Department of Medicine, Rheumatology Unit,
Karolinska Institutet, Stockholm, Sweden
Hanoi Medical University, Hanoi Vietnam

STUDIES OF NOVEL IMMUNOSUPPRESSIVE AGENTS IN EXPERIMENTAL ARTHRITIS

Dang Thi Ngoc Dzung

Karolinska Institutet

Stockholm 2006

Karolinska Institutet. Printed by Karolinska University Press
Stockholm, Sweden

ISBN 91-7357-009-5

To my family

ABSTRACT

Rheumatoid Arthritis (RA) is a chronic inflammatory disease of the joints. The hallmark of RA is persisting inflammation in diarthrodial joints with infiltration of leukocytes, thickening of the synovial lining layer and synovial pannus formation. The therapeutic aim for RA patients is to alleviate the symptoms, slow the disease progression and optimise the quality of life. In recent years the measures to achieve this goal have improved with the development of new drugs. Despite recent advances in therapies for RA, there is still no drug available that induces remission in all patients. Thus, there is still a need to screen and develop new anti-rheumatic treatments.

Because of this we investigated the effects of treatment with compounds which were extracted from three different sources in collagen-induced arthritis (CIA) in Dark Agouti (DA) rats. The constituent of the first compound is dominated by flavonoids together with other extractable compounds derived from *Artocarpus* leaves. The second compound is Glucurononxyclomannan (GXM) isolated from culture filtrates of *Cryptoccocus neoformans.* GXM is a polyssacharide with an unbranched mannose backbone. The third compound, code named Rob 803, is 9-chloro-2,3 dimethyl-6-(N,N-dimetylamino-2-oxoethyl)-6H-indolo [2,3-b] quinoxaline, an analogue of a previously reported substance, B220. B220 was originally investigated as an anti-viral drug.

Results: 1. Treatment of rats with the *Artocarpus* extract decreased arthritis incidence and severity and delayed disease onset. *In vitro,* the *Artocarpus* extract acted as a T cell modulator, inhibiting mitogen-induced T cell proliferation and inducing apoptosis of activated cells. A new flavonoid glucoside named artonkin-4'-βD-O-glucoside with an average molecular mass of 514.49 Da was isolated together with three other previously known flavonoid molecules. All four compounds were found to have anti-inflammatory effects. These effects correlated well with the inhibition of mitogen-induced T cell proliferation. Furthermore, the compounds inhibited TNF and IFNγ production of ConA stimulated T cells in a concentration-dependent manner.

2. Treatment of CIA with GXM at a dose of 50 mg/kg suppressed disease development both prophylactically and therapeutically. The effect of GXM was associated with a significant decrease of the titers of anti-CII antibodies. GXM therapy diminished MMP-2 activity but had no effect on apoptosis.

3. The anti-arthritic effect of the synthetic compound Rob803 was evaluated in the CIA model. Daily subcutaneous treatment with 40mg/kg/day of Rob803 significantly suppressed arthritis severity and delayed the onset of clinical arthritis. *In vitro* analysis of the anti-inflammatory effects of Rob803 revealed that Rob803 suppressed NO_2^- production. Interestingly, T cell proliferation was suppressed at a 1000-fold lower concentration than was NO production. Thus we report that early subcutaneous administration of the synthetic substance Rob803 has anti-rheumatic effects which are likely to be mediated via T cell suppression.

Conclusion: All of the investigated new compounds have anti-arthritic properties when tested in CIA. They have anti-T cell proliferative features which can be of benefit in modulating arthritis pathogenesis. Some of the investigated compounds also have cytokine-inhibiting properties. All compounds have low toxicity. They have the potential to be further investigated in experimental as well as clinical trials of RA and in other inflammatory diseases.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numbers:

I **Inhibition by *Artocarpus tonkinensis* of the development of collagen-induced arthritis in rats**

Dang TND, Catrina AI, Lundberg K, Erlandsson Harris H, NguyenTH, Phan TA and Larsson P.

*Scand J Immunol 2005; **61**:234-41.*

II **A novel anti-inflammatory compound, *artonkin-4'-O-glucoside,* from the leaves of *Artocarpus tonkinensis* suppresses experimentally induced arthritis**

Dang TND, Eriste E, Liepinsh E, TrinhTT, Erlandsson-Harris H, Sillard R, Larsson P. Submitted

III **Tolerability and anti-inflammatory effects of glucuronoxylomannan in collagen-induced arthritis**

Mirshafiey A, **Dang TND**, Murphy JW, Khorramizadeh MR, Saadat F, Meheabian F, Larsson P

*Scand J Immunol 2004; **60**:226-32.*

IV **Suppressive effects of *9-chloro-2,3 dimethyl-6-(N,N-dimethylamino-2-oxoethyl) 6H-indolo [2,3-b] quinoxaline* (Rob803) on pathogenic immune mechanisms in collagen induced arthritis**

Westman E , **Dang TND**, Klareskog L, Erlandsson Harris H.

Submitted

CONTENTS

LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
AIA	Adjuvant-induced arthritis
APC	Antigen presenting cell
Rob803	2,3-dimethyl-6-(2-dimethylaminoethyl)-6H-indol [2,3]quinoxaline
CTLA	T lymphocyte-associated antigen
CD	Cluster of differentiation
CCP	Cyclic Citrullinated Peptide
CIA	Collagen-Induced Arthritis
CII	Collagen type II
COX	Cyclooxygenase
DA	Dark Agouti
DC	Dendritic cell
DMARD	Disease-modifying anti-rheumatic drug
FIA	Freund´s incomplete adjuvant
FLIP	FLIP inhibitory protein
GXM	Glucuronoxylomannan
HLA	Human leucocyte antigen
HMGB1	High-mobility group B1 protein
IFN	Interferon
IL	Interleukin
Ig	Immunoglobulin
LNC	Lymph node cell
MMP	Matrix Metalloproteinase
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NF-κB	Nuclear factor-κB
NK	Natural Killer
NO	Nitric Oxide
NSAID	Nonsteroid anti-inflammatory drugs
OIA	Oil-Induced Arthritis
PAD	Peptidylarginine deiminase
PIA	Pristane-induced arthritis
PI3K	Phosphatidylinositol -3-OH kinase
PTPN22	Protein tyrosine phosphatase non-receptor 22
RA	Rheumatoid Arthritis
RF	Rheumatoid Factor
Rob803	9-chloro-2,3dimethyl-6-N,N-dimethylamino-2-oxoethyl)-6H-indolo [2,3-b]quinoxaline
ROS	Reactive oxygen species
SF	Synovial fluid
SIA	Squalene-Induced Arthritis
SM	Synovial membrane
STAT	Signal transducer and activator of transcription
TCR	T cell receptor
TGF	Transforming growth factor
TIMP	Tissue inhibitor of matrix metalloproteinase
TNF	Tumor necrosis factor
TNF-R	Tumor necrosis factor receptor
TUNEL	Terminal deoxynucleotidyl-mediated dUDP nick end labelling

1. BACKGROUND

1.1 THE REASON FOR THE THESIS

Rheumatoid Arthritis is a chronic inflammatory disease often resulting in increased morbidity, mortality and disability. During recent decades a better understanding of the pathogenesis of RA has led to the development of new strategies for disease control which have transformed the management of RA. However, none of them are effective in curing all patients. Furthermore, the potentially greater efficacy of treatment with TNF antagonists comes at a cost that is too high for the majority of the world's population. In this thesis we have studied compounds derived from traditional medicine and of microbiologic origin. In experimental models of arthritis all compounds demonstrate anti-arthritic effects and low toxicity. It is our hope and belief that these compounds can be developed to become new pharmaceutical agents that can be used at an affordable cost for people suffering from rheumatic diseases in poor and in rich countries.

1.2 RHEUMATOID ARTHRITIS

1.2.1 *Clinical features and classification*

Rheumatoid arthritis is a common chronic and systemic inflammatory disease. It targets the synovial joints and it is often accompanied by extra-articular manifestations. Its prevalence is estimated to be 0.5-1.0% (Symmons 2002). Two-thirds of the patients are women, and the peak of onset is about 60 years of age. There are classification criteria issued by the American College of Rheumatology (ACR) (table 1) which were published in 1987 (Arnett, Edworthy et al. 1988).

1.2.2 *Etiology and outcome*

RA is an inflammatory disease in which a genetically susceptible host interacts with environmental factors. The genetic influence has been reported in studies demonstrating that the disease concordance is greater in monozygotic twins (12-15%) than in dizygotic twins (3-4%) (Aho, Koskenvuo et al. 1986; Silman, MacGregor et al. 1993). The strongest genetic markers associated with RA is the HLA-DRB1 alleles that share a similar amino acid sequence that determine the specificity of the antigen-binding site termed the shared epitope (Gregersen, Silver et al. 1987). The HLA-DR

genes are associated with both disease occurrence, severity and outcome (Gorman, Lum et al. 2004; Turesson, Schaid et al. 2005). Non-HLA genes have also been identified as potential candidates to explain the genetic susceptibility to RA, e.g. PTPN22, CTLA4 and PADI4 (Plenge, Padyukov et al. 2005). A large number of risk factors potentially involved in the etiology of RA have been reported, such as smoking (Klareskog, Stolt et al. 2006), obesity (Escalante, Haas et al. 2005), blood transfusion and professional exposure to organic solvents (Symmons, Bankhead et al. 1997).

Table1. The 1987 American College of Rheumatology classification criteria for RA (Arnett, Edworthy et al. 1988)

Criterion	Definition
1. Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement
2. Arthritis of 3 or more joint areas	At least 3 joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP (proximal interphalangeal), MCP (metacarpophalangeal), wrist, elbow, knee, ankle and MTP (metatarsophalangeal) joints
3. Arthritis of hand joints	At least 1 area swollen in a wrist, MCP or PIP joint
4. Symmetric arthritis	Simultaneous involvement of the same joint areas on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry)
5. Rheumatoid nodules	Subcutaneous nodules over bony prominences, or extensor surfaces, or in juxta-articular regions observed by a physician
6. Serum rheumatoid factors	Demonstration of abnormal amounts of the serum rheumatoid factor by the any method for which the result has been positive in <5% of normal control subjects
7. Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify)
A patient shall be diagnosed to have rheumatoid arthritis if he/she has at least four of these seven criteria. Criteria 1-4 must have been present for at least 6 weeks. Patients with 2 clinical diagnoses are not excluded.	

1.2.3 *Pathogenesis*

The observation that HLA molecules may specifically bind antigenic peptides and present them to T cells suggests an antigen-driven immune response. This antigen-driven response induces antibody production. Antibodies associated with RA are RF and anti-CCP antibodies. RFs are antibodies specific for and binding to the constant part of other immunoglobulins. Anti-CCP antibodies are antibodies specific for altered peptides that have been citrullinated (van Venrooij, Hazes et al. 2002). These autoantibodies can predict future disease (Scofield 2004). The hypothesis that RA is an antigen-driven and thus T cell-mediated disease has inspired attempts to use T cell-depleting reagents as therapeutics (Hepburn, Totoritis et al. 2003) and the efficiency of treatments targeting the interaction of T cell and APCs by blocking costimulatory molecules (Kremer, Westhovens et al. 2003) have reemphasized the role of T cells in the pathogenesis of the disease.

Synovial cell-mediated inflammation has been described as an important mechanism in the disease. Synovial resident cells such as fibroblasts and macrophages produce cytokines perpetuating the joint inflammation. Binding and blocking of cytokines such as TNF and IL-1 dramatically inhibit the disease symptoms.

In summary, RA is multifactorial disease involving several genetic and environmental factors. The knowledge of effector mechanisms has increased considerably both with respect to the specific and the innate immune systems. However, it is still unclear what stimuli initiate RA.

1.2.4 *The immune system*

Basic concepts

The immune system is our defence system. It is designed to perform several tasks, for example: (1) to fight off intruders such as bacteria, viruses and parasites, (2) to clear transformed cells, (3) to remove dead cells and (4) to heal injured tissues.

The immune system is divided into two interacting compartments, the innate immune system and the adaptive immune system. The innate immune system is ancient, being present in all multicellular organisms. In general, innate immunity is a non-specific, inducible response to pathogens. It is immediate in action, yet short-lived. Cells that contribute to innate responses are dendritic cells, macrophages, neutrophils, mast

cells and natural killer cells. Conversely, the adaptive immune system is much more specific, but takes longer to become activated. T cells and B cells of the adaptive immune system are able to recognize specific antigens through specific receptors. They undergo clonal proliferation and maturation following activation. Both systems work together to provide protection against a diverse and rapidly evolving array of pathogens.

The ability to make immune responses is tightly regulated to ensure that when pathogens are eliminated the immune response tuned down to avoid unwanted damage. However, the immune system can occasionally attack self tissues and cause autoimmunity. Autoimmunity is caused by an adaptive immune response against "self" antigens resulting in tissues damage and organ dysfunction. In RA, the cartilage of the joint is damaged due to massive inflammation in the affected tissues (Stuart, Townes et al. 1984).

Triggering the immune system

The innate immune system is the first line of defence that comes into action as soon as invaders are detected. The cells involved in this response include macrophages, mast cells, neutrophils, NK and DC. The macrophages and neutrophils engulf and destroy the invaders. They also release cytokines that activate other cells to destroy pathogens by inducing a series of distinct processes. Cytokines are important in the activation, proliferation, differentiation, and chemotaxis of immune cells i.e. they orchestrate the whole immune response. DCs are extremely important for the induction of specific B and T cells, which constitute the second line of the immune defence. This is called adaptive immunity. The DCs, present in most tissues, are the most efficient APCs of the body. When activated, they differentiate and migrate to the regional lymph nodes, where they present antigens to T cells (Randolph 2001). During antigen presentation, T cells differentiate to achieve different effector functions. Type 1 T cells are characterized by type 1 cytokines production, such as IFNγ and IL-2. Type 2 T cells secrete IL-4, IL-5 and IL-10. Type 1 T cells primarily function as regulators of other immune cells either through secreted cytokines or by direct cell–cell contact. Type 2 T cells activate humoral immunity, i.e. antibody responses, but can also downregulate type 1 immune responses.

Once the pathogens are cleared many cells undergo apoptosis and a small portion of the lymphocytes become memory cells. Memory B cells respond more rapidly and

mount more effective responses on second encounter with the same antigen. All of these cells and products are important for the regulation of immune responses.

1.2.5 The immune responses in RA

The cellular network of the RA joint

The joint inflammation of the joints in RA is characterized by a complex cellular network comprising of both migrant and resident cells.

Macrophages are cells with phagocytic capacity that are abundant in the inflamed synovial tissues. They are highly activated, expressing HLA-DR (Klareskog, Forsum et al. 1982) as well as co-stimulatory molecules. They are important both for T cell activation through presentation of antigens and for production of high amounts of pro-inflammatory cytokines. Macrophages are important mediators of local inflammation and joint damage (Burmester, Stuhlmuller et al. 1997).

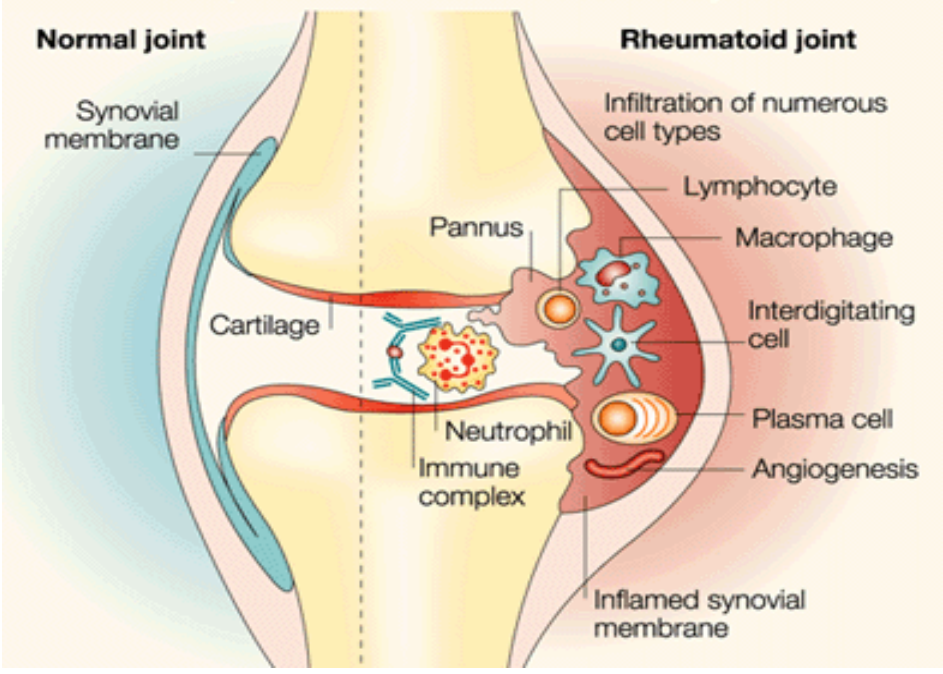


Figure1. Representation of synovial joint, normal joint (left) and RA joint (right). Adapted from Feldmann et al (Feldmann, Brennan et al. 1996)

T cells

In RA a number of observations are consistent with the hypothesis that $CD4^+$ T cells play a dominant role in the immunopathogenesis of the disease. There is a pronounced infiltration of $CD4^+$ T cells into the ST and SF of patients with RA (Van Boxel and Paget 1975). The recent findings of anti-CCP antibodies and the cytokine IL-17 in patients with RA may give new insights into the pathogenesis of RA. Anti-CCP antibodies are closely associated with the onset of RA (Schellekens, de Jong et al. 1998; van Boekel, Vossenaar et al. 2002; van Venrooij, Hazes et al. 2002). Anti-CCP antibodies can even be detected before the clinical onset of the disease (Rantapaa-Dahlqvist, de Jong et al. 2003; Nielen, van Schaardenburg et al. 2004). These reports indicate that adaptive immune responses with T cell involvement play an essential role in the initiation of the disease. Furthermore, IL-17 is produced exclusively by T cells (Rantapaa-Dahlqvist, de Jong et al. 2003) and is detected at relatively high levels in the inflamed joints. IL-17 acts as a proinflammatory cytokine in an additive way together with TNF and IL-1 (Chabaud, Fossiez et al. 1998; Katz, Nadiv et al. 2001) to enhance joint inflammation and bone and cartilage erosion (LeGrand, Fermor et al. 2001; Lubberts, van den Bersselaar et al. 2003). The pathogenic role of the T cell cytokines implies that active T cells play a role in perpetuating joint inflammation. Additionally, T cell activation is ultimately determined by positive signals from costimulatory molecules and negative signals from regulatory T cells.

B cells and autoantibodies

In RA B cells appear to have many functions such as being precursors of plasma cells producing autoantibodies (Dorner and Burmester 2003); presenting antigen to T cells and producing cytokines (Lund, Garvy et al. 2005). RF and anti-CCP correlate very well with radiological progression, providing indirect evidence that antibodies contribute to tissue destruction. Autoantibodies form immune complexes that activate B cells via Fc receptors and complement receptors. Stimulated B cells secrete TNF and IL-6 which can not only act as differentiation factors, but which can also amplify the immune responses (Dorner and Burmester 2003). The important role of B cells in RA is reflected by the good response found in patients treated with the B cell depleting antibody rituximab. Rituximab is specific for the CD20 molecule expressed on B cells. (Cohen, Emery et al. 2006). B cells may also be involved in another aspect of the inflammatory cascade by releasing cytokines that have important effects on follicular

dendritic cells and the development of germinal centers (Cyster 1999; Duddy, Alter et al. 2004)

Fibroblast-like synovial cells

These cells are non-phagocytic cells with a stellate morphology lacking HLA-DR expression. They attract, retain and stimulate lymphocytes and promote angiogenesis by secreting large amounts of pro-inflammatory cytokines (IL-6, IL-8 and IL-15) and proteolytic enzymes such as metalloproteinases capable of damaging tissues. They invade cartilage and bone, being responsible for the invasive character of the rheumatoid synovium (Davis 2003). There is evidence indicating that the high number of fibroblasts is due to both proliferation and reduced apoptosis (Pap, Muller-Ladner et al. 2000). Activated fibroblasts also contribute to the defective apoptosis of T and B cells (Salmon, Scheel-Toellner et al. 1997; Lindhout, van Eijk et al. 1999).

Neutrophils

Neutrophils are cells with high phagocytic capacity that are mainly present in the synovial fluid but only in small numbers in the synovial membrane. Neutrophils contribute to cartilage damage (Pillinger and Abramson 1995) and to local inflammation of the joint by releasing proteolytic enzymes (MMPs) and by generating oxygen radicals (Liu and Pope 2004).

The cytokine network in RA

Cytokines are a large family of small, soluble proteins which mainly serve as chemical messengers between cells. The most common mechanism through which a cytokine interacts with its receptor is through paracrine and autocrine pathways but juxtacrine and endocrine mechanisms are also involved.

Cytokines are produced by most cells. Each cell has a program of cytokines that it can synthesize. Some cell types such as macrophages, T cells and mast cells make a very wide spectrum of cytokines. Based on the cytokine production profile, a T cell is classified as being either a type 1 or type 2 T cell. It has been proposed that the T cells in the RA synovium are mainly of the type 1 phenotype with almost no type 2 cells (Simon, Seipelt et al. 1994). The local pro/anti-inflammatory and type 1/type 2

cytokine balances may affect disease progression or regression with increased inflammatory signals or downregulation of these signals.

Proinflammatory cytokines including TNF, IFNγ, IL-1, IL-17, IL-23 and HMGB1 are involved in the initiation and amplification of immune responses, working together as a network (Chabaud, Durand et al. 1999; Andersson, Wang et al. 2000; Dinarello 2000; Hunter 2005; Lubberts, Koenders et al. 2005). The proinflammatory cytokines are counter-balanced with antagonizing anti-inflammatory cytokines such as IL-4, IL-10, IL-13, TGF-β and by soluble cytokine receptors, which limit the potentially hazardous effects of sustained and/or excessive cytokine secretion (Opal and DePalo 2000; Hill and Sarvetnick 2002).

Table 2. Cytokines and their role in RA (Feldmann, Brennan et al. 1996)

Class	Cytokine	Inhibitor	RA synovial pattern	Effect in RA
Th1 cytokines	IFN-γ IL-2 IL-17	 s.IL-2R	Present in ST and SF Low amount in ST, SF Low level in ST	Pro-inflammatory Pro-inflammatory Pro-inflammatory
Th2 cytokines	IL-4 IL-13		Low level in RA No expression in RA	Anti-inflammatory Anti-inflammatory
Monokines and fibroblast-derived cytokines	IL-1 TNF IL-6 IL-8 IL-10 IL-15 IL-18 GM-CSF M-CSF	IL-1ra, Soluble IL-1R Soluble TNF-R	High amount in ST, SF Variable amount in ST and SF High level in ST and SF High level in ST and SF Present in ST and SF Present in ST Present in ST Present in ST and SF High level in ST and SF	Pro-inflammatory Promotes bone destruction See above of IL-1 Pro and anti-inflammatory Pro-inflammatory Anti-inflammatory Pro-inflammatory Pro-inflammatory Pro-inflammatory Macrophage proliferation
Growth factor	TGF-β PDGF FGF		High level in ST and SF Present in ST Present in SF	Pro and anti inflammatory Increase cell proliferation Increase proliferation and angiogenesis

Apoptosis

Cell death occurs in a variety of physiological situations in order to secure the function of the whole organism. This is called programmed or apoptotic cell death. Apoptosis is a term that was coined from a Greek word meaning ‘‘falling of’’ as leaves do in the autumn. Apoptosis is essential for the regulation of development, generation of an immune system and maintenance of homeostasis in multicellular organisms. It is a consequence of a balance between cell death and cell proliferation. A breakdown of the delicate balance has been implicated in the pathogenesis of a number of autoimmune diseases. Apoptosis can be induced via various intracellular pathways. Two major pathways are cell death receptor-mediated apoptosis (Aggarwal 2000) and mitochondrial-mediated apoptosis (Orrenius 2004). Cell death receptors include subgroups of the TNF receptor family (CD95, TRAIL-R1/2, and TNF-R1) and Fas receptors. These receptors activate intracellular adaptor molecules (caspases) that eventually lead to apoptosis. Apoptotic cells are characterized by cellular changes including 1) shrinking and condensation of the cytoplasm and nucleus, 2) aggregation of chromatin, 3) fragmentation of DNA, and 4) membrane blebbing leading to small particles called apoptotic bodies. All four mechanisms subsequently lead to cellular phagocytosis (Stadelmann and Lassmann 2000).

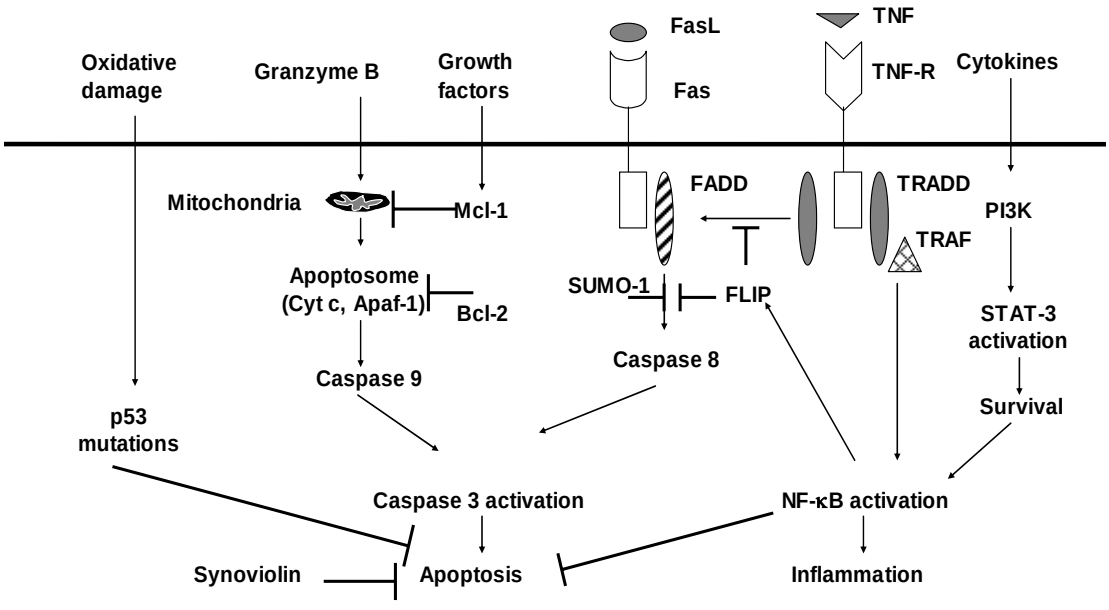


Figure2. Synovial modulation of apoptosis in rheumatoid arthritis.

(***Abbreviations: Fas ligand FasL, tumor necrosis factor TNF, TNF receptors TNF-R, Fas-associated death domain FADD, TNF receptor associated death domain TRADD, TNF receptor associated factor TRAF, cytocrome c cyt c, B-cell leukemia/lymphoma-2 Bcl-2,, myeloid cell leukemia sequence 1 Mcl-1, FLICE inhibitory protein FLIP, apoptosis protease-activating factor 1 Apaf-1, small ubiquitin-related modifier-1 SUMO-1 signal transducer and activator of transcription STAT, phosphatidylinositol-3-OH kinase PI3K, nuclear factor-κB NF-kB,***)

Figure 2 is adapted from Catrina Anca Irinel

Apoptosis has been implicated as a potential pathogenic mechanism in RA. Apoptotic cells were discovered in the synovial cells of RA patients. Several studies have examined the time course of apoptosis in the synovium using the CIA model. No apoptotic cells were detected before the onset of synovitis, but apoptotic cells increased during the early acute phase of arthritis and decreased during the later chronic stage. Low levels of apoptosis have been reported in the synovial membranes of RA (1-3% of the cells). The different apoptosis-inducing mechanisms are balanced by several pathways (fig.2) that inhibits apoptosis. For example, macrophages obtained from joints of patients with RA express high levels of FLIP (Perlman, Pagliari et al. 2001) and NF-κB (Handel, McMorrow et al. 1995) that make macrophages resistant to apoptosis. T cells that are isolated from RA joints are resistant to apoptosis and this is associated with an increased expression of Bcl-2 (Salmon, Scheel-Toellner et al. 1997; Schirmer, Vallejo et al. 1998). An even more complex anti-apoptotic network has been described in RA-derived synovial fibroblasts. These cells are characterized by STAT-3 activation and this activation is enhanced by TNF through a PI3K-mediated mechanism (Shouda, Yoshida et al. 2001; Krause, Scaletta et al. 2002). Growth factors such as TGF-β, are able to activate the PI3K pathway that induces Mcl-1 expression and blocks SF apoptosis (Kim, Jun et al. 2002).

Metalloproteinases and tissue destruction in RA

Proinflammatory cytokines such as IL-1 and TNF have been implicated in the dysregulation of bone and cartilage remodeling that is characteristic of RA. These cytokines increase the production of factors that stimulate cartilage matrix degradation such as metalloproteinases. MMPs are secreted by many cell types including chondrocytes in the synovium (Lefebvre, Peeters-Joris et al. 1990) and (Lorenzo, Pilbeam et al. 1992; Saren, Welgus et al. 1996). MMPs belong to a family of zinc-dependent endopeptidases that participate in degradation and remodulation of the extracellular matrix. The MMP family includes over 20 proteins divided into five categories: collagenases, stromelysins, gelatinases, membrane-type MMPs (MT-MMPs), and aggrecanases (Nagase and Woessner 1999). They are synthesized as pro-enzymes and their activation is induced by proteolytic cleavage of the pro-peptide domain at the N-terminal of the molecules. Stromelysin-1 (MMP-3), Collagenase (MMP-1) (Martel-Pelletier, Welsch et al. 2001), and Gelatinase A (MMP-2) (Nemec, Goldbergova et al. 2006) can degrade collagen and aggrecans. They play a central role

in degrading the extracellular matrix of the joints. Increased levels of MMP-2 are observed in the serum and synovial fluid of patients with RA. Furthermore, a polymorphism in the gene for MMP-2 may affect the development and/or severity of RA (Nemec, Goldbergova et al. 2006). MMP-1 (collagenase), which digests collagen I-IV and VII, is expressed in the superficial cartilage layers (Fernandes, Martel-Pelletier et al. 1998; Chubinskaya, Kuettner et al. 1999; Bau, Gebhard et al. 2002) (Martel-Pelletier, McCollum et al. 1994; Moldovan, Pelletier et al. 1997). MMP-1 can be detected in RA synovial fluid (Clark, Powell et al. 1993), and in serum samples (Keyszer, Lambiri et al. 1999). MMP-3, one of the three stromelysins, seems to be involved in degrading the cartilage matrix including proteoglycan, fibronectin, as well as collagen types III, IV, V and IX (Matrisian 1990). MMP-3 is increased in the synovial fluid (Ishiguro, Ito et al. 1996) and serum (Zucker, Lysik et al. 1994) of RA patients. Serum levels of MMP-3 correlates with the degree of cartilage damage (Chubinskaya, Kuettner et al. 1999).

There are naturally occurring MMP antagonists including four specific MMP inhibitors (tissue inhibitors of MMPs, TIMPs), identified as TIMPs-1–4. They form equimolar complexes with active forms of MMPs. In these complexes the N-terminal domain of TIMPs binds to the catalytic domain of MMPs, thereby blocking access to the zinc-containing binding site (Bode, Fernandez-Catalan et al. 1999). These complexes are stable and irreversible. TIMP-1 is present in RA synovium to a lesser extent than MMPs, suggesting that an imbalance exist in favor of MMPs over TIMPs (Firestein, Paine et al. 1991)

1.2.6 RA treatment

During the last 20 years there has been considerable progress in the treatment of RA. Medications that are used to treat RA are divided into three main classes: Non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying anti-rheumatic drugs (DMARDs) including traditional DMARDs and biologics.

NSAIDs

These drugs provide partial relief of pain and stiffness but they have not been shown to slow the progression of the disease (Newsome 2002). Thus for long-term care NSAIDs should be used together with DMARDs (Newsome 2002).

Corticosteroids

Corticosteroids are potent suppressors of the inflammatory response. One important effector mechanism of corticosteroids is to bind to specific cytoplasmic receptors that induce lipocortin production, resulting in changes of lymphocyte functions and downregulation of many pro-inflammatory enzymes and cytokines. Predictable but dose-dependent side-effects of corticosteroid drugs include thinning of the skin, cataract, osteoporosis, hypertension and hyperlipidemia (McDougall, Sibley et al. 1994; Saag, Koehnke et al. 1994).

DMARDs

There are two categories of DMARDs: biologic agents, such as tumor necrosis factor (TNF) antagonists, and non-biologic agents, which also are referred to as traditional DMARDs. Much of the progress to control rheumatic diseases can be attributed to more effective use of traditional DMARDs and the introduction of biologic agents.

Traditional DMARDs

DMARDs are chemically different compounds that can induce sustained suppression of inflammation. During the last 15 years extensive changes have appeared in the practical use of DMARDs in RA. Methotrexate is today drug that is most often used as the initial therapy (Mikuls and O'Dell 2000). It has demonstrated efficacy and induces long-term responses (Felson, Anderson et al. 1990; Pincus, Marcum et al. 1992). Sulfasalazine has also shown efficacy. Leflunomide, a new synthetic DMARD, has an efficacy similar to methrotrexate (Smolen, Kalden et al. 1999; Strand, Cohen et al. 1999). Recently, the concomitant administration of two or more DMARDs or in combination with a corticosteroid, has been demonstrated to be more effective than monotherpy with one DMARD alone (O'Dell 2004; Mottonen, Hannonen et al. 2006). Historically, concerns about the toxicity of DMARDs often delayed the initiation of this treatment. It is now accepted that the consequences of delaying therapy far outweigh the possible toxic effects for the majority of patients (Pincus and Callahan 1993; Felson, Anderson et al. 1995). Nevertheless, safe administration of DMARDs requires critical and careful monitoring (1996).

Biologic DMARDs

Biologics is a term that defines a new class of therapeutic agents, soluble antagonist receptors or anti-cytokine antibodies that block cytokines. Three biologic products that inhibit the action of TNF (infliximab, etanercept, and adalimumab) and one that

inhibits the action of IL-1 (anakinra) are now available for the treatment of RA. Other agents are being tested, some targeting cytokines, some targeting specific receptors of T cells or B cells (Panayi, Corrigall et al. 2001; Zhang and Bridges 2001) including anti-CTLA4 (abatacept) (Kremer, Westhovens et al. 2003) and anti-CD20 (rituximab) (Edwards, Leandro et al. 2002). This development has changed the treatment protocol as well as treatment outcomes of RA. Still one must bear in mind that one third of RA patients do not respond adequately and that adverse effects such as an increased risk for infections and autoimmune events occur not very seldom (Scheinfeld 2004).

Treating patients with RA poses significant challenges. Several issues deserve special attention: The lack of an accurate method to establish early diagnosis that permits early treatment, inadequate predictors of the different responses to available therapy and the enormous cost of new therapies. Many of our biologic therapies are expensive, with costs that may exceed 1500 USD per month. The increasing cost for effective therapy is a mounting problem. Most people suffering from chronic diseases live in poor countries and have no means to afford chronic therapy and other disease-related costs. Today there is a focus on developing treatment strategies for common infectious diseases. As the populations of the developing countries increase their life-time expectancy, the number of people suffering chronic diseases will increase. The task to provide good healthcare for these people will be no less than to find treatment strategies for malaria, tuberculosis and HIV-AIDS.

2. EXPERIMENTAL ARTHRITIS MODELS

Animal models of arthritis provide a way to control environmental factors and to manipulate the genetic influences that are involved in the pathogenesis of joint inflammation. By using inbred animal strains the polygenetic heterogeneity can be constrained and the environmental exposure can be controlled. Since RA is a multifactorial and heterogeneous disease, many different animal models available could reflect a wide range of features each contributing new insights into certain pathways leading to disease. The development of more efficient therapies for RA has been possible due to the use of animal models.

Arthritis can be induced in certain arthritis-prone animal strains by immunization with arthritogenic substances which contain either antigen emulsified in adjuvant or adjuvant components alone. Adjuvants are defined as a group of substances with the capacity to provoke endogenous signals that activate innate immunity (Gallucci, Lolkema et al. 1999). When administered together with antigen, the adjuvant serves as a depot, providing antigen to T cells and/or B cells for a prolonged time (Billiau and Matthys 2001). Adjuvant molecules trigger antigen-presenting cells by acting as danger signals (Matzinger 2002), thus inducing cytokine production and upregulating antigen-presentation capacity.

2.1 ADJUVANT-INDUCED ARTHRITIS

The first arthritis model described was ***Adjuvant-induced arthritis*** (AIA) which is induced by injection of heat-killed mycobacteria mixed with mineral oil (Pearson 1956). This model is a monophasic and self-limiting disease. It probably triggers an adaptive immune response against bacterial antigens (Joe, Griffiths et al. 1999). This model is often termed classic adjuvant arthritis. It has later been observed that arthritis can be induced by an adjuvant alone. Several adjuvants have given rise to useful models such as oil-induced arthritis (OIA), Squalene-induced arthritis (SIA) and Pristane-induced arthritis (PIA).

2.2 SPONTANEOUS ARTHRITIS

Spontaneous arthritis may develop in some species including mice and rats. Several models of spontaneously developing arthritis in genetically manipulated mice exist.

TNF transgenic mice will develop arthritis as a consequence of systemic overexpression of TNF (Butler, Malfait et al. 1997). This arthritis is both T cell and B cell-independent. Mice deficient in their IL-1 receptor antagonist (IL-1Ra$^{-/-}$) develop arthritis associated with an increased production of the pro-inflammatory cytokines IL-1β, IL-6 and TNF (Horai, Saijo et al. 2000).

2.3 COLLAGEN-INDUCED ARTHRITIS

There are several different cartilage-derived proteins that have been shown to induce arthritis in rats e.g. type II collagen (Trentham, Townes et al. 1977), type IX and type XI collagen (Cremer, Griffiths et al. 1995) and cartilage oligomeric matrix protein (Carlsen, Hansson et al. 1998).

CIA is the most common arthritis model used today. It is often employed for both pharmacological and pathological investigations. The disease was first described in rats by Trentham *et al* in 1977 (Trentham, Townes et al. 1977). In rats, both heterologous (human, bovine, porcine and chicken) and homologous collagen type II dissolved in either Freund´s complete adjuvant or incomplete adjuvants are used. CIA is inducible in mice, although disease induction requires complete Freund´s adjuvant and boosting (Courtenay, Dallman et al. 1980). However, the rat model offers several advantages compared to the mouse model in that the rat is more susceptible to the induction of the disease.

The CIA model in the rat is in many aspects similar to RA. In DA rats, one subcutaneous injection with autologous rat collagen II/FIA induces arthritis with symmetrical synovitis of peripheral joints serum titers of RF and bone and cartilage erosions. Autoantibodies to CII have been reported and both T cells and B cells are involved in the pathogenesis. Disease susceptibility is mediated by both MHC genes and non-MHC genes (Holmdahl, Vingsbo et al. 1992; Lorentzen and Klareskog 1996). The DA-congenic strain DA.1H has the same genetic setup as the DA rat except for the MHC genes and is less susceptible to CIA. Another example of genetic manipulation affecting the severity of arthritis is the LEW.1av1 congenic rats. The LEW.1av1 rats have the genetic background of the Lewis rat but have same MHC alleles (RT.1AV1) as the parental DA strain. These rats are more susceptible to CIA than are LEW rats (Griffiths, Cannon et al. 1993).

In the CIA model, the anti-CII antibodies produced are arthritogenic (Stuart, Cremer et al. 1982; Stuart, Tomoda et al. 1983). Thus the disease involves activation of both T and B cells that are antigen-specific and autoreactive. T cells and T cell-derived cytokines promote differentiation and activation of macrophages, osteoclasts and fibroblasts, leading to an aggressive erosive inflammatory process.

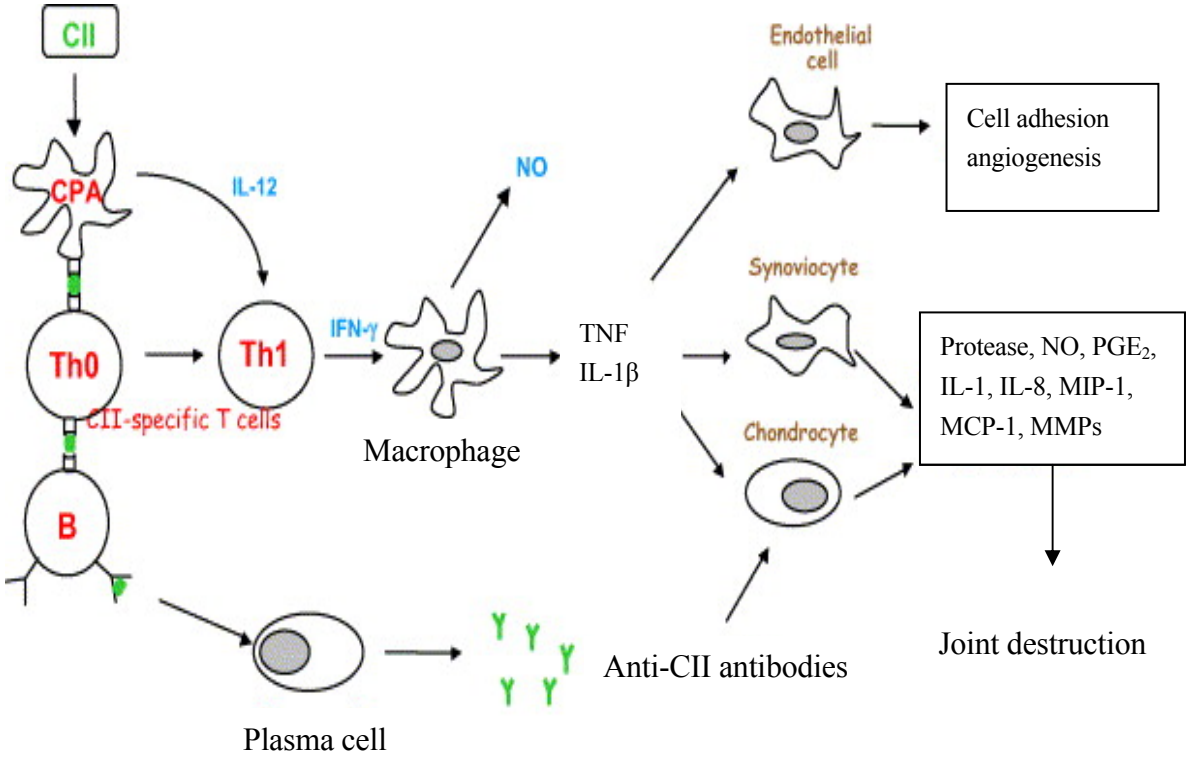


Figure 3. The pathophysiology of collagen-induced arthritis. Adapted from Catherine Fournier (Fournier 2005)

3. TRADITIONAL MEDICINE, AN INSPIRATION TO INVESTIGATE THE IMMUNOSUPPRESSIVE AND ANTI-ARTHRITIC POTENTIALS OF DIFFERENT COMPOUNDS

3. 1 MEDICINAL PLANTS AND FLAVONOIDS

3.1.1 *Traditional medicine*

Traditional Medicine (TM) is the practice of protecting and restoring health. TM existed long before the relatively recent arrival of modern medicine. TM often serves as one component of a comprehensive system of medicine that may involve the use of plant, animal and mineral based medicines, spiritual therapies, regulation of diet and exercise, and manual techniques such as acupuncture or massage to maintain health but also to prevent and treat illness.

A traditional herbal medicinal product is any medicinal product containing one or more herbal substances as active ingredients (whole, fragmented or cut plants, algae, fungi, or lichen in an unprocessed, usually dried form but sometimes fresh) or one or more herbal preparations (preparations obtained by subjecting herbal substances to processing such as extraction, distillation, fractionation, purification and concentration fermentation). The regulation of herbal medicine is characterized by large differences depending on the ethnological, medical and historical background of each country. The World Health Organization (WHO) guidelines for the assessment of herbal remedies, adopted by the international conference of Drug Regulatory authorities (Ottawa, October 1991), contain the basic elements of legislation and registration procedures for herbal medicine.

According to the WHO, up to 80% of people living in developing countries still primarily rely on Traditional Medicine for their healthcare (WHO, 2002). The uses of TM in industrialized countries are also spreading rapidly. In these countries, drugs used in TM are often referred to as alternative medicines or complementary medicines or even as dietary supplements or natural health products (Cant 2000; Kelner 2000).

Systems of TM that are widely used in national healthcare systems around the world including: Traditional Chinese medicine, Traditional Western herbal medicine,

Traditional Japanese medicine, Traditional Korean medicine and TM from other contries. In these countries an increasing number of academies, departments, associations, hospitals and institutes of TM have been established to advance research and the development of medical practice based on national cultural heritages. About ten years ago Karolinska Institutet organized its first course in TM.

3.1.2 Vietnamese traditional medicine

In Vietnam, TM can be divided into two categories: The medicine of the South and the medicine of the Northern. The medicine of the South is based on indigenous Vietnamese traditions, largely on folk herbal knowledge. The Northern medicine is influenced by Chinese TM. The development of Southern TM into a national system was effected by a Scholar and Buddhist monk, Tuetinh in the 14th century (Dung and Bodeker 2001). Tuetinh´s practical orientation towards establishing public health strategies for disease prevention and health maintenance extended beyond popularizing common remedies. He promoted the development of TM services in Buddhist monasteries throughout Vietnam. Tuetinh´s work records were collected in eleven volumes describing more than 630 remedies originally from plants of Vietnam. The volumes also contain clinical guidelines on the use of 3873 different methods of treating 184 different diseases (Do Tat Loi and Dung 1991; Dung and Bodeker 2001). In accordance with the tradition of Tuetinh, president Ho Chi Minh said in an important speech in 1955: "We must build our own medicine…Our ancestor had rich experience in the treatment of diseases using local medications and those of the north (China). To enlarge the sphere of action of medicine, it is necessary to study means of uniting the effects of oriental remedies with those of Europe" (Hoàng 1999). This initiated the establishment of a network of institutions whose mandate was to modernize, standardize and repopularize TM.

Herbal medicine has been a corner-stone of Vietnam´s national program to modernize, standardize, and re-popularize medicine. There are about 40 national and provincial TM hospitals with over 50 departments of TM. All seven of Vietnam´s medical colleges have a department of TM. The pharmacist Do Tat Loi has written a six volume series of books about the medicinal plants of Vietnam and their biochemical properties (Do Tat Loi 2001). The result of his countless journeys and conversations with traditional practitioners over a 20 year period started in 1954. The volumes have become classic reference literature, complete with botanical classifications of plants

and detailed descriptions of the plants in medicinal use (Do Tat Loi and Dung 1991). The Institute of Material Medica has also been instrumental in this task, collecting over 8000 samples of which 1850 species have been catalogued according to their vernacular names, scientific names and pharmacological properties (Nguyen 1999). Some medicinal plants in Vietnam were found to have efficacy to treat common diseases such as fever, allergy, hepatitis (Chuyen 1984), rheumatism, malaria, dysentery as well as hypertension and tumors (Do Tat Loi 1988; Do Tat Loi 2001), which are all more pervasive in developing countries. In table 3 some Vietnamese plants are listed according to the species family and purpose of treatment.

Table 3: List of some plant species and their medical values

Plant species	*Family*	*Drug/extract*	*Used in treatment of*
Achyranthus aspera[1]	*Amaranthaceae*		Hypertension
Gynostemma pentaphyllum[2]	*Cucurbitaceae*		Increased Insulin diabetes
Caesalpinia sappan[1]	*Caesalpinaceae*	"tomoc", "panma"	Epidemic dysentery
Madhuca pasquier[1]	*Sapotaceae*	Madhuxin	Burns and surgical wounds
Mangifera indica[1]	*Anacardiaceae*	-	Herpes infection
Nelium oleander[1]	*Apovynaeceae*	-	Cardiovascular disease
Orthosiphon staminacus[1]	*Laminaceae*	Othorsiphe	Oedema heart failure
Vinca rosea[1]	*Apoynaceae*	-	Tumors, leukeamia, heart failure
Artocarpus tonkinensis[3]	*Moraceae*		Rheumatic diseases, backache

Sources: 1.(Chuyen 1984); 2.(Do Tat Loi 1988);3 (Do Tat Loi 2001)

In 1996 the Ministry of Health, after consultation with the WHO and other national health authorities in the region, approved Decision 317/BYT-QD, introducing new requirements for the safety and efficacy of herbal medicines (Ministry of Health, Vietnam. 1996). These regulations require that any new industrial herbal product made for medical purposes and submitted for marketing authorization must undergo a series of tests to see whether the product meets quality, safety and efficacy standards. Product samples must be sent to the National Institute of Drug Quality Control for testing. However, the guidelines from WHO have as yet not been fully implemented in Vietnam.

The regulation of the practice of TM in Vietnam is as follows. Firstly, by making both modern and TM compulsory components of medical education and practice in

Vietnam, and secondly, by organising the apprentice-trained "herb doctors" into a national association of doctors trained at the universities in TM as well as the development of a licensing system for these practitioners. Students attending medical college in Vietnam are required to follow 16 compulsory courses altogether 8 weeks in TM covering classical theory, diagnostics, medical botany and acupuncture.

Medical professionals and consumers have often not recognized the potential toxicity of herbal products (Perharic, Shaw et al. 1993). National authorities are responsible for the control of the safety of the products used in the health-care systems. Nonetheless, since few if any pre-clinical and clinical tests have been performed there is a specific need to investigate and characterize the safety of the TM drugs. Professor Tran Khac Bao (1995), Secretary General of the Programme for preservation of genetic resource of medicinal plants, has stated that Vietnam is in need of vast and well organized scientific investments and qualified training of the personal. Thus, assurance of safety, quality control and efficacy of medicinal plant and herb products has become a priority. New processing methodologies with modern facilities and technology are needed.

The Vietnamese people have been using TM for several thousands of years. TM was widely use in Vietnam´s second war of independence against America (1965-1975) to treat burns, wounds and tropical diseases, especially since modern medical supplies were in critical shortage. Recently, scientific studies of Vietnamese herbal medicine have been performed by different international collaborative programs, for example together with Korea, Japan, France and Sweden. These programs have demonstrated efficacy of some Vietnamese traditional medicines. For example, extracts prepared from 77 Vietnamese medicinal plants had anti-proliferative activities due to induction of apoptosis that were demonstrated *in vitro* in several tumor cell lines (Ueda, Tezuka et al. 2002). Another investigation demonstrated that 24 extracts prepared from 14 medicinal plants used in Vietnamese TM to treat malaria had anti-plasmodial activity (Tran, Tezuka et al. 2003). Seven of 58 plant extracts showed strong-to-moderate inhibitory activity on the tube-like formation induced by human umbilical venous endothelial cells in an *in vitro* angiogenesis assay (Nam, Kim et al. 2003; You, Nam et al. 2003). The extract of *Ephedra sinica* can inhibit tube formation induced by human umbilical venous endothelial cells and the invasion of B16F10 melanoma cell line (Nam, Lee et al. 2003). In addition, 188/288 extracts of 96 Vietnamese medicinal plants have been demonstrated to have xanthine oxidase (XO) inhibitory activity (Nguyen,

Awale et al. 2004). In a collaboration between Sweden and Vietnam, a group of scientists have discovered a novel insulin-releasing agent, named Phanoside, that was isolated from one anti-diabetic herbal medicine (*Gynostemma pentaphyllum*) (Norberg, Hoa et al. 2004).

During this long period, the experiences and results of treatment have accumulated and resulted in a large pharmacopoeia based on actual human experiences.

3.1.3 *Future development*

Approximately 25% of modern medicines are made or developed from plants first used traditionally. Over one-third of the population in developing countries lacks access to modern medicines. The provision of safe and effective TM therapies could become a critical tool to increase access to healthcare. At least 70 countries have a national regulation of herbal medicine but the legislative control of medicinal plants has not evolved as a structured model. This is because medicinal products or herbs are defined differently in different countries and diverse approaches have been adopted with regard to licensing, dispensing, manufacturing and trading (Organization 2004).

National drug development strategies for herbal medicine should have safety as a basic premise. Even though a lot of pharmacological effects have been demonstrated in TM, the quality and experimental protocols have not been according to international standards. The central drug policy needs to review and promote clinical evaluation of existing practices.

The research of natural medicines includes novel natural products as well as newly synthesized products. The research task covers the identification of the resources of medicinal plants to clarify their action mechanisms. At the same time, we try to clarify the active constituent(s), their chemical structure, how to isolate the compounds, and the mechanisms of action of those compounds.

In fact, until medicinal herbs are standardized and properly controlled as to their extraction and manufacture, it is unlikely that the pharmacology of medicinal herbs will yield meaningful data. While Western medicine has advanced rapidly over the last 100 years, the implications of technology, and its cost, have led to a “grassroots” movement to restore some of traditional concepts of preventive care to the comprehensive public health and fitness program. Herbal medicine in one form or another is being promoted

as an alternative road leading to well being physical as weel as psycological. However, it would be more realistic to suggest that if there was ever a field of research suited for a meaningful meeting of East and West, it is the field of herbal medicine. The intiutive approach of a 5000-year-old science can surely contribute to a better understanding of the human condition, and help regain the human element of modern medicine. Our researches are central in metamorphosing medicinal herbs into viable products in the West.

3.1.4 *Artocarpus tonkinensis*

*Artocarpus tonkinensis A Cheval (*Vietnamese name*: Chay)* is a conventional Vietnamese herbal medicine. Extracts of different parts of the *Artocarpus* tree are used in folk medicine to treat back pain and rheumatic disorders (Do Tat Loi 2001). Roots of *Artocarpus* have been used both as food and medicine for hundreds of years, a tradition which has been recorded in the text books of traditional Vietnamese medicine (Do Tat Loi 2001). This tradition has stimulated a scientific interest to study possible mechanisms by which this plant may affect common inflammatory diseases.

Picture 1: the *Artocarpus tonkinensis* tree; its leaves, fruits and root (red)

Artocarpus in the perspective of traditional culture

"Trau cau" has played an important role in socio-culture and in ceremonies in Vietnam. In ancient times the Vietnamese habit to chew "trau cau" could be compared with the western habit of drinking coffee or smoking cigarettes. "Trau cau" is a combination of betel leaves (trau), areca nuts (cau) and slices of bark of the root of the *Artocarpus* tree (Chay) and lime paste. When trau cau is chewed it produces a mild stimulatory effect with a pleasant bittersweet taste. In Vietnamese there is a

saying that “the betel starts the conversation”, referring to the practice of people chewing betel on formal occasion or “to break the ice” in awkward situations. The betel is used ceremonially during traditional Vietnamese weddings based on a folk tale about the origins of these plants. The groom traditionally offers the bride´s parents betel as a betrothal gift. The betel is also used ceremonially at traditional festivals in Vietnam. It is used as a sacrifice to the deities and ancestors. The trau cau has become the symbol of Vietnamese love, marriage and literature.

Artocarpus in the perspective of traditional medicine and insight into science

Betel is used to treat headaches, arthritis and joint pains. Among the betel ingredients, *Artocarpus* has been used in Vietnamese traditional medicines. The aqueous extract and the decoction from the leaves and root of this plant are used throughout Vietnam for the treatment of backache and rheumatic disorders (Do Tat Loi 2001).

The leaves and bark of *Artocarpus* are known to contain several potentially bioactive molecules such as lectins (N.Q.Khang 1988), oxyresveratrol, catechin and triterpennoids (Lien TP 1998), and the flavonoids such as keampherol (Hien TL 2002), and ampelopsin (Dzung ND. 2002). In animal experiments of heterogenous skin grafts between Balb/C and Swiss mice, the *Artocarpus* extract inhibited skin graft rejection. The inhibitory effect was similar to the effect induced by cyclophosphamide (Ha Nguyen Thi Vinh 2004).

3.1.5 *Flavonoids*

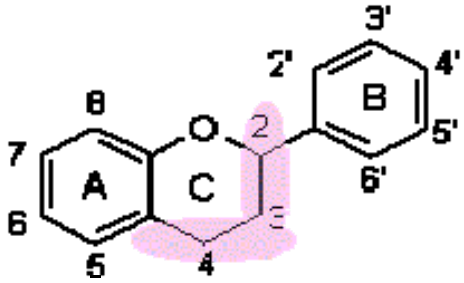
Flavonoids belong to a group of substances with variable phenolic structures that are present in plants, fruits, vegetables, grains, flowers, teas and wines (Middleton 1998). Flavonoids are the major functional components of many herbs used for medical purposes. They play an important role in reproduction and disease protection of plants (Robak and Gryglewski 1996). These natural products are known for their potential health benefits that at least in part appear to mirror their flavonoid composition. In recent years, various plant components have been isolated, chemically characterized, and in many cases the mechanisms of their biological functions have been established. Furthermore, epidemiological studies suggest a protective role of dietary flavonoids against coronary heart disease (Knekt, Kumpulainen et al. 2002). In another epidemiological study demonstrated a high flavonoid intake and the long-term effects

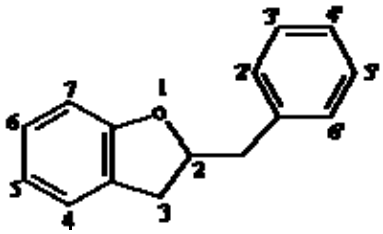
may also be correlated with a decreased risk of breast, stomach and lung several kinds of cancers (Le Marchand 2002).

The chemistry of Flavonoids

Flavonoids are polyphenolic compounds possessing 15 carbon atoms; two benzene rings joined by a linear three carbon chain. Flavonoids are a group of structurally related compounds with benzo-γ-pyrone (ring A and C in Figure 3), widely distributed in the plant kingdom. O-or C-glycosides are often present in plant flavonoids. The O-glycosides have sugar molecules bound to a hydroxyl group of aglycone, usually located at position 3 or 7, where the C-glycoside is bound to C-6 or C-8. Flavonoid – diglycosides are also frequently found in plants. Flavonoids are referred to as glycosides when they contain one or more sugar groups and as aglycone when no sugar group is present.

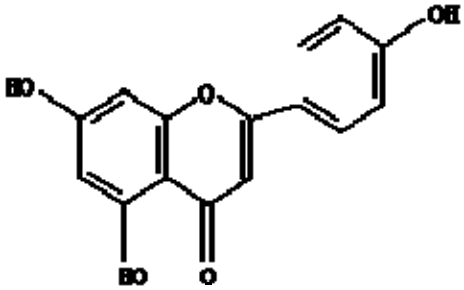
In nature 6500 derivatives have been found and classified into 14 groups (Harborne and Williams 2000). Six of the groups are particularly well known and characterized: flavones, iso-flavones, flavanones, flavanols (catechines), flavanonols and anthocyanidines (figure 3). In a few cases, the six-membered heterocyclic ring C occurs in an isomeric open form or is replaced by a five - membered ring, as in Aurones (2-benzyl-coumarone) (Figure 3).

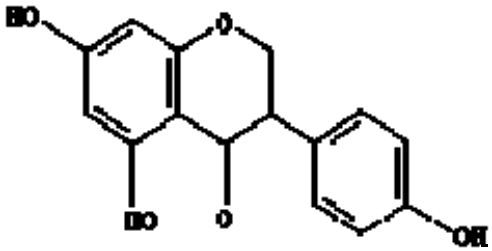




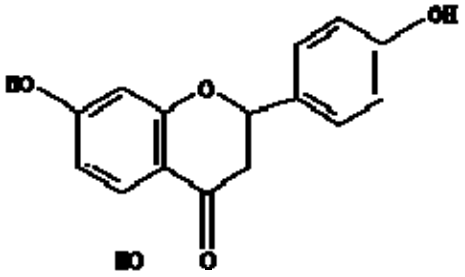
Basic structure of flavonoids

Aurone (2-benzyl-coumarone)

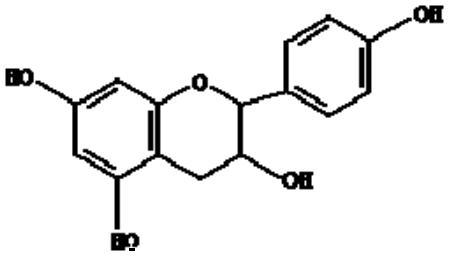




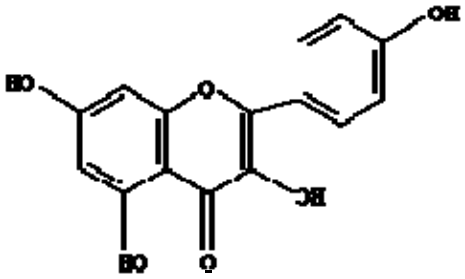
Flavone



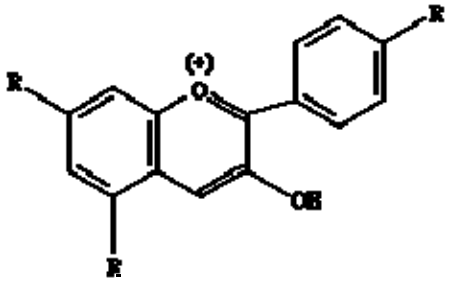
Isoflavonoid



Flavanone



Flavanol (Catechin)



Flavonol

Anthocyanidin

Figure 3. The basic structure of the six major flavonoid subgroups and aurones; *Adapted from Balch, J. F., & Balch, P. A. (2000). Prescription for Nutritional Healing. New York: Avery, Penguin Putnam Inc.*

The metabolism of flavonoids

Metabolism. Flavonoids are extensively altered during first passage metabolism. This means that the molecules that reach the peripheral circulation and tissues are different from those present in foods (Day, Mellon et al. 2001). Flavonols occur in foods mainly as glycoside conjugates. Flavonol conjugates that have been identified in plasma and urine from persons fed quercetin-containing foods are not present in food. The metabolism of most other dietary flavonoids is comparable in several ways: (1) Glycosides are generally not found in plasma or urine in the ingested form. (2) The major forms in plasma and urine are sulfate and glucuronidate conjugates of aglycones. (3) Methylation occurs in flavonoids that contain orthohydroxy functional groups. (4) Aglycones are absent or constitute only a small proportion of the total amount of flavonoids present in food. The term metabolism is used here to describe the typical

modifications that occur during or after absorption. In general, the resulting metabolites are conjugates (sulfates, glucuronidates) of parent aglycone or conjugates of methylated parent aglycones. Flavonoid glucosides are deglycosylated by β-glucosidases in the small intestine by the broad-specificity cytosolic β-glucosidases (Day, DuPont et al. 1998; Ioku, Pongpiriyadacha et al. 1998). In 1995 based on indirect evidence Hollman *et al* proposed that flavonoid glycosides actually could be absorbed intact in the small intestine using the sodium dependent glucose transporter1 (Hollman, de Vries et al. 1995). The total absorption of quercetin and quercetin.3-Glucoside, for example, across the jejunum of the isolated rat intestine are reasonably comparabale and indicate that in vivi more extensive metabolism taks place in the tissues, circulation and the liver after absorption across the intestinal wall (Choudhury, Srai et al. 1999)

Effects of flavonoids

Toxicity

There is much controversy regarding the toxic effects of flavonoids. The data concerning toxic side-effects are mainly derived from *in vitro* studies. By intravenous injection of large amounts of the flavonoid Daflon to rats, it has been possible to determine the LD_{50} value to 3g/kg of body weight (Casley-Smith 1986; Meyer 1994). An observation supporting the low toxicity of flavonoids is that many ingredients of our diet contain flavonoids. Our diet daily diets based on foods contain flavonoids such as vegetables and fruits that give us many of the essential nutrients that are necessary to preserve our heath.

In vivo anti-inflammatory activities of flavonoids

Plants and their extracts containing flavonoids have been used for treatment of different diseases as they are major constituents of Chinese and Vietnamese TM. Some extracts improve the symptoms of acute as well as chronic inflammatory disorders including RA (Do Tat Loi 2001).

Evidence collected from an epidemiological study of more than 30 thousand females 55-69 years old, conducted from 1986 to 1997, concluded that there was an inverse association between tea consumption and the onset of RA (Mikuls, Cerhan et al. 2002). Using data from The Finnish mobile clinic health examination survey, Knekt *et al*. examined the relationship between flavonoid intake and a different of chronic diseases.

A diet rich in keampferol but also other flavonoids appeared to protect against RA (Knekt, Kumpulainen et al. 2002). Many investigations have demonstrated that a variety of flavonoid molecules possess anti-inflammatory activity in various animal models of inflammation (Dai, Wei et al. 2003; Zheng and Wei 2005; Kawaguchi, Maruyama et al. 2006). This was exemplified by experiments performed in experimental arthritis such as CIA in rats (Kubo, Matsuda et al. 1984; Chen and Wei 2003; Shin, Jin et al. 2003; Verdrengh, Jonsson et al. 2003; Chang, Sung et al. 2005; Kim, Lee et al. 2005; Lee, Hyun et al. 2005; Kawaguchi, Maruyama et al. 2006).

Antioxidant activities

Flavonoids are extensively metabolized *in vivo*, resulting in a significant alteration in their redox potentials. They reduce the oxidation of low density lipoproteins (LDL) both *in vitro* (Kerry and Abbey 1997; Park, Park et al. 2006) and in human (Nigdikar, Williams et al. 1998), this most likely due to the property of these compounds to scavenge oxygen free radicals (Dugas, Morel et al. 2000). Flavonoids are chelators. For example, quercetin chelates intracellular iron (Ferrali, Signorini et al. 1997), and the chelating of iron reduces the formation of ROS. Quercetin is also able to inhibit the activity of transcription factors found in the tissue of inflammatory lesions (Rangan, Wang et al. 1999). Furthermore, baicalin, baicalien and other flavonoids affect inflammation through the inhibition of COX-2 gene expression (Woo, Lim et al. 2006).

Regulation of cellular activities

Flavonoids can regulate cellular activities of inflammatory cells such as mast cells, macrophages, lymphocytes, neutrophils, and synovial cells. These properties of flavonoids have recently been summarized by Middleton (Middleton, Kandaswami et al. 2000). Certain flavonoids modulate the enzyme activities of arachidonic acid (AA) metabolizing enzymes such as phospholipase A2 (Gil, Sanz et al. 1994; Kwak, Moon et al. 2003), cyclooxygenase (Jang, Cuendet et al. 2002; Likhitwitayawuid, Sawasdee et al. 2002; Bas, Recio et al. 2006) and the nitric oxide (NO) producing enzyme, nitric oxide synthetases (NOS) (Benito, Lopez et al. 2002). Inhibition of these enzymes by flavonoids reduces the production of AA, prostagladins, leukotrienes and NO.

Several cytokines are associated with inflammatory diseases. In particular, TNF and IL-1β are prominent contributors to chronic inflammatory disorders including RA

(Bingham 2002). Some flavonoids such as genitein, silbin and fisetin were reported to inhibit IL-1β, IL-6, TNF and INFγ production by LPS-activated human blood monocytes, the Raw 264.7 cell line and rat peritoneal macrophages (Geng, Zhang et al. 1993; Jae Youl Cho 2000; Krakauer, Li et al. 2001; Higa, Hirano et al. 2003; Verbeek, Plomp et al. 2004).

Anti-proliferation and induction of apoptosis

Although most flavonoids appear to be non-toxic to human and animals, they have been demonstrated to inhibit proliferation in many kind of cells. *In vitro* studies have concentrated on the direct and indirect actions of flavonoids on inflammatory cells and a variety of effects such as cell growth inhibition, apoptosis induction and suppression of the secretion of MMPs have been reported. One of the characteristics of RA is synovial tissue proliferation and excessive mononuclear cells infiltration that partially may be due to impaired apoptotis (Nishioka, Hasunuma et al. 1998). Flavonoids inhibit mitogen-induced lymphocyte proliferation *in vitro* (Lee, Choi et al. 1995). Quercetin and apigenin induce a reversible inhibition of activated lymphocyte proliferation. Genistein inhibits induced apoptosis of intestinal epithelial cells *in vitro* (Booth, Hargreaves et al. 1999). The growth of a human mammary tumor cell line is blocked by galangin, a naturally occurring flavonoid (Murray, Yang et al. 2006). The flavonoid quercetin is believed to play an important role in preventing bone loss by affecting osteoclastogenesis and regulating many systemic factors. For example it accelerates the TNF-induced apoptosis of osteoblastic cells (Son, Kook et al. 2006). Several mechanisms may be involved, including inhibition of DNA topoisomeraseI/II activities (Markovits, Linassier et al. 1989; Wang, Lin-Shiau et al. 1999; Sukardiman, Darwanto et al. 2000), release of cytochrome c with a subsequent activation of caspase-9 and caspase-3 (Wang, Lin-Shiau et al. 1999), downregulation of Bcl-2 and BclX(L) expression by promotion of Bax and Bak expression, nuclear transcription factor kappaB (NF-κB) and activation of endonuclease and suppression of Mcl-1 protein (Konig, Schwartz et al. 1997; Iwashita, Kobori et al. 2000; Wenzel, Kuntz et al. 2000; Lee, Shen et al. 2002)

3.2 GLUCORONOXYLOMANNAN (GXM)

3.2.1 *The GXM structure*

Cryptococcus neoformans (C. neoformans) is an encapsulated yeast that is pathogenic for humans. The capsule is a major virulence factor mainly composed of Glucuronoxylomannan (GXM). GXM represents 88% of capsular material and high levels of GXM have been detected in the serum and body fluids of patients with *cryptococcosis* (Diamond and Bennett 1974).

GXM is recognized by several effector cells such as neutrophils, monocytes, macrophages and dendritic cells, influencing the immune responses against *C. neoformans.* The structure of GXM is a (1→3)-linked, linear α-D manopyranan with β-D-xylopyranosyl (Xylp), β-D-glucupyranosuluronic acid (GlcpA), and a 6-O-acetyl constituent (Casadevall 1998).

The O-acetyl constituent is the major epitope that recognized by GXM by polyclonal antibodies (Cherniak, Reiss et al. 1980; Kozel and Gotschlich 1982) and monoclonal antibodies (Belay and Cherniak 1995).

3.2.2 *Immunosuppressive effects of GXM*

Effects ascribed to GXM include inhibition of cellular responses (Murphy and Cozad 1972). Examples of this are: 1) suppression of T cell proliferation (Retini, Vecchiarelli et al. 1998; Syme, Bruno et al. 1999) and induction of T cell apoptosis (Monari, Pericolini et al. 2005); 2) inhibition of dendritic cell activation and maturation (Vecchiarelli, Pietrella et al. 2003); 3) reduction of neutrophils and macrophages cytotoxic and chemotactic activities (Vecchiarelli 2000; Monari, Retini et al. 2003); and 4) inhibition of proinflammatory cytokine release from human monocytes (Vecchiarelli, Retini et al. 1996).

Monocytes and macrophages play a critical role in host defence to *C. neoformans.* Their involvement in the immune response is complex and characterized by ingestion of the capsular material of GXM. They act as effector cells to ingest and kill the yeast, and as regulatory cells to orchestrate the positive inflammatory cell response through accessory and secretory activities. The interaction of GXM with monocytes/macrophages regulates their cytotoxic activities due to a decrease of

superoxide anion production (Monari, Retini et al. 2003) and a reduction of pro-inflammatory cytokines such as TNF, IL-1β and IL-12 (Vecchiarelli, Retini et al. 1995);(Retini, Kozel et al. 2001).

GXM is ingested and accumulated in macrophages. The receptors for binding and uptake of GXM include CD14, TLR4 and CD18 (Shoham, Huang et al. 2001; Monari, Bistoni et al. 2005). The FcγRII also has an important role in the binding and uptake of GXM (Syme, Spurrell et al. 2002). Since FcγRII has immunosuppessive effects, binding of antibodies specific for GXM to FcγRII may explain the immunosuppressive effect of GXM (Nakamura, Malykhin et al. 2002). The inhibitory effect of GXM on T cell proliferation may be due to upregulated apoptosis through the FasL-Fas receptor pathway (Monari, Pericolini et al. 2005). Induction of the FasL is in part regulated by GXM binding to TLR4 (Hsu, Park et al. 2004). Overall, the immunosuppressive effects of GXM suggest the possibility of a clinical use of this polysaccharide for the control of the inflammatory diseases in human.

3.3 Quinoxaline-analogue (ROB 803)

Rob 803 is the coded name of 9-chloro-2,3 dimethyl-6-(N,N-methylamino-2-oxoethyl)-6H-indolo[2,3-b]quinoxaline. It is synthesized by chlorination at position 9 (X) of the ellipticine compound (fig 4).

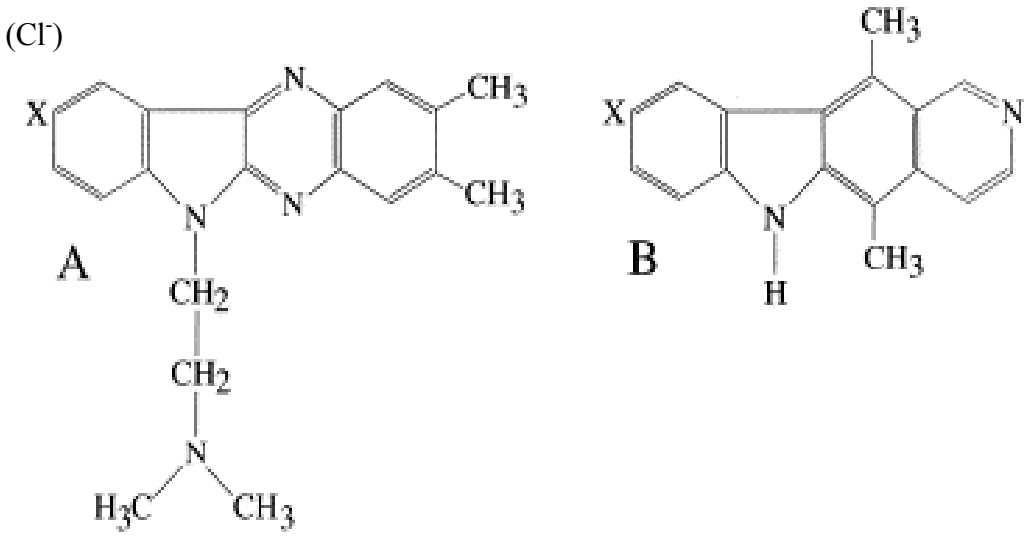


Fig.4. The structure of 2,3-dimethyl-6(2-dimethylaminoethyl)-6H-indolo-[2,3-b] quinoxaline (B220) (A); and ellipticine (B). X corresponds to position 9.

Ellipticine (fig.4B) is an alkaloid and many of its alkaloid indole derivatives have attracted attention since the 1960s when their activities against cancer cell lines were discovered. The compound 2,3-dimethyl-6 (2-dimethylaminoethyl)-6H-indolo-(2,3-b) quinixalin is one such derivative that has been given the designation B220 (fig.4A).

It is known that ellipticine binds to double-stranded DNA with high affinity by intercalation (Kohn, Waring et al. 1975; Feigon, Denny et al. 1984). Tthis causes structural damage to DNA inducing DNA degradation (Paoletti, Lesca et al. 1979; Filipski and Kohn 1982). For example, 6H-indolo-(2,3-b)quinoxaline and several other ellipticine derivatives show highly selective cytotoxicity *in vitro* against different human cancer cells (Paoletti, Le Pecq et al. 1980; Kaczmarek, Peczynska-Czoch et al. 1998; Kaczmarek, Luniewski et al. 2002). Several mechanisms have been suggested to explain the tumoricidal effect such as inhibition of cytochrome p-450 (Lesca, Lecointe et al. 1978), inhibition of p53 phosphorylation (Ohashi, Sugikawa et al. 1995), intercalation of DNA and/or interaction with the topo-isomerases II-DNA complex (Kaczmarek, Peczynska-Czoch et al. 1998; Kaczmarek, Luniewski et al. 2002).

Ellipticine has also been investigated for its antiviral capacity (Arimondo, Baldeyrou et al. 2001). In a series of synthesized derivates, it was observed the compound B220 had a considerable antiviral capacity. It down-regulated viral replication of herpes simplex virus (HSV-1) and cytomegalovirus (CMV) (Harmenberg, Wahren et al. 1988). It is likely that B220 intercalates the DNA helix and disrupts viral uncoating and replication (Harmenberg, Akesson-Johansson et al. 1991; Sehlstedt, Aich et al. 1998). Furthermore, B220 reduces oxidative stress through direct effects on human neutrophils generating reactive oxygen species (ROS), inhibiting neutrophil release of oxygen species as well as intracellular generation of ROS (Harbecke, Dahlgren et al. 1999). This effect of B220 is achieved through inhibition of the NADPH oxidase activity (Harbecke, Dahlgren et al. 1999). The inhibition of oxidative stress may have therapeutic implications, since oxidative damage is an important factor of inflammation and tissue repair as in chronic bronchitis, adult respiratory distress syndrome (Gutteridge, Quinlan et al. 1994) as well as RA (Gutteridge, Quinlan et al. 1994) and other inflammatory conditions.

4. AIMS OF THE THESIS

The aim of this thesis was to elucidate the efficacy of novel immunosuppressive agents. More specifically, we have used an experimental model of joint inflammation (CIA) to define how molecules purified from the *Artocarpus* plant, the *Cryptococus neformans* yeast, and a synthesized quinoxaline molecule (Rob803) may modify inflammation and tissue destruction.

Specific aims were:

- To investigate the immunosuppressive effect of the total extract of *Artocarpus tonkinensis* in CIA. To further characterize this effect, four purified molecules with flavonoid structures derived from the Artocarpus extract were identified and their molecular structure and biological activity were defined.

- To continue our study of immunosuppressive effects, we investigated the therapeutic efficacy of Glucuroxylomanan as well as the suppressive effects of Rob-803 in CIA

5. METHODOLOGICAL CONSIDERATIONS

5.1 CHOICE OF EXPERIMENTAL ARTHRITIS MODEL

The DA rat

DA rats are susceptible to a number of autoimmune diseases such as experimental autoimmune encephalomyelitis (Lorentzen, Andersson et al. 1997), experimental allergic neuritis (Dahlman, Wallstrom et al. 2001) and experimental autoimmune thyroiditis, as well as experimental arthritis. Among inbred rat strains the DA rat is conspicuously arthritis-prone. Disease susceptibility is mediated by both MHC and non-MHC genes (Lorentzen and Klareskog 1996). Several mouse strains are also susceptible to arthritis, such as the DBA/1 strain, but mice need to be booster-immunized.

Collagen type II induced arthritis (CIA)

Collagen-induced arthritis is used to understand elements of the arthritic process in humans. Immunization of DA rats by intradermal injection in the base of the tail with CII emulsified in FIA induces polyarthritis. Susceptibility to CIA is associated with the expression of specific MHC class II molecules. This is analogous to the situation in RA where an association is demonstrated to HLA-DR1. This feature, together with clinical, radiological, and histological joint abnormalities, makes CIA an excellent model for the investigation of the immune responses involved in RA (Boissier, Feng et al. 1987). The clinical manifestation starts 12-15 days after the CII/FIA injection. Subsequently, the rats develop a chronic arthritis with an incidence of 100%. The mechanisms underlying the joint disease include cell-mediated and humoral immune responses against CII. CII-specific T cells are found in the circulation, lymph nodes, and joint tissues of CIA affected animals (Morgan, Clague et al. 1981; Holmdahl, Klareskog et al. 1985). CII-specific T cells both produce and regulate the production of pro-inflammatory cytokines. T cells also modulate and induce B cells to produce anti-CII antibodies. When CII-specific T cells from CIA susceptible strains are stimulated with antigen *in vitro* a large amount of type 1 cytokines (INF-γ and IL-2) are produced and elevated numbers of type 1 T cells can be identified in the lymphatic tissue (Osman, Hannibal et al. 1999). Inhibition of type 1 cytokines ameliorates CIA (Nakajima, Takamori et al. 1990; McIntyre, Shuster et al. 1996; Nakajima, Seroogy et al. 2001).

The autoantibody response to CII is an important mechanism in the CIA immunopathogenesis. Passive transfer of anti-CII sera from arthritic mice induces an inflammatory arthritis not only in strains considered susceptible to CIA, but also in CIA-non susceptible strains (Watson, Brown et al. 1987). Furthermore, it has been observed that CII antibodies are found in the RA as well as in CIA cartilage (Watson, Cremer et al. 1986). Several studies have clearly demonstrated that the subclass of antibody made during the autoimmune response to CII is an important factor in disease outcome (Stasiuk, Abehsira-Amar et al. 1996; Doncarli, Stasiuk et al. 1997). The CII response in CIA is dominated by the IgG2 subclass. High levels of both IgG2a and IgG2b are present at the peak of arthritis.

5.2 PURIFICATION OF ACTIVE COMPOUNDS

Plant material: Leaves from the *A. tonkinensis* tree were collected in Bac Ninh Vietnam. A voucher specimen was deposited in the herbarium of the Institute for Materia Medica, Hanoi, Vietnam. The dried powder of the leaves (solid sample) was extracted with 70% ethanol in a Soxhlet extractor for 4 cycles. Evaporation of ethanol gave an aqueous solution. Chlorophyll was eliminated from the extract using n-hexane. The fresh extract was partitioned with ethyl acetate followed by n-butanol extraction.

Solid phase extraction (SPE): The n-butanol extract was first dissolved in ethanoland water, the solution was then filtered and mixed with an Amberlite sorbent. Compounds were eluted from the sorbant with 60% acetonitrile/water. The eluate from 60% acetonitrile/water was lyophilized and the dry material was obtained.

The first chromatography step: An HPLC system was used for the separation of the compounds. Dry material from the solid phase extraction step was dissolved in acetonitrile and filtered through a Millipore GP Express Plus filter. The filtrate was pumped onto a Vydac C18 column. The purification procedure was repeated with the rest of the material. The fractions were collected and lyophilized. To determine the bioactivity of the individual fractions each was added *in vitro* to mitogen activated LNC to determine their anti-proliferative effects. Anti-proliferative activity was detected in fractions 4, 5, 6, 15, 24 and 25 which were further purified.

Purification of compounds 3, 4, 1: fractions 15, 24, 25 from the chromatography step were diluted 1:1 with water and applied on to a Source RPC 15 column equilibrated

with water. The material with anti-proliferative activity was eluted with a linear gradient of acetonitrile/water. The purification procedure was repeated several times. The active fractions were collected and lyophilized. These materials were subjected to structural analysis.

NMR analysis: The NMR spectra were recorded using a Bruker DMX-600 spectrometer equipped with a cryoprobe in deuterated dimethylsufoxide (DMSO-d_6) solution. Chemical shifts were reported in ppm relative to the residual solvent signal ($\delta(^1H)$ 2.50 ppm, $\delta(^{13}C)$ 39.5 ppm). The two-dimensional spectrum was recorded.

Mass spectrometry – The negative-ion mass spectra was recorded using an Ettan™ ESI-ToF electrospray time-of-flight mass spectrometer. Samples of chromatographic fractions were dissolved in 75% acetonitrile/water.

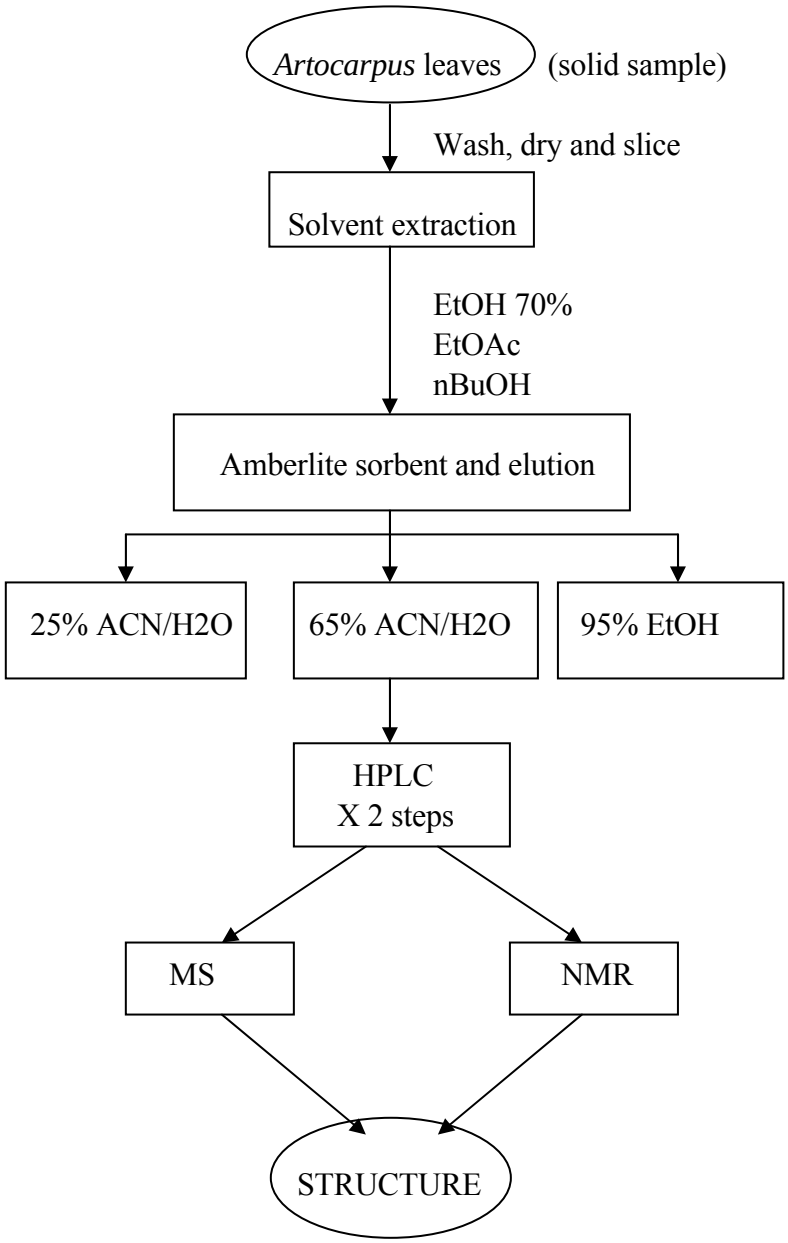


Figure 3. Purification and identification of the molecular compound structures

5.3 ELISA

Measurement of IgG and subclass specific antibodies to CII

Individual sera were collected from CII/IFA-immunised rats treated with *Artocarpus* extract, *Artonkin*, GXM, Rob-803 or with vehicle. The sera were collected on days 14, 21 and 28 p.i. and stored at -20^0C until analysed. ELISA microtitre plates were coated with native rat CII and the titers of IgG, IgG2a and IgG2b anti CII antibody levels was detemined.

Measurement of cytokines

The supernatants from LNC cultures were harvested without cells by centrifugation and stored at -20^oC until assayed. The concentrations of cytokines in the cell culture supernatants were quantified using a sandwich enzyme-linked immunosorbent assay. The rat TNF, IFN-γ and IL-6 Duoset kits were bought from R&D Systems Europe, Abingdon, UK. The sensitivity of the assay is 3 pg/ml.

5.4 EVALUATION OF APOPTOSIS

Detection of apoptosis using Annexin V and propidium iodide

Cells were treated with different agents at various concentrations and were stained with AV and PI according to the instructions of the manufacturer (R&D System, Germany) and analysed by Flow cytometry.

Detection of apoptosis using the TUNEL method

The *in situ* terminal dexoxynucleotidyl transferase (TdT) nick end labelling (TUNEL) assay was used to identify apoptosis as previously described. Single treated macrophages or LNCs were centrifuged onto slides and stained with a fluorescein labelled *in situ* cell death detection kit (Roche Diagnostics GmbH, Germany). Slides were evaluated using fluorescence microscopy followed by conversion to light microscopy using an anti-fluorescein peroxidase-labelled sheep antibody.

6. RESULTS AND DISCUSSION

During recent years considerable progress has been made to define and develop more effective treatment strategies for RA. From this perspective we have investigated the immunosuppressive efficacy of *Artocarpus tonkinensis* and flavonoids derived from *Artocarpus* (papers I and II), Glucuroxylomanan (paper III) as well as the alkaloid R803 (paper IV). Using extraction, isolation, and purification methods we produced 36 fractions from the total extract of *Artocarpus*. Four fractions were further characterized to define their capacities to inhibit T cell proliferation. Similarly, GXM and Rob803 could also inhibit the proliferation of mitogen-activated T cells. Furthermore we could demonstrate that the total *Artocarpus* extract, the four flavonoids, GXM and Rob803 all posseses anti-inflammatory properties.

6.1 ARTHRITIS SUPPRESSION BY THE NEW AGENTS

Inflammation in RA involves complex responses of chemical mediators, chemotactic factors, leukocytes and phagocytes causing injury to cartilage and other tissues. CIA has similar characteristics as RA both clinically and immunologically. The present studies demonstrated an amelioration of the signs of joint inflammation in arthritis rats treated with either the *Artocarpus* extract (paper I), artonkin-4´-βD-O-glucoside (paper II), GXM (paper III) or Rob803 (paper IV). Treatments with the soluble extract of *Artocarpus*, artonkin, GXM or Rob 803 significantly suppressed experimental arthritis (CIA) in respect to both severity and incidence of arthritis. In paper I we concentrated on the immunosuppressive efficacy of *Artocarpus tonkinensis*, a Vietnamese traditional medicinal plant which was described as being anti-inflammatory and an anti-rheumatoid drug in the pharmacopoeia of Vietnam (Do Tat Loi 2001). The inhibitory effects of the total *Artocarpus* extract and also its isolated compounds, Artonkin (paper II), reduced the severity and incidence of CIA. The total extract also suppressed the onset of the disease. The effective dose of the total *Artocarpus* extract was 150mg/kg while the corresponding dose of Artonkin was only 15mg/kg. This dose-related difference of the two anti-arthritis compounds suggested that Artonkin is the most active compound in the total *Artocarpus* extract.

In several previous investigations of CIA, the crude extracts of herbal medicines have been tested, often at doses ranging from 20-200mg/kg/day. The active doses that had

significant efficacy were between 100 and 200mg/kg, depending on herbal type used (Kim, Lee et al. 2005; Zheng and Wei 2005). These are similar to the amount of the total *Artocarpus* extract we used in our study (150mg/kg). If a single flavonoid was used the concentration of flavonoids which gave an improvement of arthritis symptoms ranged from 2 to 150 mg/kg/day (Shin, Jin et al. 2003; Verdrengh, Jonsson et al. 2003; Kawaguchi, Maruyama et al. 2006). In our experiments, artonkin was used a dose of 15mg/kg/day. Because of the cumbersomeness of the purification artonkin we were not able to purify the amount of artonkin needed to test the arthritis inhibitory effect of higher doses than 15mg. This dose reduced the incident and ameliorated the severity of arthritis.

In paper III, GXM was used to treat CIA rats using two different protocols. In the prophylactic treatment protocol with daily injection of 50mg/kg GXM, the CIA rats had significantly reduced arthritis severity and incidence and a delayed onset compared to that of the control group. Using a therapeutic protocol, GXM significantly suppressed the progression of CIA. We used the same amount of GXM in both treatment protocols. Suppression of arthritis was more obvious using the prophylactic protocol compared to treatment protocol when the GXM injections were started after the onset of arthritis.

In paper IV we studied the anti-arthritic effects of the synthetic quinoxaline substance Rob 803. This is of interest since its structural analogue B220 is a known inhibitor of reactive oxygen species (ROS) formation (Harbecke, Dahlgren et al. 1999). It has been suggested that tissue injury in RA is related to the amount of free radical reactions. The contribution of ROS in pathogenesis of RA has been confirmed *in vivo* by the use of antioxidants. The administration of superoxide dismutase (SOD) and SOD analogues suppressed the development and severity of CIA. Antioxidants such as vitamin C, vitamin E (Choi 2005) and vitamin D (Larsson, Mattsson et al. 1998) also reduced arthritis in CIA. From this point of view, in order to chracterize the arthritis ameliorating development capacity of B220 (unpublished data), we continued to examine the effect of Rob 803 on disease CIA pathogenesis. The results of this study demonstrated that Rob 803 treatment suppressed arthritic development when given subcutaneously from the day of immunization. It suppressed not only arthritis severity and incidence but also delayed the day of onset of arthritis. Oral treatment with Rob803 indicated a trend towards suppression of severe arthritis. This

may be explained by the rapid and severe course of CIA. Another possibility is that Rob803, being an oil-dissolved substance, is gradually absorbed into the target tissues.

Notably, all of the substances tested ameliorated arthritis development but had little effect on already established disease. Importantly, we could not observe any toxic effects at the doses used. The treated rats gained weight similarly to control rats and no increase of lethality was observed. The period when we are allowed to treat the rats after disease onset is only 13 days (from day 15 to 28). This is due to ethical considerations in order to limit the time rats have to suffer from arthritis.

6.2 ANTI-COLLAGEN ANTIBODIES

Although T cells play a prominent role in the regulation and development of autoimmune responses in CIA, autoantibodies to CII play also role in the immunopathogenesis of this model. CII antibodies have been reported to bind to cartilage (Watson, Cremer et al. 1986); Thus CII-specific antibodies appear to have the potential to initiate an inflammatory response to the cartilage.

In paper I the suppression of clinical arthritis was well correlated with the down-regulation of antibody production to CII. Upon immunization with CII, strong B cell responses occur in the rat, resulting in the peripheral accumulation of anti-CII antibodies. The binding of anti-CII antibodies to the cartilage surface and the subsequent activation of the complement system cascade elicits severe joint damage (Takagi and Jasin 1992). The inhibition of CIA with *Artocarpus* extract correlated with reduced levels of anti-CII IgG, suggesting that the extract suppressed B cell activation. Similar to our data, treatment of CIA with another extract called CHE (which has been extracted from the plant *Terminalia chebula*) significantly decreased the total serum IgG concentration in CHE-treated CIA mice (Lee, Hyun et al. 2005). Artonkin also affected anti-CII antibody IgG levels but less potently than did *Artocarpus* extract. It could be that the doses given to treat experimental animals were insufficient. It is our hope that we will be able to purify enough of artonkin to find if higher doses will be more effective to reduce anti-CII antibody levels as well as arthritis. That such a trial may be successful is indicated by the results from an experiment in CIA in mice. The study described that the purified flavonoid genistein was successfully at a dose of

30mg/kg (Verdrengh, Jonsson et al. 2003). Although these comparisons are difficult due to the nature of the extraction process, structure of the compound and/or experimental procedures employed, it may be possible to use similar doses or concentrations of extracts of the same groups of compound, which then may have similar therapeutic effects.

In paper III GXM therapy significantly reduced anti-CII antibody production. This effect of GXM was predictable since GXM displays anti-inflammatory activities such as inhibition of production of the pro-inflammatory cytokines IL-1β, TNF (Vecchiarelli, Retini et al. 1995) and inhibition of the migration of neutrophils (Lipovsky, Gekker et al. 1998). These effects of GXM may indirectly suppress B cell activation.

In manuscript IV subcutaneous treatment with Rob 803 had almost no effect on anti-CII antibody levels. This supports previous reports that mentioned the lack of efficacy of Rob 803 and related compounds on cell activation and LNC proliferation. However, it was surprising that we could detect a significantly increased anti-CII IgG response in rats orally treated with Rob803 compared to the control group. Thus far we have no explanation for this finding.

6.3 ANTI-ARTHRITIC AND IMMUNOSUPPRESSIVE MECHANISMS, A DISCUSSION

The induction and maintenance of joint inflammation involves many mechanisms. The innate and specific immune systems depend on eachother. Important events are the activation of antigen-presenting cells expressing transplantation antigens and producting pro-inflammatory cytokines and the antigen-spersific T cells that induces B cell maturation and antibody production. Another consequence is the production of pro-inflammatory enzymes such as metalloproteinase and the production of free oxygen radicals. Opposing this are events such as the production of Th2 cytokines and apoptosis.

In the manuscripts that are included in the thesis we have used several methods to investigate the mechanisms involved in arthritis development and maintenance. We have used methods to examine T cell activation (proliferation), B cell activation

(antibody production), apoptosis, induction of MMPs, ROS systems as well as cytokine production of activated lymph node cells. Every method was not used for each compound studied. This was due to both the limitations of materials and time that limited the range of experiments that could be performed, and to already existing experiments which mechanisms that might most likely be affected.

In paper I the effect of the total *Artocarpus* extract on ConA-induced proliferation was determined. The addition of *Artocarpus* extract inhibited ConA-induced T cell proliferation in a dose-dependent manner. To expose the relationship between the effect of *Artocarpus* on T cells and on arthritis, we isolated lymph nodes from arthritic rats and found that the increased proliferation ability of LNCs in the rats was also completely inhibited by the *in vivo* administration of *Artocarpus* extract. In this regard it is interesting that *Artocarpus* extract induced apoptosis of activated T cells at the same concentration that inhibited T cell proliferation (50-100 μg/ml) (see fig 5-paper I).

As a continuation of paper I this *in vitro* screening system was used to define substances with a selective suppressive effect on T cell proliferation. Among four purified compounds, artonkin and alphitonin exhibited the most consistent anti-proliferative effects (paper II). While this effect of artonkin was not due to induced apoptosis, alphitonin induced 4.25, 6.55, 18.6 % apoptosis of activated T cells at concentrations of 25, 50 and 100 μg/ml, respectively, compared to 1.75% cells in the control. Thus it is possible that the anti-rheumatic effects of the *Artocarpus* extract reported in paper I are caused by the apoptosis-inducing effects of altophinin (fig 5 in this section). One should remember, however, that herbal drugs contain several active ingredients that may all contribute to their pharmacological actions. It is seldom that a single purified compound can reproduce the complete pharmacological effects of the initial herb extract.

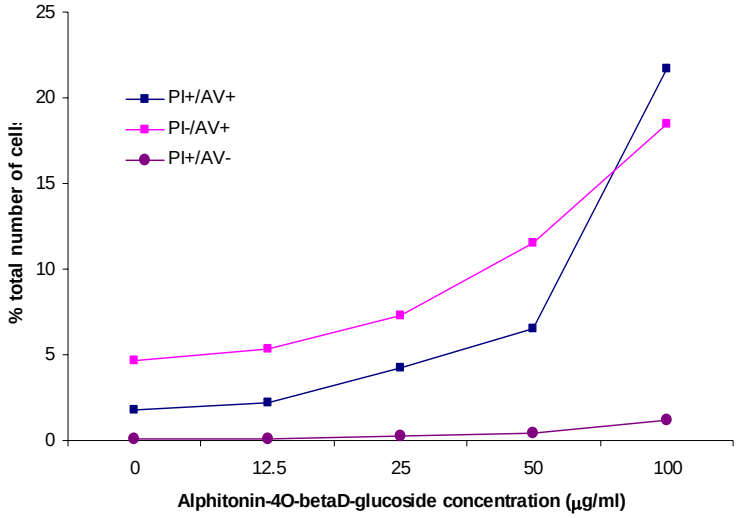


Figure 5. Alphitonin-4-O-βD-glucoside induces a dose-dependent apoptosis of LNC

It is known that CII-specific T cells maintain an activated state throughout the development of CIA and express high levels of type 1 pro-inflammatory cytokines such as IFN-γ, IL-6 and TNF (Latham, Whittington et al. 2005). These cytokines play major roles in the progression of joint destruction and proliferation of synoviocytes (Nakajima, Takamori et al. 1990; Nanki, Nagasaka et al. 2001). Our data demonstrated that CII-specific activated T cells *in vitro* produced excessive amounts of pro-inflammatory cytokines. The production of IFN-γ and TNF was significantly inhibited by the total *Artocarpus* extract, artonkin and alphitonin (fig 8-paper II).

In summary, we demonstrated the effectiveness of the *Artocarpus* extract and of the purified derivative artonkin to inhibit the development of CIA. These protective effects of *Artocarpus* appear to result from the control of key components in the pathogenesis of CIA. This may include inhibition of T cell proliferation and down-regulation of anti-CII antibody production. According to our results, *Artocarpus*, artonkin and alphitonin are important negative regulators of pro-inflammatory cytokine production of activated T cells and alphitonin also induces T cell apoptosis.

In paper IV, when analyzing the effect of Rob803 on T cell activities we could similarly detect anti-proliferative effects of this substance. Two groups of each 9 rats were simultaneously injected subcutaneously or orally fed with Rob803. The results of oral treatment with Rob 803 did not demonstrate suppressed T cell proliferation. This may be because the metabolism of Rob803 in the gastrointestinal tract reduced its

effective concentration in the tissues. Rob803 could significantly suppress Con-A induced proliferation of T cells in the subcutaneous treatment group (fig.3, 4 paper IV). This finding correlated with the arthritic inhibition of Rob803. Additionally, we found no apoptosis-inducing effect of Rob803. Several reports have demonstrated that B220 acts as an inducer of G2/M cell cycle arrest (Arimondo, Baldeyrou et al. 2001). Thus, we speculate that Rob803 exerts its anti-arthritic effects by blocking T cell proliferation through the induction of cell cycle arrest. Another possible mechanism underlying the anti-arthritic effect of Rob803 is its capacity to affect the generation of oxygen reactive species in neutrophils and macrophages. B220 reduces the capacity of neutrophils to generate reactive oxygen species (Harbecke, Dahlgren et al. 1999). We suggest that Rob803 would exert part of its effect by suppressing neutrophil and macrophage activity, cells which are present in inflamed joints in CIA. We tested the anti-oxidative effects of Rob803 by measuring a surrogate marker (NO_2^-) for NO production by macrophages. NO is an oxygen reactive molecule that works in parallel with the B220-sensitive NADPH-synthetase oxidation system. Our results revealed that Rob803 had an inhibitory effect on NO_2^- production, indicating a suppressive effect on activated neutrophils and macrophages. However, the anti-proliferative effects concentrations were a 1000-fold lower than what was needed in order to inhibite neutrophil activitiy. Thus, to conclude, we hereby report that Rob803, a chlorinated synthetic quinpxaline, has anti-arthritic properties when tested in CIA. Rob803 has both anti-T cell proliferative and anti-oxidative features which can be of benefit in modulating arthritis pathogenesis.

In paper III we studied the effect of GXM on the WEHI 164 cell line and a fibrosarcoma cell line with highly sensitivitive to cytotoxic factors which has fibroblast morphology (Espevik and Nissen-Meyer 1986). Synovial fibroblasts are evident in high numbers in the RA synovium due to proliferation and reduced apoptosis (Pap, Muller-Ladner et al. 2000). In RA, high levels of MMPs are produced, especially MMP-2. MMP-2 is implicated in the pathological processes of inflammatory joint diseases, being capable of degrading several components of the extracellular matrix (Unemori, Hibbs et al. 1991). From this point of view the agents that prevent the activity of MMPs may be effective in treatment of RA. Our findings indicated that cultured WEHI 164 cells exhibited characteristic features that resemble those of activated synovial fibroblasts in human RA. The production of MMP-2 was significantly suppressed by GXM *in vitro*. Its suppressive effect was as strong as that of conventional drugs used

for treatment of RA such as dexamethasone and piroxicam (paper III fig.5). Moreover, it has been well documented that GXM increases secretion of IL-10 and reduces the production of TNF, IL-1β, GM-CSF and NO (de Waal Malefyt, Abrams et al. 1991; Dong and Murphy 1995; Levitz, Tabuni et al. 1996; Vecchiarelli, Retini et al. 1996; Murphy, Zhou et al. 1997) as well as decreasing the expression of MHC class II molecules and reducing proliferation of T cells (Retini, Vecchiarelli et al. 1998). Additionally, the recruitment of T cells into the synovial tissue of RA is regulated by a sequence of interactions, including those between circulating T cells and the underlying basement membrane or interstitial matrix (Cid, Esparza et al. 1994; Bianchi, Bender et al. 1997). The activation-induced secretion of MMP-2 has been shown to favor T cell immigration through the endothelial basal membrane of blood vessel *in vitro* (Leppert, Waubant et al. 1995). Our results indicate a high tolerability of GXM compared with some steroidal and non-steroidal anti-inflammatory drugs. Taken together, we have come to the conclusion that MMP-2 is an important target of GXM, and that the inhibition of MMP-2 may be one of the mechanisms central for its beneficial effects in arthritis. Therefore, besides the known anti-inflammatory activities of GXM, the MMP-2 reductive effect of MMP-2 demonstrated, GXM may also be a cartilage protector.

In summary, since there are many pathways leading to inflammation and causing tissue destruction, as exemplified in arthritis, there are also many therapeutic possibilities. An important pathway affected in all of our experiments is that of T cell activation and proliferation. T cell regulation is central to most of the responses of the specific immune system. However, the role of T cells may be limited when chronic, self-perpetuating inflammation is manifested. In this situation, effector pathways of inflammation depending on the innate immune system may be more important. It is one of the strengths of our investigations that we have been able to demonstrate effects both on specific immune responses such as T cell activation and antibody production, and non-specific immune response such as MMPs and ROS.

Ideally it should be possible to be able to predict who will develop RA. Ideally we would need to know the nature of the auto-antigen(s) inducing disease. Ideally we would know what effector mechanism is most important in causing bone and cartilage destruction. Since we still do not know this we are left to develop agents that affect the mechanisms that we have been able to discern so far. Nonetheless, the situation is not so bad. Agents that affect T cell activation (inhibition of CTLA-4) and cytokine

production (TNF and IL-1 inhibitors) have injected new hope to those afflicted by rheumatism as well as their families, doctors and health providers.

Will the *Artocarpus* extract be as effective? This has not yet been tested in humans but it is my belief that the results of this thesis will lead to clinical trials to prove the efficacy of a leaf picked in the mountainous forests of Vietnam, extracted at Hanoi Medical University and purified and tested in experimental models of arthritis at Karolinska Institutet.

7. CONCLUDING REMARKS

This thesis comprises of the assessment of novel immunosuppressive agents and their efficacy in CIA. CIA isan excellent tool when exploring early pathogenic events and pathogenic changes in cartilage and bone in detail, and can be used to evaluate the mechanisms of inflammation and tissue destruction to screen for new agents with promising therapeutic capacities.

- An extract from the leaves of the herb *Artocarpus tonkinensis*, a plant traditionally used in Vietnamese folk medicine, was investigated in the CIA animal model. This extract inhibited arthritis severity and incidence. The efficacy was correlated with suppression of anti-CII IgG titres, T cell proliferation and T cell apoptosis.

- We screened 37 derived fractions from the *Artocarpus* extract for their capacity to inhibit T cell proliferation. Four molecules were structurally defined and found to inhibit T cell proliferation. Furthermore, two of them, artonkin and alphitonin, suppressed production of pro-inflammatory cytokines. Only artonkin was tested in CIA model and was found to inhibit CIA. The anti-arthritic effect of artonkin was associated with the reduction of IFN-γ and TNF.

- GXM has both prophylactic and therapeutic effects on CIA and inhibits anti-CII antibody levels. *In vitro*, GXM inhibited MMP-2 activity without causing toxicity in the treated rats.

- The synthesised quinoxalibe 9-chloro-2,3 dimethyl-6-(N,N-dimethylamino-2-oxoethyl)-6H-indolo (2,3-b) quinoxalin (Rob 803) is a chloride analogue of B-220. Rob 803 has anti-arthritic properties. *In vitro*, Rob 803 had both anti-oxidative and anti-T cell proliferative features which can be of benefit in modulating arthritis development.

8. FUTURE PERSPECTIVES

The link between flavonoids and Rheumatoid arthritis is based on the evidence that flavonoids have anti-inflammatory properties. These compounds may be effective in the treatment of RA. However, this still needs to be proven in clinical trials. Discussion with the Ministry of Health in Vietnam has been initiated and we believe that clinical trials of *Artocarpus* will become feasible. We also intend:

- To search for other unrecognized components in this plant that may have anti-inflammatory characteristics

- To determine if the active flavonoids found in *Artocarpus* may be used in other inflammatory conditions than RA.

- To introduce the concept of Good Clinical Practice in Vietnam in order to produce qualitatively data in clinical trials performed in Vietnam.

9. ACKNOWLEDGMENTS

The work presented in this thesis was performed at CMM, Karolinska Institutet and was sponsored by a grant from SIDA/SAREC and supported by Hanoi Medical University (HMU)

I would like to acknowledge all those people who have contributed to my thesis and who come to mind as having had a large impact on my academic career.

Professor *Lars Klareskog*,my mentor. He inspired my research after an interview with me that made possible for me to become a PhD student at CMM-KI. I would like to thank you for your passion for science and bringing together all the wonderful people at L8:04 that I have been working with. I am so glad I came to that first interview six years ago.

Ass. Professor *Per Larsson*, my main-supervisor who started our research by guiding my study and stimulating me to undertake and complete my research. In subsequent years, the support continuously given by you have helped me to become a creative researcher. You have also provided constant support for me to complete my thesis. Thank you for understanding my need for independence and helping me to grow as a scientist and for understanding "Vietnamese".

Ass. Professor *Helena Erlandsson Harris*, my co-supervisor; thank you for teaching me so much, initiating my works in the Lab. You have always provided valuable solutions for difficult situations. Without you this work would never have been completed. Thank you for being my mentor as a scientist and a friend when I needed your support.

Ass. Professors *NguyenThi Ha*, *Phan Thu Anh* and *Pham Thien Ngoc*, my co-supervisors and advisers from HMU who have observed my studies closely; for your encouragement and not only for your great help in my work in Vietnam, but also in many other aspects of life.

Professor *Tran Luu Van Hien, Tran Van Sung,* Dr. *Trinh Thi Thuy* and Mr. *Tung* who helped me through their comments, discussions, for preparing, storing and extracting the compounds from *Artocarpus.*

My co-authors: *Ewa Westman* and *Karin Lundberg* for exciting and fruitful collaborations, Dr. *Mirshafiey* for being the brain behind the investigations of GXM.

Anca Catrina for your intelligent advices, nice collaboration in the apoptosis experiments and your sympathy. Especially, I would thank *Rannar Sillar, Elo Eriste, Edwards Liepinsh* from the department of medical biochemistry and biophysics, Karolinska Institutet for a great collaboration in purifying and identifying the structures of the novel substances of the *Artocarpus*.

Marie Wahren Herlenius for sharing knowledge and enthusiasm, for satisfying all my questions and nice company at the lab during many weekends.

Ingeborg van der Ploeg, thank you for your arrangement of social events for Vietnamese students and helping to resolve difficult situations. Your help in coordination of Swedish-Vietnamese PhD programme has been so important.

Professor *Hans Rosling* for your intelligent advices, Ass. Professor *Nguyen Van Tuong,* Prof. *Vinod Divan*, Dr. *Tran Dung Tien*, Mrs *Monica Grangien* and *Maissa Al Aldam,* for the coordination and administrative support and *Maissa* for your warm smiles.

Taking this opportunity, I would thank all my colleagues at the Biochemistry Department of Hanoi Medical University for sharing my job during the study. Professors *Nguyen Lan Viet, Dao Van Long*, *Pham Nhat An* and *Do Doan Loi* and the management of Hanoi medical University, for their support and encouragment

I would also thank the late Professor *Ton That Bach*, former Rector of Hanoi Medical University, who supported the young medical students, for encouraging me to become a PhD student in the programme.

My special friends at the Lab**.** *Heidi* for being my friend, helping me whenever I need, sharing happiness and every minute we spent together with your family *Tony*, *Iris*, and *Vinh* at Tobu. *Therese Wallerskog, Andreas Fasth* for your spirit, for having nice lunches in the park during the weekend sometimes. *Duojia* for your inspiring company and having nice times making dumplings together,

Bob Harris, for linguistic advice and critical reading of my manuscripts and thesis, for a wonderful time at your home with *Axel* and *Helena*.

All the members in the Rheumatology group, for creating a wonderful working environment. I would like to thank; Professor *Ingrid Lundberg* for your sympathy and nice Christmas dinner with your wonderful children and your family. *Karin P, Anca*

and *Eva L* for sharing your knowledge in the field of immunochemistry and for kindly and patiently helping me with my stainings. *Lotta, Therese Östberg, and Erik* for being sweet and supportive. *Vivi Malmström* and *Tina Trollmo* for giving me interesting flow cytometry analysis lessons. The computer wizards; *Lena, Sevim, Carina, Marcus*, *Rudolf* and especially *Lasse*, who all facilitated my work. *Leonid Padyukov* for always being so nice and taking time to teach me about MHC and genetics. *Eva J* for all PBS bottles so well prepared. *Johnny* for excellent Danish cheese lessons. The others members of the rheumatology group: *Mona, Ulrika, Jian-Ping, Stina, Linn, Monica E, Wei, Fie, Adla, Åse, Barbro, Luise, Marina Korotkova, Marina S, Cissi, Dimitris, Vilija, Alex, Emilie, Karina, Monika, Sukanya* and *Aurelie.*

I would also thank all the administrative arrangements made by *Gunnel Beberfeldt* and *Susanne Karlfeldt* during my study and conferences abroad.

All present and former friends and colleges at the Neuroimmunology groups, in particular *Maja* and *Judit* for your friendship, *Britt* and *Mohsen* for being helpful, *Åsa,* and *Rux* for interesting discussions in the field of stem cells and macrophages. *Anna Lobell* for having efficient advice for my study during my half-time seminar.

More people became involved when the project was speeded up. Many have provided valuable help to me, such as everyone at the animal department; for taking such good care of our animals and ordering animals for my experiments.

My dear friends outside the lab: *Ngan,* for being like a sister to me, for taking care of me, for all you and *Tuan* have done for me. *Ngoc Lindström* and *Lennart* for being wonderful friends, for always welcoming me to your home, for nice evenings, dinners and talking. *Hang, Tram, Minh, Hanh, Ha* and *Bent* and Professor at clinical genetic department *Bui Hung* for being my real friends, bringing the Vietnamese atmosphere to Sweden.

My lovely friends, *Nguyen Vu Trung, Tran Thanh Huong, Nguyen Van Do, Hoang Thu Ha, Nguyen Khanh Hoa* for sharing the pleasure and sadness, and the difficulties during the times we have had in Sweden. *Nguyen Viet Ha*, *Ha Tran Hung, Hoang Thi Lam, Vu Thanh Huyen , Vuong Tuyet Mai, Nguyen Anh Tuan, Nguyen Van Tuan* for enjoyable times in Stockholm.

My friends back home in Vietnam *Nguyen D Tu, Hoang N Lan* for being first best friends, then part of the family. *Hang, Trang and Nguyet* for chemistry tuition for my son. *Ta Thanh Van* and *Nong Ngoc Huy* for being my colleagues and being helpful in administrative matters.

My sisters *Huong* and *Lan,* my sisters and brothers-in-law *Ngoc, Quoc, Vi, Sinh*, and *Kota* for being my family. My nephew *Trung, An, Dat* and nieces *Nhung, Van, Vi* for being nice and for the fun we have when the whole family gathers for parties.

At this moment, I would like to show my deep gratitude to my parents, *Nguyen Thi Hai*, *Dang Tran Due,* for laying the fundations for this work. A special memory to my father for being my colleague, my professor and who supported me through all my life. I would ask my father to forgive me that I was not with him when he passed away forever from home. My parents-in-law, *Le Sy Bich, Le Ngoc Hien*, for being like my own parents and always taking care of my children when I have been away from home and staying in Sweden for the study.

I am glad that my children, *Nam* and *Vinh*, can now finally read about what I have been doing all these years. Both of you are my sunshine and the real sense of my life. Thank both of you for trying to be independent when I was not at home with you due to my studies. This thesis could absolutely not have been completed were it not for my wonderful husband, *Le Trung Huu*. It is true that a woman always needs a great man for her stable stand. You encouraged and supported me at all times. And you work hard to make our life become brighter, took care of all our two boys needed during the time of this thesis, for allowing me to study and work. Thank you for always loving and for being my greatest admirer and my better half.

Stockholm, 18th October 2006.

10. REFERENCES

(1996). "Guidelines for monitoring drug therapy in rheumatoid arthritis. American College of Rheumatology Ad Hoc Committee on Clinical Guidelines." Arthritis Rheum **39**(5): 723-31.

Aggarwal, B. B. (2000). "Tumour necrosis factors receptor associated signalling molecules and their role in activation of apoptosis, JNK and NF-kappaB." Ann Rheum Dis **59 Suppl 1**: i6-16.

Aho, K., M. Koskenvuo, et al. (1986). "Occurrence of rheumatoid arthritis in a nationwide series of twins." J Rheumatol **13**(5): 899-902.

Andersson, U., H. Wang, et al. (2000). "High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes." J Exp Med **192**(4): 565-70.

Arimondo, P. B., B. Baldeyrou, et al. (2001). "DNA interaction and cytotoxicity of a new series of indolo[2,3-b]quinoxaline and pyridopyrazino[2,3-b]indole derivatives." Chem Biol Interact **138**(1): 59-75.

Arnett, F. C., S. M. Edworthy, et al. (1988). "The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis." Arthritis Rheum **31**(3): 315-24.

Bas, E., M. C. Recio, et al. (2006). "Anti-inflammatory activity of 5-O-demethylnobiletin, a polymethoxyflavone isolated from Sideritis tragoriganum." Planta Med **72**(2): 136-42.

Bau, B., P. M. Gebhard, et al. (2002). "Relative messenger RNA expression profiling of collagenases and aggrecanases in human articular chondrocytes in vivo and in vitro." Arthritis Rheum **46**(10): 2648-57.

Belay, T. and R. Cherniak (1995). "Determination of antigen binding specificities of Cryptococcus neoformans factor sera by enzyme-linked immunosorbent assay." Infect Immun **63**(5): 1810-9.

Benito, S., D. Lopez, et al. (2002). "A flavonoid-rich diet increases nitric oxide production in rat aorta." Br J Pharmacol **135**(4): 910-6.

Bianchi, E., J. R. Bender, et al. (1997). "Through and beyond the wall: late steps in leukocyte transendothelial migration." Immunol Today **18**(12): 586-91.

Billiau, A. and P. Matthys (2001). "Modes of action of Freund's adjuvants in experimental models of autoimmune diseases." J Leukoc Biol **70**(6): 849-60.

Bingham, C. O., 3rd (2002). "The pathogenesis of rheumatoid arthritis: pivotal cytokines involved in bone degradation and inflammation." J Rheumatol Suppl **65**: 3-9.

Bode, W., C. Fernandez-Catalan, et al. (1999). "Insights into MMP-TIMP interactions." Ann N Y Acad Sci **878**: 73-91.

Boissier, M. C., X. Z. Feng, et al. (1987). "Experimental autoimmune arthritis in mice. I. Homologous type II collagen is responsible for self-perpetuating chronic polyarthritis." Ann Rheum Dis **46**(9): 691-700.

Booth, C., D. F. Hargreaves, et al. (1999). "Isoflavones inhibit intestinal epithelial cell proliferation and induce apoptosis in vitro." Br J Cancer **80**(10): 1550-7.

Burmester, G. R., B. Stuhlmuller, et al. (1997). "Mononuclear phagocytes and rheumatoid synovitis. Mastermind or workhorse in arthritis?" Arthritis Rheum **40**(1): 5-18.

Butler, D. M., A. M. Malfait, et al. (1997). "DBA/1 mice expressing the human TNF-alpha transgene develop a severe, erosive arthritis: characterization of the cytokine cascade and cellular composition." J Immunol **159**(6): 2867-76.

Cant, S. a. S., U. (2000). Alternative health practices and systems., In G.L. Albrecht, R. Fitzpatrick and S. Scrimshaw (Eds.), Handbook of social studies in health and medicine.

Carlsen, S., A. S. Hansson, et al. (1998). "Cartilage oligomeric matrix protein (COMP)-induced arthritis in rats." Clin Exp Immunol **114**(3): 477-84.

Casadevall, J. R. P. A. (1998). Cryptococcus neoformans, Washington, D.C. : ASM Press,.

Casley-Smith, J. R. (1986). "high-protein oedemas and the benzo-gamma-pyrones."

Chabaud, M., J. M. Durand, et al. (1999). "Human interleukin-17: A T cell-derived proinflammatory cytokine produced by the rheumatoid synovium." Arthritis Rheum **42**(5): 963-70.
Chabaud, M., F. Fossiez, et al. (1998). "Enhancing effect of IL-17 on IL-1-induced IL-6 and leukemia inhibitory factor production by rheumatoid arthritis synoviocytes and its regulation by Th2 cytokines." J Immunol **161**(1): 409-14.
Chang, S. H., H. C. Sung, et al. (2005). "Suppressive effect of AIF, a water extract from three herbs, on collagen-induced arthritis in mice." Int Immunopharmacol **5**(9): 1365-72.
Chen, Q. and W. Wei (2003). "Effects and mechanisms of glucosides of chaenomeles speciosa on collagen-induced arthritis in rats." Int Immunopharmacol **3**(4): 593-608.
Cherniak, R., E. Reiss, et al. (1980). "Structure and antigenic activity of the capsular polysaccharide of Cryptococcus neoformans serotype A." Mol Immunol **17**(8): 1025-32.
Choi, H. K. (2005). "Dietary risk factors for rheumatic diseases." Curr Opin Rheumatol **17**(2): 141-6.
Choudhury, R., S. K. Srai, et al. (1999). "Urinary excretion of hydroxycinnamates and flavonoids after oral and intravenous administration." Free Radic Biol Med **27**(3-4): 278-86.
Chubinskaya, S., K. E. Kuettner, et al. (1999). "Expression of matrix metalloproteinases in normal and damaged articular cartilage from human knee and ankle joints." Lab Invest **79**(12): 1669-77.
Chuyen, V. V. (1984). Vietnamese medicinal plants. Hanoi, Vietnam, HAnoi Medical Pubisher.
Cid, M. C., J. Esparza, et al. (1994). "Signaling through CD50 (ICAM-3) stimulates T lymphocyte binding to human umbilical vein endothelial cells and extracellular matrix proteins via an increase in beta 1 and beta 2 integrin function." Eur J Immunol **24**(6): 1377-82.
Clark, I. M., L. K. Powell, et al. (1993). "The measurement of collagenase, tissue inhibitor of metalloproteinases (TIMP), and collagenase-TIMP complex in synovial fluids from patients with osteoarthritis and rheumatoid arthritis." Arthritis Rheum **36**(3): 372-9.
Cohen, S. B., P. Emery, et al. (2006). "Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks." Arthritis Rheum **54**(9): 2793-2806.
Courtenay, J. S., M. J. Dallman, et al. (1980). "Immunisation against heterologous type II collagen induces arthritis in mice." Nature **283**(5748): 666-8.
Cremer, M. A., M. M. Griffiths, et al. (1995). "Type XI and II collagen-induced arthritis in rats: characterization of inbred strains of rats for arthritis-susceptibility and immune-responsiveness to type XI and II collagen." Autoimmunity **20**(3): 153-61.
Cyster, J. G. (1999). "Chemokines and cell migration in secondary lymphoid organs." Science **286**(5447): 2098-102.
Dahlman, I., E. Wallstrom, et al. (2001). "Polygenic control of autoimmune peripheral nerve inflammation in rat." J Neuroimmunol **119**(2): 166-74.
Dai, M., W. Wei, et al. (2003). "Glucosides of Chaenomeles speciosa remit rat adjuvant arthritis by inhibiting synoviocyte activities." Acta Pharmacol Sin **24**(11): 1161-6.
Davis, L. S. (2003). "A question of transformation: the synovial fibroblast in rheumatoid arthritis." Am J Pathol **162**(5): 1399-402.
Day, A. J., M. S. DuPont, et al. (1998). "Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity." FEBS Lett **436**(1): 71-5.
Day, A. J., F. Mellon, et al. (2001). "Human metabolism of dietary flavonoids: identification of plasma metabolites of quercetin." Free Radic Res **35**(6): 941-52.

de Waal Malefyt, R., J. Abrams, et al. (1991). "Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes." J Exp Med **174**(5): 1209-20.
Diamond, R. D. and J. E. Bennett (1974). "Prognostic factors in cryptococcal meningitis. A study in 111 cases." Ann Intern Med **80**(2): 176-81.
Dinarello, C. A. (2000). "Proinflammatory cytokines." Chest **118**(2): 503-8.
Do Tat Loi (2001). Nhung cay thuoc va vi thuoc Vietnam (Medicine plants of Vietnam and their biochemical properties). Hanoi, Nha XB Y hoc (Medicine publisher).
Do Tat Loi and N. X. Dung (1991). "Native drugs of Vietnam: which traditional and scientific approaches?" J Ethnopharmacol **32**(1-3): 51-6.
Do Tat Loi, T. H. (1988). Hardly belieable but true : case of successful treatment of disease by traditional methods. Hanoi, Vietnam Courier.
Doncarli, A., L. M. Stasiuk, et al. (1997). "Conversion in vivo from an early dominant Th0/Th1 response to a Th2 phenotype during the development of collagen-induced arthritis." Eur J Immunol **27**(6): 1451-8.
Dong, Z. M. and J. W. Murphy (1995). "Effects of the two varieties of Cryptococcus neoformans cells and culture filtrate antigens on neutrophil locomotion." Infect Immun **63**(7): 2632-44.
Dorner, T. and G. R. Burmester (2003). "The role of B cells in rheumatoid arthritis: mechanisms and therapeutic targets." Curr Opin Rheumatol **15**(3): 246-52.
Duddy, M. E., A. Alter, et al. (2004). "Distinct profiles of human B cell effector cytokines: a role in immune regulation?" J Immunol **172**(6): 3422-7.
Dugas, T. R., D. W. Morel, et al. (2000). "Novel cell culture medium for use in oxidation experiments provides insights into mechanisms of endothelial cell-mediated oxidation of LDL." In Vitro Cell Dev Biol Anim **36**(9): 571-7.
Dung, T. N. and G. Bodeker (2001). "Tue Tinh: founder of Vietnamese traditional medicine." J Altern Complement Med **7**(5): 401-3.
Dzung ND., H. T., Ngoc PH., Trung TH. (2002). "Presence of Ampelopsis in the leave of Artocarpus tonkinensis." Revue Pharmaceutique (Vietnamese) **7**: 137-140.
Edwards, J. C., M. J. Leandro, et al. (2002). "B-lymphocyte depletion therapy in rheumatoid arthritis and other autoimmune disorders." Biochem Soc Trans **30**(4): 824-8.
Escalante, A., R. W. Haas, et al. (2005). "Paradoxical effect of body mass index on survival in rheumatoid arthritis: role of comorbidity and systemic inflammation." Arch Intern Med **165**(14): 1624-9.
Espevik, T. and J. Nissen-Meyer (1986). "A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes." J Immunol Methods **95**(1): 99-105.
Feigon, J., W. A. Denny, et al. (1984). "Interactions of antitumor drugs with natural DNA: 1H NMR study of binding mode and kinetics." J Med Chem **27**(4): 450-65.
Feldmann, M., F. M. Brennan, et al. (1996). "Role of cytokines in rheumatoid arthritis." Annu Rev Immunol **14**: 397-440.
Felson, D. T., J. J. Anderson, et al. (1995). "American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis." Arthritis Rheum **38**(6): 727-35.
Felson, D. T., J. J. Anderson, et al. (1990). "The comparative efficacy and toxicity of second-line drugs in rheumatoid arthritis. Results of two metaanalyses." Arthritis Rheum **33**(10): 1449-61.
Fernandes, J. C., J. Martel-Pelletier, et al. (1998). "Collagenase-1 and collagenase-3 synthesis in normal and early experimental osteoarthritic canine cartilage: an immunohistochemical study." J Rheumatol **25**(8): 1585-94.
Ferrali, M., C. Signorini, et al. (1997). "Protection against oxidative damage of erythrocyte membrane by the flavonoid quercetin and its relation to iron chelating activity." FEBS Lett **416**(2): 123-9.
Filipski, J. and K. W. Kohn (1982). "Ellipticine-induced protein-associated DNA breaks in isolated L1210 nuclei." Biochim Biophys Acta **698**(3): 280-6.
Firestein, G. S., M. M. Paine, et al. (1991). "Gene expression (collagenase, tissue inhibitor of metalloproteinases, complement, and HLA-DR) in rheumatoid

arthritis and osteoarthritis synovium. Quantitative analysis and effect of intraarticular corticosteroids." Arthritis Rheum **34**(9): 1094-105.
Fournier, C. (2005). "Where do T cells stand in rheumatoid arthritis?" Joint Bone Spine **72**(6): 527-32.
Gallucci, S., M. Lolkema, et al. (1999). "Natural adjuvants: endogenous activators of dendritic cells." Nat Med **5**(11): 1249-55.
Geng, Y., B. Zhang, et al. (1993). "Protein tyrosine kinase activation is required for lipopolysaccharide induction of cytokines in human blood monocytes." J Immunol **151**(12): 6692-700.
Gil, B., M. J. Sanz, et al. (1994). "Effects of flavonoids on Naja naja and human recombinant synovial phospholipases A2 and inflammatory responses in mice." Life Sci **54**(20): PL333-8.
Gorman, J. D., R. F. Lum, et al. (2004). "Impact of shared epitope genotype and ethnicity on erosive disease: a meta-analysis of 3,240 rheumatoid arthritis patients." Arthritis Rheum **50**(2): 400-12.
Gregersen, P. K., J. Silver, et al. (1987). "The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis." Arthritis Rheum **30**(11): 1205-13.
Griffiths, M. M., G. W. Cannon, et al. (1993). "Induction of autoimmune arthritis in rats by immunization with homologous rat type II collagen is restricted to the RT1av1 haplotype." Arthritis Rheum **36**(2): 254-8.
Gutteridge, J. M., G. J. Quinlan, et al. (1994). "Primary plasma antioxidants in adult respiratory distress syndrome patients: changes in iron-oxidizing, iron-binding, and free radical-scavenging proteins." J Lab Clin Med **124**(2): 263-73.
Ha Nguyen Thi Vinh, P. P. T. P., Lam Phan Chuc, Quynh Pham Thi. (2004). "Effects of Artocapus Tonkinensis extract on experimental Heterogenous skin graff and mixed lymphocytic culture of human." Vietnam medical **Number 7**(300): 27-29.
Handel, M. L., L. B. McMorrow, et al. (1995). "Nuclear factor-kappa B in rheumatoid synovium. Localization of p50 and p65." Arthritis Rheum **38**(12): 1762-70.
Harbecke, O., C. Dahlgren, et al. (1999). "The synthetic non-toxic drug 2,3-dimethyl-6(2-dimethylaminoethyl)-6H-indolo-(2,3-b)quinoxaline inhibits neutrophil production of reactive oxygen species." J Leukoc Biol **65**(6): 771-7.
Harborne, J. B. and C. A. Williams (2000). "Advances in flavonoid research since 1992." Phytochemistry **55**(6): 481-504.
Harmenberg, J., A. Akesson-Johansson, et al. (1991). "The mechanism of action of the anti-herpes virus compound 2,3-dimethyl-6(2-dimethylaminoethyl)-6H-indolo-(2,3-b)quinoxaline." Antiviral Res **15**(3): 193-204.
Harmenberg, J., B. Wahren, et al. (1988). "Antiherpesvirus activity and mechanism of action of indolo-(2,3-b)quinoxaline and analogs." Antimicrob Agents Chemother **32**(11): 1720-4.
Hepburn, T. W., M. C. Totoritis, et al. (2003). "Antibody-mediated stripping of CD4 from lymphocyte cell surface in patients with rheumatoid arthritis." Rheumatology (Oxford) **42**(1): 54-61.
Hien TL, K. P., Trung TH (2002). "Presence of Kaempferol in the leave of Artocarpus tonkinensis." Medical and pharmaceutic information (Vietnamese), **6**: 19-21.
Higa, S., T. Hirano, et al. (2003). "Fisetin, a flavonol, inhibits TH2-type cytokine production by activated human basophils." J Allergy Clin Immunol **111**(6): 1299-306.
Hill, N. and N. Sarvetnick (2002). "Cytokines: promoters and dampeners of autoimmunity." Curr Opin Immunol **14**(6): 791-7.
Hoàng, B. C., Phó, .T. and Huu, N. . (1999). Vietnamese traditional medicine. Hanoi-Vietnam, Hanoi: The Gioi Publishers.
Hollman, P. C., J. H. de Vries, et al. (1995). "Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers." Am J Clin Nutr **62**(6): 1276-82.
Holmdahl, R., L. Klareskog, et al. (1985). "T lymphocytes in collagen II-induced arthritis in mice. Characterization of arthritogenic collagen II-specific T-cell lines and clones." Scand J Immunol **22**(3): 295-306.

Holmdahl, R., C. Vingsbo, et al. (1992). "Homologous collagen-induced arthritis in rats and mice are associated with structurally different major histocompatibility complex DQ-like molecules." Eur J Immunol **22**(2): 419-24.
Horai, R., S. Saijo, et al. (2000). "Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice." J Exp Med **191**(2): 313-20.
Hsu, L. C., J. M. Park, et al. (2004). "The protein kinase PKR is required for macrophage apoptosis after activation of Toll-like receptor 4." Nature **428**(6980): 341-5.
Hunter, C. A. (2005). "New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions." Nat Rev Immunol **5**(7): 521-31.
Ioku, K., Y. Pongpiriyadacha, et al. (1998). "beta-Glucosidase activity in the rat small intestine toward quercetin monoglucosides." Biosci Biotechnol Biochem **62**(7): 1428-31.
Ishiguro, N., T. Ito, et al. (1996). "Determination of stromelysin-1, 72 and 92 kDa type IV collagenase, tissue inhibitor of metalloproteinase-1 (TIMP-1), and TIMP-2 in synovial fluid and serum from patients with rheumatoid arthritis." J Rheumatol **23**(9): 1599-604.
Iwashita, K., M. Kobori, et al. (2000). "Flavonoids inhibit cell growth and induce apoptosis in B16 melanoma 4A5 cells." Biosci Biotechnol Biochem **64**(9): 1813-20.
Jae Youl Cho, a, Pyoung Su Kima, Jisoo Parka, Eun Sook Yooa, Kyong Up Baika, Young-Kyoon Kimb and Myung Hwan Parka (2000). "Inhibitor of tumor necrosis factor- production in lipopolysaccharide-stimulated RAW264.7 cells from Amorpha fruticosa." J Ethnopharmacol. **70**((2)): 127-33.
Jang, D. S., M. Cuendet, et al. (2002). "Prenylated flavonoids of the leaves of Macaranga conifera with inhibitory activity against cyclooxygenase-2." Phytochemistry **61**(7): 867-72.
Joe, B., M. M. Griffiths, et al. (1999). "Animal models of rheumatoid arthritis and related inflammation." Curr Rheumatol Rep **1**(2): 139-48.
Kaczmarek, L., W. Luniewski, et al. (2002). "Synthesis of 6-substituted 6H-indolo[2,3-b]quinolines as novel cytotoxic agents and topoisomerase II inhibitors." Acta Pol Pharm **59**(3): 199-207.
Kaczmarek, L., W. Peczynska-Czoch, et al. (1998). "Methoxy- and methyl-, methoxy-5,6,11-trimethyl-6H-indolo [2,3-b]quinolinium derivatives as novel cytotoxic agents and DNA topoisomerase II inhibitors." Anticancer Res **18**(4C): 3133-8.
Katz, Y., O. Nadiv, et al. (2001). "Interleukin-17 enhances tumor necrosis factor alpha-induced synthesis of interleukins 1,6, and 8 in skin and synovial fibroblasts: a possible role as a "fine-tuning cytokine" in inflammation processes." Arthritis Rheum **44**(9): 2176-84.
Kawaguchi, K., H. Maruyama, et al. (2006). "Suppression of collagen-induced arthritis by oral administration of the citrus flavonoid hesperidin." Planta Med **72**(5): 477-9.
Kelner, M. a. W., B. (2000). Complementary and alternative medicine: Challenge and change. Amsterdam, Amsterdam: Harwood Academic/Abingdon: Marston .
Kerry, N. L. and M. Abbey (1997). "Red wine and fractionated phenolic compounds prepared from red wine inhibit low density lipoprotein oxidation in vitro." Atherosclerosis **135**(1): 93-102.
Keyszer, G., I. Lambiri, et al. (1999). "Circulating levels of matrix metalloproteinases MMP-3 and MMP-1, tissue inhibitor of metalloproteinases 1 (TIMP-1), and MMP-1/TIMP-1 complex in rheumatic disease. Correlation with clinical activity of rheumatoid arthritis versus other surrogate markers." J Rheumatol **26**(2): 251-8.
Kim, G., J. B. Jun, et al. (2002). "Necessary role of phosphatidylinositol 3-kinase in transforming growth factor beta-mediated activation of Akt in normal and rheumatoid arthritis synovial fibroblasts." Arthritis Rheum **46**(6): 1504-11.
Kim, K. S., S. D. Lee, et al. (2005). "Suppressive effects of a water extract of Ulmus davidiana Planch (Ulmaceae) on collagen-induced arthritis in mice." J Ethnopharmacol **97**(1): 65-71.

Klareskog, L., U. Forsum, et al. (1982). "Evidence in support of a self-perpetuating HLA-DR-dependent delayed-type cell reaction in rheumatoid arthritis." Proc Natl Acad Sci U S A **79**(11): 3632-6.
Klareskog, L., P. Stolt, et al. (2006). "A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination." Arthritis Rheum **54**(1): 38-46.
Knekt, P., R. Jarvinen, et al. (1997). "Dietary flavonoids and the risk of lung cancer and other malignant neoplasms." Am J Epidemiol **146**(3): 223-30.
Knekt, P., J. Kumpulainen, et al. (2002). "Flavonoid intake and risk of chronic diseases." Am J Clin Nutr **76**(3): 560-8.
Kohn, K. W., M. J. Waring, et al. (1975). "Intercalative binding of ellipticine to DNA." Cancer Res **35**(1): 71-6.
Konig, A., G. K. Schwartz, et al. (1997). "The novel cyclin-dependent kinase inhibitor flavopiridol downregulates Bcl-2 and induces growth arrest and apoptosis in chronic B-cell leukemia lines." Blood **90**(11): 4307-12.
Kozel, T. R. and E. C. Gotschlich (1982). "The capsule of cryptococcus neoformans passively inhibits phagocytosis of the yeast by macrophages." J Immunol **129**(4): 1675-80.
Krakauer, T., B. Q. Li, et al. (2001). "The flavonoid baicalin inhibits superantigen-induced inflammatory cytokines and chemokines." FEBS Lett **500**(1-2): 52-5.
Krause, A., N. Scaletta, et al. (2002). "Rheumatoid arthritis synoviocyte survival is dependent on Stat3." J Immunol **169**(11): 6610-6.
Kremer, J. M., R. Westhovens, et al. (2003). "Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig." N Engl J Med **349**(20): 1907-15.
Kubo, M., H. Matsuda, et al. (1984). "Studies on Scutellariae radix. VII. Anti-arthritic and anti-inflammatory actions of methanolic extract and flavonoid components from Scutellariae radix." Chem Pharm Bull (Tokyo) **32**(7): 2724-9.
Kwak, W. J., T. C. Moon, et al. (2003). "Papyriflavonol A from Broussonetia papyrifera inhibits the passive cutaneous anaphylaxis reaction and has a secretory phospholipase A2-inhibitory activity." Biol Pharm Bull **26**(3): 299-302.
Larsson, P., L. Mattsson, et al. (1998). "A vitamin D analogue (MC 1288) has immunomodulatory properties and suppresses collagen-induced arthritis (CIA) without causing hypercalcaemia." Clin Exp Immunol **114**(2): 277-83.
Latham, K. A., K. B. Whittington, et al. (2005). "Ex vivo characterization of the autoimmune T cell response in the HLA-DR1 mouse model of collagen-induced arthritis reveals long-term activation of type II collagen-specific cells and their presence in arthritic joints." J Immunol **174**(7): 3978-85.
Le Marchand, L. (2002). "Cancer preventive effects of flavonoids--a review." Biomed Pharmacother **56**(6): 296-301.
Lee, S. I., P. M. Hyun, et al. (2005). "Suppression of the onset and progression of collagen-induced arthritis by chebulagic acid screened from a natural product library." Arthritis Rheum **52**(1): 345-53.
Lee, S. J., J. H. Choi, et al. (1995). "Suppression of mouse lymphocyte proliferation in vitro by naturally-occurring biflavonoids." Life Sci **57**(6): 551-8.
Lee, W. R., S. C. Shen, et al. (2002). "Wogonin and fisetin induce apoptosis in human promyeloleukemic cells, accompanied by a decrease of reactive oxygen species, and activation of caspase 3 and Ca(2+)-dependent endonuclease." Biochem Pharmacol **63**(2): 225-36.
Lefebvre, V., C. Peeters-Joris, et al. (1990). "Modulation by interleukin 1 and tumor necrosis factor alpha of production of collagenase, tissue inhibitor of metalloproteinases and collagen types in differentiated and dedifferentiated articular chondrocytes." Biochim Biophys Acta **1052**(3): 366-78.
LeGrand, A., B. Fermor, et al. (2001). "Interleukin-1, tumor necrosis factor alpha, and interleukin-17 synergistically up-regulate nitric oxide and prostaglandin E2 production in explants of human osteoarthritic knee menisci." Arthritis Rheum **44**(9): 2078-83.

Leppert, D., E. Waubant, et al. (1995). "T cell gelatinases mediate basement membrane transmigration in vitro." J Immunol **154**(9): 4379-89.
Lesca, P., P. Lecointe, et al. (1978). "Ellipticines as potent inhibitors of aryl hydrocarbon hydroxylase: their binding to microsomal cytochromes P450 and protective effect against benzo(a)pyrene mutagenicity." Biochem Pharmacol **27**(8): 1203-9.
Levitz, S. M., A. Tabuni, et al. (1996). "Effects of interleukin-10 on human peripheral blood mononuclear cell responses to Cryptococcus neoformans, Candida albicans, and lipopolysaccharide." Infect Immun **64**(3): 945-51.
Lien TP, R. H. a. e. (1998). "Constitutients of Artocapus tokinensis." Pharmazie **5**(53): 353.
Likhitwitayawuid, K., K. Sawasdee, et al. (2002). "Flavonoids and stilbenoids with COX-1 and COX-2 inhibitory activity from Dracaena loureiri." Planta Med **68**(9): 841-3.
Lindhout, E., M. van Eijk, et al. (1999). "Fibroblast-like synoviocytes from rheumatoid arthritis patients have intrinsic properties of follicular dendritic cells." J Immunol **162**(10): 5949-56.
Lipovsky, M. M., G. Gekker, et al. (1998). "Cryptococcal glucuronoxylomannan induces interleukin (IL)-8 production by human microglia but inhibits neutrophil migration toward IL-8." J Infect Dis **177**(1): 260-3.
Liu, H. and R. M. Pope (2004). "Phagocytes: mechanisms of inflammation and tissue destruction." Rheum Dis Clin North Am **30**(1): 19-39, v.
Lorentzen, J. C., M. Andersson, et al. (1997). "Genetic analysis of inflammation, cytokine mRNA expression and disease course of relapsing experimental autoimmune encephalomyelitis in DA rats." J Neuroimmunol **80**(1-2): 31-7.
Lorentzen, J. C. and L. Klareskog (1996). "Susceptibility of DA rats to arthritis induced with adjuvant oil or rat collagen is determined by genes both within and outside the major histocompatibility complex." Scand J Immunol **44**(6): 592-8.
Lorenzo, J. A., C. C. Pilbeam, et al. (1992). "Production of both 92- and 72-kDa gelatinases by bone cells." Matrix **12**(4): 282-90.
Lubberts, E., M. I. Koenders, et al. (2005). "The role of T-cell interleukin-17 in conducting destructive arthritis: lessons from animal models." Arthritis Res Ther **7**(1): 29-37.
Lubberts, E., L. van den Bersselaar, et al. (2003). "IL-17 promotes bone erosion in murine collagen-induced arthritis through loss of the receptor activator of NF-kappa B ligand/osteoprotegerin balance." J Immunol **170**(5): 2655-62.
Lund, F. E., B. A. Garvy, et al. (2005). "Regulatory roles for cytokine-producing B cells in infection and autoimmune disease." Curr Dir Autoimmun **8**: 25-54.
Markovits, J., C. Linassier, et al. (1989). "Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II." Cancer Res **49**(18): 5111-7.
Martel-Pelletier, J., R. McCollum, et al. (1994). "Excess of metalloproteases over tissue inhibitor of metalloprotease may contribute to cartilage degradation in osteoarthritis and rheumatoid arthritis." Lab Invest **70**(6): 807-15.
Martel-Pelletier, J., D. J. Welsch, et al. (2001). "Metalloproteases and inhibitors in arthritic diseases." Best Pract Res Clin Rheumatol **15**(5): 805-29.
Matrisian, L. M. (1990). "Metalloproteinases and their inhibitors in matrix remodeling." Trends Genet **6**(4): 121-5.
Matzinger, P. (2002). "The danger model: a renewed sense of self." Science **296**(5566): 301-5.
McDougall, R., J. Sibley, et al. (1994). "Outcome in patients with rheumatoid arthritis receiving prednisone compared to matched controls." J Rheumatol **21**(7): 1207-13.
McIntyre, K. W., D. J. Shuster, et al. (1996). "Reduced incidence and severity of collagen-induced arthritis in interleukin-12-deficient mice." Eur J Immunol **26**(12): 2933-8.
Meyer, O. C. (1994). "Safety and security of Daflon 500 mg in venous insufficiency and in hemorrhoidal disease." Angiology **45**(6 Pt 2): 579-84.
Middleton, E., Jr. (1998). "Effect of plant flavonoids on immune and inflammatory cell function." Adv Exp Med Biol **439**: 175-82.

Middleton, E., Jr., C. Kandaswami, et al. (2000). "The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer." Pharmacol Rev **52**(4): 673-751.
Mikuls, T. R., J. R. Cerhan, et al. (2002). "Coffee, tea, and caffeine consumption and risk of rheumatoid arthritis: results from the Iowa Women's Health Study." Arthritis Rheum **46**(1): 83-91.
Mikuls, T. R. and J. O'Dell (2000). "The changing face of rheumatoid arthritis therapy: results of serial surveys." Arthritis Rheum **43**(2): 464-5.
Moldovan, F., J. P. Pelletier, et al. (1997). "Collagenase-3 (matrix metalloprotease 13) is preferentially localized in the deep layer of human arthritic cartilage in situ: in vitro mimicking effect by transforming growth factor beta." Arthritis Rheum **40**(9): 1653-61.
Monari, C., F. Bistoni, et al. (2005). "Glucuronoxylomannan, a microbial compound, regulates expression of costimulatory molecules and production of cytokines in macrophages." J Infect Dis **191**(1): 127-37.
Monari, C., E. Pericolini, et al. (2005). "Cryptococcus neoformans capsular glucuronoxylomannan induces expression of fas ligand in macrophages." J Immunol **174**(6): 3461-8.
Monari, C., C. Retini, et al. (2003). "Differences in outcome of the interaction between Cryptococcus neoformans glucuronoxylomannan and human monocytes and neutrophils." Eur J Immunol **33**(4): 1041-51.
Morgan, K., R. B. Clague, et al. (1981). "Native type II collagen--induced arthritis in the rat: the effect of complement depletion by cobra venom factor." Arthritis Rheum **24**(11): 1356-62.
Mottonen, T., P. Hannonen, et al. (2006). "Efficacy of combination therapy in rheumatoid arthritis: comment on the review by Smolen et al." Arthritis Rheum **54**(6): 2032-4; author reply 2034-5.
Murphy, J. W. and G. C. Cozad (1972). "Immunological unresponsiveness induced by cryptococcal capsular polysaccharide assayed by the hemolytic plaque technique." Infect Immun **5**(6): 896-901.
Murphy, J. W., A. Zhou, et al. (1997). "Direct interactions of human natural killer cells with Cryptococcus neoformans inhibit granulocyte-macrophage colony-stimulating factor and tumor necrosis factor alpha production." Infect Immun **65**(11): 4564-71.
Murray, T. J., X. Yang, et al. (2006). "Growth of a human mammary tumor cell line is blocked by galangin, a naturally occurring bioflavonoid, and is accompanied by down-regulation of cyclins D3, E, and A." Breast Cancer Res **8**(2): R17.
N.Q.Khang, A. D. S., J.Hoebeke (1988). "Purification and characterization of Artocarpus tonkinensis lectin." Lectins, Biology, Biochemistry, Clinical Biochemistry **6**: 341.
Nagase, H. and J. F. Woessner, Jr. (1999). "Matrix metalloproteinases." J Biol Chem **274**(31): 21491-4.
Nakajima, A., C. M. Seroogy, et al. (2001). "Antigen-specific T cell-mediated gene therapy in collagen-induced arthritis." J Clin Invest **107**(10): 1293-301.
Nakajima, H., H. Takamori, et al. (1990). "The effect of treatment with interferon-gamma on type II collagen-induced arthritis." Clin Exp Immunol **81**(3): 441-5.
Nakamura, K., A. Malykhin, et al. (2002). "The Src homology 2 domain-containing inositol 5-phosphatase negatively regulates Fcgamma receptor-mediated phagocytosis through immunoreceptor tyrosine-based activation motif-bearing phagocytic receptors." Blood **100**(9): 3374-82.
Nam, N. H., H. M. Kim, et al. (2003). "Inhibitory effects of Vietnamese medicinal plants on tube-like formation of human umbilical venous cells." Phytother Res **17**(2): 107-11.
Nam, N. H., C. W. Lee, et al. (2003). "Antiinvasive, antiangiogenic and antitumour activity of Ephedra sinica extract." Phytother Res **17**(1): 70-6.
Nanki, T., K. Nagasaka, et al. (2001). "Chemokines regulate IL-6 and IL-8 production by fibroblast-like synoviocytes from patients with rheumatoid arthritis." J Immunol **167**(9): 5381-5.
Nemec, P., M. Goldbergova, et al. (2006). "[Polymorphism of gene promotor region for MMP-2 in rheumatoid arthritis]." Vnitr Lek **52**(4): 348-54.

Newsome, G. (2002). "Guidelines for the management of rheumatoid arthritis: 2002 update." J Am Acad Nurse Pract **14**(10): 432-7.
Nguyen, M. T., S. Awale, et al. (2004). "Xanthine oxidase inhibitory activity of Vietnamese medicinal plants." Biol Pharm Bull **27**(9): 1414-21.
Nguyen, V. D. (1999). Traditional pharmaceutical activities in Vietnam and their possible development., Hanoi: The Gioi Publishers .
Nielen, M. M., D. van Schaardenburg, et al. (2004). "Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors." Arthritis Rheum **50**(2): 380-6.
Nigdikar, S. V., N. R. Williams, et al. (1998). "Consumption of red wine polyphenols reduces the susceptibility of low-density lipoproteins to oxidation in vivo." Am J Clin Nutr **68**(2): 258-65.
Nishioka, K., T. Hasunuma, et al. (1998). "Apoptosis in rheumatoid arthritis: a novel pathway in the regulation of synovial tissue." Arthritis Rheum **41**(1): 1-9.
Norberg, A., N. K. Hoa, et al. (2004). "A novel insulin-releasing substance, phanoside, from the plant Gynostemma pentaphyllum." J Biol Chem **279**(40): 41361-7.
O'Dell, J. R. (2004). "Therapeutic strategies for rheumatoid arthritis." N Engl J Med **350**(25): 2591-602.
Ohashi, M., E. Sugikawa, et al. (1995). "Inhibition of p53 protein phosphorylation by 9-hydroxyellipticine: a possible anticancer mechanism." Jpn J Cancer Res **86**(9): 819-27.
Opal, S. M. and V. A. DePalo (2000). "Anti-inflammatory cytokines." Chest **117**(4): 1162-72.
Organization, W. H. (2004). "WHO guilines for the developing consumer information on proper use of trasional medicine and complementary/alternative medicine."
Orrenius, S. (2004). "Mitochondrial regulation of apoptotic cell death." Toxicol Lett **149**(1-3): 19-23.
Osman, G. E., M. C. Hannibal, et al. (1999). "T-cell receptor vbeta deletion and valpha polymorphism are responsible for the resistance of SWR mouse to arthritis induction." Immunogenetics **49**(9): 764-72.
Panayi, G. S., V. M. Corrigall, et al. (2001). "Pathogenesis of rheumatoid arthritis. The role of T cells and other beasts." Rheum Dis Clin North Am **27**(2): 317-34.
Paoletti, C., J. B. Le Pecq, et al. (1980). "Antitumor activity, pharmacology, and toxicity of ellipticines, ellipticinium, and 9-hydroxy derivatives: preliminary clinical trials of 2-methyl-9-hydroxy ellipticinium (NSC 264-137)." Recent Results Cancer Res **74**: 107-23.
Paoletti, C., C. Lesca, et al. (1979). "Ellipticine and derivatives induce breakage of L1210 cells DNA in vitro." Biochem Pharmacol **28**(3): 345-50.
Pap, T., U. Muller-Ladner, et al. (2000). "Fibroblast biology. Role of synovial fibroblasts in the pathogenesis of rheumatoid arthritis." Arthritis Res **2**(5): 361-7.
Park, K. H., Y. D. Park, et al. (2006). "Anti-atherosclerotic and anti-inflammatory activities of catecholic xanthones and flavonoids isolated from Cudrania tricuspidata." Bioorg Med Chem Lett.
Pearson, C. M. (1956). "Development of arthritis, periarthritis and periostitis in rats given adjuvants." Proc Soc Exp Biol Med **91**(1): 95-101.
Perharic, L., D. Shaw, et al. (1993). "Toxic effects of herbal medicines and food supplements." Lancet **342**(8864): 180-1.
Perlman, H., L. J. Pagliari, et al. (2001). "Rheumatoid arthritis synovial macrophages express the Fas-associated death domain-like interleukin-1beta-converting enzyme-inhibitory protein and are refractory to Fas-mediated apoptosis." Arthritis Rheum **44**(1): 21-30.
Pillinger, M. H. and S. B. Abramson (1995). "The neutrophil in rheumatoid arthritis." Rheum Dis Clin North Am **21**(3): 691-714.
Pincus, T. and L. F. Callahan (1993). "The 'side effects' of rheumatoid arthritis: joint destruction, disability and early mortality." Br J Rheumatol **32 Suppl 1**: 28-37.
Pincus, T., S. B. Marcum, et al. (1992). "Longterm drug therapy for rheumatoid arthritis in seven rheumatology private practices: II. Second line drugs and prednisone." J Rheumatol **19**(12): 1885-94.

Plenge, R. M., L. Padyukov, et al. (2005). "Replication of putative candidate-gene associations with rheumatoid arthritis in >4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4." Am J Hum Genet **77**(6): 1044-60.
Randolph, G. J. (2001). "Dendritic cell migration to lymph nodes: cytokines, chemokines, and lipid mediators." Semin Immunol **13**(5): 267-74.
Rangan, G. K., Y. Wang, et al. (1999). "Inhibition of NFkappaB activation with antioxidants is correlated with reduced cytokine transcription in PTC." Am J Physiol **277**(5 Pt 2): F779-89.
Rantapaa-Dahlqvist, S., B. A. de Jong, et al. (2003). "Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis." Arthritis Rheum **48**(10): 2741-9.
Retini, C., T. R. Kozel, et al. (2001). "Interdependency of interleukin-10 and interleukin-12 in regulation of T-cell differentiation and effector function of monocytes in response to stimulation with Cryptococcus neoformans." Infect Immun **69**(10): 6064-73.
Retini, C., A. Vecchiarelli, et al. (1998). "Encapsulation of Cryptococcus neoformans with glucuronoxylomannan inhibits the antigen-presenting capacity of monocytes." Infect Immun **66**(2): 664-9.
Robak, J. and R. J. Gryglewski (1996). "Bioactivity of flavonoids." Pol J Pharmacol **48**(6): 555-64.
Saag, K. G., R. Koehnke, et al. (1994). "Low dose long-term corticosteroid therapy in rheumatoid arthritis: an analysis of serious adverse events." Am J Med **96**(2): 115-23.
Salmon, M., D. Scheel-Toellner, et al. (1997). "Inhibition of T cell apoptosis in the rheumatoid synovium." J Clin Invest **99**(3): 439-46.
Saren, P., H. G. Welgus, et al. (1996). "TNF-alpha and IL-1beta selectively induce expression of 92-kDa gelatinase by human macrophages." J Immunol **157**(9): 4159-65.
Scheinfeld, N. (2004). "A comprehensive review and evaluation of the side effects of the tumor necrosis factor alpha blockers etanercept, infliximab and adalimumab." J Dermatolog Treat **15**(5): 280-94.
Schellekens, G. A., B. A. de Jong, et al. (1998). "Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies." J Clin Invest **101**(1): 273-81.
Schirmer, M., A. N. Vallejo, et al. (1998). "Resistance to apoptosis and elevated expression of Bcl-2 in clonally expanded CD4+CD28- T cells from rheumatoid arthritis patients." J Immunol **161**(2): 1018-25.
Scofield, R. H. (2004). "Autoantibodies as predictors of disease." Lancet **363**(9420): 1544-6.
Sehlstedt, U., P. Aich, et al. (1998). "Interactions of the antiviral quinoxaline derivative 9-OH-B220 [2, 3-dimethyl-6-(dimethylaminoethyl)- 9-hydroxy-6H-indolo-[2, 3-b]quinoxaline] with duplex and triplex forms of synthetic DNA and RNA." J Mol Biol **278**(1): 31-56.
Shin, S. S., M. Jin, et al. (2003). "Suppressive effects of PG201, an ethanol extract from herbs, on collagen-induced arthritis in mice." Rheumatology (Oxford) **42**(5): 665-72.
Shoham, S., C. Huang, et al. (2001). "Toll-like receptor 4 mediates intracellular signaling without TNF-alpha release in response to Cryptococcus neoformans polysaccharide capsule." J Immunol **166**(7): 4620-6.
Shouda, T., T. Yoshida, et al. (2001). "Induction of the cytokine signal regulator SOCS3/CIS3 as a therapeutic strategy for treating inflammatory arthritis." J Clin Invest **108**(12): 1781-8.
Silman, A. J., A. J. MacGregor, et al. (1993). "Twin concordance rates for rheumatoid arthritis: results from a nationwide study." Br J Rheumatol **32**(10): 903-7.
Simon, A. K., E. Seipelt, et al. (1994). "Divergent T-cell cytokine patterns in inflammatory arthritis." Proc Natl Acad Sci U S A **91**(18): 8562-6.
Smolen, J. S., J. R. Kalden, et al. (1999). "Efficacy and safety of leflunomide compared with placebo and sulphasalazine in active rheumatoid arthritis: a double-blind,

randomised, multicentre trial. European Leflunomide Study Group." Lancet **353**(9149): 259-66.
Son, Y. O., S. H. Kook, et al. (2006). "Quercetin, a bioflavonoid, accelerates TNF-alpha-induced growth inhibition and apoptosis in MC3T3-E1 osteoblastic cells." Eur J Pharmacol **529**(1-3): 24-32.
Stadelmann, C. and H. Lassmann (2000). "Detection of apoptosis in tissue sections." Cell Tissue Res **301**(1): 19-31.
Stasiuk, L. M., O. Abehsira-Amar, et al. (1996). "Collagen-induced arthritis in DBA/1 mice: cytokine gene activation following immunization with type II collagen." Cell Immunol **173**(2): 269-75.
Strand, V., S. Cohen, et al. (1999). "Treatment of active rheumatoid arthritis with leflunomide compared with placebo and methotrexate. Leflunomide Rheumatoid Arthritis Investigators Group." Arch Intern Med **159**(21): 2542-50.
Stuart, J. M., M. A. Cremer, et al. (1982). "Type II collagen-induced arthritis in rats. Passive transfer with serum and evidence that IgG anticollagen antibodies can cause arthritis." J Exp Med **155**(1): 1-16.
Stuart, J. M., K. Tomoda, et al. (1983). "Serum transfer of collagen-induced arthritis. II. Identification and localization of autoantibody to type II collagen in donor and recipient rats." Arthritis Rheum **26**(10): 1237-44.
Stuart, J. M., A. S. Townes, et al. (1984). "Collagen autoimmune arthritis." Annu Rev Immunol **2**: 199-218.
Sukardiman, A. Darwanto, et al. (2000). "Cytotoxic mechanism of flavonoid from Temu Kunci (Kaempferia pandurata) in cell culture of human mammary carcinoma." Clin Hemorheol Microcirc **23**(2-4): 185-90.
Syme, R. M., T. F. Bruno, et al. (1999). "The capsule of Cryptococcus neoformans reduces T-lymphocyte proliferation by reducing phagocytosis, which can be restored with anticapsular antibody." Infect Immun **67**(9): 4620-7.
Syme, R. M., J. C. Spurrell, et al. (2002). "Primary dendritic cells phagocytose Cryptococcus neoformans via mannose receptors and Fcgamma receptor II for presentation to T lymphocytes." Infect Immun **70**(11): 5972-81.
Symmons, D. P. (2002). "Epidemiology of rheumatoid arthritis: determinants of onset, persistence and outcome." Best Pract Res Clin Rheumatol **16**(5): 707-22.
Symmons, D. P., C. R. Bankhead, et al. (1997). "Blood transfusion, smoking, and obesity as risk factors for the development of rheumatoid arthritis: results from a primary care-based incident case-control study in Norfolk, England." Arthritis Rheum **40**(11): 1955-61.
Takagi, T. and H. E. Jasin (1992). "Interactions of synovial fluid immunoglobulins with chondrocytes." Arthritis Rheum **35**(12): 1502-9.
Tran, Q. L., Y. Tezuka, et al. (2003). "In vitro antiplasmodial activity of antimalarial medicinal plants used in Vietnamese traditional medicine." J Ethnopharmacol **86**(2-3): 249-52.
Trentham, D. E., A. S. Townes, et al. (1977). "Autoimmunity to type II collagen an experimental model of arthritis." J Exp Med **146**(3): 857-68.
Turesson, C., D. J. Schaid, et al. (2005). "The impact of HLA-DRB1 genes on extra-articular disease manifestations in rheumatoid arthritis." Arthritis Res Ther **7**(6): R1386-93.
Ueda, J. Y., Y. Tezuka, et al. (2002). "Antiproliferative activity of Vietnamese medicinal plants." Biol Pharm Bull **25**(6): 753-60.
Unemori, E. N., M. S. Hibbs, et al. (1991). "Constitutive expression of a 92-kD gelatinase (type V collagenase) by rheumatoid synovial fibroblasts and its induction in normal human fibroblasts by inflammatory cytokines." J Clin Invest **88**(5): 1656-62.
van Boekel, M. A., E. R. Vossenaar, et al. (2002). "Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value." Arthritis Res **4**(2): 87-93.
Van Boxel, J. A. and S. A. Paget (1975). "Predominantly T-cell infiltrate in rheumatoid synovial membranes." N Engl J Med **293**(11): 517-20.
van Venrooij, W. J., J. M. Hazes, et al. (2002). "Anticitrullinated protein/peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis." Neth J Med **60**(10): 383-8.

Vecchiarelli, A. (2000). "Immunoregulation by capsular components of Cryptococcus neoformans." Med Mycol **38**(6): 407-17.
Vecchiarelli, A., D. Pietrella, et al. (2003). "The polysaccharide capsule of Cryptococcus neoformans interferes with human dendritic cell maturation and activation." J Leukoc Biol **74**(3): 370-8.
Vecchiarelli, A., C. Retini, et al. (1996). "Purified capsular polysaccharide of Cryptococcus neoformans induces interleukin-10 secretion by human monocytes." Infect Immun **64**(7): 2846-9.
Vecchiarelli, A., C. Retini, et al. (1995). "Downregulation by cryptococcal polysaccharide of tumor necrosis factor alpha and interleukin-1 beta secretion from human monocytes." Infect Immun **63**(8): 2919-23.
Verbeek, R., A. C. Plomp, et al. (2004). "The flavones luteolin and apigenin inhibit in vitro antigen-specific proliferation and interferon-gamma production by murine and human autoimmune T cells." Biochem Pharmacol **68**(4): 621-9.
Verdrengh, M., I. M. Jonsson, et al. (2003). "Genistein as an anti-inflammatory agent." Inflamm Res **52**(8): 341-6.
Wang, I. K., S. Y. Lin-Shiau, et al. (1999). "Induction of apoptosis by apigenin and related flavonoids through cytochrome c release and activation of caspase-9 and caspase-3 in leukaemia HL-60 cells." Eur J Cancer **35**(10): 1517-25.
Watson, W. C., P. S. Brown, et al. (1987). "Passive transfer studies with type II collagen antibody in B10.D2/old and new line and C57Bl/6 normal and beige (Chediak-Higashi) strains: evidence of important roles for C5 and multiple inflammatory cell types in the development of erosive arthritis." Arthritis Rheum **30**(4): 460-5.
Watson, W. C., M. A. Cremer, et al. (1986). "Assessment of the potential pathogenicity of type II collagen autoantibodies in patients with rheumatoid arthritis. Evidence of restricted IgG3 subclass expression and activation of complement C5 to C5a." Arthritis Rheum **29**(11): 1316-21.
Wenzel, U., S. Kuntz, et al. (2000). "Dietary flavone is a potent apoptosis inducer in human colon carcinoma cells." Cancer Res **60**(14): 3823-31.
Woo, K. J., J. H. Lim, et al. (2006). "Differential inhibitory effects of baicalein and baicalin on LPS-induced cyclooxygenase-2 expression through inhibition of C/EBPbeta DNA-binding activity." Immunobiology **211**(5): 359-68.
You, Y. J., N. H. Nam, et al. (2003). "Antiangiogenic activity of lupeol from Bombax ceiba." Phytother Res **17**(4): 341-4.
Zhang, Z. and S. L. Bridges, Jr. (2001). "Pathogenesis of rheumatoid arthritis. Role of B lymphocytes." Rheum Dis Clin North Am **27**(2): 335-53.
Zheng, Y. Q. and W. Wei (2005). "Total glucosides of paeony suppresses adjuvant arthritis in rats and intervenes cytokine-signaling between different types of synoviocytes." Int Immunopharmacol **5**(10): 1560-73.
Zucker, S., R. M. Lysik, et al. (1994). "Elevated plasma stromelysin levels in arthritis." J Rheumatol **21**(12): 2329-33.