ISSN 1671-4083
Shanghai Institute of Materia Medica
Chinese Academy of Sciences
http://www.ChinaPhar.com

Immunomodulatory effects and mechanisms of plant alkaloid tetrandrine in autoimmune diseases[1]

LAI Jenn-Haung[2]

Rheumatology/Immunology and Allergy, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, China

KEY WORDS tetrandrine; T-lymphocytes; autoimmune diseases; alkaloids

ABSTRACT

Autoimmune diseases characterized by activation of immune effector cells and damage of target organs are currently treated with a combination of several disease-modifying antirheumatic drugs (DMARDs) that preserve different immunomodulatory mechanisms. Such a combination treatment strategy not only provides synergistic effects but also reduces side effects from individual drug. Tetrandrine (Tet), purified from a creeper *Stephania tetrandra* S Moore, is a *bis*-benzylisoquinoline alkaloid and has been used to treat patients with silicosis, autoimmune disorders, and hypertension in Mainland China for decades. The accumulated studies both *in vitro* and *in vivo* reveal that Tet preserves a wide variety of immunosuppressive effects. Importantly, the Tet-mediated immunosuppressive mechanisms are evidently different from some known DMARDs. The synergistic effects have also been demonstrated between Tet and other DMARDs like FK506 and cyclosporin. These results highlight Tet a very potential candidate to be considered as one of DMARDs in the treatment of autoimmune diseases, especially rheumatoid arthritis. This review summarizes evidence-based *in vivo* and *in vitro* studies on this potential Chinese immunosuppressive herb.

INTRODUCTION

The immune system is a well-organized and well-regulated system and its dysregulation may lead to the development of autoimmune diseases. The proto-type of such a group of illnesses is rheumatoid arthritis (RA), a disease with progressive and massive destruction of joints accompanied with or without other organ involvement. Although many contributing factors were considered to play roles in causing rheumatoid arthritis, the etiology remains unclear. While no single agent was proven to be enough to control disease progression, current acceptable therapy for rheumatoid arthritis is aimed to attenuate disease activity with a combination of disease-modifying antirheumatic drugs (DMARDs) such as methotrexate, sulfasalazine, leflunomide, hydroxychloroquine, cyclosporin, gold, azathioprine… *etc*[1-3]. The purpose of a combination therapy is to obtain synergistic therapeutic effects of drugs with different immunomodulatory mechanisms and, in the meantime, to reduce side effects from each drug by decreasing their dosages.

Along the way of investigating the pathogenesis

[1] Project supported by National Health Research Institutes, Taiwan, China. (NHRI-GT-EX89B915C) and Tri-Service General Hospital (TSGH-C91-13).
[2] Correspondence to Prof LAI Jenn-Haung, MD, PhD. *Rheumatology/Immunology and Allergy, Tri-Service General Hospital, No. 325, Sec. 2, Cheng-Kung Rd., Neihu 114, Taipei, Taiwan, China*. Phn 886-2-8792-7135. Fax 886-2-8792-7136.
E-mail haungben@tpts5.seed.net.tw
Received 2002-09-12 Accepted 2002-10-17

of autoimmune diseases, the activated immune effector cells such as T cells, B cells, monocytes/macrophages, and dendritic cells have been consistently found in the peripheral blood and the involved organs. Among these immune effector cells, T cells have been considered to be the most important because the regulation of T cell activation requires antigen specificity and a great amount of cytokines released in autoimmune responses are from T cells[4-7].

It is clear that full activation of T cells requires the integration of two signals: one is from a T cell receptor signal and the other is from a costimulatory signal[8]. Among the molecules expressing costimulatory activities on T cells, only CD28 could in combination with the activation of T cell receptor induce detectable levels of interleukin-2 and prevent anergy, a status of un-responsiveness[9,10]. The importance of CD28 costimulation in T cell activation is substantiated by the accumulated data showing that 1) blockade of CD28 costimulatory signal leads to the reduction of disease severity and proteinuria as well as the prolongation of survival in lupus-prone mice[11]; 2) blockade of CD28 costimulation results in the increased survival of the transplanted organs such as heart, kidney, bone marrow and pancreas[12-16]; 3) enhanced activation of CD28 by immobilized anti-CD28 monoclonal antibodies (mAbs) leads to the reduction of virus load as well as the expansion of $CD4^+$ T cells in HIV-infected patients[17]; 4) blockade of CD28 costimulation causes improvement of symptoms in psoriasis patients[18]; 5) introduction of CD28 costimulation elicits tumor rejection in animal models[19-21].

Aside from the activation of immune effector cells, the defective apoptosis (programmed cell death) mechanism also plays a crucial role in disease progression of RA and other autoimmune diseases[22-25]. Apoptosis is a natural protective mechanism for embryogenesis, for thymic organ to eliminate inappropriate T cells and for immune-privilege sites to protect from inflammatory cell invasion[26,27]. Different from the necrotic process, the apoptotic process does not induce any inflammatory response because dead cells or their degraded products are rapidly phagocytosed before any leakage of cellular contents. Since the etiology of autoimmune diseases is largely unknown, the immune reaction towards the apoptotic bodies released or apoptotic antigens expressed from dead cells has been implied as one of the mechanisms leading to autoimmune diseases[28-31]. After development of autoimmune diseases, fail to execute the appropriate apoptotic program may result in sustention of inflammatory process[32-38]. In light of the significance of apoptotic process, the apoptosis-based therapy has been suggested as one of the approaches to control the progression of autoimmune diseases[39].

Altogether, both the inappropriate activation of immune effector cells and the ineffective deletion (through apoptosis) of these cells may lead to the development and progression of autoimmune disorders (Fig 1). The therapeutic approaches for autoimmune diseases may rely on both the inhibition of cell activation and the maintenance or enhancement of the apoptotic program of immune effector cells.

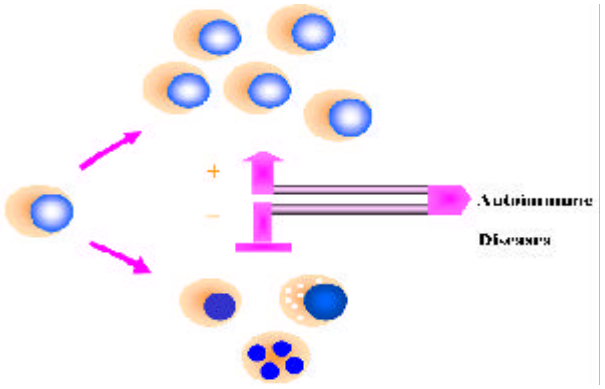


Fig 1. Regulation of immune effector cells leads to autoimmune diseases. Two major pathways contribute to the development of autoimmune diseases; one is the over-expanded proliferation or activation of immune effector cells and the other is the defective execution of the apoptotic program of immune effector cells, especially those already being activated cells.

Although the significance of T cells in autoimmune pathogenesis is very clear, the T-cell directed biological therapies for RA has been unsuccessful due to both lack of efficacy and serious side effects. The use of pharmacological agents such as cyclosporin A and leflunomide that block T cell activation and proliferation give more promising therapeutic outcomes (reviewed in [40]). Han-Fang-Chi is the dried tuberous root of the creeper *Stephania tetrandra* S Moore. The purification of Han-Fang-Chi yields an active ingredient tetrandrine (Tet) that accounts for its major biological activities[41]. Tet has been used in China for several decades to treat patients with silicosis and rheumatic diseases that are associated with the activation and infiltration of immune effector cells at lesion sites. In a clinical study of Tet effects in silicosis patients, the administration of 200 mg or 300 mg per day with aver-

agely total 120 gm(s) of Tet to silicosis patients results in significant improvement of pulmonary function and reduces the progression of silicosis[42]. Additionally, such a therapeutic strategy only causes limited side effects during the three-year follow-up. These side effects include abdominal distension, diarrhea, dry eye, itching, hyperpigmentation and mildly elevated liver enzymes. All these symptoms and signs resolve spontaneously after discontinuance of the medication[42]. This clinical observation highlights not only the effectiveness but also the acceptable tolerance of Tet for clinical use.

EFFECTS ON T CELLS

The early observation that Tet inhibits delayed-type hypersensitivity (DTH) responses in mice model suggests that Tet may have some direct effects on T cells[43]. Subsequently, Kondo *et al*[44] showed that Tet inhibited plaque-forming cell response to a T-cell-dependent antigen, sheep red blood cell, but it has no effect on such a response to a T-cell-independent antigen, lipopolysaccharide. Furthermore, the severity of arthritis is greatly reduced by late or prophylactic administration of Tet in an adjuvant-induced chronic arthritis model of rats[45].

Based upon these observations, a series of studies examining directly the effects of Tet on T cells were performed. In purified human peripheral blood T cells, Tet inhibits the CD28-costimulated T cell proliferation and cytokine production. Both T helper 1 (Th1) and Th2 cytokines are susceptible to Tet suppression[46]. However, Tet may or may not directly down-regulate CD28 signaling pathway because T cell receptor-mediated signaling events can also be inhibited by Tet. The investigation of Tet-regulated T cell receptor downstream signaling pathways clearly shows that Tet specifically inhibits protein kinase C-dependent but not calcium-dependent signaling events[47]. In addition, these observations suggest that the immunosuppressive effects of Tet may not relate to its calcium-channel blocking properties[47]. Since there are more than 11 protein kinase C isoenzymes identified so far[48], whether Tet targets one or several protein kinase C isoenzyme-mediated signaling pathways is currently not known. Because the activation of protein kinase C isoenzymes can readily be detected in many signaling pathways in a variety of immune and non-immune effector cells, these results also partly explain the broad-spectrum anti-inflammatory properties of Tet[48,49]. Molecular dissection of Tet-targeting signaling molecules showed that Tet effectively inhibited CD28-costimulated NF-κB activities and had ignored effects on AP-1 activities (unpublished observations). These observations conclude that Tet could down-regulate the activation of T cell receptor through at least blocking protein kinase C and NF-κB signaling pathways (Fig 2).

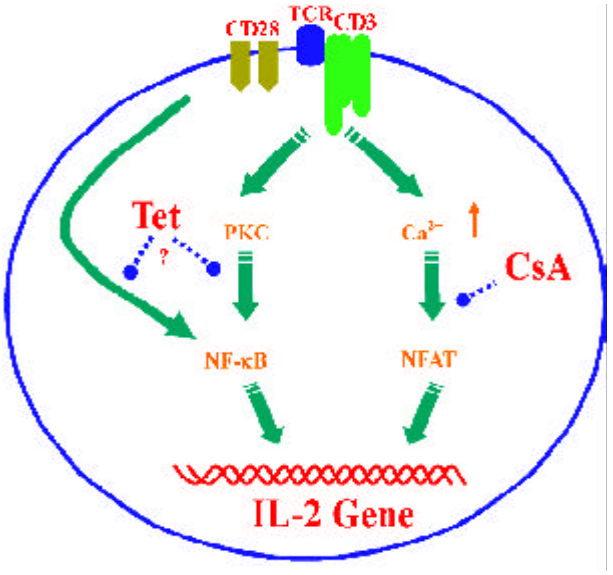


Fig 2. Tetrandrine targets protein kinase C-NF-k B signaling pathway. The T cell receptor signal is conducted via the CD3 complex that contains several subunits. This signal then causes both increase of intracellular calcium and activation of protein kinase C isoenzymes. The increase of intracellular calcium leads to the activation of nuclear factor of activated T cells (NFAT) and the activation of protein kinase C results in the stimulation of NF-k B transcription factors. CD28 costimulation has unique effects, distinct from those through T cell receptor, on the activation of NF-k B. Tet is supposed to inhibit the T cell receptor-mediated protein kinase C-NF-k B signaling pathway. Whether Tet has direct effect on CD28-mediated NF-k B activation is currently unclear. In contrast, cyclosporin is a suppressor of calcium-dependent NFAT activation.

Both *in vivo* and *in vitro* studies also suggest that the T cell receptor signaling pathway targeted by Tet is different from that targeted by cyclosporin that inhibits Ca^{2+}-dependent calcineurin activity[46,50]. Importantly, the combination of both Tet and cyclosporin provides a synergism at much lower therapeutic concentrations of each drug[47]. Indeed, in diabetes animal models, Lieberman *et al*[51] also showed that Tet in combination with FK506, an immunosuppressive agent functionally similar to but more potent than cyclosporin, synergistically prevent the development of diabetes.

EFFECTS ON OTHER IMMUNE EFFECTOR CELLS

The immunosuppressive effects of Tet could also be demonstrated in other immune effector cells, including macrophages, monocytes, B cells, neutrophils, and mast cells. At therapeutic concentrations, Tet inhibits the production of tumor necrosis factor alpha (TNF-α) from monocytes stimulated with killed *Staphylococcus aureus*[52]. Both fangchinoline and isotetrandrine, two Tet analogues, also inhibit interleukin-1 (IL-1) and TNF-α production from *Staphylococcus aureus* Cowan 1-stimulated human peripheral blood mononuclear cells[53]. Such an effect is mediated at least through transcriptional regulation of mRNA expression of these cytokine genes. Further expansion of these observations demonstrates that Tet inhibits not only IL-1 and TNF-α but also other cytokines like IL-6 and IL-8 production from activated monocytes as well as inhibits the antibody production from activated B cells[54]. Moreover, Tet significantly blocks the extent of inflammation of uveitis induced by endotoxin and IL-1α administration in rats[55]. Therefore, aside from the inhibition of cytokine production, Tet also suppresses cytokine-mediated tissue damage, a suggestion that Tet may directly block cytokine-mediated signaling pathways. The results of these studies may somehow be explained in part by an early observation showing that Tet significantly inhibits the production of nitric oxide, a critical mediator of inflammation, in lipopolysaccharide-stimulated macrophages[56]. In cultured human umbilical vein endothelial cells, Tet dose-dependently inhibits the secretion of a chemotactic factor and then blocks the migration of monocytes[57]. In addition, Tet also suppresses the IL-1α and platelet-derived growth factor (PDGF)-induced tube formation of rat vascular endothelial cells, an important step in angiogenesis[58]. The inhibitory potency is measured to be about 100-fold stronger than hydrocortisone[58].

With regard to innate immune responses, Tet has been shown to down-regulate tumor promoter and phorbol myristate acetate-induced adherence of neutrophils[59]. The phagocytic effects of neutrophils are also greatly inhibited by Tet[54]. In an animal model of myocardial ischaemia-reperfusion injury, Tet effectively suppresses the up-regulation of Mac-1 on neutrophils and therefore blocks their migration and reduces the injury-induced myocardial infarct size and ventricular tachyarrhythmia[60].

INDUCTION OF PROGRAMMED CELL DEATH (APOPTOSIS)

Since the apoptotic process is defective in autoimmune diseases[33-35], any drug that could potentially induce the apoptosis of activated immune effector cells may have its additional advantage to control the inappropriate expansion of immune responses. Several Western anti-rheumatic drugs such as corticosteroid, nonsteroidal anti-inflammatory drugs and hydroxychloroquine, and a Chinese anti-rheumatic drug, *Tripterygium wilfordii* Hook f, have been shown to preserve the capacity of inducing cellular apoptosis[61-64]. Reasonably, the apoptotic effects of Tet may also play a role in the control of autoimmune disorders.

Tet at 0.1 μmol/L to 100 μmol/L concentration-dependently reduces the viability of mouse peritoneal macrophages, guinea-pig alveolar macrophages and mouse macrophage-like J774 cells[65]. In the example of other permanent cell lines, U937 and HL-60, Tet also dose-dependently inhibits cellular proliferation and induces cellular apoptosis[66,67]. In contrast to the cellular apoptosis induced by glucocorticoids in CEM-C7 cells, the induction of apoptosis by Tet is much more rapid (40 h compared to 4 h, respectively)[68]. In addition, the Tet-induced cellular apoptosis appears not to require *de novo* protein synthesis[68]. A finding suggests that glucocorticoid-induced and Tet-induced cellular apoptosis may be mediated through different mechanisms. Aside from the results shown in immortal cell lines and murine cells, Tet could also induce the apoptotic program in human peripheral blood lymphocytes[67].

Consistent with the results in other immune effector cells, Tet effectively causes apoptosis of human peripheral blood T cells[47,69]. Importantly, compared to resting T cells, Tet preserves much stronger killing capacity towards activated T cells[69]. When drug-induced apoptosis was readily compared, the results suggest that the Tet-induced T cell apoptotic mechanism is different from the one induced by hydroxychloroquine, a commonly used Western antirheumatic drug[69]. While Tet-induced T cell apoptotic DNA damage requires caspase-3 activity, hydroxychloroquine-induced T cell apoptotic DNA damage is mediated through a caspase-3- and caspase-8-independent but Z-Asp-Glu-Val-Asp. fluomethyl ketone-sensitive signaling pathway[69]. The differential usages of caspase-mediated apoptotic

mechanisms by Tet and hydroxychloroquine may also provide a possible synergism of prescribing both drugs for the treatment of autoimmune diseases.

ANTI-OXIDATIVE EFFECTS

One of the important effects of Tet that may explain many of its biological activities is the anti-oxidative property. In measuring both hypoxanthine/xanthine oxidase and erythrocytes auto-oxidation systems, Tet effectively scavenges the generated superoxide anions at concentrations from 7.5 to 15 μmol/L[70]. Other evidence also shows that Tet inhibits freshly fractured quartz-induced lipid peroxidation[71]. In evaluation of active oxygen production from neutrophils, the results indicate that the generation of superoxide and luminol-dependent chemiluminescence induced by opsonized zymosan, arachidonic acid, formylmethionyl-leucyl-phenylalanine, or by phorbol myristate acetate decreases significantly when Tet or its analogues are added into the reaction[72]. In addition, Tet inhibits hexose-monophosphate shunt activity and hydrogen peroxide production in neutrophils[73]. The anti-oxidative reaction rate constant of Tet with *OH is determined to be 1.4×10^{10} $mol^{-1}\cdot s^{-1}$, a value that is comparable with other anti-oxidants such as ascorbate, glutathione, and cysteine [74]. When human mononuclear cells were examined, Tet greatly inhibits irradiation-induced superoxide production from these cells[75]. In animal models, via inhibiting Ca^{2+} influx and reactive oxygen species formation, Tet suppresses neutrophil adhesion to fibrinogen[76]. Part of the effects is mediated through G protein modulation that prevents Mac-1 up-regulation in neutrophil activation[77].

STRUCTURAL MODIFICATION

A great potential for Tet being one of the DMARDs is the advantage that there are several structurally similar Tet analogues. Importantly, the subtle structural difference among Tet analogues appears to have great impact on their differential immunosuppressive activities. Based upon their structural similarity and the difference in biological activity, structural modification of this group of drugs can lead to the discovery or development of more potent yet less toxic immunosuppressive drugs.

Tet and berbamine are two purified plant alkaloid analogues. Differ by only one substitution in the side chain of one of the benzene rings, these two drugs preserve quite different immunosuppressive activities. For example, Tet but not berbamine inhibits the chronic inflammation of adjuvant-induced arthritis in rats[45]. The incidence of relapsing experimental allergic encephalitis (EAE) is reduced by 41 % and 65 % for Tet and berbamine treatment, respectively[78]. Tet is shown to preserve 6-18 times more potent than berbamine in suppressing the production of IL-1 and TNF-α from the stimulated monocytes and macrophages as well as the production of TNF-β by activated lymphocytes[79]. Investigation of the direct cytokine effects in rat subcutaneous air pouch model of inflammation reveals that both compounds are equipotent in suppressing leukocyte infiltration into air pouches induced by IL-1 and TNF[80]. Nevertheless, Tet is more potent than berbamine as a suppressant of platelet activating factor-induced mononuclear cell infiltration and is less effective than berbamine in carageenen-induced polymorphonuclear cell infiltration[80]. While Tet has stronger suppressive effects on adherence and locomotion of neutrophils, berbamine preserves greater capacity for inhibition of natural killer cell cytotoxicity[81]. Along the arachidonic acid metabolizing pathway, Tet but not berbamine potently inhibits the lipooxygenase-dependent leukotriene production from activated human monocytes and neutrophils. However, both compounds were equally potent in suppressing cyclooxygenase-dependent prostaglandin generation[82]. In the context of T cell response, both Tet and berbamine are equipotent in suppressing the induction and expression phases of DTH responses to sheep red blood cell antigens in mice[83]. When human peripheral blood T cells were examined, the results also indicate that both Tet and berbamine equipotently block CD28-costimulated T cell activities, including cytokine production, cell surface activation marker expression as well as cell proliferation[46]. This study also identifies dauricine as the most potent one among Tet analogues that inhibit CD28-costimulated T cell activities[46]. Surprisingly, the accumulated evidence also suggests that Tet but not berbamine preserves strong anti-oxidative activity[72, 81]. The results in a series of these studies are summarized in Tab 1.

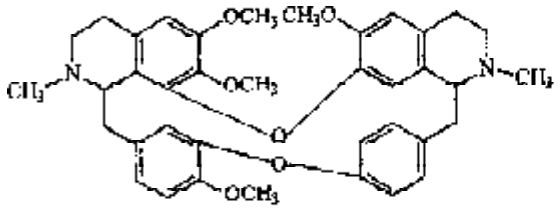
CONCLUSION

Tet preserves very extensive immunosuppressive effects through inhibiting the activation of various immune effector cells and inducing the apoptosis of these cells. The molecular target of Tet is currently unknown, but likely to be involved in T cell receptor – protein

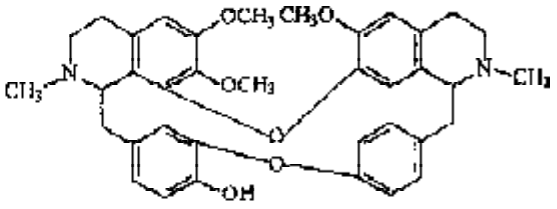
Tab 1. Comparison of the biological activities bewteen tetrandrine and berbamine.

Biological Properties	Tetrandrine	Berbamine	Ref.
Neutrophil adherence	↓	↓	81
NK cell cytotoxicity	▼	↓	81
Anti-oxidative activity	+	—	72, 81
IL 1, TNF α production	↓	↓	79
Leukotriene production	↓	↓	82
Prostaglandin production	↓	↓	82
DTH responses; T cell activation	↓	↓	46, 83
Adjuvant-induced arthritis	↓	▼	45
Experimental allergic encephalitis	↓	↓	78
IL-1 and TNF direct effects	↓	↓	89

* The symbol ↓ (strong) and ↓ (weak) indicate the suppressive intensities



Tetrandrine



Berbamine

kinase C–NF-κB signaling pathway in the example of T cells. Although only minor structural difference is present among Tet and its analogues, great biological differences exist among these analogues. These observations also give us important insights into structure-activity relationships of Tet and its analogues and the opportunity to design novel analogues that are potentially more potent and less toxic than currently available drugs for the treatment of autoimmune disorders.

Aside from its calcium-channel blocking effects, there are at least two major fields about Tet that are not covered in this review. One is its anti-cancer effect like the increase of chemotherapeutic agent drug sensitivity in multi-drug resistance cells and the other is its anti-silicosis effect like the inhibition of pulmonary macrophage and pulmonary fibroblast activation. In this context, the clinical application of this drug may not be limited to the currently known knowledge on this drug.

ACKNOWLEDGEMENTS I am grateful for the help from Dr HO Ling-Jun and for her significant contributions to the series of our publications.

REFERENCES

1 O' Dell JR, Haire CE, Erikson N, Drymalski W, Palmer W, Eckhoff PJ, *et al.* Treatment of rheumatoid arthritis with methotrexate alone, sulfasalazine and hydroxychloroquine, or a combination of all three medications [see comments]. N Engl J Med 1996; 334: 1287-91.

2 Mottonen T, Hannonen P, Leirisalo-Repo M, Nissila M, Kautiainen H, Korpela M, *et al.* Comparison of combination therapy with single-drug therapy in early rheumatoid arthritis: a randomised trial. FIN-RACo trial group. Lancet 1999; 353: 1568-73.

3 Landewe RB, Boers M, Verhoeven AC, Westhovens R, van de Laar MA, Markusse HM, *et al.* COBRA combination therapy in patients with early rheumatoid arthritis: long-term structural benefits of a brief intervention. Arthritis Rheum 2002; 46: 347-56.

4 Fox DA. The role of T cells in the immunopathogenesis of rheumatoid arthritis: new perspectives. Arthritis Rheum 1997; 40: 598-609.
5 Weyand CM. New insights into the pathogenesis of rheumatoid arthritis. Rheumatology 2000; 39: 3-8.
6 VanderBorght A, Geusens P, Raus J, Stinissen P. The autoimmune pathogenesis of rheumatoid arthritis: role of autoreactive T cells and new immunotherapies. Semin Arthritis Rheum 2001; 31: 160-75.
7 Panayi GS, Corrigall VM, Pitzalis C. Pathogenesis of rheumatoid arthritis. The role of T cells and other beasts. Rheum Dis Clin North Am 2001; 27: 317-34.
8 Linsley PS, Ledbetter JA. The role of the CD28 receptor during T cell responses to antigen. Annu Rev Immunol 1993; 11: 191-212.
9 Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 system of T cell costimulation. Annu Rev Immunol 1996; 14: 233-58.
10 Boussiotis VA, Freeman GJ, Gray G, Gribben J, Nadler LM. B7 but not intercellular adhesion molecule-1 costimulation prevents the induction of human alloantigen-specific tolerance. J Exp Med 1993; 178: 1753-63.
11 Finck BK, Linsley PS, Wofsy D. Treatment of murine lupus with CTLA4Ig. Science 1994; 265: 1225-7.
12 Lenschow DJ, Zeng Y, Thistlethwaite JR, Montag A, Brady W, Gibson MG, *et al.* Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4lg [see comments]. Science 1992; 257: 789-92.
13 Lin H, Bolling SF, Linsley PS, Wei RQ, Gordon D, Thompson CB, *et al.* Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA4Ig plus donor-specific transfusion. J Exp Med 1993; 178: 1801-6.
14 Azuma H, Chandraker A, Nadeau K, Hancock WW, Carpenter CB, Tilney NL, *et al.* Blockade of T-cell costimulation prevents development of experimental chronic renal allograft rejection [see comments]. Proc Natl Acad Sci USA 1996; 93: 12439-44.
15 Chandraker A, Azuma H, Nadeau K, Carpenter CB, Tilney NL, Hancock WW, *et al.* Late blockade of T cell costimulation interrupts progression of experimental chronic allograft rejection. J Clin Invest 1998; 101: 2309-18.
16 Via CS, Rus V, Nguyen P, Linsley P, Gause WC. Differential effect of CTLA4Ig on murine graft-versus-host disease (GVHD) development: CTLA4Ig prevents both acute and chronic GVHD development but reverses only chronic GVHD. J Immunol 1996; 157: 4258-67.
17 Levine BL, Mosca JD, Riley JL, Carroll RG, Vahey MT, Jagodzinski LL, *et al.* Antiviral effect and *ex vivo* CD4+ T cell proliferation in HIV-positive patients as a result of CD28 costimulation. Science 1996; 272: 1939-43.
18 Abrams JR, Kelley SL, Hayes E, Kikuchi T, Brown MJ, Kang S, *et al.* Blockade of T lymphocyte costimulation with cytotoxic T lymphocyte-associated antigen 4-immunoglobulin (CTLA4Ig) reverses the cellular pathology of psoriatic plaques, including the activation of keratinocytes, dendritic cells, and endothelial cells. J Exp Med 2000; 192: 681-94.
19 Bai XF, Bender J, Liu J, Zhang H, Wang Y, Li O, *et al.* Local costimulation reinvigorates tumor-specific cytolytic T lymphocytes for experimental therapy in mice with large tumor burdens. J Immunol 2001; 167: 3936-43.
20 Kwon ED, Hurwitz AA, Foster BA, Madias C, Feldhaus AL, Greenberg NM, *et al.* Manipulation of T cell costimulatory and inhibitory signals for immunotherapy of prostate cancer. Proc Natl Acad Sci USA 1997; 94: 8099-103.
21 Maccalli C, Pisarra P, Vegetti C, Sensi M, Parmiani G, Anichini A. Differential loss of T cell signaling molecules in metastatic melanoma patients' T lymphocyte subsets expressing distinct TCR variable regions. J Immunol 1999; 163: 6912-23.
22 Salmon M, Scheel-Toellner D, Huissoon AP, Pilling D, Shamsadeen N, Hyde H, *et al.* Inhibition of T cell apoptosis in the rheumatoid synovium. J Clin Invest 1997; 99: 439-46.
23 Schirmer M, Vallejo AN, Weyand CM, Goronzy JJ. Resistance to apoptosis and elevated expression of Bcl-2 in clonally expanded CD4+CD28- T cells from rheumatoid arthritis patients. J Immunol 1998; 161: 1018-25.
24 Nishioka K, Hasunuma T, Kato T, Sumida T, Kobata T. Apoptosis in rheumatoid arthritis: a novel pathway in the regulation of synovial tissue. Arthritis Rheum 1998; 41: 1-9.
25 Eguchi K. Apoptosis in autoimmune diseases. Internal Med 2001; 40: 275-84.
26 Nagata S. Apoptosis by death factor. Cell 1997; 88: 355-65.
27 Jacobson MD, Weil M, Raff MC. Programmed cell death in animal development. Cell 1997; 88: 347-54.
28 Utz PJ, Hottelet M, Schur PH, Anderson P. Proteins phosphorylated during stress-induced apoptosis are common targets for autoantibody production in patients with systemic lupus erythematosus. J Exp Med 1997; 185: 843-54.
29 Rosen A, Casciola-Rosen L. Autoantigens as substrates for apoptotic proteases: implications for the pathogenesis of systemic autoimmune disease. Cell Death Differ 1999; 6: 6-12.
30 Levine JS, Koh JS, Pender MP. The role of apoptosis in autoimmunity: immunogen, antigen, and accelerant: Genetically determined failure of activation-induced apoptosis of autoreactive T cells as a cause of multiple sclerosis. Semin Nephrol 1999; 19: 34-47.
31 Rovere P, Sabbadini MG, Fazzini F, Bondanza A, Zimmermann VS, Rugarli C, *et al.* Remnants of suicidal cells fostering systemic autoaggression. Apoptosis in the origin and maintenance of autoimmunity. Arthritis Rheum 2000; 43: 1663-72.
32 Schirmer M, Vallejo AN, Weyand CM, Goronzy JJ. Resistance to apoptosis and elevated expression of Bcl-2 in clonally expanded CD4+CD28- T cells from rheumatoid arthritis patients. J Immunol 1998; 161: 1018-25.
33 Nakajima H, Golstein P, Henkart PA. The target cell nucleus is not required for cell-mediated granzyme- or Fas-based cytotoxicity. J Exp Med 1995;181:1905-9.
34 Mountz JD, Wu J, Cheng J, Zhou T. Autoimmune disease. A problem of defective apoptosis. Arthritis Rheum 1994; 37: 1415-20.

35 Vaishnaw AK, McNally JD, Elkon KB. Apoptosis in the rheumatic diseases. Arthritis Rheum 1997; 40: 1917-27.
36 Pender MP. Genetically determined failure of activation-induced apoptosis of autoreactive T cells as a cause of multiple sclerosis. Lancet 1998; 351: 978-81.
37 Hunig T, Schimpl A. Systemic autoimmune disease as a consequence of defective lymphocyte death. Curr Opin Immunol 1997; 9: 826-30.
38 Ohsako S, Elkon KB. Apoptosis in the effector phase of autoimmune diabetes, multiple sclerosis and thyroiditis. Cell Death Differ 1999; 6: 13-21.
39 Hetts SW. To die or not to die: An overview of apoptosis and its role in disease. JAMA 1998; 279: 300-7.
40 Yocum DE. T cells: pathogenic cells and therapeutic targets in rheumatoid arthritis. Semin Arthritis Rheum 1999; 29: 27-35.
41 Feng YX, Chen H. Pharmacognostic and chemical identification of Fang Ji (Stephania tetrandra). Chin J Pharm Anal 1985; 5: 28-31.
42 Li QL, Xu YH, Zhon ZH, Chen XW, Huang XG, Chen SL, *et al.* The therapeutic effect of tetrandrine on silicosis. Chin J Tuberc Res Dis 1981; 4: 321-8.
43 Li SY, Teh BS, Seow WK, Ling LH, Thong YH. Effect of tetrandrine on immunological responses and cardiac transplant rejection in mice. Int Arch Allergy Appl Immunol 1989; 90: 169-73.
44 Kondo Y, Imai Y, Hojo H, Hashimoto Y, Nozoe S. Selective inhibition of T-cell-dependent immune responses by bisbenzylisoquinoline alkaloids *in vivo*. Int J Immunopharmacol 1992; 14: 1181-6.
45 Whitehouse MW, Fairlie DP, Thong YH. Anti-inflammatory activity of the isoquinoline alkaloid, tetrandrine, against established adjuvant arthritis in rats. Agents Actions 1994; 42: 123-7.
46 Lai JH, Ho LJ, Kwan CY, Chang DM, Lee TC. Plant alkaloid tetrandrine and its analog block CD28-costimulated activities of human peripheral blood T cells: potential immunosuppressants in transplantation immunology. Transplantation 1999; 68: 1383-92.
47 Ho LJ, Chang DM, Lee TC, Chang ML, Lai JH. Plant alkaloid tetrandrine downregulates protein kinase C-dependent signaling pathway in T cells. Eur J Pharmacol 1999; 367: 389-98.
48 Newton AC. Protein kinase C: structure, function, and regulation. J Biol Chem 1995; 270: 28495-8.
49 Hofmann J. The potential for isoenzyme-selective modulation of protein kinase C. FASEB J 1997; 11: 649-69.
50 Liu J, Farmer JD, Lane WS Jr, Friedman J, Weissman I, Schreiber SL. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell 1991; 66: 807-15.
51 Lieberman I, Heil BV, Seow WK, Thong YH. Synergy between tetrandrine and FK506 in prevention of diabetes in BB rats. Life Sci 1993; 53: PL453-8.
52 Ferrante A, Seow WK, Rowan-Kelly B, Thong YH. Tetrandrine, a plant alkaloid, inhibits the production of tumour necrosis factor-alpha (cachectin) hy human monocytes. Clin Exp Immunol 1990; 80: 232-5.
53 Onai N, Tsunokawa Y, Suda M, Watanabe N, Nakamura K, Sugimoto Y, *et al.* Inhibitory effects of bisbenzylisoquinoline alkaloids on induction of proinflammatory cytokines, interleukin-1 and tumor necrosis factor-alpha. Planta Med 1995; 61: 497-501.
54 Chang DM, Chang WY, Kuo SY, Chang ML. The effects of traditional antirheumatic herbal medicines on immune response cells. J Rheumatol 1997; 24: 436-41.
55 Xiao JG, Chiou GC. Tetrandrine inhibits breakdown of blood-aqueous barrier induced by endotoxin and interleukin-1 alpha in rats. J Ocular Pharmacol Ther 1996; 12: 323-9.
56 Kondo Y, Takano F, Hojo H. Inhibitory effect of bisbenzylisoquinoline alkaloids on nitric oxide production in activated macrophages. Biochem Pharmacol 1993; 46: 1887-92.
57 Zhou X. Tetrandrine on monocyte migration induced by endothelial cell-derived chemotactic factor. Chin Med J 1992; 72: 424-6.
58 Kobayashi S, Inaba K, Kimura I, Kimura M. Inhibitory effects of tetrandrine on angiogenesis in adjuvant-induced chronic inflammation and tube formation of vascular endothelial cells. Biol Pharm Bull 1998; 21: 346-9.
59 Seow WK, Li SY, Thong YH. Inhibitory effects of tetrandrine on human neutrophil and monocyte adherence. Immunol Lett 1986; 13: 83-8.
60 Shen YC, Chen CF, Sung YJ. Tetrandrine ameliorates ischaemia-reperfusion injury of rat myocardium through inhibition of neutrophil priming and activation. Br J Pharmacol 1999; 128: 1593-601.
61 Cohen JJ, Duke RC. Glucocorticoid activation of a calcium-dependent endonuclease in thymocyte nuclei leads to cell death. J Immunol 1984; 132: 38-42.
62 Meng XW, Feller JM, Ziegler JB, Pittman SM, Ireland CM. Induction of apoptosis in peripheral blood lymphocytes following treatment *in vitro* with hydroxychloroquine. Arthritis Rheum 1997; 40: 927-35.
63 Lu X, Xie W, Reed D, Bradshaw WS, Simmons DL. Nonsteroidal antiinflammatory drugs cause apoptosis and induce cyclooxygenases in chicken embryo fibroblasts. Proc Natl Acad Sci USA 1995; 92: 7961-5.
64 Ho LJ, Chang DM, Chang ML, Kuo SY, Lai JH. Mechanism of immunosuppression of the antirheumatic herb TWHf in human T cells. J Rheumatol 1999; 26: 14-24.
65 Pang L, Hoult JR. Cytotoxicity to macrophages of tetrandrine, an antisilicosis alkaloid, accompanied by an overproduction of prostaglandins. Biochem Pharmacol 1997; 53: 773-82.
66 Lai YL, Chen YJ, Wu TY, Wang SY, Chang KH, Chung CH, *et al.* Induction of apoptosis in human leukemic U937 cells by tetrandrine. Anti-Cancer Drugs 1998; 9: 77-81.
67 Dong Y, Yang MM, Kwan CY. *In vitro* inhibition of proliferation of HL-60 cells by tetrandrine and coriolus versicolor peptide derived from Chinese medicinal herbs. Life Sci 1997; 60: PL135-40.
68 Teh BS, Chen P, Lavin MF, Seow WK, Thong YH. Demonstration of the induction of apoptosis (programmed cell death) by tetrandrine, a novel anti-inflammatory agent. Int J

Immunopharmacol 1991; 13: 1117-26.
69 Lai JH, Ho LJ, Lu KC, Chang DM, Shaio MF, Han SH. Western and Chinese antirheumatic drug-induced T cell apoptotic DNA damage uses different caspase cascades and is independent of Fas/Fas ligand interaction. J Immunol 2001; 166: 6914-24.
70 Cao ZF. Scavenging effect of tetrandrine of active oxygen radicals. Planta Med 1996; 62: 413-4.
71 Shi X, Mao Y, Saffiotti U, Wang L, Rojanasakul Y, Leonard SS, *et al*. Antioxidant activity of tetrandrine and its inhibition of quartz-induced lipid peroxidation. J Toxicol Environ Health 1995; 46: 233-48.
72 Matsuno T, Orita K, Edashige K, Kobuchi H, Sato EF, Inouye B, *et al*. Inhibition of active oxygen generation in guinea-pig neutrophils by biscoclaurine alkaloids. Biochem Pharmacol 1990; 39: 1255-9.
73 Seow WK, Ferrante A, Li SY, Thong YH. Antiphagocytic and antioxidant properties of plant alkaloid tetrandrine. Int Arch Allergy Appl Immunol 1988; 85: 404-9.
74 Ye J, Ding M, Zhang X, Rojanasakul Y, Shi X. On the role of hydroxyl radical and the effect of tetrandrine on nuclear factor-kappaB activation by phorbol 12-myristate 13-acetate. Ann Clin Lab Sci 2000; 30: 65-71.
75 Chen YJ, Tu ML, Kuo HC, Chang KH, Lai YL, Chung CH, *et al*. Protective effect of tetrandrine on normal human mononuclear cells against ionizing irradiation. Biol Pharm Bull 1997; 20: 1160-4.
76 Shen YC, Chen CF, Wang SY, Sung YJ. Impediment to calcium influx and reactive oxygen production accounts for the inhibition of neutrophil Mac-1 Up-regulation and adhesion by tetrandrine. Mol Pharmacol 1999; 55: 186-93.
77 Shen YC, Chou CJ, Chiou WF, Chen CF. Anti-inflammatory effects of the partially purified extract of radix Stephaniae tetrandrae: comparative studies of its active principles tetrandrine and fangchinoline on human polymorphonuclear leukocyte functions. Mol Pharmacol 2001; 60: 1083-90.
78 Wong CW, Seow WK, Thong YH. Comparative effects of tetrandrine and berbamine on acute and relapsing experimental allergic encephalitis in Lewis rats. Int Arch Allergy Immunol 1992; 97: 31-6.
79 Seow WK, Ferrante A, Summors A, Thong YH. Comparative effects of tetrandrine and berbamine on production of the inflammatory cytokines interleukin-1 and tumor necrosis factor. Life Sci 1992; 50: PL53-8.
80 Wong CW, Seow WK, O' Callaghan JW, Thong YH. Comparative effects of tetrandrine and berbamine on subcutaneous air pouch inflammation induced by interleukin-1, tumour necrosis factor and platelet-activating factor. Agents Actions 1992; 36: 112-8.
81 Li SY, Ling LH, Teh BS, Seow WK, Thong YH. Anti-inflammatory and immunosuppressive properties of the *bis*-benzylisoquinolines: *in vitro* comparisons of tetrandrine and berbamine. Int J Immunopharmacol 1989; 11: 395-401.
82 Teh BS, Seow WK, Li SY, Thong YH. Inhibition of prostaglandin and leukotriene generation by the plant alkaloids tetrandrine and berbamine. Int J Immunopharmacol 1990; 12: 321-6.
83 Wong CW, Seow WK, Zeng TS, Halliday WJ, Thong YH. Comparative immunopharmacology and toxicology of the bisbenzylisoquinoline alkaloids tetrandrine and berbamine. Int J Immunopharmacol 1991; 13: 579-85.