Inflammatory markers

Introduction

Before considering markers, it is first appropriate to consider inflammation (Latin, inflamatio, to set on fire). Inflammation is a complex response by an organism to injury, either resulting from invasion by pathogenic organisms, traumatic cell damage or irritants. Inflammation is essential for tissue repair and without it, wounds would not heal but instead would continue to expand. It can be either acute (the initial response to the harmful stimulus) or chronic (a prolonged process involving continued healing and cell damage occurring simultaneously).

Acute inflammation begins very rapidly after the injury has occurred and is marked by the cardinal signs: rubor, calor, dolor, tumor et function laesa (redness, heat, pain, swelling and loss of function) as described by Celsus (30–38 BC) and Virchow (1870). These signs are caused by vasodilation, increased vascular permeability leading to fluid leakage and release of chemicals that stimulate nerve endings. Pain only occurs if the process involves pain-sensitive nerve endings; e.g. pneumonia is painless until the parietal pleura becomes involved.

The increased vascular permeability is mediated through bradykinin and other vasoactive amines, eicosanoids (prostaglandins, leukotrienes) and cytokines which allows delivery of fibrin, antibodies and complement to the site and attracts leukocytes (mostly polymorphonuclear neutrophils) whose role is to phagocytose damaged tissue and pathogens. Successful acute inflammation should terminate after a relatively short period with normalization of tissue function and minimal scarring.

Chronic inflammation represents continuation of the acute process but the balance of cells changes with an increase in mononuclear cells (macrophages, lymphocytes and plasma cells) and the activation of fibroblasts. Interferon-γ and growth factors increase in importance and, depending on the balance between tissue destruction and tissue healing, the process can continue for a very long time resulting in fibrotic scarring or continued loss of tissue (Chandrasoma and Taylor, 2005).

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Markers of inflammation

Inflammation is associated with a wide variety of disorders (*Table 1*) and therefore since there are a multiplicity of causes for inflammation and different courses for the process, there must be a range of different markers depending on which aspect of inflammation and which type of process is being examined.

One of the simplest markers is an elevated neutrophil count as a result of infection. This may be further complicated by the presence of azurophilic cytoplasmic granules ('toxic' granulations) in neutrophils observed on a blood film which correlate with other inflammatory markers (Kabutomuri, 2000). Since the available range of laboratory tests that can indicate inflammation is wide, these will be considered individually in more depth.

Table 1. Disorders associated with inflammation and selected examples

Disorder	Examples
Allergy	Hay fever
Asthma	
Autoimmune diseases	Atrophic gastritis
	Glomerulonephritis
	Systemic lupus erythematosus
	Rheumatoid arthritis
Cancer	
Infection	Appendicitis
	Meningitis
Inflammatory bowel disease	Crohn's disease
	Ulcerative colitis
Myopathies	Polymyalgia rheumatica
	Polymyositis
Pelvic inflammatory disease	
Transplant rejection	
Tuberculosis	
Vasculitis	Temporal arteritis

Erythrocyte sedimentation rate

Erythrocyte sedimentation rate is simply the rate at which a column of red cells settles in 1 hour. It is carried out by filling a narrow gauge tube with anticoagulated blood and measuring how far down the column the red cell surface has fallen in an hour. Normally erythrocyte sedimentation rate is only a few millimetres per hour because the red cells are negatively charged (the zeta potential), meaning they repel each other. When inflammation is present, the acute phase proteins in the blood, such as fibrinogen, negate the zeta potential causing red cells to stack in rouleaux, which settle faster.

Erythrocyte sedimentation rate is high in a variety of conditions including multiple myeloma, Hodgkin's disease, polymyalgia rheumatica, temporal arteritis, systemic lupus erythematosus, rheumatoid arthritis and chronic kidney disease. In these conditions erythrocyte sedimentation rate may exceed 100 mm/hr. Clinically, erythrocyte sedimentation rate may be used as a non-specific indicator of disease and for monitoring of polymyalgia and arteritis but because of the overlap with malignancies, it is not specific for inflammation and therefore can only be used as a general indicator of disease (Bridgen, 1999).

Interleukin-6

Interleukin-6 (IL-6) is both a pro-inflammatory and anti-inflammatory cytokine secreted by T-cells and macrophages in response to tissue damage and is required for resistance to bacteria. In muscle it stimulates energy mobilization causing increased body temperature. It is known to stimulate production of C-reactive protein.

C-reactive protein

C-reactive protein is composed of 10 subunits arranged as two pentameric discs. It is believed to bind to phosphocholine to assist in recognition and phagocytosis of damaged cells but is also known to

bind to C1q – an important part of the classical pathway in the complement system. It is a relatively primitive part of the immune system. It has been found in bony and cartilaginous fish but not in the lamprey, a more primitive stage in evolution which lacks complement (Matsushita et al, 2004). C-reactive protein is an acute phase reactant which increases in response to IL-6 produced by macrophages (Pepys and Hirschfield, 2003). In acute inflammation it can increase in concentration by up to 50 000-fold, with the increase beginning within 6 hours and peaking at 48 hours.

Consequently, C-reactive protein is commonly used as a marker of inflammation. Levels higher than 5–6 mg/litre are significant as an acute marker but although 5–6 mg/litre is considered to be the 'normal range' for C-reactive protein, evidence suggests that even below this level C-reactive protein is important: i.e. low level increases in C-reactive protein are associated with chronic inflammation.

The JUPITER trial evaluated treatment of apparently healthy men and women with relatively low low density lipoprotein-cholesterol (<3.4 mmol/litre) but C-reactive protein concentrations >2.0 mg/litre, and demonstrated that giving rosuvastatin 20 mg reduced vascular events (Ridker et al, 2008), which suggest that even minor elevations in C-reactive protein indicate significant chronic disease. C-reactive protein assayed in this very low range is often referred to as 'high-sensitivity C-reactive protein'.

A follow-up study of patients with heart failure undergoing cardiac resynchronization therapy showed that in those subsequently suffering major adverse cardiac events levels of the inflammatory markers C-reactive protein and IL-6 remained approximately equally elevated but in those without adverse events the levels of these markers decreased (Michelucci et al, 2007). However, in a trial comparing two drug-eluting stents (rapamycin or paclitaxel) although small differences in stent restenosis were demonstrated, no differences in C-reactive protein or IL-6 were shown (Kang et al, 2008).

C-reactive protein is commonly used as a test for infection. Many studies have been carried looking at the bone disease osteomyelitis. The relative sensitivity of white cell count, erythrocyte sedimentation rate and C-reactive protein have been estimated as 34%, 92% and 96% respectively (Unkilla-Kallio et al, 1994). Others have evaluated the use of C-reactive protein in monitoring for infection following orthopaedic procedures (Aono et al, 2007). C-reactive protein can also been used to monitor patients with chronic obstructive pulmonary disease because acute exacerbations of chronic obstructive pulmonary disease are associated with an increase in IL-6 levels (Seemungal et al, 2001), which causes C-reactive protein levels to increase.

In acute appendicitis C-reactive protein and IL-6 are both equally effective markers of severity but white blood count is effectively useless (Sack et al, 2006). Other markers considered in this study were tumour necrosis factor-alpha (TNF-α), α1-acid-glycoprotein (orosomucoid), endotoxin and erythrocyte sedimentation rate. None of these were recommended for routine use.

Amyloid A

This is an acute phase reactant that behaves similarly to C-reactive protein (Pepys and Hirschfield, 2003).

Faecal calprotectin

Calprotectin is a calcium-binding protein with bacteriostatic and fungicidal properties and is present in very high concentrations in neutrophils. It is also present in lower concentrations in monocytes and reactive macrophages. Faecal calprotectin is increased in patients with inflammatory bowel disease or colon cancer, and in patients using non-steroidal anti-inflammatory drugs (Gaya and Mackenzie, 2002).

Faecal calprotectin correlates well with endoscopic and even better with histological grading of disease activity. It is measured by an enzyme-linked immunoassay as mg calprotectin/g faeces. In a study comparing patients with ulcerative colitis and Crohn's disease, calprotectin concentrations above 150 mg/g were associated with a 2-fold increase in relapse of Crohn's disease and a 14-fold increase in relapse of ulcerative colitis, which may make it a useful tool for monitoring progress and increasing treatment intensity, particularly in colitis (Costa et al, 2005).

Eosinophil cationic protein

Eosinophil cationic protein is a new marker that has been investigated for use in monitoring asthma. In a study comparing inhaled steroid dosing based on eosinophil cationic protein or early morning peak expiratory flow, control of symptoms was similar in both groups but the mean corticosteroid dose in the eosinophil cationic protein-monitored group was significantly lower. It was not concluded that eosinophil cationic protein was better than peak expiratory flow as a monitoring tool but it did suggest that peak expiratory flow was a good surrogate marker for airway inflammation (Löwhagen et al, 2002).

Urine protein

As stated above, one of the early local effects of inflammation is enhanced vascular permeability, mediated by cytokines and other inflammatory mediators. This vascular permeability effect is not always restricted to the locality of the tissue injury, however. In a study in patients admitted to a coronary care unit, urine albumin:creatinine and immunoglobulin G (IgG):creatinine ratios were evaluated in the first urine passed following admission for infarction and on the next three consecutive days (Gosling et al, 1991). This showed that renal vascular permeability was increased as a direct consequence of myocardial infarction. Furthermore, the albumin:creatinine ratio increment was proportional to the increment in serum aspartate transaminase, and therefore proportional to infarct size. A combination of albumin and IgG gave diagnostic sensitivity and specificity as 79.6% and 96.2% respectively, and positive predictive value as 94.6%.

Enzyme markers

Not all markers of inflammation have to be direct markers of the inflammatory process. It is possible to monitor the effectiveness of management of *Helicobacter pylori* treatment by measuring the ratio of pepsinogen I:pepsinogen II. These two enzymes are secreted in different parts of the stomach: pepsinogen I comes from the oxyntic glands and pepsinogen II comes from all gastric glands. Pepsinogen II can be used as a marker of gastritis and pepsinogen I is a marker of atrophic gastritis of the body of the stomach (Di Mario et al, 2004, 2006).

Experimental markers

In addition to the markers described above there are a large number of markers used almost exclusively in research rather than clinical practice. Many of these markers correlate with age and/or lipid concentrations, which suggests they may be providing the same information as high-sensitivity C-reactive protein. These markers include interleukins (IL-6 and IL-18), chemokines (6Ckine, monocyte attractant protein (MCP-1), IP-10, macrophage inflammatory protein-1b (MIP-1b)), soluble adhesion molecules (sICam-1, sE-selectin) and adipokines (adiponectin, leptin) (Miles et al, 2006; Maskarinec et al, 2008).

In community-acquired pneumonia requiring hospitalization, elevated IL-6 and IL-10 levels at time of discharge are associated with higher 1-year all-cause mortality (Yende et al, 2008). TNF-α levels are very significantly elevated in patients with Japanese encephalitis (Babu et al, 2006).

Conclusions

The body has a complex set of systems that are designed to respond to tissue injury and prevent the injury from continuing. These systems include the immune system, the complement system, various mediators that initiate and terminate the inflammatory process and other 'acute phase reactants', whose concentrations vary as a result of the inflammatory process. There are so many potential markers of inflammation that only a few can be described in such a short article. Inflammation is complex but in general, only a small variety of markers are used to investigate it. The common markers are erythrocyte sedimentation rate, white cell count and C-reactive protein. Other markers can be measured but they are usually mainly used for research. **BJHM**

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KEY POINTS

- Inflammation is the body's response to cell injury, either as a result of trauma, infection or irritants.
- Acute inflammation begins very rapidly after the injury has occurred and is marked by the cardinal signs: rubor, calor, dolor, tumor et function laesa (redness, heat, pain, swelling and loss of function).
- Inflammation is an essential process for repair of injury. It can be acute or chronic. Chronic inflammation occurs when there is continuing cell damage at the same time as tissue repair.
- The body has many systems designed to respond to injury. Consequently there are a vast number of potential markers of inflammation.
- Commonly available markers are erythrocyte sedimentation rate, white cell count and C-reactive protein. There are many other markers available but they are usually used for research.