

**INTERDEPENDENCE BETWEEN ADHESION AND
PROLIFERATION:**

**THE ROLE OF THE α L/ β 2-INTEGRIN (LFA-1) IN
T CELL ANTIGEN RECEPTOR-DEPENDENT
PROLIFERATION OF PRIMARY HUMAN
T LYMPHOCYTES**

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Zusammenfassung

Das Thema der vorliegenden Arbeit ist die Rolle des α L/ β 2 Integrins "lymphocyte function-associated antigen-1" (LFA-1) in der T-Zell-Antigenrezeptor(TCR)-abhängigen Proliferation von primären humanen T-Lymphozyten. Die Stimulierung von LFA-1 erwies sich als notwendige Bedingung der TCR-stimulierten Proliferation in Abwesenheit anderer Rezeptor-Liganden Wechselwirkungen. Die LFA-1-abhängige Proliferation ist nicht alleine durch die adhäsionsbedingte Verstärkung der TCR-Stimulierung zu erklären, sondern beruht auf TCR-unabhängiger Signaltransduktion. Analog zu der Integrin-vermittelten Signaltransduktion in adhären Zellen beruht auch die LFA-1-vermittelte auf der zytoskelett-abhängigen Induktion einer abgeflachten Zellform ("spreading") und nicht nur auf der Rezeptoraggregation. LFA-1 beeinflusst in zwei unterschiedlichen Phasen die Zellzyklusprogression: In der G0-Phase bewirken die TCR-Stimulierung und die LFA-1-bedingte Reorganisation des Zytoskeletts eine synergistische Aktivierung von Tyrosinphosphorylierungen, die zu einer verstärkten Aktivierung der "mitogen-activated protein" (Map) kinasen und veränderter Genexpression führen. Diese resultiert dann in Zellzykluseintritt und der Fähigkeit, auf die Präsenz des T-Zell-Wachstumsfaktors Interleukin-2 mit Zellzyklusprogression zu reagieren ("Kompetenz"). Die LFA-1-bedingte Induktion des "spreading" ist aber auch eine notwendige späte Komponente der TCR-abhängigen Proliferation: Lang andauerndes "spreading" im Kontext der interzellulären Adhäsion ist eine notwendige Bedingung für die Produktion von Interleukin-2. Interleukin-2 ist notwendig und hinreichend um in kompetenten Zellen zur Expression der α -Kette des Interleukin-2-Rezeptors (CD25) und zur Aktivierung der "Cyclin-abhängigen Kinasen" (CDKs) zu führen, letzteres auf Grund der verstärkten Cyclin D3 Expression und der verminderten Stabilität des CDK-Inhibitors p27^{kip1}. Die aktivierten CDKs phosphorylieren und inaktivieren das Retinoblastom-Protein, was letztlich zur Zellzyklusprogression führt. Die Stimulierung des alternativen costimulatorischen Rezeptors CD28 resultiert in der adhäsionsunabhängigen Zellzyklusprogression. Die costimulationsbedingte Zellzyklusprogression wurde nicht von Cyclosporin A inhibiert und wurde von der verstärkten *in vitro* Bindung von Transkriptionsfaktoren zu dem Interleukin-2 Promotors begleitet. Adhäsionsabhängige ("anchorage-dependent") T-Zell Proliferation ist daher durch eine sequentielle Wirkung der integrin-vermittelten Signaltransduktion gekennzeichnet, die zusammen mit dem aktivierenden antigenen Stimulus sowohl den Zellzykluseintritt als auch die -progression reguliert. Auf Grund der erhaltenen Ergebnisse und der

zitierten Literatur wird das Modell der bedingten Adhäsionsabhängigkeit der T-Zell Proliferation vorgeschlagen: Die Stärke der TCR-stimulation, alternative costimulatorischer Wechselwirkungen und die verfügbaren Zytokine bedingen, ob LFA-1-bedingtes "spreading" für die T-Zell Aktivierung notwendig ist oder nicht.

Abbreviations

APC	antigen-presenting cell
AP-1	activator protein-1, dimerized transcription factors of the jun/fos family
ATP	adenosine triphosphate
B7-1/2	ligands of CD28
BSA	bovine serum albumin
CDK	cyclin-dependent kinase
CCD	Cytochalasin D
CD25	Cluster of differentiation 25; α -chain of IL-2 receptor
CD28RE	CD28 response element, consensus sequence of the IL-2 promoter
Cdi	CDK-inhibitor
c-jun, c-fos factors	proto-oncogenes coding for transcription of the AP-1 family
dCTP	deoxycytidine triphosphate
DNA	deoxyribonucleic acid
DTT	dithiothreitol
dA-/C-/G-/TP	deoxy-adenosine-/cytosine-/guanosine-/thymidine triphosphate
ECL	enhanced chemiluminescence
EDTA	ethylenediaminetetraacetic acid
EGTA	ethyleneglycoltetraacetic acid
ELISA	enzyme-linked immuno-sorbent assay
ERK	extracellular signal regulated kinase
FAK	focal adhesion kinase
FITC	fluorescein isothiocyanate
G0/1/2	gap phases 0,1 and 2 of the cell cycle
GST	gluthathion-S-transferase
GTP	guanosine triphosphate
HEPES	(N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid))
HRP	horseradish peroxidase
ICAM 1	intercellular adhesion molecule, ligand of LFA-1
Ig	immunoglobulin
I- κ B	cytoplasmatic Inhibitor of NF- κ B
IL-2	Interleukin 2
INK4	inhibitor of CDK4
IPTG	isopropyl- β -D-thiogalactoside

ITAM motif	immunoreceptor tyrosine-based activation
JNK	Jun-NH ₂ -terminal kinase
kD	kilo Dalton
LAD	leukocyte adhesion deficiency
IB	bacterial growth medium
LFA-1	leukocyte function antigen- 1, α L/ β 2 integrin
LDH	lactate dehydrogenase
mAb	monoclonal antibody
Map kinase	mitogen activated protein kinase
MKK	Map kinase kinase
MHC	major histocompatibility complex
MOPS	3-(N-morpholino)propanesulfonic acid
m-RNA	messenger ribonucleic acid
NF-AT transcription	nuclear factor of activated T cells, factor
NF-ATc	cytoplasmatic subunit of NF-AT
NF- κ B binding	nuclear factor kappa light-chain enhancer
NK cells	natural killer cells
NP-40	NonidetP-40,
ethylenephenylepolyethyleneglykol	
OCT	Octamer, family of transcription factors
pXY ^{abc}	proto-oncogene product of XY kD
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PD98059	Inhibitor of the ERK-cascade (MKK)
PI 3-kinase	phosphoinositide 3-kinase
PKC	protein kinase C
PLC	phospholipase C
PMA	phorbol myristate acetate
PMSF	phenylmethylsulfonylfluoride
pRb	retinoblastoma protein
Raf	proto-oncogenic threonine/serine kinase
RelA, c-Rel	members of the NF- κ B/Rel family
RNA	ribonucleic acid
rpm	rounds per minute
SB203580	Inhibitor of the p38 Map kinase
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
S/M-phase	DNA synthesis- and mitosis-phase of the cell cycle
SSC	saline-sodium citrate

src, syk	oncogenic tyrosine kinase families
TAE	Tris acetate EDTA
TBST	Tris buffered saline Tween20
TE	Tris-EDTA
TCR	T cell (antigen-)receptor
VLA-4	very late antigen 4, $\alpha 4/\beta 1$ integrin
YTA	yeast extract-tryptone-ampicillin
ZAP-70	zeta-chain associated protein 70kD

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