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MINIREVIEW

Evolution to a Chronic Disease Niche Correlates with Increased Sensitivity to Tryptophan Availability for the Obligate Intracellular Bacterium *Chlamydia pneumoniae*

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The chlamydiae are obligate intracellular parasites that have evolved specific interactions with their various hosts and host cell types to ensure their successful survival and consequential pathogenesis. The species *Chlamydia pneumoniae* is ubiquitous, with serological studies showing that most humans are infected at some stage in their lifetime. While most human infections are asymptomatic, *C. pneumoniae* can cause more-severe respiratory disease and pneumonia and has been linked to chronic diseases such as asthma, atherosclerosis, and even Alzheimer's disease. The widely dispersed animal-adapted *C. pneumoniae* strains cause an equally wide range of diseases in their hosts. It is emerging that the ability of *C. pneumoniae* to survive inside its target cells, including evasion of the host's immune attack mechanisms, is linked to the acquisition of key metabolites. Tryptophan and arginine are key checkpoint compounds in this host-parasite battle. Interestingly, the animal strains of *C. pneumoniae* have a slightly larger genome, enabling them to cope better with metabolite restrictions. It therefore appears that as the evolutionarily more ancient animal strains have evolved to infect humans, they have selectively become more "susceptible" to the levels of key metabolites, such as tryptophan. While this might initially appear to be a weakness, it allows these human *C. pneumoniae* strains to exquisitely sense host immune attack and respond by rapidly reverting to a persistent phase. During persistence, they reduce their metabolic levels, halting progression of their developmental cycle, waiting until the hostile external conditions have passed before they reemerge.

The genus *Chlamydia* contains a phylogenetically unique group of obligate intracellular parasites that successfully infect humans and a wide range of animals. They are characterized by their unique developmental cycle, which consists of two morphologically distinct forms (reviewed in reference 1). The extracellular, infectious form (elementary body [EB]) is responsible for transmitting infections between cells and between hosts and is generally considered to be metabolically inert (although this has been challenged recently [2]), whereas the intracellular, noninfectious form (reticulate body [RB]) is metabolically active and is responsible for growth and multiplication within the target host cell (Fig. 1). Once an EB attaches to and enters a host cell, it immediately hijacks several host cell pathways (3, 4), preventing phagolysosomal fusion, encasing itself in a unique double-membrane inclusion and rapidly converting to the metabolically active, RB form. The RBs multiply by binary fission around 500-fold during a 24-to-48-h period before converting back to EBs, which subsequently exit the cell to start another round of multiplication. This unique "acute" developmental cycle has both strengths and weaknesses. Once inside a suitable host cell, provided the chlamydiae can subvert several key host cell pathways, they generally have access to a rich pool of nutrients required for growth. While the chlamydiae have retained a diverse set of metabolic pathways, they have also lost many pathways, making them dependent on their host cell for a significant number of key nutrients and intermediates. Of course, eukaryotic cells have evolved a range of strategies to combat pathogens such as *Chlamydia* (reviewed in reference 5). One main line of defense is that eukaryotic cells sense foreign invaders (via pattern recognition receptor [PRR] signaling), resulting in production of the key cytokine gamma interferon (IFN-γ) (at least in humans), which in turn depletes the host cell tryptophan (TRP) pool and effectively starves the foreign bacteria of this key nutrient.

In addition to the acute developmental cycle, chlamydiae have also developed a so-called "persistent phase" (6, 7). While this persistent phase has been best studied *in vitro*, it can also be observed *in vivo* (7–12). Chlamydial persistence has been described as "a viable but non-culturable growth stage resulting in a long term relationship with the infected host cell" (13). During persistence, chlamydial metabolism is slowed and RB division, as well as differentiation into EBs, is suspended (6, 14, 15), resulting in the production of so-called aberrant bodies. Overall, this means that while the RBs are metabolically viable, they do not complete the developmental cycle and hence no infectious progeny are produced. This state is reversible. Several different stimuli have been shown to induce persistence, including (i) gamma interferon treatment, (ii) penicillin treatment, (iii) amino acid (e.g., tryptophan), glucose, and iron deprivation, (iv) chlamydiophage infection, and several others (14, 16–26). Persistence therefore appears to be a mechanism that allows the organisms to ride out hostile conditions while maintaining a long-term (chronic) infection within a host cell.

Of the nine species in the genus *Chlamydia*, *C. pneumoniae* is arguably the most successful pathogen, infecting humans and an amazingly wide range of animals. In humans, serological studies

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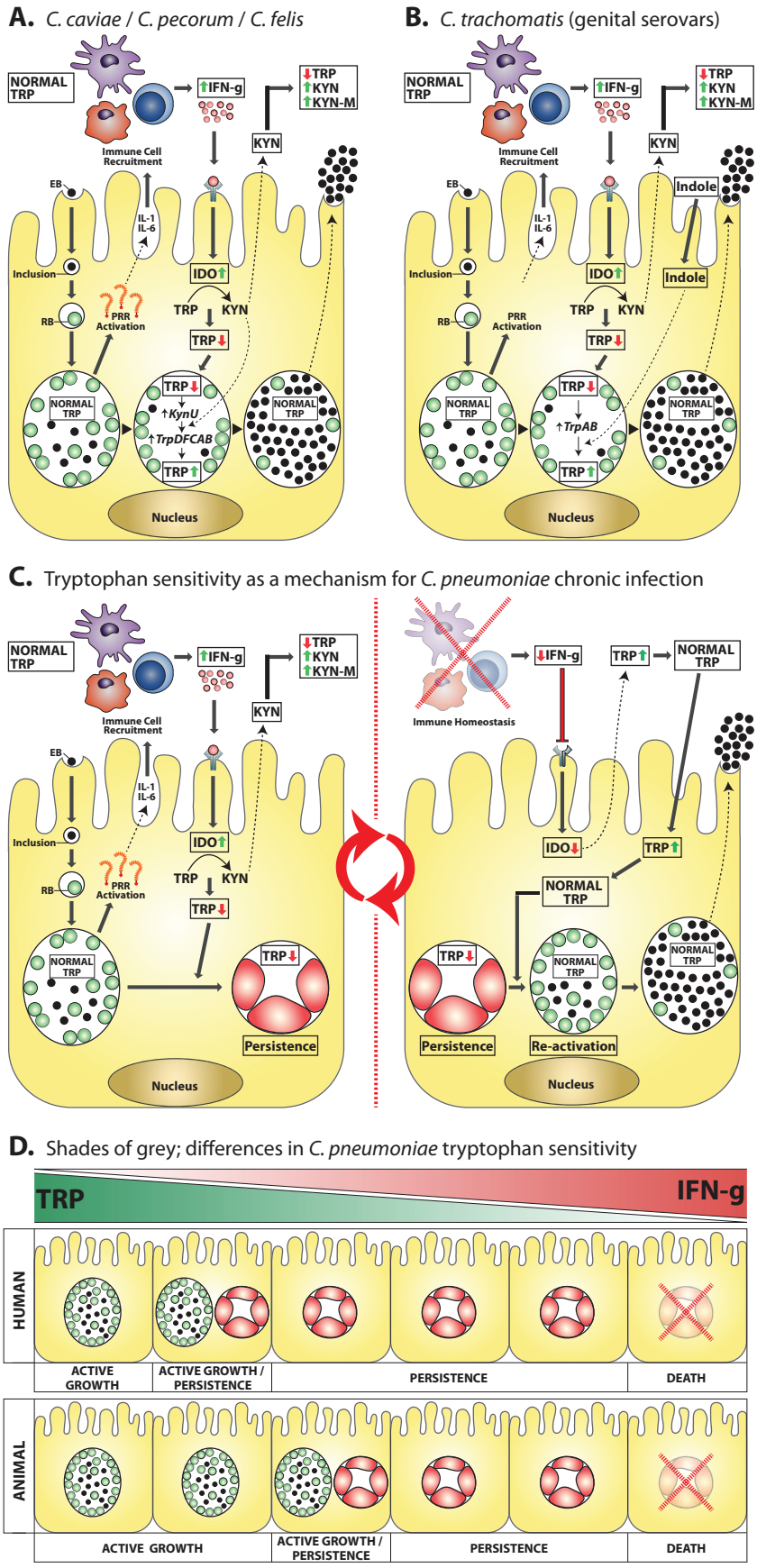
A. C. caviae / C. pecorum / C. felis
B. C. trachomatis (genital serovars)
NORMAL TRP
IFN-g
TRP
KYN
KYN-M
Immune Cell Recruitment
EB
IL-1
IL-6
Inclusion
RB
PRR Activation
IDO
Indole
TRP
KYN
NORMAL TRP
KynU
TrpDFCAB
TrpAB
Nucleus
C. Tryptophan sensitivity as a mechanism for C. pneumoniae chronic infection
Immune Homeostasis
Persistence
Re-activation
D. Shades of grey; differences in C. pneumoniae tryptophan sensitivity
TRP
IFN-g
HUMAN
ANIMAL
ACTIVE GROWTH
ACTIVE GROWTH / PERSISTENCE
PERSISTENCE
DEATH

show that almost all of the world's population is, or had been, infected with *C. pneumoniae* (27). Respiratory infections are the most common, with seroepidemiological surveys indicating that infection is both endemic and epidemic. Asymptomatic infections are common, usually manifesting as mild upper tract infections that are self-limiting, although they may progress to more-severe upper respiratory tract infections (pharyngitis, sinusitis, and otitis) and lower respiratory tract infections (acute bronchitis, exacerbations of chronic bronchitis and asthma, and community-acquired pneumonia) (28–37). The species was initially "discovered" in Finland in 1985, where it caused epidemics of pneumonia in military barracks (38), and, after its initial worldwide characterization (39–42), was thought to have declined somewhat in prevalence. However, recent reports (both serological studies and studies of outbreaks in confined populations [43, 44]) show that it is still a widespread and successful respiratory pathogen. In addition to respiratory infections, *C. pneumoniae* has been linked with several other chronic conditions, most notably cardiovascular inflammatory diseases such as atherosclerosis, abdominal aortic aneurisms, and valvular lesions (45–48). While there is a body of published evidence linking *C. pneumoniae* and cardiovascular disease, a causal link remains to be definitively proven and is considered controversial by some. In addition to the link with cardiovascular diseases, it has also been linked with asthma (49), Alzheimer's disease (50, 51), arthritis (52), lung cancer (53–55), chronic obstructive pulmonary disease (56, 57), and diabetes (58, 59). While there continue to be individual reports of severe respiratory disease associated with *C. pneumoniae* (60), it appears that the norm is now an association of *C. pneumoniae* with a range of chronic diseases.

In addition to its widespread prevalence in the human population, *C. pneumoniae* also causes infection and disease in a wide range of animals. *C. pneumoniae* infections have been reported in both cold-blooded hosts (frogs, snakes, iguanas, and crocodiles [61–63]) and warm-blooded hosts (horses and marsupials [63–67]). In these animal hosts, it infects a wide range of tissues (lung, liver, heart, eyes, and the female genital tract of koalas) and causes a wide range of disease syndromes (respiratory, vascular, conjunctival, genitourinary, and systemic).

Hypothesis: *Chlamydia pneumoniae*—a highly successful parasite with "50 shades of gray." The hypothesis which we discuss in this review is that the highly successful obligate intracellular pathogen, *C. pneumoniae*, has actually evolved an increasing susceptibility to tryptophan, the key amino acid. Rather than making this tryptophan auxotroph more vulnerable to host "attack," it has enabled the evolution of the more ancient, animal infection strains (which are less sensitive to tryptophan availability and cause more acute infections) into the currently successful human strains. The human strains not only are more sensitive to tryptophan availability but primarily cause chronic infections that are exquisitely in balance with their host.

TRYPTOPHAN IS AN ESSENTIAL AMINO ACID AND A CENTRAL REGULATOR FOR CELL BIOLOGY

Humans and animals lack the ability to synthesize the essential amino acid tryptophan. Consequently, tryptophan depletion acts as a major cellular signal. In humans, tryptophan depletion is associated with depression and other neurological consequences (68, 69). At a cellular level, reduction of intracellular tryptophan pools initiates molecular stress response pathways (via GCN2 kinase and mammalian target of rapamycin [mTOR] signaling molecules), resulting in blockage of ribosomal translation and cell cycle arrest. Localized tryptophan deprivation can also initiate these stress response pathways in local T cells, leading to arrest and promotion of regulatory T cell differentiation. Furthermore, bioactive tryptophan metabolites (produced from the kynurenine [KYN] pathway) have been found to have direct immunosuppressive capabilities, facilitating promotion of regulatory T cell differentiation (reviewed in references 70 and 71). In spite of these consequences, tryptophan is also actively depleted by animal cells in a pathogen defense strategy. Most bacteria are able to synthesize tryptophan and can partially overcome the host's efforts to starve it of tryptophan. However, some microbes, such as *Chlamydia*, are full or partial tryptophan auxotrophs. In fact, *Chlamydia* have unique host- and tissue niche-specific requirements, intrinsically based on differential abilities to synthesize tryptophan (or not) from metabolites or bioavailable intermediates.

FIG 1 The central role of tryptophan in chlamydial biology. Chlamydial elementary bodies attach to and enter a host epithelial cell, an inclusion is formed, and the elementary body (EB) converts to its replicative form, the reticulate body (RB). RBs undergo replication; however, during infection, PRRs are activated, resulting in secretion of proinflammatory cytokines from the host, which in turn recruits immune cells. Development of an immune response results in host cells being exposed to gamma interferon. IDO is activated, and tryptophan pools are depleted. (A) The species *C. caviae*, *C. pecorum*, and *C. felis* obtain kynurenine (the product of IDO activity) from the host cell and use it to recycle tryptophan via tryptophan biosynthesis genes (*kynU* and *trpD*, *-F*, *-C*, *-A*, and *-B*), meaning that these species are able to actively replicate during IDO activity and host tryptophan depletion. This tryptophan biosynthesis process is regulated by a tryptophan repressor and thus is initiated only when inclusion tryptophan levels become depleted. (B) The urogenital strains of *C. trachomatis* are believed to respond to IDO-tryptophan depletion by using indole (thought to be supplied by the vaginal microflora) as a precursor for tryptophan synthesis. Tryptophan synthesis is achieved through the tryptophan biosynthesis genes *trpA* and *-B*. As with the above-mentioned species, *C. trachomatis* tryptophan biosynthesis is also regulated via a tryptophan repressor that allows biosynthesis to occur only once inclusion tryptophan levels become depleted. Interestingly, the ocular *C. trachomatis* strains do not possess the tryptophan biosynthesis genes and are associated with more-chronic disease outcomes. (C) The key role of tryptophan in *C. pneumoniae* chronic infection. In stark contrast to the above-mentioned species, *C. pneumoniae* does not contain any tryptophan biosynthesis genes. As a result, tryptophan depletion results in conversion of an active replicating inclusion to a persistent inclusion (a viable but nonreplicating, static phase). In this state, *C. pneumoniae* becomes refractory to immune insult, allowing the organism to hide in the host cell until the immune response subsides. As the immune response abates, IFN-γ becomes depleted, IDO is no longer induced, local tryptophan pools become recharged, and tryptophan levels eventually return to normal. At this point, *C. pneumoniae* reverts to an actively replicating inclusion, resulting in the release of infectious progeny ready to reinitiate the chronic infection cycle. (D) Recent studies have found that human and animal (ancestral) strains behave very differently in the face of IFN-γ insult and tryptophan depletion. This finding suggests that the human strains have evolved to become more sensitive to IFN-γ, allowing them to enter a persistent phase at the first signs of an immune response. As a result, they have been able to develop a chronic infection cycle that has enabled them to become, arguably, one of the most successful human pathogens. EB, elementary body; RB, reticulate body; IFN-g, gamma interferon; IL, interleukin; PRR, pattern recognition receptors; IDO, indoleamine 2,3-dioxygenase; TRP, tryptophan; KYN, kynurenine; KYN-M, kynurenine metabolites.

PATHOGEN DEFENCE VIA IDO (INDOLEAMINE 2,3-DIOXYGENASE)-MEDIATED TRYPTOPHAN DEPLETION

Chlamydial infection of mucosal epithelial cells results in activation of innate, pathogen-sensing PRRs. Activation of these receptors initiates an immunological cascade, resulting in expression and secretion of cytokines and chemokines (reviewed in reference 5) and recruitment of leukocytes (NK cells, neutrophils, macrophages, and dendritic cells) to the infected tissue (72). An adaptive immune response is then initiated via antigen-processing cells presenting chlamydial antigens to T cells in local lymph nodes. Animal studies (73–76) and, to a certain degree, human studies (73–78) have found that IFN-γ-producing T cells are essential for resolution of chlamydial infections (72, 79, 80).

The antichlamydial effect of IFN-γ is attributed predominantly to direct inhibition of intracellular growth. Exposure of the host cells to IFN-γ results in induction of a myriad of responses (81). However, it is the induction of IDO (indoleamine 2,3-dioxygenase) (82) and inducible nitric oxide synthase (iNOS) (83) and downregulation of transferrin receptors (84) that is central to intracellular inhibition of chlamydial growth. IDO is a heme-containing, cytosolic, IFN-γ-inducible enzyme that catalyzes the breakdown of the essential amino acid tryptophan to kynurenine via the cleavage of the pyrrole ring. Kynurenine is then released from the cell or further metabolized to the downstream catabolites 3-hydroxykynurenine, 3-hydroxyanthranilic acid, quinolinic acid, and, finally, NAD (i.e., the kynurenine pathway).

More than 2 decades ago, tryptophan depletion, via IFN-γ induction of IDO, was described as a possible mechanism for inhibiting intracellular growth of the parasite *Toxoplasma gondii* (85). The simplistic paradigm of IDO being a host innate defense mechanism, using essential amino acid deprivation as a means to control tryptophan auxotrophic intracellular pathogen replication, has been superseded recently by a more complex model. Specifically, IDO tryptophan catabolism has been found to have far-ranging effects making it a pivotal component in immune and cellular regulation via the aforementioned signaling mechanisms. Consequently, IDO-tryptophan depletion has been found to have an important role in parasitic (86), viral (87), and bacterial (88, 89) infections. Although most reports document pathogen sensitivity to tryptophan depletion, recent studies are finding a role for IDO activity in chronic or persistent disease. In particular, IDO-tryptophan-directed immunosuppression is thought to play a role in infections by *Leishmania major* (90), human immunodeficiency virus (reviewed in reference 91), hepatitis C virus (92, 93), human papillomavirus (94), *Listeria monocytogenes* (95), *Mycobacterium tuberculosis* (96), *Candida albicans* (97), and *Aspergillus fumigatus* (98). Importantly, a recent study investigating the role IDO plays in infections by two protozoal species, *T. gondii* and *L. major*, found that IDO facilitates *T. gondii* clearance and suppresses *L. major* clearance, highlighting the opposing and pathogen-specific roles that IDO can have in infectious disease (90).

IFN-γ- and IDO-directed tryptophan depletion has had a significant effect on chlamydial evolution. *In vitro* model studies of epithelial cells, using tryptophan rescue of IFN-γ-treated cultures, indicate that tryptophan depletion is the major antipathogen activity of IFN-γ against *Chlamydia* (88, 89). The human ocular and urogenital pathogen *Chlamydia trachomatis* contains tryptophan synthesis (*trpBA*) genes allowing synthesis of tryptophan from indole (99). However, this adaptation was found to be functional only in the urogenital strains. It was proposed that the urogenital strains gain access to indole via vaginal microflora, thus identifying a tissue tropism mediator (i.e., ocular versus urogenital) (100). Interestingly, the ocular strains (serovars A to C) responsible for the chronic disease trachoma appear to be much more sensitive to the effects of IFN-γ than the urogenital strains (101). Another chlamydial species, *Chlamydia caviae* (guinea pig), has also evolved an elegant adaptation to tryptophan depletion, utilizing a more complete set of tryptophan synthesis genes (102, 103). These genes allow this species to recycle kynurenine back into tryptophan, ensuring continued propagation in the presence of IFN-γ induced IDO (104). Although not yet experimentally tested, these recycling genes are also conserved on the genomes of *Chlamydia pecorum* (sheep, cattle, koala), and *Chlamydia felis* (cats). Even though these tryptophan anabolism capabilities are present across these diverse animal-infecting species of *Chlamydia*, the situation is different for the murine strain, where IFN-γ inhibition of *Chlamydia* in murine cells is driven by p47 GTPases and not IDO (105). The murine pathogen (*Chlamydia muridarum*) produces a species-specific cytotoxin that is thought to degrade the key p47 GTPase, enabling continued propagation in the presence of IFN-γ (105).

In stark contrast, *C. pneumoniae*, arguably the most successful human pathogen in the *Chlamydia* genus, does not have any tryptophan biosynthesis genes. As we discuss below, this apparent vulnerability to tryptophan may in fact be a checkpoint system for sensing danger in the form of the presence of some *C. pneumoniae* strains (particularly the human strains). Nevertheless, *C. pneumoniae* requires a certain amount of tryptophan for survival and one possibility is that it can perhaps utilize the respiratory microflora to obtain its tryptophan. The microflora of the human respiratory tract is less well characterized than for other body sites, but it is known to be quite complex and contains bacteria that produce tryptophan, which could be transported from the microbiome environment into the *Chlamydia*-infected cell/inclusion by various *C. pneumoniae* transporters.

HAVE THE HUMAN STRAINS OF *C. PNEUMONIAE* ACTUALLY EVOLVED TOWARD AN INCREASED SENSITIVITY TO TRYPTOPHAN AVAILABILITY, ENABLING THEM TO SWITCH FROM ACTIVE GROWTH TO THE LATENT PHASE AND THEREBY BECOME MORE SUCCESSFUL CHRONIC "PATHOGENS"?

The animal strains of *C. pneumoniae* are evolutionarily older and have a slightly larger genome. Several strains of *C. pneumoniae* have now had their full genomes sequenced (106), including four human strains (107–109) and two animal strains (110, 111). While the genomes are remarkably similar (>99% identity in gene content and arrangement), there are some subtle but presumably significant differences between the human and animal strains but also between the various human strains.

One striking feature of all *C. pneumoniae* strains is that they are complete tryptophan auxotrophs. Perhaps the most interesting outcome of comparisons of the genomes of the human and animal strains of *C. pneumoniae*, though, is the evidence that the animal strains are evolutionarily more ancient and that the human strains probably evolved from the animal strains, perhaps over a relatively recent and short time period, by reducing their gene content and presumably adapting specifically to the human host (110). The animal *C. pneumoniae* genome is 1,241,024 bp in size, which is just

10 kb larger than the human *C. pneumoniae* genome, with just 6,213 single nucleotide polymorphisms (SNPs) separating the strains (110). A potentially important difference, though, is that the animal strains contain a plasmid whereas all human strains do not. While the genes in the *C. pneumoniae* plasmid have not yet been characterized, they have significant homology to the *C. trachomatis* plasmid genes. The *C. trachomatis* plasmid genes are currently the focus of considerable research, and it appears that some of these plasmid genes have roles in regulating chromosomal genes. As chlamydial transformation techniques are refined, it should become possible to test plasmidless animal strains for their response to IFN-γ and tryptophan. The other key difference is that there are several examples of genes that are full length in the animal strain but have truncations or significant disrupting fragmentations in the human strains. These facts have led investigators to conclude that the animal strains are ancestral and that the human strains evolved from them, adapting specifically to the human host as they have evolved (110).

Recent cell biology studies support the suggestion that the human strains of *C. pneumoniae* are more sensitive to exogenous tryptophan levels than the animal strains. In 2008, Mitchell et al. analyzed the *in vitro* growth of the animal strain of *C. pneumoniae* and compared it to that of the AR39 human strain (112). They observed significant growth differences, with the animal strain growing more aggressively, with a shorter doubling time (3.4 to 4.9 h versus 5.9 to 8.7 h), producing larger inclusions and a higher yield of infectious progeny than the human strain, which generally produced small inclusions with a lower infectious yield (112).

C. pneumoniae human isolates have been reported to be extremely sensitive to tryptophan depletion via IFN-γ-induced IDO (113–115). *In vitro* studies with epithelial and endothelial cell lines, utilizing IDO inhibition and tryptophan supplementation, have found human *C. pneumoniae* strains to be highly sensitive to IDO-driven tryptophan depletion (113, 115, 116). A recent study investigated the different effects that amino acid supplementation had on *C. trachomatis* and *C. pneumoniae*. Somewhat surprisingly, the addition of excess tryptophan was found to marginally inhibit *C. trachomatis* growth whereas, in contrast, it significantly enhanced *C. pneumoniae* growth (117). *C. pneumoniae* tissue tropism may also be influenced by the ability of the organism to gain access to host tryptophan, as the vascular strains only carry a single copy of the tryptophan-tyrosine permease (versus two copies in the respiratory strain), potentially resulting in reduced amino acid uptake ability in the vascular isolates (118).

Like other chlamydial species, *C. pneumoniae* can enter a state of persistence (i.e., a noninfectious but viable state) during IFN-γ insult (reviewed in references 13, 119, and 120). This persistent state can be reversed and active replication reinstated through the addition of tryptophan or via the inhibition of IDO (113, 115, 116). Unsurprisingly, due to the lack of tryptophan biosynthesis genes, only very low levels (15 to 25 U/ml) of IFN-γ are required to induce altered inclusion morphology (associated with persistent infection) in *in vitro* epithelial cell models (20, 113). However, if the organisms are exposed to IFN-γ during infection, a total bactericidal effect requires very high levels (800 U/ml) of IFN-γ (113).

A recent study investigating the cell biology differences between the ancestral animal strains and the more recently evolved human strains of *C. pneumoniae* has confirmed the sensitivity of the human strains to IFN-γ under controlled *in vitro* conditions (A. Chacko, C. J. Barker, K. Beagley, P Timms, and W. M. Huston, submitted for publication). The human strains of *C. pneumoniae* are exquisitely sensitive to the reduction in the tryptophan level via the effects of IFN-γ. The number and size of chlamydial inclusions are decreased (some appear persistent), and their infectious yield is markedly reduced (by greater than 99.99%). By comparison, the ancestral animal strain, under the same conditions, is much less affected by IFN-γ treatment (with approximately 100-fold-greater infectious yield). Interestingly, they also showed that subtle differences in IFN-γ sensitivity exist between the various human strains (comparing a respiratory isolate with a cardiovascular isolate). By using direct depletion of tryptophan from the medium, those researchers demonstrated that the IFN-γ effect was clearly associated with tryptophan depletion (Chacko et al., submitted). Furthermore, adding additional exogenous tryptophan back to the human strains actually increased their infectious yield above that produced by the non-IFN-γ-treated controls (Chacko et al., submitted). The mechanism by which the ancestral animal strains obtain sufficient tryptophan is not yet clear; however, increased host cell autophagy and effective transport from the host cell pool into the chlamydial inclusion is one possibility. This "strategy" enables the animal strains to grow basically as an acute infection, producing highly infectious outcomes, whereas the human strains prefer to sense adverse conditions (such as host immune attack via IFN-γ) by being more sensitive to lowering levels of tryptophan, diverting them from completing their infectious cycle and returning them to their persistent stage, where they wait out the unfavorable conditions.

These recent findings suggest that the ancestral strains are less "dependent" on exogenous tryptophan levels and prefer to grow as acute infections, ensuring their survival by completing their developmental cycle with the production of large numbers of infectious progeny to infect other cells or new hosts. In contrast, the evolution of the human strains has resulted in their being more "sensitive" to decreasing exogenous tryptophan levels, allowing them to sense adverse conditions (such as host immune attack via IFN-γ). These strains use the lowering levels of tryptophan to divert their infectious life cycle to a persistent phase until the adverse conditions subside. Increases in tryptophan levels then trigger reactivation of the developmental cycle, making the human strains more likely to cause chronic infections.

SUBTLE VARIATIONS IN THE TRYPTOPHAN CONTENT OF KEY CHLAMYDIAL PROTEINS COULD BE A FUNDAMENTAL DETERMINANT OF SUSCEPTIBILITY TO LIMITED TRYPTOPHAN AVAILABILITY

Several reviews have emerged recently (121, 122) suggesting that the chlamydial genus as a whole may have evolved a mechanism that mutes or downregulates selected gene products via the tryptophan levels in these key proteins. Those proteins that are required during the acute or active growth phase (i.e., normal development of *Chlamydia*) have higher tryptophan content, since tryptophan availability is less of an issue in this stage of growth. However, when the host responds to chlamydial infection by producing IFN-γ and tryptophan is depleted, these proteins become a burden to the *Chlamydia* and this triggers their switch from the acute to the persistent phase. In the persistent phase, a different subset of proteins are required and these have a lower tryptophan content (at least, the high-tryptophan-containing proteins are not highly expressed in this phase), making these proteins less of a

metabolic burden to produce during a time when tryptophan levels are limited. While this provocative hypothesis seems to fit the *C. trachomatis* data best, it may also be partly supported by the limited *C. pneumoniae* data that are available. Lo et al. (121) reported that MurA is a low-tryptophan-containing enzyme, making it more suited to expression during persistence. Indeed, when Timms et al. (123) evaluated *murA* transcription in the IFN-γ model of *C. pneumoniae*, they found that it was upregulated 2-fold under these conditions. While this hypothesis is interesting to consider, at this stage it is built entirely on bioinformatic analysis of chlamydial genomes and is not yet supported by any focused biological data. In addition, while it might be a general strategy used by chlamydiae, a comparison of the human and animal *C. pneumoniae* genomes does not support an extension of this tryptophan skew in the acute animal strains.

ARGININE AND THE IDO-TRYPTOPHAN NEXUS

C. pneumoniae also appears to have unique arginine-related adaptations. This species has a functional arginine-responsive transcriptional repressor (124) and an arginine decarboxylase and arginine-agmatine antiporter (125). In addition, excess exogenous arginine causes significant growth inhibition *in vitro* (117). The ability to take up and degrade arginine is most likely a means of protection against the host cell pathogen oxidative attack compound nitric oxide (NO) (125). Immune signaling activates transcription of iNOS, which catabolizes arginine to citrulline, producing NO. Importantly, NO directly inhibits IDO activity via binding to the active-site heme (126). An *in vitro* study found that exogenous NO is able to inhibit IDO tryptophan catabolism, resulting in nearly normal *C. trachomatis* L2 growth in epithelial cells (*C. trachomatis* L2 does not have a functional arginine decarboxylase [127, 128]). Even with these adaptations to prevent NO attack, macrophages from iNOS$^{-/-}$ mice are more susceptible to *C. pneumoniae* infections *in vitro* (129, 130). Nonetheless, it is clear that *C. pneumoniae* can reduce host arginine levels, thereby reducing NO production and, paradoxically, allowing continued IDO activity, perhaps as a direct trigger for chronic or persistent growth. These arginine-based adaptations have not been extensively investigated but seem likely to also function in the shades of gray between acute and chronic disease states and tissue adaptations that exist within the ubiquitous *C. pneumoniae* strains.

CONCLUSION

Tryptophan availability and the ability to overcome active tryptophan depletion fundamentally define host and tissue tropism and, therefore, pathogenicity for *Chlamydia*. Here we discuss the hypothesis that sensing tryptophan bioavailabilty to trigger chronic growth or persistence has been selected for as a chronic disease advantage in human *C. pneumoniae* infections. We already know that ocular *C. trachomatis* strains are more sensitive to IFN-γ than the closely related genital strains and that they are more chronic in their growth and disease states. At the other end of the spectrum, the ancestral animal *C. pneumoniae* strains appear to have been selected for a more aggressive, acute disease state. Recent data demonstrate that animal *C. pneumoniae* strains are less sensitive to tryptophan depletion than the human *C. pneumoniae* strains, are more aggressive in their growth, and present with more acute disease. The versatile and highly prevalent human *C. pneumoniae* strains are clearly advantaged by this tryptophan-responsive chronic disease trigger, which may in fact be further adapted with tissue tropism and disease states within these strains. While this model is appealing and is quite well supported by the currently published literature, further studies are needed to confirm it and to better understand the molecular mechanisms involved. As the technique of chlamydial transformation becomes more widely used, evaluating plasmidless or gene knockout strains would be one way of addressing these issues.

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