4 Inflammation and Inflammatory Diseases, Markers, and Mediators: Role of CRP in Some Inflammatory Diseases

Abstract

The knowledge of inflammation records dates back in first century AD. Initially discovered with features of rubor, tumor, calor, and dolor, scientific investigations have revealed chemical components, cells, and pathways involved in the process of inflammation. The body's initial defense in response to infection, trauma, or inflammation is through the acute-phase response (APR). APR is a multifaceted set of systemic reactions seen shortly after the experience of a triggering event. One of the many aspects of an APR is the increased hepatic synthesis of positive acute-phase proteins (APPs) leading to increased serum concentration of these proteins. The serum level of these APPs returns to base concentration when the stimulating factor is not anymore present. Today a plethora of inflammatory diseases are causing concern to global health. All the key players and mediators of inflammation change its role with the change in setup of disease and patients. The biomarkers of inflammation and inflammatory mediators are also used as therapeutic targets in under-trial animal models. Even in clinical diagnosis of an inflammatory patient, some broad-spectrum markers were analyzed without individual dissection of each mediator or biomarker. This chapter also provides a review of the acute-phase protein C-reactive protein and its possible use as inflammatory biomarker in diseases. We have highlighted case studies of some patients from Kolkata, India, revealing inflammation from disease together with their clinical history. The question which we probe in here is that whether there is a correlation with the clinical history, C-reactive protein, and inflammation and whether CRP can act as a unique diagnostic and prognostic biomarker in some diseases. The future course of this chapter lies in identifying clinical markers for inflammation, the sequential flow of inflammatory responses for a wide spectrum of diseases and its diagnostic, and therapeutic application to screen out pro-inflammatory diseases vs. anti-inflammatory conditions.

W. Ansar, S. Ghosh, *Biology of C Reactive Protein in Health and Disease*,
DOI 10.1007/978-81-322-2680-2_4

Keywords

Acute-phase response • Acute-phase proteins • Inflammation • Cytokine inflammatory mediators • Biomarkers, disease • Inflammation • Immunomodulation • Clinical diagnosis • Therapeutic treatment

Chapter Highlights

1. Inflammation initiated by infection, trauma, or injury takes place by the coacting cascade of various leukocytes and cytokines. To mediate local inflammation, pro-inflammatory cytokines like interleukin-1, tumor necrosis factor, and interleukin-6 play important roles in activating inflammatory cells like neutrophils, macrophages, and monocytes. Activated leukocytes secrete at least 15 different low-molecular-weight cytokines and also trigger acute-phase response through the manifestation of fever and leukocytosis, increased synthesis of adrenocorticotropic hormones, and production of various acute-phase proteins.
2. Acute-phase proteins are synthesized by the liver under the influence of some cytokines, which flow through the bloodstream, reach the site of inflammation, and remove the pathogens through opsonization and activating the complement pathways.
3. The changes in the serum concentrations of negative and positive acute-phase proteins are due to the altered synthesis from the liver. Three of the best renowned acute-phase proteins are C-reactive protein, serum amyloid A, and haptoglobin. Some disease statuses are actually correlated to the concentration of acute-phase proteins.
4. C-reactive protein activates complement pathways and has a major role in some forms of tissue alteration like in cardiac infarction.
5. Diagnostic routine tests are sometimes invasive. For the care of patients, the employment of noninvasive biomarkers is needed. The new biological markers are thereafter the serologic markers and acute-phase proteins.
6. Molecular biological tools have revolutionized the field of the biomarker. For the development of biomarkers, genomics and proteomics are used. To understand the pathophysiology of a disease, the most available technique is correlating serologic markers with clinical phenotypes and genotypes.

4.1 Introduction

Inflammation is a complex dynamic protective response to cell injury, infection *via* microbes, trauma, or toxins in the vascularized tissues. The causative agent is diluted, destroyed, or isolated and a sequential cascade of molecular events is set that leads to repairing, healing, and reconstituting the damaged tissue (Li et al. 2007; Iwalewa et al. 2007; Libby 2007; Delves et al. 2006; Goldsby et al. 2007; Guyton and Hall 2001). It is thoroughly characterized by the reaction in tissues and its microcirculation as clinically reflected by redness (erythema), heat (hyperemia), swelling (exudation), pain (through nerves and chemical mediators), and loss of function (pain). Pathologically, it takes place by vasoconstriction followed by vasodilation, hyperemia, stasis, accumulation of leukocytes, exudation of fluid, and finally deposition of fibrin. The combined vascular and cellular inflammatory responses are triggered by inflammatory stimulus and mediated through chemical factors derived from some cells or blood plasma. Even the injured or dead tissues release mediators (Li et al. 2007; Iwalewa et al. 2007; Libby 2007; Delves

et al. 2006; Goldsby et al. 2007; Guyton and Hall 2001).

The etiologies for inflammation are varied ranging from microbial infections (infections by bacteria, virus, fungi, etc.), physical agents (like burns, stress, trauma from cuts, radiation), and chemicals (drugs, toxins, alcohol) to immunologic reactions (e.g., rheumatoid arthritis). Inflammation involves the influx of various cells of the host immune system and release of numerous mediators to the site of assault. Assembling and regulating inflammatory responses would be impossible without the concoction of controlled leukocyte population migration, various inflammatory mediators, inflammatory biomarkers (acute or systemic inflammatory marker), and subsequent physiologic changes that carry inflammatory responses (Figs. 4.1 and 4.2). In laboratories, animal models for inflammatory diseases are being developed to understand the process of inflammation, identify inflammatory mediators, and find out their probable role in therapeutics. Currently, there are no specific markers for inflammation; rather some broad-spectrum inflammatory markers were routinely investigated in hospitals. The question we asked is, since the biochemistry of inflammation is known, is it possible to identify some potential biomarkers of inflammation for safe pharmacological treatment which may in the future be helpful for routine hospital diagnosis?

4.2 History of Inflammation

The record of inflammation dates back to nearly first century AD by Celsus. Initially it was reported as a mechanism of tissue reaction or response to injury that gave rise to *rubor* (redness, due to hyperemia), *tumor* (swelling, due to greater permeability of the microvasculature and leakage of several proteins into the interstitial space), *calor* (heat, associated with the greater blood flow and the metabolic activity of the cellular inflammatory mediators), and *dolor* (pain, in part due to alterations in the perivasculature and related nerve endings). The fifth characteristic of inflammation, known as *functio laesa* (dysfunction of the organs involved), was revealed from the writings of Rudolf Virchow in the 1850s. Then, in the late nineteenth century, Elie Metchnikoff introduced the new concept of phagocytosis, a fundamental attribute of the innate immunity, after studying the engulfment of particulate matter by protozoa and also researching the ingestion of foreign bodies by blood leukocytes. Subsequently, Metchnikoff was awarded the Nobel Prize for Physiology or Medicine in 1908, along with Paul Ehrlich, for his work and discovery of humoral immunity, a component of adaptive immunity (Li et al. 2007; Iwalewa et al. 2007; Libby 2007).

4.3 Acute and Chronic Inflammation

Inflammation is mainly divided into two types—acute and chronic inflammation. If the inflammation winds up in less than 48 h, then it is acute inflammation (e.g., abscess) (Fig. 4.3), and if it rests for greater than 48 h (i.e., weeks, months, or years), then it is chronic inflammation.

Acute inflammation is initiated by mostly resident dendritic cells (DCs), macrophages, Kupffer cells, histiocytes, and mastocytes bearing pattern recognition receptors (PRRs) on their surface, which recognize/identify pathogen-associated molecular patterns (PAMPs) expressed exclusively on the outer surface of the pathogens. These immune cells after activation release inflammatory mediators, and subsequently the cardinal signs of *rubor*, *calor*, *tumor*, and *dolor* are visible. Vasodilation and increased permeability result in an exudation/seepage (edema) of fluid and plasma proteins into the tissue space and migration and extravasations of mainly neutrophils along a chemotactic incline created by the locally inhabited cells to reach the location of injury in the tissue. Inflammatory mediators increase the sensitivity of the tissues to pain (hyperalgesia), and a resultant neurological reflex

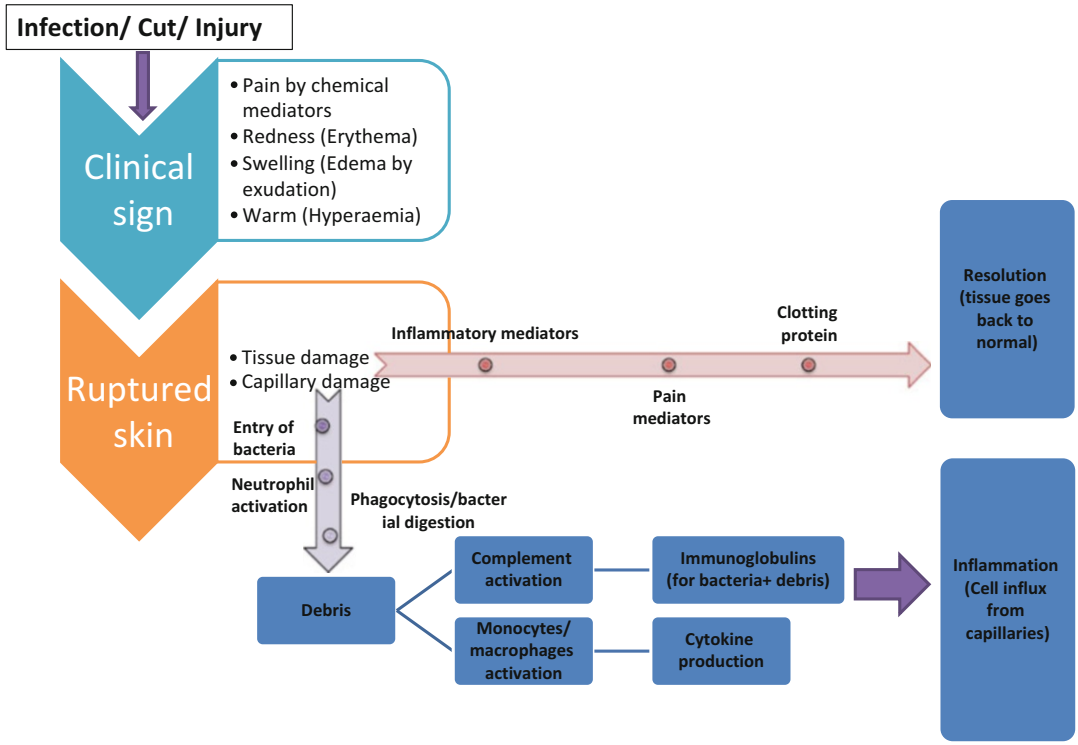


Fig. 4.1 *Mechanism of inflammation*. The process of inflammation started with physical assault of anatomical barrier with the common symptoms of pain, swelling, redness, and warmth. As the bacteria enters the host body, the activation of phagocytes, complement, and antibodies takes place in a cyclical way for both the pathogen and the debris also. At the same time, the tissue resolution goes on and further entry of pathogen is restricted

in response to pain leads to loss of function (*functio laesa*) (Fig. 4.1). A number of biochemical cascade systems, namely, the complement system (mostly activated by bacteria) and coagulation and fibrinolysis systems (mostly activated by necrosis), work in conjunction with inflammatory mediators to continue the inflammatory response (Fig. 4.2). However, the short half-life of inflammatory mediators is coterminous with the inflammation-activating signal.

Acute inflammatory outcomes like resolution, abscess, and ulcer (fistula, sinus) may lead to a chronic inflammation. An unresolved acute-phase inflammatory response leads to the development of chronic inflammation by persistent injury or infection (e.g., ulcer, tuberculosis (TB)) and prolonged exposure to toxic agents like silica and autoimmune diseases like rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Chronic inflammation leads to tissue destruction, fibrosis, and necrosis. Neutrophils are the major cell types in acute inflammation while mononuclear cells (mostly lymphocytes, macrophages, and plasma cells) participate in chronic inflammation. The outcomes of acute inflammation are resolution, abscess, and ulcer (fistula, sinus) and may expand to a chronic inflammation. In chronic inflammation, the outcomes are tissue destruction, fibrosis, and necrosis (Fig. 4.4).

Acute inflammation is perpetuated by any immune cells previously present in the tissue. Acute inflammation is initiated mostly by the cells like DCs, macrophages, Kupffer cells, mastocytes, and histiocytes. These cells bear on their outer surfaces PRRs, which recognize the PAMPs expressed exclusively on the surface of the pathogens. These immune cells after activation with the pathogen releases inflammatory mediators and subsequently showed the cardinal signs

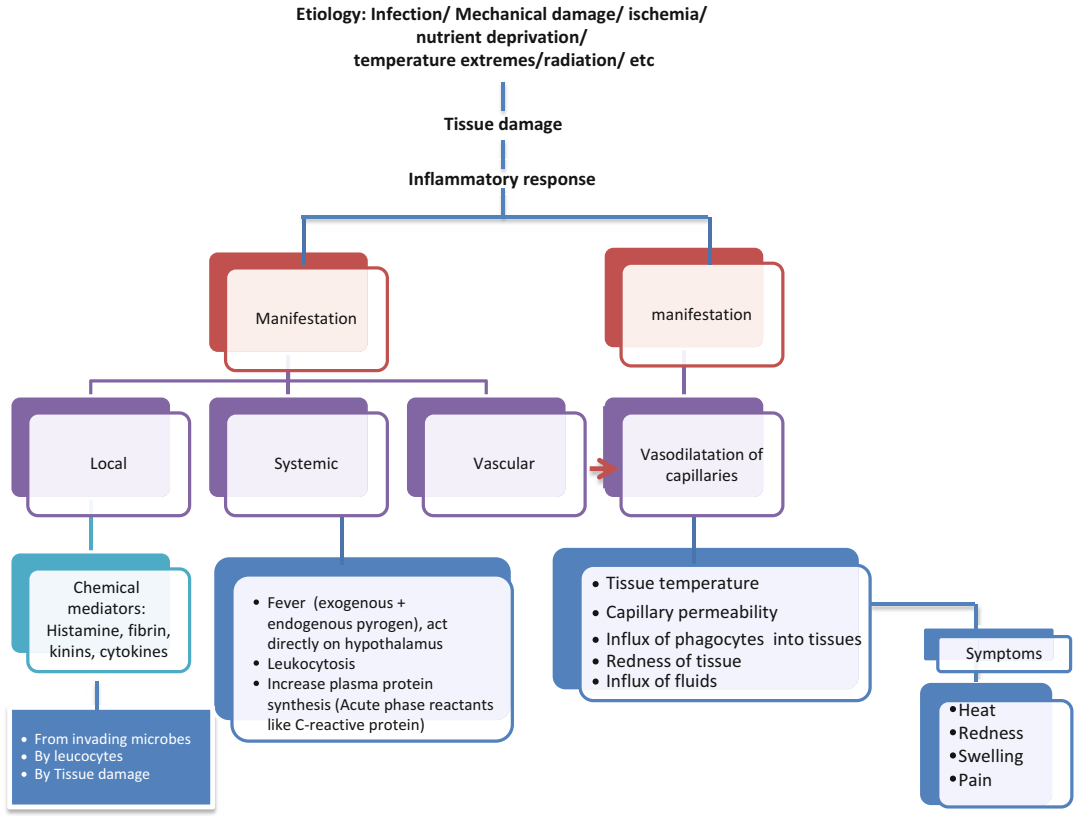


Fig. 4.2 *The mechanism of inflammatory response*. With the onset of the inflammation, the inflammatory response is manifested in local, systemic, and vascular mode. As a result, mediators are released, increasing vascularity and synthesis of plasma proteins, and finally the four symptoms of inflammation are seen. Cytokines provide the cord of connection between all these processes

(*rubor*, *calor*, *tumor*, and *dolor*) of inflammation. Vasodilation and increased permeability result in an exudation/leakage of fluid and subsequent edema. Release of plasma proteins into the tissue and migration and extravasations of leukocytes (mainly neutrophils) are the next steps of reactions. These reactions take place along a chemotactic flow generated by the locally inhabited cells to reach the locale of injury/assault in the tissue. Inflammatory mediators enhance the sensitivity of the affected tissues to pain (hyperalgesia), and a resultant neurological reflex in response to pain leads to loss of function (*functio laesa*) of the tissue (Figs. 4.1, 4.2, 4.3, 4.4, 4.5, and 4.6).

Additionally, to continue the inflammatory response with the help of the inflammatory mediators, a number of biochemical cascade systems are activated. These systems are the complement system (chiefly activated by bacteria), kinin system, and coagulation and fibrinolysis systems (chiefly activated by necrosis). Once the stimulus of acute inflammation is removed, the inflammatory mediators, having short half-lives, are disgraced in the inflamed tissue. Thus an acute inflammatory response requires the constant stimulation.

4.4 Mechanism of Inflammation

Inflammatory reactions involve a series of biochemical and cellular changes, the extent of which is associated with the spread of the initial trauma. The mechanism of inflammation takes place in four phases—vasodilation, exudation

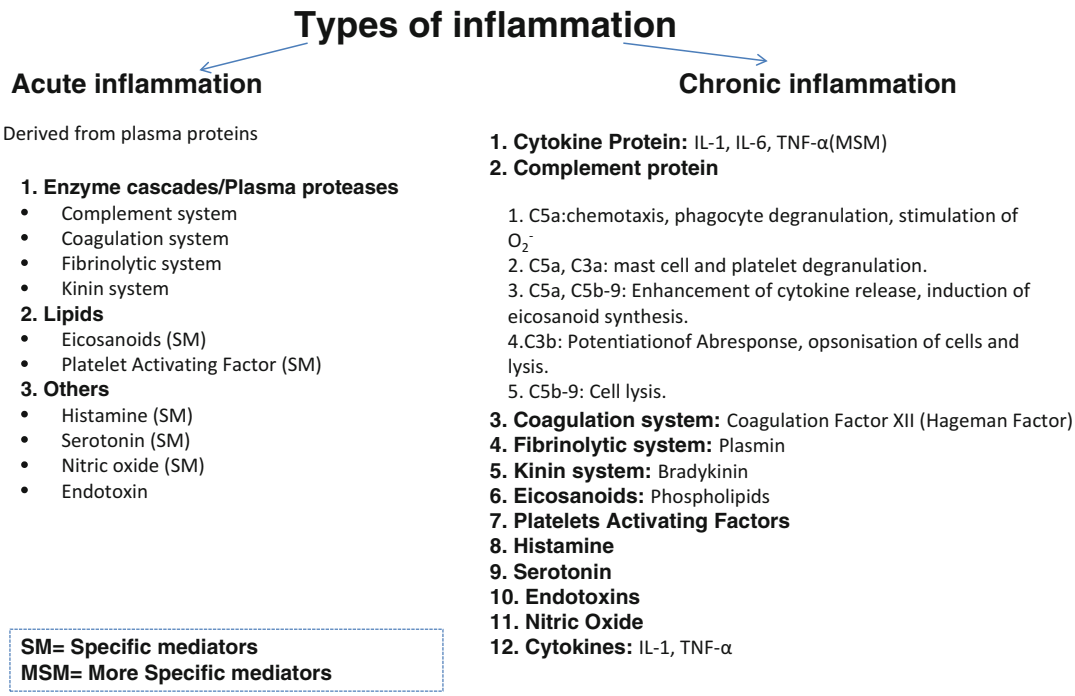


Fig. 4.3 Types of inflammation

(edema), emigration of cells, and chemotaxis (Figs. 4.1, 4.2, 4.3, 4.4, 4.5, and 4.6). Inappropriate stimulation of inflammatory responses is the primary cause of many known diseases, and inflammatory reactions are, as a result, also an imperative target for drug development (Li et al. 2007; Iwalewa et al. 2007; Libby 2007; Delves et al. 2006; Goldsby et al. 2007; Guyton and Hall 2001).

The most prolific systemic expression of inflammation is a hike of body temperature and a number of biochemical changes known as the acute-phase reaction which steers to the production of acute-phase proteins from the liver. The local inflammatory reaction is portrayed by an early increase in blood flow to the locale of injury, increased vascular permeability, and the sequential and directional influx and careful accumulation of various effector cells from the peripheral blood flow at the place of injury. Influx of nonantigen-specific but prominent destructive cells (neutrophils) is one of the initial stages of the inflammatory response/reactions (Fig. 4.1). These cells mount a phagocytic response which is rapid but nonspecific. An exudation/leakage of plasma into the lesion in the initial stage is also seen (Li et al. 2007; Iwalewa et al. 2007; Libby 2007; Delves et al. 2006; Goldsby et al. 2007; Guyton and Hall 2001). At a subsequent stage, macrophages, monocytes, and different cells of varied lineages of lymphocytes (T and B cells of specific subsets) appear at the locale of injury. These cell types are related with more tightly regulated, antigen-specific immune responses/reactions, and once they are stimulated, they also produce several protective inflammatory molecules.

Inflammatory cells articulate greater numbers of cell adhesion molecules like glycoproteins and cell surface proteins. Endothelial cells are also stimulated during the early phase of the inflammatory response/reactions and thereafter express, among many other things, several adhesion molecule counter-receptors. The controlled expression of these molecules allows for the prominent trafficking of blood-circulating leukocytes to a locale of inflammation. Cellular attachment of some immune cells to the wall of the endothelial

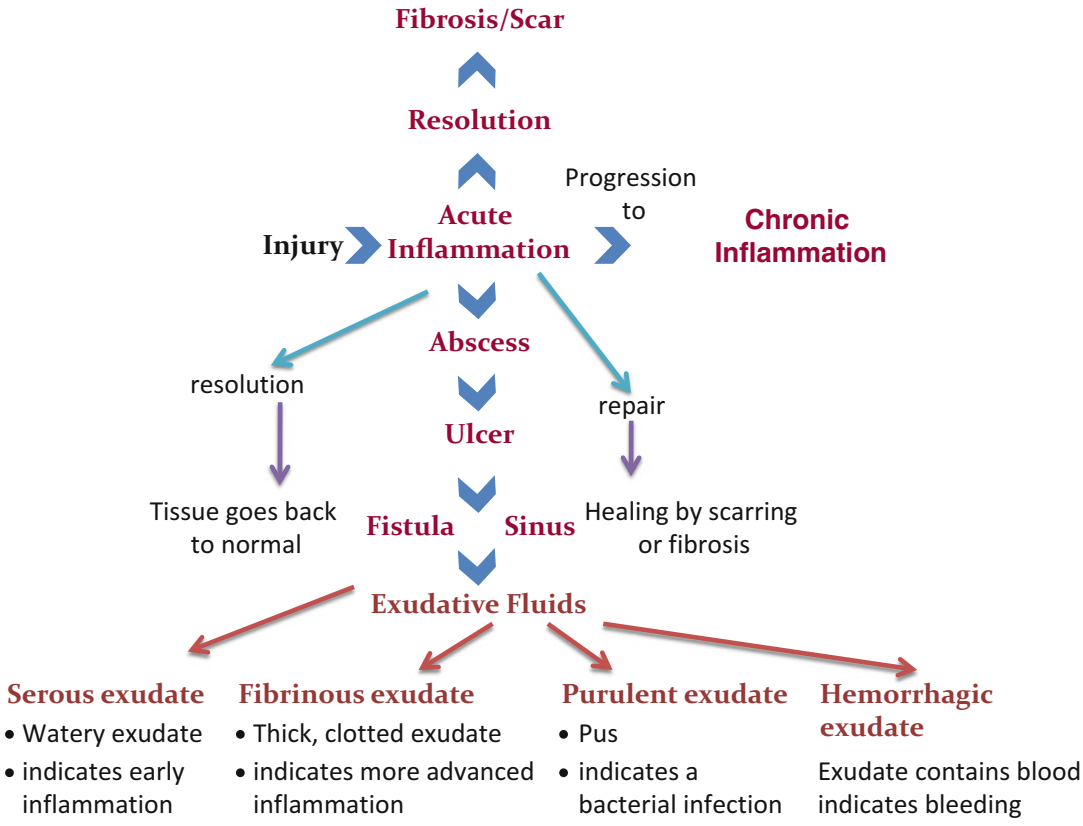


Fig. 4.4 *The outcomes of acute and chronic inflammation*. With the progression of the process of inflammation, the resolution and repair go hand in hand, and if these processes are disrupted, then it leads to exudation, pus, abscess, or its continuation as chronic inflammation

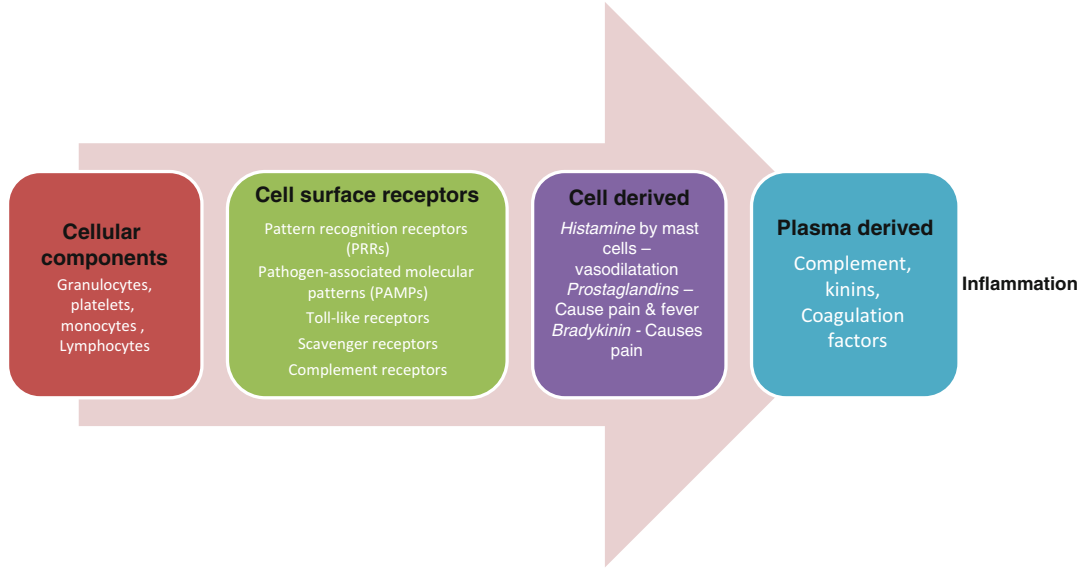


Fig. 4.5 *Components of inflammation*. The components of inflammation are different cells, cell surface receptors, and cell and plasma-derived components

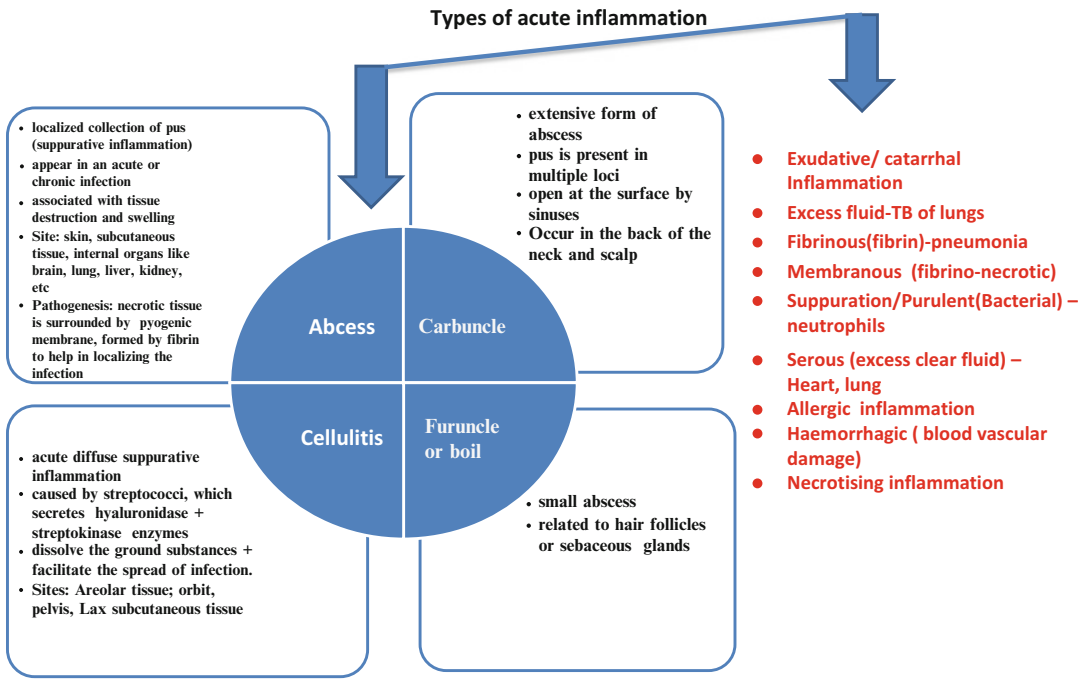


Fig. 4.6 *Types of acute inflammation*. There are different kinds of acute inflammation ranging from abscess, carbuncle, and boil to many other types

cells lining the blood vessels around the inflammatory site stops them from being swept away from the site of tissue damage or infection. This is a crucial stage required for the consequent emigration of these immune cells into the surrounding medium of inflammatory tissues (extravasation) (Li et al. 2007; Iwalewa et al. 2007; Libby 2007; Delves et al. 2006; Goldsby et al. 2007; Guyton and Hall 2001).

Once leukocytes have appeared at a locale of inflammation or infection, they release inflammatory mediators, which regulate the later accumulation and stimulation of other cells. However, in inflammatory responses initiated by the innate immune system, the final control is expressed by the antigen itself, in the identical way as it regulates the immune response itself. For this valid reason, the cellular accumulation at the locale of autoimmune reactions (antigen itself ultimately cannot be eradicated) or in chronic infection is quite distinct from that where the antigenic stimulus is rapidly cleared from the inflammatory sites (Li et al. 2007; Iwalewa et al. 2007; Libby 2007; Delves et al. 2006; Goldsby et al. 2007; Guyton and Hall 2001). In homeostasis and control of inflammation, four major plasma enzyme systems are there, which have an important role. This enzyme system includes the complement cascade, the clotting system, the plasmin (fibrinolytic) system, and the kinin system (Fig. 4.7, and 4.8).

4.4.1 Exudation of Leukocyte

The infiltration and accumulation of leukocytes past the blood vessels move through four pathways. These pathways are margination, rolling, and adhesion of leukocytes to the endothelium, diapedesis or transmigration of leukocytes across the endothelium, their migration toward a chemotactic stimulus released from the injured tissue, and finally phagocytosis.

The leukocyte starts expressing on its surface sialyl Lewis X-modified glycoproteins and integrin molecules in low-affinity state on its surface. Recruitment of leukocytes on the cell surface is through different receptors. Histamine, an inflam-

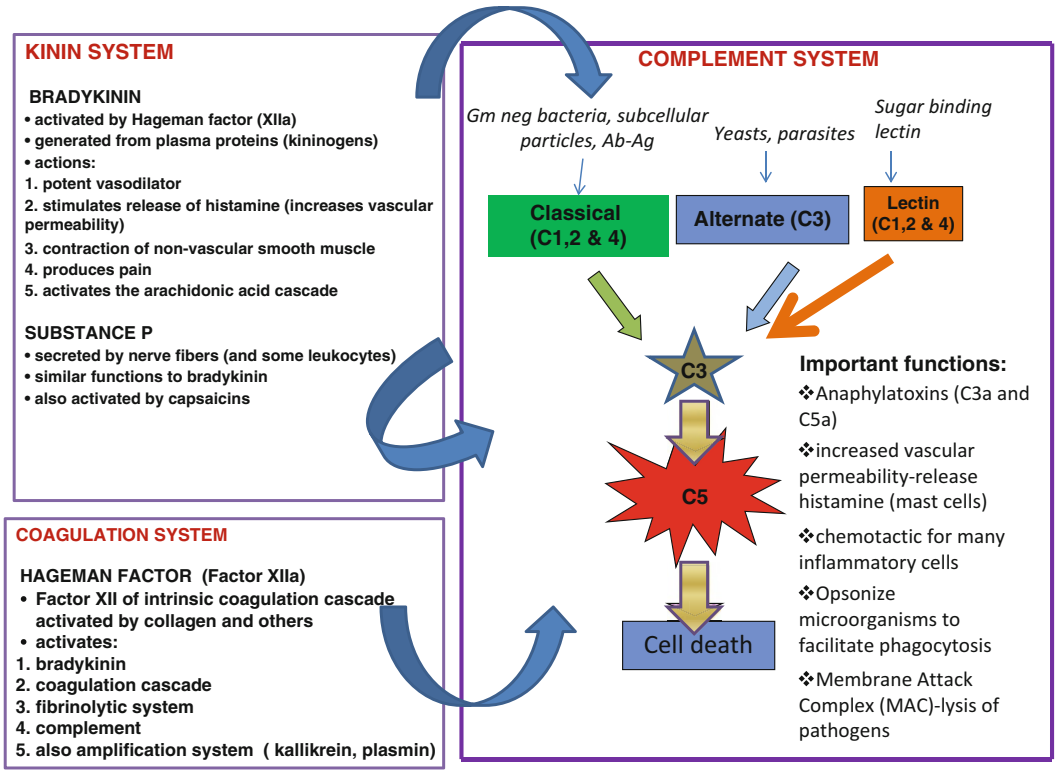


Fig. 4.7 *Relationship between kinin, clotting, fibrinolytic, and complement systems*. In inflammation, the complement, clotting, fibrinolytic, and kinin systems are activated in response to one another in an interlocked cascade

matory mediator, and cytokines liberated from injured cells promote the immediate expression of P-selectin and E-selectin of similar functions on endothelial outer cell surfaces. These receptors bind feebly to glycoprotein ligands expressed on leukocyte surfaces, and the leukocyte starts "rolling" along the endothelial surface. Other molecular expressions induced by cytokines on the outer surface of the cells are adhesion molecules, namely, vascular adhesion molecule-1 (VCAM-1) and ICAM-1 (immunoglobulin ligands), which further slow down the rolling leukocytes (Delves et al. 2006).

Leukocytes are weakly bound to these cell surface molecules and are free to detach if not properly stimulated by chemokines produced from the injured tissues. Chemokine activation starts expressing greatly the bound integrin receptors in a high-affinity state for various immunoglobulin ligands on the surface of an endothelial cell. This tightly binds the leukocytes to the endothelial surface. The chemokine gradients stimulate the adhered leukocytes to "migrate" (or transmigrate) over the endothelium and pass through the basement membrane into the tissue space through the process of diapedesis. The leukocytes start moving through the tissue through "chemotaxis." Leukocyte cells reach the tissue interstitial space and bind to extracellularly expressed matrix proteins (CD44 and integrins) to prevent their fall from the site. The flow of leukocytes toward the source of inflammation along a chemotactic gradient is mediated by these chemoattractants (Delves et al. 2006).

After reaching the locale of inflammation or infection, leukocytes released inflammatory mediators, which control the subsequent activation and later accumulation of several immune cells. Depending on the antigenic stimulus at the site of chronic infection, autoimmune reactions, or a shorter inflammatory reaction, the immune system is evoked and controlled by the antigen

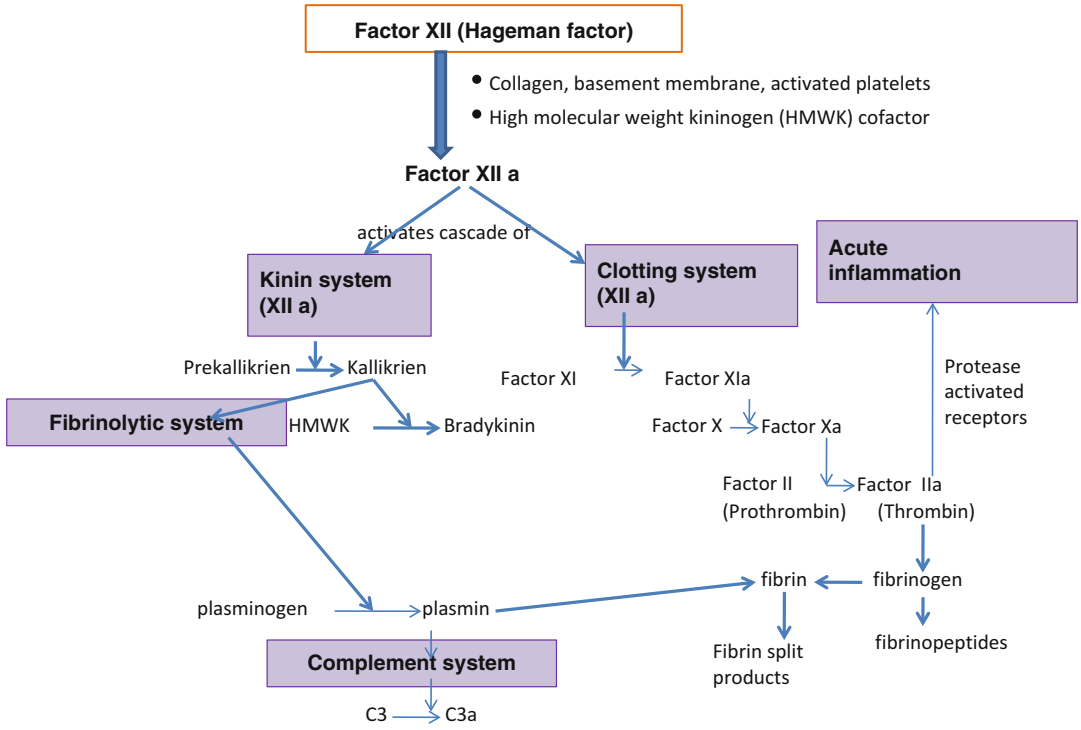


Fig. 4.8 *Three interrelated systems of inflammatory mediators*. The complement, coagulation, and kinin systems are the three interrelated and interconnected inflammatory pathways that act as mediators in both acute and chronic inflammation

itself. There are four major systems of plasma enzymes, which have a pivotal function in homeostasis and control of inflammation. These are the enzyme system of complement cascade, clotting proteins, the fibrinolytic (plasmin) system, and the kinin system (Delves et al. 2006).

Defect in leukocyte function leads to margination and adhesion of cells observed in leukocyte adhesion deficiency, whereas emigration toward a chemotactic stimulus like drugs and chemotaxis inhibitors and defective phagocytosis are observed in diseases like chronic granulomatous disease (CGD) or Chediak–Higashi syndrome.

4.4.2 Role of Lymphatics in Inflammation

Lymphatics are mainly responsible for draining the edema during inflammation. Edema is exemplified by a surplus of fluid accumulation in the interstitial tissue or serous cavities. Edema may be a transudate or exudate. A transudate is nothing but an ultrafiltrate of blood plasma and contains less plasma proteins (mostly albumin) where the permeability of endothelium is usually normal. In contrast, exudates are a filtrate of blood plasma with high plasma protein content, mixed with inflammatory cells and cellular debris; also the permeability of endothelium is usually altered. Pus is a purulent inflammatory exudate containing parenchymal cell debris and high content of leukocytes (mostly neutrophils) (Libby 2007; Delves et al. 2006).

4.5 Regulation of Inflammation

Immunoregulatory pro-/anti-inflammatory cytokines are the ones that accelerate/decelerate the process of inflammation and thus control inflammatory reactions either indirectly or directly and

stimulate the production of cell adhesion molecules or several other cytokines in diverse cell types. A collection of cytokines are known as pro-/anti-inflammatory cytokines because they accelerate/decelerate the inflammatory responses either directly or indirectly by their ability to activate the synthesis of some cellular adhesion molecules or several cytokines in some definite cell types (Libby 2007; Delves et al. 2006).

Pro-inflammatory cytokines is a common term denoted for those immunoregulatory cytokines that favor inflammation. IL-1α, IL-1β, IL-6, and TNF-α are the major pro-inflammatory cytokines responsible for early responses. LIF, IFN-γ, OSM, CNTF, TGF-β, GM-CSF, IL-11, IL-12, IL-17, IL-18, IL-8, and a group of other chemokines that chemoattract several inflammatory cells are the other collections of pro-inflammatory mediators. These cytokines either behave as endogenous pyrogens (IL-6, IL-1, TNF-α), upregulate the production of pro-inflammatory cytokines and secondary mediators by both mesenchymal cells (including fibroblasts and epithelial and endothelial cells) and macrophages, activate the production of some acute-phase proteins, or attract diverse inflammatory cells. Anti-inflammatory cytokines actually counteract the synthesis of pro-inflammatory cytokines and also exert influences on several inflammatory responses in vivo (Libby 2007; Delves et al. 2006).

Anti-inflammatory cytokines is a broad-spectrum term for those immunoregulatory cytokines that actually neutralize various aspects of inflammation, namely, the cell stimulation or the synthesis of pro-inflammatory cytokines, and thus in addition also control the magnitude of the inflammatory responses/reactions in vivo. IL-4, IL-10, and IL-13 are the prominent anti-inflammatory cytokines. Soluble receptors for TNF or IL-6 as well as IL-16, IFN-α, TGF-β, IL-1ra, and G-CSF are some anti-inflammatory mediators. These mediators act chiefly by the reticence of the synthesis of pro-inflammatory cytokines or by neutralizing/balancing many biological mechanisms of pro-inflammatory mediators in several ways (Libby 2007; Delves et al. 2006).

The final effect of an inflammatory response is decided by the stability between anti-inflammatory (e.g., IL-4, IL-10, IL-13, IL-16, IFN-α, TGF-β, IL-1ra, G-CSF, soluble receptors for TNF or IL-6) and pro-inflammatory cytokines. It should be recorded that the general and clear-cut grouping of cytokines as either a pro-inflammatory or anti-inflammatory one may be quite misleading. The duration, type, pattern, and also the expansion of cellular activities activated by one definite cytokine can be regulated considerably by the property of the target cells, the surrounding microenvironment of a cell, the type of neighboring cells, the activation state and growth of the cells, different cytokine concentrations, the presence of other accumulating cytokines, and even the sequential flow of several cytokines procuring on the same cell (Li et al. 2007; Iwalewa et al. 2007; Libby 2007; Delves et al. 2006; Goldsby et al. 2007; Guyton and Hall 2001).

4.6 Mediators of Inflammation

At the inflammatory sites, different anaphylatoxins of the complement cascade, kinin proteins of the coagulation system, some prostaglandins, leukotrienes, and several other lipid mediators act in a confined manner at the site of infection and tissue damage and at more distant locale to disperse and support the inflammation process. Inflammatory mediators serve as muscle-active molecules, edema-promoting substances, chemotaxins, and cellular activators and activators of different kinds of effector cells engaged in the inflammatory response (Rang and Dale 1999; Libby 2007; Delves et al. 2006). The advanced and efficient process of cellular inflow to inflammatory sites is carried over by a plethora of mediator molecules dispersing and supporting inflammation. These inflammatory mediators are found in the tissue fluids or serum, are discharged by degranulating cells, and are also secreted by some inflammatory cells upon stimulation or triggered endothelial cells in blood vessels at the locale of inflammatory responses. They actually serve as edema-promoting substances, muscle-active molecules, chemotaxins, and cellular acti-

vators and inducers of all kinds of effector cells involved in the inflammatory response (Figs. 4.7, and 4.8) (Rang and Dale 1999; Li et al. 2007; Iwalewa et al. 2007; Libby 2007; Delves et al. 2006; Goldsby et al. 2007; Guyton and Hall 2001). Inflammatory mediators embrace some well-researched compounds like anaphylatoxins of the complement cascade, kinin proteins of the coagulation system, prostaglandins, leukotrienes, and many other related lipid mediators (Figs. 4.7, 4.8, and 4.9).

Another group of inflammatory mediators is neuropeptides like VIP (vasoactive intestinal peptide), tachykinins, and VPF (vascular permeability factor). These substances have vasodilatory and bronchoconstrictory activity with enhanced capillary permeability and also trigger increased secretion of mucus. At the early onset of inflammatory responses, at the locale of an injury, several cells contain mediators as preformed substances within their storage granules (like histamine) or may quickly switch to produce the inflammatory mediators as required to synthesized metabolites of arachidonic acid (Delves et al. 2006).

Inflammatory mediators are varied soluble and diffusible molecules that act nearby at the locale of infection and tissue damage and at more remote sites. Mediators may be an endogenous (like lipopolysaccharide of gram-negative bacteria) and exogenous (toxins and bacterial products) type. The host immune system of higher organisms can be activated by endotoxins, which simultaneously activate the Hageman factor, coagulation proteins, the complement pathway, and kinin and fibrinolytic cascades, eliciting specialized T-cell proliferation in response to superantigens (Fig. 4.1) (Libby 2007; Delves et al. 2006; Guyton and Hall 2001).

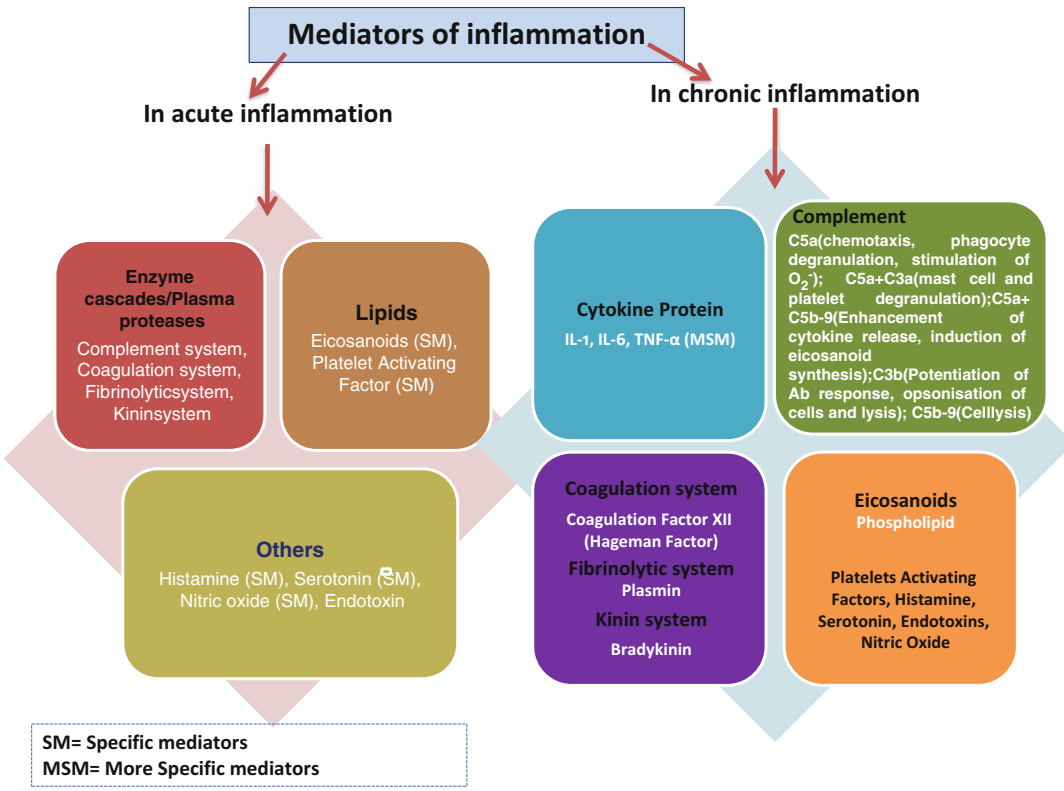


Fig. 4.9 *Types of mediators in acute and chronic inflammation*. Different subsets of mediators are released in acute or chronic inflammation. Some common mediators are present in both types of inflammation but they function differentially. *SM* specific mediators, *MSM* more specific mediators

Monocytes, macrophages, and neutrophils and a class of inflammatory mediators, controlled by cytokines, regulate different plasma enzyme systems to actively phagocytosed microbes. These cells possessing specialized granules have receptors for different complement components and also for Fc domains of immunoglobulins. NK cells and cytotoxic T lymphocytes also possess granules responsible for their cytotoxic function, involved in the adaptive and early inflammatory responses in concoction with innate immunity. Inflammatory mediators during inflammation are also discharged at the site of injury by a variety of cell types that either contain them as preformed molecules within their storage granules, like histamine, or which can quickly switch on the mechanism required to produce the mediators as and when required, for example, to produce different metabolites of arachidonic acid (Fig. 4.10) (Libby 2007; Delves et al. 2006; Guyton and Hall 2001).

Bacterial toxin products can act as exogenous mediators of inflammation. Notable among these is endotoxin or LPS of gram-negative bacteria. The immune system of higher organisms has probably evolved in a veritable sea of endotoxin, so it is perhaps not surprising that this substance evokes powerful responses. Endotoxin also activates the Hageman factor, leading to activation of the coagulation and fibrinolytic pathways as well as the kinin system. In addition, endotoxin elicits T-cell proliferation and has been described as a superantigen for T cells) (Libby 2007; Delves et al. 2006; Guyton and Hall 2001).

Endogenous mediators of inflammation are produced in both innate and adaptive immune systems. These mediators can be originated from molecules that are generally present in the plasma in an inactive form, like peptide fragments of some components of complement proteins (Figs. 4.7, and 4.8), coagulation factors, and

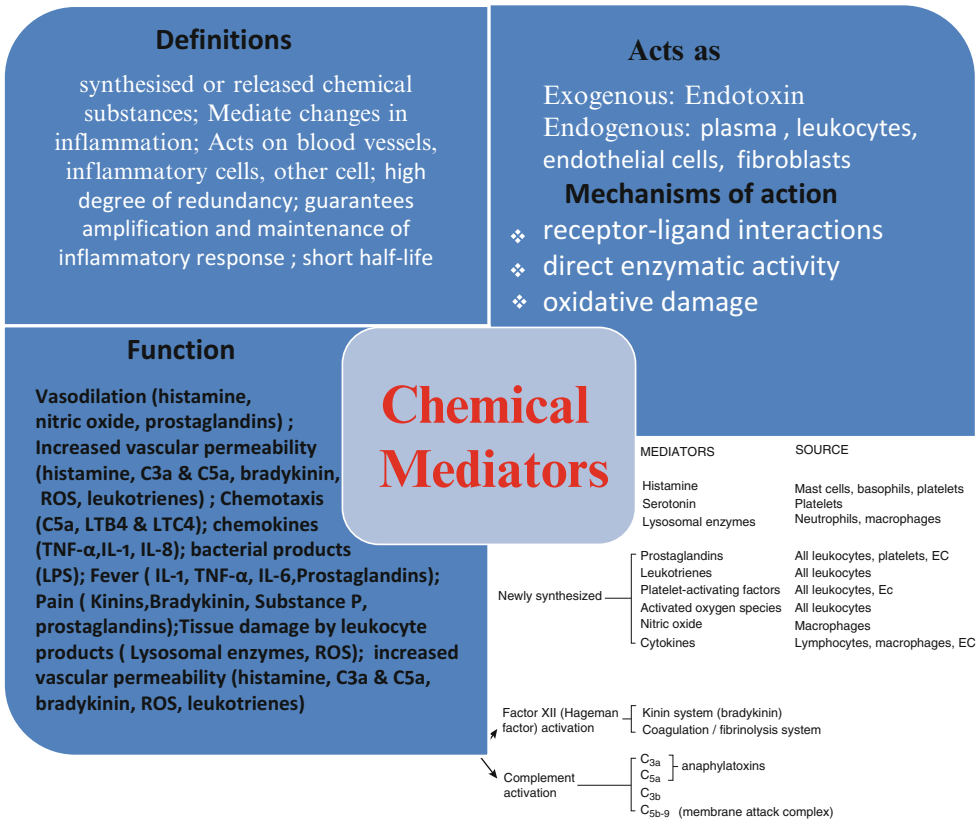


Fig. 4.10 *Chemical mediators*. The definition, types, function, and mode of action of chemical mediators are revealed

kinin systems (Bone et al. 1992; Libby 2007; Delves et al. 2006; Guyton and Hall 2001).

Mononuclear phagocytes like monocytes and macrophages play a central role in inflammation, as they produce many products which contribute in or regulate the different systems of plasma enzyme and hence control the mediators of different inflammatory responses. They are also highly phagocytic and are entailed in microbial killing, as are neutrophils. The role of these cell types is at least biased under the regulation of cytokines. All these inflammatory cells have receptors for complement proteins and for Fc domains of immunoglobulin molecules

They also possess specialized granules containing a diverse variety of products that are discharged perhaps by some general mechanisms. NK cells and cytotoxic T lymphocytes, in a wide-ranging way, also possess granules, which are significant for their cytotoxic role. In general, lymphocytes are engaged in the adaptive immune response to inflammation, and the initial events of inflammation are mediated in fraction by different molecules produced by cells of the innate immune system.

Early-phase mediators are produced generally by platelets and mast cells. They are especially significant in acute inflammation and consist mainly of serotonin, histamine, and other vasoactive substances; cytokines like IL-1, IL-6, and TNF-α; and chemoattractants like C5a. Platelets may contribute to inflammatory responses as a consequence of tissue injury, through a series of mechanisms involving: the release of permeability factors and other vasoactive amines; the release of coagulation factors; the release of lysosomal enzymes, which lead to generalized and localized fibrin deposition/accumulation; and the configuration of platelet aggregates or thrombi which causes the blocking of capillaries and vessels.

Late-phase mediators are accountable for the control of vascular events occurring later—from nearly 6–12 h after the triggering of inflammation. The later vascular events are mediated, at least in part, by various products of arachidonic acid (Bone et al. 1992; Libby 2007; Delves et al. 2006; Guyton and Hall 2001).

Mediator aggregation at local inflammatory series in skin blisters is somewhat dissimilar from the systemic effects after intravenous endotoxin. Under normal conditions, these flows of inflammatory reactions triggered by the mediators are sternly regulated. Failure to do so can direct to multiple organ failure which is known as systemic inflammatory response syndrome. Suitable inhibitors and inflammatory mediators are, therefore, of chief interest for ameliorating and modulating the effects of inflammatory responses and their sequelae (Bone et al. 1992; Libby 2007; Delves et al. 2006; Guyton and Hall 2001).

4.7 Systemic Inflammatory Response Syndrome

This syndrome is commonly seen as a systemic expression of diverse inflammatory mediators (oxygen free radicals and coagulation factors work in a juxtacrine fashion together with cytokines and related cytokine signals that generally function in an paracrine or autocrine way). Not only anti-inflammatory cytokines but also pro-inflammatory cytokines are increased in the blood and the abnormal condition has been termed as a "cytokine storm." Sepsis or septic shock syndrome is a systemic response to inflammation during an infection. It is a lethal, severe, and frequently hemodynamic breakdown caused by bacterial endotoxins during gram-negative septicemia. This toxic shock syndrome is mainly seen in younger women due to tampons contaminated with *Staphylococcus aureus* bacteria. The bacterial exotoxin, TSST-1 (toxic shock syndrome toxin-1; 23.1 kDa), triggers the synthesis of TNF and IL-1. In transgenic mice deficient in expression of CD28, the essential co-stimulatory signals of CD28 has been established in TSST-1-induced toxic shock syndrome. Chiefly due to tissue acidosis, hypoxia, and severe local modifications of metabolism, the characteristic symptoms are hypotension, insufficient tissue perfusion, and uncontrolled bleeding. The end stage of severe systemic inflammatory response syndrome is represented by multiple organ dys-

functions. The massive deterioration of homeostasis during sepsis, also known as disseminated intravascular coagulation, involves mostly the blood vessels along with fibrinolytic, complement, and blood coagulation processes, platelets, the absence or presence of inhibitors, and the kallikrein–kininogen system. Sepsis management according to symptoms is still one of the hard challenging problems faced by clinicians in intensive care patients (Bone et al. 1992; Libby 2007; Delves et al. 2006; Guyton and Hall 2001).

4.8 Inflammatory Mediators: "The Holy Grail"

In this chapter, we have tried to focus on the multiple mediators of inflammation which form a network of immune reactions. Mediators are chemical substances liberated by the endogenous trigger released from activated or injured cells during inflammatory response. They may be blocked indirectly or directly by inhibitors of inflammation. Mediators are concentrated in tissues where the observed symptoms or effects are visible. The action of mediators is the same in all species where the phenomenon occurs. It can be destroyed systemically or locally to avoid their undue concentration.

Nonimmune and immune chemical mediators are one of the many factors that control inflammation. There is long list with never-ending additions. There are mediators which suppress the inflammation whereas some others can stimulate the same. A plethora of mediators or a single mediator is responsible for most of the inflammatory signs and symptoms. By antagonizing the action or inhibiting the formation of these mediator(s), an end of treatment of most forms of inflammatory disease would be possible.

Humans and animals are continuously exposed to microbial pathogens, trauma, stress, and injury that can pose a considerable relevance in our daily livelihood. After the initiation of the etiological agent, the organism elicits a set of highly organized immunological, physiological, metabolic, and behavioral responses to represent its strategy to fight the infection. Inflammatory mediators generated at the site of assault in the peripheral tissues communicate with the brain to modify its immune response and upgrade as necessary which aid in its ability to fight and eliminate the source. The groups of mediators, pathways, and key responders/effectors during inflammation are varied and change their modus operandi in a different disease in a different organism.

As inflammation and its network of reactors form a juggling condition, thus to dissect all strings of its web, different animal models are developed in different diseases to mimic the original scenario. Inflammation is induced through varied stimulator molecules in these animals even for a single disease in a different setup to decode the mechanism of inflammation and bring out probable diagnostic and therapeutic agents.

These studies have also provided evidence of systemically generated inflammatory mediators versus local molecules both in chronic and acute inflammation. The phenomenon of inflammation is initiated by mononuclear phagocytes, which in turn synthesize diverse inflammatory mediators, the cascade of complement pathways, antibody production, and subsequent tissue repairing and resolution. Thus it traverses through both innate and adaptive immune systems. While these responses are part of our normal homeostatic mechanisms, it is quite clear that systemic inflammation has a detrimental effect in humans and also in animals.

Animal models have great inputs in our comprehending of the factors that regulate an inflammatory disease development, and they may also present valuable tools for budding novel therapeutic strategies. In inflammatory disease-induced models, chemical and cellular mediators are administered to induce acute or chronic diseases. It is useful for studying the involvement of multiple immune processes. Indeed, the diverse circulated published papers have researched on animal models revealing acute phase with mostly innate immune responses, but some are known as chronic pathologies. Thus a significant feature of these models is that the supervision of repetitive cycles of mediators leads to intestinal chronic inflammation, which allows significant revelation

about the adaptive immune responses. This permits studies of inflammatory mediators and cellular inflow associated in the severity process of inflammatory disease.

The mediators of inflammation responsible for the pathobiology of an inflammatory disease are of an assorted nature, and diverse clinical studies have reported contrasting results, especially for their cell inflow and protein expression mechanisms. Such discrepancy in observations may at least in some part be explained by the definite methodologies used, varied time points at which analyses were done, and mice strain susceptibility and/or concentration (or dose) of the administered inducer. Moreover, to the superlative of our collective research knowledge, quite a few studies aimed to elucidate the kinetics of inflammatory cascades regulating acute and chronic phases of inflammatory disease.

In this framework, the ongoing chapter aimed to judge cellular inflow and inflammatory markers during the phases of acute and chronic inflammatory disease of humans induced in mice. This chapter showed very significant differences among the stimulation phase and the consequent recovery phase which may be useful for future modeling of the experimental inflammatory diseases. This may particularly provide sturdy evidence for the recognition of inflammatory biomarkers essential for the prediction of disease pathogenesis and the designing of possible therapeutic strategies.

It is quite evident from the clinical history from the hospital that no specific mediators were investigated during the routine investigations in the diagnosis process. Rather some broad-spectrum inflammatory markers (like CRP, ESR, procalcitonin, etc.) were diagnosed which will point toward the process of inflammation and not to the pathways of activation of mediators, whereas in the laboratory people are developing inflammatory animal models to understand all possible molecules involved in inflammation. Very soon in hospitals, definite inflammatory markers for each disease will be investigated for routine diagnosis.

Over the past years, much study is ongoing to highlight the significance of understanding the pathology of inflammatory disease for the procurement of safe and efficient pharmacological treatments. In this milieu, we highlighted a few of the immunological mechanisms that occur during acute and chronic periods of inflammation, with its remission/recovery period, in some of the widely used experimental model of a spectrum of diseases. These diseases are quite common ailments and are posing quite a nuisance not only to global health but also in proving obstructions in its socioeconomic developments.

During the initial recovery phase, the undertaking of the body resolves the inflammation upshot in a complete reduction of the some inflammatory mediators, while some inflammatory mediators did not even reach normal levels. Also after the repetitive phases of administration with inducer molecules, there is a greater elevation in intensity, and some different partners of inflammatory mediators were added to the sequential immune mechanisms. Although during the succeeding recovery phase, a new homeostatic mechanism of the body to reimburse and resolve inflammation was observed, but interestingly, the clinical factors were still persisting, revealing the chronic phase.

This present chapter revealed different inflammatory mediators, the cascade of inflammatory sequences, and cell inflow in the acute and chronic period of broad spectrum of diseases. The pro-inflammatory reactions were predominant in the stimulation phase; pro-resolution mediators were revealed observed during the recovery processes. Strikingly, some important mediators were still persisting even after the initial recovery phase and thus seemed to add to the magnified inflammatory reactions elicited in the subsequent induction phase. Also, our observations revealed a balance between anti- and pro-inflammatory mediators and a contrasting disparity between anti- and pro-inflammatory responses in the consequent recovery phases which dampen the course of resolution of inflammation with the repairing of tissue. Thus, this chapter contributes to improve comprehension of the inflammatory models and emphasizes the importance of understanding the fundamental mechanisms associated with the pathophysiology of experimental animals for development of

appropriate therapeutic interferences in human inflammatory diseases.

What needs to be addressed in research in inflammation biology is that whether it is a cause of disease or the result of it or just a side effect of the disease. No clear idea exists as to whether inflammation actually causes chronic diseases or merely accompanies them. Further research needs to be conducted in the area of genes and molecules involved in causing inflammatory disorders thereby improving the diagnosis and treatment of such diseases. The question we pose here is that whether it is possible to screen the mediators of inflammation and their levels of expression in diseases associated with inflammation and correlate them with the clinical history. This would offer an advantage both from the point of view of diagnosis, therapy, and prognosis of inflammatory disorders. The future scope of this chapter remains in identifying such markers for inflammation and its correlation with disease condition.

4.9 Biomarkers of Inflammation

A biomarker is a parameter (chemical, physical, or biological) that can be applied to detect and compute the progress of disease or the results of treatment in preclinical research and clinical diagnosis. More significantly, a biomarker points out a change in state or expression of proteins, peptides, genes, and other factors that associate with the progression or risk of a disease, initial diagnosis, drug response, susceptibility of the patient to a given treatment, drug target identification, or disease intervention. The whole subset of biomarkers might be acknowledged using proteomics technologies, genomics, different imaging technologies, and invasive or noninvasive laboratory investigations using different physical parameters (Fig. 4.11) (Mayeux 2004; Kumar and Sarin 2009).

In laboratories, animal models for inflammatory diseases are being developed to understand

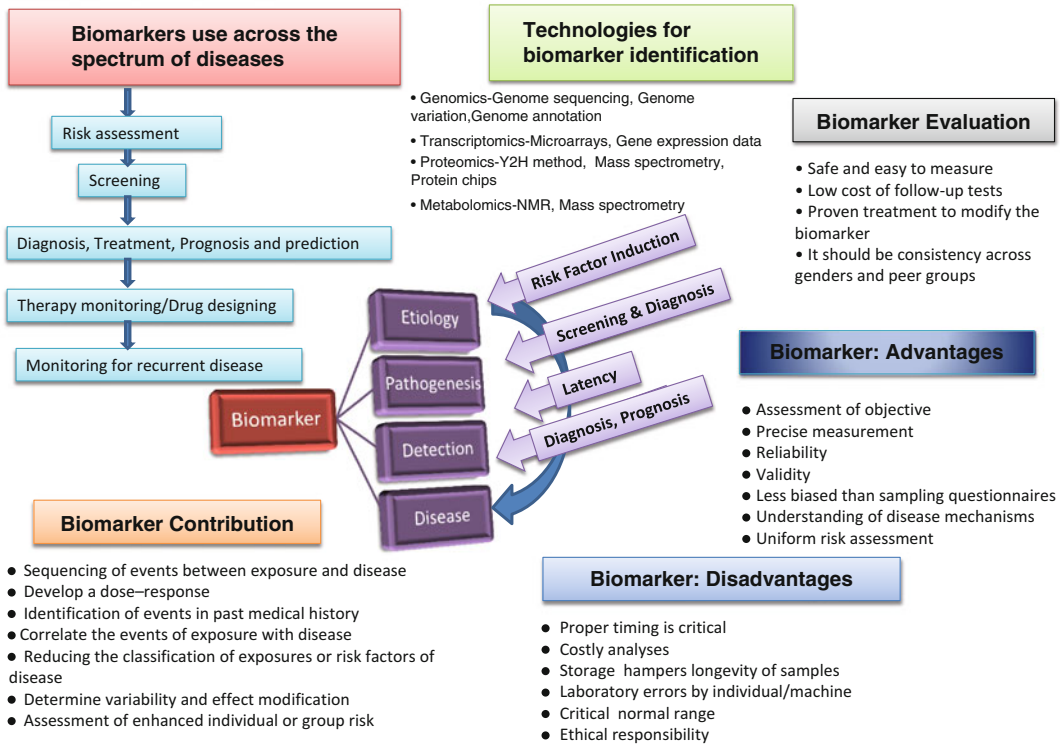


Fig. 4.11 *Disease pathogenesis and potential applications of biomarkers*. Different subsets of biomarkers are released from tissues and tumor or present in blood, urine, or other body fluids. The screening, diagnostic, prognostic, and therapeutic applications of biomarkers are presented with respect to disease pathology (Mayeux 2004; Kumar and Sarin 2009)

the process of inflammation, identify inflammatory biomarkers, and find out their probable role in therapeutics. Currently, there are no specific markers for inflammation; rather some broad-spectrum inflammatory markers were routinely investigated in hospitals. The question we asked is, since the biochemistry of inflammation is known, is it possible to identify some potential biomarkers of inflammation for safe assessment in diagnosis, treatment, and prognosis which may in the future be helpful for routine hospital diagnosis (Fig. 4.11)?

However, inflammatory biomarkers are often examined one by one; their interrelation and clear-cut aspects of their associated pathobiological mechanisms remain unclear. Explanation of these relationships could aid the suitable implementation of prognostic biomarkers in different clinical practices. Biomarkers in inflammation are gaining increasing interest given their clinical benefits. The most commonly used biomarkers are presented first, followed by a description of variable acute conditions with their relevant biomarkers. In addition to the conventional use of these biomarkers, other biomarkers are outlined in variable critical conditions that may be related to acute inflammation.

Biomarkers can be defined as any alterations in the constituents of body or tissue fluids. These markers provide a medium for uniform classification of a disease with its risk factors and can be extended in understanding the basic underlying pathophysiology of disease. Biomarkers provide a powerful and dynamic tool to grasp the spectrum of inflammatory diseases with usage in observational and analytic epidemiology, clinical trials in populations, and screening with diagnosis and prognosis. Biomarkers can also reflect the entire steps of a disease from the earliest symptoms/screening to the terminal stages. Analytical assessment of the validity of biomarkers is required to correlate with respect to the stage of disease. Variability in the measurement of biomarkers ranges from individual error in laboratory technicians, machine dysfunction, improper storage of body fluid, and other bias and confounding issues.

There has been a major sea change, in the past decade, in the way disease is diagnosed and investigated with the usage of high-throughput technologies, like proteomics, genomics, lipomics, metabolomics, microarrays, lab on a chip, etc. These advances have paved the way to the discovery of novel inflammatory disease biomarkers relating to cancers, autoimmune disorders, intestinal diseases, endocrine diseases, genetic disorders, neural damage, etc. In many cases, these developments have gone together with the discovery of biomarkers expressed via traditional or conventional methods, such as immune histopathology or clinical biochemistry. These inflammatory disease biomarkers together with microprocessor-based statistical data analysis, bioinformatics, and newer invasive/non-invasive analytical methods have been used to identify individuals with active disease versus refractory pathology versus those having distinguishing pathologies. Sometimes diagnosis relies on a cohort of biomarkers rather than a single disease biomarker. The techniques and methods of elucidating a biomarker are complex, and unfortunately an inflammatory disease biomarker cannot be readily transferable to other disease states. Thus there is a demand for a focused and comprehensive study in search of universal clinical biomarkers which is the urgent need in wide areas of cancer, nutrition, cardiology, endocrinology, immunology, addictions, birth defects, genetics, etc., to correlate with their therapeutic applications.

4.10 Inflammatory Mediators and Different Biomarkers in Different Subsets of Diseases and Related Immunomodulatory Therapies and/or Strategies

4.10.1 Intestinal Inflammation in Inflammatory Bowel Disease (IBD)

Inflammatory bowel disease (IBD) is a heterogeneous collection of inflammatory situations of the small intestine and colon which affects millions of people worldwide. These two diseases

express familiar symptoms or some common extraintestinal complication of recurrent diarrhea, abdominal pain, rectal bleeding, anemia, weight loss, vomiting, and severe internal cramps or muscle spasms in the pelvis. Generally diagnosis mainly involves assessment of inflammatory markers in stool and colonoscopy and biopsy of pathological lesions. The two main forms of IBD are ulcerative colitis (UC) and Crohn's disease (CD) characterized by massive cell influx and release of varied pro-inflammatory mediators to the intestinal tissues. By contrastingly different research groups, different experimental animal models were developed where the mediators are administered to induce different forms of IBD in the animal.

In UC, the laboratory tests most used to measure the APPs in clinical practice are the serum concentration of CRP and the erythrocyte sedimentation rate. Other biomarkers of acute-phase response include platelet and leukocyte count and serum albumin and orosomucoid concentrations. There is a significant difference in the CRP response between UC and CD as reported in literatures. A clear increase in CRP is described in CD patients, whereas in UC the response is slight or absent. For these differences, there is no satisfactory explanation. Serum IL-6 levels were significantly elevated in patients with CD compared to UC patients and normal individuals. The most sensitive serologic markers of inflammation in adult population for detecting IBD are CRP as compared to other markers. The sensitivity of CRP is quite high which ranges from 70–100 % in the differential diagnosis between CD versus irritable bowel syndrome. To differentiate between CD and UC, the higher levels of CRP in active CD than in UC might be used. For differentiation between both types of IBD, the measurements of circulating levels of CRP and ESR and platelet count are not useful at all (Cioffi et al. 2015).

Inflammatory marker levels of CRP are associated with poor sleep quality, and clinical disease activity in IBD suggested a relation between circulating inflammatory markers and sleep. The key drivers of poor sleep quality are due to the common symptoms of IBD like diarrhea and abdominal pain (Wilson et al. 2015).

Dextran sodium sulfate (DSS) can induce both acute and chronic colitis in animal models. This induction with DSS was differentiated by severe disease activity, immense colonic polymorphonuclear penetration, and elevated levels of TNF-α, IL-17, VCAM-1, and keratinocyte-derived chemokine (CXCL1/KC). In the recovery phase of intestinal inflammation, marked elevation of IL-10, IL-4, TGF-β, and cyclooxygenase 2 (COX-2) as anti-inflammatory mediators was seen. In chronic experimental colitis, nuclear factor κβ (NFκβ) and regulatory T-cell marker forkhead box P3 (FoxP3) concentrations were elevated gradually representing immune disbalance in intestinal mucosal inflammation (Bento et al. 2012).

The worldwide food-borne bacterial enterocolitis infection is caused by a zoonotic pathogen *Campylobacter jejuni*. It forms an integral part of the commensal flora in many domestic and wild animals including broiler chickens which primarily transmit this pathogen to humans. Gnotobiotic IL-$10^{-/-}$ mice were generated as a suitable vertebrate model in the laboratory by quintuple antibiotic intervention to mice just after weaning to protect these animals from chronic colitis. Then oral infection of *C. jejuni* leads to colonization of the bacteria in the gastrointestinal tract and resultant acute enterocolitis within 7 days as observed by bloody diarrhea and significant histopathological changes of the colonic mucosa. The inflamed colon was immunopathologically characterized by greater numbers of B and T lymphocytes, regulatory T cells, and apoptotic cells as well as increased concentrations of IFN-γ, TNF-α, and MCP-1 mimicking severe phases of human campylobacteriosis as a perfect animal model in vivo. In control, animal models infected with another commensal bacterium, *E. coli* indicate no symptoms of the disease. The lipoproteins and lipooligosaccharides of *C. jejuni* are the inflammatory mediators that use the toll-like receptor (TLR-2 or TLR-4) signaling pathways as mice lacking these TLRs are abated from intestinal immunopathology (Haag et al. 2012).

Enteric glial cells (EGCs) have protective functions against pathogens and play an important role in the continuation of gut homeostasis to support intestinal inflammation. EGCs may be activated and proliferated in reaction to inflammation and injury undergoing reactive gliosis (enterogliosis) due to alterations in the homeostasis of the enteric nervous system. Enterogliosis is exemplified by the massive overexpression and secretion of distinct S100B protein like astroglia-derived signaling molecules. S100B is a highly diffusible; Ca^{++}/Zn^{++} and p53-binding protein coordinate different pro-inflammatory signals thus playing a vital role during intestinal inflammation. Pentamidine directly blocks S100B activity, as an antiprotozoal drug. Acute UC was induced in mice model by inflammatory mediator DSS (4 % DSS for 4 days) as compared to control mice group and colitis groups with pentamidine treatment (0.8 mg/kg and 4 mg/kg). The anti-inflammatory role of pentamidine was evaluated in colonic tissue by evaluating the disease activity index (diarrhea, blood in the feces, animal weight loss); histopathological severity; postmortem evaluation of cyclooxygenase-2, S100B, glial fibrillary acidic protein, and iNOS; p50 and p65 protein expression; phosphorylated-p38, MAP kinase, and myeloperoxidase activity; malondialdehyde synthesis; and macrophage influx in colonic tissues. Also plasma concentrations of NO and prostaglandin E2, IL-1β, TNF-α, and S100B levels were also identified in samples. Additional in vitro quantification was done on longitudinal muscle myenteric plexus (LMMP) preparations and dissected mucosa in LPS + DSS or exogenous S100B protein induction in the absence or presence of pentamidine. The effects of pentamidine intervention on UC induced by inflammatory mediators (DSS, LPS) were implicated in histological/biochemical evaluation and macroscopic observations in colonic tissues (Mishra et al. 2012; Esposito et al. 2012).

In DSS-induced acute colitis in experimental mice deficient in IL-17C, the intestinal inflammation is exaggerated in the presence of inflammatory mediator DSS. But these mice were protected against DSS-induced colon pathology. The colons of diseased IL-17C-deficient mice showed high numbers of $\gamma\delta^+$ and $CD4^+$ T cells. Mechanistically, IL-17C directly controls the expression of the occluding or tight junction molecule in colonic epithelial cells and thus helps in perpetuation of mucosal barrier integrity (Reynolds et al. 2012).

UC is less prevalent in current smokers as compared to ex-smokers and nonsmokers. Though there were reports of smokers having lowered rates of colectomy, hospitalization, and requirement for oral corticosteroids and immunosuppressant to control this disease, other potential active mediators in smoking may pose some clinical effects. Nicotine's application in therapeutic regime in ulcerative colitis is variable as compared to conventional medicines and placebo as it modifies inflammation and risk in ulcerative colitis. The adverse events of nicotine limit its clinical relevance (Lunney and Leong 2012).

In DSS-induced mice, UC was developed resulting in acute intestinal inflammation. In colon tissues, histological alterations (eosin and hematoxylin staining), neutrophil inflow (myeloperoxidase assay), levels of iNOS (immunohistochemical staining), and mRNA expression of pro-inflammatory mediators like TNF-α, IL-1β, IL-6, and IFN-γ by RT-PCR were evaluated. Aqueous extract of chaga mushroom (*Inonotus obliquus*) (IOAE) at treatment doses to UC mice indicated suppressed mucosal damage, edema, and the loss of crypts in histological examinations in colon tissues. IOAE acts as a suggestive anti-inflammatory agent at colorectal sites due to slowing down of the expression of inflammatory mediators and thus may be useful addendum in the setting of IBD (Mishra et al. 2012).

4.10.2 Inflammatory Airway Disorder

Asthma, or bronchial asthma (BA), affects millions of people worldwide. It is a chronic inflammatory disease of the airways. It causes periodic outburst of coughing, shortness of breath, wheezing, increased contractibility of surrounding smooth muscles, bouts of narrowing of the airway, and chest tightness.

BA is associated with the interplay of various inflammatory mediators, cell infiltration (T lymphocytes, eosinophils, and mastocytes), and the liberation of many membrane bound or co-stimulatory soluble molecules (CD40, CD40L, CD30, TNF receptor, and B7-H3). The concentrations of soluble OX40L (sOX40L), the vital co-stimulatory signal molecule, increases in asthmatic children and adults and also in many diseases. Pulmonary functions by spirometer and serum concentrations of sOX40L by ELISA were detected in acute asthmatic adult patients as compared to the control group. The patients were graded according to their disease severity into stable, severe, moderate, and mild asthmatic group. In adult asthmatic patients, the serum levels of sOX40L (6.80 ± 4.95 ng/L) were distinctly elevated than that of the control population, and they were negatively associated with pulmonary function indexes. Also, serum concentrations of sOX40L showed prominent differences among severe, moderate, and mild control groups, and its concentrations reduce to the same extent as the control population after therapeutic intervention of the adult asthmatic patients. Thus sOX40L level may act as a possible inflammatory mediator in the pathology of asthma (Lei et al. 2012).

In the diagnosis of asthma, the most important cells are eosinophils and sputum eosinophilia. Apart from local inflammation, systemic inflammation in asthma can be revealed by increased concentrations of CRP. By ultrasonic nebulizer, sputum was induced, and then peripheral venous blood samples were collected to calculate the concentration of CRP (by ELISA) and to count peripheral cells. Serum levels of high-sensitivity CRP (hs-CRP) were significantly elevated in the inhaled steroid nonuser group as well as user group as compared to healthy controls. Sputum and peripheral blood eosinophilia were seen in steroid nonusers as compared to the healthy individuals. In the steroid nonuser group, serum hs-CRP levels were positively associated with sputum eosinophilia, which was not statistically significant. The levels of hs-CRP did not get affected by age, sex, and atopy status in both asthmatic groups. Thus, serum hs-CRP being a biomarker can indirectly reflect the extent of airway inflammation (Halvani et al. 2012).

The transcription factor, NK-κβ, performs a crucial role in the pathogenesis of asthma. Dehydroxymethylepoxyquinomicin (DHMEQ), a compound that inhibits NF-κB activation, was reported to abate various inflammatory diseases in animal models. In ovalbumin-induced inflamed BALB/c mice, DHMEQ was administered before induction. DHMEQ significantly reduces concentrations of TH2 cytokines in bronchoalveolar lavage fluid and lowered eosinophilic airway inflammation, mucus production, peribronchial fibrosis, eotaxin-1, and the expression of α-smooth muscle actin. Thus DHMEQ inhibits allergic responses in airway inflammation; in murine models, airway remodeling of asthma may be used in therapeutic intervention (Shimizu et al. 2012).

Chronic obstructive pulmonary disease (COPD) is a very common lung disease with inflamed lung condition associated with mild to moderate breathing problem. The two main forms of COPD are chronic bronchitis (CB) or progressive pulmonary inflammation with a mucus-laden long-term cough and destruction of the lungs for a long time by emphysema. The symptoms of COPD are cough and breathlessness to an extreme of ischemic heart disease, stroke or death in severe cardiovascular complications. Actually in COPD and in other related chronic lung inflammation, atherosclerotic plaque formation and rupture of plaque lead to cardiovascular events (Man et al. 2012).

Chronic airway inflammation is a combinational indicator of several diseases like asthma, COPD, and cystic fibrosis. The symptoms occur after the failure of immune system to combat an acute inflammation spontaneously resulting in structural and functional alterations in the parenchyma and walls of the airways, uninterrupted influx of different inflammatory cells toward the locale of inflammation, and production of protein (like chemokines, cytokines, enzymes, etc.) and eicosanoids (pro-inflammatory mediators). Eicosanoid, an n-6 polyunsaturated fatty acid (PUFA), is mainly synthesized by the metabolism of arachidonic acid in the membrane phos-

pholipids. Contrastingly, anti-inflammatory n-3 PUFA decreases the synthesis of pro-inflammatory cytokines and functions of immune cell, releases some anti-inflammatory pro-resolving mediators (protectins and resolvins), and may be used in airway disorders with an inflammatory constituent (Giudetti and Cagnazzo 2012).

hs-CRP indicates low grade of systemic inflammation in COPD. COPD associates positively with hs-CRP and smoking status and negatively related with body mass index. Apart from forced expiratory volume (FEV), the potential of CRP as biomarker to complement the present system of staging with the help of FEV can be expanded (Nillawar et al. 2012).

Acute lung injury (ALI) caused by any local or systemic inflammatory stimulus results in a dispersed heterogeneous lung injury symptomized by hypoxemia, low lung compliance, and noncardiogenic pulmonary edema with widespread capillary leakage. For treatment of ALI and pulmonary fibrosis, transplantation of bone marrow mesenchymal stem cells (MSCs) is one of the possible resorts. Experimentally, rats were exposed to cigarette smoke (CS) for nearly 11 weeks followed by administration of rMSCs into the lungs. Infusion of rMSCs mediates a reduced pulmonary cell apoptosis; a downregulation of TNF-α, IL-1β, MCP-1, and IL-6 (pro-inflammatory mediators); and an upregulation of vascular endothelial growth factor (VEGF), proteases (MMP9 and MMP12) in lung, VEGF receptor 2, and TGFβ-1 and improves destructive pulmonary function and emphysema triggered by CS exposure (Guan et al. 2013).

High plasma CRP levels within 48 h of ALI in children were tested along with its association in 28-day mortality and ventilator-free days (VFD). The CRP level in nonsurvivors was 126 mg/L which was quite high than in survivors (CRP = 56 mg/L). As cardiovascular organ failure at onset of ALI was the strongest predictor for mortality, so for every 10-mg/L rise in CRP level, mortality increased by 4.7 %. Increased CRP levels were thus associated with a decrease in VFD. Therefore, increased plasma CRP levels have no favorable outcome in ALI in children, but it is in contrast with findings in adults with ALI (Bruijn et al. 2013). CRP is used as an inflammatory biomarker to distinguish ALI or acute respiratory distress syndrome (ARDS) from cardiogenic pulmonary edema. CRP behaves as a diagnostic marker in these diseases. CRP along with brain natriuretic peptide (BNP) behaves as a stronger prognostic marker of these diseases (Komiya et al. 2011).

The ALI along with its severe form, ARDS, is exemplified by greater vascular and epithelial permeability, hypofibrinolysis, hypercoagulation, inflammation, and immunomodulation. A distinct population of an intact lipid bilayer containing small cytosolic vesicles within the microparticles (MPs) in both the alveolar and vascular compartments in respective patient groups or in ALI/ARDS animal models may serve as diagnostic and prognostic biomarkers. MPs are released in vascular, parenchymal, or blood cells containing membrane and cytosolic proteins, different organelles, lipids, and RNA derived from their relevant parental cells. MPs act as modulators, intrinsic stimulators, or even attenuators in some diseases as it can effectively interrelate with various cell types. MPs in ALI/ARDS are derived from diverse cell types of heterogeneous function and may act as a promising therapeutic agent to modulate inflammatory mechanisms by either removing/inhibiting or administrating/stimulating MPs (Mcvey et al. 2012).

Multiple-organ dysfunction syndrome and ALI are initiated by different inflammatory mediators after trauma and hemorrhagic shock (T/HS) to enter into the systemic circulation through mesenteric lymph ducts. Post-HS mesenteric lymph (PHSML) activates polymorphonuclear leukocytes (PMNs), activates red blood cell and vascular endothelial cell dysfunction, and contains biologically active lipids as pro-inflammatory mediators. In the PHSML, phosphatidylethanolamine, lysophosphatidylethanolamine (LPE), phosphatidylcholine, lysophosphatidylcholine (LPC), and sphingomyelin were detected; arachidonoyl, linoleoyl, and docosahexaenoyl LPCs and LPEs were significantly elevated in the PHSML of the T/HS group.

Elastase was also released after induction by linoleoyl and arachidonoyl LPCs. These biologically active lipids in PHSML are involved in the pathology of ALI or multiple organ dysfunction syndrome (Morishita et al. 2012).

4.10.3 Pancreatitis

Pancreatitis is an inflammatory disease of the pancreas which requires medical attention and immediate hospitalization during an attack. It occurs mainly when trypsin and other pancreatic enzymes start digesting food when activated in the pancreas instead of the small intestine. Acute pancreatitis (AP) begins suddenly and lasts for a few days, whereas in chronic pancreatitis (CP) it lasts for many years. The most common (~80 %) etiology of AP and CP is gallstones and alcohol, respectively. Pancreatitis has multiple causes including some viruses (cytomegalovirus, hepatitis B, mumps), bacteria (*Mycoplasma*, *Salmonella*), fungi (*Legionella*, *Aspergillus*), and parasites (*Ascaris*, *Cryptosporidium*) and a number of other infectious agents have been recognized.

AP if present in its severe form includes systemic organ dysfunction, local pancreatic complications (pseudocysts, necrosis, or abscess), or both. Severity of AP depends on a number of causal factors which includes both systemic organ failure and local peripancreatic necrosis. In AP, inflammatory mediators initiate the intracellular stimulation of pancreatic proenzymes and/or NF-κB. Thus, stimulated leukocytes penetrate deep into and around the pancreas and decide a central role in the severity of AP. The inflammatory reaction is initially local, which may multiply in cascade to produce excess inflammatory mediators leading to systemic and/or early organ failure (Kylänpää et al. 2012).

These immune responses are combated by the release of specific cytokine inhibitors and anti-inflammatory cytokines thus preventing the hazard for systemic infection. At present, there is no specific treatment for AP, but an enhanced understanding of the pathology of systemic inflammation and the advance of organ dysfunction may prepare for future treatment methodologies (Kylänpää et al. 2012).

In AP patients, the concentration of CRP equal or more than to 150 mg/L even at 48 h after hospital admission was relevantly associated with higher chance of receiving prophylactic antibiotics after prescription. Thus CRP was one of the most prominent biomarkers in prescribing prophylactic antibiotics in AP (Cardoso et al. 2014). In CP patients, systematic inflammation was identified. Patients with osteoporosis and higher levels of inflammatory markers had the highest systemic inflammation. Thus, in CP, the potential alteration of risk factors like CRP may provide an avenue to avert fractures and reduce bone loss in this group (Duggan et al. 2015). CRP along with serum interleukin-6 (IL-6) is used as a diagnostic marker in the differentiation between pancreatic cancer and CP (Mroczko et al. 2010).

In nonalcoholic fatty pancreas disease (NAFPD), obesity is a risk factor. During AP, IL-10 acts as an effective anti-inflammatory cytokine in downregulating the release of pro-inflammatory mediators. Obesity reduces the synthesis of pro-inflammatory cytokines in the spleen, so spleen-originated IL-10 may regulate the NAFPD pathology, caused by high-fat (HF)-diet-induced obesity. In splenectomy (SPX)-treated mice, the increased fat deposition and inflammatory responses of the pancreas in obese mice (with HF diet-induction) were reduced by systemic administration of IL-10. In IL-10 knockout mice, SPX had little effect on inflammatory responses and fat deposition in the pancreas. Thus, obesity reduces IL-10 release by the spleen which may protect against the development of NAFPD (Gotoh et al. 2012).

A biologically active portion of the plant *Nardostachys jatamansi* (NJ; NJ4) was administered intraperitoneally in mice followed by injection with the cerulein (analogue of stable cholecystokinin) for nearly 6 h. After the last cerulein injection, the morphological examination of the lung and pancreas and blood and neutrophil infiltration were done along with cytokine expression. NJ4 administration abates the AP severity and lung injury related with AP, reduces neutrophil inflow and cytokine production, and

results to in vivo elevated levels of heme oxygenase-1 (HO-1). Also, NJ4 along with NJ4-2 (another active fraction) induces HO-1 in isolated pancreatic acinar cells and thus prevents the cerulein-triggered death of acinar cells. NJ4 may be a probable therapeutic agent offering defense in AP and might also lower the severity of the disease by stimulating HO-1 expression (Bae et al. 2012).

4.10.4 Kidney Disease

The kidneys are two organs that cleanse our blood by removing excess fluid and waste, maintain the salt and mineral balance of the blood, and help to regulate blood pressure. When the kidneys are damaged, fluid and waste products can accumulate in the body, causing swelling of the ankles, with weakness, poor sleep, vomiting, and shortness of breath. Loss of kidney function is a serious and potentially fatal state and if left untreated, eventually the diseased kidneys may stop functioning totally.

The most abundant leukocytes in the kidney are DCs and macrophages which are the key players in innate immune responses as they coordinate ischemia–reperfusion injury in the kidney followed by inflammation. They directly induce sterile inflammation after reperfusion by producing pro-inflammatory cytokines, soluble inflammatory mediators, and chemokines and indirectly through activation of NK cells and effector T lymphocytes. DCs possess tolerogenic functions in normal and diseased conditions and macrophages participate in tissue repair. Governing the microenvironment of the kidney and understanding the function and phenotype of DCs and macrophages will throw light on the pathogenesis of this disease and offer novel drug targets (Okusa and Li 2012).

Chronic kidney disease (CKD) or chronic renal disease is the gradual failure in kidney function for a period of few months to some years. The worsening signs of kidney function are highly nonspecific and might normally include an uneasy feeling, anemia, some cardiovascular disease, lowered appetite, and also pericarditis. Often, people with increased blood pressure or diabetes and their blood relatives are at the risk of CKD.

The progression of CKD is aggravated by inflammation and oxidative stress. Oxidative stress leads to depletion of the most prominent endogenous intracellular antioxidant, tissue glutathione (GSH), but dilapidation of oral GSH by digestive enzymes limits its therapeutic interventions. In chronic renal failure (CRF), GSH repression reduces the oral GSH precursor, F1 (contains cystine as a cysteine carrier), which attenuates oxidative stress and inflammation and restores tissue GSH and thus reduces the severity of interstitial nephropathy. Experimental male Sprague Dawley rats were broken up into CRF (rat chow with 0.7 % adenine) group, F1-treated CRF group (rat chow containing adenine with F1) group, and control group (with regular rat chow). Finally, the animals were given regular chow and then euthanized. Consumption of adenine-containing diet causes rigorous kidney swelling; azotemia; high glomerular and tubular injury; lowered urinary concentrating capacity; massive tubulointerstitial nephropathy; severe anemia; elevated levels of markers of oxidative stress, inflammatory mediators (p-IκBα, cytoplasmic NF-κB, NF-κB, cyclooxygenase-2, p65), and plasma oxidized glutathione disulfide (GSSG) 3-nitrotyrosine; and lowered GSH/GSSG ratio and manganese superoxide dismutase. F1 cotreatment caused significantly reduced tubulointerstitial edema and inflammation; higher urinary concentrating capacity, anemia, azotemia; and normalized expression of markers of tissue oxidative and nitrosative stress. Thus, F1 acts as a unique oxidative stress modulator, prominently attenuating inflammation, renal damage, oxidative stress indicators, and renal dysfunction (Nicholas et al. 2012).

Elevated pre-procedural serum hs-CRP levels were correlated with the extended clinical outcomes of subjects with stable chronic kidney disease (CKD) who were implanted with first-generation drug-eluting stent (DES) (Ortega et al. 2014; Ogita et al. 2015). Lower sodium levels in serum had been associated in patients with CKD through their increased mortality. CRP acts

as the independent predictors of lower plasma sodium in CKD patients. Thus higher CRP levels are correlated with lower sodium levels. Thus inflammation could be one of the underlying confounding factors explaining the increased mortality in these CKD patients (Ortega et al. 2014).

4.10.5 Injury

Inflammation without any sterile inflammation or infection leads to either acute injury or chronic disease. In cerebral ischemia, the primary injury is caused by reduced blood supply and contributed by IL-1β. IL-1β is controlled by the protease caspase-1 and its related inflammasome NLRP3, the activating complex. In macrophages, the in vitro NLRP3 inflammasome-dependent responses require an early priming stimulus by a damage-associated molecular pattern (DAMP) or a pathogen (PAMP). In mouse, in the cultured brain-originated mixed glial cells (DAMP ATP), calcium pyrophosphate dehydrated crystals and monosodium urate had no effect on the expression of IL-1β or IL-1α and were released when stimulated by PAMP. Alternately, without priming, these DAMPs may trigger inflammation through the synthesis of CXCL1 and IL-6 and cathepsin B (lysosomal protease). Apart from PAMP, the acute-phase protein like serum amyloid A (SAA) may also behave as a priming stimulus. After cerebral ischemia, in vivo, synthesis of IL-1 increased the overproduction of CXCL1 and IL-6 in the ischemic hemispheres of IL-1α/β double KO mice though in these mice injury-induced cytokine responses were not abated. Thus DAMPs enhance brain inflammation by directly stimulating production of glia-originated inflammatory mediators and IL-1-dependent responses (Savage et al. 2012).

Through in vitro experiments, using necrotic and apoptotic cells revealed the binding of CRP to necrotic and apoptotic cells and also facilitated the removal of such cells. But in vivo experiments performed using animal models having ischemia/reperfusion (I/R) injury revealed that the binding of CRP to such damaged cells is detrimental to the tissue. Thus, the binding role of CRP with phosphocholine is quite unfavorable if it occurs on injured host cells as it causes more damage to the tissue by stimulating the complement pathway. So, in acute myocardial infarction and ischemia–reperfusion injury, the scenario is worsened by CRP. The consequence of the binding of CRP to damaged cells thoroughly depends on the type of tissue. In tissues like skin and subcutaneous tissue, CRP does not harm to bind the complement and hasten the death of the dead tissue. It is different and harmful to remove dead tissue having no regeneration property. In myocardial infarction, CRP will remove the necrotic part of the myocardium. But, in the ischemic part of the tissue, where the damage can be reversed, CRP may remove the tissue as described previously. Thus, the phosphocholine-binding function of CRP is quite defensive for the host because it leads to removal of necrotic tissue and also protection against pneumococcal infection. On the contrary, the phosphocholine-binding function of CRP is detrimental for the host when CRP binds reversibly to the damaged myocardial cells, as it causes more damage to the tissue by activating the complement pathways (Agrawal et al. 2014).

A heat shock protein family, αβ-crystallin, exerts cell protection under various stress-related conditions developed in the mouse model of multiple sclerosis, spinal cord injury brain ischemia, and Alexander disease. After contusion lesions, the levels of αβ-crystallin are lowered in spinal cord tissue. After contusion injury (for the first week), administration of recombinant human αβ-crystallin leads to increased granulocyte infiltration and continuous improvement in locomotor skills, modulates inflammatory responses in the injured spinal cord, reduces secondary tissue damage, and lowered recruitment of inflammatory macrophages. Thus, release of recombinant human αβ-crystallin promotes increased locomotor recovery after spinal cord injury suggesting its use as a better therapeutic agent for treating acute spinal cord injury because at present there is currently no effective treatment (Klopstein et al. 2012).

A sustained trauma (car accident, falls, sports injuries, gunshot wounds, stab wounds, a pene-

trating foreign object such as a knife, etc.) to the liver leads to liver injury, the most common abdominal injury, and constitutes 5 % of all traumas. Majority of the people who sustain this injury are also accompanied by some other injury. Nonoperative management with observation is necessary for a full recovery. Liver injury was triggered in rats by administration of acetaminophen (800 mg/kg, i.p.) followed by administration of fucoidan (extracted from various brown seaweeds, a pharmacological sulfated polysaccharide) 2 h before and after acetaminophen administration. Liver damage and cell death, overexpression of CYP2E1 (metabolizing enzymes of acetaminophen), and hepatic apoptosis (shown by the protein expression of Bax, Bcl-2, and cleaved caspase-3) induced by acetaminophen were attenuated by co-treatment of fucoidan. Fucoidan acts as an antioxidative agent and increases the production and expression of glutathione, glutathione peroxidase, and superoxide dismutase. All of them were reduced by acetaminophen. Further, fucoidan lowered the expression of inflammatory mediators (IL-1β, TNF-α, iNOS). Thus, against acetaminophen-induced liver injury, fucoidan has hepatoprotective effects through the anti-apoptotic, antioxidant, and anti-inflammatory pathways (Hong et al. 2012).

In 90 % partial hepatectomy (PH, except the caudate lobe) of rats, omega-3 PUFA acid (ω-3 PUFA) was administered intravenously before PH surgery for liver regeneration. To analyze liver regeneration, survival rates, liver weights, liver weight/body weight ratios, nuclear associated antigen Ki-67, signal transduction, and other biotechnological assays were evaluated. Survival rates in the ω-3 PUFA-induced rats were remarkable as compared to death of all in the control group (Qiu et al. 2012).

CRP is often used to assess the status of postoperative infection. Anomalous CRP values might be perceived after surgery yet in the case of noninfection due to transfusion and muscle injury, which disrupted better perioperative management. The level of CRP was evaluated after spine surgery, which was shown to be noninfection. A dramatic decrease of CRP concentration was detected on postoperative day POD3 and POD7 in lumbar open discectomy (LOD) patients of noninfection. Thus CRP would be a more sensitive and effective parameter especially in LOD patients for early evaluation of infectious complications, and the typical prototype of CRP may assist to evaluate the early postoperative course (Choi et al. 2014).

4.10.6 Cardiovascular Disease (CVD)

Cardiovascular disease (CVD) or heart diseases affects the total cardiovascular system, the heart or blood vessels (like arteries, capillaries, and veins), vascular system of the brain and kidney, and peripheral arterial disease. The reasons of cardiovascular disease are miscellaneous ranging from hypertension to atherosclerosis, aging with other allied cardiovascular dysfunction and many others causes found in even healthy asymptomatic individuals. CVD is the leading reason of deaths worldwide, though, since the 1970s, cardiovascular mortality rates have reduced in many high-income countries. At the same time, cardiovascular disease and deaths have increased at a rapid rate in middle- and low-income countries. Although CVD usually influences older adults, the antecedents of CVD, remarkably atherosclerosis, begin in initial life, making primary anticipation efforts essential from childhood. Thus there is increased stress on preventing atherosclerosis by altering risk factors, like exercise, healthy eating, and avoidance of smoking.

Reports were found pointing out that hs-CRP acts both as risk factor in the general healthy population and as prognostic factor in those with CVD. CRP was the most useful biomarker in subjects with a history of CVD and intermediate risk of events at 10 years, where adding of hs-CRP to the classical models for event risk estimation improves the risk staging. There was actually no consensus on the clinical usefulness of CRP as a prognostic marker in subjects with acute or chronic disease (Brito et al. 2015).

In asthma and in some inflammatory diseases, cysteinyl leukotrienes (CysLT) behave as immunomodulating lipid mediators and a strong spas-

mogenic agent. Apoe$^{-/-}$ mice were fed with a hypercholesterolemic diet, and the expression of some key enzymes of the CysLT pathway and their related receptors (CysLT1/CysLT2) were analyzed in the myocardium (hypoxic and normal). Chronic inflammation with increased apoptosis, fibrosis, and leukotriene C4 synthase (LTC4S) and upregulation of expression of IL-6 and CysLT1 were demonstrated in the myocardial biopsies of Apoe$^{-/-}$ in comparison to biopsies from control C57BL/6 J mice. Acute bouts of hypoxia further induce the LTC4S and CysLT1 expression, increasing LTC4S enzyme activity, with associated increased expansion of hypoxic areas in the myocardium. In acute bouts of hypoxic stress, treatment with selective CysLT1 receptor antagonist (Montelukast) inhibits CysLT signaling pathway thus reducing myocardial hypoxic areas in Apoe$^{-/-}$ mice to nearly normoxic conditions. Even in human heart biopsies from patients with chronic coronary artery disease, the mRNA expression levels of LTC4S and CysLT1 were upregulated in chronic ischemic myocardium as compared to a nonischemic one. Thus, CysLT1 antagonists may have protective function on hypoxic heart by improving the oxygen supply to myocardial ischemia areas, for example, during episodes of sleep apnea (Nobili et al. 2012).

In CVD mainly atherosclerosis and atherothrombosis are mostly controlled by immune-mediated inflammation. In CVD and in various diseases associated with an elevated cardiovascular risk (acute coronary syndrome, systemic lupus erythematosus, rheumatoid arthritis, end-stage renal disease, and severe carotid stenosis), during humoral autoimmunity, IgG autoantibodies against apolipoprotein A-1 (apoA-1) might play an emerging cardiovascular risk. Anti-apolipoprotein A-1 antibodies (anti-apoA-1 IgG) may act against the active mediators of atherogenesis and as a probable diagnostic and prognostic biomarker of cardiovascular risk (Teixeira et al. 2012).

4.10.7 Autoimmune Disease

Autoimmune disease arises from autoimmunity, which is an imperfect immune response of the body against the body's own tissues and molecules. It may be organs (autoimmune thyroiditis) or a particular tissue (Goodpasture's disease). Its treatment involves typically immunosuppressive drugs. During an infectious disease, macrophages and other immune cells due to infection release cytokines (namely, IL-1β), which in turn activate NF-kβ-dependent transcriptional pathway and inflammatory-cell recruitment via adaptor protein, myeloid differentiation factor 88 (MYD88). But these cytokines may break the tissue architecture of endothelial and reduces its cell–cell interactions. Endothelial cell blocks the inflammatory cell infiltration and in other way also acts as an inflammatory mediator. The disruptive effects of IL-1β on endothelial stability in a human in vitro cell model is through binding of MYD88 to small GTPase ADP-ribosylation factor 6 (ARF6) signaling and its activator ARF nucleotide-binding site opener (ARNO; also known as CYTH2). It is independent of the NF-kβ pathway. SecinH3, an inhibitor of ARNO, increases vascular stability significantly in animal models of acute inflammation and inflammatory arthritis (Zhu et al. 2012).

CRP along with ESR was used as an inflammatory marker in juvenile arthritis disease (Mourão et al. 2014). Delivery-related CRP levels were significantly elevated during delivery among both multiple sclerosis (MS) pregnancy patients and in control pregnant women. CRP levels were higher only during pregnancy in both study groups than during the postpartum period. Delivery-related elevated CRP levels did not correlate with postpartum disease activity. MS patients having gestational diabetes had a significantly higher level of CRP in the beginning of pregnancy as compared to nondiabetic MS patients. MS patients having fatigue had significantly higher CRP levels throughout pregnancy

as compared to patients without fatigue. Thus, higher CRP values were correlated with pregnancy-related comorbidities but not with the MS disease activity (Jalkanen et al. 2015).

In inflammatory and autoimmune disease and in CNS injury models, inflammatory mediators and DC migration are reduced by administration of cannabinoid receptor 2 (CB2R) agonists. CB2R signaling inhibits matrix metalloproteinase 9 (MMP-9) expressions and thus affects DC migration in the Matrigel migration assay (in vitro) and in draining lymph nodes (in vivo). CB2R-mediated MMP-9 expression resulted in reduced cAMP levels, decreased ERK activation, and lowered binding of c-Fos and c-Jun to MMP-9 promoter activator protein 1 sites and thus controls MMP-9-dependent DC migration to re-ensure homeostasis. In future, CB2R agonists might be targeted as potential therapeutic mediators for the treatment of different chronic inflammatory conditions activated by immune cells, including DCs (Adhikary et al. 2012).

Production of autoantibodies and breakdown of self-tolerance are the key players in SLE. Anti-dsDNA antibodies bind to cell surface receptor proteins of resident kidney cells, trigger downstream stimulation of signaling pathways, and release inflammatory mediators and fibrosis in the vascular, glomerular, and tubulointerstitial compartments of the kidney and associated acute or chronic renal failure during the pathogenesis of lupus nephritis (Yung and Chan 2012).

4.10.8 Joint Disease

Joint disease, affecting millions of people, is any disease or injury of the human joints. Arthritis and many others form the group of best-known joint diseases. Joint diseases may be acute or exceedingly chronic, causing uncomfortable, nagging, agonizing pain in one or many joints, and may affect many parts of the skeleton. Inflammatory joint diseases are represented by arthritis with inflammation of the joints, effusion of fluid into the joint cavity, stiffness, swelling, pain, and redness of the skin above the joint. In severe form, it destroys the joint cartilage and underlying bone with irreparable deformities. In ankylosis, the articulating members are adhered resulting in fusion with loss of mobility. In synovitis, inflammation is restricted to the synovial membrane/lining of the joint. Simple pains in the joints with no other accompanying evidence of arthritis are referred to as arthralgias. Rheumatism is a noninflammatory joint disease with unprecedented discomfort of the articular apparatus (joints with bursas, tendons, ligaments, and tendon sheaths). Spondylitis is the inflammation of the spine and joints.

"Spondyloarthritis" consists of an aggregation of a group of several diseases having similarities in clinical, radiological, and genetic content. Ankylosing spondylitis is the main representative of this spondyloarthritis group and is mainly characterized by a predominant axial involvement. For the diagnosis of ankylosing spondylitis, the presence of radiographic sacroiliitis is quite essential according to the newly modified New York criteria. The diagnosis of spondyloarthritis is often delayed as the occurrence of radiographic sacroiliitis takes nearly 8–11 years. In magnetic resonance imaging, the sacroiliac joint inflammation can be depicted before the appearance of radiographic damage thereby defining the new concept of "non-radiographic axial spondyloarthritis." Elevated levels of CRP at baseline have been used as biomarkers in predicting radiographic sacroiliitis progression. Thus CRP was helpful in definite diagnosis of ankylosing spondylitis (Tant et al. 2014).

The release of tenascin-C (TN-C) into knee synovial fluid after joint disease or in acute joint injury induces inflammatory mediators and matrix degradation in vitro in human articular cartilage. Human knee synovial fluid samples were collected from acute inflammatory arthritis (AIA), isolated knee meniscus injury, knee anterior cruciate ligament rupture, knee osteoarthritis (OA) with or without concomitant meniscus lesions, and knee healthy control groups for TN-C level and other cartilage markers. Same parameters were also undertaken in joints of dogs that undergone knee instability surgery. TN-C levels and its correlations with levels of matrix metalloproteinases 1 and 3 and aggrecanase-

dependent Ala-Arg-Gly-aggrecan (ARG-aggrecan) fragments were observed to be significantly higher in all the joint fluid of the human disease groups and in the dogs as compared to controls. Thus, TN-C being a marker of joint damage may also act as a stimulant of further joint degradation (Chockalingam et al. 2013).

4.10.9 Parasitic Infections

Any infectious disease caused or transmitted by a parasite is referred to as a parasitic disease. Parasitic diseases can affect all living organisms and any organ or tissue of the body. Some parasites can cause disease directly (malaria) but may also be through the toxins that they produce.

The etiology of parasitic diseases is protozoa (protozoan infection), helminths (helminthiasis), and bacteria (bacterial infection). Protozoa, helminths, and bacteria may be ectoparasites or endoparasites.

Malaria-associated acute respiratory distress syndrome (MA-ARDS) marked by pulmonary inflammation is a highly complicated disease with unknown pathophysiology. C57Bl/6 mice were infected with *Plasmodium berghei* NK65, *P. chabaudi*, and *P. berghei* ANKA, and the level of hemozoin in the lungs (in phagocytes, infected erythrocytes, and occasionally granulocytes) was correlated with the number of infiltrating inflammatory cells; alveolar edema; lung weight; increased pulmonary expression of cytokines, chemokines, and enzymes; and alveolar VEGF levels. Also, *P. falciparum*-derived hemozoin was injected intravenously in malaria-free mice. Consequently, hemozoin stimulated the pulmonary expression of cytokines (IL-6, IL-1β, TNF, IL-10, and TGF-β), pro-inflammatory chemokines (KC/CXCL1, IP-10/CXCL10, and MCP-1/CCL2), and other inflammatory mediators (iNOS, Hmox1, NOX2, and ICAM-1) (Deroost et al. 2013).

Patients infected with *P. vivax* uncomplicated and *P. falciparum* malaria in the endemic Brazilian Amazon were examined for hematological alterations and discharge of soluble mediators (CRP and NO) in acute (day 0) and convalescent phase (day 15). Laboratory inflammatory profiles reveal similar data in accordance with white blood cells, thrombocytopenia, and high band cell production during the acute phase of infection. CRP levels were higher in acute *P. vivax* infection as compared to acute *P. falciparum* infection, but higher NO was observed in acute and convalescent *P. falciparum* infections. Modifications in these mediators cannot envisage malaria infection and need further investigation (Lima-Junior et al. 2012).

Although CRP has been known to bind with varied nucleated cells, the direct binding of CRP molecule to erythrocytes in disease condition remains greatly unexplored. The binding of disease-associated CRP to erythrocytes of patients was demonstrated by flow cytometry, Western blotting, co-immunoprecipitation, ELISA, and surface plasmon resonance. A specific and strong RBC_{Mal}–CRP_{Mal} binding was observed. PC and calcium were found to be crucial for this interaction. Nearly a two- to threefold increase in RBC_{Mal}–CRP_{Mal} binding as compared to RBC_N confirmed disease specificity (Ansar et al. 2006). This binding altered the normal discoid shape of RBC_{Mal} with greater hydrophobicity and membrane fluidity. The effector function of CRP_{Mal} (10 μg/ml) has been exhibited by its greater potency to trigger the complement cascade as compared to RBC_N. Thus, these studies provide undeviating evidence for a significant phagocytic functional coaction of this acute-phase protein by activating the CRP complement cascade after binding with RBC_{Mal} (Ansar et al. 2006). Deficiencies of the complement-regulatory proteins (CD35, CD55, and CD59) on RBC_{Mal} of patients with *Plasmodium falciparum* were also reportedly known. The role of CRP_{Mal} in controlling the complement-regulatory proteins and downstream consequence on the complement cascade has been examined. In the presence of CRP, RBC_{Mal} demonstrated lowered complement-regulatory proteins with reduced affinity. These changes cause increased C3 deposition and more complement-mediated hemolysis, thus providing a novel mechanism of complement-fueled RBC_{Mal} destruction pathway refractory to eryth-

rophagocytosis, and may account for pathogenesis of anemia (Ansar et al. 2009a).

The levels of CRP were elevated in pooled serum from *P. vivax-, P. knowlesi-*, and *P. falciparum*-infected patients. CRP might represent an important marker of infection of malaria, which could be utilized as a usual diagnostic tool for detecting *P. vivax, P. knowlesi*, and *P. falciparum* infections. However, the prospective of CRP as infection marker of malaria will need to be elucidated in a larger population of malaria-infected subjects (Mu et al. 2014).

4.11 Clinical History

In our study, three patients (designated as Patient 1, 2, and 3) were included whose clinical history and photos were included in Table 4.1. The patients were admitted in KPC Medical College and Hospital, Jadavpur, Kolkata, India. Informed consents were taken from these patients and their relatives, and the clinical history was given as per the consent of the institutional review board of the hospital. There were 2 patients (Patient 1 and 2), both female and diagnosed clinically as having rheumatoid arthritis. The first patient was admitted for her cataract surgery. The second patient was admitted for shortness of breath. Another patient (Patient 3) was suffering from perianal (or perineal) abscess and he came to the hospital to have the severely painful and tender abscess operated. The clinical history and photos of the patients (Patient 1 and 3) were collected during preanesthetic checkup and the history of Patient 2 was collected from the emergency ward during critical care management.

A 56-year-old woman (Patient 2) with shortness of breath and body ache was admitted to the ITU of the hospital at the onset. She was known to be diabetic (type II) and hypothyroid and had COPD. Her primary laboratory investigations showed high ESR, high CRP levels, very high *N*-terminal pro-BNP, a positive rheumatoid arthritis (RA) factor, and mildly deranged liver functions. Her echocardiography showed left ventricular hypertrophy (LVH) with high pulmonary artery pressure (PAP) and low ejection fraction (EF) (38 %) (Table1). Patient was managed conservatively by anti-failure management by giving a furosemide injection, nitroglycerine (GTN), and ramipril tablets and discharged. For her long-term rheumatoid arthritis, she is taking methotrexate, prednisolone, and folic acid by oral route. She had heart failure.

A 52-year-old male (Patient 3) was presented with fever, soft abdomen, and severe pain, redness, and tenderness in the anal region for 3 days. He had been diagnosed previously with diabetes (type II) with high BP. His laboratory findings revealed high TLC, DLC, and ESR and raised CRP levels. His blood serology report revealed negative for both malarial and dengue antigen. He had perineal abscess (as increased risk in diabetic patient). He had a palpable inguinal lymph node and an inflamed perineal region with fever. He had neutrophilic leukocytosis with high CRP and ESR. His procalcitonin level revealed absence of bacterial infection (Table 4.1). He was treated surgically by incision and drainage of perineal abscess under general anesthesia, and intravenous followed by oral antibiotics, tight control of sugar by regular insulin, and adequate analgesics and antihypertensives were given.

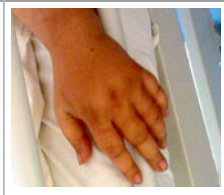
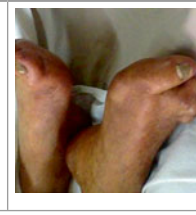
The level of inflammatory biomarkers like CRP was quite high in all the three patients.

4.12 Analysis of Biomarkers Seen in the Patients

It is quite evident from the clinical history from the hospital that no specific mediators were investigated during the routine investigations in the diagnosis process. Rather some broad-spectrum inflammatory markers were diagnosed which will point toward the process of inflammation and not to the pathways of activation of mediators, whereas in the laboratory people are developing inflammatory animal models to understand all possible molecules involved in inflammation. Very soon in hospitals, definite inflammatory markers for each disease will be investigated for routine diagnosis.

In this chapter, we have highlighted case studies of some patients from Kolkata, India, reveal-

Table 4.1 Clinical and laboratory parameters of patients

Parameters	Patients		
Sl no.	1	2	3
			
Physical parameters			
Age (years)	66	56	52
Sex	Female	Female	Male
Weight (Kg)	68	84 (obese)	73
Complaint			
1. Fever for 6 days 2. Decreased urine output for 1 day 3. Restless for the last 6 h		1. Shortness of breath for 2 days with exacerbation for the last 4 h 2. Body ache for 2 years 3. Swelling of knee joint 3 months back	1. Fever for 3 days 2. Pain and tenderness in the anal region and he can't sit for 5 days
Past medical history			
1. Diabetes mellitus type II for 4 years 2. Chronic kidney disease for 2 years		1. Diabetes mellitus type II for 2 years 2. Hypothyroid for 3 years 3. COPD	1. Hypertensive for 7 years 2. Diabetes mellitus type II for 4 years
Past surgical history			
1. Caesarian section—30 years back 2. Cholecystectomy—24 years back 3. Appendicectomy—18 years back		1. Hysterectomy and bilateral salpingo-oophorectomy—8 years back	1. Cholecystectomy—7 years back
Drug allergy			
Name not known		Not known till now	Sulfur drugs
On examination			
A. General examination			
Patient conscious, restless, and sometimes disoriented		Patient conscious, alert, and cooperative	Patient conscious, alert, and cooperative
GCS	13/15	15/15	15/15
Pulse/min	124	96	104
Temperature (°F)	101.4	98.1	101.4
Pallor	Mild	Positive	Nil
BP (mm of Hg)	136/90	140/82	152/90
JVP	Normal	Raised	Normal
Palpable lymph node	Not palpable	Not palpable	Inguinal lymph node palpable
Hydration status	Mild dehydration	No dehydration	Mild dehydration
Clubbing	Not present	Not present	Present

(continued)

Table 4.1 (continued)

Parameters	Patients		
Sl no.	1	2	3
B. Systemic examination			
Chest (air entry)	1. Adequate 2. Scattered rhonchi on both lungs 3. Maintaining SpO_2 is 94 % with 3 liters oxygen via nasal cannula with respiratory rate 29/min	1. Adequate 2. Rhonchi occasional, crepitation (++) more on left basal	Adequate
CVS (S1 and S2)	Audible, no gallop	Audible, gallop (++)	Audible
Abdomen	Soft	Soft and tender right hypochondrium, hepatojugular reflux positive	Soft
Intestinal peristaltic sound	Positive	Positive	Positive
C. Local examination			
1. No sore throat 2. No petechiae		1. *Lumbosacral spine*—tender (+) 2. *Knee joint*—tender and swollen	1. *Perineal abscess*—swollen (++), red (++), tender (++)
D. Laboratory investigations			
Hb (gm/dl)	9.8	12.3	13.4
Inflammatory and infective markers			
TLC (cells/cu.mm)	21,200	7900	22,400
DLC (%)	N (94), L (3), M (3), E (0)	N (62), L (34), M (1), E (3)	N (90), L (8), M (1) E (1)
ESR (mm/first hour)	87	96	74
CRP (mg/L)	342	126	260
Procalcitonin (ng/ml)	42	ND	12
Pro-BNP (pg/mL)	ND	35,624	ND
Malarial antigen	Negative	Negative	Negative
Dengue serology	Negative	Negative	Negative
Sugar level tests			
Blood sugar (mg/dl)	RBS-366	RBS -236	RBS -302
Glycated hemoglobin test (Hb A1c) (%)	8.6	7.6	9
Renal function tests			
Urea (mg/dl)	86	30	34
Creatinine (mg/dl)	2.8	1.3	1.2
Electrolytes			
Sodium (Na+) (mEq/L)	152	139	136
Potassium (K+) (mEq/L)	4.9	3.8	4.7
Calcium (serum) (Ca++) (mEq/dl)	8.6	9.3	8.7
Autoimmune markers			
Antinuclear antibody	ND	Negative	ND

(continued)

Table 4.1 (continued)

Parameters	Patients		
Sl no.	1	2	3
RA factor (above 20 IU/mL)	ND	Positive	ND
HLA (B-27)	ND	Negative	ND
Coagulation markers			
PTT (sec) (control—12 s)	26	13.7	14.2
APTT (sec) (control—26 s)	68	32	33
ABGA	Severe metabolic acidosis with increasing base deficit	Within normal ranges	ND
Chest X-ray	Bilateral lower zone opacities	Increased bronchovascular markings, sign of overload	Normal profile seen
Ultrasonography of the abdomen	1. *Kidney*—corticomedullary differentiation not maintained	Hepatomegaly	ND
	2. Liver mildly enlarged		
	3. Urinary bladder thickened, cystitis		
Cardiac function tests			
ECG	Sinus tachycardia with nonspecific ST changes, first-degree heart block	Sinus rhythm, rate 134/min with intermittent atrial fibrillation, nonspecific ST changes	Sinus rhythm (RBBB)
Echocardiography	Concentric LVH	LVH,dilated left atrium, PAP = 56 mm of Hg	LVH, no RWMA
EF (%)	42	38	62
Bronchoscopy and bronchoalveolar lavage (BAL)/endotracheal (ET) suction			
	1. ET suction shows MDR *Pseudomonas aeruginosa*	ND	ND
	2. MDR *Acinetobacter baumannii*		
	3. MDR *Klebsiella pneumoniae*		
Urine routine examination and culture sensitivity			
	1. Pus cells plenty	Pus cells 2–3/HPF	ND
	2. Culture sensitivity shows MDR *Klebsiella*		

(continued)

Table 4.1 (continued)

Parameters	Patients		
Sl no.	1	2	3
Treatment summary			
1. Adequate calorie was ensured (by nasogastric tube/ parenteral nutrition) 2. Deep venous thrombosis prophylaxis was done 3. Tight control of blood glucose done by insulin (regular) infusion 4. Vasopressor (noradrenaline) infusion was started as patient was hypotensive in spite of adequate fluid management 5. Patient was on mechanical ventilator (pressure controlled) and then weaning off from ventilator 6. Injection polymyxin B (loading dose of 5 lac IU i.v.) followed by maintenance dose 7. Injection meropenem (loading dose 1 gm i.v. followed by 500 mg i.v.) 8. Nebulization with Duolin after every 8 h 9. Stress ulcer prophylaxis was done 10. Hemodialysis (as renal function deteriorated) was done 11. Electrolytes were replaced as per protocol as needed 12. Fresh frozen plasma (FFP) transfusion was given 13. Vitamin K injection (i.v.) was given	1. On sliding dose of human insulin (human Actrapid) subcutaneously 2. Furosemide injection (20 mg) i.v. thrice daily with bolus of 40 mg 3. Ramipril tablet (5 mg) orally at once daily 4. Injection GTN at the dose 0.9 ml/h (50/50) 5. Eltroxin tablet (25 µg) @orally per day 6. Inhaler with ipratropium bromide thrice daily 7. Methotrexate tablet (5 mg) orally at twice a week 8. Prednisolone tablet (10 mg) orally per day	1. Incision and drainage of perineal abscess done under general anesthesia 2. Blood sugar controlled by intravenous insulin infusion followed by subcutaneous insulin 3. Ramipril (5 mg orally/day) to control BP 4. **Antibiotic** piperacillin + tazobactam (4.5 gm for every 6 h) and linezolid (600 mg i.v. every 12 h) 5. *Analgesic injection* (a) Paracetamol (1gm) i.v. after every 8 h (b) Tramadol (100 mg) i.m./ SOS
Output			
Successfully discharged	Successfully discharged	Successfully discharged	

COPD chronic obstructive pulmonary disease, *GCS* Glasgow coma scale, *°F* degree Fahrenheit, *BP* blood pressure, *JVP* jugular venous pressure, *CVS* cardiovascular system, S_1 first heart sound, S_2 second heart sound, *TLC* total leukocyte count, *DLC* differential leukocyte count, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *N* neutrophil, *M* monocyte, *L* lymphocyte, *E* eosinophil, *ND* not determined, *FBS* fasting blood sugar, *RBS* random blood sugar, *RA* rheumatoid arthritis, *HLA* human leukocyte antigen, *PTT* prothrombin time test, *APTT* activated partial thrombin time, *ECG* electrocardiogram, *RBBB* right bundle branch block, *WNL* within normal limit, *LVH* left ventricular hypertrophy, *RWMA* resting wall motion abnormality, *PAP* pulmonary artery pressure, *EF* ejection fraction, *BNP* brain natriuretic peptide, *µg* microgram, *mg* milligram, *pg* pictogram, *i.v.* intravenous, *i.m.* intramuscular, *SOS* as and when required, *GTN* glyceryl trinitrate, *CBG* capillary blood glucose, *NA* not applicable, *MDR* multidrug resistance, *ABGA* arterial blood gas analysis, *HPF* high-power field

ing acute inflammation from disease together with their clinical history (Table 4.1). All the patients had some past medical or surgical history, representing some diverse inflammatory conditions. The question which we probe in here is the search of proper biomarkers in these represented acute inflammatory conditions. All the key players and biomarkers of inflammation change its role with the change in setup of disease and patients. Even in clinical diagnosis of an inflammatory patient, some broad-spectrum markers were analyzed without individual application of a universal biomarker.

4.13 Biomarkers of Inflammation in Different Subsets of Diseases

In this chapter, we had presented three case reports in search of proper inflammatory biomarkers. CRP is a diagnostic biomarker in a varied spectrum of diseases. This is discussed in other chapters also. The role of other inflammatory biomarkers in other subset of diseases is also discussed here.

Biomarkers can be defined as any alterations in the constituents of body or tissue fluids. These

markers provide a medium for uniform classification of a disease with its risk factors and can be extended in understanding the basic underlying pathophysiology of disease. Biomarkers provide a powerful and dynamic tool to grasp the spectrum of inflammatory diseases with usage in observational and analytic epidemiology, clinical trials in populations, and screening with diagnosis and prognosis. Biomarkers can also reflect the entire steps of a disease from the earliest symptoms/screening to the terminal stages. Analytical assessment of the validity of biomarkers is required to correlate with respect to the stage of disease. Variability in the measurement of biomarkers ranges from individual error in laboratory technicians, machine dysfunction, improper storage of body fluid, and other bias and confounding issues.

Currently, there are no specific markers for inflammation; rather some broad-spectrum inflammatory markers were routinely investigated in hospitals. The clinical investigation revealed some broad-spectrum markers like C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), total leukocyte count (TLC), differential leukocyte count (DLC), sodium–potassium level, urea–creatinine level, antinuclear antibody, pro-brain natriuretic peptide (pro-BNP), and human leukocyte antigen (HLA) which were accounted.

Nearly 7896 papers from PubMed and Google Scholar fit our criterion of research, of which 2641 were review articles, 151 were letters to the editors, and the rest were original articles. In this context, we reviewed some of the immunological mechanisms and the applicability of biomarkers that occur during acute phases with its remission/recovery period in some of the widely used experimental models of varied spectrum of diseases (Table 4.2).

Invasive tests sometimes are routine for the diagnosis and care of patients. Diagnosis is mostly based on clinical symptoms combined with radiological, endoscopic, and laboratory investigations. The employment of noninvasive biomarkers is always needed to avoid an invasive diagnostic test that causes discomfort and potential complications. The ability to determine the type, severity, prognosis, and response to therapy of a disease using definite biomarkers has long been a waited goal of clinical researchers. We describe the biomarkers assessed in this chapter, with special reference to acute-phase proteins and serologic markers, and thereafter, we describe the new biological markers (Table 4.2). The biological markers could be developed in the future based on serum markers of acute-phase response. The laboratory tests mostly used to measure the acute-phase proteins in clinical practice are the serum concentration of CRP and the ESR. Other biomarkers of inflammation include platelet count, leukocyte count, and serum albumin and serum orosomucoid concentrations and serologic markers like antibodies also. To detect specific pathologies, in the last decades, serological and immunologic biomarkers have been studied extensively in immunology and have been used in clinical practice. In different diseases, the presence of antibodies can aid as surrogate markers for the aberrant host immune response and also for future biomarkers. The field of the biomarker discovery have revolutionized by the progress of molecular biology tools (microarrays, proteomics, and nanotechnology). The advances in bioinformatics coupled with cross-disciplinary collaborations have highly enriched our ability to characterize, retrieve, and analyze large amounts of data generated by the technological advances. The present techniques available for biomarkers development are proteomics and genomics (single-nucleotide polymorphism genotyping, pharmacogenetics, and gene expression analyses). In the future days, the addition of new serological markers will add significant benefit to patients and clinicians. Our understanding of the pathophysiology of a disease is based on correlating serologic markers with clinical phenotypes and genotypes.

4.14 Discussions

Humans and animals are continuously exposed to microbial pathogens, trauma, stress, and injury that can pose a considerable relevance in our daily livelihood. After the initiation of the etiological agent, the organism elicits a set of highly organized immunological, physiological, metabolic, and behavioral responses to represent its

Table 4.2 Therapeutic and diagnostic applications of inflammatory biomarkers

Assessment of biomarkers	Research study observations/results	Therapeutic/diagnostic applications
Inflammatory responses after diesel exhaust (300 μg/m(3)) exposure lasted for 1 h (Xu et al. 2013)		
1. Symptom scores 2. PEF was assessed before exposure and at 15, 75, and 135 min of exposure 3. Monocyte and total leukocyte counts in peripheral blood 4. Serum IL-6 concentrations observed 20 h postexposure	1. Self-rated throat irritation in the upper airways was significantly higher 2. PEF decreased during diesel exhaust exposure 3. Monocyte and total leukocyte counts in peripheral blood were higher 4. Serum IL-6 concentrations were increased	Diesel exhaust has induced adverse acute effects on symptom scores, lung functions, and altered levels of inflammatory markers in healthy volunteers, from 75 min postexposure
Serum activin A and B levels predict outcome in patients with ARF (de Kretser et al. 2013)		
1. ARF patients require ventilator support for more than 6 h 2. The serum levels of activins A and B (members of the transforming growth factor-β family of proteins), with their binding protein, follistatin, have reported to be important regulators of inflammation, fibrosis, and ARF as compared to normal subjects.	1. Serum activin A and B were significantly elevated in most diagnostic patients. 2. Patients who had activin A and/or B concentrations above the reference maximum level were more likely to die in the 12 months following admission	1. The measurement of serum activin A and B levels in patients with ARF therapeutically predicts the risk of death 2. Modulating the activin A and B bioactivity should be explored as potential therapeutic agent
Diagnostic differences between severe sepsis patients and ARDS by biomarkers of lung epithelial injury and inflammation (Ware et al. 2013)		
1. The bio-clinical diagnostic discrimination of ARDS patients at risk from sepsis patients by 11 biomarkers of inflammation including plasma biomarkers (IL-8, IL-6) and lung epithelium generated biomarkers (SP-D, RAGE, CC-16) 2. Fibroblast activation, endothelial injury, proteolytic injury, and lung epithelial injury were also measured in early phases of ICU admission	Altered levels of five plasma biomarkers with three lung epithelium biomarkers help for discrimination and diagnosis of ARDS patients with severe sepsis patients	Plasma biomarkers may be useful as clinic–biological diagnostic tool of ARDS with sepsis patients
Prognostic value of plasma chitotriosidase activity in acute stroke patients (Bustamante et al. 2013)		
1. The elevated plasma activity of chitotriosidase, a component of innate immunity, reflects an inflammatory response 2. Chitotriosidase constitutes a sensitive parameter of macrophage activation 3. Plasma chitotriosidase activity was serially determined in 159 acute stroke patients and age-matched controls to assess its prognostic value in acute stroke patients 4. Additional predictive value of chitotriosidase was also tested	1. The baseline levels of chitotriosidase activity were increased in stroke patients compared to controls 2. Chitotriosidase activity was an independent predictor of neurological improvement at 48 h 3. Addition of plasma chitotriosidase activity showed a better prediction of improvement at 48 h	Acute stroke patients treated with tissue plasminogen activator with baseline chitotriosidase activity may constitute a prognostic predictor of short-term outcome

(continued)

Table 4.2 (continued)

Assessment of biomarkers	Research study observations/results	Therapeutic/diagnostic applications
New-onset AF after AMI is associated with admission biomarkers (Parashar et al. 2013)		
1. AF is an independent predictor of mortality after AMI 2. The biomarkers of myocardial stretch (NT-pro-brain natriuretic peptide [NT-pro-BNP]), myocardial damage (troponin-T [TnT]), and inflammation (hs-CRP) and new-onset AF during AMI were identified in patients at high risk for AF	1. New-onset AF was noticed in patients with AMI 2. Increase in NT-pro-BNP and hs-CRP is associated with increase in the rate of AF 3. TnT was not independently associated with new-onset AF	The markers of myocardial stretch and inflammation, but not the amount of myocardial necrosis, are important determinants of AF after AMI
Relationship between CSF biomarkers for inflammation, demyelination, and neurodegeneration in acute optic neuritis (Modvig et al. 2013)		
In patients with optic neuritis with demyelinating symptom and healthy subjects, CSF levels of CXCL13, CXCL10, MMP-9, CCL-2, osteopontin, chitinase-3-like-1, MBP, and NF-L were determined	1. Leukocyte infiltration biomarkers (CXCL13, MMP-9, and CXCL10) were strongly associated with MS-risk parameters 2. Osteopontin and chitinase-3-like-1 were associated ($p<0.0001$) and correlated with tissue damage markers (NF-L and MBP) to measure dissemination in space of white matter lesions	Leukocyte infiltration biomarkers (CXCL13, CXCL10, and MMP-9) strongly predict MS risk, and osteopontin and CHI3L1 suggest tissue damage-related inflammation
SAA, phospholipase A_2*-IIA, and CRP as inflammatory biomarkers for prostate diseases* (Menschikowski et al. 2013)		
Serum levels of inflammatory markers; SAA, $sPLA_2$-IIA, and CRP with PSA were determined in patients with localized PCa, BPH, and mPCa	Patients with BPH, PCa, and mPCa have elevated serum levels of SAA, $sPLA_2$-IIA, and CRP along with elevated levels of PSA as compared to healthy subjects	Significant differences in inflammatory circulating biomarkers were found between in PCa and mPca, suggesting their prognostic value during BPH development and PCa progression
Microalbuminuria and CRP as a predictor of CVD and as inflammatory biomarkers (Lazebnik et al. 2013; Bashir et al. 2014)		
1. Microalbuminuria, a risk factor and atherogenic marker for CVD, indicates the target organ damage 2. It is a valuable tool in screening and identification of patients with CVD 3. CRP is correlated with chest pain in CVD and increased levels of CRP in atherosclerosis and reflects inflammatory condition of vessel wall 4. CRP and microalbuminuria were estimated in patients of acute chest pain and type 2 diabetes along with healthy controls	It was found that microalbuminuria and CRP was much higher in CVD patients as compared to control groups	Sensitivity, specificity, and positive predictive value of CRP and microalbuminuria in patients as compared to normal can be used as important biomarkers in screening CVD

(continued)

Table 4.2 (continued)

Assessment of biomarkers	Research study observations/results	Therapeutic/diagnostic applications
Biological therapy by cell adhesion molecules in CD (Lazebnik et al. 2013)		
1. Diagnostic value of concentration of adhesion molecules (L-selectin, E-selectin, P-selectin, integrin-sVCAM-1) in blood serum for the assessment of the effectiveness of biotherapy in patients with CD and prognosis of the disease 2. Levels of leukocytes, ESR, and CRP were also analyzed before and after the biotherapy with infliximab and transplantation of MSC	1. Biological therapy along with transplantation of MSC decreases the levels of all adhesion molecules in all patients 2. Suppression of the synthesis of the main inflammatory cytokine TNF- α	Adhesion molecules are modern markers of inflammation used to assess the effectiveness of biological therapy in CD patients and to dictate the prognosis of the disease
Profile of circulating cytokines: impact on OSA, obesity, and acute cardiovascular events (Testelmans et al. 2013)		
1. OSA induces oxidative stress, systemic inflammation, and cardiovascular morbidity 2. The circulating cytokines profiles were measured in nonobese or obese patients with or without sleep apnea and with or without an acute cardiovascular event in a case-control study 3. Patients were assessed with sleep studies and inflammatory (hs-CRP, Leptin, RANTES, MCP1, IL-6, IL-8, TNF-α) and anti-inflammatory (adiponectin, IL-1Ra) cytokine profiles 4. Cardiovascular phenotyping was performed including carotid intima-media thickness, pulse wave velocity, and 24-h blood pressure monitoring	1.In comparison with patients without sleep apnea or without comorbidities, patients with the combination of an acute cardiovascular event 2. Patients with preexisting OSA showed a higher degree of systemic inflammation and significant increase in serum levels of hs-CRP, IL1-Ra, IL-8, IL-6, TNF-α, RANTES, and sICAM 3. Serum levels of different inflammatory markers were significantly higher in patients having OSA and an acute cardiovascular event	The diagnosis and treatment of OSA is potentially more important in patients after an acute cardiovascular event because these biomarkers could be associated with worsened cardiovascular outcome
Levels of hepcidin in cord blood: A biomarker for EONS (Cizmeci et al. 2014)		
1. Acute-phase reactant, hepcidin, has a critical role in inflammation and helps in host defense by interfering with microorganism's access to iron in EONS 2. Cord blood samples of infants born having EONS were collected and the level of cord blood hepcidin was determined	Hepcidin level was found to be significantly increased in newborns with EONS	1. Hepcidin behaves as an acute-phase reactant in the pathophysiology of EONS 2. Increased level of hepcidin in cord blood may be used as a reliable biological marker of EONS

(continued)

Table 4.2 (continued)

Assessment of biomarkers	Research study observations/results	Therapeutic/diagnostic applications
Acute-phase response in patients with AP (Kusnierz-Cabala et al. 2013)		
Concentrations of PTX3, SAA, CRP, HGF, PCT, PMN-elastase, IL-6, IL-18, and sTNFR-75 were measured in plasma of patients with severe and mild form of AP with control to age- and sex-matched healthy subjects	1. On each day of the study, significant correlations were found between PTX3 with SAA, IL-6, and PMN-elastase 2. The concentrations of these inflammatory markers were higher in severe AP patients as compared to those having the mild form of AP 3. The highest concentrations of PTX3 were noted on the early phase 3. The changes in PTX3 concentration in the early phase of AP is similar to that of IL-6 4. The peak levels of PTX3 are achieved earlier than another biomarker, CRP	1. PTX3 may be useful in early evaluation and prediction of the severity of AP 2. The relationship between the patterns of changes in PTX3 concentration with other inflammatory markers was evaluated in patients with AP at the early stage of the disease

CRP C-reactive protein, *MSC* mucosal stem cell, *CSF* cerebrospinal fluid, *ICU* intensive care unit, *AMI* acute myocardial infarction, *AF* atrial fibrillation, *IBD* inflammatory bowel diseases, *UC* ulcerative colitis, *CSF* cerebrospinal fluid, *CD* Crohn's disease, *ARF* acute respiratory failure, *CVD* cardiovascular disease, *OSA* obstructive sleep apnea, *EONS* early-onset neonatal sepsis, *SP-D* surfactant protein-D, *RAGE* receptor for advanced glycation end products, *IL* interleukin, *CC-16* club cell secretory protein, *ARDS* acute respiratory distress syndrome, *PEF* peak expiratory flow, *TNF* tumor necrosis factor, *hs-CRP* high-sensitivity C-reactive protein, *MMP* matrix metalloproteinase, *MBP* myelin basic protein, *NF-L* neurofilament light chain, *ESR* erythrocyte sedimentation rate, *AP* acute pancreatitis, *PTX3* pentraxin 3, *SAA* serum amyloid A, *HGF* hepatocyte growth factor, *PCT* procalcitonin, *PMN-elastase* polymorphonuclear elastase, *sTNFR75* soluble receptor for TNFalpha, A_2 = $sPLA_2$-IIA secreted group IIA phospholipase, *PSA* prostate-specific antigens, *PCa* prostate cancers, *BPH* benign prostatic hyperplasia, *mPCa* metastatic prostate cancers

strategy to fight the infection. Inflammation generated at the site of assault in the peripheral tissues communicates with the brain to modify its immune response and upgrade as necessary which aids in its ability to fight and eliminate the source. The groups of mediators, biomarkers, pathways, and key responders/effectors during inflammation are varied and change their *modus operandi* in a different disease in a different organism. Biomarkers' applicability lies in dissecting all the steps of a disease from diagnosis, screening, treatment, and drug formulation to prognostic consequences (Fig. 4.11).

As inflammation and its network of reactors form a juggling condition, thus to dissect all strings of its web, different animal models are developed in different diseases to mimic the original scenario. Inflammation is induced through varied stimulator molecules in these animals even for a single disease in different setups to decode the mechanism of inflammation and bring out probable diagnostic and therapeutic agents.

These studies have also provided evidence of systemically generated inflammatory biomarkers versus mediators both in acute and chronic inflammation. The process of inflammation is triggered by mononuclear phagocytes, which in turn alter the concentration of various biomarkers, the cascade of complement pathways, antibody production, and subsequent tissue repairing and resolution. Thus it traverses through both innate and adaptive immune system. While these responses are part of our normal homeostatic mechanisms, it is quite clear that systemic inflammation has a detrimental effect in humans and also in animals.

The inflammatory biomarkers responsible for the pathology of an inflammatory disease are of a heterogeneous nature (Table 4.2). In Table 4.2, we cited the potential therapeutic application of some biomarkers in varied acute inflammatory conditions.

The list is always never ending. Many discrepancies in the findings may at least in some sections be demonstrated by the specific methodologies used, different time points at which investigations were done, mice strain susceptibility, and/or concentration (or dose) of the administered inducer. Moreover, to the best of our collective knowledge, a very few studies aimed to clarify the kinetics of inflammatory cascades mediating acute and chronic phases of inflammatory disease.

In this pretext, the ongoing study aimed to evaluate cellular inflow and biomarkers of inflammation during the acute and chronic phases of inflammatory disease of humans induced in mice. Our review showed very striking differences among the induction phase and its recovery period which may be useful for future modeling of the experimental inflammatory diseases. This may particularly provide strong evidence for the detection of inflammatory biomarkers essential for the calculation of disease pathology and the designing of potential therapeutic outcome.

Disease subtypes are quite often described on the basis of medical history, physical findings, different serum markers, various imaging parameters, and histopathological and endoscopic characteristics of the related disease. Modern approaches in disease systematization are often invasive and/or generally related with a lack of specificity and sensitivity. From all the patients' history (Table 4.1), we can conclude that all of them had signs, symptoms, biomarkers, and expressions of inflammation and infection that are changed in human pathophysiology. We have documented those in our case histories. The varied physical biomarkers are inclusive of physical signs like local swelling, local edema, tenderness, local increased temperature, local redness, and local palpable lymph node in localized inflammation and infection. Other physical biomarkers are respiratory distress, fever (increased temperature), anuria, increased heart rate, low BP, deteriorating sensorium, and edema (due to increased vascular permeability of vessels). Radiologically, patient had diffuse opacities or consolidations in lungs, thickened urinary bladder and signs of kidney involvement (increased cortical echogenicity), and increased pleural reaction (revealed as pleural effusion). As per laboratory investigations, TLC with preponderance of neutrophil and its toxic granulation, high ESR, high CRP, increased procalcitonin level, high random blood sugar, rising urea–creatinine, increased sodium level (due to depletion of intravascular fluid due to increased vascular permeability), deranged coagulation profile (increased prothrombin time and activated partial thrombin time), deranged liver function test, metabolic acidosis (pH less than 7.5, low bicarbonate level, and increased base deficit), increased heart rate (documented by ECG),arrhythmia (due to electrolyte imbalance), and grossly hypokinesia of walls of the heart (due to sepsis) reversed after cure of disease (no hypokinesia and EF of heart was increased normally), ET suction or BAL fluid showed growth of different bacteria, and urine showed plenty of pus cells and growth of bacteria. A few laboratory investigations showed less than normal level in inflammation like low albumin, low pH, and low bicarbonate as we documented.

Many studies on biomarkers never achieve their goal because of the failure to stick to the same rules that would apply for the use of non-biological variables. The development of any biomarker should rely on the standard design using epidemiological project or clinical trial. In formulating laboratory component, proper studies must be completed to determine reliability, accuracy, interpretability, feasibility, intra-individual or inter-individual variation, acquired or genetic susceptibility, and tissue localization, by setting the normal values with relation to variables like age, gender, and persistence of the biomarker (Sharma et al. 2013; Lazebnik et al. 2013; Testelmans et al. 2013; Cizmeci et al. 2014; Kusnierz-Cabala et al. 2013; Ansar et al. 2006, 2009a, b; Ansar and Ghosh 2013).

Advances in proteomics, genomics, metabolics, and molecular pathology have generated many potential candidate biomarkers with extensive clinical relevance. In the future, the integration of biomarkers and search for universal biomarkers, identified by high-throughput technologies, into medical science will be essential to achieve "personalization" of treatment/therapeutics and disease prevention.

The future of prevention protocol of inflammatory diseases and their detection and effective

treatment will be highly influenced by the utilization of more effective markers of inflammation with superlative performance. Given the practical problem in collecting tissue samples or blood or fluids from patients in inflammatory diseases, biomarkers obtained from body fluids have a great prospect for the value-added patient management even through the drawbacks of the abovementioned hindrances or limitations. Since the immunosuppressive therapy of different inflammatory diseases currently rotates around long-term treatment with unwanted side effects, a paradigm shift from nonspecific cytotoxic drugs to specific and selective targeted therapeutic agents/drugs is an ardent medical need.

Perhaps the most regularly used nonspecific prognostic inflammatory biomarker is CRP. CRP levels are drastically elevated within 6 h after the initiation of inflammation. The final hike can sometimes be as much as 60-fold. Moreover, CRP is much more precise than some of the other generally used inflammatory biomarkers like ESR, total leukocyte count, etc. In various bacterial infections, CRP concentrations are unusually below 10 mg/L except in the case of neonates where 10-40 mg/L value typically represents a mild inflammation condition; levels between 40–200 mg/L represent significant bacterial infection or acute inflammation state. In serious bacterial infection or burns, concentration values may rise to 300 mg/L or even higher. The assessment of disease pathology, diagnostics, prognostics, and therapeutic applications of CRP in different diseases is already cited (Ansar et al. 2006, 2009a, b; Ansar and Ghosh 2013). Most common inflammatory markers include both inhibitors and mediators of inflammation as well as scavengers of prospective dangerous substances, namely, toxins. The following protein changes are observed during the inflammatory response: a rapid fall in serum prealbumin albumin and transferrin concentration as well as an elevated level of α1- and α2-globulin levels. The selected biomarkers with therapeutic applications of bone disease are CRP, calcitonin, collagen I, collagen I telopeptide, and osteocalcin; for cardiovascular disease, they are aldosterone, angiotensin, angiotensin-converting enzyme, antidiuretic hormone, atrial natriuretic peptide, etc; for diabetes, they are glucagon, insulin, and insulin-like growth factor; for gastrointestinal diseases, they are serotonin, gastrin, somatostatin, etc.; for women's health, they are estradiol, estrone, follicle-stimulating hormone, luteinizing hormone, etc.; there are many more biomarkers in other diseases (Sharma et al. 2013; Lazebnik et al. 2013; Testelmans et al. 2013; Cizmeci et al. 2014; Kusnierz-Cabala et al. 2013; Ansar et al. 2006, 2009a, b; Ansar and Ghosh 2013).

It is quite evident from the clinical history from the hospital that no specific biomarkers were investigated during the routine investigations in the diagnosis process (Table 4.1). Rather some broad-spectrum inflammatory markers (like CRP, ESR, procalcitonin, etc.) were diagnosed, whereas in the laboratory people are developing inflammatory animal models to understand all possible molecules involved in inflammation. Very soon in hospitals, definite inflammatory markers for each disease will be investigated for routine diagnosis.

Over the recent years, much clinical research is continuing to stress the significance of understanding the pathobiology of different inflammatory diseases for the procurement of efficient and safe pharmacological treatment modalities. In this pretext, we reviewed some of the immunological mechanisms/processes and the applicability of inflammatory biomarkers that occur during acute phases with its consequent remission/recovery period in some of the widely used experimental models of varied spectrum of diseases (Table 4.2). These diseases are quite common ailments and are posing quite a nuisance not only to global health but also in proving obstructions in its socioeconomic developments. Further research needs to be conducted in the area of genes and molecules involved in causing inflammatory disorders thereby improving the diagnosis and treatment of such diseases. The question we pose here is whether it is possible to screen the biomarkers of inflammation and their levels of expression in diseases associated with inflammation and correlate them with the clinical history. This would offer advantage both from the point of view of diagnosis, therapy, and prognosis of inflammatory disorders. The future scope of this paper remains in identifying such markers for inflammation and its correlation with disease condition.