L7 Overcoming biological barriers & L8 Targeting organs & diseased tissue

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Lecture Series at the

Institute of Chemical Biology & Fundamental Medicine for Siberian Branch of Russian Academy of Sciences

Penetration & targeting of membrane barriers

Intended learning outcomes

To understand the penetration of membrane barriers in improving the bioavailability and targeting of drugs:

a) epithelial membrane barriers, including

- protein absorption *eg* insulin
- absorption enhancers
- chronic lung infection targeting
- choroid plexus CSF secretion & targeting of brain

b) endothelial membrane barriers including

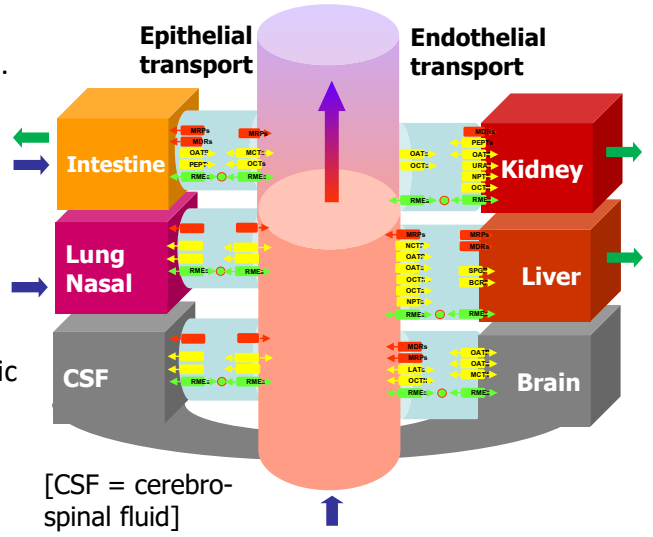
- blood brain barrier (BBB) targeting of brain
- tumour & infection targeting

Introduction: targeting tissues by selective penetration / transport at membrane barriers

Transporters at blood & tissue side of barriers selective for each organ.

Pharma's ideal 'Rule of 5' drugs unselective, penetrate all barriers, suffer drug efflux, 1st pass metabolism *etc*.

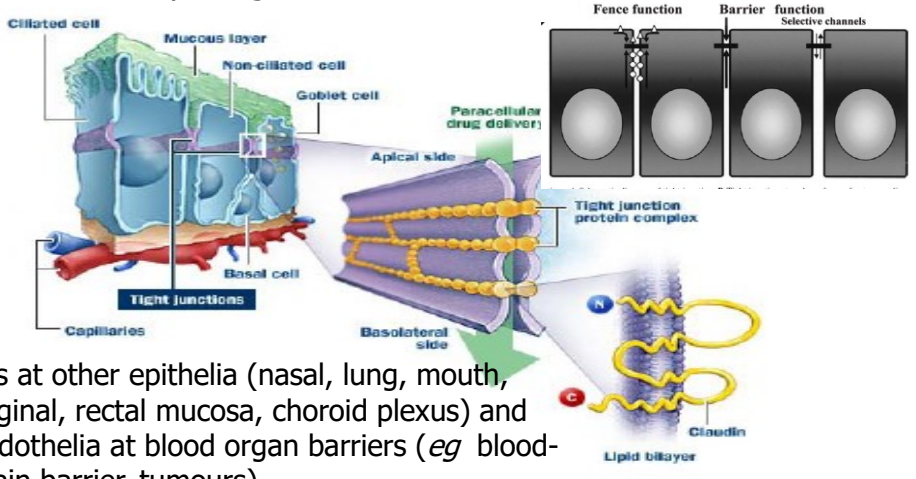
Many large & hydrophilic (*eg* viruses, proteins, hormones) fail Ro5 but selectively transport at organ & tissue barriers.



[CSF = cerebro-spinal fluid]

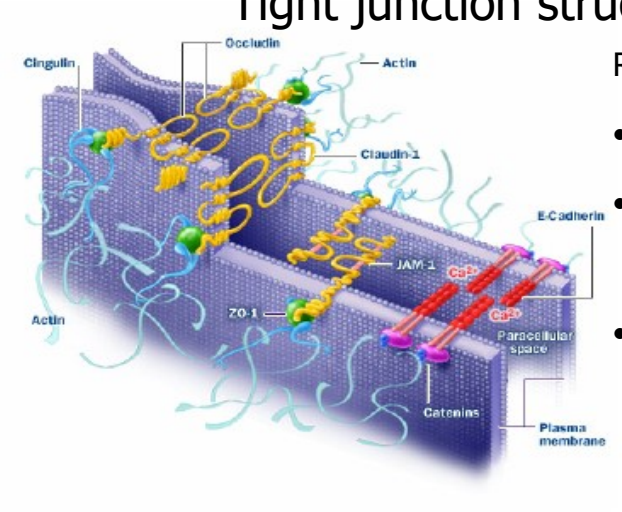
Tight junction biology

Tight junctions (TJs) connect epithelial & endothelial cells → (1) polarity fence functions, and (2) barrier functions regulating permselective passage of molecules across tissue membranes



TJs at other epithelia (nasal, lung, mouth, vaginal, rectal mucosa, choroid plexus) and endothelia at blood organ barriers (*eg* blood-brain barrier, tumours)

Tight junction structure



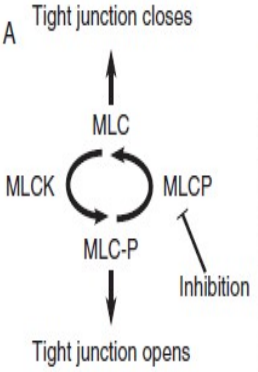
Roles of 3 families of membrane proteins:

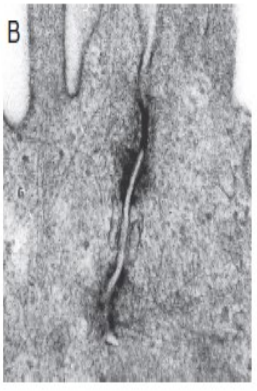
- claudins form tight junction barriers
- occludins interact with regulatory proteins (*eg* kinases), actin
- Ig superfamily member JAM involved in inflammatory reactions, including extravasation of white cells

Dysfunction of tight junctions *eg*
@ epithelia: jaundice (biliary), diarrhoea (GIT)
@ endothelia: edema (tissue swelling)

Tight junction opening & closing

- Opens phophorylation of myosin light chains (MLC) by myosin light chain kinase (MLCK) contraction & opening
- Closes dephosphorylation of MCL-P reverses & closes

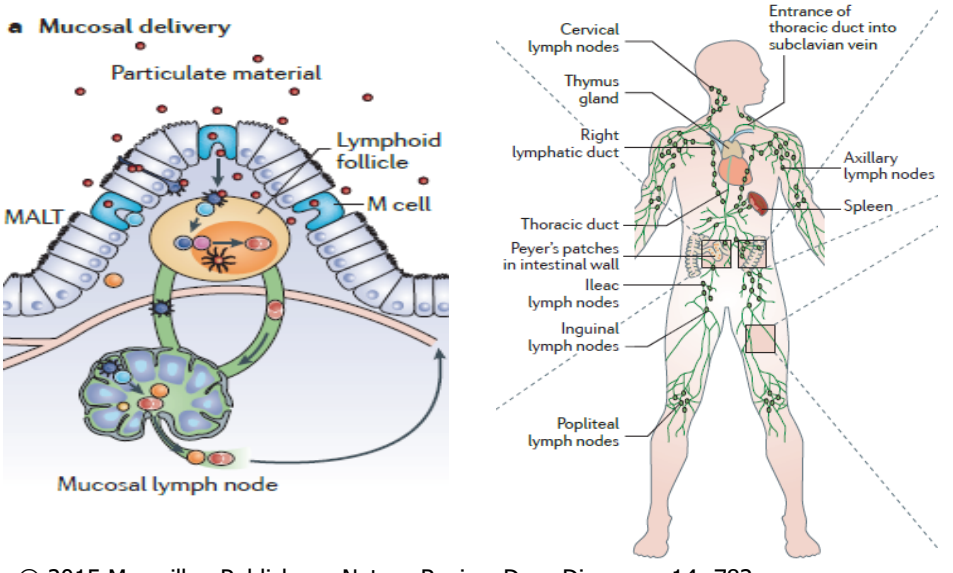




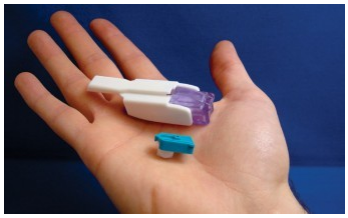
Many cytokines & pathogens affect TJ barriers

- Increasing or protecting barrier function:
  - EGF, TGF-, GDNF, neurturin,84 IL-10, IL-17
- Decreasing barrier function:
  - IFN-γ, TNF-α, HGF, TGF-α, IGF-I , IGF-II, VEGF, IL-1, IL-4, IL-13
- Functional changes (tight junction proteins targeted)
  - *Clostridium perfringens* (claudin-3, -4)
  - *Vibrio cholerae* (occludin)
  - reovirus (JAM)
  - Coxsackievirus and adenovirus (CAR)
  - *Dermatophagoides pteronyssinus* (occludin, claudin-1) dust mite allergy
- Change in actin by modulation of Rho & myosin kinase
  - *Clostridium diphtheriae & difficile,* pathogenic *E. coli*
- Indirect mechanisms such as phosphokinase C & other activations: *Bacteroides fragilis, Helicobacter pylori,* rotavirus

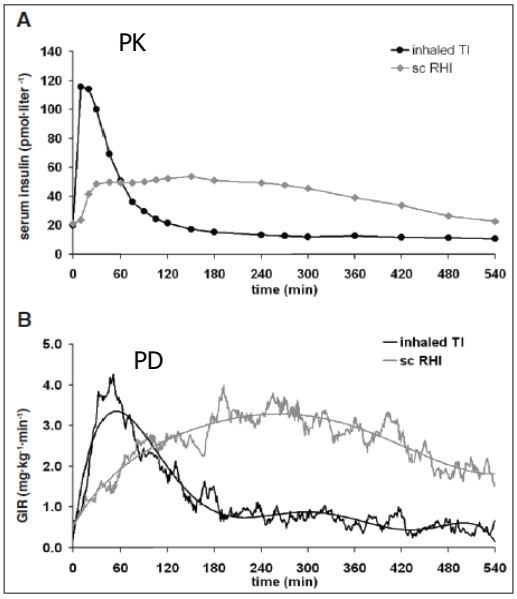
Mucosal lymphatic delivery



Case study: insulin inhalers



MannKind Dreamboat Afrezza DPI provides fast-acting mealtime insulin (Phase 3 / approved). Technosphere 2-3 μm particles of fumaryl diketopeparzine freeze dried with insulin into a dry powder.



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Epithelial permeation enhancer approaches

- Aggregation inhibitors
- Charge modification
- pH control
- Degradative enzyme inhibitors
- Mucolytic or mucous clearing
- Biomembrane penetration
- Vasodilators
- Selective transport

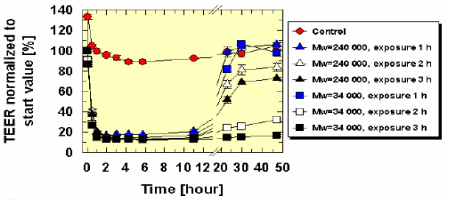
How is each likely to effect membrane permeation?

Example: chitosan permeability enhancement

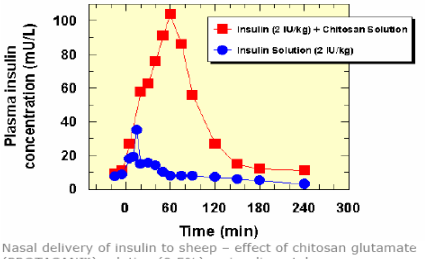
Increased bioavailability of proteins, peptides and small molecule drugs at epithelial barriers (*eg* nasal in figures across right)

Two possible effects:

- slowing clearance from mucus layers by cationic polymer binding to negatively-charged sialic acid in mucous polymer
- opening of tight junctions, which re-seal in the case of higher molecular weight chitosans



Effect of chitosan chloride (PROTASAN™, DA = 84%, 0.5% (w/v)) with high and low molecular weights on Trans-Epithelial Electrical Resistance (TEER) of Caco-2 cell monolayers after different exposure times. Each data point represents the mean of 3 replicate samples.

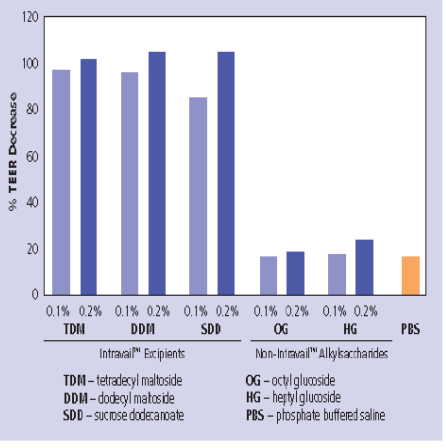


Nasal delivery of insulin to sheep – effect of chitosan glutamate (PROTASAN™) solution (0.5%) on insulin uptake.

Example: alkyl saccharide permeability enhancement

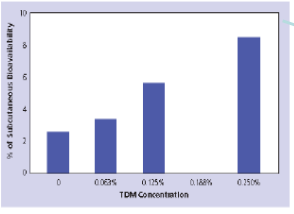
Sugar-based surfactants:

- single chain (octyl glucoside) widely used as a gentle detergent, but with much less permeability enhancement than
- multi-chain alkyl saccharides *eg* Intravail™ group (*eg* large decrease in TEER value, across right)

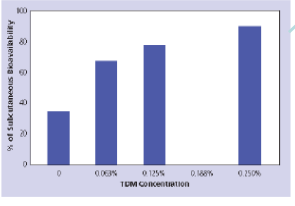


Example: alkyl saccharide enhanced bioavailability of proteins

TDM: oral/GIT heparin absorption



TDM: pulmonary heparin absorption



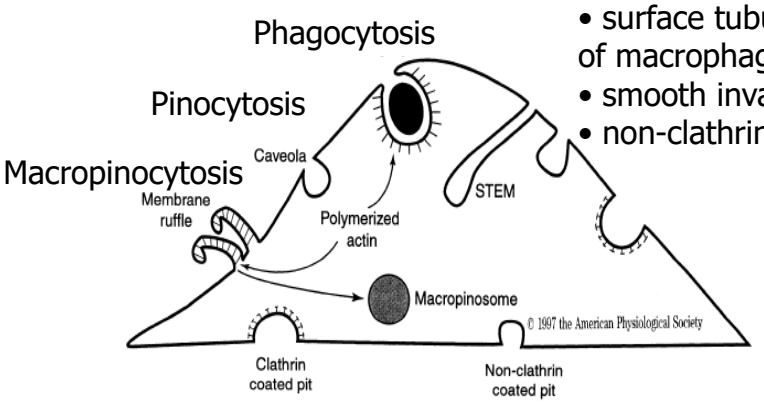
Drug	Approximate Molecular Weight	Bioavailability at 0.125% TDM	References
Intestinal LMWH	5kDa	6%	Yang *et al.*, 2005
Pulmonary insulin	6kDa	22%-24%	Hussain and Ahsan, 2005
Pulmonary LMWH	5kDa	80%	Yang *et al.*, 2004; 2005
Antisense polynucleotide	7kDa	Up to 18%	Aegis unpublished observations

Drug	Approx. Molecular weight	Intranasal bioavailability at 0.125% TDM	Intranasal bioavailability at 0.250% TDM
Calcitonin	4kDa	55%*	96%*
Insulin	6kDa	54%	62%
leptin	16kDa	58%	74%
Human growth hormone	22kDa	30%	50%
Erythropoietin	30kDa	12%	28%

*Compared to *iv* injection; all other proteins compared to sc injection.

Revision: endocytosis

Other non-clathrin modes:
- surface tubules for entry of macrophages (STEM)
- smooth invaginations
- non-clathrin coated pits.



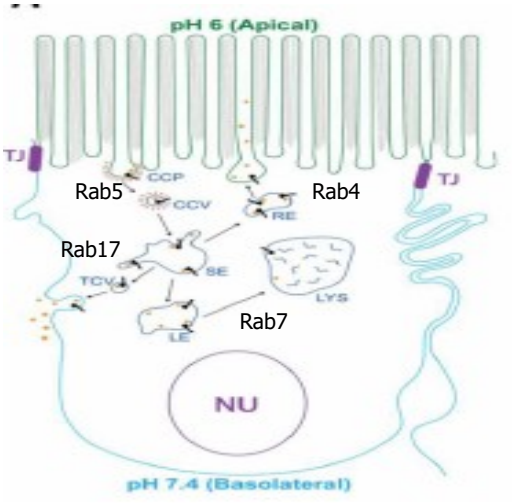
Clathrin-dependent receptor-mediated endocytosis

Clathrin-independent fluid phase endocytosis & pinocytosis

Transcytosis across membrane barriers

Transport of large hydrophilic proteins, nanoparticles *etc* across epithelial & endothelial barriers by endocytosis and exocytosis

- Endocytosis systems at each pole not shown
- Common sorting (SE) compartments
  - to recycle to poles (RE),
  - to degrade contents *via* late endosomes (LE) or
  - to sort into transcytosis vesicles (TCV), which secrete (exocytosis) across cells.
- Rab GTPase proteins associated with control of each stage of vesicle traffic



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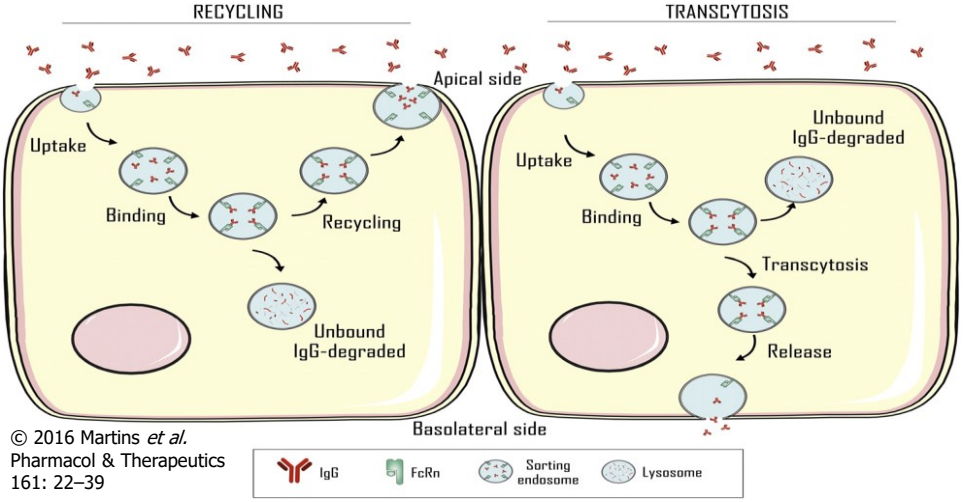
FcRn recycling & transcytosis

At mucosal barriers (gut, lung..), Fc endocytosis at the apical side (external) transfers *via* sorting vesicles to exocytosis on the basolateral side. Like recycling, FcRn binds at acidic pH & releases at neutral pH.



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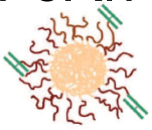
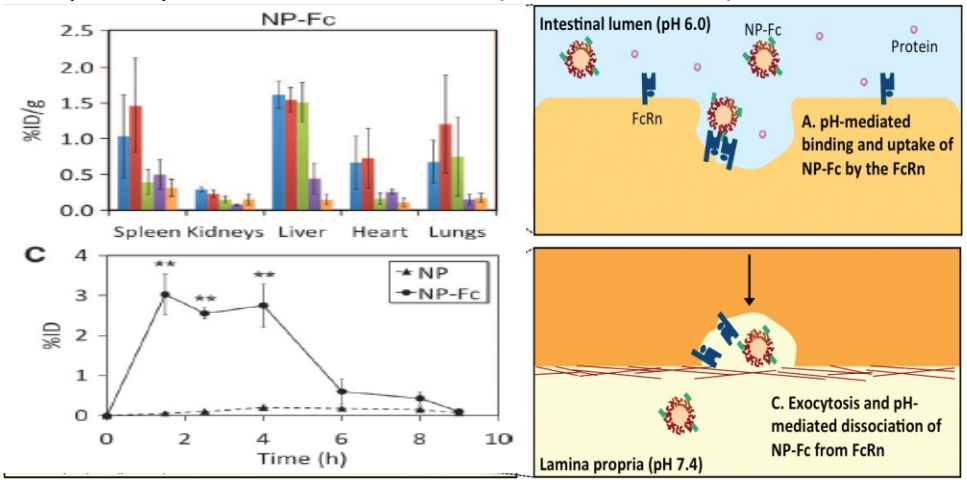
Example: transcytosis *via* FcRn

Polymer NP with PEG stealthing and Fc region of mAb, 10-15% dose absorbed per hour (cf ~1% without) *via* FcRN receptor expressed in small intestine (80% less in colon)



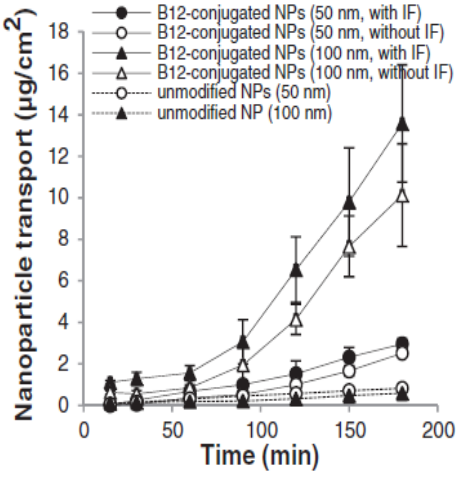


Intestinal transcytosis receptors

Receptors:	**Ligand:**
• Neonatal Fc receptor (FcRn, FcGRT)	• Fc tail region of IgG antibody
• Polymeric Ig receptor (PIGR)	• pIgA
• Lactoferrin receptor (ITLN-1)	• Lactoferrin
• Intrinsic factor receptor (CUBN, AMN)	• Vitamin B12 , folate
• Bacterial toxin receptors	• Shiga & some cholera toxins

Example: Vitamin B12 – nanoparticle transcytosis across lung epithelial cells

- NP flux very low
  - 0.047 ng/s/cm^2
- B_{12} – NP flux >25x higher
  - 1.263 ng/s/cm^2
- Blocked by endocytosis inhibitors
  - chlorpromazine (0.032)
  - filipin (0.027)



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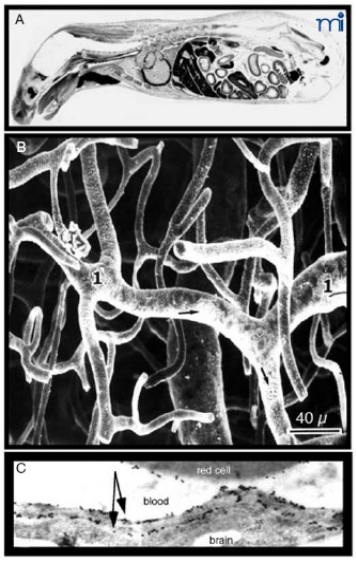
Need for drug targeting to brain

- Drug discovery falling behind advances in neuroscience :
  - 98% (pharma) drugs do not cross the blood brain barrier (BBB).
- Only small molecules with high lipid solubility and a low MW <400–500Da cross, but few diseases respond:
  - depression, chronic pain, epilepsy.
- Many conventional lipid-soluble–low-MW small-molecule drugs do not cross *eg* images across
  - A. penetration of all areas except brain in mouse
  - B. blood vessels 40 microns apart, 1 capillary per 1-2 neurones
  - C. blood - brain



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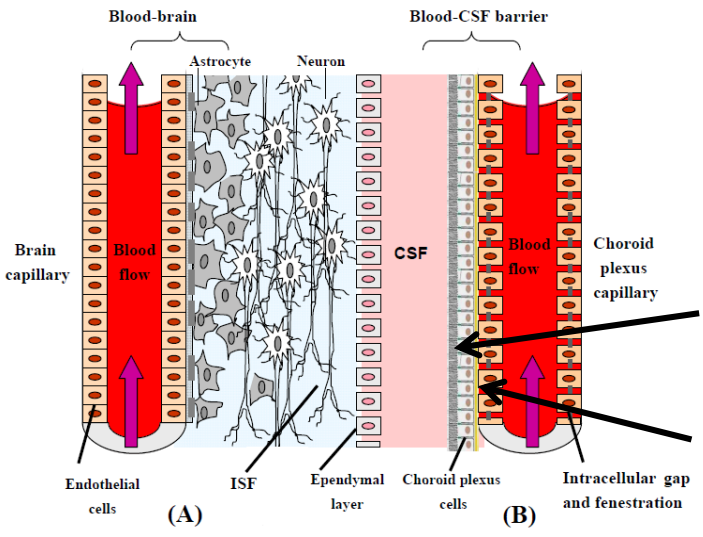
Relevance of Blood-CSF as well as BBB



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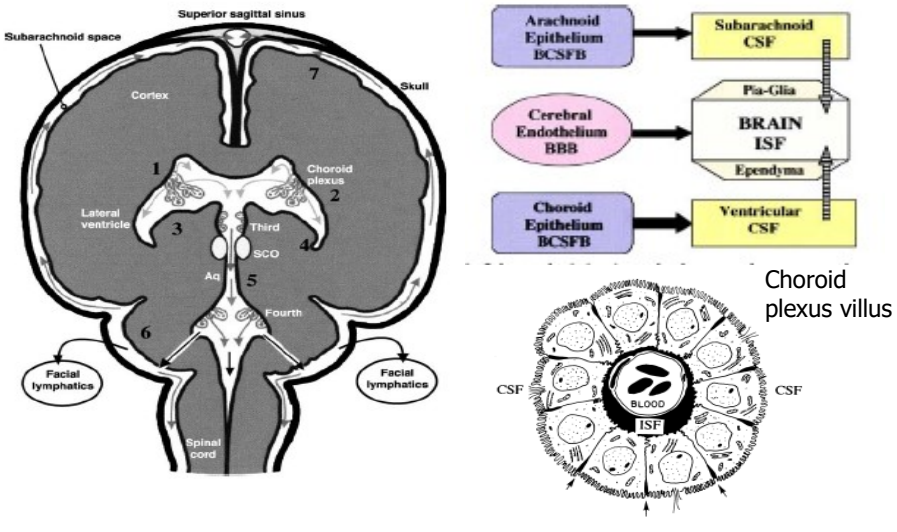
Blood→Cerebro-Spinal Fluid (CSF) barrier is more open than BBB.

BCSFB provided by the epithelial CSF-secreting cells, rather than by open blood capillary endothelium

Cerebrospinal fluid (CSF) route



Choroid plexus villus

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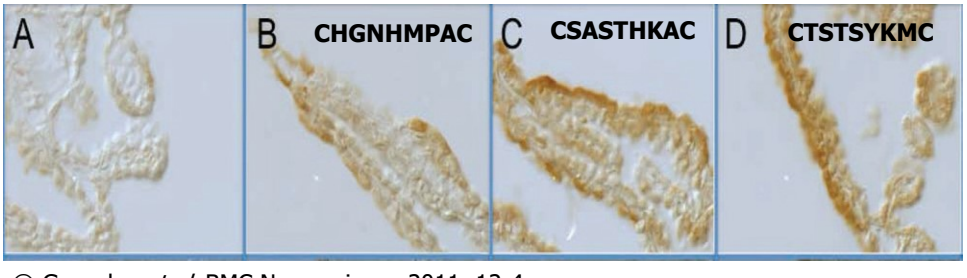
Choroid plexus (CP)

- Initial transport from blood-to-CSF not rate-limiting as in BBB.
- Choroidal vascular perfusion 5-10x mean cerebral blood flow.
- Total area for transport by the four CPs is many fold less but of the same order of magnitude as BBB.
- Macro-circulation ensures the entire CSF volume (140ml in man) secreted from and excreted back into blood every 4-5h.
- Virtually all small and large molecules in blood penetrate into CSF, at a rate inversely related to the molecular weight, but CP epithelial protection against pharma drugs (drug efflux & drug metabolism)
- Injections into CSF diffuse into brain by <0.5mm for large & 1-2mm for small molecules (*ie* not all brain) before clearance.
- Micro-circulation from CSF into brain parenchyma ~20x less than above macro-circulation exchange of CSF.
- Higher and sustained blood drug levels needed for BCSFB.

Example: CP targeting peptides

- Phage display library 'bio-panning' approach to identify peptides homing to choroid plexus and taken up by CP epithelial cells



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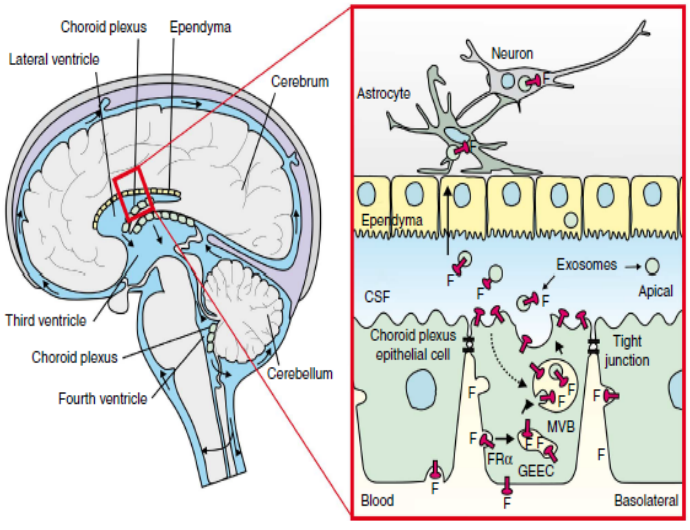
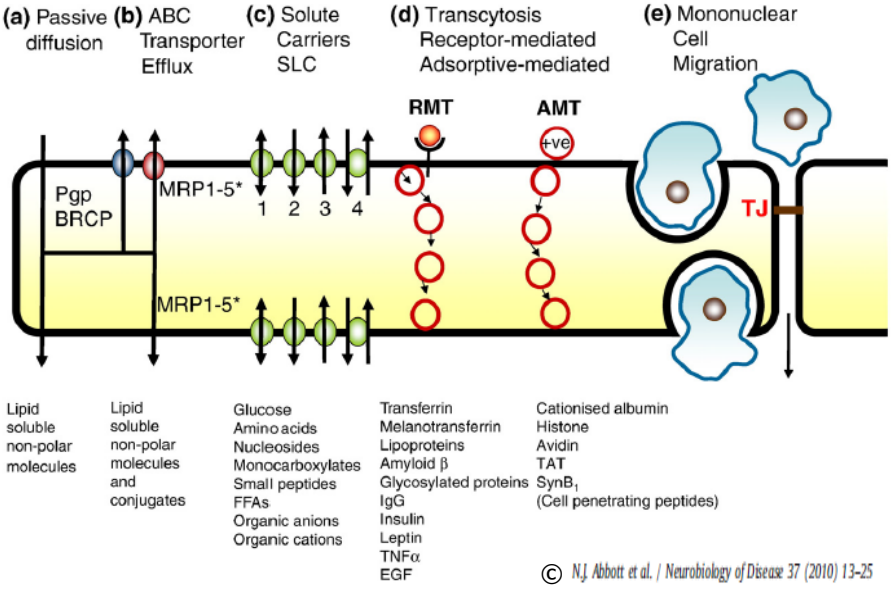
Example: CP folate transcytosis

Folate transcytosis across CP epithelia secreting exosomes into CSF

Exosomes distribute into brain



Transport across blood brain barrier (BBB)

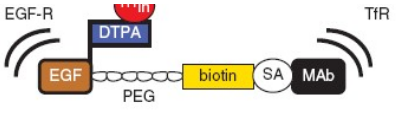


Endocytosis at BBB

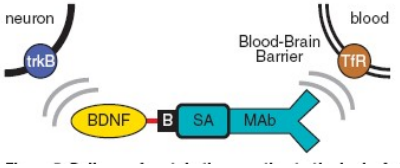
- low fluid phase endocytosis
- higher adsorptive endocytosis
- extensive receptor-mediated endocytosis:
  - **transferrin** iron uptake uses apical membrane recycling of transferrin receptor (TfR) and associated transcytosis *via* basal membrane
  - **leptin** regulates food intake (obesity factor), including by transcytosis into brain to trigger release of neuropeptides from hypothalmus
  - **insulin** facilitates glucose transport *via* insulin receptor in many tissues except brain, though insulin transcytosis across BBB arises by RME
  - **cytokine**s, although produced in brain, also appear to transcytose across BBB by RME *eg* IL-1, TNF

Example: early protein / NP across BBB

Epidermal growth factor – TfR *via* streptavidin (SA) 'lego'

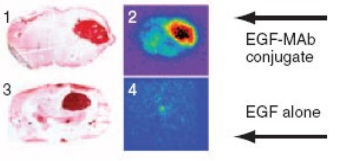


Brain derived neurotrophic factor (BDNF) conjugated to TfR Mab *via* SA–biotin (B) 'lego'



B

MAb binding human EGF-R
EGF alone does not cross BBB



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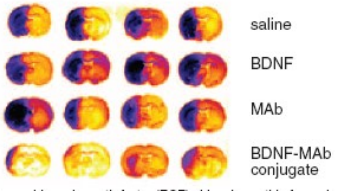


No reduction in stroke volume with BDNF alone, does not cross BBB, even in the infarcted region of brain. 65% reduction in stroke volume with BDNF chimeric peptide

Example: non-viral gene delivery to primate brain

A. PEG immunoliposome (PIL):

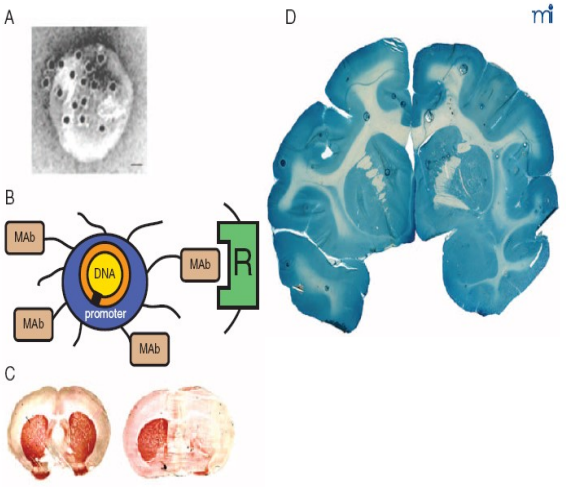
- IgG –PEG binds gold NP second antibody.

B. Plasmid DNA encapsulated in PIL

- conjugated with a receptor (R)-specific targeting Mab
- PEG inhibits uptake of PIL by reticulo-endothelial system

C. Tissue-specific gene expression *in vivo,*

D. greater in grey over white matter



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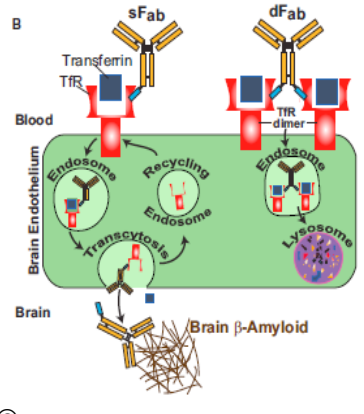
Example: Roche 'Brain Shuttle'

Roche anti-TfR sFab 'Brain Shuttle'



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Differential intracellular sorting:

- monovalent anti-TfR sFab fusions undergo transcytosis across the BBB
- bivalent anti-TfR dFab fusions lead to TfR dimerisation and lysosomal degradation

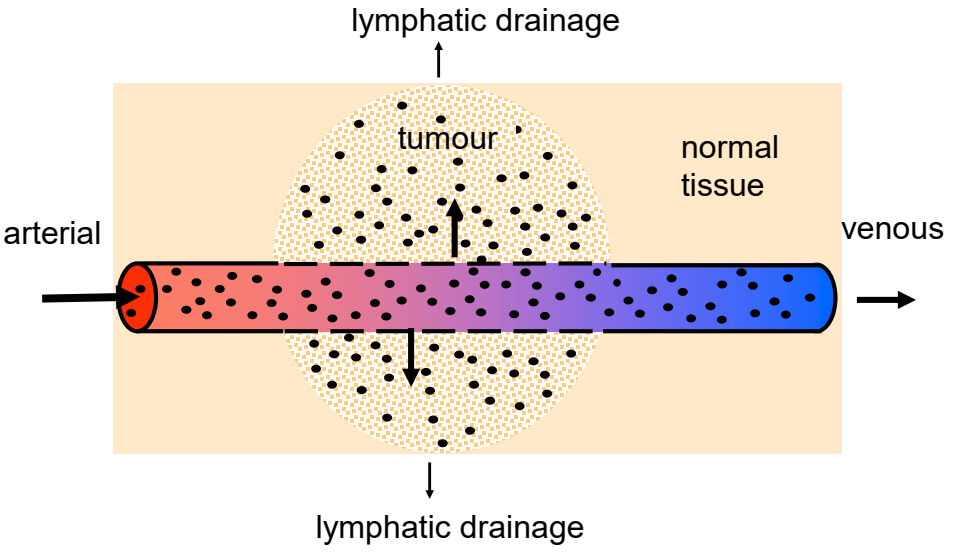
http://www.youtube.com/watch?feature=player_detailpage&v=TfJBwoQNaaw

EPR effect

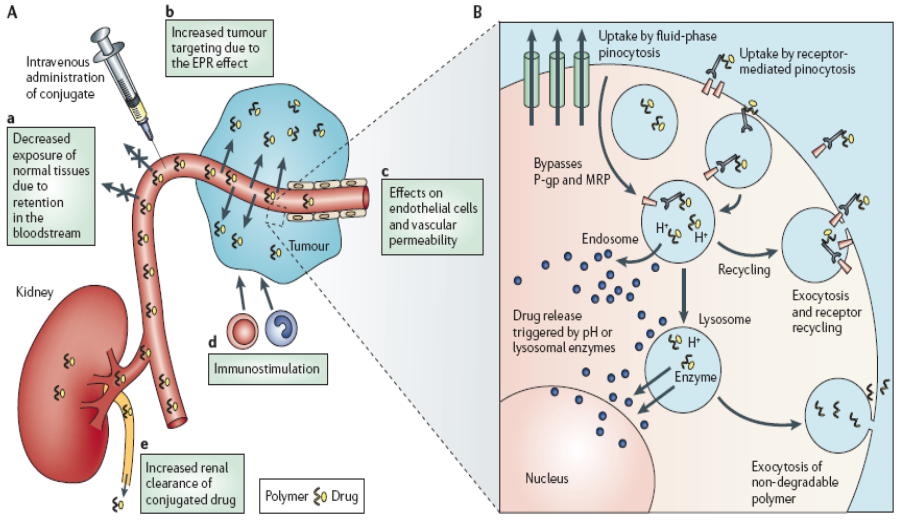
Enhanced permeability & retention (EPR) effect: mainly size-based selective accumulation of nanoparticles in diseased tissue:

1) Stealth modification (*eg* PEG combs) or Fc receptor recycling and <100 nm nanoparticles improves circulation time by reducing clearance rate by reticulo-endothelial system (RES)
2) Vascular leakiness allows extravasation (escape) of nanoparticles across inflamed (infection) and diseased (tumour) tissue capillaries *via* 'leaky' junctions between capillary endothelial cells
3) Lower rate of penetration of nanoparticles through extracellular matrices between cells in the tissue;
4) Lower rate of lymphatic drainage and clearance of nanoparticles from diseased tissue (cf small molecules).

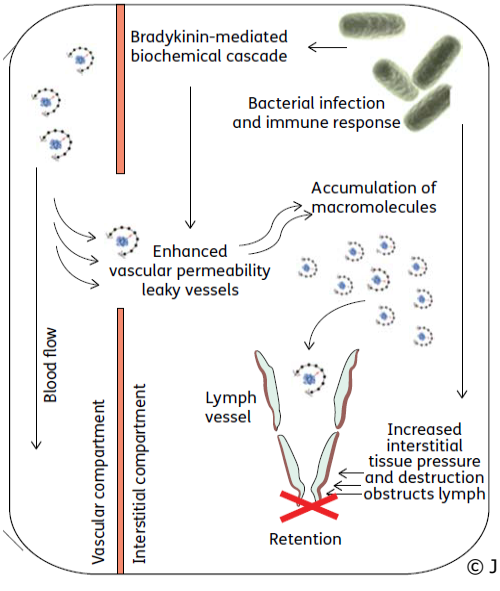
EPR effect principle



Example: polymer drug conjugates



EPR targeting of infection/inflammation sites



Enhanced permeability retention (EPR) effect:

- increased vascular permeability (*eg* inflammation - vasodilation, NO, proteases/bradykinin ...)
- Decreased lymphatic drainage – increased interstitial pressure reduces lymphatic permeability
- Accumulation of nanoparticles in infected tissue