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Oriental Pharmacy and Experimental Medicine 2005 **5(4)**, 251-261

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Review

Can herbal drug(s) meet the challenges of genomewide screen results on rheumatoid arthritis

Bholanath Paul

Immunobiology Laboratory, Industrial Toxicology Research Centre, MG Marg, Lucknow 226001, India

SUMMARY

Rheumatoid arthritis (RA) is an autoimmune/inflammatory disorder with a complex genetic component. RA is characterized by chronic inflammation of the synovial membrane in the joint, which leads to the progressive destruction of articular cartilage, ligament and bone. Several cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) have been implicated in the pathological mechanisms of synovial tissue proliferation, joint destruction and programmed cell death in rheumatoid joint. Genome wide screening of subjects suffering from autoimmune diseases especially arthritis revealed linkage to inflammatory molecules like TNF-α, IL-1β and IL-6, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), nuclear factor-kappaB (NF-κB) and human leucocyte antigen/major histocompatibility complex (HLA/MHC) locus. The status of the pharmacological mechanism of herbal drugs in the light of genome wide screening results has been discussed to reinforce the therapeutic potential and the pharmacological basis of the herbal drugs.

Key words: Rheumatoid arthritis; Herbal drugs; Genome wide screening; Autoimmune disease

INTRODUCTION

The immune system has tremendous diversity and because the repertoire of specificities expressed by B and T cell populations is generated randomly, it is bound to include many which are specific for self-components. Therefore the body must ensure a mechanism to distinguish between self and non-self determinants and avoid autoreactivity. A breakdown of this mechanism leads to an autoimmune disease (Roitt, 1993).

Autoimmune diseases are thought to affect millions of people worldwide. Despite decades of research, the underlying mechanisms of disease are poorly understood, diagnosis is often difficult, and therapies that minimize systemic side effects are lacking. Major advances in our understanding of human genetic variation and remarkable new technologies are paving the way for dramatically improving our fundamental knowledge of autoimmune diseases. Gene mapping studies have clearly illustrated the complexity of these diseases, which appear to involve many genes. Very high-throughput microarray assays that can measure the expression levels of thousands of genes simultaneously are revealing important insights into key biological pathways that appear to be perturbed in autoimmune diseases.

RA is also associated with inflammations that

*Correspondence: Bholanath Paul, Immunology Laboratory, Industrial Toxicology Research Centre, MG Marg, Lucknow 226001, India.
E-mail: bholanathpaul@yahoo.com

arise due to invasion by an infectious agent, antigen challenge or even just physical damage. The three major events occur during inflammation. a) Blood supply to the area increases. b) There is an increase in capillary permeability, caused by retraction of the endothelial cells and increased vesicular transport across the endothelium. This permits larger molecules to traverse the endothelium than would ordinarily be capable of doing so and thus allows antibody, complement and molecules other plasma enzyme system to reach the inflammatory site. c) Leucocytes, initially neutrophils and macrophages and later lymphocytes, migrate out of the capillaries and into the surrounding tissues.

The development of inflammatory reaction is controlled by cytokines, by products of the plasma enzyme system and by vasoactive mediators released from mast cells, basophils and platelets (Male, 1993).

Recent advances in genetic and genomic studies focused primarily on systemic lupus erythematosus and related rheumatic autoimmune diseases such as Sjogren's syndrome and rheumatoid arthritis. Identification of susceptibility genes and dysregulated biological pathways for these diseases is likely to foster development of novel diagnostic and therapeutic approaches that are increasingly tailored to the underlying pathological mechanisms. (Moser *et al.*, 2004).

Genome wide screening of susceptibility genes in autoimmune inflammatory diseases

It is a chronic disease that leads to progressive joint destruction (Harris, 1989) and is associated with other autoimmune diseases in families, such as thyroid diseases and insulin-dependent diabetes mellitus (Buchanan *et al.*, 1961; Thomas *et al.*, 1983). RA preferentially affects women (Harris, 1989), with a sex ratio of 2 to 4. Its mean age of onset is between 45 and 50 years of age, and its prevalence may be as high as 1% in adults (Harris, 1989). Tumor necrosis factor-alpha (TNF-α), cyclooxygenase (COX)-2, and prostaglandin (PG)E-2 play a critical role in the pathophysiology of arthritis. TNF-α mediates induction of other cytokines, COX-2, PGs, and metalloproteinases, which leads to cartilage degradation (Frondoza *et al.*, 2004). It is for this reason, RA is believed to be a multifactorial disease resulting from a T cell-driven autoimmune process aimed primarily at the joints; its pathophysiology is unknown (Feldman *et al.*, 1996). Its genetic component has been suggested by familial aggregation (Thomas *et al.*, 1983; Deljunco *et al.*, 1984), twin studies (Lawrence, 1970; Aho *et al.*, 1986; Silman *et al.*, 1993), and segregation analysis (Lynn *et al.*, 1995). Human leukocyte antigen (HLA), which is the only susceptibility locus known (Stastny, 1978; Gregersen, 1987), has been estimated to account for one-third of this component (HLA=1.8) (Wordworth and Bell, 1992; Ollier *et al.*, 1997).

The mechanisms by which HLA molecules predispose to RA have been an area of intense interest. Jawaheer and coworkers reported the first major genomewide screen of multiplex families with RA gathered in the United States and confirmed linkage of the HLA locus to RA. However, the analysis also revealed a number of non-HLA loci on chromosomes 1, 4, 12, 16, and 17 with evidence for linkage at a significance level of $P < 0.005$. Several of the regions that showed evidence for linkage in RA were also implicated in other diseases of an autoimmune nature, including systemic lupus erythematosus, inflammatory bowel disease, multiple sclerosis, and ankylosing spondylitis. Therefore, genes in the HLA complex play a major role in RA susceptibility and the non-HLA loci also contribute significantly to the overall genetic risk (Cornelis *et al.*, 1998; Jawaheer *et al.*, 2001). A recently completed genome-wide linkage analysis of rheumatoid arthritis revealed a number of possible candidate regions in addition to HLA (Tsuchiya and Tokunaga, 1999). The early components of the complement pathway, Fc receptor IIa, IIIa, mannose-binding lectin, interleukin-10 (IL-10) and tumor necrosis factor receptor-2 (TNFR-2) are the

potential non-HLA susceptibility genes to systemic lupus erythematosus. Evidence also suggested linkage of RA to the Interleukin-1 (IL-1) locus. The increased linkage to IL-1 and IL-10 in HLA-identical sibs suggests a possible interaction between these cytokines and the HLA loci. Moreover IL-10 could interact with HLA factors in predisposing to erosive disease (Barrera, 2001). Although linkage has been established between RA and HLA and non-HLA loci, absolute association is missing. However, recent work has provided some possible explanations for the lack of absolute associations between a particular disease and a particular HLA antigen. There is now some evidence to suggest that specific epitopes rather than entire class I molecules or II may be responsible for disease predisposition. Furthermore, it appears that these epitopes may be transferred between different class I and II molecules by a mechanism known as gene conversion. Work evaluating the influence of other genes, such as those for the T cell receptor, on disease susceptibility has just begun. Many of the rheumatic diseases are quite diverse in their presentation. If only one of a heterogeneous group of diseases is associated with an HLA antigen, study of the entire group of diseases will of necessity dilute the association (Schiffenbauer and Schwartz, 1987). Therefore a better definition of clinical subsets can lead to improved correlations of HLA and disease. Little is known of etiologic agents or pathogenesis. As our knowledge of the interaction of HLA antigens, T cell receptors, and etiologic agents increases, we will come closer to an understanding of the mechanisms by which these molecules predispose to disease.

It is now evident that that autoimmune inflammatory disease, RA is associated with the expression of MHC antigen, inflammatory molecules especially the cytokine profile and the expression of gene regulatory molecules. Therefore in the ongoing discussion we address these issues in the light of herbal drugs.

Can herbal drugs modulate mediators of inflammation?

The huge resurgence of interest in herbal remedies has spawned a global industry that now competes with conventional drugs as adjuncts and/or alternatives for various conditions.

Several cytokines such as TNF-α, IL-1β and IL-6 have been implicated in the pathological mechanisms of synovial tissue proliferation, joint destruction and programmed cell death in rheumatoid joint. Oral administration of water-soluble fraction of the ethanol extract of *Nyctanthes arbor-tristis* in arthritic mice showed a consistent depletion of TNF-α from the host plasma. A similar depletion of TNF-α in the plasma of soluble protein A-treated mice has been observed (Paul and Saxena, 1997). Soluble protein A treated mice has elevated level of serum TNF-α (Paul *et al.*, 1993).

The inhibitory effects of Tanshinone IIA, a diterpene isolated from *Salvia miltiorrhiza* root, on the production of nitric oxide (NO), IL-1β, IL-6 and TNF-α, and the expression of inducible nitric oxide synthase (iNOS) were investigated in activated RAW 264.7 cells. This compound markedly inhibited the production of NO, IL-1β and TNF-α, and suppressed the expression of iNOS in a dose-dependent manner suggesting the anti-inflammatory property of *S. miltiorrhiza* (Jang *et al.*, 2003). Similarly, water-soluble polysaccharides, FI0-b, and its formic acid-modified derivative, FI0-b-H, have been observed to modulate human proinflammatory cytokines. FI0-b and FI0-b-H at concentration of 4, 40, and 400 mg/l significantly downregulated interleukin-1α production by THP-1 cells induced by lypopolysaccharide (LPS) 1 or 10 mg/l and phorbol myristate acetate (PMA) 200 nmol/l. At lower stimulation with LPS 10 mg/L and PMA 200 nmol/l, both polysaccharides significantly upregulated TNF-α production by THP-1 cells. However, at higher stimulation with LPS 100 mg/l and PMA 200 nmol/l, they downregulated TNF-α production. FI0-b-H downregulated interleukin-8 (IL-8) production by THP-1 cells at a lower-dose of LPS 1 mg/l and PMA 200 nmol/l, but upregulated IL-8 production

at a higher-dose of LPS 10 mg/L and PMA 200 nmol/l. Production of cytokines (IL-1α and TNF-α) was transcriptionally or post-transcriptionally regulated by FI0-b and FI0-b-H. The water-soluble polysaccharides of *Ganoderma tsugae* mycelium have bidirectional immunomodulatory effects on cytokine production in different stimulatory conditions in a dose-dependent manner. Compared with FI0-b, FI0-b-H has more marked effects on human proinflammatory cytokine production (Gao *et al.*, 2000)

Some herbal remedies are sold as food additives and are believed to have immune-enhancing properties. The effect of five herbal remedies- "*Sambucol Black Elderberry* Extract", "Sambucol Active Defense Formula" and "Sambucol for Kids" (with known antiviral properties), "Protec" and "Chizukit N" (containing propolis and Echinacea, claimed to be immune enhancers) on the production of cytokines, one of the main components of the immune system has been studied (Barak *et al.*, 2002). The production of four inflammatory cytokines (IL-1β, TNF-α, and IL-6 and IL-8) and one anti-inflammatory cytokine (IL-10) was tested using blood-derived monocytes from 12 healthy donors. The Sambucol preparations increased the production of five cytokines (1.3 - 6.2 fold) compared to the control. Protec induced only a moderate production of IL-8 (1.6 fold) and IL-10 (2.3 fold) while Chizukit N caused only a moderate increase in IL-10 production (1.4 fold). Both Protec and Chizukit N caused moderate decreases in IL-1β, TNF-α and IL-6 production. The three Sambucol formulations activate the healthy immune system by increasing inflammatory and anti-inflammatory cytokines production, while the effect of Protec and Chizukit N is much less. Sambucol could therefore have immunostimulatory properties when administered to patients suffering from influenza (as shown before), or immunodepressed cancer or AIDS patients who are receiving chemotherapy or other treatments.

Certain irinoid-producing plants have been reported to possess anti-inflammatory remedies. Catalposide (CATP), a single compound isolated from irinoid-producing plant *Catalpa ovata*, has a potential for preventing or ameliorating diseases characterized by mucosal inflammation. Preliminary microarray-based gene expression test revealed that CATP, which alone did not significantly affect expression of any of the > 8,000 genes analyzed, attenuated the expression of TNF-α induced proinflammatory genes including interleukin-8 (IL-8) in human intestinal epithelial HT-29 cells. Down-regulation of IL-8 mRNA accumulation was also reflected by the decreased IL-8 secretion in CATP-treated HT-29 cells. The signal transduction study revealed that CATP significantly attenuates TNF-α-mediated p38 and extracellular signal-regulated kinase (ERK) phosphorylation. Further, CATP reduced Nuclear factor-kappaB (NF-κB)-mediated transcriptional activation as well as inhibitory subunit I kappaB-alpha (IκB-α) degradation. Intrarectal administration of CATP dramatically reduced the weight loss, colonic damage, and mucosal ulceration that characterize TNBS (trinitrophenyl-bovine serum) induced colitis. Moreover, CATP suppressed the expression of TNF-α, interleukin-1β, and intercellular adhesion molecule-1 along with the inhibition of NF-κB p65 translocation into nucleus in TNBS colitis. Collectively, current results demonstrate that CATP may be an effective agent for the treatment of diseases characterized by mucosal inflammation (Kim *et al.*, 2004).

The ethyl acetate (EA) extract of *Tripterygium wilfordii* Hook F (TWHF) and its major active component, "triptolide", have been reported to be effective in the treatment of rheumatoid arthritis and other autoimmune inflammatory diseases. NO has been recognized as an important mediator of inflammation. Wang and coworkers (2004) examined the effects of the EA extract and triptolide on the production of NO and inducible NO synthase (iNOS) gene expression and transcription *in vivo* and *in vitro*. Peritoneal macrophages from C57BL/6J mice treated orally with the EA extract of TWHF were assayed for NO production and iNOS mRNA expression by RT-PCR. The murine fibroblast cell

line NIH3T3 was also assessed for NO production and iNOS mRNA expression, as well as for iNOS promoter activation, Oct-1 nuclear binding capacity, and Oct-1 protein content by transient transfection, electrophoretic mobility shift assay, and immunoblotting, respectively. NO production and iNOS mRNA expression by macrophages from C57BL/6J mice immunized with trinitrophenyl-bovine serum albumin in Freund's complete adjuvant were significantly inhibited by oral administration of the EA extract (52.3% and 59.8% of control, respectively, at one-eighth of the dose that is lethal for 50% of the animals [LD_{50}] and 21.0% and 38.1% of control, respectively, at one-fourth the LD_{50}. Moreover, the EA extract and triptolide significantly inhibited NO production *in vitro* in activated peritoneal macrophages, which reflected a decreased level of iNOS mRNA. Finally, triptolide inhibited promoter activity of the iNOS gene and induction of the activity of the regulator of iNOS transcription, Oct-1. It was concluded that the EA extract of TWHF and triptolide inhibit transcription of the iNOS gene. This may contribute to the antiinflammatory effects of this traditional herbal remedy.

In a study, Yuldahansotang (YH-Tang), a Sasang Constitutional prescription composed of seven herbal mixtures, inhibited secretion of inflammatory cytokines from human astrocytes. YH-Tang regulated the cytokine secretions in astrocytes stimulated with substance P (SP) and lipopolysaccharide (LPS). YH-Tang significantly inhibited IL-1, IL-4, IL-6 and TNF-α secretion in astrocytes stimulated with SP and LPS, but did not inhibit interferon-γ and IL-2 secretion significantly. IL-1 has been shown to elevate TNF-α secretion from LPS-stimulated astrocytes while having no effect on astrocytes in the absence of LPS. IL-1 mediated inhibition of TNF-a secretion from astrocytes by YH-Tang was investigated. Incubation of human astrocytes with IL-1 antibody abolished the synergistic cooperative effect of LPS and SP. These results suggest that YH-Tang may indirectly inhibit TNF-α secretion by inhibiting IL-1 secretion. Moreover, these findings indicate that YH-Tang has regulatory effects on cytokine secretion in an acute CI patient (Choi *et al.*, 2002).

TNF, IL-1, and IL-6 are important cytokines involved in the pathogenesis of inflammatory lesions. Esculentoside A, a kind of saponin isolated from the root of the Chinese herb, *Phytolaca esculenta*, is reported to possess potent anti-inflammatory effects in acute and chronic experimental models. Ju and his group (1998) investigated the effects of esculentoside A on the production of TNF-α, IL-1 and IL-6 induced by lipopolysaccharide (LPS) in mice. *In vitro* experiments demonstrated that esculentoside A (0.1 - 10 mumol/l) significantly reduced the release of TNF from the peritoneal macrophages derived from mice pretreated with thioglycolate. IL-1 and IL-6 secretion was also inhibited in a concentration-dependent manner by esculentoside A from 0.01 to 10 mumol/l. *In vivo* experiments demonstrated that detectable TNF was observed 0.25 h after injection, was maximal at 0.5 h, and returned to baseline at 4 h. Maximal production of IL-1 and IL-6 were observed to be 1 and 2 h, respectively, after injection of LPS. Pretreatment of mice with 5, 10, or 20 mg/kg esculentoside A once a day for 7 consecutive days dose-dependently decreased the TNF, IL-1 and IL-6 levels in the sera of mice following LPS challenge. Thus we observe a large number of plant extracts are capable of modulating an array of proinflammatory cytokines and even modulate NO and iNOS that play a vital role in inflammation. The down regulation of inflammatory molecules by herbal drugs thus hold promise in curing inflammatory disease especially arthritis. It will be interesting to see whether the plant extracts that can modulate the inflammatory cytokines can modulate the regulators of gene expression like NF-κB transcription factors also. NF-κB is a central transcriptional factor and a pleiotropic regulator of many genes involved in immunological responses. Recent evidence has shown that NFκB subunits dynamically shuttle between the cytoplasm and the nucleus and IkB-α

and ensures their transport back to the cytoplasm (Chen *et al.*, 2001) and mediates inflammatory response program (Li *et al.*, 2002)

Can herbal medicine act on NF-κB dependent pathways

Certain irinoid-producing plants have been used as herbal anti-inflammatory remedies. Catalposide (CATP), a single compound isolated from irinoid-producing plant *Catalpa ovata*, has a potential for preventing or ameliorating diseases characterized by mucosal inflammation. Preliminary microarray-based gene expression test revealed that CATP, which alone did not significantly affect expression of any of the > 8,000 genes analyzed, attenuated the expression of TNF-α-induced proinflammatory genes including IL-8 in human intestinal epithelial HT-29 cells. Down-regulation of IL-8 mRNA accumulation was also reflected by the decreased IL-8 secretion in CATP-treated HT-29 cells. The signal transduction study revealed that CATP significantly attenuates TNF-alpha-mediated p38 and extracellular signal-regulated kinase (ERK) phosphorylation. Further, CATP reduced NF-κB-mediated transcriptional activation as well as IκB-α degradation. Intrarectal administration of CATP dramatically reduced the weight loss, colonic damage, and mucosal ulceration that characterize TNBS induced colitis. Moreover, CATP suppressed the expression of TNF-α, interleukin-1β, and intercellular adhesion molecule-1 along with the inhibition of NF-κB p65 translocation into nucleus in TNBS colitis. Collectively, these results demonstrate that CATP may be an effective agent for the treatment of diseases characterized by mucosal inflammation (Kim *et al.*, 2004).

Recently it has been reported that Ginger extract containing hydroxy-methoxy-phenyl compounds (HAPC) significantly inhibited the activation of TNF-α and COX-2 expression in human synoviocytes as well as suppressed production of TNF-α and PGE-2. Inhibition of TNF-α and COX-2 activation was accompanied by suppression of NF-κB and IκBα induction (Frondoza *et al.*, 2004).

Glossogyne tenuifolia (GT) is a traditional antipyretic herb used in Chinese medicine. Wu and coworkers (2004) elucidated the molecular pharmacological activity and the effective components in the ethanol extract of GT. They found that GT had potent anti-inflammatory effects on the lipopolysaccharide (LPS)-activated murine macrophages, RAW264.7. GT downregulated LPS-induced expression of inducible nitric oxide synthase (iNOS) by blocking its transcription. It also caused a dose-dependent inhibition of the release of prostaglandin E_2 by repressing the promoter activity of the inducible cyclooxygenase (COX-2) gene. Moreover, GT exerted a dose-dependent inhibition of the LPS-stimulated release of the proinflammatory cytokines, TNF-α, IL-1β, IL-6, and IL-12. They observed that GT attenuates inflammatory mediator synthesis of activated macrophages through an NFκB-dependent pathway. The active components of GT were identified as oleanolic acid and luteolin-7-glucoside. Both of these compounds inhibited LPS-stimulated inflammatory mediator production and NF-κB activation.

Thus it is evident that herbal drugs can interfere in the inflammatory response program through the regulators of gene expression like NF-κB and can be used for the treatment of RA. Genome wide screening revealed linkage between RA and the expression of MHC antigens on the immune cells, therefore it will be worthwhile to see whether herbal drugs can modulate the expression of MHC/HLA antigen on the T cell so that recognition of the antigen is down regulation.

Can herbal drug modulate MHC antigens

The modulatory effect of *Cordyceps sinensis* on MHC class II antigen expression on hepatoma cells has been studied. The extract of *Cordyceps sinensis* (VGH-CS-ME-82, 40 micrograms/ml) was found to increase the MHC class II antigen expression on human hepatoma cell line, HA22T/VGH cells in a dose dependant manner. The VGH-CS-ME-82, either alone or with IFN-gamma induction, increases the

MHC class II antigen expression on hepatoma cell line HA22T/VGH, suggestive of a possible immunotherapy, by making the host immune surveillance more effective against tumor cells with down-regulated MHC class II antigen expression (Chiu *et al.*, 1998). But for cure of autoimmune disorder we have to look for plant extracts that suppress the expression of MHC antigen.

Hochu-ekki-to (HOT), a herbal drug, was investigated to evaluate susceptibility to oral tolerance in post-neonatal infant mice, on the susceptibility were investigated. HOT increased the number of both CD4+ T cells and antigen-presenting cells expressing MHC class II as well as costimulatory molecules (CD40, CD80 and/or CD86) in the Peyer's patch of infant mice, which had fewer cells than adult mice. In the Peyer's patch HOT augmented the IL-12 p40 mRNA expression and spontaneous or CD40-stimulated IL-12 production, and increased the number of CD4+ cells expressing CD40 ligand, which is up regulated by IL-12 (Kaneko *et al.*, 2001).

Aberrant thyroid cell MHC class II antigen expression induced by IFN-gamma is suppressed by the extract of herbal medicines. These results indicate that herbal medicines inhibit cytokine-induced thyroid cell destruction, therefore, may have therapeutic potential in the treatment of autoimmune thyroid disease (Shon *et al.*, 2004).

Thus presently not much work has been done in elucidating the effect of herbal drug(s) in modulating the expression of HLA/MHC antigen in the T cells and antigen presenting cells. Nonetheless, enormous documentary evidences exist on the anti-arthritic activity of herbal drugs.

In the Korean traditional medicine, *Hominis placenta* (HP) as an herbal component of herb-acupuncture has been widely used to treat chronic inflammatory diseases such as RA. After the treatment of arthritic rats with HP, the body weights and paw volumes of arthritic rats were almost restored to the levels of normal rats whereas the evaluation by the articular index was not remarkable. The TNF-α, IL-1β and IL-6 positive cells in the immunohistological sections of subchondral bone region of the joint significantly decreased in HP-treated (ST36 acupoint) arthritic group as compared with those in non-treated or HP-treated (non-acupoint) ones, which was coincident with the behavioral studies (Yeom *et al.*, 2003).

PG201 has been formulated using 12 herbs known to have anti-inflammatory and protective effects on damaged tissue and bone among other functions. Administration of PG201 significantly suppressed the progression of collagen-induced arthritis (CIA) and inhibited the production of TNF-α and IL-1β in the paws. The erosion of cartilage was dramatically reduced in mouse knees after treatment with PG201. In the serum of PG201-treated mice, the level of TIMP-2 and the ratio of TIMP-2 to MMP-2 were significantly elevated, and the level of IL-4, but not of IL-10, was increased. Thus PG201 is a potential therapy for RA (Shin *et al.*, 2003).

Administration of *Swertia chirayita* extracts for 12 consecutive days through the oral route showed a dose dependent (0, 11.86 and 23.72 mg/kg body weight) reduction of TNF-α, IL-β and interferon-γ elevation of IL-10 in the joint homogenates of arthritic mice. IL-6 was not down regulated in joint homogenate of arthritic mice at the dose 11.86 mg/kg but at higher doses (23.72 and 35.58 mg/kg) significant reduction was observed. The aqueous extract was found to possess two polar compounds, amerogentin and mangiferin and the latter has been reported to possess potent anti-inflammatory property (Shankaranarayan *et al.*, 1979). We presume its presence in the aqueous extract of *S. chirayita* is responsible for reducing TNF-α, IL-1β, IL-6, and IFN-γ and/or elevating IL-10 in the joint homogenates of arthritic mice on day 12 (Kumar, 2003).

Ginger extract-hydroxy-methoxy-phenyl compounds (HAPC) significantly inhibited the activation of TNF-α and COX-2 expression in human synoviocytes as well as suppressed production of TNF-α and PGE-2. Inhibition of TNF-α and COX-2 activation was accompanied by suppression of NF-κB and

IκBα-alpha induction. Using *in vitro* assay, the authors discovered that the ginger extract blocks activation of proinflammatory mediators and its transcriptional regulator suggesting its mode of action. These observations indicate that ginger extract-HAPC offers a complementary and alternative approach to modulate the inflammatory process involved in arthritis (Frondoza *et al.*, 2004).

The herbal remedy *Zingiber officinale* (gingiber root) has been used for perhaps thousands of years in the far east to treat inflammatory diseases, including osteoarthritis. However, the antiarthritic effect of gingerroot has been evaluated on osteoarthritic cartilage of sow. Increasing gingiber root extacts (GRE) concentration (1 - 100 μg/ml) reduced NO production by cartilage tissue explants, and a similar pattern was observed in the production of PGE(2). The inhibitory effects of GRE on nitric oxide (NO) and PGE(2) production by sow osteoarthritic cartilage explants observed in this study suggest an important role for GRE as an anti-arthritic agent in osteoarthrosis in the sow (Shen *et al.*, 2003).

Deoxynupharidine (DON) is an alkaloid isolated from rhizome *Nuphar pumilum*, which has been extensively used in the treatment of rheumatoid arthritis and back and leg pains as a folk remedy in China. The alkaloid has potent immunosuppressive activities. Mitogens or allogen induced lymphoproliferative responses of murine splenocytes or human tonsillar mononuclear cells were markedly reduced when DON was added in the cultures. DON also inhibited the capacity of murine peritoneal macrophages to produce IL-1 and TNF, which play very important roles in the process of inflammation and immune response. This immunosuppressive action of DON may partly account for some of its potential in the treatment of chronic inflammatory diseases (Zhang *et al.*, 1995).

CONCLUSION

Using a genome-wide array screen two previously uncharacterised genes, NLF1 and NLF2 were identified that was upregulated over 30 fold by treatment with IL-1β for 2 h. They were also found to respond to TNF-α, suggesting a general role in inflammation. Expression of both genes peaked 2 h after addition of IL-1β, with similar kinetics to the fastest NF-κB induced genes. The activation of both genes by IL-1β was abrogated by the proteasomal inhibitor, lactacystin which blocks activation of NF-κB by preventing IκB degradation. Furthermore, two sequences with homology to NF-κB binding sites in the promoter of NLF1 were found to be essential for rapid elevation in expression in response to IL-1β. NLF1 and NLF2 transcripts were found predominantly in endothelial cells, and the encoded proteins were localised to the nuclear compartment suggesting a role in the regulation of transcription. Transfection of recombinant NLF into endothelial cells resulted in upregulation of the Rho kinases, Rnd1 and Gem GTPase. Warton and coworkers propose that NLF1 and NLF2 belong to a novel gene family encoding nuclear factors with a role in regulating genes which control cellular architecture. This might increase vascular permeability in acute inflammation (Warton *et al.*, 2004).

The NF-κB transcription factors are pivotal regulators of gene expression programs culminating in stress-like responses and the genesis of innate and acquired immunity. Recently, biological agents that suppress the activities of proinflammatory cytokines have shown efficacy as antiarthritic drugs, but require frequent administration. Thus, plant extract mediated modulation of inflammatory cytokines, anti-inflammatory cytokines, iNOS and transcriptional inhibitors may emerge as an alternative approach for treatment and even prevention of autoimmune inflammatory diseases. The efficacies of various plant extracts are well documented for the treatment of arthritis in laboratory animal models of disease. In this review, the current status of several plants that can reduce the burden of proinflammatory cytokines through the NF-κB and IκB-α pathway is presented

to indicate the richness of the global flora to deal with the autoimmune inflammatory diseases. In addition, if necessary, few of the related herbal extracts may be compounded to achieve the desired goal. Finally, this review reveals the possibility of managing the complexity of the disease as reflected from the genome wide screening studies using herbal drug(s).

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