COLD-FX® Stimulates Cell Mediated Immune Response of Peripheral Leukocytes *ex-vivo* to Influenza Virus in National Hockey League Players

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Abstract: A proprietary extract from North American ginseng (*Panax quinquefolium*), COLD-FX® (CVT-E002), has been found to be effective in the prevention of upper respiratory infections in a number of different groups including institutionalized seniors, community-dwelling seniors and healthy adults. These effects of COLD-FX are thought to be mediated by its ability to stimulate both the natural and acquired immune systems. Excessive exercise has been known to induce immune suppression and to increase the susceptibility for contracting recurrent upper respiratory tract infections. The purpose of the present study, therefore, was to determine if COLD-FX supplementation to players of National Hockey League caliber would be effective in altering the response of their leukocytes to influenza virus *ex vivo*. Peripheral blood leukocytes (PBLs) were isolated from 2 groups of NHL players and immediately cultured for 2.5 days in the presence of 3 strains of influenza virus. One group (n=18) had been taking COLD-FX (400 mg/day) for 30 days, while the control group (n=19) was supplement free. Cultured PBLs isolated from those taking COLD-FX (400 mg/day) and stimulated with 3 strains of influenza virus released more TNF-α and IL-2, however, moderated granzyme-B release. This indicates that COLD-FX supplementation enhanced cell-mediated immune response of PBLs to influenza viruses and likely moderated the levels of cytotoxic T-cell effector molecules, sustained release of which may lead to autoimmune conditions. Additional studies on elite athletes, whose immune systems may be compromised, are warranted.

Keywords: COLD-FX, immune response, exercise.

INTRODUCTION

A patented extract from North American ginseng (*Panax quinquefolium*), containing mainly poly-furanosyl-pyranosyl-saccharides, is commercially available over the counter as COLD-FX® (CVT-E002). This extract has been shown to have immunomodulatory effects as evidenced by many studies [1-8]. It has thus been found to enhance immune responses such as immunoglobulin production by lymphocytes and natural immune responses by peritoneal exudates macrophages [1]. The extract has also been found to be capable of increasing phagocytosis [4], induce release of interleukin-2 (IL-2), interferon-γ (IFN-γ), interleukin-1a and granulocyte macrophage-colony stimulating factor, and to activate lymphokine-activated killer cells and $CD8^+$ cells [5]. In addition the extract appears to stimulate natural killer (NK) cell cytotoxicity and cell mediated immune responses [6].

IL-2 and IFN-γ are the major T- and NK-cell cytokines involved in cell mediated immune response and thus play a significant role in the elimination of intracellular pathogens such as viruses and bacteria [9]. In recent studies, COLD-FX was found to be effective in enhancing the production of these cytokines in murine splenocytes and in human PBLs cultured with live influenza virus [8]. The results from these pre-clinical studies are in parallel to the outcomes of the clinical trials involving institutionalized seniors, community-dwelling seniors and healthy middle-aged adults [10-12], where COLD-FX has been found to be effective in the prevention of upper respiratory tract infections (URTI).

The available data, therefore, point to utilization of COLD-FX as a potential modulator of natural and acquired immune response in conditions characterized by immune suppression. Overtraining or excessive exercise is a condition that has been known to affect several components of the natural and acquired immune systems [13]. A few of these alterations include suppressed neutrophil function, lymphocyte count and proliferation, natural killer cell count and activity, and changes in polymorphonuclear cell priming potential [13, 14]. In addition, reductions in serum, nasal and salivary immunoglobulins levels have also been reported. Excessive exercise has also been found to increase plasma levels of certain Th-2 cytokines such as IL-10. This increase results in simultaneous suppression of cell mediated immunity, and in particular reductions in plasma levels of TNF-α and IL-2 [15]. Clinically, these changes predispose one to an increased susceptibility to infectious illness such as upper respiratory tract infections [13]. Therefore, the objective of

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the present study was to determine if COLD-FX supplementation to high performance athletes alters the response of peripheral blood leukocytes (PBLs) to influenza virus *ex vivo*. The study population included NHL team players and staff of the Edmonton Oilers.

MATERIALS AND METHODS

This voluntary, pilot study was administered during September 1998 to November 1998 at the University of Alberta, Edmonton, Alberta, Canada.

Participants

Following approval by the Health Research Ethics Board of the Capital Health, players and coaches from the Edmonton Oilers were recruited. Subjects were openly divided into the COLD-FX or the control group.

Preparation of COLD-FX

COLD-FX a proprietary product of Afexa Life Sciences Inc. (Edmonton, Alberta, Canada) was formulated from the roots of North American ginseng (*Panax quinquefolium* L., Fam. Araliaceae) and was found to contain approximately 90% poly-furanosyl-pyranosyl-saccharides. The freeze-dried extract was encapsulated to contain 200 mg/capsule.

Study Design

The interested volunteers attended an information session at the University of Alberta. At this session, the study details were explained. The subjects at this session also filled out a questionnaire pertaining to their general health, and use of medications and dietary supplements. The subjects then signed a written consent at this meeting. As well, during this session a baseline blood sample was also obtained from each subject. Subjects in the COLD-FX group consumed 2 capsules (400 mg) of COLD-FX every morning for one month whereas those in the control group did not receive any supplementation. All subjects were instructed not to take any other cold medication unless advised to by their family physician and to continue the use of other medications or dietary supplements. Subjects were contacted every week to ensure adherence to the study protocol.

Blood Collection and Analysis

Two blood samples were obtained from each subject. The first sample was collected at recruitment for the establishment of baseline values, and the second, collected on completion of the study. Both the samples were obtained at approximately the same time of day. Blood samples were used for the preparation of peripheral blood leukocytes (PBLs) suspensions. PBLs were isolated from heparinized blood by centrifugation over Ficoll-Paque ^{TM} Plus (Amersham Pharmacia, Piscataway, NJ). Cells were washed, and resuspended in a medium consisting of RPMI and 10% fetal bovine serum at a concentration of $1.5x10^6$ per ml. Cells were then cultured with or without live influenza virus [16]. Three different strains of influenza virus including A/Johannesberg/82/96**,** A/Nanchang/93/95, B/Harbin/07/94 were used. Culture supernatants and cells were collected after 2.5 days of culture. Supernatants were utilized for the measurement of TNF-α, IL-2, IL-10 and IFN-γ using the commercially available ELISA kits (OptEIA set, human, Pharmingen, San Diego, CA, USA). Concentrations of these cytokines were calculated relative to simultaneously run standards and expressed as ng/ml. Cell lysates were used to determine the levels of Granzyme B (Gr-B) using a previously described method based on specific cysteine aspartase cleavage of the substrate t-butyloxycarbonyl-Ala-Ala-Asp-4-nitroanaline [16]. The results were expressed as ASPase activity units/mg protein.

Statistical Analysis

The groups were compared for changes in the ratio (after supplementation: before supplementation, A/B) of the cytokines and Gr-B using Mann-Whitney analysis. This non-parametric test was used to analyze the data because the sample size of the study was small and high variability between subjects was observed. Effects were considered to be significant if the associated p value was found to be < 0.05.

RESULTS

A total of 37 members of the Edmonton Oilers team, including 32 players and 5 coaches were enrolled. Of these, 18 received COLD-FX supplementation and 19 were not given any supplementation (control group) during the study. The groups were very similar with respect to age, weight and height of the players (Table **1**). There were 3 and 2 coaches in the COLD-FX and non-supplemented groups, respectively.

Fig. (**1**) shows the effects of COLD-FX intervention on Gr-B ratios. During the study Gr-B levels in the lysates of both cells incubated with influenza virus or untreated, increased by approximately 55% in the COLD-FX group and by 80% in the control group. This difference between the groups was found to be significant in PBLs treated with B/Harbin/07/94.

The changes in TNF-α A/B ratio are shown in Fig. (**2**). In untreated PBLs cultures, A/B ratio increased in both groups and the difference between the groups was not significant.

Table 1. Demographic Characteristics of the Study Participants

Groups	COLD-FX (n=18)	Control (n=19)
Age (Y), mean ± SD	25.6 ± 2.3	23.6 ± 3.0
Weight (lbs), mean ± SD	200.4 ± 11.9	207.2 ± 17.4
Height (inches), mean ± SD	72.7 ± 1.5	73.6 ± 1.8

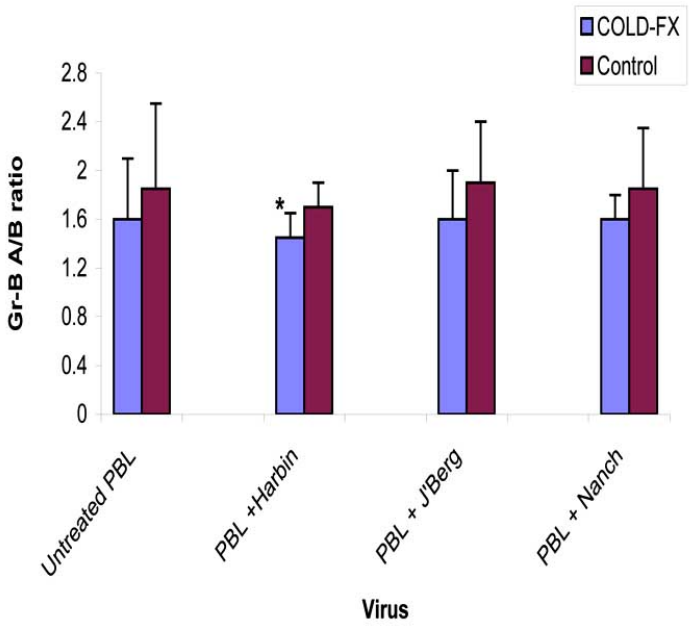

Fig. (1). Effect of COLD-FX supplementation on Granzyme-B release from PBLs treated with different strains of influenza virus.
Note PBL: Peripheral Blood Leukocytes; Gr-B: Granzyme-B; A/B ratio: After supplementation: Before supplementation; Harbin: B/Harbin/07/94; J'Berg: A/Johannesberg/82/96; Nanch: A/Nanchang/93/95.; * $p<0.05$; Bars indicate mean ± SD.

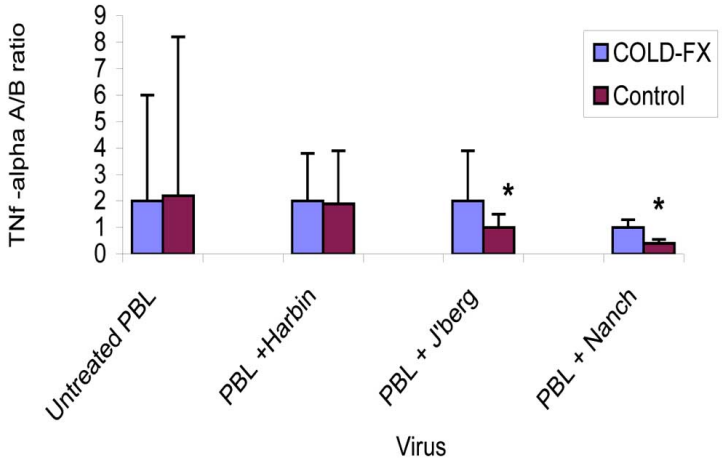

Fig. (2). Effect of COLD-FX supplementation on TNF-alpha release from PBLs in the presence of different strains of influenza virus.
Note PBL; Peripheral Blood Leukocytes; TNF-alpha A/B ratio: After supplementation: Before supplementation; Harbin: B/Harbin/07/94; J'Berg: A/Johannesberg/82/96; Nanch: A/Nanchang/93/95; * $p<0.05$; Bars indicate means ± SD.

However, antigen treatment of the cells with A/Johannesberg or A/Nanchang decreased the TNF-α release by approximately 50% in the control group whereas the cytokine release continued to increase in the COLD-FX group. This change in the A/B ratio of the two groups was found to be significant ($p < 0.05$).

Fig. (**3**) shows the effects of COLD-FX supplementation on A/B ratios for IL-2. PBLs from the control group, including both treated and untreated cells, showed a 25% decrease in their capacity to release IL-2 compared to their basal levels. In contrast, PBLs from the COLD-FX group showed a 5% increase in IL-2 production. This difference between the groups was found to be significant for untreated cells and cells treated with A/Johannesberg.

IL-10 release increased in both the groups during the study however, the difference in A/B ratio for IL-10 between the treatments was not significant. Antigen treatment had no further effect on the cytokine release. IFN-γ production from PBL cultures from both the COLD-FX and the control groups remained unchanged during the study. The antigen treatments also had no effect on IFN-γ release (data not shown).

DISCUSSION AND CONCLUSION

Our earlier studies have shown that COLD-FX is effective in the prevention of upper respiratory tract infections in institutionalized seniors, community-dwelling seniors and in middle age adults susceptible to frequent colds [10-12].

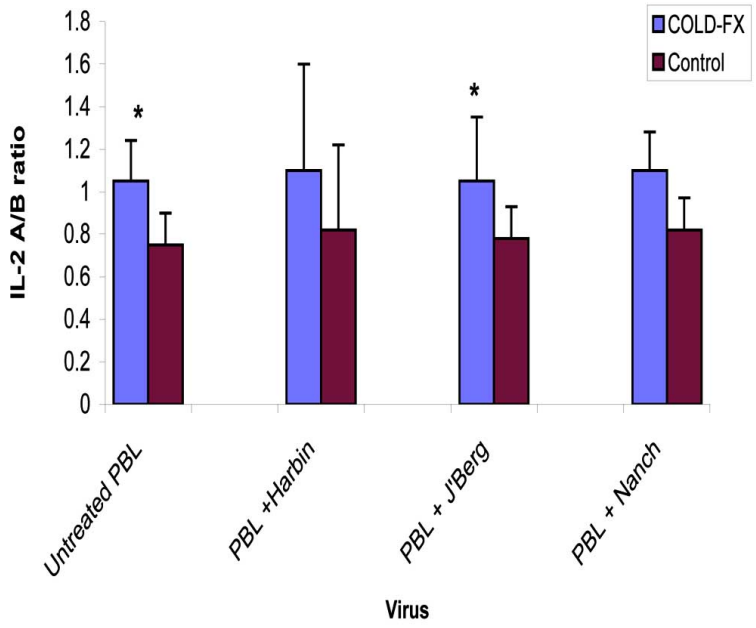

Fig. (3). Effect of COLD-FX supplementation on IL-2 release from PBLs treated with different strains of influenza virus.
Note PBL: Peripheral Blood Leukocytes; IL-2 A/B rato: After supplementation: Before supplementation; Harbin: B/Harbin/07/94; J'Berg: A/Johannesberg/82/96; Nanch: A/Nanchang/93/95; * $p<0.05$; Bars indicate mean ± SD.

These effects of COLD-FX are thought to be mediated by its ability to stimulate the cell mediated immune responses. Another group that is considered to be especially susceptible to infectious illness includes elite athletes. An extensive body of literature indicates that excessive exercise can induce tissue injury/trauma thereby leading to immune suppression [13]. In the present study, the immune suppressive effects of training were evident from the changes in systemic leukocyte cytokine profiles of players in the control group. The capacity of cells to produce the immunosuppressive cytokine, IL-10, increased during the study. In contrast, the antigen dependent cell-mediated immune responses were found to be decreased. This decrease was evident from reduction of up to 50% and 25% in the release of TNF-α and IL-2, respectively. Although COLD-FX treatment did not appear to have any effect on IL-10 release, it was effective in reducing the detrimental effects of IL-10 on production of Th-1 cytokines. Regular dosing of COLD-FX for one month was found to suppress performance-induced decreases in TNF-α and IL-2 release, that was found to be evident in the control group. These effects were more pronounced in the presence of influenza antigens. COLD-FX treatment enhanced the antigen specific TNF-α release by up to 40% and restored the capacity of the cells to produce IL-2. TNF-α and IL-2 are the major cytokines involved in innate and Th-1 related immune defense from viral infections. Increased production of these cytokines in the COLD-FX group therefore indicates greater protection from viral infections including possible protection from influenza related illnesses. These effects of COLD-FX treatment are in parallel to the outcome of a clinical open study in which 83% of the players from the Edmonton Oilers reported that a regular dosing of COLD-FX during an influenza season helped them to stay healthy [17].

IFN-γ levels did not change during the study in either of the two groups. This observation is in agreement with earlier studies reporting minimal effects of exercise on plasma IFN-γ levels [18]. However, contrary to our earlier *in vitro* study [8], the present "ex-vivo" study found no effect of COLD-FX supplementation on influenza specific release of IFN-γ.

This study also involved measurement of Gr-B levels in cell lysates. The levels of Gr-B provide an estimate of cytotoxic T lymphocyte mediated apoptosis of infected cells [19]. Gr-B expression was increased by approximately 60% in the PBLs isolated from those taking COLD-FX and 80% in the controls. Granzyme-B raising effects of exercise have been documented earlier [20]. Despite the fact that Gr-B has been known to be involved in cell-mediated immunity, a dangerously overproduction of the protease is considered undesirable as it can lead to an autoimmune state [21]. COLD-FX consumption appears to have dampened possible overproduction of Granzyme-B.

There are a number of limitations to this study. First of all, this was a small "*ex-vivo*-open" pilot study with a small sample size. There was no placebo and considerable individual variability in PBLs response was evident. However, isolated PBLs from both groups were treated identically and significant differences were clearly evident.

In conclusion, the results of this ex-vivo study on PBLs obtained from elite athletes indicate that COLD-FX supplementation may beneficially affect their cell- mediated immune responses *in-vivo*. Further long-term studies appear warranted to determine the efficacy of COLD-FX in the prevention and treatment of URTI in this susceptible group.

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