Leading Edge
Review

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Innate Immunity Gone Awry: Linking Microbial Infections to Chronic Inflammation and Cancer

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Clinical and epidemiologic studies have suggested an association between infectious agents and chronic inflammatory disorders and cancer. Better understanding of microbial pattern-recognition receptors and innate immune signaling pathways of the host is helping to elucidate the connection between microbial infection and chronic disease. We propose that a key aspect of pathogenesis is an aberrant epithelial barrier that can be instigated by microbial toxins, environmental insults, or the genetic predisposition of the host. Loss of epithelial integrity results in activation of resident inflammatory cells by microbial invaders or endogenous ligands. When coupled with a failure of normal control mechanisms that limit leukocyte activation, a cascade is established that induces chronic inflammation and its consequences. Here, we outline this mechanistic framework and briefly review how alteration of innate immune response genes in murine models can provide insights into the potential microbial origins of diverse conditions including Crohn's disease, psoriasis, atherosclerosis, diabetes, and liver cancer.

Introduction

Humans live in direct and continuous interaction with a startlingly complex microbial environment. The total number of microbes ($\sim 10^{14}$) normally inhabiting our mucosal surfaces exceeds by an order of magnitude the quantity of cells ($\sim 10^{13}$) in our bodies. Scattered amidst these commensal microflora are potential pathogens—viruses, bacteria, or parasites, intrinsically capable of producing symptomatic infectious disease. Through evolution, the mammalian innate immune system has thus been presented with a formidable task. First and foremost, the host has to mount a rapid and definitive microbicidal response to invading pathogens whose further replication threatens significant tissue injury or organ dysfunction, yet simultaneously tolerate legions of benign normal flora along with nonthreatening food, water, and airborne microbes and foreign antigens. Furthermore, the most effective defense response needs to be sufficiently lethal to rapidly kill invading pathogens while at the same time avoid collateral damage to the host.

Patterns of local immune activation due to temporary loss of barrier function, pathogen invasion, or release of endogenous mediators, followed by tissue destruction and functional impairment characterize many chronic human disease conditions (see below). The medical consequences of chronic inflammation are further compounded by potentially permanent metabolic alterations and malignant transformation, as exemplified by strong associations between chronic gastritis, hepatitis, or colitis and increased risk of primary carcinoma in the corresponding organ (Kuper et al., 2000) or the link between chronic gingivitis and insulin resistance (Pucher and Stewart, 2004). The cellular and molecular profiles of immune activation in chronic inflammatory disorders overlap considerably with those patterns observed in effective, self-limited host responses to microbial pathogens and involve similar initiating mechanisms. This realization has provided steady inspiration for investigations that seek to establish causal linkages between infectious agents and chronic inflammatory disorders or cancer. The mechanisms providing such linkages, which are just being unraveled, are the subject of the present review.

Only rarely have specific microbial pathogens been identified as critical and universal causes of a particular chronic inflammatory condition or malignancy. Rheumatic heart disease (RHD), which remains a major public health problem in developing countries, is a delayed complication of untreated *Streptococcus pyogenes* throat infections. RHD is the pathological consequence of molecular mimicry—peptide sequences in the M protein of *S. pyogenes* elicit crossreactive immune responses targeting self antigens present in myosin and tropomyosin of cardiac

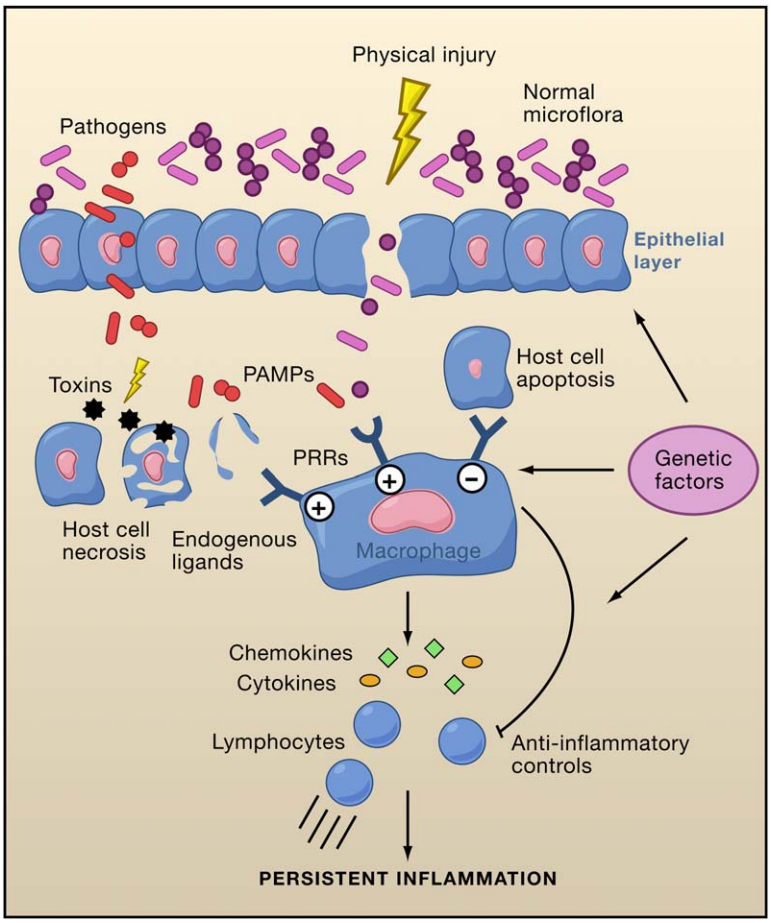

Figure 1. A Generalized Scheme to Explain the Origin of Chronic Inflammation

We propose that a key early event in chronic inflammation is the loss of epithelial barrier integrity leading to increased exposure of resident inflammatory cells (macrophages, mast cells, dendritic cells) to both pathogenic and nonpathogenic microbes. Exogenous pathogen-associated molecular patterns (PAMPs) and endogenous ligands are recognized by the macrophages through pattern recognition receptors (PRRs). Engagement of the PRRs triggers signaling pathways that lead to the release of chemokines and cytokines, the recruitment and activation of lymphocytes, and the propagation of chronic inflammation. Genetic factors can influence the system at multiple checkpoints.

valvular myocytes. Invasive cervical cancer, the second most common malignancy in women worldwide, is caused by oncogenic human papillomaviruses (HPV). HPV-mediated malignant transformation of cervical epithelial cells involves interference with cell cycle regulation by the viral oncogenes E6 and E7, inducing genetic instability and continuous selection for alterations that advance the malignant phenotype. However, as discussed below, other viral infections, for instance the hepatitis B and C viruses (HBV, HCV) can lead to cancer even though the viruses do not carry oncogenes.

A direct mechanistic link between pathogen-specific gene products and a stereotypical altered host response key to disease development is often missing. Nonetheless, clear epidemiologic associations often play out against this enigmatic backdrop. Infections with the bacterium *Helicobacter pylori*, subject of the 2005 Nobel Prize in Physiology or Medicine, can produce chronic gastritis, peptic ulcers, and appear to be a provocative factor in 60%–90% of gastric carcinomas, yet most individuals whose stomachs play host to *H. pylori* develop no clinical symptoms. Infections with the genetically unrelated HBV and HCV can result in chronic hepatitis, liver cirrhosis, and collectively are believed to trigger at least 50%–70% of cases of hepatocellular carcinoma (HCC); though humans may be lifelong asymptomatic carriers of either virus. Epstein-Barr virus (EBV) is linked to endemic Burkitt's lymphoma in Central Africa and nasopharyngeal carcinoma in Southeast Asia, but lifelong asymptomatic infection or simple mononucleosis characterize the vast majority of virus-host interactions. The flatworm *Schistosoma haematobium* infects the urinary tract and exists in endemic foci within Africa, yet a subset of patients develop a chronic granulomatous inflammatory response to parasite eggs and consequently are prone to development of bladder carcinoma.

The variable clinical manifestations of chronic infections, from inconsequential to life-threatening, make the critical contribution of host factors self evident, and in most of the diseases mentioned above it is the host response rather than toxins or oncogenes produced by the pathogen which causes the pathology. Advances in our understanding of host molecules involved in microbial recognition and innate immune signaling are beginning to provide pieces for a more general model of infection-associated chronic inflammatory disease (Figure 1). At the center of this model is the primary barrier function of skin, broncho-epithelial and mucosal epithelial surfaces,

with compromised barrier integrity representing the first step in the pathway to macrophage activation and establishment of chronic inflammation and its sequelae in the underlying tissues. The functional integrity of such epithelial barriers can be breached directly, indirectly, or mistakenly en route to inflammatory activation. Many bone fide human pathogens elaborate toxins capable of lysing host epithelial cells or promoting their demise through metabolic interference or triggering of apoptosis. Repeated exposure to noninfectious agents in our environment can also compromise barrier function, as exemplified by exposure to cigarette smoke and airborne particulates which predispose to chronic bronchitis and lung cancers. Impaired barrier function leading to microbial overgrowth on epithelial surfaces can be seen in genetic conditions (e.g., cystic fibrosis), as a complication of medical or surgical interventions (e.g., gastric hypochlorhydria or decreased intestinal peristalsis), or in the normal aging process (e.g., loss of mucin production). And perhaps the most intriguing concept lies in aberrant perception of microbial threats by host epithelium in the course of its interaction with benign normal flora. A revolution in our understanding of mammalian innate immunity has accompanied the elucidation of extracellular and intracellular pattern-recognition receptors (PRRs) or sensors (e.g., Toll-like receptors, or TLRs, and NOD proteins) recognizing small molecular sequences often referred to as "pathogen-associated molecular patterns (PAMPs)" and the signaling pathways through which they initiate inflammatory activation. In this review, we will highlight several recent advances in understanding mechanisms that link infection to chronic inflammatory disease and malignancy.

Inflammation and Its Regulation

Activation of the Inflammatory Response

One of the manifestations of the host response to infection or injury is acute inflammation characterized by a vascular response and recruitment of circulating leukocytes, classically defined by initial recruitment of polymorphonuclear granulocytes followed by monocytes, which differentiate locally into macrophages. Often, this response is triggered by mast cell degranulation releasing a battery of inflammatory mediators, including bioactive amines (histamine, 5-HT), and activation of resident macrophages through PRRs and intracellular sensors leading to release of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α. This exerts a "domino effect" leading to sequential release of lipid mediators, cytokines, and chemokines that drive recruitment and activation of additional inflammatory cells. This system has enormous capacity for synergy and redundancy, yet several key mediators have been identified, and blocking these mediators can in turn inhibit or attenuate the inflammatory response. A very critical mediator appears to be TNF-α, now established as an important therapeutic target in a range of chronic inflammatory diseases (Feldmann and Maini, 2003) that also provides an important link between inflammation and cancer (Karin and Greten, 2005).

Receptors in Acute Inflammation

Depending on the nature of the inflammatory stimulus, the cells and the receptors that trigger the inflammatory response following infection or injury do vary. PRRs of the TLR family play a critical role in detection of microbes and viruses by macrophages and dendritic cells (reviewed by Akira et al., 2006 [this issue of *Cell*]), but recent evidence suggests that members of the scavenger receptor and C-type lectin families are also important for microbial recognition and regulation of the host inflammatory response (Gordon, 2002; Figure 2). The C-type lectin Dectin-1 collaborates with TLR2 to activate macrophages exposed to β-glucans from yeast (Gantner et al., 2003). Importantly, a number of these receptors recognize endogenous ligands, for example the scavenger receptors SR-A and CD36 mediate phagocytosis of apoptotic cells (AC), which is associated with downregulation of macrophage activation (Savill et al., 2002). These receptors are also thought to mediate uptake of oxidized lipids, thus providing a trigger for development of atherosclerosis (Glass and Witztum, 2001). SR-A-deficient mice exhibit increased susceptibility to infection (Suzuki et al., 1997); however, SR-A was recently shown to negatively regulate "sterile" zymosan (a yeast cell wall extract)-induced inflammation in mice (Cotena et al., 2004), suggesting that the role of these receptors in inflammation may be context dependent. The mannose receptor (MR), another C-type lectin, has a number of endogenous ligands and is upregulated on so-called "alternatively" activated macrophages; MR is also thought to have a role in pathogen recognition and regulation of innate immunity (Chieppa et al., 2003).

Endogenous (host-derived) ligands for TLRs have also been identified (Figure 3). Heat-shock protein 70 (HSP70), described as a TLR4 agonist (Vabulas et al., 2002), and chromatin component HMG-B1, which appears to activate macrophages through the receptor for advanced glycation products (RAGE; Scaffidi et al., 2002), are released in the context of tissue injury and necrosis. This observation suggests that even in the absence of infection, TLRs and other PRRs may play a role in inflammation and immune homeostasis through recognition of endogenous ligands that are presented as a result of tissue damage. Consistent with this notion, TLR4-deficient mice are resistant to development of diet-induced atherosclerosis, a chronic inflammatory disease of the vasculature (Michelsen et al., 2004). Furthermore, polymorphisms in the human *TLR4* locus have been associated with atherogenesis (Kiechl et al., 2002), and polymorphisms in a *TLR* gene cluster encoding TRL1, TLR6, and TLR10 were linked to increased prostate cancer risk (Sun et al., 2005). In the context of chronic infection, tissue injury, and inflammation, these different families of receptors are likely to be coordinately engaged by multiple exogenous and endogenous ligands (Figures 2 and 3) giving rise to a self-propagating response. Recently, it was shown that mammalian DNA and RNA, in the form of immune complexes, act as self-antigens for TLR9 and TLR7, respectively, inducing

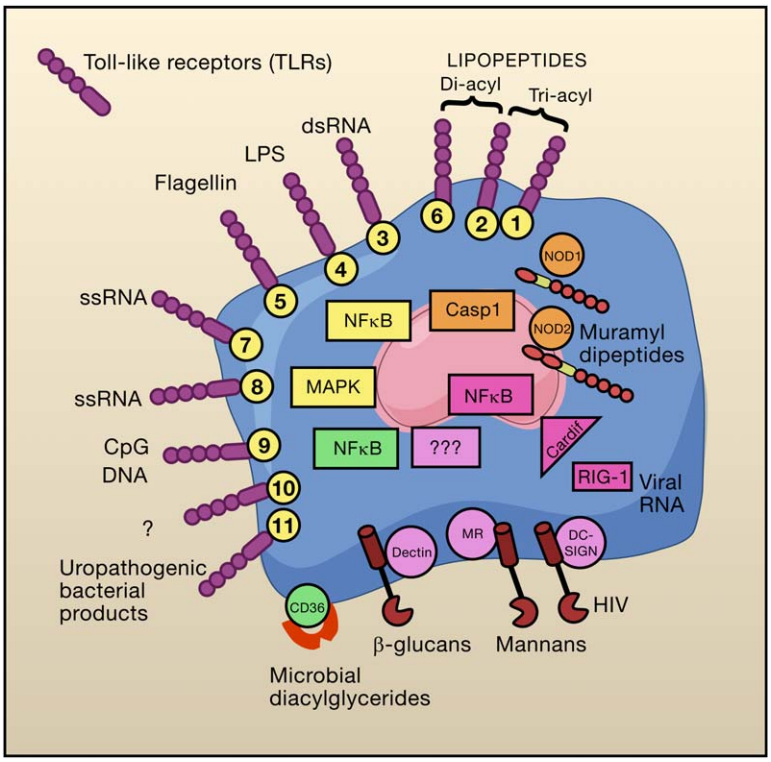

Figure 2. Surface-Exposed and Intracellular PRRs and Their Corresponding Exogenous Ligands Involved in Activation of the Macrophage Inflammatory Response

Color matching is used to indicate the major signal-transduction pathways activated by functional engagement of each PRR. The adaptor protein Cardif has also been called MAVS, IPS-1, or VISA.

IFN-α production by predendritic cells and promoting autoimmune inflammation (Barrat et al., 2005).

Resolution of Inflammation

Despite its self-amplifying nature, under normal circumstances, the acute inflammatory response is self limiting, and after a few days, inflammation is resolved. While the mediators and signaling mechanisms that initiate and promote the inflammatory response have become increasingly well understood (see below), relatively little is known about those factors that mediate resolution and thereby prevent chronic inflammatory diseases from occurring. A number of endogenous anti-inflammatory mediators have been identified, including the cytokines interleukin (IL)-10 and transforming growth factor (TGF)-β, and lipid mediators, such as lipoxins and cyclopentenone prostaglandins. Gene disruption studies and pharmacological inhibition have outlined a role for these mediators in negative regulation of inflammation and inflammatory disease (Gilroy et al., 2004). More recently, a number of anti-inflammatory receptors on macrophages were described (Figure 3). Of particular interest is CD200 (Wright et al., 2000), whose engagement by the endogenous ligand CD200L sends a "stop signal" to macrophages suppressing production of proinflammatory mediators (Hoek et al., 2000). CD200L, expressed on activated immune cells, thus provides a mechanism to dampen macrophage activation after initiation of the inflammatory response. Another receptor that plays an important role in the negative regulation of inflammation is the recepteur d'origine nantis (RON), or stem cell-derived tyrosine kinase (STK) receptor in the mouse (Wang et al., 1995). The ligand for this receptor is macrophage-stimulating protein (MSP), a serum protein generated during the coagulation cascade that may be triggered by inflammation or tissue injury, but unlike other mediators, MSP inhibits macrophage activation (Morrison et al., 2004). The recognition of AC by macrophages and dendritic cells (DC) generates anti-inflammatory signals that inhibit macrophage activation and DC maturation (Stuart et al., 2002). Different receptors have been implicated in AC recognition including CD36, $\alpha_v\beta_5$ integrin, C1q and the phosphatidylserine (PS) receptor (Fadok et al., 2001; Savill et al., 2002), but which of them generates anti-inflammatory signals that inhibit macrophage and DC activation is not clear. One such receptor could be the Mer tyrosine kinase (TK), whose absence results in defective AC clearance, development of lupus-like autoimmune disease and increased TNF-α production (Cohen et al., 2002). Related to Mer TK are Axl and Tyro3, and a compound deficiency of all three receptors results in spontaneous hyperactivation of macrophages and DC and systemic autoimmunity (Lu and Lemke, 2001). One possible ligand for these receptors is encoded by growth arrest-specific (GAS) gene 6, which binds PS presented by AC (Chen et al., 1997).

Even TLRs have the capacity of inducing expression of anti-inflammatory mediators, such as IL-10. Recent studies reveal that the signaling pathway used by TLRs to activate expression of pro- and anti-inflammatory cytokines (see below) diverges at the level of the TRAF3 and TRAF6 proteins, such that TRAF3 is critical for induction of IL-10

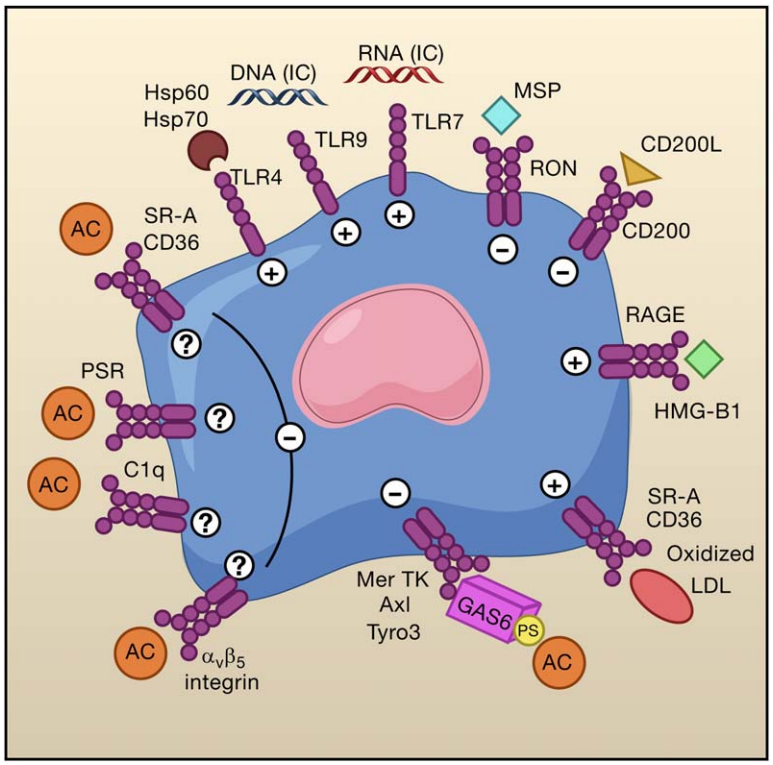

Figure 3. Endogenously Derived Ligands and Their Interaction with Macrophage Receptors in Modulation of the Inflammatory Response

Receptor-ligand interactions promoting macrophage activation are indicated by (+) while those involved in resolution of inflammation are noted by (−). AC = apoptotic cell; IC = immune complex with nucleic acid; SR = scavenger receptor; TLR = Toll-like receptor; Hsp = heat-shock protein; RON = recepteur d'origine nantis; CD200L = CD200 ligand; HMG-B1 = high mobility group box 1 protein (chromatin component); RAGE = receptor for advanced glycation products; PS = phosphotidylserine; PSR = PS receptor; GAS6 = growth arrest specific gene 6.

expression, and in its absence, expression of the TRAF6-dependent proinflammatory cytokines IL-6 and IL-12 is dramatically upregulated (Häcker et al., 2005). The balance between the TRAF3- and TRAF6-generated signals may therefore play an important role in controlling the inflammatory response and its perturbation may interfere with the proper resolution of inflammation.

Signaling Pathways that Control and Modulate Inflammation

The inflammatory response is characterized by coordinate activation of various signaling pathways that regulate expression of both pro- and anti-inflammatory mediators. Currently, most of our knowledge of signaling in inflammation and innate immunity comes form studying members of the IL-1R/TLR (Akira et al., 2006) and TNF receptor (TNFR; reviewed by Locksley et al. [2001]) families. Although structurally different, these receptors, which unlike most cytokine and growth factor receptors lack intrinsic protein kinase activity or are not directly associated with protein kinases, use similar signal-transduction mechanisms. Receptor engagement results in recruitment of adaptor proteins that posses either Toll-IL-1 receptor (TIR) domains in the case of TLRs and IL-1R (Akira et al., 2006) or death domains (DD) in the case of those members of the TNFR family that control cell survival (Muppidi et al., 2004). Once recruited to oligomerized receptors these adaptors recruit a variety of signaling proteins that belong to the TRAF family (Baud and Karin, 2001) and various protein kinases, including IRAK1 and 4 in the case of TIR signaling (Suzuki et al., 2002) and RIP kinases in the case of TNFR signaling (Kelliher et al., 1998). Some TNFR family members can directly recruit TRAF proteins without assistance from adaptor proteins (Muppidi et al., 2004). Invariably, these molecules, upon receptor engagement, activate several effector pathways, the most important of which include mitogen-activated protein kinases (MAPK), especially JNK (Karin and Gallagher, 2005) and p38 MAPK (Zarubin and Han, 2005), as well as IκB kinases (IKK) and their relatives (Ghosh and Karin, 2002). The MAPKs lead to direct and indirect phosphorylation and activation of various transcription factors, especially those that belong to the bZIP family: AP-1 (Karin, 1995) and CREB (Park et al., 2005). MAPKs also regulate gene expression through posttranscriptional mechanisms such as mRNA turnover, mRNA transport, and translation. The IKKs, which from a complex composed of two catalytic subunits, IKKα and IKKβ, and a regulatory subunit, IKKγ/NEMO, are responsible for activation of NF-κB transcription factors (Ghosh and Karin, 2002) and have emerged as central regulators of inflammatory and immune responses (Bonizzi and Karin, 2004). Two IKK-related kinases TBK1/NAK/IKKε and IKKi were found to be important activators of interferon response factors (IRFs; Fitzgerald et al., 2003) that, although not similar to NF-κB, can collaborate with it to activate important target genes (Covert et al., 2005; Werner et al., 2005). Transcriptional cooperation also occurs between NF-κB and members of bZIP family (Park et al., 2005). Collectively, these protein kinases coordinate the expression of a large number of both proinflammatory and anti-inflammatory mediators.

An important anti-inflammatory cytokine is IL-10 (Pestka et al., 2004). Although the mechanisms that control IL-10 expression are not fully understood, it was recently found that a TRAF3 deficiency prevents IL-10 induction in response to TLR engagement, resulting in increased production of the proinflammatory cytokines IL-6 and IL-12 (Häcker et al., 2005). An IL-10 deficiency also predisposes mice to inflammatory bowel disease (IBD) and colorectal cancer (Kuhn et al., 1993). These outcomes were found to be dependent on colonization of the gastrointestinal tract with *H. hepaticus* (Erdman et al., 2003), a close relative of *H. pylori* which, as discussed above, is a major risk factor for gastric cancer in humans. Interestingly, the transfer of IL-10 expressing regulatory T cells (Treg) into *H. hepaticus*-infected immunodeficient mice promoted regression of colonic adenomas (Erdman et al., 2003), illustrating the preventive and therapeutic potential of this anti-inflammatory cytokine. The role of IL-6, whose expression is negatively regulated by IL-10, has yet to be examined in the *IL-10*$^{-/-}$ mouse model of colorectal cancer; however, it should be noted that inhibition of IL-6 signaling retards development of chemically induced colitis-associated cancer (CAC) in mice (Becker et al., 2004). CAC development is also inhibited by knocking out the *Ikk*β gene in either enterocytes or myeloid cells (Greten et al., 2004). In the latter cell type, IKKβ-driven NF-κB is required for increased production of IL-6 during early stages of the carcinogenic process, when it serves as a growth factor for premalignant enterocytes that give rise to CAC. Curiously, at later stages of this malignancy, the primary source of IL-6 appear to be Th1 cells, where its production is negatively regulated by TGF-β (Becker et al., 2004).

IL-10 and the structurally-related type I interferons (IFNs), as well as IL-6, IL-12, and many other cytokines, signal via receptor associated TKs that belong to the JAK group, whose activation results in phosphorylation and nuclear translocation of STAT transcription factors (O'Shea et al., 2002). Engagement of cytokine receptors as well as TLRs can also lead to activation of phosphoinositide-3-kinases (PI3K), which in turn activate other proteins kinases such as AKT (Martin et al., 2003).

All of these pathways, which contribute to innate immunity and inflammation, are also subject to negative regulation. PI3K signaling is inhibited by the PTEN phosphatase that belongs to the protein tyrosine phosphatase (PTP) family; some of its other members, for instance SHIP, SHP1/2, and CD45, are responsible for negative regulation of TK signaling (Neel et al., 2003). MAPK kinase phosphatases (MKPs), which also belong to the PTP family, control the duration and extent of MAPK activation as shown during TNF-α-mediated JNK activation (Kamata et al., 2005). Inducible suppressors of cytokine signaling (SOCS), which function as ubiquitin ligases, are responsible for the negative feedback control of JAK-STAT signaling (Alexander and Hilton, 2004). Another inducible ubiquitin ligase which also possesses deubiquitination activity is A20, which functions as a negative feedback regulator of TLR and TNFR signaling (Boone et al., 2004). A deficiency in any one of these negative regulators can result in either "spontaneous" chronic inflammation, perhaps reflecting host cell activation by PAMPs present in endogenous microflora, or an exaggerated response to true infection that culminates in severe inflammation and damage to the host. Similar observations were recently made in mice that harbor a variant of IKKα, IKKα^{AA}, that can not be activated by upstream regulators. Although IKKα^{AA} mice do not develop "spontaneous" inflammation, they develop an exaggerated inflammatory response when challenged with bacteria, fungal cell wall particles, or even immune complexes (Lawrence et al., 2005). These observations indicate that whereas IKKβ catalytic activity is important for the activation of NF-κB through phosphorylation of inhibitor of κB (IκB) proteins (Maeda et al., 2003), IKKα is required for proper termination of NF-κB activation through phosphorylation of its RelA (p65) and c-Rel subunits (Lawrence et al., 2005). In both cases, phosphorylation results in polyubiquitination of the target protein, leading to its accelerated degradation via the 26S proteasome. However, while IκB degradation is essential for NF-κB activation and nuclear translocation, the accelerated degradation of nuclear Rel proteins is important for controlling the duration of NF-κB activation. Although all of these negative regulatory mechanisms affect different signaling pathways, genetic studies in mice have shown that even the absence of one negative regulator is sufficient for overdriving the innate immune system, resulting in serious inflammatory disorders. Undoubtedly, aberrations in such negative regulatory pathways will be found to underlie various chronic inflammatory and degenerative disorders in humans.

Mechanisms of Pathogenesis

A Link between Innate and Adaptive Immunity

In addition to fighting infections and accelerating wound healing, inflammation has an important role in shaping the adaptive immune response; a prominent aspect of this is the maturation and migration of DC from the sites of infection, where they may pick up antigens, to secondary lymphoid organs, where they can prime antigen-specific immune responses. There have been major advances in understanding DC biology and function (Mellman and Steinman, 2001). It is now clear that both proinflammatory cytokines, including TNF-α, and microbial products drive DC maturation and their migration to draining lymphoid tissue. Expression of different chemokines may differentially affect the location of DCs. Importantly, inflammatory chemokines, whose expression depends on the classical NF-κB pathway, usually promote the migration of DCs to sites of inflammation, where they take up antigens (Bonizzi and Karin, 2004). By contrast, organogenic or homeostatic chemokines that are controlled by an alternative NF-κB signaling pathway (which depends on IKKα homodimers instead of classical trimembered IKK complexes) come into play at a later point during the immune response and promote migration of antigen-loaded DCs back into lymphoid organs (Bonizzi and Karin, 2004). Crosstalk between the innate and adaptive immune

system is extensively reviewed by Pulendran and Ahmed (2006) in this issue of *Cell* and will not be discussed further here, but as outlined below, a switch from an acute but short-lived inflammatory response driven by granulocytes and macrophages to a delayed lymphocyte-dominated response is classically associated with chronic inflammation. It has also been suggested that specific TLRs are required for generation of distinct immune and autoimmune responses. Sequential engagement of the B cell receptor and TLR9 by mammalian chromatin can lead to activation of chromatin-reactive B cells and formation of anti-DNA antibodies and chromatin containing immune complexes (Leadbetter et al., 2002), a finding corroborated by the absence of such antibodies in TLR9-deficient lupus-prone mice (Christensen et al., 2005). Future studies may be warranted to examine whether RNA-recognizing TLRs (3, 7, or 8) are involved in generation of ribonucleoprotein and RNA-directed autoantibodies, as evidence exists that viral dsRNA can aggravate lupus nephritis in a TLR3-specific fashion (Patole et al., 2005). Beyond TLRs, the innate immune response to nucleic acids can be mediated by soluble intracellular receptors, such as the helicase RIG-I (for RNA) and yet-to-be-identified receptors for double-stranded DNA (Ishii et al., 2006; Yoneyama et al., 2004). Both types of receptors signal to IKK and IKK-like kinases via adaptor protein Cardif/MAVS/IPS-1/VISA (Hiscott et al., 2006). It has been suggested that these newly identified pathways may also be involved in autoimmune disease.

Autoimmunity

Chronic inflammatory diseases can encompass autoimmune disease such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Autoimmune diseases remain one of the greatest mysteries in immunobiology. Although genetic predisposition surely plays an important part in the etiology of such diseases (Rioux and Abbas, 2005), exposure to infectious agents may also play an important but currently contentious role (Benoist and Mathis, 2001). Environmental factors and customs, such as smoking, that enhance exposure to bacteria and viruses and their nucleic acids, also have an important effect on the development of course of such diseases (Benoist and Mathis, 2001; Rioux and Abbas, 2005). A thorough discussion of the pathogenic role played by autoimmunity is beyond the scope of this review and was recently addressed elsewhere (Feldmann and Steinman, 2005; Rioux and Abbas, 2005). It is worth noting, however, that in addition to the etiologic association of streptococcal infection with rheumatic fever, antigenic mimicry of microbial epitopes has gained attention as a potential provocative factor in the autoimmune tissue injury of chronic (postinfectious) Lyme arthritis (Steere and Glickstein, 2004), central nervous system demyelination in multiple sclerosis (Sospedra and Martin, 2005), and the inflammatory joint destruction of RA (Prakken et al., 2001).

Failure of Resolution

An alternative view on the etiology of chronic inflammatory disease is that a failure of endogenous anti-inflammatory mechanisms results in persistent inflammation that may also lead to autoimmunity. This has been proposed in the case of SLE, an autoimmune syndrome that is associated with presence of autoantibodies usually directed against nucleic acids (Kotzin, 1996). Studies in mice have established a role for the complement receptor C1q on macrophages in development of this disease. C1q is important for the phagocytic clearance of AC, and in its absence, mice develop a SLE-like syndrome (Botto et al., 1998). Mer TK null mice also exhibit SLE-like pathology, where persistence of AC and necrotic bodies are proposed to lead to development of an inappropriate immune response to endogenous antigens (Cohen et al., 2002). In *DNaseII*-deficient mice, failure of macrophages to degrade apoptotic DNA upon engulfing AC was shown to result in effective activation of IFN signaling (Kawane et al., 2003). The phagocytosis of AC may play an important role in the negative regulation of macrophage activation, promoting TGF-β production (Savill et al., 2003) and direct repression of the IL-12p35 gene, independent of autocrine or paracrine effects of TGF-β (Kim et al., 2004). For all these reasons, defective clearance of AC may be critical in the etiology of chronic inflammatory disease.

What Have We Learned from Mice?

In this section, we discuss recent evidence for the involvement of host defense mechanisms in development of inflammatory diseases, such as Crohn's disease (CD) and psoriasis, as well as chronic diseases such as atherosclerosis, type II diabetes, and cancer. Many of these important insights have been derived from mouse models in which genetic manipulation of innate immune and inflammatory signaling was examined for its effect on disease development and progression.

Crohn's Disease

CD is a chronic inflammatory bowel disease (IBD) with strong genetic determinants. Approximately 40% of CD cases found in Caucasians are linked to sequences variants of the *NOD2* locus, homozygosity to any of which increases disease risk by up to 40-fold (Hugot et al., 2001; Ogura et al., 2001). *NOD2* encodes a protein that belongs to the NOD-LRR superfamily, whose members are thought to be involved in pattern recognition and host defense (Inohara et al., 2004). At its N terminus, NOD2 contains two caspase activation and recruitment domains (CARD), whereas its C terminus contains a series of leucine-rich repeats (LRR), similar to those found in TLRs. In the middle of the molecule lies a nucleotide binding and oligomerization domain (NOD). Curiously, the CD-linked *NOD2* variants specify only small variations in the NOD2 primary structure, all of which are within or next to the LRR. Two of the variants, *R702W* and *G908R* result in single amino acid substitutions, whereas the third, *1007fs* (or *3020insC*), is a frameshift mutation that deletes the last 30 amino acids of the protein (Eckmann and Karin, 2005). Since no mutations that completely prevent expression of NOD2 or cause a large truncation have been found and heterozygosity for any of the disease-linked

alleles only increases the risk of CD by 2- to 4-fold, it has been argued that these mutations are likely to result in a recessive gain of function rather than a loss of function or the creation of a dominant-negative variant (Eckmann and Karin, 2005). Nonetheless, the effects of the CD-associated mutations on NOD2 function remain controversial. Furthermore, mutated NOD2 alleles are neither sufficient nor necessary for the development of CD, since they occur in healthy individuals and the majority of CD patients do not exhibit NOD2 mutations (Economou et al., 2004).

The presence of a LRR suggested that NOD2 may be a PRR. Indeed, transient NOD2 expression in heterologous cells confers responsiveness to muramyl dipeptide (MDP), a component of gram-positive and -negative bacterial cell walls, measured through activation of an NF-κB-dependent reporter (Inohara et al., 2004). Although direct binding of MDP to NOD2 remains to be demonstrated, these findings fostered the view that NOD2, a cytoplasmic protein mostly expressed by myeloid cells, is involved in recognition of intracellular bacteria. These findings fit the view that in addition to well-established genetic risk factors, pathogenesis of CD is strongly influenced by poorly defined environmental factors, the most likely of which are enteric bacteria (Isaacs and Sartor, 2004). However, apart from the strong association of *NOD2* mutations with CD and the observation that a subgroup of CD patients responds to antibiotic therapy, the role of enteric bacteria in CD development is far from established.

In an attempt to better understand the function of NOD2, mutant mouse strains were created, including complete knockouts that no longer express the protein (Kobayashi et al., 2005; Pauleau and Murray, 2003), as well as a *Nod2* knockin mutant, which mimics the human $NOD2^{1007fs}$ mutation that deletes the last 30 amino acids of the protein (Maeda et al., 2005a). Consistent with the putative innate immune function of NOD2, $Nod2^{-/-}$ mice were found to be more susceptible to oral infection with *Listeria monocytogenes*, a bacterium that replicates within macrophages (Kobayashi et al., 2005). These mice, however, do not exhibit increased colonic or intestinal inflammation nor overproduce any of the proinflammatory cytokines that characterize the human disease. Furthermore, $Nod2^{-/-}$ mice exhibit resistance to acute systemic inflammation induced by injection of LPS plus MDP (Kobayashi et al., 2005). Susceptibility to bacterial infection has not yet been tested in the *Nod2* knockin mice, but these animals were found to display an elevated inflammatory response upon administration of dextran sulfate salt (DSS), an irritant that errodes the mucosal epithelial barrier and thereby exposes lamina propria macrophages to enteric bacteria (Maeda et al., 2005a). Increased inflammation in *Nod2* knockin mice is associated with elevated secretion of mature IL-1β upon exposure to MDP or MDP combined with LPS. Inhibition of IL-1β signaling by injection of IL-1 receptor antagonist (IL-1Ra) inhibited DSS-induced colonic inflammation and abolished the phenotypic differences between the *Nod2* knockin mice and wild-type counterparts (Maeda et al., 2005a). Collectively, these results support the view that NOD2 plays a critical role in the regulation of IL-1β processing and secretion. It should be noted that IL-1β is first made as a large precursor, pro-IL-1β, that cannot be secreted by activated macrophages. Conditions and signals that promote activation of caspase 1 can induce pro-IL-1β processing and IL-1β secretion (Martinon and Tschopp, 2004). Hence, the CD-associated mutations may increase disease risk by increasing the capability of NOD2 to promote caspase 1 activation and thereby enhance pro-IL-1β processing and IL-1β secretion. This hypothesis is supported by biochemical experiments that suggest that NOD2 is an important mediator of pro-IL-1β processing in response to MDP and other stimuli (Maeda et al., 2005a). Furthermore, the resistance of $Nod2^{-/-}$ mice to systemic inflammation caused by MDP plus LPS could well be due to defective IL-1β release. Curiously, increased secretion of mature IL-1β caused by mutations in the *NALP3* gene, which encodes another member of the NOD-LRR family involved in caspase 1 activation and pro-IL-1β processing, were linked to a group of related autoinflammatory diseases: Muckle-Wells syndrome (MWS), familial cold urticaria (FCU), and chronic infantile neurological cutaneous and articular syndrome (CINCA; Martinon and Tschopp, 2004). All of these closely related diseases, whose variable signs include sensorineural deafness, rashes, systemic amyloidoses, nonspecific limb pain, skin lesions, joint inflammation, conjunctivitis, chills, and cold-induced fever, are likely to be caused by elevated IL-1β secretion and can therefore be treated by administration IL-1 receptor antagonist (Hawkins et al., 2003).

Psoriasis

Psoriasis is a chronic inflammatory disease of the skin and joints that, just like CD and RA, involves increased production of proinflammatory cytokines, such as TNF-α. Correspondingly, all three of these diseases are responsive to anti-TNF therapy (Feldmann and Maini, 2003). Curiously, *NOD2* mutations, different from those associated with CD, were found to be associated with increased risk for psoriatic arthritis (Rahman et al., 2003). This suggests that like CD, psoriasis may also be a disease of the innate immune system. However, apart from *NOD2*, several other psoriasis susceptibility loci have been identified, and their molecular nature and relationship to innate immunity remain to be elucidated (Nickoloff and Nestle, 2004).

New insights to the pathogenesis of psoriasis have been provided by a recently developed mouse model (Zenz et al., 2005). Following the observation that expression of the AP-1 subunit JunB is downregulated in psoriatic lesions relative to normal skin, Zenz et al. (2005) generated conditional knockout mice where both the *JunB* and *c-Jun* loci are inducibly deleted in the epidermis upon tamoxifen-induced Cre recombinase expression. Within 8–10 days of tamoxifen administration, affected mice, in which both JunB and c-Jun were deleted, developed skin lesions that were identical in appearance to psoriatic plaques of human patients. Appearance of psoriatic lesions was

associated with appearance in 100% of the double-knockout mice of joint inflammation, similar to psoriatic arthritis, which appears in 5%–40% of psoriasis patients. Appearance of skin and joint lesions was associated with upregulation of proinflammatory cytokines and chemokines, including TNF-α and IL-1β. Importantly, deletion of the type I TNF-α receptor (TNFR1) resulted in milder skin lesions and almost complete inhibition of joint inflammation. Inactivation of the *Rag2* locus, which prevents development of T and B lymphocytes, which are believed to be important contributors to the maintenance of psoriasis and RA (Feldmann and Maini, 2003), suppressed development of psoriatic lesions less than deletion of TNFR1 but still attenuated the development of subsequent arthritis (Zenz et al., 2005). These results suggest that psoriasis, at least in epidermal-specific JunB/c-Jun-deficient mice, is partially associated with initial activation of TNFα-producing myeloid cells, which contribute to some of the skin pathology, and that activation of these cells results in longer lasting activation of lymphoid cells, which mediate the arthritic lesions.

The key question, however, is what causes the initial and deregulated activation of myeloid cells in the skin of epidermally deleted JunB/c-Jun knockout mice. Although the full mechanism remains to be determined, Zenz et al. offer a few important clues. First, the development of the disease is attenuated by application of a broad-spectrum antibiotic, suggesting the involvement of bacteria, most likely commensals that live on mouse skin. Second, one of the first detectable changes in gene expression by epidermal keratinocytes following inducible JunB/c-Jun deletion is upregulation of S100 proteins (S100A8 and S100A9), which have been shown to serve as chemotactic factors for neutrophils (Rioux and Abbas, 2005). Rapid upregulation of S100A8 and S100A9 was also seen upon deletion of JunB and c-Jun in cultured keratinocytes, suggesting a close link between the two (Zenz et al., 2005). As the two Jun proteins are important regulators of keratinocyte proliferation (Zenz et al., 2003), it is possible that their absence may result in increased exposure to commensal bacteria due to accelerated loss of barrier function. Together with the upregulation of S100 proteins and other yet-to-be identified factors, this results in an exacerbated inflammatory response that fails to resolve and eventually leads to activation of lymphocytes that may propagate psoriatic arthritis through autoimmune mechanisms. While it will be important to validate this hypothesis in human subjects, it should be recognized that a patient suffering from psoriasis may be far removed from the initial episode that exposed its inflammatory cells to skin-born bacteria and therefore unlike the inducible JunB/c-Jun knockout mice may no longer be responsive to antibiotic therapy.

Atherosclerosis and Type II Diabetes

Atherosclerosis and type II diabetes are very common and chronic metabolic diseases that although not strongly associated with microbial or viral exposure are dependent on activation of macrophages. The role of activated macrophages in formation of atherosclerotic plaques is well established and extensively reviewed (Glass and Witztum, 2001). Most likely, the major cause of persistent macrophage recruitment and activation within developing atherosclerotic plaques are oxidized forms of LDL taken up by scavenger receptors (Febbraio et al., 2000). While the signaling mechanisms used by scavenger receptors remain to be identified, they likely function as PRRs, whose occupancy leads to macrophage activation and subsequent production of inflammatory mediators, including growth factors that cause hyperproliferation of smooth muscle and endothelial cells in blood vessels, culminating in formation of atherosclerotic plaques (Glass and Witztum, 2001).

Type II diabetes is a metabolic disease caused by decreased sensitivity to insulin, thus creating an increased demand for insulin production by pancreatic β cells, which are eventually destroyed through a mechanism that may depend on persistent endoplasmic reticulum (ER) stress (Ozcan et al., 2004). An important clue for the role of inflammation in the pathogenesis of type II diabetes was provided by the finding that TNF-α leads to insulin resistance through activation of signaling pathways, such as the JNK MAPK cascade (Hirosumi et al., 2002), that decrease the ability of insulin receptors to activate effector pathways (Hotamisligil et al., 1996). For many years, the source of TNF-α and other inflammatory cytokines capable of inducing insulin resistance was thought to be the adipocyte. However, recent studies based on selective inactivation of the classical NF-κB pathway in myeloid cells suggest that migratory macrophages may be the major source of proinflammatory cytokines in obesity-induced insulin resistance (Arkan et al., 2005). How obesity leads to activation of macrophages is a matter of conjecture, but it is tempting to speculate that it is the uptake of certain circulating lipids or lipoproteins through scavenger receptors or engagement of other types of PRRs that may be involved. Alternatively, excessive lipid accumulation and metabolism may result in necrotic cell death of adipocytes or hepatocytes, for instance, through elevated production of reactive oxygen species (ROS). As discussed above, necrotic cells release a variety of mediators that can lead to macrophage recruitment and activation through PRRs. Thus, although not a direct response to microbial infection or exposure, both atherosclerosis and type II diabetes can be viewed as diseases caused by excessive and recurrent tissue injury that exposes inflammatory cells to endogenous PRR ligands (Figure 2). This hypothesis can be tested by examining the effect of targeted disruptions of key elements of innate immune signaling such as the MyD88 adaptor protein.

Cancer

The relationships and mechanisms through which infection and inflammation increase cancer risk and promote tumor development were recently reviewed (Karin and Greten, 2005). It is well established that chronic infections with hepatitis B and C viruses (HBV, HCV) represent major risk factors for development of hepatocellular carcinoma

(HCC), while chronic *H. pylori* infection is the major risk factor for gastric cancer (Kuper et al., 2000). It was proposed that in both cases and others the infectious agent leads to activation of NF-κB in myeloid cells (and eventually in lymphoid cells), resulting in production of growth and survival factors that stimulate tumor progression and development (Karin and Greten, 2005). Interestingly, HCV was recently found to encode a nonstructural protein (NS5A) that actually inhibits the activation of NF-κB by TNF-α and, as a result, potentiates TNF-α-induced JNK activation (Park et al., 2003). Another HCV protein, NS3/4A, is a protease that cleaves TRIF and Cardif, thereby preventing IKK activation and induction of antiviral innate immunity (Meylan et al., 2005). Importantly, inhibition of NF-κB activation in hepatocytes, the cells that are infected by HCV, was found to increase their susceptibility to carcinogen-induced death (Maeda et al., 2005b) However, hepatocyte death also triggers through inflammation-propagated mechanisms the compensatory proliferation of surviving hepatocytes. The latter depends both on JNK activation within hepatocytes (Maeda et al., 2005b) and on production of proinflammatory cytokines, such as TNF-α and IL-6, by inflammatory cells (Fausto, 2000). It thus seems that HBV and HCV infections, for instance, stimulate the development of HCC through both nonspecific injury caused by the cytolysis of virally-infected hepatocytes and through expression of specific proteins that alter the balance between TNF-α-triggered survival and death pathways. The necrotic death of hepatocytes is likely to cause acute activation of liver myeloid cells (Kupffer cells), thereby stimulating the production of growth factors that trigger the compensatory proliferation of surviving hepatocytes, which otherwise spend most of their life in the G_o phase of the cell cycle. Compensatory hepatocyte proliferation results in propagation of oncogenic mutations, induced by exposure to exogenous or endogenous carcinogens, leading to clonal expansion of transformed cells that eventually give rise to HCC. As in other inflammatory diseases, chronic viral hepatitis eventually results in persistent activation of inflammation-maintaining T cells (Chisari and Ferrari, 1995). Such T cells can induce the development of HCC when transferred to naive mice.

Thus, even in cancer, especially HCC, one can invoke the general model of repeated tissue injury associated with chronic exposure to a pathogen, resulting in acute and then sustained chronic inflammation as the major mechanism underlying the development of a long-lasting chronic disease. Recent work on a mouse model of gastric cancer suggests that repeated tissue injury caused by bacterial infections (in this case caused by a relative of *H. pylori—H. felis*) is associated with an aberrant regenerative response that may account for this type of cancer as well (Houghton et al., 2004). Given the well-established tumor-promoting function of chronic inflammation (Karin and Greten, 2005) and the requirement of tumor promotion for enhancing cancer development after carcinogen exposure, it is not too farfetched to suggest that chronic airway inflammation caused by repeated exposure to irritants found in tobacco smoke, which enhance exposure to airborne microbes and viruses, plays an important tumor-promoting function in the development of lung cancer as well.

Summary

Nearly one and half centuries ago, Rudolf Virchow extended the conceptual framework of the cell theory to provide the cornerstones of modern clinical pathology: diseases of organs reflected abnormal physiology of cells, and propagations of physiologically altered cells were the root of chronic inflammation and cancer. Insistent on the universality of his model, Virchow vigorously rejected the germ theory of disease proposed by his contemporaries Robert Koch and Louis Pasteur. In the era of modern molecular medicine, clarification of the intricacy of normal innate immune system function and its specific vulnerabilities suggest that integrated "germ-cell" theories are required to adequately understand many chronic disease conditions and cancers with an eye toward improved therapy and cure. Indeed, the 2005 Nobel Prize in Physiology or Medicine awarded to Barry J. Marshall and J. Robin Warren acknowledged this point. As cited in the press release of the Nobel Assembly: "Many diseases in humans such as Crohn's disease, ulcerative colitis, rheumatoid arthritis, and atherosclerosis are due to chronic inflammation. The discovery that one of the most common diseases of mankind, peptic ulcer disease, has a microbial cause has stimulated the search for microbes as possible causes of other chronic inflammatory conditions. Even though no definite answers are at hand, recent data clearly suggest that a dysfunction in the recognition of microbial products by the human immune system can result in disease development. The discovery of *Helicobacter pylori* has led to an increased understanding of the connection between chronic infection, inflammation, and cancer."

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