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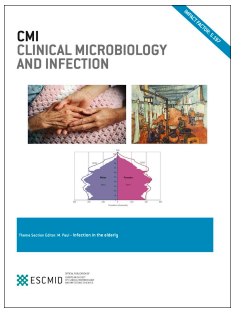

Evolution of Koch's postulates: towards a twenty-first century understanding of microbial infection

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Evolution of Koch's postulates: towards a twenty-first century understanding of microbial infection.

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From the conception of what became known as Koch's postulates (or Henle-Koch's postulates in recognition of Henle's prior conceptualisation of infection theory) through to our current era, microbiologists have wrestled with the problem of infectious agent attribution. Robert Koch himself appreciated that their might be exceptions, or even flaws, in his proposed guide for establishing causality for microbial infections, in particular when dealing with the requirement for the infectious agent to grow and to "produce the disease anew" in experimental vertebrates [1]. Indeed, some researchers of the time struggled to fulfil these postulates resulting in delay in the publication of their findings such as, for instance, the causal relationship of *Borrelia recurrentis* for louse-borne relapsing fever. Here laboratory animals proved refractory to this human-adapted infection, which coupled with the inability to cultivate this spirochaete, challenged fulfilment of the above postulates [2].

Birth of the field of virology confirmed the inadequacy of a stringent adherence to the criteria postulated by Koch. Determination of causality had to be relaxed to accommodate asymptomatic carriage, but also the complexity of recovering these infectious agents and successfully demonstrating infection in animal models. Further evidence of causality was gleaned from use of serological criteria whereby appearance of antibodies against the proposed pathogenic virus could corroborate its role in disease aetiology and was indeed instrumental, for instance, in elucidating the role of Epstein-Barr virus in causing infectious mononucleosis [1].

Attempts to update Koch's postulates were introduced using a molecular variant of these [3]. These pragmatic guidelines were essential to prevent falsely incriminated microbes being correlated with pathological causality through "guilt by association" (see table 1).

The paradigm was further challenged by our increasing understanding of microbial infection including, for examples: modulation between latency and overt infective episodes; appreciation of mixed polymicrobial biofilm infections; the certainty that one organism might have a causative role in various clinically distinct infections whilst others show a more narrow clinical presentation; the ability of some pathogens to cause differing clinical pathologies and similarly, unrelated infectious agents to produce indistinguishable disease consequences. Host factors must not be overlooked, with different immunological predispositions, efficacies resulting in a gradient of clinical consequences following infection. Furthermore, existing prior infections might have provoked an immune response that is poorly aligned to tackle the current pathogen, thus such immunological dissonance might facilitate heightened spread or indeed provoke pathological destructive consequences.

Intertwined with these host factors, we must also consider the composition of our normal flora and its ability to deter the ingress of pathogens either directly or indirectly through colonisation resistance [4]. Our appreciation of complex microbial communities and their influence over development of disease is still in its infancy.

Currently disease attribution demands that complex interactions whereby location, host factors and possession of virulence genes combine contributory influences regarding pathological consequences. Others suggest that successful intervention should also be included, however given the increasing levels of antimicrobial resistance, this may now be a less informative criteria.

Singh et al. eloquently review these challenging dilemmas within this issue [5]. They detail the microbial dysbiosis observed in patients with inflammatory bowel disease and explore our current knowledge of the underpinning factors that facilitate this condition. They argue the need for expansion of Koch's postulates to accommodate the multitude of microbial triggers and highlight that a common profile for this dysbiosis is probably unrealistic, however, with such modifications, Koch's postulates still offer value in demonstrating causality. Importantly, they note that though experimental models can reproduce inflammatory bowel responses, these are rarely followed for sufficient duration to demonstrate the chronic and relapsing picture typified by Crohn's disease or ulcerative colitis.

Additional issue with which we have to deal with when the evolution of Koch's postulates is addressed come from virology. It is now clear that, together with the major known pathogenic viruses, many other viruses are present in clinical samples and that the totality of these agents, defined as human virome, is an integral part of the microbiotic universe that makes us healthy [6]. In this framework, we know that human Torquetenovirus TTV), the most abundant virus within the virome, has a remarkable ability to produce chronic infections with no clearly associated clinical manifestations, gaining the status

of orphan virus. Focosi et al. in this issue summarize recent findings about TTV and review its characteristics [7].

The review by Gentile and Micozzi deals with further emerging concepts regarding viral infections whereby some cause a life-threatening illness whilst in others with similar risks, present with only a limited or benign illness, if indeed any consequences of infection are noted. The authors speculate that some viruses, other than those causing viral diseases, and beyond those existing as chronic commensal long-lived infectious accrued throughout lifetime, may exert a physiological protective effect on the host through “trans-kingdom interactions” and immunomodulatory effects, also potentially providing anti-tumour protection [5].

Now that we have embarked upon the whole genomic sequencing era, further adaptations of causality guidelines for infectious diseases are warranted. The powerful combination of cultivation approaches assessed against the backdrop of the local microbial consortia in its entirety might collectively provide deeper insights into causality for infectious diseases [4]. We can further embed this approach within the emerging iterative computational field of systems biology whereby a milieu of molecular interactive networks can potentially predict pathological interactions and outcomes between microbes and their host. Though still in its infancy, the technological impacts of this emerging discipline could revolutionise diagnostics and furnish us with new intervention possibilities [6].

In the light of the above considerations, it is tempting to speculate that, finally, we still fail to have consensus by which infectious disease causation can be established without doubt. Largely this dilemma arises from the continuum of interactions demonstrated between microbes and their host, necessitating our understanding of this complex interplay and underpinning factors that influence whether this will manifest as asymptomatic carriage or development of life-threatening pathological consequences.

120 Table: Criteria for causality over the ages

Suggested causality indicators	Year	Reference
• Diseases might be caused by microorganisms	1838	Henle cited by [1]
• Parasite occurs in every case of the disease under circumstances that could account for observed pathology • Parasite is absent from those without the disease • It can be reproducibly grown in pure culture • It can induce the disease anew	1891	Koch cited by [1]
• Specific virus must regularly be found associated with a disease. • Virus must be shown to occur in the sick individuals but not as an incidental or accidental finding, instead being the cause of the disease under investigation.	1937	[7]
• New virus established by laboratory passage (animal/tissue culture) • Repeatedly isolated from human specimen and not a contaminant derived from host used to propagate the virus • Antibody response increasing as a result of infection • Agent compared with other similar viruses • Constant association with specific illness • Double blind studies with human volunteers should reproduce clinical disease • Cross-sectional and longitudinal studies to identify patterns of disease • Preventable by use of specific vaccine	1957	[8]
• Nucleic acid sequence belongs to a putative pathogen and is present in most cases of an infectious disease preferentially associated with pathology • Lower copy number or absence of these sequences from those without disease • Decrease or absence following treatment/recovery • Detection predates disease or sequence copy number correlated with severity • Congruence with biological knowledge • Correlation with areas of tissue pathology • Reproducible findings	1996	[3]
• Sequencing microbial community • Computational models to assess presence and	2016	[4]

proportion for resulting pathology
- Isolation of microbes of interest from diseased host
- Testing of fresh isolates and consortia in relevant disease model

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