Self/Nonself 2:3, 142-146; July–December 2011 © 2011 Landes Bioscience

Are you my friends or are you my enemies?

Amy H. Tang
Department of Microbiology and Molecular Cell Biology; Eastern Virginia Medical School; Norfolk, Virginia

Keywords: microbe-host interaction, pathogen-host antagonism, Drosophila innate immune activation, pattern recognition, protease-induced innate immune activation, PGRP-LC receptor, elastase, matrix metalloproteinase (MMP)

Abbreviations: AMP, antimicrobial peptide; DAP-type PGN; meso-Diaminopimelic Acid-type Peptidoglycan; IMD, immune deficiency; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; PAMP, pathogen-associated molecular pattern; PGN, peptidoglycan; PGRP, peptidoglycan-recognition protein; TLR, Toll-like receptor; TNF; tumor necrosis factor

Submitted: 03/13/12

Accepted: 03/13/12

http://dx.doi.org/10.4161/self.20137

Correspondence to: Amy H. Tang; Email: tangah@evms.edu

One hot summer morning, a little fly named "Buzz" was wandering around the dusty New York City looking for food. He was desperately hungry and thirsty and gradually becoming dizzy as the search dragged on. All of a sudden, his attention fell onto a banana peel on the sidewalk. The scent of a rotten banana made his stomach rumble and chirp. He examined the banana longingly and carefully. The banana had been stepped on by several pedestrians who left muddy footprints on it. Under the sun, the rotten banana was dotted with a colorful collection of hundreds of microorganism colonies that had begun to take over the food source—during the 12–16 hour period since Buzz's last meal. Should he eat the food right away or should he sterilize and clean the banana before indulging himself? Would he get sick from the countless colorful bacterial and fungal colonies if he ate this rotten fruit? Unfortunately, Buzz simply couldn't wait any longer. The hungry little fly dived into the food without any hesitation, drank the fermented juice without stopping, and shoveled down the microbe-infested food greedily without a second thought. After a few minutes, Buzz was comfortably full, felt happy and he flew away into the wilderness of the bustling city. Buzz worked hard and died at a ripe old age of 60 days without getting sick from the millions of microbes he encountered in the filthy environment of rotten fruits. Was he just lucky?

All species on Planet Earth have to learn to live with the billions of diverse microbes in their environments. The vast majority of animals go on with their daily business without succumbing to the omnipresence of "invisible" microorganisms everywhere including on their bodies, inside their digestive tracts and in their food. Should the host immune cells engage in the pre-emptive strikes due to the presence of microbe-specific patterns alone? Is it evolutionarily advantageous and economical to do so? How can the host immune cells distinguish omnipresent "good bugs" i.e., the nonpathogenic microbes, vs. the rare "bad bugs"—the pathogenic microbes. During these encounters, how do immune cells differentiate their deadly microbial foes from those harmless microbial friends? Should they wait until the microbes attack them first before mounting a vigorous counter-attack, i.e., a robust innate immune response? Should they attack microbes non-discriminatively? Can they afford to do so when microbes are everywhere?

To understand how a host cell differentiates pathogenic microorganisms from nonpathogenic ones is a fundamental question in cell biology and immunology. It is well known that innate immunity receptors can be activated via pathogen-associated molecular pattern (PAMP) binding and microbial pattern recognition.[1] The Drosophila IMD pathway exhibits homology to the mammalian tumor necrosis factor (TNFα) signaling pathway.[2] It has been shown that the IMD receptor, PGRP-LC, is activated via pattern recognition of monomeric peptidoglycan (DAP-type PGN) in response to Gram-negative bacteria.[3-5] The Toll-like receptors (TLR1-9) can also be activated by pattern recognition in mammalian cells. However, many questions remain: First, these

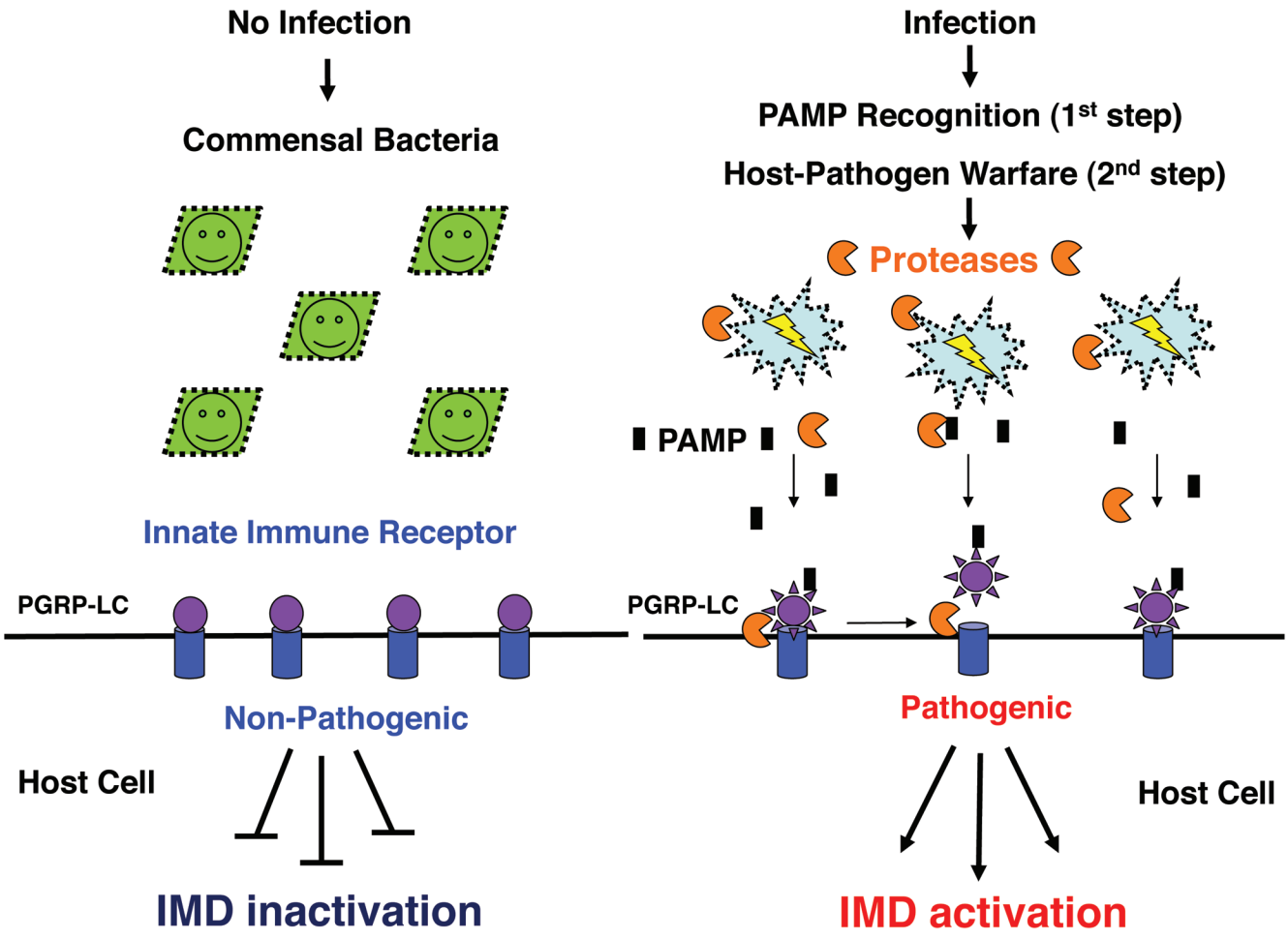

Figure 1. A working model illustrating that the release of proteases (or other virulence factors) during pathogen-host antagonism is the key signature of pathogenic microbes. A schematic illustration of how host cells differentiate pathogenic microbes from nonpathogenic ones, is shown. The left panel is an illustration of host-non-pathogen interaction that signals peaceful co-existence (no IMD activation) or physiological inflammation (low level IMD activation). The right panel is an illustration of host-pathogen antagonism resulting in active warfare. In the first step, host-microbe engagement starts with microbial pattern recognition that may or may not lead to innate immune activation. In the second step, the infection-induced protease (or other virulence factor) release is a "pathogenic" signature that will alert the host cells of the onset of pathogen invasion and tissue damage. In the third step, the release of proteases from both pathogens and host cells will lead to the cleavage of innate immunity receptors/sensors that will elicit an irreversible and robust activation of host immune responses in Drosophila.

common microbial patterns exist on commensal bacteria as well as pathogenic ones. Second, it is unknown how these common PAMPs, such as LPS and DAP-PGN, are generated during pathogenic bacterial infection. Third, how can these commensal-derived but commonly-shared immune agonists activate the host innate immune response while normal cell wall metabolites/byproducts of rapidly dividing commensal bacterial colonies trigger a basal level activity of innate immune response? Lastly, with limited innate immunity receptor diversity, how the pattern recognition receptors such as PGRPs/Toll/TLRs recognize specific pathogens amidst billions of nonpathogenic microbes and elicit an appropriate immune response, remains to be determined. These mechanistic gaps in our understanding of innate immune activation suggest that our current model of the PAMP recognition may admit expansion.

The human body contains 20 times more microbes than it does human cells in sheer number. Our body is like a planet in that as much as 2% of our body mass is not even "us." It is trillions of microorganisms (10^{14} microbes) coexisting peacefully in a complex and dynamic ecosystem.[6] It has been reported that all multicellular animals, including Drosophila, co-exist peacefully with millions or billions of commensal bacteria.[7-10] How Drosophila controls its resident microbiota communities and how Drosophila differentiates pathogenic microbial infection in the midst of commensal bacteria is an extremely interesting question.[11,12] The discovery of commensal bacteria in our body suggests that "PAMP pattern recognition" alone may not be sufficient to explain the peaceful symbiosis between host animals and their commensal flora in vertebrate and invertebrate animals. It is conceivable that no organism can afford to attack all microorganisms all the time because of the presence of common

microbial pattern molecules like LPS and PGN.

Drosophila has an innate immune system similar to humans but lacks the adaptive immune system. We used Drosophila as the model organism to determine how innate immunity is activated upon pathogen invasion, in particular to understand how a host immune cell differentiates pathogenic microorganisms from nonpathogenic ones. We have provided preliminary experimental evidence to demonstrate that the Drosophila IMD pathway can also be activated by proteases (i.e., Elastase and Mmp2). Using transgenic fly models, we demonstrate that protease release after pattern recognition provides a "pathogenic signature" and/or "tissue damage" signal that could alert host cells to the onset of endogenous tissue damage and exogenous pathogen invasion.[13] The sentinel receptor PGRP-LC is activated via proteolytic cleavage in response to Gram-negative bacterial infection. The protease-dependent cleavage of the receptor PGRP-LC is the signal for the IMD activation. We show that the PGRP-LC receptor is cleaved in response to live Salmonella/E. coli infection in vivo. A PGRP-LC receptor cleavage intermediate can be detected during live Gram-negative bacterial infection in Drosophila cells and in live animals in vivo. In contrast, no cleavage of the receptor PGRP-LC is detected in the presence of massive amounts of chemical-fixed, structurally intact but dead bacteria. No cleavage of the receptor PGRP-LC is detected in the presence of protease-deficient *E.coli* or Gram-positive bacteria *Staphylococcus carnosus*. We show that the cleaved PGRP-LC is a constitutively active receptor, i.e., an ectodomain-deleted PGRP-LC common to the three PGRP-LC isoforms and lacking the extracellular peptidoglycan recognition domain functions as a constitutively active receptor.[13] Our data suggest that PGRP-LC is a sentinel receptor that has dual roles in regulating and integrating two host defense systems: antimicrobial peptide (AMP) production and the melanization cascade. Our observations suggest a simple alternative explanation for how innate immunity receptors are activated by the action of proteases commonly deployed in pathogen-host antagonism, tissue injury and inflammation. Thus, the infection-induced protease-dependent PGRP-LC cleavage and the IMD pathway activation may provide one of many simple, efficient and elegant "working" models to explain how the Drosophila innate immune system can be activated in response to pathogen invasion, inflammation and tissue damage.

This concept is highly efficient and pathogen-specific because it provides a simple explanation for how innate immune pathways are activated by the action of proteases commonly deployed in pathogen-host antagonism, tissue injury and inflammation. We propose that the release of proteases (or other virulence factors) during pathogen-host antagonism is the key signature of pathogenic microbes. The release of digestive enzymes (or other pathogenic factors) that is common during pathogen-host antagonism may provide an important cue to alert host cells about the presence of pathogenic microorganisms. This concept is simple in which the irreversible cleavage of the innate immunity receptors (PGRP-LC in the IMD pathway) and ligands (Spätzle in the Toll pathway) by injury-induced protease release may signal the onset of tissue damage and pathogen invasion. By sensing generic proteases (or other pathogenic factors) commonly released during pathogen-host warfare through the integrity of the innate immunity receptors/sensors/signaling molecules, this explains why a small number of sentinel receptors, such as PGRP-LC/TOLL, could recognize and activate the host defense system in response to a diverse array of pathogenic microbes in Drosophila. Moreover, we present evidence suggesting that the cleaved sentinel receptors can irreversibly activate the innate immunity pathways so that host cells can respond rapidly to pathogen infection and mount an effective host defense and initiate tissue repair.[13] Our model complements the current model of pathogen-associated molecular pattern (PAMP) recognition in explaining how innate immune receptors are activated in response to pathogen invasion, inflammation and tissue damage.

We promote the notion that microbial pattern recognition is one of the most important, early and universal mechanisms of innate immune recognition and activation. We believe that pattern recognition is only the first step of host-microbe engagement. We question the conventional wisdom of how a few pattern recognition receptors, with the limited receptor diversity, recognize specific PAMPs and pathogens amidst billions of nonpathogenic microbes, and elicit an appropriate immune response specifically against pathogenic microbes. These mechanistic gaps in our understanding of innate immune activation suggest that our current model of PAMP recognition may need expansion. Thus, we propose a second step in host-microbe interaction, i.e., protease-mediated (cleavage-dependent) activation of innate immune receptors/sensors after pattern recognition engages pathogens and host cells. We suggest that pattern recognition (1st step) and the subsequent antagonistic interaction between pathogen and host cells may induce a highly localized release of proteases or other virulence factors (2nd step) during the initiation (onset) and progression (intensified antagonistic phase) of pathogen-host warfare, resulting in a highly specific and localized cleavage of the innate immunity receptor (3rd step). As a result, the induction of proteases (other virulence factors) during host-microbe "warfare" should be a defining feature of pathogenic microbes. A simple model would place protease-dependent innate immunity receptor cleavage after pattern recognition. Pathogenic microbes will elicit protease release to send a "pathogenic" signal to the host cells while nonpathogenic microbes will not elicit protease release and therefore promote peaceful co-existence.

We suggest that protease activation is like the "bullet" whose release initiates pathogen-host warfare. The induction of these virulence factors is the key determinant that allows host cells to differentiate pathogenic from nonpathogenic microbes. We hypothesize that protease release is just one example among many other possible pathogenic determinants that host cells can use to distinguish "friends—not to attack" from "enemies—to attack."

It is conceivable that proteases released during host-pathogen antagonism might play a role in differentiating pathogenic vs. non-pathogenic microbes to specifically activate innate immunity pathways and mount an effective and localized host response against invading pathogenic microorganisms. This model is simple, elegant and specific, and it complements the current model of pattern recognition in explaining how PGRP-LC receptors and the IMD pathway can be activated by pathogens in Drosophila.

We propose that protease release (or other pathogenic-virulent factors) during pathogen-host antagonism is the key feature of pathogenic microbes. The recent report that avian TLR15 is cleaved and activated by microbial protease provides another example that is consistent with our hypothesis.[13,14] There is increasing evidence that the innate immune system can be activated by proteases or pathogen-induced proteolytic cleavage activity.[15-17] Our new model is attractive because mammalian innate immunity and pattern recognition receptors lack the receptor diversity to match the amazing diversity of potential pathogens.[1,18-21] By detecting infection-induced "loss of well-being" signal through the monitoring of structural integrity of a small number of innate immunity receptors (PGRP-LC in the IMD pathway) and ligands (Spätzle in the TOLL pathway), it can send an unambiguous and irreversible "pathogenic" signal to host cells in response to infection and tissue damage by a diverse array of pathogenic microbes. A locally activated proteolytic cascade initiating from a small number of host innate immune receptors/sensors would allow for selective activation of specific innate defense pathways, and confinement of host defense to specific sites of infection/injury to initiate tissue repair, limit tissue damage, and fight off infection in a temporally and spatially tightly controlled manner. In addition, irreversible cleavage of the innate immunity receptors/sensors would also preclude a microorganism's ability to evade innate immune pattern recognition receptors through adaptive mutations and evolution. The commensal bacteria that are living in our digestive tracts have the same PAMP patterns as the pathogenic bacteria. Are the host cells proactively monitoring all the microbes constantly or are they monitoring the microbes rather passively until being provoked by pathogenic infection and invasion? Even if this theory may not be completely correct, we believe that this novel concept has several merits that may lead to a potential paradigm shift in the field. First of all, it helps to explain how inflammatory pathways contribute to tissue regeneration and repair in wound-healing in the absence of pathogen infiltration. Second, the model helps to explain how a small number of sentinel receptors like PGRP-LC can be so effective in activating the host defense system to combat specific pathogenic microbes while remaining unaffected by a diverse array of commensal microbes continually present in the environment. Lastly, it may help explain the roles of infiltrating immune cells in promoting tumor growth, cancer cell dissemination, invasion and metastasis.[22-24]

We would like to propose a new working model in which we suggest that protease (or other virulence factors) release may be a "pathogenic" signature for host cells to distinguish pathogenic microbes from nonpathogenic ones (Fig. 1). We encourage cross-examination, data validation and additional experimentations by experts in the field. Through this commentary, we hope to suggest an interesting idea and innovative concept to stimulate scientific discussions that will allow new ideas, unconventional data and creative approaches to emerge in a heatlhy debate to understand how host cells differentiate pathogenic microbes from nonpathogenic microorganisms in vivo.

Acknowledgments

Correspondence should be addressed to A.H.T. I am grateful to Dr. Stephen I. Deutsch, Dr. Julie Kerry, Dr. Ann Campbell, Dr. Richard Ciavarra, Dr. Ralf Janknecht, Dr. Edward Leof, Dr. Margaret Wortman, Dr. Marc Silverberg, Dr. Vasilena Zheleva and Ms. Zena Urban for their critical reading and encouraging comments about this manuscript. I am particularly grateful to my Ph.D. graduate student, Miss. Zena Urban and Miss Angela Tang-Tan, for their critical reading and encouraging comments about this manuscript. A.H.T is supported by a startup fund to A.H.T. from the Eastern Virginia Medical School and in part by National Institutes of Health Grants (GM069922–06S1 and R01 NIH CA140550).

References

1. Medzhitov R, Janeway CA Jr. Decoding the patterns of self and nonself by the innate immune system. Science 2002; 296:298-300; PMID:11951031; http://dx.doi.org/10.1126/science.1068883.
2. Lemaitre B, Hoffmann J. The host defense of Drosophila melanogaster. Annu Rev Immunol 2007; 25:697-743; PMID:17201680; http://dx.doi.org/10.1146/annurev.immunol.25.022106.141615.
3. Leulier F, Parquet C, Pili-Floury S, Ryu JH, Caroff M, Lee WJ, et al. The Drosophila immune system detects bacteria through specific peptidoglycan recognition. Nat Immunol 2003; 4:478-84; PMID:12692550; http://dx.doi.org/10.1038/ni922.
4. Kaneko T, Goldman WE, Mellroth P, Steiner H, Fukase K, Kusumoto S, et al. Monomeric and polymeric gram-negative peptidoglycan but not purified LPS stimulate the Drosophila IMD pathway. Immunity 2004; 20:637-49; PMID:15142531; http://dx.doi.org/10.1016/S1074-7613(04)00104-9.
5. Kaneko T, Yano T, Aggarwal K, Lim JH, Ueda K, Oshima Y, et al. PGRP-LC and PGRP-LE have essential yet distinct functions in the drosophila immune response to monomeric DAP-type peptidoglycan. Nat Immunol 2006; 7:715-23; PMID:16767093; http://dx.doi.org/10.1038/ni1356.
6. Mazmanian SK, Kasper DL. The love-hate relationship between bacterial polysaccharides and the host immune system. Nat Rev Immunol 2006; 6:849-58; PMID:17024229; http://dx.doi.org/10.1038/nri1956.
7. Ryu JH, Kim SH, Lee HY, Bai JY, Nam YD, Bae JW, et al. Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in Drosophila. Science 2008; 319:777-82; PMID:18218863; http://dx.doi.org/10.1126/science.1149357.
8. Sansonetti PJ. War and peace at mucosal surfaces. Nat Rev Immunol 2004; 4:953-64; PMID:15573130; http://dx.doi.org/10.1038/nri1499.
9. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science 2005; 307:1915-20; PMID:15790844; http://dx.doi.org/10.1126/science.1104816.
10. Sansonetti PJ. The innate signaling of dangers and the dangers of innate signaling. Nat Immunol 2006; 7:1237-42; PMID:17110939; http://dx.doi.org/10.1038/ni1420.
11. Silverman N, Paquette N. Immunology. The right resident bugs. Science 2008; 319:734-5; PMID:18218861; http://dx.doi.org/10.1126/science.1154209.
12. Muyskens JB, Guillemin K. Bugs inside Bugs: what the fruit fly can teach us about immune and microbial balance in the gut. Cell Host Microbe 2008; 3:117-8; PMID:18329607; http://dx.doi.org/10.1016/j.chom.2008.02.011.
13. Schmidt RL, Trejo TR, Plummer TB, Platt JL, Tang AH. Infection-induced proteolysis of PGRP-LC controls the IMD activation and melanization cascades in Drosophila. FASEB J 2008; 22:918-29; PMID:18308747; http://dx.doi.org/10.1096/fj.06-7907com.

14. de Zoete MR, Bouwman LI, Keestra AM, van Putten JP. Cleavage and activation of a Toll-like receptor by microbial proteases. Proc Natl Acad Sci U S A 2011; 108:4968-73; PMID:21383168; http://dx.doi.org/10.1073/pnas.1018135108.
15. Wills-Karp M, Nathan A, Page K, Karp CL. New insights into innate immune mechanisms underlying allergenicity. Mucosal Immunol 2010; 3:104-10; PMID:20032970; http://dx.doi.org/10.1038/mi.2009.138.
16. Sokol CL, Barton GM, Farr AG, Medzhitov R. A mechanism for the initiation of allergen-induced T helper type 2 responses. Nat Immunol 2008; 9:310-8; PMID:18300366; http://dx.doi.org/10.1038/ni1558.
17. McKerrow JH, Caffrey C, Kelly B, Loke P, Sajid M. Proteases in parasitic diseases. Annu Rev Pathol 2006; 1:497-536; PMID:18039124; http://dx.doi.org/10.1146/annurev.pathol.1.110304.100151.
18. Janeway CA Jr., Medzhitov R. Innate immune recognition. Annu Rev Immunol 2002; 20:197-216; PMID:11861602; http://dx.doi.org/10.1146/annurev.immunol.20.083001.084359.
19. Beutler B, Rietschel ET. Innate immune sensing and its roots: the story of endotoxin. Nat Rev Immunol 2003; 3:169-76; PMID:12563300; http://dx.doi.org/10.1038/nri1004.
20. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. Nat Immunol 2004; 5:987-95; PMID:15454922; http://dx.doi.org/10.1038/ni1112.
21. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 2006; 124:783-801; PMID:16497588; http://dx.doi.org/10.1016/j.cell.2006.02.015.
22. Rakoff-Nahoum S, Medzhitov R. Toll-like receptors and cancer. Nat Rev Cancer 2009; 9:57-63; PMID:19052556; http://dx.doi.org/10.1038/nrc2541.
23. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008; 454:436-44; PMID:18650914; http://dx.doi.org/10.1038/nature07205.
24. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002; 420:860-7; PMID:12490959; http://dx.doi.org/10.1038/nature01322.