and transmission of the hosts. Based on the transmission modes, vertical or horizontal, and the function of the endosymbionts, the host–symbiont dependence can be divided into primary or secondary. In dermatology, the role of endosymbionts in skin ectoparasitosis has aroused great interests in the past years. *Riesia pediculicola* is a primary bacterial endosymbiont in body lice *Pediculus humanus*, and supplement their hosts with vitamin B, especially pantothenic acid. In cimicosis, the Gram-negative *Wolbachia* can synthesize biotin and riboflavin, which are crucial for the growth and reproduction of the bedbug *Cimex lectularius*. In human demodicosis and rosacea, further study is required to prove the pathogenic role of the Gram-negative bacteria *Bacillus oleronius* or the Gram-positive bacteria *Bacillus cereus* demonstrated in the *Demodex* mites. The high infection rate of adult female ticks *Ixodes ricinus* with the Gram-negative bacteria *Midichloria mitochondrii* present in the mitochondria in diverse ovarian cells, with the high seroprevalence rate in tick-exposed subjects, raises the possibility that this non-pathogenic endosymbiont may play a role in immune response and successful transmission of the tick-borne pathogen. The anaerobic protozoan *Trichomonas vaginalis* and bacteria *Mycoplasma hominis* are two obligate parasites in the urogenital epithelium, with partially overlapping symptoms. Intracellular localization of *Mycoplasma hominis* can avoid host immune response and penetration of antibiotics, while *Trichomonas vaginalis* infected with *Mycoplasma hominis* seems to have a higher cytopathic activity and amoeboid transformation rate. Further study on the biology and pathogenesis of different endosymbionts in dermatological parasitosis will help for the development of new treatment modalities.

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Introduction

Endosymbiosis is a type of interaction between different species in which one symbiont is living within the tissues or cells of another organism (host). The endosymbiotic theory was first proposed by Russian botanist Konstantin Mereschkowski in 1905, then developed by American biologist Lynn Margulis in 1967 to explain the origin of chloroplasts, mitochondria and other organelles from bacterial endosymbionts.[1,2]

Nowadays, endosymbiosis is defined by various interaction patterns, including beneficial (mutualism), neutral (commensalism) and harmful (parasitism), between organisms from distinct domains (viruses, archaea, bacteria or eukaryotes). Their interactions can generate totally new combinations of biochemical capabilities and enable the two intertwined species to thrive in unfavourable environments.[3] The term endosymbionts normally refers to bacteria identified in a large variety of eukaryotes, best characterized in arthropods, particularly insects, and can be broadly divided into primary and secondary forms.[2,4] Primary endosymbionts are transmitted vertically from parents to their offspring and necessary for host reproduction.[4,5] Phylogenetic studies indicated that these relationships are often ancient. During the long co-evolutionary history with their hosts, primary endosymbiont genomes have undergone drastic size reduction through losing genes and pathways that are no longer necessary for the unique metabolic niches provided by the hosts.[3,6] Secondary or facultative endosymbionts are neither essential for

host survival nor required for host reproductive competency and can spread vertically or horizontally via vectors or through the environment. Their presence can be neutral, beneficial or detrimental to the host.[4,6] As compared to primary endosymbionts via simple transmission, they can manipulate the host reproduction to get transmitted to as more offspring as possible and benefit their hosts by providing absolute or conditional fitness.[5,7]

Bacterial endosymbiosis

The most widespread endosymbionts with varying life strategies according to the host group are bacteria from the genus *Wolbachia*. *Wolbachia* are maternally inherited intracellular α-proteobacteria of the Anaplasmataceae family.[8] The hosts of *Wolbachia* include numerous terrestrial arthropod species as well as filarial nematodes.[9] In most arthropods, *Wolbachia* exist as facultative endosymbiont and are not required for host survival. *Wolbachia* can also act as reproductive parasites and maintain an extraordinary ability to influence host reproduction or sex determination. They facilitate proliferation in infected females, ensuring their own vertical transmission to the next host generation by male killing, feminization, induction of asexual reproduction parthenogenesis, or cytoplasmic incompatibility.[7,10] In filarial nematodes, *Wolbachia* maintain a fixed obligate relationship with the hosts required for their development, fertility and survival.[7,11,12] *In vitro* and *in vivo* studies indicated that depletion of *Wolbachia* in filarial parasites by doxycycline treatment can kill adult worms and block embryogenesis, microfilariae output and worm development.[13]

The impact of *Wolbachia* on the productivity, viability and development time in some hosts makes *Wolbachia* the subject of intensive research regarding its potential microbial role as 'natural enemy', or as vector for spreading desirable genetic modifications in insect populations, and in the biocontrol of insect-borne parasitosis, such as malaria, dengue fever, Chikungunya, yellow fever and West Nile fever. *Wolbachia* also represent a promising new drug target and therapeutic strategy for filarial disease.[13–15]

Viral endosymbiosis

Viruses with mutualistic symbiotic relationships with their hosts have been very well documented in plants and insects, but also identified in fungi, mammals including humans and bacteria.[16–18] In contrast to bacterial endosymbionts, viruses are obligate endosymbionts and cannot replicate outside their hosts. Vertical transmission of viral endosymbionts is well recognized in insect hosts, and different to bacteria, viruses can be transmitted maternally as well as paternally, which allows them to rapidly invade and spread through the host populations, even when infection is costly to the host.[18]

Polydnaviruses (PDVs) of the endoparasitoid wasps are the most well studied mutualistic viral endosymbionts, in which the viral genes have integrated into the wasp genome. The successful development of the wasp eggs in the parasitized insects host and the survival of wasp offspring depends on the PDV genes expression, because PDVs suppress the host immune system, preventing host from killing wasp offspring.[16,19] In human, certain mutualistic viruses can attenuate viral and non-viral diseases, for example human non-pathogenic GB virus C (related to hepatitis C virus) or cytomegalovirus can slow down full-blown AIDS in HIV-1-positive patients.[20–22] Infection of lymphotropic virus in mouse could prevent type 1 diabetes,[23] while murine gammaherpesvirus 68 or murine cytomegalovirus protects infections from *Listeria monocytogenes* or *Yersinia pestis*.[24] It is demonstrated that the capsid of endosymbiotic *Leishmania* RNA virus 1 (LRV1), identified in the cytoplasm of several *Leishmania* species, is a promising vaccine component to reduce clinical complications in endemic areas with LRV1 co-infected *Leishmania* species.[25]

Endosymbiosis in dermatology

In the following, we will discuss the advance in the understanding of endosymbiosis and endosymbionts in certain human skin diseases to illustrate their roles in pathogenesis and potentials in treatment and disease control (Table 1).

Lice, *Riesia pediculicola* and pediculosis

Human pediculosis is caused by blood-sucking lice, including the head louse (*Pediculus humanus capitis)*, the closely related body louse (*Pediculus humanus corporis*) and the less in common pubic louse *Pthirus pubis*.[26] Many parasitic lice species harbour several different lineages of endosymbionts from γ-proteobacteria potentially engaged in nutritional provision.[27–30] The

Table 1 Examples of endosymbiosis in human skin diseases

Skin Disease	Arthropod/Vector	Endosymbionts	Relationship	Functions of Endosymbionts
Pediculosis	*Pediculus humanus capitis* *Pediculus humanus corporis*	Candidatus *Riesia pediculicola*	Primary	Pantothenic acid synthesis and supplementation
Cimicosis	*Cimex lectularius*	*Wolbachia spp.*	Primary	Biotin and riboflavin supplementation
Demodicosis	*Demodex folliculorum* *Demodex brevis*	*Bacillus oleronius* *Bacillus cereus*	Unclear	Unclear
Borreliosis	*Ixodes ricinus*	*Midichloria mitochondrii*	Unclear	Unclear
Trichomoniasis	*Trichomonas vaginalis*	*Mycoplasma hominis*	Unclear	A common arginine dihydrolase pathway

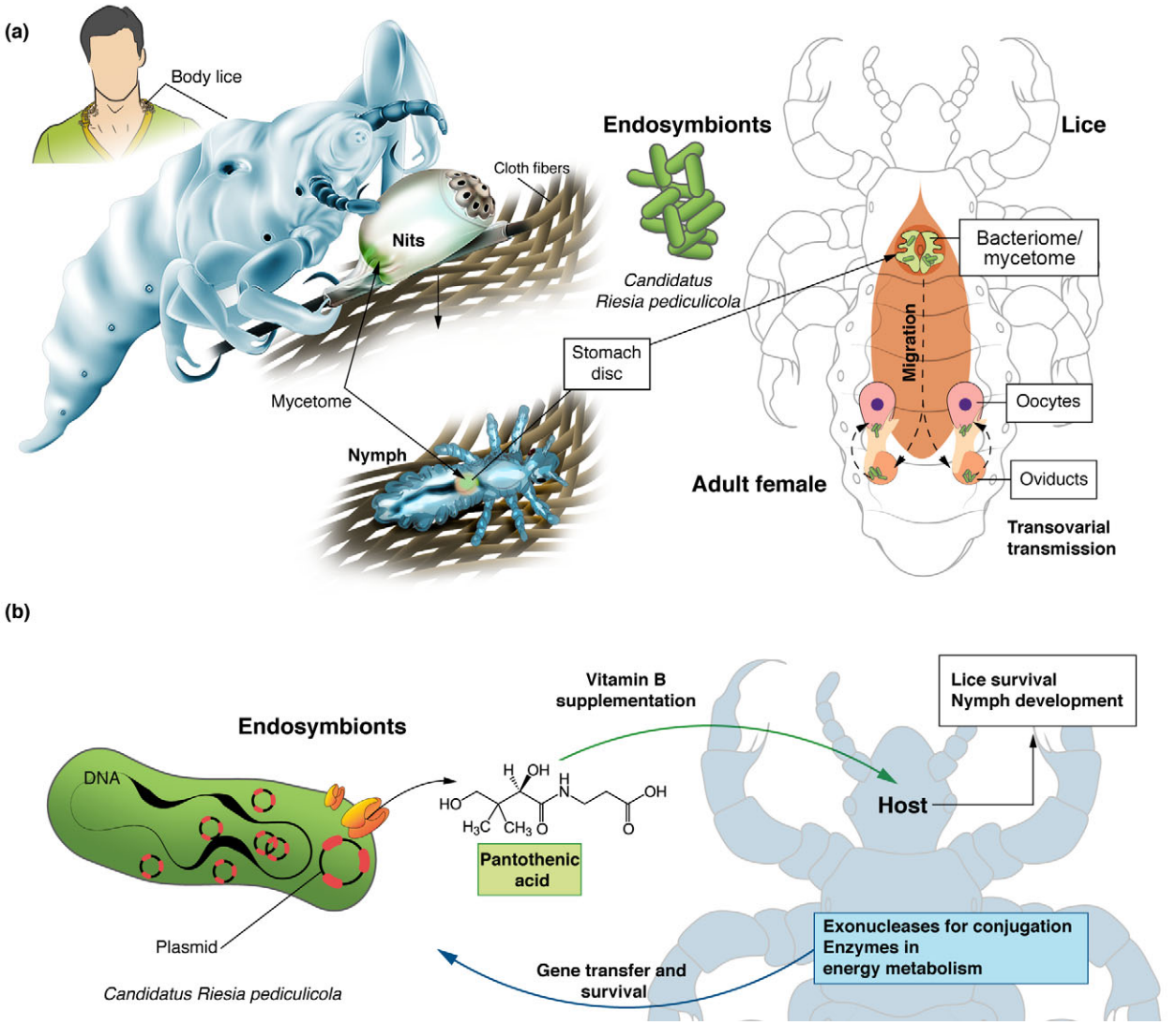

Figure 1 (a) *Candidatus Riesia pediculicola* is a primary endosymbiont of human body louse *Pediculus humanus humanus,* existing intracellularly in mycetome (bacteriobiome), which are visible as rings of enlarged midgut cells in the lice abdomen. Symbiosis between the two species is characterized by migration of the endosymbionts from the stomach disc in nymphal stages to the ovarial ampullae of adult females to infect the oocytes. (b) Endosymbionts are essential for host survival and growth, by providing vitamin B supplementation, especially pantothenic acid, which is lacking in the lice natural diet. The genes of the key enzymes for synthesis of pantothenic acid are located extrachromosomally on a multicopy plasmid to reduce the risk of genome degradation and to increase enzyme expression level, whereas the endosymbionts depend on the louse host for the lacking exonucleases required for conjugation and enzymes involved in energy metabolism.

better-known body lice primary endosymbiont is *Riesia pediculicola* (*Candidatus Riesia pediculicola*), a member of the family Enterobacteriaceae.[27,29] They are located intracellularly in specialized structures called bacteriome (mycetome), which are visible in the lice abdomen as a ring of enlarged midgut cells, and divided internally by a series of septa, with endosymbionts living in-between.[31–33] Symbiosis between *P. humanus* and *R. pediculicola* is characterized by sequential development of different mycetomal stages, with migration of the endosymbionts from the stomach disc in nymphal stages to the ovarial ampullae of female, causing infection of oocytes.[30,33,34] Removal of endosymbionts from human body lice demonstrated that they are essential for host survival and growth, because they supplement lice with vitamin B,[30,32] which is lacking in diet composed of blood, keratin-rich epidermal components and host secretions.[31] Pantothenic acid exerts the greatest effect on lice survival, and its

lack leads to the death of nymphs during their first moult.[35] The genes of three key enzymes for synthesis of pantothenic acid are located extrachromosomally on a multicopy plasmid, which can reduce the risk of genome degradation and increase expression level to secure sufficient acquirement of pantothenic acid. As compared with other endosymbionts, there are several genes unique to *Riesia* coding for transport and binding proteins as well as for enzymes involved in lipopolysaccharide biosynthesis. On the other hand, *Riesia* lacks exonucleases required for conjugation and enzymes involved in energy metabolism, which reflects its dependence on the louse host for nutrients.[36] The lack of antibiotic resistance genes in *Riesia* renders possible to target this obligatory endosymbiont in the design of louse-control strategies.

Bedbugs, *Wolbachia* and cimicosis

Growing concern over bedbugs *Cimex lectularius* has been raised since their sudden worldwide resurgence in the late 1990s, probably due to widespread resistance to insecticides, increased foreign travel, changing climatic conditions and extermination of their natural consumer cockroach.[37,38] They are haematophagous ectoparasite arthropods living only on vertebrate hosts by release of salivary proteins, like nitrophorin or apyrase, to increase blood flow.[39,40]

Symbiotic bacteria were first described in bedbugs in 1921 as a pair of specialized organs, now called bacteriomes, located intracellularly close to gonads in both sexes.[37,41] Three morphological forms were distinguished and localized in gut, Malpighian tubules, spermalege, ovaries, testes or eggs.[37,41,42] The suppressive effect of heat treatment on the microbial levels in bacteriomes, the oviposition and egg hatching rate led to the conclusion that proper bacterial load was required for host condition.[43] Molecular analysis of 16S rDNA gene identified *Wolbachia* as the dominant microorganisms in ovary tissue of *C. lectularius*.[44] Phylogenetically they are F monophyletic bacteria and also live in termites, weevils and filarial nematodes, with a more frequent and flexible evolution through horizontal transfer.[37,45] The prevalence of *Wolbachia* in *C. lectularius* was estimated at 75–100% in natural regions of North America, Africa and India,[46,47] and at 63% and 38.1% in urban areas of USA and France, respectively.[48,49] In transcriptome analysis, there are 59 presumed *Wolbachia* sequences in laboratory strains of bedbugs.[50]

Administration of blood meal added with antibiotics was found to completely remove or heavily reduce the bacteria-residing bedbugs.[51] Eggs laid by the tested specimens were characterized by considerably lower rate of development, deformations and darker colour, as compared to the controls. Nymphs treated with antibiotics displayed not only extended immature stage, but also significantly lower percentage of adults evolved. Experimental supplementation of blood meal with vitamin B caused opposite effects by restoring the normal development and number of eggs, shortening the nymph stage and increasing the adult emergence rate.[51] The existence of a whole operon encoding biotin synthesis in the genome of *Wolbachia* was most likely acquired via lateral gene transfer from unrelated but jointly infecting species like *Cardinium* or *Rickettsia,* which enables *Wolbachia* to evolve from facultative symbionts to obligate mutualists.[52] Riboflavin was also recognized as crucial agent for the growth and reproduction of *C. lectularius*, while the genes responsible for its metabolism, in contrast to biotin synthesis, were highly conserved and permanently maintained within *Wolbachia* genome during evolution.[53] In female bedbugs, *Wolbachia* can stimulate host condition via increasing egg hatching, body size of offspring and percentage of adult forms, as well as via reducing survival costs of traumatic insemination. In male bugs, *Wolbachia* can enhance their fitness and thus female oviposition rate after mating.[37]

Demodex mites, *Bacillus oleronius, Bacillus cereus* and demodicosis

Since its first identification by Jacob Henle in 1841 and classification as mite by Carl Gustav Theodor Simon in 1842, the role of human *Demodex* mites (*Demodex folliculorum* and *Demodex brevis*) in the pathogenesis of skin and ocular diseases remains incompletely understood.[54] As the only permanent human ectoparasite, its causal link to rosacea is still debating.[54] A new light thrown on this old controversy was the isolation of *Bacillus oleronius* from one *Demodex folliculorum* in the face of a patient with papulopustular rosacea in 2007.[55] *Bacillus cereus*, instead of *B. oleronius* or other bacillus species, was later identified in a patient with secondary demodicosis associated with steroid-induced rosacea-like facial dermatitis, but only in one positive culture.[56] *B. oleronius* is a sporing Gram-negative bacterium originally identified as endosymbiont from the hindgut of termite,[55] which does not have anus and needs to decompose organic substances by bacterial enzymes.[57] Two bacterial surface antigens (83 and 62 kDa) were found to have proliferation-stimulating activity on peripheral blood mononuclear cells and identified to be homologous to heat shock protein and protease engaged in carbohydrate metabolism and signal transduction, respectively. The serum reactivity against both antigens was confirmed in patients with facial rosacea and ocular rosacea.[57,58] Correlation between serum reactivity to *Bacillus* proteins, lower sebum level and higher *Demodex* count was shown in erythematotelangiectatic rosacea.[59] The *Demodex*-associated bacterial proteins could enhance neutrophil migration and release of metalloproteinase-9 (MMP-9), cathelicidin, interleukin 8 (IL-8) and tumour necrosis factor α (TNF-α) *in vitro*, which were implicated in the inflammation induction. It was supposed that metronidazole or tetracycline therapy could kill the bacteria to stop additional proteins release.[59,60]

In ocular rosacea, the exposure of corneal epithelial cells to *Bacillus* proteins caused not only increased secretion of MMP-9

and expression of MMP-3, but also decreased levels of β-integrin and vinculin, leading to improper wound healing and corneal ulcer formation.[61] Exposure of human telomerase-immortalized corneal epithelial cells to *Bacillus* proteins induced reduction in cell proliferation and increase in the expression of genes coding for IL-6, IL-1β, IL-8, TNF-α, defensins, CCL20 and S100A7.[62] In vitro study demonstrated the ability of *B. oleronius* proteins to activate neutrophils via the inositol 1,4,5-trisphosphate (IP3) pathway, and the activated neutrophils displayed increased levels of IP1 production, F-actin formation, chemotaxis and production of pro-inflammatory cytokines IL-1β and IL-6.[62] The sensitivity of *B. oleronius* to ciprofloxacin, doxycycline and gentamicin isolated from *Demodex* mites residing eyelashes in patients with blepharitis,[63] but the lack of correlation between blepharitis severity and intensity of mites infestation doubted the primary role of *B. oleronius* in the pathogenesis of certain blepharitis.

Ixodes ricinus, Midichloria mitochondrii and tick-borne diseases

Interactions of ticks with several different vertebrate hosts (mammals, birds and reptiles) during life cycle promote the opportunity to acquire a large range of various viruses, bacteria and protozoa present in the host blood, making ticks an excellent reservoir and vector.[64] In the northern temperate climate zones of the world, *Ixodes ricinus* plays the key role as reservoir and vector of a broad range of human pathogenic microorganisms, including bacteria *Borrelia burgdorferi sensu lato* complex, the causative agent of Lyme borreliosis, *Rickettsia* spp. and *Anaplasma* spp., the flavivirus responsible for tick-borne encephalitis and the protozoan agents for babesiosis.[64,65] Besides pathogenic agents, *Ixodes ricinus* harbours intracellular non-pathogenic bacteria, which are vertically transmitted in ticks in an obligate symbiotic association. Most of them (*Coxiella*-, *Francisella*- and *Rickettsia*-like) are phylogenetically close to the tick-transmitted pathogenic bacteria species, but they have not been isolated in pure culture.[66,67] Studies suggested their possible roles in the reproductive fitness and vectorial competence of ticks.[67–72]

One of the dominant bacterial endosymbionts is *Midichloria mitochondrii* (*Candidatus Midichloria mitochondrii*) in the class α-proteobacteria.[69,73,74] They are present in the mitochondria in diverse cell types of the ovary, including oocytes, both within the inner and outer membranes as well as in the cytoplasm, and have the unique ability to invade and destroy the mitochondrial matrix.[68,69,73,74] The interaction between *Midichloria mitochondrii* and the organelle is clearly parasitic, but it does not involve the whole mitochondrial population of the oocytes. A balanced equilibrium between the bacterial population and the infected mitochondria may exist under the control of host cells, which will not affect the reproduction and survival of the ticks.[68] In Europe and North Africa, 94–100% natural population of adult female *Ixodes ricinus* were infected with *Midichloria mitochondrii,* while DNA of this bacteria was detected only in 44% of the males.[73,75] High female-specific prevalence of *Midichloria mitochondrii* has no connection with the ability of bacteria to influence host reproduction, sex determination or the inequality between the sex ratios. An infected female *Ixodes ricinus* can give birth to both male and female larvae. All of female larvae and nymphs derived from a single engorged female infected, while no males contained the bacteria.[76] It is likely that when an infected egg develops into a female, the bacteria can continue to survive in ovarian tissues, but most or all of the bacteria will get lost, if the egg develops into a male. Even with a 100% transovarial transmission rate, the prevalence of *Midichloria mitochondrii* in long-term laboratory colonies of *Ixodes ricinus* was considerably lower, probably dependent on specific laboratory conditions like temperature and antibiotic treatment.[75] *Midichloria mitochondrii* has also been detected in the salivary glands of *Ixodes ricinus*. Seropositivity against *Midichloria mitochondrii* antigens was found in 58% of the patients parasitized by *Ixodes ricinus*, in contrast to only 1.2% of the healthy individuals.[77,78] Antibodies to *Midichloria mitochondrii* were also detected in other mammalian tick hosts.[77] The high seroprevalence recorded in tick-exposed subjects raises the possibility that *Midichloria mitochondrii* may play a role in the immune response and immunomodulation determined by the *Ixodes ricinus* saliva, which is important both for success of the tick blood meal and for establishment of the infection by the pathogens transmitted by the tick.[78] Useful approach to investigate the role of microbial endosymbionts in the vector fitness is through antibiotic administration.[79,80] Tetracycline treatment of engorged adult female *Ixodes ricinus* did not eliminate the *Midichloria mitochondria* population from the host ticks, but reduced the multiplication of endosymbionts in the progeny.[81] There is no evidence that *Midichloria mitochondrii* can replicate in human host and cause any pathological changes.

Trichomonads, *Mycoplasma hominis* and trichomoniasis

Trichomonas vaginalis, an anaerobic, flagellated protozoan, is an obligatory ectoparasite in mucous membranes of human urogenital tract inducing trichomoniasis, the most common sexually transmitted disease worldwide.[82] Comparison of vaginal flora in *T. vaginalis*-positive and *T. vaginalis*-negative women confirmed that the parasite occurrence was correlated with low percentage of protective *Lactobacillus spp.* but high proportion of *Mycoplasma*, in particular, *Mycoplasma hominis*.[83–86] *M. hominis*, one of the class *Mollicutes* without cell wall, is self-replicating and dependent on host metabolism.[87] It is a human opportunistic pathogen causing mycoplasmosis associated with spontaneous abortion, endometritis, postpartum fever and low birth weight.[82,88]

Little is known about the interaction between these two obligate parasites in the same anatomical region with partially overlapping symptoms. The endosymbiotic relationship between

M. hominis and *T. vaginalis* was presumed by the bacteria detection in protozoan cells, their isolation from patients of various geographic origin and a high level of infection up to 80%.[87] *M. hominis* was the only common genital microflora capable of entering, surviving and multiplying inside the *T. vaginalis* cells.[85,89] Transmission from infected to uninfected trichomonads as well as to epithelial cells suggested that *T. vaginalis* can function as a 'Trojan horse' during the disease process.[82,88,89] Intracellular localization of *M. hominis* avoids host immune response and antibiotic penetration.[82,85] Internalization of *M. hominis* through endocytosis has been demonstrated by their use of special cellular tip structures as an anchor, mycoplasma digestion in experimentally infected trichomonads, prolonged intracellular maintenance of mycoplasmas and their leaving from the vacuole to the cytosol in trichomonad cells.[90] *T. vaginalis* infected with *M. hominis* was observed to have a higher amoeboid transformation rate, phagocytic properties and cytopathic activity towards vaginal epithelial cells.[91] Mycoplasma-infected trichomonads can stimulate human macrophages inducing a pronounced upregulation of pro-inflammatory cytokines IL-1β, IL-23, IL-8 and TNF-α.[92] Intensification of inflammatory response through endosymbiosis of *M. hominis* in *T. vaginalis* can contribute to cancer development or HIV acquisition associated with parasitosis.[92] Both symbionts have a common arginine dihydrolase pathway as a primary energy source, which is used by *T. vaginalis* at 10% under anaerobic conditions.[82,87] The *T. vaginalis–M. hominis* consortium exhibited increase in arginine dihydrolase activity, substrate (arginine) utilization, intracellular production of ornithine, putrescine and ATP, as well as faster growth rate as compared to mycoplasma-free isolates.[82,92,93] This metabolic interaction between *T. vaginalis* and *M. hominis* seems to be fundamental in their endosymbiotic relationship, in which mycoplasma benefit from the intracellular localization and enrichment in putrescine, whereas trichomonads profit from high ATP levels and growth rate. An impaired host defence causes decreased nitric oxide synthesis by phagocytes due to arginine shortage.[82,87,92]

It is well-known that metronidazole and tinidazole are effective against *T. vaginalis* infection,[87] whereas the resistance of *M. hominis* to both antibiotics is noted.[82] However, association between *M. hominis* infection of *T. vaginalis* and metronidazole resistance could not be confirmed.[94] Further investigation is needed to clarify the pathogenic properties of *T. vaginalis–M. hominis* endosymbiotic relationship and to develop new therapeutic strategies.

Conclusions

Advances in the understanding of host–endosymbiont relationship and interaction have revealed new strategies for the treatment and control of diseases induced by arthropods or filarial nematodes. The re-examination of host–endosymbiont dependence in the pathogenesis of relevant skin diseases, such as pediculosis, cimicosis, demodicosis, borreliosis and trichomoniasis, will help us to better understand the medical as well as the evolutionary significance of these ancient human infectious diseases.

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