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The Use of a Whey Protein Concentrate in the Treatment of Patients with Metastatic Carcinoma: A Phase I-II Clinical Study

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Abstract. *Glutathione (GSH) concentration is high in most tumour cells and this may be an important factor in resistance to chemotherapy. Previous in-vitro and animal experiments have shown a differential response of tumour versus normal cells to various cysteine delivery systems. More specifically, an in-vitro assay showed that at concentrations that induce GSH synthesis in normal human cells, a specially prepared whey protein concentrate, Immunocal™, caused GSH depletion and inhibition of proliferation in human breast cancer cells. On the basis of this information five patients with metastatic carcinoma of the breast, one of the pancreas and one of the liver were fed 30 grams of this whey protein concentrate daily for six months. In six patients the blood lymphocyte GSH levels were substantially above normal at the outset, reflecting high tumour GSH levels. Two patients (#1,#3) exhibited signs of tumour regression, normalization of haemoglobin and peripheral lymphocyte counts and a sustained drop of lymphocyte GSH levels towards normal. Two patients (#2,#7) showed stabilisation of the tumour, increased haemoglobin levels. In three patients (#4,#5,#6,) the disease progressed with a trend toward higher lymphocyte GSH levels. These results indicate that whey protein concentrate might deplete tumour cells of GSH and render them more vulnerable to chemotherapy.*

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Feeding a specially formulated whey protein concentrate, Immunocal™, appears to exert an inhibitory effect not only on the initiation of cancer but also on its progression (1). Indeed, *in vitro* studies have confirmed a direct inhibitory effect of whey protein concentrate on human cancer cell replication (2,3). In other human cancer cell studies the inhibitory effect was found to be related to the serum albumin component of whey (4) and, most recently to alpha-lactalbumin, another major component of whey protein concentrate (5). Feeding lactoferrin to mice inhibited the growth of solid tumours and, in addition, reduced lung colonisation by melanomas (6). Serum albumin was found to exhibit, unlike other proteins, a strong antimutagenic effect in an *in vitro* assay using hamster cells (7).

A possible explanation for these newly discovered properties of dietary whey protein may be found in recent findings on the role of glutathione (GSH) in tumour biology. GSH, (L-γ-glutamyl-L-cysteinyl-glycine) is the principal cellular antioxidant and detoxifying agent (8).

The whey protein concentrate used in our studies (Immunocal™) contains substrates for GSH production as it was found in normal animals to enhance cellular GSH synthesis (9). We have succeeded in concentrating in Immunocal™ serum albumin, alpha-lactalbumin and lactoferrin containing a significant number of cystine residues (9).

Free cysteine cannot be used to deliver this necessary

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substrate, as it is toxic, and is rapidly metabolised. In the peptide form, however, the disulphide cystine of undenatured Immunocal™ released during gastrointestinal digestion can be effectively transferred to target cells where it is readily cleaved to cysteine for GSH synthesis.

Unlike commercial whey concentrates, Immunocal™ is produced using specially designed techniques aimed at preserving the bioactive cystine rich components in their undenatured form. Recent experimental evidence revealed an intriguing differential response of tumour vs. normal cells to GSH synthesis promoting compounds.

Cellular GSH levels have been found to be several times higher in human cancer cells than in the adjacent normal cells (10). This finding is presumably related to their proliferative activity. In fact, cancer is the only condition in which an elevation of a tightly regulated system such as GSH has been reported. However, when a cysteine and GSH-promoting compound such as oxothiazolidine-4-carboxylate (OTZ) was added to cultured human lung cancer cells exhibiting very high levels of GSH at the outset, no intracellular GSH increase was noted, whereas GSH increased substantially in normal cells (10.) This differential response is even more pronounced *in vivo*. In tumour bearing rats OTZ treatment was found to actually deplete GSH in the tumours (11).

More specifically, an *in vitro* assay showed that at concentrations that induce GSH synthesis in normal human cells, Immunocal™ caused GSH depletion and inhibition of proliferation of human breast cancer cells (submitted for publication by Baruchel S. 1995).

On the basis of these experimental data a Phase I-II clinical trial was undertaken to test the effect of Immunocal™ in five patients with metastatic breast cancer, one with pancreatic cancer and another with metastatic adenocarcinoma to the liver of unknown primary.

Materials and Methods

In testing a new approach to cancer treatment, patient selection and accrual are difficult issues. The selected patients must have a reasonable life expectancy, and must have measurable disease. They must not be denied available standard therapy and must understand the rationale of the proposed treatment in order to be able to sign an informed consent. Thus the number of subjects in this pilot study was small and, for ethical reasons, a control group was not included.

The inclusion criteria for our patients were as follows.

1. Histology was available for review
2. Measurable disease was present in soft tissues, lymph nodes, lungs, liver or bones.
3. No chemotherapy, radiotherapy or hormonal therapy had been given within three months of starting therapy with Immunocal™.
4. Informed consent was obtained.

Each patient had a full clinical assessment at the time of entry into the study and at three and six months after initiation of therapy. Relevant imaging studies were done at the same time intervals. They were also seen bi-weekly for the assessment of their general condition, weight, tolerance to and compliance with the Immunocal™ therapy. Complete blood counts, serum albumin, total protein and standard liver function tests (bilirubin, SGOT, alkaline phosphatase) were obtained. An aliquot of blood was drawn for lymphocyte isolation and measurement of intracellular glutathione.

Intracellular GSH in lymphocytes was measured by enzymatic assay using niacinamide diphosphate, dithio nitrobenzene and glutathione reductase. Briefly, the isolated lymphocytes were lysed with ice cold water and 6% sulfosalicylic acid, centrifuged at 600g for ten minutes and the supernatant collected and processed. Spectrophotometric analysis was performed for 2 minutes at 412^{nm} and the results expressed in nmol of GSH/10^7 cells.

Immunocal™ was administered as a daily dose of 30 gram of powder dissolved in a liquid of the patient's choice. The patient's diet was assessed to rule out excessive protein intake which has been shown to potentially enhance tumour growth. Immunocal™, a bovine whey protein concentrate, was prepared for us by Immunotech Research Corporation. It had the following characteristics: pure protein content 77% with a solubility index of 99% at pH 4.6. Protein composition as percent of total whey protein measured by polyacrylamide gel electrophoresis was: beta-lactoglobulin 56.3%, alpha-lactalbumin 22.8%, serum albumin 11.1%, lactoferrin 0.7% and immunoglobulin 9.2%. Standard bacteriological tests were negative. A brief description of the individual patients' history, clinical course, radiological and laboratory results follow.

Only the parameters showing significant and relevant changes are reported in the individual tables. The pattern of change in lymphocyte glutathione concentrations is shown separately. The haemoglobin values are reported in g/l, white blood and lymphocyte counts as bil/l and the protein and albumin levels as g/l.

Results

Patient (1)

This 37 year old woman received standard therapy for a Stage II carcinoma of the breast in 1990. Three years later she was investigated for increasing fatigue and shortness of breath. Chest X-rays showed a large right pleural effusion with a pathological fracture of the right eleventh rib. Cytology of the pleural fluid and a pleural biopsy were positive for metastatic breast cancer. In June 1994, when there was evidence of mild disease progression she was started on Immunocal™.

After starting Immunocal™ she reported an increased sense of well being and her weight remained stable at 61 kg. After three months of therapy, her chest X-ray demonstrated significant clearing of the effusion, and evidence of healing of the pathological fracture of her eleventh rib. A corresponding bone scan showed the lesion to be less intense. After six months her clinical condition was stable, her chest X-ray essentially unchanged. A repeat bone scan suggested slight distal deterioration in her bone metastases.

The relevant laboratory data are shown on Table I and Figure 1.

The data prove to be very interesting. Her pre-treatment haemoglobin was low and her platelet count abnormally high; abnormalities which are both seen with malignancy. As her treatment continued these values both came into normal range. The overall leukocyte count did not change but the lymphocyte count rose. Serum protein and albumin values remained stable, so it appears that the amelioration in the other parameters cannot be explained by nutritional factors alone. The range of normal values for human lymphocyte

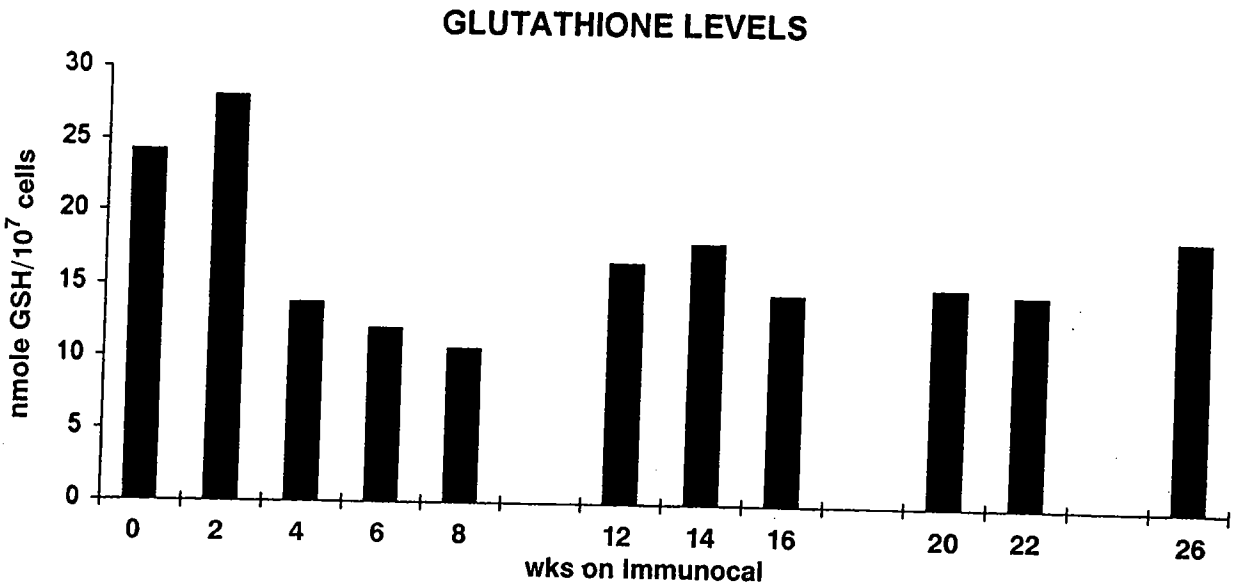

Figure 1. *Changes in blood lymphocyte glutathione levels during treatment.*

Table I. *Changes in blood cell counts and blood chemistry during treatment.*

	0 wks	4 wks	8 wks	12 wk	16 wk	20 wk	24 wk	28 wk	32 wk	36 wk
HB	108	113	118	126	131	130	123	125	125	
WBC	8.6	6.4	6.4	6.9	8.0	6.6	6.4			
PLT	564	424	342	342	339	339	326	315	300	
LYM	1.1	1.1	1.2	1.4	1.5	1.5	1.6	1.4	1.5	
PROT		76	75	75		77	85	75	75	77
ALB		41	40	40		39	41	39	37	38

glutathione levels is 13±2 nmol/10^7cells. In agreement with her clinical improvement and the improvements in her basic blood data, this patient's GSH levels fell from abnormally high values at the start of the therapy to be within normal range.

Patient (2)

This 49 year old woman in 1986 underwent a left modified radical mastectomy for a Stage II invasive ductal carcinoma. She received 12 cycles of adjuvant chemotherapy with cyclophosfamide, methotrexate, and 5-fluorouracil (CMF). In 1990 she developed a persistent cough and a chest X-ray revealed two nodular densities in the left lung compatible with metastatic breast cancer. Her chest X-rays showed progressive disease but the patient refused to consider standard therapy.

In June 1994 she was started on Immunocal™, which she tolerated well. Her first set of chest X-rays taken three months after therapy showed an approximate 10% increase in the size of the lesions.

The subsequent X-ray in January 1995 showed no further interval change with apparent arrest of the progression of the pulmonary metastases. The patient remained completely asymptomatic with a stable weight at 49kg.

In this patient the haemoglobin showed a minimal rise into normal range, while the leukocyte, platelet and lymphocyte counts, which were normal at the initiation of therapy, remained so. The serum proteins remained singularly unchanged during the study.

Although this patient appeared to have a good clinical response, the GSH levels did not consistently fall. The levels however even at their highest levels were almost within normal range, so a significant drop could not really be expected.

Patient (3)

This 64 year old woman had an invasive ductal carcinoma

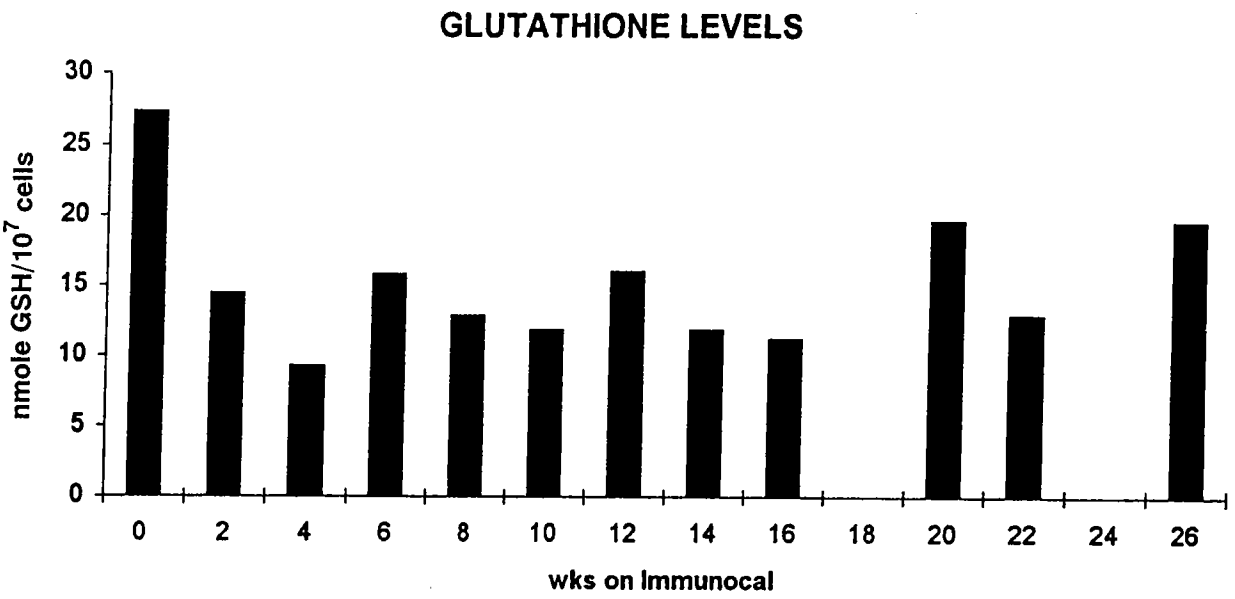

Figure 2. *Changes in blood lymphocyte glutathione levels during treatment.*

Table II. *Changes in blood cell counts and blood chemistry during treatment.*

	0 wks	4 wks	8 wks	12 wks	16 wks	20 wks	24 wks	28 wks	32 wks
HB	116	125	125	123	122	118	121	119	124
WBC	5.3	6.5	5.3	4.4	5.5	5.4	5.6	5.8	8.3
PLT	207	228	215	221	206	224	219	223	223
LYMP	1.6	2.4	1.7	1.4	2.0	1.9	1.8	2.1	1.9
PROT		65	67			66	67	68	69
ALB		43	40	40		37	39	38	39

resected from her breast in November 1989. The patient was extremely thin and there were no palpable axillary or supraclavicular lymphadenopathy. At her request she did not undergo a staging axillary lymphadenectomy. In June 1994, she presented with a palpable right supraclavicular lymph node of 2cm in diameter which upon fine needle aspiration cytology was found to contain metastatic carcinoma. A routine metastatic assessment otherwise was negative.

She was started on Immunocal™ at the same time and after six months of therapy the metastatic lymph node decreased in size and measures one cm in diameter. A repeat fine needle aspiration remains positive for viable adenocarcinoma cells. Clinically she remains quite active and healthy. Her weight remains unchanged at 43 kg. during the course of the study. She tolerates the Immunocal™ with no untoward side-effects.

The relevant blood data findings are shown in Table II. Glutathione levels are shown in Figure 2.

Patient 3 was a clinical responder. Her haemoglobin reflects this, rising into normal range with treatment. The platelet and lymphocyte counts started off normal and remained so. Note that the improvement cannot be explained by the nutritional effects of Immunocal™ as the serum protein levels did not reflect significant nutritional improvement.

The GSH levels fell from a significantly elevated level to within normal range, a trend that follows her clinical course as well as the changes in her haemoglobin.

Patient (4)

This 48 year old woman initially presented in September 1990 with a carcinoma of the left breast. A left modified radical mastectomy was performed for a Stage II carcinoma. She received adjuvant CMF chemotherapy and at her request, Tamoxifen.

In February 1994, she experienced fatigue and on investigation was found to have widespread metastatic

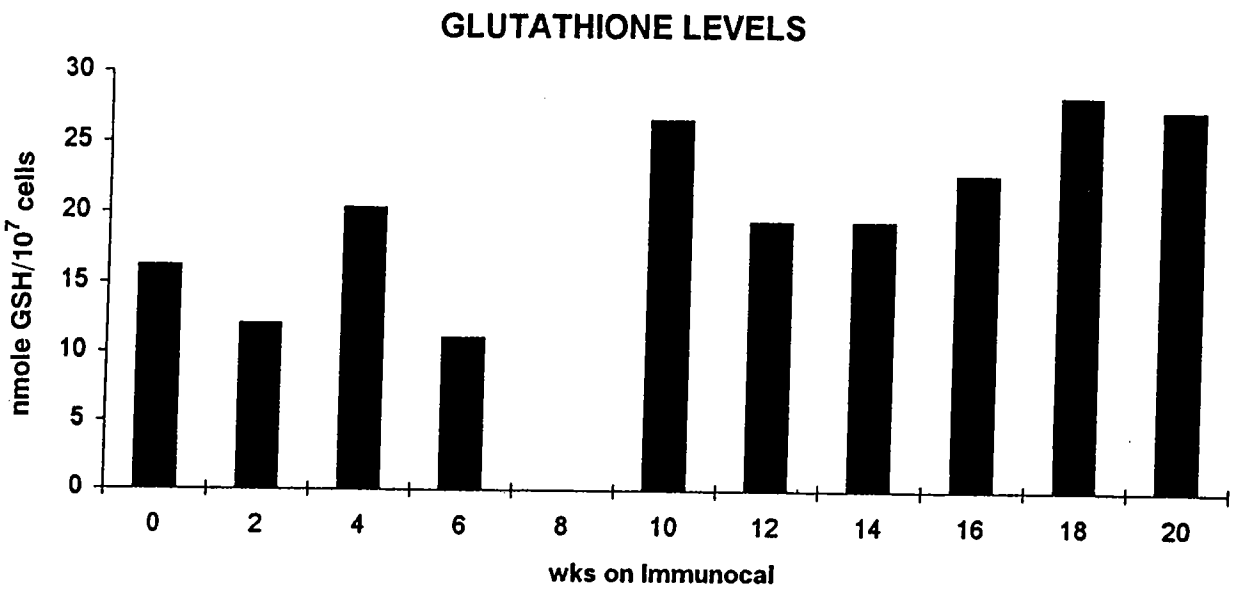

Figure 3. *Changes in blood lymphocyte glutathione levels during treatment.*

Table III. *Changes in blood cell counts and blood chemistry during treatment.*

	0 wks	4 wks	8 wks	12 wks	16 wks	20 wks	24 wks	28 wks
HB	125	122	123	122	122	117	125	122
WBC	8.0	8.2	8.3	7.2	7.9	6.1	5.6	6.8
PLT	388	330	324	280	295	226	232	326
LYMPH	2.5	1.5	1.8	1.3	1.3	1.4	1.3	1.3
PROT	56	65	70	72		69	69	66
ALB	30	34	40	39		36	35	33

disease. She was started on Immunocal™ and initially, for the first eight to twelve weeks she had an excellent clinical response with a remarkable feeling of well-being. She was able to take a physically demanding vacation. After this initial period of improvement her condition gradually deteriorated with the development of diffuse musculoskeletal pain. Her initial GSH was high, then it fell with therapy but thereafter followed a rising trend.

Patient (5)

This 73 year old woman presented in June 1994 with obstructive jaundice secondary to a carcinoma in the head of the pancreas. At laparotomy it was found to be unresectable and a palliative choledochojejunostomy and gastrojejunostomy were performed. She had an uneventful post operative course and, at her request she was started on Immunocal™ in July 1994.

The CT scan of the abdomen at three months of treatment showed stabilisation of her disease. At six months, she was feeling slightly tired, having just recovered from a flu-like illness, but her CT scan showed evidence of progressive disease with an increased tumour size, and the development of mild ascites.

After an initial response with an increased sense of well being, clinically she started to deteriorate and this deterioration was associated with a steady increase in her lymphocyte GSH levels and a progressive fall in peripheral blood lymphocyte count, as shown in Table III and Figure 3.

Patient (6)

This 72 year old woman in 1985 had a modified radical mastectomy for a Stage I invasive ductal cancer. In 1989 she developed metastatic bone disease and received radiotherapy as well as chemotherapy with CMF for six cycles. When her disease progressed she was offered only supportive care and pain relief as she had refused second line chemotherapy.

In July 1994 she was offered Immunocal™. Initially, she did very well with increased strength and energy, becoming noticeably more mobile. This period lasted about two and a half months after which she became progressively more fatigued and inactive. Her imaging studies showed at that time, progressive disease.

The GSH levels were elevated at the start of therapy, and there was a brief decline to normal range, but as with her overall status, this did not remain so and they rose to even higher than pre-treatment values as her disease progressed. Her blood haemoglobin and lymphocyte count progressively declined.

Patient (7)

This 76 year old woman presented with abdominal pain. On investigation she was found to have biopsy proven metastatic adenocarcinoma of the liver. A primary site was never found. As there were no specific treatment options open to this patient she was started on Immunocal™ in August 1994. Her clinical course remained stable until January 1995 when she developed increased nausea and vomiting. She was admitted to hospital where her medications were readjusted and she once again is thriving clinically and has almost regained her usual weight of 74 kg, after having lost 4kg. Her blood profile has been stable and her abdominal CT scan has shown minimal progression of her liver lesion. Her GSH levels show an initial fall with a slow subsequent rise probably heralding an impending clinical progression of her disease. Blood haemoglobin and lymphocyte count remained stable within normal range.

Discussion

An interesting phenomenon was observed in that every patient experienced a period of improved sense of well-being, which while difficult to quantify, was appreciable in all patients. In some, it led to a perceived improvement in the quality of life for at least a short duration, and some patients were able to perform activities they could not previously. The number in the study are too small to draw firm conclusions from this, especially without matched controls, but the results are encouraging.

Most of the patients can be characterised as "responders", or "non-responders" based on the correlation between their clinical course and their blood GSH levels. Six patients started with substantially elevated values compared to known normal values as well as a simultaneously performed normal control. Those who showed only a brief response had an initial drop in their lymphocyte GSH levels which corresponded to their clinical picture, however, as their disease progressed, the GSH levels began to rise, once again (patients 4-6). Those who had a favourable, and more protracted clinical response to Immunocal™, had noticeable and sustained drops in their GSH levels (patients 1,3). In some patients, positive clinical results were associated with increased haemoglobin (patients 1-3) and peripheral blood lymphocytes (patients 1,3) and normalization of platelet counts (patients 1,5), all of which are indirect measures of disease regression.

A major problem in the use of chemotherapeutic agents in cancer treatment is the protection offered by the defence mechanisms of the tumour cells. An important element of protection is represented by GSH which is an effective detoxification agent, relatively abundant in tumour cells. A line of human ovarian tumour cells with known drug resistance was examined and found to contain elevated levels of GSH(12). Others have shown that GSH depletion of tumour cells can render them sensitive to anthracycline, alkylators, and radiation. This depletion can be accomplished experimentally with Butathionine Sulfoximine (BSO), that inhibits GSH synthesis, and therefore reduces intracellular GSH levels(13). Treating melphalan-resistant cells with this agent, thus lowering tumour GSH levels, results in re-sensitisation to this chemotherapeutic agent. Unfortunately, BSO also depletes concomitantly the host's normal cells of GSH, which they require for normal reparative processes, and also causes neuro- and nephrotoxicity. Immunocal™ favourably influences the GSH synthesis in normal cells(9). Hence, this nutritional supplement while exerting an inhibitory effect per se on cancer cell replication, could also be viewed as an adjunct to chemotherapy. Lower GSH levels could in fact, render cancer cells more vulnerable to chemotherapeutic agents. The validity of this assumption is substantiated by the observation that cancer patients are more likely to respond to chemotherapy if their erythrocyte GSH, and by inference tumour GSH, concentrations are low(14).

Given the technical and ethical difficulties in monitoring tumour tissue GSH during cancer treatment, peripheral blood lymphocyte GSH levels are taken, in our study, as a reflection of those in tumour cells. This assumption is substantiated by a previous study of a large series of cancer patients in whom erythrocyte GSH concentration was found to reflect tumour cell GSH on the basis of differential tumour responses to chemotherapy(14).

In view of the advantages of reduced intracellular levels of GSH in tumour cells and increased levels in the host, it seems noteworthy to have found in the patients who performed well on Immunocal™ (#1,#3), the highest levels of initial lymphocyte GSH. These values fell into normal range soon after Immunocal™ was initiated and remained so through the six months of treatment. In patients exhibiting disease progression the GSH levels tended to rise. It is felt that the high lymphocyte levels of GSH occur as a result of a leaching effect from the tumour to the lymphocytes. These lymphocyte values are therefore felt to be an indirect measure of tumour levels, as are the erythrocytes in the previously quoted study(14).

Conclusions

This preliminary study indicates that this newly discovered property of whey proteins may be a promising adjunct in the nutritional management of cancer patients about to undergo chemotherapy. Selective depletion of tumour GSH may in fact render the malignant cells more vulnerable to the action of chemotherapeutic agents.

Acknowledgements

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