REVIEW ARTICLE

Accepted 29 June 2003
Revised 5 May 2003
Received 28 September 2001
Indian J. Pharm. Sci., 2003, 65(6): 565-575

Inflammation and Novel Therapeutic Approaches for its Management

D. HARI KRISHNA, M. SRIPAL REDDY[1], K. RAJNARAYANA[1], D. R. KRISHNA[1], M. C. PRABHAKAR*
Department of Pharmacology, University College of Pharmaceutical Sciences,
Kakatiya University, Warangal-506 009.
[1]Drug Metabolism and Clinical Pharmacokinetics Division, University College of Pharmaceutical Sciences,
Kakatiya University, Warangal-506 009.

Inflammation is a biological response to a series of chemical reactions whose major function is protection from infection and the resolution of tissue damage caused by injury. There are several mediators released during the process of inflammation. Activation of phospholipase-A_2 (PLA_2) family is a key step in the production of precursors in biosynthesis of inflammatory lipid mediators. Platelet activating factor, cyclooxygenase-2, leukotrienes, nerve growth factor, inducible nitric oxide synthase, bradykinins, adhesion molecules, nuclear factor-kB and cytokines are also important mediators for inflammation. There are several agents, which inhibit the endogenous mediators that can be used for the treatment of inflammation. Apoptosis is another important mechanism, where several agents that act by different mechanisms of action can treat the inflammation.

The rapid development in our understanding of the molecular processes controlling the activation and function of the cells has opened up a wide variety of new potential targets for drug intervention. This is particularly true in the fields of oncology, immunology and inflammation[1]. Inflammation is a biological response to a series of chemical reactions whose major function is protection of the body from infection and the resolution of tissue damage caused by injury. During these reactions, toxic materials and cellular debris are removed by means of increased capillary permeability and the migration of leucocytes to the injured area. As a result of cell injury, an intricate system is activated causing the release of numerous inflammatory mediators such as histamine, serotonin, bradykinin, Hageman factor, lysosomal enzymes, prostaglandins, and leukotrienes. These mediators initiate a three-phase process consisting of: vasodilatation, increased vascular permeability, and leukocytic exudation, all of which occur simultaneously in a multiple interaction process resulting in the characteristic clinical signs of heal, redness, swelling, pain and diminished function[2,3].

*For correspondence
E-mail: mc_prabhakar@yahoo.com

MEDIATORS OF INFLAMMATION

Phospholipase A2 (PLA_2):

Activation of the phospholipase A2 (PLA_2) family is a key step in the production of precursors for the biosynthesis of inflammatory lipid mediators. Inhibition of this enzyme could result in the suppression of three important classes of inflammatory lipids prostaglandins, leukotrienes, platelet activating factor), which offer an attractive therapeutic approach to the design of novel agents for the treatment of inflammation and tissue injury. The PLA_2 family is a series of enzymes involved in phospholipid catabolism. More than 150 PLA_2 amino acid sequences are currently available in protein sequence database[4]. PLA_2 exist in both extracellular and intracellular forms. Secretory PLA_2 are Ca^{+2} dependant enzymes (molecular weight 14 kD, regulated by millimolar amounts of Ca^{+2}), which are classified into two groups on the basis of their primary structures. Secretary non-pancreatic PLA_2 has been implicated in the pathogenesis of par-

ticular inflammation in rheumatoid arthritis, whereas pancreatic PLA_2 contributes to the tissue damage associated with acute pancreatitis. The cytosolic PLA_2 ($cPLA_2$) is an 85.2 kD enzyme with substrate specificity for arachidonic acid (AA). It is found in most cells and tissues and is regulated by Ca^{+2} in the macro molar range. $cPLA_2$ plays an important role in both the rapid and prolonged cellular responses occurring during inflammatory process[5-7].

Platelet activating factor (PAF):

Platelet activating factor (PAF), a PLA_2–dependant phospholipid, is an extremely potent mediator of shock and inflammation. It exerts the biological effects by activating the PAF receptor, consequently stimulating protein kinase-C and increasing intracellular Ca^{+2}. PAF is found in most biological fluids and is synthesized in most cell types. Micro vascular permeability is markedly increased by PAF, allowing fluids to leak out of the circulation. In the area of haemodynamic effects, PAF has negative ionotropic property and lowers arterial blood pressure. It is a potent platelet aggregator, leukocyte activator, and it strongly promotes AA metabolism. It has been proposed to play a crucial role in pathogenesis of rheumatoid arthritis, asthma, endotoxin shock and acute renal transplant rejection.

Cyclooxygenase-2 (COX-2):

The 1990s has been a new dawn in inflammation research, with several studies demonstrating increasing COX activity of cells after exposure to endotoxins, inflammatory cytokines, growth factors, hormones and tumor promoters. This gave rise to the new concept that there might be a constitutive COX activity, further referred to as COX-1 and an inducible one, further referred to as COX-2. Because of the substantial risks involved with the long-term use of NSAIDs, there is an increasing demand for the development of newer agents with better pharmacological profile i.e., to improve NSAID tolerability, particularly gastric tolerability. The discovery of COX isoenzymes has given rise to a better understanding of the inhibition of COX by classical NSAIDs and offers the prospect of devising new and potentially safer drugs[8]. The main reason for labeling COX-1 and COX-2 as physiological and pathological respectively is because pro-inflammatory cytokines like INF-γ, TNF-α, IL-1β and IL-1, induce COX-2 in macrophages or endothelial cells. Thus, COX-2 is the main isoform associated with inflammation, however, there is constitutive expression of either/both the isoforms in specific tissues[9].

Leukotrienes (LTs):

Leukotrienes (LTs) are potent pro-inflammatory agents involved in the pathophysiology of various inflammatory diseases such as asthma, psoriasis, rheumatoid arthritis, chronic bronchitis, ulcerative colitis and inflammatory bowel disease. Therefore many leukotriene compounds have been developed which selectively block either LT receptor sites or enzymes related to their biosynthesis. The first step in the generation of leukotrienes is catalyzed by the calcium and ATP-dependant enzyme 5-lipoxygenase (5-LO). This is one of a family of LO enzymes that metabolize AA to hydroperoxy-eicosatetraenoic acids (HPETEs). Each enzyme catalyses the insertion of an oxygen moiety at a specific position in the AA backbone, 5-LO forms 5-HPETE, the precursor of the LTs.

When cells are activated, cytosolic 5-LO is translocated to the nuclear membrane. A nuclear membrane protein, 5-LO activating protein (FLAP), is required before 5-LO can synthesize 5-HPETE from AA. The rearrangement of 5-HPETE to form the unstable LTA_4 is the rate-limiting step in the synthesis of LTs. This step is catalyzed by LTA_4 synthase. LTA_4 is then converted to either LTB_4 or LTC_4. LTC_4 is actively transported out of cells, rapidly metabolized to LTD_4, and then to LTE_4 (fig. 1). LTC_4, LTD_4 and LTE_4 are referred to as the cysteinyl (cys) leukotrienes because of their chemical structure. LTE_4 is either excreted in the urine or metabo-

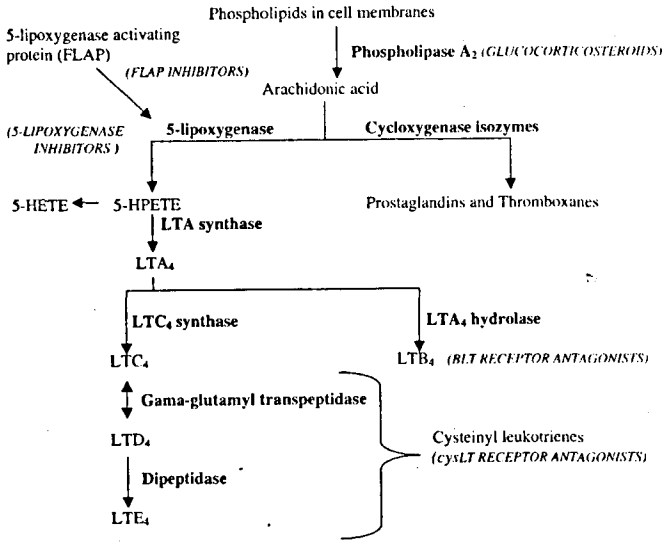
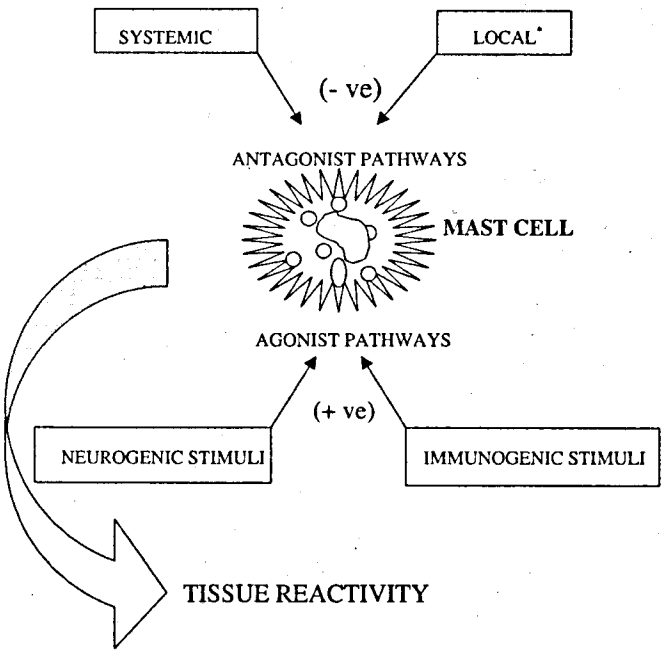


Fig. 1: The main pathways for the formation of leukotrienes and the sites of action of the current groups (brackets) that can attenuate responses Normal: main pathway, Bold: enzymes, Italic bracket: drug groups.

lized to a variety of biologically less active or inactive metabolites including LTF_4[10].

Nerve growth factor:

Recent discoveries have shown that nerve growth factor (NGF) plays a role in inflammation via mast cells, and a novel class of lipid amides has been identified that exhibits a very interesting anti-inflammatory action[11]. A new lipid amide acting on the peripheral cannabinoid receptors (CB_2 receptors) may offer all the beneficial effects of cannabis without unwanted side effects[12] (fig. 2). There was a local feed back mechanism that negatively modulated mast cell behavior during inflammatory processes. Veneto *et al.* coined the term acronym ALIA (autacoid local inflammatory antagonism) to describe this sequence of events. They were able to show that these same mast cells express the peripheral CB_2 subtype of cannabinoid receptors, which exhibits differential sensitivity to the anandamide and to the lipid N-acylethanolamine derivative N-(2-hydroxy ethyl) hexadecanamide. The later has been demonstrated in peripheral tissue, and suggested that, this compound may be the endogenous ligand for CB_2 receptor, acting as a local autacoids, down-regulating mast cell activation and inflammation[13].



* ALIA: Autocoid Local Inflammatory Antagonism

Fig. 2: Nature's way for the physiological modulation of mast cells in the inflammatory process.

Although anandamide and N-(2-hydroxy ethyl) hexadecanamide bind to the CB_2 receptors, only the later is able to down-regulate mast cell activation *in vitro*[14]. This ethanol amide and its close derivatives are potent antiinflammatory agents acting specifically on the mast cells. The prototype of these aliamides is the putative endogenous ligand for the CB_2 receptors, N-(2-hydroxy ethyl) hexadecanamide, given the code LG 2110/1 by research group, was able to produce a variety of effects following oral administration. If the clinical trials support the efficacy of LG 2110/1, and related aliamides, it will offer the possibility of obtaining all the beneficial effects previously attributed to cannabis such as analgesic, anticonvulsants, antiasthmatic, hallucinogenic and antihypertensives.

Inducible nitric oxide synthase (iNOS):

An important property pertinent to the role of nitric oxide (NO) in inflammation is its ability to activate COX, resulting in larger production of proinflammatory PGs. This discovery led to the demonstration that the dual inhibition of the release of proinflammatory NO and PG contributes to the antiinflammatory property of NOS inhibitors. NO is synthesized in mammalian systems from L-arginine by a family of enzymes known collectively as nitric oxide synthases (NOSs; EC 1.14: 13.39). With regard to regulation, the NOS enzymes fall into two distinct categories: constitutively expressed isoforms ecNOS and ncNOS, which are regulated primarily by Ca^{2+} and calmodulin and a cytokine-inducible isoform (iNOS), which is regulated primarily at the level of *de novo* protein synthesis.

The distinct properties of each of the NOS isoforms have important implications, since as summarized in fig. 3, it is the magnitude, duration and the cellular sites of the NO production which determine the overall physiological or pathophysiological effects of NO. In view of the critical importance of NO produced by constitutive enzymes in a variety of physiological processes, a major emphasis in inflammatory research is the development of potent and selective inhibitors of the iNOS isoforms. Selective inhibition of iNOS offers the potential advantage of blocking the synthesis of a major cytotoxic agent namely NO, and ultimately reduces the tissue damage during chronic inflammatory diseases, unfaltering the beneficial effects of NO[15].

NO and Inflammation:

The potential for the production of sustained, high levels of NO from the iNOS isoforms, as well as an increased understanding of the cytotoxic and/or cytostatic action of

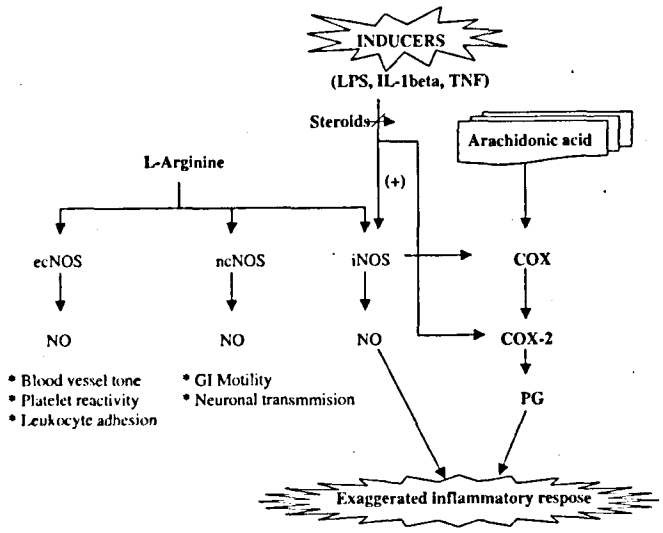


Fig. 3: Biosynthetic pathways of Nitric oxide and its effects.

NO, has led many investigations to examine the role of NO in a variety of pathophysiological conditions[16]. Excessive production of NO plays a pathogenic role in both acute and chronic models of inflammation[17]. As indicated previously, NO is a potent vasodilator and its involvement during an inflammatory response may be related to its ability to increase vascular permeability and edema through changes in local blood flow[18]. Like COX, NOS has both constitutive and inducible isoforms, synthesis of iNOS is triggered by those cytokines that also induce COX-2, and this may explain why iNOS and COX-2 are often expressed together in a variety of pathological states. iNOS and COX-2 induction is blocked by potent anti-inflammatory steroids, including dexamethasone, and this property of steroids explains their potent antiinflammatory effects[15].

Bradykinin:

The kinins, particularly bradykinin (BK), are important mediators involved in both the initiation and progression of an inflammatory response. The pro-inflammatory effects of BK are brought about by its interaction with specific G-protein coupled receptors. At present, there are two clearly defined and cloned kinin receptors; B_1 and B_2[19]. B_2 receptors are constitutively present on many cell types involved with the inflammatory response; endothelial cell, mast cells[20] and sensory neurons[21]. The natural endogenous ligand for these receptors is BK, with Lys-BK in most cases possessing a lower affinity for the receptors. In contrast, B_1 receptors are not normally expressed in basal conditions but are indeed in situation of stress, such as shock and inflammation. The classical, natural ligand for this receptor is des-Arg BK (DABK) a cleavage product of the activity of carboxypeptidases on BK. (although the endogenous B_1 receptor ligands have no effect at B_2 receptors BK and Lys-BK bind to human B_1 receptors)[19].

Activation of B_1 receptors produces a range of pro-inflammatory effects that include edema, pain and promotion of blood-borne leukocyte trafficking. There is support for the concept that not only all B_1 receptors up-regulated by specific inflammatory stimuli, but there is also an increase in the levels of DABK produced. This elevation could be due to an increase in the activity of CPM, an enzyme involved in the synthesis of DABK[22].

Thus, during inflammation it is apparent that both agonist and receptors are up-regulated. These lines of evidence suggest that B_1 receptor activation might be functionally involved in mediating the cardinal signs of inflammation. For inflammatory pain, the sensory neurons might be the site of expression because this is essentially neurogenic in nature and sensitive to agents that modify sensory C-fiber activity. These fibers contain pro-inflammatory peptides Substance-P (SP) and calcitonin-gene related peptide (CGRP)[23]. There is evidence however that B_1 receptor activation by DABK results in the release of hyperalgesic prostanoids[24].

Adhesion molecules:

Cell-to-cell interactions and the adhesion of cells to extra cellular matrix components have a key role in important physiological phenomena as well as in the pathogenesis of diverse conditions such as tumor cell metastasis, graft rejection, ischemia/reperfusion injury and immune mediated hypersensitivity. Inflammation is a phenomenon that involves the accumulation of leukocytes in a given tissue leading to varying degrees of cell damage, extra cellular matrix disruption and organ dysfunction. The migration of leukocytes towards inflammatory foci and the interactions of inflammatory cells at these sites are mediated by cell adhesion molecules (CAM). Although inflammation is primarily a defense mechanism, this phenomenon frequently becomes undesirable condition that requires therapy[25-27]. Adhesion molecules involved in inflammation belong mainly to the families of selectins, integrins and the immunoglobin [Ig] gene super family.

Selectins:

This family comprises of 3 members that are L, P and E-selectins. L-selectins (CD62L) are expressed by leukocytes. There are lectin-like molecules that mediate leuko-

cyte rolling under conditions that induce cell activation; the basal expression of P- and E-selectin is increased where as that of L-selectin is down regulated[28]. P-selectins (CD62P) are expressed on the surface of activated endothelial cells and platelets. It is stored in weibel-palade in EC and in á-granules in platelets. During inflammation EC P-selectin promotes the aggregation of leukocytes with platelets to form thrombin[29].

Integrins:

Integrins that are involved in leukocyte-EC interactions and the inflammatory phenomena belong mainly to the β1 and β2 subfamilies. The α4β1 integrins have an important role in leukocyte-EC interactions; where as other β1 integrins are primarily involved in the adhesion of leukocytes to extra cellular matrix components (laminin, collagen, vitronectin and fibronectin). It is expressed by lymphocytes, monocytes, and eosinophils[30]. The β2 integrin subfamily comprises the α1β2 (leukocyte function associated antigen-1 (LFA-1) or CD11a/CD18), αM22 heterodimers. LFA-1 is expressed by all leukocytes and interacts with ICAM-1,2 and 3. αX22 binds to fibrinogen and the component fragment-1C3b, whereas αDβ2 mainly interacts with ICAM-3. The β3 integrins (GPIIb/IIIa) and αV23 are expressed by platelets and are involved in important phenomenon (platelet aggregation EC-platelet adhesion) that also occurs in inflammatory conditions[31].

An adhesion molecule that belongs to the Ig gene super family possesses one or more domains homologous to those found in immunoglobins. Members of this super family are expressed by EC (e.g.: MadCAM-1) vascular cell adhesion-1 and –2 (ICAM-1 and 2 or CD54 and CD102 respectively) and platelet endothelial cell adhesion molecule-I (PECAM-I or CD31). There are additional intercellular adhesion molecules that also participate in the inflammatory phenomenon. Cadherins are calcium-dependant adhesion proteins that mainly interact homotypically by themselves. Cadherins found at intercellular endothelial junctions seems to have a key role in the extravasations of inflammatory cells[32].

Nuclear factor-kB (NF-kB):

NF-kB was first identified as a regulator of the expression of the kappa light chain gene in murine B lymphocytes. The activated form of NF-kB is a heterodimer, which usually consist of two proteins, a P_{65} (also called relA) subunit and a P_{50} subunit. Other subunits, such as rel, relB, v-rel, and P_{52} may also be part of activated NF-kB, and it is likely that the different forms of NF-kB may activate different sets of target genes[33]. Many stimuli activate NF-kB, including cytokines, activators of protein kinase-C, viruses, and oxidants. Several signal transduction pathways may be involved, but all these stimuli act by means of protein kinase that phosphorylate (and thus degrade) IkB. The activation of NF-kB therefore leads to a coordinated increase in the expression of many genes whose products mediate inflammatory and immune response. For example, the coordinated stimulation of expression of the genes of E-selectin, IL-8 & TNF-α results in the recruitment and activation of neutrophils[34].

Cytokines:

Cytokines constitute a family of proteins selected from a variety of cell types during the host response to infection and injury that coordinately regulate both haemopoiesis and the protective immune and inflammatory responses. These pleiotropic molecules regulate the production, differentiation and function of effector cells which mediate multiple processes, including the recognition of self verses non-self, the targeted removal of foreign pathogens, the killing of tumor cells and an effective wound healing process[35,36]. The various cytokines involved in inflammatory condition are IL-2, 3, 4, 5, 6, 8, GM-CST, MCP-1, RANTES, MIP-1α, TNF-α, and. Steroids inhibit the transcription of several proinflammatory cytokines and cytokine receptor genes, and in addition, can inhibit the function of transcription factors including AP-1 and NF-kB, through which many proinflammatory cytokines exert their effects[37]. However, the adverse systemic side effect profile, and the resistance of some patients to steroid therapy limit their use.

Apoptosis and inflammation:

Following tissue injury, inflammatory cells invade the lesion, then fibroblasts migrate, proliferate and synthesize extra cellular matrix component participating in the formation of granulation tissue. As they would close and evolve into a sear, there is an important decrease in cellularity and in particular myofibroblasts disappear. This cellular loss is brought about by apoptosis. In chronic inflammatory conditions like RA, T-cells migrate into the synovial cells in RA patients undergo extensive DNA fragmentation. However, these cells fail to complete apoptosis thus permitting chronic proliferative arthritis to continue unabated. Since apoptosis can be induced by several mechanisms, many attractive approaches could serve as potential therapeutic strategies[38,39].

NOVEL THERAPEUTIC AGENTS IN TREATING INFLAMMATION

PLA_2 Inhibitors:

The natural compounds that inhibit the PLA_2 are steroids like lipocortin-1, manoalide, BMS-181162, scalaradial, aristolochic acid, YM-265671, YM-267334, thielocin A_1beta, thielocin $beta_3$, whereas the synthetic compounds which includes 3-acrylic acid derivatives and dehydroabietylamine derivatives such as WAY-121520[40-42].

AntiPAF:

It is elucidated that PAF antagonists such as rocepafant, apafant, lexipafant, and bepafant can reach significance in the therapy of critically ill patients. The natural compounds include terpenes (ginkolides A, B, C, M and J), lignans (kadsurenone), gliotoxins (PR-900452, PR-49175) and the synthetic compounds include the compounds structurally related to PAF (CV-3988, SR-27417, ONO-6240, RO-193704), whereas compounds unrelated to PAF includes thiazoles (RP-48740, RP-52629) triazolobenzodiazepines (apafant [WEB-2086], rocepafant [BN-50730]), flumazenil (TCV-309)[4].

Cyclooxygenase-2 inhibitors:

There are several categories of compounds that inhibit COX-2, that include diclofenac, indomethacin, naproxen, and ibuprofen, those that selectively inhibit the enzyme such as nimesulide, meloxicam, celecoxib, rofecoxib, and those that are highly selective include etodolac, DFU, and L745337[43-45].

Antileukotrienes:

The compounds that act as antileukotriens include LT receptor antagonists and 5-LO inhibitors, which are represented in the Table-1[4,10,46].

NOS Inhibitors:

L-NMMA, L-NAME (non selective), L-NIO (irreversible), L-NOARG (slowly dissociating), L-NIL, amino guanidine (AG), 7-propyl-homoiminopiperidinium chloride (selective)[47,48].

B_1 receptor inhibitors:

The first BK receptor antagonists produced were peptidic in nature, easily prone to enzyme attack and therefore had short half-lives[49]. Among the best of the peptidic antagonists is B9958 a compound highly selective for the human B_1 receptor. To date there are no nonpeptidic B_1 receptor antagonists.

A significant advance came with the generation of the stable peptidic B_2 receptor selective antagonist, HOE 140 (icatibant)[50] and more recently the development of non-peptide blockers. The non-peptidic antagonist to be described was WIN 64338, shown to be an effective antagonist in guinea pig tissues but with little activity in human tissues[51].

Antiadhesion therapy:

As stated above the cell-to-cell interaction that takes place in the inflammatory phenomenon is mediated by CAM. Therefore the blockade of expression and/or function of CAM may be a potential target for inhibiting the inflammatory response. Blockade of the activity of pro-inflammatory cytokines is another (indirect) approach to the anti-adhe-

TABLE 1: ANTILEUKOTRIENES ACTING BY LT RECEPTOR ANTAGONISM AND 5-LO INHIBITION.

LT receptor antagonists			5-LO inhibitors
BLT (LTB_4)	LT_1 (LTD_4)	Cys LT_2 (LTC_4)	
LY 293111	Montelukast	BAY49773	Zileuton
SC 53228	Iralukast	nonselective at $CysLT_1$ & $CysLT_2$	Bay-X-1005
SB 209247	Pobilukast		BW-755C
CP 105696	Zafirlukast		Guiflapon
CGS 25019C	Pranlukast		MK 896
	Verleukast		Pinprost
	Tomelukast		Ionapalene
	Cinalukast		R-65151
			ZD 2138

sion therapy in inflammatory conditions. The neutralization of cytokines and TNF-α with monoclonal antibodies, or the pharmacological inhibition of the synthesis of the later cytokine, has a noticeable antiinflammatory effect[52,53].

Several integrins interact with RGD (Arg-Gly-Asp) motifs, whereas VLA-4 interacts with the fibronectin fragment CS-I and the LDV (Leu-Asp-Val) motifs. This provides a rational basis for the development of reagent that specifically blocks the formation of integrins. AntiCAM Mab and peptides that contain integrin binding motifs (e.g.: RGD or LDV sequences) or soluble counter-receptors of CAM, are able to effectively interfere with the function of these molecules[54,55]. AN 100226M, a MAb to α4 integrin has been shown to reverse the clinical and histologic signs of experimental allergic encephalomyelitis (EAE) by blocking this interaction[56]. A significant advance in this field has been the generation or isolation of synthetic and natural substances that bind to integrins and block their function. On the other hand, soluble carbohydrates, that specifically reacts with selectins, e.g.: sulfatide, fucoidins or sialyl Le are also able to block the function of these CAM[57].

Leumedins are novel compounds with anti-inflammatory properties that inhibit the adhesion of neutrophil mediated by Mac-1[58].A new approach that emerged in recent years is to inhibit CAM expression. Antisense therapy for the blockade of both cell adhesion molecules and pro-inflammatory cytokine may be a significant advance in the treatment of inflammatory conditions. However, several issues require clarification before these can enter wide clinical use. These include toxicity, site-specific delivery, *in vivo* stability and clearance of metabolites. The second generation of synthetic antisense oligonucleotides should facilitate this therapeutic approach[59,60]. Another interesting approach to anti-adhesion therapy is the use of synthetic double-stranded DNA molecules possessing specific consensus sequence that compete for the binding of transcription factors necessary for gene expression. Lately, gene expression can also be selectively blocked with triplex forming oligoneucleotides[61].

NF-kB inhibitors:

Glucocorticoids are effective inhibitors of the NF-kB, the activation that may account for most of their anti-inflammatory actions, but they have endocrine and metabolic side effects when given systemically. These side effects may not occur with more specific NF-kB inhibitors. There may be a direct protein-protein interaction[61] between glucocorticoid receptor and AP-1 and between the receptor and NF-kB and present it from binding to kB sites on genes that have a role in inflammatory process[63].

Antioxidants that inhibit the activation of NF-kB represent a class of compounds that has not yet been extensively investigated. In view of the fact that currently available antioxidants, such as vitamin-C and E and acetylcysteine are relatively week, more potent and long lasting antioxidants are needed[64]. Some naturally occurring inhibitors of NF-kB have been identified; gliotoxin derived from aspergillin, is a potent and relatively specific inhibitor[65]. The anti-inflammatory cytokine IL-10 also inhibits the action of NF-kB, through an effect on IkBα[66].

It may be unwise to block the activation of NF-kB for prolong periods, because the factor plays a very crucial role in the immune response and other defensive responses. The target disruption (a knockout) of the P_{65} compound of the NF-kB is lethal because of the associated developmental abnormalities, whereas the lack of the P_{50} component results in immune deficiencies and increased susceptibility to infection[67,68].

Anticytokine therapy:

In considering strategies for the discovery of novel anticytokine therapies the paracrine/autocrine nature of cytokine action affords various opportunities for intervention of multiple cellular and molecular levels (fig. 4). One means of inhibition of cytokine action is by decreasing their production, since many cytokines are upregulated at the transcriptional, translational and post-translational levels. Production of IL-1β and TNF-α from LPS, stimulated monocytes can be inhibited at the translational level by a series of anti-inflammatory compounds, the pyridinyl imidazoles[69].

There are also opportunities for inhibiting these proinflammatory cytokines at the post-translational level, since both IL-1 and TNF-α are translated as precursor proteins that are sequentially cleaved by proteinases or converting enzymes prior to secretion from the cell as mature, biologically active cytokines[70,71].

Protein antagonists:

One of the first such antagonist was the mutation of a single critical residue of murine IL-2, where substitution of the polar Gly with the acidic Asp functionality resulted in a protein that was a week partial agonist but was capable of antagonizing IL-2 activity. Furthermore substitution of the aromatic residue Tyr with an acidic Asp in IL-4 resulted in a antagonist of IL-4 activity with picomolar potency.

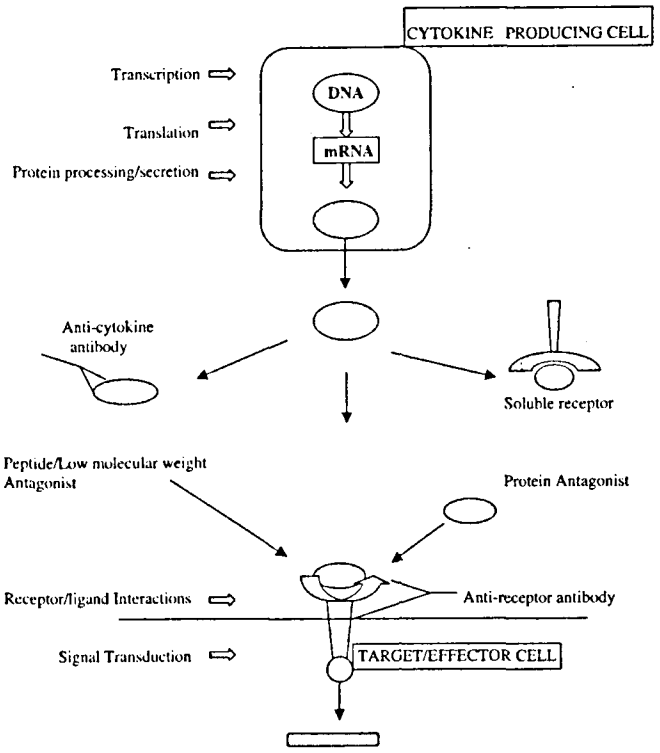


Fig. 4: Potential cellular and molecular levels for inhibition of cytokine action.

The eosinophilopoietic cytokines IL-3, IL-5, and GM-CST bind to receptors composed of a ligand specific α-chain and a common β-chain. Reversal of this at position Glu in GM-CST results in an antagonist protein and substitution of Glu residue of IL-5 with the polar but unchanged residue Glu similarly results in an IL-5 antagonist. The predicted third helix of IL-6 and the loop linking it to the fourth have been shown experimentally responsible for signaling, since specific mutation in this region have produced protein that can antagonize IL-6 action. IL-8 a member of the cytokine family (α-sub class), where the truncation of the ECR tripeptide at the aminoterminals, which is essential for the receptor binding and activation results in a mutant protein that exhibits week antagonistic activity towards IL-8. Monocyte chemoattractant protein-1 (MCP-1) is a member of the b-subclass, where either deletion of the first eight residues or an internal deletion of the residues 2-9 results in protein capable of antagonizing the monocyte chemo attractant properties of MCP-1. The most potent cytokine antagonist described to date is that created by the production of the recombinant *E coli*-expressed form of RANTES, where the initiating methionine molecule is not removed and the protein albeit correctly folded, exhibits no agonist activity. However, it is able to bind to the RANTES receptor CCCKR-1 without signaling and antagonize the responses elicited by both RANTES and macrophage inflammatory protein-1α (MCP-1α) with nanomolar potency.

ANTITNF-α THERAPY

Various approaches against TNF-α includes

TNF-α converting enzyme (TACE) inhibitors:

TACE has been found to be a specific Zn containing metal proteinase that cleaves peptide chains between alanine and valine residues. Metalloproteinase inhibitors nonspecifically inhibit the release of TNF-α, inhibits the shedding of TNF-α receptor protein p55 and p75, and receptors for IL-6, IL-1 and IL-2 from cell surfaces. Examples of TACE inhibitors include matrix metalloproteinase (MMP) inhibitors like marimastat, barimastat, TAPI (TNF-α processing inhibitor), BB-1101, BB-2284, BB-3241, BB2275, RO-31-9790, CBS-27023A, 5B-20358.

Phosphodiesterase (PDE) type–IV inhibitors:

Enhancement of intracellular cyclic AMP (cAMP) level has long been associated with anti-inflammatory and anti-allergic activity. The recognition of the cyclic neucleotide PDE isozymes has generated considerable interest in the possibility of selective inhibitors of inflammation. PDE type-IV is the predominant cAMP-specific PDE in inflammatory cells. PDE inhibition increases intracellular cAMP concentrations there by inhibit the release of TNF-α and related infiltration of cells as well as the production of ILs. Examples include rolipram, piclamistat, isobutylxanthine, RU-20-1724, SB-207499, SKGF-95654 and CDP-840[72,73].

Thalidomide:

Thalidomide, once known for its marked teratogenecity, is being viewed at with renewed interest because of its ability to down-regulate TNF-α It has been successfully used in the treatment of rheumatoid arthritis, systemic lupus erethematosus and Crohn's disease[74-76].

TNF-α inhibitors:

The severity of the disease in experimental encephalomyelitis (EAE) to be reduced by treatment with TNF-α inhibitor pentoxyfylline. A similar result was also seen in the animal model for SLE with pentoxyfylline[77,78].

Monoclonal antibodies:

Antibodies (Abs) to TNF-α have been found to reduce disease activity in Crohn's disease. Antibodies to TNF-α have

also shown to prevent death in animal models of specific shock and cerebral malaria. Examples of TNF-α Antibodies include anti-TNF-α, CD-006 and CA-2[79].

Soluble TNF-α receptors:

Soluble TNF-α receptors bind both membrane bound and soluble TNF-α. Clinical results with these agents in RA and allergic inflammation are promising. Examples include soluble P^{75} TNF-α receptor, human IgG fusion protein (sTNF-α RFC), PEG linked P^{55} (PEG-sTNF-RI) and RO-45-208 (Tenefuse)[80].

THERAPEUTIC AGENTS FOR APOPTOSIS

FAS mimetics:

FAS is a cell surface protein expressed on the T-cells when it encounters an antigen. They also temporarily make another surface molecule called FAS ligand. In activated T-cells FAS bind FAS ligand, thereby signaling the cell to undergo apoptosis.

Anti FAS/APO-1 Antibody:

Monoclonal antibodies against FAS or APO-1 (apoptosis inducing protein-1) react with cell surface molecule, APO-1 and trigger signals inducing apoptosis.

Cytokine growth factor analogs:

Drugs inhibiting the synthesis or receptor binding of cytokine/growth factors can trigger passive apoptosis.

Integrin inhibitors:

Integrins are primarily responsible for adhesion to extracellular matrices. Extracellular matrix interaction blockers like integrin inhibitor drugs anchorage and induce apoptosis.

Poly (ADP) ribose polymerase activators:

Activation of poly (ADP) ribose polymerase results in consumption of NAD in course of polymer formation leading to decline in ADP levels resulting in breakdown of glycolysis, ultimately leading to cell death.

Lamin and actin blockers:

Lamin and Actin are proteins involved in chromatin condensation and maintaining cell membrane architecture. Drugs that block these would however induce apoptosis indiscriminately.

Topoisomerase inhibitors:

These stabilize enzyme DNA complex leading to the production of single or double strand breaks that ultimately precipitate cell death.

Nucleoside analogues:

These also induce DNA strand breaks although their mechanism of action may additionally involve cellular signaling network. This has been shown by purine nucleoside analogue in chronic lymphocyte leukaemia (CLL) cells.

Antiapoptosis gene inhibitors:

Bcl-2, Bcl-Xc, Bcl-Xβ Bcl-W, Bc-ab1, V-ab1 are examples for genes that inhibit apoptosis. Inhibition of expression of these genes would facilitate the induction of apoptosis[8]. The discovery of new methods of selectively inducing apoptosis may lead to entirely new therapies for chronic inflammatory diseases.

CONCLUSIONS

Although substantial progress has been made over the last decade concerning the relationship between variety of inflammatory mediators and inflammation, a great deal remains to be learned about these complex physiological systems. The remarkably diverse actions and interrelationships of these mediators with an intricate process such as inflammation have resulted in slow and tedious progress toward understanding of these systems. Nevertheless, great research accomplishments have been made in this area during recent years. Certainly the future will unfold many effective therapeutic applications for inflammatory mediators and their inhibitors.

REFERENCES

1. Parnham, M.J., **Drugs News and Perspectives,** 1996, 9, 631.
2. Ford, S.W., King, E.K. and Pento, T.J., **Drugs of Today,** 1984, 20, 175.
3. Robins, S.C. and Cotran, R.S., In; Pathologic basis of disease, 2nd Edn., WBS authors, Philadelphia 1979, 55
4. Axel Heller, A., Koch,T., Schmeck, J. and Klaus Van Ackern, K.V., **Drugs,** 1998, 55, 482.
5. Arni, R.K. and Ward, R.J., **Toxicon,** 1996, 34, 827.
6. Kramer, R.M. and Sharp, J.D., **Agents Actions Suppl.,** 1995, 46, 65.
7. Marschall, L.A. and Douglas Morgan, D.W., **Drug News and Perspectives,** 1998, 11, 82.
8. Kulkarni, S.K. and Varghese, N.P., **Indian Drugs,** 1998, 35.
9. Sengupta, S., **Indian J. Pharmacol.,** 1999, 31, 322.
10. Donnell, R. O., **Aust. Prescriber,** 1999, 22, 55.
11. Leon, A., Buriani, A., Dal Toso, R., Fabris, M., Romanello, S., Aloe, L. and Montalcini, L.R., **Proc. Natl. Acad. Sci.,** 1994, 91, 3739.
12. Jack, D.B., **Drugs News and Perspectives,** 1996, 9, 93.
13. Aloe, L., Leon, A. and Levi-Montalcini, R., **Agent Action.,** 1993,

39, c145.
14. Natarajan, V., Reddy, V.P., Schmid, P.C. and Schmid, H.H.O., **Biochem. Biophy. Acta,** 1982, 712, 342.
15. Salvemini, D., Seibert, K. and Marino, M, H., **Drugs News and Perspectives,** 1996, 9, 204.
16. Gross, S.S., **Annu. Rev. Physiol.,** 1995, 57, 737.
17. Clancy, R.M. and Abramson, S.B., **Soc. Exp. Biol. Med.,** 1995, 23, 93.
18. Ialenti, A., Ianaro, A., Moncada, S. and Di Rosa, M., **Eur. J. Pharmacol.,** 1992, 211, 177.
19. Ahluwalia, A. and Perretti, M., **Trends Pharmacol. Sci.,** 1999, 20, 100.
20. Bhoola, K.D., Figueroa, C.D. and Worthy, K., **Pharmacol. Rev.,** 1992, 44, 1.
21. Steranka, L.R., **Proc. Natl. Acad. Sci.,** 1988, 85, 3245.
22. Schremmer, D.E., Offner, A., Siebock, M. and Roscher. A.A., **Biochem. Biophys. Res. Commun.,** 1998, 243, 246.
23. Dray, A. and Perkin, M., **Trends Neurosci.,** 1993, 16, 99.
24. Davis, C.L., **Brit. J. Pharmacol.,** 1996, 118, 1469.
25. Anaro, G.R., Gonzalez, D.F. and Madrid, S. F., **Drugs,** 1998, 56, 977.
26. Frenette, P.S. and Wagner, D.D., **N. Engl. J. Med.,** 1996, 334, 1526.
27. Frenette, P.S. and Wagner, D.D., **N. Engl. J. Med.,** 1996, 335, 43.
28. Rosen, S.D. and Bertozzi, C.R., **Curr. Opin. Cell Biol.,** 1994, 6, 663.
29. Steegmaier, M., Levinorit, A. and Isenmman, S., **Nature,** 1995, 373, 615.
30. Gorbi, A.L., In; Leukocytes, Integrins, Structure, Expression and Function, Springer Verlag, Heidelberg, 1996, 65.
31. Phillips, D.R., Charo, F.L. and Parise, L., **Blood,** 1988, 71, 831.
32. Takeichi, M., **Development,** 1988, 102, 639.
33. Barnes, P. J. and Karin, M., **N. Engl. J. Med.,** 1997, 1066.
34. Seibenlist, U., Franzoso, G. and Brown, K., **Annu. Rev. Cell Biol.,** 1994, 10, 405.
35. Murray, M.C., Kinnun A.E.J., Proudfoot, T., Wells, N.C. and Robertosolari, **Drugs News and Perspectives,** 1996, 9, 389.
36. Arai, K., Lee, F., Miyajima, A., Miyatake, S., Arai, N. and Yokota, T., **Annu. Rev. Biochem.,** 1990, 59, 783.
37. Banes, P.J. and Adcock, I., **Mol. Mech. Trend. Pharmacol. Sci.,** 1993, 14, 436.
38. Winkler, J. and Cochran, F., **Inflamm. Res.,** 1997, 46, 3.
39. Frisch, S. M. and Francis, H., **J. Cell Biol.,** 1994, 124, 619.
40. Marschall, L.A., Bolognese, B. and Raymond, H., **Pharm. Comm.,** 1993, 5, 27.
41. Jacobson, P.B., Marshall, L.A., Sung, A. and Jacobs, R.S., **Biochem. Pharmacol.,** 1990, 39, 1557.
42. Miyake, A., Yamamoto, H., Kubota, E., Hamaguchi, K, Kouda, A., Honda, K. and Kawashima, A., **Brit. J. Pharmacol.,** 1993, 110, 447.
43. Botting, J., **Drugs News and Perspectives,** 1996, 9, 123.
44. Michael, J., **Drugs News and Perspectives,** 1997, 10, 182.
45. Dewitt, D.L., **Mol. Pharmacol.,** 1999, 55, 625.
46. Seale. J.P., **Aust. Prescriber,** 1999, 22, 58.
47. Salvemini, D., Wang, Z.G., Wyatt, P., Mavino, M.H. and Currie, M.G., **Endothelium,** 1995, 3, 205.
48. Connor, J., Manning, P.T., Settle, S.L., Moore, W.M., Jerome, G.M., Webber, R.K., Tjueng, F.S. and Currie, M.G., **Eur. J. Pharmacol.,** 1995, 273, 15.
49. Varrek, R. and Stewart, J.M., **Peptides,** 1985, 6, 161.
50. Hock, F.L., **Brit. J. Pharmacol.,** 1991, 769, 769.
51. Regoli, D., Allogha, S.N., Rizzi, A. and Gobeil., **Eur. J. Pharmacol.,** 1998, 348, 1.
52. Sekido, N., Mukaida, N. and Harada, A., **Nature,** 1993, 365, 654.
53. Eigler, A., Sinha, B. and Hartmann, G., **Immunol Today,** 1997, 18, 487.
54. Buckley, C.D. and Simmons, D.C., **Mol. Med. Today,** 1997, 3, 449.
55. Cornejo, C.J., Winn, R. K. and Harlan, J.M., **Adv. Pharmacol.,** 1997, 39, 99.
56. Kent, S.J., Karlik, S.J., Rice, G.P. and Horner, H.C., **J. Magn. Reson. Imaging,** 1995, 5, 535.
57. Shjmaoka, M., Ikeda, M. and Lida, T., **Amer. J. Res. Unit Care Med.,** 1996, 153, 307.
58. Endemann, G., Feng, P. and Bryant, C.M., **J. Pharmacol. Exp. Ther.,** 1996, 276, 5.
59. Bennett, C.F. and Kornbrust, H. D., **J. Pharmacol. Exp. Ther.,** 1997, 280, 988.
60. Agarwal, S. and Iyer, R.P., **Pharmacol. Ther.,** 1997, 76, 151.
61. Clande, H., **Anticancer Drug Res.,** 1991, 6, 569.
62. Yang Yen, H.F., Chambard, J. C. and Sun, Y. C., **Cell,** 1990, 62, 1205.
63. Ray, A. and Prefontaine, K.E., **Proc. Natl. Acad. Sci.,** 1994, 91, 752.
64. Schreek, R., Richer, P. and Baeuere, P.D., **EMBO J.,** 1991, 10, 2247.
65. Pahl, H.L., Krauss, B. and Schulze-Osthott, K., **J. Exp. Med.,** 1996, 183, 1829.
66. Wang, P., Siegel, M., Wu, P., Egan, R.W. and Billahm, M., **J. Biol. Chem.,**1995, 270, 9558.
67. Beg, A.A., Sha, W.C., Bronson, R.T., Ghosh, S. and Baltimore, D., **Nature,** 1995, 376, 167.
68. Sha W.E., Liou, H.C., Tuomanen, E. and Baltimore, D., **Cell,** 1995, 80, 321.
69. Lee, J.C., Laydon, J.T., McDonnell, P.C., Gallagher, J.F., Kumar, S., Green, D., Mc Nulty, D., Blumenlthal, M.J., Heys, J.R., L. and Vatter, S.W., **Nature,** 1994, 372, 739.
70. Kriefler, M., Perez, C., Defay, K., Albert, I. and Lu, S.D., **Cell,** 1988, 53, 45.
71. Black, R.A., Kronheim, S.R., Cantrell, M., Deeley, M.C., March, C.J., Prickett, K.S., Wignall, J., Cohlon, P.J., Cosman, D. and Hopp, T.P., **J. Biol. Chem.,** 1988, 263, 9437.
72. Gimbeyz, M.A. and Dent, G., **Clin. Exp. Allergy.,** 1992, 22, 337.
73. Cohan, V.L. and Naclerio, B.A., **J. Immunol.,** 1993, 150, 2124.
74. Sommer, C., **Drugs of the Future,** 1999, 24, 67.
75. Rudrizuez, G.O., Bacal, S. P. and Montes, M.O., **J. Rheumatol.,** 1989, 16, 158.
76. Bessis, D., Guillof, B., Monpoint, S., Dandurand, M. and Guilhou, J., **Lancet,** 1992, 339, 549.
77. Nataf, S., Louboutin, J.P., Chabannes, D, Feve, J.R. and Muller,

J.Y., **Acta Neurol. Scand.,** 1993, 88, 97.
78. Hecht, M., Muller, M., Matlher, L. M.C. and Emmendorffer, A., **J. Leukocyte. Biol.,** 1995, 57, 242.
79. Dullemen,V.H.M., Deventer, V.S.J.H., Hommes, D.W., Bijl, H.A., Jansen, J., Tytgal, G.N.J. and Woody, J., **Gastroenterology,** 1995, 109, 129.
80. Lukacs, N.W., Strieter, R.M., Chensue, S.W., Widmer, M. and Kunkel, S.L., **J. Immunol.,** 1995, 154, 5411.