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COMMENTARY

Rickettsial Entry into Host Cells: Finding the Keys To Unlock the Doors

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The global impact of rickettsial infections (inclusive of infections by all members of the order *Rickettsiales*, including both families, *Anaplasmataceae* and *Rickettsiaceae*) is illustrated by both the historical record and the emergence of new pathogens. Epidemic typhus, caused by *Rickettsia prowazekii*, has been responsible for the loss of millions of human lives (18), while investigation of *Rickettsia rickettsii*, the etiologic agent of Rocky Mountain spotted fever, led to the development of what is now the National Institutes of Health Rocky Mountain Laboratories (6). More recently, *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* emerged to be recognized as major causes of tick-borne human illness (3). Furthermore, the last year alone has seen several key publications revealing the widespread importance of rickettsial infections as truly neglected tropical diseases (8, 13, 15). Given this global impact and the rapidity of progression to severe morbidity and mortality, there is a compelling need for safe and effective vaccines. Although crude vaccines have been used and shown to protect against disease, development of standardized, safe, and effective vaccines has been limited by a lack of understanding how these obligate intracellular pathogens invade cells, and specifically the required bacterial molecules.

In the current issue of *Infection and Immunity*, Ojogun et al. (10) present compelling evidence that *A. phagocytophilum* outer membrane protein A (OmpA) is required for efficient entry into host myeloid cells. Using classical approaches, this team of investigators led by Jason Carlyon at Virginia Commonwealth University shows that entry can be blocked by specific antibody to OmpA and competitively inhibited by both full-length OmpA and the predicted N-terminal extracellular domain. In this discovery, their data add to the identification of outer membrane proteins shown to mediate rickettsial binding with and/or entry into specific target host cells (1). Where the work by Ojogun et al. stands out is in the dissection of the interaction between the bacterial surface protein and the specific receptor components. In a series of seminal publications, beginning over 10 years ago, P-selectin glycoprotein 1 (PSGL-1) was identified as the receptor for *A. phagocytophilum* entry into human myeloid cells, using both the HL-60 cell line as a model and mature neutrophils, the natural host cell (5, 7, 17). PSGL-1 is capped on its N terminus by an O-glycan with exposed sialyl Lewis x (sLe^x), a tetrasaccharide composed of α1,3 fucose and α2,3 sialic acid. Notably, receptor function in human myeloid cells has been linked to three structural elements: (i) the N terminus of PSGL-1 itself, (ii) α1,3 fucose of sLe^x, and (iii) α2,3 sialic acid of sLe^x. In the present work, OmpA, and specifically the extracellular domain, has been shown to bind the α2,3 sialic acid of sLe^x, and this receptor-ligand interaction is required for efficient cellular entry. Competitive inhibition of binding and entry using OmpA (and, separately, the extracellular domain) essentially mimics the inhibition afforded by either a monoclonal antibody directed against the sLe^x α2,3 sialic acid or sialidase treatment of the host cell.

The number of years between initial demonstration of PSGL-1 and sLe^x α2,3 sialic acid as being critical components of the host cell receptor and the present identification of OmpA as the ligand likely reflects the impact of a relatively poor genetic toolbox available for manipulation of rickettsial pathogens to date. On the positive side, the recent identification of OmpA and ligands for other rickettsial pathogens has been greatly facilitated by the availability of complete genome sequences and comparative approaches by multiple investigators (4). Indeed, an initial clue that *A. phagocytophilum* OmpA may play a critical role in cell invasion was the evidence that antiserum against the *E. chaffeensis* OmpA orthologue blocked monocyte entry (2).

Several questions remain for this recent identification of outer membrane proteins as ligands to be translated into effective rickettsial vaccines. At the cellular level, it is unclear whether the degree of antibody-mediated inhibition of rickettsial entry would be sufficient to protect against infection. Antibody against OmpA reduced the percentage of *A. phagocytophilum*-infected cells by only approximately 25%. While this may be improved by specific targeting of antibody to the OmpA binding domain, competitive inhibition using the binding domain reduced infection by only approximately 50%. This is consistent with redundancy in binding and entry mechanisms, both with alternative ligands that bind sLe^x α2,3 sialic acid (12) and in binding to other components of sLe^x and PSGL-1. Evidence from research with the spotted fever group rickettsia has clearly shown a significant level of redundancy, with binding and/or entry being mediated via several outer membrane proteins (1). Indeed, prior work in the Carlyon laboratory has identified PSGL-1-independent invasion and positive selection on the *A. phagocytophilum* population for this capacity (14, 16), raising the question as to whether this selection may also occur in the face of vaccine-induced immunity blocking PSGL-1-dependent entry. Importantly, the investigators' careful dissection of the structural elements involved in binding and entry as reported in the present study has made it clear that additional ligand-receptor interactions are required. The identification of

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such interactions will likely provide a set of targets for more complete inhibition.

Immunization studies using OmpA in animal models of *A. phagocytophilum* have not yet been reported; the recognition of OmpA by serum antibody obtained from experimentally infected mice and naturally infected humans does indicate that the protein is immunogenic during infection and that the recombinant OmpA at least partially recapitulates the B cell epitope structure of the native protein. From a comparative standpoint, studies in our laboratory with the closely related *Anaplasma marginale* have demonstrated that a surface protein complex containing the OmpA orthologue, designated AM854, induces protection in cattle, a natural host (9, 11). Whether this protection can be ascribed to AM854/OmpA or the inclusion of additional ligands as well is currently unknown. However, the present report implicating OmpA in cellular entry has both provided the most refined molecular basis for rickettsial cell invasion to date and established direction for vaccine development against a subset of rickettsial pathogens in which OmpA is highly conserved.

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