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Anti-inflammatory Interventions – What Has Worked, Not Worked and What May Work in the Future

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Abstract

Our Introductory Commentary relates to many topics that are linked to inflammatory responses and how these responses are regulated in order to promote healing of damaged tissues and bring about effective clearance of infectious agents. In non-infectious situations, cells and tissues release products (danger associated molecular patterns) that can trigger damaging inflammatory responses. These products must be effectively dealt with in order to avoid serious tissue injury. We provide a perspective about many decades of research into the inflammatory response and describe strategies that have achieved success in restraining inflammatory responses, as well as many approaches that have not been clinically effective. With development of new technologies such as advanced genomic analysis, highly sensitive and sophisticated mass spectrometry and related approaches, as well as the ability to employ mutagenesis induction, we are beginning to define highly sophisticated molecular pathways that previously were opaque. This progress may well have clinical relevance, and we may be on the edge of a scientific revolution in the broad area of inflammation.

INTRODUCTION

The inflammatory response is central to effectively dealing with invasive infectious agents (bacteria, viruses, protozoa) as well as in the setting of tissue/organ “sterile” injury (e.g. ischemia-reperfusion injury, hemorrhagic shock, non-penetrating trauma, etc.) and in repair of damaged tissues. With infectious agents, activation of the acute inflammatory response occurs via the innate immune system, featuring local buildup of neutrophils (PMNs) from the bone marrow and blood, as well as accumulation of blood mononuclear cells, which migrate into extravascular sites and mature into tissue macrophages. The emergence of new technologies over the past many decades has propelled research into the inflammatory response along many new directions. These new technologies include mass spectroscopy and related technologies, cloning and expression of peptides, new methods to rapidly purify peptides, proteins and lipids, as well as break-through technologies for sequencing of proteins, DNA and RNA as well as their “editing”. The appearance of molecular biology along with the ability to clone and express purified peptides has allowed access to products that are homogenous and not contaminated with substances that could cause experimental

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artifacts. In the current issue, there is a wide range of mechanisms that can be linked to inflammatory injury. The heme oxygenase pathway generates CO which, together with NO•, has been used in humans to suppress lung inflammatory responses (1). Products of tryptophan metabolism may amplify inflammatory responses (2). Various anti-inflammatory strategies have been used to treat chronic obstructive pulmonary disease (COPD) and asthmatic conditions (3). Several years ago it was discovered that CCR5 as well as CXCR4 play key roles in entry of HIV into CD4⁺ T cells (4, 5). Part of this discovery process was related to mutations occurring in CCR5 of humans, which impaired entry of HIV into T cells. Numerous synthetic inhibitors of CCR5 have been developed which impair HIV entry into T cells, and some have been approved by the FDA for clinical application. Based on this type of strategy, chemokine receptor antagonists may find application in the area of inflammatory diseases, such as inflammatory bowel disease, for which there is a dearth of clinically effective anti-inflammatory drugs. The “metabolic syndrome” can be manipulated by targeting the inflammatory system, including the NLRP3 inflammasome (6). Even in sickle cell disease, inflammatory targets have been suggested (7). Anti-inflammatory interventions after immune reconstitution in humans following bone marrow transplantation may provide clinically desirable outcomes (8). It is also clear that in acute lung injury both in mice and in humans, new targets (histones) have been suggested (9). Similar strategies may be used in Type I diabetes (10), chronic renal disease (11), graft-versus-host disease (12), and in acute ischemic injury involving the heart (13). Another poorly understood condition is non-alcoholic fatty liver disease which may be emerging to be the most prevalent liver disease, with increased risk of liver-related and cardiovascular related mortality (14). As this disease progresses, inflammation and fibrotic changes in liver appear and engagement of toll-like receptors may be linked to cytokine production by hepatocytes. Obviously, much more information is needed before a rational therapeutic approach can be considered. There are many new review articles describing numerous inhibitors and neutralizing mAbs that block complement activation products, cytokines or chemokines, to treat various inflammatory conditions (15–18). The relationship between obesity, insulin-resistance, and pancreatic β cell dysfunction suggests a linkage between cytokine release from adipocytes and residential macrophages and signaling pathways activated by the cytokines, all of which results in eventual apoptosis of pancreatic β cells and onset of Type 2 diabetes (19). Assuming these pathways cause the diabetic condition, it may be possible to intervene early on with targeted anti-inflammatory interventions.

OVER 50 YEARS, LIMITED PROGRESS IN DEVELOPING NEW, EFFECTIVE ANTI-INFLAMMATORY DRUGS

In general, one might conclude that, in spite of huge financial investments by the federal government (NIH, NSF, DoD, etc.) along with large expenditures by “big pharma” over the past 50 years, the returns on these investments has been quite limited. Evidence for “breakthroughs” related to development of innovative and highly effective anti-inflammatory drugs has been sparse and efforts to develop new, effective anti-inflammatory drugs have evolved slowly. In the 1980s and 1990s, there was a focus on the role of oxidants (oxygen and nitrogen-based), proteases released from a variety of cell types, and new drugs that might inactivate these products. A considerable body of new data emerged to link

inflammatory damage to free radical molecules and we learned how to block these reactive species with enzymes or relatively specific inhibitors. The bulk of these “reactive oxygen and nitrogen species” were derived from phagocytes (PMNs, blood mononuclear cells and macrophages), but were also produced by other cell types including hepatocytes. Catalase, which neutralizes H_2O_2 , and superoxide dismutase, which converts $O_2^\bullet$ to oxygen (O_2) and H_2O_2 , emerged as being therapeutically effective in a variety of experimental inflammatory models. N-acetyl cysteine (NAC), an antioxidant, appeared in preclinical studies to be protective in many disorders associated with oxidants. However, numerous clinical trials with these compounds, especially in patients with stroke, myocardial ischemia, chronic lung inflammatory disorders, etc., did not show clinical efficacy. During the same period of time, a great deal of effort went into defining how non-steroidal anti-inflammatory drugs (NSAIDs) affect proinflammatory products derived ultimately from arachidonic acid. While NSAIDs were often useful in controlling pain associated with the inflammatory response, in general they were not especially effective anti-inflammatory drugs.

At the turn of the 20th century, cloning related strategies allowed scientists to define the roles of chemokines and cytokines in inflammatory responses. With some exceptions, in spite of promise in preclinical settings, clinical trials have produced many disappointing results when inhibitors of or neutralizing antibodies for these peptides or their receptors were used in situations such as ischemia-reperfusion injury, acute respiratory distress syndrome, sepsis, etc. Part of this problem may be the numerous similar overlapping proinflammatory activities of cytokines and chemokines, as well as “promiscuity” of receptors for these receptors. There have been some impressive successes. Clinical intervention with products that neutralize TNF or its receptor has been highly successful in treatment of patients with rheumatoid arthritis (20). One of the current reports suggests that blockade of TNF or its receptor may reduce cardiovascular disease developing in patients with inflammatory arthritis (20).

INHIBITION OF ADHESION-PROMOTING MOLECULES AND CELL-ACTIVATING FACTORS

In the 1990s and beyond, there was a strong focus on adhesion molecules related to leukocytes (PMNs, blood mononuclear cells, lymphocytes) and endothelial cells. The biological activities of these adhesion promoting molecules were identified as distinct molecular entities. Early work by several different groups established the paradigm of leukocyte rolling in post-capillary venules, adhesion to endothelial cells, and transmigration of leukocytes to extravascular sites. There was much expectation that these studies would result in effective strategies for employment of blocking agents in various inflammatory disorders, especially in acute ischemic conditions. To date, with only a few exceptions, clinical trials have not validated such expectations. Another strategy has been to block activation of cells (phagocytes, T cells) whose products trigger inflammatory responses. In the case of psoriasis, a skin inflammatory disorder (sometimes also causing joint inflammation), mAbs that block leukocyte function associated antigen 3 (LFA-3), CD2, or CD11a (a β integrin adhesion molecule) were FDA approved and appear to be effective in the treatment of psoriasis.

In the setting of inflammatory bowel disease (ulcerative colitis, Crohn's disease), the use of mAbs to neutralize proinflammatory cytokines as well as broad immunosuppressive and antiinflammatory drugs has given way to strategies unique to the inflamed gut or blockade of cell adhesion pathways involving enterocytes (21). Fecal microbiota transplantation or use of phosphatidylcholine, which alters properties of intestinal mucous to reduce permeability of the intestinal barrier, show promise. mAbs to p40 of IL-12 and IL-23 or use of a new JAK inhibitor has produced encouraging results, as have mAbs to integrins (α 4B7, B7). Time will tell if these strategies will replace current clinical strategies for treatment of IBD. The studies also suggest that cell adhesion pathways may vary from organ to organ (e.g. gut, heart, lung).

LIPID-DERIVED PRODUCTS WITH ANTI-INFLAMMATORY ACTIONS

There is reason to be optimistic about novel and effective anti-inflammatory strategies, as suggested in several papers in this issue. One area of promise involves pathways that generate derivatives of arachidonic acid, leading to generation of anti-inflammatory lipid products broadly described as "resolvins" (22, 23). In preclinical studies, receptors for these lipid products have been defined, and resolvins have appeared to be effective as anti-inflammatory compounds at nM concentrations. There has been little evidence of toxicity, and resolvins did not impair innate immune responses of phagocytes nor did they interfere with phagocyte clearance of infectious agents (23). It is possible that resolvins may turn out to be effective, novel anti-inflammatory products with a wide margin of safety, if supported by data in clinical trials.

COMPLEMENT INHIBITORS AS ANTI-INFLAMMATORY DRUGS

A few comments about complement and its inhibitors are in order. Complement is a major fortress in the innate immune system (24), providing protection against invasive microorganisms (bacteria, viruses, protozoa) as well as promoting tissue recovery after trauma, burn, ischemia-reperfusion, etc (25–28). Generation of C3 convertases results in C3a (anaphylatoxin) and C3b, the latter being a major promoter for uptake of complement-coated microorganisms, resulting in their killing by phagocytes. The next downstream key activation factor in the complement system is the C5 convertase, which generates C5a (anaphylatoxin) that strongly activates PMNs with resulting chemotaxis and activation of NADPH oxidase 2 (which generates $O_2^\bullet$ and H_2O_2 , which are important in the killing of microorganisms (29–31). C5b interacts with C6, 7, 8 and 9 to form the membrane attack complex (C5b-9) which can kill microbes but can also injure or damage mammalian cells if generated on the cell surface (32, 33). Theoretically, avoiding excessive blockade of the C3 convertase could preserve C3b generation. Blockade of either C5a or C5b (or intact C5) might be an optimal strategy to block unwanted proinflammatory responses initiated by C5a and C5b-9. Complement inhibitors that have been used in preclinical studies include purified cobra venom factor (which causes consumptive depletion from C3 and beyond), compstatin (which blocks C3 activation), C1 esterase inhibitor (which blocks the classical pathway of complement activation), and neutralizing antibodies to C5a and C5b. Of all of these inhibitors, C1INH is safe but studies have shown conflicting results related to clinical efficacy. It is not obvious that C1INH has a clear-cut benefit in the setting of human sepsis

(34–38). Anti-C5a (known as IFX-1), which neutralizes the phlogistic activities of C5a (30, 39–41), is now being used in phase II clinical trials in sepsis in Germany (*InflaRx*, <http://www.inflarx.de>), while anti-C5 is effective in the treatment of patients with paroxysmal nocturnal hemoglobinuria (PNH), a rare autoimmune disease that is C5b-9 dependent and results in periodic massive hemolysis that cause renal damage (42–44). The mAb has also been approved by the FDA for use in atypical hemolytic uremic syndrome, a renal glomerular disorder that is associated with uncontrolled activation of the complement system and is often associated with clot formation within small vessels throughout the body. As many as 30–40% of these patients develop end-stage renal disease.

THE NLRP3 INFLAMMASOME

Several articles in this issue describe the NLRP3 inflammasome, its activation in PMNs and macrophages, and formation in PMNs of neutrophil extracellular traps (NETs) as the innate immune system responds to contain infectious agents (45, 46). Contributions of the NLRP3 inflammasome to inflammatory disorders are also described. Signaling pathways in human autoimmune diseases as well as in mouse autoimmune diseases have been delineated (47). Vaccination of patients who are afflicted with autoimmune diseases, using clinically safe and effective vaccines, may lead to protective outcomes (48). While there are several structural versions of the NLRP inflammasome, the most intensely studied is the NLRP3 inflammasome, which is considered to be an important player in the innate immune system, providing capture and killing of bacteria associated with NET formation (see below). Inflammasome activation occurs both in PMNs (49) and in macrophages (50, 51). The NLRP3 inflammasome consists of four components: NLRP3 protein, pro-caspase 1, ASC, and the precursor version of IL-1 β . The inflammasome can be “primed” by LPS and activated by numerous factors (histones, ATP, C5a, PMA, and crystals [urate, cholesterol, etc.]) resulting in caspase 1 activation and release of the mature form of IL-1 β . The activation process in the inflammasome of PMNs, but not in macrophages, results in formation of DNA strands along with appearance of PMN products (elastase, MPO, NOX2) and extracellular histones. Formation of NETs causes bacterial immobilization and killing of a variety of bacterial species, both gram+ and gram-. NETs have also been found in a variety of acute inflammatory responses and in autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus). In these disorders there may also be autoantibodies reactive with citrullinated histones (52–54) which can result in additional autoimmune responses (55, 56). The NLRP3 inflammasome has also been found in tissue following ischemia-reperfusion injury, in cardiomyocytes (57–61), in Kupffer cells in liver (62–64), in the kidney (65–67), and in the central nervous system after trauma (68–70) as well as in other conditions (reviewed, (71, 72)). The biological activity of IL-1 β may be neutralized with either IL-1 receptor antagonist or anti-IL-1 β monoclonal antibody, both of which have been approved by the FDA for use in rheumatoid arthritis (18).

ROLE OF INFLAMMATION IN THE SETTING OF CANCER AND PREGNANCY

The role of inflammation related to malignant tumors is controversial and poorly understood (73). There is also a controversy related to the roles of the innate immune system and adaptive immunity, and whether products of these two pathways might cause propagation or

regression of malignant cells. Use of corticosteroids, NSAIDs, and other interventions have been studied extensively in humans and animals where effects on inflammatory responses have been determined. However, in the setting of cancer cells, very little is known about how the same interventions may affect tumor progression. There are numerous reports of activation of JAK/STAT pathways in malignant tumors, but whether blockade of such pathways will be therapeutically beneficial remains to be determined.

With respect to inflammation and pregnancy, in uncomplicated pregnancies, there is a very carefully balanced series of inflammatory responses that promote normal fetal development (74). If “aberrant inflammation” develops, this may lead to preterm labor, infection, fetal loss, pre-eclampsia, maternal obesity gestational diabetes mellitus or other complications of unregulated inflammation. Therapeutic interventions in such cases are under consideration.

FUTURE DIRECTIONS

In spite of many failed clinical trials using a variety of anti-inflammatory interventions in a variety of conditions, we seem to be making progress in the development of new, effective and non-toxic anti-inflammatory drugs. The expansion, development and employment of advanced genomic approaches has resulted in new approaches to define the biochemical nature of inflammatory responses and how such information can be used to develop new strategies for therapeutic interventions. A better molecular understanding of details regarding cell signaling pathways in phagocytic cells and in their targets (endothelial cells, epithelial cells, etc.) may permit development of new, highly specific compounds that selectively block the chain of events that leads to cell and organ damage. Perhaps we are entering into a new and exciting phase of anti-inflammatory interventions.

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