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REVIEW

Multidisciplinary perspectives on mechanisms of activity of popular immune-enhancing herbal supplements used by athletes

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Abstract **This paper examines cellular and molecular mechanisms that may underpin the purported effects of five herbal supplements in the context of athlete immune function. Ginseng and echinacea are used frequently by athletes, whereas astragalus and elderberry are used infrequently and pequi is just emerging as a possible supplement. *In vivo* studies of these products on athlete immune function have yielded heterogeneous results, likely due to experimental design differences. Ginseng, echinacea, elderberry, and pequi are considered asterids *sensu lato*. Ginseng appears to exert strongest effects on components of adaptive immunity, in particular maintaining Th1/Th2 balance of $CD4^+$ T cells and their downstream effects, via its ginsenosides, flavonoids, and polysaccharides. Echinacea alkamides, caffeic acid derivatives, and polysacchardies may target both innate and adaptive immunity, though perhaps the former more consistently. Elderberry harbors anthocyanins and lectins which may modulate innate immunity. Data on pequi is limited but suggests that carotenoids, phenols, and fatty acids may alter circulating leukocyte populations. More phylogenetically distant, astragalus is a rosid *sensu lato* and may influence the innate immune system through flavonoids, polysaccharides, and saponins. Supplements generally demonstrate no effects on physiologic parameters such as lactate, oxygen dynamics, or athletic performance. Bioavailability studies indicate that purported bioactive molecules of these supplements may reach circulation in low but therapeutically-relevant quantities. Difficulties in cross-comparisons due to study design differences, coupled with an overall dearth of research on the topic, currently hamper any formal conclusions regarding the efficacy of these supplements as immunoregulators for athletes.**

Keywords athlete, dietary supplement, exercise, herb, immunity, mechanism

Introduction

Athletes train to maximize their performance yet minimize the deleterious effects of chronic training. Although damage to the musculoskeletal system is an obvious and important consequence of practice and competition, many other body systems are oftentimes affected including the immune system. Studies regarding the effects of exercise on immunity are ongoing and have yielded a complex picture due to variations in training or subject factors and the interplay between the immune system and other body systems (Walsh et al., 2011a, b), most notably neurological/psychological and hormonal (endocrine) systems (Mackinnon, 1994).

Regular, moderate exercise is associated with lower incidence of upper respiratory infections as compared to a sedentary lifestyle, whereas frequent, strenuous exercise is associated with higher incidence of infections compared to either a sedentary lifestyle or one of moderate exercise (Nieman, 1994; Martin et al., 2009). Intense exercise is also associated with inflammatory conditions, possibly through exaggerated pro-inflammatory cytokine release (Main et al., 2010). Therefore to stay healthy for competition, athletes must balance rigorous training schedules with exercise-associated effects on the immune system.

To help achieve this balance, athletes are increasingly using herbal supplements that claim to strengthen immunity (Senchina et al., 2009c). Several studies have documented

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that herbal supplement use among athletes is higher than among the general population and may be growing (Rosenfield, 2005; Senchina et al., 2009c), though evidence suggests athletes often use supplements without always understanding their purported function to improve performance (Petroczi et al., 2007b) or health (Petroczi et al., 2007a). The numbers and types of herbal supplements available to athletes are increasing as supply meets demand and novel herbs or formulae are introduced to the market. Despite this growth, little research exists regarding the clinical effects of herbal supplements on athlete immune function and, with few exceptions, even less is known about how such supplements may interact with the human body at cellular and molecular levels.

The purpose of this paper is to critically review current knowledge on mechanisms of activity for herbal supplements that claim to improve immune function in athletes. Five herbal supplements representative of the current state of the field were selected given either their (a) current use or potential use among athletes and (b) the existence of at least three peer-reviewed references that were suitable for evaluating such use. [Many if not most herbal supplements used by athletes are used primarily for ergogenic and not immunomodulatory purposes; a summary has been provided elsewhere (Kundrat, 2005).] Ginseng, echinacea, and astragalus represent herbs already highly used by athletes, whereas elderberry meets all the same criteria as the three preceding supplements even though it has not been studied in athletic contexts per se, and pequi is an emerging herbal supplement studied in athletes but which little is known. First, a brief overview on the botany and phytochemistry of each supplement is provided. Second, known mechanisms of activity are discussed. Third and finally, some general conclusions are drawn from the accumulated data and directions for future research are offered. Evaluating claims of efficacy is beyond the scope of this paper and will not be discussed here.

Overview of exercise and immunity

The human immune system can be thought of in two branches: the innate (non-specific) and the adaptive (specific) (Murphy et al., 2011). Both are functionally intertwined, composed of cellular and molecular components, and critical for proper immune function. Innate immunity includes those elements of the immune system that are capable of recognizing broad categories of pathogens without any previous exposure whereas adaptive immunity includes those elements that require "priming" with initial exposure to the pathogen before manifesting effector functions, but are consequently capable of engineering a highly-specific response.

Markers of innate immunity often studied by exercise immunologists include cells such as neutrophils and monocytes (macrophages). Neutrophils exhibit several diverse functions (phagocytose invaders, release cytotoxic granules to destroy cells, secrete cytokines) and serve as a representative example of exercise effects on innate immunity. Acute exercise is associated with an increase of functional neutrophils in the bloodstream, but these neutrophils may not function at full capacity (Robson et al., 1999). Chronic exercise may be associated with diminished neutrophil activity (Pyne et al., 1995). Neutrophils are important in many capacities but especially bacterial clearance; any exercise-induced changes in neutrophil number or function may compromise athlete immunity.

Markers of adaptive immunity include cells such as B cells and T cells and antibody (a marker of "mucosal immunity"). B cells mature into plasma cells and produce antibody, whereas T cells may exhibit killer ($CD8^+$ T cell) or helper ($CD4^+$ T cell) functions; helper T cells may be categorized as Th1 or Th2 if they are better at activating macrophages or B cells, respectively. Acute exercise is associated with a transient reduction in numbers and functions of adaptive immunity markers; chronic exercise may be damaging if there is unsufficient rest between acute exercise bouts (Walsh et al., 2011b). Secretory IgA (sIgA; an antibody) protects against infections. Acute exercise does not appear to alter sIgA levels and even chronic, moderately-training athletes have similar sIgA levels as non-athletes; however, intensively-training athletes have lower sIgA levels (Bishop and Gleeson, 2009) which may compromise their ability to handle infections.

These representative examples illustrate why athletes (often under rigorous training programs) consume herbal supplements as immune-enhancers. Since chronic, intense exercise often deleteriously alters immunity, any herbal supplements that purport to offset exercise effects are of obvious utility for maintaining general health and possibly providing an edge over competitors.

Overview of selected herbs

Botanical aspects of the five herbal supplements selected for this paper are summarized in Table 1. Although all five plants are angiosperms and more specifically belong to the core eudicotyledons, they are phylogenetically diverse (Angiosperm Phylogeny Working Group, 2011). Ginseng, echinacea, and elderberry are members of a group classically named "asterids II" but each belong to different orders and consequently families, with elderberry and ginseng being more closely related to each other than either is to echinacea. Pequi belongs to a family that is basal within the clade of asterids yet not considered as part of either the asterid I nor the asterid II groups. Sister to the asterid clade is the rosid clade. Astragalus belongs to a group of rosids known as "rosids I" (Fabidae). Not only is astragalus most distantly related from the other plants encompassed here, but the rosid clade is regarded as more evolutionarily basal ("primitive")

Table 1 Botanical characteristics of herbs in this review

Common name	Scientific name	Family (Order)	Habit	Endemic region
Astragalus, locoweed, or milk vetch	*Astragalus* spp. L.	Fabaceae (Fabales)	Herbaceous perennial, rarely shrub	Northern hemisphere
Echinacea or coneflower	*Echinacea* spp. Moench	Asteraceae (Asterales)	Herbaceous perennial	North America
Elderberry	*Sambucus* spp. L.	Adoxaceae* (Dipsicales)	Shrub or tree	Global
Ginseng	*Panax* spp. L.	Araliaceae (Apiales)	Herbaceous perennial	Eastern Asia (most species) and North America
Pequi or souari	*Caryocar brasilense* Cambess.	Caryocaraceae (Ericales†)	Tree	South America

Note that the common name for most of the herbs can refer to multiple species within a single genus. * = Elderberry was formally in Caprifoliaceae. † = syn. Theales.

compared to the asterid clade.

Many supplement names (or plant common names) can refer to more than one species. It is important to note the differences in name application because different species within a group often exhibit subtle yet significant differences in biochemical composition, which in turn yield differences in medicinal activities. "Ginseng" is a term applicable to any of the approximately dozen species within genus *Panax* and most commonly refers to *P. ginseng* C. A. Meyer (Chinese or Asian ginseng), but sometimes *P. pseudoginseng* Wall. (Korean ginseng, syn. *P. notoginseng*) or *P. quinquefolius* L. (American ginseng). *Panax ginseng* is the most common species of ginseng used in herbal supplements but *P. quinquefolius* is sometimes used. In exercise science studies the name is also extended to so-called Siberian ginseng [*Eleutherococcus senticosus* (Rupr. and Maxim.) Maxim.], though Siberian ginseng will not be discussed here. "Echinacea" is both a genus name (e.g., *Echinacea*) and a common name (e.g., echinacea) and can refer to any of nine classically-recognized species (Blumenthal and Urbatsch, 2006; Wu et al., 2009), though *E. angustifolia* DC., *E. pallida* (Nutt.) Nutt., and *E. purpurea* (L.) Moench are most commonly marketed. Similar to echinacea, "astragalus" can be used as both a genus name (e.g., *Astragalus*) and common name. There are hundreds of species within *Astragalus* and the taxonomy is in flux, but *A. propinquus* Schischkin (syn. *A. membranaceus*) is almost exclusively used in the supplement industry. "Elderberry" is a common name referring to members of genus *Sambucus* but most often *S. nigra* subspecies *nigra* L. from Europe (syn. *S. nigra*, black elderberry), *S. nigra* subsp. *canadensis* (L.) R. Bolli from America (syn. *S. canadensis*, American elderberry), and *S. racemosa* subsp. *sielboldiana* (Mig.) H. Hara from Japan (syn. *S. sieboldiana*, Japanese elderberry). "Pequi" is an exception to the general trend in Table 1 because this name refers to a single species, *Caryocar brasiliense* Cambess.

Ranges and habits of these five plants are also quite different (Mabberley, 1997). Ginseng, astragalus, and elderberry have relatively wide natural global ranges whereas echinacea and pequi have more limited endemic ranges. Ginseng, echinacea, and astragalus are herbaceous perennials whereas elderberry and pequi are shrubs or trees. For the purposes of supplement production, different parts of each plant are used (Ody, 2000; Shealy, 2009) as illustrated in Fig. 1. Ginseng, echinacea, and astragalus supplements are traditionally made from underground structures such as roots, although echinacea supplements are increasingly produced from aboveground parts. Both elderberry and pequi supplements are made from each respective plant's fruit (berry in the case of elderberry, nut in the case of pequi).

Purported bioactive compounds found in each of the five supplements are also diverse biochemically and are presented in Fig. 2. They are commonly classified as secondary metabolites (Buchanan et al., 2002). Major categories of secondary metabolites included in this paper are terpenoids, flavonoids, and phenols. Terpenoids can themselves be divided into numerous subgroups such as steroids and carotenoids. One group of steroids is the saponins, and both ginsenoside (from ginseng) and the astragalosides (from astragalus) belong to this group (Song and Hu, 2009). One group of carotenoids is the xanthophylls including zeaxanthin and violaxanthin, purported bioactive molecules from pequi (Ferreira et al., 2011). Flavonoids are another major category of secondary metabolites with quercetin or anthocyanins (from elderberry; also characterized as polyphenols) being representative examples (Roschek et al., 2009). Other phenols include caffeic acid and its derivatives (including caftaric acid, chrlogoenic acid, and cichoric acid), echinacoside, and various ketones, all found in echinacea (Barnes et al., 2005). Echinacea also contains a rich diversity of fatty acid amides derived from lauric acid termed alkamides (alkylamides), while pequi produces fatty acids with purported immunomodulatory properties. Two other molecule types deserve mention though they do not easily fit into the above classification scheme. Numerous polysaccharides have been identified from the five supplements in this review and are probable immunomodulators, at least *in vitro*. Non-enzymatic proteins called lectins are found in elderberry (Haas et al., 1999).

Considering Table 1, Figs. 1 and 2, and associated text, it is clear that the five supplements encompassed in this review are diverse both botanically and phytochemically. The ways in which bioactive compounds from these supplements interact with the human immune system at the cellular and molecular levels is equally complex and will be the focus of the rest of this paper.

Figure 1 Plants discussed and their most commonly-utilized organs. (A) Ginseng (*Panax* spp.; pictured is *P. ginseng*). The root is most commonly used. (B) Echinacea (*Echinacea* spp.; pictured is *E. purpurea*). Although the underground parts of *Echinacea* spp. contain the highest concentrations of purported bioactive molecules, many contemporary commercial operations harvest aboveground parts and allow belowground parts to regenerate to yield multiple harvests. (C) Astragalus (*Astragalus* spp.; pictured is *A. membranaceus*). Dried roots are most commonly used. (D) Elderberry (*Sambucus* spp.; pictures is *S. nigra*). Most supplements are produced from berry clusters. (E) Pequi (*Caryocar brasiliense*). The nut is used in supplement production.

Mechanisms of supplement activity

Before proceeding to a review of current knowledge on mechanisms of activity for each supplement, some precautions should be acknowledged. First, this review considers both *in vivo* and *in vitro* studies, although the preponderance of relevant studies were conducted *in vitro* or *ex vivo*. Given inherent differences in the models, there are sometimes discrepancies in findings when the same immunological parameters are measured for the same supplements. Compounding this concern are issues related to bioavailability (e.g., what and how much of a supplement makes it into the bloodstream post-digestion, addressed later). Second, findings for the same immunological parameters may vary across

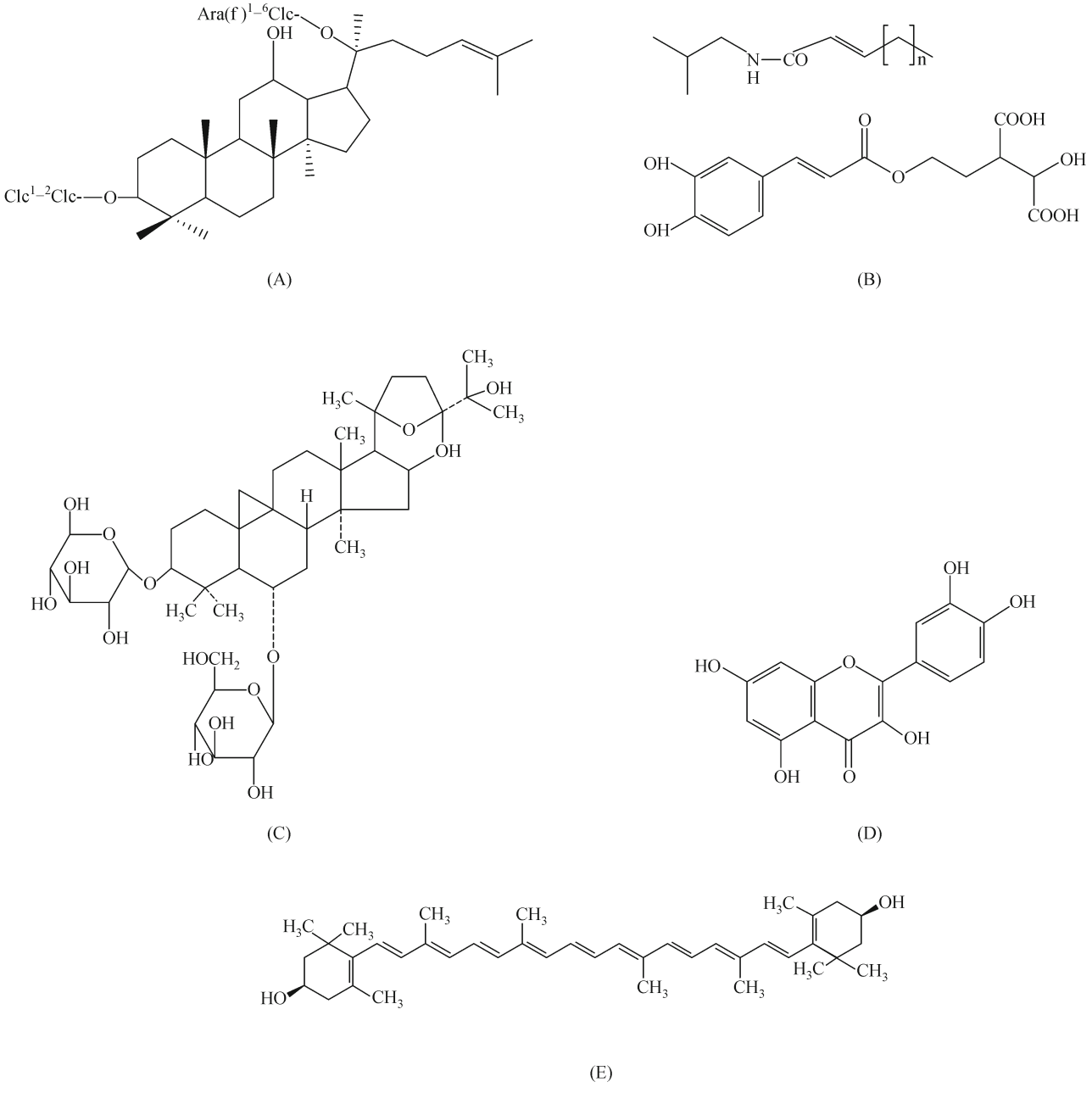

Figure 2 Selected bioactive molecules from the specimens in Figure 1. (A) Ginsenoside Rc from ginseng. (B) Alkamides and caffeic acid derivatives (such as caftaric acid) from echinacea. (C) Astragaloside IV from astragalus. (D) Quercetin from elderberry. (E) Zeaxanthin from pequi.

animal versus human studies, or among cell types within the same organism. Third, some studies utilize isolated compounds from supplements whereas other studies use combinations of individual compounds or whole-tissue extracts; each yields differing results. The method of extraction or solvent may also influence results, as some solvents may extract more hydrophilic constituents (e.g., aqueous solvents) whereas others extract more hydrophobic molecules (e.g., alcoholic solvents). Fourth, any number of pre-clinical or pre-experimental factors not reported (e.g., species, subspecies, or accessions chosen within a taxon; growing conditions; storage or drying conditions pre-processing; tissues or organs used) complicate comparisons across studies. It is very difficult (if not impossible) to generate models that will take into account all possible factors or every conceivable experimental outcome.

The goal of this manuscript is to present larger concepts or example models related to each supplement rather than to be exhaustively comprehensive, sometimes erring on the side of simplifying more complicated topics to promote understanding. Some limitations have been implemented to focus its scope (limiting our review to only those articles written or

summarized in English, using only data from human or rodent models, and restricting the number of studies utilizing combinational therapies where the effects of one agent are inseparable from others). Databases used to locate articles included PubMed, Google Scholar, and Biologic Abstracts; primary searches were performed using search strings including the plant's name (both vernacular and Latin), and the words "athlete" or "exercise" and "immune." Oftentimes subsequent references were located by consulting bibliographies of articles found in the primary search. We apologize to any authors whose work was not included given those constraints.

Ginseng

As one of the most globally recognized elements of traditional Chinese medicine (TCM), Chinese ginseng (*Panax ginseng*) has been used for thousands of years to improve immunity, nerve function and cognition, and metabolism. It is extensively employed among athletes for all of the preceding reasons, and many studies have been conducted on its use in exercise contexts. The preponderance of evidence suggests ginseng supplements may not possess strict ergogenic properties (Bahrke and Morgan, 2000; Palisin and Stacy, 2006; Senchina et al., 2011); this finding extends to energy drinks that contain ginseng, where observed ergogenic effects may be more attributable to caffeine and glucose (Ballard et al., 2010). Other studies indicate it may improve reaction time during exercise (Ziemba et al., 1999) or alter immune variables.

Table 2 presents a summary of studies that examined the effects of ginseng supplementation *in vivo* on exercise-associated immune parameters. The complexity in comparing ginseng research becomes quickly apparent because studies differed in: ginseng species, supplement type, and dosage; exercise intensity, duration, and mode; and training status, though all but one used males. One message that emerges from Table 2 is that ginseng supplements appear to target components of adaptive immunity moreso than innate immunity.

The majority of immunomodulatory activity from ginseng supplements has been ascribed to ginsenosides (Leung and Wong, 2010), a subclass of saponins (terpenoids). Individual ginsenosides are distinguished by the presence or absence of different functional groups and each may have different immunomodulatory properties. Ginsenoside content varies between species with twice as many ginsenosides identified from *P. ginseng* compared to *P. quinquefolius* (Choi, 2008). Two of the seven studies from Table 2 (Biondo et al., 2008; Biondo et al., 2010) noted their extract contained 8% weight/volume ginsenosides, but ginsenoside identity was not reported. To gain a better understanding of how ginsenosides may influence athlete immune function, non-exercise studies must be considered.

Figure 3 provides a summary of representative studies that examined effects of isolated ginsenosides on immune cell activity. As noted earlier with Table 2, ginsensodies appear to influence components of adaptive immunity more strongly than innate immunity and current research reflects this. More pointedly, most recent work has been dedicated to unraveling the role ginseng supplements may play in influencing the Th1/Th2 balance. Briefly, CD4 + T cell-mediated immune responses can be categorized as either Th1 (primarily involving macrophages and the destruction of intracellular

Table 2 Summary of exercise-associated studies of ginseng supplements and immune function in humans

References	Population*	Treatment†	Exercise‡	Immune outcome§
Engels et al., 2003	TR ♂	PG 8 wk	Three 30 s Wingate tests	No differences in salivary IgA between GT and C
Gaffney et al., 2001	TR ♂	PG 8 wk	Normal in-season training	No differences in circulating leukocyte populations between GT and C
Hsu, 2010	TR ♂	PQ 4 wk	60 min treadmill run at 60% VO_2max, 10% downhill grade	Plasma IL-4 ↑ post-exercise in GT compared to C; no differences in TNF, IL-1β, IL-10
Jung et al., 2011	TR ♂	RG 7 d	Two 45 min treadmill runs	Plasma IL-6 ↓ at 2 h post-exercise in GT compared to C; plasma CK ↓ 72 h post-exercise in GT compared to C
Park et al., 2008	TR ♂	RG 7 d	Two 45 min treadmill runs	Plasma IL-6 ↓ at 2 h post-exercise in GT compared to C
Biondo et al., 2008	UT ♂	PQ 35 d	36 min on cycle ergometer (various % VT based on VO_2max)	Blood $CD8^+$ T cells ↑ and *in vitro* PHA-stimulated PBMC proliferation ↑ in GT compared to C; no differences in other cell subsets, proliferation, or neutrophil oxidative burst
Biondo et al., 2010	UT ♀	PQ 4 wk	36 min on cycle ergometer (various % VT based on VO_2max)	Blood $CD4^+CD45RO^+$, $CD4^+CD28^+$, and $CD28^+$ T cells ↓ post-exercise in GT compared to C; *in vitro* neutrophil oxidative burst ↑ in GT compared to C; no differences in other cell subsets, proliferation, or NK cell activity
Lau et al., 2011	UT ♂	PP 3 d	30 min treadmill run at 60% VO_2max	No differences in plasma IL-6 between GT and C

* Population abbreviations: TR = trained, UT = untrained; † Treatment abbreviations: PG = *Panax ginseng*, PP = *P. pseudoginseng*, PQ = *P. quiquefolius*, RG = red ginseng (a heat-treated form of PG); ‡ Exercise abbreviations: VO_2max = maximal voluntary oxygen consumption, VT = ventilatory threshold; § = Immune outcome abbreviations: C = control, GT = ginseng-treated; NK = natural killer; PBMC = peripheral blood mononuclear cells; PHA = phytohemagluttinin.

pathogens, but to a lesser extent involving B cells) or Th2 (primarily involving B cells/plasma cells and the destruction of extracellular pathogens, but to a lesser extent involving macrophages).

Ginsenosides appear to augment both Th1 and Th2 aspects of immunity in a balanced way as represented by their effects on antibodies, cytokines, splenocytes, and $CD4^+$ T cells (Fig. 3). Evidence suggests ginsenosides stimulate production of both Th1-associated antibodies (IgG2a and IgG3) and Th2-associated antibodies (IgG1 and IgG2b) (Rivera et al., 2005; Yang et al., 2007; Sun et al., 2008; Song et al., 2009; Song et al., 2010). Ginsenoside-treated rodents displayed elevated serum levels of Th1-associated cytokines (IFN-γ, IL-2, TNF-α) and Th2-associated cytokines (IL-4, IL-5, IL-10) (Rivera et al., 2005; Yang et al., 2007). Splenic-derived $CD4^+$ T cell activities were modulated after ginsenoside treatment. Th1 $CD4^+$ T cells had augmented IL-2 and IFN-γ mRNA and protein levels whereas Th2 $CD4^+$ T cells had augmented IL-4 mRNA and protein and IL-10 protein (Lee et al., 2004; Lee and Han, 2006). Ginsenoside treatment uprgulated Th1 $CD4^+$ T cell expression of the surface molecule CD69 (Lee et al., 2004), proliferation (not pictured) (Lee et al., 2004), and overall percentage within the total lymphocyte pool (Kenarova et al., 1990). Splenocytes harvested from ginsenoside-treated rodents exhibited increased levels of Th1-associated IL-2 and IFN-γ mRNA (Yang et al., 2007) and protein (Rivera et al., 2005; Sun et al., 2008; Song et al., 2009; Song et al., 2010), as well as TNF-α protein (Rivera et al., 2005), and increased levels of Th2-associated IL-4 and IL-10 mRNA (Yang et al., 2007) and protein (Rivera et al., 2005), and additionally IL-5 protein (Sun et al., 2008; Song et al., 2009; Song et al., 2010). Splenocyte proliferation was increased (Luo et al., 1993; Yang et al., 2007; Song et al., 2009, 2010).

Although not illustrated in Fig. 3, on the Th1 side ginsenosides increase splenocyte natural killer cell activity (Kenarova et al., 1990), peritoneal macrophage IL-1β protein production (Kenarova et al., 1990) and phagocytosis (Luo et al., 1993), and IL-12 production by stimulated bone marrow-derived dendritic cells (Tung et al., 2011). Regarding the innate immune system, evidence suggests that saponins

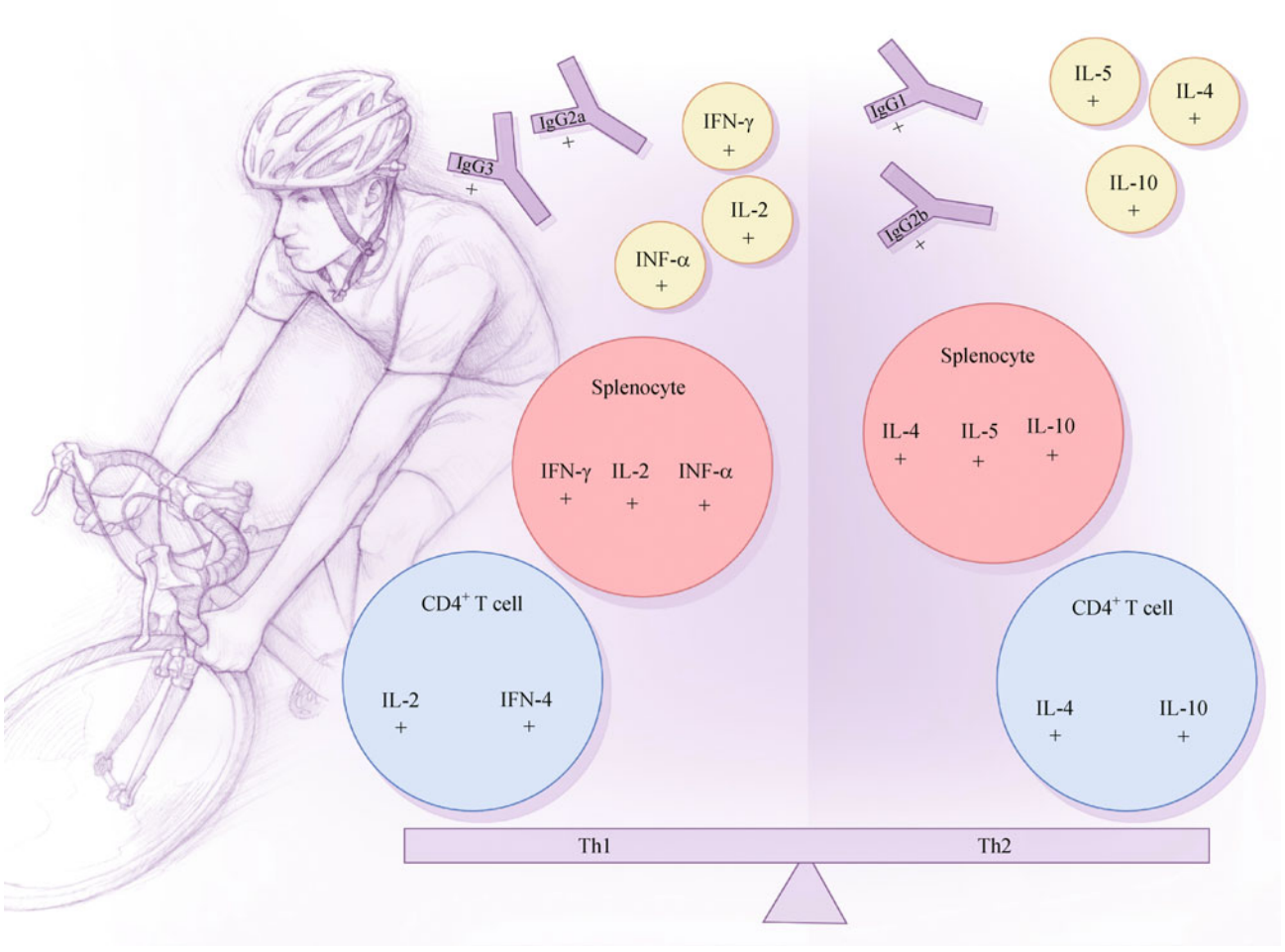

Figure 3 Representative model of current mechanisms research on ginseng supplements with a focus on ginsenoside interactions with rodent splenocyte and blood models. (Bottom) Endurance athletes including runners, cyclists, or triathletes are the populations that consume ginseng most often. (Top) CD4 T-cell-mediated immune responses can be categorized as either Th1 (top left panel) or Th2 (top right panel; see text). Ginsenosides appear to augment both Th1 and Th2 in a balanced way (represented by the see-saw).

reduce inflammatory cytokine expression by working through the PPAR (peroxisome proliferator-activated receptor) pathway in dendritic cells (Su et al., 2010) or by inhibiting transcription factors IKK-β, IRAK-1, NF-κB, or various mitogen-associated protein (MAP) kinases (p38, ERK, JNK) in macrophages (Joh et al., 2011).

Polysaccharides may also play an important role in ginseng bioactivity though they have only been recognized recently and their mechanisms of activity are unclear. They are more likely to be found in aqueous (versus alcoholic) extractions given their hydrophilic properties. Some teams have reported that polysaccharide fractions from *P. quinquefolius* extracts upregulate inflammatory or Th1 cytokine production including tumor necrosis factor (TNF), interleukin-6 (IL-6), IL-12, interferon-γ (IFN-γ), granulocyte-macrophage colony stimulating factor (GM-CSF), and nitric oxide (NO) *in vitro* in macrophages (Jang and Shin, 2010; Azike et al., 2011) and dendritic cells (Ilarraza et al., 2011), concomitant with an upregulation of cell surface activation markers in dendritic cells and upregulation of both inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) in macrophages. A similar effect on dendritic cell surface markers was observed for a specific polysaccharide, ginsan, from *P. ginseng* (Kim et al., 2009). Conversely, other teams demonstrated reductions in *in vitro* inflammatory cytokines (TNF, IL-1β, IL-6, IL-12, IL-18, IFN-γ) from rodent macrophages treated with ginsan from *P. pseudoginseng* (Ahn et al., 2006) or reductions in both inflammatory/Th1 (TNF, IL-1β) and Th2 (IL-5, IL-5) cytokines *in vivo* in lung epithelial tissue from asthmatic mice treated with polysaccharides from *P. ginseng* (Kim and Yang, 2011). These latter two studies further demonstrated that the polysaccharides worked by reducing phosphorylation of the transcription factors p38, ERK, and JNK, and NF-κB; this is similar to some effects seen from saponins (Joh et al., 2011). Ginseng polysaccharides may also play anticancer roles by promoting macrophage phagocytosis or NO production, natural killer cell killing, and lymphocyte proliferation (Ni et al., 2010).

Echinacea

Echinacea is one of the more frequently consumed herbal supplements by athletes and there are a goodly number of studies examining possible mechanisms of immunomodulatory activity. Echinacea supplements are purported to reduce the incidence and severity of upper respiratory infections (URI's) such as colds or influenza, though there are studies both supporting (Linde et al., 2006) and refuting (Barrett et al., 2011) its efficacy in that capacity. Most associations of echinacea and athletics involve endurance-type athletes and general reviews have been provided elsewhere (Senchina et al., 2009c; Bergeron et al., 2010; Walsh et al., 2011a), but limited evidence suggests some efficacy in athletes (Berg et al., 1998; Schoop et al., 2006; Hall et al., 2007). Echinacea may also benefit endurance athletes by improving erythropoietin and associated factors (Whitehead et al., 2007a) or oxygen dynamics (Whitehead et al., 2007b).

Since many symptoms of URI's are associated with inflammation, much echinacea research has been focused on cell models involving inflammatory pathways such as those associated with cytokine (e.g., TNF, IL-1β), NO, or prostaglandin synthesis. Rodent macrophages are often used in this capacity and will serve as a representative model for echinacea mechanisms research (Fig. 4). Macrophage biology may be one of the key elements that many immunomodulatory herbal supplements target (Groom et al., 2007). Accumulated research indicates that echinacea supplements more strongly influence innate immune system components than adaptive.

Various echinacea constiuents dock with and signal through different macrophage surface receptors. Alkamides dock with CB2 receptors and an as-yet unrecognized secondary receptor (Gertsch et al., 2004; Gertsch, 2008), whereas polysaccharides may enter through TLR (Sullivan et al., 2008); receptors for phenols are not reported. Through these receptors echinacea constituents influence transcription factors, one example being NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells; despite its name, NF-κB is found in most animal cells and is important in regulating DNA expression). One study in rodent macrophages has shown NF-κB levels decrease upon administration of alkamides (Stevenson et al., 2005), but other studies in human cells suggest various effects depending on experimental variables (Gertsch et al., 2004; Matthias et al., 2008). Transcription factors ultimately influence protein levels via regulating mRNA (mRNA) expression, though in some instances a constituent may upregulate mRNA levels but not protein levels because other cellular processes interfere with mRNA translation (Gertsch et al., 2004). Echinacea constituents are known to differentially influence levels of inflammatory cytokines including TNF (Goel et al., 2002b; Stevenson et al., 2005; Zhai et al., 2007; Cech et al., 2010), IL-1β (Sullivan et al., 2008), IL-12 (Sullivan et al., 2008), and IFN-γ (Goel et al., 2002a, b), but have no effect on IL-2 (not shown) (Goel et al., 2002a). One lone alkamide has been purported to downregulate GM-CSF, monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1 (MIP-1α), and RANTES (not illustrated) (Cech et al., 2010). The effects of echinacea constituents on iNOS (the enzyme responsible for processing L-arginine into NO) are unclear (Chen et al., 2005; Stevenson et al., 2005; Zhai et al., 2007; Sullivan et al., 2008; LaLone et al., 2009; Zhai et al., 2009; Senchina et al., 2010). Additionally, echinacea may: upregulate arginase activity to yield more ornithine (Zhai et al., 2009); diminish prostaglandin E_2 (PGE_2) production, possibly by influencing COX levels (Rininger et al., 2000; LaLone et al., 2007; LaLone et al., 2009; Cech et al., 2010); and increase phagocytic activity (Goel et al., 2002a, b).

Figure 4 suggests there is much contradiction and perhaps confusion in our current understanding of how echinacea

interacts with macrophages. Some of these apparent contradictions may be resolved by considering differences in experimental factors between studies from immunological, chemical, or botanical vantages, as assisted by Table 3. Taking the NO pathway from Fig. 4 as an example, those teams that used isolated or combined alkamides, caffeic acid derivatives, and ketones reported reductions in (Chen et al., 2005; LaLone et al., 2009) or no effect on (Stevenson et al., 2005) iNOS levels, while the team that used a polysaccharide-enriched fraction (Sullivan et al., 2008) found higher iNOS levels. Those teams that used whole-tissue alcohol tinctures reported differing results, with one team finding decreased iNOS levels (Zhai et al., 2007, 2009) and the other team increased levels (Senchina et al., 2010). Both teams used plant material harvested from the same common garden and both teams used the same three species (*E. angustifolia*, *E. pallida*, and *E. purpurea*); however, both reported species-specific differences within their own studies and their extraction techniques were quite different, likely explaining differences in results. NO findings from Fig. 4 thus demonstrate how an understanding of botanical and chemical factors is critical to understanding current mechanisms literature. Notably, with one exception (Sullivan et al., 2008), all of the teams who have contributed to NO mechanisms in Fig. 4 used the same cell (a murine macrophage cell line, RAW264), thus largely eliminating cell type (but not necessarily culture conditions) as an additional confounding variable.

Although an exhaustive discourse about all known echinacea-immune system mechanisms is beyond the scope of this review, some general comments about ways those mechanisms can be influenced by chemical or botanical

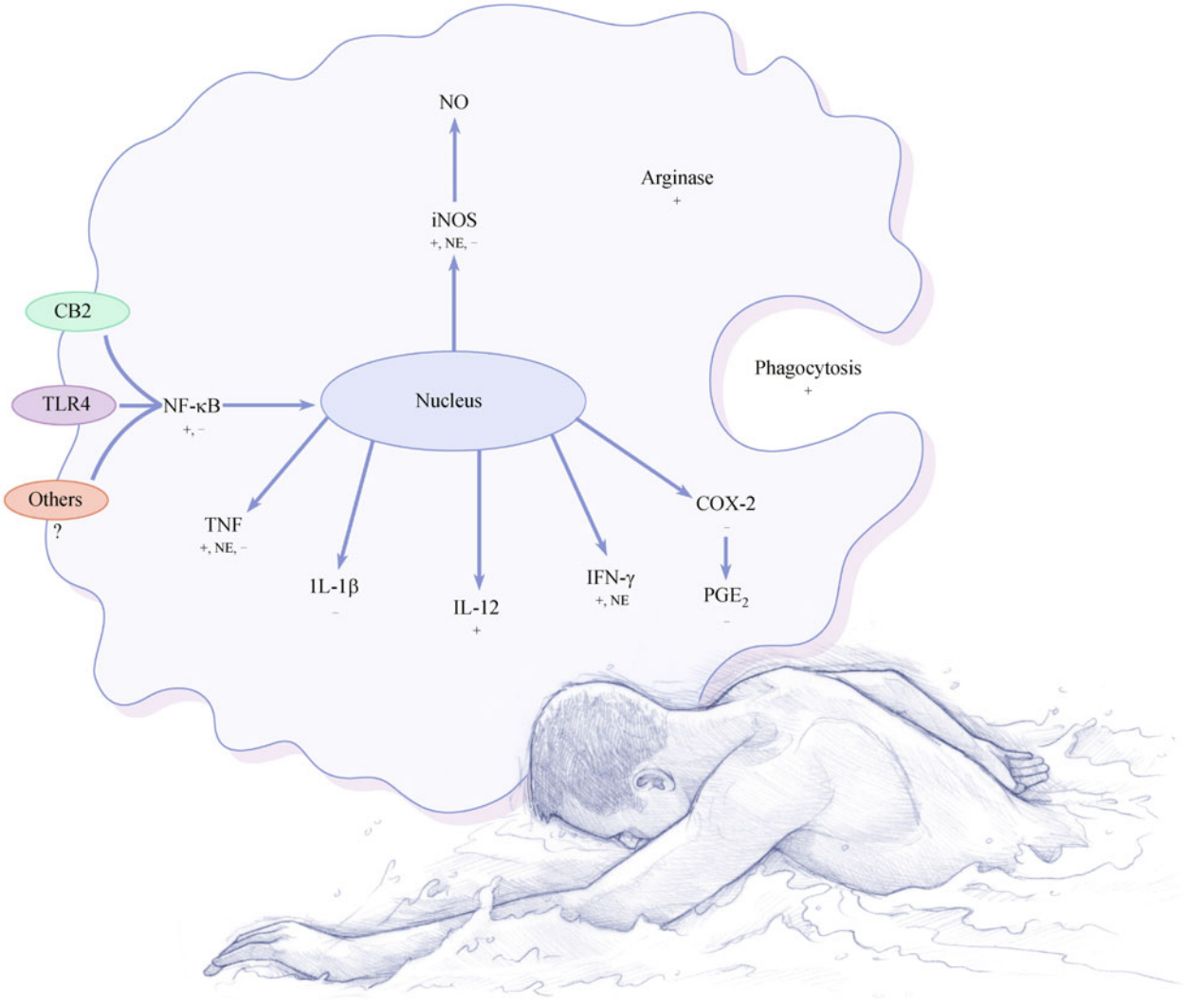

Figure 4 Representative model of current mechanisms research on echinacea supplements with a focus on the rodent macrophage. (Right) Echinacea supplements are typically consumed by aerobically-trained athletes such as swimmers. (Left) Summary of selected current research on the effects of echinacea on rodent macrophage inflammation-associated pathways (see text). Positive signs (+) indicate upregulation, negative signs (−) indicate downregulation, and "NE" stands for "no effect."

Table 3 Experimental variables for the data presented in Figure 4

Reference	Species*	Organ	Extraction	Constituent (Solvent)†	Stimulant‡	MΦ Type§
Cech et al., 2010	PUR	Root	Ethanol	ALK (ethanol)	H1N1	RAW 264.7
Chen et al., 2005	ANG, PAL, PUR	Root	Ethanol, methanol, chloroform, hexane	ALK (methanol)	LPS	RAW 264.7
Goel et al., 2002a	PUR	Aerial	Ethanol-water	ALK, CAD, POLY (ethanol-water)	LPS	ALV
Goel et al., 2002b	PUR	Aerial	Ethanol-water	ALK, CAD, POLY (ethanol-water)	LPS	ALV
LaLone et al., 2007	ANG, PAL, PUR	Root	Ethanol, chloroform, hexane	ALK (ethanol, DMSO)	None	RAW 264.7
LaLone et al., 2009	ANG, PAL, PUR, TEN	Root	Ethanol, water	ALK, KET (ethanol, water)	LPS	RAW 264.7
Rininger et al., 2000	Unknown	Aerial, Root	Unknown	Unknown	None	RAW 264.7
Senchina et al., 2010	ANG, PAL, PUR, TEN	Root	Ethanol, ethanol-water	Ethanol, ethanol-water	HSV	RAW 264.7
Stevenson et al., 2005	ANG, PUR	Root	Ethanol-water	ALK, CAD (ethanol-water)	LPS	RAW 264.7
Sullivan et al., 2008	PUR	Aerial, root	Unknown	POLY (C-RPMI)	None	PER
Zhai et al., 2007	ANG, PAL, PUR	Root	Ethanol, chloroform, hexane	ALK, CAD (ethanol, water)	LPS, *Salmonella enterica*	PER, RAW 264.7
Zhai et al., 2009	ANG, PAL, PUR	Root	Ethanol, chloroform, hexane	ALK, CAD (ethanol, water)	LPS	RAW 264.7

All data is derived from protein (and not mRNA) analyses. * Species abbreviations: ANG = *Echinacea angustifolia*, PAL = *E. pallida*, PUR = *E. purpurea*, TEN = *E. tennesseensis*. † Constituent/solvent abbreviations: ALK = alkamides, CAD = caffeic acid derivatives, CRPMI = complete RPMI medium, DMSO = dimethyl sulfoxide, KET = ketones, POLY = polysaccharides. ‡ Stimulant abbreviations: H1N1 = influenza A strain, HSV = herpes simplex virus, LPS = lipopolysaccharide. § Macrophage abbreviations: ALV = alveolar, PER = peritoneal.

factors will be proffered. Three major classes of echinacea compounds have been explored for their potential immunomodulatory activities: alkamides, caffeic acid derivatives and associated molecules, and polysaccharides. More is known about alkamides than other echinacea components. Alkamides are structurally similar to human endogenous cannabinoids (endocannabinoids). Since peripheral immune cells express a receptor for endocannabinoids, the CB2 receptor (Gertsch et al., 2004), docking of alkamides with CB2 receptors is one likely mechanism for how alkamides may modulate cell signaling (Woelkart et al., 2005; Raduner et al., 2006; Woelkart and Bauer, 2007; Gertsch, 2008). Phenols such as caffeic acid derivatives also have immunomodulatory and antioxidant properties (Pellati et al., 2004; Birt et al., 2008), though how they interact with cells is presently unclear. One study has shown that cynarin binds to CD28 on human immortalized T cells, preventing its binding to the CD80 molecule on stimulating B cells and possibly explaining observed immunosuppressive effects (Dong et al., 2009). Different caffeic acid derivatives may have opposite effects on cell activities, as demonstrated experimentally in Table 4. Polysaccharides from echinacea may also contribute to its immunomodulatory properties (Sullivan et al., 2008), possibly by mimicking the binding properties of "danger signal" molecules such as LPS (lipopolysaccharide, a bacterial endotoxin present on the cell surfaces of Gram–bacteria) and binding to and signaling through Toll-like receptors (TLR) or other receptors. The relative contribution of each compound class to overall echinacea supplement activity remains under investigation.

Different *Echinacea* spp. exhibit different immunomodulatory effects both *in vitro* and *in vivo* (Barnes et al., 2005; Senchina et al., 2006a) as has been shown for all but one of the nine species. Table 5 provides a practical example of this phenomenon using some of the cytokines included in Fig. 4. Concentrations and presence/absence of all three classes of biochemical compounds vary by plant species (Barnes et al., 2005), providing an obvious explanation for such differences and underscoring the need to acknowledge which species is used in each investigation. Biochemistry can vary based on biologic (organ or tissue harvested; plant age) and ecological (soil composition; drought stress) factors as discussed elsewhere even within the same species (Senchina et al., 2009c; Rininger et al., 2000; Hou et al., 2010).

Cumulative evidence suggests that echinacea supplements contain a large and diverse panoply of bioactive molecules that modulate immune function through multiple and distinct pathways. Alkamides are believed responsible for the majority of observed effects, though caffeic acid derivatives likely play important roles, and it is possible under the right experimental conditions polysaccharides are influential. Emerging genomic (Wang et al., 2006; Altamirano-Dimas et al., 2007), proteomic (Wang et al., 2008; Yin et al., 2010a), and metabolomic (Hou et al., 2010; Modarai et al., 2010) studies of echinacea supplements should better clarify the role that specific constituents play mechanistically, but robust comparison across experiments are not currently possible.

Astragalus

Astragalus membraraceous is a common component of TCM, most often in the form of dried root powder or shavings (Anonymous, 2003), and oftentimes combined with other planta medica instead of being used in isolation. Astragalus is purported to be a general immune stimulant (Tan and Vanitha, 2004), sometimes indicated specifically for upper respiratory infections (such as colds) but other times indicated more generally to improve immune system vigor and spleen function (an important immunoregulatory organ).

There are several reports concerning astragalus supplementation in human athlete or rodent models of exercise. Using a population of 33 cyclists and runners, one team reported that one month of daily supplementation with ISSI (a traditional Chinese preparation including *A. membranaceous* among other components) improved natural killer cell and T cell activity, as well as augmenting plasma antibody (immunoglobulin, specifically IgA and IgG) levels and reducing soluble interleukin-2 receptor (sIL-2R) (Fan et al., 2005), but it is unclear which role astragalus specifically may have played. Another study followed 12 basketball players during two weeks of intense training. For six of the subjects who underwent treatment, researchers reported the astragalus preparation "improved the body's immune system," though no further details were available in English (Gu et al., 2009). In studies of basketball players (Chen et al., 2002), judo athletes (Su et al., 2001), and track-and-field athletes (Yin et al., 1995), a multi-herbal preparation (including *A. membranaceous*) was shown to improve vitality (and not immune

Table 4 Demonstration of how individual caffeic acid derivatives may have different effects on immune cell proliferation

Compound	High dose	Middle dose	Low dose
Vehicle control	765.8±21.9	N/A	N/A
Caftaric acid	790.0±29.1	771.8±19.8	728.3±6.1
Chlorogenic acid	695.8±20.8	742.6±30.2	798.6±36.2
Cichoric acid	746.4±23.2	748.8±44.9	789.2±47.1

Values are expressed as average optical impedance measurements±standard error. One hundred μL of Jurkat T cells (at 5.0×10^5 cells/mL) were plated in 96-well plates and stimulated with 5 μL of one of three different caffeic acid derivatives (caftaric acid, chlorogenic acid, cichoric acid). Ten-fold dilutions were performed with starting doses ("high dose") being 0.15 mg/mL, 0.05 mg/mL, and 1.0 mg/mL for each acid, respectively, based on levels normally found in whole tissue extracts prepared using lay herbalist methods (Senchina et al., 2006b). All conditions were done in triplicate. Effects on proliferation were measured using a formazine salt assay as described previously (Senchina et al., 2009b).

Table 5 Demonstration of how different *Echinacea* species grown and processed under identical conditions can exert different effects on *in vitro* cytokine production by human peripheral blood mononuclear cells

Cytokine	Solvent control	*E. angustifolia*	*E. pallida*	*E. paradoxa*	*E. purpurea*	*E. tennesseensis*
IFN-γ	1.00±1.00	4.70±3.32	15.19±5.10	12.36±4.12	16.56±7.79	15.52±4.06
IL-2	16.53±3.79	16.14±1.70	22.58±3.75	24.11±8.95	29.82±4.98	25.64±1.86
IL10	0.52±0.33	4.44±3.78	34.03±9.83	13.32±0.81	114.83±20.25	4.04±2.37
TNF	5.93±3.93	167.72±69.34	1971.53±397.84	1081.39±225.90	2230.02±514.64	571.48±155.35

Cytokine amounts are expressed as pg/mL, mean±standard error. An asterisk (*) indicates a significant difference in treatment compared to solvent vehicle control ($p < 0.05$). Approval to conduct the research was granted by the Iowa State University Institutional Review Board. Root material from five different *Echinacea* species was dried for six-and-a-half years and used to produce extracts according to methods described previously (Senchina et al., 2006b). Peripheral blood mononuclear cells were taken from the blood of four male human volunteers at rest, cultured *in vitro* with extracts, and tested for their abilities to modulate cytokine production as described elsewhere (Senchina et al., 2009a) using ELISA on supernatants collected at 24 h (TNF), 48 h (IFN-γ), and 72 h (IL-2, IL-10) incubation. Endotoxin levels varied by extract (all stock extracts $p \leqslant 2.88$ EU/mL) but were too small to elicit any effect in our model as demonstrated experimentally (Senchina et al., 2006b), suggesting the effects observed in this table are due purely to plant constituents and not bystander LPS. To the best of our knowledge, this is the first report to demonstrate that *Echinacea* material dried at room conditions for 6.5 years is capable of *in vitro* immunomodulation.

function), but as above it is difficult to ascertain what contribution astragalus by itself may have made. Astragalus supplementation may also be associated with improved hematocrit and hemoglobin (Yin et al., 1995), VO_2max, or peak power output (Fan et al., 2005). In a study of forced swimming in rats which compared sedentary (control), exercised, and exercised rats supplemented with astragalus polysaccharides, researchers reported that the exercise-alone rats had lower T cell mRNA levels for the IL-2 receptor (mIL-2Rα) and serum IL-2 levels but higher serum sIL-2R, whereas the exercised rats supplemented with astragalus did not show similar effects (Zhou et al., 2005). Other studies have reported improved endurance or recovery (Li et al., 1986; Wu et al., 2007; Yin et al., 1995).

As with echinacea, the rodent macrophage serves as a representative model for understanding astragalus mechanisms (Fig. 5) because of its role in inflammation. Astragalosides, isoflavonoids, and polysaccharides are all known constituents of astragalus extracts; of these, the only compound-receptor relationship known for this model is the binding of polysaccharides to TLR4 (Shao et al., 2004; Yin et al., 2010b). Astragalus may even induce upregulation of TLR4 (Yin et al., 2010b). Likely other receptors are also involved for the other molecules.

Astragalus can exert its effects on one of two signaling pathways. First, encompassed by a dashed box in Fig. 5, signal transduction elements of the mitogen-activated protein kinase (MAPK) family are heterogeneously effected; p38 and Erk 1/2 appear to be downregulated whereas JNK is unaffected (Ryu et al., 2008). MAP kinase phosphatase-1 (MKP-1) can itself regulate the activity of MAPK elements and astragalus has been shown to increase both activity (through enhanced phosphorylation) and total protein quantity of MKP-1 (Ryu et al., 2008). Second, NF-κB levels may increase or decrease upon astragalus administration (Ryu et al., 2008; Zhao et al., 2011); related, levels of p65 (the molecule responsible for translocating the NF-κB complex into the nucleus) may be inhibited or simulated (Lee and Jeon, 2005; Ryu et al., 2008).

Cytokines may be variously upregulated or downregulated based on experimental conditions, and data has been collected for GM-CSF (Zhao et al., 2011), TNF-α (Yoshida et al., 1997; Shao et al., 2004; Xu et al., 2007; Clement-Kruzel et al., 2008; Ryu et al., 2008; Chao et al., 2009; Zhao et al., 2011), IL-1α and -1β (Shao et al., 2004; Lee et al., 2005; Xu et al., 2007; Ryu et al., 2008), IL-6 (Yoshida et al., 1997; Lee et al., 2005; Xu et al., 2007; Clement-Kruzel et al., 2008; Ryu et al., 2008; Chao et al., 2009), IL-10 (Clement-Kruzel et al., 2008), and IL-12 (Clement-Kruzel et al., 2008). PGE_2 synthesis via COX-2 is inhibited (Ryu et al., 2008; Chao et al., 2009). Astragalus treatment can have either an inhibitory or stimulatory effect on iNOS levels, in turn having heterogeneous effects on NO production (Lee and Jeon, 2005; Lee et al., 2005; Ryu et al., 2008; Chao et al., 2009; Zhang et al., 2011; Zhao et al., 2011).

Additionally, astragalus supplements increase macrophage trafficking (Cho and Leung, 2007), proliferation (Lee et al., 2005), phagocytosis (Cho and Leung, 2007; Xu et al., 2007), and cleavage of complement component C3 (Wang et al., 1989).

Astragalus treatment can have either pro- or anti-inflammatory effects depending on context; some experimental variables are presented in Table 6 and may explain the discrepancies in Fig. 5. Limited data suggest that similar effects are seen when astragalus saponins are used in a human macrophage model (Bedir et al., 2000). Effects on other cell populations including splenocytes (Yoshida et al., 1997; Cho and Leung, 2007; Chao et al., 2009), dendritic cells (Liu et al., 2011b), and lymphocytes (Yoshida et al., 1997; Shao et al., 2004; Brush et al., 2006; Cho and Leung, 2007) have been reported; curiously, some studies show that astragalus causes a Th1 → Th2 shift (Liu et al., 2011b) whereas other studies show the opposite (Kang et al., 2004; Liu et al., 2011a). Upregulating regulatory T cell function may be another important activity induced by astragalus supplements (Qu et al., 2010).

Flavonoids, polysaccharides, and triterpene saponins are the three major classes of bioactive molecules from astragalus. The saponins from astragalus are named astragalosides and are structurally glycosides of epoxycycloartanes

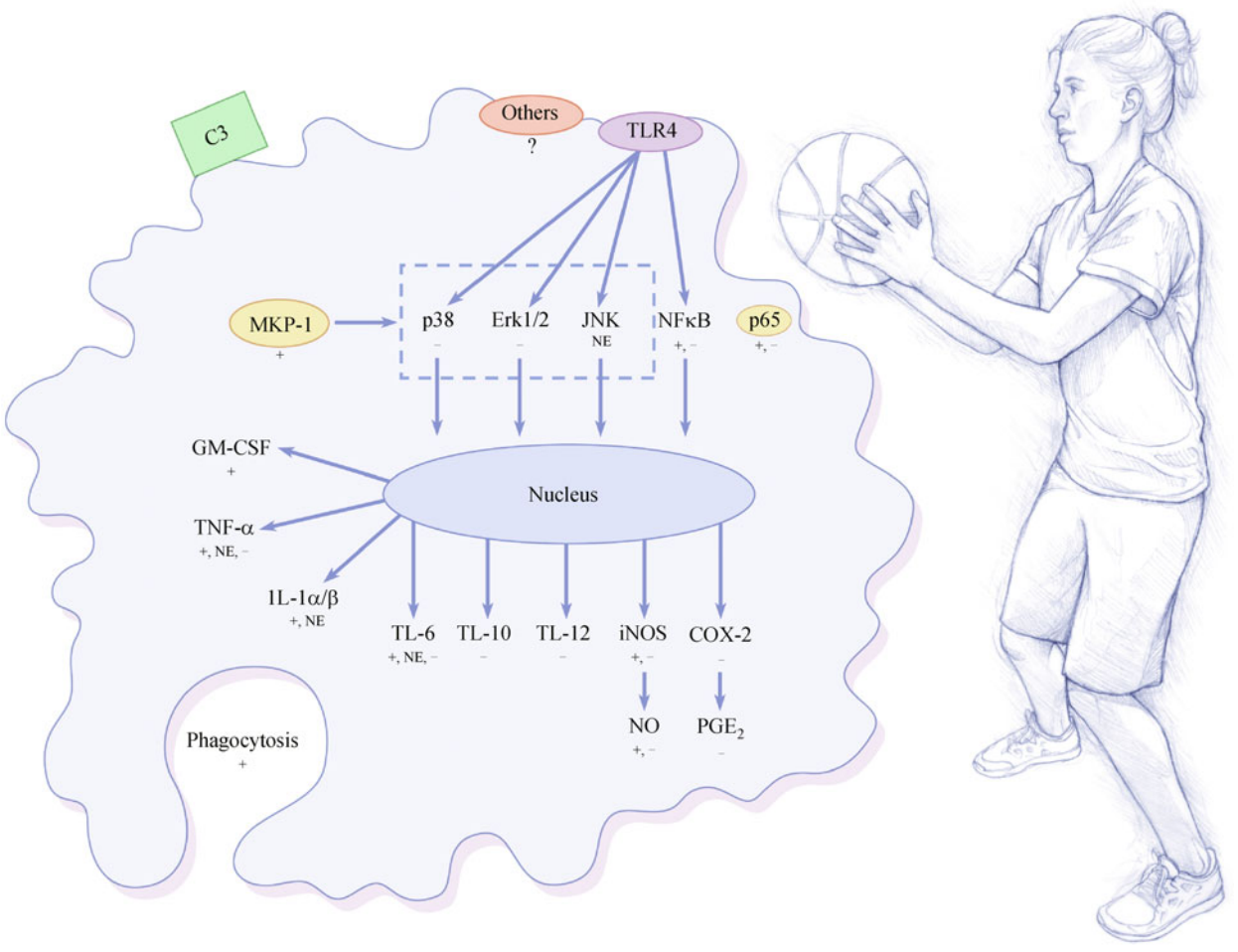

Figure 5 Representative model of current mechanisms research on *Astragalus membranaceous* supplements with a focus on the rodent macrophage. (Right) Astragalus supplements are consumed by a wide range of athletes for general immune-enhancing effects. (Left) Summary of selected current research on the effects of astragalus on rodent macrophage inflammation-associated pathways. Positive signs (+) indicate upregulation, negative signs (–) indicate downregulation, and "NE" stands for "no effect."

(Zhang et al., 2011). Much recent work has investigated whether or not saponins may be good vaccine adjuvants, reviewed elsewhere (Rajput et al., 2007; Song and Hu, 2009; Nalbantsoy et al., 2011); however, saponins alone may not explain all adjuvant activity (Hong et al., 2011). Astragalus flavonoids such as formononetin are still poorly understood and most current efforts are targeted toward elucidating their identities with various chemical methods. Some evidence suggests they may have anti-inflammatory (Zhang et al., 2011) or antitumor (Auyeung and Ko, 2010) capacities. Polysaccharides may interact with multiple types of leukocytes (Liu et al., 2011a, b).

Current genomic efforts have substantiated the results gleaned from immune studies (Denzler et al., 2010). There is also evidence that other species within genus *Astragalus* have cytokine-manipulating properties (Yesilada et al., 2005).

Elderberry

Unlike the four other supplements discussed in this review, to the best of our knowledge there are no clinical studies evaluating the efficacy of elderberry (*Sambucus* spp.; most commonly *S. nigra*) on athlete immune function specifically, though it has been recommended specifically for that capacity (Kundrat, 2005). One study examined how a single bolus of elderberry concentrate influenced physiologic parameters after a 1 h run and reported that elderberry-treated athletes exhibited lower post-exercise blood lactate levels than controls, but no differences in blood glucose, bicarbonate (HCO_3), or partial pressure of carbon dioxide (pCO_2) (Porta et al., 2007). There are some *in vitro* studies of its interactions with influenza (Zakay-Rones et al., 1995; Roschek et al., 2009; Krawitz et al., 2011), and clinical studies of elderberry supplements on immune function both within and outside of influenza contexts (Zakay-Rones et al., 2004; Guo et al., 2007; Vlachojannis et al., 2010). Thus, elderberry meets many of the same preliminary criteria that echinacea does in terms of candidacy for use among athletes.

Elderberry constituents interact directly with influenza virus, interfering with its normal activities (Fig. 6, top left).

Table 6 Experimental variables for the data presented in Figure 5

References	Organ	Extraction	Constituent (Solvent)*	Stimulant†	mRNA or protein	MΦ type‡
Chao et al., 2009	Root?	Ethanol	Unknown (ethyl acetate, hexane, water)	LPS/IFN-γ	Protein	PER
Cho and Leung, 2007	Root	Ethanol, water	Unknown (ethanol, water)	None	Protein	PER
Clement-Kruzel et al., 2008	Root	Alcohol, water	Unknown (alcohol, water)	LPS	Protein	J774A.1
Lee and Jeon, 2005	Root	Water, saline	POLY (ethanol)	None	Both	PER, RAW 264.7
Lee et al., 2005	Root	Water	Unknown (water)	MTX	Both	RAW 264.7
Ryu et al., 2008	Root	Water	Unknown (PBS)	LPS, ZYM	Both	RAW 264.7
Shao et al., 2004	Root	Water, saline, ethanol	POLY (water)	None	Both	PER
Wang et al., 1989	Root	Unknown	POLY (saline)	None	Protein	PER
Xu et al., 2007	Unknown	Unknown	POLY, SAP (unknown)	GCM	Protein	PER
Yin et al., 2010b	Unknown	Unknown	POLY (unknown)	LPS	Both	PER
Yoshida et al., 1997	Unknown	Water	Unknown (water)	THIO then LPS	Protein	PER
Zhang et al., 2011	Root	Ethanol	FLAV (ethanol, methanol)	LPS	Protein	RAW 264.7
Zhao et al., 2011	Root	Ethanol, water	POLY (water)	LPS	Protein	RAW 264.7

* Constituent/solvent abbreviations: FLAV = flavonoids, PBS = phosphate-buffered saline, POLY = polysaccharides, SAP = saponins. † Stimulant abbreviations: GCM = gravy culture medium, IFN-γ = interferon-γ, LPS = lipopolysaccharide, MTX = methotrexate, THIO = thioglycolate, ZYM = zymosan. Note that stimulation sometimes occurred *in vivo*, sometimes *in vitro*, or sometimes using both methods sequentially (see individual papers).‡Macrophage abbreviation: PER = peritoneal.

Influenza viruses use hemagluttinin molecules on their cell surface to bind target cells. Numerous elderberry consistuents have been shown *in vitro* to block influenza hemagluttinin including flavonoids such as a methylquercetin and a dihydromyricetin (Roschek et al., 2009), and lectins such as *Sambucus nigra* agglutinins or SNA's (Shibuya et al., 1987), via its interactions with sialic acid residues.

Little is known about the interactions of elderberry extracts with leukocytes. Figure 6 (top right) illustrates possible mechanisms in both human monocytes/macrophages and basophils, and reflects our paltry knowledge on this supplement. On macrophages, elderberry lectins may bind to CD68 (Baldus et al., 1995) as inferred based on structural similarity of SNA's to other lectins, or possibly other molecules. Through unascertained pathways, production of pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8, as well as the anti-inflammatory cytokine IL-10 are upregulated (Barak et al., 2002; Waknine-Grinberg et al., 2009). On basophils (Haas et al., 1999), lectins may bind to IgE molecules which themselves are bound to FcεRI receptors, thereby cross-linking the Fc receptors and promoting cell signaling, or may bind to FcεRI receptors directly. They may possibly also bind CD88. Through unknown pathways production of the inflammatory mediators IL-3, IL-14, and histamine is upregulated.

The macrophage/monocyte work (Barak et al., 2002; Waknine-Grinberg et al., 2009) used a proprietary elderberry extract (Sambucol) which also contained slight amounts of raspberry (*Rubus* spp., Rosaceae) standardized for flavonoid content (Zakay-Rones et al., 2004) and a mixture of proteins (including lectins) and other molecules, whereas the basophil work (Haas et al., 1999) used SNA. The leukocyte membrane receptors for elderberry flavonoids are not known. Lectins are non-enzymatic proteins that bind sialic acid residues which are abundant on glycoproteins. The basophil research team reasoned that SNA may bind to IgE molecules which are themselves bound to FcεRI receptors of basophils, thereby cross-linking receptors and initiating cell signaling; alternatively, they also suggested lectins may bind directly to FcεRI receptors or CD88 (Haas et al., 1999). The monocyte research teams did not suggest a possible receptor in their studies, but the available literature reveals some possibilities. SNA's can bind to macrophages directly (Petryniak et al., 1992) and have binding properties similar to a lectin from *Griffonia simplicifolia* (Fabaceae) dubbed GSA-I-B4. Since GSA-I-B4 can bind to CD68 molecules on macrophages (Baldus et al., 1995), CD68 may be one portal of entry. Macrophages also express an Fc receptor (FcεRIII; also known as CD23) with low affinity for IgE (Murphy et al., 2011), making CD23 another candidate. The cytokine-modulating effects in Fig. 6 are reminiscent of a Toll-like receptor pathway suggesting TLR's (possibly TLR4) as a third possible receptor. Given lectins' binding promiscuity coupled with the macrophage's infamy for expressing a bewildering lineup of both functional and nonfunctional receptors, it is likely that many receptors may interact with lectins. Glycoproteins themselves are abundant across the cell membrane and on viral peplomers. As illustrated in Fig. 6, some studies have shown direct binding of lectins to influenza hemagluttinin (Shibuya et al., 1987), a molecule necessary for binding to target cells; this likely explains how lectins prohibit influenza infectivity. Sialic acid residues are so abundant that it is equally plausible lectins could bind to other influenza peplomers (e.g., neuraminidase) and through stearic hindrance interfere with hemagluttinin binding activities. The same reasoning could be applied to the eukaryotic cell surface.

Elderberry has two main bioactive fractions: flavonoids and lectins. Elderberry flavonoids primarily take the form of anthocyanins (a group of polyphenols), prized for their antioxidant capacities (Schmitzer et al., 2010). Given that exercise produces oxidative stress (Schippinger et al., 2002), elderberry extracts may provide antioxidant benefits to athletes also. Flavonoid quantities are highest in the fruits. Elderberry lectins are located in multiple tissues of the plant including the bark (Shibuya et al., 1987), berries (Vlachojannis et al., 2010), and flowers (Serkedjieva et al., 1990). Lectin diversity and quantity varies among the three species used medicinally (Shibuya et al., 1989). The traditional use of medicinal elderberry began in Europe, hence most supplements are produced from *S. nigra* subsp. *nigra* and most research has focused on this taxon. Species-specific differences in lectin composition (and possibly also anthocyanin composition, though this remains to be demonstrated) and specific constituent identities are important variables to consider when making comparisons across studies as each lectin has slightly different physical and chemical properties.

Pequi

Compared to the preceding supplements, much less is known about pequi (*Caryocar brasiliense*) in the context of athlete immune function because its use in this capacity has only been explored recently despite a long tradition of use. Pequi represents a potential herbal immunomodulator which is just emerging scientifically.

The fruit, known both as pequi nut or souari nut, is rich in a subclass of carotenoids called xanthophylls including lutein, violaxanthin, and zeaxanthin, with trace amounts of other xanthophylls (Azvedo-Meleiro and Rodriguez-Amaya, 2004). It also contains other phenols and acids (Ferreira et al., 2011), particularly fatty acids (Miranda-Vilela et al., 2009b). Oils from the plant have demonstrated modest antibacterial activity and some antioxidant activity, yet these same oils exhibited cytotoxic activity as well (Ferreira et al., 2011).

Only four studies could be located pertaining to pequi extracts and athletes or exercise, all of which were authored by the same research team and all of which focused exclusively on a group of endurance runners (~75 males and ~50 females) who maintained their training regimen during two weeks of pequi oil capsule supplementation (Fig.

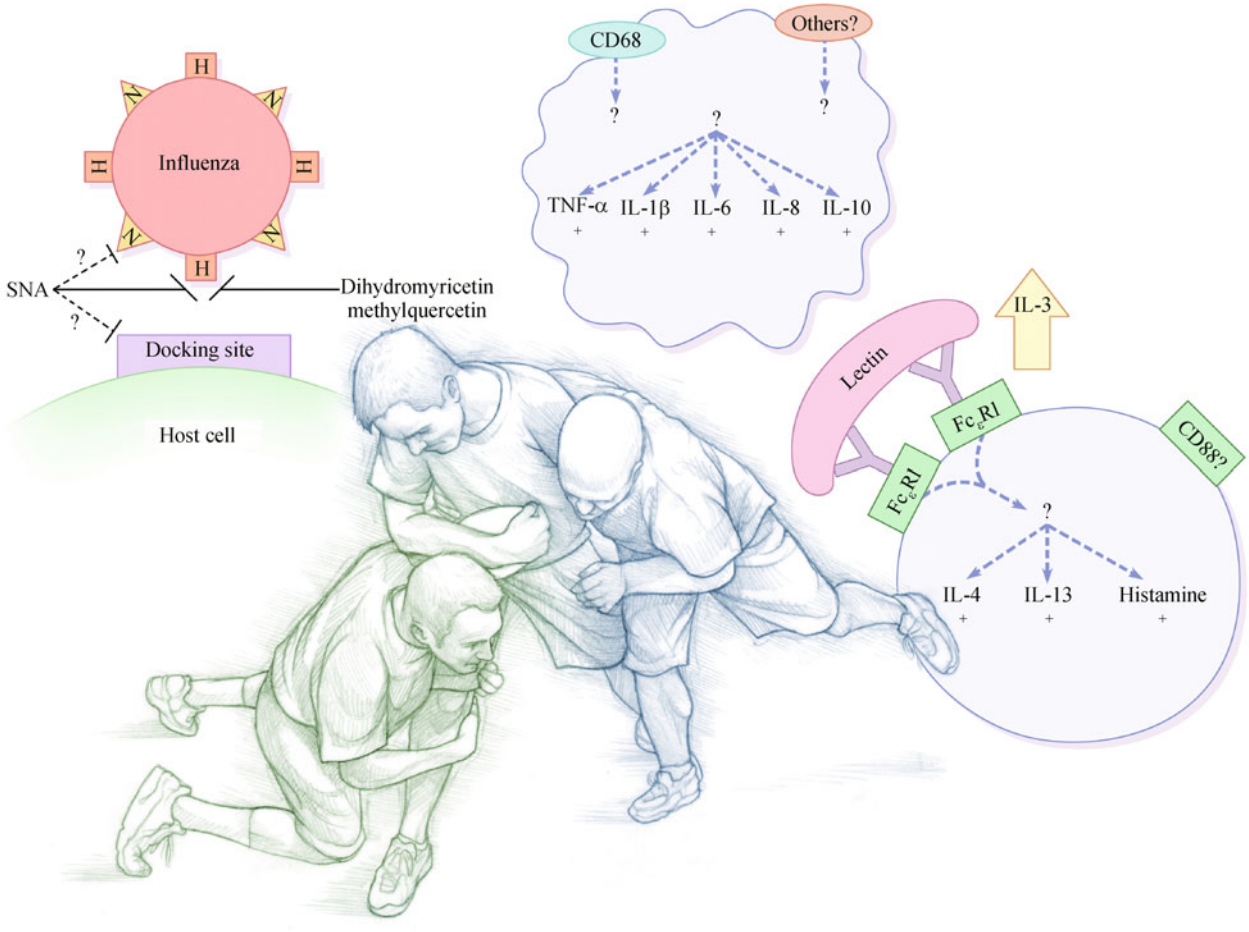

Figure 6 Representative model of current mechanisms research on elderberry supplements. (Center) No studies of elderberry supplements on athlete immune function exist, but given its immunostimulatory, antioxidant, and antiviral properties, elderberry supplements may be of benefits to athlete groups like football players. (Top, Left): Interactions between elderberry supplement components and influenza virus. (Top, Right) Effects of elderberry on macrophage and basophil activities.

7). In their first study, the research team examined peripheral blood lymphocyte populations from pre- to post-supplementation and analyzed their results both by gender and age groupings. Monocyte counts increased for both genders with more pronounced effects in females, and basophil counts increased in males (Miranda-Vilela et al., 2009b). There was a non-significant decline in lymphocytes and mature neutrophils. No differences were seen in immature neutrophils or eosinophils. When the same data was considered by age groups instead of gender, eosinophil counts increased among individuals aged 15–19 years, whereas monocyte counts increased among individuals aged 15–19 and 30–34. C-reactive protein (CRP; an inflammatory mediator found in the blood) levels were reduced in individuals aged 30–34, but not in other groups (Miranda-Vilela et al., 2009b); however, in a follow-up study it was demonstrated that there was an increase in CRP levels among some individuals with a specific genotype for the methylenetetrahydrofolate reductase (MTHFR) (Miranda-Vilela et al., 2011). Levels of cytosolic proteins including alanine and aspartate aminotransferases (ALT and AST) and creatine kinase (CK) were measured as indexes of tissue and muscle damage (respectively); while levels of all three markers decreased post-treatment, the differences were only significant for ALT and AST and were more pronounced in females (Miranda-Vilela et al., 2009a). Effects of pequi oil supplementation on erythrocytes and plasma varied and were influenced by athletes' genotypes (Miranda-Vilela et al., 2010). Taken together, the results tentatively suggest that pequi oil supplements have varied effects on the peripheral leukocyte counts in runners and may have an overall anti-inflammatory effect. Limited evidence suggests pequi oil supplements warrant further investigative attention as therapeutic immunomodulators in athletes.

Discussion and conclusions

From an immunological standpoint alone, the five herbal supplements reviewed here appear to exert different effects on the immune system. Astragalus and elderberry supplements predominantly interact with innate immunity whereas

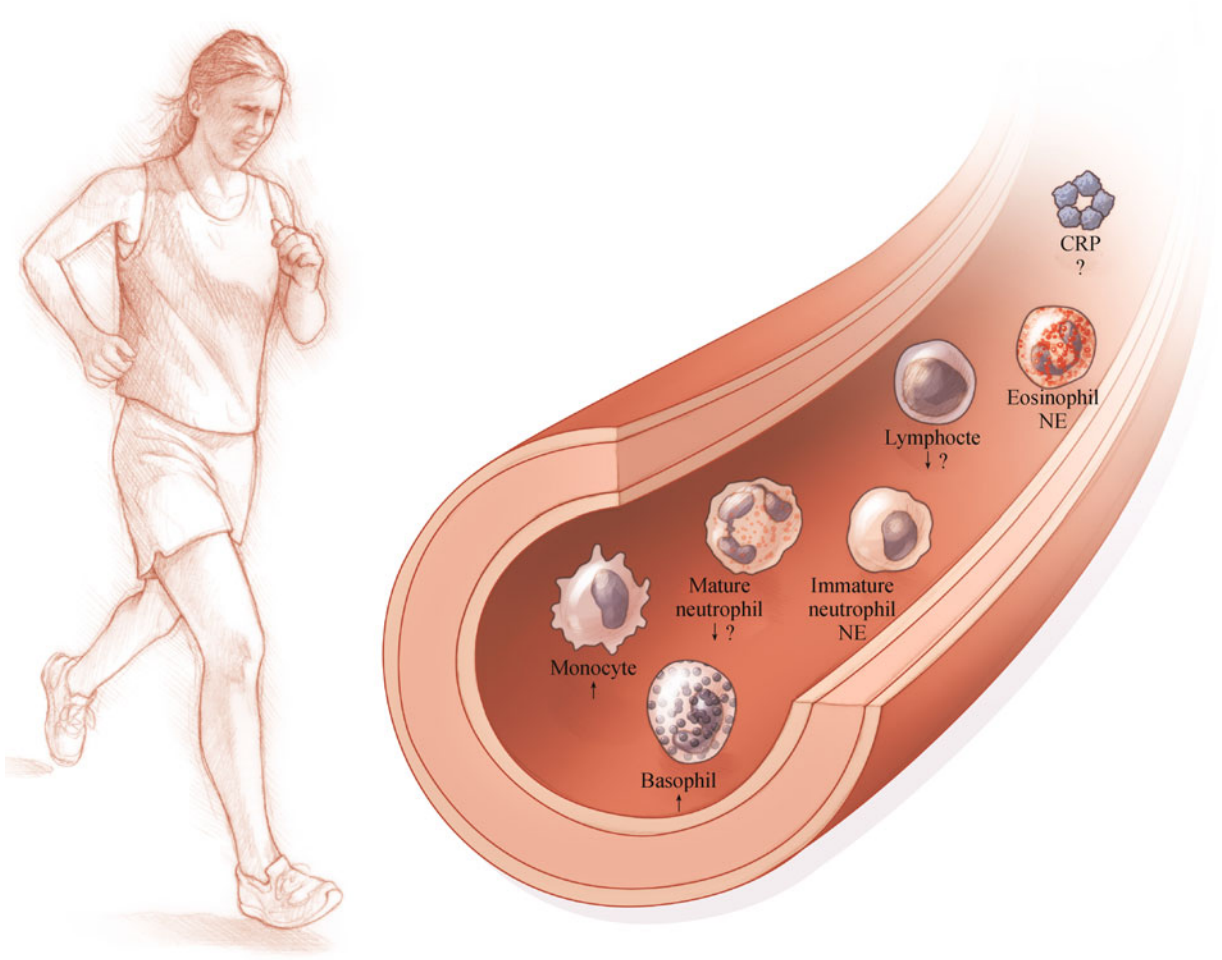

Figure 7 Representative model of current mechanisms research in pequi supplements. (Left) To date, all studies on pequi supplements have been performed in runners. (Right) Cut-away view of a blood vessel showing possible effects of pequi supplement ingestion on leukocyte subsets from one study (Miranda-Vilela et al., 2009b). Arrows indicate direction of cell counts from pre- to post-treatment and "NE" stands for "no effect."

ginseng supplements predominantly interact with adaptive immunity; echinacea and pequi supplements may interact with both, though echinacea seems to influence innate immunity more robustly. It is unclear if these generalizations are accurate reflections of each supplement's activity or confounded by a wholly inadvertent "research bias" due to the small number of studies that can be considered. Comparing bioactive compound classes (e.g., flavonoids, terpenoids, etc.) broadly across the different supplements, there are no apparent associations between the presence or absence of a given class and the type of immune effect. Similarly, there are no apparent associations between evolutionary relatedness (phylogenies) of the plants and observed immune effects.

Two important concerns in herbal supplement studies are bioavailability (e.g., if a purported bioactive molecule can pass through digestion unaltered into the bloodstream) and pharmacokinetics (e.g., if a molecule can circulate systematically and how long it lingers). Data are available for most but not all of the supplements. For ginseng, bioavailability studies suggest saponins can enter systemic circulation without modification in low but physiologically-relevant quantities (Leung and Wong, 2010) and additive molecules may improve their pharmacokinetics (Feng et al., 2011). For echinacea, alkamides are absorbed into the bloodstream, possibly quite rapidly through the oral mucosa (Woelkart et al., 2006; Guiotto et al., 2008), but phenols and polysaccharides may not be able to enter the bloodstream post-ingestion (Stevenson et al., 2005). Alkamides oftentimes demonstrate weak effects when used in isolation, or only partial effects that are restored when they are used in combination (Stevenson et al., 2005), suggesting they may act synergistically (LaLone et al., 2007; Chicca et al., 2009). One study reported that caffeic acid derivatives could not be identified in plasma samples from individuals who ingested echinacea tablets (Matthias et al., 2008). Contrasting this, another team

studying a mouse colitis model reported that not only was caffeic acid absorbed by the mouse cecum but it had an anticolitic effect and differences in inter-mouse absorption rates were explained by differences in individual gut microbiota (Ye et al., 2011). Astragalus data are limited to non-human models but suggests that both astragalosides (Zhang et al., 2005) and flavonoids (Xu et al., 2006; Wang et al., 2007) reach circulation. Strict bioavailability studies for elderberry lectins could not be located, but lectins can bind to and pass through gut epithelial cells (Pusztai et al., 1990) and in the bloodstream bind to serum glycoproteins and red blood cells (Haas et al., 1999), suggesting ample opportunities for leukocytes to interact with lectins. Studies suggest most elderberry anthocyanins are absorbed at low yet therapeutically-relevant quantities (Frank et al., 2007). Rates of anthocyanin absorption may be lower than for other flavonoids (Yang et al., 2011). Bioavailability data could not be located for pequi. Finally, whether or not polysaccharides (from any supplement or species) survive the digestive process without alteration before entering circulation is still contentious.

One polysaccharide of potentially great concern in *in vitro* studies is LPS, mentioned previously. LPS is recognized by germline-encoded TLR found on some cell surfaces, including macrophages. LPS may be present in botanical extracts from any species because of bacteria growing on plant material that inadvertently become incorporated into the extract, and some have opined that background LPS levels present in many herbal supplements may partially (if not exclusively) explain *in vitro* immunomodulatory activity (Pugh et al., 2008). While endotoxin is a legitimate concern and is not always addressed by scientists, many researchers working with herbal supplements have proactively examined possible endotoxin effects and often report findings that suggest no effect of endotoxin. Concerning ginseng, those teams that reported upregulated inflammatory or Th1 cytokine production by ginseng polysaccharides also demonstrated that LPS was not responsible for activities reported in their model, with one also demonstrating absence of signaling through TLR4 (Azike et al., 2011; Ilarraza et al., 2011). Another team demonstrated ginseng polysaccharides reduced expression of TLR2 and MyD88, a molecule that binds to its cytoplasmic domain (Ahn et al., 2006). These data suggest that the effects attributed to ginseng polysaccharide fractions are due to ginseng compounds and not LPS. Echinacea research teams have provided experimental data arguing against LPS effects (Senchina et al., 2006b; Sullivan et al., 2008; Senchina et al., 2010), indicating the observed effects are due to echinacea constituents alone. In those astragalus studies where LPS was controlled for (Denzler et al., 2010; Yin et al., 2010b) findings show the most parsimonious explanation of observed effects is from astragalus components, possibly acting through the same receptors as echinacea.

Directions for future research are many. Increasing the number of immunology studies using *in vivo* supplementation of athletes is paramount. Regardless of model chosen, studies should examine multiple compartments of immunity and should not only report but endeavor to control for all known botanical, biochemical, immunological, and experimental factors that may influence results (Senchina et al., 2009c) so comparisons might be more efficiently made across studies.

Scientists should not be discouraged by the current patchwork appearance of the cumulative evidence nor conclude that herbal supplements are generalizeably ineffective (as was disproven multiple times throughout this paper). It is promising to note that herbal supplement research is advancing from the basic and misleading approach of "does it work"? to a more sophisticated and accurate approach of understanding under which experimental conditions certain outcomes accrue. The burgeoning use of these supplements by athletes provides encouragement and purpose for future endeavors.

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