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Dedicated to

Natasha

The Relationship of Structure to Function of the Superantigen
Staphylococcal enterotoxin A

Keith R. Hudson

A thesis submitted in partial fulfilment of the requirement
for the degree of Doctor of Philosophy

Department of Molecular Medicine
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Abstract

The relationship of structure to function in the superantigens of *Staphylococcus aureus*

- 1 Enterotoxins were isolated from expression in *E. coli* using the pGeX vector. Yields of up to 20 mg/litre of culture of purified toxin were achieved.
- 2 Recombinant SEA, SEB, SED and SEE produced by this system were identical to the wild type enterotoxins by several biological and biochemical criteria.
- 3 The unique T-cell receptor binding site of SEA and SEE was identified by the use of a panel of hybrids between these two toxins. Four different assays showed that the different V β specificity of these toxins was determined by G200, S206 and N207 in SEA and at P206 and D207 in SEE.
- 4 Residues on both SEA and HLA-DR were identified that directly interact upon binding, thus defining the MHC class II binding site of SEA. The requirement of zinc for SEA binding to HLA-DR allowed the identification of the MHC class II binding site on SEA. This requirement was shown to be due to zinc bridging between these proteins. The zinc binding residues in SEA were demonstrated to be H187, H225 and D227.
- 5 Histidine 81 in the β -chain of HLA-DR1 was shown to be the fourth residue in the tetravalent coordination of zinc.
- 6 This investigation in conjunction with the crystal structure of SEB and other mutational studies has collectively identified the T-cell receptor binding site of the enterotoxins. This study also located the MHC class II binding site of SEA and on the crystal structure of SEB the zinc coordinating residues cluster together, well separated from the T-cell receptor binding site. Thus proving that these superantigens cross link the T-cell receptor and MHC class II antigen.
- 7 The location of the zinc coordinating residues together with the crystal structures of SEB and HLA-DR1 allowed the construction of a structural model of how SEA binds to HLA-DR1. This model predicts that SEA binds across the end of the peptide groove and significantly that the orientation of the T-cell receptor in the recognition of the SEA-MHC class II complex is different to the orientation used to recognise peptide-MHC. This non conventional recognition of superantigen is compatible with the influence of polymorphism in both the T-cell receptor and MHC class II on superantigen recognition.

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Abbreviations and definitions

$^{\circ}\text{C}$	degree Celsius
2ME	2-mercaptoethanol
AIDS	acquired immunodeficiency syndrome
anchor $\text{V}\beta$ PCR	PCR technique to assess mRNA $\text{V}\beta$ enrichment
APC	antigen presenting cell
bp	base pair
BSA	bovine serum albumin
bSag	bacterial superantigen
bt	bovine thrombin
C-terminus	carboxy-terminus
$\text{C}\beta$	TCR β -chain constant region
cDNA	complementary DNA
CDR	complementarity determining region
CM	carboxymethyl
cpm	counts per minute
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
ddATP	dideoxyadenosine triphosphate
ddCTP	dideoxycytidine triphosphate
ddGTP	dideoxyguanosine triphosphate
ddTTP	dideoxythymidine triphosphate
dGTP	deoxyguanosine triphosphate
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphates
DTT	dithiothreitol
dTTP	deoxythymidine triphosphate
EBV	Epstein barr virus
EDTA	ethylenediaminetetra-cetic acid
ELISA	enzyme linked immunosorbent assay
FCS	foetal calf serum
FPLC	fast protein liquid chromatography
g	gram

glutathione	3 amino acid peptide (sequence-ESG)
GST	glutathione-s- transferase
h	human
Hepes	N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HLA-DR	Human leukocyte antigen-DR
HPLC	High performance liquid chromatography
HV4	4th hypervariable loop of the TCR β -chain
IFN- γ	interferon gamma
Ii	Invariant chain
IL	interleukin
IPTG	isopropylthio- β -galactosidase
kb	kilobases
K_d	dissociation constant
kD	kilodalton
l	litre
LTR	long terminal repeat
M	molar
m	murine
μ g	microgram
MHC	Major histocompatibility complex
ml	millilitre
μ l	microlitre
mM	millimolar
mRNA	message RNA
MW	molecular weight
N-terminus	amino-terminus
ng	nanogram
orf	open reading frame
pM	picomole
PBL	peripheral blood lymphocytes
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PHA	phytohaemagglutinin

pI	isoelectric point
PMA	Phorbol 12-Myristate 13-Acetate
PMSF	phenylmethylsulphonylfluoride
PVDF	polyvinylidenedifluoride
r	recombinant
RIA	radioimmunoassay
RNA	ribonucleic acid
rpm	revolutions per minute
Sag	superantigen
SDS	sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SE	staphylococcal enterotoxins
SEA	Staphylococcal enterotoxin A
SEB	Staphylococcal enterotoxin B
SEC	Staphylococcal enterotoxin C
SED	Staphylococcal enterotoxin D
SEE	Staphylococcal enterotoxin E
soPCR	single oligonucleotide PCR
t	trypsin
TBE	Tris borate EDTA
TCA	trichloroacetic acid
TCR	T-cell receptor
Temed	N, N, N', N'-teramethylethylenediamine
TNF	tumour necrosis factor
toxin	superantigens secreted by <i>S. aureus</i> or <i>S. pyogenes</i>
Tris	2-amino-2 (hydroxymethyl) propane-1,3-diol
TSS	Toxic shock syndrome
TSST	Toxic shock syndrome toxin
V	volt
V β	TCR β -chain variable region
vSag	viral superantigen
W	watt
wtSEA	SEA derived from <i>S. aureus</i>