Dedicated to

Natasha

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

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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 Vβ 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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# LIST OF PUBLICATIONS

## Refereed Journals :

Irwin, M. J., Hudson, K. R., Fraser, J. D., Gascoigne, N. R. J. (1992). Enterotoxin residues determining T cell receptor Vβ binding specificity. Nature 359, 841-843.

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Fraser, J. D. and Hudson K. R. (1993). Superantigens-remnants of a past process ? Res. Immunol. 144, 188-193.

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## Book Chapters in press :

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Fraser, J. D., Lowe, S., Irwin, M. J., Gascoigne, N. and Hudson K. R. A structural model of staphylococcal enterotoxin A interaction with MHC class II antigens. Superantigens.

## Publications submitted :

Hudson, K. R., Urban, R. G., Lowe, S. and Fraser, J. D. Location of the zinc bridge between Staphylococcal enterotoxin A and major histocompatibility class II antigen: a structural model for superantigen T-cell activation. Cell (submitted).

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## Abbreviations and definitions

| | |
|---|---|
| $^0$C | degree Celsius |
| 2ME | 2-mercaptoethanol |
| AIDS | acquired immunodeficiency syndrome |
| anchor Vβ PCR | PCR technique to assess mRNA Vβ enrichment |
| APC | antigen presenting cell |
| bp | base pair |
| BSA | bovine serum albumin |
| bSag | bacterial superantigen |
| bt | bovine thrombin |
| C-terminus | carboxy-terminus |
| Cβ | 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 |
| μg | microgram |
| MHC | Major histocompatibility complex |
| ml | millilitre |
| μl | 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 | isolectric 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 *S. aureus* or *S. pyogenes* |
| Tris | 2-amino-2 (hydroxymethyl) propane-1,3-diol |
| TSS | Toxic shock syndrome |
| TSST | Toxic shock syndrome toxin |
| V | volt |
| Vβ | TCR β-chain variable region |
| vSag | viral superantigen |
| W | watt |
| wtSEA | SEA derived from *S. aureus* |