Interventions with impact on the immune system

- Immunomodulation
  - It is not clearly possible to distinguish stimulation from suppression

- Overreacting IS → Immunosuppression
- Insufficient IS → Immunostimulation

- Antigen specific
- Antigen non-specific
Legend

- effect specificity
- speed of effect
- frequency of clinical usage
Effect of glucocorticosteroids

- interaction with gene transcription
- reduction of transcription of genes for pro-inflammatory cytokines
- limiting activity and presence of immunocompetent cells in the inflammed region (effect on endothelium, decrease of chemotaxis)
- influence of number and function of immunocompetent cells

main usage:
- autoimmunity, GvHD prevention and malignancy
# Effect of Glukocorticosteroids

<table>
<thead>
<tr>
<th>Effect</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cellular transport</strong></td>
<td></td>
</tr>
<tr>
<td>Neutrophils in circulation</td>
<td>Release from BM but limited access into the tissues</td>
</tr>
<tr>
<td>Monocytes in circulation</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (mainly CD4+)</td>
<td>Apoptosis of CD4+ T cells, sequestration within BM</td>
</tr>
<tr>
<td><strong>Cellular function</strong></td>
<td>Maturation of M0 from monocytes</td>
</tr>
<tr>
<td>Activity of macrophages</td>
<td></td>
</tr>
<tr>
<td>Production of proinflammatory cytokines (IL-1, 6, TNF-alfa)</td>
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<tr>
<td>Chemotaxis</td>
<td></td>
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<tr>
<td>Bactericidial activity</td>
<td></td>
</tr>
<tr>
<td>T cell activation</td>
<td>Transcription of genes for IL-1, 2, 3, 4, 6, IFN-gamma</td>
</tr>
<tr>
<td>Function of endothelium</td>
<td>Expression of adhesive molecules</td>
</tr>
<tr>
<td>Function of NK cells</td>
<td>Activity of NO synthase</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td></td>
</tr>
<tr>
<td>Prostaglandins synthesis</td>
<td>Inhibition of phospholipase A2 and cyclooxygenase</td>
</tr>
</tbody>
</table>
Adverse effects of glucocorticosteroids

depression, mood changes
skin atrophy
cataract
acne
hirsutism

proximal myopathy
hypertension
gastric ulcer
diabetes mellitus
suppression of adrenal glands
aseptic necrosis
osteoporosis

Cushingoid habitus
increased infection rate
decreased growth dynamics

impaired wound healing
Effects of NSAID on IS

- Inhibition of cyclooxygenase (COX-1,2) → ↓ prostaglandin E2
- ↑ late sensitivity reaction
- ↑ rejection of skin grafts as well as tumours in experimental animals
- ↓ serum concentration of RF IgM in patients with RA

- Main usage: analgesics, antipyretics
- One of the most spread drugs in the world
  - acetylsalicylic acid, ibuprofen, coxib (Vioxx, Celebrex, GIT bleeding, myocardial infarction...)
Antihistaminics

- **generation I**
  - dithiaden

- **generation II**
  - cetirizin, loratadin (Zodiac, Zyrtec, Claritine...)
  - decreased transport through hemato-encephalic barrier

- **generation II-III**
  - antihistaminics with immunomodulatory effect – decrease of adhesive molecules, anti-inflammatory effect
  - desloratadin, levocetirizin (Xyzal, Aerius)

použití: alergie, sedace
Antihistaminics

allergen
IgE
Fc-ε receptor
degranulation
histamin
mast cell
Antileukotriens

- inhibition of leukotriene production (or its receptors)
  - zafirlukast, montelukast (Singulair)

- usage:
  - mild form of bronchial asthma
  - activity-induced asthma
  - ACP-sensitive asthma
Antileukotriens

phospholipase A2

arachidonic acid

cyclooxygenase

X

lipoxygenase

prostaglandins

leukotriens
tromboxans
Immunosuppressive drugs

a) **immunomodulatory drugs**
   - antagonists of folic acid - methotrexate
   - purin analogs – azathioprin, mykofenolate mofetil
   - alkylating agents - cyklophosphamid
   - sulfasalazin
   - antimalarics

b) **drugs binding to immunofilins**
   - cyklosporin A
   - tacrolimus (FK 506), sirolimus

c) **anti-T, anti-B**
   - anti-T:
     - anti-thymocytic globulin
     - monoclonal antibodies against CD3, CD4, CD52, CD25
     - organ transplantation – rejection, GvHD
   - anti-B
     - anti-CD20 (Rituximab)
     - lymphoma, autoimmune disease
Purine analogs

**Azathioprine (Imuran)**
- inhibition of synthesis DNA
- metabolites are active (after metabolism in liver)
- effects are seen after several weeks
- bone marrow toxicity (granulocytopenia, trombocytopenia)
- homozygotic deficiency of TPMT (thiopurine-methyl transferase) – life-threatening bone marrow aplasia

**Mycofenolate mofetil (CellCept)**
- inhibition of inosine-monophosphate dehydrogenase = key enzyme in de novo synthesis of purines for T and B cells
Alkylating agents

- interference with DNA duplication in pre-mitotic phase
- DNA reparation after alkylation is different in particular tissues

**Cyclophosphamide**
- metabolites are active (after metabolization in liver)
- clear mechanism of action is not known
- reduced response on antigen stimulation
- after discontinuation return to normal takes weeks and months
- long-term application connected with urinary bladder carcinoma

**Chlorambucil**
- directly affects B cells
- B-cell tumours, leukaemias
Agents binding immunophilins

**Cyclosporine A**
- binds to intracellular receptors – cyclophilin – calcineurin
- inhibition of translocation of transcription factors into nucleus – inhibition of calcium-dependent processes
- main effect is decreased production of IL-2 (affects CD4+ dependent processes)
- main usage
  - prolongs survival of grafts after transplantation
  - in autoimmune diseases where CD4+ play major role – psoriasis, uveitis, severe RA, AD
- effect seen after in 2-12 weeks, sometimes rebound phenomenon
- nephrotoxicity, hypertension, hepatotoxicity, gingival hyperplasia, tremor, hirsutism, lymphoma

**FK506 (tacrolimus)**
- binds to intracellular protein, similar mechanism as CyA, but 10-100x more potent
- higher nephrotoxicity than in CyA

**Rapamycine (Sirolimus)**
- similar to FK506, transcription of cytokines not influenced
- T-bb inhibition of proliferation after stimulation by IL-2, 4
Anti-cytokine therapy

Monoclonal antibodies against TNF-α
- infliximab (Remicade) - chimeric
- adalizumab (Humira) - humanized
- etanercet (Enbrel) – humanized, receptor inhibitor

- anti-dsDNA Ab induction, increased incidence of tuberculosis
- RA, JCA, Crohn, Bechtěrev

Inhibition of IL-1 - Interleukin1-RA = Anakinra (Kineret)
- frequent usage, extremely expensive
Immunostimulation

- bacterial lysates – non-specific activation of macrophages
  - Bronchovaxom, Ribomunyl, Luivac etc.
- chemical immunostimulation
  - not widely used
  - Isoprinosine, Levamisol
- vaccination
Immunoglobulin therapy

- substitution
  - primary antibody immunodeficiency
  - secondary antibody immunodeficiency
- immunomodulation of autoimmune diseases
- 1 g approx. $4 \times 10^{18}$ IgG molecules
- different dosing
Mechanisms of IVIg effect
- Fc fragment dependent

- blockade of Fc receptors on phagocytes (similar effect as MoAb anti-FcgR, lasts approx. 30 days)
- inhibition of proinflammatory cytokines by macrophages (in vitro)
- diminishing of NK cells function
- effect on Fc receptors on B cells (CD32)
Mechanisms of IVIg effect
- Fab fragment dependent

- different antigen neutralization
- anti-idiotypic activity
- inhibition of B cell differentiation and activation
- creation of rheumatoid factors (anti-Ig Ab)
Clinical use of IVIg

**Effect proven by RCT**
- immune thrombocytopenia
- Guillain-Barré syndrome
- chronic demyelinizing neuropathy
- Kawasaki disease
- Dermatomyositis
- Lambert-Eaton myastenic syndrome
- Multifocal neuropathy

**Effect not proven by RCT**
- viral induced malaise
- rheumatoid arthritis
- juvenile rheumatoid arthritis
Monoclonal antibodies in anti-cancer therapy

- conjugate of MoAb and cytotoxic drug (methotrexate, vincristine), toxins (ricin, abrin), radioisotope (iod-131, yttrium-90)
- immunolocalization of tumour – radioisotope-labeled MoAb (indium-111, technecium-99)
FDA approved MoAb in cancer

![Table 2. Monoclonal Antibody Products](image)

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Target</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab</td>
<td>Chimeric</td>
<td>CD20</td>
<td>NHL</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>Humanized</td>
<td>Erb B2</td>
<td>Breast</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>Humanized</td>
<td>VEGF</td>
<td>Colorectal</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>Humanized</td>
<td>CD52</td>
<td>CLL</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>Chimeric</td>
<td>EGFR</td>
<td>Colorectal</td>
</tr>
<tr>
<td>Panitumumab</td>
<td>Human</td>
<td>EGFR</td>
<td>Colorectal</td>
</tr>
</tbody>
</table>

Abbreviations: NHL, non-Hodgkin’s lymphoma; VEGF, vascular endothelial growth factor; CLL, chronic lymphocytic leukemia; EGFR, epidermal growth factor receptor.
Immunostimulation by cytokines

**IFN alpha**
- malignancy, hepatitis B and C
- flu-like symptoms, malaise, anorexy, mood changes, bone marrow suppression, hepatotoxicity, cardiotoxicity

**IFN beta**
- multiple sclerosis
- possible effect due to inhibition of expression of HLA-DR on glial cells

**IFN gamma**
- lepromatous lepra, leishmaniasis, chronic granulomatosis

**IL-2**
- PID, HIV, increases number of CD4+ T cells

**GM-CSF, G-CSF**
- production of new granulocytes, monocytes and macrophages
Immuno modulation with antigen

1. immunotherapy of allergic diseases – use of defined exoallergen
2. cancer immunotherapy – DC, T cells
3. adoptive immunotherapy
4. immunotherapy of autoimmune diseases – hypothetical use of defined autoantigen
Allergen-specific immunotherapy

- hyposensitization – repeated application of gradually growing doses of allergen
- lasts for 3-5 years
- isotype switch, degranulation of mast cells
- subcutaneous, inhalation, ingestion
Allergy – senzitization and memory

Larche, Nat Rev Immunol, 2006
Immediate phase of allergic inflammation

Larche, Nat Rev Immunol, 2006
Late phase of allergic inflammation

Smooth-muscle-cell activation and hyper-reactivity for contraction, and release of chemokines and pro-inflammatory cytokines

Increased endothelial-cell adhesion and inflammatory-cell transmigration

**Histamine**

**Mast cell**

**Basophil**

Eosinophil activation and release of mediators, chemokines and pro-inflammatory cytokines

IL-5

IL-13

**Eosinophil**

**IFNγ, TNF**

**T_{H1} cell**

**IL-4, IL-13, CCL5**

**Increased**

**Memory B cell**

**T_{H2} cell**

**TCR**

**MHC class II molecule**

**Allergen**

**IL-4, IL-13**

**IL-9, IL-13**

**Allergic rhinitis and asthma**

- T_{H2}-cytokine-mediated induction of increased mucus production
- Local production of IgE
- T_{H1}-cell-mediated induction of bronchial epithelial-cell apoptosis

**Atopic dermatitis**

- T_{H1}-cell-mediated induction of keratinocyte apoptosis
- T_{H1}-cell-mediated epithelial-cell activation, and release of chemokines and pro-inflammatory cytokines

**IFNγ, TNF**

**CD95L**

**T_{H1} cell**

**T-cell activation and proliferation by IgE-facilitated and non-IgE-facilitated presentation of allergens by inflammatory DCs**

Larche, Nat Rev Immunol, 2006
Proposed role of regulatory T cells in IT

Larche, Nat Rev Immunol, 2006
Effects of allergen-specific IT

Larche, Nat Rev Immunol, 2006
Dendritic cells based vaccines

- TLR receptors (Toll-like) – essential for the initiation of immune response
- If no TLR costimulation – T cell anergy, expansion of regulatory T cells
- minimal residual disease
- need for costimulation
- inadequate activation
- role of tumour environment
DC vaccines in anti-cancer IT

Tacken, Nat Rev Immunol, 2007
## Different DC loading techniques

<table>
<thead>
<tr>
<th>DC loading technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCs loaded with defined tumour antigens</td>
<td>• Induces CTL responses against leukaemia cells alone, not stem cells</td>
<td>• Antigen drift/loss</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• HLA restriction (for LAA-derived peptides)</td>
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<tr>
<td></td>
<td></td>
<td>• Differing responses/ avidity of CTLs to peptides</td>
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<tr>
<td></td>
<td></td>
<td>• T-cell exhaustion if peptide already strongly expressed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Difficult to induce pure apoptosis or necrosis — overlap in stages of cell death</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• May stimulate tolerogenic DCs/danger signals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Broad range of antigens may induce autoimmunity</td>
</tr>
<tr>
<td>DCs loaded with undefined tumour antigens</td>
<td>• Broad range of tumour antigens expressed including undefined antigens</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Less likely for antigen drift to be relevant</td>
<td></td>
</tr>
<tr>
<td>AML-DCs/fusion hybrids</td>
<td>• Combines features of both leukaemic cells and DCs</td>
<td>• Low conversion efficiency</td>
</tr>
<tr>
<td></td>
<td>• Broad range of tumour antigens expressed including unknown antigens</td>
<td>• Broad range of antigens may induce autoimmunity</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

CTL, cytotoxic T lymphocyte; AML, acute myeloid leukaemia; HLA, human leukocyte antigen; LAA, leukaemia-associated antigen.
Sites of action DC IT in cancer

Melief, Immunity, 2008
Adoptive cell therapy in cancer

Rosenberg, Nat Rev Immunol, 2008
Tregs in GvHD

Rocarolo, Nat Rev Immunol, 2007
Clinical use of Tregs
Hematopoetic stem cell transplantation

- inborn errors
- mega-chemotherapy leading to BM ablation
- GvL effect
- autoimmune diseases
- first HSCT and first gene therapy – in PID
Hematopoetic stem cell transplantation in immunodeficiency
Gene therapy

- selective growth advantage
- ADA (no use of PEG-ADA), SCID
- site of integration may influence cell’s fate
- proto-oncogene LMO2
Gene therapy – viral vectors

A Retroviral vector used for the SCID clinical trials

B Self-inactivated vectors

C Self-inactivated vector containing 2 x (250 bp) cHS4 insulators

D Self-inactivated vector containing insulator and a suicide gene (TK)

Figure 2
Schematic representation of retroviral vectors and their modifications to improve safety. (A) The transcription of the therapeutic gene is driven by the enhancer-promoter activity of the U3 region of the retroviral LTR. (B) The transcription of the therapeutic gene is driven by the addition of an internal promoter. The U3 region of the retroviral LTR has been almost completely deleted. (C) The provirus contains the cHS4 element (i.e., insulator) in order to protect the transcriptional cassette against position effects. (D) This provirus contains 2 cassettes: (a) the therapeutic gene driven by a first internal promoter and (b) a suicide gene (e.g., thymidine kinase, TK) that could allow the elimination of gene-corrected cells if an adverse event such as a monoclonal proliferation occurs. EF-1α, elongation factor-1α; IRES, internal ribosome entry site; PGK, phosphoglycerate kinase; R, repeats; SA, site acceptor; SD, site donor.
Gene therapy – homologous recombination

Cavazzana-Calvo, JCI, 2007
Gene therapy - problems

- low effectivity of gene transfer
- low expression of the target protein
- mutagenesis - oncogenesis
- immunogenicity of the gene/vector
Future of immunotherapy

- antigen specific immunosuppression
- lower toxicity and side effects
- the more we know about etiology, the more focused could be the attack
Psychological aspects

- beta-endorphins during exams
  - lymphopenia
  - activity of NK cells
  - production of IFN-gamma

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