Published in final edited form as:

J Cell Physiol. 2013 July ; 228(7): 1413–1422. doi:10.1002/jcp.24299.

Disruption of Immune Regulation by Microbial Pathogens and Resulting Chronic Inflammation

Kenneth Barth¹, Daniel G. Remick², and Caroline A. Genco^{1,*}

¹Department of Medicine Section of Infectious Disease, Boston University School of Medicine, Boston, Massachusetts

²Department of Pathology and Laboratory Medicine, Boston University School of Medicine, Boston, Massachusetts

Abstract

Activation of the immune response is a tightly regulated, coordinated effort that functions to control and eradicate exogenous microorganisms, while also responding to endogenous ligands. Determining the proper balance of inflammation is essential, as chronic inflammation leads to a wide array of host pathologies. Bacterial pathogens can instigate chronic inflammation via an extensive repertoire of evolved evasion strategies that perturb immune regulation. In this review, we discuss two model pathogens, *Mycobacterium tuberculosis* and *Porphyromonas gingivalis*, which efficiently escape various aspects of the immune system within professional and non-professional immune cell types to establish chronic inflammation.

Acute inflammation results from resident cell recognition of irritants that gain entry into a host or exogenous microorganisms that actively invade host tissues and cells. Following bacterial recognition, a number of complex biological responses ensue that initiates changes in the vasculature, cell trafficking and movement of the exudative component to attempt to eradicate invading pathogens. Clearance leads to discontinuation of an inflammatory response, signifying a critical step in preventing unnecessary or excessive damage to the host, whereby homeostasis reasserts itself as the host recovers. In the event that long-term continual stimulation occurs, a condition termed chronic inflammation may last for weeks, months, or even years. The cause may be an irritant, self-antigens, or infectious agents, all of which promote increased inflammatory cellular infiltrate, predominately consisting of macrophages. A number of bacterial pathogens maneuver themselves into microenvironments conducive for their survival. In these environments the bacteria are protected from removal and their continued stimulation of the inflammatory response induces chronic immune stimulation. The ability of bacteria to mediate chronic inflammation is in part a result of bacterial evasion of host immune responses.

Bacteria have co-evolved with humans throughout our history, as reports of bacterial-induced diseases exist back into ancient times. These intimate interactions have driven

selective pressures that tailor microbe genomics and proteomics to harbor and utilize several kinds of virulence factors which allow for colonization and transmission to new hosts. A key determinant in bacterial evolution is to strike the balance between promoting bacterial proliferation without compromising host integrity as death of the host generally represents an end point for the microbe. This balance must be achieved within hosts who have evolved complex, fully functional and highly specific immune systems. When working properly, immune activation is tightly regulated and through sustained activation, results in the control or eradication of bacteria, without eliciting significant collateral damage. This confrontation with the human immune system defines the major obstacles bacterial pathogens act to overcome. To this end, successful pathogens have developed a huge array of evasion strategies in the continuing effort to seek a niche that allows for their survival and transmission. A subset of microbes, such as *Mycobacterium tuberculosis* and *Porphyromonas gingivalis*, employ detailed coordinated efforts that allow these organisms to develop persistent human infections, promoting chronic inflammation via the dysregulation of inflammatory pathways. This review will detail the breadth of tactics utilized by *M. tuberculosis* (Fig. 1) and *P. gingivalis* (Fig. 2) to preclude eradication and induce chronic inflammation.

M. tuberculosis (Mtb) is an acid-fast species that causes an initial disease without complete clearance from the host, where they persist within a particular niche for long-periods of time as a facultative intracellular pathogen. Other examples include *Salmonella enterica* serovar Typhi, and *Helicobacter pylori*. Mtb specifically is capable of surviving within lesions for many years, effectively hiding from active immune surveillance and maintaining a state of chronic inflammation. There exists a sensitive balance between bacterial replication, direct immune response intervention and alterations in the immune response itself. Immune evasion by this organism contributes in its ability to be transmissible. *P. gingivalis* is a gram-negative species that colonizes the oral cavity of a significant number of adults within a subclinical state. Circumstances arise that allow bacterial access to the bloodstream, enabling the attachment, invasion and persistence within endothelial cells. Intracellular survival is a key determinant for pathogen-induced chronic inflammation and both model organisms tailor efforts to acquire and maintain replicative niches.

***Mycobacterium tuberculosis*: Microbe and Disease**

Mtb can easily be considered as one of the most successful human pathogens with recent estimates by the World Health Organization suggesting that one-third of the world's population harbors this microorganism and in 2011 there were 8.7 million incident cases (World Health Organization, 2012). Mtb is the etiological cause of tuberculosis (TB), the second leading cause of death from an infectious agent. With the recent emergence of highly resistant Mtb strains, in addition to associations with HIV and active TB disease, there has been a reemergence of Mtb as a serious health concern. Primarily a pathogen of the human respiratory system, Mtb represents a model organism that effectively utilizes intervention strategies that modulate and/or exploit various aspects of host innate and adaptive immunity. To this end, TB most commonly presents as an asymptomatic latent infection, occurring in approximately 90% of infected individuals. Generally, infection is insufficiently controlled by the innate immune system, requiring adaptive immunity to constrain the primary

infection. Consequently, persistence allows for reactivation and development of chronic inflammation.

The primary site of Mtb infection is the lung, where transmission involves the inhalation of bacteria from an individual with active disease, followed by deposition into the alveolar spaces where the bacteria are contained within aerosolized droplet nuclei 1–5 μm in diameter. Initial infection occurs within this microenvironment, as Mtb interacts with resident phagocytic immune cells and pulmonary epithelial cells. Mtb is either engulfed (via alveolar macrophages and/or dendritic cells (DC)) or active invasion of the organism is triggered by interactions with host receptors, such as complement receptor CR1 and CR3 (Korbel et al., 2008). Proper uptake and bactericidal activity by professional phagocytes promotes antigen presentation and represents a fundamental link to the development of the adaptive response (Fig. 1A). Consequently, it is not surprising that Mtb, like many pathogens, has developed mechanisms to interfere with internalization and/or phagolysosomal maturation, both of which are critical stages in functioning phagocyte-dependent clearance (Fig. 1B).

Innate Immune Recognition and Mtb Evasion Strategies

Initial innate immune signaling pathways mediate a critical role in pathogen recognition via detection of pathogen-associated molecular patterns (PAMPs); conserved bacterial components recognized by a collection of host pattern recognition receptors (PRR) that when engaged, coordinate a variety of signals to induce defense mechanisms. PRR include surface expressed Toll-like receptors (TLR), representing the main component of the innate immune response to Mtb (Harding and Boom, 2010), in addition to C-type lectins and cytosolic nucleotide-binding oligomerization domain-like receptors (NLR). TLR-2, -4, -9 and NLR member NOD2 activation in response to Mtb has been described, dependent on the recognition of Mtb lipoproteins, LPS, CpG-containing DNA, and peptidoglycan, respectively (Korbel et al., 2008). Successful recognition leads to cellular activation to promote a harsh environment tailored to clear infectious agents. Professional phagocytes engulf microbes, containing them within intracellular vesicles called phagosomes that undergo a detailed maturation process. Systematic steps include the lowering of pH, acquisition of both degradative enzymes and reactive oxygen/nitrogen species (ROS/RNS), followed by lysosomal fusion. Exposure to this bactericidal environment is the primary mechanism by which phagocytes kill internalized microbes (Russell, 2001).

In spite of expressing innate immune receptor ligands, Mtb is capable of primarily persisting within macrophages, the main reservoir of this pathogen in vivo. Successful establishment of a replicative niche within these cell types is considered important for generating an infectious cycle of the organism. Persistence, that is latent infection, is one of the least understood facets of TB, however it is known that preventing eradication allows for dissemination into extrapulmonary organs, including lymph nodes, pleura, and osteoarticular areas. Mtb achieves continued survival within its host because of a plethora of evolved mechanisms to evade and suppress innate and adaptive immunity. The major evasion mechanisms pertinent to Mtb disease will be discussed below.

Arrest of Phagosomal Maturation

Mtb produces glycolipid and protein factors that arrest phagolysosomal fusion and maturation in non-activated macrophages, thus avoiding the antimicrobial effects of lysosomes (Fig. 1B) (Tischler and McKinney, 2010). Particular proteins involved in the regulation of vesicle fusion, maturation and sorting have been used as markers to define the remodeling of intracellular vesicles containing Mtb. Specifically, Mtb containing phagosomes have prolonged retention of early endosomal markers, such as RAB5, a GTPase involved in endosome homotypic fusion. Furthermore, retention of the host tryptophan aspartate rich coat protein (TACO) appears to inhibit normal lysosomal fusion (Jayachandran et al., 2007). In contrast, there is selective exclusion or diminished expression of late endosomal and lysosomal markers, including GTPase RAB7 and early endosome antigen 1, representing two regulators that facilitate vesicular trafficking and phagosome maturation (Fratti et al., 2001). Further exclusion occurs for vacuolar-proton transporting ATPase, which normally functions to lower the internal pH to allow for efficient hydrolase activity to perform microbicidal tasks (Sturgill-Koszycki et al., 1994). Vesicle maintenance by Mtb avoids pH-altering systems as a means of limiting the adverse effects of an acidic environment, while also establishing continued interaction with recycling endosomes, providing access to important nutrients, such as iron (Clemens and Horwitz, 1996). Overall, prolonged early endosome arrest serves as a niche for the microbe and is one of the hallmarks of Mtb infection.

The mycobacterial cell wall components appear to play critical roles in the arrest of phagosome maturation. Mannosylated lipoarabinomannan (ManLAM) and trehalose dimycolate (TDM) are key lipoglycans in slow-growing *Mycobacteria* species. Vacuoles containing either ManLAM or TDM coated beads fail to acidify and acquire late endosomal markers (Indrigo et al., 2003; Kang et al., 2005). Their precise activities remain unclear, however, studies have suggested they act by inserting into host membranes, particularly into lipid rafts to interfere with endosomal tethering and fusion machinery, which promotes persistence within macrophages (Kang et al., 2005; Welin et al., 2008). Similarly, bacterial proteins have proposed roles in prevention of phagosome maturation and include a serine/threonine kinase (PknG), a tyrosine phosphatase (PtpA), a lipoamide dehydrogenase (LpcD), a nucleoside diphosphate kinase (Ndk), a Zn²⁺ metalloprotease (Zmp1), and finally a lipid phosphatase (SapM) (Philips, 2008). In the case of Ndk, the protein exhibits GTPase activity toward RAB5 and RAB7, resulting in their inactivation, subsequent phagosome arrest and increased bacterial survival (Sun et al., 2010). It is likely that bacterial factors work in conjunction and in a redundant manner to arrest maturation, as these factors alone are incapable of completely blocking vesicle maturation (Diacovich and Gorvel, 2010). For instance, SapM activity is thought to contribute to ManLAM disruption of phosphatidylinositol-2-phosphate (PI3P) accumulation, thereby preventing PI3P effector recruitment, causing a stall in phagosome maturation (Philips, 2008).

Resistance to RNS and ROS

Efficient bacterial clearance often relies on the ability of phagocytes to generate ROS and RNS (Fig. 1A). Following phagocytosis, NADPH oxidase components are translocated to

the phagosome in order to generate a short-term oxidative burst, generating superoxide radicals (O_2^-). Dismutation or decomposition products of O_2^- produce other reactive species, such as H_2O_2 and hydroxyl radicals. A multitude of signals meanwhile activate RNS systems, including proinflammatory cytokines (IL-1 β , TNF α , and IFNs) or stimulation of PRR (Fang, 2004). This incurs *de novo* synthesis of inducible nitric oxide synthase (iNOS), responsible for generating high concentrations of nitric oxide (NO). RNS activation is instead characterized as a more prolonged response, with the ability to reach increasing distances due to the ability of NO to act as a diffusion-ready free radical. In the event of both oxidative and nitrosative stress generation, concurrent O_2^- and NO production allows for the production of more potent reactive intermediates, including peroxynitrite ($ONOO^-$). Oxidation of lipids, DNA, and modification of particular amino acids can occur from $ONOO^-$ and its decomposition products.

With respect to Mtb infection, when this pathogen is found within IFN γ -activated macrophages, Mtb become increasingly routed to mature phagolysosomes, compartments capable of both RNS and ROS onslaught (Via et al., 1998). Despite this, Mtb remains capable of survival due to evolved adaptation mechanisms to counteract the killing effects of RNS and ROS (Fig. 1B). RNS defense has been well established in murine models as studies demonstrate RNS are essential for containment of both acute and chronic infection, and iNOS inhibitors lead to reactivation of disease (Flynn et al., 1998). Similarly, mice deficient in iNOS demonstrate increased susceptibility to Mtb infection with increased bacterial loads in the lung and other organs, as well as greatly elevated mortality (MacMicking et al., 1997; Jung et al., 2002).

An established mechanism of RNS resistance is reported for the detoxification of $ONOO^-$ by the action of peroxiredoxins, a class of enzymes found within a broad spectrum of bacterial species (Bryk et al., 2000). Specifically, Mtb utilizes alkyl hydroperoxide reductase subunit C (AhpC) to reduce $ONOO^-$ to nitrite rapidly enough to forestall the oxidation of bystander molecules such as DNA, lipids, and proteins (Bryk et al., 2000). Mtb is remarkably resistant to $ONOO^-$, with 100% survival following exposure to 1mM $ONOO^-$, while *Mycobacteria* avirulent strains and other bacterial pathogens show high sensitivities (Yu et al., 1999). The role of AhpC is thought to be critical in survival of the microbe from exiting the stationary phase during dormancy that occurs within latent infection (Diacovich and Gorvel, 2010).

A variety of Mtb genes and expressed structural components have been implicated in RNS defense without identification of direct mechanism. Two genes, *noxR1* and *noxR3* have been proposed to be involved in RNS resistance and cross-protection from oxidative stresses. Expression of a mycobacterial library within *E. coli* and *S. typhimurium* identified *noxR1* and *noxR3* as inducing protection from NO, acidified nitrite and H_2O_2 (Ehrt et al., 1997; Ruan et al., 1999). Expression of recombinant *noxR1* in *M. smegmatis* further conferred increased survival within peritoneal macrophages (Ehrt et al., 1997). The mechanism of action remains to be determined, however these may prove to be interesting and unique defense proteins, considering both are only found within pathogenic *Mycobacteria* genomes. Furthermore, no homologies or motifs at the nucleotide or amino acid sequence levels exists for *noxR1* in other organisms (Ehrt et al., 1997). Another report has identified *cysH*, a

metabolic gene, as having a secondary role in protection from ROS/RNS during the chronic phase of Mtb infection (Senaratne et al., 2006). The protection against ONOO⁻ and H₂O₂ conferred by CysH is thought to be a result of reductive scavenging by cysteine or downstream reduced sulfur-containing metabolites. Similarly, Mtb increases production of TDM in response to an intracellular environment and because of this, the protective efforts of TDM are hypothesized to be a result of its ability to scavenge ROS/RNS (Diacovich and Gorvel, 2010).

The role of ROS in controlling Mtb infection appears to be more controversial. Deletion of NADPH oxidase (*phox*) causes mice infected with Mtb to have increased bacterial loads and alterations in granuloma formation (Cooper et al., 2000). Reduced bacterial control was transient however, as once the adaptive response emerged within these animals; bacterial burdens were similar to wild-type (Cooper et al., 2000). Based on human case reports, patients with chronic granulomatous disease have higher rates of Mycobacterial disease (Bustamante et al., 2007). In contrast, conflicting data using *phox*^{-/-} mice have shown that these animals effectively control infection with virulent Mtb similarly to wild-type mice (Jung et al., 2002). One important observation involves OxyR, the central regulator of peroxide stress response, which contains multiple mutations and frame shift insertions and is dysfunctional in Mtb. Additionally, the SoxRS regulon, responsible for controlling the transcription profile responding to oxidative stress, is absent from the genomes of *Mycobacteria* spp. (Zahrt and Deretic, 2002). Despite these deletions, Mtb retains a number of defense mechanisms that function similarly to RNS subversion and rely primarily on detoxification methods. Mtb produces the ROS scavenging enzymes catalase (KatG), peroxidase reductase complex (AhpCD, SucB, and Lpd), and superoxide dismutase (SodA/C), while also harboring cellular antioxidants (mycothiol) to maintain a reducing environment within the cytoplasm (reviewed in Ehrt and Schnappinger, 2009). Furthermore, the physiology of the thick mycobacterial cell wall containing ManLAM, mycolic acids, in addition to phenolic glycolipid I (PGL-1), provide scavenging of oxygen radicals (Chan et al., 1989).

With the ability of immune activation to generate highly reactive oxidants that can modify structure and function of biomolecules, comes the requirement of pathogens to either limit reactive species generation or repair the damage resulting from reactive species activities. Normally, cellular localization of iNOS requires a functional actin cytoskeleton for delivery to the phagosome (Miller et al., 2004). In contrast to *E. coli* containing phagosomes, those containing Mtb fail to colocalize with iNOS, thus avoiding accumulation of the enzyme and its noxious products, suggesting changes to the efficacy of NO and its derived reactive species (Miller et al., 2004). In the event of oxidant production, proteins remain susceptible to damage, particularly at specific amino acids. Mtb express two methionine sulfoxide reductases (MsrA and MsrB), responsible for reducing oxidized methionines, effectively preventing excessive accumulation of non-functional proteins (Moskovitz, 2005; Lee et al., 2009).

Disruption of Cytokine Networks

The binding of pathogens to surface expressed receptors, such as PRR, primarily induces a pro-inflammatory response. Mtb has been shown to bind both dendritic cell-specific intercellular adhesion molecule-3 (DC-SIGN) and mannose receptor (MR). Both receptors are C-type lectins present on macrophages and DC that initiate phagocytosis, CD4⁺ T cell activation, and complement activation upon receptor engagement. However, in the case of Mtb, binding to DC-SIGN instead causes induction of IL-10 expression, known to diminish Th1 cell development, favoring Mtb replication (Fig. 1B) (Geijtenbeek et al., 2003). Similarly, MR engagement by Mtb induces expression of anti-inflammatory cytokines (IL-4, IL-13), while preventing expression of IL-12, a powerful signal for Th1 response generation (Fig. 1B) (Nigou et al., 2001).

In addition to PRR, intracellular pathogens may contain PAMPs or stimulate danger associated molecular patterns (DAMPs: such as external ATP and DNA) that can activate a multiprotein complex known as the inflammasome. Under this scenario, innate immune signaling induces the expression of IL-1 β and IL-18 (Fig. 1A). IL-1 β in particular, has a critical role in Mtb infection, as its expression allows for macrophages to overcome phagosome arrest (Master et al., 2008). Interestingly, Mtb actively prevents inflammasome activation in host cells, suppressing IL-1 β production via the secretion of a predicted zinc metalloprotease, ZmpA (Fig. 1B) (Master et al., 2008).

Adaptive Immune Evasion—Modulation of Antigen Presentation Pathways

The innate and adaptive immune responses commonly fail to eradicate Mtb infection. Generation of a granuloma ensues, a characteristic feature of latent TB, indicating the involvement of the adaptive response to maintain a chronic inflammatory state. The same proinflammatory cytokines (TNF α , IL-1 β , and IFN γ) that activate macrophages are produced by Th1 lymphocytes, enabling efficient macrophage killing of Mtb (Fig. 1A) (Flynn and Chan, 2003). There also exists the reciprocal importance of antigen presentation via alveolar macrophages and DC to prime CD4⁺ T cells, signifying a crucial event in the control of Mtb infections since CD4⁺ T cell responses are central to host resistance to Mtb (Fig. 1A) (Flynn and Chan, 2001). Consequently, evasion strategies by Mtb focus on preventing antigen processing/presentation with the intention of reducing cellular activation (macrophage and T cell) (Fig. 1B).

Mtb interferes with MHC class II processing/presentation at a variety of stages (reviewed in Gupta et al., 2012) via several mechanisms that include: (1) reduced expression or intracellular sequestration of MHC class II molecules on antigen presenting cells, (2) altered peptide loading, and (3) inhibition of IFN γ signaling in macrophages. In vitro studies indicate Mtb-induced inhibition of CIITA, the master regulator of MHC class II molecules (Pai et al., 2003). ManLAM, TDM, and the 19 kDa lipoprotein (LpqH) all act to downregulate IFN- γ inducible genes including those involved in MHC class II processing/presentation (Chan et al., 1991; Noss et al., 2001; Kan-Sutton et al., 2009). Downstream effects benefit the pathogen via decreased T cell activation, abrogated IFN γ and TNF α expression, in addition to the inability of the macrophage to directly kill Mtb as well as the

dysregulation of T cell detection of infected cells (Fig. 1B). Not only is survival and persistence of Mtb enhanced, but the likelihood of further bacterial dissemination and transmission increases. Secondary effects of adaptive immune evasion highlight the protective role of the immunoregulatory cytokines IFN γ and TNF α on the immune response to Mtb, particularly on cellular activation, RNS production, granuloma formation, and bacterial killing (Ahmad, 2011). Recent reports suggest that Mtb may also exploit the recruitment of mesenchymal stem cells, known for their ability to inhibit T-cell activation via production of NO and TGF- β , to Mtb-containing granulomas (Raghuvanshi et al., 2010).

Subversion via Immunological Decoys, APC Infection and Apoptosis Disruption

Mtb coordinates the expression and secretion of a subset of proteins as immunological decoys (Fig. 1B). As one example, immunodominant CD4⁺ T cell responses are generated against the secreted Mtb protein, Ag85b. Expression remains high at initial stages of infection, while tight regulation shuts off expression once establishment of infection occurs (Rogerson et al., 2006). Hence, T cell priming develops towards a decoy antigen that is no longer expressed by Mtb-containing cells. Infection of antigen-presenting cells should also be considered as a mechanism of reducing APC function and subverting T cell responses. Infection of DC with Mtb alters the normal maturation process of these cells, a critical step in efficient T cell priming (Fig. 1B) (Hanekom et al., 2003). Inhibitory effects on T cell priming likely contributes to the slow development of adaptive responses to Mtb in the lung. In mouse models, Mtb is capable of uncontrolled growth for approximately 21 days, where a delay in adaptive responses allows establishment of a protective niche. This is in stark contrast to the kinetics of adaptive immunity that occurs with other intracellular bacterial infections (Baena and Porcelli, 2009). Finally, apoptosis can be an effective immune strategy to control and eradicate bacterial infected host cells. It is well established that Mtb inhibits apoptosis, while instead promoting necrosis, a response that is strain dependent (Fig. 1B) (Chen et al., 2006; Hinchey et al., 2007). Behar et al. (2010) has hypothesized that bacteria regulate the induction of necrosis. Specifically, Mtb are able to postpone cell death during the initial phase of intracellular replication to maintain an advantageous environment for growth/survival. When this niche is no longer conducive for the organism, an active mechanism is initiated to induce necrosis, allowing phagosome exit and increased potential for infection of additional cell populations.

Porphyromonas gingivalis: Microbe and Disease

A second model microorganism that has evolved sophisticated mechanisms to evade host defenses, enabling the establishment of chronic and persistent infections is *P. gingivalis*. This oral pathogen is the primary etiological agent of periodontal disease (PD), characterized by the erosion of the hard and soft tissues of the periodontium. Although there are over 500 microbial species found within the oral cavity (Paster et al., 2006), relatively few have been linked to PD, the most common being *P. gingivalis* (Yang et al., 2004). Patients with active periodontal inflammation develop a periodontal pocket, where regions of ulcerations that contain bacterial biofilms are located proximal to the circulation. *P. gingivalis* is well adapted to and appears to benefit within this inflammatory environment

(Socransky et al., 1999) and despite a profound inflammatory response, the host immune system is typically unable to eliminate the organism (Danser et al., 1996). Numerous epidemiological human studies support a link between PD, a condition characterized as a local site infection, with chronic inflammatory disease found at sites distant to infection, such as atherosclerosis. For example, there have been 18 reports using clinical or radiographic measures of PD that report increased odds ratio or an increased relative risk for atherosclerotic vascular disease related outcomes (Lockhart et al., 2012). A variety of murine models have highlighted the ability of *P. gingivalis* to induce chronic inflammation, representing a risk factor for the long-term progression of atherosclerosis, validating human studies (Li et al., 2002; Gibson et al., 2004). Our group has established that *P. gingivalis* oral infection in ApoE^{-/-} mice accelerates inflammatory plaque deposition in atherosclerotic lesions. Furthermore, only invasive bacteria that are capable of invading endothelial cells are capable of inducing this chronic inflammatory response (Gibson et al., 2004).

Several mechanisms by which *P. gingivalis* can accelerate the chronic inflammatory disease atherosclerosis have been proposed (Gibson et al., 2006). *P. gingivalis* is initially located within the oral cavity, usually as a member of a polymicrobial biofilm. Of interest, the shift from healthy periodontal status to active state of periodontal disease does not appear to be a direct function of number of microorganisms, but instead is a function of the growth of a subset of pathogenic microbes. Development of inflammatory lesions in PD initially involves high numbers of neutrophils, followed by a shift to primarily monocyte and lymphocyte populations within chronic lesions. At these sites, *P. gingivalis* maintains intimate interactions with epithelial cells, responsible for providing barrier function within the oral mucosa to both resident and invading pathogens. Provided dissemination occurs, *P. gingivalis* comes into contact with endothelial cells. *P. gingivalis* is capable of invading both gingival epithelial and endothelial cells, granting an intracellular niche for the pathogen that avoids circulating leukocytes. Activation of the epithelial cells and the endothelium via PRR mediates vascular dilation, recruitment of leukocytes, and release of inflammatory mediators, all of which are important in facilitating resolution of infection. We propose that the organism enters the bloodstream, disseminates and persists within endothelial cells as a mechanism for the induction of low-grade chronic inflammation. To this end, the pathogen must overcome immune responses within a diverse set of microenvironments. Here we focus on a variety of evasion strategies that promote *P. gingivalis* survival that permits the induction of chronic inflammation at sites local and distant from an initial infection. Specifically, *P. gingivalis* evasion of innate immunity has been the most well studied area and thus will be discussed in detail below and represented as a model in Figure 2.

Inhibition of TLR Signaling—Agonist Modification and Coreceptor Degradation

Detection of LPS via TLR4 plays a fundamental role in innate immune recognition of gram-negative bacterial pathogens. Activation of this surface expressed PRR results in the expression of a collection of inflammatory mediators that function to limit and eradicate invading pathogens. To avoid this inflammatory response, *P. gingivalis* modifies its lipid A structure, the biologically active core of LPS. *P. gingivalis* exploits endogenous phosphatase

activity to generate nonphosphorylated, immunologically silent lipid A to evade TLR4-mediated signaling and killing by cationic antimicrobial peptides (Fig. 2B) (Coats et al., 2009). The functional characteristics of modified lipid A are increasingly detrimental to the host in the context of a polymicrobial microenvironment, since it may affect other bacterial PAMPs, as demonstrated with antagonism of *E. coli* LPS-mediated TLR4 activation (Coats et al., 2009). Our group has further defined *P. gingivalis* lipid A modification as important for intracellular survival within bone marrow derived macrophages (Barth and Papadopolous, unpublished results). Expression of atypical Lipid A moieties is not unique to *P. gingivalis* as a number of pathogens have also been reported to modify lipid A structures (Miller et al., 2005). Other TLR-directed strategies attempt to interrupt the communication of the immune system. Bacterial proteases degrade CD14, an important coreceptor in LPS recognition via TLR4, rendering leukocytes hyporesponsive to LPS (Fig. 2B) (Sugawara et al., 2000).

Degradation of Receptor-Interacting Protein Kinases (RIPK)

We have recently reported on a novel mechanism by which *P. gingivalis* evades intracellular immune signaling via cysteine proteases (gingipains) which likely contributes to pathogen persistence. Due to the breadth of interactions gingipains are involved in, one could argue that they are the most influential virulence factor that *P. gingivalis* harbors. These proteases are unique to *P. gingivalis*, having no significant sequence similarity to any known protein, and represent a separate family (C25) of cysteine proteases. They are transcribed from three genes, *rgpA*, *rgpB*, and *kgp*, having specificity for arginine (RgpA and RgpB) or lysine (Kgp) in the P1 position of substrates. Gingipains contribute collectively to three primary functions associated with *P. gingivalis* pathogenesis: (1) bacterial survival, (2) colonization, and (3) evasion of host defenses. With respect to the host-microbe interface, the majority of studies conducted on gingipain function have focused on the ability of these proteases to degrade extracellular host cell proteins, particularly “end point” targets involved in host immunity (cytokines, chemokines, complement factors, and immunoglobulins), where induction of their expression is required (Fig. 2A).

We recently identified bacterial-mediated interference with intracellular RIPK1 and RIPK2 (Madrigal et al., 2012), essential kinases that function upstream of inflammatory mediators via their role in TLR, TNF α , and NLR signaling (Meylan and Tschopp, 2005). We demonstrate that *P. gingivalis* infection of primary human aortic endothelial cells results in the rapid, dose-dependent cleavage of RIPK1 and RIPK2. Within the context of immune activation, targeting RIPK represents a very early stage of activation interference, consequently offering additional benefits to the pathogen. We speculate that evasion of innate immune signaling (TNF-R and NLR) is exploited by the microbe to allow persistence within target cells (Fig. 2D). This strategy would potentially affect *P. gingivalis* survival within initial (oral cavity) and distant (cardiovascular system) sites of infection. Although the effects of RIPK degradation on *P. gingivalis* intracellular survival remains undefined, *P. gingivalis* infection of endothelial cells impairs TNF α -dependent signaling, likely a direct result of RIPK1 degradation (Barth, unpublished results). It is logical that *P. gingivalis* infection similarly impairs NOD1- and NOD2-dependent signaling due to their specific interactions and requirement for RIPK2 function.

As a result of RIPK degradation, two other factors offer intriguing opportunities for increased bacterial persistence. RIPK degradation does not occur strictly within endothelial cells, as professional immune cells are also likely targets in vivo considering macrophages are prone to degradation in vitro (Madrigal et al., 2012). How it is that *P. gingivalis* disseminates from the oral cavity to infect cells of the vasculature is largely unknown. It may be that intracellular innate immune signaling is vastly abrogated within macrophages via gingipain intervention, establishing a safe niche for the pathogen that permits transit to sites distant from infection. Furthermore, all gram-negative bacteria identified to date bleb outer membrane vesicles (OMV), and it is common to establish these vesicles as a reservoir of virulence factors, such as toxins, adhesins, and proteases. *P. gingivalis* OMV specifically harbor proteolytic activity and our in vitro experiments indicate they possess similar capability to degrade intracellular RIPK (Barth, unpublished results). Utilization of OMV as delivery machines would overcome the fact that *P. gingivalis* lacks common bacterial secretion systems. We envision that OMV may act as “bacterial bombs” to effectively induce RIPK modification in surrounding, uninfected cells, thus facilitating invasion and persistence within a new population of cells. Reports have indicated that *P. gingivalis* is capable of cell-to-cell transmission within primary endothelial and smooth muscle cells (Li et al., 2008). Therefore, bacterial colonization of other sites may be dependent on impairing these innate signaling networks.

Disruption of Cytokine Networks—Gingipain-Dependent Degradation

Deepening of the gingival sulcus and development of a periodontal pocket results from cell infiltration into the junctional epithelium and underlying connective tissues. Prolonged inflammation stemming from bacterial colonization is clearly evident, as crevicular fluid obtained from periodontal disease sites have been shown to contain high levels of cytokines (IL-1 β , IL-6, TNF α , and IFN γ) and chemokines (IL-8, RANTES) (Salvi et al., 1998; Gamonal et al., 2000). The spectrum of gingipain roles in *P. gingivalis* survival also includes their ability to degrade inflammatory mediators. A large number of reports demonstrate that gingipains proteolytically degrade IL-8, IL-6, TNF α , IL-1 β , and IFN γ , among others found within the surrounding milieu, thus reducing chemoattractant and immunoregulatory function of these critical mediators of immunity (Fig. 2A) (Imamura, 2003).

Inhibition of Phagocyte Killing

CR3 is a β 2 integrin highly expressed by phagocytes that maintains a variety of roles in inflammation, including iC3b-mediated phagocytosis and induction of cytokine responses. In a complicated subversion protocol, *P. gingivalis* fimbriae (of which the organism expresses two types: major and minor), surface expressed appendages with high adhesive proficiency to host cells, interact with CD14/TLR2 signaling complex, causing induction of CR3 expression within minutes. Advantageous to the pathogen, major fimbriae can bind CR3, activating “outside-in signaling” resulting in numerous benefits to the pathogen. First, reduction in IL-12 expression inhibits in vivo immune clearance of the microbe (Fig. 2B) (Hajishengallis et al., 2007). Furthermore, uptake into macrophages and long-term survival within these phagocytic cells has been shown in vitro to be partly CR3-dependent

(Hajishengallis et al., 2006; Wang et al., 2007). In the context of chronic inflammatory pathologies such as periodontal disease, in vivo CR3 blockade inhibits the ability of *P. gingivalis* to persist and to induce periodontal bone loss in the mouse (Hajishengallis et al., 2007). Interestingly, it appears that CR3 is exploited as a potential gate of entry for several bacterial pathogens, *M. tuberculosis* included (Sendide et al., 2005). The particular bacterial factor that binds CR3 varies among species, yet the overall entry mechanism via CR3 binding is a relatively safe portal of entry considering activation of microbicidal mechanisms does not occur with CR3 activation, in contrast to Fc γ receptors (Lowell, 2006). The same bacterial fimbriae also appear to bind CXCR4, a G protein coupled receptor found to co associate with TLR2 (Hajishengallis et al., 2008). CXCR4 binding suppresses TLR2-mediated NF κ B activation by inducing cAMP-dependent PKA signaling, impairing iNOS-dependent killing of *P. gingivalis* in macrophages in vitro and in vivo (Fig. 2B) (Wang et al., 2010).

Inhibition of Complement

The complement system is a collection of innate immune amplificatory cascades that generate protein products whose function is to control and clear infections. Downstream complement activation can directly enhance opsonization of bacteria, induce the recruitment of leukocytes to the site of infection, or directly kill the bacteria via the formation of the membrane attack complex. Relevant to *P. gingivalis* disease, complement components are present within gingival fluids at 70% of serum concentrations, thus circumvention of complement function is critical (Schenkein and Genco, 1977). *P. gingivalis* is inherently resistant to serum-mediated killing, proposed to be partly a function of its LPS structure containing anionic polysaccharide repeat units (Slaney et al., 2006). Gingipains also offer assistance, as they are capable of destroying the bactericidal activity of complement in human serum, allowing for survival of other normally sensitive bacterial species such as *E. coli* (Popadiak et al., 2007). Mechanistically, *P. gingivalis* gingipain activity degrades C3, the central complement component, plus several other complement members, C4 and C5 included, resulting in the inhibition of classical, alternative and lectin pathways (Fig. 2A) (Popadiak et al., 2007). A separate strategy exists that is independent of proteolytic function, where gingipain adhesion domains bind C4bp, a mechanism of hijacking a complement regulatory protein to downregulate complement attack function (Potempa et al., 2008).

Hijacking of Intracellular Trafficking

Similar to *Mtb*, reports suggest a mechanism by which *P. gingivalis* interferes with phagosome maturation within non-professional immune cells. Following attachment and invasion within endothelial cells, initiation of autophagy occurs, causing the bacterium to be sorted to a vacuole similar in characteristics to late autophagosomes (Bip⁺, Lamp1⁺). These vacuoles fail to mature into autolysosomes, thus allowing the microbe to reside within a “replicating vacuole” (Fig. 2D). This compartment has characteristics devoid of digestive enzymes but contains sequestered host cell proteins, providing a nutrient source for the asaccharolytic pathogen (Belanger et al., 2006). It should be noted that intracellular trafficking of *P. gingivalis* may be strain-dependent and vary between host cell endothelial type, considering *P. gingivalis* has been shown to be localized within autolysosomes

(Yamatake et al., 2007). Comparing strategies, *P. gingivalis* traffics from early phagosome vesicles to the autophagic pathway, while *Mtb* remains within phagosomes, two varying approaches to avoid lysosomal killing.

Adaptive Immune Evasion—Disruption of Cytokine Networks and Immunoglobulin Function

A number of cytokines maintain essential roles in promoting cell-mediated immunity, such as IL-12 which actively promotes Th1 differentiation. *P. gingivalis* gingipains inherently contain C5-convertase activity that converts C5 to C5a, mediating C5aR activation (Fig. 2A,B). Subversion of C5aR from its normal role of inflammatory and regulatory functions occurs via the down regulation of IL-12, promoting reduced immune clearance of the pathogen (Liang et al., 2011). Other indirect effects on the adaptive response are likely a result of bacterial protease degradation of cytokines themselves. Those cytokines that participate in the cell growth and proliferation (IL-6), or activation of lymphocytes (IL-1), could potentially slow or limit the status of the adaptive response to an active infection.

Proper activation of the adaptive response initiates humoral immunity, generating immunoglobulin secretion that can protect the host by neutralization and opsonization of invading pathogens, in addition to activation of complement. Patients with periodontitis generate detectable serum levels of *P. gingivalis*-specific IgG, IgA and to a lesser extent, IgM (Ogawa et al., 1990). Within saliva, IgA is the main immunoglobulin subtype and *P. gingivalis* isolation from subgingival plaque has been correlated with detection of IgA-specific antibodies in gingival crevicular fluid (Condorelli et al., 1998). IgG, IgA, and IgM are all-susceptible to cleavage via gingipain activity (Fig. 2A) (Gregory et al., 1992).

Disruption of T Cell Responses

A recent study has identified the role of *P. gingivalis* fimbriae on altering DC function and subsequent T cell responses to bacterial adhesins (Zeituni et al., 2009). Minor fimbriae-dependent binding to monocyte derived DC led to internalization, reduced secretion of inflammatory cytokines (IL-1 β , IL-6, IL-8, IL-12, and TNF α) and halting of DC maturation (Fig. 2C). Furthermore, *P. gingivalis* pulsed DC were capable of promoting a Th1 or Th2 effector response, dependent on bacterial expression of either major or minor fimbriae. Considering fimbriae expression is modulated during various growth conditions, it is intriguing to speculate *P. gingivalis* surface expressed proteins modulate T cell responses in vivo. Additional effects on T cell responses comes from the identification of gingipain-dependent cleavage of CD27 ligand on resting T cells and CD70 on activated B cells (Yun et al., 2007). Considering lymphocyte co-stimulation is partly mediated by CD27-dependent binding to CD70, these data suggest pathogen down-regulation of a co-activation loop to alter T-cell mediated adaptive responses.

Microbial Ligands Predisposes the Immune System to Chronic Inflammation

The extent of *P. gingivalis* evasion mechanisms collectively allow intracellular survival of the organism via the prevention of innate immune activation (degradation of coreceptors or essential protein members within signaling pathways), establishment of an intracellular niche (hijacking of vesicular trafficking), or counteracting downstream inflammatory mediators (cytokine/chemokine/immunoglobulin degradation). Regardless of long-term bacterial persistence, active invasion by a bacterial pathogen can induce initial cellular activation (upregulation of cell adhesion molecules, TLRs, pro-inflammatory mediators, and chemoattractants) by innate immune recognition of exogenous microbial ligands. Under these conditions, the immune response may or may not clear the infection; however, the microenvironment becomes predisposed to heightened responsiveness to endogenous ligands. Dietary lipids, such as oxidized LDL, have been identified to be recognized by PRR while endogenous cholesterol crystals appear within early atherogenic lesions and activate the NLRP3 inflammasome (Duewell et al., 2010). Furthermore, cholesterol crystal-dependent induction of IL-1 β response was seen only in primed PBMC (Duewell et al., 2010). Consequently, we propose that perturbation of immune regulation originates by the activities of *P. gingivalis* but may be perpetuated by other known or unidentified endogenous ligands, to result in chronic and sustained inflammatory disease.

Conclusions

Historically, examinations of bacterial-induced human diseases were descriptive in nature, without having the ability to dissect the complicated interface between host and pathogen. Advancing technology has fueled research to identify novel and complicated strategies that single cell microorganisms have evolved to sway their advantage within their respective hosts. These methods act to avoid, manipulate, or subvert the human immune system at the level of both the innate and adaptive responses. *M. tuberculosis* and *P. gingivalis* are just two examples of pathogens that coordinate evasion tactics to provide for intracellular persistence. The inability of the host immune system to achieve eradication opens the door for long-term chronic stimulation, thus limiting resolution and recovery. With the age of genomics and proteomics upon us, we can be confident that additional and novel examples of evasion strategies that disrupt immune regulation will be identified. A number of considerations would allow for an increase in relevant findings. Research should move towards evaluating the importance of bacterial factor expression kinetics in the context of varying host microenvironments, rather than focusing on singular roles of these factors. Additionally, studies should acknowledge that defined pathogens often share the same real-estate with commensal organisms where microbial survival is influenced by both the pathogen's own activities, but also other microorganisms within the microbiome. Taking into account the host, pathogen and commensals will present a better understanding of the network of intervening factors affecting immune regulation. Utilizing this broader outlook, the outcome of infection is a consequence of all the inputs towards regulation of inflammation, some of which have been overlooked or disregarded as important in previous studies.

Acknowledgments

We thank Ryan McClure for critical review of the manuscript. This work was supported by National Institutes of Health grants RO1-HL80387, T32AI089673 (C.A.G.) and PO1-AI078894 (C.A.G. and K.B.).

Contract grant sponsor: National Institutes of Health;

Contract grant numbers: RO1-HL80387, T32AI089673, PO1-AI078894.

Literature Cited

- Ahmad S. Pathogenesis, immunology, and diagnosis of latent *Mycobacterium tuberculosis* infection. Clin Dev Immunol. 2011; 2011:814943. [PubMed: 21234341]
- Baena A, Porcelli SA. Evasion and subversion of antigen presentation by *Mycobacterium tuberculosis*. Tissue Antigens. 2009; 74:189–204. [PubMed: 19563525]
- Behar SM, Divangahi M, Remold HG. Evasion of innate immunity by *Mycobacterium tuberculosis*: Is death an exit strategy? Nat Rev Microbiol. 2010; 8:668–674. [PubMed: 20676146]
- Belanger M, Rodrigues PH, Dunn WA Jr, Progulski-Fox A. Autophagy: A highway for *Porphyromonas gingivalis* in endothelial cells. Autophagy. 2006; 2:165–170. [PubMed: 16874051]
- Bryk R, Griffin P, Nathan C. Peroxynitrite reductase activity of bacterial peroxiredoxins. Nature. 2000; 407:211–215. [PubMed: 11001062]
- Bustamante J, Aksu G, Vogt G, de Beaucoudrey L, Genel F, Chappier A, Filipe-Santos O, Feinberg J, Emile JF, Kutukculer N, Casanova JL. BCG-osis and tuberculosis in a child with chronic granulomatous disease. J Allergy Clin Immunol. 2007; 120:32–38. [PubMed: 17544093]
- Chan J, Fan XD, Hunter SW, Brennan PJ, Bloom BR. Lipoarabinomannan, a possible virulence factor involved in persistence of *Mycobacterium tuberculosis* within macrophages. Infect Immun. 1991; 59:1755–1761. [PubMed: 1850379]
- Chan J, Fujiwara T, Brennan P, McNeil M, Turco SJ, Sibille JC, Snapper M, Aisen P, Bloom BR. Microbial glycolipids: Possible virulence factors that scavenge oxygen radicals. Proc Natl Acad Sci USA. 1989; 86:2453–2457. [PubMed: 2538841]
- Chen M, Gan H, Remold HG. A mechanism of virulence: Virulent *Mycobacterium tuberculosis* strain H37Rv, but not attenuated H37Ra, causes significant mitochondrial inner membrane disruption in macrophages leading to necrosis. J Immunol. 2006; 176:3707–3716. [PubMed: 16517739]
- Clemens DL, Horwitz MA. The *Mycobacterium tuberculosis* phagosome interacts with early endosomes and is accessible to exogenously administered transferrin. J Exp Med. 1996; 184:1349–1355. [PubMed: 8879207]
- Coats SR, Jones JW, Do CT, Braham PH, Bainbridge BW, To TT, Goodlett DR, Ernst RK, Darveau RP. Human Toll-like receptor 4 responses to *P. gingivalis* are regulated by lipid A 1- and 4'-phosphatase activities. Cell Microbiol. 2009; 11:1587–1599. [PubMed: 19552698]
- Condorelli F, Scalia G, Cali G, Rossetti B, Nicoletti G, Lo Bue AM. Isolation of *Porphyromonas gingivalis* and detection of immunoglobulin A specific to fimbrial antigen in gingival crevicular fluid. J Clin Microbiol. 1998; 36:2322–2325. [PubMed: 9666013]
- Cooper AM, Segal BH, Frank AA, Holland SM, Orme IM. Transient loss of resistance to pulmonary tuberculosis in p47 (phox^{-/-}) mice. Infect Immun. 2000; 68:1231–1234. [PubMed: 10678931]
- Danser MM, Timmerman MF, van Winkelhoff AJ, van der Velden U. The effect of periodontal treatment on periodontal bacteria on the oral mucous membranes. J Periodontol. 1996; 67:478–485. [PubMed: 8724705]
- Diacovich L, Gorvel JP. Bacterial manipulation of innate immunity to promote infection. Nat Rev Microbiol. 2010; 8:117–128. [PubMed: 20075926]
- Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, Abela GS, Franchi L, Nunez G, Schnurr M, Espevik T, Lien E, Fitzgerald KA, Rock KL, Moore KJ, Wright SD, Hornung V, Latz E. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Nature. 2010; 464:1357–1361. [PubMed: 20428172]

- Ehrt S, Schnappinger D. Mycobacterial survival strategies in the phagosome: Defence against host stresses. *Cell Microbiol.* 2009; 11:1170–1178. [PubMed: 19438516]
- Ehrt S, Shiloh MU, Ruan J, Choi M, Gunzburg S, Nathan C, Xie Q, Riley LW. A novel antioxidant gene from *Mycobacterium tuberculosis*. *J Exp Med.* 1997; 186:1885–1896. [PubMed: 9382887]
- Fang FC. Antimicrobial reactive oxygen and nitrogen species: Concepts and controversies. *Nat Rev Microbiol.* 2004; 2:820–832. [PubMed: 15378046]
- Flynn JL, Chan J. Immunology of tuberculosis. *Ann Rev Immunol.* 2001; 19:93–129. [PubMed: 11244032]
- Flynn JL, Chan J. Immune evasion by *Mycobacterium tuberculosis*: Living with the enemy. *Curr Opin Immunol.* 2003; 15:450–455. [PubMed: 12900278]
- Flynn JL, Scanga CA, Tanaka KE, Chan J. Effects of aminoguanidine on latent murine tuberculosis. *J Immunol.* 1998; 160:1796–1803. [PubMed: 9469439]
- Fratti RA, Backer JM, Gruenberg J, Corvera S, Deretic V. Role of phosphatidylinositol 3-kinase and Rab5 effectors in phagosomal biogenesis and mycobacterial phagosome maturation arrest. *J Cell Biol.* 2001; 154:631–644. [PubMed: 11489920]
- Gamonal J, Acevedo A, Bascones A, Jorge O, Silva A. Levels of interleukin-1 beta, -8, and -10 and RANTES in gingival crevicular fluid and cell populations in adult periodontitis patients and the effect of periodontal treatment. *J Periodontol.* 2000; 71:1535–1545. [PubMed: 11063385]
- Geijtenbeek TB, VanVliet SJ, Koppel EA, Sanchez-Hernandez M, Vandenbroucke-Grauls CM, Appelmek B, Van Kooyk Y. Mycobacteria target DC-SIGN to suppress dendritic cell function. *J Exp Med.* 2003; 197:7–17. [PubMed: 12515809]
- Gibson FC III, Hong C, Chou HH, Yumoto H, Chen J, Lien E, Wong J, Genco CA. Innate immune recognition of invasive bacteria accelerates atherosclerosis in apolipoprotein E-deficient mice. *Circulation.* 2004; 109:2801–2806. [PubMed: 15123526]
- Gibson FC III, Yumoto H, Takahashi Y, Chou HH, Genco CA. Innate immune signaling and *Porphyromonas gingivalis*-accelerated atherosclerosis. *J Dental Res.* 2006; 85:106–121.
- Gregory RL, Kim DE, Kindle JC, Hobbs LC, Lloyd DR. Immunoglobulin-degrading enzymes in localized juvenile periodontitis. *J Periodontal Res.* 1992; 27:176–183. [PubMed: 1318974]
- Gupta A, Kaul A, Tsolaki AG, Kishore U, Bhakta S. *Mycobacterium tuberculosis*: Immune evasion, latency, and reactivation. *Immunobiology.* 2012; 217:363–374. [PubMed: 21813205]
- Hajishengallis G, Shakhathreh MA, Wang M, Liang S. Complement receptor 3 blockade promotes IL-12-mediated clearance of *Porphyromonas gingivalis* and negates its virulence in vivo. *J Immunol.* 2007; 179:2359–2367. [PubMed: 17675497]
- Hajishengallis G, Wang M, Harokopakis E, Triantafilou M, Triantafilou K. *Porphyromonas gingivalis* fimbriae proactively modulate beta2 integrin adhesive activity and promote binding to and internalization by macrophages. *Infect Immun.* 2006; 74:5658–5666. [PubMed: 16988241]
- Hajishengallis G, Wang M, Liang S, Triantafilou M, Triantafilou K. Pathogen induction of CXCR4/TLR2 cross-talk impairs host defense function. *Proc Natl Acad Sci USA.* 2008; 105:13532–13537. [PubMed: 18765807]
- Hanekom WA, Mendillo M, Manca C, Haslett PA, Siddiqui MR, Barry C III, Kaplan G. *Mycobacterium tuberculosis* inhibits maturation of human monocyte-derived dendritic cells in vitro. *J Infect Dis.* 2003; 188:257–266. [PubMed: 12854081]
- Harding CV, Boom WH. Regulation of antigen presentation by *Mycobacterium tuberculosis*: A role for Toll-like receptors. *Nat Rev Microbiol.* 2010; 8:296–307. [PubMed: 20234378]
- Hinchey J, Lee S, Jeon BY, Basaraba RJ, Venkataswamy MM, Chen B, Chan J, Braunstein M, Orme IM, Derrick SC, Morris SL, Jacobs WR Jr, Porcelli SA. Enhanced priming of adaptive immunity by a proapoptotic mutant of *Mycobacterium tuberculosis*. *J Clin Invest.* 2007; 117:2279–2288. [PubMed: 17671656]
- Imamura T. The role of gingipains in the pathogenesis of periodontal disease. *J Periodontol.* 2003; 74:111–118. [PubMed: 12593605]
- Indrigo J, Hunter RL Jr, Actor JK. Cord factor trehalose 6,6'-dimycolate (TDM) mediates trafficking events during mycobacterial infection of murine macrophages. *Microbiology.* 2003; 149:2049–2059. [PubMed: 12904545]

- Jayachandran R, Sundaramurthy V, Combaluzier B, Mueller P, Korf H, Huygen K, Miyazaki T, Albrecht I, Massner J, Pieters J. Survival of mycobacteria in macrophages is mediated by coronin 1-dependent activation of calcineurin. *Cell*. 2007; 130:37–50. [PubMed: 17632055]
- Jung YJ, LaCourse R, Ryan L, North RJ. Virulent but not avirulent *Mycobacterium tuberculosis* can evade the growth inhibitory action of a T helper 1-dependent, nitric oxide Synthase 2-independent defense in mice. *J Exp Med*. 2002; 196:991–998. [PubMed: 12370260]
- Kan-Sutton C, Jagannath C, Hunter RL Jr. Trehalose 6,6'-dimycolate on the surface of *Mycobacterium tuberculosis* modulates surface marker expression for antigen presentation and costimulation in murine macrophages. *Microbes Infection/Institut Pasteur*. 2009; 11:40–48. [PubMed: 19007905]
- Kang PB, Azad AK, Torrelles JB, Kaufman TM, Beharka A, Tibesar E, DesJardin LE, Schlesinger LS. The human macrophage mannose receptor directs *Mycobacterium tuberculosis* lipoarabinomannan-mediated phagosome biogenesis. *J Exp Med*. 2005; 202:987–999. [PubMed: 16203868]
- Korbel DS, Schneider BE, Schaible UE. Innate immunity in tuberculosis: Myths and truth. *Microb Infect/Institut Pasteur*. 2008; 10:995–1004.
- Lee WL, Gold B, Darby C, Brot N, Jiang X, de Carvalho LP, Wellner D, St John G, Jacobs WR Jr, Nathan C. *Mycobacterium tuberculosis* expresses methionine sulphoxide reductases A and B that protect from killing by nitrite and hypochlorite. *Mol Microbiol*. 2009; 71:583–593. [PubMed: 19040639]
- Li L, Messas E, Batista EL Jr, Levine RA, Amar S. *Porphyromonas gingivalis* infection accelerates the progression of atherosclerosis in a heterozygous apolipoprotein E-deficient murine model. *Circulation*. 2002; 105:861–867. [PubMed: 11854128]
- Li L, Michel R, Cohen J, Decarlo A, Kozarov E. Intracellular survival and vascular cell-to cell transmission of *Porphyromonas gingivalis*. *BMC Microbiol*. 2008; 8:26. [PubMed: 18254977]
- Liang S, Krauss JL, Domon H, McIntosh ML, Hosur KB, Qu H, Li F, Tzekou A, Lambris JD, Hajishengallis G. The C5a receptor impairs IL-12-dependent clearance of *Porphyromonas gingivalis* and is required for induction of periodontal bone loss. *J Immunol*. 2011; 186:869–877. [PubMed: 21149611]
- Lockhart PB, Bolger AF, Papapanou PN, Osinbowale O, Trevisan M, Levison ME, Taubert KA, Newburger JW, Gornik HL, Gewitz MH, Wilson WR, Smith SC Jr, Baddour LM. Periodontal disease and atherosclerotic vascular disease: Does the evidence support an independent association? A scientific statement from the American Heart Association. *Circulation*. 2012; 125:2520–2544. [PubMed: 22514251]
- Lowell CA. Rewiring phagocytic signal transduction. *Immunity*. 2006; 24:243–245. [PubMed: 16546092]
- MacMicking JD, North RJ, LaCourse R, Mudgett JS, Shah SK, Nathan CF. Identification of nitric oxide synthase as a protective locus against tuberculosis. *Proc Natl Acad Sci USA*. 1997; 94:5243–5248. [PubMed: 9144222]
- Madrigal AG, Barth K, Papadopoulos G, Genco CA. Pathogen-mediated proteolysis of the cell death regulator RIPK1 and the host defense modulator RIPK2 in human aortic endothelial cells. *PLoS Pathog*. 2012; 8:e1002723. [PubMed: 22685397]
- Master SS, Rampini SK, Davis AS, Keller C, Ehlers S, Springer B, Timmins GS, Sander P, Deretic V. *Mycobacterium tuberculosis* prevents inflammasome activation. *Cell Host Microb*. 2008; 3:224–232.
- Meylan E, Tschopp J. The RIP kinases: Crucial integrators of cellular stress. *Trends Biochem Sci*. 2005; 30:151–159. [PubMed: 15752987]
- Miller BH, Fratti RA, Poschet JF, Timmins GS, Master SS, Burgos M, Marletta MA, Deretic V. Mycobacteria inhibit nitric oxide synthase recruitment to phagosomes during macrophage infection. *Infect Immun*. 2004; 72:2872–2878. [PubMed: 15102799]
- Miller SI, Ernst RK, Bader MW. LPS, TLR4 and infectious disease diversity. *Nat Rev Microbiol*. 2005; 3:36–46. [PubMed: 15608698]
- Moskovitz J. Methionine sulfoxide reductases: Ubiquitous enzymes involved in antioxidant defense, protein regulation, and prevention of aging-associated diseases. *Biochim Biophys Acta*. 2005; 1703:213–219. [PubMed: 15680229]

- Nigou J, Zelle-Rieser C, Gilleron M, Thurnher M, Puzo G. Mannosylated lipoarabinomannans inhibit IL-12 production by human dendritic cells: Evidence for a negative signal delivered through the mannose receptor. *J Immunol.* 2001; 166:7477–7485. [PubMed: 11390501]
- Noss EH, Pai RK, Sellati TJ, Radolf JD, Belisle J, Golenbock DT, Boom WH, Harding CV. Toll-like receptor 2-dependent inhibition of macrophage class II MHC expression and antigen processing by 19-kDa lipoprotein of *Mycobacterium tuberculosis*. *J Immunol.* 2001; 167:910–918. [PubMed: 11441098]
- Ogawa T, Kusumoto Y, Hamada S, McGhee JR, Kiyono H. Bacteroides *P. gingivalis*-specific serum IgG and IgA subclass antibodies in periodontal diseases. *Clin Exp Immunol.* 1990; 82:318–325. [PubMed: 1978704]
- Pai RK, Convery M, Hamilton TA, Boom WH, Harding CV. Inhibition of IFN-gamma-induced class II transactivator expression by a 19-kDa lipoprotein from *Mycobacterium tuberculosis*: A potential mechanism for immune evasion. *J Immunol.* 2003; 171:175–184. [PubMed: 12816996]
- Paster BJ, Olsen I, Aas JA, Dewhirst FE. The breadth of bacterial diversity in the human periodontal pocket and other oral sites. *Periodontology.* 2006; 42:80–87.
- Philips JA. Mycobacterial manipulation of vacuolar sorting. *Cell Microbiol.* 2008; 10:2408–2415. [PubMed: 18783482]
- Popadiak K, Potempa J, Riesbeck K, Blom AM. Biphasic effect of gingipains from *Porphyromonas gingivalis* on the human complement system. *J Immunol.* 2007; 178:7242–7250. [PubMed: 17513773]
- Potempa M, Potempa J, Okroj M, Popadiak K, Eick S, Nguyen KA, Riesbeck K, Blom AM. Binding of complement inhibitor C4b-binding protein contributes to serum resistance of *Porphyromonas gingivalis*. *J Immunol.* 2008; 181:5537–5544. [PubMed: 18832711]
- Raghuvanshi S, Sharma P, Singh S, Van Kaer L, Das G. *Mycobacterium tuberculosis* evades host immunity by recruiting mesenchymal stem cells. *Proc Natl Acad Sci USA.* 2010; 107:21653–21658. [PubMed: 21135221]
- Rogerson BJ, Jung YJ, LaCourse R, Ryan L, Enright N, North RJ. Expression levels of *Mycobacterium tuberculosis* antigen-encoding genes versus production levels of antigen-specific T cells during stationary level lung infection in mice. *Immunology.* 2006; 118:195–201. [PubMed: 16771854]
- Ruan J, St John G, Ehrt S, Riley L, Nathan C. noxR3, a novel gene from *Mycobacterium tuberculosis*, protects *Salmonella typhimurium* from nitrosative and oxidative stress. *Infect Immun.* 1999; 67:3276–3283. [PubMed: 10377101]
- Russell DG. *Mycobacterium tuberculosis*: Here today, and here tomorrow. *Nat Rev Mol Cell Biol.* 2001; 2:569–577. [PubMed: 11483990]
- Salvi GE, Beck JD, Offenbacher S. PGE2, IL-1 beta, and TNF-alpha responses in diabetics as modifiers of periodontal disease expression. *Ann Periodontol.* 1998; 3:40–50. [PubMed: 9722689]
- Schenkein HA, Genco RJ. Gingival fluid and serum in periodontal diseases. I. Quantitative study of immunoglobulins, complement components, and other plasma proteins. *J Periodontol.* 1977; 48:772–777. [PubMed: 73582]
- Senaratne RH, De Silva AD, Williams SJ, Mougous JD, Reader JR, Zhang T, Chan S, Sidders B, Lee DH, Chan J, Bertozzi CR, Riley LW. 5'-Adenosinephosphosulphate reductase (CysH) protects *Mycobacterium tuberculosis* against free radicals during chronic infection phase in mice. *Mol Microbiol.* 2006; 59:1744–1753. [PubMed: 16553880]
- Sendide K, Reiner NE, Lee JS, Bourgoin S, Talal A, Hmama Z. Cross-talk between CD14 and complement receptor 3 promotes phagocytosis of mycobacteria: Regulation by phosphatidylinositol 3-kinase and cytohesin-1. *J Immunol.* 2005; 174:4210–4219. [PubMed: 15778383]
- Slaney JM, Gallagher A, Aduse-Opoku J, Pell K, Curtis MA. Mechanisms of resistance of *Porphyromonas gingivalis* to killing by serum complement. *Infect Immun.* 2006; 74:5352–5361. [PubMed: 16926430]
- Socransky SS, Haffajee AD, Ximenez-Fyvie LA, Feres M, Mager D. Ecological considerations in the treatment of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* periodontal infections. *Periodontology.* 1999/2000; 20:341–362.

- Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK, Allen RD, Gluck SL, Heuser J, Russell DG. Lack of acidification in *Mycobacterium phagosomes* produced by exclusion of the vesicular proton-ATPase. *Science*. 1994; 263:678–681. [PubMed: 8303277]
- Sugawara S, Nemoto E, Tada H, Miyake K, Imamura T, Takada H. Proteolysis of human monocyte CD14 by cysteine proteinases (gingipains) from *Porphyromonas gingivalis* leading to lipopolysaccharide hyporesponsiveness. *J Immunol*. 2000; 165:411–418. [PubMed: 10861079]
- Sun J, Wang X, Lau A, Liao TY, Bucci C, Hmama Z. Mycobacterial nucleoside diphosphate kinase blocks phagosome maturation in murine RAW 264.7 macrophages. *PLoS ONE*. 2010; 5:e8769. [PubMed: 20098737]
- Tischler AD, McKinney JD. Contrasting persistence strategies in *Salmonella* and *Mycobacterium*. *Curr Opin Microbiol*. 2010; 13:93–99. [PubMed: 20056478]
- Via LE, Fratti RA, McFalone M, Pagan-Ramos E, Deretic D, Deretic V. Effects of cytokines on mycobacterial phagosome maturation. *J Cell Sci*. 1998; 111:897–905. [PubMed: 9490634]
- Wang M, Krauss JL, Domon H, Hosur KB, Liang S, Magotti P, Triantafilou M, Triantafilou K, Lambris JD, Hajishengallis G. Microbial hijacking of complement-toll-like receptor crosstalk. *Sci Signal*. 2010; 3:ra11. [PubMed: 20159852]
- Wang M, Shakhatreh MA, James D, Liang S, Nishiyama S, Yoshimura F, Demuth DR, Hajishengallis G. Fimbrial proteins of *porphyromonas gingivalis* mediate in vivo virulence and exploit TLR2 and complement receptor 3 to persist in macrophages. *J Immunol*. 2007; 179:2349–2358. [PubMed: 17675496]
- Welin A, Winberg ME, Abdalla H, Sarndahl E, Rasmusson B, Stendahl O, Lerm M. Incorporation of *Mycobacterium tuberculosis* lipoarabinomannan into macrophage membrane rafts is a prerequisite for the phagosomal maturation block. *Infect Immun*. 2008; 76:2882–2887. [PubMed: 18426888]
- World Health Organization. Geneva, Switzerland: 2012. Global Tuberculosis Report. http://www.who.int/tb/publications/global_report/en/.
- Yamatake K, Maeda M, Kadowaki T, Takii R, Tsukuba T, Ueno T, Kominami E, Yokota S, Yamamoto K. Role for gingipains in *Porphyromonas gingivalis* traffic to phagolysosomes and survival in human aortic endothelial cells. *Infect Immun*. 2007; 75:2090–2100. [PubMed: 17296756]
- Yang HW, Huang YF, Chou MY. Occurrence of *Porphyromonas gingivalis* and *Tannerella forsythensis* in periodontally diseased and healthy subjects. *J Periodontol*. 2004; 75:1077–1083. [PubMed: 15455734]
- Yu K, Mitchell C, Xing Y, Magliozzo RS, Bloom BR, Chan J. Toxicity of nitrogen oxides and related oxidants on mycobacteria: *M. tuberculosis* is resistant to peroxynitrite anion. *Int J Tuberc Lung Dis*. 1999; 79:191–198.
- Yun LW, Decarlo AA, Hunter N. Blockade of protease-activated receptors on T cells correlates with altered proteolysis of CD27 by gingipains of *Porphyromonas gingivalis*. *Clin Exp Immunol*. 2007; 150:217–229. [PubMed: 17937677]
- Zahrt TC, Deretic V. Reactive nitrogen and oxygen intermediates and bacterial defenses: Unusual adaptations in *Mycobacterium tuberculosis*. *Antioxid Redox Signal*. 2002; 4:141–159. [PubMed: 11970850]
- Zeituni AE, Jotwani R, Carrion J, Cutler CW. Targeting of DC-SIGN on human dendritic cells by minor fimbriated *Porphyromonas gingivalis* strains elicits a distinct effector T cell response. *J Immunol*. 2009; 183:5694–5704. [PubMed: 19828628]

Fig. 1.

Evasion strategies utilized by *Mycobacterium tuberculosis* disrupts immune regulation to promote bacterial transmission. *M. tuberculosis* is considered a pathogen capable of persistence within the human host. There are, however, differences in the extent to which the immune system is able to limit transmission to new hosts. A: Within 90% of *Mtb* infected individuals, infection is insufficiently controlled by the innate immune system, thus requiring adaptive responses. The coordination of both arms of immunity results in granuloma formation and containment of the organism. Eradication is not achieved, yet transmission is limiting. A number of interactions involving several cell types are critical for host containment of *Mtb*. APC actively prime T cells as Th1 effector cells, specialized to activate macrophages via TNF α and IFN γ secretion, allowing for phagocyte-dependent clearance of bacteria. Macrophage-derived cytokines importantly activate other macrophages (TNF α) and actively promote Th1 differentiation (IL-12). Internalized bacteria become sensitive to phagocytic killing mechanisms (ROS/RNS), provided vascular sorting remains unaltered, allowing for maturation and fusion of lysosomes, both key steps in generating bactericidal activity of these cells. Inflammasome activation may ensue with detection of DAMP or PAMP, inducing the expression of IL-1 β , involved in macrophage and T cell activation. Lastly, *Mtb* infected cells can undergo apoptosis. B: Approximately 10% of infected individuals fail to contain the infection, which offers potential opportunities for pathogen transmission. The lack of containment can be partly attributed to *Mtb* subversion of both innate and adaptive immune responses. Infection of APC in concert with inhibition of antigen processing/presentation greatly subverts APC function in T cell priming. Direct *Mtb* binding to C-type lectins induces anti-inflammatory cytokines (IL-4, IL-13), diminishes Th1 development via IL-10 induction and prevents IL-12 expression, a powerful mediator for Th1 responses. Failure to generate appropriate Th1 responses leads to decreased TNF α and IFN γ , cytokines known to be protective against *Mtb* infection with critical functions in cellular activation and granuloma formation. Bacterial secretion of immunological decoys skew CD4⁺ T cell responses to non-expressed antigens. *Mtb* prevents phagosomal maturation and arrests lysosomal fusion. For those bacteria that encounter ROS/RNS killing mechanisms, three distinct evasion strategies provide assistance. Inhibition of species generation, detoxification of reactive species and repair of damaged macromolecules affords protection for the pathogen. *Mtb* actively inhibits Inflammasome activation, preventing IL-1 β expression, which impairs the ability of host cells to overcome phagosome arrest. Lastly, *Mtb* postpones host cell death

during the initial phases of intracellular replication, instead promoting necrosis that allows phagosome exit by the bacterium, thus increasing the potential for infection of other cell populations.

Fig. 2.

Model of *P. gingivalis* evasion strategies preferentially targeting innate immunity to promote pathogen persistence. One of the major virulence factors expressed by *P. gingivalis* are gingipains, which proteolytically degrade (denoted by scissors) a wide range of substrates critically involved within host immunity (A). Since gingipains are both membrane anchored and secreted in OMV, alternative opportunities exist for immune interference at sites proximal and distant to infection, as denoted by the gradient encircling the bacterium. Targets include complement components (C3 and C5), pro-inflammatory cytokines/chemokines generated from both immune and nonimmune cells (IL-1 β , IL-6, IL-8, and TNF α), immunoglobulins (IgA, IgM, and IgG), in addition to PRR coreceptors (CD14). A variety of subversion techniques exploit host cell receptors within the macrophage, providing disruption of its cell activation, expression of inflammatory mediators and microbicidal potential (B). *P. gingivalis* modifies its lipid A structure to express LPS with unique characteristics. It is not only immunologically silent, but harbors the ability to antagonize TLR4 signaling, leading to increased bacterial survival within macrophages. Following gingipain cleavage of complement factor C5 and C5aR is activated in concert with fimbriae-dependent activation of CXCR4. Receptor engagement activates protein kinase A (PKA), impairing iNOS-dependent killing of the pathogen. Major fimbriae-dependent binding to CR3 induces ERK1/2 signaling, repressing IL-12 expression, impairing immune clearance in vivo. *P. gingivalis* also expresses minor fimbriae that binds DC-SIGN to promote bacterial internalization that affects DC function (C). DC maturation is uncoupled from the cytokine response resulting in decreased expression of IL-1 β , IL-6, IL-8, IL-12, and TNF α . The other important cell type involved in chronic inflammatory diseases of the vascular system is the endothelial cell. A number of reports have proposed *P. gingivalis* strategies that attempt to create an intracellular niche for the organism within the cells of the endothelium (D). Autophagy is initiated following attachment and invasion of endothelial cells, causing sorting to

vacuoles that fail to mature into autolysosomes, thus establishment of a “replicating vacuole.” Furthermore, intracellular PRR signaling pathways involved in endothelial cell activation (TNF-R/RIPK1) and intracellular recognition of pathogens (NOD/RIPK2) is targeted by gingipain-dependent activity. We propose degradation of RIPK allows for intracellular survival of the pathogen within endothelial cells. The majority of these evasion strategies have only been tested within specific cell types, as described within the text, and future studies will likely increase the breadth of cell types sensitive to the specific examples of immune intervention. Consequently, *P. gingivalis* utilizes its own protein factors or exploits host pathways to subvert immune responses. These strategies act to repress cellular function (endothelial, macrophage, dendritic, T cell, etc.) and disrupt communication of the immune system, effectively offering an opportunity for persistence and chronic immune stimulation.