COMMENTARY

The beginning of an era of functional genomics in Rickettsiology is steeped in history

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Human diseases caused by bacteria in the genus *Rickettsia*, commonly referred to as typhus or spotted fevers, are among the oldest and most severe scourges of humankind. All of the diseases' agents utilize blood-sucking arthropods as vectors, which influences disease epidemiology. Epidemic typhus continues to cause outbreaks today in situations of war and unrest (1), and the incidence of Rocky Mountain spotted fever is growing in the Americas (2). Despite an urgent need, broadly protective vaccines are unavailable, although previously used vaccines proved this an achievable goal (3). It has long been known that recovered patients mount protective immune responses, producing antibodies that have been used for diagnosis of rickettsioses through the so-called Weil–Felix reaction since the early 1900s (4). However, until the research by Kim et al. (5), the identity of the genes encoding the molecular machinery responsible for producing the cognate antigen in all species of rickettsiae was unknown.

The history of the Western world reflects the power of rickettsial disease agents. Epidemic typhus, which may kill over 30% of infected people, has shaped history profoundly at least since medieval times (6), when hapless inhabitants of cities beleaguered by enemy armies were forced to live in crowded quarters and under unsanitary conditions. This created favorable conditions for human body lice, the vectors for the causative agent, *Rickettsia prowazekii* (7). Soldiers of the occupying armies or on the move were not spared, either, as they marched and slept in their clothes for weeks and longer. Thus, Napoleon's "Grande Armée" was reduced to a few thousand men from a starting strength of 600,000 through epidemic typhus exasperated by the severe Russian winter (8). During World Wars I and II, the devastating effects of epidemic typhus spurred vaccine development (3). The epidemiology of tick-borne rickettsial diseases, such as Rocky Mountain spotted fever and boutonneuse fever caused by *Rickettsia conorii*, the subject of the present article by Kim et al. (5), is likewise dictated by their vectors. Tick-borne rickettsial pathogens are zoonotic and involve wild (e.g., rodents) or domestic animals such as dogs. In the late 1800s, Rocky Mountain spotted fever, feared for its ability to kill 4 of 5 people who became ill (9), was a big deterrent to people who considered settling in sparsely populated but resource-rich Montana. This was enough of a problem for citizens to call for help from their governor. Research stimulated by this public demand eventually resulted in the identification of the tick vector and disease agent through the seminal work of Howard Ricketts, after whom the genus of bacteria was named, while employed as a professor of pathology at the University of Chicago (10) (Table 1). This is also where the team of Kim and Schneewind conducted their groundbreaking research to identify the molecular machinery responsible for producing the antigen that elicits protective bactericidal antibodies in patients (5).

Once researchers started to investigate anti-*Rickettsia* immune responses, they found that survivors of infection were protected, and that there was substantial cross-reactivity (11), not only among rickettsiae but also with nonpathogenic *Proteus vulgaris* strains OX2 and OX19 (4). This puzzling feature of a family of obligately intracellular bacteria having this much immunological homology in common with commensal intestinal bacteria was later linked to shared structure and composition of the lipopolysaccharide (LPS) layer of both rickettsiae and *P. vulgaris* strains OX2 and OX19 (12). First described by Weil and Felix in 1916 (4), it became widely used for the diagnosis of rickettsial diseases, even today in resource-limited settings, due to its simple and cheap design, although superior diagnostics are now available.

With the continued and increasing threat from rickettsioses (2), vaccine development has been a priority. A number of different targets have been identified, including surface proteins and metabolic enzymes (11, 13). Despite the long-standing knowledge that Weil–Felix antibodies were linked to immunity (14), the identity of the gene(s) coding for the enzymes producing the protective antigen(s) remained obscure. Kim et al. (5), in the laboratory of Schneewind at the

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Table 1. Timeline of milestones leading to the discovery of the *R. conorii pso* operon

Year	Milestone
1906	Taylor H. Ricketts, professor of pathology at the University of Chicago, determines that the agent of Rocky Mountain spotted fever is transmitted by ticks (20)
1907	Ricketts discovers the agent of Rocky Mountain spotted fever in ticks and in blood of experimentally infected animals (20)
1909	Ricketts identifies the agent of epidemic typhus in body lice and patient blood, and demonstrates louse transmission to rhesus monkeys and development of protective immunity (21)
1916	Weil and Felix (3) publish diagnosis of typhus using *P. vulgaris* ("Bacillus proteus") strain X 19
1930	Weigl (22) demonstrates that Weil–Felix antibodies are correlated with immunity to epidemic typhus
1993	Amano et al. determine the chemical properties that underlie cross-reactivity of *P. vulgaris* OX19 and spotted fever rickettsiae LPSs (12)
1997	Ziolkowski et al. (23) establish the structural properties of the O antigen of *P. vulgaris* OX strains
2019	Kim et al. (5) identify the LPS synthesis operon (*pso*) of *R. conorii* as comprising the genes encoding the enzymes used by rickettsiae to synthesize the O antigen that elicits rickettsiacidal antibodies cross-reactive with *P. vulgaris* O antigens OX2 and OX19

Howard T. Ricketts Laboratory, recognized the importance of these observations in providing a rational basis for the development of a broadly protective vaccine against these serious diseases. Vaccine development could be accelerated by methods to analyze gene function through knock-out followed by molecular restoration of function to unequivocally prove that the defect induced by the mutation was attributable to the target being disrupted, and not an unrelated off-target effect. Although transposon mutagenesis of rickettsiae and complementation of gene function using a shuttle vector has been achieved (15, 16), these methodologies have primarily been used to investigate rickettsial mechanisms of motility and spread among cultured cells. In their research, Kim et al. identify the genes of the polysaccharide synthesis operon involved in producing the antigen that elicited the bactericidal Weil–Felix antibodies, and determine that transposon insertion in *Rc0457* encoding UDP-GlcNac 4,6-dehydratase/3,5-epimerase abolishes O-antigen production, resulting in extensive reorganization of the rickettsial outer cell wall. Notably, this increased the amounts of rOmpA and rOmpB in the rickettsial outer membrane, 2 protective antigens identified previously (13). Complementation of mutants with a plasmid carrying *Rc0457* and downstream sequences partially restored rickettsial cell walls to wild-type composition and significantly improved mutant growth rates. Sadly, Olaf Schneewind, the lead investigator of the team, passed away in May after losing his battle against cancer, while the article was under review. He was only 58 years old. While insertional mutagenesis and restoration of function using complementation with a plasmid-encoded wild-type gene is standard practice in research with bacteria that can be propagated axenically, this is still far from routine with rickettsiae, especially those that require BSL3 containment. Significantly, the *kkaebi* transposon created by Kim et al. adds a tool to the functional genomics toolbox for rickettsiae which is sorely in need of additional technologies (17), and, through analysis of the HK2 mutant phenotype, the authors have contributed fundamental advances in *Rickettsia* immunology. Designed for random insertion, *kkaebi* transposons appear to be of comparable efficiency to the himar1 transposon originally designed for use with *Anaplasma phagocytophilum* (18), but are smaller, which is an advantage in the world of small-genome rickettsiae. This mutagenesis system has the potential to accelerate rickettsial molecular genetics to gain insights into the function of annotated and hypothetical genes in rickettsial genomes. Approximately 30 to 40% of rickettsial genes have no known role (19), making random mutagenesis a preferred approach over targeted mutagenesis for discovery of gene function.

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