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Koch's Postulates and Infectious Proteins

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Abstract

Koch's postulates were formulated in the late nineteenth century as guidelines for establishing that microbes cause specific diseases. Because the rules were developed for living agents—particularly bacteria—their applicability to inanimate pathogens such as viruses and infectious proteins has been problematic. The unorthodox mechanism by which prion diseases are transmitted, involving specific physicochemical characteristics of the protein as well as susceptibility traits of the host, has made these disorders refractory to analysis within the context of the original Koch's postulates. In addition, evidence is accumulating that other proteopathies, such as AA amyloidosis, apolipoprotein AII amyloidosis, and cerebral A β amyloidosis, can be induced in vulnerable recipients by cognate proteinaceous agents. In light of the salient differences in the mode of disease-transmission by microbes and proteins, we propose modifications of Koch's postulates that will specifically accommodate presumed infectious proteins.

Keywords

Alzheimer's disease; amyloid; apolipoprotein AII; conformational disease; prion

Introduction

The misconformation and polymerization of specific proteins is now recognized as an important disease process in a variety of neurological and systemic disorders, including Alzheimer's disease, Parkinson's disease, Huntington's disease, prion diseases and the systemic amyloidoses [3, 18, 22, 32, 40, 41]. The prion diseases have been considered to be the only "infectious" form of cerebral proteopathy [8, 9, 16], but, despite decades of productive research [1, 6, 39], only recently has the proteinaceous nature of the infectious agent passed the point of reasonable dispute [4, 24, 44, 47]. A complicating feature of the prion diseases is that they also can emerge *de novo* in genetic and idiopathic forms [33], and transmissibility is highly dependent on the characteristics of both the source and recipient

of the agent. Surprisingly, it is becoming apparent that other proteopathies can be induced by seeding-like mechanisms that share intriguing commonalities with prionoses [reviewed in 35, 42]. These disorders include systemic amyloid A (AA) deposition, mouse senile (apolipoprotein AII) amyloidosis, and cerebral A β -amyloidosis. The fact that disparate amyloidoses can be precipitated by exposure to diseased tissue extracts suggests the possibility of common molecular mechanisms of induction. Verifying that a transmissible molecular template is involved in the instigation of these disorders will require general agreement about the evidence that is necessary and sufficient to establish causality by exogenous agents. Koch's postulates, tailored to accommodate inanimate pathogens, are well-suited for this purpose.

Koch's postulates

Despite considerable circumstantial evidence linking microbes to infectious illnesses, the germ theory of disease remained disputable until late in the 19th century. Then, Friedrich Loeffler (a protégé of Robert Koch) explicitly outlined a series of steps for establishing with a high degree of certainty that a specific microorganism causes a specific disease [2, 26]. These steps are usually known today as "Koch's postulates", and, in their modern incarnation, they differ little from those articulated by Loeffler:

1. *The organism must be shown to be invariably present in characteristic form and arrangement in the diseased tissue.*
2. *The organism, which from its relationship to the diseased tissue appears to be responsible for the disease, must be isolated and grown in pure culture.*
3. *The pure culture must be shown to induce the disease experimentally.*
4. *The organism should be re-isolated from the experimentally infected subject [this postulate was added after Loeffler].*

Koch's postulates were devised as general guidelines to identify infectious microbes that could be detected with the available methods and that were demonstrably alive (i.e., capable of independent metabolism, growth, and reproduction). In many instances, one or more of the rules could not be fulfilled, such as when a pathogen could not be cultured, when a usually innocuous microbe becomes pathogenic, or when a valid animal model of the disease was unavailable for experimental transmission [12]. Subsequent, futile attempts to apply the precepts to viruses, which at that time could neither be seen nor cultured in isolation, may actually have impeded the early development of virology [2, 13]. More recently, the postulates have been strained further by putative infectious agents that consist solely of conformational variants of normally produced protein molecules.

Causation postulates and proteinaceous agents

In the transmissible proteopathies, the disease state can be initiated by exposure of a host to pathogenic material from an afflicted animal. However, the ease with which different diseases can be induced by exogenous agents varies greatly. For example, whereas prion disease and mouse senile amyloidosis can be communicated between compatible organisms even by relatively inefficient routes, there is, as yet, no evidence that A β -amyloidosis can

be transmitted except by direct introduction of pathogenic material into the brain [42]. Moreover, in complex tissue extracts, it is difficult to isolate and identify unequivocally the substance or substances that are necessary and sufficient to actuate protein conformational diseases. The problem is further complicated by the fact that a potentially pathogenic protein must be generated by the host to sustain replication; hence, in instances where a conformational change arises spontaneously, the disease emerges in the apparent absence of “infection”. Finally, the proteopathic agent may not transmit disease except within an accommodating milieu, such as an excess of susceptible host protein that might result from inflammation, deficient clearance, the presence of molecular chaperones, or genetic factors. Transmissibility, then, is regulated by the complementarity of host- and agent-specific traits. While microbiologists and physicians have long been aware of the critical role that host factors play in the transmission of conventional infectious agents [12, 14], mention of the host is conspicuously absent from Koch’s postulates.

In light of these considerations, and of the growing list of proteopathies that can be induced by exogenous material, it may be worthwhile to establish the basic conditions that must be met to demonstrate, beyond reasonable doubt, that a protein alone is able to transmit a particular disease. With a nod (and apologies) to the Kochian tradition, we suggest the following guidelines for substantiating the transmission of a proteinaceous agent:

1. *The protein must be invariably present in a disease-specific form and arrangement in the diseased tissue.*
2. *The physicochemical characteristics that confer infectivity on a specific protein must be established.*
3. *The characteristics that render the host susceptible to infection by a specific proteinaceous agent must be established.*
4. *The disease process must be induced in a susceptible organism by the pure agent in its infectious form.*
5. *The protein must be recovered in its infectious form from the animal that was experimentally infected with the pure agent.*

The *first* step - to establish a link between a specific protein and a specific disease – is essentially the same as that proposed for microbes by the Koch school and its predecessors. In all proteopathies, a particular protein accumulates in certain tissues, often forming distinctive lesions such as inclusion bodies or extracellular protein deposits. The presence and distribution of these lesions can vary, and in some instances, they are not apparent histologically, so it is necessary to confirm protein accumulation using quantitative biochemical methods. Additionally, it is important to recognize that soluble forms of the protein, such as small oligomers, may have a greater cytotoxic effect than the histologically detectable lesions *per se* [5, 7, 40–42]. The *protein-disease* connection can be strengthened by the discovery of genetic mutations affecting the precursor protein (or proteins involved in the production, degradation, modification, or transport of the pathogenic product) that cause hereditary forms of the disorder.

Second, the primary amino acid sequence, the secondary, tertiary, and quaternary structure, and post-translational modifications or processing that make a protein infectious must be unambiguously characterized, since strain differences appear to be encoded in the multidimensional configuration of conformationally transmissible proteins [8, 20, 23, 30, 36, 37, 44]. Additionally, infectious and toxic protein particles may not necessarily be identical [5, 7, 8]. Full structural characterization of endogenous proteins in their inductive state is a significant unmet objective, but one that is beginning to yield to the creative application of new methods [11, 21, 23, 28, 34, 46].

The *third* requirement is to determine the genetic, biochemical, and cellular features that govern host vulnerability to infectious proteins. This step is indispensable for demonstrating protein-based transmission, as the host supplies the raw material for proteopathic amplification, as well as the integrated physiological context in which the disease is nurtured [31]; failure of disease transmission to an unsuitable host is not confirmation that a protein particle is non-infectious. The possible participation of intermediate cells (such as the lymphoid system) in transmission must also be considered [19, 38, 43]. While the list of *potential* host factors is long, the endogenous characteristics that render an organism vulnerable to a specific infection are likely to be few, a crucial one, of course, being the amino acid sequence of the host protein [10].

The *fourth* step is to induce the disease *in a susceptible organism* by a purified or synthetic agent *in its infectious form*. A pure agent would be one meeting the requirements set forth in postulate 2, and that is unadulterated by other substances. As a corollary of this postulate, any alteration of the optimal pathogen or host susceptibility factors should diminish or abolish the infectivity of the protein. Producing a purified and infectious proteinaceous agent remains a challenge.

Finally, the abnormal protein should be recovered in its infectious form from the animal that was infected with the purified agent. In this case, it is important to recognize the possibility that re-transmission can be influenced by host/species traits that select, or conformationally modify, the strain of pathogen in the new host [8, 25, 45]. Hence, if infectivity is diminished after passage through a particular host, it is necessary to determine if critical features of the agent have been altered (postulate 2).

These conditions for demonstrating the transmissibility of protein-based diseases depart from those of the Koch school mainly in the explicit recognition of host factors that govern infection, and in requiring a more complete characterization of the pathogen. Koch asserted that “The pure culture is the foundation for all research on infectious diseases” [2], and this premise, in a slightly modified sense, applies to inducible protein diseases as well. Even a pure agent, however, is only pathogenic in a receptive host; the synergy of host and pathogen is thus essential to the meaningful assessment of protein infectivity. Finally, it is worth noting that the structural corruption of normal proteins by permissive templating [17] allows for the possibility that any exogenous agent with the appropriate conformational complementarity with the pathogenic protein could induce disease [15, 27, 29, 35, 41, 42].

Conclusions

Koch's postulates were developed in the late 19th century to facilitate sound research on the microbial causation of infectious diseases. The postulates have unquestionably proven their mettle in the bacterial domain, but to enhance their contemporary utility, they must be adapted to accommodate etiologically atypical diseases. Growing evidence suggests that several proteopathies might share with prion diseases some fundamental pathogenic features, including inducibility by abnormally conformed proteins. Koch's postulates, properly matched to such unconventional "infectious" disorders, can help to guide the experimental assessment of this hypothesis.

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