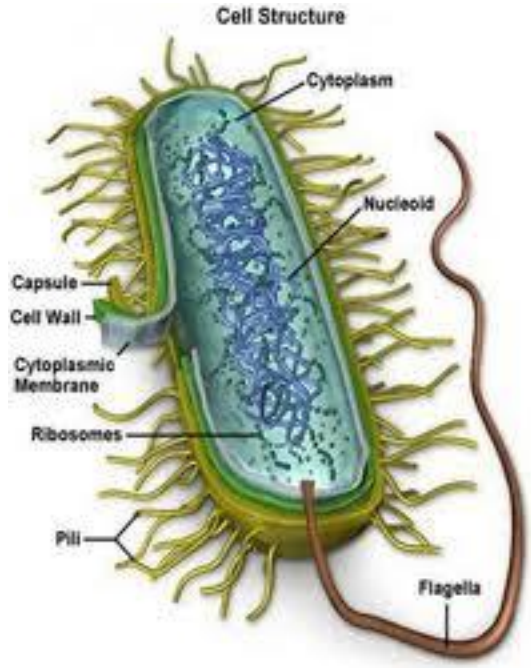
Neonatal Meningitis: Pathogenesis and Prevention of Central Nervous System Injury



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Outline

- Which infants need a lumbar puncture?
- Diagnosis of neonatal meningitis (what are normal/abnormal cerebrospinal fluid values?)
- Pathogenesis of meningitis and pathophysiology of brain (cellular) injury
- Treatment of infants with meningitis (antibiotics)
- Management of cerebral edema

The Case Begins

- G.S. was a 2.6 Kg male infant born to a 23 year old gravida 6, para 5 woman after a 36 week gestation complicated by PPROM. Membranes were ruptured for 18 hours. Mrs. S was afebrile until two hours prior to delivery when she developed a temperature of 37.8 degrees centigrade. A vaginal culture done at 35 weeks gestation was positive for GBS and intrapartum ampicillin was administered four hours prior to delivery.

The Case Continued

- The baby was delivered by cesarean section. Apgar scores were 7 & 8 and he was sent to the NICU because of grunting and tachypnea. The estimated gestational age was 36 weeks. The initial chest x-ray appeared wet and the care providers debate whether antibiotics are indicated and if a lumbar puncture should be done. What would you do?

Clinical Decisions

A. Observation (no additional testing)

B. Antibiotics following blood culture & lumbar puncture

C. Antibiotics following blood culture (no lumbar puncture)

Should the Lumbar Puncture Be Part of the Sepsis Workup in the Healthy-Appearing Infant with Suspected *Early-onset Sepsis*?

Probably not!

Lumbar Puncture in the Asymptomatic Infant

	Risk factors *Symptomatic*	No risk factors *Symptomatic*	Risk factors *Asymptomatic*
Fielkow (1991)	1.5%	2.0%	0%
Kumar	3.4%	3.3%	0%
Johnson (1997)	3.2%	---	0%

Should the Lumbar Puncture be Part of the Sepsis Workup in the *Symptomatic Infant* with Possible *Early-onset Sepsis*?

- It is difficult to distinguish infants with non-infectious conditions from those with early-onset sepsis.
- Meningitis is present in 10-25% of bacteremic infants.
- Up to *1/3* of Infants with meningitis have negative blood cultures. Therefore the decision to do an LP cannot be based on blood culture results.

Should the Lumbar Puncture be Part of the Sepsis Workup in the *Symptomatic Infant* with Possible *Early-onset Sepsis*?

- Meningitis is very unlikely in infants with obvious non-infectious conditions (e.g., RDS or TTN) when there are no risk factors for sepsis.
- An LP should not be a routine part of the sepsis workup, *but ought to be done selectively*.

Should the Lumbar Puncture Be a Routine Part of the Sepsis Workup with Suspected Late Onset Sepsis?

- Retrospective study of *9641* infants: *6056* had ≥ 1 blood culture & 2877 ≥ 1 LP beyond day 3.
- Meningitis was present in at least 5% of infants with suspected late onset sepsis; *One third of infants with meningitis had negative blood cultures.*
- *Lumbar puncture ought to be done selectively.*

Stoll BJ et al Pediatrics 113: 1181-86, 2004

Selective Use of Lumbar Puncture

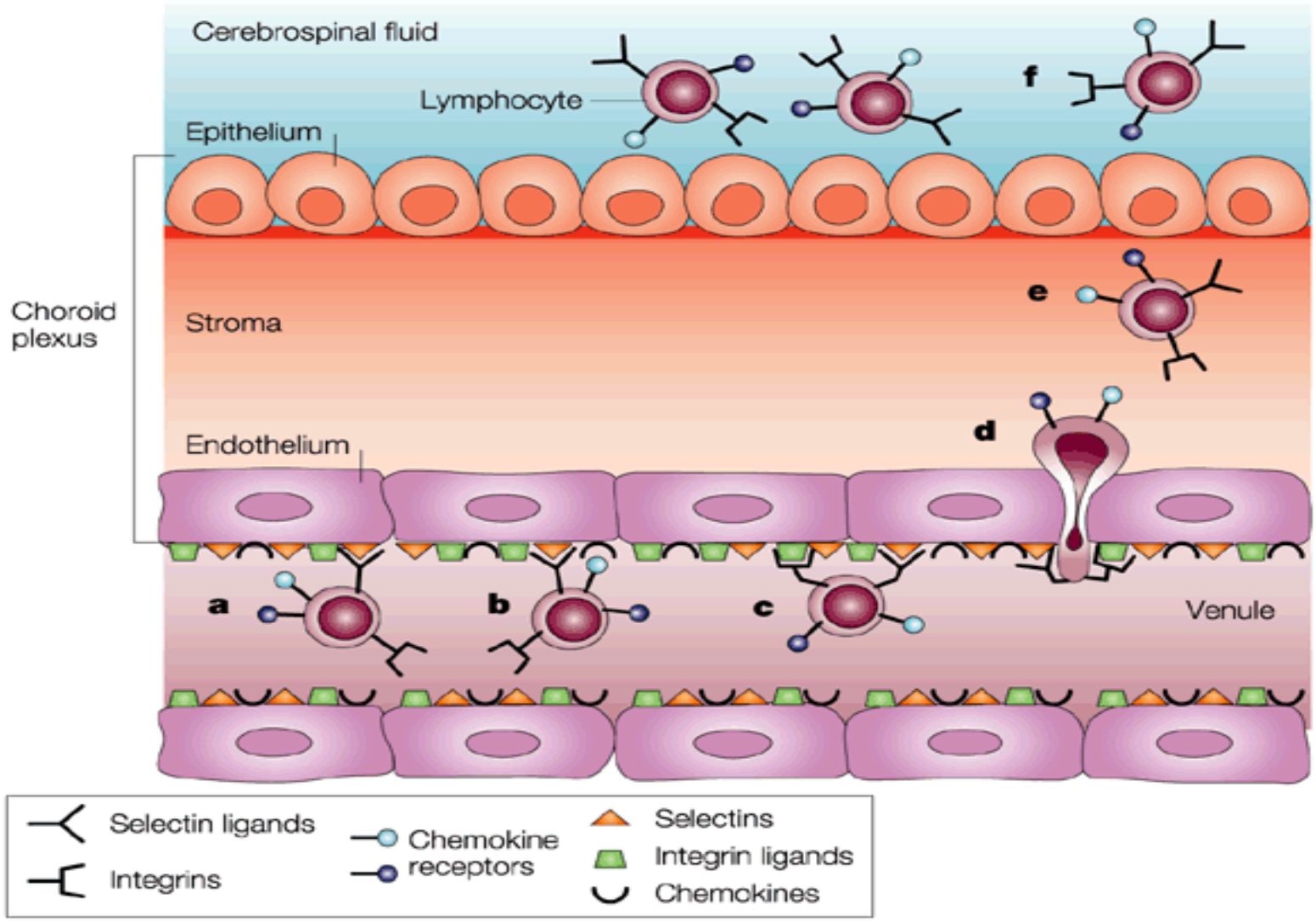
- Lumbar puncture ought to be used selectively for infants *either* with a *positive blood culture* or a *high probability of bacterial or fungal sepsis (based on clinical signs or laboratory data) or infants that do not respond to conventional antibiotic therapy in the usual fashion.*

The Case Continued

- The initial laboratory testing is abnormal and the decision is made to do a lumbar puncture
  - WBC 24/ul (35% PMN)
  - Protein 120 mg/dl
  - Glucose 45 mg/dl

- *How Should the CSF Values be Interpreted?*

The Blood-CSF Barrier Allows Physiological Movement of Leukocytes into CSF



Nature Reviews | Immunology

Cell content of CSF Under Physiological Conditions

- T cells 80% (increased CD4+ cells)
- Monocytes 5%
- B cells 1%
- Neutrophils-rare

*CSF Values in Healthy Term & Preterm Infants**

	Cell count (Mean)	Protein (mg/dl)
Ahmed (Term)	*7.3 (90th percentile 11.0)*	*64 ± 24*
Bonadio (Term)	*11.0 (90th percentile 22.0)*	*84 ± 45.1*
Martin-Ancel (Term)	*1.0 (range 0-5)*	*No data*
Nascimento-Carvalho :Term@	*4.5 (95th percentile 11.7)*	*77.6 ± 31.5*
:Preterm@	*5.1 (95th percentile 16.7)*	*101.2 ± 45.7*
Kestenbaum (Term & Preterm (n =142)	*9.2 (95th percentile 19.0)*	*No data*
Srinivasan: Term (n = 148)	*Median 3.0 (upper IQR + 1.5x IQR 14.0)*	*74 (median)*
: Preterm (n = 229)	*Median 3.0 (upper IQR + 1.5x IQR 14.0)*	*104 (median)*

* *All studies excluded traumatic lumbar puncture and were sterile; Ahmed and Bonadio did viral cultures*

Excluded enteroviruses, HSV, Syphilis, seizures, non-CNS bacterial infection and traumatic LP (< 500 RBCs)

@ Cisternal puncture

CSF Values in the Healthy Infant

- More than 15 cells in a CSF sample should probably be considered suspect and more than 20 cells elevated; most cells should be mononuclear
- The CSF glucose concentration should be 70-80% of the blood glucose concentration.
- Protein concentrations in excess of 100 mg/dl in a term infant should be viewed as suspect.
- Protein concentrations in preterm infants often exceed 100 mg/dl and there is an inverse correlation with gestational age.

CSF Values

- With a *delay in analysis* (> 2 hours) CSF WBCs & glucose decrease significantly
- *Pretreatment with antibiotics* does not modify CSF cell counts (but increases CSF glucose levels and lowers protein concentrations)
- Adjusting the CSF white blood count for a traumatic tap does not improve the diagnostic utility (loss of sensitivity with marginal gain in specificity)
- The number of bands in the CSF does not predict meningitis

What is the Correlation Among CSF Cultures, Blood Cultures and CSF Parameters (> 34 weeks gestation)?

- Retrospective study of 95 neonates with positive CSF cultures felt to represent meningitis.
- *Only 62% had a positive blood culture*
- Neonates *without meningitis* had a median WBC of 6/mm^3 &
- neonates *with meningitis* had a median WBC of 477/mm^3
- Protein and glucose concentrations were highly variable.

- *Meningitis can occur with normal WBC, protein & glucose levels.*

Garges et al 117: 1095, 2006

Correlation Among CSF Cultures, Blood Cultures and CSF Parameters

	Sensitivity(%)	Specificity(%)
WBC		
1-8	*97 (88-99)*	*11 (10-12)*
9-21	*83 (71-91)*	*61 (60-63)*
22-100	*79 (67-88)*	*81 (80-82)*
>100	*66 (52-78)*	*94 (93-95)*
Glucose		
< 20	*44 (30-58)*	*98 (97-99)*
20-60	*89 (78-96)*	*20 (18-21)*
Protein		
41-90	*100 (84-100)*	*2 (1-3)*
90-120	*84 (71-92)*	*28 (27-29)*
>120	*76 (63-87)*	*63 (62-64)*

- *12.6% if infants with meningitis had a CSF WBC < 21 mm^3.*
- *There were 12 infants with meningitis who had CSF counts ranging from 1-13/mm^3.*
- *Protein concentrations were <100 mg/dl in 6/12 infants*
- *Glucose concentration were normal in 12/12.*

Garges et al 117: 1095, 2006

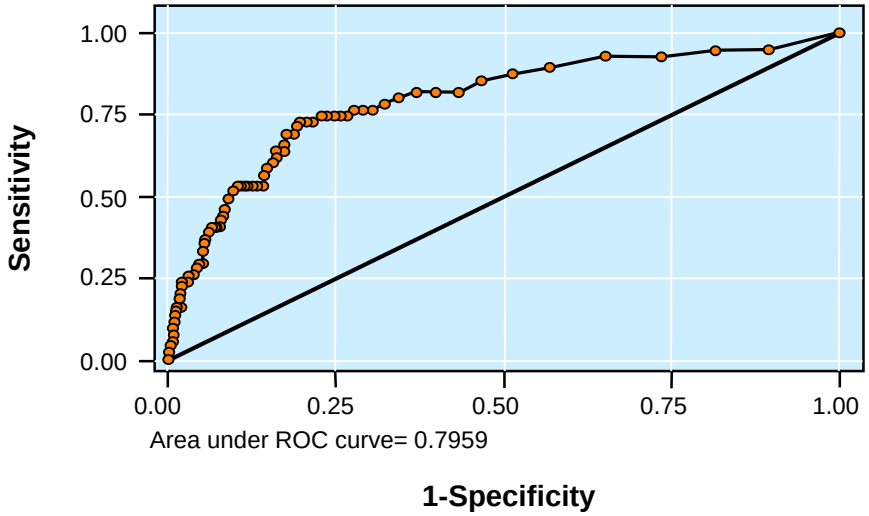
What is the Correlation Among CSF Cultures, Blood Cultures and CSF Parameters (≤ 34 weeks gestation) ?

- Retrospective study of 95 neonates with positive CSF cultures felt to represent meningitis.
- *30%* of the blood cultures were negative, and 5% were discordant.
- Staphylococcus aureus and Candida were the most common pathogens
- Neonates *without meningitis* had a median WBC of 6/mm^3 & neonates *with meningitis* had a median WBC of 110/mm^3
- The median CSF protein concentration was higher (217 vs. 130 mg/dl) and the median CSF glucose concentration was lower (43 vs. 49 mg/dl) in infants with meningitis.

Smith et al Am. J. Perinatol. 25:421, 2008

CSF values in preterm infants with meningitis

	Sensitivity	Specificity	PPA
WBC > 20	73	79	7
WBC > 25	69	82	8
Protein > 170	61	75	5
Glucose <24	32	96	14



CSF White Blood Count

Area under the ROC curve

WBC	.80
Protein	.72
Glucose	.63

★ 22% of preterm neonates with meningitis had normal WBC, glucose and protein levels.

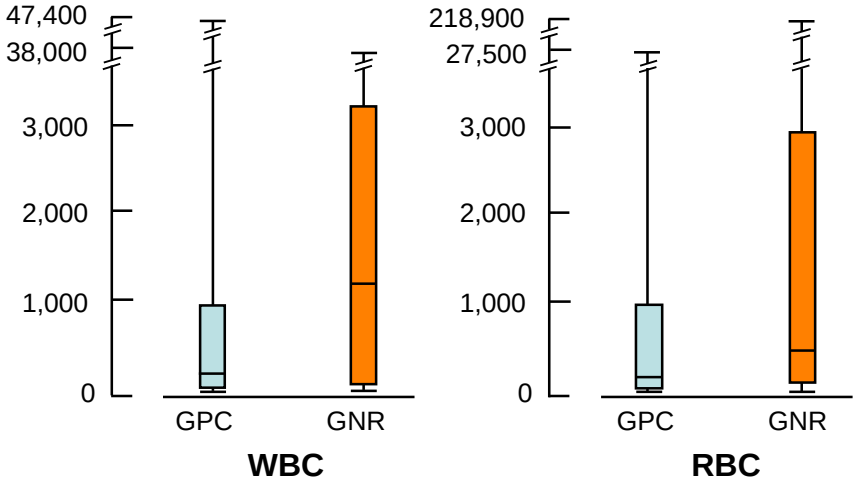
Retrospective Study of Bacterial Meningitis in Infants

- 63 cases of proven bacterial meningitis; and 50 cases of suspected meningitis; in those 50 cases 80% were after antibiotics in babies with positive blood cultures
- E coli and GBS were the most common pathogens.
- Blood cultures were positive in 2/3 of cases
- CSF WBC counts: 2.7% of infants with culture proven meningitis had no pleocytosis. An additional 3.5% had CSF WBCs < 25

Ouchenir et al Pediatrics 2017

A Comparison of Gram positive and Gram negative Meningitis in Neonates.

★ CSF cell counts from 163 infants with meningitis (77 gram negative & 86 gram positive) *Coagulase negative staphylococci were excluded*



Median counts/mm³

Species	N	Median	Range
All GNRs	46	*1217*	(1–38 000)
Escherichia coli	22	2525	(1–15 900)
Pseudomonas	4	757	(7–7480)
Enterobacter	4	657	(4–5850)
All GPC	41	*187*	(0–47 400)
GBS	28	271	(2–13 000)
Staph aureus	9	35	(0–980)
Enterococcus	2	23 701	(3–47 400)

No difference in protein or glucose values

Smith PB et al J. Perinatol. 26: 11, 2006

Pathogenesis of Bacterial Meningitis

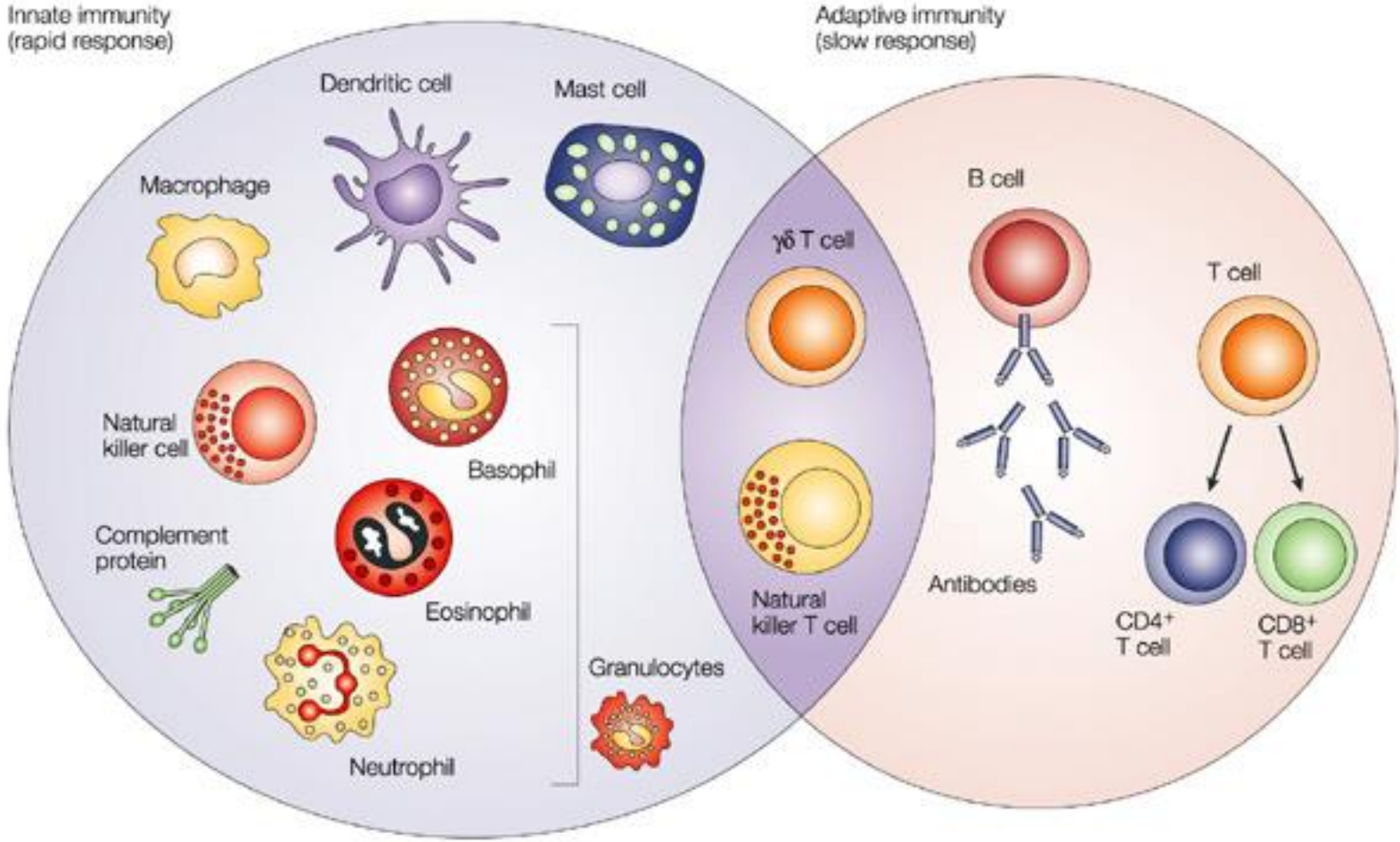
- Nasophayngeal colonization with subsequent seeding of the CSF
- Bacterial multiplication and induction of inflammation in the subarachnoid and ventricular systems
- Progression of inflammation
- Neuronal damage

Nasopharyngeal Colonization

- Bacteria causing meningitis usually colonize the nasopharynx
- Bacterial components which facilitate attachment include *fimbriae and pili*
- Bacteria penetrate the mucosal barrier through or between epithelial cells depending on the pathogen

Intravascular Survival

- Specific antibody offers the best protection, but newborn infants often lack protective antibody.
- In the absence of antibody, the *innate immune system* is the main host defense.



Nature Reviews | Cancer

Skin
Mucous membranes

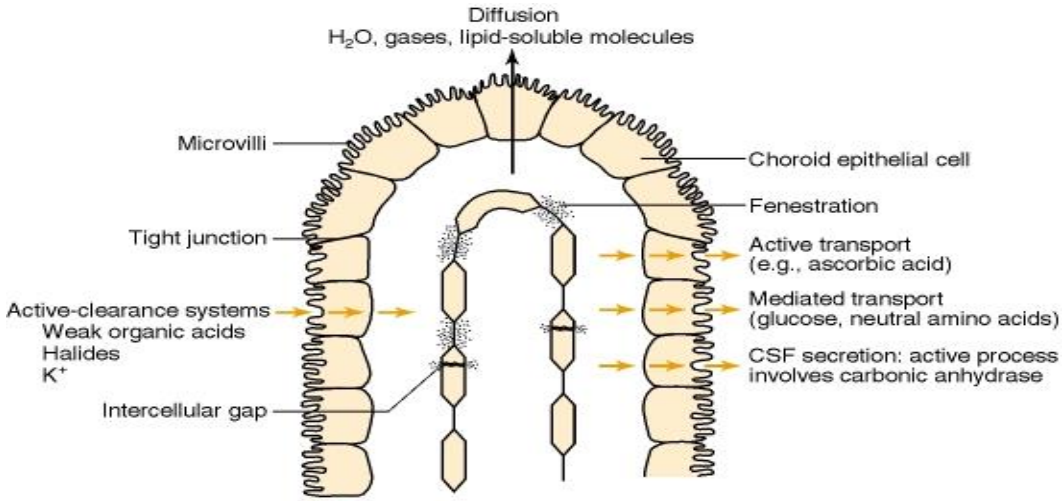
Tears and Saliva
Antimicrobial Peptides

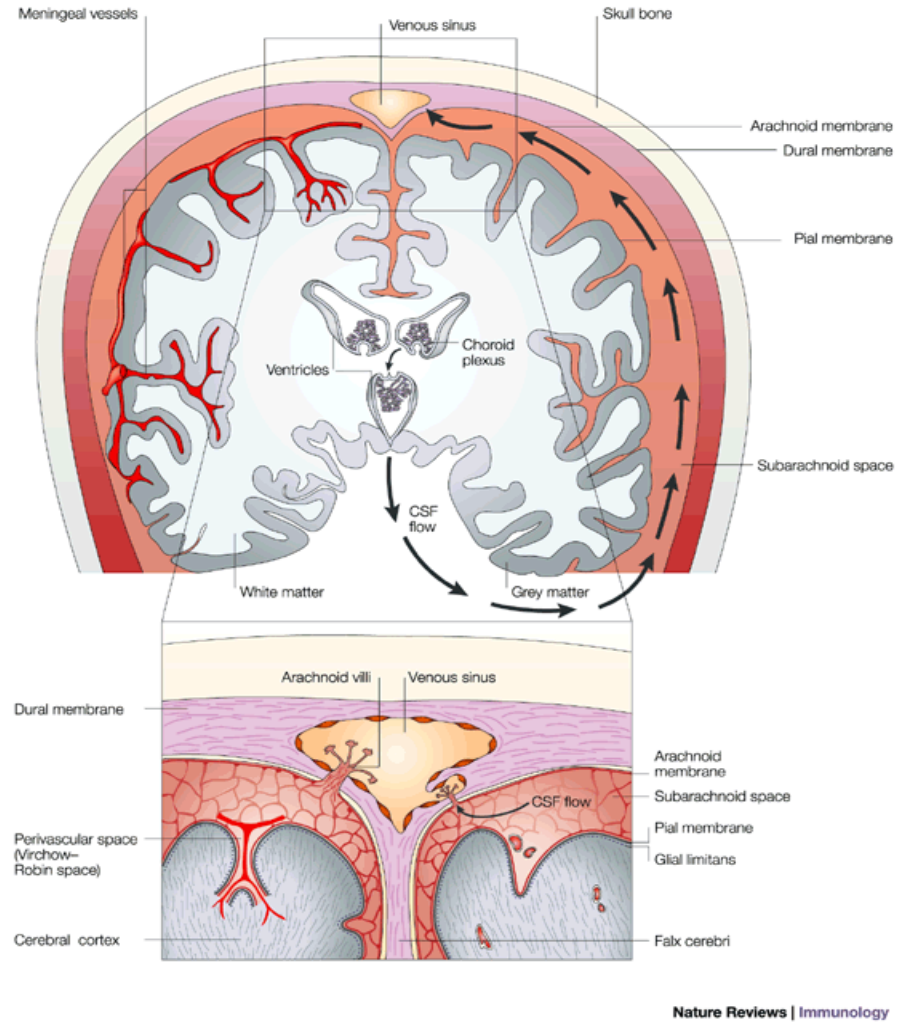
Microbial Entry into the CSF

- To invade the meninges the bacterial pathogen must cross the physiological barriers between the blood and CSF (*Blood-CSF barrier (choroid plexus) & Blood-brain barrier*

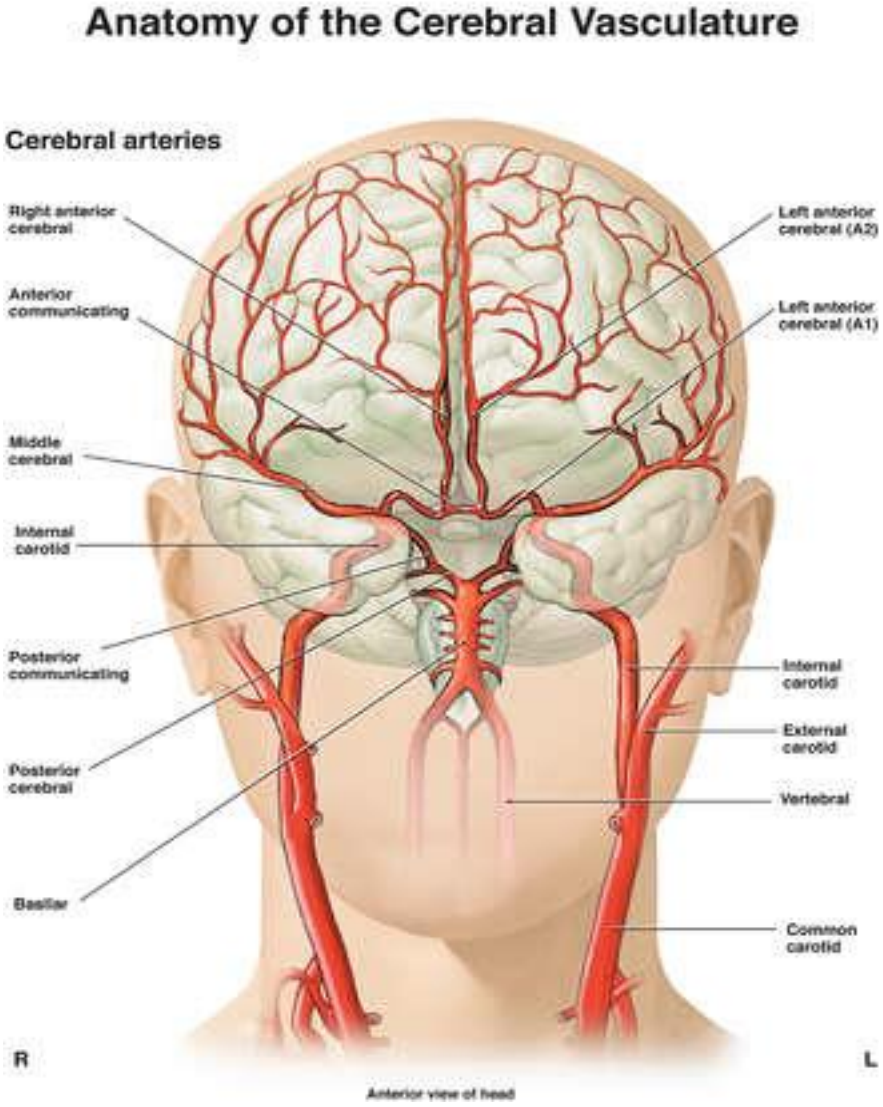
Meningeal Invasion & the Blood-CSF Barrier

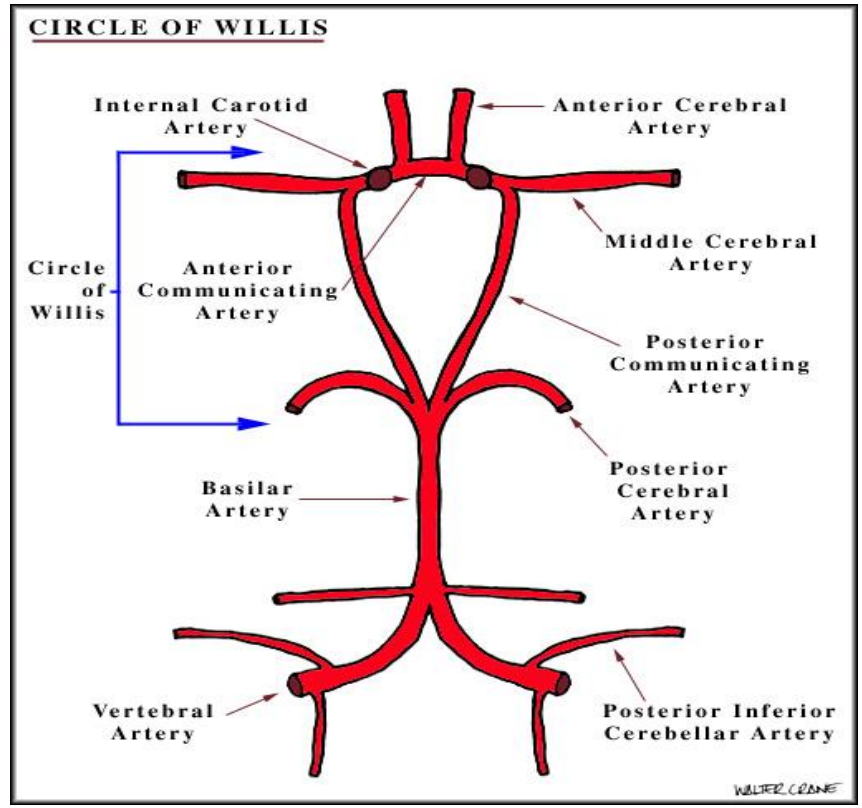
- The blood-CSF barrier consists of choroid plexus epithelial cells are arranged in villi surrounding vascular stroma
- The capillaries of the choroid plexus stroma are *fenestrated and lack tight junctions*

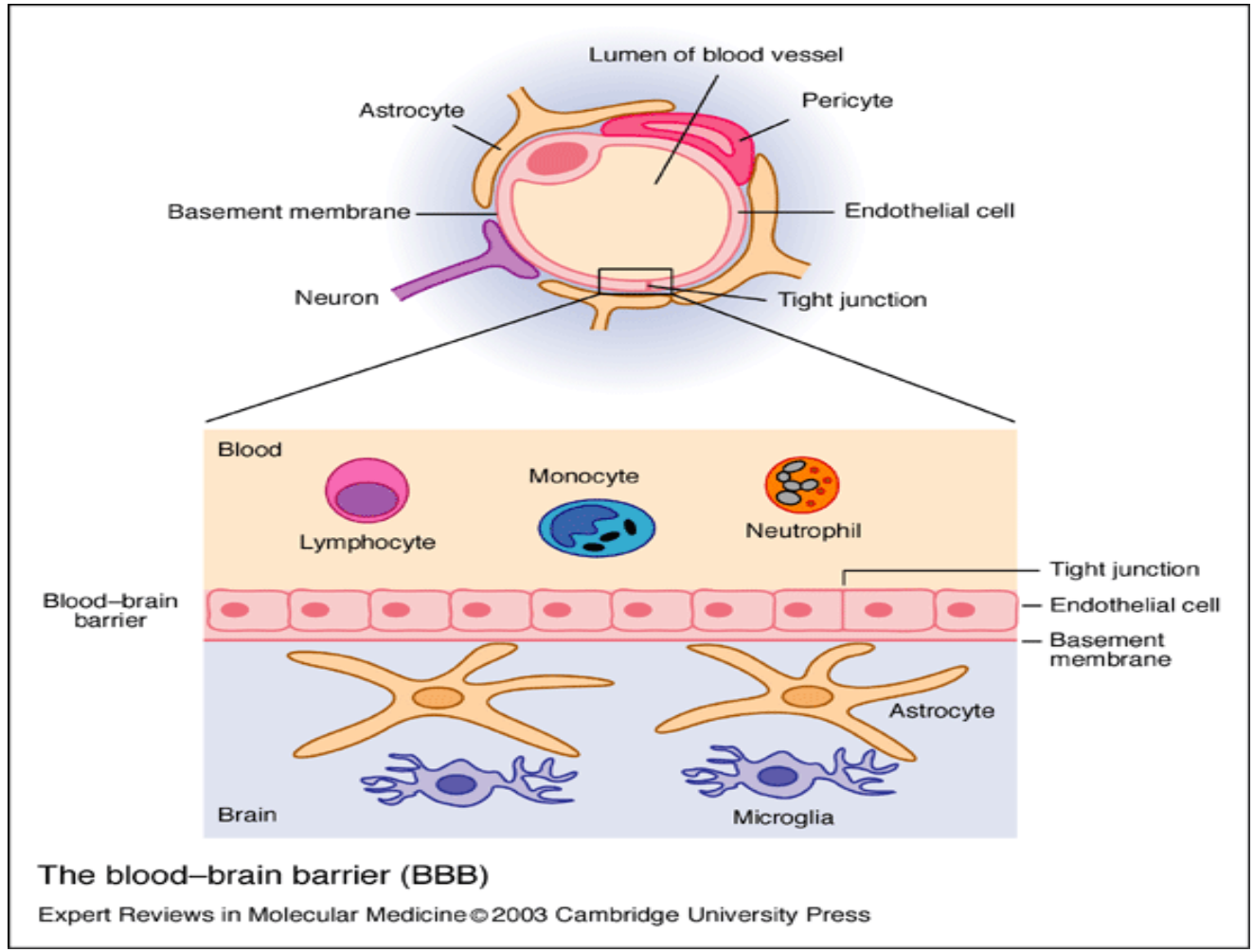




The Blood-Brain Barrier







The blood–brain barrier (BBB)

- The blood-brain barrier (BBB) restricts passage of pathogens and macromolecules
- The cells lining the BBB have a poor capacity for pinocytosis, but have specific transport systems

- Blood-borne pathogens cross *the blood brain barrier* by a receptor mediated, *transcellular* mechanism.

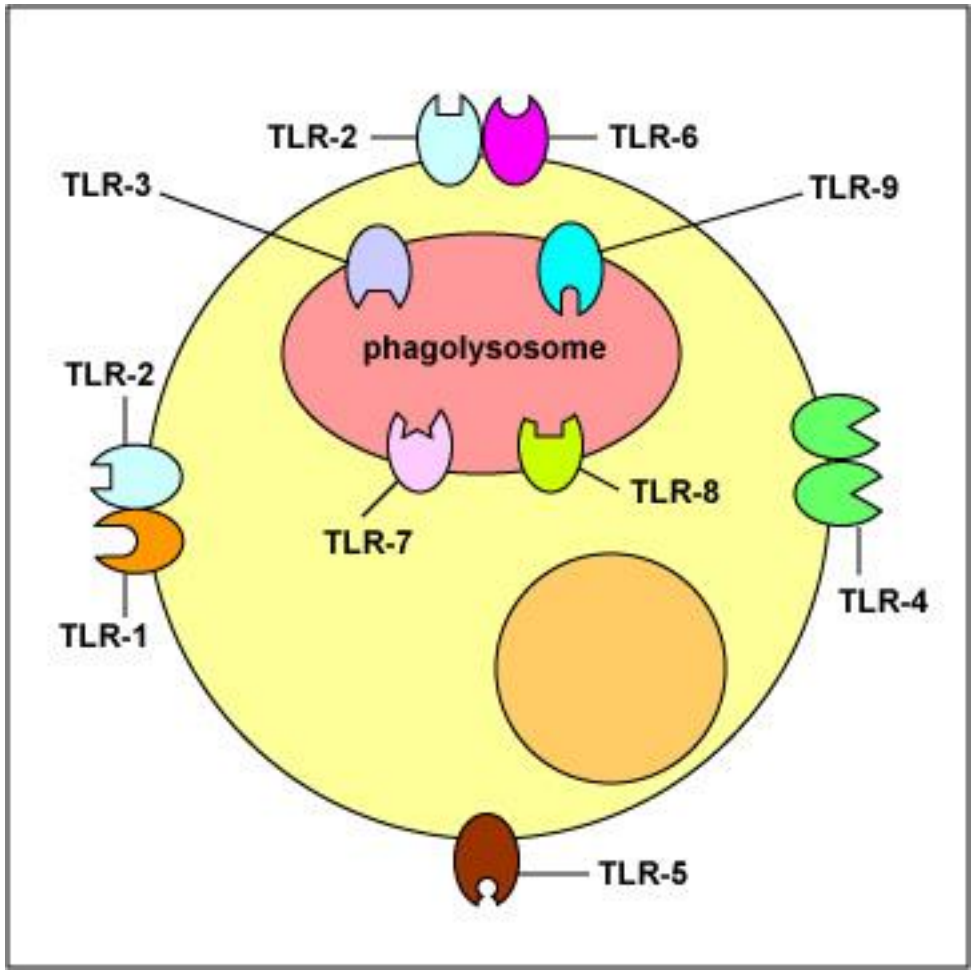
Microbial Entry into the CSF- A Ligand Receptor Process

- *Adhesion*: ligand-receptor interaction that activates the endothelial cell
- *Uptake* of bacteria into a vacuole.
- *Exocytosis:* Cytoskeletal mechanics transfer the pathogen to the basolateral surface where it exits.

Bacterial Multiplication and Induction of Inflammation

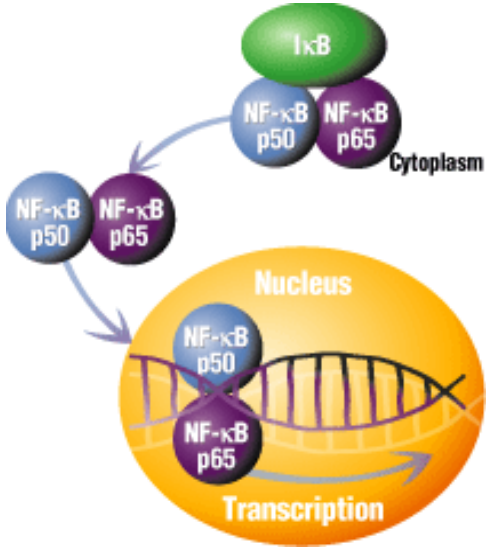
- Host defense mechanisms are severely limited in the CSF (localized area of host immunodeficiency)
- Most blood components do not enter the CSF under physiological conditions.
- Replication and autolysis of bacteria leads to the release of bacterial components (LPS, teichoic acid & peptidoglycans *PAMPs and DAMPS*) which promote release of proinflammatory proteins through activation of pattern recognition receptors (PRRs).

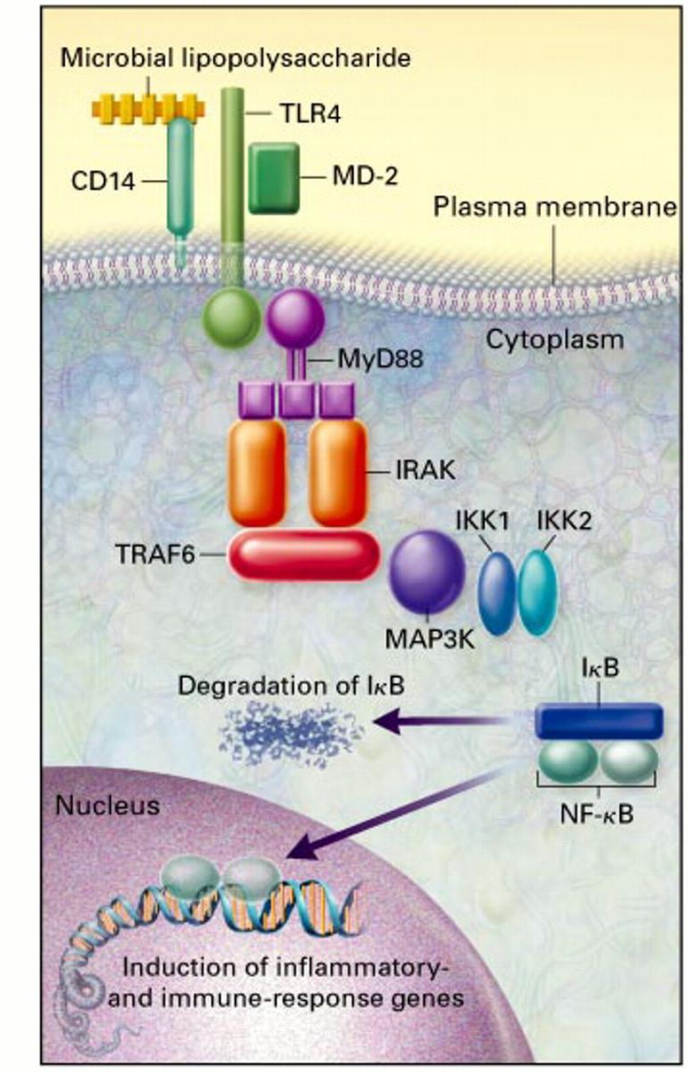
PAMPS Bind to Toll-like Receptors



- Toll like receptors are widely distributed in the CNS (brain & surrounding tissue)
- TLR 1-9 are expressed on microglia.
- Neurons express TLRs 3,7 8 & 9
- Astrocytes express TLRs 2, 3 & 9
- Gram positive bacteria signal through TLR-2 and gram negative bacteria through TLR-4.

Transcription Factors: Molecular Events





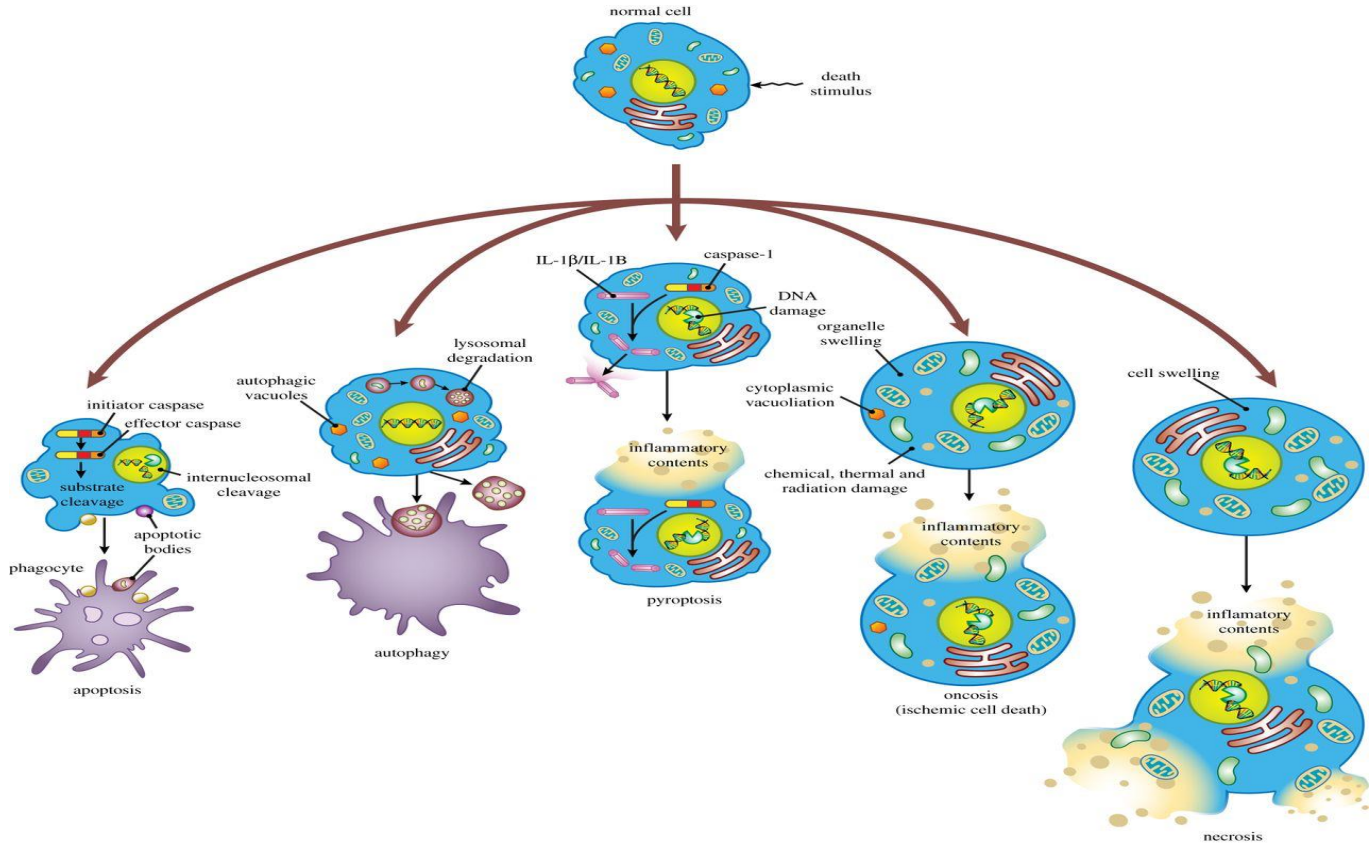
Mechanisms Leading to Neuronal Injury in Meningitis

Neurotoxicity

- *Oncosis* (cell swelling and death with ATP depletion)
- *Apoptosis* Death receptor mediated and mitochondrial mediated pathways
- *Pyroptosis (Caspase 1 mediated >> Inflammasomes - sense microbes in the cytosol)*
- *Autophagy* (degradation of intracellular components through the action of lysosomes)
- *Necroptosis* (triggered by death receptors (TNF binding to TNFR) or TLRs

**Necrosis signifies the postmortem observation of dead cells, but does not indicate a mechanism*

Pathways of Cell Death



Regulated necrosis or Necroptosis

The Case Continued

- G.S. is started on broad spectrum antibiotics. He remains somewhat obtunded; however vital signs are urine output are normal. A head ultrasound reveals slit like ventricles and cerebral edema is suspected. Which of the following interventions is indicated at this time?

A. Fluid restriction
B. Intravenous dexamethasone
C. Intravenous mannitol or glycerol
D. None of the above

Causes of Increased Intracranial Pressure in Meningitis

- Cerebral edema secondary to increased vascular permeability
- Hydrocephalus secondary to obstruction of CSF flow. (ventriculitis or arachnoiditis)
- Abscess or extra-cerebral collection (subdural empyema or effusion)
- Water intoxication: Syndrome of inappropriate secretion of antidiuretic hormone (SIADH).

A Primer on Cerebral Edema

- In adults and older children, the skull is rigid and increased intracranial pressure may be accompanied by herniation.
- The skull of the neonate is expandable and cerebral edema is unlikely to result in herniation, but may result in brain injury secondary to decreased perfusion.
- Brain injury is less likely to occur when arterial blood pressure and perfusion are kept in a normal range.
  - *Maintenance of cerebral perfusion pressure (CPP = MAP – ICP)*

Management of Cerebral Edema

- Fluid restriction
- Dexamethasone
- Mannitol
- Glycerol
- Hyperventilation

- No proven benefit, unless there is SIADH
- No benefit for neonatal meningitis
- Potentially dangerous (contraindicated)
- Limited efficacy data in older children
- No proven benefit and might worsen ischemia

Best Intervention to Lessen Neuronal Injury

- Maintain cerebral perfusion
- Administer appropriate antimicrobial agents

Treatment of Bacterial Meningitis

- The goal of treatment is to achieve bactericidal concentrations of the appropriate antibiotic in CSF, so as to sterilize the fluid as quickly as possible.

Factors Influencing the Bactericidal Activity of Antibiotics in CSF.

- *Penetration* (CSF/blood concentration)
- *Activity* of the antibiotic in CSF
- *Concentration* of the antibiotic itself in the CSF

Factors Influencing Penetration of Antibiotics into CSF

- "*Protein binding*" High degree of protein binding limits entry (e.g., ceftriaxone)
- "*Lipo-solubility*"Solubility in lipids promotes entry (rifampin)
- "*Molecular weight*" Low molecular weight antibiotics (rifampin) and those with a simple structure enter the CSF more readily
- "*Meningeal inflammation*" (increased penetration of hydrophilic agents (e.g., β lactams and vancomycin) but not lipophilic agents.
- *Transport systems*" may eliminate some drugs (cephalothin) from CSF

Factors Influencing Antibiotic Activity in Purulent CSF

1. The decreased CSF pH in meningitis may limit the effectiveness of some antibiotics (aminoglycosides),
2. Highly protein-bound antibiotics may exhibit diminished activity as a result of high CSF protein concentrations
3. The slow growth of bacteria in CSF may limit the effectiveness of antibiotics that depend on brisk growth (β-lactams)
4. Some antibiotics may be metabolized to less active compounds
5. High bacterial densities may limit the effectiveness of antibiotics by an “inoculum effect”

CSF to Blood Concentration Ratios

	AUC_{CSF}/AUC_{serum}
Ampicillin*	0.32 (*with intense inflammation*)
Cefotaxime*	0.12
Aminoglycosides	0.24
Vancomycin	0.30
Meropenem*	0.39

** CSF concentration with uninflamed meninges close to the MICs for moderately susceptible pathogens*

Nau, clinical Microbiology reviews 2010

Treatment of Bacterial Meningitis

- *A regimen of ampicillin and cefotaxime is recommended as empiric therapy of infants with suspected early-onset meningitis.*
- *In a retrospective case series from Canada* all cases of early onset meningitis were susceptible to ampicillin or cefotaxime, but 2/15 were resistant to gentamicin*
- *Gentamicin and cefotaxime resistant pathogens were more frequently recovered with late onset meningitis (10%). Therefore, meropenem might be a better alternative to cefotaxime.*

**Ouchenir et al Pediatrics 2017*

Treatment of Gram-Negative Bacterial Meningitis

- For gram negative meningitis cefotaxime is often paired with an aminoglycoside. (meropenem is recommended for resistant organisms)
- Gram negative meningitis should be treated for at least three weeks (after a documented negative culture)

Treatment of Gram-Positive Meningitis

- Since there is synergism with ampicillin and gentamicin from most GBS, *Listeria monocytogenes* and enterococci, combination therapy is recommended until the CSF is sterilized.
- Gram positive infections should be treated for 14 days from negative CSF cultures.

Intraventricular Antibiotics for Neonatal Meningitis

- In a mixed population of neonates (69%) and infants (31%) with gram negative meningitis (and ventriculitis), the group that received intraventricular antibiotics plus gentamicin exhibited a significantly higher mortality compared with a group receiving only gentamicin.
- Further trails are not justified.

Cochrane Review 2007

Should there be a repeat LP in neonates with meningitis?

- Retrospective observational study of neonates with meningitis who had a repeat LP.
- Infants who had a repeat "positive" LP were more likely to die.
- In a retrospective series from Canada*, 11% of babies had a positive culture 3-8 days after starting antibiotics.
- Repeat LP to determine protein glucose or WBC counts is not useful.
- Repeat LP should be considered > 7 days into treatment in infants that are not responding to treatment in the expected fashion or in infants with fungal meningitis or those with meningitis due to gram negative organisms (where the duration of therapy is often calculated on sterilization).

Greenberg et al J Perinatol 2011

**Ouchenir et al Pediatrics 2017*

Should there be Head Imaging at the end of Treatment?

- In a retrospective series from Canada*, 82% of infants had an abnormal study
- CT and MRI were more sensitive studies than ultrasound
- Clinically significant sequelae (hearing loss, motor problems and developmental delay) were documented in 74% of infants

**Ouchenir et al Pediatric2 2017*

Neonatal Meningitis: Conclusions

- Neonatal meningitis remains a devastating disease.
- Improvements in outcome will result from
  - Interventions which decrease the incidence of sepsis
  - A better understanding of the mechanisms responsible for brain injury.