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Xenotransplantation

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Outline

- **Mechanism of xenograft rejection**
- **Immune system**
- **Immuno suppressants in xenotransplantation area**
- **Organ-specific barriers and challenges**

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Introduction

- Transplantation is often the last resort for end-stage organ failures.
- The shortage of donor organs is the main limiting factor for successful transplantation in humans.
- The WHO estimates that only 10% of the worldwide need for organ transplantation is being met.
- Therefore except living donations, other alternatives are needed, e.g., xenotransplantation of pig organs.

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xenotransplantation

- Since 2009, porcine models with new genetic modifications have been constantly implemented to improve molecular compatibilities.
- As gene editing techniques such as zinc finger nucleases, TALEN, and CRIPSR/Cas9 genome editing system improve, the production of multiple-gene edited pigs has become easier and faster.

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Types of xenograft rejection

- **Three different xenogeneic types of rejections, based on the responses and mechanisms involved. It includes :**

1-Hyperacute rejection (HAR),

2-Delayed xenograft rejection (DXR)

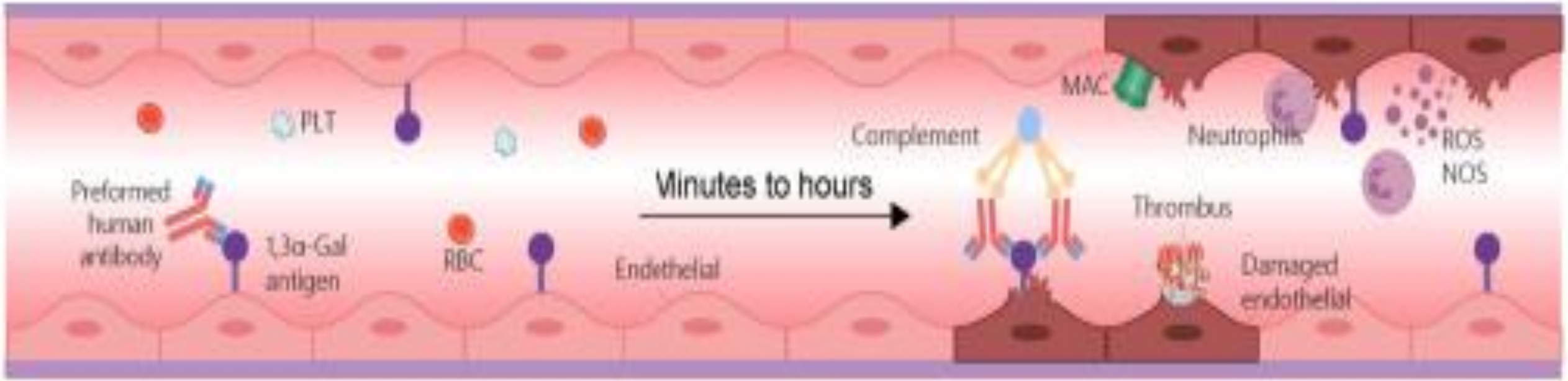
3-Chronic rejection.

Hyperacute rejection

- HAR occurring within 24 hours and lasts for few minutes to hours.
- It is caused by pre-existing antibodies against graft antigens .
- Among these antibodies, most frequent IgMs and IgGs recognize galactose-a (a-Gal) residues.
- Humans, Old World monkeys lack a-Gal epitopes because their a1,3GT gene is affected by a loss-of-function mutation.

Hyperacute rejection

- When a pig organ is transplanted into a human or a NHP, the pre-existing anti-Gal antibodies bind to a-Gal epitopes present on the graft's vascular endothelium, and induce complement.
- These reactions cause endothelial cells lysis, destruction of the vasculature, and ultimately, graft rejection.



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Two ways to prevent HAR

1- Knocking out the a-Gal gene in pigs.

Reported : averted HAR in baboons receiving hearts from pigs and increased the pig heart survival by 2– 6 months.

2-Inhibiting complement activation by inducing the expression of hCD46, hCD55, and hCD59, on pig cells.

Reported : was associated with a survival time of 7 to 9 days while the wild-type (WT) liver graft did not extend over 3 days.

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Delayed xenograft rejection

- Causes immunological destruction within a few days to a few weeks
- Refers to post-HAR and is also called AHXR from a mechanistic prospect, or AVXR from a pathophysiological prospect.

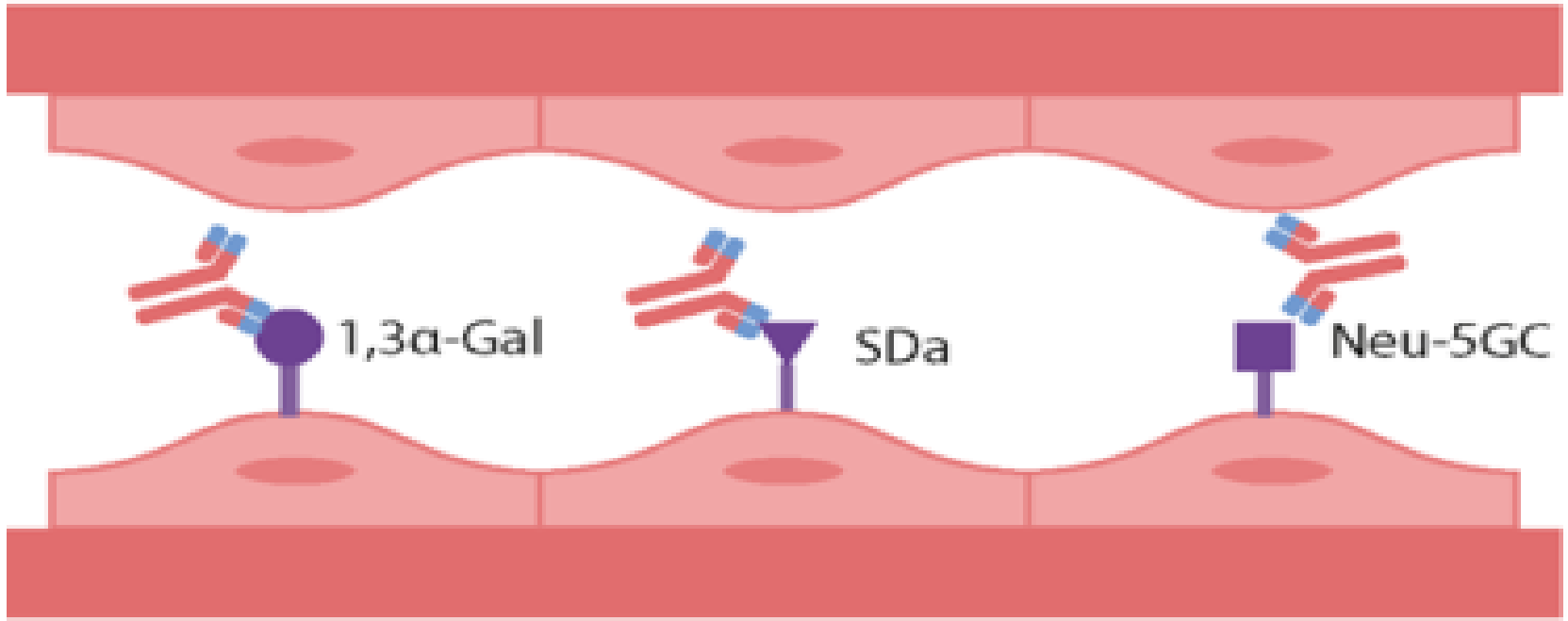
1- AHXR or AVXR define xenograft injuries occurring within the vasculature and involving antibodies, while complements play a minor role during this type of rejection .

2- CXR(cellular xenograft rejection) refer to antibody and complement-independent.

AHXR

- **Gal-specific antibodies were implicated in AHXR, in addition to HAR.**
- **Pre-existing antibodies against non-Gal epitopes, such as carbohydrate**

N-glycolyl neuraminic sialic acid (Neu5Gc), glycan SDa was also implicated in rejection.



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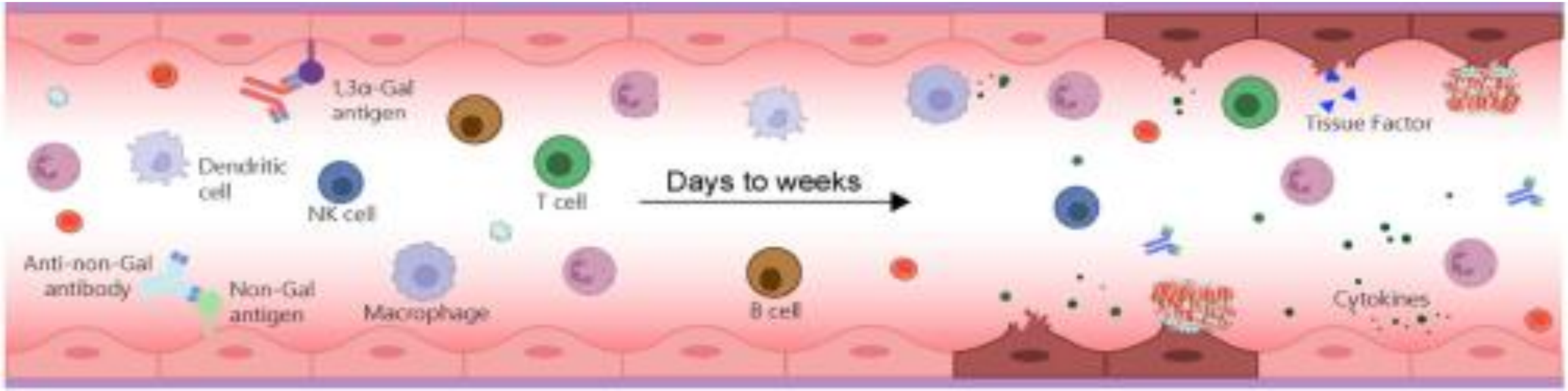
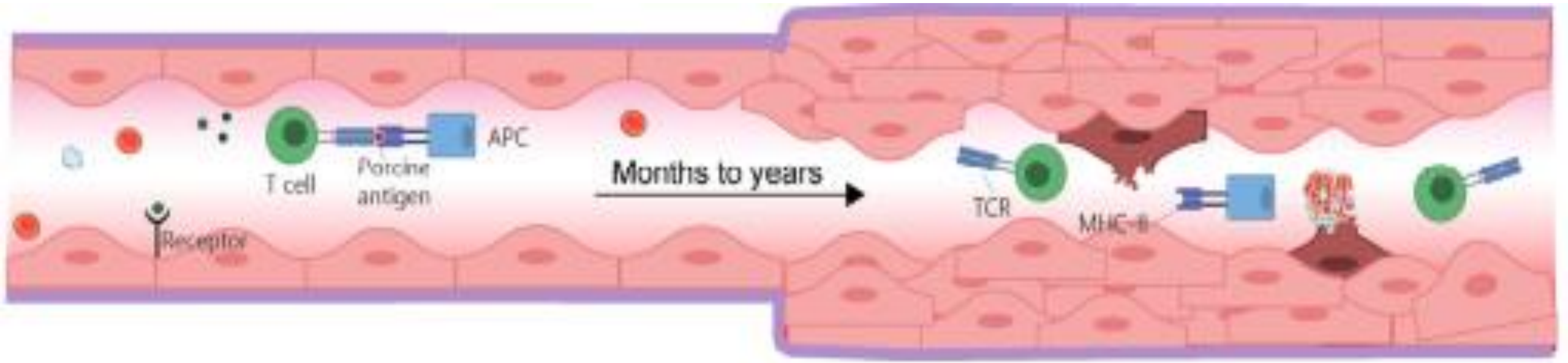
CXR

- If both HAR and AHXR are overcome, but immunosuppressive therapy is insufficient, CXR may occur and lead to graft rejection within days or weeks following transplantation.
- CXR can be mediated by the innate and/or the adaptive immune system, and may involve NK cells, macrophages, neutrophils, dendritic cells (DCs), T cells, and B cells.



Chronic rejection

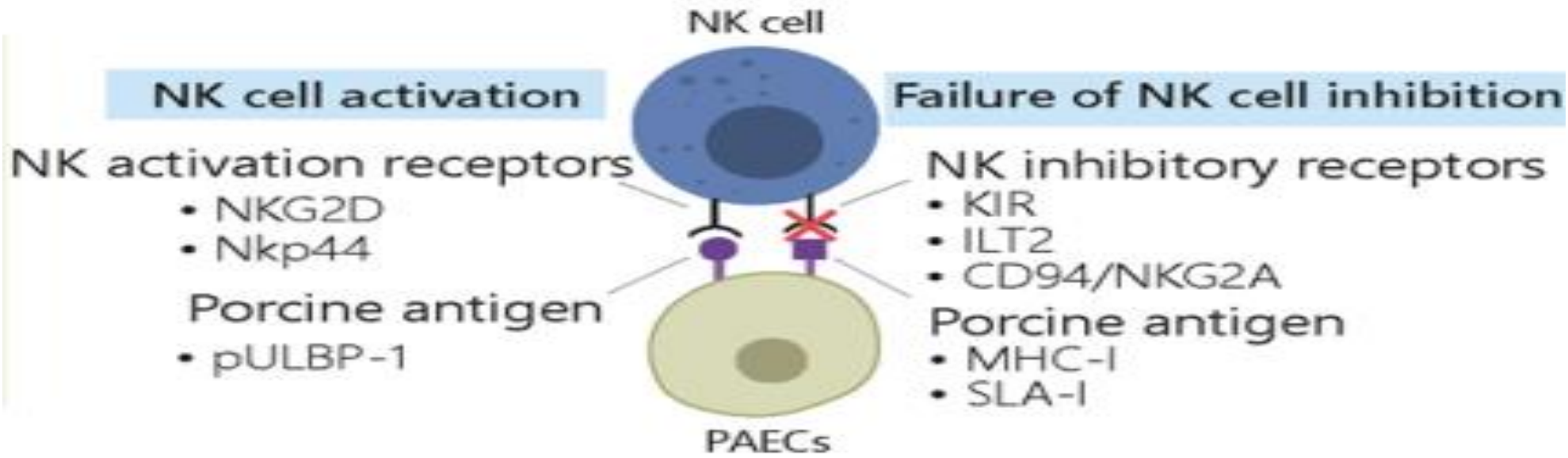
- Usually occurs several months to years after organ transplantation.
- Proliferation of graft vascular endothelial cells, vessel narrowing, interstitial fibrosis, which ultimately, result in graft failure.
- Achieved a long-term survival of 499 days by depleting CD4+ T cells that indicating that these cells are responsible for chronic rejection.



Immune system

NK cells

- NK cells mediate xenograft rejection by ADCC.
- NKG2D and NKp44 can bind porcine pULBP-1 and an unidentified ligand.
- KIRs , ILT2, and CD94/NKG2A mainly recognize MHC-I molecules.
- Expression of HLA-Cw3 and/or G, and/ or E, on porcine cells could protect the xenograft from human NK cytotoxicity.



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Macrophages

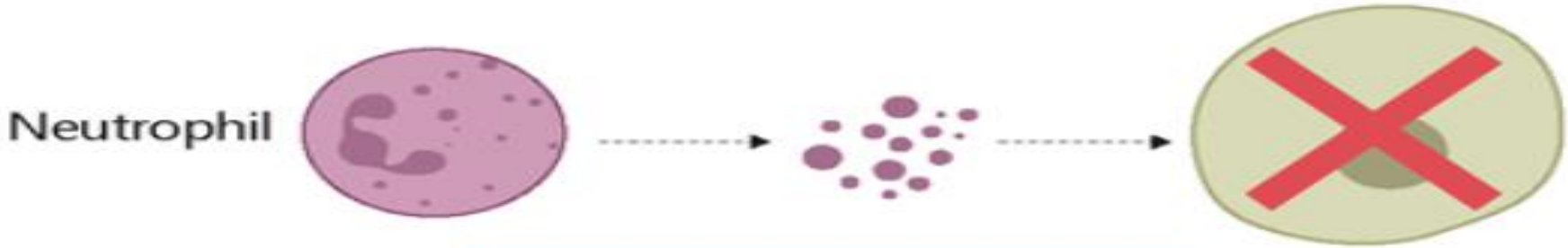
- Synergistic effect of TLR and IFN-γ cause macrophages licensed to process and present xenoantigens, and exert direct cytotoxicity by TNFa, IL-1, IL-6, and nitric oxide.
- Interspecies incompatibility of CD47 was also reported to contribute significantly to macrophage-mediated rejection of xenogeneic cells.
- Phagocytose porcine red blood cells independently of the presence of antibodies or complement activation, even in setups where the a-Gal epitopes were absent from the porcine cells.

Neutrophils

- **Three mechanisms activated neutrophils can induce tissue damage:**

  (i) ROS generation (ii) release of tissue-digesting enzymes (iii) NETs.

- **Leukocyte proteases have been implicated in neutrophil-mediated graft tissue damage, For instance neutrophil elastase breaks degrading the ECM.**

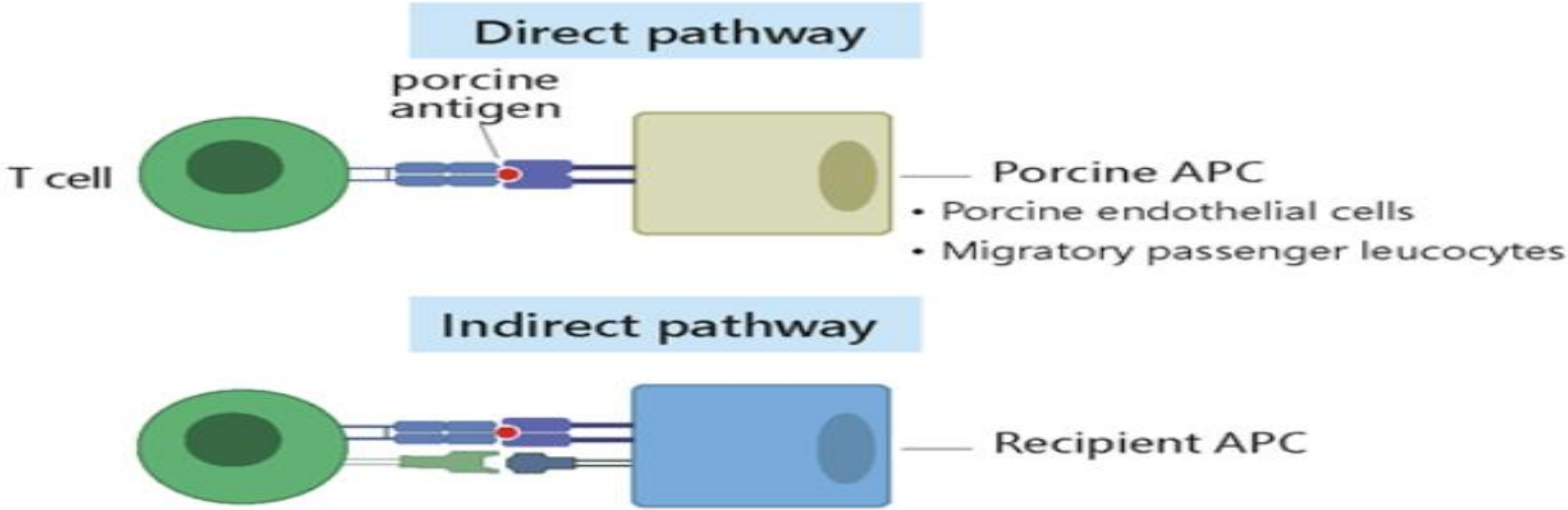


Neutrophil dependent tissue damage

- ROS
- Tissue damaging enzymes
- nuclear extracellular traps

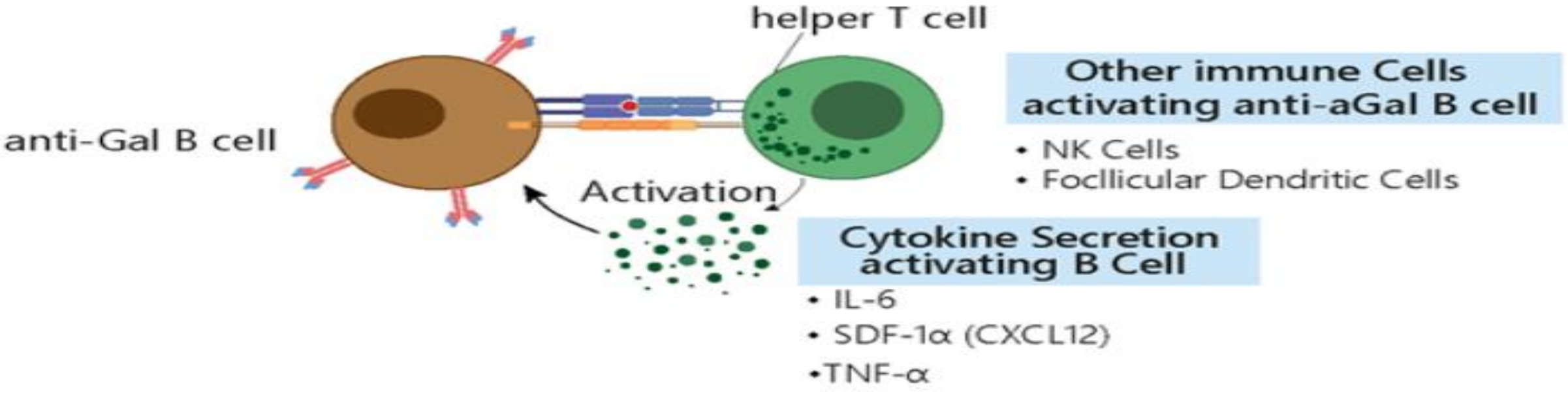
T cells

- T cells are activated by both direct and indirect pathways.
- Drugs targeting these pathways: anti-CD40 mAb and CTLA4Ig.



B cells

- The cells producing **anti-Gal antibodies** reside mainly in the **spleen** and to a lesser degree in lymph nodes and bone marrow.
- **Depletion** of secondary lymphoid organ-resident **B cells** by **anti-CD20** antibody at the time of transplant **prevents** anti-pig humoral responses and resulting **graft injury**.



Immuno suppressants in xenotransplantation area

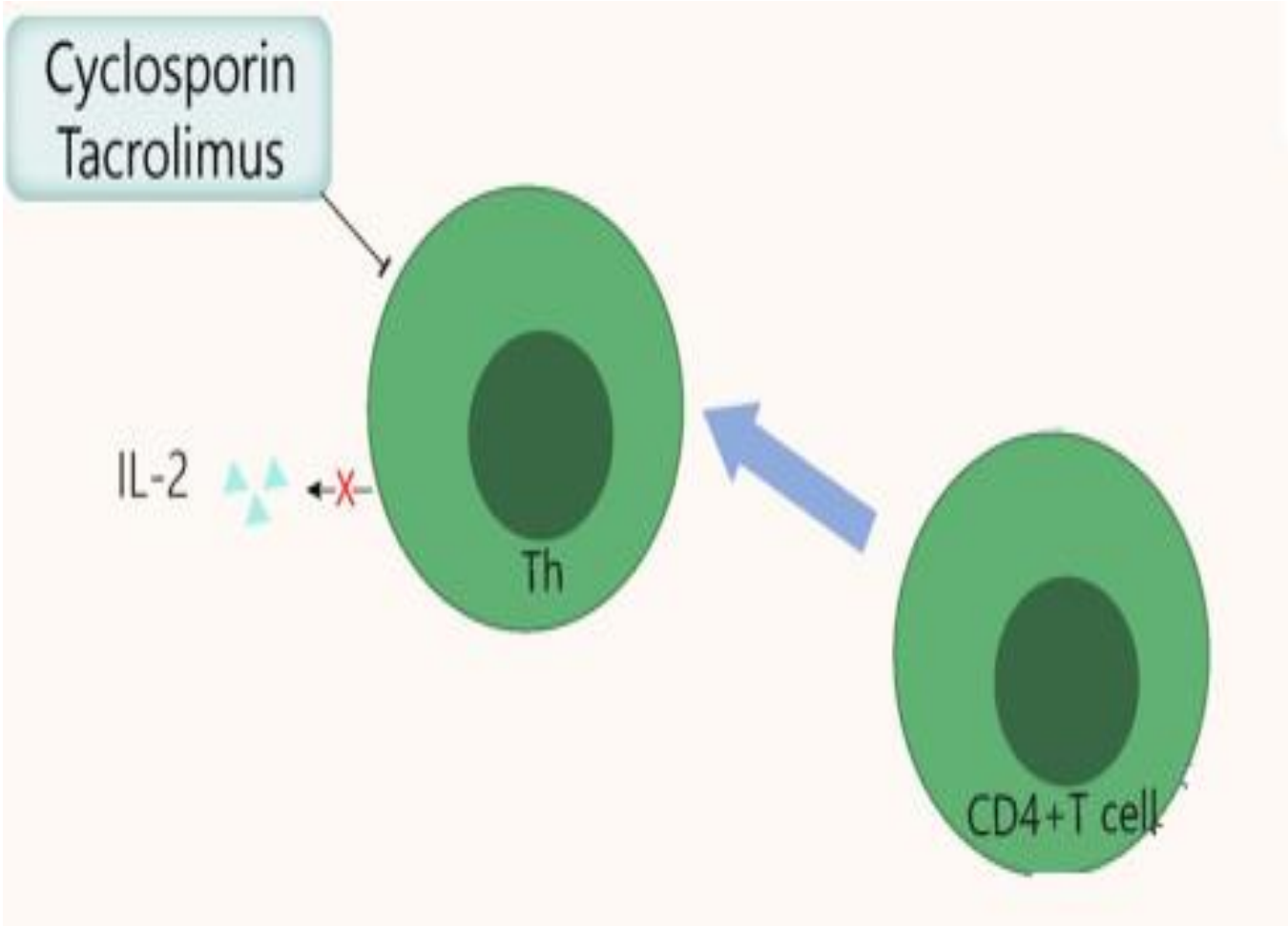
Glucocorticoids

- Glucocorticoids (GCs) were discovered in the 1940s .
- GCs have been used as first-line medication during the induction and maintenance phases after transplantation to prevent acute rejection.
- Three immunosuppressive mechanisms triggered by GCs include:

(i) T cell depletion via inhibition of IL-2 (ii) prevention of B cell clonal expansion through inhibition of the production of IL-2 and related peptides, which reduces antibody production; (iii) induction of eosinophil apoptosis either directly or through IL-5 inhibition

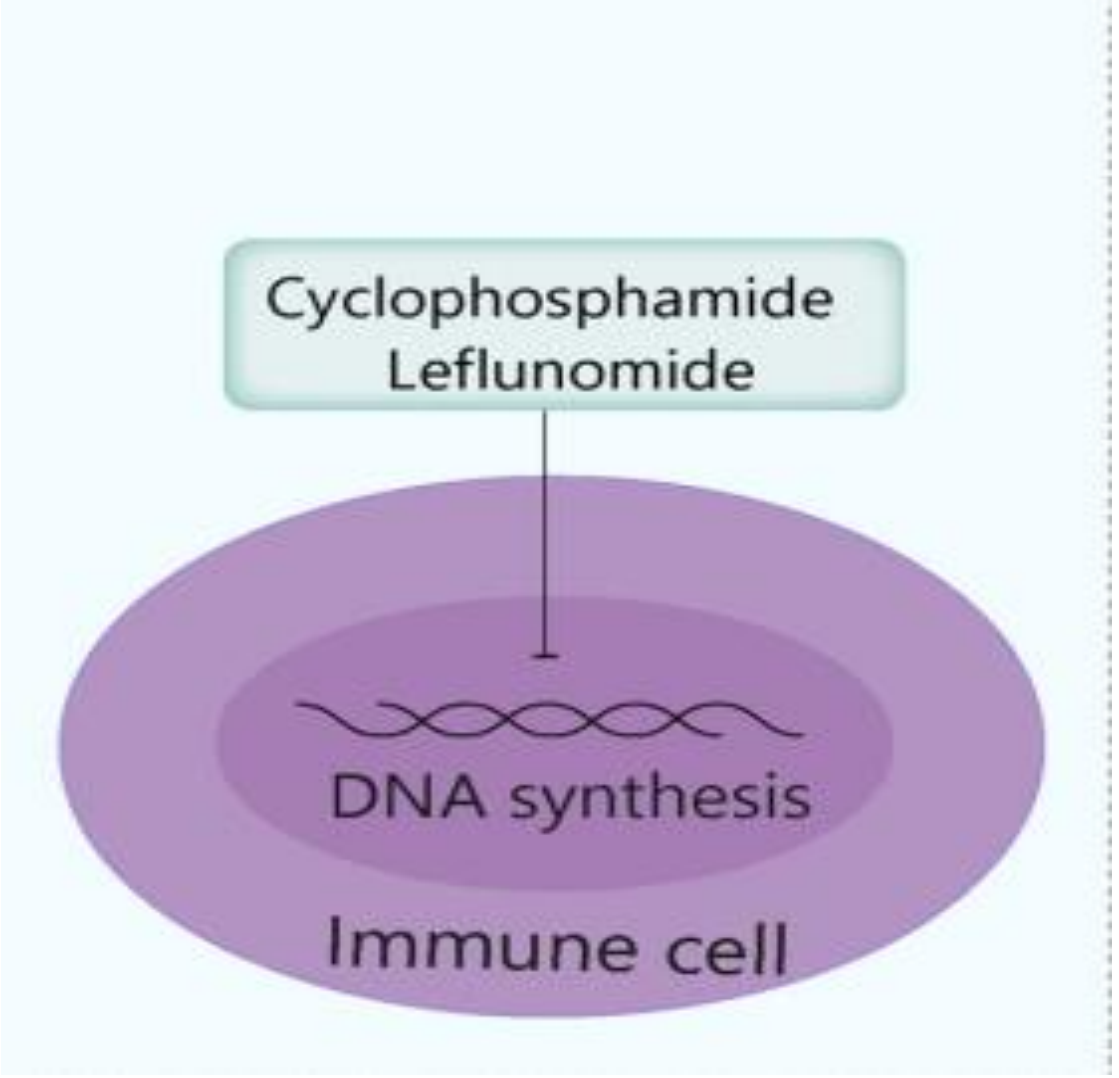
Calcineurin inhibitors

- **Calcineurin inhibitors: cyclosporin and tacrolimus, inhibit the dephosphorylation of NFAT.**
- **This inhibition results in Decreased T cell maturation and IL-2.**



Anti proliferative agents

- Cyclophosphamide
- Rapamycin
- Leflunomide
- Mycophenolate mofetil

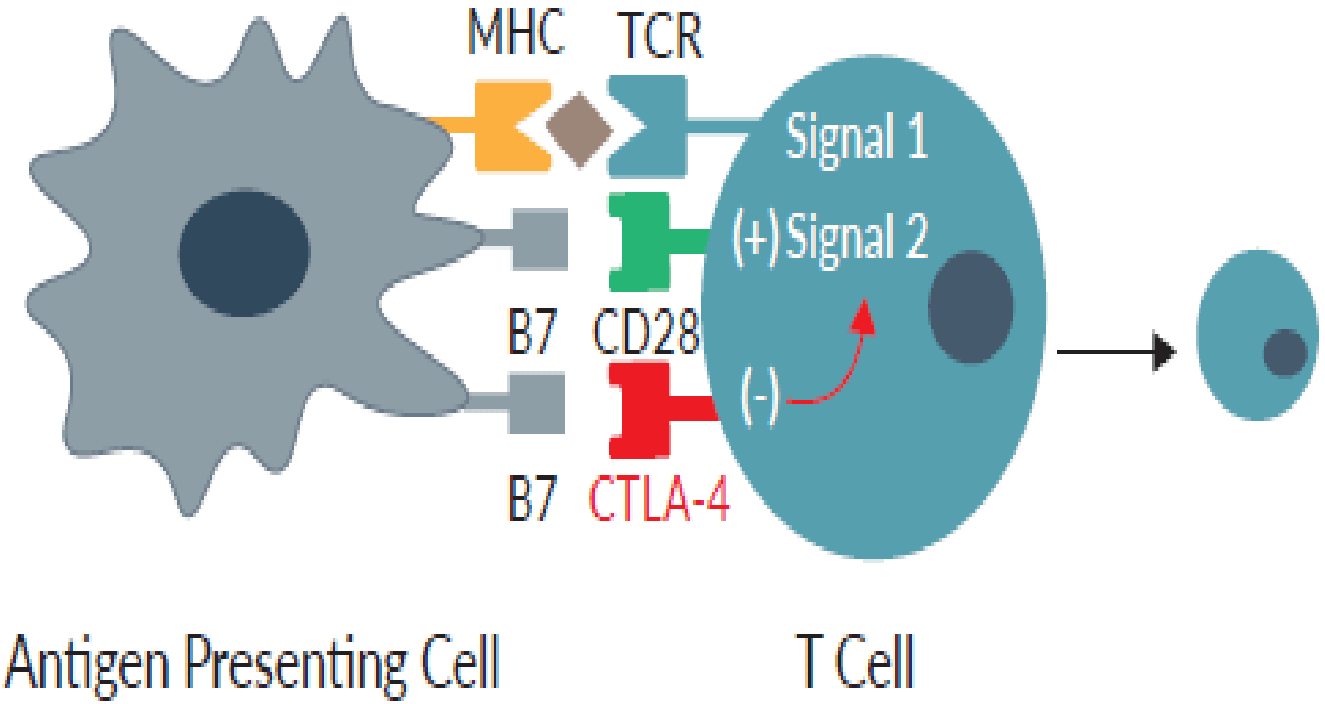


Monoclonal or polyclonal antibodies

- **AntiCD3.**
- **Tocilizumab: blocks human IL-6Ra.**
- **Anti-CD20(rituximab) that leads to B cell depletion.**
- **Polyclonal anti-thymocyte globulins (ATGs) primary mechanism of action consist in promoting lymphocyte depletion through T cell activation-induced apoptosis and complement dependent lysis.**

Blockade of costimulatory signals

- **Blockade of CD80/86:CD28 costimulatory pathway by CTLA4Ig.**
- **Targeting of CD154:CD40 costimulatory signal with anti-CD40mAb.**



Organ-specific barriers and challenges

Liver xenotransplantation

- From chimpanzee to human was first held in the 1960s.
- The transplanted patients either died from sepsis due excessive immunosuppression, or from hepatic failure with clear rejection
- Two major problems must be overcome in liver xenotransplantation:

1-lethal thrombocytopenia 2-antibody-mediated rejection targeting antigens such as a1,3GT, N- glycolylneuraminic acid and b4GALNT2.

- If the severe and immediate thrombocytopenia could be prevented, pig liver xenotransplantation could be used as a bridge towards allotransplantation.

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Cardiac xenotransplantation

- First attempt of pig-to-NHP cardiac xenotransplantation started in the mid-1980s.
- The longest survival of heterotopic heart xenograft, reaching up to 945 days, has been achieve in baboons, with ATG and anti-CD20 antibody treatments, followed by maintenance with MMF and high-dose anti-CD40 immunosuppressive regimen.
- The first clinical trial of pig to human was carried out with genetically modified pig heart transplanted into a 57-year-old man in the USA in 2022, and the patient survived for two months without signs of rejection, while the cause of death is unknown.

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Conclusion and perspectives

- **Although porcine-to-human xenotransplantation of kidneys and hearts have been carried out, it is not known when xenotransplantation of liver, small intestine, and even pancreas will become possible.**
- **Until now, the survival of transplanted organs on the long term largely depends on high doses of different immuno suppressants, which would expose the recipients to high infection risks and other side effects.**

Reference

- Current status of xenotransplantation research and the strategies for preventing xenograft rejection

Thanks for your attention