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MINIREVIEW

Interaction between *Yersinia pestis* and the Host Immune System▽

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The mammalian immune system comprises multiple physical, chemical, and cellular components that are traditionally classified as innate and adaptive immunity. The innate immune response is the first line of host defense against pathogens, depending on barrier structures, phagocytic cells (neutrophils, macrophages, and dendritic cells [DCs]), natural killer (NK) cells, and molecules such as complement proteins, cytokines, and antimicrobial peptides. Once the bacterium infects the host, the innate immunity provides immediate protection. After 4 to 5 days, the T-cell- and B-cell-mediated adaptive immune response begins to provide organism-specific protection and has a long-lasting immunological memory. In most cases, the bacterium will be eliminated from the host by the synergistic effect of both innate and adaptive immunity.

Yersinia pestis, a gram-negative bacterium and plague pathogen, is classified as a category A agent of bioterrorism (18, 19). It gains its notoriety from causing three massive pandemics in history that killed hundreds of millions of people (66). If not treated with proper antimicrobial drugs, the bacteria rapidly escape from containment in the lymph node, spread systemically through the blood, and cause fatal sepsis (66). One of its forms, pneumonic plague, is difficult to treat because of the speed of the disease's progress (a typical incubation period is 1 to 3 days), and by the time individuals are symptomatic, they are often close to death.

To survive inside of the host and maintain a persistent infection, *Y. pestis* uses a variety of mechanisms to evade or overcome the host immune system, especially the innate immune system. Since the interaction of *Y. pestis* and host immune system is such a large area of research that it is difficult to cover all aspects in full detail, this review will focus on the following subtopics.

HOW *Y. PESTIS* OVERCOMES THE INNATE IMMUNE RESPONSE

The innate immune system (nonspecific immunity) is able to discriminate between self and a variety of pathogens by recognizing the highly conserved sets of molecular structures specific to microbes (pathogen-associated molecular patterns [PAMPs]) via a limited number of germ line-encoded pattern recognition receptors (PRRs). Different PRRs react to specific pathogen-associated molecular patterns, exhibit distinct expression patterns, and activate immune cells directly to induce the expression of a variety of genes involved in the innate and adaptive immunity (for reviews, see references 3, 64, and 85). PRRs activate the complement pathway of innate immunity and induce production of cytokines such as interleukin-1β (IL-1β), IL-6, tumor necrosis factor (TNF), and chemokines to collectively induce inflammatory responses to pathogens, recruit neutrophils to the infection site, and activate macrophages to kill the microbes.

By the bite of an infected rodent flea, *Y. pestis* may invade directly into the host through the barrier structure of the host skin and encounter phagocytes such as polymorphonuclear leukocytes (PMNs) (predominantly neutrophils) and macrophages at the site of invasion. Most of them might be killed by neutrophils. However, the facultative intracellular *Y. pestis* preferentially infects host macrophages, possibly via recognition of specific surface-associated CCR5 molecules (33), and survives inside of macrophages at the early stage of infection. After proliferation and expression of various virulence determinants in macrophages, *Y. pestis* can be released into the extracellular compartment and spread systemically with acquisition of phagocytosis resistance. During this process, *Y. pestis* may circumvent destruction by the components of the host innate immune system (Fig. 1).

DEFENSE MECHANISMS OF THE HOST INNATE IMMUNITY AT AN EARLY STAGE OF INFECTION

Inhibition of TLR4-mediated activation in the mammalian host. Lipopolysaccharide (LPS) is a major component of the outer membrane in gram-negative bacteria and a ligand for Toll-like receptor 4 (TLR4), one kind of PRR (85). The expression of potent LPS by some pathogens might be beneficial for the host by providing early recognition of infection and effective initiation of immune signaling. The number and length of fatty acid side chains of LPS lipid A are diverse, and functional structure analysis indicates that the number and length of acyl side chains are critical for TLR4 signaling in humans. Hexa-acylated lipid A with side chains 12 to 14 carbons in length maximally stimulates immunological responses in humans, whereas altering the number or length of the attached fatty acids reduces the magnitude of the signal (55).

At different host-specific environments, the expression and formation of LPS in *Y. pestis* can change accordingly (74, 86).

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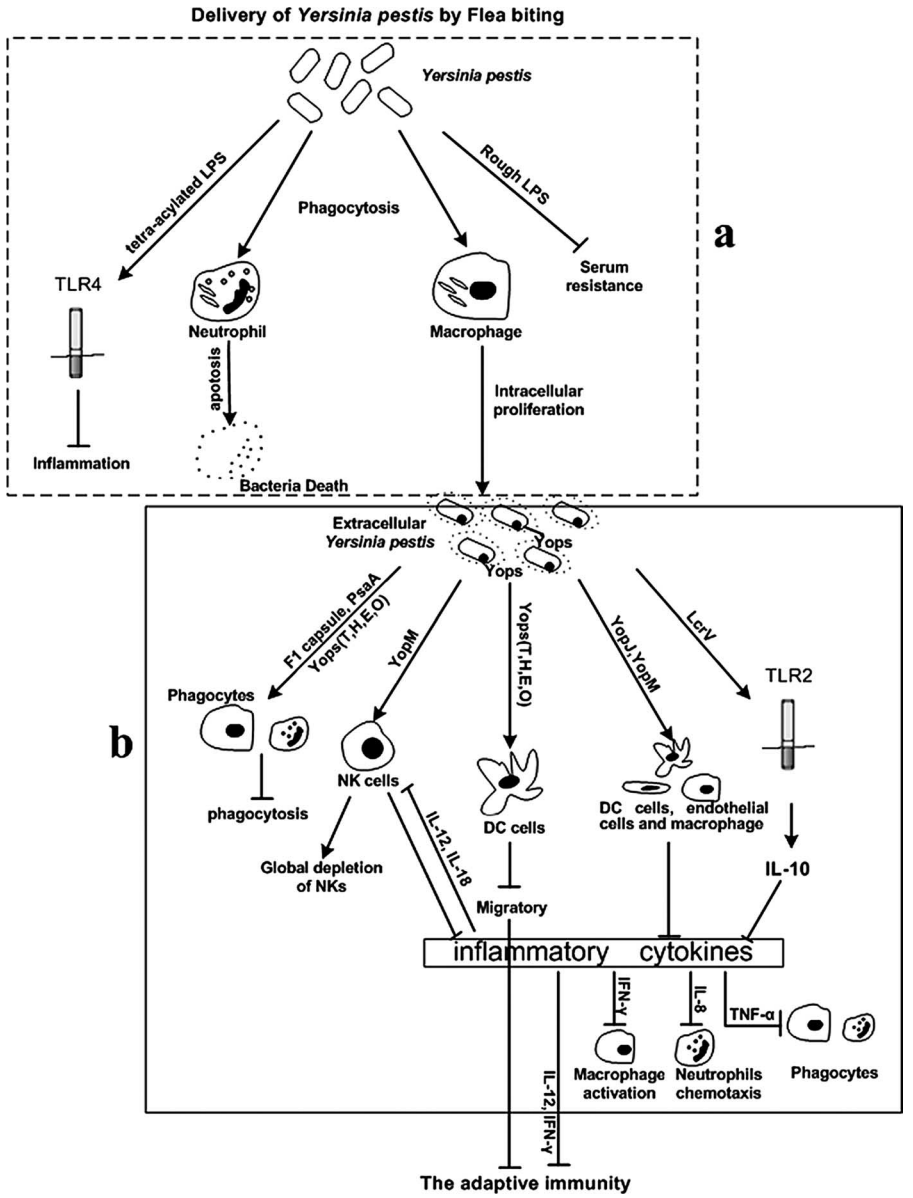

FIG. 1. *Y. pestis* defense mechanisms against host innate immunity. (a) Defense mechanisms at the early stage of infection. The LPS structure diversities of *Y. pestis* during transition between flea and host temperatures make the bacteria resistant to the serum-mediated lysis and suppress the proinflammatory response. Meanwhile, the bacteria phagocytosed by macrophages can proliferate and express various virulence determinants to act on host immune responses. (b) Defense mechanisms after the release of *Y. pestis* from macrophages. The bacteria released from macrophages acquire the ability to resist phagocytosis and can inhibit the production of proinflammatory cytokines, which also attenuate the host's adaptive immunity.

Lien and coworkers demonstrated that *Y. pestis* may well contain a mixture of stimulatory and nonstimulatory LPS species, especially during transition between flea and host temperatures (56). The bacteria grown in flea gut (21 to 26°C) produce a typical hexa-acylated LPS, which activates the TLR4-mediated immune signaling to induce proinflammatory cytokines such as TNF, IL-1, IL-6, and IL-8. However, after the temperature transition from the flea (26°C) to the mammalian host (37°C), *Y. pestis* immediately begins to produce tetra-acylated LPS, which is not only nonstimulatory for TLR4 but also acts as an antagonist for the stimulatory hexa-acylated form of LPS (30, 56). These changes may prevent activation of macrophages, secretion of proinflammatory cytokines, and activation and maturation of DCs that are required for the induction of adaptive immunity.

Serum resistance. In order to survive and/or grow in blood for transmission between its flea vector and the mammalian host, *Y. pestis* has developed resistance to complement-mediated lysis (serum resistance) (65, 89). In contrast to enteropathogenic yersiniae, which are resistant to complement when grown at 37°C but not when grown at 26°C (66), *Y. pestis* is resistant to complement at both 26 and 37°C (9). In enteropathogenic yersiniae, this function is mediated by YadA, Ail, and LPS. *Y. pestis* does not express YadA (14, 15). LPS seems

to play a major role in the resistance to serum-mediated lysis by elevating its content of *N*-acetylglucosamine (9). However, the *Y. pestis* strains deficient in the expression of Ail are extremely sensitive to complement. Alteration in the structure of LPS may also interfere the expression and the surface exposure of Ail. The study performed by Plano and coworkers suggested that Ail might be the sole complement resistance factor due to its requirement for full protection of *Y. pestis* from complement-mediated lysis in vitro (12).

Replication in macrophages and acquisition of phagocytosis resistance. Phagocytes are essential effector cells of innate immunity by ingestion (engulfment) and destruction of microorganisms. At the early stage of infection, *Y. pestis* is phagocytosed by $CD11b^+$ macrophages and Ly-$6G^+$ neutrophils at the infection site (50). Histological evidence indicates that *Y. pestis* was killed within neutrophils, and bacterial cultivation and flow cytometric analyses also suggest that at up to 2 days postinfection host neutrophils were able to control *Y. pestis* growth (50). However, *Y. pestis* can survive and proliferate in the phagolysosome of macrophages to express various virulence determinants (72, 83). This intracellular growth is essential for the pathogenesis of *Y. pestis*. First, macrophages provide a niche, allowing bacteria to proliferate and acquire the ability to evade phagocytosis. Second, intracellular growth in macrophages provides a protected environment for the bacteria to avoid contact with other components of the host immune system. Furthermore, the bacteria within macrophages can be trafficked to the local draining lymph node (73, 96). Between 4 and 5 days of postinfection, the numbers of *Y. pestis* increase rapidly in vivo, and the bacteria can escape from macrophages into the extracellular compartment with phagocytosis resistance. The two-component system PhoP/PhoQ may regulate one or more genes important for the intracellular survival of *Y. pestis* in macrophages (61).

DEFENSE MECHANISM OF BACTERIA RELEASED FROM MACROPHAGES

After 1 to 4 h infection of the macrophage cell line, *Y. pestis* demonstrated the rapid expression of virulence markers such as Yops, F1 antigen, and V antigen (66). At 1 to 2 days postinfection, the virulence markers begin to disturb the host immune responses by different mechanisms, such as inducing apoptosis, suppressing the production of proinflammatory cytokines (e.g., TNF-α), inhibiting Fc receptor-mediated phagocytosis, and preventing neutrophil chemotaxis.

F1- and PsaA-mediated resistance to phagocytosis. When *Y. pestis* replicates within macrophages, F1 protein (fraction 1 antigen) is expressed and forms a capsule around the bacterium. This capsule increases bacterial resistance to engulfment by both macrophages and neutrophils through a mechanism different from that of the type III secretion system (T3SS), presumably by preventing interactions of receptors that could potentially result in the uptake of pathogens (29).

The fimbrial structure of *Y. pestis*, PsaA (pH 6 antigen) has been shown to be induced at 37°C in acidic media, an environment close to the phagolysosome of macrophages (49, 70). A recent report demonstrated that purified PsaA selectively bound to apolipoprotein B (*apoB*)-containing lipoproteins (LDL) in human plasma (51). At concentrations close to the physiological concentration in human blood (250 μg of human LDL/ml), LDL nearly abolished the interaction of the purified PsaA with macrophages. This process could prevent recognition of pathogens by the host defense systems (38, 51).

Roles of Yops against host immune system. The T3SS proteins encoded by pCD1 plasmid are important factors in the progression of acute systemic infection by *Y. pestis*. When *Y. pestis* replicates within macrophages at 37°C, the expression of T3SS proteins is increased and forms a needle-like complex on the surface of the bacterium (23). Once released from macrophages and interacted with target cells, the bacterium injects six different T3SS effector proteins (YopE, YopJ/YopP, YopM, YopH, YopT, and YpkA/YopO) into the cells and inhibits the responses of the host immune system. Macrophages, DCs, and granulocytes/neutrophils are early target cells for the injection (for details, see references 1, 22, 27, 36, 40, and 58).

(i) Direct effects on the activation of immune cells. . The NK cell is one kind of T lymphocyte that can arrive at the inflammatory sites and directly kill the infected cells without the recognition of antigenic peptides. *Y. pestis* can cause a global depletion of NK cells and decrease the secretion of IFN-γ, resulting in a reduced production of reactive nitrogen intermediates by macrophages. These effects depend on the presence of the effector YopM and are manifested by the second day after infection (41).

Phagocytes (macrophages and neutrophils) are the main target cells by bacterial T3SS for the injection of Yops. At least four Yops (YopH, YopE, YopT, and YopO) are involved in inhibiting the phagocytosis of yersiniae, either by interfering with the host cell actin regulation of Rho GTPases (YopE, YopT, and YopO) or specifically and rapidly inactivating host proteins associated with signaling from the receptor to actin (YopH) (1, 2, 6, 39, 77). Moreover, YopH can suppress the production of reactive oxygen intermediates by macrophages and PMNs (35).

(ii) Suppression of the production of proinflammatory factors. Besides paralyzing phagocytic cells, Yops also inhibit the proinflammatory responses elicited by infected cells. YopP has been shown to inhibit TNF-α release by macrophages and IL-8 release by epithelial and endothelial cells (17). TNF-α is a proinflammatory cytokine, which is primarily released by activated macrophages and plays a crucial role in limiting the severity of the bacterial infection. In addition to YopP, YopM interacts with protein kinase C-like 2 and ribosomal protein S6 kinase, which are also involved in proinflammatory signaling (54). The suppression of the production of proinflammatory factors not only reduces the activation of NK cells and phagocytes, but also destroys the inflammatory environment for the adaptive immunity.

Role of LcrV on host immune system. LcrV is a multifunctional protein that is involved in the formation of a tip complex at the tip of the T3SS needle. It activates the secretion and translocation of effectors by binding to the negative regulator LcrG (42) and works together with Yops B and D for delivering Yops into eukaryotic cells (24). It is also exposed on the bacterial cell surface prior to contact with mammalian cells and may play a role in cell-cell adhesion (67). After being secreted into the environment, LcrV exploits TLR2 and CD14 to trigger the release of IL-10 by host immune cells and suppresses the

production of proinflammatory cytokines such as TNF-α and IFN-γ, as well as some other innate defense components required to combat the pathogenesis of plague (81, 82). An LcrV mutant strain with a deletion close to the C terminus (amino acid residues 271 to 300) protected animals from lethal plague infection and did not block proinflammatory capacity (60). However, recent studies showed a weak interaction of LcrV and TLR2, and LcrV did not contribute to the virulence of *Y. pestis* (69, 75). These studies suggest that the LcrV of *Y. pestis* does not efficiently activate TLR2 signaling and that TLR2-mediated immunomodulation is unlikely play a significant role in plague (69).

Inhibition of chemotaxis. PMNs are one kind of leukocytes which can migrate to the infection site by chemotaxis and destroy the invading bacteria. It has been shown that the effector YopP/YopJ of T3SS can be injected into endothelial cells and reduce the expression of adhesion molecules, such as ICAM-1 and E-selectin, on endothelial and bronchial epithelial cells, which might inhibit the recruitment of PMNs to the infection site (16, 28, 97). In addition to YopP, LcrV also inhibits the chemotaxis of neutrophils in both in vivo and in vitro (91).

HOW *Y. PESTIS* DEFEATS THE ADAPTIVE IMMUNE RESPONSES

The adaptive immune responses use selective and clonal expansion of the immune cells (T and B cells) to recognize antigens from a pathogen, providing the specificity and long-lasting immunological memory. Activation of the T and B cells not only depends on the TCR-MHC/peptide interaction and the costimulatory signals, but also the induction of costimulatory molecules and secretion of cytokines and chemokines by the cells of the innate immune system. *Y. pestis* reduces the host adaptive immunity by both influencing the cytokine induction and acting directly on the immune cells involved in the adaptive immune responses (Fig. 2).

Inhibiting the maturation of DCs and destroying the antigen presentation of DCs. DCs play a key role at the interface of innate and adaptive immunity. They reside in peripheral epithelial tissues in an immature state, where they serve as sentinels for the invading microorganisms. Contact with the microorganisms triggers a series of programmed events that are initiated by internalization of pathogens and a concomitant stimulation of PRRs such as TLRs, followed by the conversion of immature DCs into mature ones. This occurs together with the degradation of pathogens within the phagosome and the presentation of microbial antigens to the major histocompatibility complex (MHC) molecules. This in turn causes DCs to activate T cells by an MHC-specific manner (11). In addition to the control of the costimulatory pathway, DCs seem to contribute to T-cell activation by overcoming suppression mediated by CD4 and CD8 regulatory T cells by secretion of IL-6 (46).

Many pathogens have developed a variety of mechanisms to disarm the host defenses, such as impairing DC maturation, promoting the apoptosis of DCs, and inhibiting cytokine secretion to modify its functions. During the infection of *Y. pestis*, DCs are one of the early targets of T3SS effectors (52). Unlike the interaction of *Y. enterocolitica* and DCs, which abolishes

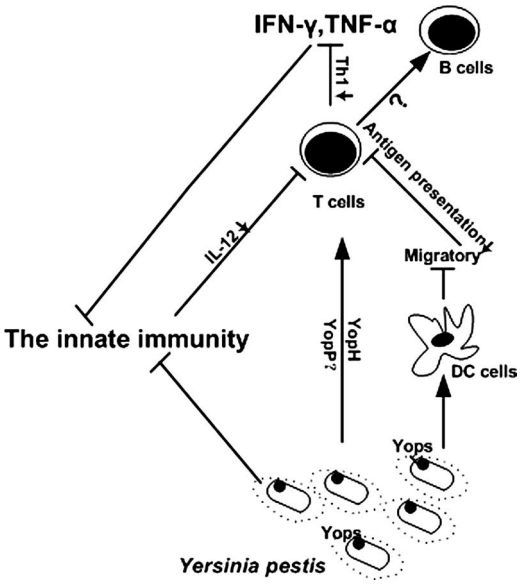

FIG. 2. Defense mechanisms of *Y. pestis* against the host's adaptive immunity and the link between innate immunity and adaptive immunity. The adaptive immunity against *Y. pestis* was influenced not only by suppression of cytokines induction provided by the innate immunity but also by the action of Yops directly on the immune cells involved in the adaptive immune responses. In contrast, the inactivation of T cells, which reduce the production of IFN-γ and TNF-α, influences the activity of the innate immunity.

the surface presentation of MHC class II and costimulatory molecules (79), the most pronounced effect of *Y. pestis* on DCs appears to be the paralysis of DCs movement by impairing the cytoskeleton rearrangement function. This effect can be destructive to the function of DCs in the presentation of *Y. pestis* antigens (90).

Impairing the T-cell activation. *Yersinia* has the ability to influence adaptive immunity by directly suppressing T-lymphocyte activation. YopH is the first documented effector protein to inhibit the adaptive immune responses in a cell culture model (4, 94). It paralyzes T cells rapidly by inducing cells to undergo mitochondrially regulated programmed cell death but also ensures that the cells will not be recovered to induce a protective immune responses (21). The functional analysis of *Y. pseudotuberculosis* YopP shows that it can suppress the development of a CD8 T-cell response in a mouse infection model (88).

Unfortunately, our current understanding of the interaction of *Yersinia* and the immune system is mostly based on studies of *Y. pseudotuberculosis* and *Y. enterocolitica*, two species closely related to *Y. pestis*. Both yersinia species are enteropathogenic and are usually limited to lymphoid tissue, where they cause a chronic infection characterized by prolonged local inflation mediated by shared pCD/pYV-encoded T3SS (20). Although these species are closely related, one should be cautious in applying data from *Y. pseudotuberculosis* and *Y. enterocolitica* studies to *Y. pestis* because only the latter species causes systematic infection (20). For example, T3SS effector YopJ can cause apoptosis of professional phagocytes according to studies on *Y. enterocolitica*, but this effector is not delivered via the T3SS in *Y. pestis* (95). Moreover, the studies of *Y. pestis*

TABLE 1. Proteins that have been proved to be immunogenic

Protein	Function	Reference(s)
LcrV	V antigen	5, 45, 92
YscF	Type III secretion apparatus component	53, 84
YscC	Type III secretion apparatus component	34
YscJ	Type III secretion apparatus component	37
VirG	Targeting protein of the YscC complex	37
YopN	Type III membrane-bound Yop targeting protein	8
YscO	Type III secretion apparatus component	37
YscP	Type III secretion apparatus component	37
TyeA	Type III secretion and targeting protein	37
YopD	Type III targeting component	8, 13
YopH	T3SS effector	8, 13
YopE	T3SS effector	8, 13, 43
YopK	Type III virulence determinant protein	8, 13, 43
YopM	T3SS effector	8, 13
YpkA	T3SS effector	8
OppA	Oligopeptide periplasmic binding protein	87
Pla	Coagulase/fibrinolysin precursor	13, 31
PsaA	pH6 antigen	47
LPS	Lipopolysaccharides	71
YadC	Outer member protein	57
F1	F1 capsule antigen	92

and host immune system were mostly focused on T3SS proteins. Further understanding the interaction between *Y. pestis* and immune systems should be explored to better understand its unique pathogenesis.

HOST IMMUNE RESPONSES TO *Y. PESTIS*

The process of an infectious disease is a complex interaction between pathogen and host. To survive inside the host, bacteria must have mechanisms (such as the secreted T3SS proteins) to counteract the defense mechanisms possessed by the host, especially the host immune system. On the contrary, the host also utilizes its immune system to eliminate the invading bacteria. During *Y. pestis* infection, the host redeploys its specific humoral and cellular immunity and establishes the protective immunity. Classical humoral immune mechanisms could directly combat extracellular *Y. pestis* organisms and simultaneously aid cell-mediated immunity by neutralizing *Y. pestis* virulence factors that dampen cellular responses and delivering antibody/antigen complexes to B cells, macrophages, and/or DCs, thereby promoting T-cell activation. At the same time, classical cell-mediated immune mechanisms may aid humoral defense by eradicating intracellular *Y. pestis* reservoirs.

Therefore, characterization of the antimicrobial immune responses in the host will provide a wealth of information for illustrating bacterial virulence and promoting the development of specific countermeasures.

Humoral immune responses to *Y. pestis*. After infection, the complex antigen structure of *Y. pestis* induces the production of a number of antibodies in plague patients and infected animals (Table 1). Proteomic technologies, such as protein microarray and antigenomics, represent powerful methods for discovering novel immunogens and protective antigens via profiling antibody responses to the invading pathogen (10, 59, 76). We have used an antigen microarray containing more than 140 *Y. pestis* virulence-associated proteins to detect the antibody responses in plague patients. Apart from F1, YopD, YopE, and pH6 antigens, which have been described previously as immunogens, we provided experimental evidence for the immunogenicity of 10 other novel proteins (YPO2090, YPO2091, YPO2102, YPO2112, YPO2118, YPO2131, YPO2190, YPMT1.12c, YPMT1.24c, and YPMT1.75c) (48).

The serum samples collected from plague convalescent patients can transfer passive protection to naive mice, indicating that humoral immunity plays an important role against *Y. pestis* challenge. Two proteins, F1 and LcrV, provide a high degree of protection, and subunit vaccines based on these proteins have demonstrated efficacy in small animal models (5, 7, 26, 44, 80). The mechanism of protection conferred by the vaccines is principally antibody mediated, and the antibody titers to F1 and LcrV in the mouse are correlated with the protection (78, 93). Besides F1 and LcrV, YopD, YpkA, YscF, YadC, and OppA are the only proteins providing partial protection against *Y. pestis* challenge (8, 43, 53, 57, 87).

Although the vaccines containing F1 and/or LcrV can provide protection in small animals, studies by the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, in October 2004 found that a significant number of nonhuman primates immunized with the F1-V fusion protein vaccine succumbed to aerosol *Y. pestis* infection despite their possession of high-titer antibody at the time of challenge (M. L. Pitt, Animal Models and Correlates of Protection for Plague Vaccines Workshop, Gaithersburg, MD [http://www.fda.gov/cber/minutes/workshop-min.htm]). Thus, antibodies may not suffice in protection against pneumonic plague.

Cellular immune responses to *Y. pestis*. More and more evidence shows that the cell-mediated immune responses also play an important role in defense against *Y. pestis* (32, 62, 63). Cell-mediated protection against bacteria often relies upon the development of type 1 immune responses, characterized by the expansion of pathogen-specific T cells that secrete IFN-γ and TNF-α and by the $CD8^+$ cytotoxic T-lymphocyte response. Studies from the Trudeau Institute showed that IFN-γ, TNF-α, and nitric oxide synthase 2, key elements of cellular immunity, provided critical protective functions during humoral defense against lethal pulmonary *Y. pestis* infection. Vaccination with live *Y. pestis* (KIM5 $pCD1^+$, pMT^+, $pPCP^+$, pgm^-) primes CD4 and CD8 T cells that synergistically protect against lethal pulmonary *Y. pestis* infection (63, 68). Moreover, the transfer of the *Y. pestis*-primed T cells to naive μ-MT mice protects against lethal intranasal *Y. pestis* challenge. These studies confirm that cellular immunity, in the absence of antibody, can protect the animal against pulmonary *Y. pestis* infection (62, 63).

Thus, the development of more efficient subunit vaccines should aim at priming both humoral immunity and cellular immunity. It is not reasonable to assume the same antigens could prime both antibody- and T-cell-mediated immunity effectively. The results from the Trudeau Institute indicate that protective T cells likely recognize antigens distinct from those previously defined targets for humoral immunity and F1 is not a dominant T-cell antigen (68).

Identification of *Y. pestis* antigens that stimulate protective T cells would be one of the major goals for vaccine development. Advances in whole-genome sequencing, bioinformatics, and proteomics provide a unique opportunity to define T-cell an-

tigens. Belisle and coworkers used a proteomic approach by analyzing the subcellular protein fractions of *Mycobacterium tuberculosis* against splenocytes of C57BL/6 mice infected with *M. tuberculosis* to identify the fractions that stimulate a dominant T-cell response by measuring the production of IFN-γ. A total of 17 novel T-cell antigens were identified (25). In order to recognize the T-cell antigens in *Y. pestis*, we used in silico computer analysis and an in vitro IFN-γ assay to identify potential T-cell antigens. In all, 34 individual proteins that stimulated a strong IFN-γ response from splenocytes of mice immunized with *Y. pestis* live attenuated vaccine EV76 has been identified, and their protection efficiencies are currently under evaluation (unpublished data).

PERSPECTIVES

Pathogen-host interaction is an important topic for understanding pathogenesis and hence developing effective countermeasures. *Y. pestis* is a notorious pathogen causing plague pandemics throughout history and is a selected agent of bioterrorism threatening public health. Understanding the complex interaction between *Y. pestis* and the host immune system will enable us design more effective vaccines. Both humoral and cellular immunity contribute to host defense against *Y. pestis* infection. The proteins F1 and LcrV have been found to be major protective antigens in protecting against bubonic plague in both mouse and nonhuman primate models. However, cellular immunity seems necessary for protection against pneumonic plague. Currently, we have limited information about cellular immunity during plague development. New technologies, including genomics, proteomics, antigenomics, bioinformatics, pharmacogenomics, and reverse vaccine methodologies, have provided us with a wealth of opportunities for developing more effective countermeasures against plague.

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