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Review

The Art of War: Innate and adaptive immune responses

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Abstract. Research over the last several years has greatly advanced our understanding of the mechanisms by which the immune system functions. There exist two main branches of immunity, termed innate and adaptive immunity. Innate immunity uses the genetic memory of germline-encoded receptors to recognize the molecular patterns of common pathogens. Adaptive immunity, akin to somatic memory, is a complex system by which the body learns to recognize a pathogen's unique antigens and builds an antigen specific response to destroy it. The effective development of the overall immune response depends on careful interplay and regulation between innate and adaptive immunity. Here we review our current understanding of how these integrated systems distinguish targets against which a response is appropriate and neutralize potentially pathogenic challenges.

Key words. Pathogen recognition; innate activation; host defense; antigen presentation; immunoregulation.

[I]t is said that one who knows the enemy and knows himself will not be endangered in a hundred engagements. … The combat of the victorious is like the sudden release of a pent-up torrent down a thousand-fathom gorge. This is the strategic disposition of force.

– Sun Tzu *The Art of War* [1]

Introduction

Since the first millennium B.C., the study of strategic warfare has proven that a thorough understanding of the enemy and one's own disposition is critical to victory – through organization, tactics and carefully directed execution. Similarly, in the constant war between metazoans and invading pathogens, the evolutionary mind has devised a powerful defense system based upon discrimination between self and infectious/pathogenic non-self. A highly effective arsenal of weapons awaits any intruder, through integrated systems broadly categorized as 'innate' or 'adaptive' immunity. Innate immunity, a teleologically ancient system of microbial recognition, activates defenses through a fixed number of germ-line-encoded receptors that recognize structural components of microorganisms and viruses. The adaptive immune system is only present in vertebrates and cartilaginous fish and relies on the clonal expansion of antigen-specific effector cells selected for by receptor gene rearrangement.

While it is clear that these two classes of responses evolved independently, it is now accepted that innate immune signals play a critical role in initiating and instructing the development of adaptive effector mechanisms. This is illustrated by the use of adjuvants composed of innate activators (such as the inclusion of heat-killed

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Mycobacterim tuberculosis in complete Freund's adjuvant) that greatly increase the adaptive memory response to protein antigens. However, only some of the mechanisms by which these systems are intertwined are well understood. The purpose of this review will be to discuss our current understanding of the innate-adaptive paradigm, with emphasis on the most recent studies and their implications for the dogma of the field. This is of necessity an incomplete survey, as much a comment on our perspective of the field. There exists an extraordinary amount of information on innate and adaptive immune responses, and unfortunately, due to space limitations readers will be referred to more focused reviews in key subjects of interest as necessary. As will be discussed, the intersection of innate and adaptive immunity is intricate and finely balanced, and deregulation of these responses can also lead to various forms of immunopathology.

Pathogen Recognition

The interior of the host organism is a sterile environment, and pathogens often gain initial access through the skin, respiratory or gastrointestinal routes. Due to microbial life cycles, pathogens invariably produce some conserved molecules that are not present in the internal eukaryotic environment. While there is much variation, related species of microbes contain similar structural motifs that can be readily identified as molecular patterns of infectious non-self. These include components of microbial membranes, cell walls, proteins and DNA, and are termed pathogen-associated molecular patterns (PAMPs) [2]. Therefore, the detection strategy of innate immunity is based on the principal of 'pattern recognition' (reviewed in [2–6]). Indeed, germ-line-encoded pattern recognition receptors (PRRs) that recognize microbial products are highly conserved and were present before the evolutionary divergence of the plant and animal kingdom [7]. Key aspects of PRRs are that they only recognize a fixed structural motif and do not undergo rearrangement. Additionally, there are a limited number of such germ-line-encoded proteins in any genome. Knowledge of this family has expanded in mammals, and dedicated PRRs have been found with a variety of functions, including intracellular signaling, opsonization, chemotaxis and endocytosis [4] (see table 1).

Table 1. Examples of major pattern recognition receptors and their respective ligands.

Receptor	Ligand	Reference
Toll-Like:		
TLR1	triacylated lipoproteins (with TLR2)	[161, 162]
TLR2	peptidoglycan, lipoproteins, zymosan	[163, 164]
TLR3	double-stranded RNA, poly I:C	[165]
TLR4	lipopolysaccharide, RSV F protein	[10, 166]
TLR5	flagellin	[167]
TLR6	diacylated lipoproteins (with TLR2)	[162, 168]
TLR7	imidazoquinolines (natural ligand unknown)	[169]
TLR8	imidazoquinolines	[192]
TLR9	bacterial DNA, unmethylated CpG motifs	[126]
TLR10	unknown	–
Soluble:		
CD14	lipopolysaccharide	[170, 171]
LBP	lipopolysaccharide	[172]
MBL	terminal mannose residues	[40]
CRP	phosphocholine determinant of teichoic acids	[173, 174]
PTX3	fungal motifs	[44]
Scavenger:		
MSR1 (SR-AI/II)	lipid A, lipoteichoic acid, whole bacteria	[175, 176]
MARCO	Gram-positive, Gram-negative bacteria	[177]
Mannose Receptor	α-mannan from yeast cell walls	[178, 179]
Dectin-1	β-glucan from yeast cell walls	[180]
Complement/Opsin:		
FcR	IgG-opsinized particles	[181]
CR1	C1q, C4b, C3b, MBL	[182–184]
CR2	iC3b, C3d, C3dg	[39, 185] 186]
CR3	iC3b	[187]
CR4	iC3b	[188]
Intracellular:		
Nod2	muranyl dipeptide, lipoproteins?	[56, 57]
PKR	double-stranded RNA	[189]
OAS	double-stranded RNA	[190, 191]

Toll-like Receptors

Toll-like receptors (TLRs) are PRRs that have been conserved from insects to humans and have a unique and important role in signaling the presence of infection. *Toll* was originally identified as a *Drosophila* gene required for dorsoventral axis formation and was later shown to be essential for antifungal immunity [8]. The discovery of a human homologue for the *toll* gene inaugurated the field of PAMP recognition by the TLR receptors in mammals [9]. The TLRs make up a family of type I transmembrane receptors which contain an extracellular leucine-rich repeat (LRR) domain and an intracellular Toll/interleukin IL-1 receptor (TIR) domain [5]. The LRR is believed to mediate ligand-binding specificity, while the TIR domain is responsible for initiating intracellular signaling pathways through protein-protein interactions.

The first definitive evidence that mammalian TLRs facilitate recognition of PAMPs came from genetic experiments. C3H/HeJ is a strain of mouse that is resistant to endotoxic shock triggered by injection of lipopolysaccharide (LPS), a molecular component of bacterial cell wall. Positional cloning of this defect led to the identification of

a missense mutation in the *Tlr4* gene of C3H/HeJ mice [10]. TLR4 was thus proposed to be the critical receptor for LPS detection, and this was later confirmed by studies in genetically engineered TLR4-deficient mice [11]. Subsequently, many additional TLRs have been identified, and the gene-targeting approach has established that unique PAMPs are recognized by each TLR. To date, 10 TLRs have been identified in mammals and have been shown to recognize a diverse array of PAMPs derived from bacteria, viruses, protozoa and fungi. In addition, some studies have indicated that endogenous ligands, such as heat shock proteins and fibronectin, may use TLR4 as a receptor [12–14]. However, the contribution of these ligands to a biological role for TLR4 is unclear. TLRs are variously expressed on immune system cell types, such as macrophages and dendritic cells (DCs), but have also been reported in other tissues such as endothelial, epithelial and muscle tissues [3].

Numerous studies have carefully mapped out the signaling pathways that are activated within the cell upon TLR-ligand recognition (reviewed in [5, 15, 16]). A simplified model of the current view of TLR signaling is depicted in figure 1. Recognition of the PAMP ligand leads to recruitment of the adaptor proteins, such as Myeloid differentiation factor-88 (MyD88), through the TIR domains. The affinity of the interaction between a TLR and its PAMP can be significantly augmented by coreceptors, such as CD14, which cooperates in TLR4 activation by LPS [17]. The association of MyD88 with the intracellular domain of TLR provides a death domain that nucleates assembly of the other death domain-containing signaling molecule, the IL-1 receptor associated kinase-1 (IRAK1) and IRAK4 [18]. Additional scaffolding and signaling molecules that assemble on this nucleus include the TNF-receptor-associated factor-6 (TRAF6) [19]. This leads finally to activation of the nuclear factor κB (NF-κB) and members of the mitogen-activated protein kinase (MAPKs) family such as the stress-activated protein kinase (SAPK) and p38. These signaling pathways directly regulate gene expression profiles following TLR stimulation. While TLRs were originally described to activate a common set of signaling pathways, recent studies have indicated that the functional roles of TLRs are more complex. Key to this regulation are the alternative roles of MyD88 homologues TIRAP and TRIF [20–22]. Recently it was demonstrated that TLR3 and TLR4 can specifically induce innate antiviral responses by selective activation of the transcription factor, Interferon-regulated factor-3 (IRF3) [23]. This indicates that at least TLR3 and TLR4 are capable of directing unique downstream signals – it still remains to be determined whether other TLR-specific pathways exist.

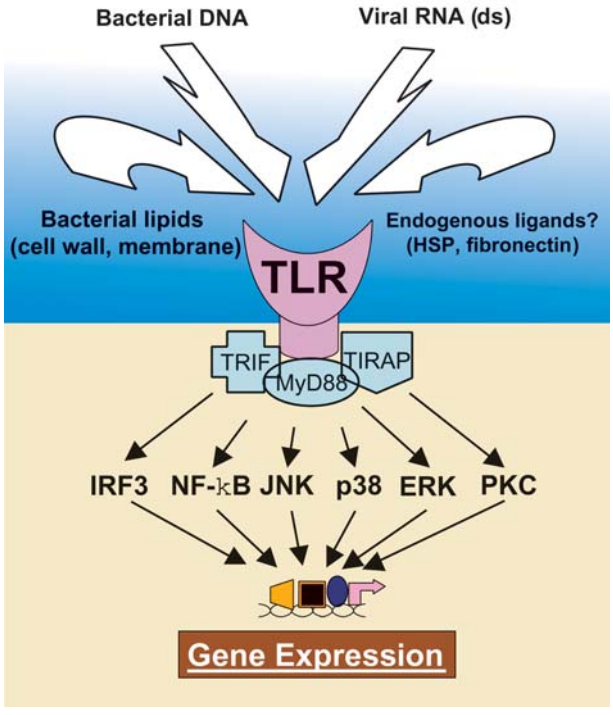

Figure 1. Schematic representation of the signaling pathways activated upon pattern recognition by the TLRs.

The consequence of these TLR-induced signals is ultimately upregulation of different sets of genes that regulate distinct functional events. The initiation of innate and inflammatory responses depends on TLR activation of processes such as antigen presentation, phagocytosis and apoptosis. TLR also rapidly alters cell trafficking and recruitment by inducing expression of a number of inflammatory cytokines and chemokines. In addition, TLR activation promotes DC maturation by upregulation of major histocompatibility complex (MHC) proteins and costimulatory molecules, such as CD80 and CD86 [3], thus providing the two necessary signals for T cell activation.

The multi-faceted role of the TLRs in innate and adaptive response is as enigmatic as it is essential. However, several questions stand out and are currently the subject of a considerable amount of research. Certain TLRs, such as TLR4, can recognize a diverse array of seemingly unrelated ligands (LPS, viral proteins). It has been hypothesized that unknown coreceptors exist which mediate binding, as CD14 does for LPS, but the true nature of the TLR-ligand interaction is still unclear. While a wealth of knowledge is available about TLR-mediated signaling pathways and components, there is a curious scarcity of studies that find a susceptibility to infection in TLR-deficient mice. This may be due to redundancy in TLR receptor recognition, or to the low sensitivity of attempted experiments. Regardless, more careful studies of multiple TLR knockout mice using relevant disease models will be required to understand the unique functional role of each TLR.

Complement

The complement system is composed of more than 20 serum proteins and cell surface receptors. What has emerged from analysis of knockout strains of mice and patients with specific lesions is a more coherent picture of an innate immune system with an extremely efficient effector system. Complement uses a cascade of serum proteases to assemble a membrane attack complex (MAC) that introduces pores into the membranes of activator surfaces such as bacteria. In assembling a MAC, the proteases release complement fragments that function as inflammatory regulators and attract lymphocytes. Initiation of the complement cascade depends on generation of an enzyme complex known as a C3 convertase. This is achieved, in classical pathway activation, when natural immunoglobulin (Ig)M antibodies activate and fix the C1, C2 and C4 proteins. Alternative pathway activation results from the direct activation of C3. The efficiency of complement activation is such that all cells bear receptor inhibitors such as CD35 and CD59 to prevent inappropriate activation. Absence of this regulation results in rapid and complete rejection of the organ or surface as seen in xenograft rejection.

During initial exposure to an infectious agent, the complement system helps to contain microorganism numbers. Control of acute infections is clearly seen in C3-, C4- or soluble IgM-deficient mice that are completely unable to control a murine model of acute peritonitis [24, 25]. The pentameric structure of IgM allows binding of the antibody to low-affinity targets such as bacterial cell wall structures. This initiates classical pathway activation of complement and MAC formation, which itself serves to restrict the bacterial numbers. Additionally, complement activation is detected by peritoneal mast cells that release intracellular stores of TNF-α [25]. This induces chemotaxis of neutrophils that can directly kill bacteria by phagocytosis and oxidative shock. The detection of complement activation by mast cells is mediated largely by the release of the anaphylotoxin C5a during classical pathway activation. This fragment can also be detected by neutrophils. Additionally, complement-coated targets can be opsonized very efficiently by complement receptor type 3 (CR3) recognition of iC3b fragments.

Activation of complement has long been known to be critical for the generation of an antibody response. Pepys first observed an impaired immune response in animals transiently depleted of C3 protein [26]. A series of elegant experiments with soluble receptor constructs [27], knockout mice [28, 29] and blocking antibodies [30] have clearly defined the interaction between activated fragments of C3 with complement receptor type 2 (CR2, CD21) as the necessary interaction for complement support of an antibody response. Upon activation, C3 becomes covalently bound to hydroxyl or amino acid side chains on target antigens. The complement-tagged antigen can then enhance the humoral immune response by a number of methods. By cross-linking the antigen receptor with the CD19/CD21 coreceptor complex on the B cell, complement tagged antigen can decrease the stimulation threshold in a dose-responsive manner [31]. This cross-linking also upregulates costimulatory receptors CD80 and CD86 [32–34], and enhances by two orders of magnitude peptide presentation by B cells [35–37]. Targeting antigen to the follicular dendritic cell in the B cell area of lymphoid organs has also been shown to prolong the memory and enhance the affinity selection of B cells [38]. These combined effects make it easy to understand how complement tagging generates such effective B cell antigens [39].

Other Complement Activators

Access to complement activation is also achieved through mannose-binding lectin (MBL). This member of the collectin family recognizes various structures such as Gram-negative oligosaccharides and lipopolysaccharides (reviewed in [40]). In conjugation with the associated MBL-associated serine proteases (MASPs)-1, 2 and 3 (MASP1–3), C3 convertase is generated, resulting in complement activation. MBL also enhances phagocytosis, although this may be related to its ability to fix complement. Critically, patients with MBL deficiencies show increased susceptibility to a wide variety of infectious agents [41].

The pentraxins are another evolutionarily conserved family of acute-phase proteins regulating immediate early host responses. Mice expressing the short human pentraxin, C-reactive protein (CRP) are protected against fatal streptococcal and salmonella infections [42, 43]. CRP, like MBL, is an oligovalent ligand for bacterial lipoproteins. Recognition by CRP also activates complement and therefore supports opsonization. The long pentraxin PTX3, when knocked out in mice, left them susceptible to opportunistic pulmonary infections with the fungal pathogen *Aspergillus fumigatus* [44]. This is one of the few examples where a specific PRR has been shown to be primarily responsible for pathogen recognition and response.

Scavenger Receptors

Scavenger receptors (SRs) are found chiefly on macrophages and dendritic cells. These are a family of receptors with loosely related structure based on a trimerizing coiled-coil domain and a ligand-binding collagenous domain. Although recent interest in atherosclerosis has implicated the class A SR in the recognition of low-density lipoproteins, their innate function is based on their ability to bind polyanionic ligands such as lipid-A [45]. SR-A deficient animals are more susceptible to infection with *Listeria* and *Staphylococcus aureus* [46, 47]. Using

fluorescence-activated cell sorting (FACS); direct binding to Gram-negative and positive bacteria has been demonstrated by SR-A I and II and to *S. aureus* by the related collagenous SR, MARCO. Consistent with this pattern of carbohydrate recognition, the mannose receptor is distributed on macrophages and dendritic cells throughout the body and recognizes a range of mannosylated molecular patterns. While there is clearly a functional role for mannose receptors in particulate clearance, mannose receptor ligation additionally regulates the release of cytokines such as IL-6 [48] and IL-12 [49] and macrophage activation.

Intracellular Receptors

The LRR domain, as described in TLR family proteins, has become synonymous with pattern recognition. Examples of LRR in innate immunity can be found throughout evolution. In plants, disease resistance genes (R) are composed of repeated LRR domains. The mammalian nucleotide-binding oligomerigation domain (NOD) family of proteins contain LLR repeats but were originally cloned based on the presence of a caspase activation and recruitment domain (CARD) [50, 51]. This family, numbering 33 members to date, contains carboxy-terminal LLR domains that are responsible for ligand binding. All members of this family have a nucleotide-binding domain whose function is not well defined. Their amino-terminal domain is varied depending presumably on the function of each protein. The family includes members with amino-terminal death domains, pyrin domains, or CARDs. The CARD domain is found in two members of this family, Nod1 and Nod2 [52]. These proteins have been implicated, by dominant-negative and overexpression experiments, in the recognition of cytoplasmic LPS. While confirmation awaits description of the knockout phenotype, patients with a frameshift deletion of the terminal LRR of Nod2 present with Crohn's disease, a chronic inflammatory disease of the gastrointestinal tract [53, 54]. Point mutations or deletions in the LRR domain of Nod2 are also associated with Blau's syndrome, another disease marked by chronic inflammatory symptoms such as arthritis and uveitis [55]. It has been suggested that Nod mutations prevent recognition of intracellular pathogens that therefore persist, leading to a chronic infection state. This persistent infection causes inflammation characterized by Crohn's and Blau syndromes. Enthusiastic support for this model is provided by the recent description of the muramyl dipeptide, the minimal immunostimulatory component of bacterial peptidoglycan, as the specific ligand for Nod2 [56, 57].

In contrast to bacterial infections, awareness of such intracellular innate receptors has a long history in host defense to viral infection. As for bacterial infection, innate immunity plays a critical role in immediate early responses to viral infection. The double-stranded RNA (dsRNA)-dependent protein kinase (PKR) is expressed in latent form in the cytoplasm of most cells. Upon exposure to viral genomic dsRNA, PKR phosphorylates a large number of substrates. Phosphorylation of the translation initiation factor eIF2α inhibits protein synthesis in the cell [58]. Activation of PKR also leads to induction of apoptosis by activation of the caspase-8 pathway. Thus the virus is contained by destruction of the host cell. Consistent with this, PKR-deficient animals are extremely sensitive to viral infection [59]. The 2′, 5′ oligoadenylate synthetases (OASs) represent another family of interferon (IFN)-inducible antiviral enzymes (reviewed in [60]). Upon exposure to dsRNA, OAS oligomerizes ATP, in turn activating IFN-inducible RnaseL. RnaseL-deficient animals show a suppressed interferon-α antiviral response, possibly due to alterations in the apoptosis mechanisms accessed by RnaseL. An indication of the importance of these pathways is measured by 100% mortality following West Nile virus infection in mice that carry a nonsense mutation in exon 4 of OAS and a significantly diminished morbidity or mortality in animals bearing wild-type OAS alleles [61].

Innate Immune Responses

Upon detection of infection, the first lines of host defense are quickly initiated (see fig. 2). Cellular mediators of innate immunity include tissue macrophages, neutrophils, and natural killers (NK) cells. These cells migrate towards the source of the infection. Macrophages are phagocytic cells that reside in many tissues and produce high levels of cytokines and chemokines that function as the 'red alert' of infection [62]. Immediate changes at the site of infection include increased vascular permeability, increased expression of adhesion markers and recruitment

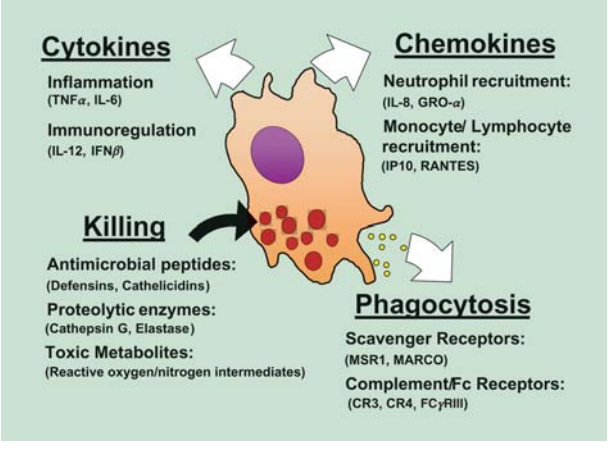

Figure 2. Overview of the multiple mechanisms used by innate immune cells in response to pathogenic challenge.

of leukocytes. In humans, neutrophils represent ~70% of peripheral blood leukocytes and mediate effector functions against many classes of pathogens [63]. Neutrophils have the shortest life span among leukocytes (only 6–10 h in the circulation), but are equipped with an intimidating array of antimicrobial weaponry, including toxic metabolites, antimicrobial proteins and proteolytic enzymes. NK cells also possess intrinsic killing ability by production of apoptosis-inducing proteins such as perforin and FasL [64]. Although initially found to kill certain tumor cell lines in vitro, it is now known that NK cells can also detect and kill virus-infected cells through an complex system of 'activating' and 'inhibitory' receptors, as well as MHC class I interactions [65]. Eosinophils remain among the most enigmatic of cells as our appreciation of their detrimental activities, such as asthma and allergic disease, far outweighs our understanding of their beneficial effects in viral and filarial infections [66]. Thus, there exists an elaborate panel of unique immune cell types with nonredundant functions that provides a 'basal' level of host defense against commonly encountered pathogens. For more severe infections, innate immune effector cells also provide a considerable degree of protection in the several days required to mount the adaptive response.

Inflammation I: Cytokines

Recognition of pathogens by TLRs and other PRRs leads to the production of inflammatory cytokines that have local and systemic effects that explain some of the signs and symptoms of acute disease. Systemic effects include changes in temperature, blood flow, cell trafficking and serum protein levels. These coordinated events, collectively termed the 'acute phase' response, are attributed to the flood of inflammatory cytokines produced. While many studies have focused on TNF-α, IL-1β, and IL-6, recent reports have also described a role for IL-12, IL-18 and lymphotoxin-α in pathogen-induced inflammation (reviewed in [62, 67–69]). The acute phase cytokine milieu is responsible for effects on nervous tissue (hypothalamic temperature regulation) through prostaglandin synthesis [70]. In addition, liver secretes high levels of acute phase proteins such as CRP, serum amyloid proteins and secretory phospholipase A_2 ($sPLA_2$) into the circulation [68]. These acute phase products facilitate the activation of complement, opsonization of pathogens and the clearance of apoptotic cells.

While the acute phase cytokines are critical for survival, activation of this response also causes some level of host injury. One of the best-described examples is endotoxin release from dead and dying Gram-negative bacteria during infection that can result in septic shock. It is characterized by hypotension, vascular collapse, multiorgan dysfunction syndrome (MODS), and can be mimicked by injection with purified endotoxin alone [68, 71]. On a cellular level, both the acute phase response and pathogenic stimuli often cause cell death and apoptosis. While this has been shown to be very protective in viral infections, the role of apoptosis in bacterial infection is less clear. *Yersinia enterocolitica* was shown to specifically induce apoptosis in macrophages through the outer protein YopP [72]. However, *Bartonella*, a Gram-negative pathogen that induces angio-proliferative lesions, could inhibit both early and late events in apoptosis of endothelial cells in vitro [73]. Thus some apoptosis could inadvertently help to extend bacterial infection.

Inflammation II: Chemokines

Awareness of the pathogen and redirection of innate immune cell types towards the source of detection is a critical process in limiting infection. Examined histologically, it is readily apparent that the nature of the pathogen determines the type of infiltration. Generally, infiltration during bacterial infection is dominated by neutrophils, during viral infection, by mononuclear leukocytes and helminth infections by eosinophils [4]. Chemokines are small secreted molecules that function to direct cell trafficking and movement in diverse functions such as innate immune responses, dendritic cell maturation, lymphocyte development, angiogenesis and generation of T-helper type 1 (T_H1) and T_H2 adaptive responses (reviewed in [74–76]). The discrimination of the pathogen by TLRs and the subsequent production of a specific subset of chemokines may be the first point at which the immune system tailors its response to specific pathogens.

Using bioinformatic approaches, the chemokines have been classified into four subfamilies according to the specific cysteine motifs in their amino acid sequence: CXC, CC, C and CX3C [75]. Several members of the CXC and CC chemokine family have been demonstrated to play a role in cell trafficking during acute inflammation, and distinct chemokines attract specific leukocyte subpopulations. The CXC subfamily has been divided into two groups depending on the presence of an ELR amino acid motif preceding the first cysteine. CC and non-ELR CXC chemokines, such as macrophage inflammatory protein-1α (MIP-1α) and monocyte chemotactic protein-1 (MCP-1), and interferon-γ (IFN-γ) inducible protein-10 (IP-10), have been shown to preferentially attract monocytes and lymphocytes [76]. In contrast, CXC chemokines with an ELR domain, such as IL-8 and growth-related oncogene-α (GRO-α), are major neutrophil chemoattractants [77]. In models of urinary tract infection, mice deficient in the IL-8 receptor (IL-8Rh) were unable to clear bacteria from kidneys and bladders, and developed bacteremia and symptoms of systemic disease due to failure of neutrophil recruitment [78]. These data suggest that disease progression can be linked to the failure of the appropriate subpopulation of innate immune cells to find their target.

Inflammation III: Matrix Changes

The influx of cytokine and chemokine production also has numerous effects on other surrounding cell types, which respond by appropriately altering expression of key surface receptors and matrix proteins. During an inflammatory response, the vascular endothelium directs the accumulation of leukocytes through various means, particularly by upregulation of specific cell surface molecules that are adhesive for ligands on circulating leukocytes. Examples of such molecules are E-selectin and intercellular adhesion molecule-1 (ICAM-1) [79]. Initial slowing of leukocytes on the vascular endothelium by selectins is followed by adherence of leukocyte adhesion proteins (e.g. CD11) to vascular endothelial ligands (e.g. ICAM-1). Extravasation of leukocytes into tissues through tight junctions of endothelial cells is mediated by platelet and endothelial cell adhesion molecule-1 (PECAM-1). Blocking PECAM-1 can block transendothelial migration but not chemotaxis [80].

In addition, multiple changes take place in the extracellular matrix (ECM) at the site of pathogen detection. Often responsible are the matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases targeting ECM compounds as well as a number of other proteins [81]. Their proteolytic activity acts as a mechanism for modulation of inflammation, as well as wound healing. Neutrophils rapidly release numerous collagen-matrix proteases, such as neutrophil collagenase (MMP-8) and gelatinase B (MMP-9) from prestored granules after activation by IL-8 or other cytokines [77]. A related molecule, neutrophil gelatinase-associated lipocalin (NGAL), was recently reported to tightly bind bacterial catecholate-type ferric siderophores, thereby adding an antibacterial iron depletion strategy to the innate response [82].

Innate Defense Mechanisms I: Anti-Microbial Peptides

Once cells are recruited to the site of infection, several mechanisms exist for actual destruction of the pathogenic organism or infected cell. The phylogenetically oldest innate immune response is production of antimicrobial peptides, such as defensins and cathelicidins reviewed in [83–86]. These antibiotic-like molecules have been shown to be directly toxic to a particular spectrum of bacteria, fungi or some enveloped viruses in vitro, and are therefore considered direct effectors of innate immunity. Antimicrobial peptides are small, cationic proteins (2–6 kDa), and are believed to function by disruption of negatively charged components of microbial membranes [83]. Recently, mice deficient in the cathelicidin protein CRAMP were shown to be susceptible to severe necrotic skin infection caused by group A *Streptococcus* (GAS) [87]. The critical role of this system in human disease is further highlighted by the recent understanding of the molecular basis of high susceptibility to upper-respiratory infection by bacterial and fungal pathogens in patients with cystic fibrosis (CF). CF patients have a mutation in a respiratory chloride ion transporter channel (CFTR), and the abnormally high salt concentrations in airway surface fluid causes inactivation of antimicrobial peptides and abnormal growth of lung commensals [88, 89].

Much of the interest in this field has focused on the defensin family of antimicrobial peptides, which produce toxic effects in a wide variety of pathogens. Defensins have been isolated from species as diverse as plants, insects and arthropods, and two major classes have been studied in mice and humans [84]. α-Defensins are produced and stored as pre-propeptides in granules of mature neutrophils and some intestinal Paneth cells. β-Defensins are constitutively expressed in the epithelial compartment but can also be amplified by inflammatory stimuli, such as LPS. In addition, β-defensins have also been reported to be chemotactic for immature dendritic cells and memory T cells, postulating a role in the development of adaptive responses [90]. In a reciprocal functional overlap, some CXC chemokines have been shown to have microbicidal activity [91]. The overlap between the antimicrobial and chemotactic properties of these molecules is currently the subject of intense study, and many hypotheses await corroboration by gene-disruption techniques.

Innate Defense Mechanisms II: Killing

The frontline of any war is gruesome, and the pathogen-host interaction during a major infection is no different. By the millions, innate immune cells migrate towards the source of infection, equipped with versatile antimicrobial machinery. While microbes are often killed by constitutively produced antimicrobial peptides and complement, efficient elimination of the pathogen requires direct and concentrated exposure to toxic metobolites. Therefore, innate immune cells use the mechanisms of phagocytosis and receptor-mediated endocytosis in order to ingest particles and 'sample' their environment. This effect is multiplied by recognition by secreted PRRs such as MBL, complement and CRP, which act as tags for particle uptake [92].

If the cell is in an activated state or receives activation signals, phagocytosed particles can be killed by a number mechanisms initiated simultaneously. Macrophages and neutrophils produce toxic metabolites derived from both oxygen and nitrogen, termed reactive oxygen intermediates (ROIs) and reactive-nitrogen intermediates (RNIs), respectively [93]. The critical role of ROI in microbial killing has been long-accepted since the finding that chronic granulomatous disease (CGD), a recessive disorder characterized by life-threatening pyogenic infections and inflammatory granulomas, is due to defective phagocyte respiratory burst [94]. RNIs also exhibit potent microbiocidal activity. However, recent studies with transgenic mice have shown that the production of nitric oxide

(NO) is linked to many diverse functions in the immune system [95]. Of note, several conflicting reports have appeared regarding whether NO production is beneficial or detrimental in models of pathogenic infection. In addition, evidence exists for a surprising role for NO in suppression of immune responses and thymic development [96].

Neutrophil granules contain several serine proteases, such as elastase and cathepsin G, which have direct microbicial activity, degrade extracellular matrix and can cleave other protein products [97]. Upon phagocytosis, granules filled with toxic metabolites, antimicrobial peptides and/or serine proteases fuse with the vacuole and release their contents directly onto the pathogen. In an impressive study by Reeves and colleagues, pathogen-induced influx of ROI into the vacuole was associated with a surge of potassium ions which compensated the buildup of anions [98]. This increase in ionic strength allowed for the release and activation of the serine proteases elastase and cathepsin G. However, intracellular pathogens hiding inside infected cells often escape this mechanism of the innate immune response. These pathogens are killed when the infected cell is ultimately forced into apoptosis by recognition mechanisms of NK cells and cytotoxic T lymphocytes [65]. This killing is mediated by Fas-FasL interactions, perforin (pore-forming protein) and granzymes (activators of caspases) [64].

Linking Innate and Adaptive immunity

Any conversation between innate and cellular immunity requires accurate and effective translation of the innate signals of infection or damage. Central to this translation is the dendritic cell. The dendritic cell is a heterogeneous population of cells found in an immature state at sites of interaction with the environment such as the skin and mucosa. Here, they sample the environment by macropinocytosis. Both self and non-self proteins are degraded into peptides, loaded into newly synthesized class II MHC molecules in a post-Golgi compartment and trafficked to the cell membrane, a process known as antigen presentation. Upon pathogen challenge, the dendritic cells interpret innate system activation as a maturation signal. This results in the migration of the dendritic cell to a draining lymph node site. Here the stability of the class II-peptide complex is increased to reflect the peptide experience of the dendritic cell at the site of inflammation. Costimulatory molecule expression is also induced by maturation, making dendritic cells the best antigen-presenting cells (APCs) capable of activating a naive CD4+ T cell. Critical to the nature of the immune response is the ability of dendritic cells to influence the outcome of immune activation. Thus different dendritic cell populations and different maturation signals produce varied profiles of cytokines and costimulatory receptors. The outcome of T cell activation is the activation and differentiation of T cells into (T_H1 and T_H2 phenotypes. Simplistically, T_H1 cells produce IFN-γ and support cell-mediated immunity and inflammation. T_H2 cells produce IL-4 and provide help to B cells for isotype switching during a humoral response (see fig. 3).

This outline of acquired immune activation presents a large number of sites of regulation. From antigen selection to peptide processing, the exact specificity of responding antigen is dependent on many factors ranging from the ability of MHC to bind a particular peptide to the specificity of antigen receptor randomly generated by the process of variable gene fragment recombination. The panel of processing proteases available and epitope masking effects of immune complexes also influence the peptide produced by the APC.

Coreceptors on T cells

If the antigen receptor were to the lymphocyte what the growth factor receptor is to a fibroblast, then simple ligation of the antigen receptor would engender all the necessary signals for proliferation and differentiation. By separating the necessary signal transduction pathways over different receptor systems, lymphocytes provide

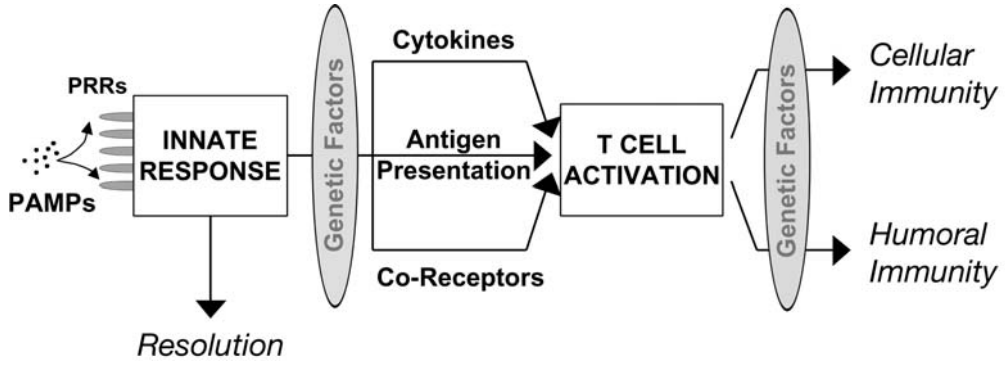

Figure 3. Schematic representation of the levels of regulation affecting acquired immune responses. Like a telescope, a compound lens of innate responses and genetic factors focus the appropriate acquired immune responses on specific targets of infectious agents.

themselves with multiple levels of checks and balances to ensure the selected antigen is an appropriate target.

During T cell activation, formation of an immunological synapse depends on the coordinated interaction of the TCR with peptide MHC class II complex, CD4 interaction with MHC class II molecules and LFA-1/ICAM-1 interaction. A calcium response can be detected with as few as one TCR/MHC interaction in the synapse. Inclusion of 20–30 specific complexes in the synapse leads to a sustained and strong calcium signal [99]. The stability of this signal is dependent on CD4, as blocking antibodies decrease the sensitivity of stimulation by 10–100-fold.

Effective activation of CD4+ T cells requires a second signal from the APC to ligate CD28. CD28 is a necessary coreceptor that, in conjunction with the TCR signal, stimulates transcription at the IL-2 locus [100]. Ligands for CD28 include CD80 and CD86, receptors that are expressed on APCs following innate activation. CD80 and CD86 expression is restricted to lymphoid tissues and therefore probably regulates activation of naive T cells. In the absence of these CD28 ligands, stimulation of T cell via the TCR is tolerogenic. The B7 homologous receptor (B7h) was described as a TNF-α-responsive receptor expressed by B cells and LPS-stimulated fibroblasts [101]. B7h binds to the CD28 homologue ICOS (inducible costimulator) and costimulates the T cell for proliferation and production of cytokines such as IL-4 and IFN-γ [102]. It does not, however, enhance IL-2 production, leading to the suggestion that B7h-ICOS interaction regulates reactivation of T cells. Animals with a targeted deletion of ICOS show reduced T_H2-mediated lung inflammation [103]. Critically, however, blockade of ICOS interaction during the later effector phase of inflammation blocked T-cell-dependent pathology. In contrast, it was necessary to block CD28 interaction during the priming stage to ameliorate inflammation. These data support a role in secondary stimulation for B7h in contrast to a role for CD28 in priming [104].

An additional member of this costimulator family was described with the cloning of programmed death-1 (PD-1). PD-1 contains an inhibitory signaling motif and is inducibly expressed on T cells. PD-1 binds to PD-L1, which is expressed by monocytes, dendritic cells and peripheral tissues such as the lung [105]. A second ligand, PD-L2, is expressed chiefly by dendritic cells. Ligation of PD-1 inhibits T cell proliferation and cytokine expression. Consistent with this phenotype, animals with a targeted deletion of PD-1 present with an autoimmune disorder, suggesting that negative regulation of T cells by PD-1 is important in maintaining peripheral tolerance [106, 107].

This description of negative regulators is an important idea in cellular immunity. The reemergence of the concept of suppressor or regulatory T cells is provoking a shift in the understanding of the relationship between inflammation and autoimmune pathologies. A number of different phenotypes have been described, although the relationships between them are not yet clarified. T regulatory (Tr) cells produce high levels of IL-10 and little or no IL-1 or IFN-γ [108]. They are suppressive of inflammatory T_H1 cell subsets in a cell contact-independent manner. In the human system, Tr1 cells can be derived by activation with reagents specific to CD3 and the complement regulator CD46 [109]. Conversely, CD25+CD4+ Tr cells are regulatory in a manner that seems to require cell-to-cell contact [110]. However, experiments with IL-10 and TGF-β-receptor-deficient mice suggest CD25+ cells may use multiple mechanisms to suppress T cell activation.

Coreceptors on B cells

The biologic concerns for inappropriate activation of the B cell differ from those of the T cell. This is reflected in a panel of coreceptors that focus, to a greater extent, on maintaining B cell viability rather than modifying the activation of the antigen receptor complex, as seen for the T cell. Many surface proteins have been shown to regulate B cell activation, including but not limited to CD19, CD22, CD40, MHC class II, integrins and Fc receptors.

CD22 is a glycoprotein expressed on B cells that binds to sialoglycoconjugates expressed on T cells. Upon stimulation of B cells, CD22 becomes tyrosine phosphorylated and associates with the tyrosine phosphatase SHP-1. This association inhibits signaling through the phosphotyrosine-dependent antigen receptor complex signaling [111]. The suppressive effect of CD22 SHP-1 association can be mitigated by sequestering CD22 away from the antigen receptor complex, as when a B cell is surrounded by T cells expressing a CD22 ligand. This negative regulatory role is supported by hyposensitivity to antigen receptor signaling [112] and development of autoimmunity in animals lacking functional CD22 [113]. CD22 may thus serve as a molecular switch distinguishing sites of appropriate activation for the B cell [114].

CD19 coligation decreases, by two orders of magnitude, the number of antigen receptors needed for activation of a proliferative stimulus through the antigen receptor [115]. Phosphotyrosine-dependent association with phosphoinositol 3-kinase (PI3-K) and Vav achieves this costimulation, although primary association with PI3-K reconstitutes most of the biological functions of CD19 [116]. Animals with targeted deletions of CD19 lack the ability to generate T-dependent B cell responses. They are unable to undergo affinity maturation and germinal center formation [117, 118]. This similarity to CD40 deficiency suggests a possible overlap between CD19 and CD40 function or reduced CD40 signaling ability in CD19-deficient mice [119]. CD19 exists in the membrane in association with other membrane receptors, including CD21. The more severe phenotype of CD19 deficiency as com-

pared with CD21 deficiency suggests there are ligands of CD19 other than the complement ligands of CD21. In addition to being a rheostat for innate activation by virtue of CD21 association, IgM and basement membrane-expressed ligands for CD19 are expressed in germinal centers and are reminiscent of the CD4 receptor of T cells detecting the contextually appropriate presentation event [120].

CD40 is a member of the tumor necrosis receptor family of proteins. CD40 function was initially described in terms of B cell biology (reviewed in [121]). This was due to the hyper-IgM presentation of patients bearing a mutation in the gene for the CD40 ligand, CD154. A similar phenotype was observed in mice with targeted deletions of the genes for either CD40 or CD154. Ligation of B-cell-expressed CD40 by activated T-cell-expressed ligand CD154 is thus responsible for B cell proliferation, differentiation into antibody-producing cells, germinal center formation and isotype switching. Consistent with this role as a general activation receptor, CD40 ligation also induces expression of cytokines such as IL-6 and TNF-α. It can upregulate adhesion molecules such as ICAM-1 and the costimulatory receptors CD80 and CD86. In addition, increased levels of expression of MHC class I and class II as well as upregulation of the TAP transporter ensures provision of T cell ligands [122]. CD40 thus focuses much of its B cell function on interaction with the T cell. This variety of effects can be explained by the ability of CD40 to rescue the cell from apoptosis as well as induce germline transcription of switch isotypes. CD40 acts as a facilitator or coordinator. The specificity of switching, however, is determined in large part by the cytokine milieu.

Coreceptors on APCs

The major advance in CD40 biology, however, has been the description of CD40 function in APCs such as monocytes and DCs. Here CD40 function is reminiscent of that in B cells. So CD40 ligation prevents apoptosis and upregulates cytokines such as IL-6, IL-12 and MIP-1α, as well as adhesion and costimulatory receptors. In addition, CD40 ligation results in activation to a mature APC. Therefore, knockouts of CD40 and CD154 also show significantly decreased CD4 activation. For instance, CD40 stimulation of DC enhances expression of the TNF-R family member APRIL. In combination with IL-10 or TGF-β, APRIL induces class switch recombination to IgG or IgA isotypes. This remains, however, just one of sundry ways in which DC can be activated.

Alternatively, CD80 and CD86 upregulation following TLR stimulation on DCs, combined with the production of IL-12, a cytokine with an important role directing the T_H1 response, suggests a mechanism by which TLR could regulate adaptive responses. Compelling evidence was recently presented using mice deficient in the adaptor protein MyD88 [123]. It was demonstrated that in absence of MyD88, mice had a major deficit in mounting T_H1 responses; however, T_H2 responses were unaffected. These results suggest that TLRs have a decisive role in adaptive effector responses. However, the data must be interpreted with caution, as MyD88 also serves as the nonredundant adaptor required for IL-1β and IL-18 signaling.

Whether the effective response is cellular or humoral, the nature and scale of the immune response is in large part determined by the type of DC activated. Although all DCs are hematopoietic in origin, they have myeloid and plasmacytoid or lymphoid and myeloid origins in humans and mouse, respectively. Their regulatory role became apparent due to the large amount of T_H1-biasing cytokine IL-12 that they could produce. Without classifying either lineage as strictly T_H1 or T_H2 biasing, it has become clear that DCs have an elastic ability to respond to inflammatory differences in their environment. This functional flexibility has been observed in many different ways. Microbial PAMPs such as dsRNA and Helminth protein extract could induce the same population of DCs to mature into a T_H2- or T_H1-supporting phenotype respectively [124]. This may in part be due to the different panels of TLRs expressed by DCs. So immature DCs preferentially express TLR1, 2 and 3. In contrast, plasmacytoid pre-DCs express high levels of TLR7 and TLR9. Thus, immature DCs express inflammatory cytokines such as TNF-α, IL-6 and IL-12 in response to lipoproteins from Gram-positive bacteria [125]. In contrast, plasmacytoid DCs produce type I interferons in response to bacterial CpG motifs [126]. These differences presumably reflect different microenvironmental challenge profiles [127]. Additionally, the TLRs are downregulated as the DCs develop from pre-DCs to the immature and then mature phenotype. This accompanies a switch from antigen responder to antigen presenter function. Additional factors that alter DC effector types include the stimulator/responder ratio and antigen dose, although these have been shown only in vitro. Murine DC subsets can support the development of either T_H1 or T_H2 effector cells with high doses of antigen in general selecting the T_H1 phenotype and low doses, T_H2 [128]. Similarly, at low DC to T cell ratios, T_H2 differentiation is supported, consistent with low-dose stimulation. Increasing DC:T cell ratios increases the degree of T_H1 differentiation [129].

DCs also perform negative regulatory functions. In a dramatic example of how two negatives make a positive, TLR-mediated production of IL-6 by dendritic cells and macrophages has been shown to be primarily responsible for inhibition of regulatory CD25+ cells. This blockade of the suppressive effect of Tr cells released the activation of antigen-specific T_H cells [130].

Nature vs. Nurture:
Factors affecting immune activation

The activation of DCs bears important implications for regulation of the immune response. It has been appreciated for a long time that certain means of immunization were more effective than others. So complement-activating IgG2a immune complexes are up to 1000-fold more immunogenic than antigen alone in part because of the ability of complement to stimulate the humoral immune response [131]. It is also due to the ability of IgG2a to target APCs expressing Fc receptors [132]. Mannosylated antigens are targeted to class II compartments and presented to CD4+ T cells by DCs with 100- to 1000-fold greater efficiency than pinocytosed antigens. Also, the inclusion of LPS in an immunization can convert a tolerogenic signal to an immunogenic one [133]. Similarly, immunization with plasmids encoding viral antigens can protect against viral challenge [134]. This occurs in part because IL-12 secretion in response to bacterial CpG motifs induces IFN-γ production by NK cells [135]. These cytokines select T_H1 differentiation even on a background predisposed to T_H2 activation. Targeting TLR5 activation by inclusion of bacterial flagellin in an immunization has a significant adjuvant effect [136]. Indeed, in vivo activation of APC with antibody to CD40 or TLR9 ligand is sufficient to release tolerance of myelin-reactive CD4+ T cells [137]. These observations suggest mechanisms to explain the link between infectious illness and autoimmune events. Microbial pathogens could produce molecules that bind to TLRs, or activate T cells to express CD154, thus instructing APC-bearing peptides of both microbial and self-origin to release tolerance.

To some degree, the panel of stimulatory receptors activated during a microbial challenge controls the nature of an immune response. However, genetic determinants also have a significant effect. One of the best-studied examples is infection of mice with *Leishmania major*. Resistant mouse strains, such as C57Bl/6, when infected with *L. major*, develop a T_H1-mediated inflammation which includes production of IFN-γ [138]. The IFN-γ activates macrophages harboring the microbe and induces them to produce NO, thus limiting infection to a cutaneous leishmaniasis. In Balb/C mice however, *L. major* induces a T_H2 response characterized by the production of IL-4. IL-4 inhibits expression of the IL-12 receptor, preventing T_H1 differentiation. Thus, little or no IFN-γ is produced, and the infection continues. Treating with cytokines or blocking antibodies at the point of infection can alter this balance between T_H1 and T_H2 responses in C57Bl/6 and Balb/C animals. So treating Balb/C animals with IL-12 or anti-IL-4 allows development of a T_H1 response and clearance of the infection. In mixing experiments using resistant C3H and susceptible Balb/K cells, C3H T cells did not produce IL-4 unless stimulated by Balb/K APCs. Conversely, Balb/K T cells produced IL-4 whether stimulated by C3H or Balb/K APC [139]. Clearly, there are genetic factors that can affect both the APC and T cell populations. This is most obvious in the case of animals with a targeted deletion of the gene for TNF-α. TNF-α is critical for successful resolution of an *L. major* infection [140].

The effects of other cytokine mediators on leishmaniasis, especially those produced by APCs, such as IL-6, is not clear. IL-6 is simplistically thought to favor T_H1 responses. It can promote the expression of IL-4 and inhibit IFN-γ production [141]. Despite this, IL-6 deficiency does not alter the outcome of infection on a Balb/C background [142].

Immune Responses: A balancing act

Lessons from Chronic Inflammation

The immunologists dirty little secret has finally been, in large part, demystified. For many years the use of complete Freund's adjuvant (CFA) as a carrier for immunization disguised the fact that the adjuvant was spiked with preparations of mycobacteria whose mechanism was unknown but whose effect was clearly immunostimulatory. Although incomplete Freund's adjuvant (IFA) mimics the depot effect of CFA, only the latter is sufficient to release tolerance to self-antigens. It has thus been a tool central to numerous mouse models of autoimmunity for many years. So animals immunized with spinal cord homogenate in CFA stimulate chronic encephalomyelitis [143]. T cells reactive with murine cytochrome C are activated by immunization with the homologous human cytochrome C in CFA [144]. Arthritis-like symptoms are recapitulated by immunization of joints with type II collagen [145]. These in vitro models of autoimmunity make more sense in light of the progress made on the role of PAMPs in stimulating immune responses. The combined effects of receptor-specific recognition of the presence of mycobacterial proteins, APC activation and upregulation of antigen presentation and T cell costimulation markers, and cytokine-mediated maturation of activated T cells are all processes directly regulated by PAMP recognition.

The link between these somewhat artificial models and real pathologies associated with infectious events is now a much smaller leap. The other point worth reiterating about these effects of receptor-specific recognition is the impact of genetics on the scale and nature of immune responses. Although these examples all rely on examination of systems where the balance between the presence and absence of a response is artificially altered, they serve to emphasize the very real genetic effects on this balance in human responses to infectious agents. From the initial characterization of immune response genes as major histocompatability antigens to the preference of systemic lupus erythematosus for the female population or the asso-

ciation of mutations in the gene for Nod2 with Crohn's disease, genetics has a profound effect on immune responses. Many different animal models provide evidence for this such as murine models for inflammatory bowel disease (IBD). Animals with a targeted deletion of the gene for IL-10 develop colitis similar to human IBD condition [146]. When these animals are raised in specific pathogen-free (spf) conditions, colitis is absent. Infection of SPF animals with a single bacterial agent, *Helicobacter hepaticus* or *Enterococcus faecalis*, is sufficient to induce colitis in these animals [147, 148]. The IL-10 knockout background emphasizes that genetic background determinants can play a significant role. In the absence of IL-10, IFN-γ and IL-12 production is unchecked, so T_H1 regulation is lacking. While these conventional intestinal flora provide the PAMPs necessary for inflammation, whether self antigens become targets of inflammation is not yet clear. In a clever elaboration of the murine colitis model, *Escherichia coli* inducibly expressing ovalbumin could induce subacute colitis in Balb/C animals. Chicken-egg albumin or ovalbumin OVA-specific T cell transfer either inhibited or induced colitis, depending on whether an IFN-γ or IL-10 secreting line was transferred, emphasizing the importance of T_H cell distinction [149]. Consistent with these data, colitis in IL-10 knockout animals can be prevented by treatment with *Lactococcus lactis* engineered to secrete murine IL-10 [150]. Targeted IL-10 expression may thus be a useful tool in other reactive models of inflammation such as arthritis.

Emphasizing the distinction between infection and inflammation, the recently developed murine model of arthritis in K/BxN mice relies on production of pathogenic immunoglobulins specific for glucose-6-phosphate isomerase (GPI). GPI is accumulated on joint surfaces which, lacking complement regulators such as CD59 and CD35, cannot regulate alternative pathway activation. This complex activation results in Fc receptor ligation and C5a production [151]. The combined effects of mast cell degranulation and neutrophil localization due to C5aR stimulation explains the observed pathology and supports the postulate that an uncontrolled innate response can result in activation of the host acquired response [152].

Lessons from Viruses

The utility of these immunoregulatory mechanisms is also supported by the variety of mechanisms evolved to specifically inhibit or disrupt their function. These mechanisms are possibly clearer in the context of viral infections, as the obligate intracellular nature of this parasite predicts targeted apoptosis as a singularly effective means of containing a viral infection. Obligate parasitism also suggests that viruses are under selective pressure to assume immunoevasive mechanisms. Indeed, apoptosis of virally infected cells either by activation of death receptors such as Fas, or by targeted killing by CD8 cells, is inhibited in multiple ingenious ways by virus. Fas ligation on an infected cell leads to apoptosis by activation of a caspase cascade and mitochondrial depolarization. In opposition to these responses, herpesviruses express vFLIP, a dominant-negative caspase inhibitor [153]. CrmA expressed by poxvirus [154], and the inhibitors of apoptosis (IAP) family of proteins, originally described in insect viruses, all inhibit efficient caspase activation [155]. In addition, many of these viruses activate anti-apoptotic pathways, such as NF-κB activation by the LMP-1 protein of Epstein-Barr virus EBV [156]. NF-κB activation stimulates a panel of endogenous survival factors that protect the cell from apoptosis.

Viruses have also selected a large variety of ways of thwarting CD8 cell activation and recognition. Most of these mechanisms involve regulation of antigen presentation. By masking the presence of viral proteins, the acquired immune system remains blind to their presence. Of the viruses that contain mechanisms for interfering with antigen presentation, the herpesvirus provide the greatest catalogue of mechanisms. By way of example, the EBNA-1 protein of EBV contains a sequence that prevents proteosomal degradation of proteins, thus blocking peptide presentation of viral antigens in MHC complexes [157]. The US6 protein of human CMV virus binds to the TAP peptide transporter complex and prevents peptide translocation into the MHC class I loading endosome [158]. The US2 and US11 proteins of CMV bind MHC class I molecules in the endoplasmic reticulum and cause their degradation, thereby preventing presentation of any peptides that have escaped previous inhibitory mechanisms [159].

Evolutionarily wise to the many alternatives available to the innate immune systems, viruses have also picked up on endogenous regulatory mechanisms for their own survival. For instance, vaccinia virus encodes a receptor with sequence and functional homology to complement regulator proteins [160]. Virally produced soluble cytokine receptors have been described that compete with endogenous receptors, thereby inhibiting their activation. Soluble receptors with specificity for IL-1, TNF-α and type I interferons are known.

Future Studies

Our current understanding of innate regulation is based in large part on the very specific analysis that has been performed with purified PAMPs, such as LPS and CpG, on purified populations of cells using defined antigens. During any infectious event, however, a wide variety of pathogen-associated molecules are recognized, each with its unique pattern of inflammation and gene regulation. This makes the response to any infectious agent a fingerprint of overlapping innate activation pathways. Each as-

pect of innate activation is interpreted by the acquired immune reponse cumulatively to generate a different profile of acquired immune response. One of the major challenges facing immunologists is understanding how multiple innate responses make a rheostat for acquired activation. This toolbox of responses will provide us with the next generation of vaccine adjuvants. Furthermore, the immune activation variations between different individuals can often be ascribed to allotypic or mutant alleles in innate or acquired response genes. Defining the interplay between innate and acquired immunity at this genetic level will eventually provide avenues of therapy for inappropriate activation in autoimmune conditions.

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