Transplantation

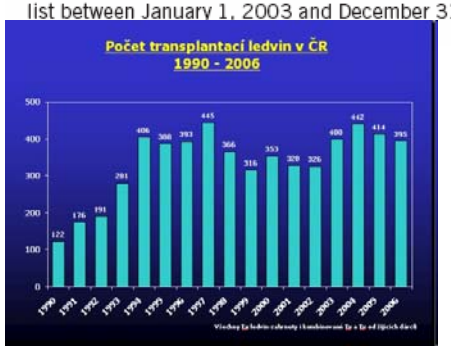
Tomáš Kalina

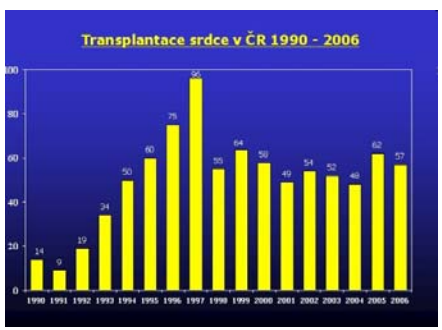
Transplantation rates in U.S. and in CR

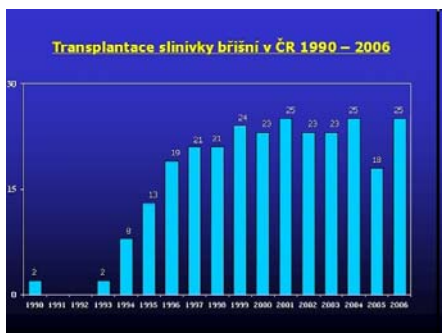
Table 1 2003 organ transplant data for the United States[a]

Organ or tissue	Transplants	Patients on waiting list[b]	Patients added to list[c]	1-year graft survival	5-year graft survival
Kidney	13,765	57,211	24,493	94.3% (Live donor) 88.7% (Cadaver donor)	78.6% (Live donor) 65.7% (Cadaver donor)
Pancreas	527	1,390	926	78.8%	45.4%
Kidney + pancreas	1,236	2,472	1,653	92.0% (Kidney) 85.1% (Pancreas)	74.2% (Kidney) 69.8% (Pancreas)
Liver	2,239	17,515	10,331	80.6%	64.2%
Heart	2,065	3,529	2,942	85.3%	70.6%
Lung	1,110	3,874	1,954		

[a]Data were obtained from the US Transplant website (www.ustransplant.org). [b]The number of total patients on the waiting list on December 31, 2003. [c]The number of new patients added to the list between January 1, 2003 and December 31, 2003.







Česká Transplantační Společnost

www.transplant.cz/

MAJOR CONCEPTS IN TRANSPLANT IMMUNOLOGY

- How does the immune system deal with a transplant, i.e. What are the mechanisms of rejection?
- What are the current clinical strategies to block rejection?
- What are the new and future strategies to promote specific immune tolerance?
- What is the role of xenotransplantation?
- What is graft versus host disease?

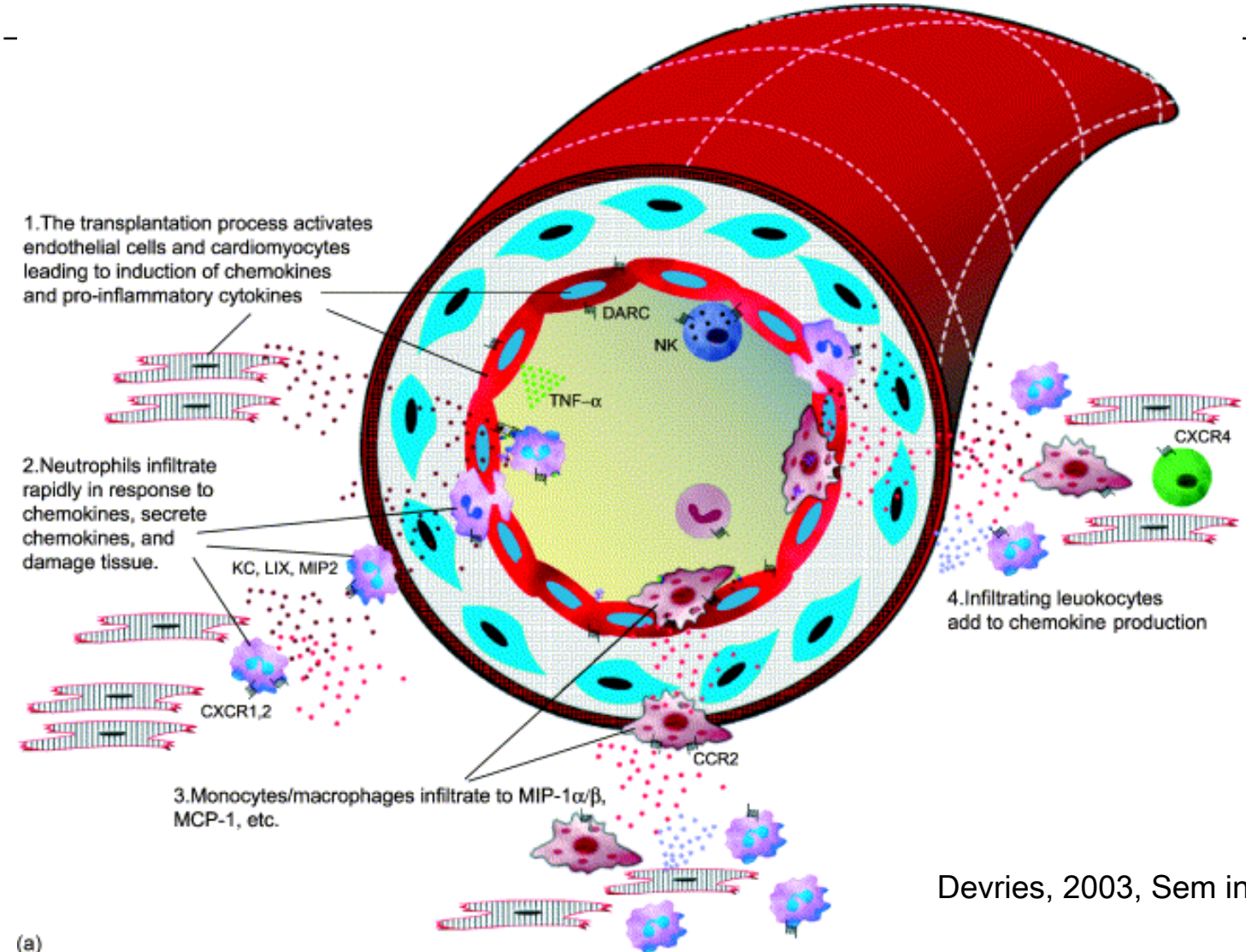
Content

- Mechanisms
- Rejection types
  - Hyperacute
  - Acute
  - Chronic
- Laboratory tests
- Management of rejection
- Bone marrow transplantation, GvHD and GvL effect
- In vivo imaging – *cool images for those who are patient*

ANTIGEN INDEPENDENT MECHANISMS

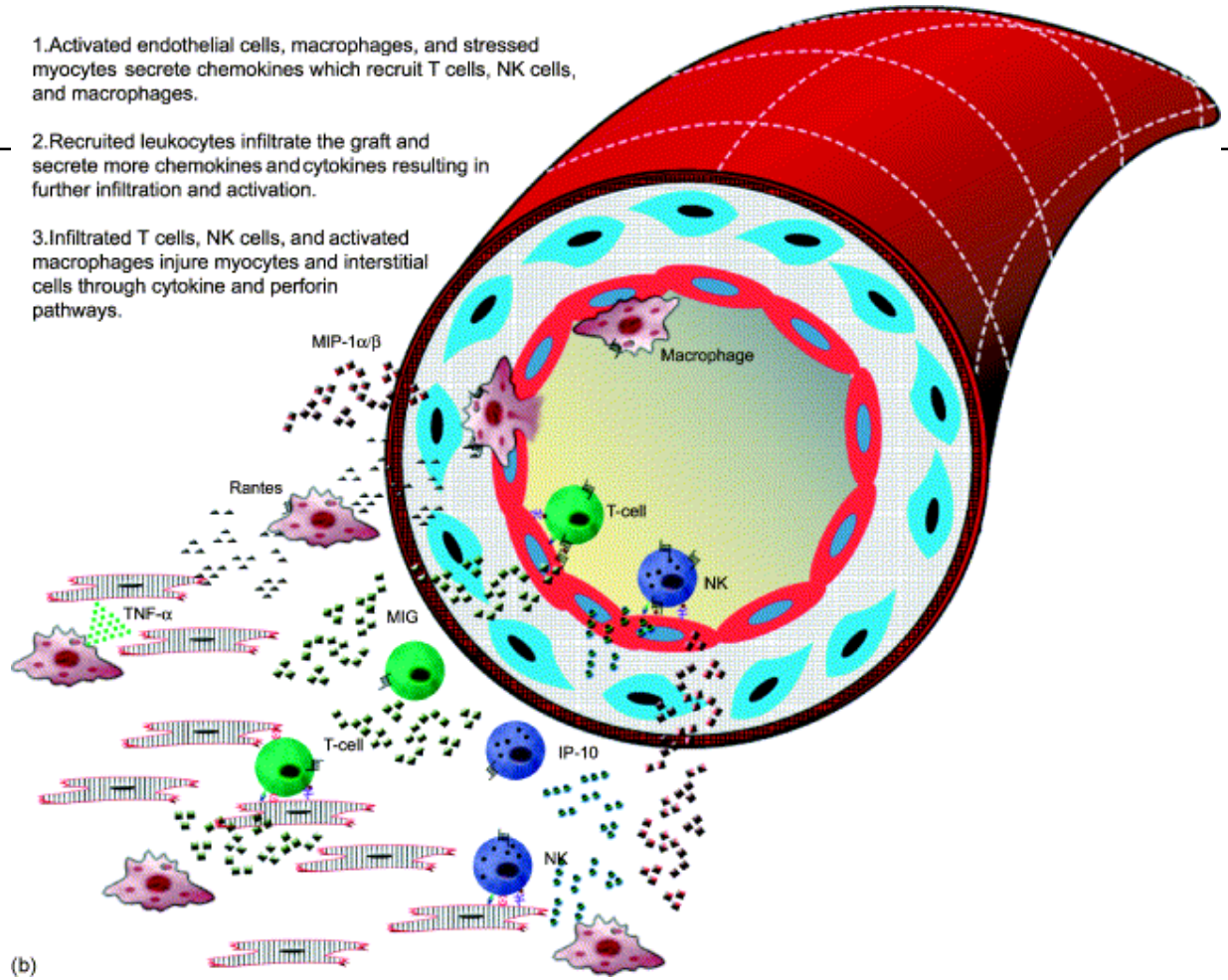
- PERITRANSPLANT ISCHEMIA
- MECHANICAL TRAUMA
- REPERFUSION INJURY

Peritransplant injury induces chemokines that increase inflammation and immunity



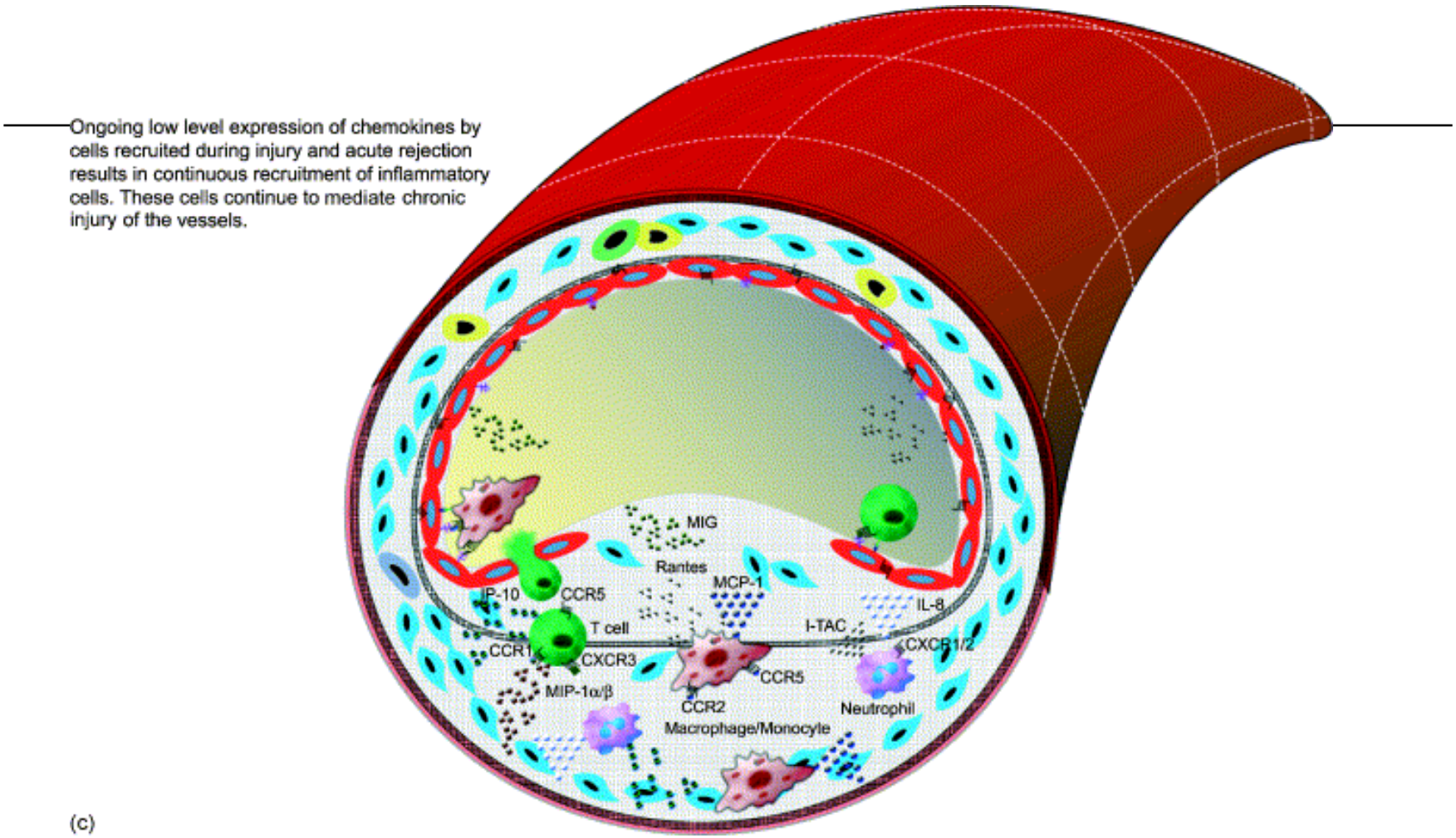
Devries, 2003, Sem in Imm 15:33-48

Peritransplant injury as a risk factor for Acute Rejection



Early inflammatory injury to graft promotes continued chemokine expression that recruits lymphocytes and macrophages

Peritransplant injury as a risk factor for Chronic Rejection



Early inflammatory injury to graft promotes continued chemokine expression that persists and contributes to and chronic rejection

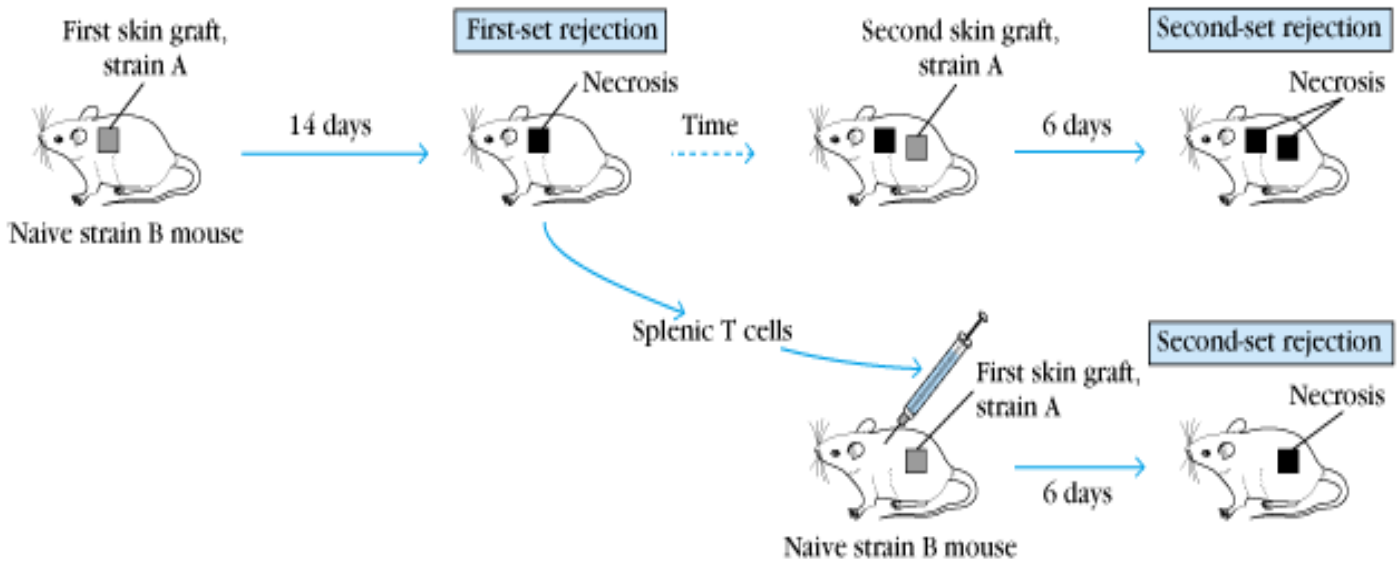
Transplants and the immune system

- Discrimination between self/nonself
- This is not good for transplants
- At first the only possible transplants were blood transfusions
- Otherwise the grafts were disastrous

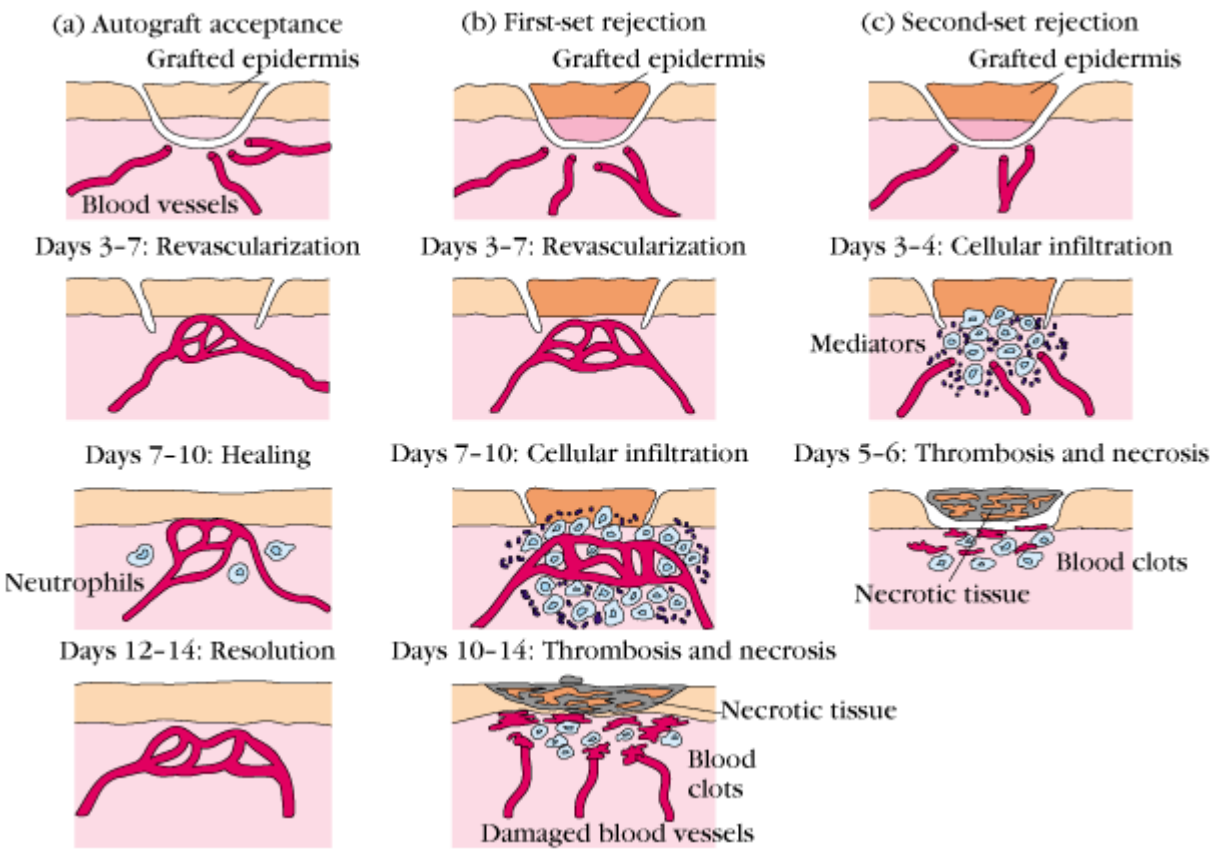
Why are blood transfusions tolerated?

Immune mechanisms

- Skin is transplanted to genetically different organisms



Graft


(a) Autograft acceptance
Grafted epidermis
Blood vessels
Days 3–7: Revascularization
Days 7–10: Healing
Neutrophils
Days 12–14: Resolution
(b) First-set rejection
Grafted epidermis
Days 3–7: Revascularization
Days 7–10: Cellular infiltration
Days 10–14: Thrombosis and necrosis
Necrotic tissue
Blood clots
Damaged blood vessels
(c) Second-set rejection
Grafted epidermis
Days 3–4: Cellular infiltration
Mediators
Days 5–6: Thrombosis and necrosis
Blood clots
Necrotic tissue

Cellular and Molecular Understandings

•Associated with graft rejections and immunosuppressive therapies

•Rejection has not been eliminated only reduced

- Hyperacute rejection
- Acute rejection
- Chronic rejection

Hyperacute Rejection

- Occurs within a few minutes to a few hours
- Result of destruction of the transplant by performed antibodies (cytoxic antibodies)
- Some produced by recipient before transplant
- Generated because of previous transplants, blood transfusions, and pregnancies
- Antibodies activate the complement system then platelet activation and deposition causing hemorrhaging and swelling

Cell-mediated immunity is not involved at all in these reactions

Acute Rejection

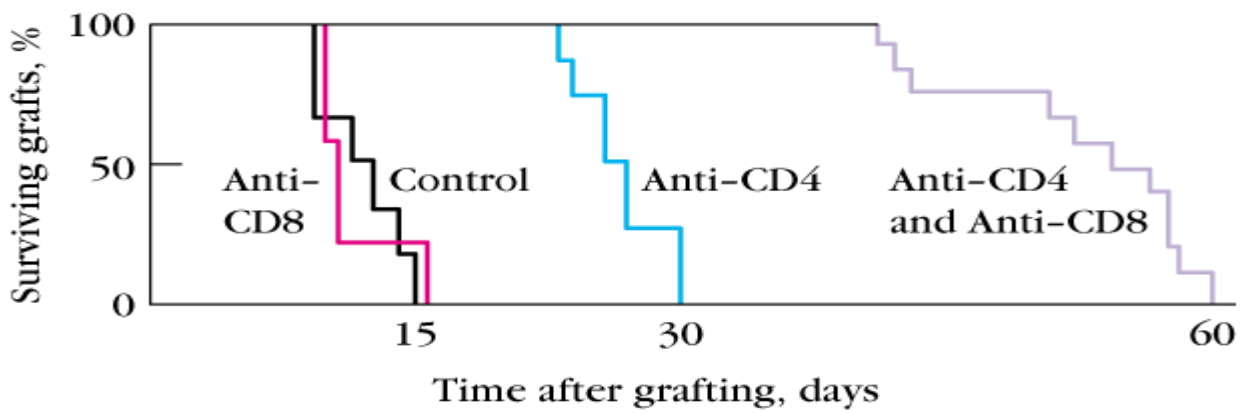
- Seen in recipient that has not been previously sensitized to the transplant
- Mediated by T cells and is a result of their direct recognition of alloantigens expressed by the donor
- Very common in mismatched tissue or insufficient immunosuppressive treatment
- Reduced by immunosuppressive therapy

Cell-mediated immunity is happening here

Cell-mediated immunity is happening here

Allograft

1. Exam of rejection site reveals lymphocyte and monocytic cellular infiltration reminiscent of the delayed type hypersensitivity reaction
2. Animals that lack T lymphocytes do not reject allograft or engrafts
3. Rejection doesn't occur at all in immunosuppressed individuals



Chronic rejection

- Caused by both antibody and cell-mediated immunity
- May occur **months to years** down the road in allograft transplants after normal function has been assumed
- Important to point out rate, extent, and underlying mechanisms of rejection that vary depending on tissue and site
- The recipients circulation, lymphatic drainage, expression of MHC antigens and other factors determine the rejection rate
- **Inflammation, smooth muscle proliferation, fibrosis**
- **Tissue ischemia**

Histology of graft rejection

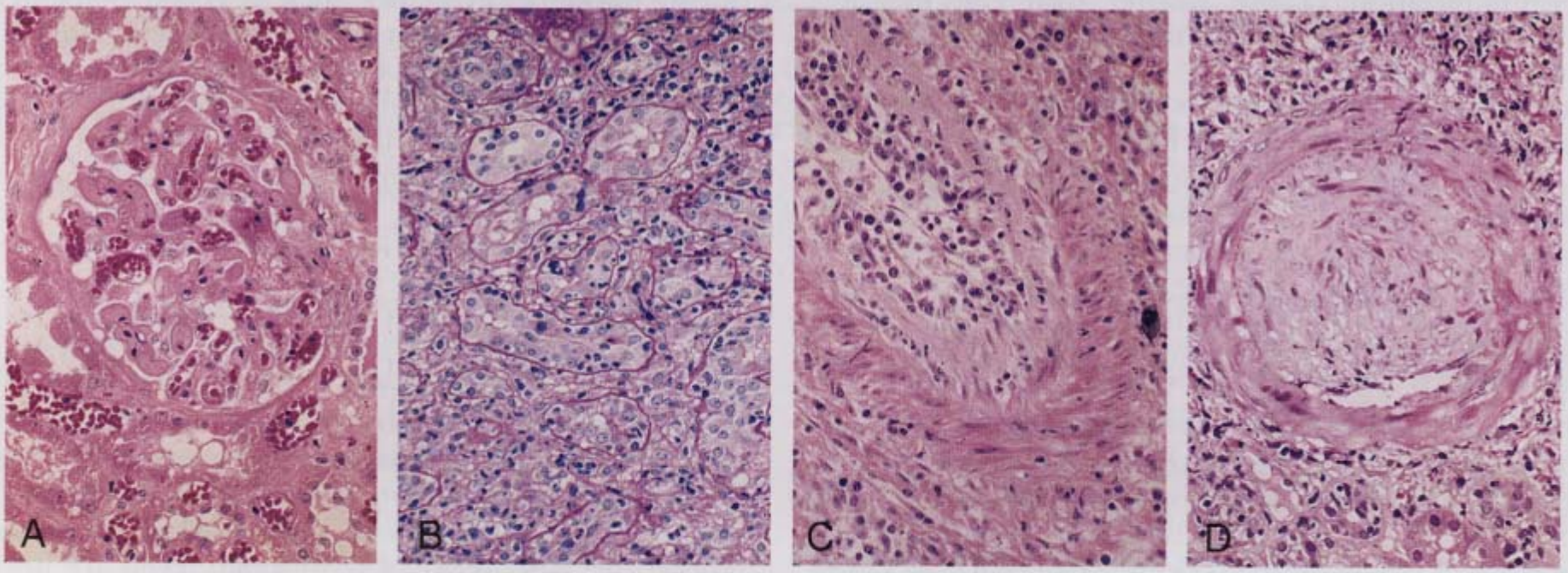


Figure 16–6 Histopathology of different forms of graft rejection.

A. Hyperacute rejection of a kidney allograft with endothelial damage, platelet and thrombin thrombi, and early neutrophil infiltration in a glomerulus.

B. Acute rejection of a kidney with inflammatory cells in the interstitium and between epithelial cells of the tubules.

C. Acute rejection of a kidney allograft with destructive inflammatory reaction destroying the endothelial layer of an artery.

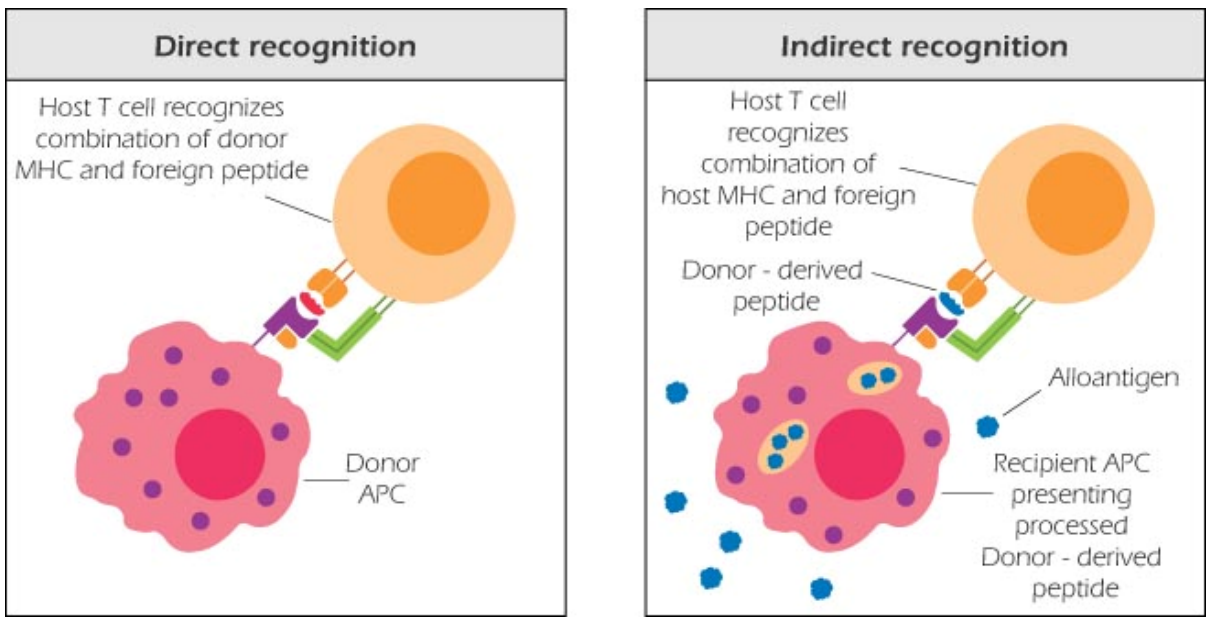
D. Chronic rejection in a kidney allograft with graft arteriosclerosis. The vascular lumen is replaced by an accumulation of smooth muscle cells and connective tissue in the vessel intima. (Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston.)

Role of MHC molecules

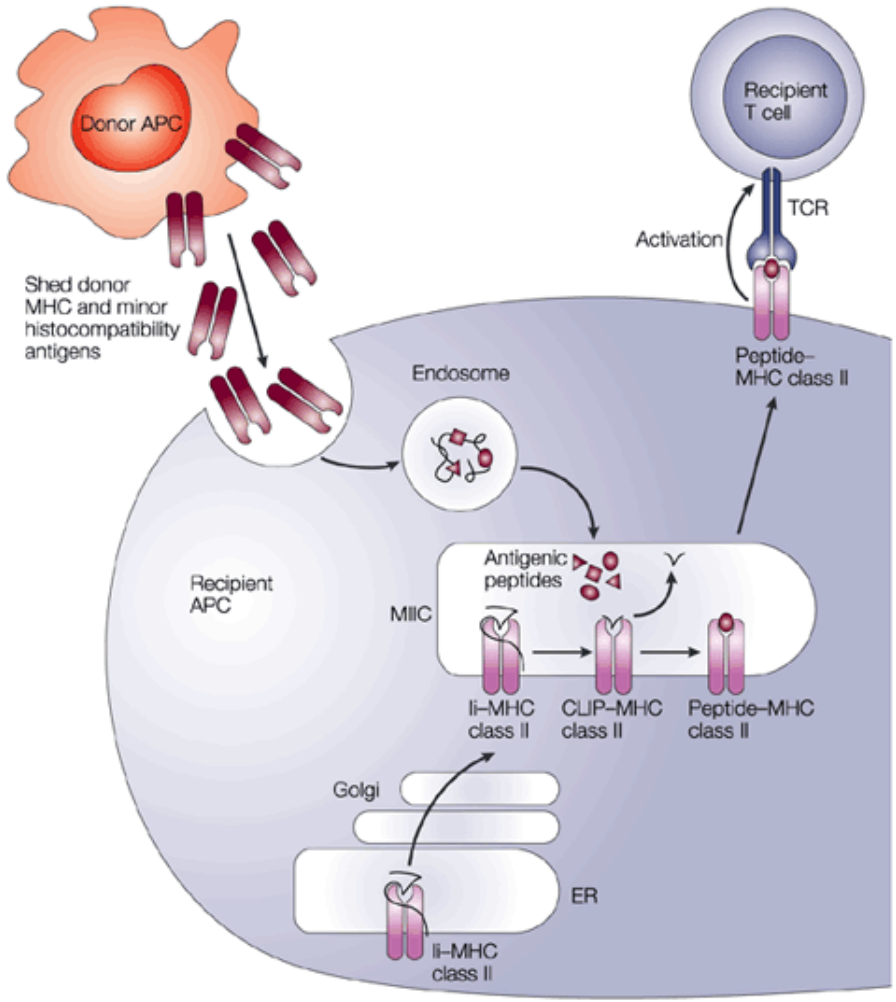
- When T cells are exposed to foreign cells expressing non-self MHC, many clones are tricked into activation - their TCRs bind to foreign MHC-peptide complex's presented
- T cells are reacting directly with the donor APCs expressing allogeneic MHC in combination with peptide. These donor APCs also have costimulatory activity to generate the second signal for the second reaction to occur
- Minor H antigens are encoded by genes outside the MHC

T Cells and Cytokines

- CD4+ and CD8+
- DTH



Indirect – donor APC shed MHC that activate immune system that then reacts to transplanted organ



Laboratory Tests

- ABO Blood typing
- Tissue typing (HLA Matching)
- (Lymphocytotoxicity test)
- (Mixed leukocyte reaction)
- Screening for Presence of Preformed Antibodies to allogeneic HLA
- Crossmatching

Prolonging Allograft Survival

- Anti-inflammatory Agents
- Cytotoxic Drugs
- Agents that interfere with Cytokine production and signaling
- Immunosuppressive Therapies
- New Immunosuppressive strategies

Prolonging Allograft Survival

- Cyclosporine and Tacrolimus (FK-506)
- Azathioprine
- Mycophenolate Mofetil
- Rapamycine
- Corticosteroids
- Anti-CD3, Anti-CD52, Anti-IL-2, Anti–CD25

Prolonging Allograft Survival

Table 2 Sites of action of antirejection drugs in clinical use

Category	Molecular target
Inhibitors of DNA synthesis	
Azathioprine	Inhibitor of purine synthesis; blocks phosphorybosyl pyrophosphate synthase, inosinate-monophosphate dehydrogenase and inosine monophosphate dehydrogenase
Mycophenolate mofetil	Inhibitor of purine synthesis; blocks inosine monophosphate dehydrogenase
Mizoribine[a]	Same as mycophenolate mofetil
Inhibitors of cytokine production	
Cyclosporine	Binds to cyclophilin and blocks calcineurin, thus preventing activation of the transcription factor NFAT
Tacrolimus	Binds to FKBP-12 and blocks calcineurin
Inhibitors of cytokine binding	
IL-2 receptor–specific monoclonal antibody	Binds the IL-2 receptor α chain and blocks IL-2 from binding to the receptor
Inhibitors of cytokine receptor signal transduction	
Rapamycin	Blocks mammalian target of rapamycin (mTOR)
Inhibitor of antigen presenting cell differentiation or function	
Deoxyspergualin[a]	Blocks activation of NF-κB; may have other as yet unidentified actions

Prolonging Allograft Survival

BOX 1 PROMISING AGENTS FOR CLINICAL DEVELOPMENT

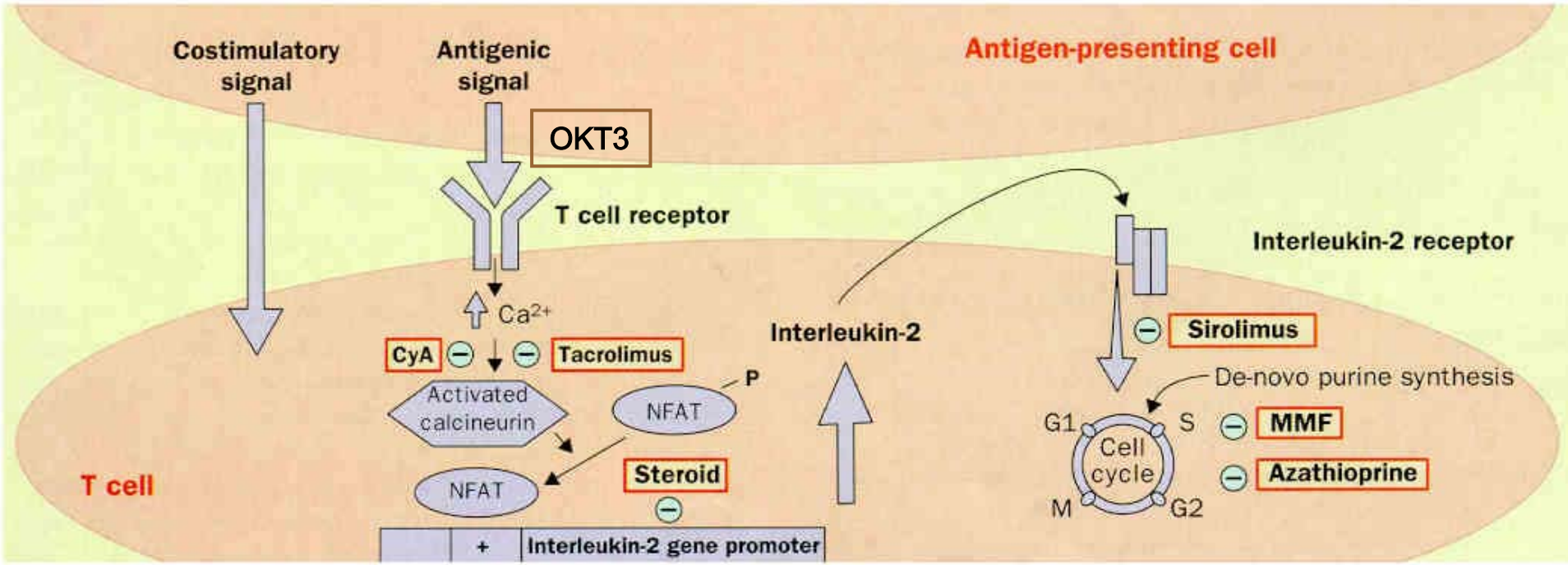
T-cell depletion or TCR signal transduction
- Anti-CD3 immunotoxin
- Campath-1H (CD52-specific monoclonal antibody)
- CD45RB-specific monoclonal antibody

Costimulatory blockade
- CD154:CD40 pathway (CD40-specific monoclonal antibody)
- CD28:B7 pathway (B7-specific monoclonal antibodies, CTLA4Ig, LEA29Y)
- Cytokine receptor signaling (JAK 3 kinase inhibitors)

B-cell depletion
- CD20-specific monoclonal antibody

Lymphocyte trafficking
- LFA-1–specific monoclonal antibody
- FTY720 (sphingosine-1-phosphate receptor modulator)
    - causes lymphocyte sequestration in lymphoid tissue
- CXCR3/CCR1/5 antagonists
    - inhibits lymphocyte trafficking to rejection site

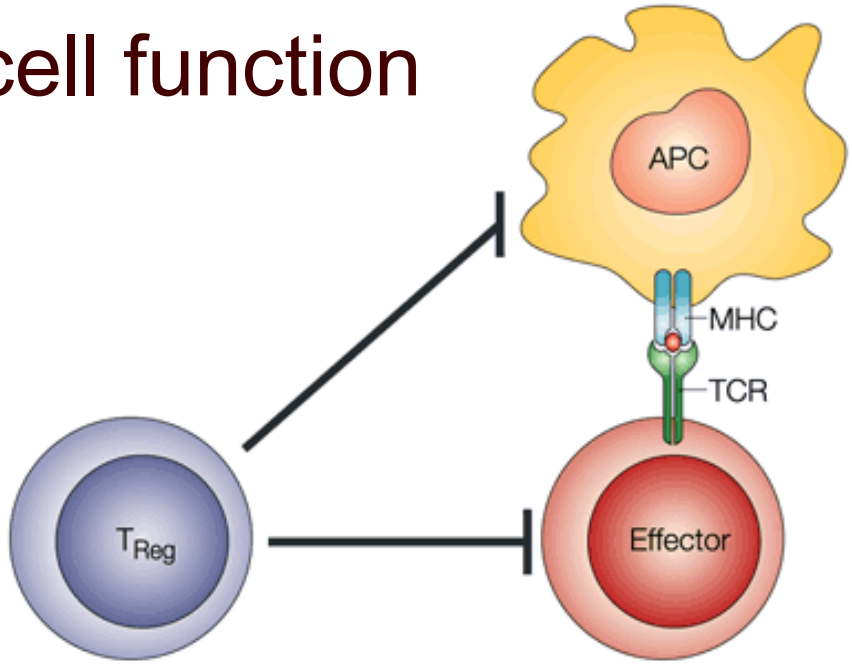
SITES OF ACTION OF MAJOR IMMUNOSUPPRESSIVE DRUGS



ANTIGEN SPECIFIC TOLERANCE (VS GENERAL IMMUNOSUPPRESSION)

- Decreases risk of infections and secondary cancers
- Enhance allospecific T regulatory cell stimulation
- Monoclonal antibodies or protein blockers for costimulatory molecules
- Myeloablation followed by reconstitution with chimeric marrow - as T cells mature in the thymus, the immune system is recreated
- Decrease graft immunogenicity
- Transplant to privileged sites
- Inject thymus with alloantigen to induce clonal deletion with tolerance for donor antigens

T -regulatory cell function



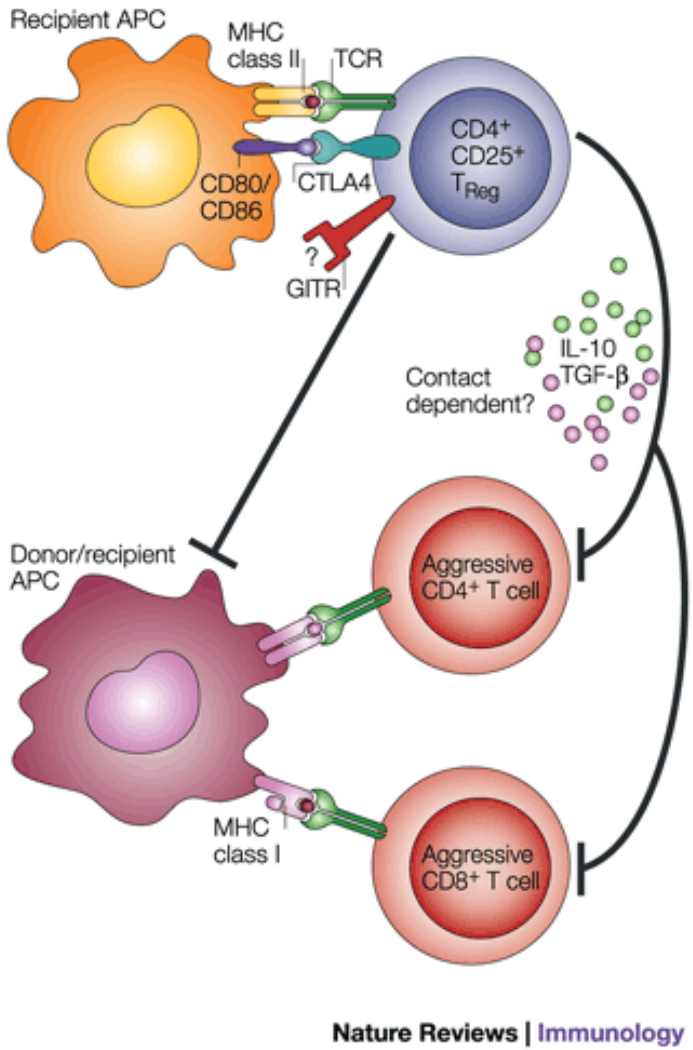
Benefits:
- T-cell homeostasis
- prevents autoimmune disease
- tolerance after transplantation
- prevents GVHD
- prevents allergy
- prevents hypersensitivity

Detrimental effects:
- down-regulation of tumour immunity
- down-regulation of immunity to infection

Nature Reviews | Immunology

Wood Nature Reviews Immunology **3**; 199-210 (2003)

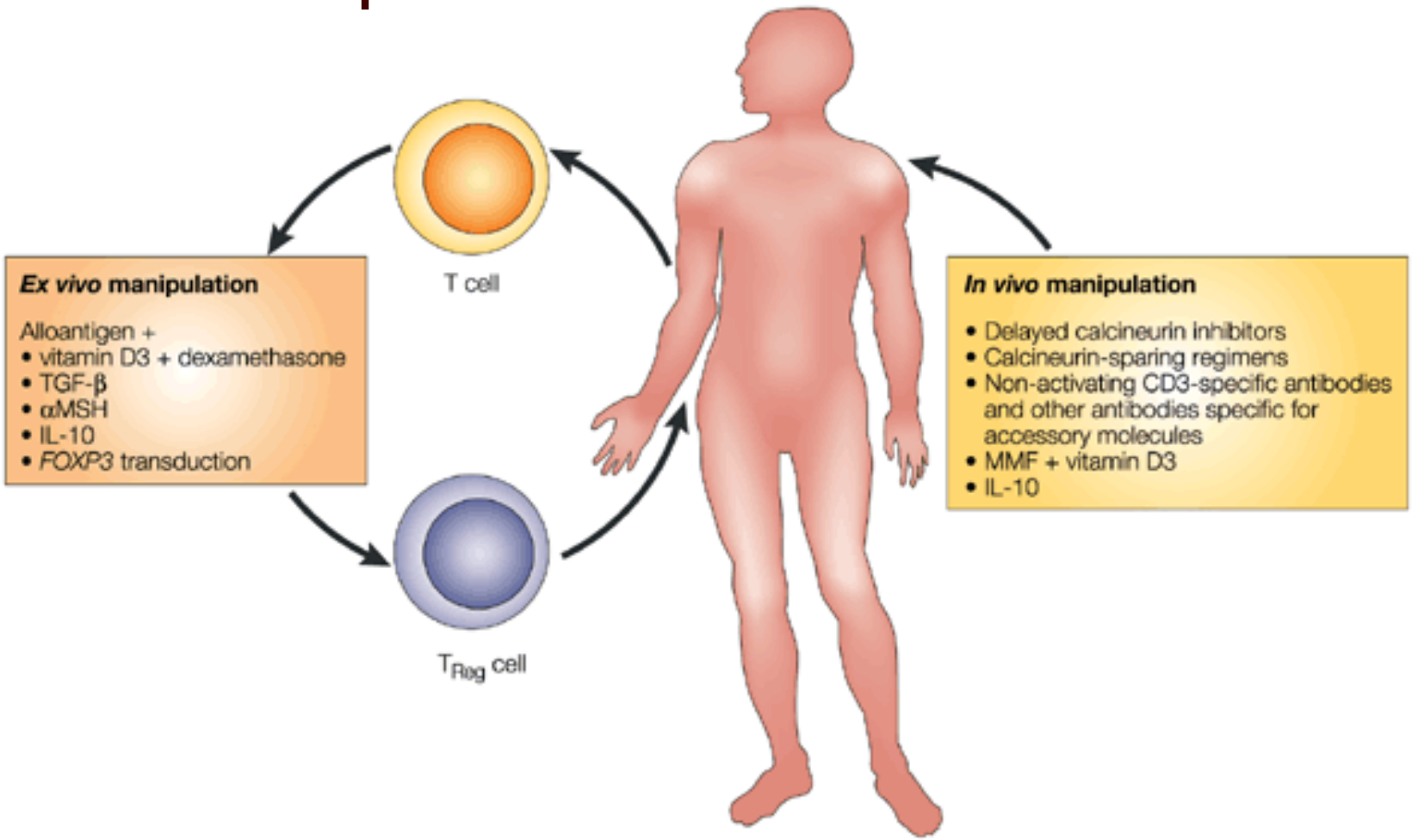
Induction of tolerance – Enhance allospecific T regulatory cell activity



If T reg cells can be induced to recognize the indirect antigen presentation, they exert a powerful suppressive effect on both indirect and direct CD4 and CD8 cell activity through the secretion of IL-10 and TGF-β

Wood, 2003, Nature Reviews Immunology 3:199-210

How to manipulate T reg activity to induce transplant tolerance?



Wood, 2003, Nature Reviews Immunology 3:199-

Bone Marrow

- Attempts to use these cells have been around for at least 60 years
- Explored intensely since world war II
- Used for treating blood diseases, severe combined immunodiffency and leukemia
- This type of transplant is also called a form of gene therapy

Types of Transplants

- Autologous Transplant
  - Patient's own stem cells
- Allogeneic Transplant
  - Stem cells from someone else=donor stem cells

In 2004, there were 22 216 hematopoetic stem cells (HSCT), 7407 allogeneic (33%), 14 809 autologous (67%) and 4378 additional re- or multiple transplants reported from 592 centres in 38 European and five affiliated countries.

Bone Marrow Transplant. 2006 Jun;37(12):1069-85.

Early Allogeneic Transplants

- Toxicity noted in early allogeneic studies:
  - "2° disease of diarrhea, liver necrosis & skin"
  - Termed *Graft Versus Host Disease (GVHD)*
  - *Now well recognized toxicity of alloBMT*

- GVHD pts had less leukemic relapse
  - in 1968, of 14 AlloBMT patients
  - 10/20 died of GVHD w/o evidence of leukemia
  - 4/20 had no GVHD, died of recurrent leukemia
  - Same donor cells causing toxicity were anti-leukemic
  - Termed the *Graft Vs Leukemic Effect (GVL)*

GVL & GVHD is Immune Mediated

- Donor Immune cells recognize Recipient cells as non-self
- T-cell & NK cell response
  - Attack host cells: malignant and normal host cells
- Balance of this immune response:
  - Minimize GVHD + Maximize GVL
  - 1) Immunosuppressive Therapy with BMT
  - 2) HLA-Match Donor & Recipient
    - Match major antigens to decrease GVHD
    - Mismatch of minor antigens results in GVL

Source of stem cells for Transplants

- Bone Marrow graft
- Peripheral Blood Stem Cells (PBSCT)
- Umbilical cord

Source of stem cells for Transplants

- Peripheral Blood Stem Cells (PBSCT)
- Stem cells collected peripherally using apheresis (cell separator machine)
  - Less invasive; less discomfort; less morbidity than BM
- Outpatient procedure
- PBSCT results in more rapid hematopoietic recovery than BM
- No difference in treatment outcome
- Quickly replacing traditional BM

  - Using cytokine stimulation (G-CSF injections)
  - BM releases large number CD34 stem cells into circulation
  - Stem cells harvested via peripheral line

Complications

- Infections
  - Early:
    - Potentially life threatening
    - Main complication in first 30 days
    - CMV infections have high mortality (so prophylaxis and early intervention important)
  - Late:
    - Immune function takes 1 year (autologous) to 2 years (allogeneic) to fully recover
    - Later opportunistic infections common, including pneumocystis carinii (PCP) and herpes zoster
    - Prophylaxis required for 6-12 months

Complications (Con'd)

- GVHD
  - Allogeneic complication
  - Donor T cell response against recipient tissue cells
  - Prophylaxis against GVHD begins day +1 with immunosuppressive agents
    - Cyclosporine, methotrexate, mycophenelate
  - Acute GVHD first 3-6 months:
    - Skin, GI (especially diarrhea) or obstructive Liver dysfunction
    - >60% develop
  - Chronic GVHD develops 12-18 months post transplant:
    - Autoimmune manifestations of Skin especially, as well as GI, Liver and Lung
    - 30-40% develop

Complications (Con'd)

- Veno-Occlusive Disease (VOD)
  - Obstructive liver disease due to microthrombi in liver venules
  - Patients with previous liver disease at greater risk
  - No good treatments
- Graft Rejection
  - Rare in present day (<1%)

INNOVATION

In vivo imaging using bioluminescence: a tool for probing graft-versus-host disease

Robert S. Negrin and Christopher H. Contag

Abstract | Immunological reactions have a key role in health and disease and are complex events characterized by coordinated cell trafficking to specific locations throughout the body. Clarification of these cell-trafficking events is crucial for improving our understanding of how immune reactions are initiated, controlled and recalled. As we discuss here, an emerging modality for revealing cell trafficking is bioluminescence imaging, which harnesses the light-emitting properties of enzymes such as luciferase for quantification of cells and uses low-light imaging systems. This strategy could be useful for the study of a wide range of biological processes, such as the pathophysiology of graft-versus-host and graft-versus-leukaemia reactions.

Box 1 | **Molecular imaging in immunology: watching and waiting for an immune response**

Imaging modalities. Two-photon intravital microscopy offers resolution of cells *in vivo*[11], but it is constrained by small fields of view and motion artefacts. Non-invasive measures of immunological processes *in vivo* have been accomplished using positron emission tomography (PET)[49–51], single photon emission computed tomography (SPECT)[52], magnetic resonance imaging (MRI)[53], bioluminescence imaging (BLI) and fluorescence imaging (FLI)[49,54–56]. Ultrasound and X-ray computed tomography (CT) provide anatomical information, and when used in combination with other modalities, this information improves localization of the signals obtained by PET, SPECT or optical imaging[57]. PET, SPECT, ultrasound MRI and CT have potential clinical uses, and therefore are useful in translational studies.

Optical methods. BLI and FLI can be used to refine and accelerate studies of animal models, but they have limited clinical application. Imaging times for optical imaging methods are generally short, which facilitates the analysis of greater numbers of animals. Optical methods also allow a range of image resolutions from microscopic to macroscopic, produce images without the use of ionizing radiation, offer the choice of many reporters and dyes, and benefit from user-friendly and inexpensive instrumentation[58]. Signal-to-noise ratios (SNRs) for BLI are excellent[59,60], enabling detection of subtle changes non-invasively, thereby obviating the need to remove overlying tissue.

Reporter genes. The use of dyes and contrast agents allows visualization of the early events, but they are diluted by cell division. To prevent loss of labels during cell division, genes that encode reporter proteins can be integrated into the genome. Reporter genes are available for PET, SPECT, MRI and optical imaging[49,57], each with strengths and weaknesses. The radiotracers used for PET and SPECT often produce signals from kidney, liver and bladder that can obscure the target tissue, and MRI is generally less sensitive than imaging of reporter-gene expression with PET or SPECT. In summary, optical imaging of reporter-gene expression *in vivo* offers the greatest versatility, sensitivity and SNR of all of the modalities used for small animals.

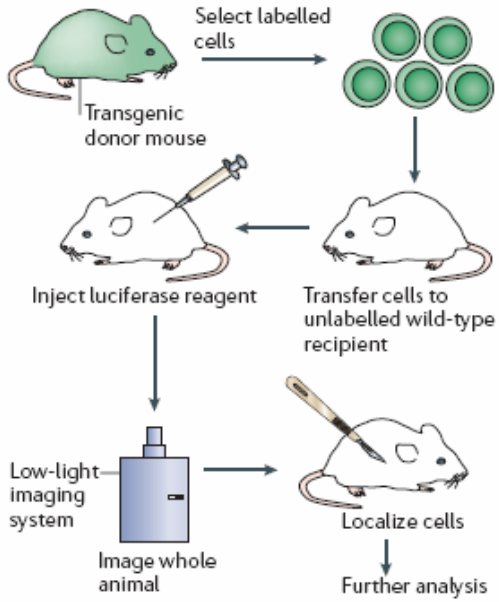


Figure 1 | **Schematic representation of a bioluminescence imaging strategy using cells from a transgenic donor mouse.** Cells expressing the transgene encoding a luciferase–GFP–green fluorescent protein (GFP) fusion protein are isolated from a transgenic donor animal and selected by cell-sorting technologies, using the GFP signal or fluorescent antibodies specific for selected cell-surface markers. Luciferase–GFP-positive cells are then transferred to recipient syngeneic or allogeneic animals. Recipient animals are then injected with the luciferase substrate to allow serial imaging of the bioluminescent signal *in vivo*. The tracking of effector cells involved in graft-versus-host disease or graft-versus-leukaemia reactions can be carried out in recipient animals that have been prepared using myeloablative or non-myeloablative regimens.

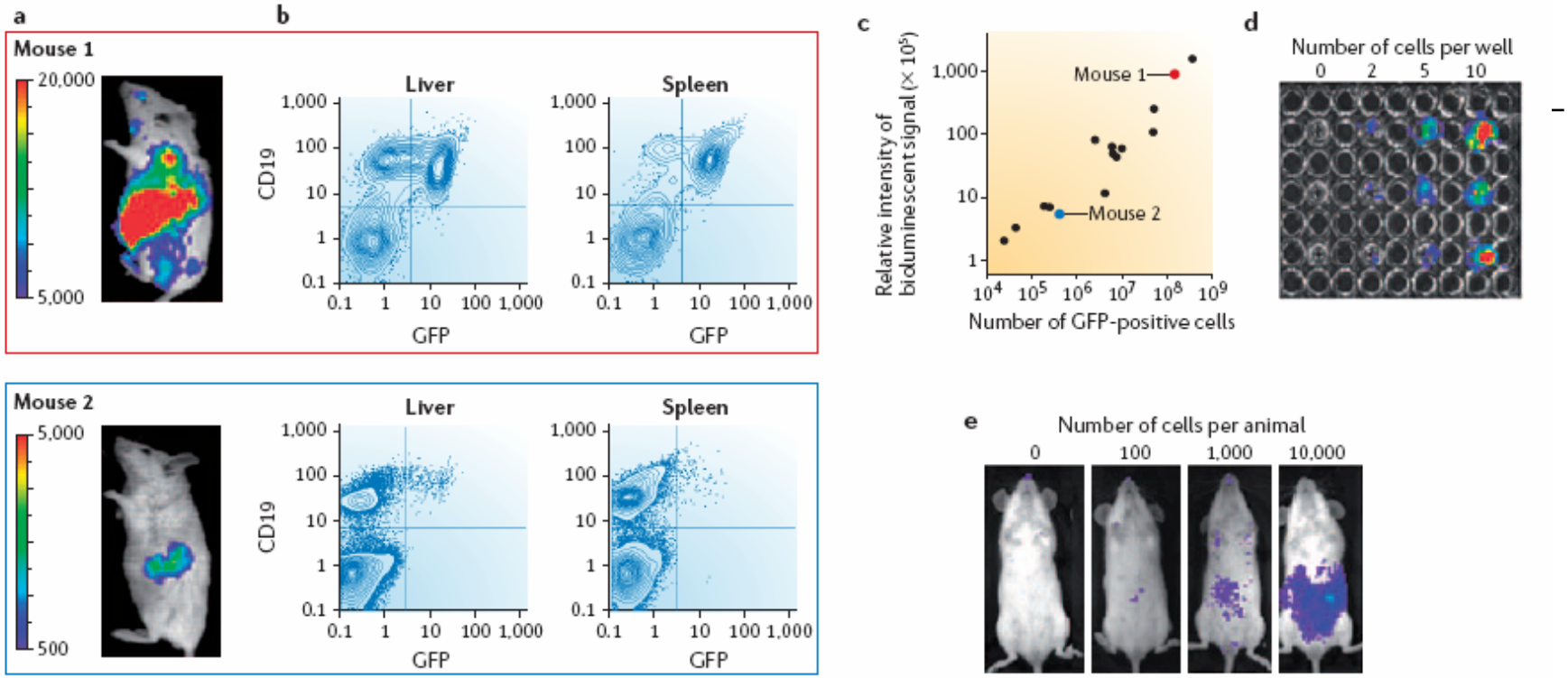


Figure 2 | **Sensitivity of detection in bioluminescence imaging studies.** The detection of weak optical signals, such as those generated during bioluminescence imaging (BLI), from inside small animals is influenced by several factors that include: the level of cell brightness (photon flux from source), the depth at which the bioluminescent source is located in the tissue, the wavelength of the emitted light, the quantum efficiency and noise of the detector, the nature of the collection optics and the background emission levels from the live animal. **a** | In this example, studies of the detection sensitivity of BLI were carried out in a mouse B-cell lymphoma model, in which the tumour cells were labelled with luciferase and green fluorescent protein (GFP), by imaging whole animals at various times during the disease course. One time point is shown for two mice. **b** | After recovery from the animals, tumour cells from the liver and spleen of these animals can then be quantified by flow cytometry using the GFP signal and fluorescent antibodies specific for the B-cell marker CD19; the results for the two animals in part **a** are shown. **c** | The quantity of GFP-positive cells detected by flow cytometry correlated well with the bioluminescent signals detected *in vivo*, showing that BLI is a sensitive and reliable measure of cell number *in vivo*. **d** | Using the same detector, the detection sensitivity of BLI can also be analysed by measuring the bioluminescent signal emitted from known numbers of cells in culture or following transfer *in vivo* (**e**). Images are adapted, with permission, from REF. 24 © (2003) the American Society of Hematology.

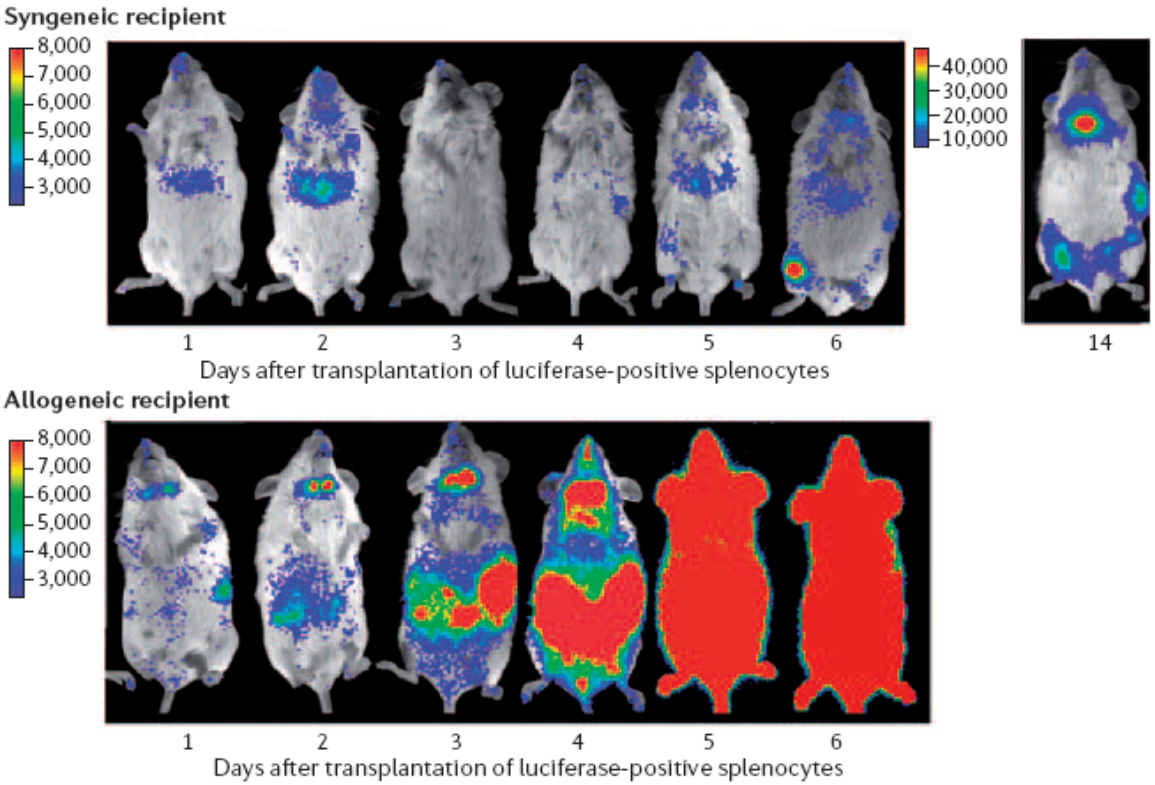


Figure 3 | **Imaging of graft-versus-host disease.** Bioluminescence imaging of luciferase-positive splenocytes transplanted to either irradiated syngeneic (top panels) or allogeneic (bottom panels) animals are shown. Serial images show markedly different patterns of lymphocyte trafficking, proliferation and tissue infiltration. At defined time points, tissue sites of interest, as determined by bioluminescence imaging, can then be further analysed. Images are reprinted, with permission, from REF. 25 © (2005) the American Society of Hematology.

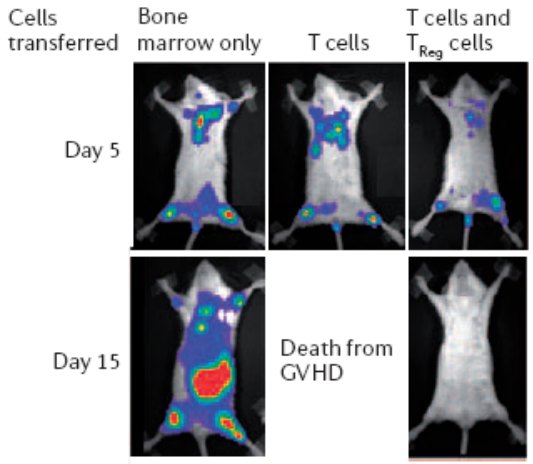


Figure 4 | **Effect of transfer of conventional CD4⁺ and CD8⁺ T cells with and without CD4⁺ CD25⁺ regulatory T cells on tumour progression.** Leukaemia cells expressing a transgene encoding the fusion protein luciferase–yellow fluorescent protein were injected into recipient mice and, using bioluminescence imaging, can be observed infiltrating the bone marrow. Recipient mice that received irradiation and T-cell-depleted bone marrow only have progressive tumour growth at day 5 and 15 (left panels). Animals that received T-cell-depleted bone marrow and conventional T cells die rapidly due to acute lethal graft-versus-host disease (GVHD). By contrast, recipient mice that received both conventional T cells and $CD4^+CD25^+$ regulatory T cells (T_{Reg} cells) in equal proportions retain the ability to reject the tumour without significant GVHD. Images are reproduced, with permission, from *Nature Medicine* REF. 27 © (2003) Macmillan Publishers Ltd.

Xenogenic Transplantation

- >50,000 people that need organs die while waiting for a donor
- Studies are underway involving nonhuman organs
- Attention has been focused on the pig but the problem is the existence of natural or preformed antibodies to carbohydrate moieties expressed in the grafts endothelial cells
- As a consequence activation of the compliment cascade occurs rapidly and hyperacute rejection ensues
- Concern has given to debate about the safe use of xenografts and animal tissues that the tissues might harbor germs