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Immunomodulatory Properties Of Bovine Whey Proteins And Whey Protein Concentrates

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy
In
Nutritional Science and Immunology

at Massey University, Palmerston North, New Zealand

Pauline Ping Lin Low

2004



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ABSTRACT

In recent years, partly due to advances in protein separation technology, many studies have focused on the immunomodulatory activity of bovine milk and colostrum protein components. Individual milk proteins have been purified for the purpose of studying their physical properties and physiological functions. Today there is substantial evidence to indicate that the major components of bovine milk, such as whey protein and several highly purified whey protein isolates, can regulate immune function in heterologous species. Intense research has focused on identifying biologically active components within bovine milk whey, as well as characterising the mode by which mammalian immune function is modulated by these components. However, information regarding the effect of bovine whey proteins on immune responses to orally and parentally-administered antigens is currently conflicting and far from exhaustive. Consequently, this thesis sought not only to investigate the immunomodulatory ability of previously untested bovine whey products on general immunoresponses but also to investigate the ability of bovine whey proteins to modulate murine immune responses to vaccines currently in routine medical use. Initially, individual whey proteins (α -lactalbumin, β -lactoglobulin and lactoferrin) were screened for their *in vitro* effects on lymphoid cell function and phagocytic function. These *in vitro* studies found that the individual whey protein components had a positive immunomodulatory effect, providing evidence that these components have the potential to enhance immune function, and investigation into their immunomodulatory capabilities in an *in vivo* murine model was consequently undertaken. The results of the *in vivo* studies demonstrated that the dietary whey protein isolates and whey protein concentrate tested in this study could not only enhance two important indices of *ex vivo* lymphoid and non-lymphoid cell function (lymphocyte proliferation and phagocytic function) but could enhance mucosal and systemic antibody responses to orally and systemically administered human vaccines. The demonstrated benefits to the immune system of dietary whey proteins in the murine model could result in the production of immune-boosting, nutritionally and physiologically advantageous food supplements suitable for human consumption. Of particular relevancy to modern human health is the use of whey proteins as dietary adjuvants or immunopotentiators to increase immune responses to commonly administered vaccines.

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ABBREVIATIONS

α	alpha
ACE	angiotensin converting enzyme
ANOVA	analysis of variance
β	beta
B cell	a lymphocyte that matures in bone marrow and when activated differentiate into plasma cells that secrete antibodies
BCG	<i>Mycobacterium bovis</i> , strain BCG
BCM-7	β -casomorphin-7
BrdU	5-bromo-2'deoxyuridine
C	Celsius
CLA	conjugated linoleic acid
CO ₂	carbon dioxide
Con A	concanavalin A
CGP	caseinoglycopeptide
CT	cholera toxin (B subunit) vaccine
DHA	docosahexanoic acid
Dip	diphtheria toxoid vaccine
DMH	dimethylhydrazine
DNA	deoxyribonucleic acid
DTH	delayed-type hypersensitivity
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme linked immunosorbent assay
EPA	eicosapentaenoic acid
FACS	fluorescence-activated cell sorter
FCS	foetal calf serum
FITC	fluorescein isothiocyanate
Flu	influenza virus vaccine
γ	gamma
g	grams

GALT	gut-associated lymphoid tissue
GF	growth factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GSH	glutathione (L-gamma-glutamyl-L-cysteinyl-glycine)
hrs	hours
Ig	immunoglobulin
IU	international units
kDa	kilo Daltons
L	litre
LF	lactoferrin
LPS	lipopolysaccharide
μ	micro
m	milli
M	molar
max	maximum
MHC	major histocompatibility complex
min	minute
mM	milli molar
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
n	nano
NK	natural killer
NZDB	New Zealand Dairy Board (now known as Fonterra)
NZDRI	New Zealand Dairy Research Institute (now known as Fonterra Marketing and Innovation)
ω	omega
OD	optical density
OV	ovalbumin
PBL	peripheral blood leucocytes
PBS	phosphate buffered saline
<i>pers. comm.</i>	personal communication
PHA	phytohaemagglutinin
PWM	pokeweed mitogen
RNA	ribonucleic acid

RPMI 1640	Roswell Park Memorial Institute 1640
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SEM	standard error of the mean
SRBC	sheep red blood cells
<i>S. typhi</i>	<i>Salmonella typhi</i>
<i>S. typhimurium</i>	<i>Salmonella typhimurium</i>
T cell	a thymus-derived lymphocyte that differentiates after activation into a cytotoxic T cell or a helper T cell
TCID ₅₀	log ₁₀ 50% tissue culture infective dose
<i>T. cruzi</i>	<i>Trypanosoma cruzi</i>
TT	tetanus toxoid vaccine
U	units
UHT	ultra high temperature
WPC	whey protein concentrate
WPI	whey protein isolate
w/w	weight/weight
v/v	volume/volume

PUBLICATIONS

Publications arising from this work:

Enhancement Of Mucosal Antibody Responses By Dietary Whey Protein Concentrate.

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Responses In Immunized BALB/c Mice. Low PPL, Rutherford KJ, Gill HS & Cross ML (2003) *International Immunopharmacology* 3, 393-401.