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■ STRUCTURAL BIOLOGY

Taking Aim at Bioterrorism

Phan J, Lee K, Cherry S, Tropea JE, Burke TR Jr, and Waugh DS. High-resolution structure of the *Yersinia pestis* protein tyrosine phosphatase YopH in complex with a phosphotyrosyl mimetic-containing hexapeptide. *Biochemistry* 42: 13113–21, 2003.

Yersinia pestis, the causative agent of plague, is arguably the deadliest pathogen in history. At least 200 million deaths have been attributed to plague in recorded history. Plague outbreaks are rare in developed countries and can readily be controlled by antibiotics when they occur. However, some recent clinical isolates of *Y. pestis* were reportedly resistant to all of the drugs commonly used for plague prophylaxis and therapy, indicating that the spread of drug resistance may soon become a serious problem.

A more ominous threat, however, is posed by weaponized forms of *Y. pestis*, such as those developed in the Soviet Union during the late 1980s. Reportedly more virulent than wild-type *Y. pestis*, these strains are also reputed to be resistant to all of the antibiotics clinically effective against plague. It would not be surprising if weaponized strains of *Y. pestis* were currently being developed outside the former Soviet Union, in some cases by the same Soviet scientists who were previously engaged in offensive biological weapons research.

Vaccines are clearly one line of defense against weaponized plague, and it is

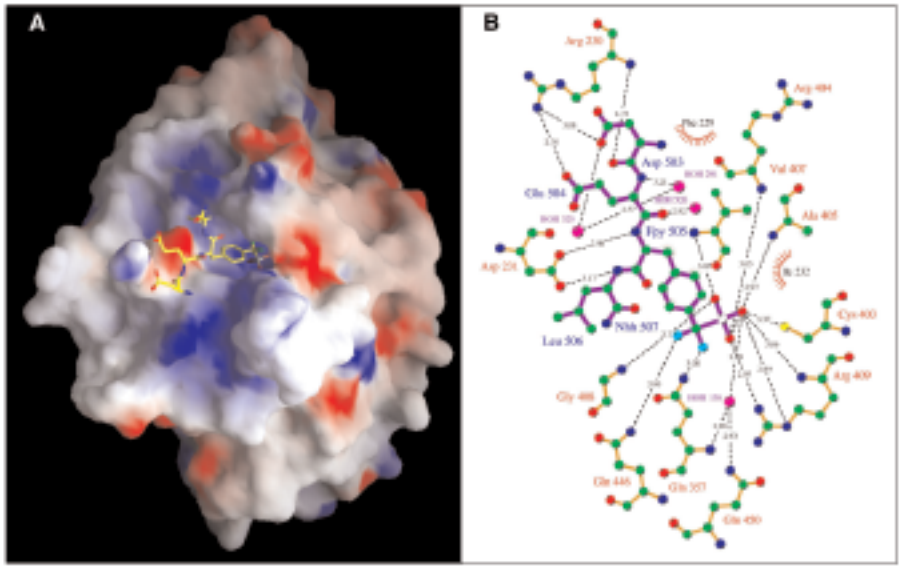

Figure 1. Crystal structure of YopH in complex with a nonhydrolyzable hexapeptide substrate analog. (*A*) Electrostatic potentials mapped onto the molecular surface of YopH with the ligand depicted as a ball-and-stick model. Blue and red represent positively and negatively charged regions, respectively. (*B*) Hydrogen bonding interactions between the enzyme (brown) and the ligand (purple). Carbon, nitrogen, oxygen, sulfur, fluorine, and phosphorus atoms are colored green, blue, red, yellow, light blue, and white, respectively.

reasonable to envision immunization of all military personnel who might be exposed during a conflict. However, vaccination is not a viable strategy to guard against potential civilian casualties of an attack. The current vaccine requires periodic boosters, and repeated exposure results in adverse effects in a considerable number of people who receive it. Besides, the sheer size of the population makes this approach untenable.

Therefore, an antidote for infection is needed—a new kind of drug that cannot be neutralized by genetic engineering or spontaneous mutation as easily as traditional antibiotics. An obvious strategy for developing effective countermeasures against plague is to target the essential virulence factors directly. Because these "molecular terrorists" play a critical role in the pathogenicity of the disease, it may be difficult or even impossible to obtain variants of *Y. pestis* that are resistant to anti-virulence drugs but that can still cause disease. The long-range goal of our research is to facilitate the development of anti-plague drugs via structure-based inhibitor design by attempting to solve the crystal structures of key virulence factors, both alone and in complex with their targets.

The hallmark of *Y. pestis* and many other gram-negative bacterial pathogens is the contact-dependent or type III secretion system (TTSS), which serves to direct the translocation of a small number of cytotoxic proteins, termed effectors, across three membranes from the bacterium into the cytosol of a eukaryotic cell. Collectively, these effectors enable the pathogen to disarm the innate immune response of the infected organism by interfering with crucial signal transduction pathways that regulate actin cytoskeleton dynamics and inflammation. One of the cytotoxic effector proteins that *Y. pestis* injects into mammalian cells via the TTSS is YopH, a potent eukaryotic-like protein tyrosine phosphatase (PTPase). YopH dephosphorylates several proteins associated with the focal adhesion in eukaryotic cells, thereby enabling *Y. pestis* to avoid phagocytosis and destruction by macrophages. Because the PTPase activity of YopH is essential for virulence and the enzyme crystallizes readily, we view it as a particularly promising target for therapeutic intervention.

To establish a framework for the structure-based development of YopH inhibitors, we recently determined the crystal structure of YopH in complex with a nonhydrolyzable hexapeptide substrate analog (Figure 1). The cocrystal structure suggests that the tetrapeptide analog DE-F_2Pmp-L-NH_2, which encompasses the bulk of the interactions with the enzyme active site, would be a good starting point for further optimization. The side chain of the C-terminal leucine residue appears to be a particularly good prospect for modification because it projects into, but does not make optimal contacts with, the body of the enzyme. The crystal structure of the YopH/hexapeptide complex also suggests additional ways in which the inhibitor could be modified to take advantage of adjacent hydrophobic and polar pockets on the surface of the protein.

The challenges that lie ahead are formidable. As in any drug development project, specificity, toxicity, and bioavailability are major concerns. Yet, with the structure of YopH in complex with a nonhydrolyzable substrate analog now in hand, we are potentially one step closer to the eradication of this deadly bioterroristic threat.

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TRANSLATIONAL RESEARCH

A Novel Role for Endothelium-bound SDF-1 Chemokine in Kaposi's Sarcoma Pathogenesis

Yao L, Salvucci O, Cardones AR, Hwang ST, Aoki Y, De La Luz Sierra M, Sajewicz A, Pittaluga S, Yarchoan R, and Tosato G. Selective expression of stromal-derived factor-1 in the capillary vascular endothelium plays a role in Kaposi sarcoma pathogenesis. *Blood* 102: 3900–5, 2003.

Kaposi's sarcoma (KS) is the most common neoplasm in patients with AIDS (Boshoff C et al. *Nat Rev Cancer* 2: 373–82, 2002). The cancer typically involves the extremities and presents as multiple skin lesions containing tumor cells that are referred to as spindle cells because of their microscopic shape. KS is caused by Kaposi's sarcoma–associated herpesvirus (KSHV) (Moore P et al. *N Engl J Med* 332: 1181–5, 1995). A proportion of individuals with HIV infection harbor the virus in a fraction of circulating cells—identified as B lymphocytes and monocytes—and a direct correlation exists between the presence of such cells and the subsequent development of KS. The spindle cells are infected with KSHV, but the surrounding normal tissue is not. Given the predominantly cutaneous location of KS lesions, we investigated how the herpesvirus takes residence in the skin and contributes to the development of this malignancy.

Recently, the expression of selected chemokines and their receptors have been implicated in the recruitment of cells from the bloodstream to specific tissues. Proposed mechanisms involve the generation of chemokine gradients