Group A Streptococci

- Nonmotile gram positive cocci with fermentative metabolism
- Beta-hemolytic
- Mostly members of *Streptococcus pyogenes* (Lancefield grouping)
- several Genomes sequenced (M1, M3, M18) - 1.85-1.9 Mb, 1752-1895 ORFs, each serotype – 100-200 unique ORF for each serotype

Group A Streptococci- diseases

- Usually mild infection of skin and mucosal surfaces of upper respiratory tract
- Occasionally
  - Severe systemic infection
    - Toxic shock-like syndrome, Necrotizing fasciitis
  - Autoimmune disease
    - Acute rheumatic fever
- Changing spectrum of the diseases

  Puerperal fever – Scarlet fever, rheumatic fever - STSS

Classification of group A streptococcal infection

1. Streptococcal toxic shock syndrome (STSS): defined as a definite case by isolation of group A streptococci from a normally sterile site (e.g. blood, cerebrospinal, pleural or peritoneal fluid, tissue biopsy, surgical wound and so on) or a probable case if isolated from a nonsterile site (e.g. throat, sputum, vagina, superficial skin lesion and so on) in conjunction with combinations of the following signs of clinical severity; hypotension, renal impairment, coagulopathy, increased liver activity, adult respiratory distress syndrome, generalized erythematous rash or soft-tissue necrosis.
2. Other invasive infections: defined by isolation of group A streptococci from a normally sterile site in patients not meeting the criteria for STSS.
    (a) Bacteremia with no identified focus.
    (b) Focal infections with or without bacteremia. Includes meningitis, pneumonia, peritonitis, puerperal sepsis, osteomyelitis, septic arthritis, necrotizing fasciitis, surgical wound infections, erysipeas and cellulitis.
3. Scarlet fever: defined by a scarletina rash with evidence of group A streptococcal infection, most commonly pharyngotonsillitis.
4. Non-invasive infections: defined by the isolation of group A streptococci from a non-sterile site.
    (a) Mucous membrane: includes pharyngitis, tonsillitis, otitis media, sinusitis and vaginitis
    (b) Cutaneous: includes impetigo.
5. Non-suppurative sequelae: defined by specific clinical findings with evidence of a recent group A streptococcal infection.
    (a) Acute rheumatic fever.
    (b) Acute glomerulonephritis.

Disease diversity

- Strain-to-strain variation
- Regulation of virulence genes

- The major human host defence against invasive GAS infection is that of phagocytosis and killing by polymorphonuclear leucocytes (PML)
- Susceptible to most antibiotics

Known or postulated GAS virulence factors in people

Antiphagocytic
- M protein
- M-protein-like
- M-related protein (Mrp)
- Enn and others
- Hyaluronic acid capsule
- C5a peptidase
- IdeS

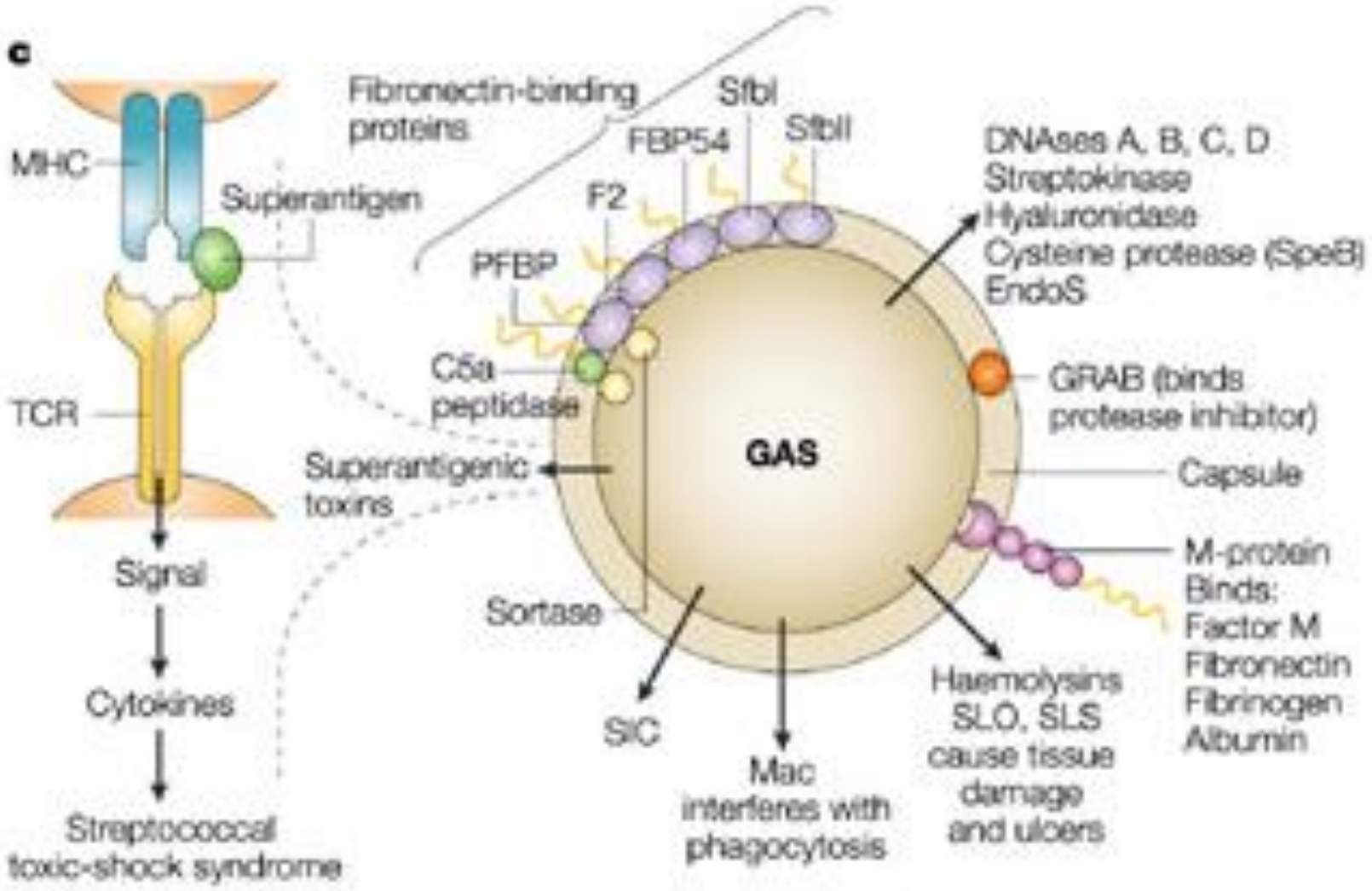
Adherence to epithelial cells
- Lipoteichoic acid (oral epithelial cells)
- Fibronectin binding proteins (oral epithelial cells, cutaneous Langerhans cells)
- M protein (skin keratinocytes)
- Hyaluronic acid capsule (CD44-positive keratinocytes)

Internalisation
- M protein
- Protein F1

Invasion
- Hyaluronic acid capsule
- M protein

Spread through tissues
- Hyaluronidase
- Streptokinase
- SpeB
- DNAses A-D

Systemic toxicity
- Streptolysin O
- Streptolysin S
- Superantigenic exotoxins

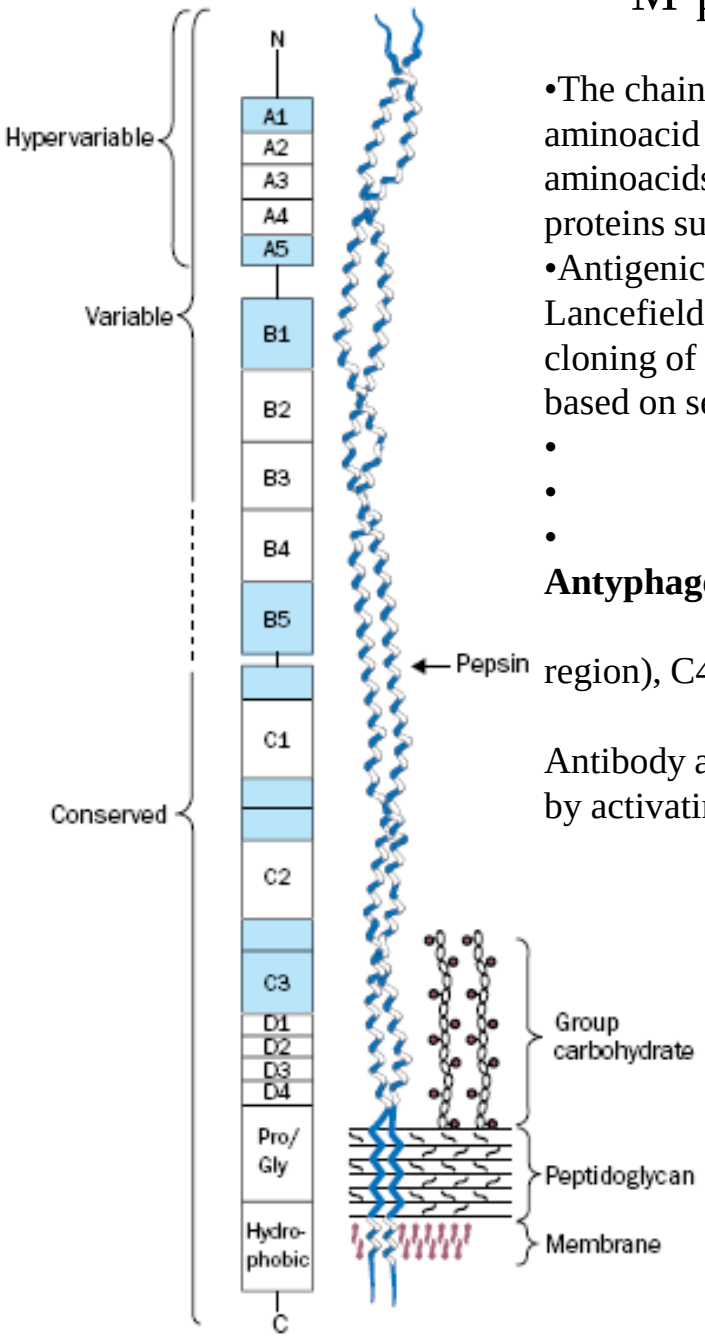

c
MHC
Superantigen
TCR
Signal
Cytokines
Streptococcal
toxic-shock syndrome
Fibronectin-binding
proteins
SfbI
SfbII
FBP54
F2
PFBP
C5a
peptidase
Superantigenic
toxins
GAS
Sortase
SIC
Mac
interferes with
phagocytosis
DNAses A, B, C, D
Streptokinase
Hyaluronidase
Cysteine protease (SpeB)
EndoS
GRAB (binds
protease inhibitor)
Capsule
M-protein
Binds:
Factor M
Fibronectin
Fibrinogen
Albumin
Haemolysins
SLO, SLS
cause tissue
damage
and ulcers

Adhesion

- Unspecific – LTA
- Specific – adhesins targeting specific tissue types
    - host target molecules – fibronectin, collagen
        - present in every tissue, yet in tissue-specific composition concerning amounts and molecular types
    - MSCRAMMs (microbial surface components recognizing adhesive matrix molecules)
        - Comon structural motifs
            - N-terminal signal sequence – outbound transport
            - Variable N-terminus, with mostly undefined function
            - ECM-binding region (one to many), closer to C-term
            - LPxTG motif – covalent binding to bacterial peptidoglycan by sortase
        - ~ 15 MSCRAMMs
            - Only few present in all serotypes
            - Growth phase regulation
            - Host cell type specific

Kreikemeyer B, Klenk M, Podbielski A, International Journal of Medical Microbiology 294 (2004) 177–188

M-protein



•The chains comprise four repeat blocks (labelled A–D), each differing in size and aminoacid sequence, within which there are seven-residue repeats of non-polar aminoacids. This heptad periodicity is characteristic of alpha-helical coiled-coil proteins such as mammalian tropomyosin

•Antigenic differences in the hypervariable region constitute the basis for the Lancefield serological classification of GAS, which has been expanded after the cloning of the M-protein (*emm*) gene and the standardisation of an *emm* typing system based on sequencing of the N-terminal nucleotide residues.

- There are over 150 recognised M genotypes
- M18 – accute rheum fever
- M1, M3 – severe invasive disease, increased mortality

Antyphagocytic

- binding of factor H (C-region) and factor H-like protein (hypervarible region), C4b (hyperavariable region)
- binding of fibrinogen (close to N-terminus)

Antibody against variable region override the antiphagocytic effect by activating classic complement pathway

employing chromosomal mutants expressing M5 proteins with internal deletions demonstrated that only the B-repeat region is essential for phagocytosis resistance. However, only antibodies to the HVR were opsonic.

Sandin C et al, Mol Microbiol. 2006 Jan;59(1):20-30

M-like proteins

emm, mrp, fcrA, arp, protH, Mac (binds to CD16 on surface of PML)
binds IgA, IgG, albumin, fibrinogen, plasminogen etc.

- orchestration of antiphagocytic activities of GAS

PrtF1/SfbI – fibronectin binding protein

•dual binding site close to C-term – one unique, one consisting of repeated motifs

–**adherence** of 30 kDa N-term domain of fibronectin with repeated motifs domain followed by unfolding of unique domain that target 45 kDa of fibronectin

•triggers **internalization** through integrins

– Formation of focal complex (signaling cascade involving FAK, Rho fam. Proteins) – cytoskeleton rearrangement – **phagocytic vacuole**

» Can lead to resting or multiplication of the bacteria

Prerequisite for GAS persistance

- Related proteins

–SbfII, FBP54, F2, PFBP

Plasmin(ogen)
crucial host factor for invasive GAS infection

- GAS interacts with plasminogen to acquire surface plasmin, that can not be regulated by alpha-antiplasmin
  - Mechanism to hijack the host fibrinolytic system
  - Can be the cause of human specificity of GAS
  - (Plasmin is an important enzyme (EC 3.4.21.7) present in blood that degrades many blood plasma proteins, including fibrin clots. The degradation of fibrin is termedfibrinolysis.)
- Plg binding proteins
  - PAM - Plasminogen-binding group A streptococcal M-like protein
  - SEN – surface alpha enolase
  - Plr/SDH/GAPDH
  - Streptokinase

Chhatwal GS, McMillan DJ, Tr Mol Med, Vol11 No4 april 2005
Wlaker MJ et al, Tr Microbiol Vol 13 No7 July 2005

PAM - Plasminogen-binding group A streptococcal M-like protein

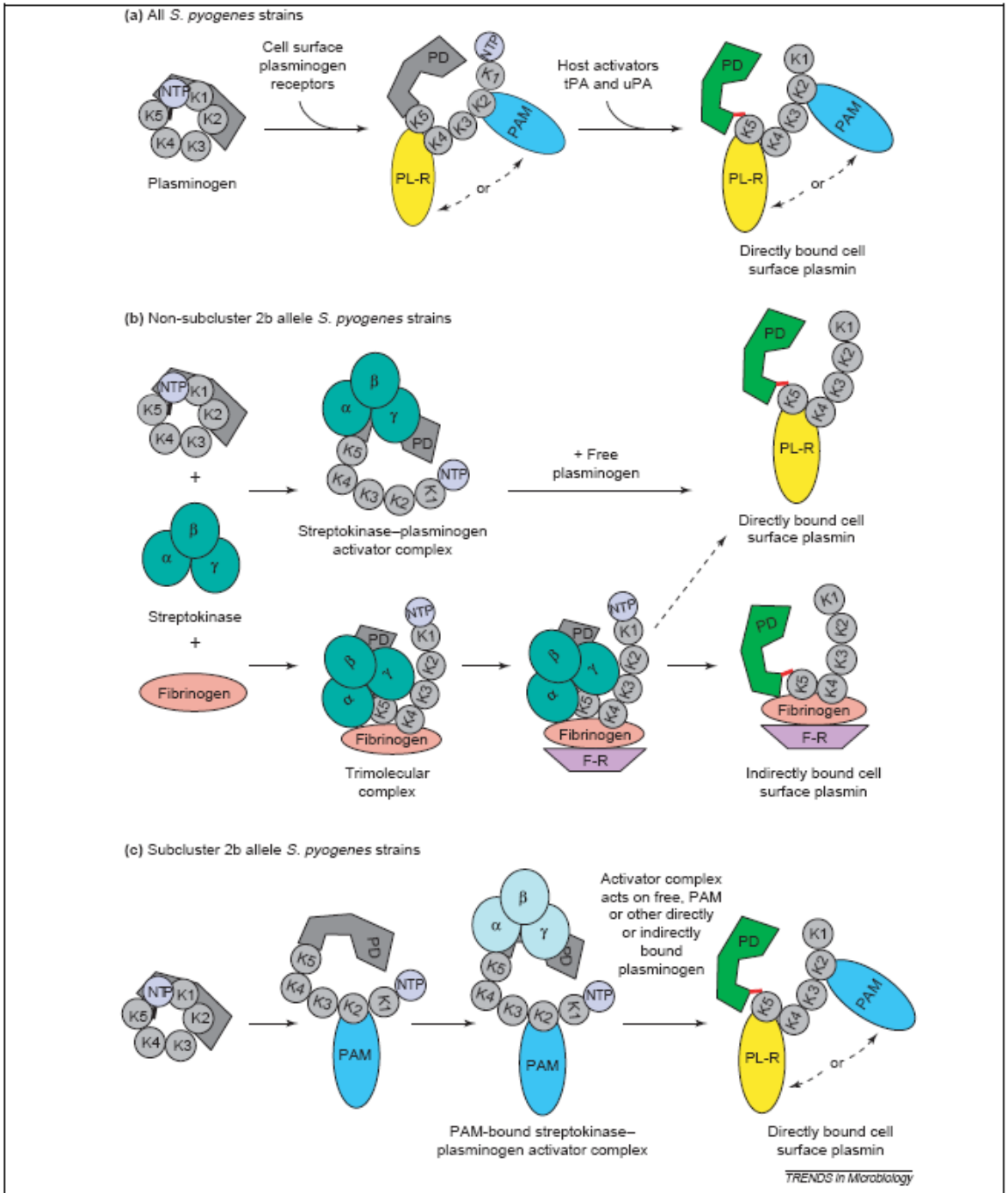
- High affinity to Plg
- PAM-positive strains bind significantly higher level of Plg than PAM-negative isolates
- Binding mediated by N-terminal plasminogen binding tandem repeat motifs containing K residues – interaction with lysin binding kringle 2 domain in Plg

Streptokinase

- Interaction between plasminogen and streptokinase promotes bacterial invasion of tissues
  - Streptokinase has limited specificity for other than human plasminogen
    - Transgenic mouse expressing human plasminogen
      - Increased mortality even for strains non-virulent to mice before
      - No diff. if infected intravenously
    - GAS mutant in streptokinase decreased back the susceptibility

Sun et al(2004) Science 305, 1283-1286

- 2 sequence clusters – 1, 2
  - Most PAM-positive strains - 2b
    - Streptokinase-dependent surface plasmin activity, but low levels of secreted streptokinase activity


(a) All S. pyogenes strains
Cell surface plasminogen receptors
Host activators tPA and uPA
Plasminogen
PD
PAM
PL-R
or
Directly bound cell surface plasmin
(b) Non-subcluster 2b allele S. pyogenes strains
+ Free plasminogen
Streptokinase–plasminogen activator complex
Streptokinase
Fibrinogen
Trimolecular complex
F-R
Indirectly bound cell surface plasmin
(c) Subcluster 2b allele S. pyogenes strains
Activator complex acts on free, PAM or other directly or indirectly bound plasminogen
PAM-bound streptokinase–plasminogen activator complex
TRENDS in Microbiology

Plr/SDH/GAPDH

- anchorless, multifunctional protein displayed on the surfaces of GAS
- In mutant strain – hydrophobic tail - the protein was not secreted in the medium but was retained in the cytoplasm and to some extent trapped within the cell wall
  - cell wall extracts of the mutant strain showed 5.5-fold less GAPDH activity than the wild-type strain. The mutant strain, M1-SDH(HBtail), bound significantly less human plasminogen, adhered poorly to human pharyngeal cells, and lost its innate antiphagocytic activity

    Roel G et al. Infect Immun. 2005 Oct;73(10):6237-48
- C5a binding activity
  - necessary for C5a cleavage on the cell surface together with C5a peptidase

    Terao et al, JBC 2006 May19

Capsule

Hyaluronic acid

Operon - hasA (hyaluronate synthase), hasB (UDP-glucose dehydrogenase), hasC (UDP-glucose pyrophosphorylase)

Variation in degree of encapsulation – difference in resistance to phagocytosis

acapsular isogenic mutant – 100-fold decreased virulence

link between mucoid strains and invasive diseases

Human-like composition – poor immunogen

Capsule impedes adherence and interanlization to keratinocytes

Hyaluronidase

- Hyl digests tissue HA and facilitates spread of large molecules but is not sufficient to cause subcutaneous diffusion of bacteria or to affect lesion size
- Hyl may permit the organism to utilize host HA or its own capsule as an energy source

Starr CR, Engleberg NC, Infect Immun. 2006 Jan;74(1):40-8.

Lytic enzymes important to streptococcal virulence

Plasmin: Plasminogen bound to the bacterial surface forms complexes with streptokinase, resulting in the formation of plasmin. Unregulated plasmin activity results in the breakdown of tissue barriers, thereby enabling the dissemination of streptococci.
SpeB: A generalist protease that cleaves host human proteins such as fibrin and fibronectin, in addition to human immunoglobulins, assisting in bacterial dissemination. SpeB activity at the bacterial surface results in the cleavage of many virulence factors, including the M protein.
C5a peptidase: A specific protease that degrades the chemostatic complement factor C5a, resulting in the impaired recruitment of polymorphonuclear leukocytes to the site of infection.
IdeS: A serine protease , interferes with phagocytic killing by specifically cleaving the heavy chain of immunoglobulin G, Mac is identical to IdeS
IL-8 protease: A specific protease that degrades IL-8, the chemokine that is responsible for the recruitment of neutrophils to the site of infection. IL-8 has a key role in group A streptococcal necrotising soft-tissue infections.

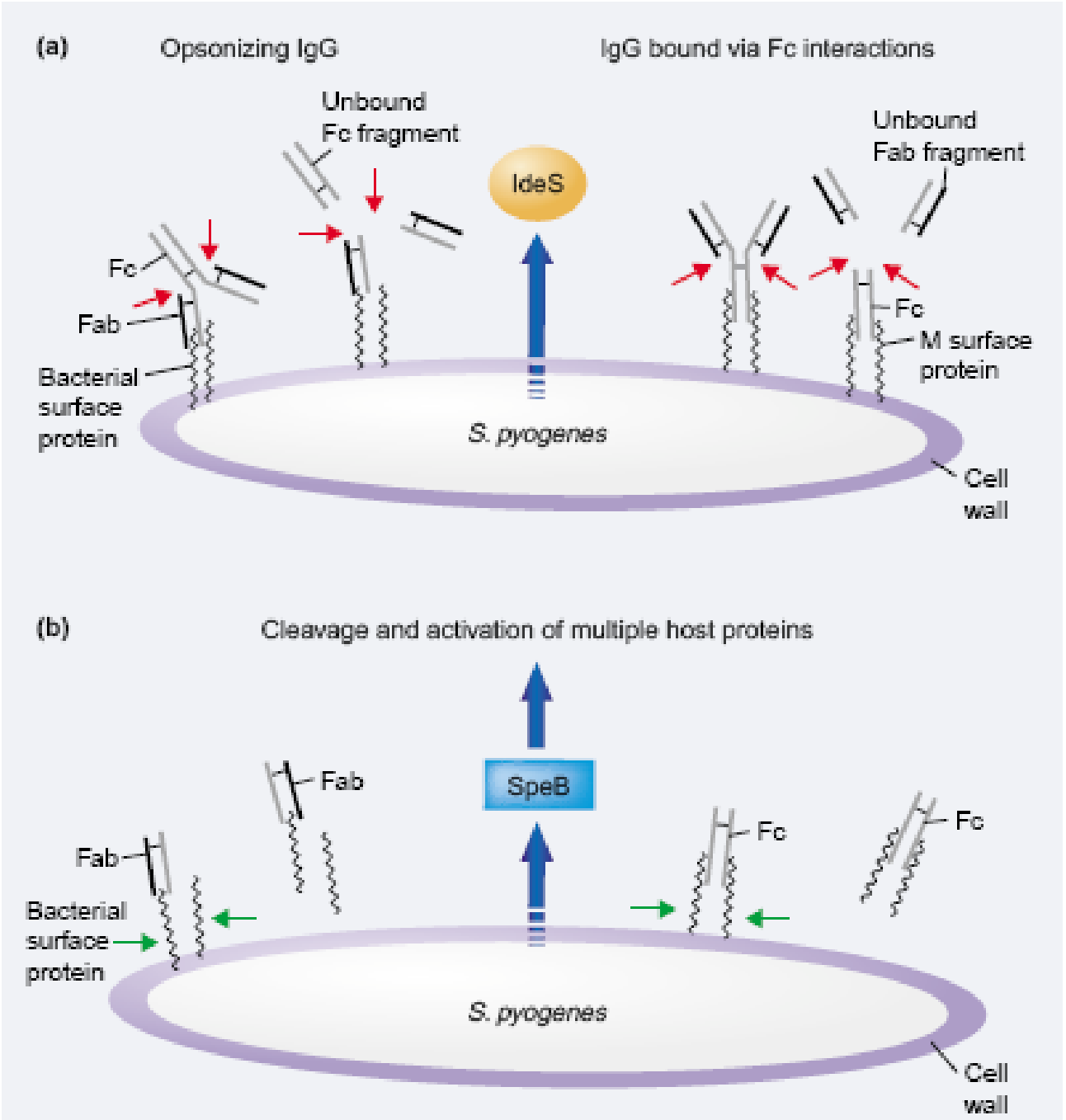
EndoS: (secreted endoglycosidase) hydrolysis of conserved N-linked oligosacharides on IgG. Fc glycosylation is critical for Ig recognition by FcR
Hyaluronidase

DNases –A, B, C, D

DNase Sda1 is both necessary and sufficient to promote GAS neutrophil resistance and virulence in a murine model of necrotising fascities

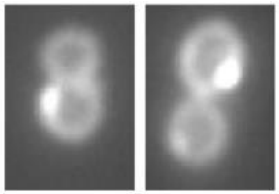
Defence against neutrophil extracellular traps (NETs) composed of DNA and histones

Buchanan JT, Curr Biol. 2006 Feb 21;16(4):396-400

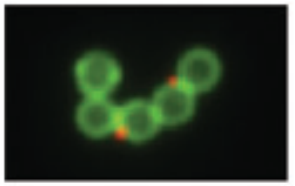


Current Opinion in Microbiology 2003, 6:50-55

Current Opinion In Microbiology



ExPortal



- a unique single microdomain of the cellular membrane specialized to contain the Sec translocons
- biogenesis of secreted proteins by coordinating interactions between nascent unfolded secretory proteins and membrane-associated chaperones
- provides a mechanism by which Gram-positive bacteria can coordinate protein secretion and subsequent biogenesis in the absence of a specialized protein-folding compartment
- positioned at a hemispherical position distal to either cell pole.
- *S. pyogenes* secretes Sec substrate proteins exclusively through the ExPortal
- SpeB maturation
- GAPDH, SEN assymetrically distributed following SpeB distribution
  - Could be also exported through ExPortal into extracelular space

Rosch J, Caparon M, ScienceVol 304 4 June 2004

Streptolysin S

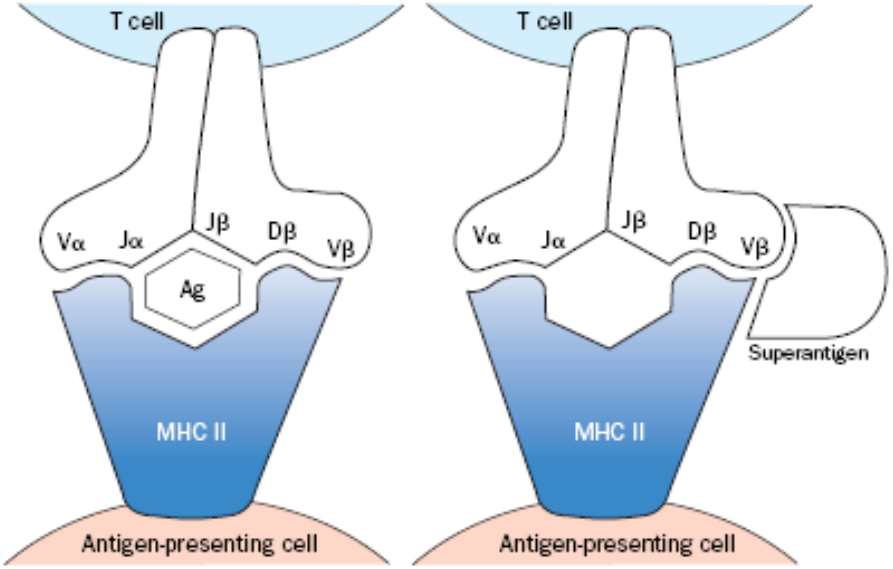
- the principal factor responsible for β-hemolysis in GAS
- oxygen-stable cytolysin
- not immunogenic in the course of natural infection
- broad cytolytic spectrum
    - the membranes of erythrocytes, leukocytes, platelets, tissue-culture cells and sub-cellular organelles such as lysosomes and mitochondria.
- can be synthesized continuously by stationary-phase cells in the presence of a minimal energy source, for example, glucose and Mg2+ ions
- exists primarily in a cell-bound form, presumably linked to the streptococcal surface by lipoteichoic acid
- One of the most potent cytotoxins known

Streptolysin O

- 57 kDa oxygen-labile prototype of the cholesterol-binding, ‘thiol-activated’cytolysin family
- elicits antibodies useful for documenting recent exposure to GAS
- Lytic effect on erythrocytes, PML, platelets, tissue culture cells, lysosomes, mammalian and amphibian hearts
- Facilitate the translocation of NAD-glycohydrolase into kerationocytes

Superantigens

SAgs bind, as intact molecules to the class II major histocompatibility complex **MHC antigens** expressed on professional antigen presenting cells (APCs) outside the peptide-binding groove then sequentially bind the T cell receptor (TcR) via the variable region of the TcR b-chain. **Every SAg binds a subset of TcR Vb domains** and as the number of different Vb regions in the human T cell repertoire is restricted to approximately 50, comprising about 24 major types of Vb elements, a substantial number of T cells are activated by SAgs. This can be as high as 20% compared with only 1 in 10^5-10^6 naive T cells that are responsive to conventional peptide antigen. This results in **massive systemic release of pro-inflammatory cytokines**, such as tumour necrosis factor-alpha (TNFa) and interleukin-beta (IL-1b), and T cell mediators, such as IL-2, which can **lead to fever and shock**

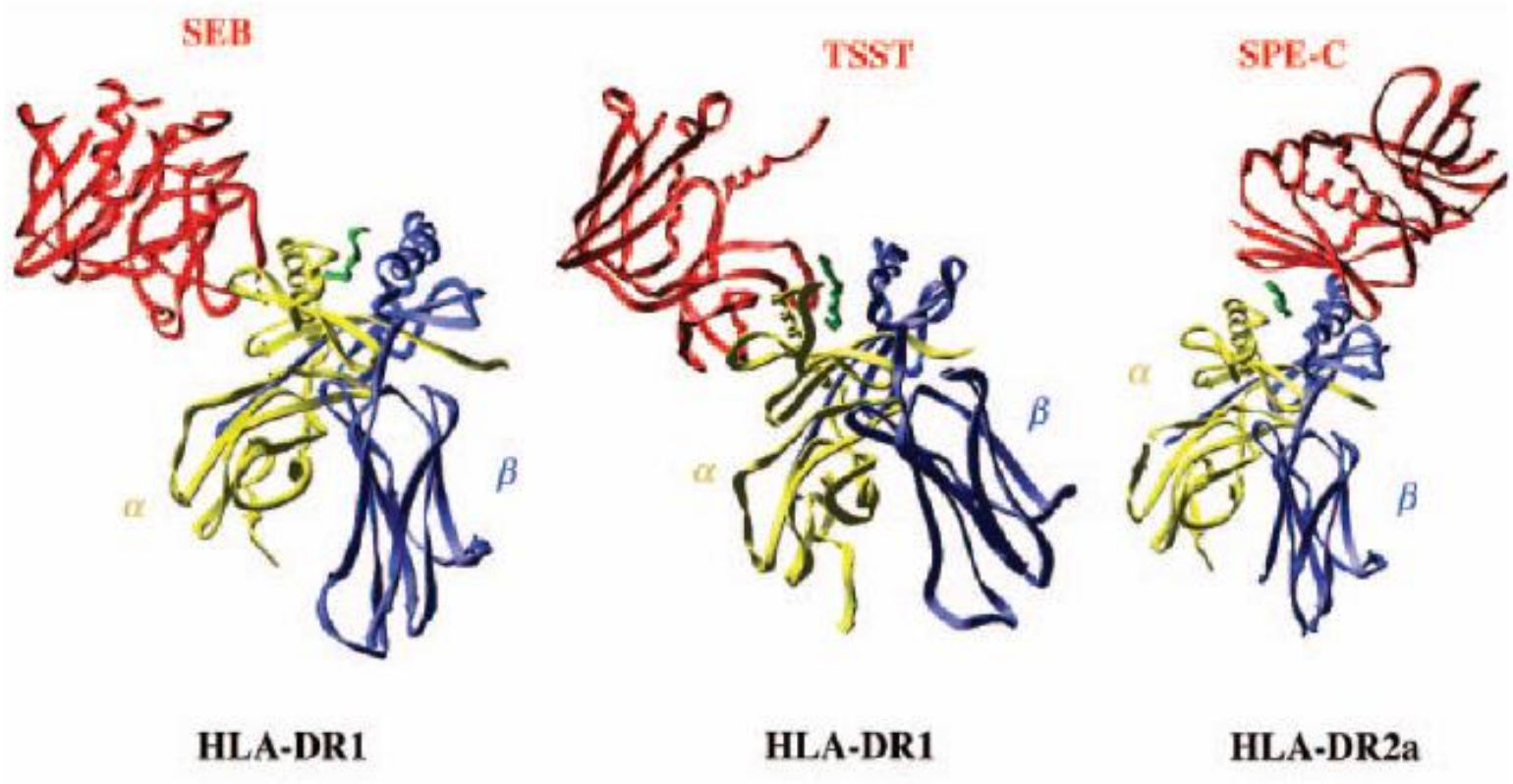


12 SAgs identified in GAS

- streptococcal pyrogenic exotoxins (SPEs) A, C, G-M
- streptococcal superantigen (SSA)
- streptococcal mitogenic exotoxin (SMEZ) 1 and 2.
- many new SAgs have been identified by screening the completed *S. pyogenes* genomes

*T. PROFT, J. D. FRASER; Bacterial Superantigens; Clinical and Experimental Immunology, **133**:299–306*

a common core-fold based on two globular domains: a smaller N-terminal pseudo b-barrel domain, which is most similar to the classical oligosaccharide/oligonucleotide-binding fold (OBfold) found in many bacterial proteins that bind oligomeric molecules and a larger C-terminal b-grasp domain. The two domains are separated by a long solvent-accessible a-helix that extends down the centre of the molecule



By structural comparison, the staphylococcal and streptococcal superantigens build a large protein family, indicating that they have all evolved from a single primordial superantigen

GAS diseases related to SAgs activity

Streptococcal toxic shock syndrome (STSS)

The *spe-a* and *spe-c* genes were found at higher frequencies inisolates from STSS patients compared to control groups, lack of protective anti-SAg antibodies was found to be associated with an increased risk for STSS and circulating SAgs were found in several patients suffering from STSS

Acute rheumatic fever (ARF)

ARF is a cross-reactive immune response to the host's cardiac tissue and it has been proposed that the reactive T cells might be driven by SAgs. Recently, several novel streptococcal SAgs have been identified from ARF-associated serotypes. The genes for SPE-K/L were found in high frequencies on serotypes M3 (USA and Japan) and on M89 (New Zealand), while SPE-M and SPE-M* were found in M18 (USA)

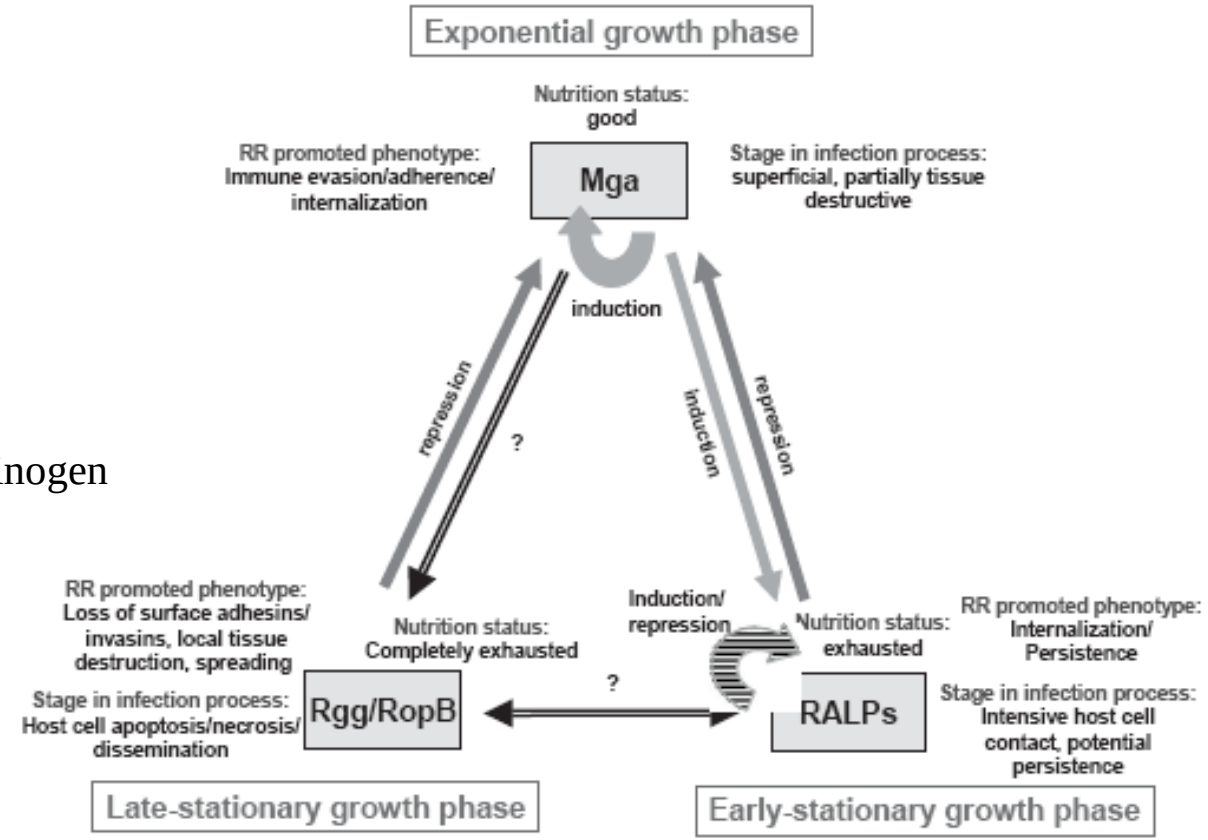
Regulation of Virulence Factors

- 11 conserved two-component regulators, 2 serotype specific, 38 putative transcription factors without sensor kinase
- Regulation of M-protein and capsule, evaluation of importance of other putative factors based on coregulation
- **Mga** – multiple gene regulator
  regulates M-protein, Sic, C5a peptidase
  regulated by CO2 concentration
  regulated by Nra (regulates also ***prtF2***, ***cpa*** – colagen binding protein Cpa)

RALPs family of regulators
RofA, Nra

Rgg/RopB
global regulator

FasBCA – three component system
controls expression of fibrinogen
and fibronectin binding



Two-component regulatory system

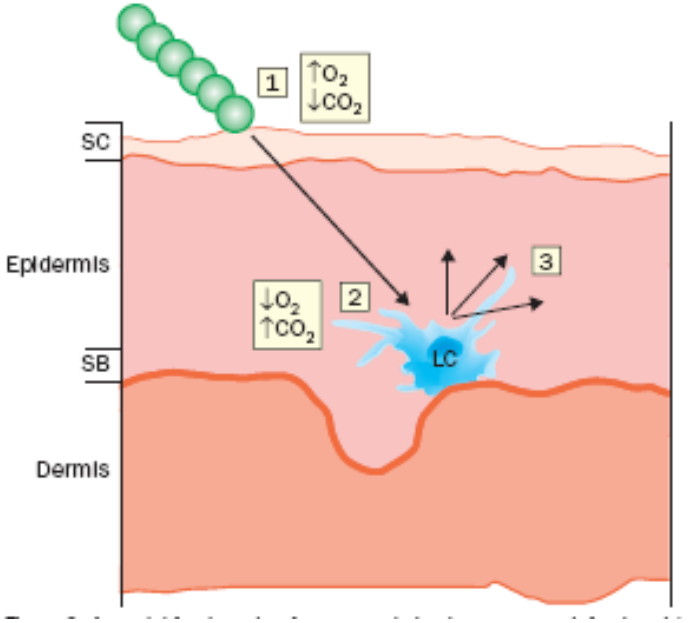
CovR – CovS

Regulates about 15% of all genes
repression of ***has***
regulation of Streptolysin, Streptokinase
trigger unknown – blood factor ???, growth phase regulation

SilA – SilB

Expression of proteins responsible for spreading into deeper tissues

A model for the role of gene regulation in cutaneous infection. *(1)* Streptococcus pyogenes *initially attaches to the O2-rich ,* ***CO2-poor outer surface of the skin*** *(SC, stratum corneum). This environment* ***will stimulate expression of protein F****. (2) The traumatic implantation of* S pyogenes *into the epidermis will direct interaction with dendritic Langerhans cells (LC) located in the basal epidermis (SB, stratum basale) via protein F. (3) Multiplication in this more O2-poor,* ***CO2-rich environment will stimulate expression of M protein*** *and promote interaction of the bacterium with epidermal keratinocytes. The significance of these events is unknown; however, both types of host cells are thought to be important in the defence of cutaneous tissues from infection.*



Genetic plasticity

•Comparison of the surface proteins with respective proteins of closely related streptococcal species supports the hypothesis that the encoding genes were acquired through horizontal gene transfer events.

- •Alpha C protein family of *S. agalacticae* – similarity to surface proteins of *S. pyogenes*
  - •Similar multiple tandem repeats – chimeric structure derived from *S. agalacticae*

G. Broker, B. Spellerberg, International Journal of Medical Microbiology 294 (2004) 169–175

•The acquisition of prophage is the major source of genetic diversity within GAS isolates of M3-type

- •Carrying novel virulence factors – pyrogenic exotoxins (speA)
- •Pleiiotropic effect on other genes altering virulence potential
- •Variation of the M-protein N-term – M3.1, M3.2 – first four amino acid duplicated

Beres SB et al (2004) Proc.Natl. Acad. Sci U.S.A. 101 11833-11838

•4 – 6 phage genomes identified per bacterial chromosome

•The streptococcal SAgs SPE-A, SPE-H, SPE-I and SSA

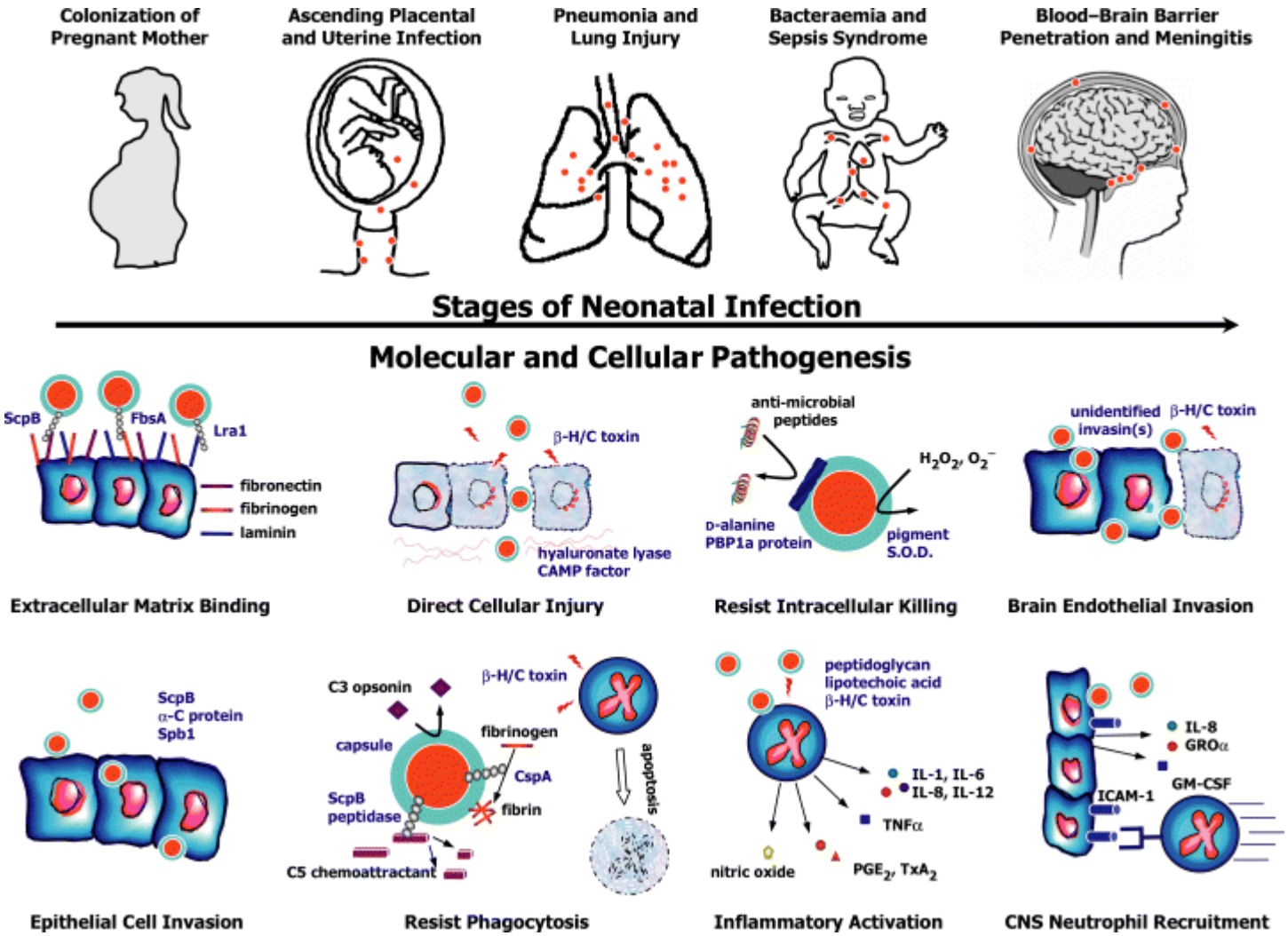
- • related more closely to the staphylococcal SAgs than to any other streptococcal SAg
- • the genes are all located on mobile elements, so it is likely that this SAg subgroup in *S. pyogenes* arose through the horizontal transfer from *S. aureus* rather than evolving from existing streptococcal superantigen genes

*T. PROFT, J. D. FRASER; Clinical and Experimental Immunology, **133**:299–306*

Vaccine

- M protein-derived vaccines have shown promise in murine vaccine models and a recent phase 1 human clinical trial
- SpeB, C5a peptidase

Group B streptococci



Stages in the molecular and cellular pathogenesis of neonatal group B *Streptococcal* (GBS) infection. -H/C, beta-haemolysin/cytolysin; S.O.D., superoxide dismutase; IL, interleukin; TNF , tumour necrosis factor-alpha; PGE2, prostaglandin E2; TxA2, thromboxane A2; GRO , growth-related oncogene-alpha; ICAM-1, intercellular adhesion molecule 1; GM-CSF, granulocyte-macrophage colony-stimulating factor.

Table 1. Key virulence factors of group B *Streptococcus.*

Virulence factor	Genetic basis	Chemical nature	Molecular or cellular action(s)	Proposed contribution(s) to disease pathogenesis
Exopolysaccharide surface capsule	*cps*A-L, *neu*A-D	High-molecular-weight polymer with terminal sialic acid residues	Impairs complement C3 deposition and activation	Blocks opsonophagocytic clearance
			Decreases immune recognition, perhaps through molecular mimicry of host sialic acid epitopes	Delays neutrophil recruitment
-Haemolysin/cytolysin	*cyl*E	CylE protein (79 kD)	Forms pores in cell membranes	Direct tissue injury
			Induces apoptosis	Penetration of epithelial barriers
			Promotes cellular invasion	Induction of sepsis syndrome
			Triggers iNOS, cytokine release	Phagocytic resistance
+ linked pigment	*cyl* locus	Carotenoid	Antioxidant effect blocks H_2O_2, singlet oxygen	Impairment of oxidative burst killing
Hyaluronate lyase	*hyl*B	HylB enzyme (110 kD)	Cleaves hyaluronan and chondroitin sulphate	Spread through host tissues
				Impairment of leukocyte trafficking
C5a peptidase	*scp*B	ScpB protein (120 kD)	Cleaves human C5a	Inhibit PMN recruitments
			Binds fibronectin	Extracelluar matrix attachment
				Epithelial adherence and invasion
CAMP factor	*cf* b	CAMP protein (24 kD)	CAMP reaction (co-haemolysin)	Direct tissue injury
			Binds to Fc portion of IgG, IgM	Impairment of antibody function
Lipotechoic acid	Complex	Amphiphilic glycerol phosphate polymer of complex lipids and short-chain fatty acids	Binds host cell surfaces	Epithelial cell attachment
			Binds host pattern recognition receptors (TLRs)	Activation of the sepsis syndrome
			Alanylation inhibits host anti-microbial peptides	Resistance to neutrophil killing
C protein (alpha and beta components)	*bca* (alpha) *cba* (beta)	Alpha: protein with mutiple identical tandem repeats (14-145 kD); beta: 84-94 kD variants	Binds cervical epithelial cells	Epithelial cell adherence
			Blocks intracellular killing by neutrophils non-immune binding of IgA	Epithelial cell invasion
				Resistance to phagocytic clearance
Serine protease	*csp*A	CspA protein (142 kD)	Cleaves fibrinogen to fibrin-like fragments	Resistance to phagocytic clearance?
				Promotes tissue spread
Fibrinogen receptor	*fbs*A	FbsA protein (44.2 kD)	Binds fibrinogen through repetitive structure motifs	Extracellular matrix attachment
				Epithelial adherence
				Resistance to opsonophagocytic killing