



Persistent response to pneumococcal vaccine in individuals supplemented with a novel water soluble extract of *Uncaria tomentosa*, C-Med-100®

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Summary

A human intervention study was carried out using male volunteers attending a General Practice Clinic in New York City involving comparison of individuals supplemented with 350 mg × 2 C-Med-100® daily dose for two months with untreated controls for their abilities to respond to a 23 valent pneumococcal vaccine. C-Med-100® is a novel nutraceutical extract from the South American plant *Uncaria tomentosa* or Cat's Claw which is known to possess immune enhancing and antiinflammatory properties in animals. There were no toxic side effects observed as judged by medical examination, clinical chemistry and blood cell analysis. However, statistically significant immune enhancement for the individuals on C-Med-100® supplement was observed by (i) an elevation in the lymphocyte/neutrophil ratios of peripheral blood and (ii) a reduced decay in the 12 serotype antibody titer responses to pneumococcal vaccination at 5 months.

Key words: *Uncaria tomentosa*, immune response, pneumococcal, vaccine, human trial

Introduction

C-Med-100® is a proprietary water extraction of plant parts (primarily inner and outer bark sources) from *Uncaria tomentosa* (Willd.) DC, commonly called Una de Gato or Cat's Claw. Cat's Claw has been used historically as a medicinal plant source by the native Indians of South America for many years. Cat's Claw is a vine which is shredded and prepared traditionally as a hot tea to be drunk either hot or cold as a supplement in the treatment of many human disorders including inflammations, cancer and infections (Jones 1995; Reinhard 1999). Because of its historical medicinal use, Cat's Claw products have been offered commercially in the USA and abroad for hundreds of years in preparations of pulverized plant parts, water and ethanol extractions (Keplinger et al. 1999).

The most consistent medicinal property related to Cat's Claw products has been immune modulation characterized by immune enhancement and/or anti-inflammatory effects. The immunoactive ingredients

have been attributed to ingested pulverized plant parts, water and ethanol extracts containing oxindole alkaloids, polyhydroxylated triterpenes, and quinovic acid glycosides (Aquino et al. 1989, 1990, 1991; Cerri et al. 1988; Reinhard 1999; Keplinger et al. 1999; Sandoval et al. 1998, 2000). Clearly these identified active components of Cat's Claw differ dramatically in their water solubility, and consequently in their bioavailability when administered as nutraceuticals to modulate immunity in the human population. Therefore, one way to clarify what is important to immune modulation in humans is to carry out clinical trials on chemically well defined Cat's Claw nutraceuticals where bioavailability and active components are controlled.

Abbreviations: C-Med-100® – a novel water extract from *Uncaria tomentosa*; NF-kB – nuclear transcription factor kappa B; CAE – carboxy alkyl esters; WBC – white blood cells

A straight forward approach to control bioavailability of nutraceuticals is to prepare water soluble extracts of the active components for human use which is standard practice in traditional medicine. C-Med-100® is such a Cat's Claw preparation. It is 100% water soluble containing 8–10% carboxy alkyl esters, less than 0.05% oxindole alkaloids and ultrafiltrated to reduce high molecular weight toxic side effect components (Sheng et al. 2000b). It has been shown to possess multiple modes of action such as modulation of apoptosis, DNA repair and chemotherapy induced leukopenia (Sheng et al. 1998, 2000a, 2000b). Other research teams have also characterized water extracts of *Uncaria tomentosa* as having immune stimulating and anti-inflammatory effects useful in protecting against mutation (Rizzi et al. 1993), chronic intestinal inflammation (Sandoval et al. 1998) and inhibition of TNF α production (Sandoval et al. 2000).

Pneumococcal infections are widespread, and despite the availability of effective antimicrobial drugs and vaccines, these infections remain a leading global cause of morbidity and mortality (Butler et al. 1999). Currently pneumococcal vaccines are about 76–92% effective in preventing pneumococcal infections in high risk populations, and so there is a significant number of individuals who escape protection from vaccination. Because of the epidemiology surrounding pneumococcal infections in humans, the hypothesis that C-Med-100® is a general immune enhancer in humans has been tested by evaluating antibody responses to broad spectrum pneumococcal vaccines in healthy volunteers before, during and after C-Med-100® supplement.

Materials and Methods

Volunteers

A human volunteer study was randomized with cross-sectional and longitudinal comparison between control and supplement groups, and between baseline and after supplement. The randomization, medical examinations, administration of vaccine, and collection of samples and medical data were carried out by Dr Steven Lamm and his General Practice Staff located at 12 East 86th Street in New York City between July 1999 and February 2000. Caucasian male volunteers between 40–60 years with exclusion criteria of no previous pneumococcal vaccination, no active chronic disease, and no concurrent medications or nutritional supplements were included into the study. Twenty three patients were randomized into 2 groups, one supplemented with 350 mg C-Med-100® \times 2 times daily for 2 month ($n = 11$) and the other received no supplement ($n = 12$). The schedule and events for clinical evaluation are presented in Table 1.

Basically the design was to baseline the medical examination, clinical chemistry and blood analysis on day 1 visit, followed by 2 months of C-Med-100® supplement in the treatment group. On day 30, the level of natural immunization was characterized by estimation of pneumococcal antibody titers in both the C-Med-100® supplement group and the no supplement group, before they were vaccinated with pneumococcal vaccine. Day 60 was the sample collection point at which the C-Med-100® treated group supplemented for 60 days and including the first 30 day post vaccination period was compared to the non-supplemented control group treated in the same manner. In all cases C-Med-100® treatment was discontinued on day 60, but blood analyses and medical examinations were performed on day 1, day 30 and day 60 for comparison of before and after effects from C-Med-100® supplementation. Finally on day 180 pneumococcal antibody titers were again determined to provide a basis for analysis of persistence of pneumococcal vaccination in the supplemented and non-supplemented groups.

Pneumococcal antibody titers

Serum blood samples were collected by venal puncture by Dr Lamm and his staff for all study subjects on their day 30, day 60, and day 180 scheduled visits. The pneumococcal antibody assay tests were performed by Quest Laboratories (One Malcolm Avenue, Teterboro, NJ 07608–1070) in order to estimate the IgG responses to 12 purified pneumococcal antigens (Serotypes 1,3,4,6,8,9N,12,14,19F,23F,51 and 56) included in the 23 valent pneumococcal vaccine, Pneumovax. The tested sera were pre-absorbed with cell wall polysaccharide to provide accurate capsule-specific IgG measurements.

Pneumococcal vaccination

All study subjects were administered intramuscularly 0.5 ml of Pneumovax on their day 30 visit by Dr Lamm. Pneumovax is a mixture of capsular polysaccharides of 23 pneumococcal types that account for 85–90% of all pneumococcal infections.

Blood analyses

Blood samples were also collected by Dr Lamm and his staff from all subjects on day 1, day 30 and day 60 scheduled visits, and sent to the Quest Laboratories for analysis of serum clinical chemistry and differential blood cell status.

Medical examination

A case report form was developed for this study containing demographic data, disease history and concomitant medication at baseline evaluation on day 1

visit. Medical parameters during the trial period were evaluated by personal interview with Dr Lamm at day 30, day 60 and day 180 scheduled visits.

The schedule of times and events outlining the conduct of this trial are listed in Table 1. Informed consent was obtained from each participant and the study was conducted in accordance with the recommendations guiding physicians in pharmaceutical research involving human subjects decided by the Declaration of Helsinki.

C-Med-100® preparation

C-Med-100® is a patented extract (U.S. patent 6,039,949) from Cat's Claw, *Uncaria tomentosa*, manufactured by Laboratório Centroflora (Sao Paulo, Brazil) and distributed in North America by AF Nutraceutical (Morristown, NJ). It is formulated and based on the historical medicinal use of Cat's Claw. Basically it is a water soluble extract ultrafiltrated to remove high molecular weight toxic conjugates (>10,000 MW), containing 8–10% carboxy alkyl esters (CAE) as active ingredients, and it is essentially free of oxindole alkaloids

(<0.05%, Sheng et al. 2000b). The active ingredients of C-Med-100® (85% of them) absorb onto charcoal, have a UV absorption maximum of 200 nm, and react with hydroxylamine and ferric chloride thus characterizing them as esters (e.g. CAE). The natural product extract used in the human trial was provided as 350 mg tablets taken twice a day (morning and evening).

Statistical analysis

One case in the supplement group was excluded from final analysis because of lack of complete data on day 30 and 60. Case report and clinical test results were analyzed by SPSS program software package (SPSS Inc.). Comparison of means between control and supplement groups was made by two-tailed t-test. Comparison before and after supplement for the supplement group was made by paired t-test. Comparison of the percentage of the immunized levels of pneumococcal antibody titers was made by chi square test. The significant level was set at 0.05.

Results

Study population

The subjects for the trial were enrolled from male patients attending a clinic in New York between 40–60 years (average 50). Although no apparent acute diseases were reported for these subjects, it should be pointed out that this population might differ from the general population. However, great efforts have been made to randomize the groups at initiation of the study, so that the supplement group was comparable to the control group before in regard to ethnicity, sex, body weight and body high (body surface area), percentages of active disease and concomitant medications ($p > 0.05$ by t-test or chi square analysis). For example, body mass index (body weight in kg/ height in m²), percentages of active disease and concomitant medications for the control group were 26.8 ± 3.5 , 16% (2/12) and 66.7% (8/12), respectively, and the corresponding values for the C-Med-100® supplemented group were 26.3 ± 3.3 , 20% (2/10) and 50% (5/10). The only active disease was cardiovascular involving high blood pressure and cholesterol elevation. These parameters were determined at baseline examination on day 1, and they were taken as evidence of successful randomization of medical factors. Moreover, the medical condition of the study subjects were found not to significantly differ throughout the trial period. There were also no documented side effects attributed to C-Med-100® supplementation or pneumococcal vaccination during the trial evaluation period of 180 days.

Table 1. Schedule and events for clinical evaluation of C-Med-100® as enhancer of response to pneumococcal vaccination.

Time	Control group (No supplement)	C-Med-100® Group (350 mg × 2 daily)
Day 1	Case Report Form Examination #1 WBC analysis	Case Report Form Subject information and consent Examination #1 WBC analysis T-cell studies C-Med-100® tablets (130) Start supplement
Day 30	Examination #2 WBC analysis T-cell studies Pre-vaccination titers Pneumococcal vaccination	Examination #2 WBC analysis T-cell studies Pre-vaccination titers Pneumococcal vaccination Continue C-Med-100® supplement
Day 60	Examination #3 WBC analysis Post-vaccination titers	Examination #3 WBC analysis Post-vaccination titers Compliance Record Remaining drug count
Day 180	Post-vaccination titers	Post-vaccination titers

Clinical chemistry

A complete profile of clinical parameters were determined in sera from the study subjects on day 1 and day 60 in order to assess the effects of C-Med-100[®] supplementation on liver function, kidney function, mineral balance and nutrient status. The data are recorded in Table 2. There were no statistically significant differences between the C-Med-100[®] supplemented group and controls for the serum chemical markers estimating liver and kidney function or nutrient status. The mineral status did indicate a statistically significant but clinically insignificant decrease in serum sodium and a comparable increase in iron while the rest of the minerals measured remained unchanged. In general these data strongly support the clinical findings of a lack of any observable medical changes that may reflect supplement-related side effect episodes. Even at the biochemical level there was no evidence of supplement-induced abnormalities except with regard to the minor effects observed on mineral status. It was concluded

that C-Med-100[®] supplementation at 350 mg × 2 daily was a safe dose for a two month intervention period.

Blood cell analysis

The effect of C-Med-100[®] on hematological parameters was also evaluated for the two month intervention. Baseline values collected at day 1 were compared to the day 60 values for the group receiving 350 mg × 2 daily doses of C-Med-100[®] in Table 2. It can be seen that most of the blood cell parameters were unaffected by C-Med-100[®] supplementation including red blood cells (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC distribution width (RDW), mean platelet volume (MPV), platelet count (PLT), and numbers/percentages of monocytes (MONO), eosinophils (EOS), basophils (BASO) and lymphocyte subset analyses (i.e. absolute numbers and percentages of CD3, helper, suppressor T-lymphocytes and the

Table 2. Clinical chemistry and biochemistry tests before and after C-Med-100[®] supplement (n=10) for 2 months at the daily dose of 350 mg × 2. The clinical chemistry relevant to biological screening consisted of (i) Liver function including total bilirubin, direct bilirubin, alkaline (alk) phosphatase, gamma glutamyl transpeptidase (GGT), serum glutamic oxaloacetic transaminase (SGOT, or AST), and serum glutamic pyruvic transaminase (SGPT or ALT), (ii) Kidney function including blood urea nitrogen (BUN), creatine, BUN/creatinine ratio, uric acid (iii) Heart disease screening including triglycerides, cholesterol, high density lipoprotein, low density lipoprotein, cholesterol/HDL ratio, lactic dehydrogenase (LD, or LDH), (iv) Minerals including iron, calcium, sodium, potassium, and (v) Miscellaneous indicators including glucose, total protein, albumin, globulin. * by two-tail t-test (n = 10 for before and after groups).

Test	Before Supplement	After Supplement	p-value *
Sodium (mmol/l)	141 ± 0.6	139.7 ± 1.6	0.045
Potassium (mmol/l)	4.40 ± 0.27	4.45 ± 0.26	0.702
Calcium (mg/dl)	9.74 ± 0.33	9.82 ± 0.24	0.448
Iron (µg/dl)	76.3 ± 24.3	105.4 ± 28.4	0.021
Chloride (mmol/l)	103.9 ± 3.2	103.6 ± 2.5	0.697
BUN (mg/dl)	18.4 ± 5.6	18.5 ± 4.8	0.915
Creatine (mg/dl)	1.13 ± 0.18	1.13 ± 0.18	1.000
BUN/Creatine Ratio	16.5 ± 5.1	16.6 ± 4.5	0.893
Uric Acid (mg/dl)	4.43 ± 0.68	4.05 ± 0.92	0.146
Glucose (mg/dl)	96.3 ± 10.0	92.8 ± 4.6	0.354
Total Protein (g/dl)	7.25 ± 0.56	7.35 ± 0.33	0.401
Albumin (g/dl)	4.42 ± 0.18	4.46 ± 0.14	0.443
Globulin (g/dl)	2.83 ± 0.48	2.89 ± 0.41	0.496
Albumin/ Globulin Ratio	1.60 ± 0.24	1.57 ± 0.24	0.646
Cholesterol (mg/dl)	198.8 ± 29.5	208.8 ± 27.8	0.185
HDL Cholesterol (mg/dl)	55.2 ± 12.2	55.1 ± 10.4	0.956
CHOL/HDL Ratio	3.71 ± 0.70	3.88 ± 0.70	0.154
LDL Cholesterol (mg/dl)	120 ± 25.3	132 ± 24.1	0.086
Triglycerides (mg/dl)	146.2 ± 106.9	113.8 ± 59.1	0.108
Bilirubin, total (mg/dl)	0.73 ± 0.28	0.84 ± 0.28	0.061
Bilirubin, direct (mg/dl)	0.152 ± 0.052	0.149 ± 0.057	0.862
ALK Phosphatase (Units/L)	64.1 ± 14.1	60.1 ± 13.5	0.183
GGT (Units/L)	20.7 ± 9.5	21.9 ± 11.7	0.347
AST (SGOT) (IU/L)	21.7 ± 4.7	24.0 ± 7.6	0.150
ALT (SGPT) (IU/L)	24.3 ± 7.5	25.5 ± 7.2	0.360
LD, total (IU/L)	159.1 ± 24.1	150.0 ± 18.2	0.091

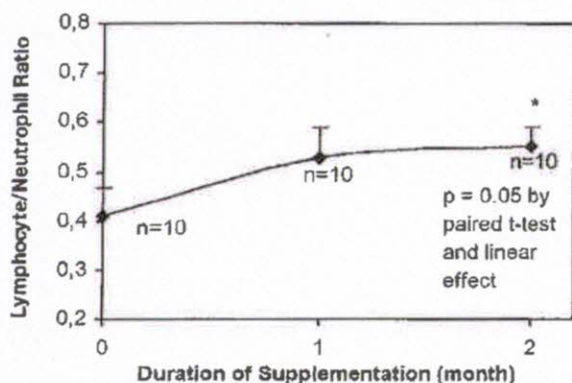


Fig. 1. The enhancement of lymphocyte to neutrophil ratios in peripheral blood from humans supplemented with C-Med-100® at an oral daily dose of 350 mg \times 2 for the indicated time periods. Differential WBC analyses were performed by direct collection and submission by the principle Investigator (Dr Steven Lamm, New York City) to Quest Laboratories (Teterboro, NJ).

helper/suppressor ratio). However, the total white cell counts (WBC) and the numbers/ percentages of neutrophils (POLY) and lymphocytes (LYMPH) tended to be statistically altered from C-Med-100® treatment ($p < 0.1$, Table 2). This tendency was confirmed by a more detailed analysis whereby the lymphocyte/neutrophil ratios were compared by a group analysis of the data col-

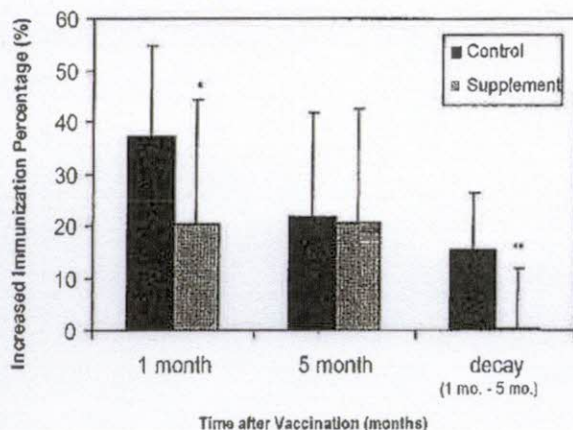


Fig. 2. The net increase in the percentage of 12 pneumococcal antibody titers (serotypes 1, 3, 4, 6, 8, 9N, 12, 14, 19F, 23F, 51 and 56) estimated 1 month and 5 months after pneumococcal vaccination in 12 controls and 10 C-Med-100® supplemented subjects. Natural immunization at day 30 before vaccination visit has been subtracted from the 1 and 5 month values to yield data on the net increase and decay of pneumococcal antibody responses to pneumococcal vaccination. Immunization was determined by comparison to the manufacturer's reference values. * $p < 0.05$, ** $p < 0.01$ comparing C-Med-100® to control groups by non-parametric Mann-Whit-

lected on day 1, day 30 and day 60. The data reported in Fig. 1 add to the hypothesis that lymphocyte/neutrophil ratios which represent the bulk of WBC are significantly increased in a duration of exposure manner by C-Med-100® supplementation. Because an increased percentage of lymphocytes is important to enhancing immune response, these results support the potential of an enhanced immune function related to C-Med-100® via a greater proportion of immune competent lymphocytes being present among the supplemented individuals. Moreover, due to the fact that no other blood cell parameter other than lymphocyte/neutrophil ratios showed any effect from C-Med-100® supplementation, these data also strongly suggest a selective influence on this immune modulating parameter without any observable toxicological effects on the other hematological parameters.

Pneumococcal antibody response to vaccination

The primary aim of this study was to determine if the immune modulating properties seen in other studies of C-Med-100®, could be confirmed in humans by measuring pneumonia antibody titer responses to pneumonia vaccination over time. For this purpose we have used a 23 valent pneumococcal polysaccharide vaccine (Pneumovax) and estimated 12 serotype antibody responses which are also present as antigens in the vaccine. These data were quantified in two ways as either

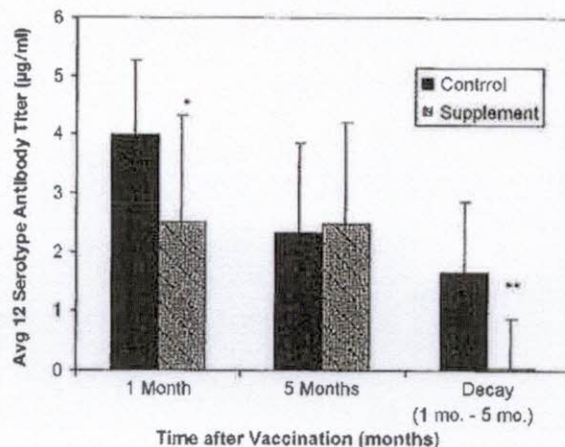


Fig. 3. The net increase in the average of 12 pneumococcal antibody titers (serotypes 1, 3, 4, 6, 8, 9N, 12, 14, 19F, 23F, 51 and 56) estimated in (g/ml) 1 month and 5 months after pneumococcal vaccination in 12 controls and 10 C-Med-100® supplemented subjects. Natural immunization at day 30 before vaccination has been subtracted from the 1 and 5 month values to yield data on the net increase and decay of pneumococcal antibody responses to pneumococcal vaccination. The actual concentrations of the the various serotype antibody titers were measured and analyzed in (g/ml). * $p < 0.05$, ** $p < 0.01$ by two-tailed t-tests for comparing C-Med-100® to con-

(i) the % of the 12 serotype antibody responses which were above the company recommended cut off values for immunization, or (ii) the average of the actual 12 serotype antibody levels expressed as $\mu\text{g/ml}$.

The control and C-Med-100[®] supplemented groups were quantified for natural immunization to pneumococcal infection on day 30, and then measured again at day 60 and day 180 (or 1 and 5 months) post vaccination. Unfortunately the two groups were not randomized for natural immunization to pneumococcal infection, and the controls had 44.8 (23.5% natural immunization compared to the C-Med-100[®] group which had only 31.3 (33.9% ($p < 0.05$)). This fact introduces a serious bias into the study, since the control group could be expected to have a greater immunological boost to responding to pneumococcal vaccine, because of the increased priming from natural immunization.

Despite the potential bias nature of the pneumococcal antibody titer data in favor of pneumococcal vaccination response in the control group, we have analyzed the 1 and 5 month post-vaccination pneumococcal 12 serotype titers for comparison of controls to the C-Med-100[®] group. The data reported in Figure 2–3 have been adjusted to minimize the bias due to differences in natural immunization by subtracting the day 30 values from the day 60 and day 180 values. The 1 month after vaccination level for both control and C-Med-100[®] treated groups demonstrated that immunization was achieved; i.e. the reported pneumococcal immunization levels of 60–80% (Fine et al. 1994) for the 12 serotype antibody titers were comparable to the observed levels in both groups (calculated from natural immunization + data in Figure 2 as 72.8 and 52.3%, respectively). As expected, the 1 month response to pneumococcal vaccination was significantly greater in the controls than the C-Med-100[®] treated group, presumably contributed to by the bias in primed pneumococcal immunological response. However, when decay in pneumococcal antibody response was estimated by again quantifying the pneumococcal antibody titers at 5 months, there was no loss of pneumococcal immunity in the C-Med-100[®] group whereas the controls experienced a highly significant decrease in pneumococcal immunity. This was true whether % immunization level against the 12 serotypes was used for the analyses, or the actual levels of the 12 serotype antibody responses measured in $\mu\text{g/ml}$ (compare Figures 2 and 3).

Discussion

The dose of C-Med-100[®] previously administered to humans has been 350 mg total daily dose for 1.5 months (Sheng et al. 2000a). The dose in this study was increased to 350 mg \times 2 daily C-Med-100[®] for 2 months.

This was justified because rat studies have indicated that C-Med-100[®] is extremely safe having no toxicity at doses up to 8 gm/kg (Sheng et al. 2000a). Here we report no toxic side effects of the 350 mg \times 2 dose of C-Med-100[®] as judged from medical interviews by the attending physician, clinical chemistry, and differential blood cell analysis (Tables 2–3). It was concluded that even though efficacious effects of C-Med-100[®] have been shown at lower doses, it is quite safe to double the daily human dose to 350 mg \times 2 in an effort to achieve even better efficacy.

The mode of action of water extracts from *Uncaria tomentosa* has not been clearly defined although important biological modulation has been documented such as induction of apoptosis, inhibition of NF- κ B and TNF(production, protecting against mutation, reducing DNA damage and enhancing DNA repair (Rizzi et al. 1993; Sheng et al. 1998, 2000a, 2000b; Sandoval-Chacon et al. 1998, 2000). These processes are known to be important to regulation of human disease conditions relating to immune suppression, inflammation and cancer prevention. C-Med-100[®] has also been reported to increase peripheral blood WBC without any compromise to lymphocyte immune responsiveness (Sheng et al. 2000a, 2000b). One overall explanation for these data is that because C-Med-100[®] inhibits NF- κ B, there is inhibition of oxidative stress originating from proinflammatory cytokine production, which in turn prevents oxygen-radical inhibition of DNA damage and repair (Pero et al. 1996). This postulated effect would predict that WBC could survive in circulation longer because they have enhanced DNA repair and reduced DNA damage, resulting in an increased lifespan of WBC in circulation thus elevating the number of immune competent cells. This interpretation is strongly supported by the facts that (i) C-Med-100[®] enhances the recovery of WBC from chemotherapy-induced leukopenia (Sheng et al. 2000b) and (ii) the primary enhanced immune response to pneumonia vaccination was an increased persistence of the pneumococcal antibody titers (i.e. longer lived immunized lymphocytes would increase the effective half life of the vaccine, Fig.2–3).

Pneumococcal vaccine analyzed by meta-analysis of randomized trials is about 60–80% effective in immuno-competent middle-aged, elderly adults, and the immunological responses to the 23 serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F) vary considerably in intensity and persistence (Fine et al. 1994). Moreover, there are several well recognized high risk groups where the effectiveness of pneumococcal vaccine is much lower such as children, the elderly, HIV-seropositive patients, non-Hodgkin lymphoma, and patients having recurrent infections (Loeliger et al. 1995;

Table 3. Complete blood count, differential white blood cell count and lymphocyte subset analysis before and after 2 months C-Med-100® supplement (n = 10). Abbreviations are total white blood cells (WBC), red blood cells (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC distribution width (RDW), mean platelet volume (MPV) and platelet count (PLT), as well as differential WBC count including the absolute numbers and percentages of neutrophils (POLY), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EOS), basophils (BASO), and lymphocyte subset analyses (i.e. absolute numbers and percentages of CD3, helper, suppressor T-lymphocytes and the helper/suppressor ratio).

* by two-tail t-test (n = 10 for before and after groups).

Blood Indicators	Before Supplement	After Supplement	p-value*
Complete blood count:			
WBC ($10^3/\text{mm}^3$)	6.46 ± 1.04	5.76 ± 0.66	0.089
RBC ($10^6/\text{mm}^3$)	4.93 ± 0.28	4.94 ± 0.31	0.970
HGB (g/dl)	15.2 ± 0.63	15.3 ± 0.51	0.787
HCT (%)	45.09 ± 1.97	44.87 ± 1.95	0.805
MCV (fl)	91.5 ± 4.2	91.0 ± 4.4	0.797
MCH (pg)	30.8 ± 1.67	31.0 ± 1.63	0.810
MCHC (%)	33.7 ± 0.46	34.1 ± 0.86	0.211
RDW (%)	13.1 ± 0.52	12.9 ± 0.52	0.360
MPV (fl)	8.68 ± 0.81	8.85 ± 0.81	0.645
Platelet ($10^3/\text{mm}^3$)	204.8 ± 31.5	205.2 ± 30.3	0.977
Differential WBC Count			
Poly (mm^3)	4084 ± 1121	3243 ± 836	0.036
Poly (%)	62.5 ± 7.98	56.7 ± 4.70	0.065
Lymph (mm^3)	1549 ± 516	1791 ± 470	0.285
Lymph (%)	24.6 ± 8.6	30.7 ± 4.6	0.064
Mono (mm^3)	622 ± 226	521 ± 171	0.275
Mono (%)	9.88 ± 4.08	8.96 ± 2.28	0.542
EOS (mm^3)	174 ± 126	169 ± 124	0.930
EOS (%)	2.58 ± 1.62	2.96 ± 2.41	0.684
Baso (mm^3)	30.1 ± 14.1	34.4 ± 13.1	0.489
Baso (%)	0.48 ± 0.23	0.61 ± 0.24	0.230
Lymphocyte Subset			
CD3 (mm^3)	1137 ± 425	1244 ± 341	0.540
CD3 (%)	70.9 ± 6.0	69 ± 6.1	0.492
T-helper (mm^3)	796 ± 280	874 ± 272	0.536
T-helper (%)	50.0 ± 7.9	47.7 ± 6.5	0.484
T-suppress (mm^3)	298 ± 163	339 ± 128	0.545
T-suppress (%)	18.7 ± 6.4	18.3 ± 6.6	0.892
Helper/suppressor Ratio	3.15 ± 1.7	2.8 ± 1.2	0.632

Petrasch et al. 1997; Raby et al. 1996; Shelly et al. 1997; Sorensen et al. 1998). In addition, nutritional factors also influence successful pneumococcal vaccination such as vitamin B12 and vitamin E (Fata et al. 1996; Meydani et al. 1997). Hence, there is a great need to enhance the effectiveness of pneumococcal vaccine in order to improve the morbidity and mortality attributed to pneumococcal infections.

One key factor to increasing the effectiveness of pneumococcal vaccination is to eliminate the resistance of high risk groups to vaccination by increasing the persistence of pneumococcal antibody responses over time (Musher et al. 1993). C-Med-100® has been identified by this study as a nutritional factor where

significant enhancement in the persistence of protection from pneumococcal vaccination could be expected (Fig. 2-3).

Finally, it should be pointed out that the immune enhancing properties of C-Med-100® have been attributed to a new general class of active ingredients called CAE. These are compounds having at least one carboxy ester in their structure. In fact there have been CAE identified in Cat's Claw as esters of quinovic acid and their glycosides (Aquino et al. 1989, Cerri et al. 1988). Quinovic acid derivatives are also known to possess antiinflammatory properties, and as such may have contributed to the efficacious effects observed for C-Med-100®.

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References

- Aquino, R, De Simone, F, Pizza, C, Conti, C, Stain, ML. Plant metabolites. Structure and in vitro antiviral activity of quinovic acid glycosides from *Uncaria tomentosa* and *Guettarda platypoda*. *J. Nat. Prod.* 52(4): 679-85, 1989.
- Aquino R, De Simone F, Vincieri, C, Pizza C, Gacs-Baitz, E. New polyhydroxylated triterpenes from *Uncaria tomentosa*. *J. Nat. Prod.* 53(3): 559-564, 1990.
- Aquino R, De Feo V, De Simone F, Pizza C, Cirino G. Plant metabolites. New compounds and anti-inflammatory activity of *Uncaria tomentosa*. *J. Nat. Prod.* 54(2): 453-9, 1991.
- Butler, JC, Shapiro, ED, Carlone, GM. Pneumococcal vaccines: History, current status, and future directions. *Am. J. Med.* 107(1A): 69S-76S, 1999.
- Cerri R, Aquino R, De Simone F, Pizza C. New quinovic acid glycosides from *Uncaria tomentosa*. *J. Nat. Prod.* 51: 257-261, 1988.
- Fata, FT, Herzlich, BC, Schiffman, Ast, AL. Impaired antibody responses to pneumococcal polysaccharide in elderly patients with low serum vitamin B12 levels. *Ann. Intern. Med.* 124(3): 299-304, 1996.
- Fine, MJ, Smidl, MA, Carson, CA, Meffe, F, Sankey, SS, Weissfeld, LA, Detsky, AS, Kapoor, WN. Efficacy of pneumococcal vaccination in adults. A met-analysis of randomized controlled trials. *Arch. Intern. Med.* 154: 1666-2677, 1994.
- Jones, K. Cat's Claw: Healing vine of Peru, Slyvan Press, 152 pages, Seattle, Washington, 1995
- Keplinger, K, Laus, G, Wurm, M Dierich, MP, Teppner, II. *Uncaria tomentosa* (Willd.) DC. - Ethnomedicinal use and new pharmacological, toxicological and botanical results, *J. Ethnopharmacology* 64: 23-34, 1999.
- Loeliger, AE, Rijkers, GT, Aerts, P, Been-Tiktak, A, Hoepelman, AI, van Dijk, H, Borleffs, JC. Deficient antipneumococcal polysaccharide responses in HIV-seropositive patients. *FEMS Immunol. Med. Microb.* 12(1): 33-41, 1995.
- Meydani, SN, Meydani, M, Blumberg, JB, Leka, LS, Siber, G, Loszewski, R, Thompson, C., Pedrosa, MC, Diamond, RD, Stollar, BD. Vitamin E supplementation and in vivo immune response in healthy elderly subjects. A randomized controlled trial. *JAMA* 277(17): 1380-1386, 1997.
- Musher, DM, Groover, JE, Rowland, JM, Watson, DA, Struewing, JB, Baughn, RE, Mufson, MA. Antibody to capsular polysaccharides of *Streptococcus pneumoniae*: prevalence, persistence, and response to revaccination. *Clin. Infect. Dis.* 17(1): 66-73, 1993.
- Petrascch, S, Kuhnemund, O, Reinacher, A, Uppenkamp, M, Reinert, R, Schmiegel, W, Luticken, R, Brittinger, G. Antibody responses of splenectomized patients with non-Hodgkin lymphoma to immunization with polyvalent pneumococcal vaccines. *Clin. Diagn. Lab. Immunol.* 4(6): 635-638, 1997.
- Pero, RW, Sheng, Y, Olsson, A, Bryngelsson, C, Lund-Pero; M. Hypochlorous acid/N-chloramines are naturally produced DNA repair inhibitors. *Carcinogenesis* 17: 13-18, 1996.
- Raby, R, Blaiss, M, Gross, S., Herrod, HG. Antibody response to conjugated haemophilus influenzae b and pneumococcal polysaccharide vaccines in children with recurrent infections. *J. Allergy Clin. Immunol.* 98(2): 451-459, 1996.
- Reinhard, K-H. *Uncaria tomentosa* (Willd.) D.C.: Cat's Claw, Una de gato, or Saventaro. *J. Altern. Complem. Med.* 5(2): 343-351, 1999.
- Rizzi, R, Re, F, Bianchi, A, De Feo, V, de Simone, F, Bianchi, L, Stivala, LA. Mutagenic and antimutagenic activities of *Uncaria tomentosa* and its extracts. *J. Ethnopharmacol.* 38(1): 63-77, 1993.
- Sandoval-Chacon, M, Thompson, JH, Zhang, XJ, Liu, X, Mannick, EE, Sadowicka, H, Charbonet, RM, Clark, DA, Miller MJ. Anti-inflammatory actions of Cat's Claw: the role of NF-kappa B. *Aliment. Pharmacol. Ther.* 12(12): 1279-89, 1998.
- Sandoval M, Charbonnet RM, Okuhama NN, Roberts J, Krenova Z, Trentacosti AM and Miller MJ. Cat's Claw inhibits TNFalpha production and scavenges free radicals: role in cytoprotection. *Free Radic Biol Med* 29(1): 71-78, 2000.
- Shelly, MA, Jacoby, H, Riley, GJ, Graves, BT, Pichichero, M, Treanor, JJ. Comparison of pneumococcal polysaccharide and CRM197- conjugated pneumococcal oligosaccharide vaccines in young and elderly adults. *Infect. Immun.* 65(1): 242-247, 1997.
- Sheng, Y, Pero, RW, Amiri, A, Bryngelsson, C. Induction of apoptosis and inhibition of proliferation in human tumor cells treated with extracts of *Uncaria tomentosa*. *Anti-cancer Research* 18(5): 3363-3368, 1998.
- Sheng, Y, Pero, RW, Bryngelsson, C. Enhanced DNA repair and reduced toxicity of C-MED-100®, a novel aqueous extract from *Uncaria tomentosa*. *J. Ethnopharmacol.* 69(2): 115-126, 2000a.
- Sheng, Y, Pero, RW, Wagner, H. Treatment of chemotherapy-induced leukopenia in the rat model with aqueous extract from *Uncaria tomentosa*. *Phytomedicine* 7(2): 137-143, 2000b.
- Sorensen, RU, Leiva, LE, Javier, FC, Sacerdote, DM, Bradford, N, Butler, B, Giangrosso, PA, Moore, C. Influence of age on the response to streptococcus pneumoniae vaccine in patients with recurrent infections and normal immunoglobulin concentrations. *J. Allergy Clin. Immunol.* 102(2): 215-221, 1998.

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