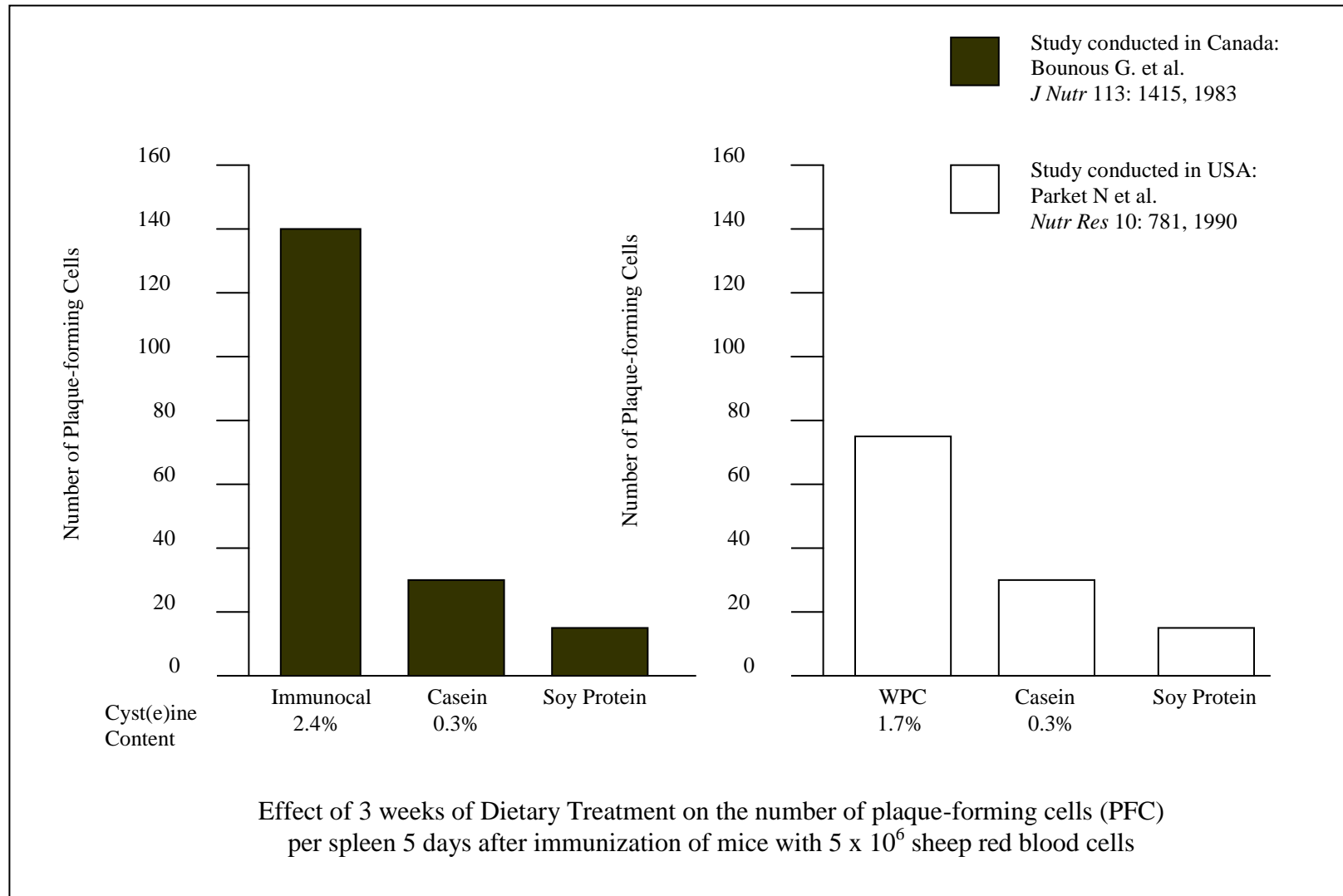

**Modulation of Cellular Glutathione
by Immunocal™**

A Milk Serum Protein Isolate:

APPLICATION IN VIRAL INFECTIONS

Gustavo Bounous, MD

Immunocal: a pharmaceutical grade undenatured whey protein concentrate (WPC)



The immunoenhancing effect is dependent upon a critical concentration of the thermolabile cystine-rich proteins

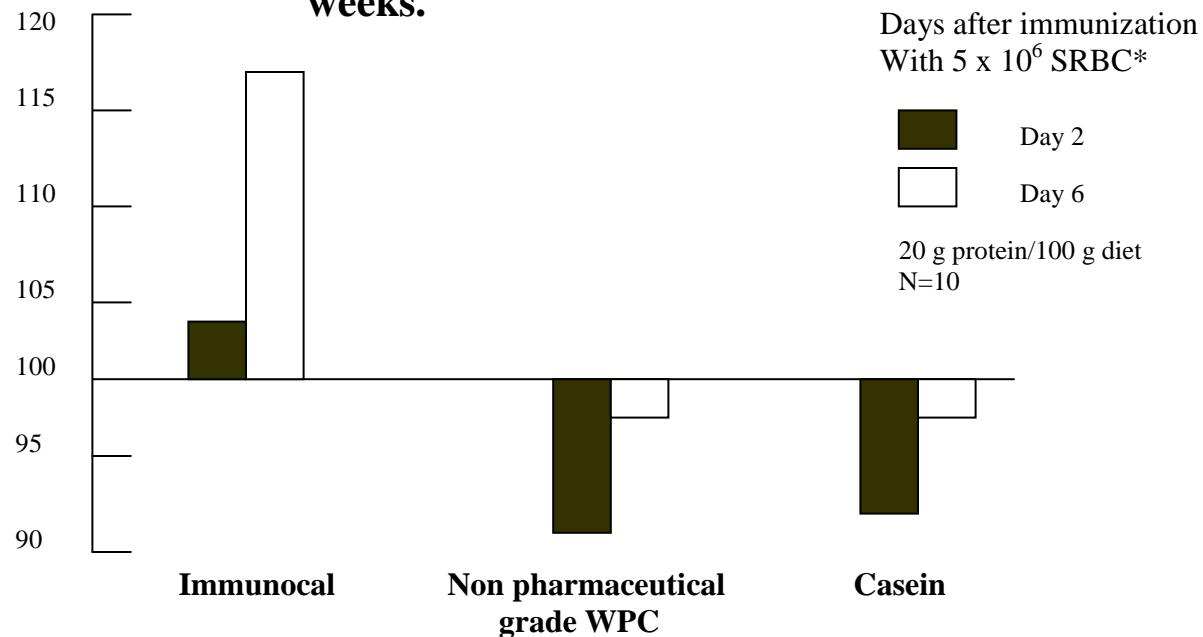
What is the role of cysteine/cystine in the immune response?

It has been demonstrated that the ability of lymphocytes to offset oxidative damage during their oxygen requiring clonal expansion, and following that expansion in the production of antibodies, is measured by determining the capacity of these cells to regenerate intracellular stores of the antioxidant [glutathione](#), thus allowing them to respond more fully to the antigenic stimulus (5,6).

*Cysteine is the crucial component and limiting factor
In the synthesis of glutathione (7)*

Optimization of the immune response in animals fed [Immunocal™](#), a pharmaceutical grade undenatured whey protein concentrate (WPC), is attributed (Bounous G., Gold P., *Clin Inves Med* 14-296-1991) to a greater production of [glutathione](#) (GSH) in the lymphocytes through dietary provision of supplementary doses of the GSH precursor cystine.

Lymphocyte GSH as Percentage of Values in Unimmunized Mice Fed the Corresponding Diet (Immunocal, a Commercial WPC or Casein) for three weeks.



*Sheep red Blood cells

This table is derived from *Clin Invest Med Vol 14 1991*

Physical-chemical characteristics and biological activity of different types of whey protein concentrate

	<u>Undenatured Conformation</u>	PFC¹ x 10 ⁻³	<u>Effect of 3 Weeks dietary treatment</u>	
	Solubility Index (pH 4.6)		Glutathione (μmol/g)	
			Liver	Heart
Immunocal	99.5%	148 ± 16	7.95 ± 0.40	1.15 ± 0.7
Product 1	97.0%	65 ± 14	6.64 ± 0.41	1.0 ± 0.7
Product 2	97.1%	66 ± 17	6.04 ± 0.36	-
Product 3	96.0%	44 ± 15	6.70 ± 0.20	1.01 ± 0.5
Product 4	95.0%	67 ± 16	-	-
Product 5	98.0%	31 ± 8	-	-
Product 6	90.1%	65 ± 20	-	-
Casein	-	35 ± 9	-	1.0 ± 0.8

Values are expressed as mean ± SD;

¹ Number of plaque-forming cells/spleen (antibodies) 5 days following immunization with 5 x 10⁶ sheep red blood cells, 20 g protein/100 g diet fed to mice during three weeks before and following immunization.

This table illustrates the immunoenhancing (antibody production) and glutathione promoting activity of different types of whey protein concentrate (WPC). The above data compare the activity of Immunocal to other commercially available WPC.

1. **Most human cells do not absorb the glutathione molecule.**
2. Glutathione is synthesized **inside** the cell from exogenous precursors, most critically, cysteine.
3. However, **oral administration of cysteine is ineffective** because:
 - a) It is toxic in the plasma;
 - b) It is rapidly catabolized before it can reach the target cells.
4. Undenatured cystine (two cysteine molecules) released [by Immunocal](#) digestion travel **safely** in the plasma and it is cleaved to the two cysteine components upon cell entry.

Synthesis of glutathione: the cell's own antioxidant.
ImmunocalTM as a cysteine delivery system”

(voir tableau CENTRAL ROLE OF CYST(e)ine6.10.98)

Milk is the only obligatory food for newborn mammals. It represents nature's choice in terms of evolution and disease prevention.

Indeed breast-feeding protects human babies against otitis and pneumonia, and children against several types of cancer. (Bounous G. et al. Evolutionary traits in human milk proteins. *Med. Hypotheses* 27; 133-140, 1988) Using advanced technology it has been possible to preserve in their native form, the specific cow's milk proteins which share with the predominant human milk proteins the same rare glutathione promoting component namely cystine.

Protein Composition of Cow's and Human Milk			
Protein	Composition (g/L)		
	Cow's Milk	Human Milk	Cystine/Molecule
Casein	26	3.2	0*
Beta-lactoglobulin	3.2	Negligible	2
Alpha-lactalbumin	1.2	2.8	4
Serum albumin	0.4	0.6	17
Lactoferrin	0.14	2.0	17
Total cystine (mol/L)	8.19×10^{-4}	13.87×10^{-4}	
Total cystine (mg/g of proteins)	6.4	38.7	

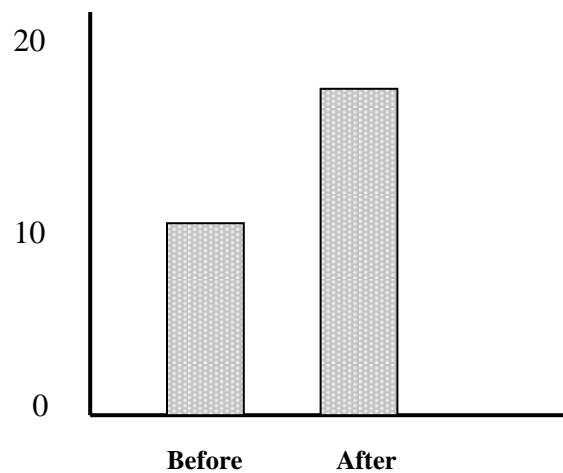
*Casein has 0 to 2 cysteine/molecule.

Adapted from: Jennes R. Inter-species comparison of milk proteins. In: *Developments in Dairy Chemistry-1*. Fox W. (Ed.). 4ASP NY : 87, 1982; and Eigel WN, Butler JE, Ernstrom CA, Farrell HM et al. Nomenclature of proteins of cow's milk. Fifth revision. *J Dairy Sci* 67 : 1599-631, 1984.

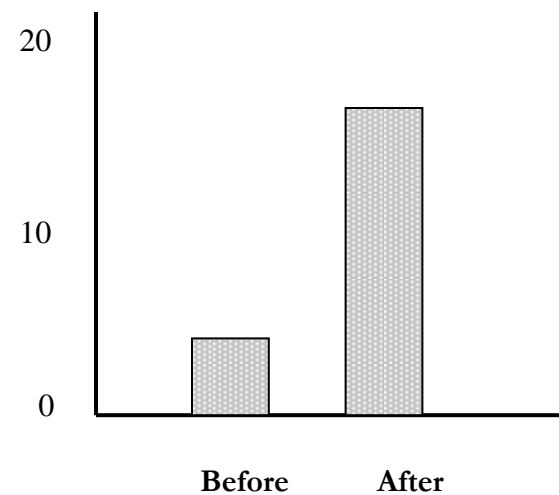
A cysteine delivery system is expected to increase cellular [Glutathione](#) (GSH) but only to a value determined by feedback inhibition of gamma-glutamylcysteine synthetase by GSH.

The best evidence that [Immunocal](#)® is indeed a cysteine delivery system comes from studies in AIDS patients.

Effect of Immunocal Treatment on lymphocyte Glutathione (nmol-10⁷ cells) in patients with AIDS and Wasting



Bounous G, Baruchel S et al
Clin Invest Med 164: 204-209, 1993



Baruchel S, Bounous G
Case Report

Normal values in healthy seronegative individuals: 17.05 ± 1.4 (mean ± SD)

Immunocal® (Milk Serum Protein Isolate) increases
lymphocyte Glutathione and body weight in
children with AIDS.

[Immunocal](#)® was administered over a six month period to 10 children with AIDS and wasting syndrome ranging in age from 8 months to 15 years. At the end of the study all patients experienced a weight gain ranging from 5.2% to 22% over their pretreatment weight. No correlation was found between the weight gain and any significant increase in the nutrient intake (expressed as the mean % of requirement) suggesting reduced catabolism rather than an anabolic effect of Immunocal®. Patients who started with low [glutathione](#) in the lymphocytes, exhibited a significant increase to but not above normal values, ranging from 7% to 305%. A positive correlation was found between an increase in weight and an increase in glutathione.

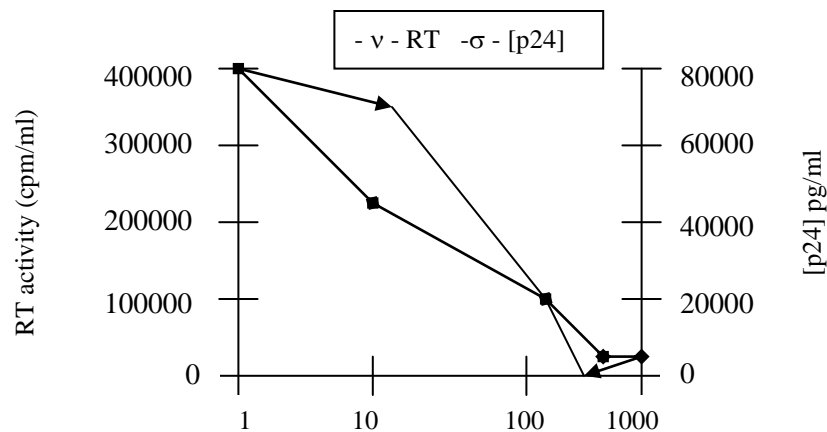
Baruchel S., Viau G., Olivier R., Bounous G., Wainberg M.A. "Nutriceutical modulation of glutathione with a humanized native milk serum protein isolate, Immunocal™: application in AIDS and cancer" in Oxidative Stress in Cancer, AIDS and Neurodegenerative Diseases. Ed. Montagnier L., Olivier R., Pasquier C. Publ. Dekker M. Inc., New York pp. 447-461, 1998.

Herzenberg demonstrated that glutathione deficiency in CD4 T cells is a key determinant of survival in HIV disease.

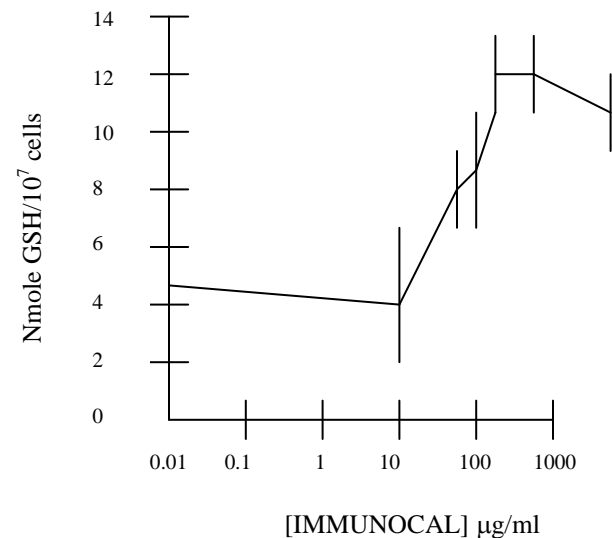
Herzenberg L.A. et al: "[Glutathione](#) deficiency is associated with impaired survival in HIV disease.

Proc. National Acad. Sci. USA 96/1967-72/1997

Immunocal® enhances glutathione synthesis by peripheral blood mononuclear cells (PBMC) and inhibits HIV replication *in vitro* as measured by reverse transcriptase (RT).



Immunocal® has been shown to inhibit HIV replication.



Incubation of PBMC for 72 h in the presence of various amounts of Immunocal®. Each point represents the mean ±SD of 3 measurements of intracellular glutathione. **p* < 0.05.

Major glutathione synthesis occurred at the same concentration as inhibition of HIV replication (100-1000 µg/ml)

Baruchel S., Viau G., Olivier R., Bounous G., Wainberg M.A. "Nutriceutical modulation of glutathione with a humanized native milk serum protein isolate, Immunocal®: application in AIDS and cancer" in *Oxidative Stress in Cancer, AIDS and Neurodegenerative Diseases*. Ed. Montagnier L., Olivier R., Pasquier C. Publ. Dekker M. Inc., New York pp. 447-461, 1998.

In collaboration with Dr. R. Olivier at the Pasteur Institute, S. Baruchel utilized the *in vitro* assay as in page 12 to compare the [glutathione](#) promoting activity of most currently available serum milk protein products.

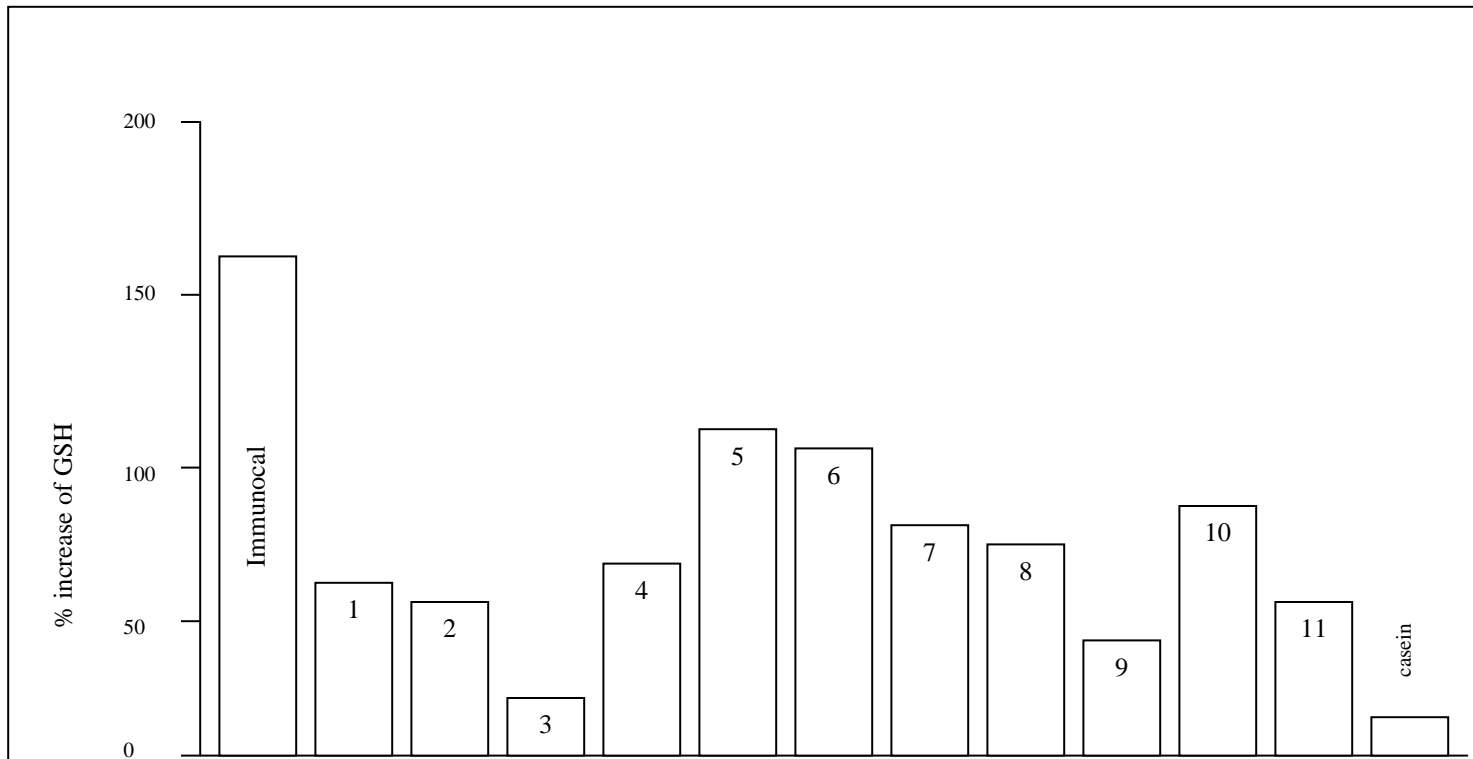


Figure 2. Incubation of PBMCs for 72h in the presence of Immunocal® and other serum milk products: Percentage increase in glutathione.

Baruchel S., Viau G., Olivier R., Bounous G., Wainberg M.A. “Nutriceutical modulation of glutathione with a humanized native milk serum protein isolate, [Immunocal](#)®: Application in AIDS and Cancer” in *Oxidative Stress in Cancer, AIDS and Neurodegenerative Diseases*. Ed. Montagnier L., Olivier R., Pasquier C. Publ. Dekker M. Inc., New York pp. 447-461, 1998.

An important antagonistic relation appears to exist between the HIV and cellular [glutathione](#) which may well determine the final outcome. This relation is not limited to HIV.

Non specific antagonistic relation between the viral illness and cellular glutathione (GSH).

In chronic hepatitis B liver cell, plasma and lymphocyte GSH levels are all low. The administration of Immunocal® for 12 weeks to these patients produced a restoration of GSH to normal values and increased IL-2 and NK activity with significant clinical improvement.

(Watanabe A, Higuchi K, Yasumura S, Shimizu Y et al. Nutritional modulation of glutathione level and cellular immunity in chronic hepatitis B and C. Hepatology 24: 1883, 1996).

In conclusion, an important antagonistic relation appears to exist between the viruses and cellular glutathione. This relation is not limited to the retrovirus of AIDS as it also involves the virus of hepatitis B.

While a clinical trial of [Immunocal™](#) in AIDS sponsored by the “Canadian HIV trial network” is under way, a substantial number of HIV positive individuals in the Montreal area are currently taking Immunocal™ and report increased energy and sense of well being. The following 4 cases are presented because they are the only members of this group in which laboratory investigations were available to us. All patients received Immunocal™, a milk serum protein isolate (90% protein).

1. The Father

This previously healthy 46 year old man was hospitalized on March 1995 for bacterial pneumonia that resolved with antibiotics. In April 1995 he was diagnosed HIV-1 positive and developed bilateral axillary adenopathy. He was infected through a heterosexual extramarital relation. On May 1995, he was treated with AZT for one week but the drug was discontinued due to strong adverse reaction. He was constantly fatigued and had to stop work. No wasting was seen.

On April 1997, oral treatment with Immunocal was initiated, 25 g a day. Two weeks later the patient felt stronger and was able to resume his work on a regular basis. The lymphadenopathy had significantly improved. However, in early August 1997, he was advised to take 5 g a day of vitamin C. A few days later he experienced extreme fatigue and for the first time in 4 months he was forced again to quit his work. He discontinued Vitamin C intake on September 10th. Two weeks later his general condition had improved and he was feeling stronger. The time course of laboratory investigation is shown on the next table:

		← Immunocal – From April 1997 →				
		← Vitamin C: Aug-Sept/97 →				
		February 97	June 97	August 97	Sept. 97	Oct. 97
Viral load	copies/mL	440,000	170,000	243,000		208,000
WBC	K/emm	1.7	1.7	1.6		2.1
Lymphocytes	K/emm	0.26	0.3	0.5		0.3
Hb	Gm/dl	10.9	11.5	11.6		
CD4+μL		-	81	40		35
CD4/CD8		0.23	-	0.12		0.18

- The Mother

This 35 year old woman was diagnose HIV-1 positive a few days after her husband was tested positive in April 1995. She presented at the time with right cervical lymphadenopathy. She tried AZT for one month but was forced to stop it because of vomiting and an intolerable headache driving her to the point of suicidal ideation. This incidentally was the reason why she refused AZT treatment for her son. No weight loss was documented.

She was started on [Immunocal](#)® 20g a day, on April 1997 and 2 weeks later noticed increased energy and strength. Her lymphadenopathy cleared and has not reappeared since. Time course of laboratory investigation in the following table:

Immunocal – From April 1997

		Feb. 97	June 97	August 97	Sept. 97	Oct. 97
Viral load	Copies/mL	19,000	9,007	-	7,071	3,009
WBC	K [^] /cmm	3.0	3.1	5.1		6.8
Hb	Gm/dl	11.4	11.8	12.4		
CD4 +/μL		410	420	400		
CD4/CD8		1.32	1.31	1.35		

This young woman showed a progressive decline in the virus load and an increase in WPC with slight Hb increment. These positive lab data are associated with a major improvement in strength and sens of well being, persisting after 6 months of therapy.

- The Son

The 2 year old boy was tested positive about the same time as the parents in April 1995. He did not appear to suffer major inconvenience other than decreased exercise tolerance and he exhibited no failure to thrive. He was started on [Immunocal](#)® (10g daily) from April/97. He showed increased energy in playing and six months later he is doing very well. Because of the magnitude of the improvement after only 2 month therapy, the October cell blood count results were verified at the “Fletcher Allen Health Care” in Burlington, VT and the Mayo Clinic Laboratory.

The time course of laboratory investigation is in the following table:

← Immunocal – From April 1997 →

		Feb. 97	June 97	Sept. 97	Oct. 97
Viral load	Copies/mL	140,000	1,104	5,000	
WBC	K/cmm	4.7	4.8		7.23 <small>Sept 95 value: 7.3 (at age 15 month)</small>
Lymphocytes	K/cmm	2.5	3.0		3.33
Neutrophils	K/cmm	1.6	1.1		3.65
CD4+/ μ L		1,025	1,200		1,450
CD4/CD8		1.78	2.35		2.2

- This 44 year old homosexual male was first tested HIV-1 positive in 1986. In the following years he felt progressively weaker, had recurrent episodes of diarrhea and frequent skin lesions such as eczema and herpes. He always refused antiretroviral drug therapy. No wasting developed. He began Immunocal treatment on February 1, 1997 at a daily dose of 10g. Two weeks after, he noticed increased energy and physical strength which has remained to date. He has not experienced diarrhea during the last nine months and the skin lesions have greatly improved.

The time course of laboratory investigation is shown in the next table:

← Immunocal Feb/97 →

		June 1993	Oct. 1993	1994	1995	1996	June 1997	Oct. 1997
Viral load	Copies/mL					140,000	17,900	30,210
CD+/ μ L		1.015	726	684	666	344	448	490
WBC	K/cmm	5.5		5.6	5.8	5.1	6.8	

CONCLUSION

Unlike specific antiretroviral drugs which may induce viral mutation hence resistance of the virus to therapy, the normalization of the lymphocyte glutathione levels and redox status through a cysteine delivery system represents a totally different approach by which the natural cellular defense system is boosted. In this manner the virus cannot build up resistance by mutation.

[The Role of Glutathione in Health and Disease In People and Animals](#)

Glutathione is continuously defending our body against attacks from disease, toxins, poisons, viruses, pollutants, radiation and oxidative stress. Low glutathione levels are linked to diseases such as Cancer, Multiple Sclerosis, AIDS, Alzheimer's, Parkinson's, Atherosclerosis, pregnancy complications, male infertility and Cataracts. A Glutathione deficiency can cause a lack of coordination, mental disorders, tremors, and difficulty maintaining balance. Without Glutathione our liver would soon become overwhelmed with the accumulation of toxins, resulting in organ failure and death. The level of Glutathione in our cells is predictive of how long we will live.

[Immucol, A Natural Source of the Glutathione GSH Building Block](#) [Immucol Science – Research and Articles](#)

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