

Immunologic Tolerance
The Search for the Holy Grail

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Limitations to the Field of Transplantation

- Drug treatment-related complications
- Chronic rejection
- Availability of organs

Issues

- Transplantation: treatment of choice for end stage organ failure
- Specific immunologic tolerance to transplanted organs: goal for >50 years, since first described by Medawar
- Data have been accumulating since the 50s regarding strategies, but has not been achieved
- Graft survival is better than ever

Why Tolerance?

- Side effects of immunosuppressive drugs
- Consequences of immunosuppression
- Costs of drugs to patients/society
- Problem of non-compliance
- Grafts are eventually rejected
- Shortage of organs—cannot afford to give more than one to a patient

Clinical Definition of Tolerance

- Achieving long-term, drug-free graft acceptance with normal organ function
- Lack of destructive immune response toward the graft in presence of generalized immune competence

Tolerance - Definitions

- State of immune system unresponsiveness to an antigen.
- Failure to Respond to an antigen
- Tolerance to self antigens-Essential feature of the immune system
- Loss of tolerance to self: Destruction of self tissues-Autoimmune disease

Tolerance

□ Central Tolerance

- Tolerance established in lymphocytes developing in central lymphoid organs

□ Peripheral Tolerance

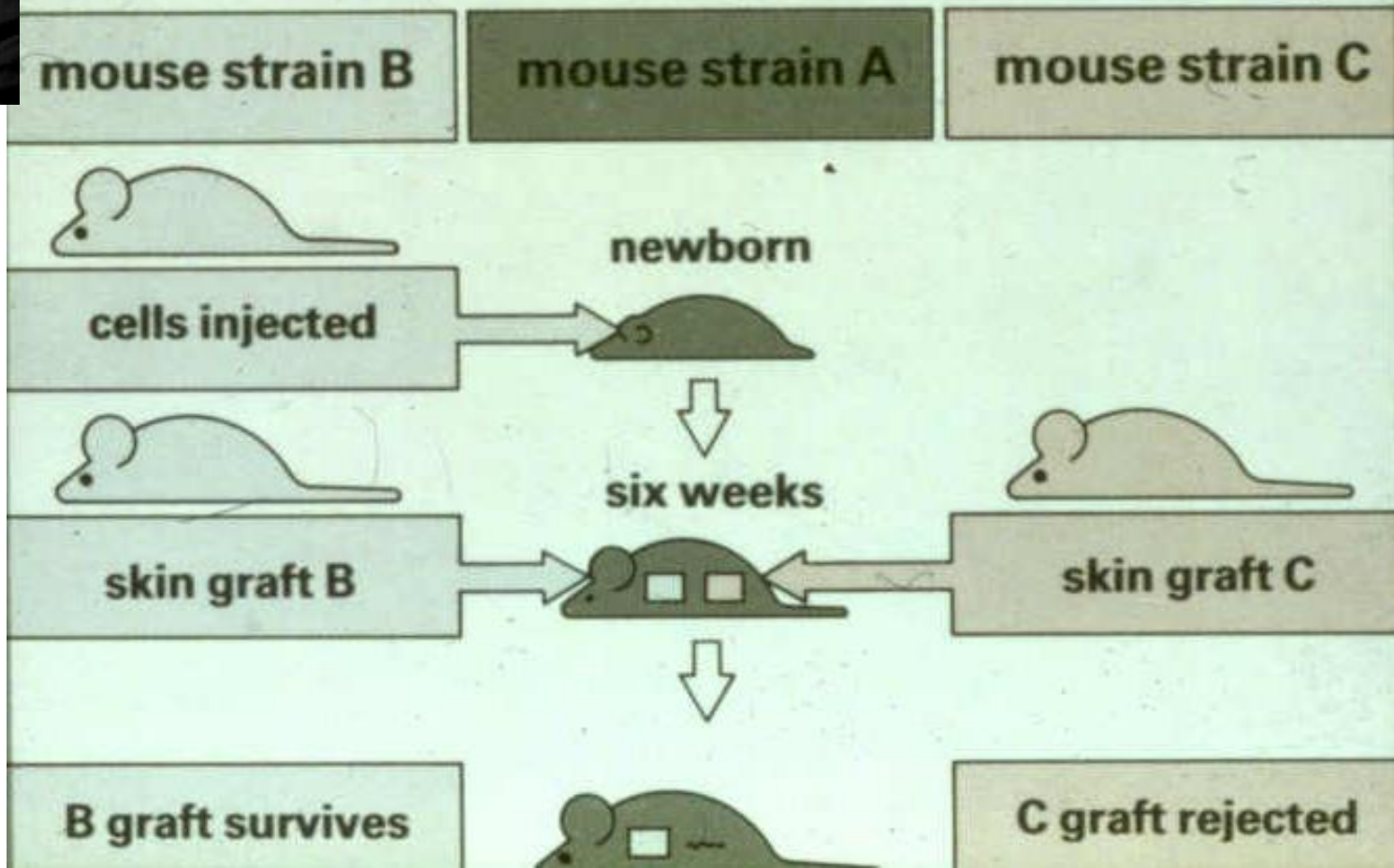
- Tolerance acquired by mature lymphocytes in the peripheral tissues.

Bone Marrow Induces Tolerance

- Animal studies >45 years ago showed that mixed bone marrow chimerism educates the immune system to make it tolerant of the donor - Central Tolerance
- We have aimed at making this approach less toxic and therefore clinically applicable



Billingham, Brent and Medawar's neonatal grafting experiment



Regulation of Alloreactive T cells

- Key regulators of the immune response are T cells
- T cells act both directly and indirectly (this is the physiologic pathway)
- Individual T cells responding to their specific antigen can undergo a number of different responses
- T cells can be physiologically silenced

Peripheral tolerance

- Clonal deletion
 - cells are physically eliminated (apoptosis)
- Clonal anergy
 - cells are functionally silenced
- Clonal ignorance
 - cells are there but do not respond to the antigen
- Regulation
 - an ongoing immune response with a great degree of complexcity involving various immunue cells

Mechanisms of T Cell Tolerance

- **Clonal Deletion** Deletion of self-reactive clones of CD4+ and CD8+ T cells in the thymus by Negative Selection
Immature lymphocytes eliminated by apoptosis during T or B cell maturation.
- **Clonal Anergy** -Peripheral lymphoid organs
Naive T and B cells exposed to foreign /self Ag are inactivated or rendered unresponsive to restimulation-Functional Inactivation-Tolerogens
- **Regulatory Lymphocytes-Suppress T Cell Activation**
- **Clonal Ignorance** - Privileged sites

- Antigen recognition by T cells in the absence of costimulation leads to T cell Tolerance
- Naïve T cells recognizing self peptides on tissue cells are not activated, instead they enter a state of anergy/unresponsiveness.



Signals required for Treg homeostasis

Signal 3

Cytokines

B7/CD28

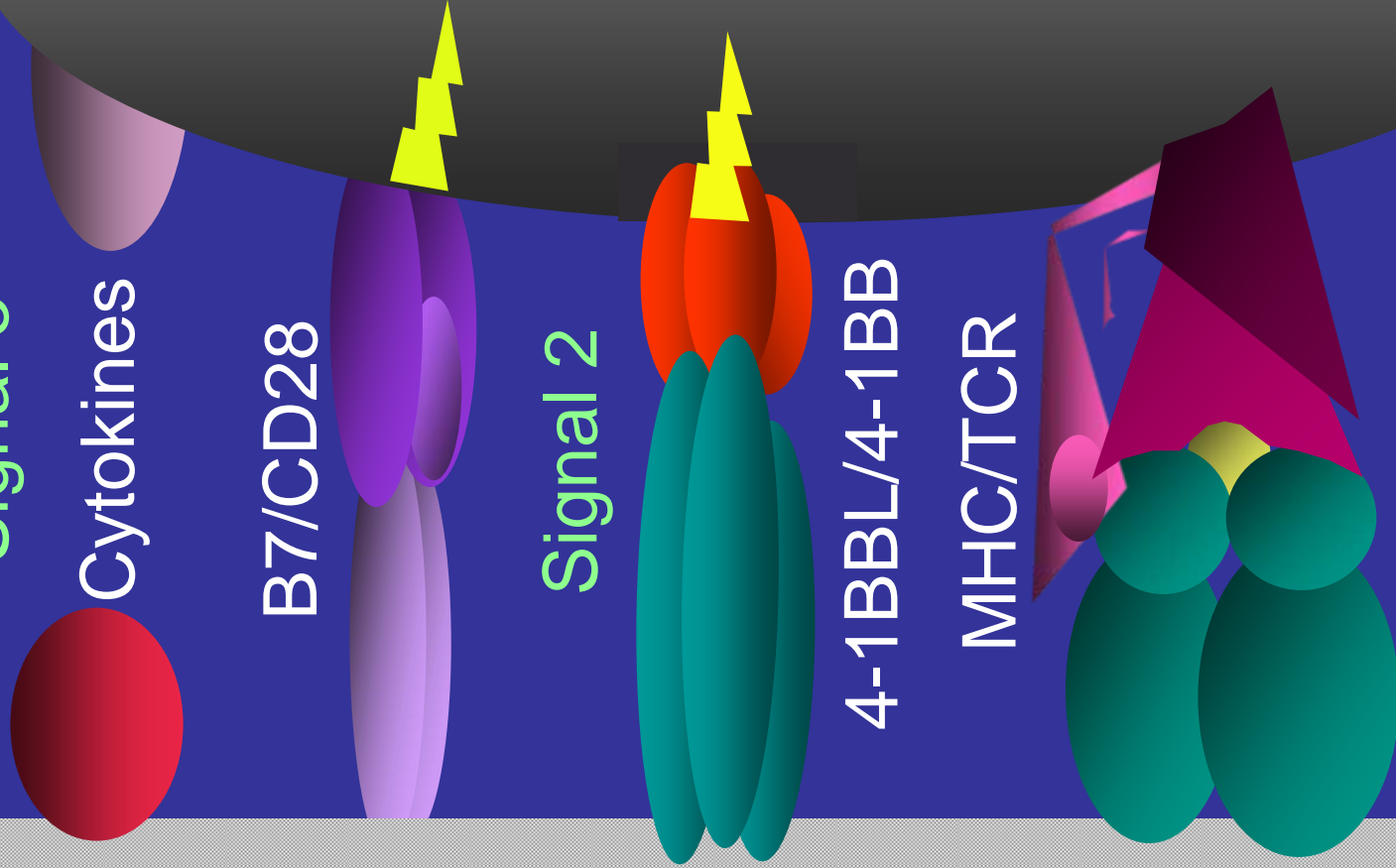
Signal 2

4-1BBL/4-1BB

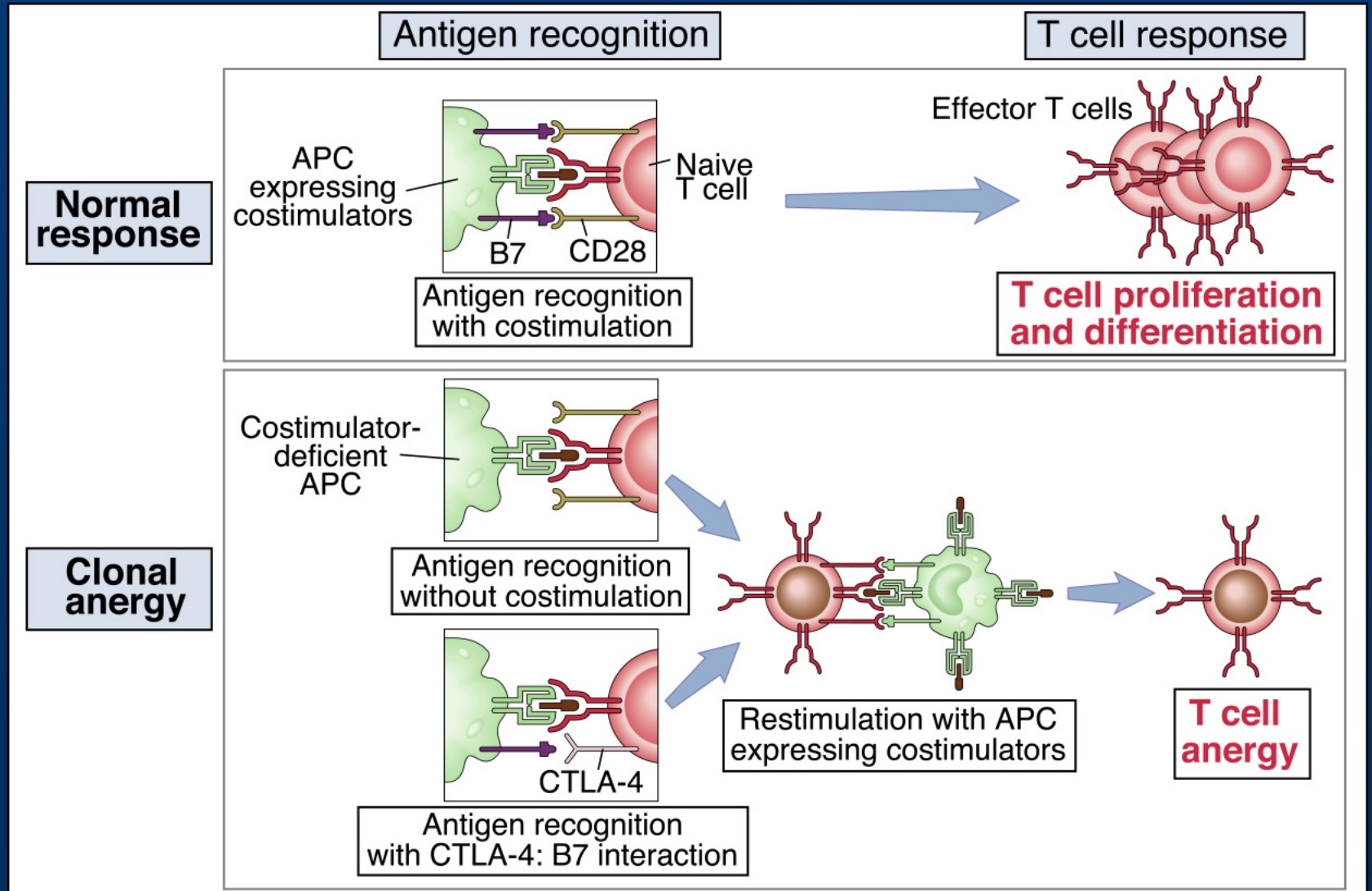
MHC/TCR

Signal 1

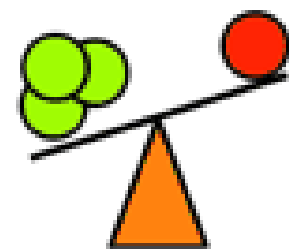
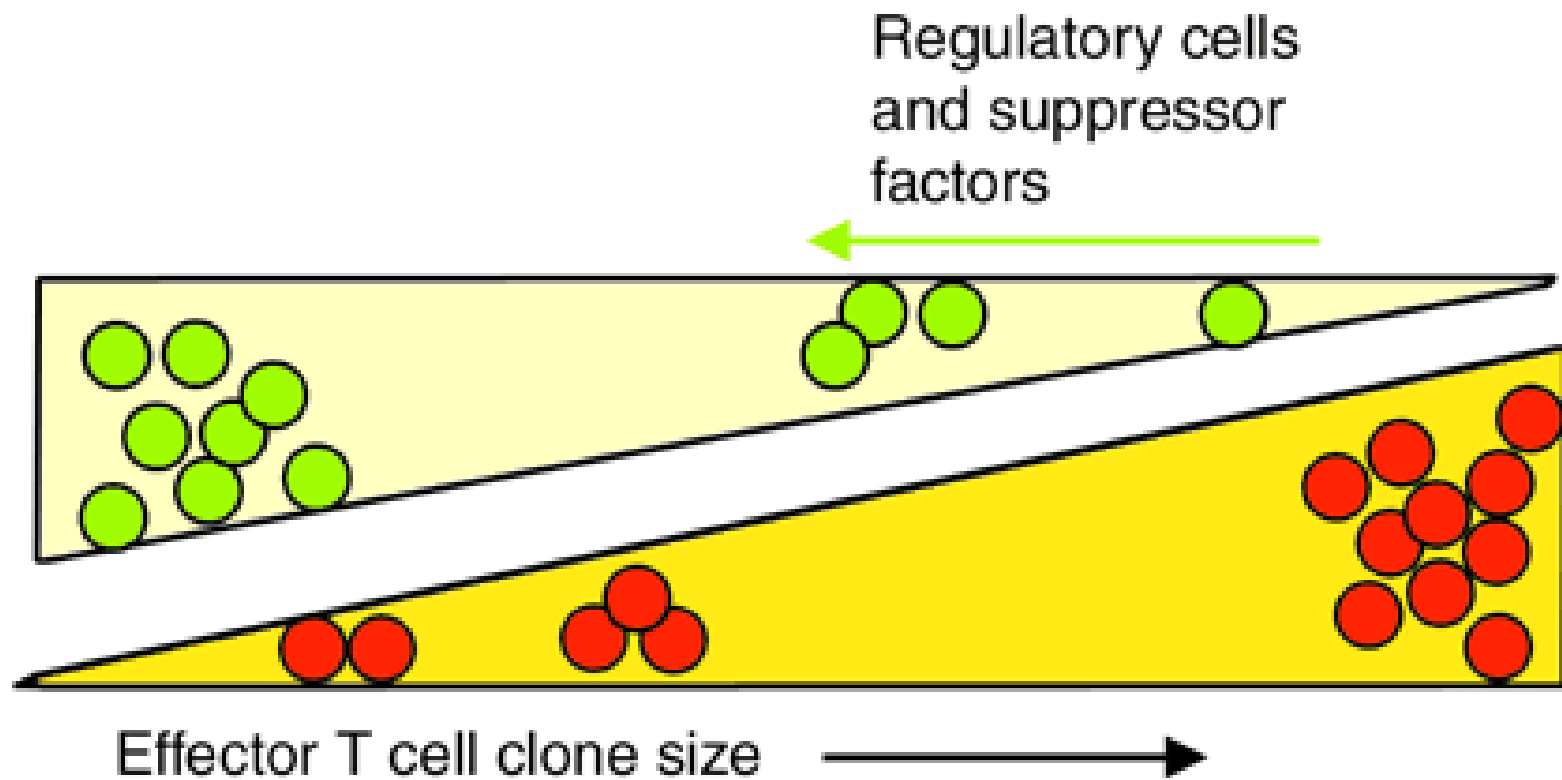
APC



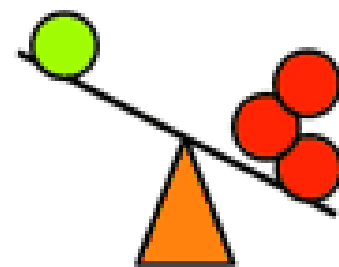
T cell anergy



From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 10-4



Tolerance



Rejection

Immunologically Privileged Sites

- Tissue grafts placed in these sites are not rejected

- Antigens are sequestered in immunologically privileged sites
 - Brain
 - Anterior chamber of Eye
 - Testis
 - Hamster Cheek Pouch

Experimental Induction of Allograft Tolerance

- Use of bone marrow from donor to induce mixed chimerism – Starzl and many others
- Use of *in vitro*-manipulated or immature donor dendritic cells which can induce both peripheral and central tolerance – Thompson and Hardy
- Gene targeting of transplanted organs— immunomodulatory molecules
- Use of T cell co-stimulatory blockade - Larsen
- Use of T cell depleting agents
 - Humanized, non-mitogenic anti CD3 - Bluestone
 - Campath-1H – Calne, Knechtle

Tolerance in Clinical Transplantation

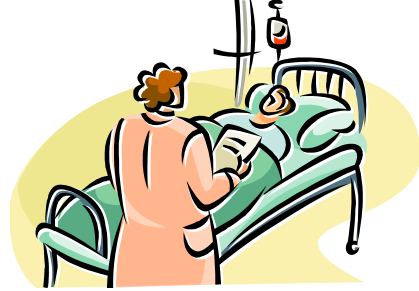
- Patients have stopped maintenance immunosuppression –Ken Newell registry at Emory (PTLD, Non-compliance)
- Patients received bone marrow transplants for hematologic disease and subsequently were transplanted with kidneys from same donor – MGH experience

Immune Tolerance Network

- hOKT3 γ -1 (ala-ala) and sirolimus immunotherapy in Type 1 diabetic islet allograft recipients –B. Hering
- Pilot Study Using Donor Stem Cells and Campath-1H to Induce Renal Transplant Tolerance – J. Miller
- Campath-1H/tacrolimus/sirolimus in renal transplantation (tacrolimus and sirolimus withdrawal) – S. Knechtle
- Mixed chimerism with non-myeloablative conditioning in renal transplant patients with ESRD due to multiple myeloma or living donor transplants (CsA withdrawal) D.Sachs & B. Cosimi

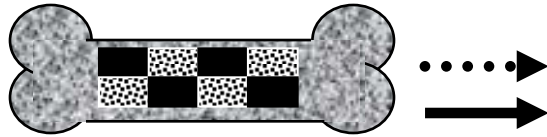
Rationale: Combined Matched Related Donor Bone Marrow and Kidney Transplantation in Multiple Myeloma With Kidney Failure

- **Allogeneic BMT is the only known cure for MM. Complication rates are high with standard allogeneic BMT.**
- **Kidney failure is a common complication of MM, but the malignancy usually precludes kidney transplantation.**
- **Successful allogeneic BMT with less toxic conditioning induces transplantation tolerance (animal models).**
- **MGH investigators have developed a less toxic BMT protocol that is safe and effective in MM.**
- **Less toxic BMT combined with kidney transplantation from the same donor might induce tolerance while curing the myeloma.**



Step 1: Bone marrow transplant with less toxic recipient treatment that includes antibodies.

Donor marrow is T cell depleted



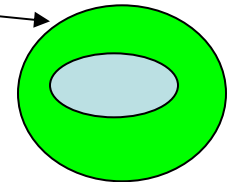
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Blood cells are a mixture of donor and host: **Mixed chimerism is achieved without GVHR**

↓
Wait 1-2 months. Inflammation from preparative treatment subsides.



Step 2: Infuse donor T cells.

Donor T cells interact with “presenting cells” of mixed chimera to maximize GVHR



Donor T cells are armed to kill tumor cells that express recipient antigens. They stay inside the blood and lymph, where tumor is.



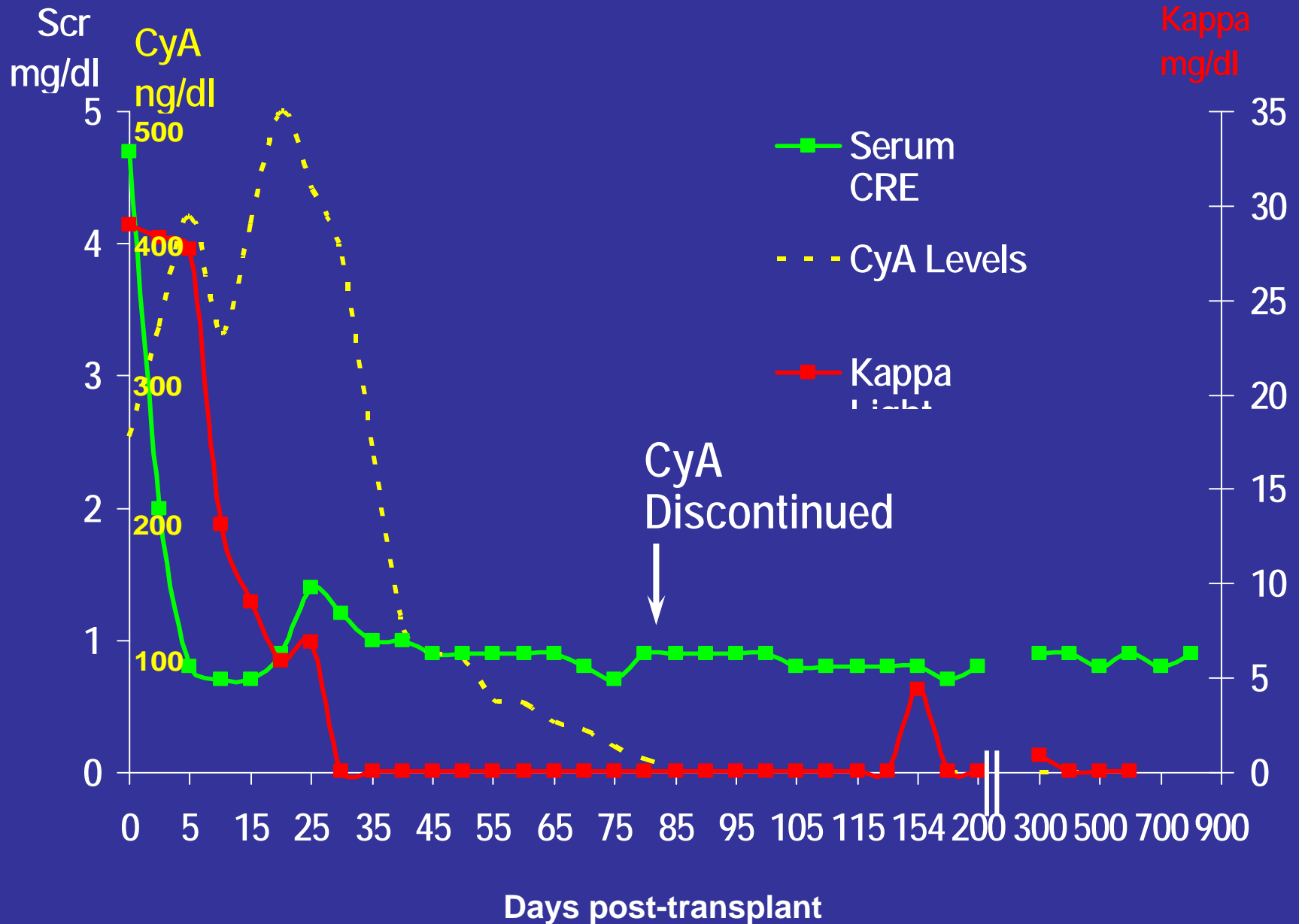
Tumor is killed



T cells don't go to skin/gut/liver. There is no GVHD.

Sykes et al

Clinical course of patient 1



Role of Immunosuppressive Drugs

- Some may promote tolerance – Thymoglobulin, Sirolimus
- Some may block tolerance – CI's, Antiproliferatives
- When and how should they be withdrawn? ???

- Organ Hierarchy to Tolerance -
Liver, Kidney, Heart, Lungs, Intestine
- Older patients and may be easier to tolerize because of because immunologic aging or fewer alloreactive T cells

Measures of Tolerance Induction and Longevity

Need to predict:

- **if rejection is imminent** - Biopsy findings, graft dysfunction
Alloantibody production, cytotoxic gene expression by PCR
- **when/if immunosuppression can be withdrawn**
 - MLR non-reactivity
 - CTL non reactivity
 - Absence of indirect responses in vitro
 - Microchimerism
 - Trans vivo DTH (with regulation)
 - Microarrays
 - Macrochimerism
 - Complete Chimerism

Clinical Tolerance Strategies to be Tested

- Mixed allogeneic chimerism (using bone marrow or bone marrow stem cell, or immature DC's transplantation) with non-ablative immunosuppression
- Antilymphoid polyclonal sera and/or T cell co-stimulatory blockade, with donor antigen (bone marrow, bone marrow stem cells, immature dendritic cells, or donor-specific blood transfusions)

Risks of Tolerance Induction Strategies

- Will not prevent allograft disease due to non alloantigen-dependent mechanisms?
- Will also tolerize a patient to infectious agents that accompany the graft (e.g. CMV)?
- Will predispose certain tissues to malignant transformation (T cell depletion, whole body irradiation)?



Thank you. Are there any other questions?