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Is Lyme disease always poly microbial? — The jigsaw hypothesis

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Summary Lyme disease is considered to be caused by Borrelia species of bacteria but slowly evidence is accumulating which suggests that Lyme disease is a far more complex condition than Borreliosis alone. This hypothesis suggests that it may be more appropriate to regard Lyme disease as a tick borne disease complex. Over recent years numerous different microbes have been found in ticks which are known to be zoonotic and can coinfect the human host. The hypothesis suggests that multiple coinfections are invariably present in the clinical syndromes associated with Lyme disease and it is suggested that these act synergistically in complex ways. It may be that patterns of coinfection and host factors are the main determinants of the variable clinical features of Lyme disease rather than Borrelia types. An analogy with a jigsaw puzzle is presented with pieces representing Borreliae, coinfections and host factors. It is suggested that many pieces of the puzzle are missing and our knowledge of how the pieces fit together is rudimentary. It is hoped that the hypothesis will help our understanding of this complex, enigmatic condition.

Introduction

Lyme disease is a condition associated with spirochete bacteria of the Borrelia genus transmitted by ticks of the Ixodes species. This association was made in 1982 [1]. An expanding clinical entity of Lyme disease was already recognised [2]. Since its recognition Lyme disease has caused controversy within the medical profession with frequent lack of agreement about diagnosis and treatment. This paper suggests that a possible source of the confusion has been the preoccupation with Borrelia as the cause of Lyme disease. A hypothesis is presented that Borrelia alone does not result in the clinical syndromes we associate with Lyme disease.

The prevalence of Lyme disease in the USA is reported to be increasing [3]. This may partly reflect a true increase in incidence but is also likely to reflect increasing recognition of the disease. The CDC recognises that the reported cases of Lyme disease underestimate the true prevalence of Lyme disease. The true prevalence remains unknown [3].

The CDC criteria for reporting Lyme disease includes two tier serological testing [3] but it is accepted that this may not be appropriate for diagnosis in a clinical setting [3]. Both false posi-

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tive and false negative results can occur using serologically based tests and laboratories have been found to yield inconsistent results when reporting results of serological testing [4,5].

Culturing of spirochetes is difficult and Borrelia PCR testing has been suggested to hold promise for diagnosis [6] but the sensitivity of the test is low especially if blood is used as the material for testing [6].

Where the main clinical hallmark of Lyme disease, the Erythema migrans rash, is present diagnosis of Lyme disease in an endemic area is relatively straightforward and the diagnosis should be purely clinical [7]. Lyme disease may be present in the absence of EM however [8] and while some fear this may result in over-diagnosis of Lyme disease [8] it should be recalled that the true prevalence of Lyme disease is not known.

In Lyme disease classical inflammatory indices may be normal and objective features traditionally associated with infection such as temperature are usually absent although subjective symptoms such as chills are common [9]. As we have noted diverse presentations of Lyme disease occur and the encephalopathy may be the presenting feature [9,10].

The optimum treatment for Lyme disease has not been established [9]. Courses of treatment recommended in many medical textbooks may be adequate for early infection but will frequently fail in chronic Lyme disease. Retrieval of Borrelia by culture and PCR has been reported from Lyme disease patients even after repeated antibiotic courses [11,12].

Coinfections are being increasingly recognised in Lyme disease but their precise role is proving difficult to elucidate. There are many different micro-organisms found in ticks which are capable of causing a Zoonosis. They include species of Anaplasma (Erhlichia) [13], Babesia [14], Bartonella [15] Mycoplasma [16] and Rickettsiae [17] as well as others. All may be transmitted by other vectors as well as ticks and all have a very wide geographic distribution.

The hypothesis

This hypothesis suggests that Lyme disease only results when certain synergistic combinations of micro-organisms occur in the host. Borreliosis alone may be an insufficient condition for the development of Lyme disease.

It is postulated that only in the presence of other micro-organisms in addition to Borrelia can Lyme disease develop. These other micro-organisms may be tick borne, other vector borne or non vector borne microbes and may be bacterial, protozoan, viral or even fungal.

Pathogenic micro-organisms which may be chronic and are not associated with traditional markers of infection have been termed stealth organisms but this term has not been widely used in the medical literature to date.

An analogy with a jigsaw puzzle is suggested whereby symptomatic disease only occurs when certain pieces of the jigsaw puzzle are in place, the pieces representing coinfections, known and unknown, and host factors.

Evidence for the hypothesis

(1) *Coinfections*. Coinfections are recognised to be important in Lyme disease [18] and it is not possible to consider the topic of Lyme disease without also considering coinfections although some authors have attempted to do this [7].

In one study of 240 patients diagnosed with Lyme disease 11% were found to have evidence of concurrent Babesia infection [19]. In another study of 27 Lyme disease patients 100% were found to have evidence of persistent Mycoplasma fermentans infection by PCR analysis despite previous lengthy antibiotic treatment [20].

The frequent finding of coinfections in Lyme disease patients is one piece of evidence suggesting that Borrelia alone may be insufficient for the development of Lyme disease. Just as it can be difficult to confirm the presence of Borrelia in Lyme disease it can also be difficult to confirm the presence of coinfections. This implies that we are likely to underestimate the frequency with which coinfections occur in Lyme disease.

Attempts to distinguish which symptoms are due to Borrelia and which are due to coinfections in a patient can be difficult. For example it has been reported that the symptoms of Babesia and Borrelia may be difficult to distinguish [19,21]. Presentation of Anaplasma infection is usually non specific with flu like symptoms similar to those which may occur in early Borrelia infection [22].

Since it is likely that there are coinfections which remain to be discovered a picture emerges that in Lyme disease multiple coinfections may be present in most if not all cases and attempting to ascribe different symptoms to different organisms will prove difficult.

Detection of the presence of the organisms associated with Lyme disease can be difficult for the following reasons:

(a) The organisms are few in number.
(b) The organisms are difficult to culture.
(c) There may be failure to elicit an antibody response due to insufficient antigen numbers, immunomodulation or immune evasion by the organisms.

(2) *Known carrier state for Borrelia*. There is evidence for a carrier state of Borreliosis [23] whereby an individual may harbour Borrelia but be asymptomatic. The frequency with which this occurs is not known since patients who are asymptomatic rarely have tests which would indicate the presence of viable Borreliae. In the context of this hypothesis latent Borreliosis may exist because of the absence of other necessary micro-organisms although many alternative mechanisms could be postulated to account for the phenomenon.

(3) *Variable clinical manifestations of Lyme disease*. We have already noted that the presentation of Lyme disease can be highly variable and there could be many possible reasons for this. One possible reason is that the variable manifestations correlate with variations in type and load of coinfections. As we have noted attempts to distinguish which symptoms are due to which coinfection in Lyme disease are difficult as would be expected if a complex, variable, poly-microbial eco-system is present.

One aspect of the variable clinical features of Lyme disease is the apparent difference in presentation between different geographic areas. For example differences have been noted between the American and European forms of the disease [7]. While this may in part be due to differences between strains of Borreliae it may also be that the differences reflect a differing coinfecting load in the two groups.

(4) *Multi-system nature of Lyme disease*. Lyme disease is a multi-system illness and this is recognised in the CDC criteria for diagnosis. Objective criteria for multi-system involvement may be absent and patients with confirmed Borreliosis on occasion have symptoms of multi-system involvement without signs [24]. Lyme disease patients frequently have differing symptoms [3,9,25] and this might lead one to question how it is that one condition can result in such a vast array of symptoms. This hypothesis suggests that the answer is that Lyme disease is not one condition but a disease complex caused by a variable system of interdependent micro-organisms.

(5) *Difficulty in treating Lyme disease*. It is known that Lyme disease can be difficult to treat [9,12] and there is evidence that Lyme disease treatment is more likely to be successful when undertaken early on [26]. Various reasons have been suggested for the difficulties in treating the condition and there is evidence for a cystic, dormant form of Borrelia which is unresponsive to antibiotics [27]. In the context of the present hypothesis another possible reason for the difficulties in treatment could be the complex, variable microbial load.

(6) *Lyme like illness with Erythema migrans in the apparent absence of Borrelia burgdorferi — Masters or STARI disease*. The condition often referred to as Masters disease is a condition which may follow the bite of a Lonestar tick, *Amblyomma americanum* [28]. Masters Disease resembles Lyme disease but despite intensive efforts Borreliae have so far not been recovered from patients with this condition and the putative organism *Borrelia lonestari* has not been cultured [29,30]. Within lonestar ticks Borreliae have been found but their role in the pathogenesis of masters disease remains unproven [31]. Erythema migrans does occur following lone star tick bites [28] and Erythema migrans is normally considered to be pathognomonic of Borreliosis. However it may be the case that other types of micro-organisms found in the lonestar tick can produce Erythema migrans in the absence of Borrelia and this is consistent with the present hypothesis. Using a jigsaw puzzle analogy there may be sufficient pieces in place for Erythema migrans to develop in the absence of the jigsaw piece which represents Borrelia. The suggestion that Borrelia may sometimes not be necessary for the development of a Lyme disease like illness goes beyond the current hypothesis but it is plausible. In a recent study [32] it was found that the clinical features associated with Erythema migrans in Missouri where Lone star ticks are common were quite different to patients presenting with Erythema migrans from New York State where *B. burgdorferi* is prevalent in Ixodes ticks. While different Borrelia types may explain the differences in clinical features an alternative explanation is that the differences reflect the presence of different complements of coinfection.

Evidence for synergism between Borrelia and coinfections

Implicit in this hypothesis is the concept that in Lyme disease Borrelia acts in concert with other micro-organisms for their mutual benefit but to the detriment of the host. It should be appreciated that if this hypothesis is true it will be virtually impossible to study synergism within the human

host since by the time people become symptomatic they will invariably be multiply coinfected.

In one study in humans [19] it was found that clinical symptoms appeared to be more severe in patients who had both Borrelia and Babesia infection than in either condition alone.

Using a mouse model it has been found that coinfection with *Babesia microti* and *B. burgdorferi* results in greater severity of carditis and arthritis than by either infection alone [33].

A similar finding of increased severity of Lyme arthritis has been found in mice coinfected with *Anaplasma phagocytophilum*, the agent of HGE, and *B. burgdorferi* [34]. In another study in mice coinfected with Anaplasma and Borrelia it was shown that an increase in spirochaete numbers occurred in the presence of Anaplasma [35]. It was postulated that the ability of *A. phagocytophilum* to functionally impair neutrophils was a possible mechanism for the observed synergy [35].

Beyond modification of the host immune response there are many possible mechanisms in which synergism between tick borne pathogens may occur but such discussion is beyond the scope of this article. Borrelia is a highly complex microbe [36] and certainly has the genetic capacity for complex interactions with the host, other tick borne and possibly non tick borne microbes.

Implications of the hypothesis

If it is true that Lyme disease is invariably due to the presence of a complex of different micro-organisms then the implications are profound. The role played by Borrelia in the condition is diminished and it may become necessary to redefine Lyme disease in terms of a tick borne disease complex rather than a condition caused by Borrelia.

The hypothesis implies that Lyme disease as a tick borne disease complex will remain an illness which is diagnosed clinically. Difficulties will arise when tick borne micro-organisms are found in a patient who exhibits features of another illness and it may be that dual diagnosis will become more common.

An interesting implication of the hypothesis is that Lyme disease may develop in stages analogous to adding pieces to a jigsaw puzzle. Latent Borreliosis from an earlier tick bite may not become symptomatic until the necessary coinfections have been introduced.

An important implication of the hypothesis relates to treatment. The hypothesis suggests that treatment may need to be tailored to patients according to the complement of coinfecting organisms. This tailoring will be difficult to achieve until methods of detection of Borrelia and its coinfections are improved. In the meantime the hypothesis suggests that it is unlikely a definitive treatment protocol for Lyme disease will be found. If synergism is occurring between pathogens the hypothesis lends support to either a monotherapy or combination therapy approach as being reasonable.

The hypothesis has implications for clinical research into Lyme disease. It will be difficult to produce matching controls of Lyme disease patients for clinical trials as it is unlikely the full complement of the infecting load will be the same for any two cases. At the scientific level the hypothesis suggests that research into Lyme disease should focus on mechanisms of microbial interaction within the host. Such an ecological approach will be difficult technically but modern molecular biological techniques have enabled this work to begin [37].

Using the jigsaw puzzle analogy it is likely that numerous pieces of the puzzle are yet to be found: It is highly likely new coinfections and host factors will be discovered in this truly complex and highly evolved disease.

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