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Title	Effects of triptolide, an alcohol extract of a Chinese herb, Thunder God Vine, on peripheral blood mononuclear cell protein synthesis and signal transduction in rheumatoid arthritis(RA)
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EFFECTS OF TRIPTOLIDE, AN ALCOHOL EXTRACT OF A CHINESE HERB, *THUNDER GOD VINE*, ON PERIPHERAL BLOOD MONONUCLEAR CELL PROTEIN SYNTHESIS AND SIGNAL TRANSDUCTION IN RHEUMATOID ARTHRITIS (RA).

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Objective: *Thunder God Vine (Tripterygium Wilfordii Hook, TWH)*, a Chinese herb, is widely used in China in the treatment of RA. We have previously shown that triptolide (Tr), a purified ethanol extract of *TWH*, has potent immunosuppressive effects. This study investigated the mechanism of immunosuppression of Tr.
Methods: Peripheral blood mononuclear cells (PBMC) from controls and patients with RA were obtained by centrifugation over lymphoprep. Purified T cells were obtained by negative selection by anti-CD14 and anti-CD19 respectively. Intracellular calcium changes in purified T cells in response to PHA stimulation following 3 days incubation with various concentrations of Tr (0 - 15 nM) were determined by flowcytometry using the Ca^{2+} dependent dye Fluo-3. Protein biosynthesis by PBMC following stimulation by PMA/ionomycin and Tr incubation was measured by H^3-leucine amino acid labelling and immunoprecipitation.
Results: PHA induced an immediate influx in intracellular Ca^{2+}. The rise in Ca^{2+} was unaltered in cells from both controls and RA patients pre-incubated with Tr at doses (>7.5 nM) that already blocked lymphoctye responses. On the other hand, Tr had significant in vitro inhibitory effects on PBMC protein biosynthesis at concentration ≥7.5 nM in both controls and patients with RA.

	No PMA/I	PMA/I only	PMA/I + 2 nM Tr	PMA/I + 7.5 nM Tr
Controls	0.85 ± 0.32	7.39 ± 0.37	6.68 ± 0.64	3.23 ± 0.59 *
RA	0.67 ± 0.21	8.31 ± 0.78	8.05 ± 0.67	1.89 ± 0.27*

Results are expressed as mean ± sem H^3-leucine positive cells (10^3 cpm)

Conclusion: Our results showed that the inhibitory actions of triptolide on lymphocyte activation was not through Ca^{2+} mobilisation and the early signal transduction process. However, inhibition of PBMC protein biosynthesis may be one of the mechanisms of action of triptolide.

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EFFECTS OF TRIPTOLIDE, AN ALCOHOL EXTRACT OF A CHINESE HERB, *THUNDER GOD VINE*, ON PERIPHERAL BLOOD T CELL PROLIFERATION AND B CELL IMMUNOGLOBULIN PRODUCTION IN RHEUMATOID ARTHRITIS (RA).

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Objective: *Thunder God Vine (Tripterygium Wilfordii Hook, TWH)*, a Chinese herb, is widely used in China in the treatment of RA. 2 major crude preparations of *TWH* have been extracted - an ethanol extract and a methanol extract (T2). Previous studies have demonstrated the immunosuppressive effects of T2 on normal T and B lymphocytes. In this study, we tested the effects of triptolide, a purified ethanol extract of *TWH*, on the activity and viability of T cells, B cells and synoviocytes from patients with RA.
Methods: Peripheral blood mononuclear cells (PBMC) from control subjects and patients with RA were obtained by centrifugation over lymphoprep. Synovial tissue was obtained from RA patients at the time of surgery for total knee replacement. PBMC were incubated at 1 x 10^6 cell/ml in complete medium with or without various concentrations of triptolide (0 - 15 nM). PBMC proliferation following stimulation by T cell stimulants PHA, IL2 and PMA/ionomycin, was measured by thymidine incorporation. Synovial cell proliferation was determined by crystal violet staining. B cell production of IgG and IgM following stimulation by SAC and PWM was measured by ELISA. Cell viability was determined by trypan blue exclusion test.
Results: Triptolide significantly reduced PBMC proliferation in response to PHA, IL2 or PMA/ionomycin in a dose dependent manner. The half maximum inhibitory effects of triptolide on healthy donor derived PBMC was obtained at 2.7, 3.7 and 6.7 nM in reponse to PHA, IL2 and PMA/ionomycin respectively. The corresponding figures for RA derived PBMC were 3.2, 3.5 and 6.1 nM. Similar effects were observed in synovial cell proliferation. Control/RA derived IgG and IgM production following stimulation by SAC was significantly inhibited by triptolide at ~3 nM concentration. Inhibitory effects were observed during the first 3 days of a 10-day culture period suggesting that triptolide inhibits the initial stages of B cell activation. Using the trypan blue exclusion test, cell killing by triptolide was observed at a dose ≥15 nM.
Conclusion: Triptolide has significant immunosuppressive effects on both healthy subject and RA patient derived T and B cells. These effects are probably not due to non-specific cytotoxicity.