Immunotherapy

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Interventions with impact on the immune system

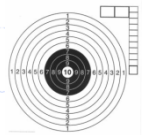
- immunomodulation
  - it is not clearly possible to distinguish stimulation from suppression

- overreacting IS ⇒ immunosuppression
- insufficient IS ⇒ immunostimulation

- antigen specific
- antigen non-specific

Legend

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



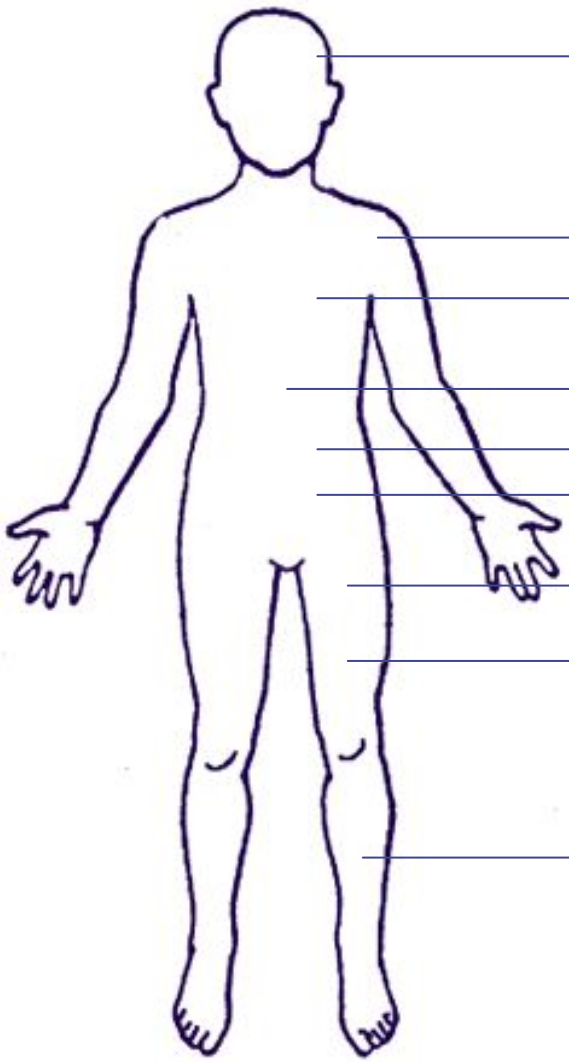
Effect of glukocorticosteroids

- 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

	Effect	Mechanism
Cellular transport	↑ neutrophils in circulation	release from BM but limited access into the tissues
	↓ monocytes in circulation	
	↓ lymphocytes (mainly CD4+)	apoptosis of CD4+ T cells sequestration within BM
Cellular function	↓ activity of macrophages	↓ maturation of M0 from monocytes
	↓ production of proinflammatory cytokines (IL-1, 6, TNF-alfa)	
	↓ chemotaxis	
	↓ baktericidia	
	↓ T cell activation	↓ transcription of genes for IL-1,2,3,4,6, IFN-gamma
	↓ function of endothelium	↓ expression of adhesive molecules
	↓ function of NK cells	↓ actoivity of NO synthese
Inflammation	↓ prostaglandins synthesis	↓ inhibition fof phospholipase A2 and cyclooxygenáse

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

impaired wound helaing

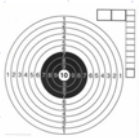
Cushingoid habitus
increased infection rate
decreased growth dynamics

Obrázek převzat z www.bfawu.org

Effects of NSAID on IS

- inhibition of cyklooxygenase (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

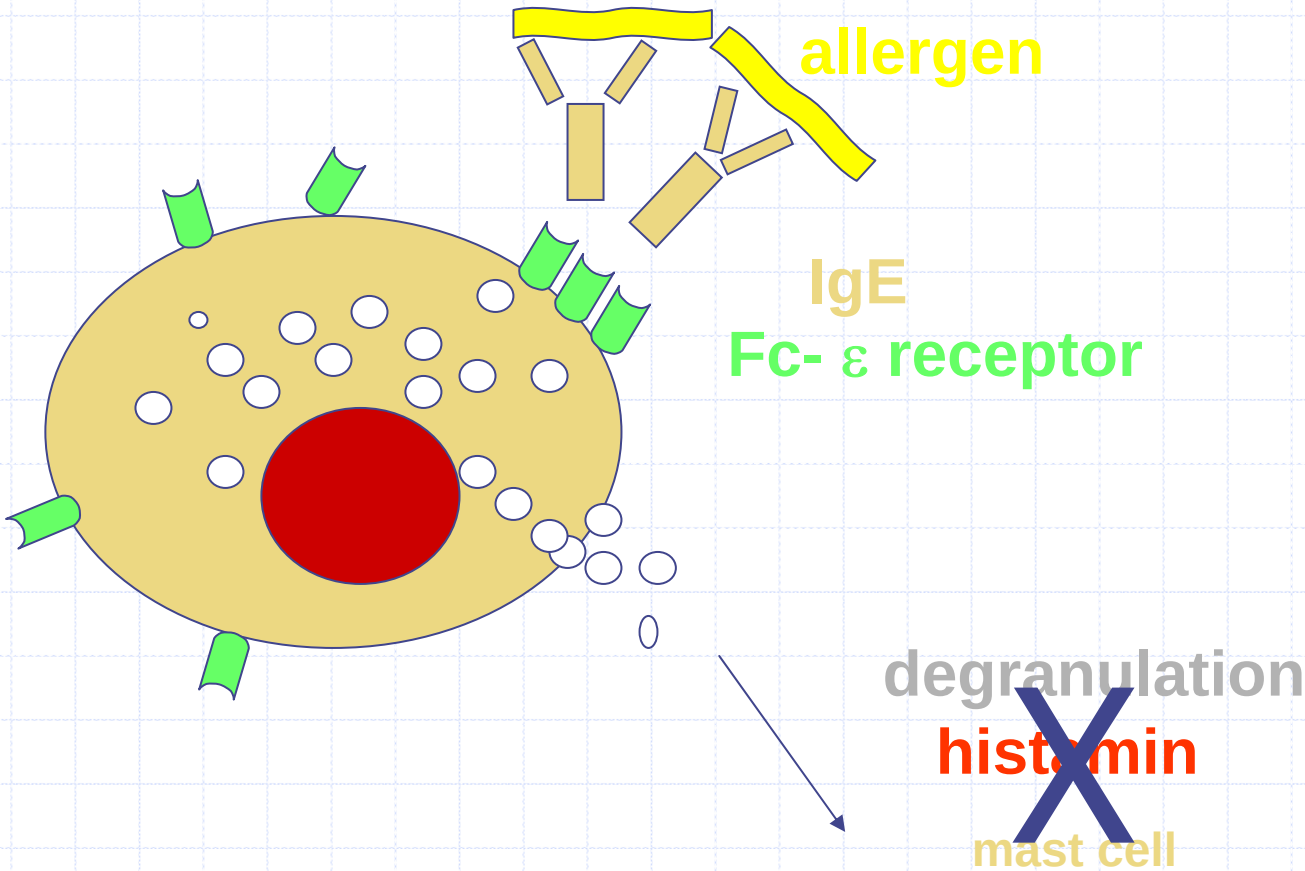
- main usage: analgesics, antipyretics
- one of the most spread drugs in the world
  - :acylpyrin, ibuprofen, coxiby (Vioxx, Celebrex, GIT bleeding, myocardial infarction...)



Antihistaminics

- generation I
  - dithiaden
- generation II
  - cetirizin, loratadin (Zodac, 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)

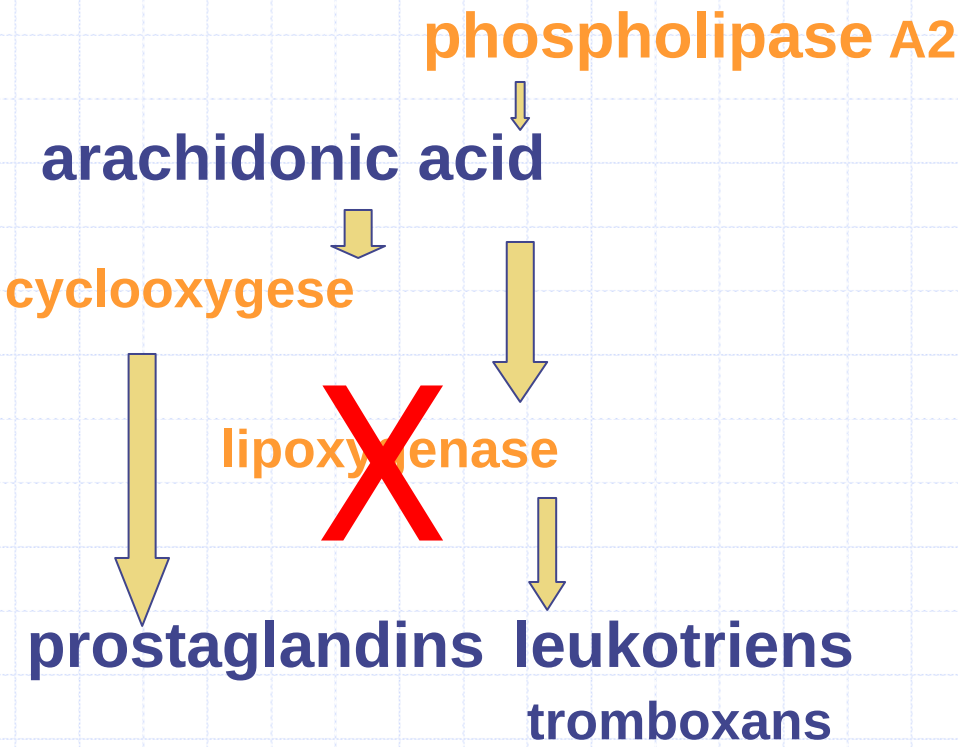
Antihistaminics



Antileukotriens

- inhibition of leukotrien production (or its receptors)
  - zafirlukast, montelukast (Singulair)
- usage:
  - mild form of bronchial asthma
  - activity-induced asthma
  - ACP-sensitive asthma

Antileukotriens



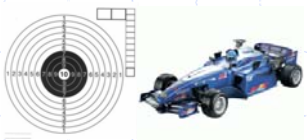
Immunosuppressive drugs

- 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 metabolization in liver)
  - effects are seen after several weeks
  - bone marrow toxicity (granulocytopenia, trombocytopenia)
  - homozygotic deficiency of TPMT (thiopurine-methyl transferase) – life-threatning 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 agens

- 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 phenomen
- 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 - alpha

- 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)

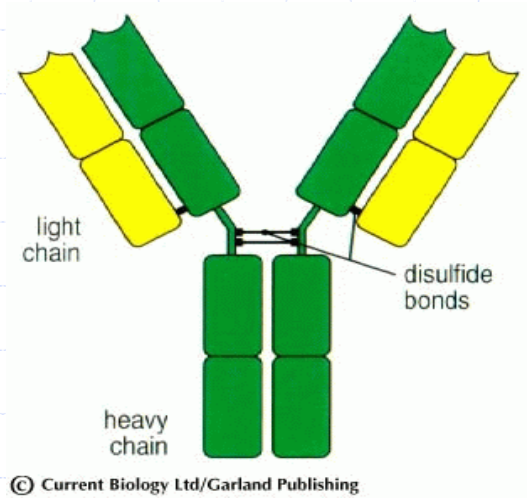
- frequent usage, extremely expensive

Immunostimulation

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

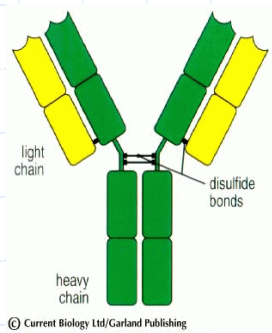
Immunoglobulin therapy

- substitution
  - primary antibody immunodeficiency
  - secondary antibody immunodeficiency
- immunomodulation of autoimmune diseases
- 1 g approx. $4x10^{18}$ IgG molecules
- different dosing



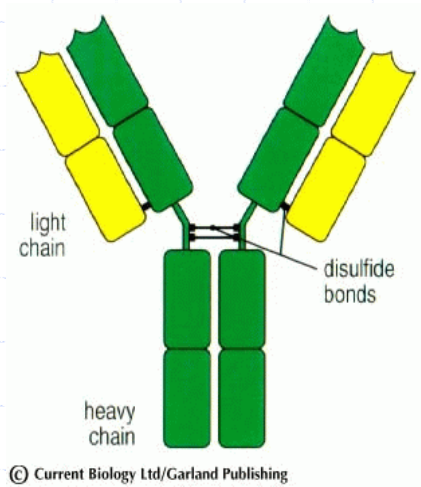
Mechanisms of IVIg effect - Fc fragment dependent

- 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-idiotype activity
- inhibition of B cell differentiation and activation
- creation of rheumatoid factors (anti-Ig Ab)



Clinical use of IVIg

effect **proven** by RCT

- immune trombocytopenia
- Guillain-Barré syndrome
- chronic demyelinizating 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, vinkristine), toxins (ricin, abrin), radioizotope (iod-131, yttrium-90)
- immunolocalization of tumour – radioisotope-labeled MoAb (indium-111, technecium-99)

FDA approved MoAb in cancer

Table 2. Monoclonal Antibody Products

Name	Type	Target	Clinical
Rituximab	Chimeric	CD20	NHL
Trastuzumab	Humanized	Erb B2	Breast
Bevacizumab	Humanized	VEGF	Colorectal
Alemtuzumab	Humanized	CD52	CLL
Cetuximab	Chimeric	EGFR	Colorectal
Panitumumab	Human	EGFR	Colorectal

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

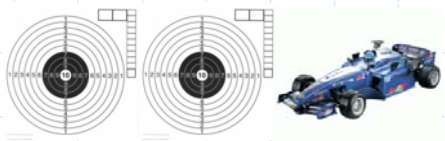
Oldham, J Clin Oncol, 2008

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

Immunomodulation with antigen

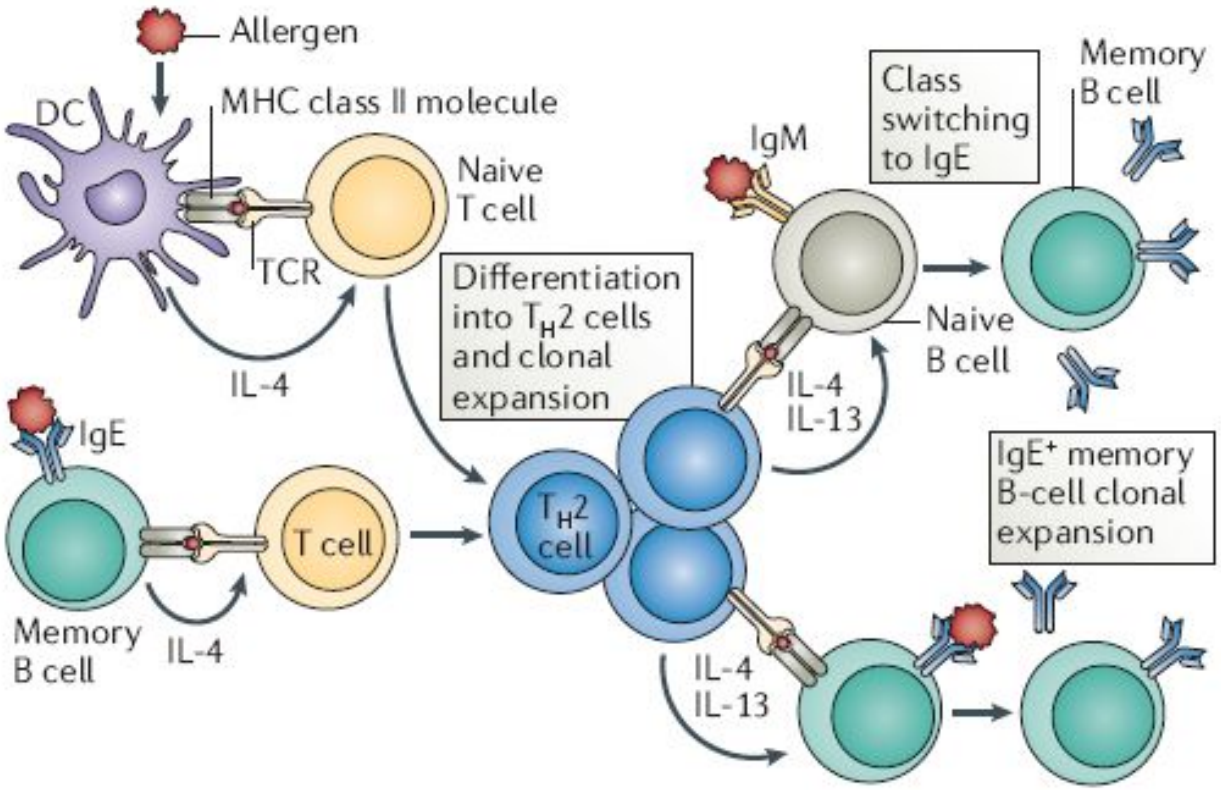
1. immunotherapy of allergic diseases – use of defined exoallergen
2. cancer immunotherapy – DC, T cells
3. adoptive immunotherapy
4. immunotherapy of autoimmune diseases – hypothetical use of defined autoantigen



Allergen-specific immunotherapy

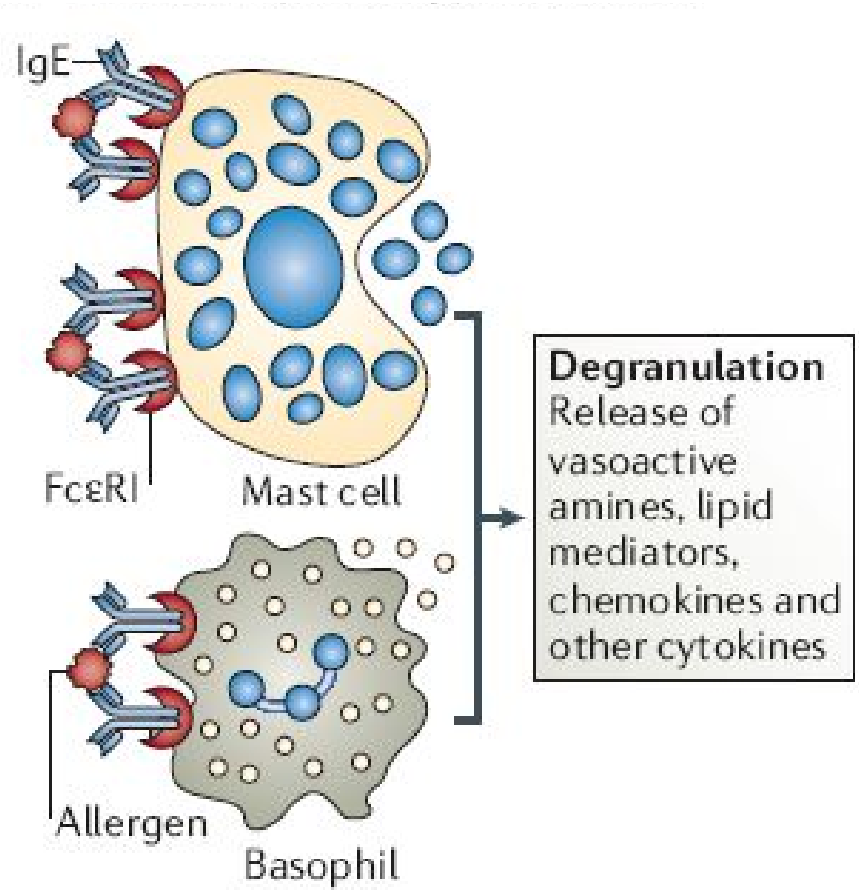
- hyposensitization – repeated application of gradually growing doses of allergen
- lasts for 3-5 years
- isotype switch, degranulation of mast cells
- subcutaneous, inhalation, ingestion

Allergy – senzitization and memory



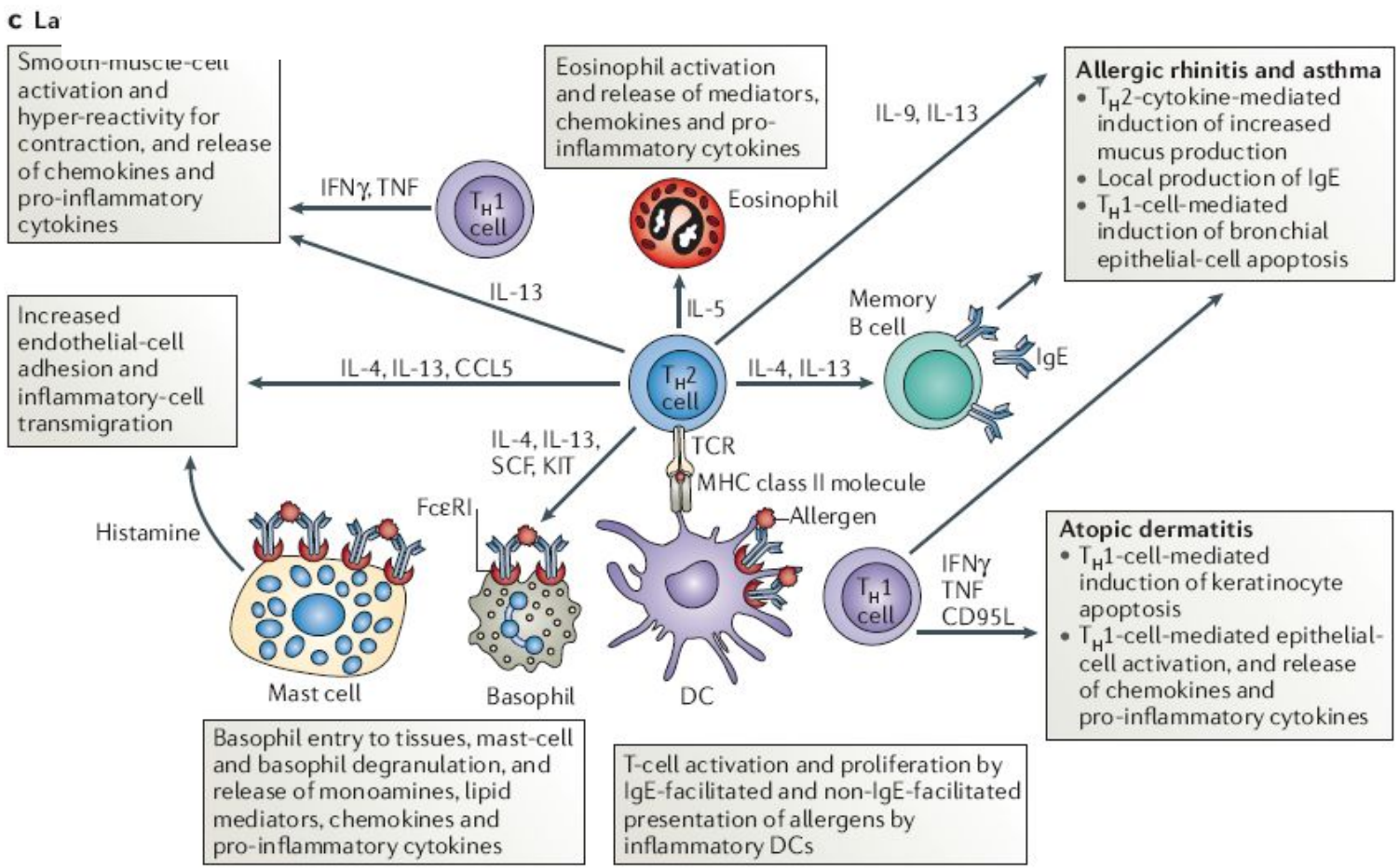
Larche, Nat Rev Immunol, 2006

Immediate phase of allergic inflammation



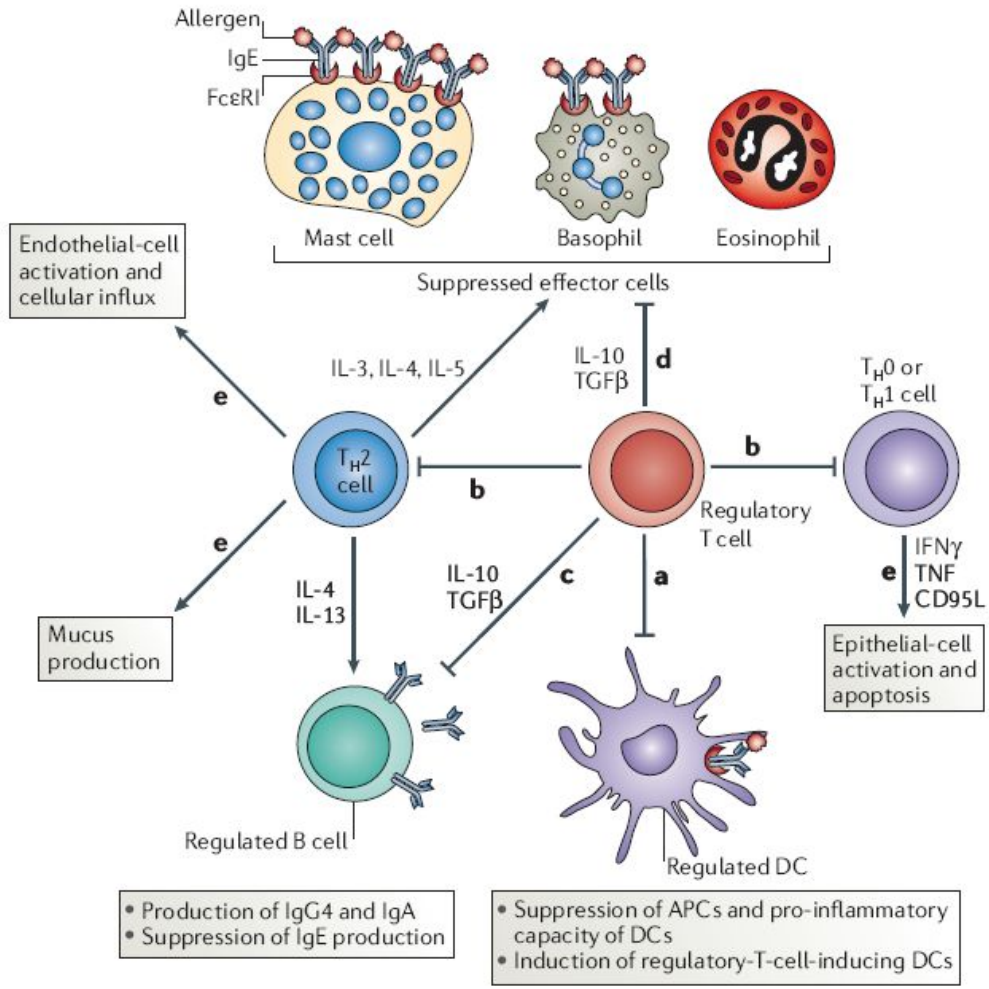
Larche, Nat Rev Immunol, 2006

Late phase of allergic inflammation



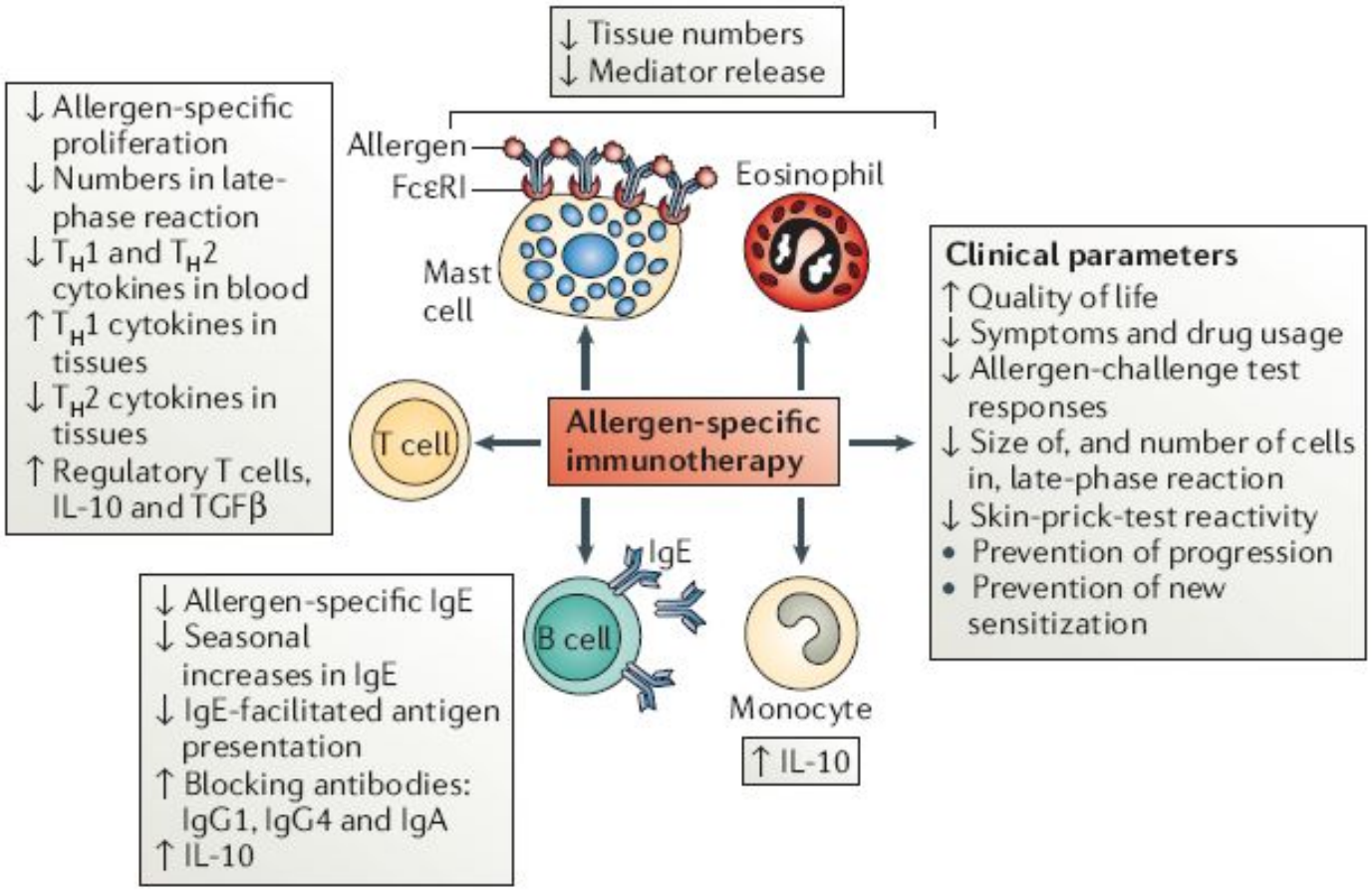
Larche, Nat Rev Immunol, 2006

Proposed role of regulatory T cells in IT



Larche, Nat Rev Immunol, 2006

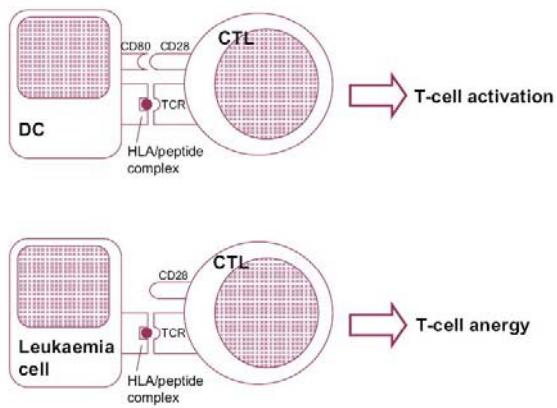
Effects of allergen-specific IT



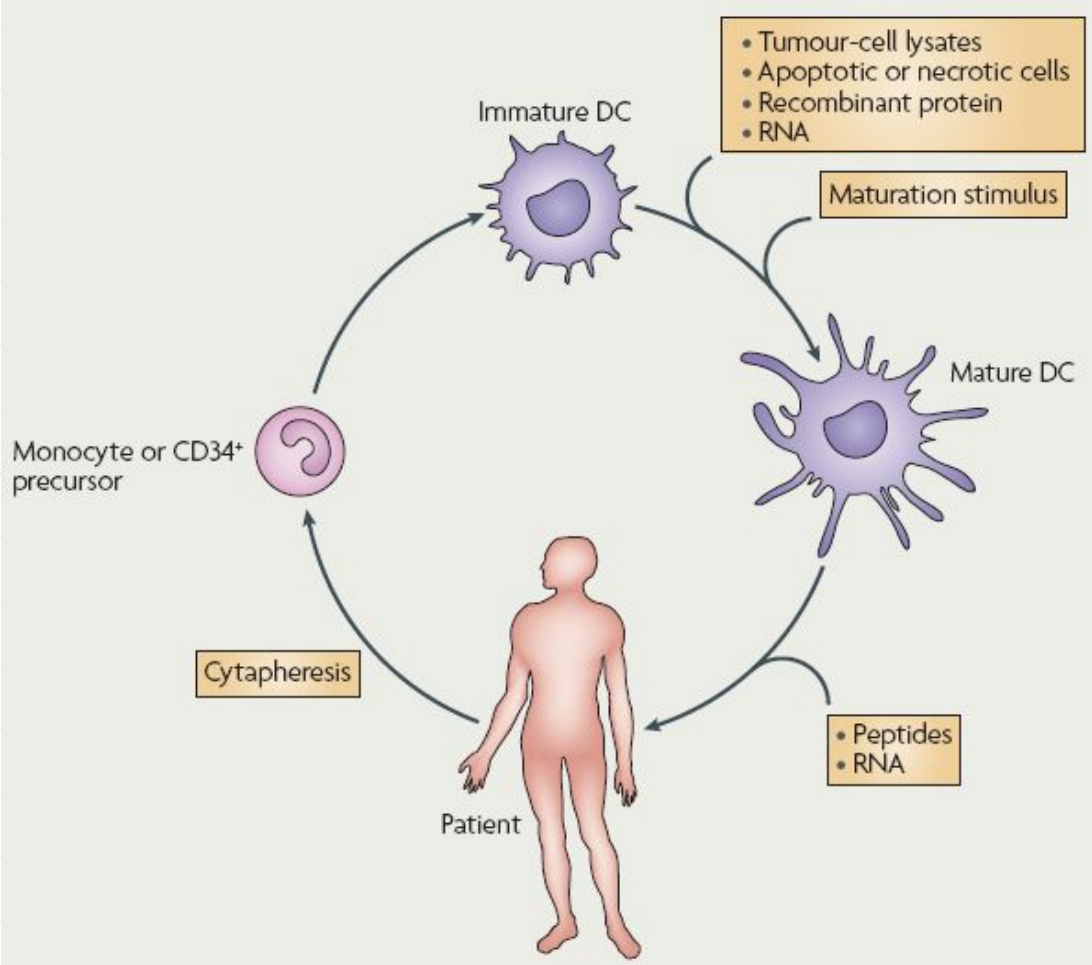
Larche, Nat Rev Immunol, 2006

Dendritic cells based vaccines

- TLR receptors (Toll-like) – essential for the initiation of immune response
- If no TLR costimulation – Tcell 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

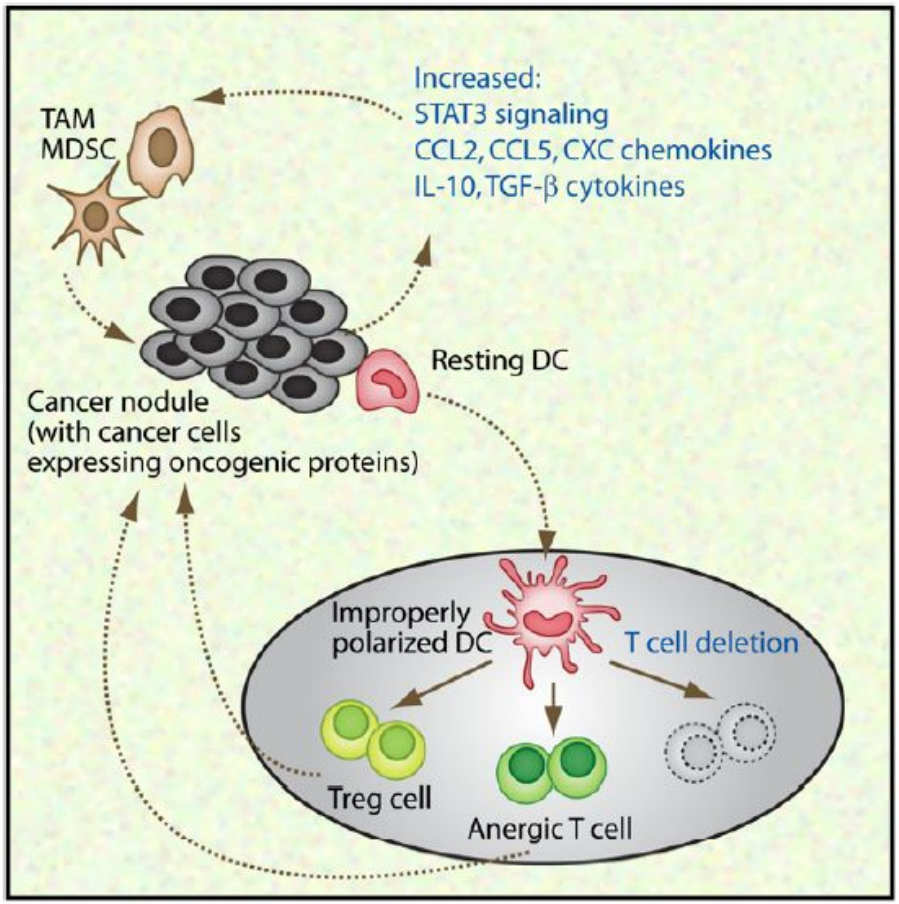
Different DC loading techniques

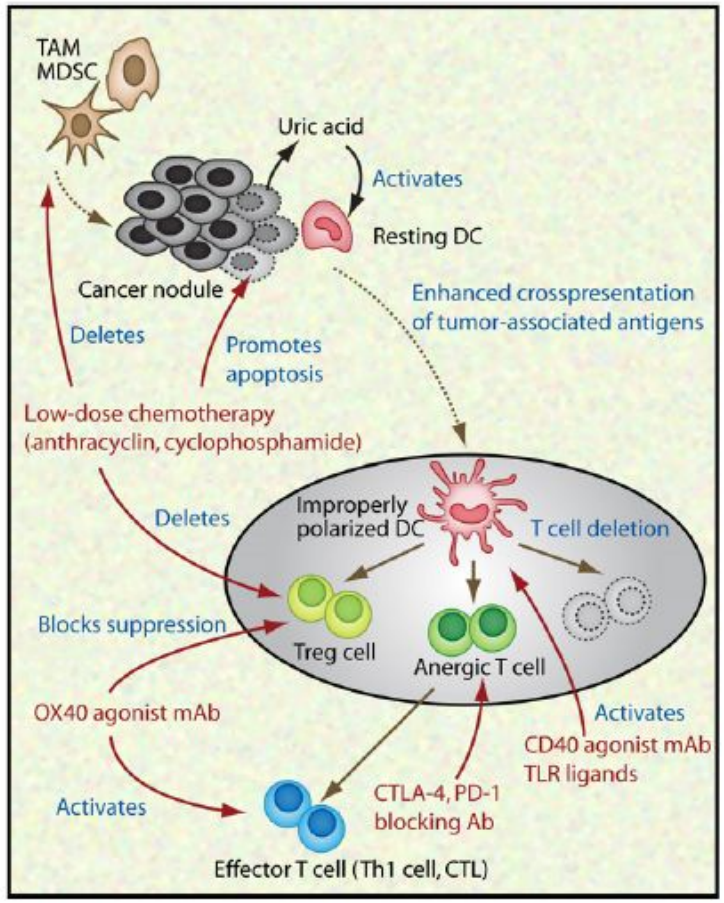
DC loading technique	Advantages	Disadvantages
DCs loaded with defined tumour antigens	• Induces CTL responses against leukaemia cells alone, not stem cells	• Antigen drift/loss • HLA restriction (for LAA-derived peptides) • Differing responses/avidity of CTLs to peptides • T-cell exhaustion if peptide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

CTL, cytotoxic T lymphocyte; AML, acute myeloid leukaemia; HLA, human leukocyte antigen; LAA, leukaemia-associated antigen.

Duncan, Best Practice and Clinical Haematology, 2008

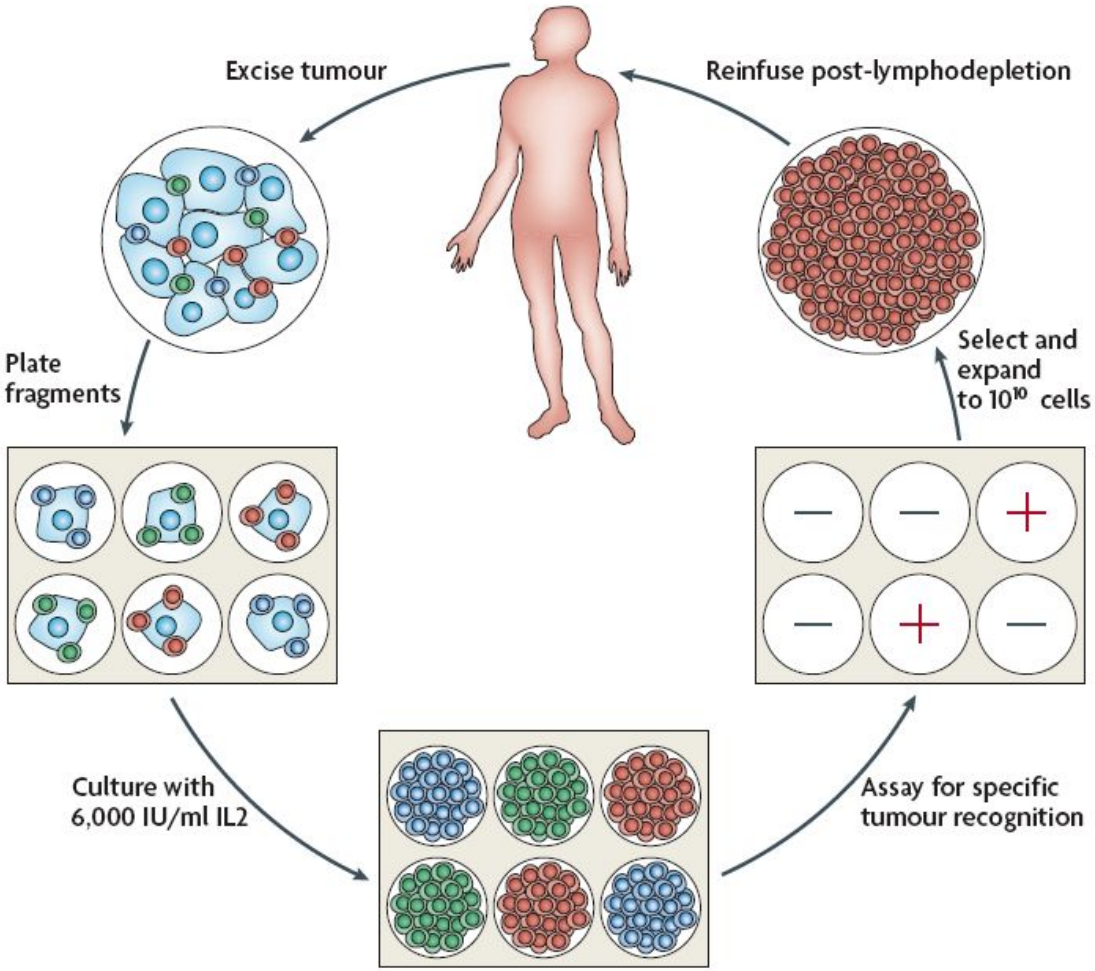
Sites of action DC IT in cancer





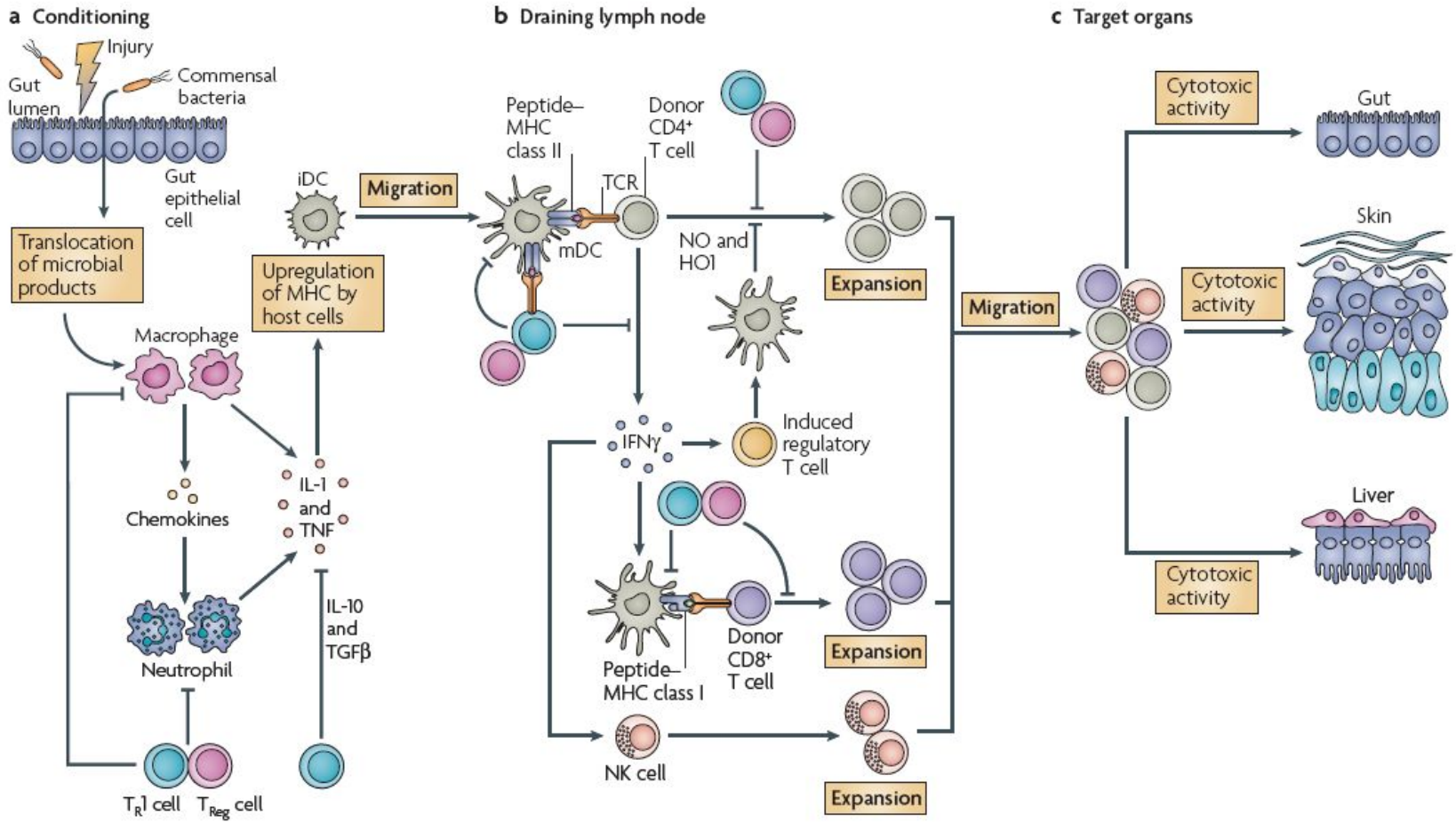
Melief, Immunity, 2008

Adoptive cell therapy in cancer



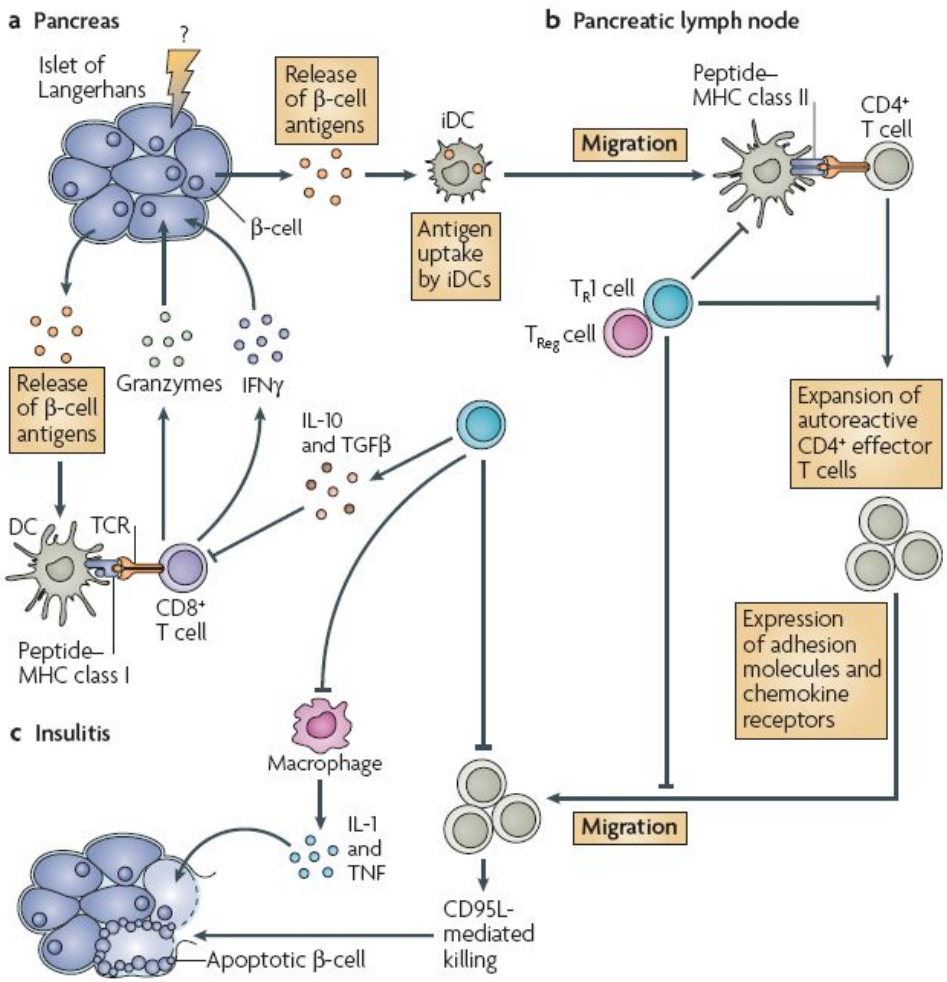
Rosenberg, Nat Rev Immunol, 2008

Tregs in GvHD

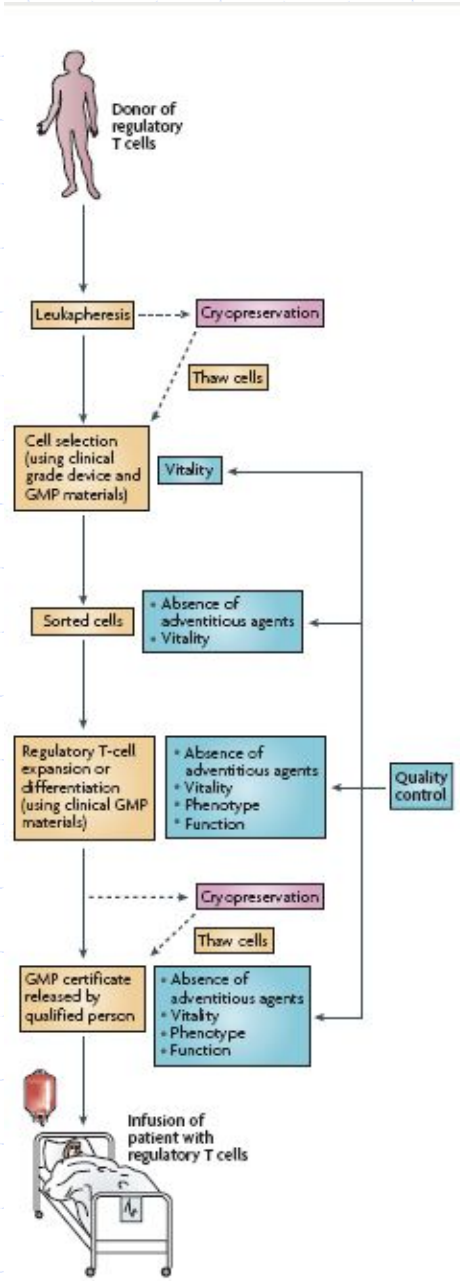


Rocarolo, Nat Rev Immunol, 2007

Clinical use of Tregs



diabetes

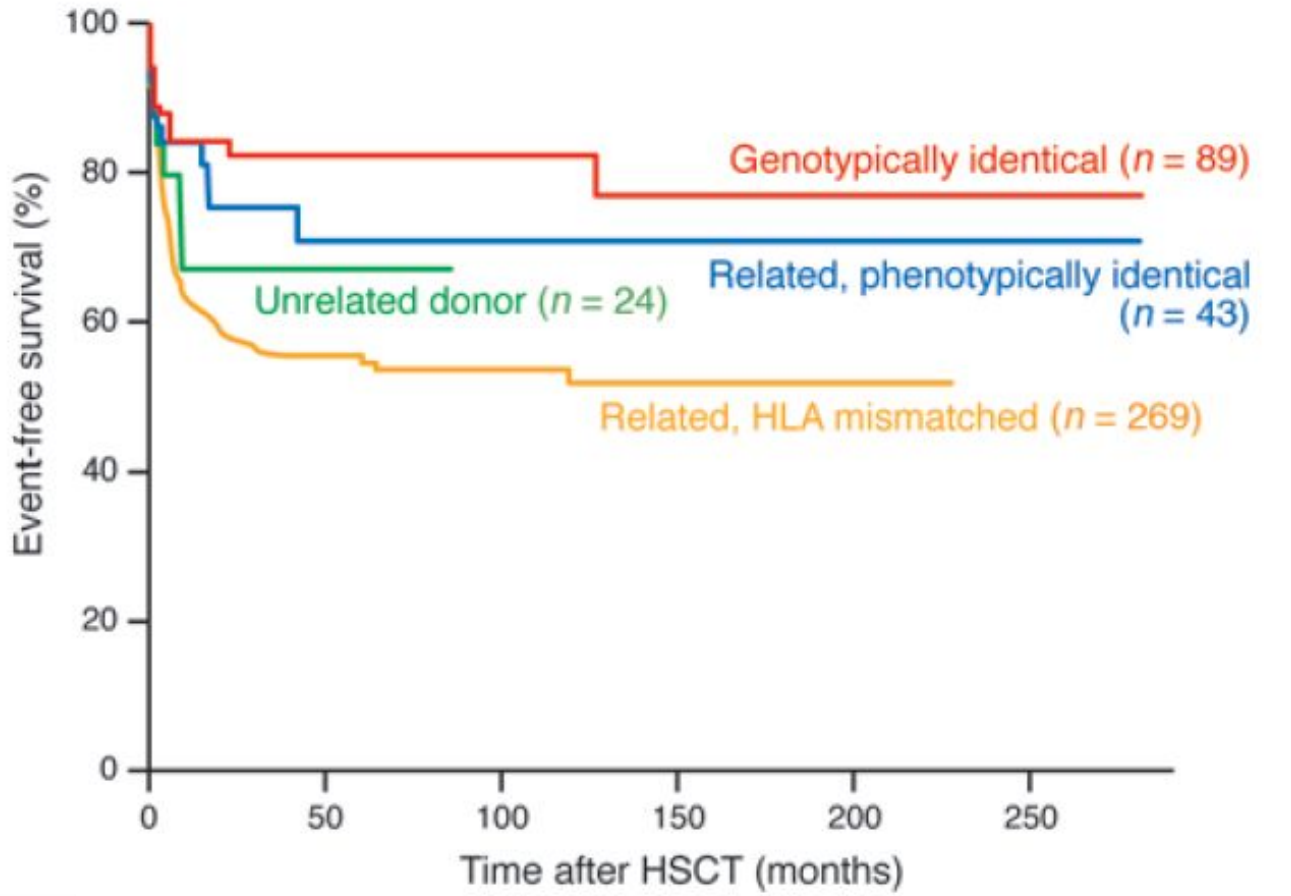


Rocarolo, Nat Rev Immunol, 2007

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



Lancet

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

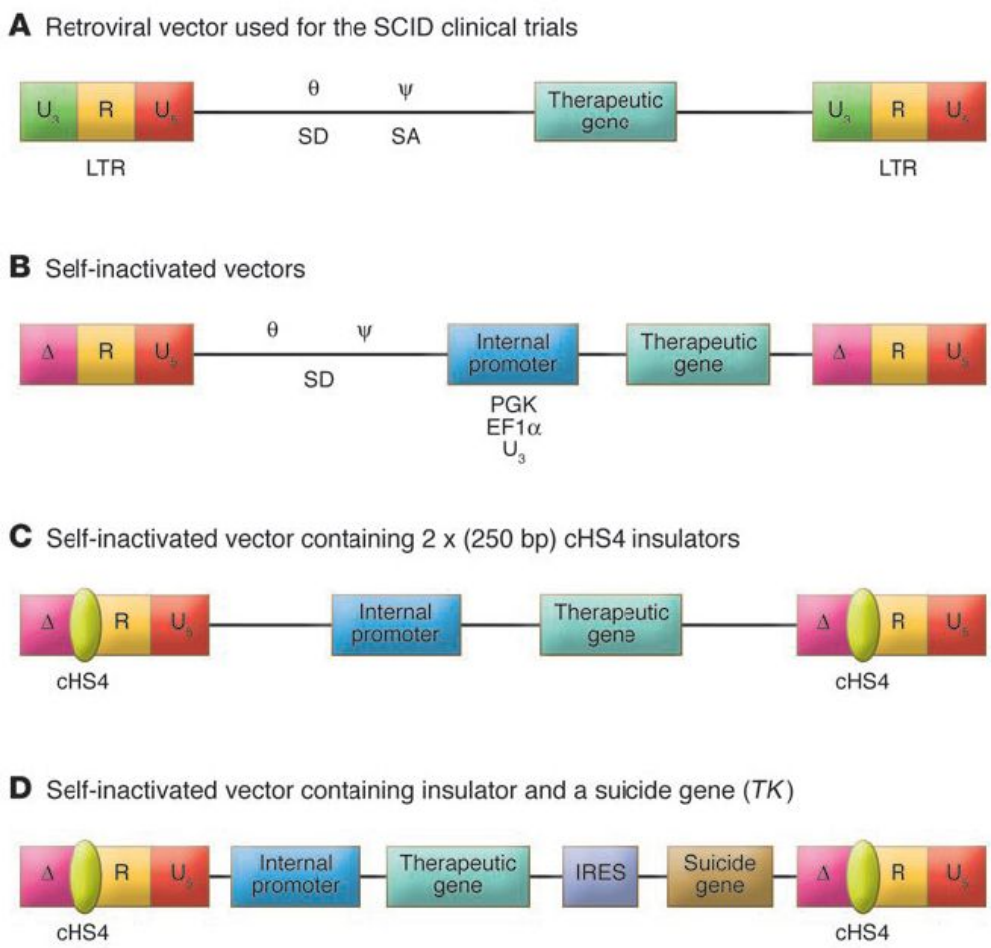
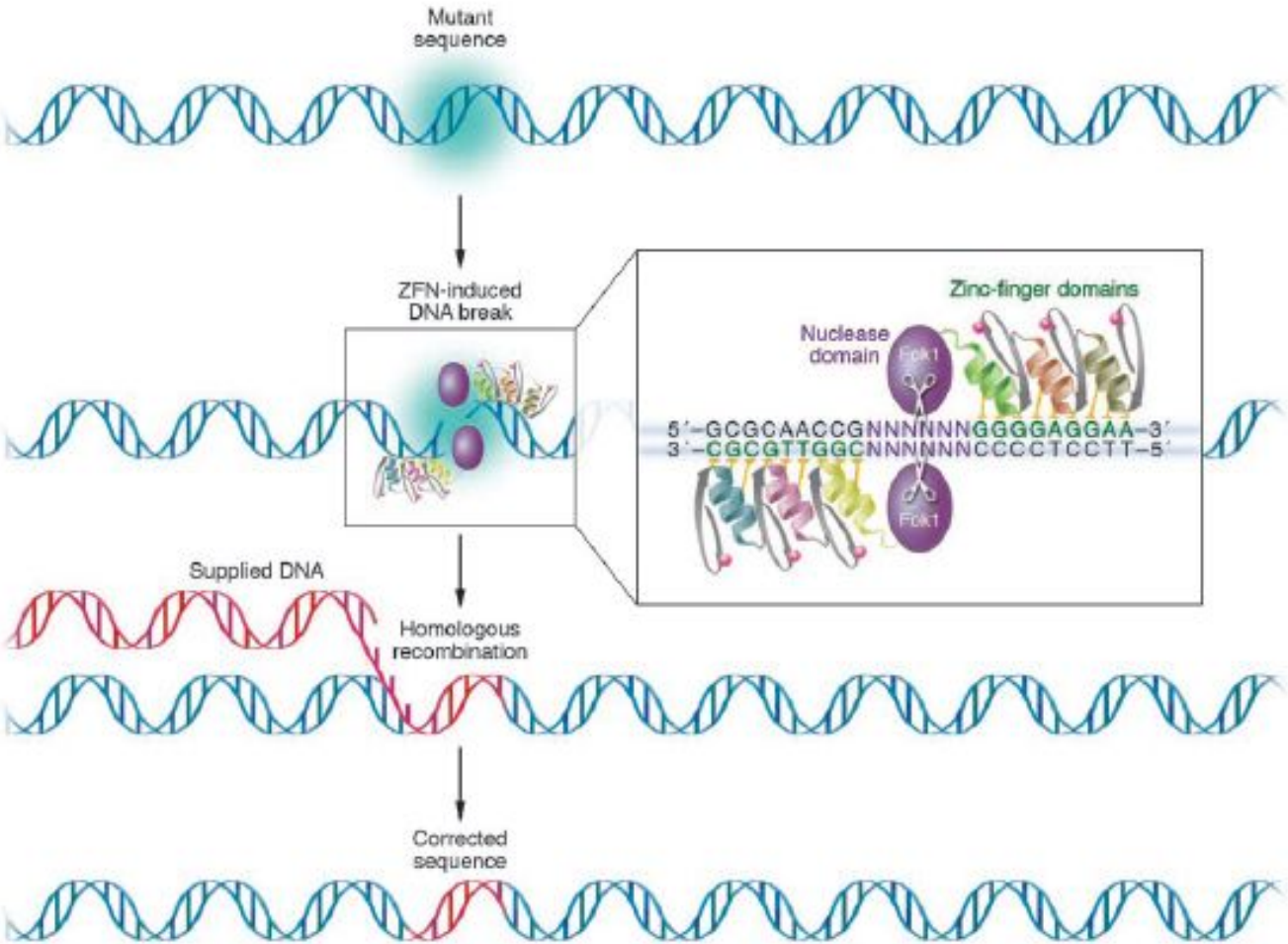


Figure 2
Schematic representation of retroviral vectors and their modifications to improve safety. (**A**) The transcription of the therapeutic gene is driven by the enhancer-promoter activity of the U_3 region of the retroviral LTR. (**B**) The transcription of the therapeutic gene is driven by the addition of an internal promoter. The U_3 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-1a, elongation factor–1a; IRES, internal ribosome entry site; PGK, phosphoglycerate kinase; R, repeats; SA, site acceptor; SD, site donor.

Cavazzana-Calvo, JCI, 2007

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-endorfins
- during exams
  - lymphopenia
  - ⬇ activity of NK cells
  - ⬇ production of IFN-gamma

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www.glcyd.org

www.thecamreport.com