Modulation of Cytokine Expression by Traditional Medicines: A Review of Herbal Immunomodulators

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Abstract

INTRODUCTION: Modulation of cytokine secretion may offer novel approaches in the treatment of a variety of diseases. One strategy in the modulation of cytokine expression may be through the use of herbal medicines. A class of herbal medicines, known as immunomodulators, alters the activity of immune function through the dynamic regulation of informational molecules such as cytokines. This may offer an explanation of the effects of herbs on the immune system and other tissues. For this informal review, the authors surveyed the primary literature on medicinal plants and their effects on cytokine expression, taking special care to analyze research that utilized the multi-component extracts equivalent to or similar to what are used in traditional medicine, clinical phytotherapy, or in the marketplace. METHODOLOGY: MEDLINE, EBSCO, and BIOSIS were used to identify research on botanical medicines, in whole or standardized form, that act on cytokine activity through different models, i.e., *in vivo* (human and animal), *ex vivo*, or *in vitro*. RESULTS: Many medicinal plant extracts had effects on at least one cytokine. The most frequently studied cytokines were IL-1, IL-6, TNF, and IFN. *Acalypha wilkesiana, Acanthopanax gracilistylus, Allium sativum, Ananus comosus, Cissampelos sympodialis, Coriolus versicolor, Curcuma longa, Echinacea purpurea, Grifola frondosa, Harpagophytum procumbens, Panax ginseng, Polygala tenuifolia, Poria cocos, Silybum marianum, Smilax glabra, Tinospora cordifolia, Uncaria tomentosa*, and *Withania somnifera* demonstrate modulation of multiple cytokines. CONCLUSION: The *in vitro* and *in vivo* research demonstrates that the reviewed botanical medicines modulate the secretion of multiple cytokines. The reported therapeutic success of these plants by traditional cultures and modern clinicians may be partially due to their effects on cytokines. Phytotherapy offers a potential therapeutic modality for the treatment of many differing conditions involving cytokines. Given the activity demonstrated by many of the reviewed herbal medicines and the increasing awareness of the broad-spectrum effects of cytokines on autoimmune conditions and chronic degenerative processes, further study of phytotherapy for cytokine-related diseases and syndromes is warranted. (*Altern Med Rev* 2006;11(2):128-150)

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Introduction

Cytokines, a large group of soluble extracellular proteins or glycoproteins, are key intercellular regulators and mobilizers. Classified into family groups (e.g., interleukins, interferons, and chemokines) based on the structural homologies of their receptors, these proteins were initially believed to act primarily as antiviral[1] or antineoplastic[2] agents. They are now seen to be crucial to innate and adaptive inflammatory responses, cell growth and differentiation, cell death, angiogenesis, and developmental as well as repair processes.[3] Their secretion, by virtually every nucleated cell type, is usually an inducible response to injurious stimuli.[3] In addition, cytokines provide a link between organ systems, providing molecular cues for maintaining physiological stability.[4] Medical literature of the last several decades reveals an array of conditions, from cardiovascular disease to frailty, whose onset and course may be influenced by cytokines.[5]

The diverse and far-reaching influences of these proteins can be seen in the central nervous system (CNS); cytokines cause the brain to produce neurochemical, neuroendocrine, neuroimmune, and behavioral shifts.[6] Abnormal cytokine production has been demonstrated in neuropsychiatric disorders such as attention deficit hyperactivity disorder, obsessive-compulsive disorder, and anorexia nervosa.[6,7] Cytokines also appear to play a role in depression, schizophrenia, and Alzheimer's disease,[7] and may be a common link between insomnia and depression.[8,9] In addition, there appears to be an involvement of cytokines in anhedonia (the inability to experience pleasure) and learned helplessness.[10]

The understanding of stimuli that invoke cytokine secretion has expanded. Besides chronic infections, negative emotions and stressful experiences have been shown to stimulate production of proinflammatory cytokines.[5] In addition to involvement in neuropsychiatric disorders, these diverse glycoproteins have activity in all body systems. As models of physiology continue to develop beyond compartmentalized organ systems, elucidation of the global activity of cytokines offers further support to an expanding understanding of cell-to-cell communication. The inflammatory processes of cardiovascular disease are one such example. Beyond leukocytes, the liver, heart, vessel walls, and adipose tissue are known to produce cytokines; thus any of these tissues may potentially contribute to the inflammatory nature of cardiovascular disease.[11]

As a result of the growing recognition of cytokine activities, altering cytokine expression and targeting their receptors may offer therapeutic potential. Current pharmacological strategies include cytokine antagonist, agonist, inhibition, and stimulation models.[12] Therapeutic application of cytokines in clinical medicine has rapidly surpassed the FDA's 1986 approval of an interferon (IFN) agonist for the treatment of hairy cell leukemia. In 2001, an antagonist to tumor necrosis factor (TNF), a pivotal cytokine in the pathogenesis of rheumatoid arthritis (RA), was described as one of the most important advances in RA treatment.[13] In addition, interleukin-1ß (IL-1ß) and TNF antagonists offer options for the treatment of periodontal disease.[14] A novel approach in the treatment of asthma is the inhibition of T-helper 2 (TH2) derived cytokine expression, resulting in downstream effects on IgE and eosinophils.[15] Interleukin-10 (IL-10) demonstrates modulation of brain inflammation, which may have application for conditions such as Alzheimer's disease.[16] In additional, interleukin-2 (IL-2) and interleukin-12 (IL-12) in combination may provide a potential therapeutic approach for neuroblastomas.[17]

Due to their diverse and pleiotropic activities, cytokine treatments may prove promising for disorders seemingly unrelated to immune function. However, much of their therapeutic effect relies on direct influence of immune activity. For example, in the field of oncology, progress has been made in the therapeutic use of several interleukins, including IL-4, -6, -11 and -12.[18] In combination with surgery, pretreatment with IL-2 may enhance survival rates in patients with renal cell carcinoma.[19] IL-18 demonstrates antitumor effects in leukemia.[20] The interferons are used in the treatment of hepatitis B and C, malignant melanoma, follicular lymphoma, and AIDS-related Kaposi's sarcoma.[21]

However, as with the development of many nascent pharmacological strategies, the occurrence of adverse events generates barriers to successful therapeutic applications. Such obstacles have delayed progress in the use of several synthetic cytokines.

Treatment with recombinant cytokines has yielded a number of adverse effects, such as transient lymphopenias induced by IFN, IL-2, and TNF. Monocytopenia has been reported with the use of interferon-gamma (IFN-γ) and TNF, while IL-2, IFN-α, and TNF induce neutrophilia.[22] Patient experience of flu-like symptoms with the use of interferons makes adherence to a therapeutic protocol a challenge. Both IL-2 and IFN-α, used for the treatment of hepatitis C and some cancers, are known to evoke depression, fatigue, sleepiness, irritability, and loss of appetite.[23] These toxic side effects have limited the clinical value of such therapies.[24]

In light of the adverse events experienced with cytokine-targeted therapy, it could prove useful to consider the use of phytotherapy in the modulation of cytokine expression. Immune-related illnesses have long been treated with herbal medicines. The primary literature suggests many of the effects of botanicals may be via cytokine modulation.[25] The term immunomodulator has been used in the phytotherapy literature to describe botanical medicines believed to influence immunity.[26] In regard to phytotherapy, immunomodulators may be defined as botanical medicines that alter the activities of the immune system via the dynamic regulation of informational molecules – cytokines, hormones, neurotransmitters, and other peptides.

This article provides an informal review of the scientific literature regarding the effects of botanical medicines on cytokines. Islam and Carter point out that therapy based on medicinal plants, such as the immunomodulators, is based on diverse constituents or groups of constituents and therefore, researching isolated constituents to reveal modes of activity disregards the principles of phytotherapy.[27] In addition, when clinicians use medicinal plant preparations in practice, they often do not treat with isolated constituents. Therefore, in order to maintain relevance for clinical phytotherapy, this informal survey was limited to herbal medicines available in the marketplace or preparations that represent multi-component botanical medicines.

Methodology

Search Strategy

The databases MEDLINE, EBSCO, and BIOSIS were searched for appropriate studies. Titles were screened for all hits to the terms "herbs and cytokines" and "Chinese medicine and cytokines" and "Ayurveda and cytokines." A language restriction of English was observed.

Criteria for Inclusion

The following parameters were necessary for study inclusion:

- Investigations on whole herbs (e.g., seed, leaf, root, stem, flower, or entire plant), standardized extracts, or extractions of whole herbs not reduced to one constituent were accepted. Research on isolated constituents or multiple herbal formulations were generally rejected. Fungi, although technically not plants, were included as they are commonly used in phytotherapy.

- All study model types were accepted – *in vitro*, *ex vivo*, and *in vivo* (both animal and human) models were accepted.

- Information on methods of herbal preparation, concentration of the plant preparation, and dose/exposure time were required.

- Only studies demonstrating activity with regard to cytokines were included.

One hundred thirty-nine titles and abstracts were reviewed for inclusion criteria. Ninety-five studies were eliminated due to single constituent-based research or insignificant results. Forty-nine papers met the criteria.

Results

Information collected as a result of searches is listed in Tables 1-5. The majority of the research used *in vitro* models, but *in vivo* animal models were also utilized. Data in Tables 1A and 1B catalog *in vivo* results, noting the genus and species of the plants, the

Table 1A. *In vivo* Effects of Botanicals on Cytokine Expression

Genus species	Plant Part	Preparation Used	Dose	Duration of Exposure	Model	Cytokines Affected	Author/Date
Aloe secundiflora	Sap	Aqueous	200 mg/kg (pre) 400 mg/kg (post) infection	2 weeks (pre) + 7 days (post)	*In vivo,* Fowl	IL-6	(Waihenya et al, 2002)[28]
Angelica sylvestris	Root	Aqueous	640 mg/kg PO	2 weeks	*In vivo,* Murine	TNF	(Haranaka et al, 1985)[29]
Asparagus racemosus	Root	80% Ethanol	100 mg/kg QD PO	17 weeks	*In vivo,* Murine	IL-1α TNF	(Dhuley, 1997)[30]
Bupleurum falcatum	Root	Aqueous	640 mg/kg PO	2 weeks	*In vivo,* Murine	TNF	(Haranaka et al, 1985)[29]
Cinnamomum cassia	Bark	Aqueous	560 mg/kg PO	2 weeks	*In vivo,* Murine	TNF	(Haranaka et al, 1985)[29]
Cnidium monnieri	Rhizome	Aqueous	560 mg/kg PO	2 weeks	*In vivo,* Murine	TNF	(Haranaka et al, 1985)[29]
Coptis spp.	Rhizome	1% Coptis pwd in standard mouse diet	1% *C. rhizoma* in diet	7 days	*In vivo,* Murine	IL-6	(Iizuka et al, 2000)[31]
Panax ginseng	Root	40% Ethanol	150 mg/kg QD injection	7 days	*In vivo,* Murine	IL-4	(Song et al, 2002)[32]
Panax ginseng	Root	40% Ethanol	150 mg/kg QD injection	21 days	*In vivo,* Murine	IL-4 TNF IFN-γ	(Song et al, 2002)[32]

Table 1B. *In vivo* Effects of Botanicals on Cytokine Expression

Genus species	Plant Part	Preparation Used	Dose	Duration of Exposure	Model	Cytokines Affected	Author/Date
Perilla frutescens	Leaf	Aqueous	0.4 mL/murine x2 PO	2 doses 18 hrs+1 hr	*In vivo,* Murine	TNF	(Ueda and Yamazaki, 1997)[33]
Picrorhiza kurroa	Rhizome	80% Ethanol	100 mg/kg QD PO	17 weeks	*In vivo,* Murine	IL-1α TNF	(Dhuley, 1997)[30]
Polygala tenuifolia	Root	Aqueous	$2x10^3$ mg/kg QD PO	9 days	*In vivo,* Murine	IL-4 IFN-γ	(Hong et al, 2002)[34]
Silybum marianum	Seed and Fruit	Silymarin	10 mg/kg QD IP injection	5 days	*In vivo,* Murine	IL-2, -4	(Johnson et al, 2003)[35]
Silybum marianum	Seed	Silymarin	250 mg/kg QD IP injection	5 days	*In vivo,* Murine	IL-1β, -6 TNF	(Johnson et al, 2003)[35]
Silybum marianum	Seed	Silymarin	250 mg/kg QD IP injection	5 days	*In vivo,* Murine	IL-2, -4	(Johnson et al, 2002)[36]
Smilax glabra	Rhizome	Aqueous	400 mg/kg QD PO	14 days	*Ex vivo,* Murine	IL-1, -2 TNF	(Jiang and Xu, 2003)[37]
Tinospora cordifolia	Root and Herb	80% Ethanol	100 mg/kg QD PO	17 weeks	*In vivo,* Murine	IL-1α TNF	(Dhuley, 1997)[30]
Withania somnifera	Root and Leaf	80% Ethanol	100 mg/kg QD PO	17 weeks	*In vivo,* Murine	IL-1α TNF	(Dhuley, 1997)[30]
Withania somnifera	Root	70% Methanol	20 mg QD IP	5 days	*In vivo,* Murine	IL-2 TNF IFN-γ	(Davis and Kuttan, 1999)[38]
Withania somnifera	Root	70% Methanol	20 mg QD IP	10 days	*In vivo,* Murine	IL-2 IFN-γ	(Davis and Kuttan, 1999)[38]

Table 2A. *In vitro* (human cell) Effects of Aqueous Botanical Extracts on Cytokine Expression

Genus species	Plant Part	Preparation Used	Dose	Duration of Exposure	Model	Cytokines Affected	Author/Date
Acanthopanax gracilistylus	Bark	Aqueous	1.08 x 10^3 µg/mL incubation	24 hours	*In vitro,* Human	IL-1	(Shan et al, 1999)[39]
Acanthopanax gracilistylus	Bark	Aqueous	20 µg/mL incubation	3 days	*In vitro,* Human	IL-1, -6 IFN-γ, TNF	(Shan et al, 1999)[40]
Astragalus membranaceus	Root	Aqueous	1.5x10^5 µg/mL incubation	24 hours	*In vitro,* Human	IL-6	(Shon et al, 2002)[41]
Astragalus membranaceus	Root	Aqueous	1.08x10^3 µg/mL incubation	24 hours	*In vitro,* Human	IL-1	(Shan et al, 1999)[39]
Cinnamomum cassia	Bark	Aqueous	1.08x10^3 µg/mL incubation	24 hours	*In vitro,* Human	IL-1	(Shan et al, 1999)[39]
Codonopsis pilosula	Root	Aqueous	1.08x10^3 µg/mL incubation	24 hours	*In vitro,* Human	IL-1	(Shan et al, 1999)[39]
Derris scandens	Stem	Aqueous	10 µg/mL incubation	5 days	*In vitro,* Human	IL-2	(Sriwanthana and Chavalittumrong, 2001)[42]
Epimedium brevicornum	Herb	Aqueous	1.08x10^3 µg/mL incubation	24 hours	*In vitro,* Human	IL-1	(Shan et al, 1999)[39]
Oldenlandia diffusa	Herb	Aqueous	1.08x10^3 µg/mL incubation	24 hours	*In vitro,* Human	IL-1	(Shan et al, 1999)[39]
Rauwolfia serpentina	Root	Aqueous	5 µg/mL incubation	42 hours	*In vitro,* Human	TNF	(Jin et al, 2002)[43]
Rauwolfia serpentina	Root	Aqueous	10 µg/mL incubation	42 hours	*In vitro,* Human	IFN-γ	(Jin et al, 2002)[43]
Schisandra chinensis	Fruit	Aqueous	1.08x10^3 µg/mL incubation	24 hours	*In vitro,* Human	IL-1	(Shan et al, 1999)[39]
Sinomenium acutum	Stem	Aqueous	0.1µg/mL incubation	1 hour	*In vitro,* Murine	TNF	(Kim et al, 1999)[44]
Smilax glabra	Rhizome	Aqueous	400 mg/kg QD PO	14 days	*Ex vivo,* Murine	IL-1, -2 TNF	(Jiang and Xu, 2003)[37]
Typhonium sp.	Rhizome	Aqueous	1.08x10^3 µg/mL incubation	24 hours	*In vitro,* Human	IL-1	(Shan et al, 1999)[39]

Table 2B. *In vitro* (human cell) Effects of Ethanolic Botanical Extracts on Cytokine Expression

Genus species	Plant Part	Preparation Used	Dose	Duration of Exposure	Model	Cytokines Affected	Author/Date
Curcuma longa	Rhizome	Acetone or Ethanol, % unspecified	1.8 µg/mL incubation	4 hours	*In vitro,* Human	TNF	(Chan, 1995)[45]
Curcuma longa	Rhizome	Acetone or Ethanol, % unspecified	1.8 µg/mL incubation	18 hours	*In vitro,* Human	IL-1	(Chan, 1995)[45]
Harpagophytum procumbens	Root	60% Ethanol	>100 µg/mL incubation	0.5 hour	*In vitro,* Human	IL-1β, -6	(Fiebich et al, 2001)[46]
Harpagophytum procumbens	Root	60% Ethanol	100 µg/mL incubation	0.5 hour	*In vitro,* Human	TNF	(Fiebich et al, 2001)[46]
Sparassis crispa	Fruit	Defatted by Ethanol, Aqueous	100 µg/mL incubation	48 hours	*In vitro,* Murine	IL-6	(Harada et al, 2002)[47]
Tripterygium wilfordii	Bark	50% Hot Ethanol	0.6 µg/mL incubation	48 hours	*In vitro,* Human	IL-2	(Chou and Chang, 1998)[48]
Zingiber officinale	Rhizome	50% Ethanol	$2x10^4$ µg/mL incubation	18 hours	*In vitro,* Human	IL-1β GM-CSF*	(Chang et al, 1995)[49]
Zingiber officinale	Rhizome	50% Ethanol	$1x10^4$ µg/mL incubation	18 hours	*In vitro,* Human	IL-6	(Chang et al, 1995)[49]

* granulocyte/macrophage-colony stimulating factor

Table 2C. *In vitro* (human cell) Effects of Other Botanical Extracts on Cytokine Expression

Genus species	Plant Part	Preparation Used	Dose	Duration of Exposure	Model	Cytokines Affected	Author/Date
Acalypha wilkesiana	Seed	6.8% Ethanol 5.9% Water 42.5% Hexane	10 μg/mL incubation	2 hours	*In vitro,* Human	IL-5, -6, IFN-γ, TNF	(Bussing et al, 1999)[50]
Allium sativum	Bulb	Crushed extract	≥0.1 μg/mL incubation	24 hours	*In vitro,* Human	IL-12	(Hodge et al, 2002)[51]
Allium sativum	Bulb	Crushed extract	≥10^4 μg/mL incubation	24 hours	*In vitro,* Human	IL-1α, -2, -6, -8, -10, IFN-γ, TNF	(Hodge et al, 2002)[51]
Ampelopsis brevipedunculata	Unspecified	Methanol, then DMSO	100 μg/mL incubation	3 days	*In vitro,* Human	IL-1β TNF	(Kuo et al, 1999)[52]
Curcuma longa	Rhizome	Curcumin in DMSO 0.1%	18 μg/mL incubation	2 hours	*In vitro,* Human	IL-8	(Hidaka et al, 2002)[53]
Echinacea purpurea	Flower and Herb	Dried or fresh juice in 20% ethanol	0.012 μg/mL incubation	18 hours	*In vitro,* Human	IL-1	(Burger et al, 1997)[54]
Echinacea purpurea	Flower and Herb	Dried or fresh juice in 20% ethanol	0.012 μg/mL incubation	72 hours	*In vitro,* Human	IL-6	(Burger et al, 1997)[54]
Echinacea purpurea	Flower and Herb	Dried or fresh juice in 20% ethanol	0.025 μg/mL incubation	36 hours	*In vitro,* Human	IL-10	(Burger et al, 1997)[54]
Echinacea purpurea	Flower and Herb	Fresh juice in 20% ethanol	0.012 μg/mL incubation	36 hours	*In vitro,* Human	TNF	(Burger et al, 1997)[54]
Echinacea purpurea	Flower and Herb	Dried juice in 20% ethanol	0.025 μg/mL incubation	36 hours	*In vitro,* Human	TNF	(Burger et al, 1997)[54]
Ludwigia octovalvis	Unspecified	Methanol, then DMSO	100 μg/mL incubation	3 days	*In vitro,* Human	IL-1β TNF	(Kuo et al, 1999)[52]
Rhus semialata	Unspecified	Methanol, then DMSO	100 μg/mL incubation	48 hours	*In vitro,* Human	IL-1β TNF	(Kuo et al, 1999)[52]
Tabernaemontana divaricata	Unspecified	Methanol, then DMSO	100 μg/mL incubation	3 days	*In vitro,* Human	IL-1β TNF	(Kuo et al, 1999)[52]

Table 3A. *In vitro* (animal cell) Effects of Aqueous Botanical Extracts on Cytokine Expression

Genus species	Plant Part	Preparation Used	Dose	Duration of Exposure	Model	Cytokines Affected	Author/Date
Acanthopanax senticosus	Seed	Aqueous	10^3 µg/mL	0.5 hour	*In vitro*, Murine	TNF	(Yi et al, 2002)[55]
Acer nikoense	Flower	Aqueous	260 µg/mL	1 hour	*In vitro*, Murine	TNF	(Fujiki et al, 2003)[56]
Cnidium monnieri	Rhizome	Aqueous	560x10^3 µg/kg PO	2 weeks	*In vivo*, Murine	TNF	(Haranaka et al, 1985)[29]
Dichroa febrifuga	Root	Aqueous	500 µg/mL incubation	20 hours	*In vitro*, Murine	TNF	(Kim et al, 2000)[57]
Ixeris dentata	Whole plant	Aqueous	100 µg/mL incubation	24 hours	*In vitro*, Murine	TNF	(Chung et al, 2002)[58]
Panax quinquefolius	Root	Aqueous	100 µg/mL incubation	20 hours	*In vitro*, Murine	TNF	(Assinewe et al, 2002)[59]
Polygala tenuifolia	Root	Aqueous	1 µg/mL incubation	18 hours	*In vitro*, Murine	IL-1 TNF	(Kim et al, 1998)[60]
Rosa davurica	Fruit	Aqueous	100 µg/mL incubation	16 hours	*In vitro*, Murine	TNF	(Kim et al, 1999)[44]
Sinomenium acutum	Stem	Aqueous	0.1 µg/mL incubation	1 hour	*In vitro*, Murine	TNF	(Kim et al, 2000)[61]
Smilax glabra	Rhizome	Aqueous	400 mg/kg QD PO	14 days	*Ex vivo*, Murine	IL-1, -2 TNF	(Jiang and Xu, 2003)[37]
Uncaria guianensis	Bark	Aqueous	9.5x10^{-3} µg/mL incubation	19 hours	*In vitro*, Murine	TNF	(Sandoval et al, 2002)[62]
Uncaria tomentosa	Bark	Aqueous	1x10^{-3} µg/mL incubation	19 hours	*In vitro*, Murine	TNF	(Sandoval et al, 2002)[62]
Uncaria tomentosa	Bark	Aqueous	100 µg/mL incubation	24 hours	*In vitro*, Murine	IL-1, -6	(Lemaire et al, 1999)[63]
Uncaria tomentosa	Bark	Aqueous	14.1x10^{-3} µg/mL incubation	2 hours	*In vitro*, Animal	TNF	(Sandoval et al, 2002)[62]

plant parts used, methods of preparation, dose, duration of exposure, model utilized, cytokines affected, and references. Tables 2A-C list the *in vitro* results utilizing human cells, categorized by solvents used for the medicinal plant extractions (A, aqueous; B, ethanolic; and C, other extractions). Tables 3A-C, similar to Tables 2A-C, list the *in vitro* results utilizing animal cells, categorized by solvents used for the medicinal plant extractions (A, aqueous; B, ethanolic; and C, other extractions). Table 4 illustrates the

research conducted on medicinal mushrooms. Tables 5A-E are categorized by cytokine, matching the cytokine and the direction of effect (upregulation or downregulation) exerted by the particular plant.

A large volume of research was disregarded due to the inclusion criteria. Much of the rejected research was based on isolated constituents. Some research on semi-purified compounds, such as curcumin or bromelain, was included due to their frequent use and availability in commerce.

Discussion

The majority of the research presented in this review relies on *in vitro* and/or animal models; the authors acknowledge the inadequacies of information derived from such research. Both *in vitro* and animal models may be misleading and often prove to be poor representations of human physiology. The lack of pharmacokinetics in an *in vitro* model brings up questions of the relevance of data gathered from such methodology. In addition, animal models often are misrepresentative of human physiology. Nevertheless, data drawn from such sources, coupled with empirical data from traditional uses of botanical medicines, may provide an insight, however limited, to the mode of activity for many of these herbs. *In vivo* and *in vitro* studies for the listed herbs do suggest that the immunomodulating effects of the botanical medicines reviewed may be due, at least in part, to cytokine modulation. Furthermore, given the broad-spectrum effect of cytokines on cell-to-cell communication, it seems likely some of the other organ systems and tissue effects of these herbal immunomodulators are due to modulation of cytokine expression.

Astragalus membranaceus

The root of *Astragalus membranaceus* is traditionally used in Chinese medicine as a "spleen chi tonic" and for various deficiency and wasting conditions.[79] *A. membranaceus*, in an *in vitro* human model, has been shown to lower IL-6.[41] IL-6 is implicated in a number of inflammatory disorders and as a global marker of impending deterioration.[5] The decrease of IL-6 activity provides a possible rationale for thousands of years of use of this plant in deficiency and wasting diseases. In addition, Astragalus is also indicated in shortness of breath and edema,

Table 3B. *In vitro* (animal cell) Effects of Ethanolic Botanical Extracts on Cytokine Expression

Genus species	Plant Part	Preparation Used	Dose	Duration of Exposure	Model	Cytokines Affected	Author/Date
Cissampelos sympodialis	Leaf	80% Warm Ethanol	12.5 µg/mL incubation	24 hours	*In vitro*, Murine	IL-4, IFN-γ	(Piuvezam et al, 1999)[64]
Cissampelos sympodialis	Leaf	80% Warm Ethanol	6.25 µg/mL incubation	24 hours	*In vitro*, Murine	IL-10	(Piuvezam et al, 1999)[64]
Emblica officinalis	Fruit	70% Ethanol	250 µg/mL incubation	48 hours	*In vitro*, Murine	IFN-γ	(Sai Ram et al, 2003)[65]
Sparassis crispa	Fruit	Defatted by Ethanol Aqueous	100 µg/mL incubation	48 hours	*In vitro*, Murine	IL-6	(Harada et al, 2002)[47]

Table 3C. *In vitro* (animal cell) Effects of Other Botanical Extracts on Cytokine Expression

Genus species	Plant Part	Preparation Used	Dose	Duration of Exposure	Model	Cytokines Affected	Author/Date
Ananus comosus	Stem	Bromelain	50 µg/mL incubation	24 hours	*In vitro*, Murine	TNF	(Engwerda et al., 2001)[66]
Ananus comosus	Stem	Bromelain	50 µg/mL incubation	24 hours	*In vitro*, Murine	IFN-γ	(Engwerda et al., 2001)[66]
Echinacea purpurea	Unspecified	Simulated digestion	80 µg/mL incubation	24 hours	*In vitro*, Murine	IL-1α, IL-1β	(Rininger et al., 2000)[67]
Echinacea purpurea	Unspecified	Simulated digestion	320 µg/mL incubation	24 hours	*In vitro*, Murine	IL-6	(Rininger et al., 2000)[67]
Echinacea purpurea	Unspecified	Simulated digestion	5 µg/mL incubation	24 hours	*In vitro*, Murine	TNF	(Rininger et al., 2000)[67]
Paeonia suffruticosa	Bark	95% Methanol	100 µg/mL incubation	1 hour	*In vitro*, Bovine	IL-8	(Oh et al, 2003)[68]
Pinus maritima	Bark	Pycnogenol	50 µg/mL incubation	6 hours	*In vitro*, Murine	IL-1β, -2	(Cho et al, 2001)[69]
Scutellaria baicalensis	Root	70% Methanol	10 µg/mL incubation	24 hours	*In vitro*, Murine	TNF	(Kim et al, 2001)[70]
Silybum marianum	Seed and Fruit	Methanol, then Hexane	25 or 250 µg/mL	60 hours	*In vitro*, Murine	IL-4, -10, IFN-γ	(Wilasrusmee et al, 2002)[71]
Silybum marianum	Seed and Fruit	Silymarin	10 mg/kg QD IP injection	5 days	*In vivo*, Murine	IL-2, -4	(Johnson et al, 2003)[35]
Silybum marianum	Seed	Silymarin	250 mg/kg QD IP injection	5 days	*In vivo*, Murine	IL-1β, -6, TNF	(Johnson et al, 2003)[35]
Silybum marianum	Seed	Silymarin	250 mg/kg QD IP injection	5 days	*In vivo*, Murine	IL-2, -4	(Johnson et al, 2002)[36]
Terminalia chebula	Fruit	50% Methanol	1.0 mg/mL incubation	6 hours	*In vitro*, Murine	TNF	(Shin et al, 2001)[72]
Tylophora asthmatica	Leaf	Methanol	$19x10^{-6}$ µg/mL and $19.5x10^{-3}$ µg/mL incubation	48 hours	*In vitro*, Murine	IL-2	(Ganguly et al, 2001)[73]
Tylophora asthmatica	Leaf	Methanol	$39x10^{-3}$ µg/mL incubation	18 hours	*In vitro*, Murine	IL-1	(Ganguly et al, 2001)[73]

symptoms that could be suggestive of cardiovascular effects. Notably, increased levels of IL-6 and C-reactive protein are associated with a significant increase in cardiovascular-related death.[5,11] Thus, a possible mechanism for the cardiovascular effects of *A. membranaceus* could be due to its reduction of IL-6.

Table 4. The Effect of Medicinal Mushrooms on Cytokine Expression

Genus species	Plant Part	Preparation Used	Dose	Duration of Exposure	Model	Cytokines Affected	Author/Date
Cordyceps cicadae	Fruiting body	50% Methanol, then DMSO	100 µg/mL incubation	3 days	*In vitro, Human*	IL-2 IFN-γ	(Weng et al, 2002)[74]
Cordyceps cicadae	Larvae	50% Methanol, then DMSO	100 µg/mL incubation	3 days	*In vitro, Human*	IL-2 IFN-γ	(Weng et al, 2002)[74]
Coriolus versicolor	Mycelia	70% Ethanol	5 µL/mL incubation	3 days	*In vitro, Human*	IL-1β	(Hsieh et al, 2002)[75]
Coriolus versicolor	Mycelia	70% Ethanol	3 µL/mL incubation	3 days	*In vitro, Human*	IL-6	(Hsieh et al, 2002)[75]
Coriolus versicolor	Mycelia	Aqueous	5 µL/mL incubation	3 days	*In vitro, Human*	IL-1β, -8	(Hsieh et al, 2002)[75]
Coriolus versicolor	Mycelia	Aqueous	3 µL/mL incubation	3 days	*In vitro, Human*	IL-6	(Hsieh et al, 2002)[75]
Ganoderma lucidum	Fruiting body	Aqueous Ethanolic precipitation	12.8 µg/mL incubation	48 hours	*In vitro, Murine*	IL-12	(Cao and Lin, 2002)[76]
Grifola frondosa	Fruiting body	Ethanol	$1x10^3$ µg/mL incubation	18 hours	*In vitro, Murine*	IL-12	(Kodama et al, 2002)[77]
Grifola frondosa	Fruiting body	Ethanol	$5x10^3$ µg/kg body wt	14 days	*In vivo, Murine*	TNF IFN-γ	(Kodama et al, 2002)[77]
Poria cocos	Sclerotium	50% Hot Ethanol	800 µg/mL incubation	6 or 24 hours	*In vitro, Human*	IL-1β	(Yu and Tseng, 1996)[78]
Poria cocos	Sclerotium	50% Hot Ethanol	400 µg/mL incubation	6 or 24 hours	*In vitro, Human*	IL-6	(Yu and Tseng, 1996)[78]
Poria cocos	Sclerotium	50% Hot Ethanol	400 µg/mL incubation	3, 6 or 12 hours	*In vitro, Human*	TNF	(Yu and Tseng, 1996)[78]
Poria cocos	Sclerotium	50% Hot Ethanol	200 µg/mL incubation	3, 6 or 24 hours	*In vitro, Human*	TGF-β	(Yu and Tseng, 1996)[78]

Table 5A. Botanical Influences on IL-1

Cytokine	Plant	Model	Direction of Effect	Author/Date
IL-1	*Acanthopanax gracilistylus*	*In vitro*, Human	Increase	(Shan et al, 1999)[39]
	Astragalus membranaceus	*In vitro*, Human	Increase	(Shan et al, 1999)[39]
	Cinnamomum cassia	*In vitro*, Human	Increase	(Shan et al, 1999)[39]
	Codonopsis pilosula	*In vitro*, Human	Increase	(Shan et al, 1999)[39]
	Curcuma longa	*In vitro*, Human	Decrease	(Chan, 1995)[45]
	Echinacea purpurea	*In vitro*, Murine	Increase	(Burger et al, 1997)[54]
	Epimedium brevicornum	*In vitro*, Human	Increase	(Shan et al, 1999)[39]
	Oldenlandia diffusa	*In vitro*, Human	Increase	(Shan et al, 1999)[39]
	Polygala tenuifolia	*In vitro*, Murine	Decrease	(Kim et al, 1998)[60]
	Schisandra chinensis	*In vitro*, Human	Increase	(Shan et al, 1999)[39]
	Smilax glabra	*Ex vivo*, Human	Decrease	(Jiang and Xu, 2003)[37]
	Tylophora asthmatica	*In vitro*, Murine	Increase	(Ganguly et al, 2001)[73]
	Typhonium sp.	*In vitro*, Human	Increase	(Shan et al, 1999)[39]
	Uncaria tomentosa	*In vitro*, Murine	Decrease	(Lemaire et al, 1999)[63]
IL-1α	*Acanthopanax gracilistylus*	*In vitro*, Human	Increase	(Shan et al, 1999)[40]
	Allium sativum	*In vitro*, Human	Decrease	(Hodge et al, 2002)[51]
	Echinacea purpurea	*In vitro*, Murine	Increase	(Rininger et al, 2000)[67]
	Picrorhiza kurroa	*In vivo*, Murine	Increase	(Dhuley, 1997)[30]
	Tinospora cordifolia	*In vivo*, Murine	Increase	(Dhuley, 1997)[30]
	Withania somnifera	*In vivo*, Murine	Increase	(Dhuley, 1997)[30]
IL-1β	*Ampelopsis brevipedunculata*	*In vitro*, Human	Decrease	(Kuo et al, 1999)[52]
	Coriolus versicolor	*In vitro*, Human	Increase	(Hsieh et al, 2002)[75]
	Echinacea purpurea	*In vitro*, Murine	Increase	(Rininger et al, 2000)[67]
	Harpagophytum procumbens	*In vitro*, Human	Decrease	(Fiebich et al, 2001)[46]
	Ludwigia octovalvis	*In vitro*, Human	Decrease	(Kuo et al, 1999)[52]
	Pinus maritima	*In vitro*, Murine	Decrease	(Cho et al, 2001)[69]
	Poria cocos	*In vitro*, Human	Increase	(Yu and Tseng, 1996)[78]
	Rhus semialata	*In vitro*, Human	Decrease	(Kuo et al, 1999)[52]
	Silybum marianum	*In vivo*, Murine	Increase	(Johnson et al, 2003)[35]
	Tabernaemontana divaricata	*In vitro*, Human	Decrease	(Kuo et al, 1999)[52]
	Zingiber officinale	*In vitro*, Human	Increase	(Chang et al, 1995)[49]

Table 5B. Botanical Influences on IL-2, -4, and -5

Cytokine	Plant	Model	Direction of Effect	Author/Date
IL-2	*Allium sativum*	*In vitro,* Human	Decrease	(Hodge et al, 2002)[51]
	Cordyceps cicadae (Fruit body)	*In vitro,* Human	Increase	(Weng et al, 2002)[74]
	Cordyceps cicadae	*In vitro,* Human	Decrease	(Weng et al, 2002)[74]
	Derris scandens	*In vitro,* Human	Increase	(Sriwanthana and Chavalittumrong, 2001)[42]
	Pinus maritima	*In vitro,* Murine	Decrease	(Cho et al, 2001)[69]
	Silybum marianum	*In vivo,* Murine	Decrease	(Johnson et al, 2003)[35]
	Silybum marianum	*In vivo,* Murine	Decrease	(Johnson et al, 2002)[36]
	Smilax glabra	*Ex vivo,* Murine	Increase	(Jiang and Xu, 2003)[37]
	Tripterygium wilfordii	*In vitro,* Human	Decrease	(Chou and Chang, 1998)[48]
	Tylophora asthmatica	*In vitro,* Murine	Decrease	(Ganguly et al, 2001)[73]
	Tylophora asthmatica	*In vitro,* Murine	Increase	(Ganguly et al, 2001)[73]
	Withania somnifera	*In vivo,* Murine	Increase	(Davis and Kuttan, 1999)[38]
IL-4	*Cissampelos sympodialis*	*In vitro,* Murine	Increase	(Piuvezam et al, 1999)[64]
	Polygala tenuifolia	*In vivo,* Murine	Increase	(Hong et al, 2002)[34]
	Panax ginseng	*In vivo,* Murine	Decrease	(Song et al, 2002)[32]
	Panax ginseng	*In vivo,* Murine	Increase	(Song et al, 2002)[32]
	Silybum marianum	*In vivo,* Murine	Decrease	(Johnson et al, 2003)[35]
	Silybum marianum	*In vivo,* Murine	Decrease	(Johnson et al, 2002)[36]
	Silybum marianum	*In vitro,* Murine	Increase	(Wilasrusmee et al, 2002)[71]
IL-5	*Acalypha wilkesiana*	*In vitro,* Human	Increase	(Bussing et al, 1999)[50]

Allium sativum

Allium sativum (garlic), like many of the plants highlighted in this review, demonstrates effects on multiple cytokines. Garlic lowered IL-6 in an *in vitro* human model.[51] Besides the hypocholesterolemic, antioxidant, and ACE-inhibition activity of garlic,[80] the effect on IL-6 may offer further insight into garlic's well-known cardiovascular activity.

In the same model, garlic also lowered the proinflammatory cytokine IL-1. IL-1 has been postulated to be involved in the destruction of pancreatic ß-cells[80] and garlic demonstrates hypoglycemic action and amelioration of alloxan-induced diabetes in murine models.[81] IL-1 inhibition may be partially responsible for this activity.

Because of the potential for garlic to reduce the proinflammatory cytokines IL-1, TNF, and IL-8, and stimulate IL-10 secretion (an antagonist of proinflammatory cytokines), Hodge et al[51] concluded that this effect, along with garlic's antimicrobial activity, may provide potential mechanisms for garlic's use in inflammatory bowel disease.[80] IL-10 demonstrates modulation of the immunopathology of brain inflammatory diseases such as Alzheimer's disease, providing another potential use for garlic as a cytokine modulator.[16]

Table 5C. Botanical Influences on IL-6, -8, -10, and -12

Cytokine	Plant	Model	Direction of Effect	Author/Date
IL-6	*Acalypha wilkesiana*	*In vitro,* Human	Increase	(Bussing et al, 1999)[50]
	Acanthopanax gracilistylus	*In vitro,* Human	Increase	(Shan et al, 1999)[40]
	Allium sativum	*In vitro,* Human	Decrease	(Hodge et al, 2002)[51]
	Aloe secundiflora	*In vitro,* Murine	Increase	(Waihenya et al, 2002)[28]
	Astragalus membranaceus	*In vitro,* Human	Decrease	(Shon et al, 2002)[41]
	Coptis spp.	*In vivo,* Murine	Decrease	(Iizuka et al, 2000)[31]
	Coriolus versicolor	*In vitro,* Human	Decrease	(Hsieh et al, 2002)[75]
	Coriolus versicolor	*In vitro,* Human	Increase	(Hsieh et al, 2002)[75]
	Echinacea purpurea	*In vitro,* Human	Increase	(Burger et al, 1997)[54]
	Echinacea purpurea	*In vitro,* Murine	Increase	(Rininger et al, 2000)[67]
	Harpagophytum procumbens	*In vitro,* Human	Decrease	(Fiebich et al, 2001)[46]
	Poria cocos	*In vitro,* Human	Increase	(Yu and Tseng, 1996)[78]
	Silybum marianum	*In vivo,* Murine	Increase	(Johnson et al, 2003)[35]
	Sparassis crispa	*In vitro,* Murine	Increase	(Harada et al, 2002)[47]
	Uncaria tomentosa	*In vitro,* Murine	Increase	(Lemaire et al, 1999)[63]
	Zingiber officinale	*In vitro,* Human	Increase	(Chang et al, 1995)[49]
IL-8	*Coriolus versicolor*	*In vitro,* Human	Decrease	(Hsieh et al, 2002)[75]
	Curcuma longa (Curcumin)	*In vitro,* Human	Decrease	(Hidaka et al, 2002)[53]
	Paeonia suffruticosa	*In vitro,* Bovine	Decrease	(Oh et al, 2003)[68]
IL-10	*Allium sativum*	*In vitro,* Human	Increase	(Hodge et al, 2002)[51]
	Cissampelos sympodialis	*In vitro,* Murine	Increase	(Piuvezam et al, 1999)[64]
	Echinacea purpurea	*In vitro,* Human	Increase	(Burger et al, 1997)[54]
	Silybum marianum	*In vitro,* Murine	Increase	(Wilasrusmee et al, 2002)[71]
IL-12	*Allium sativum*	*In vitro,* Human	Decrease	(Hodge et al, 2002)[51]
	Ganoderma lucidum	*In vitro,* Murine	Increase	(Cao and Lin, 2002)[76]
	Grifola frondosa	*In vitro,* Murine	Increase	(Kodama et al, 2002)[77]

The Case for Multi-Component Remedies: A Hypothesis

One of the criticisms of botanical medicines is they are "crude drugs" representing a dilute mixture consisting of hundreds of compounds, not concentrated to contain any single active constituent. Laboratory studies clearly elucidate that the overall pharmacological effects and therapeutic efficacies of medicinal plants often do not derive from a single compound, but from several compounds generating synergic activity.[82-86] A number of researchers have proposed that multi-component pharmacological agents that hit multiple targets impact the complex

Table 5D. Botanical Influences on TNF

Cytokine	Plant	Model	Direction of Effect	Author/Date
TNF	*Acalypha wilkesiana*	*In vitro,* Human	Increase	(Bussing et al, 1999)[50]
	Acanthopanax gracilistylus	*In vitro,* Human	Increase	(Shan et al, 1999)[40]
	Acanthopanax senticosus	*In vitro,* Murine	Decrease	(Yi et al, 2002)[55]
	Acer nikoense	*In vitro,* Murine	Decrease	(Fujiki et al, 2003)[56]
	Allium sativum	*In vitro,* Human	Decrease	(Hodge et al, 2002)[51]
	Ampelopsis brevipedunculata	*In vitro,* Human	Decrease	(Kuo et al, 1999)[52]
	Ananus comosus (Bromelain)	*In vitro,* Murine	Increase	(Engwerda et al, 2001)[66]
	Angelica sylvestris	*In vivo,* Murine	Increase	(Haranaka et al, 1985)[29]
	Asparagus racemosus	*In vivo,* Murine	Increase	(Dhuley, 1997)[30]
	Bupleurum falcatum	*In vivo,* Murine	Increase	(Haranaka et al, 1985)[29]
	Cinnamomum cassia	*In vivo,* Murine	Increase	(Haranaka et al, 1985)[29]
	Cnidium monnieri	*In vivo,* Murine	Increase	(Haranaka et al, 1985)[29]
	Curcuma longa	*In vitro,* Human	Decrease	(Chan, 1995)[45]
	Dichroa febrifuga	*In vitro,* Murine	Decrease	(Kim et al, 2000)[57]
	Echinacea purpurea	*In vitro,* Human	Increase	(Burger et al, 1997)[54]
	Echinacea purpurea	*In vitro,* Murine	Increase	(Rininger et al, 2000)[67]
	Grifola frondosa	*In vivo,* Murine	Increase	(Kodama et al, 2002)[77]
	Harpagophytum procumbens	*In vitro,* Human	Decrease	(Fiebich et al, 2001)[46]
	Ixeris dentate	*In vitro,* Murine	Increase	(Chung et al, 2002)[58]
	Ludwigia octovalvis	*In vitro,* Human	Decrease	(Kuo et al, 1999)[52]
	Panax ginseng	*In vivo,* Murine	Increase	(Song et al, 2002)[32]
	Panax quinquefolius	*In vitro,* Murine	Increase	(Assinewe et al, 2002)[59]
	Perilla frutescens	*In vivo,* Murine	Decrease	(Ueda and Yamazaki, 1997)[33]
	Picrorhiza kurroa	*In vivo,* Murine	Increase	(Dhuley, 1997)[30]
	Polygala tenuifolia	*In vitro,* Murine	Decrease	(Hong et al, 2002)[34]
	Polygala tenuifolia	*In vitro,* Murine	Decrease	(Kim et al, 1998)60
	Poria cocos	*In vitro,* Human	Increase	(Yu and Tseng, 1996)[78]
	Rauwolfia serpentina	*In vitro,* Human	Increase	(Jin et al, 2002)[43]
	Rhus semialata	*In vitro,* Human	Decrease	(Kuo et al, 1999)[52]
	Rosa davurica	*In vitro,* Murine	Decrease	(Kim et al, 1999)[44]
	Scutellaria baicalensis	*In vitro,* Murine	Decrease	(Kim et al, 2001)[70]
	Silybum marianum	*In vivo,* Murine	Increase	(Johnson et al, 2003)[35]
	Silybum marianum	*In vitro,* Murine	Increase	(Wilasrusmee et al, 2002)[71]
	Sinomenium acutum	*In vitro,* Murine	Decrease	(Kim et al, 1999)[44]
	Smilax glabra	*In vivo,* Murine	Decrease	(Jiang and Xu, 2003)[37]
	Tabernaemontana divaricata	*In vitro,* Murine	Decrease	(Kuo et al, 1999)[52]
	Terminalia chebula	*In vitro,* Human	Increase	(Shin et al, 2001)[72]
	Tinospora cordifolia	*In vivo,* Murine	Decrease	(Dhuley, 1997)[30]
	Uncaria tomentosa	*In vitro,* Murine	Decrease	(Sandoval et al, 2002)[62]
	Withania somnifera	*In vivo,* Murine	Increase	(Dhuley, 1997)[30]
	Withania somnifera	*In vivo,* Murine	Decrease	(Davis and Kuttan, 1999)[38]

Table 5E. Botanical Influences on TGF-ß, IFN-γ, and GM-CSF

Cytokine	Plant	Model	Direction of Effect	Author/Date
TGF-β	*Poria cocos*	*In vitro,* Human	Decrease	(Yu and Tseng, 1996)[78]
IFN-γ	*Acalypha wilkesiana*	*In vitro,* Human	Increase	(Bussing et al, 1999)[50]
	Acanthopanax gracilistylus	*In vitro,* Human	Decrease	(Shan et al, 1999)[40]
	Allium sativum	*In vitro,* Human	Decrease	(Hodge et al, 2002)[51]
	Ananus comosus (Bromelain)	*In vitro,* Murine	Increase	(Engwerda et al, 2001)[66]
	Cissampelos sympodialis	*In vitro,* Murine	Decrease	(Piuvezam et al, 1999)[64]
	Cordyceps cicada (Fruit Body)	*In vitro,* Human	Increase	(Weng et al, 2002)[74]
	Cordyceps cicada (Larvae)	*In vitro,* Human	Decrease	(Weng et al, 2002)[74]
	Emblica officinalis	*In vitro,* Murine	Increase	(Sai Ram et al, 2003)[65]
	Grifola frondosa	*In vivo,* Murine	Increase	(Kodama et al, 2002)[77]
	Panax ginseng	*In vivo,* Murine	Increase	(Song et al, 2002)[32]
	Polygala tenuifolia	*In vivo,* Murine	Decrease	(Hong et al, 2002)[34]
	Rauwolfia serpentina	*In vitro,* Murine	Increase	(Jin et al, 2002)[43]
	Withania somnifera	*In vivo,* Murine	Increase	(Davis and Kuttan, 1999)[38]
GM-CSF*	*Zingiber officinalis*	*In vitro,* Human	Increase	(Chang, 1995)[49]

* granulocyte/macrophage-colony stimulating factor

equilibrium of whole cellular networks more favorably than drugs that act on a single target.[87-93] Keith and Zimmerman[91] suggest many genes might need complementary action to modify disease processes. In other words, efficacious therapy might depend on perturbing more than one target. In addition, multi-target agents need affect their targets only partially, which corresponds well with the presumed low-affinity, substrate/enzyme interactions of medicinal plants.[87-91] The partial "perturbations" of medicinal plants on a pharmacological network may accurately mimic physiological scenarios where hundreds of different enzyme systems and receptor types and subtypes are triggered simultaneously.[87] This is compared to the complete elimination of a single network node (enzyme or receptor system), which is a rather unusual phenomenon not typically found in a physiological scenario.[87] Clinicians have historically overcome such single target insufficiency by using combination drug therapy; for example, therapeutic application of drug cocktails are increasingly utilized in AIDS, cancer, and resistant infections.

Substantial historical, empirical, and scientific evidence demonstrates that whole plants, not just isolated constituents, have immunomodulating activity. Combinations of phytochemicals and cytokines may also provide a novel approach to clinical medicine. Engwerda et al[66] demonstrated the potential for combination therapy using bromelain, a mixture of cysteine proteases from the stems of pineapple plants. In this model, bromelain alone showed limited activity on cytokine secretions. However, if combined with cytokines, a synergic effect was observed. Bromelain with IFN-γ significantly enhanced TNF production beyond the effect of IFN-γ alone. In addition, when bromelain was combined with IL-12, a significant increase of IFN-γ was demonstrated compared to that of only IL-12.[66] Since such responses could enhance acquired immune responses in addition to innate immune responses, critical for first-line defense against many infectious agents, such combinations are likely important.[94] For example, combination therapies could act as vaccine adjuvants, enhancing their efficacy.[95-97]

The combination of medicinal plants with one another or other pharmacological agents fits well into a phytotherapeutic paradigm. Commonly, many of these constituents have additive or synergic activity, while a class of constituents or a single constituent may potentiate a single pharmacologically active molecule.[83,98]

Csermely[89] suggests that a pharmacological strategy directed toward multiple targets could result in more efficient therapeutic outcomes. Broader specificity, lower affinity, multi-component compounds, as found in botanical medicines, can be more efficient than high affinity, high specificity compounds.[87] Moreover, the use of whole plants, instead of isolated chemicals, may offer a safer clinical strategy in the treatment of many diseases.[85,99] Network models of pharmacology, which view human physiology as a complex web of molecular interactions, strongly imply that herbal remedies serve clinical therapy efficaciously, efficiently, and safely.

Equally, this web-like nature is reflected in the immune system by the concerted signaling of cytokines. Cytokines operate both as a cascade and as a network, regulating the production of other cytokines and cytokine receptors, while stimulating the production of acute-phase proteins.[100] Endogenous levels of cytokines are in the nanomolar to picomolar range, suggesting that dilute mixtures of biologically active compounds may provide therapeutic benefit. Illustrating the therapeutic potential for dilute mixtures of biologically active compounds, a group of researchers found subclinical doses of oral IFN-α can provide powerful, broad-spectrum benefits.[1]

In another study, when cytokine levels were compared to symptoms in individuals with cardiovascular disease, Testa et al[101] demonstrated that circulating levels of cytokines increased with severity of symptoms. Considering the variety of adverse events listed for recombinant cytokine therapies, perhaps subtle perturbations of the cytokine network should be considered. The dilute nature of botanical immunomodulators may offer a reasonable strategy for subtle induction of a variety of cytokines.

Most likely, cells are seldom exposed to only a single cytokine. Rather, combinations of cytokines and other messenger molecules generate biologically relevant informational cues.[100] This is demonstrated by the synergic antitumor effects observed from combining IL-12 gene therapy with other cytokines, chemokines, or co-stimulatory molecules.[24] The effects of cytokines on their target cells and tissues may be inhibited or enhanced by other cytokines, hormones, and cytokine-receptor antagonists and circulating receptors. Just as pharmacological activity by specific plant constituents is suggested to be affected by combinations of constituents,[83,98,102] combinations of cytokines have been found to have additive, inhibitory, or synergic effects.[100] Further research may find that the herbal immunomodulators affecting multiple cytokines can each generate a unique signature of immune perturbation dependent on the concerted effect on arrays of cytokines.

Biphasic Effects

Both exogenous and endogenous compounds can have opposing, dose-dependent biological effects. For example, Calabrese and Baldwin discuss biphasic aortic smooth muscle response to an adrenergic agonist; low doses of isoproterenol bind ß-adrenergic receptors, inducing relaxation of aortic smooth muscle. However, at higher doses, where the ß-receptors are saturated and the α-receptors are also bound, isoproterenol induces aortic constriction.[103] Similarly, Sapolsky[104] discusses the effects of glucocorticoids (GCs) on performance of hippocampal-dependent memory, suggesting low-to-moderate levels of endogenous GCs, saturating mineralocorticoid receptors (and some GC receptors), could enhance this process, while higher doses of GCs impair memory.

This biphasic effect can be noted in Table 5D. For example, *Withania somnifera* (ashwagandha) has been found to influence the expression of TNF. Dhuley et al[30] found *W. somnifera* increased TNF expression, while Davis and Khutan[38] showed it decreased TNF.

The models used in these two laboratory investigations are disparate. Dhuley[30] used the carcinogen ochratoxin A (OTA) against murine macrophages to suppress chemotactic activity induced by IL-1 and TNF. Ashwagandha, at an oral dose of 100 mg/kg daily, countered the immunosuppressive effects of OTA, raising TNF expression and theoretically restoring chemotactic activity.

In contrast, Davis and Khutan,[38] using a dose of 20 mg/animal daily by IP injection, found TNF was lowered in the *W. somnifera* group without an inducer. Although the two models are unrelated by dose, duration of exposure, and method of administration, the question still arises as to the paradoxical effects on TNF secretion. The inconsistency in the TNF results could lie in the utilization of divergent models, although these authors suggest the possibility of a biphasic dose response.

TNF is believed to be a key factor in cancer anorexia-cachexia syndrome.[105] Ashwagandha has a history of thousands of years of use in the treatment of wasting syndromes and general debility,[106] and is often currently used clinically as an adjuvant to cancer treatments.[26,107]

Known as anthrapachaka in the Ayurvedic system, *Tylophora asthmatica* is traditionally used in the treatment of asthma, allergies, and autoimmune disorders.[108] Tylophora demonstrates a biphasic effect on IL-2 secretion. Ganguly et al demonstrated this effect in an *in vitro* model. Using the same model throughout their investigations, a lower dose of *T. asthmatica* increased IL-2 levels, while more than a thousand-fold increase in dose reduced IL-2 levels, demonstrating a paradoxical response to the same exogenous stimulus.[73]

Conclusion

Although many of the plants listed in this review appear to affect only a few cytokines, it is the lead author's opinion that future research will further demonstrate the broad-spectrum activity of herbal medicine. Currently, the research on the influence of botanical medicines on cytokines and other messenger molecules is limited. Informational molecules and many of their receptors may likely turn out to be modulated by plants, both herbal medicines and foods, providing potential for future therapeutics.

Despite the fact that the majority of research in this review was performed with *in vitro* or animal models, there is substantial historical, empirical, and scientific evidence that whole plants, not just isolated constituents, have immunomodulating activity. The *in vitro* and *in vivo* research suggests that the reviewed botanical medicines modulate cytokines, and that such modulation may provide the mechanism of action for many of their therapeutic effects. Further research (particularly clinical studies) is indicated to elucidate the effects of botanical medicines and to support or refute the hypotheses presented in this article.

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