Regulation of the beta7 integrin gene in T cells- Role of the MAPK signalling pathways

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To my dear parents, for without their love and support, this would not have been possible.

Abstract

Members of the integrin superfamily of adhesion molecules are involved in cell to cell, cell to ECM, and cell to pathogen interactions, and are of fundamental importance in many biological processes. The β7 integrins α4β7 and αEβ7 have evolved to play specialized roles in gut mucosal immunity. α4β7 mediates the homing of lymphocytes to intestinal Peyer's patches, mesenteric lymph nodes, and the lamina propria by binding to the vascular addressin MAdCAM-1 (Fong *et al.*, 1997). αEβ7 binds epithelial E-cadherin retaining lymphocytes at the intraepithelial compartment of the mucosa. The expression of αEβ7 is induced on migratory lymphocytes by TGF-β secreted by gut enterocytes. The signalling mechanisms responsible for basal and TGF-β-induced expression of β7 integrins are not well understood. Previous studies identified two TGF-β1 response regions in the β7 gene promoter termed TGFBRR-1 and TGFBRR-2 encompassing nucleotides −509 to −398 and −121 to −34. Here, TGF-β1 activation of the β7 gene proximal promoter is shown to be mediated by MAPK family members. TGF-β1 stimulation of TK-1 T cells increased the steady-state mRNA levels of the β7 gene relative to α4 transcripts, and led to enhanced phosphorylation of c-Jun. TGF-β1 stimulation induced rapid increases in the binding of nuclear protein complexes to TGFBRR-1 and -2. Sp1 and Sp3 which mediate TGF-β1 signalling were shown to bind to an Sp1 cis-element encompassing nucleotide positions −67 to −60 within TGFBRR-2. The interaction of Sp1 with its cognate binding site was c-Jun dependent. In contrast, there was no evidence for involvement of the Smad proteins. ATF-2 was identified to bind to a region encompassing nucleotide positions −699 to −689 just upstream of TGFBRR-1. Sp1 and ATF-2 expression vectors co-transfected into Sp1-deficient SL-2 cells synergized to drive the activity of a β7 gene luciferase reporter. Mutation of the ATF-2-binding site modestly reduced β7 gene reporter activity.

The involvement of c-Jun in TGF-β signalling and interaction of Sp1 with the β7 gene promoter suggested that MAP kinase pathways might mediate β7 gene transcription. Specific chemical inhibitors were used to ascertain which of the three MAPK pathways namely p38, JNK, and ERK were involved. Results obtained by nuclear run-on transcription analysis which measures nascent RNA synthesis showed that both the p38 and JNK pathways regulate β7 gene expression in TK-1 cells, whereas only the p38 pathway regulates α4 gene expression. Thus, treatment of TK-1 cells with the p38 inhibitor SB203580 and the JNK inhibitor SP600125 inhibited the synthesis of β7 transcripts, whereas only SB203580 inhibited the synthesis of α4 transcripts. Conversely, sodium arsenite which induces JNK and p38 upregulated nascent synthesis of α4 and β7 RNA transcripts. SB203580 blocked the binding of nuclear factors to TGFBRR-1, and ATF-2 binding to nucleotide position –699 to –689. Similarly, SP600125 blocked the binding of Sp1 and Sp3 to TGFBRR-2, whereas unexpectedly SB203580 enhanced their binding. Furthermore, both SB203580 and SP600125 decreased cell-surface expression of the β7 subunit and SB203580 inhibited TK-1 cell adhesion to MAdCAM-1. In contrast, the MEK inhibitor PD98059 had no effect on the expression of nascent β7 RNA transcripts and cell-surface expression of the β7 subunit, suggesting that the ERK pathway is not involved in regulation of β7 gene expression in TK-1 cells. In contrast to the results obtained with TK-1 cells, SB203580, SP600125, and PD98059 each inhibited the nascent synthesis of α4, β7, and αE transcripts in peripheral blood lymphocytes.

In conclusion, this study has revealed for the first time that both the p38 and JNK pathways mediate TGF-β1-induced expression of the integrin β7 gene. Expression of the β7 gene is Sp1-dependent, and involves the synergistic cooperation of c-Jun and ATF-2. It is proposed that the p38 and JNK pathways play a role by triggering the activation and translocation of c-Jun and ATF-2 to the nucleus.

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Abbreviations

A	absorbance
Act D	actinomycin D
ATP	adenosine triphosphate
AMP^r	ampicillin resistance
ATCC	American Type Culture Collection
bp	base pair
cDNA	complementary DNA
Ci	curies
©	copyright
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
dNTP	deoxynucleoside triphosphate
dTTP	deoxythymidine triphosphate
DEPC	diethylpyrocarbonate
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
ECM	extracellular matrix
EDTA	ethylenediaminetetra-acetic acid
ERK	extracellular signal-regulated protein kinase
EtBr	ethidium bromide
FACS	fluorescence-activated cell sorter
FMLP	fmethionine-leucine-phenylalanine
FN	fibronectin
fpu	foot print unit
G3	glyceraldehyde-3-phosphate dehydrogenase
h	hour
HBS	hepes-buffered saline
HEV	high endothelial venules
HBSS	Hanks balanced salt solution
ICAM-1	intercellular adhesion molecule-1
IFN-γ	interferon-γ
IEL	intraepithelial lymphocytes
IL	interleukin
IMAGE	integrated molecular analysis of genomes and their expression
JNK	c-Jun N-terminal kinase
kb	kilobase
kDa	kilodalton

L	litre
LB	luria-bertani
LCPS	luminescent counts per second
LPAM-1	lymphocyte Peyer's patch adhesion molecule-1
MAdCAM-1	mucosal addressin cell adhesion molecule-1
μ	micro
m	milli
M	molar
MAPK	mitogen activated protein kinase
MOPS	morpholinol propane sulfonic acid
ml	millilitre(s)
μl	microlitre(s)
mM	millimolar
mAb	monoclonal antibody
mRNA	messenger RNA
min	minute(s)
NAD	nicotinamide adenine dinucleotide
NK	natural killer
ng	nanogram(s)
nm	nanometre(s)
nt	nucleotide
nM	nanomole(s)
pM	picomole(s)
P	p-value
PBS	phosphate-buffered saline
PKC	protein kinase C
PMA	phorbol 12-myristate 13-acetate
PMSF	phenylmethyl sulphonyl fluoride
RGD	argnine-glycine-asparagine
rh	recombinant human
RT	room temperature
RNA	ribonucleic acid
rRNA	ribosomal RNA
Rpm	revolutions per minute
®	registered
S	second
SDS	sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SL-2	*Drosophila* Schneider cell line
TAE	tris-acetate-EDTA
TAB1	TAK1-binding protein 1
TAB2	TAK1-binding protein 2

TAK1	TGF-β activated kinase 1
TBE	tris-borate-EDTA
TEMED	tetramethylethylenediamine
TfbI	transformation buffer I
TfbII	transformation buffer II
TGF-β	transforming growth factor-β
TNF-α	tumour necrosis factor-α
U	unit(s)
UTP	uridine triphosphate
UV	ultraviolet
V	volt(s)
v	volume
VCAM-1	vascular cell adhesion molecule-1
w	weight
WWW	world wide web