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Targeting the production of monocytes/macrophages-derived cytokines by anti-inflammatory herbal drugs

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Abstract

Macrophages and their immature undifferentiated predecessors, monocytes, are part of the innate immune system with primary function in defense against infection, malignancy and in immunity. Of the various protein mediators produced by monocytes/macrophages are proinflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1β) and IL-6. The regulated release of these cytokines result in the initiation of inflammation through activation of immune cells and upregulation of expression of adhesion molecules that eventually lead to leucocyte infiltration to extravascular sites. Hence, one of the most attractive approaches of alleviating the severity of chronic inflammatory diseases is suppressing the production of monocytes/macrophages-derived TNF-α, IL-1β and IL-6. This review highlights some common targets of the monocytes/macrophage-derived cytokines expression, experimental models of proinflammatory cytokines release, and mechanism of action of some exemplary antiinflammatory herbal drugs. The effects of crude drug preparations on transcription factors (e.g. nuclear factor-κB), signalling pathways including the mitogen-activated protein kinase cascades and induction of immunosuppressive proteins (IL-10) are among the common targets discussed.

Keywords: Monocytes; macrophages; inflammation; herbal drugs; proinflammatory cytokines; TNF-α; IL-1β; IL-6; IL-8; IL-10; HMGB1

Introduction

Monocytes are members of the granulocytes (neutrophils, basophils, eosinophils, mast cells, natural killer cells and monocytes) cell populations that constitute the innate immune system (Papatriantafyllou, 2011**)**. Being the first line of defense of the body, their primary function is that of cell mediated immunity against infection and malignancy. Monocytes are formed in the bone marrow from a common monocyte, macrophage and dendritic pluripotent stem cell precursors (Gordon and Taylor, 2011). Following the differentiation process that lasts less than 24 hours, mature monocytes leave the bone marrow and enter the bloodstream

as quiescent cells. Circulating monocytes then migrate into tissues such as spleen, liver, central nervous system, lymph nodes, lungs, peritoneal cavity, and the subcutaneous tissue (Strauss-Ayali et al., 2007). The spleen is considered as a secondary reservoir where about half of the monocyte population in the body are stored.

Circulating monocytes are recruited to sites of inflammation, injury or antigen deposition where they are activated by a variety of stimuli. Once entered into tissues, monocytes differentiate into cells that express the macrophage phenotype and also into dendritic cells (Gordon and Taylor, 2011; Strauss-Ayali et al., 2007). In general, the macrophage population in tissues are classified as 'resident' when they are present in tissues at all times or 'newly recruited'. Undoubtedly, the major function of the monocytes/macrophages is phagocyteosis. They are equipped with an array of sensory recognition and removal mechanisms for invading pathogens as well as senescent, damaged or dead host cells. In addition to their routine phagocytosis role, monocytes/macrophages are further involved in specific immunity by presenting antigens to T-cells (Parkin and Cohen, 2001). They are also by far the most well known secretary cells which are capable of responding to a variety of stimuli. Of the various mediators produced by monocytes/macrophages are enzymes, enzyme inhibitors, cytokines, chemokines, complement components, coagulation factors, and arachidonic acid metabolites. They are also influenced by their own products in a paracrine and autocrine manner. The crucial role of monocytes/macrophages in physiological and pathological conditions, including chronic inflammatory disorders, have been reviewed in the various literatures (Gui et al., 2012; Lebre and Tak, 2012). The main focus of this communication is to review the monocyte/macrophage inflammatory cytokines production as a valued target for herbal drug preparations.

Major Monocytes/macrophages-derived proinflammatory cytokines

Tumour necrosis factor-α (TNF-α)

TNF-α is one of the major proinflalamatory cytokenes secreted by monocytes/macrophages. The human TNF-α is known to be expressed as a 26-kDa precursor monomer protein which is anchored to a plasma membrane. Further proteolytic cleavage by TNFα-converting metalloprotease enzyme releases a mature 17-kDa soluble TNF-α protein. Both secreted and membrane-bound TNF-α are assembled in trimeric structural form that induce a range of biological activities (Aggarwal, 1992; Bazzoni & Beutler, 1995). It is now well established that TNF-α gene expression is primarily regulated at transcriptional level by nuclear factor κB (NFκB) and other transcription factors though regulation at translational level has also been reported (Pauli, 1994; Sariban et al., 1988).

The release of TNF-α from monocytes/macrophages is triggered by a variety of agents including physical trauma, infection, bacterial cell wall components and cytokines. TNF-α does also act on monocytes/macrphages in an autocrine fashion to induce a more sustained release of inflammatory mediators such as protein (cytokines including TNF-α) and lipid-borne mediators. Not surprisingly, TNF-α is now considered as a major target for chronic inflammatory diseases including rheumatoid and osteo arthritis, ankylosing spondylitis, Crohn's disease and ulcerative colitis, psoriasis, etc. (Wong et al., 2008; Vinay and Kwon,

2012). Etanercept and infliximab are two examples of protein based clinically useful drugs that neutralise TNF-α. The therapeutic potential of these drugs in various chronic inflammatory conditions have been extensively reviewed in recent years (Hauwermeiren et al., 2011; Furst et al., 2007; Griffoul et al., 2009; Markham & Lamb 2000; Mortensen et al., 2011; Tracey, 2008; Weinberg et al., 2003).

Interleukin-1β (IL-1β)

The second most prominent proinflammatory cytokine of monocyte/macrophage origin is IL-1β (Gabay et al., 2010; Dinarello, 2011). As with TNFα, IL-1β is formed as a precursor protein that requires proteolytic processing by IL-1β converting enzyme (Dinarello, 1997). The role of IL-1β in the pathology of chronic inflammatory conditions is well establishhed. An IL-1 receptor antagonist (e.g. anakinra) and protein that neutralize IL-1 (e.g. rilonacept) have been clinically used to treat various chronic inflammatory conditions (Llord et al., 2007; Furst, 2004; McDermott MF, 2009; Scott, Terkeltaub, 2010). Other approaches of targeting IL-1 family cytokines have also been reviewed recently (Dinarello et al., 2012**).**

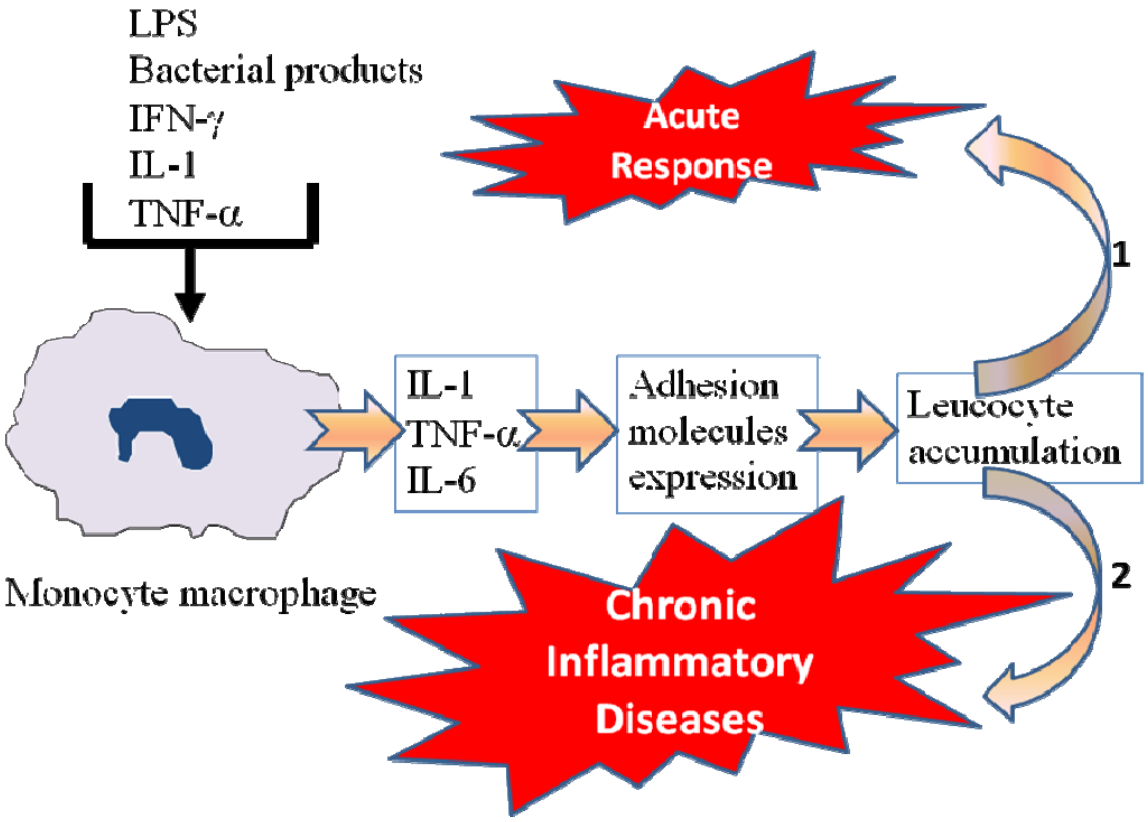
Interleukin-6 (IL-6)

IL-6 is the late addition of proinflammatory cytokine of monocyte origin that gained some attention in recent years. In view of its possible therapeutic potential, receptor antagonist approaches have also been developed (Jalal et al., 2010). Some clinical studies demonstrated that a monoclonal antibody against IL-6 showed beneficial effect in treating rheumatoid arthritis (Alonso and Bilbao, 2009). The true potential of targeting IL-6 for inflammatory diseases however is yet to be fully assessed.

The therapeutic approach of targeting monocytes/macrophages-derived proinflammatory cytokines

Once released, TNF-α, IL-1β and IL-6 activate vascular endothelial cells and lecocytes leading to the expression of various adhesion molecules. The receptor-ligand type interaction by the various adhesion molecules on leucocyte and endothelial cell surface further mediate leucocytes arrest and infiltration to extravascular sites of injury and/or antigen deposition. As shown in Scheme 1, the regulated acute inflammatory process is part of the body's defense mechanism and is necessary for the overall maintenance of tissue repair and architecture. Under chronic inflammatory condition however, over expression of inflammatory cytokines coupled with continuous leucocyte recruitment lead to a disease state. The various approaches of targeting cytokines-induced expression of adhesion molecules by natural products is reviewed recently (Habtemariam, 2010).

A decade ago, a comprehensive review article from this laboratory (Habtemariam, 2000) also outlined the potential role of natural products as inhibitors of by far the most potent inflammatory mediator, TNF-α. The present review is intended, to shade some light on advances in herbal medicine research since then with particular emphasis to monocyte/macrophage-derived proinflammatory cytokines release.



Scheme 1**.** Inflammation is a necessary body defense mechanism against infection and injury. Among other granulocytes, monocytes contribute in phagosytosis and elimination of microbes and injured cell debris. As a major producers of proinflammatory cytokines (TNF-α, IL-1β and IL-6), monocytes orchestrate the inflammatory reaction though activation of endothelial and lecucytes leading to adhesion molecules expression and eventually leucocyte accumulation at extravascular sites. This acute normal physiological phenomenon (shown as 1) however became exaggerated under pathological condition (route 2) and lead to chronic inflammatory condition.

Experimental models of monocyte/macrophage-derived cytokines release

The use of human peripheral blood mononuclear cells (PBMC) as an *in vitro* method for studying monocyte immune functions has been extensively reviewed (Reddy et al., 2004). In this study protocol, whole blood from healthy donors is collected in sterile tubes containing anticoagulants such as heparin or EDTA. After dilution, PBMC can be isolated by density gradient centrifugation over leukocyte separation media (e.g. Ficoll-Hystopaque). PBMC often at a higher density (~ 10^6/ml) are cultured in RPMI-1640 medium containing 10% heat-inactivated foetal bovine serum. Various other literatures have also described cytokines release study from PBMS of rodent's origin (Treffkorn et al., 2004).

The other common source of primary culture for cytokines release study is mice/rats-derived peritoneal macrophages. In this classical model of monocyte/macrophage *in vitro* cell culture, animals are sacrificed and surface steralisation of the skin achieved by using 70% alcohol. Phosphate buffered saline can then be injected into the peritoneal cavity and residential macrophages collected after vigorous shaking. In order to increase the yield of peritoneal macrophages, mice/rats are often treated with thioglycollate (Ezeamuzie & Khan, 2007)

and macrophages recovered few days (3-4 days) later. Bronchoalveolar lavage fluid (Joubert et al., 2011) and bone marrow (dos Santos et al., 2011) from mice/rats have also been used as sources of primary monocyte/macrophages culture for cytokines studies. One could therefore adopt a primary culture-based cytokines release from either human or animal sources depending on availability of volunteers and available resources. In all cases, isolated cells can be seeded in a flat-bottomed 96-well culture plate at 10^5 -10^6 cells/well in a final volume of 100-200 µl. Cell culturing for 2-3 days in RPMI medium (supplemented with 10% serum) at 37 °C will then allow macrophages to adhere to the plate while other blood cells (e.g. lymphocytes) are still suspended in the culture medium. Non-adherent cells are removed by repeated washing with warm buffer (e.g. Hank's balanced salt solution). Macro-phages can be distinguished by their morphology and expression of a marker lipopolysaccharide (LPS)-binding protein, CD14.

The vast majority of cytokine release studies from monocytes/macrophages came through the use of transformed cell lines. THP-1 and U937 are human leukaemia derived monocytic cells that are routinely used for *in vitro* cytokine release studies. These cells can be readily differentiated to macrophage phenotypes when stimulated by appropriate simply such as phorbol esters. Perhaps the most convenient method of cytokine release inhibition study is that based on the murine macrophage-like cell line, RAW 264.7 cells. These adherent cells can easily be maintained and activated by a variety of inflammatory stimuli including bacterial LPS or proinflammatory cytokines. Treatment of cultured macrophages results in the release of IL-1, IL-6 and TNF-α that can be quantified by ELISA or other bioassay methods. For further details of each bioassay model, readers are directed to the various literature cited in Table 1.

Herbal drugs with inhibitory effect against targets in monocyte/macrophage-derived proinflammatory cytokines: progress in the last decade

Cytokines expression in macrophages requires activation of specific genes that can be regulated at various levels. The promotor genes of these cytokines (e.g. TNF-α) have been shown to have specific binding sites for transcription factors, including NF-κB, AP-1 (activator protein-1), CRE, and CCAAT/enhancer binding protein β (C/EBPβ, also called NF-IL6) (Zagariya et al., 1998). Numerous studies suggest that the transcriptional control of proinflammatory cytokines is primarily mediated by NF-κB. It is now unequivocally established that NF-κB exists in macrophages as a heterodimeric inactive form in the cytosol by binding with an inhibitory IκB protein subunit. NF-κB activation thus requires phosphorylation, ubiquitination, and subsequent proteolytic degradation of IκB through the action of the IκB kinase (IKK) (Karin, 1999). The liberated NF-κB subsequently translocates into the nucleus and binds to the κB motif in the promoters region of target genes, leading to the induction of inflammatory cytokine (TNF-α, IL-1β and IL-6) genes. A number of inflammatory stimuli including reactive oxygen species are now known to trigger inflammatory response through the activation of NF-κB (Gloire et al., 2006). As shown in Table 1, the vast majority of anti-inflammatory herbal drugs have been shown to inhibit proinflammatory cytokines release through inhibition of NF-κB activation.

In addition to the NF-κB system, cytokine production from macrophages involves multiple signalling pathways, including the phosphatidylinositol-3 protein kinase/Akt, and the

mitogen-activated protein kinases (MAPKs) (Zhang & Ghosh, 2000; Ojaniemi et al;., 2003; Yang et al., 2000). The MAPKs are a group of serine–threonine protein kinases that mediate signal transduction from the cell surface to the nucleus. Numerous recent reports highlighted the role of MAPK signaling cascade in cell survival and proliferation, differentiation, and apoptosis. In macrophages, the three well-defined MAPK cascades involved in proinflammatory cytokines expression are ERK (extracellular signal-related kinase), p38 MAPK, and JNK/SAPK (C-Jun N-terminal kinase), ERK1/2, JNK, and p38 MAP kinase (Morel and Barenbaum, 2004). Activation of the MAPK system is achieved by a variety of inflammatory stimuli, including mitogens, proinflammatory cytokines and reactive oxygen species, UV radiation and physical stress (Morel and Barenbaum, 2004; Raingeaud et al., 1995). It is also widely accepted that MAPK signalling is a prerequisite to transcription factors activation such as NF-κB. Non-receptor type protein tyrosine kinases including Syk and Src have also been shown to play a role in monocyte-derived proinflammatory cytokines release (Pan et al., 1999). Since all of these signalling events eventually lead inflammatory cytokines gene expression in macrophages, they are considered as valuable anti-inflammatory targets for new drugs (Kaminska 2005). As with the NF-κB, the MAPK system has been shown to be a validated target for a number of herbal medicines (Table 1). As described in section 2, both IL-1β and TNF-α are produced as inactive molecules and need processing by converting enzymes. It remains to be seen however if this mechanism is the primary target for crude anti-inflammatory herbal drug preparations.

IL-8 is a pleotropic cytokine product of not only monocytes/macrophages but also a variety of other cell types including lymphocytes, neutrophils, vascular endothelial cells, fibroblasts, keratinocytes, airway epithelial cells and hepatocyles. The role of IL-8 in the pathogenesis of inflammatory diseases has now been well established and high level of this cytokine has been detected in patients suffering from psoriasis, rheumatoid arthritis, osteoarthritis, gout, respiratory distress syndrome or idiopathic pulmonary fibrosis (Aherne et al., 2009; Ferran et al., 2010; Mihailidou et al., 2010). The major pathological role of IL-8 that gained considerable attention in recent years was however in airway inflammation (e.g. asthma) where a good correlation between IL-8 level and severity of the disease was established (Mihailidou et al., 2010). As a potent leucocyte activator and chemotaxin product of airway epithelial cells, IL-8 is often used as a biological marker of environmentally induced pulmonary inflammation (Strieter, 2002; Tal et al., 2010). The selective recruitment of eosinophils to accumulate in the airways of asthmatic patients is also shown to be attributed to IL-8 (Nakagome & Nagata, 2011). Agents that inhibit the secretion of IL-8 are thus likely to benefit asthmatic and other patients suffering from airway inflammation. As shown in Table 1, some crude plant extract preparations have been shown to inhibit the release of IL-8 from monocytes *in vitro,* suggesting their potential use for the above mentioned disease conditions.

Iterleukin-10 (IL-10) is another pleiotropic cytokine that is released from monocytes and other activated immune cells. IL-10 was initially named as cytokine-synthesis inhibitory factor due to its ability to reduce the production of cytokines including TNF-α (Mocellin, et al., 2004). This cytokine is thus regarded as immunosuppressive and its level of production is associated with susceptibility to infection that is common in diseases like AIDS (Clerici et al., 2000). Under chronic inflammatory conditions where overproduction of proinfllamatory cytokines is the major pathological hallmark, however, administration of IL-10 could be seen as an attractive therapeutic approach. Hence, IL-10 administration benefits patients suffering

from soriasis, Crohn's disease, rheumatoid arthritis and many other chronic inflammatory diseases (Mocellin et al., 2003). Agents that increase the production of IL-10 can also offer immunosuppressive effect and the identification of *Cucumis melo* (Vouldoukis et al., 2004) and *Vitex trifolia* (Matsui et al., 2009) as anti-inflammatory agents that act through this mechanism are interesting findings (Table 1).

High mobility group box 1 (HMGB1) is a highly conserved, ubiquitous protein present in the nuclei and cytoplasm of nearly all cell types. As a late-stage proinflammatory cytokine, HMGB1 is secreted by immune cells to induce the expression of adhesion molecules and production of pro-inflammatory cytokines (Park et al., 2004). High level of HMGB1 has been shown to be associated with sepsis and a number of chronic inflammatory diseases like atherosclerosis while blocking monoclonal antibodies against HMGB1 and other inhibitory drugs could ameliorates the severity of these disease conditions (de Souza et al., 2012; Schierbeck et al., 2011). *Angelica sinensis* (Table 1) appears to be one good example of an anti-inflammatory herbal drug that work through HMGB1 inhibition.

Table 1. Crude plant preparations with reported inhibitory activities towards monocyte/macrophage-derived proinflammatory cytokines.

Plant Name (Family)	Plant Part (Extract)	Study model	Stimuli	Cytokines	Proposed mechanisms: Inhibition of -	References
Angelica sinensis (Apiaceae)	Root (Aqueous)	RAW 264.7	LPS	HMGB1	cytokine cytoplasmic translocation	Wang et al., 2004; Wang et al., 2006
Artemisia vestita Wall (Asteraceae)	Herb (EtOH)	RAW 264.7	LPS	TNF-α, IL-1β	MAPKs (p38, ERK1/2, JNK); NF-κB	Sun et al., 2006
Boerhaavia diffusa (Nyctaginaceae)	Herb (EtOH)	RAW 264.7; hPBMCs	LPS	TNF-α		Mehrotra et al., 2002
Boswellia serrata (Bruseraceae)	Resin (hexane, CH_2Cl_2, EtOAc, MeOH)	hPBMC; RAW 264.7	LPS	TNFα, IL-1β, IL-6	MAPKs (JNK, p38)	Gayathri et al., 2007
Centella asiatica (Umbelliferae)	Whole plant (80% EtOH or Aqueous)	J774.2	LPS	TNF-α	Gene expression	Punturee et al., 2004
*Crinum asiaticum Linne var. Japonicum (*Amaryllidaceae)	(95% EtOH)	RAW 264.7	LPS	IL-6, IL-8		Kim et al., 2008
Cryptolepis buchanani Roem. & Schult. (Asclepiadaceae)	(50% EtOH)	THP-1	LPS	TNF-α		Laupattarakasem et al., 2006
Curcuma comosa Roxb. (Zingiberaceae	Rhizome (Hexane or EtOH)	U937, hPBMC	PMA	TNF-α , IL-1β	NF-κB ; IκB kinase	Sodsai et al., 2007
Cucumis melo LC., Cucurbitaceae)	Fruit (Aqueous)	Peritoneal macrophages (PM)	IFN-gamma and IgGl/anti-IgG1 immune	TNF-α; induction of IL-10		Vouldoukis et al., 2004

			complexes (IgG1IC)			
Cymbopogon citrates (lemon grass; Poaceae)	Leaves (50% EtOH)	Alveolar macrophages (AM)	LPS	TNF-α		Tiwari et al., 2010
Daemomorops draco BL. (Palmae). (Arecaceae)	Resin (EtOAc)	RAW 264.7	LPS	IL-1β, TNF-α, IL-8, IL-6	Possibly ROS production	Heo et al., 2010
Daphne gnidium (Thymelaceae)	Leaves (EtOAc)	PM	LPS	IL-1β, TNF-α		Harizi et al., 2011
Dionysia termeana (Primulaceae)	Aerial parts (MeOH)	PM	LPS	IL-1β		Amirghofran et al., 2011
Dracocephalum kotschyi (Labiatae)	Aerial parts (MeOH)	PM	LPS	IL-1β		Amirghofran et al., 2011
Duchesnea indica (Andr) Focke	(EtOH)	RAW264.7	LPS	TNF-α	NF-κB	Zhao et al., 2008
Enicostema axillare (Lam.) A. Raynal (Gentianaceae)	Whole plant (MeOH)	PM	LPS	TNF-α, IL-1β		Saravanan et al., 2012
Eupatorium perfoliatum L. (Asteraceae)	Aerial parts (MeOH, EtOH, CH_2Cl_2)	hPBMC; RAW 264.7	LPS	TNF-α, IL-1β		Maas et al., 2011
FAHF-2	Mixed herbal formula (Butanol)	RAW 264.7		TNF-α		Yang et al., 2009
Ferulago angulata (Apiaceae)	Aerial parts (MeOH)	PM	LPS	IL-1β		Amirghofran et al., 2011
Ginkgo biloba (Ginkgoaceae)	Standardised commercially available extract	RAW 264.7	LPS	TNF-α	NF-κB; MAPKs (ERK1/2, p38 MAPK)	Wadsworth et al., 2001
Glossogyne tenuifolia (Hsiang-Ju) (Asteraceae)	Aerial parts (EtOH)	hPBMC; RAW264.7 PM	LPS	TNF-α, IL-1β, IL-6, IL-12	NF-κB; IκB- α phosphorylation	Wu et al., 2005; Wu et al., 2004; Ha et al., 2006
Glycyrrhiza glabra (Fabaceae)	Root (Aqueous $(CH_3)_2CO$)	J774A.1	LPS	IL-1β		Thiyagarajan et al., 2011
Harpagophytum procumbens (Pedaliaceae)	Root (50 or 60% EtOH; standardised extract (SteiHap 69; MeOH)	RAW 264.7; PM	LPS	TNFα , IL-6, IL-1β	Gene expression; No effect on NF-κB and MAPK.	Fiebich et al., 2012; 2010; 2001; Gyurkovska et al., 2011; Inaba et al., 2010
Hibiscus sabdariffa (Malvaceae)	Calyces (Aqueous)	U937	Cadmium	TNFα , IL-6, IL-1β		Okoko and Ere, 2012

Hippophae rhamnoides (Elaeagnaceae)	(Supercritical CO_2 extract)	hPBMCs	LPS	IL-6, TNF-α	NF-κB translocation; p38 MAPK	Jayashankar et a., 2012
HMCO5	Comprises of eight different herbs (Aqueous)	RAW264.7	LPS	TNF-α, IL-1β	NF-κB	Kim et al., 2007a
Hypericum triquetrifolium (Hypericaceae)	(Aqueous)	RAW 264.7; THP-1	LPS	TNF-α, IL-6	NF-κB; IκB-α degradation; MAPK (ERK, JNK, p38)	Choi et al., 2012; Saad et al., 2011
Inula japonica Asteraceae)	Flowers (EtOH)	RAW264.7	LPS	TNF-α; IL-6	NF-κB; ERK, JNK, and p38 MAP kinases	Choi et al., 2010
Justicia gendarussa Burm. F. (Acanthaceae)	Roots (MeoH, EtOAc)	hPBMCs	LPS	IL-6	NF-κB	Kumar et al., 2012
Kochia scoparia (L.) Chard (Chenopodiaceae)	Fruits (MeOH)	RAW264.7	LPS	TNF-α	NF-κB; IκB-α degradation	Shin et al., 2004
Mangifera indica L. (Anacardiaceae)	Stem bark (Aquous)	RAW264.7	LPS	TNF-α		Garrido et al., 2004s, b; 2006
Ligustrum lucidi (Oleaceae)	Fruit (EtOH)	PM; J774 cells	LPS	TNF-α	NF-κB; IκB-α degradation; IκB- α phosphorylation	An et a., 2007 Lee et al., 2011
Linum persicum (Linaceae)	Aerial parts (Aqueous)	PM *	LPS	TNF-α, IL-1β		Amirghofran et al., 2011
Paeonia suffruticosa (Paeoniaceae)	Root (MeOH)	PM*; U937	LPS/rIFN-gamma	TNF-α; IL-8	NF-κB	Chung et al., 2007; Oh 2003
Panax notoginseng Burk. (Araliaceae)	Root (EtOH)	RAW264.7	LPS	TNF-α, IL-1β IL-6		Rhule et al., 2006
Phlebodium decumanum (Polypodiaceae)	Fronds (aqueous)	hPBMC; J774; RAW	LPS	TNF-α	No effect on NF-κB	Punzón et al., 2003
Phyllanthus amarus (Euphorbiacea)	(Standardised commercially available extract; Hexane, aqueous EtOH, Aqueous)	RAW 264.7; PM; BMDM*	LPS	TNF-α	NF-κB	Kiemer et al., 2003 Nworu et al., 2010
Platycladus orientalis Linn. (Cupressaceae)	Leaves (95% EtOH)	PM; RAW264.7	LPS	TNF-α		Fan et al., 2012
Polygonum hydropiper L. (Polygonaceae)	Leaves (MeOH)	RAW264.7; PM*	LPS	TNF-α	NF-κB activator protein (AP-1) and cAMP responsive element binding protein (CREB), Syk, Src, and IRAK1	Yang et al., 2012

Pteris ensiformis Burm (Pteridaceae)	Aerial parts (Aqueous)	RAW264.7	LPS	TNF-α, IL-6	NF-κB	Wu et al., 2005
Rhinacanthus nasutus (L.) Kurz (Acanthaceae)	Whole plant (Aqueous or 80% ethanol)	J774.2	LPS	TNF-α	Gene expression	Punturee et al., 2004
Salix spp (Willow bark, Salicaceae)	Bark (Aqueous)	hPBMC	IFN-γ/LPS	TNF-α	NF-κB	Bonatera et al., 2010
Sasim	seven herbal mixtures (aqueous)	hPBMCs; THP-1	LPS	TNF-α	Strongly induced HO-1	Kim et al., 2008
Sclerocarya birrea (Anacardiaceae)	Stem bark (MeOH)	BMDM*	LPS; BCG	TNF-α, IL-1β, IL-6, IL-12	NF-κB	Fotio et al 2010
Sedum telephium ssp. *maximum* (Crassulaceae)	(MeOH)	PM	LPS	TNF-α , IL-1β, IL-6	MAPK (ERK1/2, JNK)	Altavilla et al., 2008
Si-Miao-San	Mixture of 4 herbal medicines (Aqueous)	RAW264.7 cells	LPS	TNF-α , IL-6	NF-κB; IκB-α degradation	Fan et al., 2010
SKI306X	Mixture of 3 herbal drugs (30% EtOH)	hPBMC	LPS	TNF-α, IL-1β		Hartog et al., 2007
So-Pung-Tang (Sopung)	composed of 14 herbal mixtures	THP-1; hPBMCs	LPS	TNF-α, IL-6; but not IL-1β	MAPK (ERK1/2, JNK but not p38)	Kim et al., 2007
Thuja orientalis L. (Cupressaceae)	Leaves (EtOH)	RAW 264.7	LPS	TNF-α, IL-6	p38 MAPK; NF-κB	Kim et al., 2011
Vitex trifolia L. (Labiatae)	Leaves (Aqueous)	RAW 264.7	LPS	IL-1β, IL-6 TNF-α; induce IL-10 production		Matsui et al., 2009; 2012
Wen-Pi-Tang-Hab-Wu-Ling-San	Herbal mixture formula (Aqueous)	RAW264.7	LPS	TNF-α, IL-1β, IL-6	MAPKs (ERK1/2 and JNK); NF-κB	Jung et al., 2007
Yi Shen Juan Bi	A patented Chinese herbal mix	PM	LPS	TNF-α		Perera et al., 2011

*Common abbreviations used in the table: Bone-marrow derived macrophages –BMDM, Peritoneal macrophages – PM, Phorbol-12-myristate-13-acetate – PMA, Human peripheral blood mononuclear cells - hPBMC

Traditional herbal medicines have been used as a primary healthcare medicine by mankind for thousands of years. Numerous reports also claim that the vast majority of the world population today still relay on such crude drug preparations for treating various diseases (Habtemariam, 2010 and references there in). While scientific data are available to justify the claimed traditional uses of some medicinal plant, more research is needed to prove the quality, safety and efficacy of many herbal preparations. In this communication, the release of proinflammatory cytokines from monocytes/macrophages is evaluated as a target for anti-inflammatory crude herbal preparations. The use of *in vitro* monocyte/macrophage culture methods allows rapid screening and identification of plant extracts that inhibit cytokine production/release. Further validation of anti-inflammatory activity can be obtained by employing *in vivo* studies as secondary assays. For example, the popular Chinese herbal medicine,

Angelica sinensis, which has been shown to inhibit proinflammatory cytokines release (Table 1) can protect mice against lethal endotoximia and sepsis (Wang et al., 2006). Similarly, lemongrass extract has been shown to inhibit the LPS-induced IL-6 *in vivo* (Bachiega and Sforcin, 2011) while *Mangifera indica* demonstrated to have anti-inflamm-atory activity in a variety of *in vivo* inflammation models (Garrido vet al., 2004a; b; Garrido et al., 2006).

Conclusion

The production and release of proinflammatory cytokines (TNF-α, IL-1β and IL-6) from monocytes/macrophages play critical role in the pathogenesis of chronic inflammatory diseases. The anti-inflammatory effect of many herbal drugs are now shown to be attributed to their potent suppressive effect of TNF-α, IL-1β and/or IL-6 release. Although the exact mechanism of action has not been studied for all of these crude herbal drugs, many have been shown to inhibit the activation of NF-κB and/or the MAPK signalling system that are crucial for cytokines gene expression in monocytes/macrophages. Few herbal preparations have been shown to enhance the production of an immunosuppressive cytokine, IL-10. Other interesting development in this field is the identification of HMGB-1 as a target for chronic inflammatory diseases.

References

Aggarwal BB & Vilcek J. (1992). Protein and gene structure of TNF-α and TNF-β. In: Aggarwal BB, Vilcek J, eds. *Tumor necrosis factors. Structure, function and mechanism of action*. New York: Marcel Dekker. pp 239-589.

Aherne CM, McMorrow J, Kane D, FitzGerald O, Mix KS, Murphy EP. (2009). Identification of NR4A2 as a transcriptional activator of IL-8 expression in human inflammatory arthritis. *Molecular Immunology* 46, 3345-3357.

Altavilla D, Polito F, Bitto A, Minutoli L, Miraldi E, Fiumara T, Biagi M, Marini H, Giachetti D, Vaccaro M, Squadrito (2008). Anti-inflammatory effects of the methanol extract of *Sedum telephium* ssp. *maximum* in lipopolysaccharide- stimulated rat peritoneal macrophages. F. *Pharmacology* 82, 250-6.

Alonso RB & Bilbao MA. (2009). Inhibition of interleukin 6, a new therapeutic option in rheumatoid arthritis *Reumatología Clínica* 5, 121-127.

Amirghofran Z, Malek-Hosseini S, Golmoghaddam H, Kalantar F, Shabani M. (2011). Proinflammatory Cytokines by Several Medicinal Plants**.** *Iranian Journal of Immunol*ogy 8, 159-169.

An HJ, Jeong HJ, Um JY, Park YJ, Park RK, Kim EC, Na HJ, Shin TY, Kim HM, Hong SH. (2007). *Fructus Ligustrum lucidi* inhibits inflammatory mediator release through inhibition of nuclear factor-kappaB in mouse peritoneal macrophages. *Journal of Pharmacy and Pharmacology* 59, 1279-1285.

Bachiega TF, Sforcin JM. (2011). Lemongrass and citral effect on cytokines production by murine macrophages**.** *Journal of Ethnopharmacology* 137, 909-193.

Bazzoni F, Beutler B. (1995). How do tumor necrosis factor receptors work? *Journal of Inflammation* 45, 221–238.

Bonaterra GA, Heinrich EU, Kelber O, Weiser D, Metz J, Kinscherf R. (2010). Anti-inflammatory effects of the willow bark extract STW 33-I (Proaktiv(®)) in LPS-activated human monocytes and differentiated macrophages. *Phytomedicine* 17, 1106-1113.

Choi JH, Park YN, Li Y, Jin MH, Lee J, Lee Y, Son JK, Chang HW, Lee E. (2010). Flowers of *Inula japonica* Attenuate Inflammatory Responses. *Immune Network* 10, 145-52.

Chung HS, Kang M, Cho C, Parvez S, Park CH, Kim D, Oh J, Kim H, Shin M, Hong M, Kim Y, Bae H. (2007). Inhibition of nitric oxide and tumor necrosis factor-alpha by moutan cortex in activated mouse peritoneal macrophages. *Biological & Pharmaceutical Bulletin* 30, 912-916.

Clerici M, Galli M, Bosis S, Gervasoni C, Moroni M, Norbiato G. (2000). Immunoendocrinologic abnormalities in human immunodeficiency virus infection. *Annals of the New York Academy of Sciences* 917, 956–961.

de Souza AWS, Westra J., Limburg PC, Bijl M, Kallenberg CGM. (2012). HMGB1 in vascular diseases: Its role in vascular inflammation and atherosclerosis. *Autoimmunity Reviews* 11, 909-917.

Dinarello CA, Simon A, van der Meer JWM. (2012). Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nature Reviews Drug Discovery* 11**,** 633-652

Dinarello CA. (2011). Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* 117, 3720-3732.

Dinarello CA. (1997). Interleukin-1. *Cytokine and Growth Factor Reviews* 8, 253-265.

dos Santos DF, Bitencourt CS, Gelfuso GM, Pereira PAT, de Souza PRM, Sorgi CA, Nicolete R, Faccioli LH. (2011). Biodegradable microspheres containing leukotriene B_4 and cell-free antigens from *Histoplasma capsulatum* activate murine bone marrow-derived macrophages. *European Journal of Pharmaceutical Sciences* 44, 580-588.

Ezeamuzie CI, Khan I. (2007). The role of adenosine A_2 receptors in the regulation of TNF-α production and PGE_2 release in mouse peritoneal macrophages. *International Immunopharmacology* 7, 483-490

Fan SY, Zeng HW, Pei YH, Li L, Ye J, Pan YX, Zhang JG, Yuan X, Zhang WD. (2012). The anti-inflammatory activities of an extract and compounds isolated from *Platycladus orientalis* (Linnaeus) Franco *in vitro* and *ex vivo*. *Journal of Ethnopharmacology* 141, 647-652.

Fan J, Liu K, Zhang Z, Luo T, Xi Z, Song J, Liu B. (2010). Modified Si-Miao-San extract inhibits the release of inflammatory mediators from lipopolysaccharide-stimulated mouse macrophages. *Journal of Ethnopharmacology* 129:5-9.

Ferran M, Galván AB , Giménez-Arnau A, Pujol RM, Santamaría-Babia LF. (2010). Production of Interleukin 8 by Circulating CLA^+ T Cells With Skin Tropism in Patients With Psoriasis and in Healthy Controls. *Actas Dermo-Sifiliográficas* 101, 151-155.

Fotio AL, Olleros ML, Vesin D, Tauzin S, Bisig R, Dimo T, Nguelefack TB, Dongo E, Kamtchouing P, Garcia I. (2010). *In vitro* inhibition of lipopolysaccharide and mycobacterium bovis bacillus Calmette Guérin-induced inflammatory cytokines and *in vivo* protection from D-galactosamine/LPS-mediated liver injury by the medicinal plant *Sclerocarya birrea*. *International Journal of Immunopathology and Pharmacology* 23, 61-72.

Fiebich BL, Muñoz E, Rose T, Weiss G, McGregor GP. (2012). Molecular targets of the antiinflammatory Harpagophytum procumbens (devil's claw): inhibition of TNFα and COX-2 gene expression by preventing activation of AP-1. *Phytotherapy Research* 26, 806-811.

Fiebich BL, Heinrich M, Hiller KO, Kammerer N. (2001). Inhibition of TNF-alpha synthesis in LPS-stimulated primary human monocytes by *Harpagophytum* extract SteiHap 69. *Phytomedicine* 8:28-30.

Furst, D. E., Breedveld, F. C., Kalden, J. R., Smolen, J. S., Burmester, G. R.,Sieper, J., et al. (2007). Updated consensus statement on biological agents for the treatment of rheumatic diseases, 2007. *Annals of Rheumatic Diseases* 66, iii2−iii22.

Furst DE. (2004). Anakinra: Review of recombinant human interleukin-I receptor antagonist in the treatment of rheumatoid arthritis. *Clinical Therapeutics* 26, 1960-1975.

Gabay C, Lamacchia C, Gaby Palmer (2010) IL-1 pathways in inflammation and human diseases. *Nature Reviews Rheumatology* 6, 232-241

Garrido G, González D, Lemus Y, Delporte C, Delgado R. (2006). Protective effects of a standard extract of *Mangifera indica* L. (VIMANG) against mouse ear edemas and its inhibition of eicosanoid production in J774 murine macrophages. *Phytomedicine* 13, 412-418.

Garrido G, González D, Lemus Y, García D, Lodeiro L, Quintero G, Delporte C, Núñez-Sellés AJ, Delgado R. (2004a). In vivo and in vitro anti-inflammatory activity of *Mangifera indica* L. extract (VIMANG). *Pharmacology Research* 50, 143-149.

Garrido G, Delgado R, Lemus Y, Rodríguez J, García D, Núñez-Sellés AJ. (2004b). Protection against septic shock and suppression of tumor necrosis factor alpha and nitric oxide production on macrophages and microglia by a standard aqueous extract of *Mangifera indica* L. (VIMANG). Role of mangiferin isolated from the extract. *Pharmacology Research* 50, 165-172.

Gayathri B, Manjula N, Vinaykumar KS, Lakshmi BS, Balakrishnan A. (2007). Pure compound from *Boswellia serrata* extract exhibits anti-inflammatory property in human PBMCs and mouse macrophages through inhibition of TNF alpha, IL-1beta, NO and MAP kinases. *International Immunopharmacology* 7, 473–482

Gloire G, Legrand-Poels S, Piette J. (2006). NF-κB activation by reactive oxygen species: Fifteen years later. *Biochemical Pharmacology* 72, 1493-1505.

Gordon S & Taylor PR. Monocyte and macrophage heterogeneity. *Nature Reviews* 5, 953-964.

Griffoul I, Giraudeau B, Mulleman D, Benhamou C-L, Valat LP, Goupille P. (2009) Infliximab for treating axial spondylarthropathy in everyday practice. *Joint Bone Spine* 76, 39-43.

Gui T, Shimokado A, Sun Y, Akasaka T, Muragaki Y. (2012). Diverse Roles of Macrophages in Atherosclerosis: From Inflammatory Biology to Biomarker Discovery. *Mediators of Inflammation* Volume 2012 (2012), Article ID 693083, *In press.*

Gyurkovska V, Alipieva K, Maciuk A, Dimitrova P, Ivanovska N, Haas C, Bley T, Georgiev M. (2011). Anti-inflammatory activity of Devil's claw *in vitro* systems and their active constituents. *Food Chemistry* 125,171-178.

Ha CL, Weng CY, Wang L, Lian TW, Wu MJ. (2006). Immunomodulatory effect of *Glossogyne tenuifolia* in murine peritoneal macrophages and splenocytes. *Journal of Ethnopharmacology* 107, 116-25.

Habtemariam S. (2010). Applying new science for old medicines: targeting leukocyte-endothelial adhesions by antiiflammatory herbal drugs. *Natural Product Communications* 5**,** 1329-1336.

Habtemariam S. (2000). Natural inhibitors tumour necrosis factor-a production, secretion and function. *Planta Medi*ca 66, 303-313.

Harizi H, Chaabane F, Ghedira K, Chekir-Ghedira L. (2011). Inhibition of proinflammatory macrophage responses and lymphocyte proliferation *in vitro* by ethyl acetate leaf extract from *Daphne gnidium*. *Cellular Immunology* 267, 94-101

Hartog A, Hougee S, Faber J, Sanders A, Zuurman C, Smit HF, van der Kraan PM, Hoijer MA, Garssen J. (2007). The multicomponent phytopharmaceutical SKI306X inhibits in vitro cartelage degradation and the production of inflammatory mediators. *Phytomedicine* 15, 313-320.

Hauwermeiren FV, Vandenbroucke RE, Libert C. (2011). Treatment of TNF mediated diseases by selective inhibition of soluble TNF or TNFR1. *Cytokine & Growth Factor Reviews* 22, 311-319.

Heo S-K, Yi H-S, Yun H-J, Ko C-H, Choi J-W, Park S-D (2010). Ethylacetate extract from Draconis Resina inhibits LPS-induced inflammatory responses in vascular smooth muscle cells and macrophages via suppression of ROS production. Food and Chemical Toxicology 48, 1129 1136.

Inaba K, Murata K, Naruto S, Matsuda H. (2010). Inhibitory effects of devil's claw (secondary root of *Harpagophytum procumbens*) extract and harpagoside on cytokine production in mouse macrophages**.** *Journal of Natural Medicine* 64, 219-222.

Jayashankar B. Mishra KP, Kumar MSY, Udayasankar K, Misra K, Ganju L. Singh SB. (2012). A supercritical CO_2 extract from seabuckthorn leaves inhibits pro-inflammatory mediators *via* inhibition of mitogen activated protein kinase p38 and transcription factor nuclear factor-κB. *International Immunopharmacology* 13, 461-467.

Jalal A. Jazayeri, Graeme J. Carroll, Ann B. Vernallis (2010). Interleukin-6 subfamily cytokines and rheumatoid arthritis: Role of antagonists *International Immunopharmacology* 10, 1-8.

Joubert P, Cordeau M-E, Lavoie J-P. (2011). Cytokine mRNA expression of pulmonary macrophages varies with challenge but not with disease state in horses with heaves or in controls. *Veterinary Immunology and Immunopathology* 142, 236-242.

Kaminska B. (2005). MAPK signalling pathways as molecular targets for anti-inflammatory therapy—from molecular mechanisms to therapeutic benefits. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1754, 253-262.

Karin M. (1999). How NF-κB is activated: the role of the IκB kinase (IKK) complex *Oncogene* 18, 6867–6874.

Jung HW, Yoon CH, Kim YH, Boo YC, Park KM, Park YK. (2007). Wen-Pi-Tang-Hab-Wu-Ling-San extract inhibits the release of inflammatory mediators from LPS-stimulated mouse macrophages. *Journal of Ethnopharmacology* 114, 439-445.

Kiemer AK, Hartung T, Huber C, Vollmar AM. (2003). *Phyllanthus amarus* has anti-inflammatory potential by inhibition of iNOS, COX-2, and cytokines via the NF-κB pathway. *Journal of Hepatology* 38, 289-297.

Kim JY, Kim HJ, Kim SM, Park KR, Jang HJ, Lee EH, Jung SH, Ahn KS. (2011). Methylene chloride fraction of the leaves of *Thuja orien*talis inhibits in vitro inflammatory biomarkers by blocking NF-κB and p38 MAPK signaling and protects mice from lethal endotoxemia. *Journal of Ethnopharmacology* 133, 687-695.

Kim YH, Kim KH, Han CS, Park SH, Yang HC, Lee BY, Eom SY, Kim YS, Kim JH, Lee NH. (2008). Anti-inflammatory activity of *Crinum asiaticum* Linne var. *japonicum* extract and its application as a cosmeceutical ingredient. *Journal of Cosmetic Science* 59, 419-430.

Kim KM, Choi JY, Yoo S-E, Park MY, Lee B-S, Ko YH, Sung SH, Shin H-M, Park JE. (2007a). HMCO5, herbal extract, inhibits NF-κB expression in lipopolysaccharide treated macrophages and reduces atherosclerotic lesions in cholesterol fed mice. *Journal of Ethnopharmacology* 114, 316-324.

Kim Y, So H-S, Youn M-J, Kim ES, Song MS, Chai KY, Woo WH, Cho K-H, Moon B-S, Park R. (2007b). Anti-inflammatory effect of So-Pung-Tang, a Korean traditional prescription for cerebral infarction patients. *Journal of Ethnopharmacology*, *114, 425-431.*

Kim SH, Park HH, Lee S, Jun CD, Choi BJ, Kim SY, Kim SH, Kim DK, Park JS, Chae BS, Shin TY. (2005). The anti-anaphylactic effect of the gall of *Rhus javanica* is mediated through inhibition of histamine release and inflammatory cytokine secretion. *Internaltional Immunopharmacology* 5, 1820-1829.

Kim JH, Ryu KH, Jung KW, Han CK, Kwak WJ, Cho YB. (2005). Effects of SKI306X on arachidonate metabolism and other inflammatory mediators. *Biological & Pharmaceutical Bulletin* 28, 1615-1620.

Kim Y, So H-S, Moon B-S, Youn M-J, Kim H-J, Shin Y-I, Moon S-K, Song MN, Choi, Jeho Song KY, Park R. (2008). Sasim attenuates LPS-induced TNF-α production through the induction of HO-1 in THP-1 differentiated macrophage-like cells. *Journal of Ethnopharmacology* 119, 122-128.

Kumar KS, Vijayan V, Bhaskar S, Krishnan K, Shalini V, Helen A. (2012). Anti-inflammatory potential of an ethyl acetate fraction isolated from *Justicia gendarussa* roots through inhibition of iNOS and COX-2 expression via NF-κB pathway. *Cellular Immunology* 272, 283-289.

Laupattarakasem P, Wangsrimongkol T, Surarit R, Hahnvajanawong C. (2006). *In vitro and in vivo anti-inflammatory potential of Cryptolepis buchanani*. *Journal of Ethnopharmacology* 108, 349-354.

Lebre MC & Tak PP. (2010). Macrophage subsets in immune-mediated inflammatory disease: Lessons from rheumatoid arthritis, spondyloarthritis, osteoarthitis, behçet's disease and gout. *The Open Arthritis Journal* 3, 18-23.

Lee S-J, Lee JH, Lee HH, Lee S, Kim SH, (2011). Effect of mung bean ethanol extract on pro-inflammtory cytokines in LPS stimulated macrophages. *Food Science and Biotechnology* 20, 519-524.

Llord JDLM, Crespo RG, Manzano JM. (2007). Treatment of rheumatoid arthritis with anakinra: A systematic review. *Reumatología Clínica* 3, S153-158.

Maas M, Deters AM, Hensel A. (2011). Anti-inflammatory activity of *Eupatorium perfoliatum* L. extracts, eupafolin, and dimeric guaianolide via iNOS inhibitory activity and modulation of inflammation-related cytokines and chemokines. *Journal of Ethnopharmacology* 137, 371-381.

Markham A, Lamb HM. (2000). Infliximab: a review of its use in the management of rheumatoid arthritis. *Drugs* 59, 1341-59.

Matsui M, Adib-Conquy M, Coste A, Kumar-Roiné S, Pipy B, Laurent D, Pauillac S. (2012). Aqueous extract of *Vitex trifolia* L. (Labiatae) inhibits LPS-dependent regulation of inflammatory mediators in RAW 264.7 macrophages through inhibition of Nuclear Factor kappa B translocation and expression. *Journal of Ethnopharmacology* 143, 24-32.

Matsui M, Kumar-Roine S, Darius HT, Chinain M, Laurent D, Pauillac S. (2009). Characterisation of the anti-inflammatory potential of *Vitex trifolia* L. (Labiatae), a multipurpose plant of the Pacific traditional medicine. *Journal of Ethnopharmacology* 126, 27-33.

McDermott MF. Rilonacept in the treatment of chronic inflammatory disorders. *Drugs Today* 45, 423-30.

Mehrotra S, Mishra KP, Maurya R, Srimal RC, Singh VK. (2002). Immunomodulation by ethanolic extract of *Boerhaavia diffusa* roots. *International Immunopharmacology* 2, 987-996.

Mihailidou EM, Papadopouli EP, Spanaki MS, Paspalaki PP. (2010). The role of cytokines TNF-α, IL-6 and IL-8 in children with severe bronchial asthma (BA). Evaluation in broncho-alveolar fluid (BALF). *Paediatric Respiratory Reviews* 11, S84-S85.

Mocellin S, Marincola F, Rossi CR, Nitti D, Lise M. (2004). The multifaceted relationship between IL-10 and adaptive immunity: putting together the pieces of a puzzle. *Cytokine & Growth Factor Reviews 15, 61-76.*

Mocellin S, Panelli MC, Wang E, Nagorsen D, Marincola FM. (2003). The dual role of IL-10. *Trends in Immunology* 24, 36-43.

Morel J & Barenbaum F. (2004). Signal transduction pathways: new targets for treating rheumatoid arthritis. *Joint Bone Spine* 71, 503 -510.

Mortensen C, Caspersen S, Christensen NL, Svenningsen L, Thorsgaard L, Christensen LA, Bendtsen F. (2011). Treatment of acute ulcerative colitis with infliximab, a retrospective study from three Danish hospitals . *Journal of Crohn's and Colitis* 5, 28-33.

Nakagome K, Nagata M. (2011). Pathogenesis of airway inflammation in bronchial asthma. *Auris Nasus Larynx* 38, 555-563.

Nworu CS, Akah PA, Okoye FB, Proksch P, Esimone CO. (2010). The effects of *Phyllanthus niruri* aqueous extract on the activation of murine lymphocytes and bone marrow-derived macrophages. *Immunological investigations* 39, 245-267.

Oh GS, Pae HO, Choi BM, Jeong S, Oh H, Oh CS, Rho YD, Kim DH, Shin MK, Chung HT. (2003). Inhibitory effects of the root cortex of Paeonia suffruticosa on interleukin-8 and macrophage chemoattractant protein-1 secretions in U937 cells. *Journal of Ethnopharmacology* 84, 85-9.

Ojaniemi M, Glumoff V, Harju K, Liljeroos M, Vuori K, Hallman M. (2003). Phosphatidylinositol 3-kinase is involved in Toll-like receptor 4-mediated cytokine expression in mouse macrophages. *European Journal of Immunology* 33, 597–605.

Okoko T, Ere D. (2012). *Hibiscus sabdariffa* extractivities on cadmium-mediated alterations of human U937 cell viability and activation. *Asian Pacific Journal of Tropical Medicine 5, 33-36.*

Pan X-Q, Darby C, Indik ZK, Schreiber AD. (1999). Activation of Three Classes of Nonreceptor Tyrosine Kinases Following Fcγ Receptor Crosslinking in Human Monocytes. *Clinical Immunology* 90, 55-64.

Papatriantafyllou M. (2011). Monocytes: Nudged out of the niche. *Nature Reviews Immunology* 11, 368-369.

Park JS, Svetkauskaite D, He Q, Kim JY, Strassheim D, Ishizaka A, Abraham E. (2004). Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. J. Biol. Chem., 279, . 7370–7377.

Parkin J & Cohen B. (2001). An overview of the immune system. *The Lancet* 357, 1777-1789.

Pauli U. (1994). Control of tumor necrosis factor gene expression. *Critical Reviews in Eukaryotic Gene Expression* 4, 323-434.

Perera PK, Peng C, Xue L, Li Y, Han C. (2011). *Ex vivo* and *in vivo* effect of Chinese herbal pill Yi Shen Juan Bi (YJB) on experimental arthritis. *Journal of Ethnopharmacology* 134, 171-175.

Punturee K, Wild CP, Vinitketkumneun U. (2004). Thai medicinal plants modulate nitric oxide and tumor necrosis factor-α in J774.2 mouse macrophages. *Journal of Ethnopharmacology* 95, 183-189.

Punzón C, Alcaide A, Fresno M. (2003). In vitro anti-inflammatory activity of *Phlebodium decumanum*. Modulation of tumor necrosis factor and soluble TNF receptors. *International Immunopharmacology* 3,1293-1299.

Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, Ulevitch RJ, Davis RJ. (1995). Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. *Journal of Biological Chemistry* 270, 7420–7426.

Rhule A, Navarro S, Smith JR, Shepherd DM. (2006). *Panax notoginseng* attenuates LPS-induced pro-inflammatory mediators in RAW264.7 cells. *Journal of Ethnopharmacology* 106, 121-128.

Saad B, Atta BSA, Basha W, Hmade A, Kmail A, Khasib S, Said O. (2011). *Hypericum triquetrifolium*—Derived Factors Downregulate the Production Levels of LPS-Induced Nitric Oxide and Tumor Necrosis Factor-α in THP-1 Cells. *Evidence-Based Complementary and Alternative Med*. Article ID 586470, 7 pages doi:10.1093/ecam/nen056.

Saravanan S, Prakash Babu N, Pandikumar P, Karunai Raj M, Gabriel Paulraj M, Ignacimuthu S. (2012). Immunomodulatory potential of *Enicostema axillare* (Lam.) A. Raynal, a traditional medicinal plant. *Journal of Ethnopharmacology* 140, 239-46.

Sariban S, Imamura K, Luebbers R, Kufe D. (1988). Transcriptional and posttranscriptional regulation of tumor necrosis factor gene expression in human monocytes. *Journal of Clinical Investigation* 81, 1506–1510.

Sheng BW, Chen XF, He DL. (2008). The protective effect of Chinese herbs for supplementing shen to eliminate stone on renal injury induced by extracorporeal shockwave lithotripsy in patients with renal calculus. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. 28, 694-697.

Sae-wong C, Tansakul P, Tewtrakul S. (2009). Anti-inflammatory mechanism of Kaempferia parviflora in murine macrophage cells (RAW 264.7) and in experimental animals. *Journal of Ethnopharmacology* 124, 576-580.

Schierbeck H, Lundbäck P, Palmblad K, Klevenvall L, Erlandsson-Harris H, Andersson U, Ottosson L. (2011). Monoclonal Anti-HMGB1 (High Mobility Group Box Chromosomal Protein 1) Antibody Protection in Two Experimental Arthritis Models. *Molecular Medicine* 17, 1039-1044.

Scott IC, Hajela V, Hawkins PN, Lachmann HJ. (2011). A case series and systematic literature review of anakinra and immunosuppression in idiopathic recurrent pericarditis. *Journal of Cardiology Cases* 4, e93-e97.

Sekine Y, Yumioka T, Yamamoto T, Muromoto R, Imoto S, Sugiyma K, Oritani K, Shimoda K, Minoguchi M, Akira S, Yoshimura A, Matsuda T. (2006). Modulation of TLR4 signaling by

a novel adaptor protein signal-transducing adaptor protein-2 in macrophages. *Journal of Immunology* 176,380–389.

Shin KM, Kim YH, Park WS, Kang I, Ha J, Choi JW, Park HJ, Lee KT. (2004). Inhibition of methanol extract from the fruits of *Kochia scoparia* on lipopolysaccharide-induced nitric oxide, prostaglandin [correction of prostagladin] E2, and tumor necrosis factor-alpha production from murine macrophage RAW 264.7 cells. *Biological and Pharmacological Bulletin* 27, 538-543.

Sodsai A, Piyachaturawat P, Sophasan S, Suksamrarn A, Vongsakul M. (2007). Suppression by *Curcuma comosa* Roxb. of pro-inflammatory cytokine secretion in phorbol-12-myristate-13-acetate stimulated human mononuclear cells. *International Immunopharmacology* 7, 524-531.

Strauss-Ayali D, Conrad SM, Mosser DM. (2007). Monocyte subpopulations and their differentiation patterns during infection. *Journal of Leukocyte Biology* 82, 244-252.

Strieter RM. Interleukin-8: a very important chemokine of the human airway epithelium Am. J. Physiol., Lung Cell Mol. Physiol., 283 (2002), pp. L688–L689.

Sun Y, Li YH, Wu XX, Zheng W, Guo ZH, Li Y, Chen T, Hua ZC, Xu Q. (2006). Ethanol extract from *Artemisia vestita*, a traditional Tibetan medicine, exerts anti-sepsis action through down-regulating the MAPK and NF-kappaB pathways. *International Journal of Molecular Medicine* 17, 957-62.

Tal TL, Simmons SV, Silbajoris R, Dailey L, Cho S-H, Ramabhadran R, Linak W, Reed W, Bromberg PA, Samet JM. (2010). Differential transcriptional regulation of IL-8 expression by human airway epithelial cells exposed to diesel exhaust particles. *Toxicology and Applied Pharmacology* 243, 46-54.

Terkeltaub R. (2010). Update on gout: new therapeutic strategies and options. *Nature Reviews Rheumatology* 6, 30-38.

Thiyagarajan P, Chandrasekaran CV, Deepak HB, Agarwal A. (2011). Modulation of lipopolysaccharide-induced pro-inflammatory mediators by an extract of *Glycyrrhiza glabra* and its phytoconstituents. *Inflammopharmacology* 19, 235-241.

Tiwari M, Dwivedi UN, Kakkar P. (2010). Suppression of oxidative stress and pro-inflammatory mediators by *Cymbopogon citratus* D. Stapf extract in lipopolysaccharide stimulated murine alveolar macrophages. *Food and Chemical Toxicology* 48, 2913-2919.

Tracey D. Klareskog L, Sasso EH, Salfeld JG, Tak PP. (2008). Tumor necrosis factor antagonist mechanisms of action: A comprehensive review. *Pharmacology & Therapeutics* 117, 244–279.

Treffkorn L, Scheibe R, Maruyama T, Dieter P. (2004). PGE_2 exerts its effect on the LPS-induced release of TNF-α, ET-1, IL-lα, IL-6 and IL-10 via the EP2 and EP4 receptor in rat liver macrophages. *Prostaglandins & Other Lipid Mediators*, 74, 113-123.

Vinay DS, Kwon BS (2012). Targeting TNF superfamily members for therapeutic intervention in rheumatoid arthritis. *Cytokine* 57, 305-312.

Vouldoukis I, Lacan D, Kamate C, Coste P, Calenda A, Mazier D, Conti M, Dugas B. (2004). Antioxidant and anti-inflammatory properties of a *Cucumis melo* LC. extract rich in superoxide dismutase activity. *Journal of Ethnopharmacology* 94(1):67-75.

Wadsworth TL, McDonald TL, Koop DR. (2001). Effects of Ginkgo biloba extract (EGb 761) and quercetin on lipopolysaccharide-induced signaling pathways involved in the release of tumor necrosis factor-alpha. *Biochemical Pharmacology* 62, 963-974.

Wang JM, Sit WH, Lee CL, Fu KH, Chan DK. (2006). Protection of lethal toxicity of endotoxin by *Salvia miltiorrhiza* Bunge is via reduction in tumor necrosis factor alpha release and liver injury. *Int Immunopharmacol*. 6(5):750-8.

Wang H, Ma G, Ochani M, Li J. (2004). Ancient Chinese herbal medicine as a modern hope for the treatment of sepsis: Extract of *Angelica sinensis* as an antagonist for a newly discovered late mediator of sepsis, HMGB1. *Annals of Emergency Medicine* 44, S52-S52.

Wang H, Li W, Li J, Rendon-Mitchell B, Ochani M, Ashok M, Yang L, Yang H, Tracey KJ, Wang P, Sama ES. (2006). The aqueous extract of a popular herbal nutrient supplement, *Angelica sinensis*, protects mice against lethal endotoxemia and sepsis. *Journal of Nutr*ition 136, 360-365.

Wong M, Ziring D, Korin Y, Desai S, Kim S, Lin J, Gjertson D, Braun J, Reed E, Singh RJ. (2008). TNFα blockade in human diseases: Mechanisms and future directions. *Clinical Immunology* 126, 121-136.

Weinberg M. (2003). An overview of infliximab, etanercept, efalizumab, and alefacept as biologic therapy for psoriasis *Clinical Therapeutics* 25, 2487-2505.

Wu SJ, Tsai JY, Lai MN, Ng LT. (2007). Armillariella mellea shows anti-inflammatory activity by inhibiting the expression of NO, iNOS, COX-2 and cytokines in THP-1 cells. *Americal Journal of Chinese Medicine* 35, 507-516.

Wu MJ, Weng CY, Ding HY, Wu PJ. (2005). Anti-inflammatory and antiviral effects of *Glossogyne tenuifolia*. *Life Science* 76, 1135-46.

Wu M-J, Weng C-Y, Wang L, Lian T-W. (2005). Immunomodulatory mechanism of the aqueous extract of sword brake fern (*Pteris ensiformis* Burm.). *Journal of Ethnopharmacology* 98, 73-81.

Wu MJ, Wang L, Ding HY, Weng CY, Yen JH. (2004). Glossogyne tenuifolia acts to inhibit inflammatory mediator production in a macrophage cell line by downregulating LPS-induced NF-kappa B. *Journal of Biomedical Science* 11, 186-199.

Yang Y, Yu T, Jang HJ, Byeon SE, Song SY, Lee BH, Rhee MH, Kim TW, Lee J, Hong S, Cho JY. (2012). *In vitro and in vivo anti-inflammatory activities of Polygonum hydropiper* methanol extract. *Journal of Ethnopharmacology* 139, 616-25.

Yang N, Zhan J, Li X. (2009). Effect of Butonal-extracted Food Allergy Herbal Formula 2 (FAFH2) and its sub-fractions on TNF-alpha production by macrophage cells. *Journal of Allergy and Clinical Immunology* 123, S182-S182.

Yang H, Young DW, Gusovsky F, Chow J.C. (2000). Cellular events mediated by lipopolysaccharide-stimulated toll-like receptor 4. MD-2 is required for activation of mitogen-activated protein kinases and Elk-1. *Journal of Biological Chemistry* 275, 20861–20866

Wang H, Li W, Li J, Rendon-Mitchell B, Ochani M, Ashok M, Yang L, Yang H, Tracey KJ, Wang P, Sama AE. (2006). The aqueous extract of a popular herbal nutrient supplement, *Angelica sinensis*, protects mice against lethal endotoxemia and sepsis. *Journal of Nutrition* 136, 360-365.

Zagariya, S. Mungre, R. Lovis, M. Birrer, S. Ness, B. (1998). Thimmapaya, R. Pope Tumor necrosis factor alpha gene regulationenhancement of C/EBPβ-induced activation by c-Jun. *Molecular and Cellular Biology* 18, 2815–2824.

Zhao L, Tao JY, Zhang SL, Pang R, Jin F, Dong JH, Guo YJ. (2007). Inner anti-inflammatory mechanisms of petroleum ether extract from *Melilotus suaveolens* Ledeb. *Inflammation* 30, 213-223.

Zhang g, Ghosh sj. (2000). Molecular mechanisms of NF-kappaB activation induced by bacterial lipopolysaccharide through Toll-like receptors. *Journal of Endotoxin Research* 6, 453–457

