A Genetic Approach To Identify *Mycobacterium bovis* Exported Protein Antigens

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Abstract

A novel approach, combining *phoA*-fusion technology with T cell screening of a recombinant cosmid library, was used to detect *Mycobacterium bovis* exported T cell antigens. An *M. bovis* BCG library of *phoA*-fusions was constructed in *Escherichia coli* and *Mycobacterium smegmatis* using the plasmid vector pJEM11. The *M. bovis BCG* DNA inserts from ten PhoA+ clones were partially sequenced and used to search databases for similarities to known genes. These revealed similarities to a family of genes coding for high temperature-requirement serine proteases and a *Mycobacterium leprae* putative exported lipoprotein gene (*pel*).

The DNA inserts from PhoA+ clones were used to probe an *M. bovis* cosmid library expressed in *M. smegmatis* to identify cosmids containing the full-length genes coding for these exported proteins. Culture filtrates (CFs) prepared from selected *M. smegmatis* recombinants (cosmids) were assayed for their ability to induce proliferation and IFN-γ-production from peripheral blood mononuclear cells (PBMCs) taken from *M. bovis* BCG-immunised and non-immunised control cattle. Culture filtrates from two recombinant *M. smegmatis* (cosmids 44 and 56) induced significant IFN-γ-production and proliferation by PBMCs from immunised animals.

An exported protein gene, identified using the *phoA*-fusion technology, was subcloned from cosmid 56 and its sequence determined and analysed. Database searches using the deduced amino acid sequence of this gene revealed similarities to an *M. leprae* putative exported lipoprotein (Pel) and a family of MalE maltose-binding proteins. The *M. bovis pel* gene was shown to be expressed by recombinant *M. smegmatis*. Preliminary evidence from this study indicates that the *M. bovis* Pel protein is recognised by antigen-specific lymphocytes from *M. bovis* BCG-immunised animals. The PBMCs taken from

M. bovis challenged and *M. bovis* BCG vaccinated / challenged cattle also recognised CF from recombinant *M. smegmatis* expressing the *pel* gene in *in vitro* immunoassays.

The combined strategy of using *phoA*-gene fusions and T cell screening of CFs from a recombinant *M. bovis* cosmid library proved a sensitive and rapid method for the detection of potential *M. bovis* T cell antigens.

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2-D	two dimensional
AM	alveolar macrophage
APC	antigen presenting cell
ATCC	American type culture collection
BCA	bicinchoninic acid
BCG	bacillus Calmette-Guérin
BLAST	basic local alignment search tool
bp	base pairs
CCT	comparative cervical test
CF	culture filtrate
CFT	single intradermal caudal fold test
c.f.u.	colony forming units
CIE	crossed immuno-electrophoresis
CMI	cell-mediated immunity
cpm	counts per minute
dCTP	deoxycytosine triphosphate
DEPC	diethylpyrocarbonate
DNA	deoxyribose nucleic acid
DTH	delayed-type hypersensitivity
EDTA	ethylenediamine tetraacetic acid
ELISA	enzyme-linked immunosorbant assay
G+C	guanine and cytosine
hr	hour
IFN-γ	interferon-gamma
kb	kilobases
kDa	kilodaltons
Km^R	kanamycin resistant

LB	Luria-Bertani
MCS	multiple cloning site
min	minute
MM	minimal medium
MOPS	3-(*N*-morpholino)-propanesulfonic acid
MycDB	mycobacterial database
OD	optical density
ORF	open reading frame
PBS	phosphate buffered saline
PBS-T	phosphate buffered saline / 0.1% tween-20
PCR	polymerase chain reaction
PBMC	peripheral blood mononuclear cells
pI	isoelectric point
PPD-a	avian purified protein derivative
PPD-b	bovine purified protein derivative
RNA	ribonucleic acid
rpm	revolutions per minute
RT	room temperature
SD	Shine and Dalgarno
SDS-PAGE	sodium dodecyl sulphate - polyacrylamide gel electrophoresis
sec	second
SI	stimulation index
SICT	single intradermal cervical test
stdev	standard deviation
TE	tris-EDTA
XP	5-bromo-4-chloro-3-indolyl phosphate