

# Bacterial-Metal Interactions: The Potential Role of Aluminum and Other Trace Elements in the Etiology of Crohn's Disease

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■ *Hypothesis: We suggest that bacteria colonizing the intestinal wall of patients with Crohn's disease incorporate aluminum and other trace elements through their siderophore iron-uptake system and that as a result of the adjuvant properties of aluminum, they induce an exuberant granulomatous inflammatory response.*

Despite the recent burst of excitement about the genomics of Crohn's disease, the critical importance of environmental factors in this condition has been recognized ever since the disease was described.<sup>1-4</sup> The chief suspects for this role have always been microorganisms and dietary constituents. Many candidate microorganisms have been proposed, evaluated and discarded over the decades, but the most persistent theories have revolved around mycobacterial infection.<sup>5,6</sup> Among potential dietary agents, however, none has yet emerged as a favorite.<sup>7,8</sup> An offending dietary constituent, of course, need not be conventional food-stuff; additives, preservatives, and even microparticle contaminants are plausible alternatives.<sup>9</sup> With this in mind, we have arrived at an etiologic hypothesis for Crohn's disease based on our study of a fatal outbreak of granulomatous enteritis in a group of horses sharing a common environment.<sup>10</sup> The horses suffered from a disorder with many histologic similarities to human Crohn's disease. In the evaluation of the cluster, an unexpected finding was the presence of excess aluminum in affected tissues. Microprobe elemental analysis demonstrated that aluminum was concentrated within microorganisms in the intestinal wall. Based on these observations, we propose a novel approach to etiologic concepts of Crohn's disease.

Our hypothesis is that the capability of certain microbial organisms, in particular mycobacterial species, to take up certain trace elements from environmental and host sources, may alter the pathogenicity of these organisms and exacerbate the host's responses to them. As we will outline, mycobacteria possess potent metal chelators that provide essential metallic elements, especially iron, required for bacterial growth and virulence. We propose that in addition to iron, other metals, particularly aluminum, may use these same metal chelating systems to gain access to the organisms and this may alter their pathogenicity and ability to induce an exuberant granulomatous response.

It is well established that the virulence and even survival of many bacterial organisms during infection depends on the ability of the pathogenic microbe to compete for essential nutrients, especially iron.<sup>11</sup> However, in vertebrates iron remains sequestered within a number of high molecular weight binding proteins, such as transferrin, lactoferrin and ferritin. In what is essentially an iron-poor environment, microorganisms have evolved to produce ferric ion chelating agents, called siderophores, that are capable of solubilizing and transporting iron into the microbe.<sup>12</sup> Siderophores contain some of the most efficient iron-binding ligands in nature and are capable of securing the metal from transferrin, ferritin, ferric hydroxide as well as from synthetic chelators such as EDTA. The affinity of siderophores for iron is remarkably strong with dissociation constants (Ks) in the range of  $10^{22}$  to  $10^{50}$ .<sup>11</sup>

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Access to iron by microorganisms is carefully regulated by complex systems that respond to situations of either iron excess or deficiency. Iron-dependent regulators (IdeRs) are metal-activated DNA-binding proteins found in Gram-positive and acid-fast bacteria. It is critical to our hypothesis to recognize that these proteins are also transcriptional regulators of virulence factors. For example, in many gram-negative and a few gram-positive organisms, the synthesis of siderophores and their receptors is controlled by the ferric uptake regulator protein, Fur.<sup>12</sup> When complexed with iron, Fur binds to a consensus nucleotide sequence in the operator region of Fur-related proteins, which is related to expression of the iron-uptake system proteins as well as other virulence factors. In situations where the intracellular iron concentration is sufficiently high, transcription of virulence factors and iron import pathways is repressed by the binding of Fe(II)-IdeR complexes to the promoter/operator sequences of their genes.

Mycobacteria require iron for their growth and also possess important iron-regulatory factors. Mycobacterial elaborate mycobactins, a form of siderophore localized to the cell envelope. They also elaborate exochelins, which are water-soluble extracellular siderophores. This iron-binding system is critical for the survival of mycobacteria in the iron-limited environment of the host. A null mutation of *mbtB* introduced into *M. tuberculosis* severely impairs its growth in the human macrophage cell line THP-1.<sup>13</sup> It has been shown that IdeR from *M. smegmatis* strains regulates siderophore synthesis in an iron-dependent manner and that IdeR-deficient *M. smegmatis* strains no longer repress synthesis of exochelins and mycobactins under high-iron conditions.<sup>11</sup> Further data implicate IdeR as a regulator of genes that are essential for full virulence of *M. tuberculosis*.<sup>14</sup> In vitro, the presence of a wide variety of other iron-binding ligands significantly changed the growth index of *M. paratuberculosis*.<sup>15</sup>

It is clear that bacteria, in general, and mycobacteria specifically, have systems for obtaining and internalizing iron, an essential element for their growth and virulence. However, the chelating and internalizing molecules of this system are not specific for iron and may attract a wide variety of other cation species.<sup>16</sup> Such siderophore systems have been effectively used to solubilize and incorporate a wide variety of toxic metals in environmental bioremediation approaches to clean up metal-contaminated wastes.<sup>17</sup> Further, marine organisms have been shown to make siderophores with an affinity for a wide variety of transition metals.<sup>16</sup> Recently, the pharmaceutical industry has begun to use siderophore-antibiotic conjugates as a means of entry of the drug to target bacteria through the iron-siderophore system.<sup>18</sup>

We therefore propose that under certain conditions, aluminum (and possibly other metals) may be carried into mycobacterial, and other microbial species through their iron-uptake systems. This same siderophore pathway would allow aluminum to enter the organism and become incorporated into

its biologic constituents in a manner analogous to the Trojan Horse of Greek mythology. Once aluminum gained access to invading bacteria, it could alter both the pathogenicity of the organism and the nature of the host's response to the infection. Aluminum, after all, has been used for many years as a vaccine adjuvant<sup>19</sup> and in many settings is known to stimulate prominent granuloma formation.<sup>20-23</sup>

There are ample precedents to support the idea that aluminum could accompany iron into micro-organisms. Iron and aluminum are commonly encountered together in biologic systems. A wide variety of human disorders and animal models with iron overload and tissue deposition (e.g., hemosiderosis, hemochromatosis) demonstrate the simultaneous presence of both iron and aluminum accumulation in affected tissues.<sup>24</sup> Transferrin and lactoferrin are capable of carrying iron and aluminum simultaneously.<sup>25-27</sup>

Host factors for iron-dependent functions also play an important role in determining biologic response, and may even account for some of the genetic susceptibility data in Crohn's disease. Macrophages are the principal cells that sequester microbial organisms, particularly mycobacteria. Macrophages possess their own iron-uptake systems and produce proteins called Nramps to alter their own iron status. Susceptibility to mycobacterial infection in mice is controlled by a gene, *Bcg*<sup>28</sup> that codes for natural resistance associated macrophage protein 1 (Nramp1).<sup>29</sup> Nramp1 is believed to provide a means for efflux of a variety of metals, especially iron, from the phagosome membrane. Mycobacteria make their own form of Nramp, which is generally referred to as Mramp. Both Nramp and Mramp are divalent cation transporters and during mycobacterial infection compete within macrophages for various metal ions.<sup>30</sup> Strong associations have been noted for intronic and extragenic polymorphic variants of human Nramp1 and tuberculosis susceptibility.<sup>31,32,33</sup> Linkage of Nramp1 alleles to susceptibility to leprosy has also been reported.<sup>34,35</sup> We are aware of a single study of Nramp polymorphisms in patients with Crohn's disease. This study showed a significant ( $P < 0.05$ ) association of Nramp markers D2S434 and D2S1323 and the occurrence of the disease.<sup>36</sup>

In sum, we propose that the iron-related trace element uptake and/or regulatory system of either the putative mycobacteria or of the human host, or both, are involved in the pathogenesis of Crohn's disease. We further propose that the iron-uptake/regulatory system allows access to mycobacteria of metal ions other than iron (especially aluminum) resulting in an alteration in the organism's virulence and/or the host's ability to contain it. Once the aluminum-loaded organism is incorporated into the host, aluminum enhances the organism's ability to induce a prominent granulomatous immune response, thus giving rise to the pathologic features of the disease.

The implications of such a hypothesis, of course, extend well beyond Crohn's disease alone. Over the past twenty years, it has been demonstrated that microbial organisms can sur-

vive—indeed, in some instances flourish—under extreme environmental conditions, such as extremes of temperature, high and low pH, or high concentrations of trace metals and other potentially toxic substances. Bacterial organisms have a remarkable capacity to evolve and adapt successfully to such harsh conditions.

Our hypothesis is testable in both human specimens and analogous naturally occurring animal disorders as well as in experimental animals. For example, this concept could be explored in Johne's disease, a focally endemic disorder of sheep with many similarities to Crohn's disease. Further, experimental infection by aluminum-enhanced microbial species might be employed as means for developing new animal models of Crohn's disease. Finally, genetic testing in Crohn's disease patients for host-related metal ion-dependent functions might also prove a fruitful avenue of research.

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