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# Immunosuppressive and Anti-Inflammatory Mechanisms of Triptolide, the Principal Active Diterpenoid from the Chinese Medicinal Herb *Tripterygium wilfordii* Hook. f.

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## Abstract

Extracts of *Tripterygium wilfordii* hook. f. (leigong teng, Thundergod vine) are effective in traditional Chinese medicine for treatment of immune inflammatory diseases including rheumatoid arthritis, systemic lupus erythematosus, nephritis and asthma. Characterisation of the terpenoids present in extracts of *Tripterygium* identified triptolide, a diterpenoid triepoxide, as responsible for most of the immunosuppressive, anti-inflammatory and antiproliferative effects observed *in vitro*. Triptolide inhibits lymphocyte activation and T-cell expression of interleukin-2 at the level of transcription. In all cell types examined, triptolide inhibits nuclear factor-κB transcriptional activation at a unique step in the nucleus after binding to DNA. Further characterisation of the molecular mechanisms of triptolide action will serve to elucidate pathways of immune system regulation.

For a historical perspective on the emergence of *Tripterygium* extracts in allopathic practice in China, and a comprehensive listing of Chinese clinical experiences with *Tripterygium* extracts, the reader is referred to the excellent review by Tao and Lipsky.[1] *Tripterygium wilfordii* hook. f. is a vine-like plant that grows in southern China and Taiwan. The *Tripterygium* vine has been used in China for the treatment of arthritis for several centuries, and toxicities, predominantly intestinal, have been recognised.[2-4] Recently, ethyl acetate and chloroform/methanol extracts of *Tripterygium* plants have re-emerged in allopathic practice for the treatment of rheumatoid arthritis, systemic lupus erythematosus, nephritis, leprosy and asthma. The principal active compound present in these *Tripterygium* extracts has been identified as triptolide, a diterpenoid triepoxide. In this review, we will focus on mechanistic studies that reveal novel properties of highly enriched or pure triptolide in cellular and animal studies.

## 1. Chemistry of Triptolide and *Tripterygium* Extracts

The vines and roots of *T. wilfordii* hook. f. contain terpenoids, alkaloids and glycosides. The most

potent anti-inflammatory and immunosuppressive compounds are highly oxygenated diterpenoids. Triptolide has been identified as one of the most potent immunosuppressive compounds isolated from *Tripterygium* vine (figure 1). Other compounds closely related to triptolide and with similar activities that may be isolated from *Tripterygium* include tripdiolide, triptonide, 16-hydroxytriptolide, tripchloride and triptriolide.[1] The diterpene lactone, tripterifordin, and sesquiterpene alkaloids, including triptonine from *Tripterygium*, exhibited potent anti-HIV effects *in vitro*.[5,6] Epoxide moieties in triptolide appear to be necessary for the potent immunosuppressive properties.[1]

The extracts of *Tripterygium* that have been used for treatment of patients include a hot water extract (decoction), an ethyl acetate extract and a chloroform/methanol extract (T2). The diterpenoid compositions of the ethyl acetate and chloroform/methanol extracts have been described.[7] These studies revealed that the *Tripterygium* extracts contain relatively low amounts of pure triptolide. Numerous clinical studies have been reported from China, and one from the US,[8] that describe anti-inflammatory and immunosuppressive properties associated with the ethyl acetate and T2 extracts of *Tripterygium*.[1]

Recently, a succinate ester of pure triptolide, PG490-88, has been developed at Pharmagenesis (Palo Alto, CA, USA). PG490-88 is water-soluble and orally active and is hydrolysed in the serum to PG490, which is pure triptolide (figure 1).

The complete chemical synthesis of triptolide was described.[9] Recently, Yang reported the enantioselective total synthesis of (–)-triptolide and congeners.[10] These elegant studies indicate that, as the mechanisms and targets of triptolide and congeners become better characterised, synthetic-organic strategies exist to optimise the medicinal properties of these potent diterpenoid triepoxides.

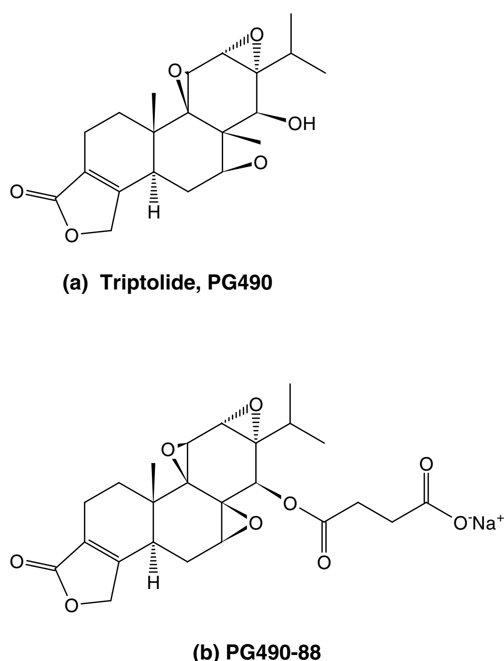


**Fig. 1.** Chemical structures of (**a**) triptolide and (**b**) PG490-88. PG490-88 is a succinate ester prodrug of triptolide.

## 2. Clinical Studies Using *Tripterygium* Extracts

Clinical studies performed in China using extracts of *Tripterygium* for the treatment of autoimmune and inflammatory disorders have been reviewed.[1] A prospective, double-blind, crossover study of the T2 chloroform/methanol extract of *Tripterygium* for the treatment of rheumatoid arthritis was published by Tao et al.[11] This study reports more impressive cure rates than in previous open-label trials. A phase I dose-ranging study to explore toxicities was performed in the US using the ethyl acetate extract of *Tripterygium* in patients with rheumatoid arthritis, and was published later by Tao et al.[8] Thirteen patients with established rheumatoid arthritis received escalating doses of the ethyl acetate extract of *Tripterygium* ranging from 180 to 570 mg/day for up to 76 weeks. Eight of nine patients treated with 300 mg/day showed improvements in clinical manifestations and laboratory measurements.[8]

Other diseases for which *Tripterygium* extracts have been reported to show efficacy in China in-

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**Table I.** Effects of *Tripterygium* extracts in animal models of disease

| *Tripterygium* extract | Solvent | Animal | Disease model | Ref. |
|---|---|---|---|---|
| Triptolide (PG490) | Ethanol, 97% pure triptolide in ethanol | Rat | Pulmonary hypertension | [13] |
| | | | Cardiac allograft | [14] |
| | | | Renal allograft | [12] |
| | | | Collagen-induced arthritis | [15] |
| PG27 | Refined extract in ethanol | Rat | Cardiac and renal allograft | [12] |
| | | Mouse | Bone marrow transplantation, GVHD | [16] |
| PG490-88 | 14-Succinyl triptolide sodium salt (98% pure), semisynthetic derivative of PG490, water soluble | Rat | Lung fibrosis | [17] |
| | | Mouse | Cardiac and renal allograft | [12] |
| | | | Bone marrow transplantation and prevention of GVHD | [16,18] |
| T2 | Chloroform/methanol extract | Rat | Liver transplantation | [15,19] |
| | | Mouse | MRL-*lpr/lpr* nephritis and arthritis | [20] |
| | | | Skin allograft | [21] |
| | | Guinea-pig | Allergic encephalomyelitis | [22] |
| | | Rabbit | Cornea allograft | [23] |
| | | Pig | Intestinal allograft | [24,25] |
| Others | Ethyl acetate extract | Rabbit | Nephritis | [26] |
| | Chloroform | Mouse | Chronic GVHD | [27] |
| | Decoction | | Collagen arthritis | [28] |
| | | | MRL-*lpr/lpr* nephritis and arthritis | [29] |
| | | | Cardiac allograft | [30] |

**GVHD** = graft-versus-host disease.

clude systemic lupus erythematosus, nephritis, asthma and leprosy.[1]

## 3. Animal Studies Involving Triptolide and *Tripterygium* Extracts

### 3.1 Solid Organ Transplantation

#### *3.1.1 Allo- and Xenograft Survival*

Improvements in survival after cardiac and renal allografting were demonstrated using a *Tripterygium* extract, PG27 (contains 0.36 ± 0.04% PG490/Triptolide)[12] [table I], PG490 (97% pure triptolide), and PG490-88, a water-soluble succinate ester prodrug of PG490/triptolide. In addition, Fidler's group demonstrated synergy between PG27 and cyclosporin in improving allograft survival. This synergy suggested that PG27 and cyclosporin exert their immunosuppressive effects through distinct mechanisms.[12]

Fidler's group performed heterotopic cardiac allografts in which hearts from Brown Norway donor rats were placed into the abdomens of recipient Lewis rats and graft survival was measured in days of persistent palpable cardiac contraction of the allograft. PG27 administration at dosages of 10, 20 or 30 mg/kg/day intraperitoneally, or 5, 10 or 30 mg/kg/day orally, produced significant increases in allograft survival beyond the control result of 7 days with vehicle treatment alone. Notably, long-term cardiac allograft survival beyond 100 days was achieved when PG27 was administered at 30 mg/kg/day for 50 days following transplantation.[12]

The renal allograft experiments by Fidler's group involved transplantation of donor kidneys from Brown Norway rats into the abdomen of recipient Lewis rats, followed by bilateral nephrectomies of the native Lewis rat kidneys. Renal allograft function was monitored by measurements of serum blood urea nitrogen and creatinine. PG27 at 20 and 30 mg/kg/day intraperitoneally signifi-

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cantly prolonged allograft survival compared with control animals.[12]

Combination treatment with PG27 and cyclosporin demonstrated synergistic effects in prolonging cardiac and renal allograft survival (table I). The allograft mean survival times were significantly prolonged when low dosages of cyclosporin (0.25 mg/kg/day) were combined with PG27 at 5 or 10 mg/kg/day, dosages that were ineffective alone. The addition of PG27 at 5 or 10 mg/kg/day reduced by 50–75% the requirement of cyclosporin needed to prevent cardiac allograft rejection. Measurement of BUN reflects renal allograft rejection, and the combinations of cyclosporin 0.25 mg/kg/day with PG27 5 or 10 mg/kg/day were more effective than any dosage of cyclosporin tested and as effective as PG27 at 30 mg/kg/day. Haematology and serum chemistry studies of normal Lewis rats and Lewis allograft recipient rats revealed no significant abnormalities associated with PG27 administration. Dosages of PG27 alone that preserved renal allograft function produced a smaller increase in BUN than effective dosages of cyclosporin, suggesting that PG27 was less nephrotoxic than cyclosporin. When loss of bodyweight was used as a criterion of drug toxicity, the combination treatment regimens of low dosage cyclosporin and PG27 showed the best toxicity profile.[12]

PG27 contains 0.36 ± 0.04% PG490/Triptolide as its active component. Allograft survival experiments were conducted using PG490 (97% pure triptolide), and PG490-88, a water-soluble, succinyl ester prodrug of PG490. PG490 at 0.25 and 0.5 mg/kg/day increased renal allograft survival, as did the combination of cyclosporin 0.125 mg/kg/day with PG490 at 0.0625 mg/kg/day[12] PG490-88 at 0.335 mg/kg/day significantly increased cardiac allograft mean survival time from 7 to >47 days, and significantly increased cardiac allograft survival when used in combination with cyclosporin.[12]

Xenotransplantation of animal organs into human recipients has been proposed as a potential solution to the problem of scarcity of human donor organs. Barriers to xenotransplantation include hyperacute rejection, mediated by inappropriate activation of the complement system by preformed antibodies, acute rejection involving humoral immune responses, and chronic rejection mediated by cellular immune responses. Fidler and co-workers characterised the efficacy of combination therapy with PG27 and cyclosporin for maintenance of hamster to rat cardiac xenografts[31] (table I). Neither PG27 nor cyclosporin as monotherapy was effective in prolonging xenograft survival significantly beyond the control duration of 3 days. However, combination therapy with PG27 at 30 mg/kg/day and cyclosporin 10 mg/kg/day on days –1 to 7, followed by PG27 at 30 mg/kg/day and cyclosporin 5 mg/kg/day on days 8–35, followed by cyclosporin 5 mg/kg/day on days 36–100, resulted in long-term xenograft survival and function.[31] Once therapy was discontinued, the xenografts underwent acute rejection. Acute rejection in this cardiac xenograft model was accompanied by rapid increases in the production of anti-hamster IgM and IgG antibodies. Effective immunosuppressive regimens completely suppressed this acute humoral antibody response, and the response was restored when immunosuppressive therapy was discontinued. This study demonstrated that the combination of PG27 with cyclosporin could completely inhibit production of xenoantibodies and allow cardiac xenotransplantation in a rodent model.[31]

#### 3.1.2 Vascular Occlusion with Neointimal Formation

Coronary artery occlusion due to accelerated atherosclerosis and neointimal formation involves chronic inflammation, and may represent a form of chronic rejection after cardiac allografting. Hachida et al. performed heterotopic cardiac transplantation of Wistar-King donor hearts into Lewis rat recipients and treated the rats for 60 days with either an extract of *Tripterygium* 30 mg/kg/day or with cyclosporin[14] (table I). The extent of graft atherosclerosis was decreased in the animals treated with the *Tripterygium* extract compared with cyclosporin, and this was accompanied by a

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decrease in platelet-derived growth factor mRNA in the cardiac allografts.[14] Extending these prevention studies, Hachida et al. demonstrated that late treatment with *Tripterygium* extracts can rescue graft atherosclerosis, and that the active compound responsible for these effects is triptolide.[32] These studies indicate that triptolide can inhibit vascular neointimal formation, and suggests anti-angiogenic properties of triptolide.

### 3.2 Bone Marrow Transplantation

Bone marrow transplantation in humans has become a curative therapy for certain malignancies and non-malignant diseases. A major limitation to broader successes in human bone marrow transplantation is the development of graft-versus-host disease (GVHD), in which immune cells of donor graft origin attack and damage host recipient organs. Although diverse and extensive immunosuppressive therapies are utilised to prevent and suppress GVHD, the incidence of GVHD in humans remains at 30% on average, with a mortality rate as high as 50%.[33]

Investigators in Chao's laboratory demonstrated that PG27 was very effective in preventing GVHD in a mouse model[34] (table I). Additionally, PG27 induced host-specific immune tolerance while preserving third-party immunoreactivity and graft versus leukaemia effects. The initial experiments used a primary mixed lymphocyte reaction (MLR) across a major MHC barrier: Balb/c spleen cells were co-incubated with irradiated C57BL/6 spleen cell stimulators, and proliferation was assayed by [$^3$H]thymidine incorporation assay. Treatment with PG27 was able to completely inhibit the MLR, and 100% inhibition occurred at 1.25 μg/mL.[34] A secondary MLR was evaluated across a minor MHC barrier: B10.D2/nSnJ mice were immunised intraperitoneally with irradiated Balb/c spleen cells, then 21 days later the B10.D2/ nSnJ spleen cells were restimulated by co-incubation with irradiated Balb/c spleen cells. Again, PG27 inhibited the proliferative response in a dose-dependent manner, and 100% inhibition occurred at 2.5 μg/mL.[34]

In a murine model of GVHD, lethally irradiated Balb/c mice were reconstituted with B10.D2/nSnJ bone marrow and spleen cells, and were then treated to prevent GVHD for 5 weeks with the vehicle alone or PG27 at 40 mg/kg/day intraperitoneally. By 90 days, mortality was 100% in recipient mice treated with the vehicle. Recipient mice treated with PG27 showed 100% survival beyond 100 days without any signs of GVHD. When weight was used as a marker of GVHD, vehicle-treated mice showed an irreversible loss of bodyweight, from 30 to 21g, whereas PG27-treated mice regained baseline weight (30g) from day 40 to day 100.[34]

Analysis of reconstitution of the bone marrow confirmed long-term engraftment of the B10.D2/ nSnJ donor cells in the Balb/c recipients. The recipient mice, free of GVHD as a result of treatment with PG27, were used as marrow and spleen donors into Balb/c (same-party) or C3H (third-party) lethally irradiated recipient mice. Severe GVHD occurred in all third-party recipients, and the animals died within 40 days. In contrast, there was 100% survival beyond 100 days, with no evidence of GVHD, after adoptive transplant into Balb/c mice.[34] This experiment demonstrates the establishment of immunological tolerance in a murine model of GVHD after 5 weeks of treatment with PG27 during the initial allogeneic bone marrow transplantation. Extending these studies, Chen et al. demonstrated preservation of the graft-versus-leukaemia effect in animals rendered tolerant and free of GVHD by treatment for 5 weeks with PG27.[34]

As PG27 contains 0.36% triptolide as its active immunosuppressive compound, Chao and coworkers investigated suppression of GVHD using PG490-88, a water-soluble semisynthetic derivative of triptolide.[16] Using the same model of allogeneic bone marrow transplantation from B10.D2 to Balb/c mice, Chen et al. showed complete inhibition of fatal GVHD with PG490-88 at 0.535 mg/kg/day intraperitoneally. Analysis of interleukin (IL)-2 production was performed using intracellular cytokine staining on CD4+ splenic

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lymphocytes. PG490-88-treated mice showed nearly 50% inhibition of IL-2-producing cells compared with vehicle-treated mice developing GVHD. Treatment with PG490-88 did not inhibit expression of the CD25 chain of the IL-2 receptor. PG490-88 treatment also decreased the numbers of spleen cells that expressed tumour necrosis factor (TNF)-$\alpha$ and interferon (IFN)-$\gamma$ following stimulation with phorbol 12-myristate 13-acetate (PMA).[16] Taken together, these results indicate that triptolide is highly effective in suppressing T-cell activation that is responsible for fatal murine GVHD.

Chao's group went on to characterise the mechanisms of long-term tolerance induction by PG490-88 at 0.535 mg/kg/day following allogeneic bone marrow transplantation[16] (table I). Analysis of the reconstituted immune system demonstrated complete donor chimaerism. Thus, the absence of GVHD implied tolerance of the bone marrow to the recipient solid organs. Host-specific tolerance was further demonstrated by transferring two neonatal hearts, one from recipient Balb/c H2d mice, and one from third-party C3H/HeJ H2k mice, into subcutaneous pouches in the right and left ears. The third-party heart from the C3H/HeJ animal was rejected in 10 days, whereas the Balb/c hearts were accepted indefinitely by the animals that had been reconstituted with bone marrow and spleen cells from B10.D2 mice.[16] Chen et al. demonstrated that the mechanisms that underlie this long-term tolerance involve the induction of T helper 2 ($T_H2$) cytokines such as IL-4.[18] Host-reactive T cells were present in a state of anergy. There was no evidence that tolerance was associated with suppressor cells. Thus, host-specific tolerance induced by PG490-88 was associated with induction of antigen-specific anergy, or through induction of $T_H2$ immunity.[18]

## 3.3 Proliferative, Non-Malignant Lung Diseases

### *3.3.1 Pulmonary Arterial Hypertension with Neointimal Formation*

Primary pulmonary hypertension (PPH) is a rare, severe and ultimately fatal disease characterised by progressive obliteration of the lumen of small pulmonary arterioles and death from right congestive heart failure.[35] The pathology of small vessel obliterative pulmonary hypertension in humans involves proliferation of vascular endothelial and smooth muscle cells.[36] The best currently available medical therapies for proliferative and obliterative pulmonary vascular disease involve treatment with vasodilators, such as prostacyclin.[37]

We established a model of experimental hypertensive pulmonary vascular disease in rats that reproduces many of the pathological features of human PPH. We used this rat model of hypertensive pulmonary vascular disease to characterise antiproliferative treatments without acute vasodilator activities. Rats undergo left pneumonectomy, which produces increased shear stresses in the remaining right lung; they then receive a single subcutaneous injection of the plant alkaloid monocrotaline.[13,38,39] Monocrotaline is metabolised in the liver to a toxic pyrrole, which then enters the pulmonary circulation and induces first-pass endothelial cell injury.[40,41] The endothelial injury initiates an inflammatory and proliferative response that results in proliferation of vascular smooth muscle cells, which leads to luminal obliteration and pulmonary hypertension.[40,41]

We characterised the effects of triptolide on the development of experimental pulmonary hypertension. The rats received vehicle or triptolide (0.25 mg/kg intraperitoneally every other day from day 5 to day 35)[13] [table I]. At day 35, the rats underwent physiological measurements of mean pulmonary artery pressure (mPAP), and were then sacrificed for analysis of organ weights, right ventricular hypertrophy and histopathological analyses of the lungs. Normal rats show an mPAP of 18 ± 2mm Hg, a right ventricle/(left ventricle + septum) [RV/(LV + S)] weight ratio of 0.30, and a vascular occlusion score (VOS) of 0. Compared with vehicle-treated rats, triptolide-treated rats showed significant decreases in the development of pulmonary arterial hypertension (mPAP = 21 ± 3mm Hg versus 42 ± 5mm Hg), right ventricular

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hypertrophy [RV/(LV + S) = 0.39 ± 0.10 versus 0.69 ± 0.18], and vascular occlusion (VOS = 1.27 ± 0.61 versus 1.92 ± 0.11). Triptolide, even when administered only from day 21 to day 35, partially rescued animals from the development of pulmonary arterial hypertension, right ventricular hypertrophy and vascular occlusion. When all the organs were weighed, triptolide-treated rats showed significant decreases in the weights of the thymus and the testis. This study demonstrated that triptolide, a non-vasodilator, with potent immunosuppressive and antiproliferative properties, effectively prevented the development and progression of experimental pulmonary hypertension in rats.

The neointimal proliferation observed in this model of pulmonary vascular disease demonstrates histopathological features similar to those seen in accelerated coronary atherosclerosis following cardiac allograft transplantation. The mechanisms through which triptolide is effective in preventing neointimal formation and vascular obliteration in pulmonary arterioles are likely to be similar to the mechanisms through which triptolide attenuated the development of coronary atherosclerosis after cardiac allografting in experimental models.[14]

The inhibition of neointimal proliferation in pulmonary arterioles and in coronary arteries by triptolide suggests its anti-angiogenic properties. A related nortriterpenoid from *Tripterygium*, demethylzeylasteral, was shown to potently inhibit the growth of vascular endothelial cells and expression of urokinase-type plasminogen activator *in vitro*, and inhibited tumour angiogenesis *in vivo*.[42] Thus, the antiangiogenic properties of *Tripterygium* components may represent useful modalities for the treatment of cancer.

#### 3.3.2 Bleomycin-Induced Lung Fibrosis

In a mouse model of pulmonary fibrosis after intratracheal instillation of bleomycin, Krishna et al. showed that treatment with PG490-88 (0.25 mg/kg/day on days 1–15 or days 5–15) improved histology, decreased hydroxyproline content and suppressed transforming growth factor (TGF)-β expression compared with untreated controls[17] (table I).

## 4. Cellular Studies Using *Tripterygium* Extracts and Pure Triptolide

Kupchan showed in 1972 that one of the active components in extracts of *Tripterygium* is the diterpene triepoxide, triptolide, which possesses antileukaemic properties.[43] In addition to suppressing the growth of leukaemic cells, triptolide inhibits the proliferation of transformed cell lines.[44,45] In 1974, Kupchan and Schubert described the importance of the 9,11-epoxy-14-β-hydroxy system in triptolide for biological activity.[46] They proposed that this system may be involved in selective alkylation of nucleophilic groups such as thiols present in key target enzymes involved in growth regulation.[46]

Lymphocytes are exquisitely sensitive to the growth inhibitory effects of triptolide[47,48] (table II). Activation of lymphocytes to produce cytokines and growth factors is very susceptible to inhibition by the choroform/methanol extract of *Tripterygium*, T2.[48] Expression of IL-2 and IFNγ are potently inhibited by triptolide. Other cytokines that are inhibited by triptolide are IL-8, IL-3, 4, 5, 6, and TNFα (table II). Expression of prostaglandin $E_2$ was reported to be suppressed by triptolide[49](table II). These effects of triptolide result in a potent immunosuppressive activity of triptolide. The T2 extract also inhibited the production of the immunoglobulins IgM, IgG and IgA by stimulated B-lymphocytes[48] (table II).

In our *in vitro* assays, we showed that PG490 inhibits IL-2 expression by normal human peripheral blood lymphocytes stimulated with PMA and antibody to CD3 (50% inhibitory concentration [$IC_{50}$] of 10 μg/L) or with PMA and ionomycin ($IC_{50}$ of 40 μg/L) [table II].

Other primary cells that are very sensitive to growth inhibitory effects of triptolide are spermatogonia. The maturation of sperm is potently inhibited by triptolide, and this drug has been explored as a male antifertility agent.[61,62]

Transformed cell lines are also sensitive to the growth inhibitory effects of triptolide[44,63] (table II). The growth inhibitory effects of triptolide on 60 tumour cell lines has been determined

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**Table II.** Inhibitory effects of *Tripterygium* extracts on cellular expression of signalling molecules

| Molecules | Cell sources | Stimuli | *Tripterygium* extracts | Effects | Ref. |
|---|---|---|---|---|---|
| **Cytokines** | | | | | |
| IL-1α | Human monocytes | None | T2 | No inhibition | [48] |
| IL-1β | Human monocytes | None | T2 | No inhibition | [48] |
| IL-2 | Human PBLs | PMA/ionomycin | Triptolide | Inhibition | [47] |
| | | PMA/CD3 | Triptolide | Inhibition | [47] |
| | Human Jurkat T cells | PMA/ionomycin | Triptolide | Inhibition | [47] |
| | Mouse spleen cells | ConA/alloantigen | Triptolide | Inhibition | [50] |
| IL-6 | Normal and transformed human bronchial epithelial cells | PMA, TNFα, IL-1β | Triptolide | Inhibition | [51] |
| IL8 | Normal and transformed human bronchial epithelial cells | PMA, TNFα, IL-1β | Triptolide | Inhibition | [51] |
| IFNγ | Human T cells | PMA/PHA | Triptolide | Inhibition | [52] |
| TNFα | Mouse macrophage | LPS | Triptolide | Inhibition | [53] |
| TGFβ | Human lung fibroblasts | Bleomycin | Triptolide | Inhibition | [17] |
| VEGF | Human endothelial cells | TPA | Triptolide | Inhibition | [54] |
| **Cell surface receptors** | | | | | |
| IL-2 receptor | Mouse spleen cells | ConA or PHA | Triptolide | Inhibition | [55] |
| | Human T cells | PMA/CD28 | T2 | No inhibition | [56] |
| Transferrin receptor (CD71) | Human T cells | PMA/CD28 | T2 | No inhibition | [56] |
| **Transcription factors** | | | | | |
| NF-AT | Jurkat T cells | PMA/ionomycin | Triptolide | Inhibition | [47] |
| NF-κB | Jurkat T cells | PMA/ionomycin | Triptolide | Inhibition | [47] |
| AP-1 | Jurkat T cells | PMA/ionomycin | Triptolide | Inhibition | Fig. 2 |
| Oct-1 | Jurkat T cells | PMA/ionomycin | Triptolide | No inhibition | Fig. 2 |
| p53 | Breast and colon cancer cells | None | Triptolide | Induction | [57,58] |
| **Mediators** | | | | | |
| COX-2 | Human synovial cells | IL-1β | Ethanol extract | Inhibition | [59] |
| | | | | | [49] |
| $PGE_2$ | Human monocytes | LPS | T2 or triptolide | Inhibition | [49] |
| MMP1 | Human monocytes | LPS | T2 or triptolide | Inhibition | [49] |
| MMP3 | Human synovial fibroblasts | LPS | Triptolide | Inhibition | [53] |
| ICAM-1 | Human synovial fibroblasts | LPS | Triptolide | Inhibition | [53] |
| VCAM-1 | Human synovial fibroblast | IL-1 | Triptolide | Inhibition | [60] |
| | Human vascular endothelial cells | IL-1 | Triptolide | | [60] |
| **Immunoglobulins** | | | | | |
| IgA, IgG, IgM | Human B cells | *Staphylococcus aureus* + IL-2 | T2 | Inhibition | [48] |

**AP-1** = activator protein; **ConA** = concanavalin A; **COX-2** = cyclo-oxygenase 2; **ICAM-1** = intercellular adhesion molecule 1; **IFNγ** = interferon-γ; **IL** = interleukin; **MMP** = matrix metalloproteinase; **NF-AT** = nuclear factor of activated T cells; **LPS** = lipopolysaccharide; **PBLs** = peripheral blood lymphocytes; **$PGE_2$** = prostaglandin $E_2$; **PHA** = phytohaemagglutinin; **PMA** = phorbol 12-myristate 13-acetate; **TGFβ** = transforming growth factor β; **TNFα** = tumour necrosis factor α; **TPA** = tissue plasminogen activator; **T2** = chloroform/methanol extract of *Tripterygium*; **VCAM** = vascular cell adhesion molecule; **VEGF** = vascular endothelial growth factor.

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by the NCI Developmental Therapeutics program.[64] The colon cancer line HCT-116 and breast cancer line MCF-7 were most sensitive, and the central nervous system lines SNB-19 and U251 were least sensitive, to growth inhibition by triptolide.

An extension of the antiproliferative effects of triptolide is to induce cell death at higher doses. The mechanisms of triptolide-induced cell death involved apoptosis in lymphocytes[65] and in tumour cells.[57,58,63,66] Melanoma lines were consistently very sensitive to triptolide-induced cell death.[64] Triptolide shows synergy with ligands of the TNF family of receptors in inducing tumour cell apoptosis, and this was demonstrated through TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling) assays, annexin-staining, fluorescent-activated cell sorting and demonstration of DNA fragmentation.[63] Triptolide also shows synergy with chemotherapeutic agents to induce apoptosis of tumour cells.[57]

Primary cultured prostatic epithelial cells are susceptible to apoptosis induced by triptolide.[67] At triptolide concentrations of 50–100 μg/L, there was nuclear accumulation of p53 and reductions in the cell-cycle inhibitors $p21^{Waf/Cip1}$ and $p27^{Kip1}$ and in the anti-apoptotic protein bcl-2. Low doses of triptolide (1 μg/L) were effective at inducing cellular senescence of primary cultured prostatic epithelial cells.[67]

## 5. Molecular Mechanisms and Targets of Triptolide

### 5.1 Triptolide Inhibits Activation of Transcription Factors Associated with the Inflammatory Response

Immune activation of T and B lymphocytes leads to expression of cytokines, including IL-2, 3, 4, 5, 6 and 8, granulocyte-macrophage colony-stimulating factor (GM-CSF) and TNFα.[68] A major level of regulation of cytokine gene expression in lymphocytes occurs at the level of transcription.[68,69]

Triptolide potently inhibits lymphocyte activation *in vivo* and *in vitro*.[7,56,70] The process of T-cell activation, triggered at the T-cell antigen receptor and leading to transcriptional activation of IL-2 and other cytokines, has been extensively characterised.[68,71,72] Immunosuppressants such as cyclosporin and tacrolimus inhibit T-cell activation at the early stages of transcriptional activation of the IL-2 gene.[47,73,74]

Triptolide inhibits transcriptional activation of the IL-2 gene[47,56] (table II), and thus triptolide is one of the few immunosuppressants that act at this early stage of signalling.[47] This characteristic identifies triptolide as operating in a class of immunosuppressants similar to cyclosporin and tacrolimus,[47] and as different from immunosuppressants that block proliferation, such as leflunomide, mycophenolic acid or brequinar.[75]

The analysis of IL-2 transcriptional regulation has been advanced through the use of IL-2 reporter gene assays. The IL-2 promoter/enhancer regulatory sequences (300bp 5′ to the transcription start site) are cloned upstream of an easily assayed reporter gene, and the plasmid construct is transiently or stably transfected into cultured T lymphocytes, such as Jurkat T cells.[47] Activation of the reporter gene is characterised in resting vs stimulated T cells, and the effects of different doses of the immunosuppressive drug are characterised. Such studies demonstrated that the T2 extract of *Tripterygium* inhibited IL-2 chloramphenicol acetyltransferase reporter gene activity[56] and that pure triptolide inhibits IL-2 luciferase activity.[47]

T cells can be activated through the T-cell receptor, which involves calcium fluxes, and may also be activated through co-stimulatory receptor pathways such as the CD28 receptor, which do not involve dramatic calcium fluxes.[47,76] T-cell receptor and calcium-dependent activation of T cells is inhibited by triptolide, cyclosporin and tacrolimus.[47] In contrast, T-cell activation through CD28 co-stimulation can be inhibited by triptolide, but is resistant to inhibition by cyclosporin and tacrolimus.[47] This result indicates that triptolide is broader in its immunosuppressant

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effects than cyclosporin or tacrolimus. The potential of triptolide to inhibit T-cell activation triggered through co-stimulatory receptors suggests its utility in the treatment of states that involve cyclosporin-resistant T-cell activation, such as may occur in autoimmune diseases or GVHD after bone marrow transplantation.[16,33]

#### *5.1.1 Nuclear Factor of Activated T Cells*

The IL-2 enhancer has been extensively analysed, and is the site of action of a set of transcription factors associated with the inflammatory response. These inflammatory transcription factors include nuclear factor κB (NF-κB) and activator protein 1 (AP-1), consisting of Fos and Jun proteins.[68,71] In addition, nuclear factor of activated T cells (NF-AT) was first described as a T-cell nuclear protein complex with high DNA-binding affinity for the purine-rich antigen receptor response element in the IL-2 enhancer.[77] This purine-box/antigen receptor response element (ARRE) regulates the calcium-dependent and cyclosporin-sensitive activation of the IL-2 enhancer.[77] The central role of the ARRE regulatory element was identified by studies of the chromatin reorganisation of the IL-2 enhancer during T-cell activation.[78] The purine-box/ARRE sequence was suggested to nucleate the melting of the DNA, thereby controlling access of the other required transcriptional activators, such as NF-κB and AP-1 proteins.[79] The laboratories of Rao and Crabtree have purified proteins from the cytoplasm of T cells, termed NF-ATp and NF-ATc proteins, which have been shown to cooperate with AP-1 for binding the purine-box/ARRE sequences in *in vitro* electrophoretic mobility shift assays (EMSA).[72,80] The NF-AT family proteins translocate from the cytoplasm to the nucleus in response to calcium signalling in lymphocytes and epithelial and endothelial cells.[81] Knockout mice deficient in NF-ATp or NF-ATc proteins show modest alterations in immune functions that do not include severe combined immunodeficiency or defects in lymphocyte maturation.[82,83] Targeted disruption of NF-ATc revealed unexpected defects in cardiac valve development.[83]

We previously showed that alternative proteins Ku70, Ku80, NF45, NF90 and DNA-dependent protein kinase contribute to a nuclear DNA-binding complex with affinity for the purine-box/ARRE in the IL-2 enhancer.[84-86] Targeted disruption of Ku70,[87,88] Ku80[89,90] or DNA-dependent protein kinase[91-93] are each associated with defective lymphopoiesis and severe combined immunodeficiency.

We characterised the effects of triptolide on specific purine-box/ARRE DNA-binding activity in activated Jurkat T-cells. Triptolide potently inhibits the development of high affinity nuclear purine-box/ARRE DNA-binding activity in T cells (figure 2).[47] The inhibition of specific purine-box/ARRE DNA-binding activity by triptolide is more complete than with high doses of cyclosporin (figure 2). We used NF-AT luciferase reporter gene constructs to characterise the inhibitory effects of triptolide on transcription initiated through the purine-box/ARRE regulatory DNA sequences. Triptolide potently inhibited the activation of NF-AT luciferase triggered by all stimuli tested.[47]

Calcineurin is a calcium-activated serine-threonine protein phosphatase, the function of which is inhibited by cyclosporin and tacrolimus.[94] The inhibition of calcineurin activity by cyclosporin and tacrolimus causes NF-AT proteins to remain in the cytoplasm instead of shuttling to the nucleus, and has been proposed as the mechanism for the T-cell immunosuppression by these drugs.[81] We investigated whether calcineurin phosphatase activity was involved in triptolide immunosuppression. Jurkat T-cells were transfected with expression constructs for calcineurin A (catalytic) and calcineurin B (regulatory) subunits, together with IL-2 luciferase or NF-AT luciferase reporter genes.[47] Changes in the dose-response curves for inhibition of luciferase reporter gene activity by cyclosporin, tacrolimus and triptolide were determined. T cells transfected with expression constructs for calcineurin showed diminished sensitivity to immunosuppression by cyclosporin and tacrolimus. In contrast, T cells transfected with expression constructs for calcineurin showed no

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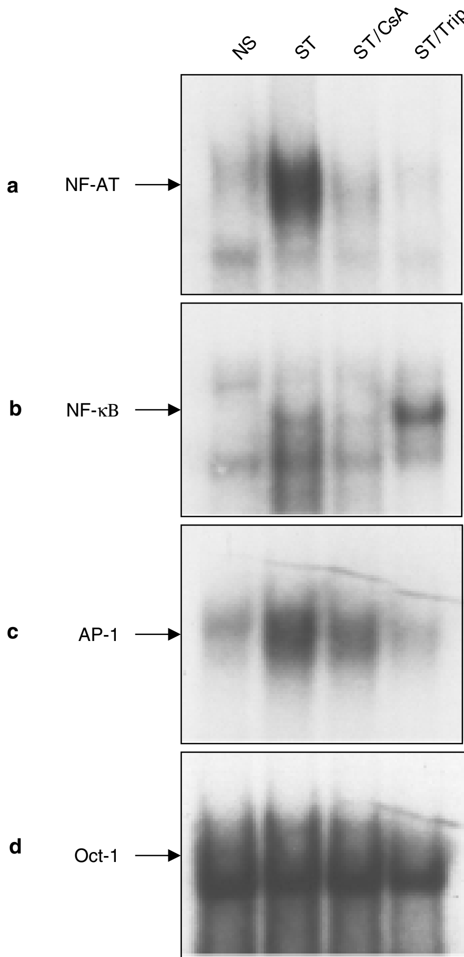


**Fig. 2.** Triptolide inhibits nuclear DNA-binding activity of the transcription factors purine-box/nuclear factor of activated T cells (NF-AT), regulator and activator protein 1 (AP-1), but not of nuclear factor-κB (NF-κB) or Oct-1. Electrophoretic mobility shift assays (EMSAs) showing inhibition of DNA-binding activity in stimulated Jurkat T cells. Nuclear extracts were prepared from nonstimulated T cells (NS), T cells stimulated for 6 hours with phorbol 12-myristate 13-acetate (PMA) + ionomycin (ST), T cells stimulated with PMA + ionomycin in the presence of cyclosporin (1000 μg/L; ST/CsA) or T cells stimulated with PMA + ionomycin in the presence of triptolide (1000 μg/L; ST/Trip). Specific double-stranded oligonucleotide probes were radiolabelled with $^{32}P$ and EMSAs were performed as described.[47] (**a**) Purine-box regulator binding at NF-AT target DNA sequence, (**b**) NF-κB, (**c**) AP-1, (**d**) Oct-1.

change in susceptibility to immunosuppression by triptolide.[47] These results indicate that the mechanisms of immunosuppression by triptolide differ from those of cyclosporin and tacrolimus, and probably do not involve calcineurin signalling.

#### *5.1.2 Nuclear Factor κB*

Further clues to the cellular site of action of triptolide came from the mechanisms of triptolide inhibition of NF-κB transcriptional activation.[47] The immunosuppressants cyclosporin and tacrolimus cause partial inhibition of T-cell NF-κB activation triggered by the combination of PMA + ionomycin.[47] However, cyclosporin and tacrolimus produce essentially no inhibition of NF-κB activation triggered by PMA alone. In contrast, triptolide completely suppressed NF-κB transcriptional activation triggered by PMA alone, or by PMA + ionomycin.[47]

In resting cells, the NF-κB p65/Rel A subunit exists in an inactive form tethered in the cytoplasm by a specific inhibitor, I-κB. Cell activation triggered through TNF receptors or exposure to PMA stimulates IκB phosphorylation and degradation, releasing p65 for translocation into the nucleus. In the nucleus, p65/Rel A combines with p50 to form an NF-κB heterodimer that can bind with high affinity to NF-κB target DNA elements in cytokine enhancer sequences.[69,95,96]

We showed that triptolide treatment of cells led to augmented nuclear DNA-binding activity of NF-κB p65/Rel A (figure 2), yet triptolide caused complete inhibition of NF-κB-regulated transcriptional activation.[47] The implication of this result is that triptolide exerts its immunosuppressive effects on NF-κB activation in the nucleus, after the development of high-affinity specific DNA binding. We proposed that triptolide might inhibit a post-translational modification of DNA-bound NF-κB, which is necessary for transcriptional activation to occur. Post-translational phosphorylation of NF-κB Rel A by protein kinase A has been shown to modulate interaction with a transcriptional coactivator protein with acetyltransferase activity, p300/CBP.[97,98] Other laboratories have confirmed that triptolide inhibits NF-κB transcrip-

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tional activation without inhibiting nuclear NF-κB DNA-binding activity.[58,63] Recent studies have revealed that NF-κB transcriptional activation is regulated in the nucleus by variable interactions between co-repressor histone deacetylases and co-activating acetyltransferases.[98,99]

On the basis of the ability of triptolide to inhibit calcium-independent NF-κB transcriptional activation, we hypothesised that triptolide would exert anti-inflammatory effects and suppress chemokine expression in non-lymphoid cells. IL-8 is an important chemokine for neutrophils, and the transcriptional regulation of IL-8 expression is predominantly dependent on NF-κB.[51,100] We showed that triptolide inhibited IL-8 transcription, NF-κB transcription, and IL-8 mRNA and protein expression in 16HBE human bronchial epithelial cells.[51] These results demonstrated the anti-inflammatory effects of triptolide, and suggested that triptolide would inhibit the expression of numerous inflammatory genes known to be regulated by NF-κB, such as IL-8, macrophage inflammatory protein (MIP)-1α, monocyte chemoattractant protein (MCP)-1, RANTES, eotaxin, inducible nitric oxide synthase, inducible cyclo-oxygenase-2, 5-lipoxygenase, cytosolic phospholipase $A_2$, intercellular adhesion molecule-1 (ICAM-1), vascular-cell adhesion molecule 1 and E-selectin.

NF-κB has been shown to play a significant role in cell survival. Inhibition of NF-κB activity can promote cell death by apoptosis.[101] Our discovery that triptolide interferes with NF-κB transcriptional activation may represent one mechanism through which triptolide sensitises cells to apoptosis.[47,63]

#### *5.1.3 Activator Protein 1*

AP-1, a heterodimer of Fos and Jun subunits, is important for transcriptional activation of genes associated with the inflammatory response.[69] We showed that triptolide inhibited the induction of AP-1-specific DNA-binding activity in Jurkat T cells stimulated with PMA + ionomycin (figure 2). Thus, the inhibition of AP-1 DNA-binding activity by triptolide was similar to that observed for the binding to the purine-box/ARRE/NF-AT target DNA sequences (figure 2), but different from the binding to the NF-κB target DNA sequences (figure 2). Jiang et al. demonstrated that triptolide inhibits transcriptional activation of an AP-1 luciferase reporter gene.[58] Triptolide showed no significant inhibition of Oct-1 DNA binding (figure 2).

### 5.2 Triptolide Inhibits Expression of Genes Associated with Cell-Cycle Progression

The synergistic effects of triptolide with chemotherapeutic agents were characterised in detail by Chang et al.,[57] who demonstrated that triptolide caused translational induction of the p53 tumour suppressor gene. However, the increased cellular p53 protein was transcriptionally inactive in the presence of triptolide. As a consequence, a target gene regulated by p53 transcription, the $p21^{Waf/Cip}$ cell-cycle inhibitor, was inhibited in the presence of triptolide. The inhibition of p21 expression by triptolide is proposed as the mechanism through which triptolide cooperates with doxorubicin to induce tumour cells to undergo apoptosis.[57] Triptolide treatment caused HT1080 cells to accumulate at the late $G_1$/S phase of the cell cycle.[57] A mechanism for this accumulation was proposed to involve triptolide inhibition of cyclins A, D or E necessary for the $G_1$/S transition.[57]

Jiang et al.[58] also showed that triptolide induced p53 protein expression, but reported that triptolide treatment led to induction of $p21^{Waf/Cip}$ and $G_0/G_1$ cell cycle arrest in MKN gastric cancer cells. These differing results of triptolide effects on $p21^{Waf/Cip}$ may arise from the different cell types examined.[57,58]

### 5.3 Use of DNA Microarrays to Identify Novel Gene Targets of Triptolide

DNA microarrays are useful tools for characterising the effects of triptolide on the expression of numerous genes involved in cell-cycle regulation and the inflammatory response. We characterised the effects of triptolide on gene expression *in vitro* in resting and stimulated Jurkat T cells, and in 16HBE bronchial epithelial cells.[51] More recently, we characterised changes in patterns of

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**Table III.** Genes on the Atlas array inhibited by triptolide in stimulated Jurkat T cells

| Position | Gene name(s) | GenBank | Comments |
|---|---|---|---|
| A61 | Cyclin B1, $G_2$/M-specific | M25733 | Associates with p34cdc2 to regulate cell cycle |
| B5b | MARCKS-related protein | X70326 | Integrates $Ca^{2+}$ and PKC signals in nerves |
| B5g | MAP kinase kinase 3 (MAPKK3) | L36719 | Stress-activated, phosphorylates MAP kinase |
| B5m | PKA, cyclic AMP-dependent protein kinase, regulator R1β | M65066 | Enhanced expression in cancer cells, tumours |
| B7c | Colon carcinoma kinase-4 | U33635 | Catalytically inactive receptor tyrosine kinase |
| C3n | Lymphotoxin-β (tumour necrosis factor C) | L11015 | Homotrimer signals lymphoid development |
| C4h | Bcl-2-related Al protein (Bfl-1) | U29680 | Anti-apoptotic protein, induced by stimulation |
| C4l | Apoptosis inhibitor HIAP1 | U45878 | Induced by NF-κB, signals TNF receptor to caspases |
| C4l | Cysteine protease ICE-LAP6 (caspase 9) | U56390 | Apoptotic caspase, target of cytochrome c, granzyme |
| C7g | Growth arrest, DNA-damage induced (GADD153) | S40706 | CHOP: C/EBP, bzip transcription factor |
| D3b | DB 1 (zinc finger protein 161) | D28118 | Binds to and regulates IL-3 promoter |
| D4f | Transcription factor ETR 103 (EGR-1) | M62829 | Immediate early inducible transcription factor |
| D5f | Nucleobindin | M96824 | Secreted DNA-binding protein |
| D6f | Cyclin-dependent kinase inhibitor (p21, Cip1) | U09579 | Negative regulator of cell cycle |
| D7h | Homeobox protein HOX-D3 | D11117 | Transcription factor in development and endothelium |
| E5e | CD27L receptor (T-cell active antigen, CD27) | M63928 | TNF receptor homologue regulates lymphoid growth |
| E5l | Leukosialin (sialophorin, CD43) | J04536 | *O*-Glycosylated lymphoid adhesion molecule |
| E7k | Integrin α L (LFA-1 α chain, CD11a) | Y00796 | Integrin binds to ICAM-1, regulates T-cell activation |
| E2m | IL-2 receptor, α chain (CD25) | X01057 | IL-2 receptor mediates cell growth, differentiation |
| Fle | GM-CSF | Ml 1220 | Stimulates growth of neutrophils, eosinophils, monocytes |
| F2c | T-cell activation protein (Act-2, MIP-1β) | J04130 | Monocyte CC chemokine |
| F2n | MIP-1α | M23452 | Monocyte, lymphocyte CC chemokine |
| F4b | Endothelin-2 (ET-2) | M65199 | Vasoconstrictor peptide |
| F5n | IL-3 (mast cell growth factor) | M14743 | Haemopoietic growth factor |
| F6b | IL-6 (IFN$\beta_2$, B-cell differentiation factor) | X04602 | Coordinates acute phase of inflammation |
| F5f | IL-8 (neutrophil activating protein) | Y00787 | Chemokine and growth factor for neutrophils |
| Flg | Transforming growth factor-$\beta_2$ | M19154 | Controls development, inflammation immunity |
| F3g | Fibroblast growth factor receptor 1 (FGFRI) | M37722 | BFGF receptor tyrosine kinase, epithelial growth |
| F6g | IL-13 | L06801 | IL-4-like activity on B-cells (promotes IgE) |
| F2l | RANTES protein, T-cell specific | M21121 | CC chemokine for monocytes, up in allergy |
| F5k | IL-2 | A14844 | Controls growth and differentiation of T cells |

**BFGF** = basic fibroblast growth factor; **cAMP** = cyclic adenosine monophosphate; **GM-CSF** = granulocyte-macrophage colony-stimulating factor; **ICAM-1** = intercellular adhesion molecule 1; **IFN** = interferon; **IL** = interleukin; **LFA** = lymphocyte function-assisted antigen; **MARCKS** = myristoylated alanine-rich C kinase substrate; **MAP** = mitogen-activated protein; **MIP** = macrophage inflammatory protein; **PK** = protein kinase.

gene expression *in vivo* in triptolide-treated rats with monocrotaline-induced pulmonary hypertension.

For the *in vitro* analyses, RNA was prepared from Jurkat T cells or 16HBE cells, resting or stimulated for 6 hours with PMA + ionomycin, in the absence or presence of triptolide. Total RNA was prepared and used for preparation of $^{32}$P-radiolabelled first strand cDNA. The labelled cDNAs were hybridised to nylon membranes that contained 588 immobilised genes involved in cell signalling (Clontech Atlas array).

In activated Jurkat T cells, triptolide inhibited the expression of 31 genes (table III), including

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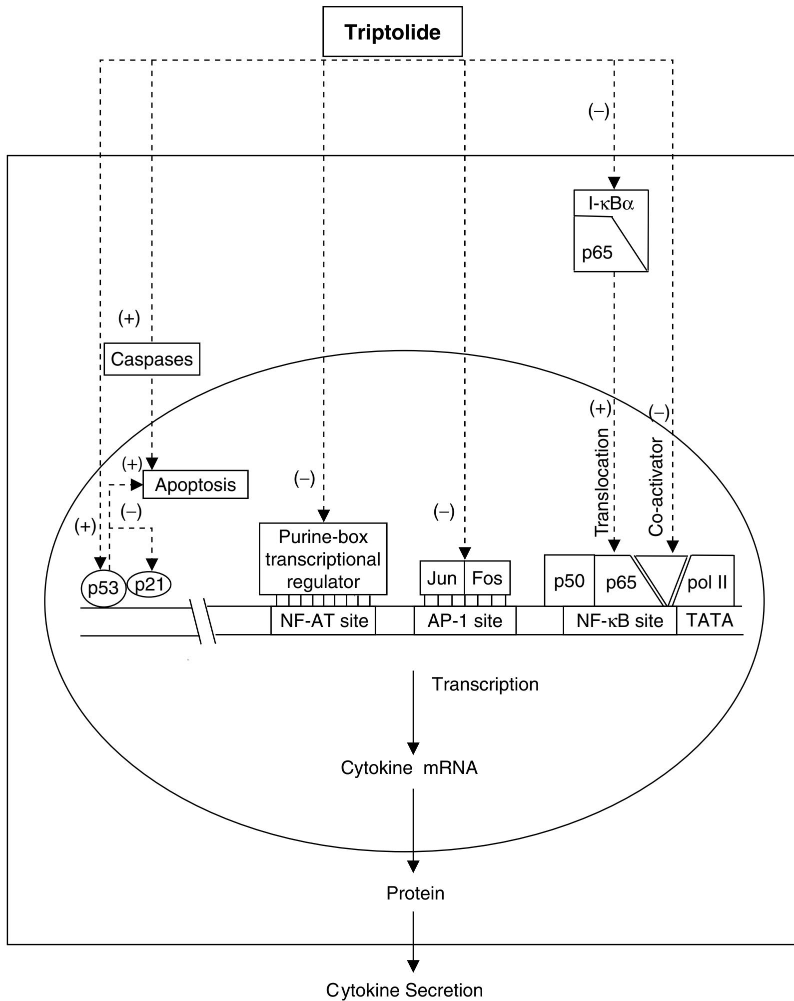


**Fig. 3.** Schematic representation of postulated mechanisms of nuclear inhibition of transcriptional activation and enhancement of apoptosis by triptolide.
**AP-1** = activator protein 1; **I-κBα** = inhibitor of κB; **NF-AT** = nuclear factor of activated T cells; **NF-κB** = nuclear factor κB; **pol II** = DNA polymerase II; **p65** = subunit of NF-κB.

those for IL-2, IL-3, IL-6, IL-8, IL-13, TNFα, TGFβ, MIP-1α, MIP-1β, GM-CSF and RANTES. Triptolide also inhibited expression of cell surface receptors, including IL-2 receptor α-chain (CD25), lymphotoxin, CD27, CD43 and nucleobindin. Several apoptosis inhibitor-related genes were inhibited by PG490. Bfl-1 is a bcl-2 homologue that promotes cell survival. Bfl-1 expression is strongly induced following activation of NF-κB, and its expression can block TNFα-induced apoptosis.[102] Many genes are unaltered in the presence of triptolide, which provides reassurance that triptolide is not acting as a general transcriptional or cellular poison.

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In 16HBE human bronchial epithelial cells, we characterised the anti-inflammatory effects of triptolide on PMA-stimulated gene transcription.[51] We showed that triptolide inhibits expression of the PMA-induced genes TNFα, IL-8, MIP-2α, ICAM-1, integrin β6, vascular endothelial growth factor (VEGF), GM-CSF, GATA-3, fra-1 and NF45. Triptolide also inhibited the constitutively expressed cell-cycle regulators and survival genes cyclins D1, B1, A1, cdc-25, bcl-x and c-Jun.

## 6. Conclusion

We propose that triptolide inhibits transcriptional activation through NF-AT, NF-κB and AP-1 factors at a unique step in the nucleus (figure 3). For NF-κB, triptolide inhibition occurs after the step of high-affinity binding of p65/Rel A to target DNA sequences.

What are the precise molecular mechanisms through which triptolide inhibits the transcriptional activation of nuclear transcription factors such as NF-κB, the purine-box regulator at the NF-AT target site, AP-1 and p53? How does triptolide inhibit NF-κB and p53 transcriptional activation without inhibiting specific DNA binding? Does triptolide interact with and block each transcription factor? Or does triptolide inhibit the activity of an upstream modifying enzyme such as a kinase or phosphatase? Does triptolide affect a nuclear co-activator or co-repressor involved in the activation of selective transcription factors associated with cellular inflammation and survival? These represent some of the unresolved questions that need to be answered in order to elucidate the anti-inflammatory and pro-apoptotic mechanisms of triptolide.

Kupchan et al.[46] predicted that the epoxide moieties that are essential for the potency of triptolide might interact covalently with key cellular targets. Such targets covalently modified by triptolide might represent signalling intermediates whose (enzymatic) functions may be inhibited by covalent inactivation of active sites. Identification of the cellular target(s) covalently labelled by triptolide represents one of the high priorities in elucidating the molecular mechanisms of action of triptolide.[48]

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