REVIEW
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Microbes, pathogens pathogenesis, and host folate metabolism: What is the connection?

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Abstract

Folate (FA) is an essential micronutrient of vitamin B group for growth, development, and reproduction through participating in the nucleotide synthesis and methyl donation reactions. The changes of FA level have been linked to dietary insufficiency (e.g., poor diet, etc.) malabsorption (e.g., FA-associated gene mutation, etc.), increased demand (e.g., pregnancy, etc). or medication (e.g., antifolates drugs), or bad habits (e.g., smoking, alcoholism, etc.). Recently, epidemiological data showed that the levels of the host FA typically changed in patients with infectious diseases. Interactions between pathogens, including bacteria, parasites and viruses, and their hosts are complex, in particular, pathogenic infection-mediated changes of the host FA levels can affect the utilization and uptake of limited FA resources of the host. Therefore, FA supplementation or the use of antifolate agents may be a potential antimicrobial strategy for managing infectious diseases. Furthermore, given that the gut microbiota is a primary source of FA in the human body, the association between gut microbiota and pathogenic infections warrants investigation. To date, little is known about how FA status and its biochemistry function affect the course of infectious diseases. In this review, we focus on the roles of FA in the interaction between the host and microbe, and briefly discuss the potential of FA and antifolates agents in the treatment of infectious diseases.

Keywords: folate; bacteria; parasites; viruses; infectious diseases

Author contributions

Yajie Qian, Jing Wu and Deyan Chen contributed to the conception and design of the current review article. Yajie Qian, Jing Wu and Ruining Lyu were responsible for drafting the manuscript. Hongji Tao help to draw the figures in the paper. Xiancheng Chen, Changyuan Yao, Fang Zhang and Waqas Nawaz performed the manuscript review and writing-review and editing. All authors contributed to the article and approved the submitted version.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

FA, folate; RFC, reduced folate carrier; FOLR, folate receptor; 1C, one-carbon; THF, tetrahydrofolate; DHF, dihydrofolate; DHFR, dihydrofolate reductase enzyme; MTHFR, methylene tetrahydrofolate reductase; *H. pylori*, *Helicobacter pylori*; *S. aureus*, *Staphylococcus aureus*; *Mtb*, *Mycobacterium tuberculosis*; BT1, biopterin transporters; *C. elegans*, *Caenorhabditis elegans*; *T. gondii*, *Toxoplasma gondii*; HSV-1, herpes simplex virus type 1; HPV, human papillomavirus; CIN, cervical intraepithelial neoplasia; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; BV, bacterial vaginosis; ZIKV, Zika virus.

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Introduction

Folate (FA) was discovered by Lucy Wills in 1930 and became known as "the Wills Factor" [1]. FA was subsequently isolated from spinach in 1941 and then identified as a member of vitamins named vitamin B9 [2]. Finally, the chemical formula of FA was determined to be $C_{19}H_{19}N_7O_6$ and folic acid was further synthesized industrially in 1945 [3]. Since then, researchers have tried to understand the roles of FA in human health and disease [4].

FA is an essential nutrient, mainly derived from exogenous supplies, which include foods and gut microbiota [5]. FA is mostly abundant in animal liver and kidney, mushrooms, spinach, yeast, green leaves, and grasses [6]; bacteria residing in the small intestine are also involved in FA de novo synthesis and genes implicated in FA biosynthesis have been found across 512 gastrointestinal microbial genomes, including *phyla Bacteroidetes*, *Fusobacteria*, *Proteobacteria*, and *Actinobacteria*, of which *Bifidobacterium* exhibits a high capacity for FA synthesis [7, 8]. FA is mainly absorbed in the proximal small intestine, which includes the duodenum and jejunum [9]. FA absorption is maximized at a pH between 5.5 and 6.5, and further transported to liver for maintaining critical live activities [10–12]. For the cellular uptake of FA, reduced FA carrier (RFC) and FA receptor (FOLR) are the main transport receptors mediating the extracellular to intracellular transport of FA [13–15]. RFCs are ubiquitous transporters, expressed in the liver, kidney, lung and small intestine, etc.; RFCs mediate bidirectional transport of FA according to the transmembrane anion gradient caused by organic phosphates and FA [16]. FOLRs, including three members: α, β, γ, are only detected in organs with high metabolic activity, such as placenta, kidney, choroid plexus, and neurons [17]. FOLRs have a high affinity to FA (dissociation constant, Kd was 1–10 nM, and KD was 12 ± 6 pg/mL) and mediate FA entry into cells through endocytosis [18, 19].

Members of the FA family are well known for their capacity to mediate one-carbon (1C) metabolism [20]. FA is initially reduced to tetrahydrofolate (THF); and then 1C derived from serine is transferred to THF to mediate the production of 5, 10-methylene THF; subsequently, dUMP is converted into dTMP by thymidylate synthase, which provides substrates for nucleic acid synthesis and repair [21]. Meanwhile, the dihydrofolate (DHF) generated by this reaction is converted into THF by dihydrofolate reductase enzyme (DHFR); moreover, methylene tetrahydrofolate reductase (MTHFR) involves in the reduction of 5, 10-methylene THF to 5-methyl THF, which is important for the conversion from homocysteine to methionine that is further converted into S-adenosylmethionine, which acts as a methyl donor to nucleic acid, protein, and others [22]. Nucleic acid synthesis and repair, as well as methylation modifications are vital activities for the maintenance of cellular survival [23]. A typical example is that pregnancy elevates the consumption of FA to ensure the development of fetal [24, 25]. Understandably, FA plays vital roles in human health, growth and development.

FA deficiency is associated with multiple pathologies and developmental abnormalities, including fetal neural tube defects, megaloblastic anemia, cardiovascular disease, cancer, cognitive dysfunction, and hyperhomocysteinemia, etc [26, 27]. Therefore, several countries required the fortification of the food supply with folic acid, which is a oxidized synthetic form of FA [28]. FA deficiency is typically caused by dietary insufficiency (e.g., poor diet, etc.), malabsorption (e.g., alcoholism; FA-associated gene mutation, etc.), increased demand (e.g., pregnancy, etc.), or medication (e.g., antifolates drugs) [5, 29]. However, it has been reported that excessive FA intake is potentially associated with cancer promotion including colorectal cancer, and has been linked to masking of vitamin B12 deficiency [5, 30]. However, recent epidemiological or clinic studies showed that infectious diseases of bacterial, viral, or parasitic etiology are associated with altered serum FA levels and that the growth of pathogens requires the involvement of FA metabolism, though, with some controversies [31–38]. In this review, we focus on the relationship between FA and infectious disease, and briefly discuss the prospects of FA and antifolates agents in the treatment of infectious diseases.

Relationship between host FA and microbes

Host FA and bacteria

Symbiotic bacteria. Some bacteria inhabiting the human gut may synthesize FA, as genes encoding enzymes required for FA biosynthesis have been identified in the genomes of gut microorganisms [39, 40]. It is estimated that FA produced by commensal bacteria residing in the human gut account for 17%–37% of the dietary reference intake [41]. Recent studies have shown that the potential for FA production derived from bacteria in the gut is positively correlated with FA levels in the intestinal environment [41]. In addition, supplementation with probiotics (*Bifidobacterium spp.*, *L. acidophilus*, *B. infantis*, *B. longum*, *L. plantarum*, *S. thermophilus*, *B. breve, L. delbrückii subsp. Bulgaricus*, *L. paracasei*, etc.) can significantly improve FA levels in human plasma [42–44]. Interestingly, some strains of *Bifidobacteria* can also supply FA to host [45]. The above evidence suggests that commensal bacteria play an important role in host FA replenishment and uptake. However, several studies reported that the level of FA synthesized by symbiotic bacteria is much higher than that required for human growth, which means that FA overload can occur in the human body under certain circumstances [46, 47]. Although FA is considered non-toxic for the host, excessive FA intake has been identified as a risk factor for FA-sensitive cancers and masking vitamin B12 deficiency [5, 48, 49]. Furthermore, it has been established that high local FA levels in the intestine increase the risk of colorectal cancer [50, 51]. However, these results should be interpreted with caution, none of these studies took into account of interactions among different bacteria. Actually, little is known about how FA synthesized by bacteria affects the host (Figure 1).

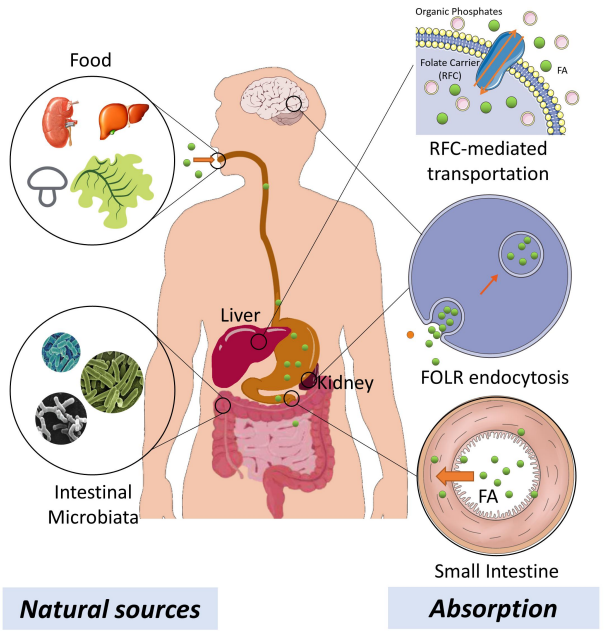

Figure 1 The natural sources and absorption of FA.
FA, folate; RFC, reduced folate carrier; FOLR, folate receptor.

Pathogenic bacteria. To date, the relationship between pathogenic bacterial infections and host FA levels has been well studied. *Helicobacter pylori* (*H. pylori*) is a type of bacteria that infects the stomach lining and is a major cause of chronic gastritis and peptic ulcers [52, 53]. It is also associated with an increased risk of developing gastric cancer [54]. Epidemiological studies in several countries and regions have reported significantly lower serum FA concentrations in *H. pylori*-positive patients than in negative patients, and that FA levels in serum and red blood cells rebound in patients after *H. pylori* eradication [55–59]. Cai et al., identified 48 studies and found that *H. pylori*-positive patients had lower serum FA levels than negative patients [60]. In patients with *H. pylori*-associated gastritis, FA levels show an inverse correlation with bacterial density [61]. Above evidence indicates that *H. pylori*-infection may be associated with host's FA levels reduction. The low FA levels observed in *H. pylori*-infected patients may result from multiple factors. First, *H. pylori* competitively consumes large amounts of FA from the host during biofilm formation [62]. Second, infection alters gastrointestinal pH, impairing FA absorption [63]. Third, infection-associated gut inflammation further disrupts absorption [61]. Finally, *H. pylori* decreases gut microbiota diversity, interfering with microbial FA synthesis and reducing overall FA production [64, 65]. However, establishing a clear relationship between *H. pylori* and low FA levels requires careful consideration of confounding factors such as age, gender, and dietary habits. Therefore, expanding the scope of research is necessary to further clarify this association.

In addition to *H. pylori*, there is no direct evidence documenting the impact of other common pathogenic bacteria on host FA levels; however, some indirect findings suggest a potential link between bacterial infection and alterations in host FA status. *Staphylococcus aureus* (*S. aureus*), a commensal gram-positive bacterium, can cause multiple diseases ranging from skin infections to fatal sepsis. Recent evidence reports that although *S. aureus* possesses the capability for *de novo* FA synthesis, it may exploit host FA pools to fulfill its own metabolic demands, particularly during intracellular persistence or nutrient-limited conditions [66]. However, these findings require further validation through in vivo studies. *Mycobacterium tuberculosis* (*Mtb*), a major global bacterial infectious disease primarily affecting the lungs, has claimed millions of lives throughout human history [67]. *Mtb* possesses a functional FA biosynthesis pathway and has a limited capacity to utilize exogenous FA, making endogenous production essential for its survival [68]. Interestingly, *Mtb* lung infection can disrupt the homeostasis of the gut microbiota, which is crucial for maintaining FA balance in the host [39, 69]. Therefore, *Mtb* infection does not appear to directly alter host FA levels.

In short, the relationship between pathogenic bacteria and host FA levels is complex. On one hand, pathogens may directly deplete host FA stores. On the other hand, they could disrupt the gut microbiota or alter the intestinal microenvironment, thereby impairing FA absorption. Further studies are needed to validate these mechanisms and account for potential confounding factors. And the understanding of the relationship of FA and bacterium-induced pathogenesis and the related mechanisms is of pivotal importance to bacterial infectious disease prevention and intervention (Figure 2).

Host FA and parasites. Parasites are an important cause of human infectious diseases and a major public health challenge affecting millions of people worldwide [70]. The clinical severity and outcome of parasitic diseases caused by parasitic infections typically depend on the immune response of the host [71]. When parasitic infections were identified as a risk factor for malnutrition in humans, it became clear that the outcome of parasitic infections also depends on the interaction of nutritional status between the host and the parasite [72–75]. FA, an essential nutrient, is often observed to be reduced in parasitic infectious diseases, and it is clear that the interaction between parasite and host FA environment impacts the progression of parasite-FA-diseases [32, 76]. In this section, we focus on reviewing the evidence of interactions between parasites and the FA of the host (Figure 3).

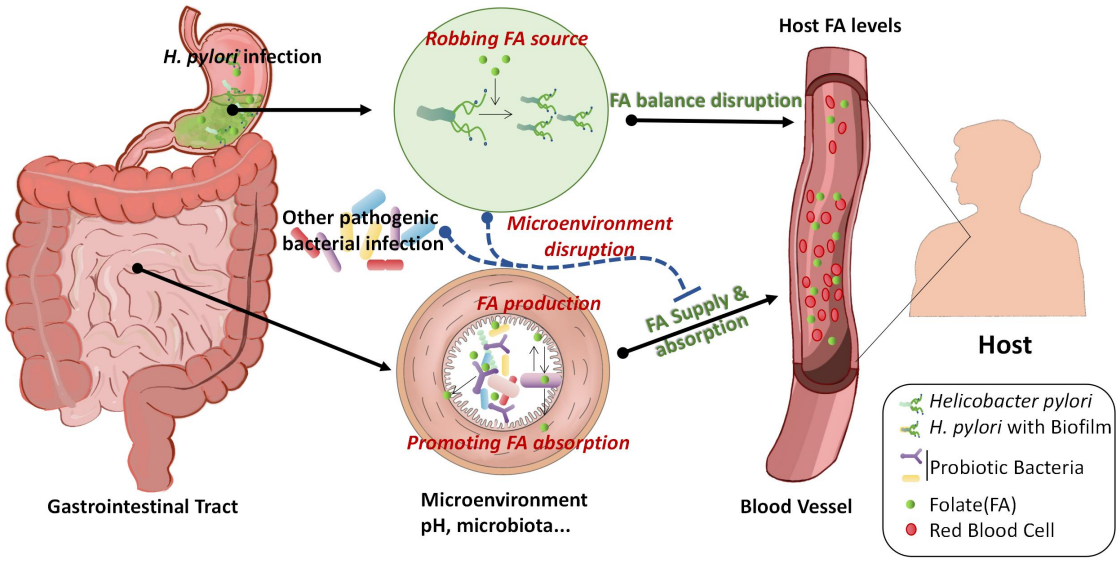

Figure 2 The interaction between bacteria and FA. FA, folate; *H. pylori*, *Helicobacter pylori*.

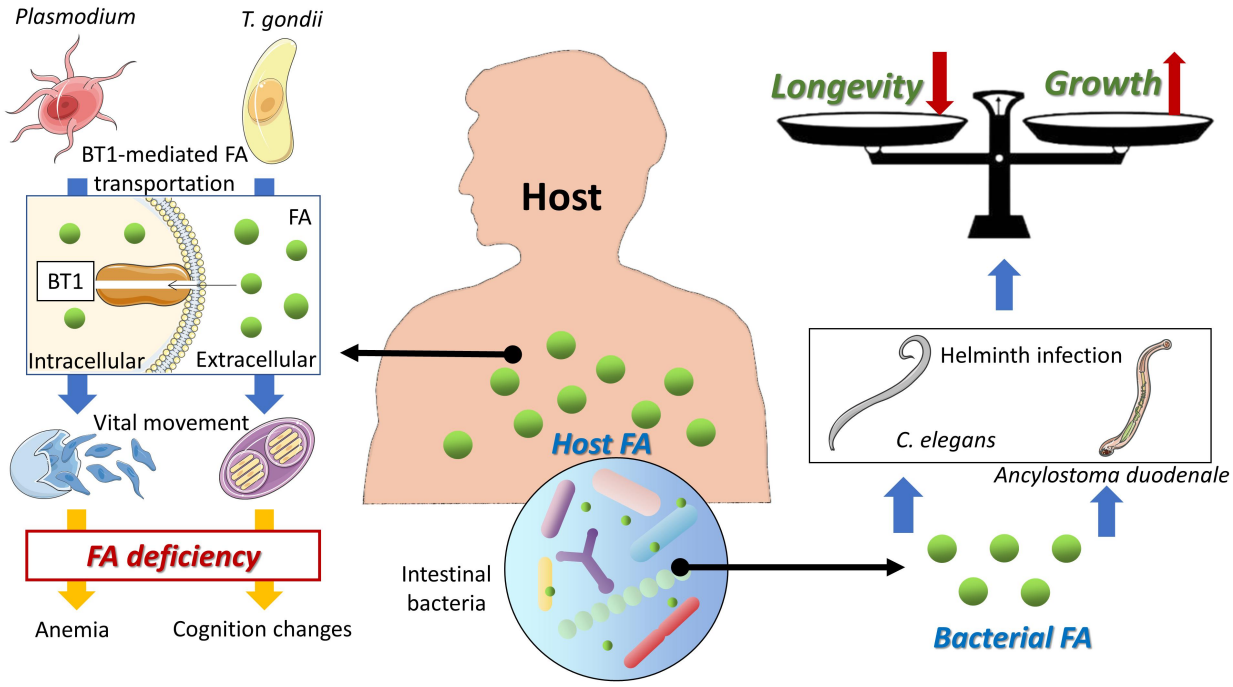

Figure 3 The interaction between parasitic infections and FA of the host.
FA, folate; BT1, biopterin transporters; *T. gondii*, *Toxoplasma gondii*; *C. elegans*, *Caenorhabditis elegans*.

Malaria, the most important human protozoan parasite can cause FA-deficient-related diseases, such as anemia, low birth weight, and neural tube defects [32, 77]. Indeed, lower FA levels are commonly observed in malaria-infection patients, for instance, malaria-positive had lower FA levels than negative children with stunting in Uganda [78]. Previous studies reported that malaria proliferation requires FA during important stages of the malaria life cycles, therefore, folic acid supplementation or increased FA intake may increase the risk of malaria infection [76, 79, 80]. Although certain strains of *Plasmodiumare* capable of *de novo* FA synthesis, this endogenous production is insufficient to support their rapid proliferation, consequently, they must acquire FA by scavenging from the host [81, 82]. Several potential mechanisms may explain how malaria acquire FA from their host. Membrane proteins known as the biopterin transporters (BT1) family, are a high-affinity FA transporter for a variety of parasites including *Plasmodium*, *Leishmania*, and *Trypanosoma brucei ESAG10*, and have been reported to be responsible for exogenous FA transportation; this notion is further supported by studies that the ability of *Plasmodium* to take up FA can be notably inhibited by P218, an inhibitor of BT1 activity [83, 84]. It has been confirmed that DHF, THF, and MTHFR play a key role in the FA metabolic network of malaria, which is more conducive to the uptake of FA from the host [85, 86]. Furthermore, the association between *Plasmodium* infection and low FA levels may be indirect. For instance, hemolysis caused by malaria-infected erythrocytes is a major pathological manifestation of malaria-associated anemia, which leads to an increased host demand for FA to support bone marrow hematopoiesis, and then leads to FA levels decreased [87–91]. It is noteworthy that malaria frequently occurs in regions with poor socioeconomic and sanitary conditions, where FA deficiency is also common due to insufficient dietary intake. In summary, the relationship between malaria infection and low FA levels is a highly noteworthy issue that warrants further in-depth investigation.

Helminth infections are one of the most common parasitic infectious diseases in developing nations [92–94]. Almost all helminths do not support de novo FA synthesis and have to uptake FA from environment [95]. Studies have found that intestinal helminth infection could increase the risk of anemia, which might be associated with helminth-mediated potential FA deficiency [96–101]. However, the other relative contributions of etiologic factors of anemia including iron deficiency and hemoglobinopathies should also be fully taken into account [102]. Nematode *Caenorhabditis elegans* (*C. elegans*), a powerful model for characterizing helminth *in vitro*, offers an avenue to dissect the cause and effect of the host FA status and helminth infection on the host. Free-living *C. elegans* are likely to encounter multiple bacteria from environment, and bacterial FA promotes the proliferation of *C. elegans* germline stem cells, suggesting that bacterial FA is important for helminth growth. In contrast to its beneficial role in development, bacterial FA but not animal FA is a negative regulator of the longevity of *C. elegans* [47, 103–106], which is associated with AMP-dependent protein kinase pathways and nitric oxide-induced oxidative stress pathways [107]. Further study identified that FA of bacterial origin is excessive for *C. elegans* required for growth and development [108]. Ortbauer et al. found that either folic acid over-supplementation or FA deficiency could impair *C. elegans* longevity [109]. In short, intestinal bacteria are the major providers of helminthic FA but not the host, and the evidence of relevance between helminth infection and host FA deficiency-associated diseases are insufficient.

Toxoplasma gondii (*T. gondii*) is an apicomplexan parasite and approximately one-third of the world population were infected by *T. gondii*, which usually affects muscle and neural tissues in humans [110]. *T. gondii* does not produce FA de novo, and has to harvest FA directly from the host cell to help its proliferation [111]. *T. gondii* further utilizes host FA through DHFR-TS, an important enzyme for FA metabolism in *T. gondii*, to maintain its own survival [112]. FA deficiency in the nervous system resulted in cognitive impairment [113–115]. For instance, data from the third National Health and Nutrition Examination Survey by Berrett et al. found that *T. gondii* might deplete FA in the brain thereby affecting cognitive functions in adults [111]. BT1 protein family is responsible for the transport of pterins and FA in multiple parasites such as *Plasmodium falciparum*, *Leishmania tarentolae*, *Leishmania major*, *Cryptosporidium parvum*, *Cryptosporidium hominus*, which may be associated with the predation of the host FA by *T. gondii* [83]. Based on this, the inhibition of FA-related pathways serve as one of the major targets of toxoplasmosis

treatment in clinic, but the relationship and mechanisms between *T. gondii* and FA-related diseases are largely unknown [116]. In-depth investigation of the interaction between *T. gondii* and host FA will help to provide a clear picture of the pathogenic mechanism and facilitate the development of new drugs targeting *T. gondii* infection.

Until now, knowledge about the roles of FA in the processes of symbiosis between parasites and the host has remained incomprehensive. The epidemic of parasitic infections is closely associated with FA deficiency, which is common in underdeveloped countries and regions [117, 118]. The study of the causal relationship between FA deficiency and parasitic infections will have important implications for the prevention and control of parasitic diseases worldwide.

Host FA and viral infections. Viruses are intracellular parasitic pathogens that depend on cellular metabolic processes to complete their replication cycles [119, 120]. On the one hand, viruses reshape and hijack the metabolic machineries of the host to facilitate their replication. For instance, adenovirus infection reshapes the host cell metabolism including a shift to aerobic glycolysis in the Warburg format to aid its replication and increases the risk of obesity [121, 122]. On the other hand, host cells need metabolic reprogramming to fight against viral infections. For example, the OGDH-itaconate pathway reprograms cellular metabolism to inhibit the replication of a variety of viruses, such as vesicular stomatitis virus, herpes simplex virus type 1 (HSV-1), encephalomyocarditis virus [123]. It is suggested that the investigation of the relationship between cellular metabolic processes and viral infectious diseases is important for the development of new drugs [124].

FA metabolism, one of the vital cellular metabolic processes for cell proliferation, methylation, and nucleic acid repair, is also important for viral infections [125]. For example, FA is enriched in intestinal tissue of the mice during enterovirus-A71 infection [126]. Early studies reported that FA deficiency was usually observed during the active phase of viral hepatitis [127, 128]. Similar phenomenon of low FA levels has been observed in patients infected with influenza A, influenza B, and human parvovirus [129]. Lower FA levels have also been observed in the sera of human papillomavirus (HPV)-positive patients with cervical intraepithelial neoplasia (CIN) [130, 131]. Low levels of FA are also common in HIV-positive patients, for instance, mean FA levels are low in HIV-positive African mothers [132–136]. Decreased serum FA levels are also common in hospitalized COVID-19 patients [33], caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), particularly in mild and severe COVID-19 patients [137, 138]. Since viruses completely depend on host cell machinery for replication, they have to hijack specific metabolism of host cell for their replicative needs, and FA metabolism is no exception. Interestingly, different viruses possess distinct mechanisms of host FA hijacking, for example, human cytomegalovirus activates cellular DHFR to support viral replication and DNA synthesis; murine cytomegalovirus-induced folylpolyglutamate synthetase converted FA to polyglutamated forms, which bound with DHFR to initiate 1C metabolism and provide substrates for viral DNA replication [139–141]. MTHFR is responsible for the conversion of THF, the catalytic product of DHFR, into biologically active L-methylfolate to support purine/pyrimidine synthesis. *Mthfr*-deficient mice and fibroblasts resisted murine cytomegalovirus infection [142]. FA is depleted in SARS-CoV-2-infected cells because SARS-CoV-2 hijacks FA and 1C metabolism for viral replication by targeting serine hydroxy methyl-transferase (SHMT1 and SHMT2) [125, 143]. Newcastle disease virus relied on the FA metabolic pathway to support viral replication [144]. And we also found that FA depletion suppressed vesicular stomatitis virus replication, and FA metabolism negatively modulated 2'-5'oligoadenylatessynthesis- mediated antiviral innate immunity [145, 146]. Above studies suggested that the FA metabolism of host was essential for the viral replication, as well as antiviral innate immunity modulation (Figure 4).

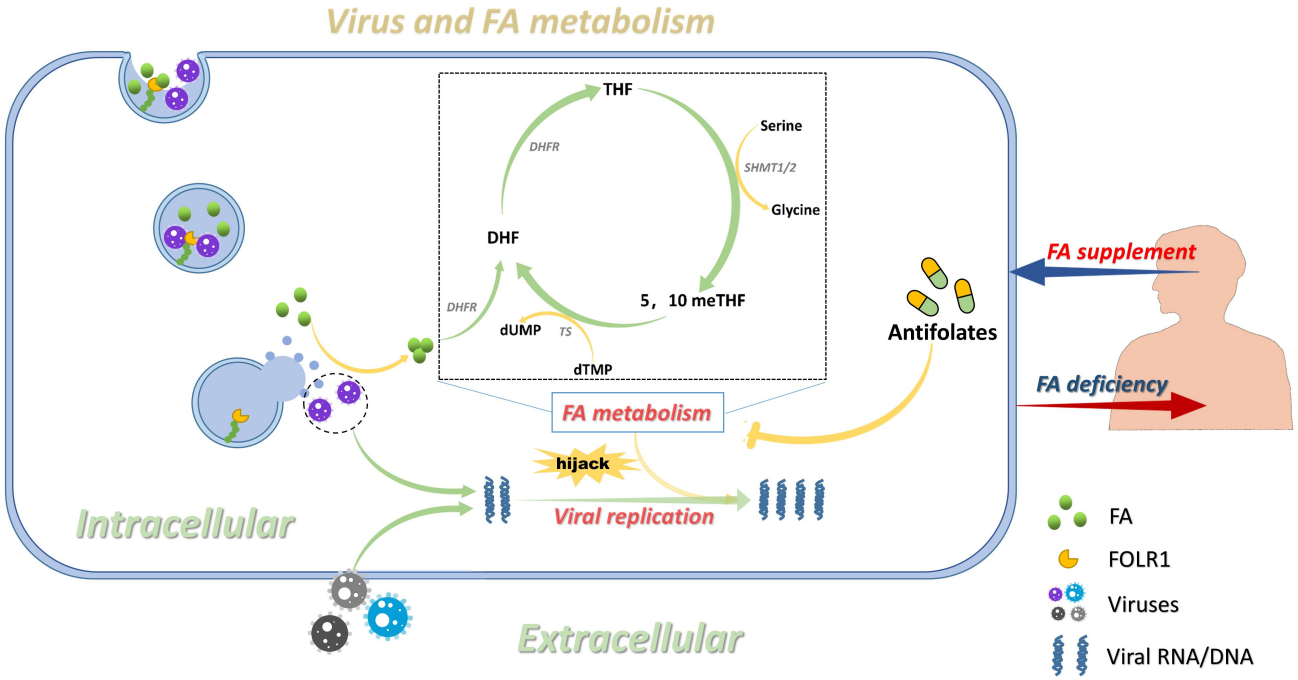

Figure 4 The interaction between viral infections and FA of the host.
FA, folate; FOLR, folate receptor; THF, tetrahydrofolate; DHF, dihydrofolate; DHFR, dihydrofolate reductase enzyme; dTMP, deoxythymidine monophosphate; dUMP, 2'-deoxythymidine 5'-monophosphate; TS, thymidylate synthase.

The host FA participates in the development of viral infectious diseases, and two main distinct views coexist currently. One of them is that host FA metabolism is vital for the survival of viruses. For instance, Topless et al reported an association of increased risk for COVID-19-related death in people prescribed folic acid supplementation [147]. HPV16-FA deficiency-organotypic rafts contain less HPV16 viral particles and viral DNA load [148]. Methotrexate, a classical FA antagonist shares a similar structure with FA and allowing competitive inhibition of DHFR, has shown an excellent broad-spectrum antiviral activity, including SARS-CoV-2, ZIKV, HSV-1, and Epstein-Barr virus, etc [149–154]. The above evidence is easily understood, as the synthesis and methylation modification of nucleic acids is a vital step of the intracellular replication of viruses, and FA deficiency interrupts the supply of substrates of nucleic acid synthesis and methylation [155, 156]. However, another viewpoint claims that low levels of FA might promote viral infections and its pathogenic process. Infant mice with low FA levels are more likely to develop severe manifestations of rotavirus infection compared to infant mice with normal FA levels [157]. And low levels of FA in the blood appear to increase the risk of CIN and have a potential synergistic effect with HPV in the development of cervical cancer [158–161]. In Egyptian patients with HCV-related hepatocellular carcinoma, FA deficiency induces high frequency of deletion of mitochondrial (mt)DNA, a molecular biomarker for the progression of HCV infection [162]. FA deficiency before and after the time of Friend's virus-infection increased the incidence of leukemia [163]. These seemingly opposite effects can be explained by the context of FA metabolism in viral infection: although FA inside cells helps viruses replicate, low FA levels in the body can weaken immune function and damage DNA, leading to more severe disease. Therefore, the role of FA is not contradictory but dual-sided, varying by location, timing, and virus-host interactions. Future research should clarify these context-specific mechanisms to guide nutritional and treatment strategies.

FOLR1 is one of pivotal receptors for the cellular entry of FA. Chan et al. discovered that FOLR1 acted as a significant cofactor for cellular entry of Marburg and Ebola viruses via FOLR1 binding Marburg glycoproteins and mediating syncytia formation [164]. FOLR1 usually highly expresses in multiple cancers and contributes to the progression of cancers [15]. Several viruses have been identified as a contributor to the pathogenesis of some human cancers, including HPV-CIN, Epstein-Barr virus-lymphoma and HBV-related hepatocellular carcinoma, etc., which indicates the potential link between FOLR1 and virus-associated-tumor development [165].

In conclusion, the metabolism of FA and 1C as substrate providers to support viral replication may lead to an excessive depletion of the limited FA in the host. Low levels of FA may contribute to the development of multiple diseases associated with viral infections. However, solid evidence remains lacking for the relationship between FA deficiency and viral pathogenesis and the understanding of the mechanisms of virus-induced FA-related metabolic reprogramming is limited.

Folic acid and treatment of infectious diseases

Folic acid supplementation

FA is one of the natural products that have been identified to have antimicrobial activities. For bacterial infections, folic acid treatment alleviated pneumococcal meningitis-mediated inhibition of brain-derived neurotrophic factor in hippocampus tissues of rats, leading to the improvement of memory functions [166]. Bacterial vaginosis (BV) is a condition of altered vaginal flora, resulting in multiple adverse reproductive outcomes. Neggers et al. and Dunlop et al. found that FA deficiency was strongly associated with the development of BV and increased intake of FA may decrease the risk of severe BV [167, 168]. However, the exact mechanism is unknown. For parasitic infections, a six-year program showed that weekly iron-folic acid supplementation and regular deworming were effective for the prevention of soil transmitted helminth and notably decreased the anemia rates in Vietnam [169]. Recent studies showed that FA inhibited furin protease through which SAR-CoV-2 enters its host cell; FA inactivated virus-encoded protease $3CL^{pro}$ to inhibit the replication of SARS-CoV-2; furthermore, FA treatment resulted in a lower Zika virus (ZIKV) load in mice [170–175]. Recently, a clinical study showed that folic acid, epigallocatechin gallate, vitamin B12, and hyaluronic acid supplementation demonstrated a viral clearance in 85.8% of enrolled patients with HPV infection [176]. Above evidence implies that FA may have antimicrobial activity in specific infectious diseases, however, most of these findings come from *in vitro* or animal studies, with weak clinical evidence. Therefore, caution should be exercised when interpreting the antibacterial activity of folic acid, particularly its antiviral effects.

Despite the benefits of folic acid supplementation in preventing anemia, its use in patients with infectious diseases requires careful consideration. There is evidence that indiscriminate increase in FA intake in the population can also aggravate infectious disease conditions [177]. For instance, clinical trial data indicated that in malaria-infected patients higher FA levels were associated with higher parasitemia; although data from experimental studies supported that FA inhibited SARS-CoV-2 entry into the host and viral replication, contradictory data from observational studies indicated that folic acid supplementation increased the risk of post-infection mortality [178–180]. Meanwhile, folic acid supplementation promoted the development of *Plasmodium cynomolgi* infection in rhesus monkeys [181]. Kupka reported that supraphysiologically, and possibly even physiologically, FA may favor the growth of *Plasmodium falciparum* [182]. Ersöz et al. suggested that both low iron and FA levels should be controlled first in patients diagnosed with COVID-19 [183]. And people prescribed folic acid supplementation had positive association with death after a diagnosis of COVID-19 [147]. Those evidence may be just the 'tip of the iceberg' on the adverse effects of indiscriminate folic acid supplementation on infectious diseases. Therefore, more studies are called for on the roles of folic acid supplementation in infectious diseases to evaluate the cons and pros of FA.

Antifolate treatment

The antifolates were the first antimetabolites to enter the clinic 75 years ago, which were initially used in the treatment of acute granulocytic leukemia by disrupting the FA-mediated 1C metabolic pathways [184]. Subsequently, antifolates were widely used as chemotherapeutics of multiple cancers [185]. However, until now, only a few antifolates have been developed and used clinically for the treatment of infectious diseases. Sulfa powder is one of the sulfonamides, the first inhibitor of the bacterial FA biosynthetic pathway and used on soldier's wounds to prevent infection on the battlefield [186]. Due to the idiosyncratic hepatotoxicity of sulfonamides, sulfamethoxazole was synthesized to improve pharmacokinetics and reduce side effects [187, 188]. Trimethoprim, the first inhibitor of DHFR, was synthesized for the treatment of *S. aureus* and *Escherichia coli* [189]. Subsequently the combination of trimethoprim and sulfamethoxazole was used to treat infections caused by both Gram-positive and Gram-negative bacteria in clinic [190]. For parasitic infections, the antifolate agent fansidar (sulphadoxine-pyrimethamine) was identified as an effective and cheap antimalarial alternative to chloroquine as characterized by excellent compliance, good tolerance, and safety profile and has been used as a first-line antimalarial drug targeting DHFR of *P. falciparum* [191]. Furthermore, pyrimethamine is also a first-line and the only approved drug for *T. gondii* treatment in the United States [192]. Notably, pathogens resistant to the traditional antifolates are spreading all over the world, which is a matter of concern because there are no other approvable antifolates available [193, 194]. For the treatment of viral infectious diseases, although experimental studies have reported the excellent antiviral activity of antifolates, there are no antifolates being approved for viral therapy [150, 195–198]. Unlike bacteria and parasites, viral FA metabolism depends on the metabolic pathways of the host cells [125], which means that the termination of viral FA metabolism can also lead to cytotoxicity [199].

Thus, it is necessary to consider the benefits of antifolate treatment in viral infectious diseases [200, 201]. In addition, it will also be important to understand the mechanisms with which viruses hijack the host FA metabolism, and identify novel targets along the 'viral FA metabolism'.

While traditional antifolates like trimethoprim-sulfamethoxazole have long been employed, widespread drug resistance and toxicity issues have necessitated the development of novel, targeted agents. For example, compounds sulfaguanidine hybrid with some new pyridine-2-one derivatives demonstrate potent activity against multidrug-resistant bacteria by inhibiting bacterial DHFR enzymes activity and modulating host's immunity [202]. P218 was engineered as a highly selective inhibitor of bifunctional DHFR-TS to overcome antifolate resistance in *Plasmodium falciparum* [203]. Similarly, new inhibitors targeting FA pathways in other parasites, such as *T. gondii* and *Leishmania* species, are under active investigation, offering hope for treating neglected tropical diseases with improved efficacy and safety profiles [204, 205]. The exploration of antifolates as antiviral agents is a more recent and cautiously optimistic development. Certain viruses, particularly cytomegalovirus and other herpesviruses, encode specific proteins that reprogramming of host FA metabolism, a mechanism essential for their efficient replication [141]. Therefore, novel, selectively targeted antifolates are being explored to target these viral proteins. By leveraging structural biology and rational drug design, these novel antifolates show remarkable potential in addressing the critical challenges of drug resistance across bacterial, parasitic, and viral domains. Future success will hinge on continued efforts to optimize selectivity, minimize off-target effects, and thoroughly evaluate the complex role of host FA metabolism during infection. The FA pathway remains a fertile ground for the discovery of much-needed antimicrobial agents.

In short, existing research on the relationship between microbial infections and host folate metabolism remains limited and fragmented. However, it is certain that infections or colonization by microorganisms, including bacteria, parasites, viruses, and others, can influence host folate metabolism (Table 1). These effects may be either positive or negative. Further extensive studies are still needed to clarify these relationships.

Conclusion

According to current evidence, microbes are closely associated with alterations in host FA levels, influenced both by the FA-producing capacity of commensal bacteria and the FA consumption by pathogens. This interplay suggests that microbial activity may contribute to diseases linked to aberrant FA levels, whether deficiency or excess. However, robust experimental models establishing causality between infectious processes and FA dynamics remain scarce. Furthermore, FA exerts a dual role in infectious contexts: while FA deficiency impairs pathogen proliferation by disrupting one-carbon metabolism essential for nucleic acid synthesis and methylation, excessive FA also exhibits antimicrobial effects through mechanisms yet to be fully elucidated. This dual response highlights the importance of a physiologically balanced FA level for pathogen survival, urging further investigation to determine optimal FA thresholds across different infections. Notably, certain viruses manipulate host intracellular FA pathways to support their replication, though the molecular mechanisms remain poorly defined. Deciphering these processes could reveal novel therapeutic targets. Moving forward, future research should prioritize the development of targeted antifolate agents optimized for antimicrobial efficacy and host safety, as well as evidence-based nutritional strategies to guide FA supplementation in infected patients. Such efforts will not only clarify the microbial–host FA interplay but also promote translational applications in the management of infectious diseases.

Table 1 Summarized the connection between FA metabolism and microorganism

Type	Pathogens	The relationship between infection and FA
Bacteria	*H. Pylori* [57, 60, 206]	FA values of HP-positive patients were significantly lower than HP-negative patients.
	Symbiotic bacteria [42–44]	*Bifidobacterium spp.*, *L. acidophilus*, *B. infantis*, *B. longum*, *L. plantarum*, *S. thermophilus*, *B. breve, L. delbrückii subsp. Bulgaricus*, *L. paracasei*, etc., is capable of de novo folate synthesis, can significantly improve FA levels in human plasma.
Parasite	*Malaria* [78, 101]	Malaria-positive patients had a lower FA levels than malaria-negative patients.
	T. gondii [111]	In subjects seropositive for *T. gondii,* as FA levels decreased and as homocysteine levels increased.
Virus	CIN-HPV$^+$ [130, 131]	Comparing to patients with normal cytological smears without HPV infection, notably lower levels of FA were observed in CIN-HPV$^+$ patients's serum.
	HIV [132–135]	Lower levels of FA were frequent observed in HIV patients.
	SARS-CoV-2 [33, 138]	Decreased serum FA levels are common among hopitalized patients with COVID-19.

FA, folate; RFC, reduced folate carrier; *H. pylori*, *Helicobacter pylori*; *T. gondii*, *Toxoplasma gondii*; HPV, human papillomavirus; CIN, cervical intraepithelial neoplasia; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; HIV, human immunodeficiency virus; HP, *Helicobacter pylori*; *L. acidophilus*, *Lactobacillus acidophilus*; *B. infantis*, *Bifidobacterium infantis*; *B. longum*, *Bifidobacterium longum*; *L. plantarum*, *Lactobacillus plantarum*; *S. thermophilus*, *Streptococcus thermophilus*; *B. breve*, *Bifidobacterium breve*; *L. delbrückii subsp. Bulgaricus*, *Lactobacillus delbrueckii subsp. Bulgaricus*; *L. paracasei*, *Lactobacillus paracasei*.

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