

## AUTOIMMUNE MECHANISMS AND SUPERANTIGENS IN TRANSVERSE MYELITIS

Jorens PG  
Dept Intensive care medicine  
UZA-UIA

## GOALS

- Acute demyelination: antigen-superantigen
- Streptococcal exotoxins - acute demyelination
- epidemiology
- therapy

## ACUTE DEMYELINATION

- inflammatory reaction: infiltrating, perivascular CD4+ T lymphocytes and activated macrophages, local synthesis of inflammatory mediators

## TWO THEORIES

- viral: a virus enters the CNS
- autoimmune
  - viral or bacterial agents may play a role in the activation of autoreactive T cells
  - migration of T cells in CNS: activated T cells migrate quickly in the CNS (*Hafler and Weiner, Ann Neurol 1987*)
  - become reactivated by myelin antigens in the brain, initiating the pathogenic autoimmune cascade (*Stinissen et al, Crit Rev Immunol 1997*)

## DEMYELINATION

- autoimmune process mediated by autoreactive T lymphocytes with specificity for myelin antigens
  - myelin basic protein (MBP)
  - proteolipid protein (PLP)
  - myelin oligodendrocyte glycoprotein (MOP)

## PROBLEM 1

- autoimmune T cells directed against various myelin antigens can be isolated from most individuals. However, in most individuals they do not lead to autoimmune mechanisms: either anergized or present in low numbers (*Kotb, Clin Microbiol Rev, 1995*)

## PROBLEM 1

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- in the periphery of patients with MS, autoimmune myelin-specific cells are activated; IL-2 responsive (*Zhang et al, J Exp Med, 1994*)

## PROBLEM 2

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- how are pathogenic autoreactive T cells activated as there is no contact with the CNS myelin ?

## MECHANISM 1: MOLECULAR MIMICRY

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- a specific peptide, carbohydrate or lipid epitope of an infecting (viral or bacterial) agent shares sequence homology to an antigenic epitope of myelin
  - ganglioside GQ1b and *Campylobacter jejuni*
  - sequences from Herpes simplex, Epstein-Barr virus, adenovirus, influenza A, *Pseudomonas aeruginosa* ... (*Wucherpfennig et al, Cell, 1995*)

## MOLECULAR MIMICRY IS NOT THE ANSWER ...

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- activated autoreactive T cells (cross blood-brain barrier- become reactivated by myelin antigens in the brain) lead to autoimmunity in only a minority of sensitized individuals (*Stinissen et al, Crit Rev Immunology, 1997*)

## MECHANISM 2: SUPERANTIGEN

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- Myelin-reactive T-cells can be activated in vitro by several superantigens in an unconventional manner (*Zhang et al, J Autoimmun 1995*), once activated autoreactive T cells migrate into CNS

## "NORMAL" ANTIGENS

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- antigen presenting cell ( dendritic cells, macrophages, B cells ...)
  - cells that activate T-cells by processing antigen and displaying it on their surfaces along with class II major H antigens (MHC)

## "NORMAL ANTIGENS"

- two known T-cell receptors (TCR),  $\alpha\beta$  and  $\gamma\delta$
- given cell only one type of receptor, normal antigens react with both  $\alpha$  and  $\beta$
- constant (C) and variable (V) region

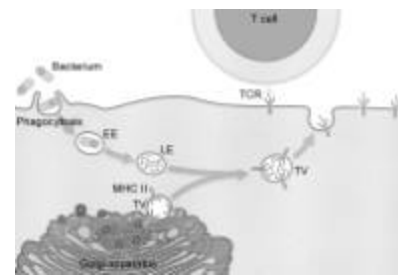
## "NORMAL" ANTIGENS

- protein antigens are processed in small peptides within the lysosomes of the APC, associate/ complexes with MHC class II molecules, cell surface, sampled by T cells, each expressing a unique  $\alpha\beta$  TCR specific for a particular MHC-antigen complex

## "NORMAL" ANTIGENS

- together the junctional regions between TCR regions create the hypervariable CDR3 (complementarity determining region) domain of the TCR recognizing MHC-peptide complexes with a high degree of specificity (*Davis and Bjorkman, Nature, 1988*), CDR3 necessary for signal transduction

## "Normal" antigen interaction



<http://www.finchcms.edu/anatomy/histology>

## SUPERANTIGENS...

- group of microbial proteins
- can be produced by viruses, bacteria, Mycoplasma
- interact with the immune system in an unconventional manner

## SUPERANTIGENS ...

- bifunctional molecules, utilize (at least) 2 types of receptors expressed on different mononuclear cells of the immune system (*Kotb, Clin Microbiol Rev, 1995*)

## SUPERANTIGENS ...

- on T cells:  $\alpha\beta$  heterodimeric TCR for antigen (*White et al, Cell, 1989*)
- capable of binding to the beta chain (Vbeta) of a characteristic set of T-cell receptors (with little contribution from the other variable regions of the TCR)

## SUPERANTIGENS ...

- on B cells, monocytes, dendritic cells: MHC class II molecules (*Fleischer and Schrezenmeier, J Exp Med, 1988*)
- first engaging the class II major histocompatibility complex and then the complex binds to the T-cell receptor (TCR) in a beta-chain variable-region
- also integrins ? (*Beharka et al, Infect Immun, 1994*)

## SUPERANTIGENS ...

- binding on TCR triggers intracellular biochemical signals, activation, differentiation, proliferation, release of inflammatory cytokines
- unique; do not require processing by the antigen presenting cell (APC)

## SUPERANTIGENS

- unique: can interact with a large number of T cells that share particular sequences within the variable region of the beta chain of the TCR, known as Vbeta elements (*Marrack and Kappler, Science 1990*), up to 5 to 20 % of resting cells

## ANTIGEN-SUPERANTIGEN (*Kotb Clin Microb Rev 1995*)

	antigen	superantigen
■		
■		
■ % responding cells	0.00001	5-20
■ MHC II dependence	+	+
■ MHC restriction pres.	+	-
■ restricted Vbeta usage	-(+)	+

## SUPERANTIGENS: three criteria (1)

- reproducible pattern of selective Vbeta interaction ( flow cytometry, PCR ...), independent of other variable elements of the TCR, = stimulation of murine and human T cells with a given toxin leads to selective expansion of T cells carrying certain Vbeta

## SUPERANTIGENS: three criteria (2)

- dependence of the response of APC that express either autologous or allogeneic class II molecules, T cells recognize superantigens bound to MHC class II molecules in an MHC-unrestricted manner, MHC class II molecules specific receptors for superantigens

## SUPERANTIGENS: three criteria (3)

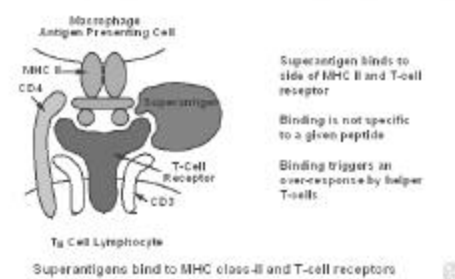
- lack of requirement for complex processing by APC

## Moreover ...

- some superantigens activate CD4 and CD8 T cells, or gamma d T cells
- can cause apoptosis (programmed cell death) of lymphocytes (*Kawabe and Ochi, 1991, Nature*)

## SUPERANTIGEN [www.thalassa.ca.sandia.gov](http://www.thalassa.ca.sandia.gov)

CMR can be used to understand protein-protein interactions as well as protein-small molecule interactions



## PYROGENIC EXOTOXINS

- *Staphylococcus aureus*:
  - enterotoxins A- E, toxic shock syndrome toxin-1
  - Enterotoxin: a substance that causes fluid secretion in the intestine
- *Streptococcus pyogenes* (group A Streptococci)
  - streptococcal pyrogenic exotoxins (Spe A, B, C, F...)
- *Mycoplasma*

## (BACTERIAL) PYROGENIC EXOTOXINS

- *Pseudomonas aeruginosa*
- *Yersinia pseudotuberculosis*, enterocolitica
- *Clostridium perfringens*
- group B and C Streptococci
- viruses: EBV, Herpes, HIV, rabies ...

## BACTERIAL PYROGENIC EXOTOXINS

- interact with large number of T cells
- elicit strong inflammatory response
- enhance susceptibility to shock
- globular proteins, obvious sequence homology, but no obvious primary structural feature predicts superantigenicity

## And moreover ...

- new types of superantigens
- interaction T-cell with superantigen
  - costimulation: activation/proliferation
  - excessive TNF and IFN: apoptosis/deletion
  - no costimulation: anergy/paralysis

## Multiple sclerosis

- preferential TCR beta-chain variable gene use in
  - 1. Brain lesions (*Oksenberg et al, Nature 1993*)
  - 2. In MBP-reactive T-cell clones from patients with MS (*Kotzin et al, PNAS, 1991*) Vb5.2 region

## Indications in experimental models ...

- superantigens can cause reactivation of experimental allergic encephalomyelitis (*Schiffenbauer et al, PNAS, 1993*)
- onset and relapses in EAE by selective expansion of the encephalitogenic T-cell populations expressing TCR Vbeta8 (*Brocke et al, Nature, 1993*)

## Question

- does it play a role in vivo in humans ?
- despite compelling indirect evidence that superantigens are involved in autoimmunity, a direct link is missing ...

## CASE HISTORY (1)

- 3-year-old-child
- history: recurrent otitis, developmental milestones
- no vaccination

## CASE REPORT (2)

- lethargy, vomiting, meningeal syndrome
- otitis media en externa
- cyanotic, hypotonic, anisocoria, intubated
- CT-scan: right temporal lesion
- transfer UZA

## CASE REPORT (3)

- tetraplegic, anisocoria, purulent discharge from the ear
- biochemistry: normal, polyclonal increase of IgG, lymphopenia
- toxicology-immunology: negative, GC-MS organic acids (urine-CSF)
- L.P (2): 10, 13 WBC, total protein 81 mg/dl (nl 15-45), increased  $\gamma$ , no malignant cells

## CASE REPORT (4)

- cultures of blood, nasopharynx, endotracheal asp., urine ...: negative
- middle ears: Streptococcus pyogenes, type M6
- CSF: negative, including viral (herpes, entero, RSV, (para)influenza, CMV, adeno and mumps). PCR: Herpes simplex and type 6, toxoplasma, Mycoplasma and CMV

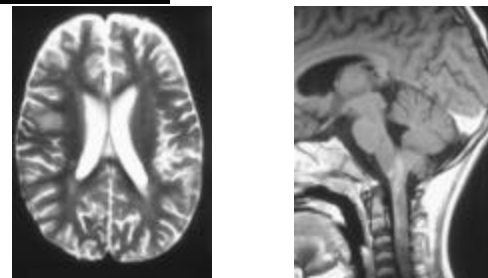
## CASE REPORT (5)

- na raised antibody titers (admission and after 3 weeks) : "28" from adeno to toxoplasma
- HLA-DR : DR2,6;DQ1;DR52; 36% CD8+ cells ( nl 25-35%), normal ratio

## MRI : day 1 and 8 after admission

- T2-weighted images multiple scattered and confluent areas subcortical and deep white matter, assymetric, ranging 2-20 mm
- large lesion lower medulla oblongata, cervical spinal cord up to C4
- confluent areas of demyelination?
- after 8 days: breakdown blood-brain barrier

## MRI: day 8 after admission



### CASE REPORT (6)

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- EMG: normal; evoked potentials: delayed latencies

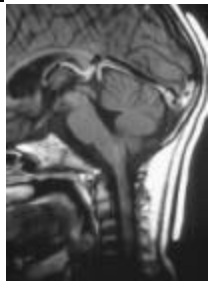
### CASE REPORT (7)

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- therapy acyclovir (Herpes?) , erythromycin (Mycoplasma?) and ceftriaxone, 10 days
- corticosteroids (30 mg/kg 3 days, tapered) and 0.4 g/kg/d IVIG 5 days, five monthly courses
- periods arrhythmia

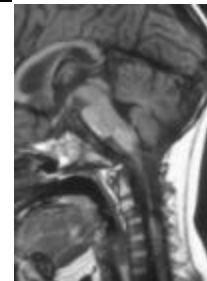
### MRI: 35 days after admission

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### MRI: 5 months after admission

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### CASE REPORT (8)

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- 6 months after admission: high titers of antibodies against SPEA and SPEB

### CASE REPORT (9)

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- tetraplegic, ventilator-dependent
- tracheostomy, port-a-cath, suprapubic urinary catheter, enterogastrostomy
- only the expression of its facial musculature has been improving, learned to speak and eat

## CASE REPORT (10)

- returned home after 30 months of hospitalisation
- died 6 months later: hyperthermia, new lesions on MRI, status epilepticus, cerebral edema

## HYPOTHESIS

- in vivo exposure to streptococcal exotoxins ( superantigens) in this child may have induced activation of pathogenic myelin reactive T cells, contributing to this dramatic inflammatory demyelination

## Superantigen genes in Streptococcus pyogenes

- genotyping method
- SPEA, SPEB, SPEC, SPEF

## CONCLUSION A

- this M6 type produces different superantigens

## PHENOTYPIC ANALYSIS of PBMC

- after Ficoll, flow cytometry
- increased percentage of CD 3 T cells (both CD4 and CD8)

## TCR profile analysis

- in vivo activation by superantigens will lead to expansion or deletion of T cells in a Vbeta gene-specific manner, induce TCR gene skewing
- semiquantitative PCR-ELISA (*VanderBorghet et al, J Immunol Methods, 1999*)

### TCR profile analysis

- TCR BV 4, 7 and 14 overexpressed, especially in CD 8 population
- in vitro stimulation by streptococcal exotoxins: expansion of BV4, BV7 and BV14 genes (*Watanabe-Ohnishi et al, J Immunol, 1994*)
- expansion of TCR BV 1,2,4 and 10 after supernatant

### CONCLUSION B

- a restricted number of BV genes were expanded among the CD8 T cells of our patient
- at least some of the expansions caused by superantigens released by this *S. pyogenes*

### GENERATION of MBP-specific T-cell lines and clones

- *Zhang et al, Science, 1993*
- MBP-reactive T cells, comparable to MS or healthy subjects (*Stinissen et al, Mult Scler, 1998*)
- 5 independent CD8 MBP-reactive T cell lines were cloned, 4 identical CDR3 amino acid sequence in TCR beta chain

### CONCLUSION C

- clonally expanded MBP-reactive T cells found in MS patients, not healthy subjects (*Vandevyver et al, Eur J Immunol 1995*)
- clonal expansion, a consequence of in vivo activation, had taken place, as identical CDR3 sequences of the clones found

### MBP-reactive T-cells: cross reactivity

- 4 out of 11 lines showed proliferative activity (3H-thymidine-uptake) to the supernatant of this *S. pyogenes*, but not to control supernatant
- all 4 tested clones responded to stimulation with SPEA, SPEC and supernatant of *S. pyogenes*

### CONCLUSION D

- the expansion might have been caused by an interaction with toxins produced by the isolated *S. pyogenes* strain

### CYTOKINE m RNA in unstimulated PBMC

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- 20 fold increase of IL-4 and IL-10, Th2 bias

### CYTOKINE PRODUCTION By clones

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- high levels of TNF- $\alpha$ , IFN- $\gamma$ , IL-4 and IL-10 .. after stimulation with *S. pyogenes*

### CONCLUSION E

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- Th2 MBP-reactive T cells previously found in ADEM (*Pohl-Kope et al, J Neuroimmunol 1998*)
- Th1 to Th2 shift leads to exacerbating of autoimmune processes such as EAE (*Genain et al, Science, 1996*)
- glucocorticoids ? (*Franchimont et al, Regul Pept 1998*)

### GENERAL CONCLUSION

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- human T cells showing in vitro reactivity to myelin antigens may be pathogenic in vivo (*Jorens et al, Neurology, 2000*)

### HYPOTHESIS: ADEM

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- young age, fever, areflexia, multifocal lesions (*Storch-Hagenlocher, Neurocritical care, 1994*)

### Superantigens - acute demyelination

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- meningo-encephalitis *Streptococcus pyogenes*: 3 cases (*Munn et al, Neuropediatrics 1992; Pergami et al, Ital J Neurol Sci 1996, Hall et al, J Child Neurol, 1998*)
- transverse myelitis - *Mycoplasma*

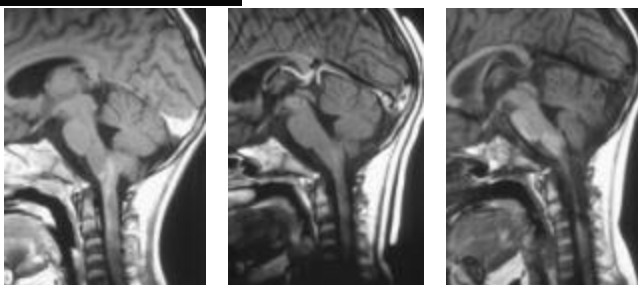
## Superantigens- acute demyelination

- role of bacterial infection in exacerbation of MS (*Rapp et al, Am J Phys Med Rehabil, 1995*)
- encephalomyelitis: herpes, EBV, Streptococcus pneumoniae ..

## OTITIS?

- "gerbils"; inoculation ear ( *S. pneumoniae, Listeria monocytogenes*) meningeal invasion, encephalitis (*Muffat-Joly et al, Arch Otol Head Neck 1994; Blanot et al, Microb Pathol, 1997*)

## SUPERANTIGEN IN VIVO-DEMYELINATION



## Therapeutic strategies

- antibiotics ? once acute manifestations are triggered, ineffective
- anticytokine therapies?
- vaccination? synergizing with other virulence factors, not effective?
- immunoglobulins

## Why should it work?

- modulation of cytokine release and production) by T cells, B cells and monocytes: decreased IL-2, increased IFN- $\gamma$  (*Amran et al, Clin Immunol Immunopathol, 994*), decreases LPS-induced production of TNF and IL-1 by increasing intracellular cAMP levels (*Shimozato et al, Immunology, 1991*)

## Why should it work?

- modulation of T and B lymphocyte function:
  - inhibits proliferation of B and T lymphocytes
  - inhibits antibody production by B-cells , dependent on Fc
- ...

## IMMUNOGLOBULINS

- intravenous immunoglobulins contain
  - neutralizing antibodies not only against several cytokines, but also to epitopes of superantigens (*Takei et al, J Clin Invest, 1993*), IgM and IgA higher inhibitory activity than IgG (*Norrby-Teglund et al, Clin Infect Dis 2000*)

## IMMUNOGLOBULINS

- contain soluble HLA molecules (*Blasczyk et al, Lancet, 1993*), competitively inhibiting the binding of superantigens?
- antibodies against the Vbeta 3,8,17 gene families of the T-cell receptor peptides (*Marchalonis et al, PNAS, 1992*)

## GLUCOCORTICOIDS

- endogenous and exogenous GC regulate T cell responses to Staphylococcal superantigens (*Gonzalo et al, J Exp Med, 1993*)
- glucocorticoids can inhibit superantigen mediated deletion of mature T lymphocytes (*Ayrolidi et al, Immunology, 1995*)

## GLUCOCORTICOIDS

- superantigens induce glucocorticoid insensitivity by induction of glucocorticoid receptor beta in human PBMCs (*Hauk et al, J Allergy Clin Immunol 2000*)

## Why do bacteria produce superantigens ?

- render the host immunocompromised in preparation for a takeover by the organism, as is manifested in cases of superantigen-associated toxic shock
- effects of superantigen will vary considerably depending on the host, the interplay between immune systems and the pathogen

## Why do bacteria produce superantigens ?

- a particular superantigen may produce starkly diverse effects in different individuals
- *Hippocrates: Inflammation, if limited, is a prerequisite for survival.*

## Transverse myelitis ...

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- T -lymphocytes
- R eceptor glucocorticoids-superantigen
- A utoimmune
- N ew studies, antibody titers
- S treptococcus, Staphylococcus
- V beta
- E xacerbations ? superantigens
- R eceptor T-cell
- S hock: toxic
- E xotoxins and enterotoxins

## Transverse myelitis ...

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- M HC II
- Y es, superantigens
- E pidemiology
- L isteria, Streptococcus pneumoniae
- I nflammation
- T hree criteria: superantigen
- I nterleukins, IL-2
- S econd international transverse myelitis symposium