

Biological treatment - principles, technology, examples



Biological drug

- „Biodrugs, biologics, targeted treatment “

Recombinant proteins, peptides,
antibodies, hormones

substances derived from blood / plasma and recombination variants

- „Biologicals, Biopharmacy,
Biopharmaceuticals“

- recombinant proteins, peptides,
antibodies, hormones

+ Stem cells, xenotransplantation, gene and
antisense therapy

Biological drugs

- designed to specifically target a biological phenomenon, gene, protein, or group of genes or proteins thought to be involved in the disease.



Traditional/Classical vs. Biological drug

Small molecule <1kDa
Production of chemical synthesis
/ isolation of plant
Less critical steps in the synthesis
Very well characterized
Molecular mechanism of action
usually better described
Linear dose-response
relationship
Mostly non-immunogenic

Usually with pharmacokinetic
interactions at P450

large, complex molecules, commonly
proteins > 50 kDa
manufactured using living organisms
/ cells

- risk contamination
- own "inherited,, activity

Complex heterogeneous structure
matrix from which was drug isolated
More difficult to characterize
(3D conformation)
mechanism of action is complex,
sometimes not fully understood

Usually immunogenic
Mostly without interaction at P450



Traditional/Classical vs. Biological drug

Often a small molecule, different from endogenous substances

Expose its effects throughout the whole body

Limited efficacy for some states
Easy handling and formulation of DDF

Often very similar / identical with the molecules of the human body.

Usually targeting the center of disease/anormality.

Can be used to treat previously difficult-to-treat diseases.

The difficult adjustment to DDF



Research and development of Biological drugs

- The development of biologics is 10 - 15 years, costs 1.5 billion USD
- Biologics are produced by the genetically modified host cells (bacterial, yeast, mammalian and plant) into which was inserted the genetic information stored in DNA.
- The first drug produced by biotechnological procedure was insulin (in 1978, registering 1982).
- The discovery of biotechnological production of pharmaceuticals, respectively. monoclonal antibodies, was in 1984 awarded the Nobel Prize.



Targeted effects of biologics

- A specific effect of biologics is targeted - a specific target structure, antigen, enzyme, signal path (e.g. tumor cells).
- Biologics are able to identify the damaged cells for destruction by the immune system.
- Can prevent the growth and proliferation of cells that cause disease.
- Can deliver drug directly to the **target** which increases the effectiveness of treatment.



History

- 1972 - obtained recombinant deoxyribonucleic acid (rDNA)
- 1975 - first monoclonal antibody (MAb).
- This was followed by the establishment of the first biotech pharmaceutical companies. USA - Genentech
Europe - Biotech
- 1982 - The first biotech product was - recombinant insulin (Genentech, marketed by Eli Lilly)
- 1986 monoclonal antibody OKT3 (OrthoCloneR) recombination. IFN

Examples of Biological drugs

1) Immunomodulating biologics

MAB (Infliximab) and fusion proteins (etanercept), IFN

2) Hormones – insulin, GH

3) Vaccines – e.g. HVB, HPV

4) Growth factors – erythropoetin, trombopoetin, CSF

5) Enzymes for the treatment of hereditary diseases (monogenic) (e.g. Imiglucerase for the treatment of Gaucher disease)

6) Biologics influencing homeostasis- f. VII, F VIII, F IX, other inh. Of coaglation or activators of fibrinolysis

7) Gene therapy (e.g.. Alipogen tiparvovek – LPL gene)



Advantages for the patients

- Better efficiency vs. "Classical" drugs
- Biologicals are used under the supervision of experts in specialized centers.
- targeted, personalized treatment, which is always personalized
- the patient undergoes a more detailed examination before medication
- better understanding of the basic properties of the drug and its effects
- better solution of possible ADRs, their early detection

Risks and disadvantages of biologic drugs in general



- carcinogenicity
- allergenic potential
- contaminants from the source cells
- stabilizing additives (cryopreservation stabilizers)
- sterility
- stability, variability of drugs (biotechnology products)
- adverse effects - dead teenager in first stage CT - liver toxicity after gene therapy of congenital deficiency of the enzyme using an adenoviral vector (1999)



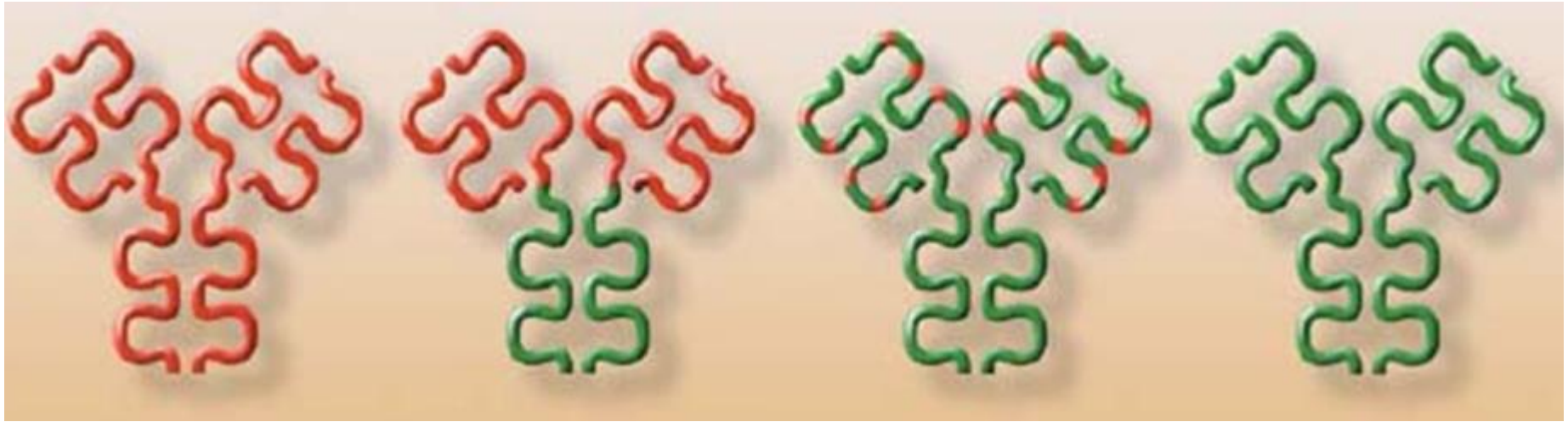
Nomenclature

- Derived from biochemical name (Pegasys -PEG IFN)
- The name given by the manufacturer - unrelated to the effect of the origin
- Hormone with different tradename (Serostim, Saizen, Zorbitive)
- MAB- system root words and suffixes



Nomenclature of MAB

- Generally: suffix -mab
- Letter before suffix:
 - o – of mice origin
 - a - rat origin
 - e - hamster origin
 - i - of primates
 - u – of human origin (human cell line production)
 - zu – humanized
 - xi - chimeric



Mouse MAb

100% of the mice orig.

Hypersensitivity

High levels of Ab

(not used clinically)

Chimeric MAb

34% of mice orig.

Hypersensitivity

Low levels of

circulating Ab

(rituximab

infliximab)

Humanized

5-10% mice orig.

MAb

Hypersensitivity

Low levels of

Neutralizing

Antibodies

(Trastuzumab

Certolizumab)

Human MAb

100% human

Hypersensitivity

Low levels of

Neutralizing

Antibodies

(panitumumab

adalimumab)



Nomenclature of MAB

Sometimes encoding indication

- lim - immune
- bac - bacterial
- cir- cardiovascular
- tu - malignity

E.g. rituximab - chimeric MAB to treat Non-Hodgkin.
lymphomas

alemtuzumab - humanized antibody to the CD52 glycoprotein
CLL



Production of biological drugs

Isolation from natural sources - historically:

- insulin from the pancreas of cattle, pigs (recombinant today)
- h-choriogonadotropin - from the urine of pregnant women (today recombinant)
- hirudin - Medical leeches (*H. officinalis*) (today synthetic / recombinant)

The production of biological drugs - recombinant technology

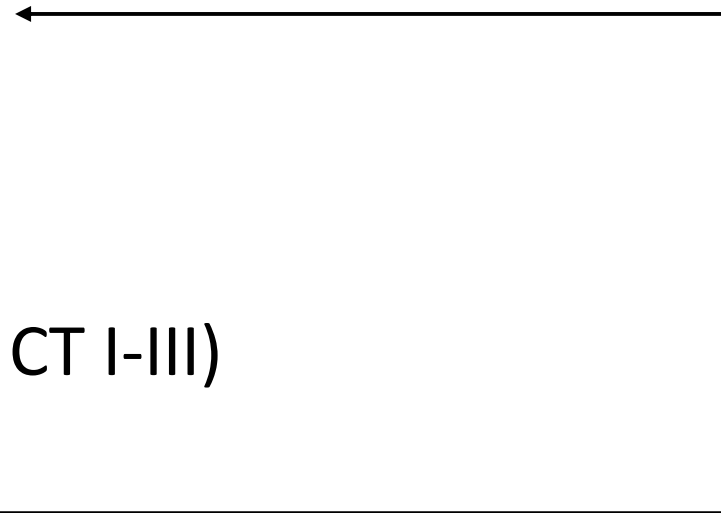


- DNA extraction
- product / synthesis according to library
- transformation / DNA transfection into producer cells
- production
- purification
- stabilization
- testing (biological activity - CT I-III)
- registration (RCT + IV)

The production of biological drugs - recombinant technology



- DNA extraction
- product / synthesis according to library
- transformation / DNA transfection into producer cells
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- purification
- stabilization
- testing (biological activity - CT I-III)
- registration (RCT + IV)



The production of biological drugs - recombinant technology



Transformation/transfection with use of

plasmid

start of replication

marker of selection

polycloning site (enables insertion of DNA of
foreign origin)

transcription promoter and terminator

selection

The production of biological drugs - recombinant technology



- production microorganisms
 - bacteria
 - yeast
- Tissue cultures of higher organisms
- Cell-free expression systems
- Genetically modified animals, plants

The production of biological drugs - recombinant technology



- Production by microorganisms
 - Bacteria - optimized *E. coli* strains (mutations of periplasmatic and membrane protease)
 - Yeasts - *S. cerevisiae* - *Pichia pastoris* ?

The production of biological drugs - recombinant technology



E. Coli

- The synthesis of proteins **without posttranslational modifications**
 - Cheap medium, mutated forms of E. coli with advantageous properties - increase the stability of the gene product ...
 - Modification of wall, transformation of plasmid (DNA product introduction) - thermal shock, electroporation
 - Selection - resistance to antibiotics / cell culture media
 - Production of the peptide together with peptide sequences which ensure binding to an affinity carrier
 - Renaturation
- E.g. IFN , GSM, insulin, growth hormone ...

The production of biological drugs - recombinant technology



S. Cerevisiae

- suitable for the synthesis of proteins **with posttranslational modifications**
- Haploid yeast - the possibility of hybridization
- Easy , economical cultivation generation time of 2h
- GRAS - " Generally regarded as safe"
- Mutants with advantageous properties - increase the stability of the gene product ...

The production of biological drugs - recombinant technology



S. cerevisiae

- modification of cell wall, transformation of plasmid (various vectors)
- selection - auxotrophic strains (disabled biosynthetic pathway for AA, NA); plasmid introduce this gene – only transformed yeast are viable in selection media (ATB)
- production of the peptide together with peptide sequences which ensure binding to an affinity carrier
- renaturation
- E.g. insulin, growth hormone ...

The production of biological drugs - recombinant technology



Pichia pastoris

- higher genet. stability, possibility of similar glycosylation of proteins as in humans

The production of biological drugs - recombinant technology



Tissue cultures of higher organisms

- About 60% of recombinant proteins

positives: same way of modifications as in humans
a wide variety of products
eliminates ethical / technical problems
(isolation, animal cells, the lack of material)

negatives: higher risk of contamination
(rich medium, slower growth, expensive,
difficult cultivation)

The production of biological drugs - recombinant technology



Tissue cultures of higher organisms

primary cultures (subculturing or passaging not possible)/ cell lines (tumor)

mostly adherent cell lines - release trypsin

Medium: ions , glucose, vitamins, nucleotides , lipids, calf serum (source of growth factors, hormones + PDGF, EGF, FGF, ...)

pH control, morphology

Vectors (details are kept secret): plasmids , viral plasmids (retroviruses), polycations (DEAE dextran)

Part of the transfected DNA are regions of DNA increasing production (in units of g / L medium)

Selection (principles similar to those of *S. cerevisiae* .)

The production of biological drugs - recombinant technology



Tissue cultures of higher organisms

CHO – chinese hamster ovary

NS0, Sp2/0-Ag14 – mouse plasmacytome cells (leukocytes) –
auxotrophic for L-glutamine

BHK 21 (baby hamster kidney – syrian hamster)

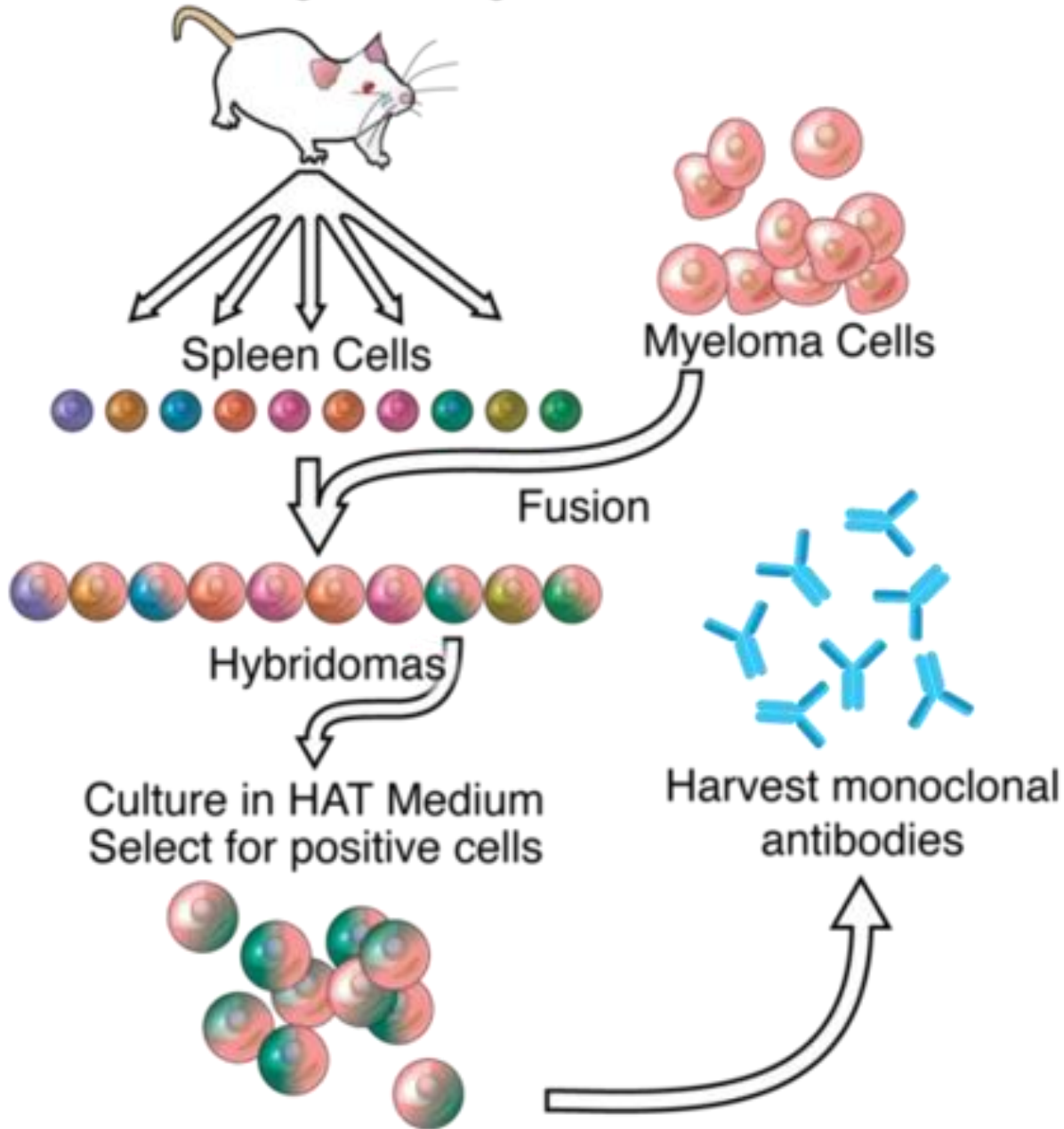
The production of biological drugs - recombinant technology



Tissue cultures of higher organisms

production of „hybridomes“

Mouse challenged with antigen



The production of biological drugs - recombinant technology



Transgenic plants

„**Edible vaccines** " - production of the immunogenic protein (like the polio vaccine)

culturing plant tissue culture in agar

Agrobacterium transfection (+ recombinant plasmid)

selection, planting

tobacco (*Nicotiana tabacum*), *Arabidopsis thaliana*



PRX - 112 - 06/2014 - 1st patient treated with recombinant protein from the plant (Protalix Biotherapeutics) Gaucher disease - deficit of glucocerebrosidase

„...active recombinant proteins systemically through oral administration of plant cells expressing biotherapeutic proteins...“

The production of biological drugs - recombinant technology



3 generation of biologicals

- 1) " copies " of human proteins
- 2) modified proteins (AAs substitution, glycosylation, PEGylation) - better pharmacokinetics, pharmacodynamics - e.g. glargine, PEG-IFN
- 3) de novo designed proteins / MAB



Contaminants from manufacture process

- Microorganisms - antigenic structures, pyrogenicity , sepsis
- Viruses
- DNA - ? Consequences?
- Custom product in improper 3D structure
- Contaminating proteins
 - antigenicity
 - stability (protease)
 - safety (growth factors, hormones, toxins)



Purification - affinity gel / permeation chromatography



Contaminants from manufacture process

Purification - affinity gel / permeation chromatography

Purity \pm 98-99 %

→ Verification of the biological activity of each batch !

- Biochemical methods , cell lines or animal (e.g. Epoetin)
= Time-consuming , cost , accuracy

The risk of functional damage of biologicals

- denaturation
 - precipitation
 - deamination
 - mismatch of SH groups (= incorrect 3D)
 - oligomerization, aggregation, covalent binding
 - hydrolysis
 - isomerization
 - racemisation
 - formation imides
 - oxidation
- Multiple stabilizers
cryopreservation
metal chelation
checking the pH, osmolarity ,
strengthening the hydrophobic
bonds

Costs of the treatment



- Biological therapy is more expensive than "traditional" drugs
- Reasons - significantly higher development costs
 - Demanding and complex testing
 - The nature of products and higher costs after launch
 - Higher costs for production, storage, transportation, shorter expiration

consequences: **lower numbers of treated patients** (up to 2 orders !!!)

Despite that: effective and in many cases can save money in terms of direct and indirect costs

direct costs: shorter hospitalization, reducing the number of surgical procedures, reduce the cost of follow-up treatment , ...

indirect costs : accelerating the patient's self-sufficiency, reducing the costs of absenteeism, cost reductions in social support and care allowances , reducing the cost of informal care and nursing

Clinical use of biological drugs

(selected examples)

Recombinant hormones

Insulin the first biological drug (1982)

- *S. cerevisiae* , *E. coli* - modified insulins (aspart, glulisine, detemir, glargin)

Glucagon - peptide, 29 AMK, *E. coli* i *S. cerevisiae*

GH - 191 AMK, 2x S-S bridge

- before biotechnological synthesis it was isolated from hypophysis of dead people

- I: Turner sy. (caryotype 45, XO)

- 1977 (Nutropin, P. Seeburg, Genentech, UCSF)

IGF – growth failure

Recombinant hormones

Parathyroid hormone - 115 AA

Calcitonin - originally isolated from salmon

Gonadotropins - for IVF (RG Edwards , Nobel. Prize , 2010)

FSH - follitropin α (CHO cells) - hyperovulation

LH - maturation of oocytes, together with FSH

hCG - (CHO) cells - follicular maturation, support for ovulation

Biological treatment of autoimmune diseases

1. rheumatic diseases
2. psoriasis
3. inflammatory bowel disease
4. asthma bronchiale
5. multiple sclerosis
6. ophthalmology

1. Biological Treatment of rheumatic diseases

Rheumatoid arthritis, juvenile idiopathic arthritis,
psoriatic arthritis , ankylosing spondylitis

Early intervention prevents irreversible changes;

Some of patients stay in sustained remission even after
treatment has stopped !

1. Biological Treatment of rheumatic diseases

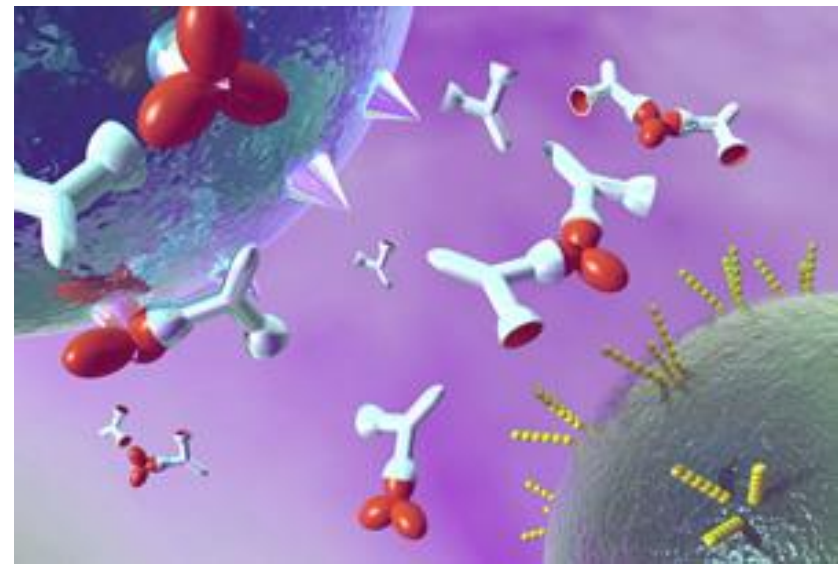
Anti-TNF drugs

Infliximab – chimeric mAbs, IgG, 75 % of human,
25% of the murine antibody

high affinity binding to human TNF α

formation of stable complexes which bind complement

induction of apoptosis and lysis of the cells that produce TNF α , the
disintegration of granulomas



1. Biological Treatment of rheumatic diseases

Anti-TNF drugs

Adalimumab

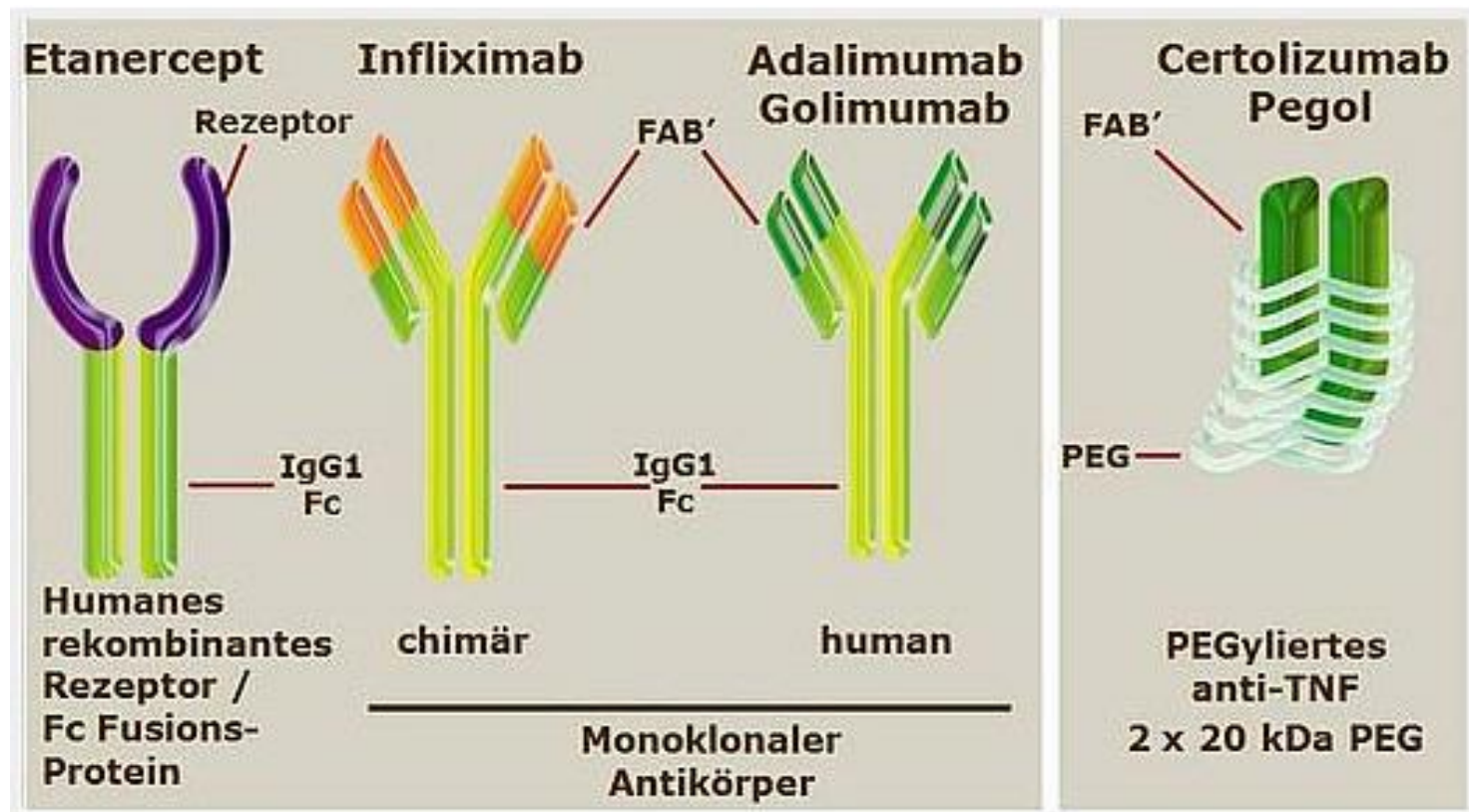
- human MAB binds specifically to TNF and neutralizes the biological function of TNF by blocking its interaction with the p55 and p75 cell surface TNF receptors

Golimumab

- human MAB which bind to both soluble and membrane bound TNF α
- the same mechanism action as infliximab

1. Biological Treatment of rheumatic diseases

Certolizumab – humanized Fab fragment of TNF α antibody, konjugated with PEG



1. Biological Treatment of rheumatic diseases

Etanercept – binds TNF α

= soluble dimeric fusion protein – extracellular domain of receptor for TNF α and Fc chain of human IgG₁

Mechanism of action: competitive inhibition of TNF α , decreased effect of TNF β

does not bind complement, but leads to the disintegration of granulomas

1. Biological Treatment of rheumatic diseases

Anakinra – IL1 receptor antagonist („IL-1 RA“)

weaker effect than anti-TNF drugs

Rituximab - binds to the transmembrane antigen CD20

(on pre-B and mature B lymphocytes)

expressed on > 95% of all non-Hodgkin lymphomas of B cell origin.

in combination with MTX , cyclophosphamide

Adverse effects of anti -TNF agents

opportunistic infections –

higher risk for: combination of immunosuppressive
drugs (with 2 - combined to 14x !)

malnutrition

Age > 50 years

mycobacteria , listeria , fungal , viral infections

Adverse effects of anti -TNF agents

Paradoxical autoimmune reactions

anti-idiotypic antibodies (in addition prevents binding of the antibody to TNF)

TB –activation of latent forms

Late carcinogenicity - lymphoproliferative disease
(2-3 times higher versus the healthy population) , inconsistent data

Others - specific ADR for specific substances

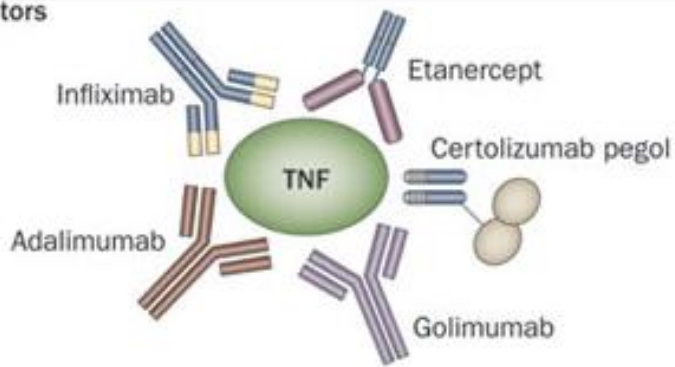
1. Biological Treatment of rheumatic diseases

Abatacept - recombinant fusion protein from the extracellular CTLA4 (Cytotoxic T - lymphocyte - assoc . Antigen 4) ; competitively binds to CD80 , thereby preventing T cell proliferation –

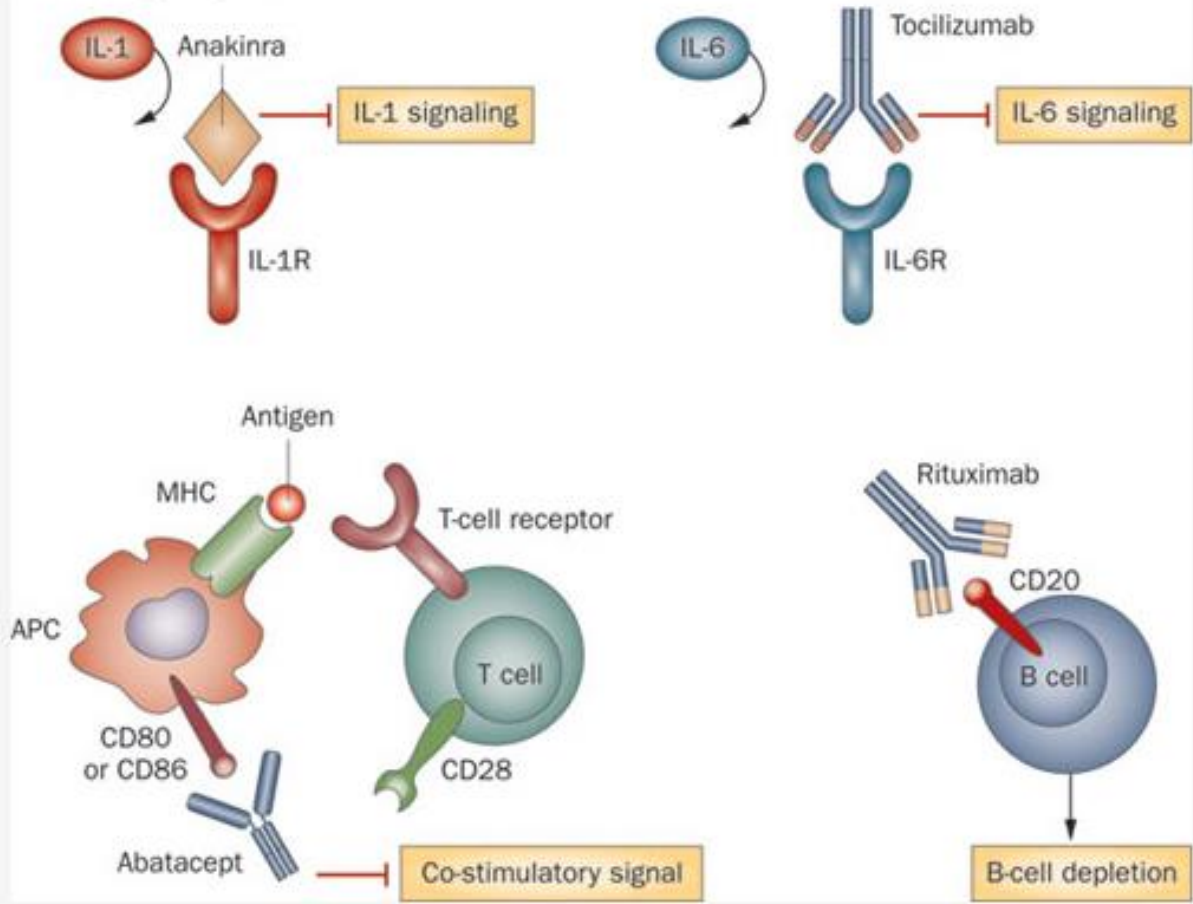
in children not to be combined with anti-TNF therapy
(infectious complications)

Tocilizumab - humanized mAb against conventional IL6
AE: (infection) + lipids increase (CHOL , LDL, TAG)

TNF inhibitors



Other biologic agents



2. The biological treatment of psoriasis

Etanercept - see above

- Only one biological treatment of psoriasis for children 8-18 years

Infliximab - see above – s.c. admin 0th , 2nd, 6th week , then after 8 weeks

Ustekinumab - MAB fully human IgG1 anti-IL -12/ 23 (important in the pathogenesis of psoriasis) inhibition of cytokine cascade sc at 0, 4 weeks, 12 weeks

AE : nasopharyngitis, headache, arthralgia, local irritation at the injection site

3. Biological treatment of inflammatory bowel disease

inflammatory bowel disease

Crohn's disease

ulcerative colitis

Anti-TNF drugs (see above)

infliximab

adalimumab

certolizumab

3. Biological treatment of inflammatory bowel disease

Natalizumab - IgG4 humanized MAB against integrin α (on the surface of leukocytes, ensures migration across the capillary wall)

Rescue treatment in Crohn D. (only in the US , in Czech Republic registered for MS)

Vedolizumab - humanized mAb IgG1 against $\alpha 4\beta 1$ integrin (on activated leukocytes , provides adhesion to the endothelium and the penetration into the circulation from the gastrointestinal tract)

4. Biological treatment of bronchial asthma

Adjunctive/supplementary treatment in patients with more serious disease which do not respond to other treatments

–**Omalizumab** – humanized anti ab, bis IgE , s.c.

AE: malignancies?, parasitoses ... data not convincing

Mepolizumab - anti IL5 antibody

Infliximab/Golimumab/etanercept – anti TNF α

Off label, clinical benefit not convincing

4. Biological treatment of bronchial asthma

Other „problematic“ treatment

keliximab – MAB anti- CD4 , smaller study demonstrated effect on pulmonary parameters (PEF) ; studies have been stopped because of concerns about the reduction in the number of CD4 cells

pitrakinra - inhibits the binding of IL- 4 and IL -13 to IL- 4R α subunit; may prevent worsening of FEV1 after allergen exposure ; tested also in EA; requires further study

daclizumab - binds to CD25 inhibits the binding of IL- 2 receptor; may decrease T-cell activation , improves FEV1 , in one study alleviate symptoms ; 3 patients had serious adverse reactions

5. Biological treatment of multiple sclerosis

- demyelinating disease
- onset 20-40 yrs of age, women more frequently
- usually begins first clinical event (clinically isolated syndrome , CIS) includes visual, sensory or motor symptoms

Relaps-remitent form

Progressive form

- If untreated - after 10-20 years swithes in secondary progression - ↑ neurological disability in about 50 % after 15 years of physical disability and cognitive dysfunction with serious socioeconomic consequences

Total direct + indirect costs of invalidized is 3 times higher than biol. treatment

5. Biological treatment of multiple sclerosis

The attack of the immune system against its own tissues CNS

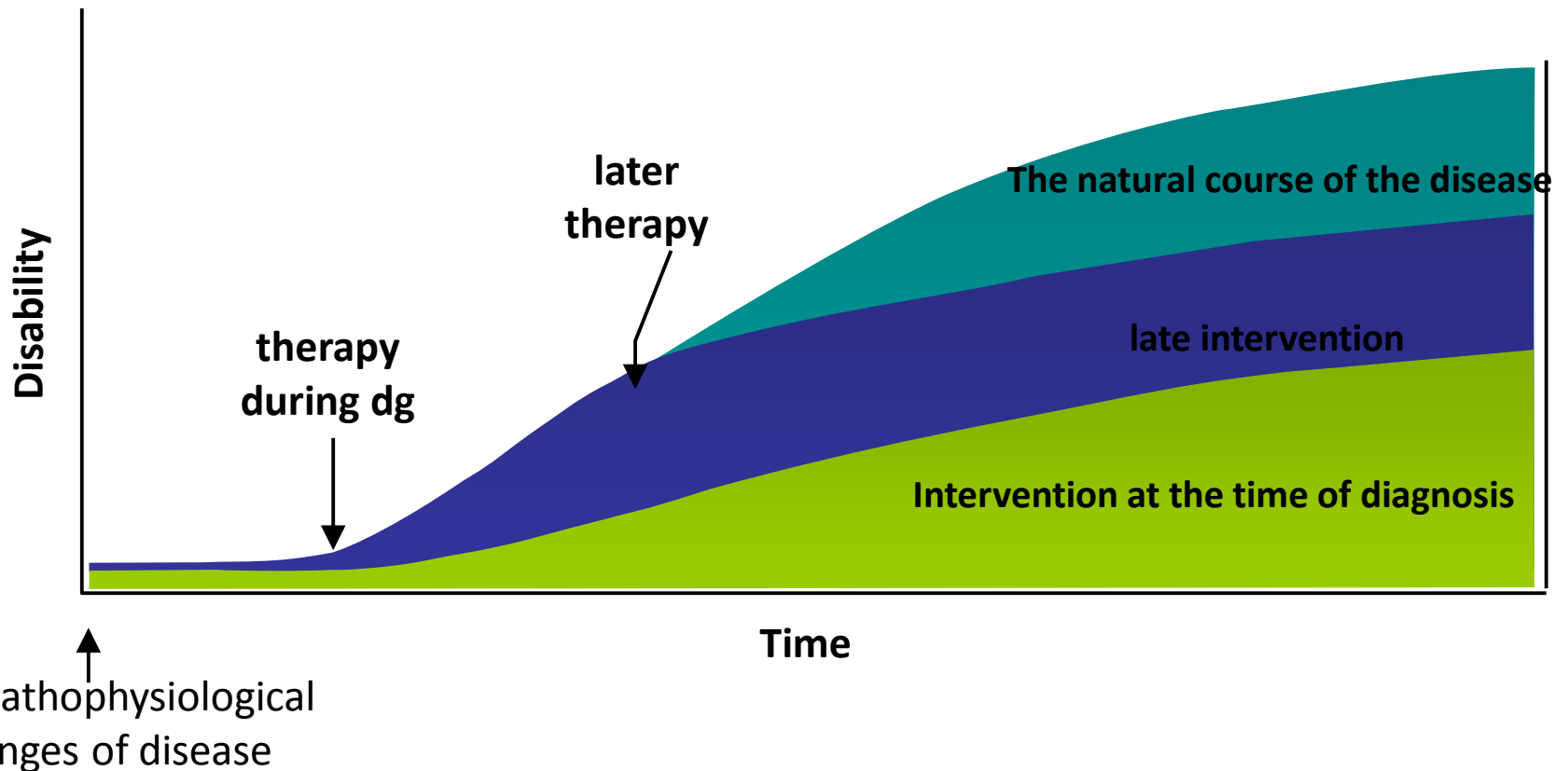
Inflammation focus, in which there is :

Disintegration of myelin (surrounds the axon dielectric)

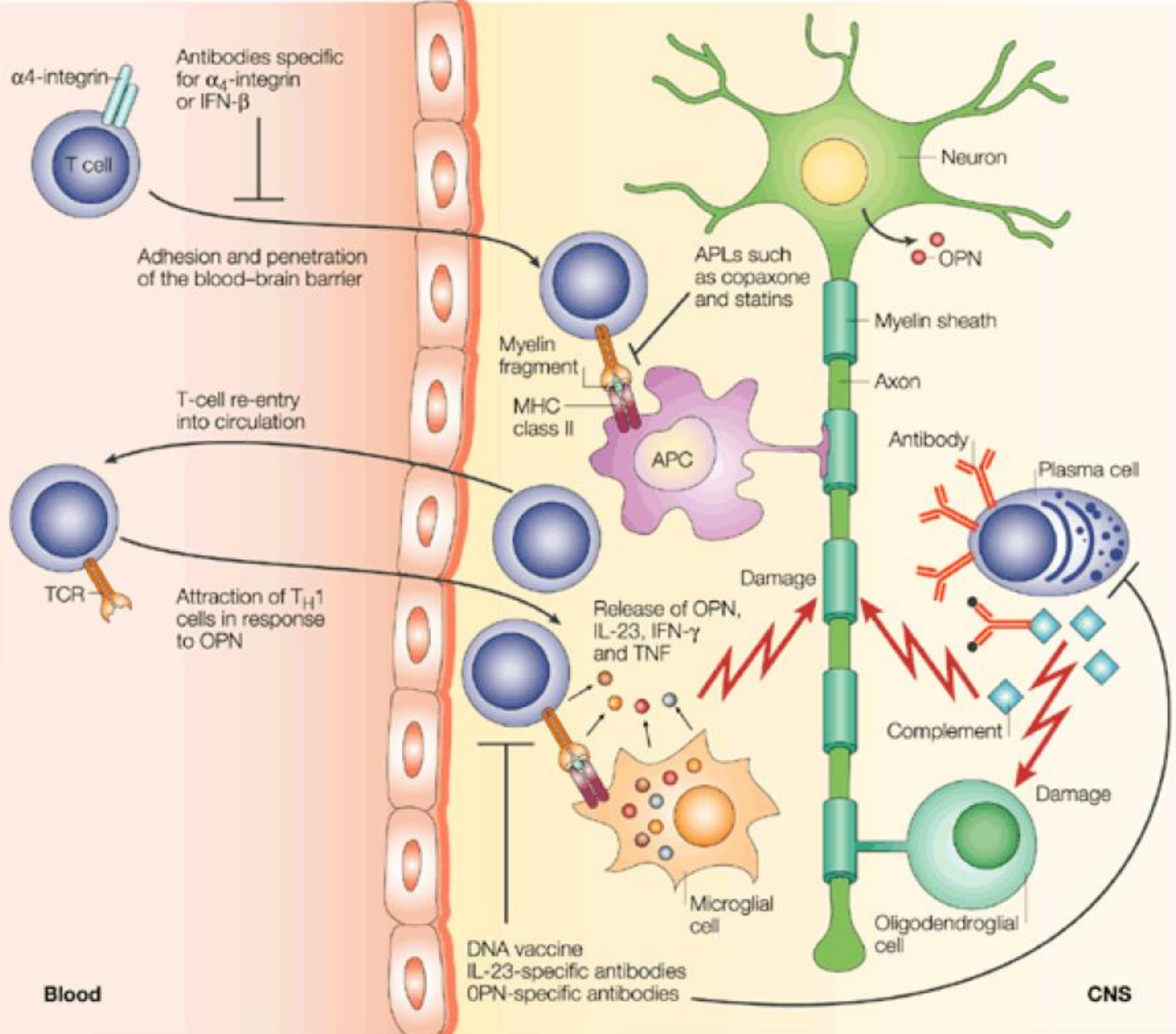
the destruction of nerve fibers

The theoretical importance of early therapy MS

Early medical intervention has the potential to slow the progression of MS



Adapted from Trapp et al. *Curr Opin Neurol.* 1999;12:295; Trapp et al. *Neuroscientist.* 1999;5:48; Trapp et al. *N Engl J Med.* 1998;338:278; Jeffery. *J Neurol Sci.* 2002;197:1; Cohen et al. *J Neuroimmunol.* 1999;98:29.



α_4 -integrin
 T cell
 Antibodies specific for α_4 -integrin or IFN- β

Adhesion and penetration of the blood-brain barrier

APLs such as copaxone and statins

T-cell re-entry into circulation

Myelin fragment
 MHC class II
 APC

Antibody
 Plasma cell

Attraction of T_H1 cells in response to OPN

TCR

Release of OPN, IL-23, IFN- γ and TNF

Damage

Complement

Damage

Microglial cell

Oligodendroglial cell

DNA vaccine
 IL-23-specific antibodies
 OPN-specific antibodies

Blood

CNS

5. Biological treatment of multiple sclerosis

Drug development:

60's : ACTH, corticosteroids

70.-80's: azathioprine, cyklofosamid

1993 –IFN β

1996 –glatiramer acetate

2002 –mitoxantrone

2004 –US/ 2006- Europe –natalizumab

2010 – fingolimod

5. Biological treatment of multiple sclerosis

IFN beta - MoA: through receptors - phosphorylation of kinases ,
changes in the amount and localization of signal molecules
(MHC I), neopterin , $\beta 2$ microglobulin

assumed immunosuppressive effect

IFN : cytokines with antiviral , antiproliferative and
immunomodulatory activity.

IFN α + β = " type I interferons " γ " type II "

partially overlapping but clearly distinguishable biological activities.

IFN - β 1a = glycosylated protein ; Expression in CHO cells

IFN - β 1b = 1 exchange AA (prevents from SS bridges) , expressed in
E. coli .

5. Biological treatment of multiple sclerosis

Natalizumab - AB binds to $\alpha4\beta1$ integrin of lymphocytes which allow them to enter into the CNS (bind integrin subunit on endothelial adhesion molecules before penetration of vascular wall)

It has a preventive effect on relapses and reduce the symptoms of ongoing relapse

Adverse reactions: Serious opportunistic infections , rare (1 : 1000) progressive multifocal leukoencephalopathy (**PML**) – risk factors- JCV viral infection/antibodies, immunosuppressant treatment

iv at intervals of 4 weeks

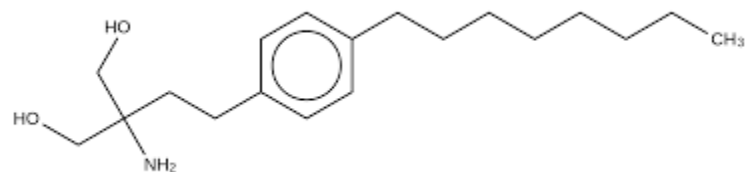
5. Biological treatment of multiple sclerosis

Fingolimod – active metabolite fingolimod phosphate is antagonist of receptor for sphingosine - 1 -phosphate (S1P) on lymphocytes .

Passes easily BBB, blocks the ability of cells output from the lymph nodes, resulting in redistribution of lymphocytes (rather than depletion)

redistribution reduces the infiltration of pathogenic cells, including proinflammatory Th17 cells into the CNS , where they are involved in inflammatory processes and nervous tissue damage .

! Not a biological treatment !



5. Biological treatment of multiple sclerosis

Glatiramer acetate – a mixture of synthetic polypeptides derived from four AA (Glu, Ala , Tyr , Lys)

- Peptide analog of myelin basic protein - suppresses inflammation and also has neuroprotective properties

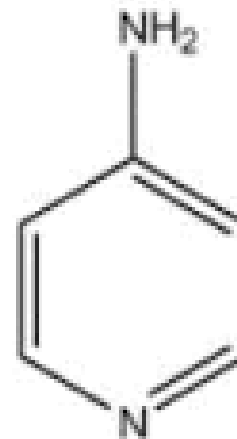
.

influences lymphocyte populations in the circulation by induction of immunoregulatory Th2 lymphocytes



5. Biological treatment of multiple sclerosis

Fampridin – blockade of K⁺ channels , prolongs the repolarization and increases the formation of action potentials in demyelinated axons and neurological function



! Not biological drug!

5. Biological treatment of multiple sclerosis

Anti-CD20 MAB (CD 20 lymphocytes)

rituximab

ocrelizumab

ofatumumab

alemtuzumab

Anti CD 25 MAB

daclizumab – humanized monoclonal antibody of IgG1 subtype that binds to the Tac epitope on the interleukin-2 (IL-2) receptor α -chain (CD25), thus, effectively blocking the formation of the high-affinity IL-2 receptor.

For severe attacks of MS



6. the treatment of viral hepatitis

IFN α , IFN β –chronic infection HBV, HCV

Antivirotics - tenofovir, entecavir, adefovir, lamivudin

IFN α – „conventional“ is already not used

CIFN – consensual IFN - prim . structure according to the most frequently occurring AAs in the sequence of all the known IFN

PEGylated – PolyEthylenGlycol – polymer of ethylene oxide - prolongs the half (7-10x) , increases the plasma concentrations do not change the absorption rate

- Protection against proteases



6. the treatment of viral hepatitis

PEG-IFN α 2a – substituted monomethoxyPEG + IFN α 2a

PEGylované – PolyEthylenGlycol – polymeric etylendioxid
– prolong halflife (upo to 100x)

PEG-IFN α 2b – linear PEG + IFN α 2b

Albumin-IFN α 2b - recombinant protein, a fusion of two
genes ; efficiency , NA similar to the previous one,

1 application every 14 days (longer half-life of albumin) ,
higher price



6. the treatment of viral hepatitis

General ADRs IFN

- **Frequent** - flu-like syndrome . , chronic fatigue syndrome
 - myelosuppression
 - dysthymia , depression, anorexia
 - weight loss, diarrhea
- **Rare** - inflammation at the injection site
 - psychotic disorder , suicidal tendencies
 - disorders of sex . functions
 - acute heart failure
 - Interstitial nephritis

The use of targeted/biological therapy in ophthalmology

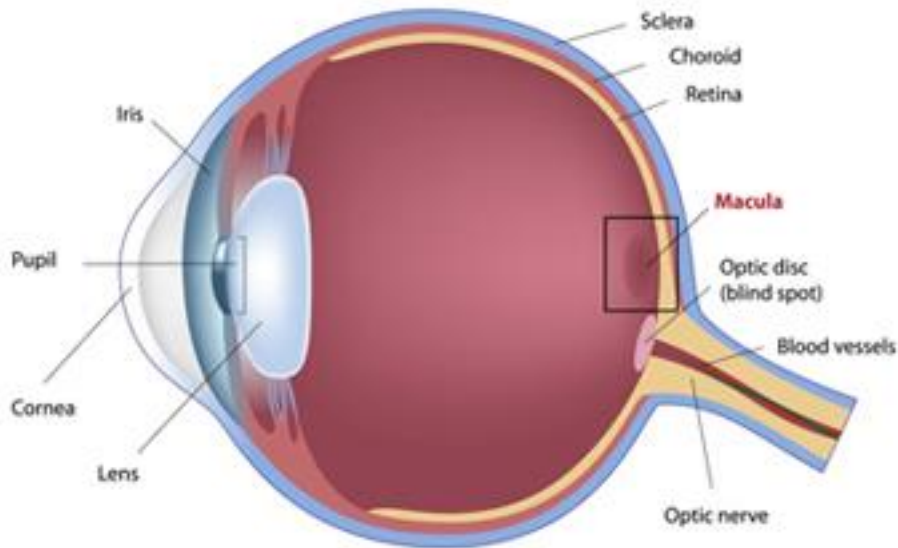
- **Indications:** age-related macular degeneration (AMD) , diabetic eye disease based on
- **neovascularization** (diabetic retinopathy, neovascular glaucoma , diabetic macular edema, ...)
- **dysregulated neovascularization** = → complex process of creating new blood vessels from existing vascular bundles
uncontrolled neovascularization → irreversible damage and blindness
- **Pathogenesis:** newly formed blood vessels are formed from retinal blood vessels and cause ocular vitreous hemorrhage → → conversion to retinal detachment

AMD

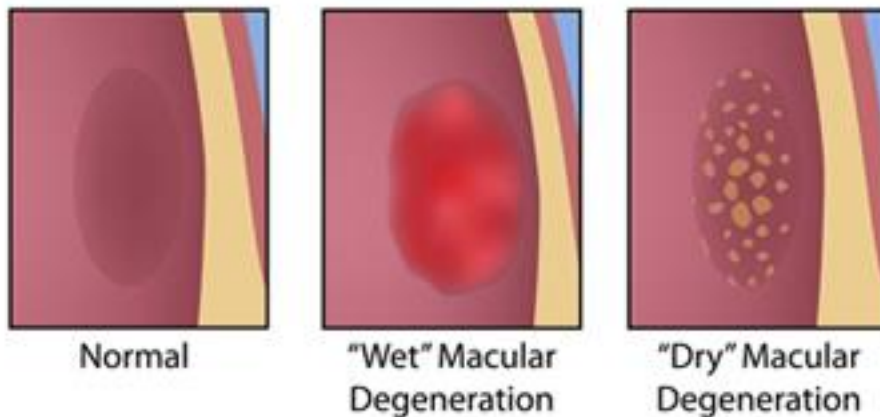


-in developed countries a major cause of vision loss in the elderly

Macular Degeneration



Wet AMD (10% of patients) → new blood vessels grow into the space between the retinal pigment epithelium or rods and cones , retinal bleeding , swelling and scarring in the macular region



Dry AMD (90 % of patients) → gradual loss of central vision , peripheral vision is preserved

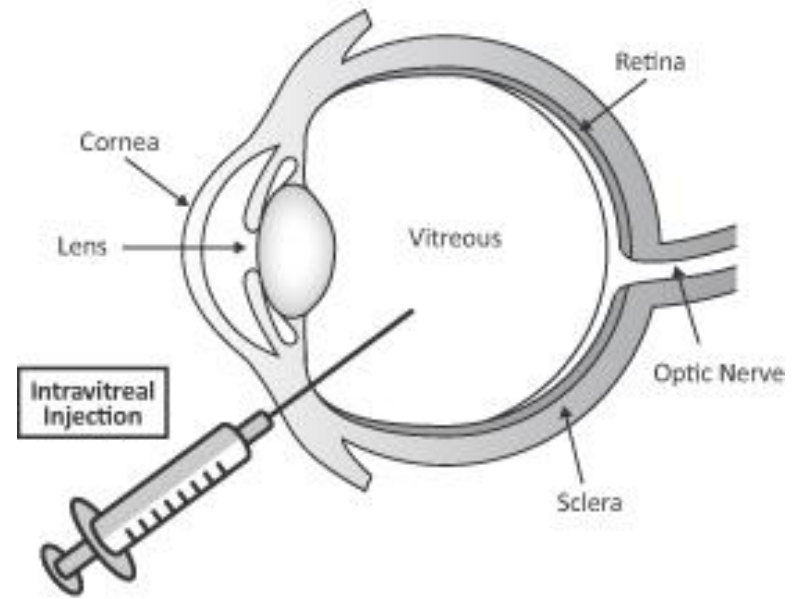
The use of targeted/biological therapy in ophthalmology

- used primarily substances with ***antiangiogenic activity*** (ie. block of neovascularization)
- most importance for the process of angiogenesis , VEGF = **vascular endothelial growth factor**
 - important physiological functions
 - located on the lining of blood and lymph vessels in the body
 - regulates the proliferation and vascular permeability
 - several types (A - E)
- **drugs used**
 - monoclonal antibodies (**bevacizumab, ranibizumab**)
 - small drugs (**pegaptanib, vortepofin**)
 - **aflibercept**= fusion protein

Administration of targeted treatment in ophthalmology



- intravitreal administration
- therapy usually takes a year or more
- **advantages:** fewer adverse systemic effects of drugs
- **cons:** clogging, intraocular infection , atrophy of the retinal pigment sheet , haemophthalmus..



The use of targeted/biological therapy in ophthalmology



- **Bevacizumab** - was developed for the treatment of colorectal cancer, indication AMD is used off-label

MA : monoclonal antibody against VEGF (binds VEGF1 and VEGF2 , blocks the interaction of DP.)

AE: acceleration of hypertension, proteinuria, thromboembolic events, poor wound healing...

- **ranibizumab**

- Indications : AMD , CNV (chorioidal neovascularization)
- MA : fragment of a monoclonal antibody against VEGF - A
- short plasma half-life
- Administration 1x per month until the patient's visual acuity is stable (three consecutive monthly assessments)

Targeted therapy



- **pegaptanib**
 - synthetic oligonucleotide
 - the indication wet AMD
 - MA : binds to receptor VEGF - A₁₆₅
- **vorteporfin**
 - as a single infusion is administered systemically
 - always in combination with photodynamic therapy
- **aflibercept**
 - MA : recombinant fusion protein; false receptor binds VEGF-A and PlGF

BIOSIMILARS



BIOSIMILARS

" Copy" of biotechnology drugs

- produced after the expiry of patent protection on the original biotechnology drugs
- In the US, for the same group uses the term „Follow -on Biologics“ , abbreviated „fobs“ .

The standard procedure for the registration of generic medicines with defined structure (ie . bioequivalence study) is inapplicable



BIOSIMILARS

- Biosimilars drugs are similar, but **not identical** with the original biological drug.
- Biosimilars are **not automatically therapeutically interchangeable** with the original biological drug .
- **small change** process in biosimilars may cause an **entirely different drug**.
- Biosimilars pass before entering the market or **shorter simplified clinical trials** , but disproportionately more complex than with generics

BIOSIMILARS



- after completing „Centralised registration Procedure“ (mandatory for the registration of medicinal products manufactured with sophisticated technology, particularly biological drugs)
- compared the effectiveness and quality in studies similar to those that are required for orig. preparation
- included in European Directives
- determining whether the product was evaluated in the same dosage form, strength, by the same route of administration as the ref. product and whether it was used in the same indication e.g. growth hormone: 12 months
- comparative study of efficacy - safety within the RCT stage III
The price is about 10 times higher than for generic medicines

Biological drugs in a broader context

- Gene therapy
- Anti-sense therapy
- Immunization with vaccines

Biological drugs in a broader context

Gene therapy

- Incorporation of a gene sequence into a target tissue by an appropriate vector approach
- treating or preventing gene-related illnesses by changing the expression human genes

Gene therapy - vectors

- biological vectors of viral origin
 - the most effective method of therapy
 - retroviruses , lentiviruses , adenoviruses
- injection of naked DNA directly into the tumor
- genetic cannon - DNA wraps around gold particles and scored into the epidermis - strong helium gun
- lipofections of DNA -coated liposome and delivered into the tissue
- intravascular , intratracheal , oral...

Gene therapy SCID

- SCID severe combined immunodeficiency
- 1990 my first attempt at gene therapy on 4-year girl Ashanti DeSilva
- the T cells have been modified ex vivo
- normal ADA (adenosine deaminase) by a vector derived from MoMLV (Moloney murine leukemia virus).

- The girl is still alive .

- 2002 -5 boys with severe X-linked SCID underwent gene therapy ex vivo, with four times a year the state has improved significantly, with two of them unfortunately died leukemia .
- Attempts suspended and perfecting the methodology

Gene therapy - the risks

- Adverse immune response
- Infections vector - natural activation of virus
- Genetic influence on gametes
- Risk of malignancy- activation of protooncogenes ,
suppression of regulatory genes

Biological drugs in a broader context

Anti-sense therapy

- Incorporation of complementary oligonucleotides to the initiation codon / promoter to DNA
- block the effects of action of proteins that are not transcribed
- **Olimersen** - lowering expression of Bcl -2 (overexpressed in many cancer) - withdrawn registration

The antisense and gene therapy in practice

- **Fomivirsen** - antisense sequences to the mRNA of human CMV - Ophthalmic applications for patients with HIV + to reduce CMV infection
- **Pegaptanib** - oligonucleotide binding to the VEGF protein - for the treatment of wet AMD
- **Gencidin** - adenovirus carrying the gene for p53 - First registered in China in 2004
- **Glybera** - 3 x 10¹² genome copies of human lipoprotein lipase in a viral vector (adeno-associated virus serotype 1 (AAV1)) to treat hyperlipoproteinemia I

Thank you for attention

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