The responsiveness of the bovine lactoferrin promoter to cytokines and glucocorticoids

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Dedication

This thesis is dedicated to my parents, Sandra and Gary Allen, and to my twin sister, Kim Allen, for all their support and encouragement during the past two years.

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Abstract

Lactoferrin is an iron-binding protein found in many bodily secretions and in the secondary granules of polymorphonuclear leukocytes. While there are many proposed functions for the lactoferrin protein - e.g. for iron storage, antibacterial properties, or a role in inflammation, the specific function(s) of lactoferrin have yet to be elucidated.

Evidence that lactoferrin may be involved in inflammation was observed by Harmon *et al.* (1976) where after the induction of bovine mammary infections, a significant increase in secreted lactoferrin protein was seen during the early phase of the infection. As this increase was during the period of the acute phase response, this suggested that lactoferrin, as was the case with other proteins induced during this time, may have a role in the inflammatory response. The bovine lactoferrin (bLf) promoter contains many putative binding sites for inflammatory modulators, which suggests that the increases in lactoferrin seen during inflammation may be due to activation of lactoferrin gene transcription by these specifically-induced transcription factors. Substantiation of this suggestion would provide further evidence for a specific role for lactoferrin during inflammation.

To investigate the cytokine-responsiveness of the bLf promoter, constructs corresponding to various lengths of the putative bLf promoter were linked to the luciferase reporter gene and introduced, by transient transfection, into RL95-2 human endometrial carcinoma cells. Cytokines, glucocorticoids or expression vectors for transcription factors were added to the cells, or potential 'masking' factors in the media such as phenol red or insulin were removed. The luciferase activity of the transfected cells was monitored for significant variation from the basal levels.

The addition of cytokines with or without phenol red or insulin did not cause any significant changes in bLF promoter activity. In phenol red-free media, increases in luciferase reporter gene activity were observed after the co-transfection of an expression vector for NF-IL6, the addition of dexamethasone and also the addition of dexamethasone together with the co-transfection of a glucocorticoid receptor

expression vector. These data provided evidence that lactoferrin transcription may be induced by inflammatory factors which support the suggestion that lactoferrin has a role in the inflammation process.

Abbreviations

A	adenine
AP-1	activator protein-1
APR	Acute Phase Response
ATP	adenosine triphosphate
bLf	bovine Lactoferrin
bp	base pair
BSA	bovine serum albumin
BRL	Bethesda Research Laboratories
C	cytosine
C-terminal	carboxyl terminal
cAMP	cyclic adenosine monophosphate
CAT	Chloroamphenicol acetyl transferase
cDNA	complementary DNA
CDP	CCAAT displacement protein
C/EBP	CCAAT enhancer binding protein
COUP-TF	chicken ovalbumin upstream promoter - transcription factor
CO_2	carbon dioxide
cpm	counts per minute
CRE	cyclic AMP response element
CREB	CRE binding protein
DBD	DNA-binding domain
dex	dexamethasone
DMEM	Dulbecco's modified Eagle's media
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
DNaseI	deoxyribonuclease I
DTT	dithiothreitol
E. coli	*Escherichia coli*
EDTA	ethylene diamine tetra-acetate
EGF	epidermal growth factor

EGFRE	epidermal growth factor response element
EMSA	Electrophoretic Mobility Shift Assay
ER	Estrogen Receptor
ERE	Estrogen Response Element
FCS	Fetal calf serum
G	guanine
GCG	Genetics Computing Group
GM-CSF	granulocyte-monocyte-colony stimulating factor
GR	Glucocorticoid Receptor
GRB-2	Growth factor bound-2
GRE	Glucocorticoid Response Element
GTP	Guanosine triphosphate
HCMV	human cytomegalovirus
HEPES	N-2-hydroxyethyl piperazine-N'-2-ethane sulfonic acid
hGH	human growth hormone
HIV	human immunodeficiency virus
hLf	human lactoferrin
HNF4	hepatocyte nuclear factor 4
HRP	horse radish peroxidase
HSF-1	hepatocyte stimulating factor-1
hsp90	heat shock protein 90
IFN-β2	Interferon-beta2
IFN-γ	Interferon-gamma
IFN-γR-α	Interferon-gamma receptor-alpha
IGF-I	Insulin Growth Factor-I
IGF-II	Insulin Growth Factor-II
IL-1α or β	Interleukin one-alpha or beta
IL-1R1	Interleukin-one receptor type I
IL-1RII	Interleukin-one receptor type II
IL-6	Interleukin-six
IL-6RE	Interleukin-six response element
IL-6Rα	Interleukin-six receptor α chain

IL-8	Interleukin-eight
IL-11	Interleukin-eleven
IRE	Insulin Response Element
IRS-1	Insulin Receptor Substrate-1
JAK	*Janus* kinase
JNK	jun N-terminal kinase
kb	kilobase
kDa	kiloDalton
LAK	lymphokine killer cell
LBD	Ligand Binding Domain
Lf	lactoferrin
LPS	lipopolysaccharide
MAPK	mitogen activated protein kinase
MCF-7	mammary gland carcinoma cell line
MEK	Map Kinase Kinase
mERM	mouse Estrogen Response Module
mLf	mouse lactoferrin
mRNA	messenger ribonucleic acid
mSOS	mouse Son of Sevenless
MW	molecular weight
NADH	nicotinamide adenine dinucleotide
NFIL-6	nuclear factor interleukin-six
N-terminal	amino terminal
oligo	oligonucleotide
OPNG	o-Nitrophenol β-D-Galacto-pyranoside
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PBSE	phosphate buffered slaine EDTA
PC12	rat pheochromocytoma cells
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
pGL2E	pGL2-Enhancer

pGL3B	pGL3-Basic
pGL2C	pGL2-Control
PI-3	phosphatidyl inositol-3
PMN	polymorphonuclear leukocytes
PMSF	phenyl methane sulfonyl fluoride
pSV-β-gal	pSV-β-galactosidase expression vector
RA	retinoic acid
RAR	retinoic acid receptor
RARE	retinoic acid response element
RNA	ribonucleic acid
RNase	ribonuclease
RT-PCR	reverse transcriptase polymerase chain reaction
S-S	disulphide bond
SDS	sodium dodecylsulphate
SH2	src homology domain 2
SH3	src homology domain 3
Stat	Signal Transducers and Activators of Transcription
SV40	Simian virus 40
T	thymine
T75	75 cm^2 tissue culture vented flasks
TAE	Tris acetate EDTA
TAT	tyrosine aminotransferase
TBE	Tris Boric acid EDTA
TBST	Tris buffered saline triton X-100
TBP	TATA-box binding protein
TEMED	N,N,N',N'-Tetramethylethylenediamine
TNF	Tumour Necrosis Factor
TGF-β	transforming growth factor-β
Tris	Tris-(hydromethyl) aminomethane
tsp	transcription start point
UV	ultra violet light

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