Current Clinical Microbiology Reports (2018) 5:261–267
https://doi.org/10.1007/s40588-018-0108-8

BACTERIOLOGY (N BOREL, SECTION EDITOR)

Chlamydia psittaci in Ocular Adnexal MALT Lymphoma: a Possible Causative Agent in the Pathogenesis of This Disease

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Published online: 16 November 2018

Abstract

Purpose of Review To elucidate the role and to summarize the current literature of *Chlamydia psittaci (Cp)* as a causative factor in ocular adnexal extranodal marginal B cell lymphomas (OAEMZLs).

Recent Findings The association of *Cp* and OAEMZLs varies according to geographic location. Antibiotic therapy is an effective therapeutic approach in *Cp*+ OAEMZLs. *Cp*+ OAEMZLs are significantly associated with chronic conjunctivitis and prolonged household animal contact. Furthermore, somatic hypermutation, intraclonal microheterogeneity, genetic aberrations, and gene promotor methylation can be found in *Cp*+ OAEMZLs.

Summary It has been shown that antibiotic therapy is an effective therapeutic approach in *Cp*-positive OAEMZLs, therefore raising the question if *Cp* is an important etiologic agent of this disease. Since there is evidence pointing in this direction, we summarize the recent findings and support the pathophysiological model of *Cp* as a causative agent in OAEMZLs.

Keywords *Chlamydia psittaci* · Ocular adnexa · MALT lymphoma · Pathogenesis · Antibiotic treatment · Extranodal marginal B cell lymphoma

Introduction

Lymphomas are the most frequent malignancies of the ocular adnexa. According to the Florida Cancer Registry, they make up 55% of all orbital tumors [1]. Moreover, it has been shown that the incidence of ocular Non-Hodgkin lymphomas (NHL) among Caucasians rose steadily since 1975 up to 2001 with an annual increase of 6.2% [2]. Ocular adnexal extranodal marginal B cell lymphomas (OAEMZLs) of the MALT type make up the majority of ocular adnexal lymphomas (OAL), with percentages ranging from 36% to 89.7% of all OALs [3–11]. Taking these facts into consideration, it is of high interest to elucidate the etiology, pathogenesis, and therapeutic approaches of OAEMZL. Research suggests that infection with *Chlamydia psittaci (Cp)* could be one causative factor of this disease [12, 13•, 14, 15, 16•, 17, 18, 19•, 20, 21] and antibiotic drugs have shown to be an effective therapeutic approach [22–28, 29••]. Therefore, this review summarizes the most compelling current literature by proposing a pathophysiological model of *Cp* as a causative factor in OAEMZLs and discusses the therapeutic approach of antibiotic therapy in this disease.

This article is part of the Topical Collection on *Bacteriology*

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Chlamydia psittaci

Chlamyida psittaci, broadly known as the etiologic agent of psittacosis [30], was first defined as an own species of the genus *Chlamydia* in 1968 by Page LA [31]. It is a gram-negative [31], obligately intracellular bacterium that can be recognized in two different forms during its life circle [32]; its extracellular infectious form called elementary body (0.2- to 0.3-μm diameter) and its metabolically active form the reticulate body (0.6- to 0.8-μm diameter) [33]. During infection, chlamydial elementary bodies are attached to the host cells, and enter them via host cell-derived phagocytic vesicles. Within the host cells, the elementary bodies undergo morphological changes and transform into the larger reticulate bodies. These metabolically active forms start to divide and become

visible as microcolonies within endosomes of the host cells, which are referred to as chlamydial inclusion. The reticulate bodies transform back into the infectious elementary bodies, after time, and the developmental cycle ends, when the *Chlamydiae* leave the infected host cell, either through exocytosis or lysis of the cells [32]. Feral pigeons are a regularly reported source of *Cp* infection [34], with prevalence rates of seropositivity to *Cp* antibodies in 19.4 to 95.6% of European feral pigeons [35]. Despite these facts, *Cp* infection seems to be a rare condition within the European population [36].

Chlamydia psittaci as a Causative Factor of OAEMZL

In 2004, Ferreri et al. hypothesized that *Cp* might be a causative factor for OAEMZLs [12]. This hypothesis was based on findings that suggest that extranodal marginal cell lymphomas arise from chronic inflammation due to chronic antigen stimulation [37]. As it has been shown that the presence of *Cp* is linked to chronic conjunctivitis [38]. Tissues of OAEMZL patients were screened for *Cp* by multiplex touchdown enzyme time-release polymerase chain reaction (TETR-PCR) and *Cp*-DNA could be detected in 80% ($n = 40$) of the patients' tissue samples, which was significantly higher than that in control tissues ($p < 0.001$) [12]. Since then, *Cp*-DNA has been detected in OAEMZL tissue samples of various patients all over the world (Fig. 1), with frequencies ranging from 11 to 80% [12, 13•, 14–18, 19•, 20, 21]. However, there is a considerable number of reports which could not find any association of *Cp* (*Cp*-DNA in patient tissue) and OAEMZL [3, 39–48] raising the questions whether these disparities are due to different methodical approaches of *Cp*-DNA detection or geographical differences. The current literature suggests that it is rather due to the latter, as Carugi et al. detected *Cp*-DNA in tissue samples from Italy (Siena), whereas no *Cp*-DNA was detected in tissues of OAEMZL patients from Kenya (Nairobi) using the same methodical approach [21]. Furthermore, *Cp*-DNA prevalence differs significantly in tissue samples of OAEMZLs in regard to the geographic location. As *Cp*-DNA prevalence was significantly higher in samples of German patients (47% $n = 19$) compared to samples from the UK (12% $n = 33$), Italy (13% $n = 15$), and Southern China (11% $n = 37$) ($p = 0.007$; $p = 0.039$; $p = 0.004$; respectively) [13•]. As the mere epidemiological association between *Cp* and OAEMZL is not enough to establish a causative relationship, following Koch's postulates, it has been shown that the frequencies of *Cp*-DNA were significantly higher in tissues from OAEMZLs compared to controls with p values ranging from < 0.001 to 0.042 [12, 13•, 14]. Moreover, *Cp* has been detected in patients with OAEMZL using different diagnostic approaches [16•, 20, 23, 26] and *Cp* could be cultivated from conjunctival swabs and PBMCs of patients with OAEMZL, whereby 100% of the *Cp*-positive cultures originated from patients of *Cp*-DNA-positive OAEMZLs [20]. In addition, *Cp* was found in a single case of a diffuse large B cell lymphoma (DLBCL) in a woman prior successfully treated for (and in remission for 44+ months) *Cp*-positive OAEMZL. By employing DNA sequence analysis, the authors showed that both tissue samples (OAEMZL and DLBCL) contained the same chlamydial strain and were able to identify a potential animal vector (the patient's canary) harboring the same chlamydial strain in its feces and organs [49]. However, it would be too early to conclude that *Cp*-infection is indeed a causative etiological agent of OAEMZL based solely on this report. Nonetheless, it can be argued, considering the aforementioned facts, that the first and second postulates of Koch have been fulfilled. It remains unclear whether the infection of *Cp* causes OAEMZL in formerly healthy individuals since to our knowledge, no study exists which truly elucidates this question. Beside the facts stated above, the most compelling evidence that *Cp* is a causative factor of OAEMZL is the efficiency of antibiotic therapy in the treatment of OAEMZL [22–28, 29••]. This will be further discussed in the following paragraphs.

A Pathophysiological Model of *Chlamydia psittaci* as a Causative Agent in OAEMZL

Extranodal marginal zone B cell lymphomas (EMZLs) in the ocular adnexa (OA) develop at an anatomical site, which is believed to be devoid of MALT tissue from birth (this has been shown for the conjunctivae) [50]. However, there are recent studies that challenge this common point of view [51, 52]. Since EMZLs are thought to develop from marginal-zone B cells, due to the similarity in morphology and immunophenotyping [53], the development of MALT-tissue seems mandatory for the pathogenesis of OAEMZL. It has been shown that lymphoma cells of patients with OAEMZL show somatic hypermutations in their V_H segments [37, 54]. As this hypermutations seem to be restricted to germinal centers of lymph follicles [55] caused by antigenic stimulation [56], it can be deduced that both (germinal centers/MALT tissue and antigenic stimulation) might be necessary for the pathogenesis of OAEMZL. That gives putative evidence for the hypothesis that inflammatory processes play a considerable role in the pathogenesis of OAEMZLS. These findings are followed by the fact that ongoing mutation (intraclonal microheterogeneity) could be observed in the V_H genes of OAEMZL, which might suggest that some OAEMZL are still under the influence of hypermutational mechanism [37], making chronic Ag stimulation plausible. Furthermore, the hypothesis of inflammation as a causative factor in the pathogenesis of MALT lymphoma has been extensively investigated in gastric MALT lymphoma with *Helicobacter pylori* as its

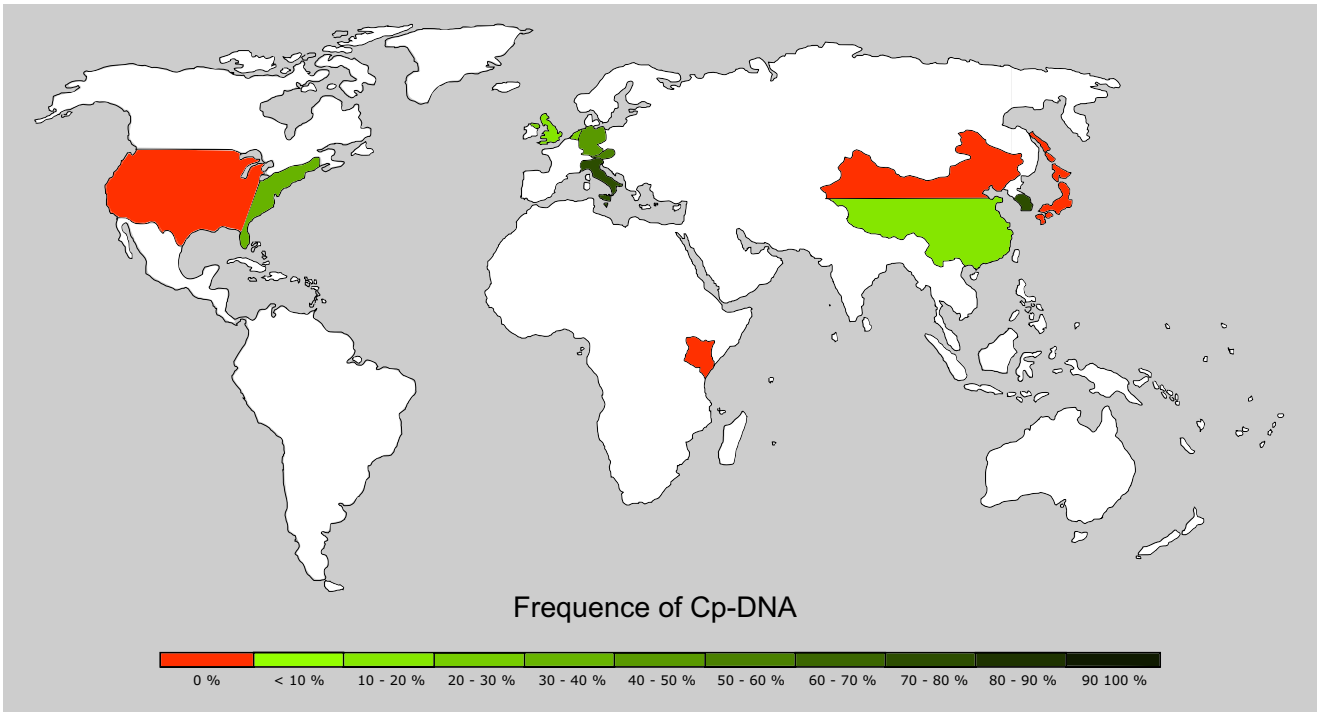

Fig. 1 Geographic differences in the detection of *Chlamydia psittaci* (Cp)-DNA in ocular adnexal extranodal marginal B cell lymphoma: drawing based on all Cp-positive studies [12, 13•, 14, 15, 16•, 17, 18, 19•, 20, 21] as well as studies that could not find *Cp*-DNA [3, 39–48]. The lines drawn in the USA and China are arbitrary; however, the studies that found *Cp*-DNA were located at the East Coast and the south, respectively. If more than one study was available in one geographic region, the highest frequency was used

etiological driving factor [57]. Considering the available evidence at that time, Suarez et al. proposed a pathophysiological model of antigen-derived marginal cell lymphomagenesis in which indirect transformation of lymphoid cells due to microbial pathogens plays one of two central hypotheses (versus direct transformation due to microbial pathogens). The model is comprised of three stages: an early phase where microbial infection, persistence, and proliferation of polyclonal Ag-specific B cells takes place, followed by an antimicrobial-sensitive phase where the MALT lymphoma is Ag dependent for further expansion and transformation of a B cell clone that eventually leads to an antimicrobial-insensitive phase, where the lymphoma cells are fully transformed and do not require Ag stimulation anymore [58]. In line with this model, it has been shown that:

- *Cp* is associated with chronic follicular conjunctivitis [38] and patients with OAEMZL have a significantly higher prevalence of chronic conjunctivitis compared to healthy individuals (50% ($n = 20$) vs 5% ($n = 42$) $p = 0.00001$).
- The prevalence of prolonged contact with household animals (i.e., cats, birds, dogs, fishes, chickens, horses, and others) is significantly higher in OAEMZL patients (5% ($n = 20$) vs. 0% ($n = 42$) $p = 0.006$) [20] as well as the multi-logistic regression odds ratio (MLR-OR) between *Cp*-positive OAEMZL and non-OAEMZL OAL (i.e., nodal marginal-zone lymphoma, diffuse large B cell lymphoma, follicular lymphoma, small lymphocytic lymphoma) was significantly higher in OAEMZL OALs regarding occupational exposure to animals and slaughtering (MLR-OR (95% CI) 7.69 (2.65–22.34) and 16.65 (3.47–80.02), respectively) [59] .
- *Cp* infection can be acquired through contact with household animals (i.e., dogs [60] and birds [61]).

Taking these facts into consideration, it can be hypothesized that *Cp* serves as an etiological factor of chronic infection or recurrent infection through contact to household animals and thereby provides the possibility of chronic antigen stimulation. This is supported by the fact that somatic hypermutation and intraclonal diversity (ongoing mutation) has been found in *Cp*-positive OAEMZL [62•]. Which might indicate that the lymphoma derived from B cell clones, which have undergone Ag selection and are still under the influence of hypermutational mechanism due to chronic Ag stimulation. However, V_H genes expressed in high proportions within OAEMZL can be linked to autoreactivity [54, 63]. Raising the questions whether *Cp* serves as the antigen source itself or induces auto-immunoreactivity. This could either be through molecular mimicry, since it has been shown that *Chlamydia* species share immunoreactivity with eukaryotic heat shock proteins [64] or indirectly acting upon the

malignant B cells, similar to what could be shown for *Hp*, where the B cell receptor expressed by neoplastic B cells is directed against auto-Ags [65, 66] and *Hp* acts indirectly by providing an inflammatory milieu were lymphoma cells react to *Hp*-specific T cells and their products instead of the bacteria [67]. Evidence that supports the latter hypothesis has been provided by Dagklis et al. who did not find a statistically significant difference in V_H-gene usage between *Cp*-positive and *Cp*-negative OAEMZL nor were they able to find IGs similarities between all OAEMZL and Abs specific to *Chlamydiae*. However, they found that V_H-CDR amino-acid sequence cluster analysis of three *Cp*-positive OAEMZL showed homology to B cell clones in a case of rheumatoid arthritis, Sjögren's Syndrome, and a rheumatoid factor from a healthy individual immunized with mismatch red blood cells [62•]. Besides these findings, it has been shown that OAEMZL harbor ≥ 1 genetic aberrations in 62,2% of cases [68] (Genetic aberrations found in OAEMZLs are as follows: *t*(11;18)(q21;21) [21, 68, 69], *t*(14;18)(q32,q21) [68], *t*(3;14)(p14.1;q32) [70], trisomy 3+, and 18+ [46, 68, 70], raising the question if these mutations can also be explained by a *Cp*-based model. It can be hypothesized that *Cp* serves as the etiological agent of a persistent infection, thereby inducing chronic inflammation, and generating a milieu in which reactive oxygen and nitrogen species are produced by leukocytes, serving as mutagenic agents [71], similar to what has been shown for *Chlamydia trachomatis* infection [72]. However, evidence for *Cp* regarding this hypothesis is still lacking. Furthermore, Cargui et al. was not able to find any association between *Cp*-positive OAEMZL and all FISH probes used, testing for chromosomal aberrations (i.e., BCL10, CCND1, MALT1, IGH, IGL, BCL6, BCL2, and MYC-split signal) [21]. This could be due to the very low sample size of *Cp*-positive OAEMZL ($n = 4$) limiting any statistical analysis. Nonetheless, the hypothesis of *Cp* as a direct mutagenic agent still needs to be investigated in more detail. Beside genetic aberrations, it has been shown that DNA methylation in OAEMZLs is significantly associated to *Cp* status [19, 27], with *Cp*-positive OAEMZLs displaying CpG promotor methylations in regions of known or suspected tumor suppressor genes (DAPK, ECAD, MT1G, THBS1,RAR-beta, MGMT), out of which ECAD hypermethylation is significantly associated to *Cp* positivity ($p = 0.041$) [19]. These findings are limited since it was not investigated if these methylations result in altered gene transcription. Nevertheless, promotor CpG methylation of ECAD (a protein maintaining cell-to-cell contacts due to being one of the cadherin molecules) has shown to be significantly associated with gastric *Hp* infection, ($p = 0.002$) as well as with depth of tumor invasion ($p = 0.02$) and regional nodal metastasis ($p = 0.05$) in gastric cancer [73]. Furthermore, the decrease of ECAD was found to be correlated to malignant transformation from chronic gastritis, to chronic atrophic gastritis, intestinal metaplasia, up to gastric cancer and further to lymph node metastasis (i.e., Spearman's rho $r = -0.81$, ($p < 0.0001$)) [74]. Moreover, *Hp*-eradication therapy could reverse ECAD methylation in patients with chronic gastritis [75]. These facts indicate that ECAD gene promotor methylation is an early event in cancer development, which is initiated by microbial infection and can be reversed by antimicrobial therapy (i.e., gastric cancer, *Hp* infection, *Hp*-eradication therapy). Given this evidence, it might be possible that ECAD gene promotor methylation in *Cp*-positive OAEMZLs has a similar role in the development of OAEMZLs, from which can be hypothesized that chronic *Cp* infection serves as a causative agent in the initial development of OAEMZLs, and that its progression relies on continuous stimulation by *Cp* either through the bacterium itself or the inflammatory microenvironment it provides. Evidence that strengthens this hypothesis is that *Cp* infection has been shown to be significantly associated with early-stage disease ($p = 0.035$) [19], as well as the efficiency of antibiotics in the treatment of OAEMZL [22–28], which will be discussed in the following paragraph.

Antibiotic Therapy in *Cp*-Positive OAEMZLs

The use of antibiotic therapy in patients with *Cp*-positive OAEMZL has been evaluated in several studies [22–27], since Ferreri et al. first described its effectiveness (complete remission in 71.4% of treated cases ($n = 7$)) in 2004 [12]. These studies showed overall response rates ranging from 33 to 65% [22–28]. However, only three studies assessed the response to antibiotic therapy stratified to *Cp*-positive and *Cp*-negative cases [23, 25, 26]. Only one study could show an improved response rate for *Cp*-positive OAEMZL (86% vs 47%; $p = 0.02$ ($n = 34$)) [25], whereas the other studies failed to detect a statistically significant difference ($p = 1.000$ ($n = 36$) [26]; $p = 0.25$ ($n = 27$) [23]), even after stratifying for complete remission $p = 0.18$ [23]. Interestingly, also patients suffering from *Cp*-negative OAEMZL did benefit from an antibiotic therapy with response rates ranging from 38 to 60% [23, 25, 26]. This could be due to eradication of other unknown microbes or via direct antitumor activity which has been shown for clarithromycin in animal models [76]. Possible evidence for the later hypothesis could be shown in two trials in which relapsed or refractory EMZLs were treated with clarithromycin (over all response ranging from 38 to 52% (95% CI, 12–64% and 32–72%) [28, 29••]. The authors concluded that the observed regression was rather due to a direct antitumor or immunomodulatory effect of clarithromycin than bacterial eradication since there was no evidence of bacterial infection at the time of study entry and antibiotic therapy received prior to enrollment was either refractory or a relapse occurred [28, 29••]. However, a

retrospective study evaluating the benefit of antibiotic therapy (doxycycline) regardless of the *Cp* status could not show any effect of lymphoma regression whatsoever [77], thus leaving the question unanswered whether antibiotic therapy should be used in patients regardless of their *Cp* status. Nevertheless, treatment with doxycycline [22–28, 29••] or clarithromycin [28, 29••] has been shown to be an effective treatment for OAEMZL, especially in *Cp*-positive cases [25], and can therefore be seen as an optional therapeutic approach for patients with *Cp*-positive OAEMZL.

Conclusion

Based upon the aforementioned evidence, we propose a similar model as Suarez et al. [58] regarding the pathogenesis of OAEMZL that comprises four phases:

1. *Cp* infection takes place establishing an inflammatory microenvironment in which specific Ag selection to either *Cp* or auto-antigens occurs leading to the proliferation and somatic hypermutation of specific polyclonal B cells.
2. This is followed by a phase of persistent or recurrent infection (due to exogenic factors, e.g., animals) that leads to neoplastic transformations, either due to mutagenic agents (e.g., ROS), epigenetic changes (e.g., promoter methylation), or both.
3. These changes in a monoclonal Ag-specific B cell lead to increased proliferation and the overgrowth of other Ag-specific B cells but the *Cp*-induced microenvironment is still mandatory to proliferate substantially.
4. One monoclonal Ag-specific B cell acquires further oncogenic mutations that lead to *Cp*- and Ag-independent proliferation.

However, further pathophysiological studies and prospective multi-centered clinical trials are needed to validate this model and elucidate the question whether all patients with OAEMZL should receive antibiotic therapy regardless of their *Cp* status or not.

Acknowledgements Open access funding provided by Medical University of Graz.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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- •• Of major importance

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