The Biology of IL-15 and IL-2: Implications for Cancer Therapy and Vaccine Design

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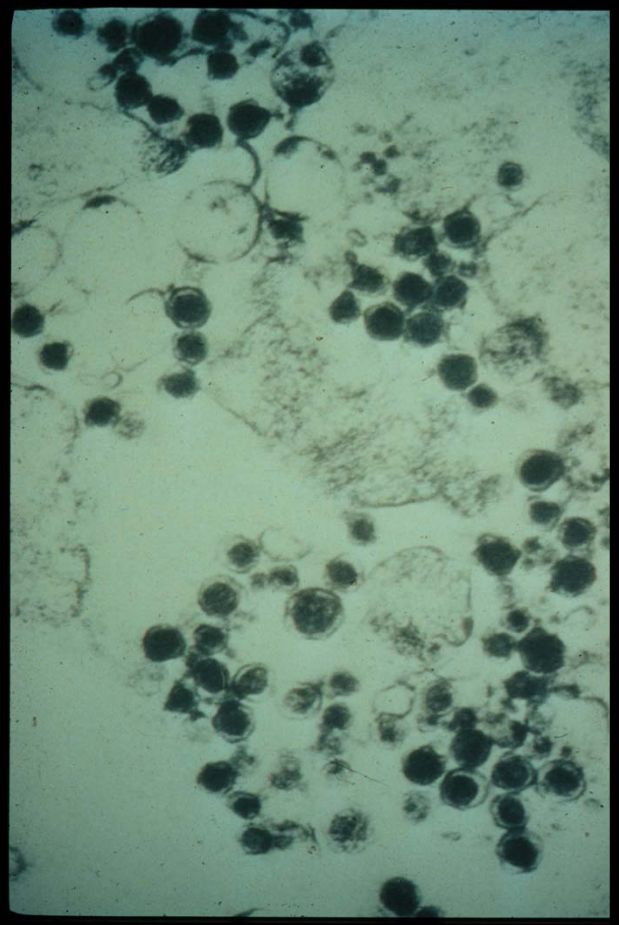
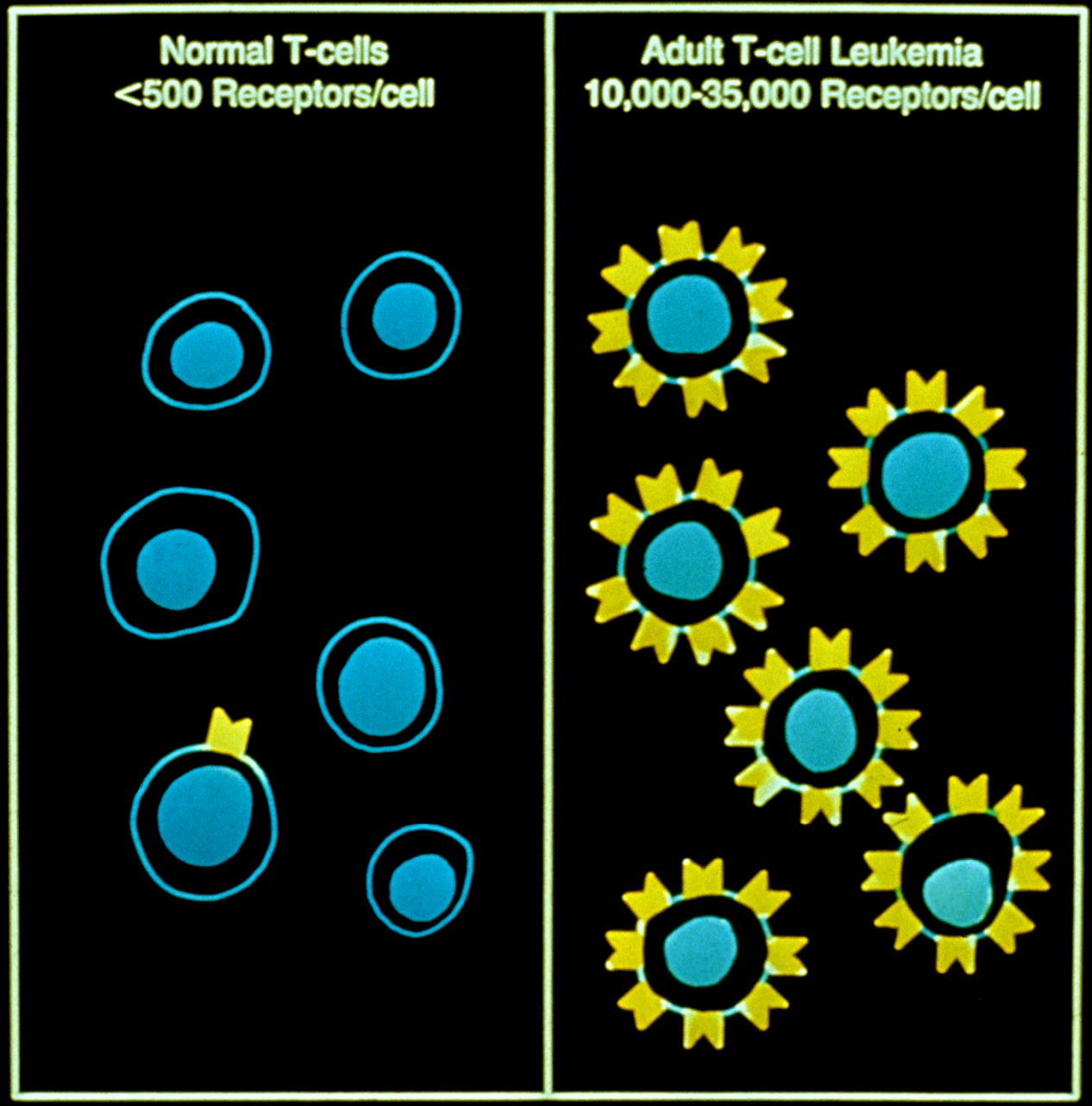
Adult T Cell Leukemia/Lymphoma

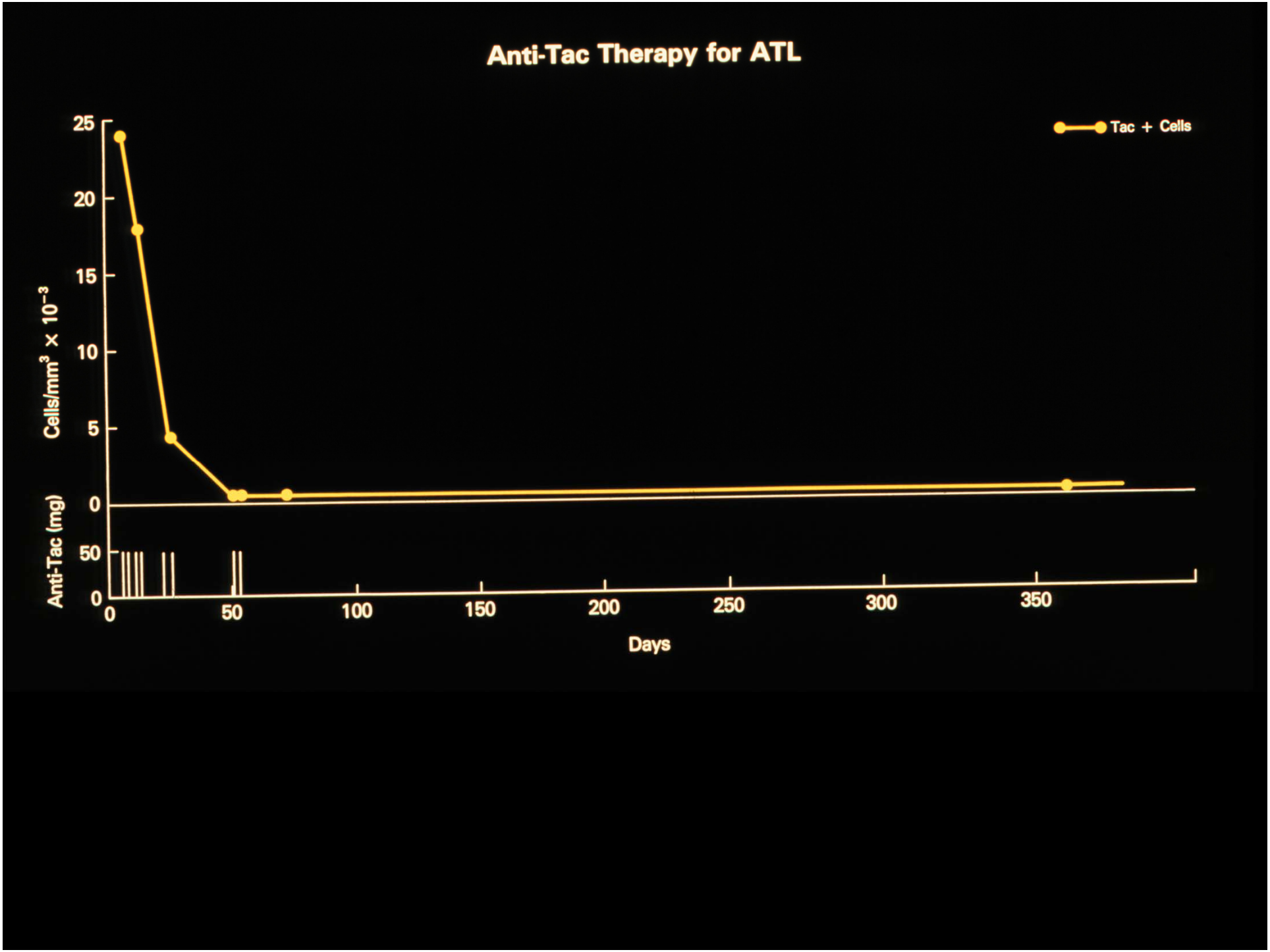


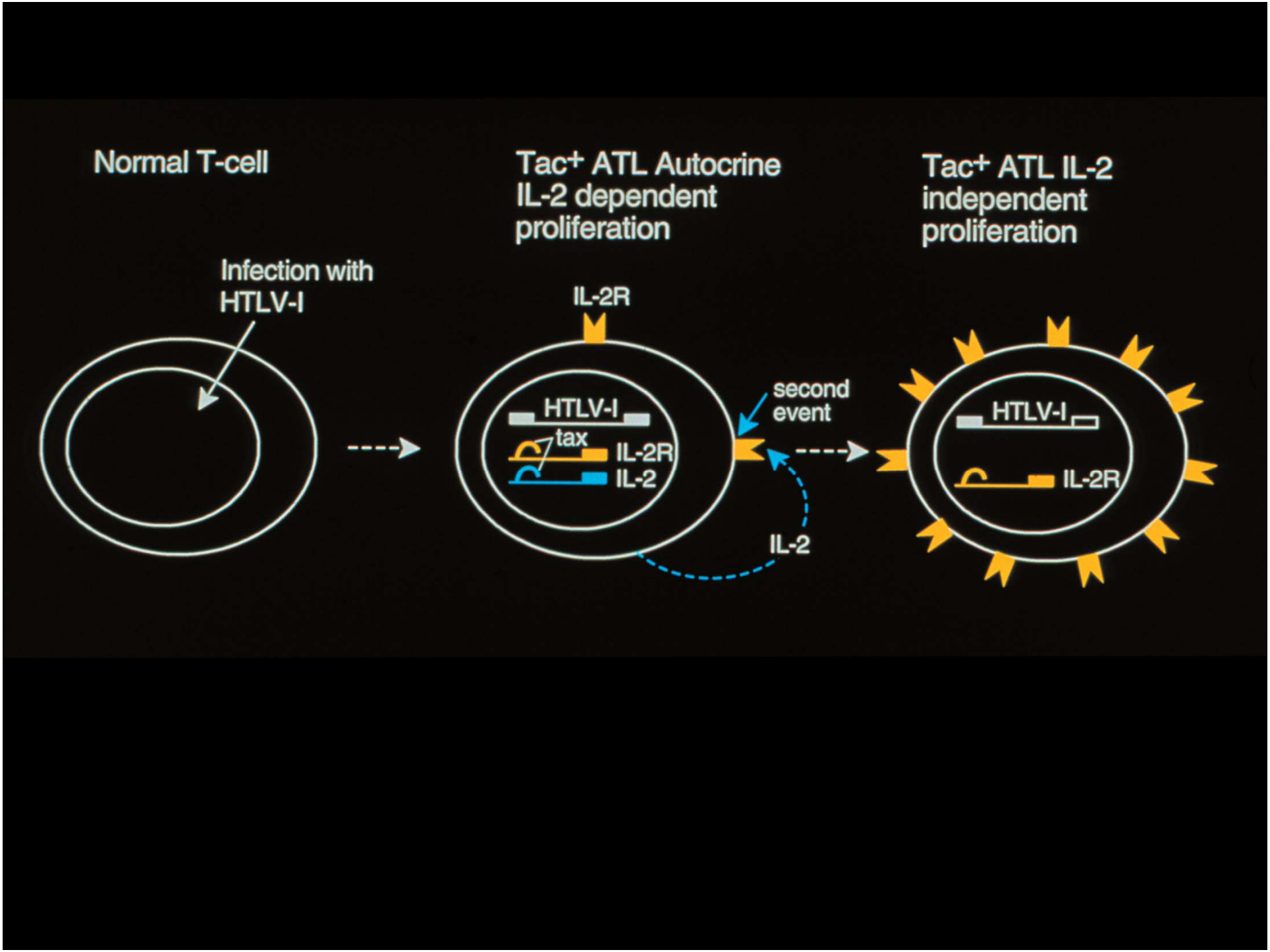
- Aggressive lymphoproliferative disorder caused by human T-cell lymphotrophic virus Type-1.
- Geographic distribution follows that of HTLV-1.
- Malignant proliferation of T cells, usually T-helper cells (CD4+).
- Characterized by high WBC counts, hypercalcemia, skin and lytic bone lesions. Lung, liver, CNS and G.I. involvement are also common.
- Limited treatment.
- Poor prognosis- survivals of 4 to 10 months.

NATIONAL CANCER INSTITUTE

IL-2 RECEPTOR EXPRESSION IN ATL

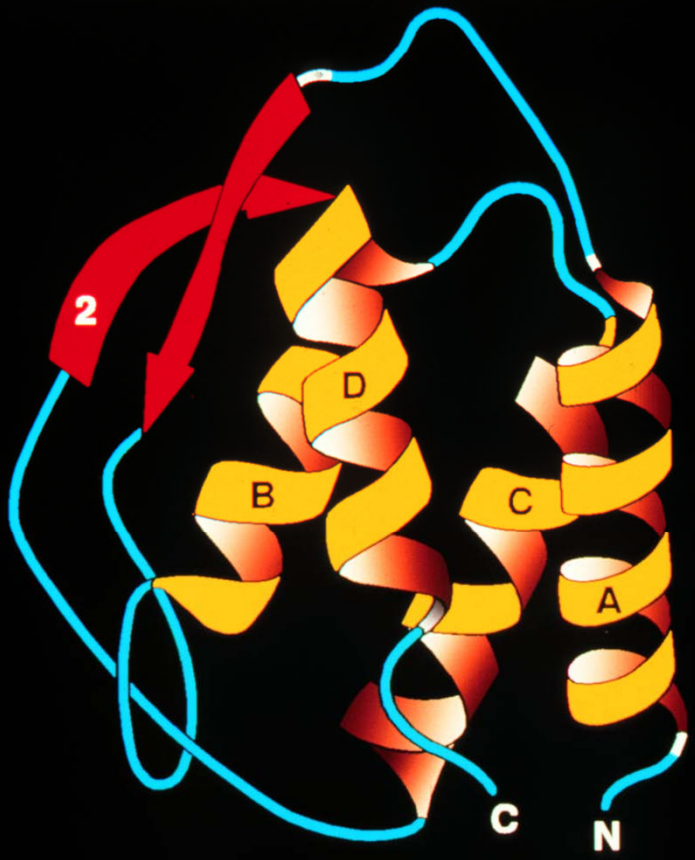



Anti-Tac Therapy for ATL
Tac + Cells
Cells/mm³ × 10⁻³
25
20
15
10
5
0
Anti-Tac (mg)
50
0
0
50
100
150
200
250
300
350
Days

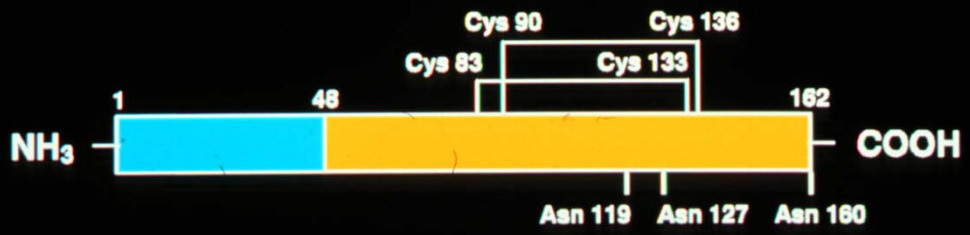

Normal T-cell
Tac+ ATL Autocrine IL-2 dependent proliferation
Tac+ ATL IL-2 independent proliferation
Infection with HTLV-I
IL-2R
HTLV-I
tax
IL-2R
IL-2
second event
HTLV-I
IL-2R
IL-2

The Structure of Human IL-15

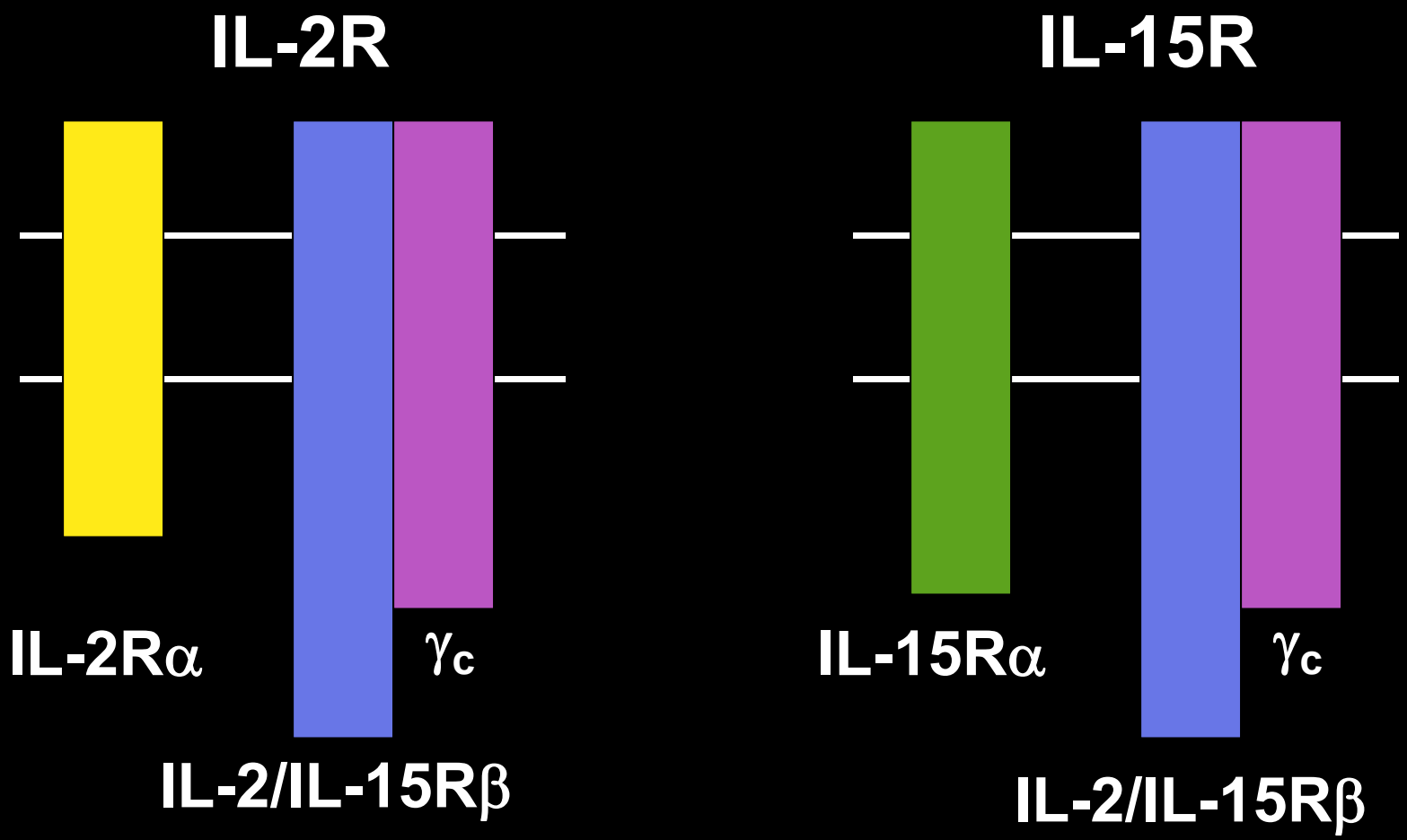
The IL-15 protein is a 14-15 kDa member of the 4α helix bundle cytokine family.



IL-15



The IL-2 and IL-15 Receptor Systems



The Contrasting Roles of IL-2 and IL-15 in the Life and Death of Lymphocytes

1. Both IL-2 and IL-15 stimulate the proliferation of T and B-cells and the generation and maintenance of NK cells.

2. IL-2 is pivotally involved in AICD and the maintenance and fitness of CD4+CD25+ T regs, actions that prevent a T-cell immune response to self.

3. IL-15 inhibits IL-2 mediated AICD and stimulates the development of NK cells and CD8+ memory phenotype T-cells that maintain an immune response to invading pathogens.

Phenotype of IL-2 and IL-2Rα Gene Targeted Mice

1. Massive enlargement of peripheral lymphoid organs.

2. High levels of IgG1 and IgE.

3. Autoimmune disease with hemolytic anemia, inflammatory bowel disease, and infiltrative granulopoiesis.

4. This phenotype is associated with impaired activation-induced cell death (AICD) and reduction in T regs.

Phenotype in IL-15 and IL-15R Alpha Gene Targeted Mice

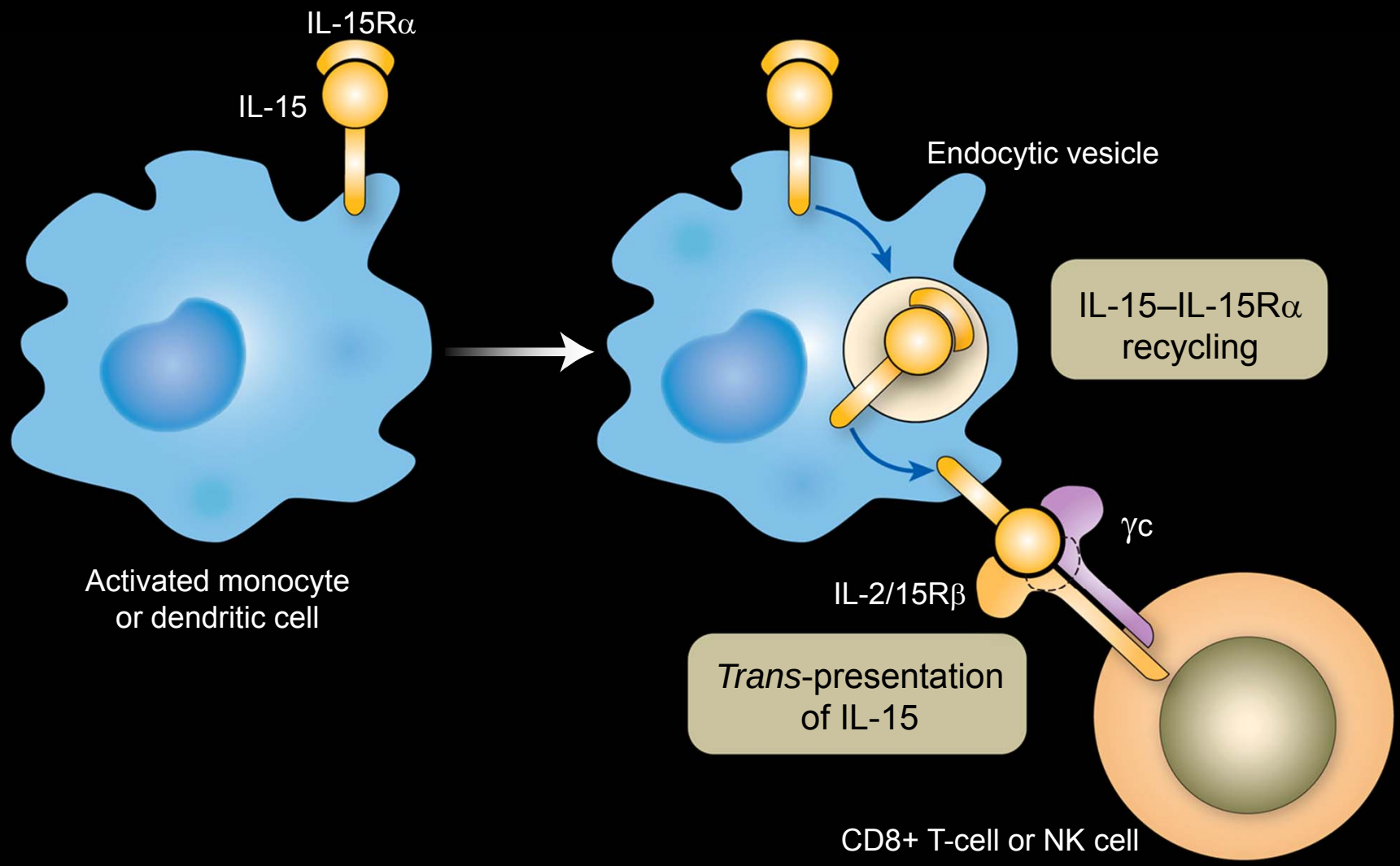
1. No lymphoid enlargement, autoimmune disease or impaired AICD or T reg function.

2. Marked reduction in the number of NK cells and memory phenotype CD8+ T cells.

How Can IL-2 and IL-15 Manifest Different Functions?

1. There are distinct cellular distributions for the private alpha subunits and different signals mediated by these subunits or by peptides non-randomly associated within them.
2. IL-15 signaling occurs as part of an immunological synapse in association with other co-stimulatory signals.

The interleukin-15 Receptor α-chain Presents Interleukin-15 *in trans* to Neighboring Natural Killer Cells and CD8+ T-cells



Adoptively transfered NK cells survive in normal but not in IL-15Rα-deficient mice

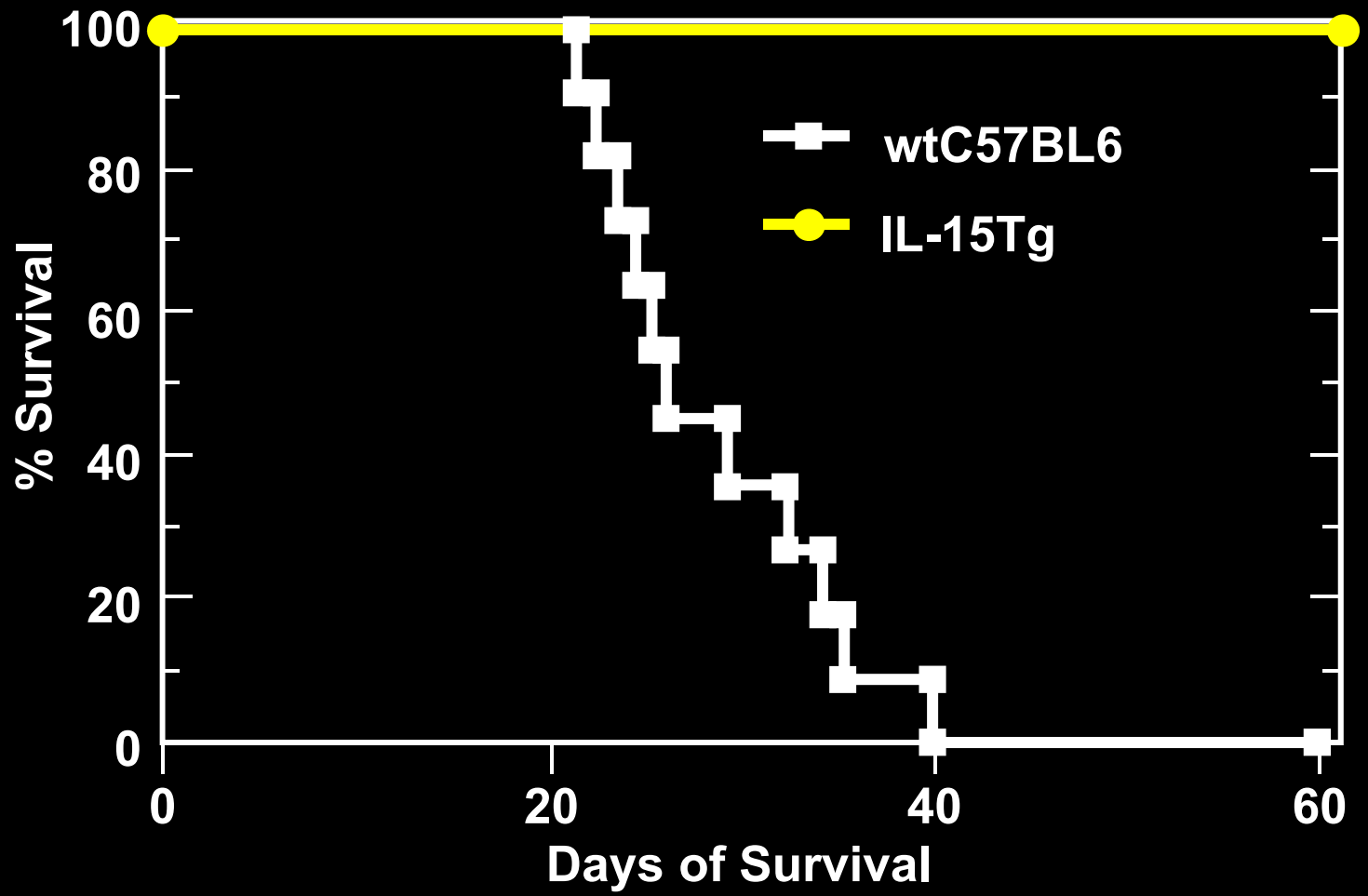
Koka et al. *J. Exp. Med.* 197: 1-9, 2003

Opposing Effects of IL-2 and IL-15: Implications for Their Use in Cancer Therapy and as a Component of Vaccines

1. IL-2 — IL-2 has been approved for use in metastatic renal cell carcinoma; however in the presence of IL-2 the CTL generated may interpret the tumor cells as self and may die by AICD or may be inhibited by T-regs.

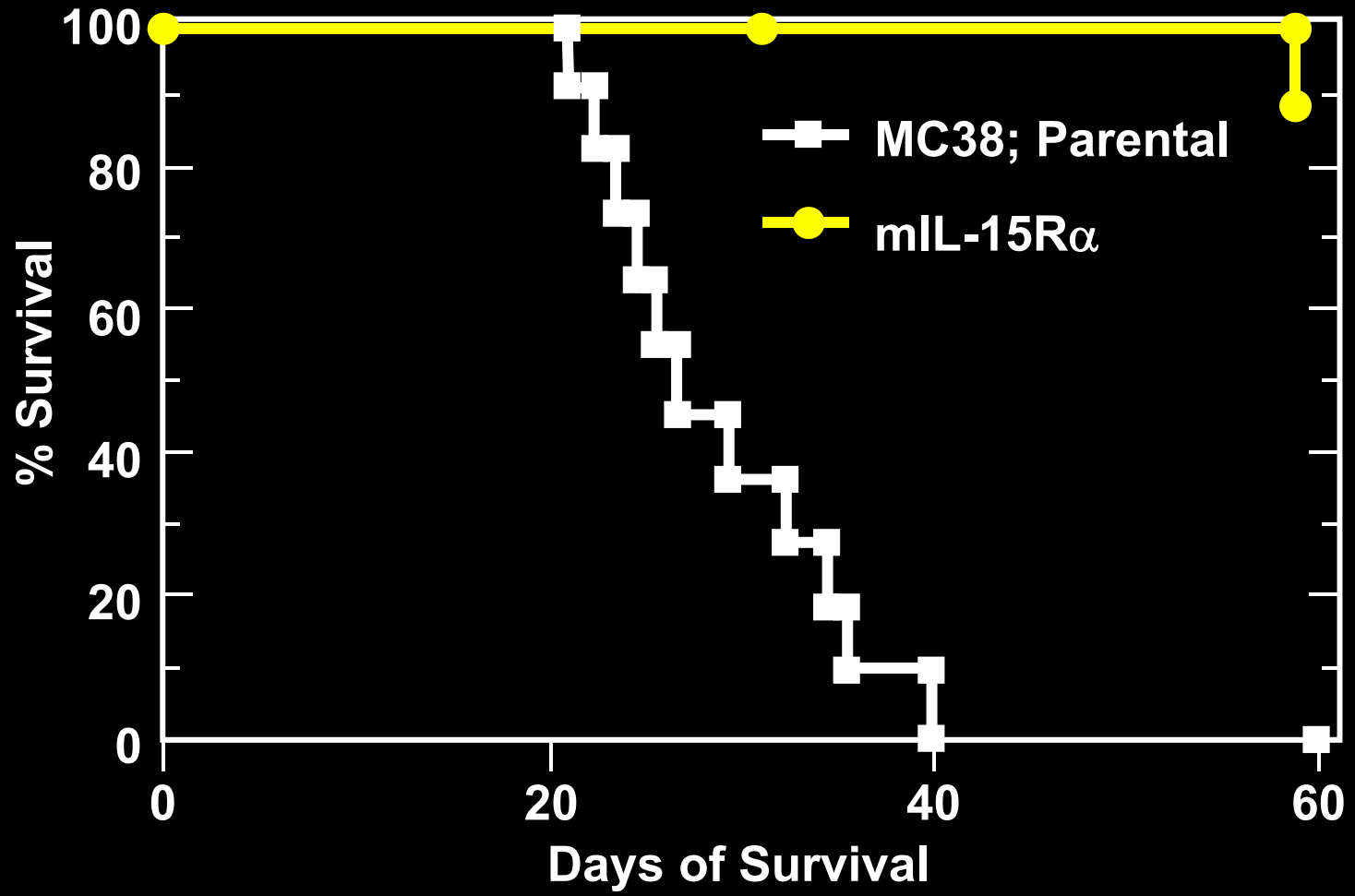
2. IL-15 — With its activation of T-cells its inhibitory action on AICD and its facilitation of the persistence of memory CD8+ T-cells, IL-15 may be superior to IL-2 in the treatment of cancer and as a component of vaccines.

IL-15 Tg Mice Survive Following Injection of MC 38 Cells



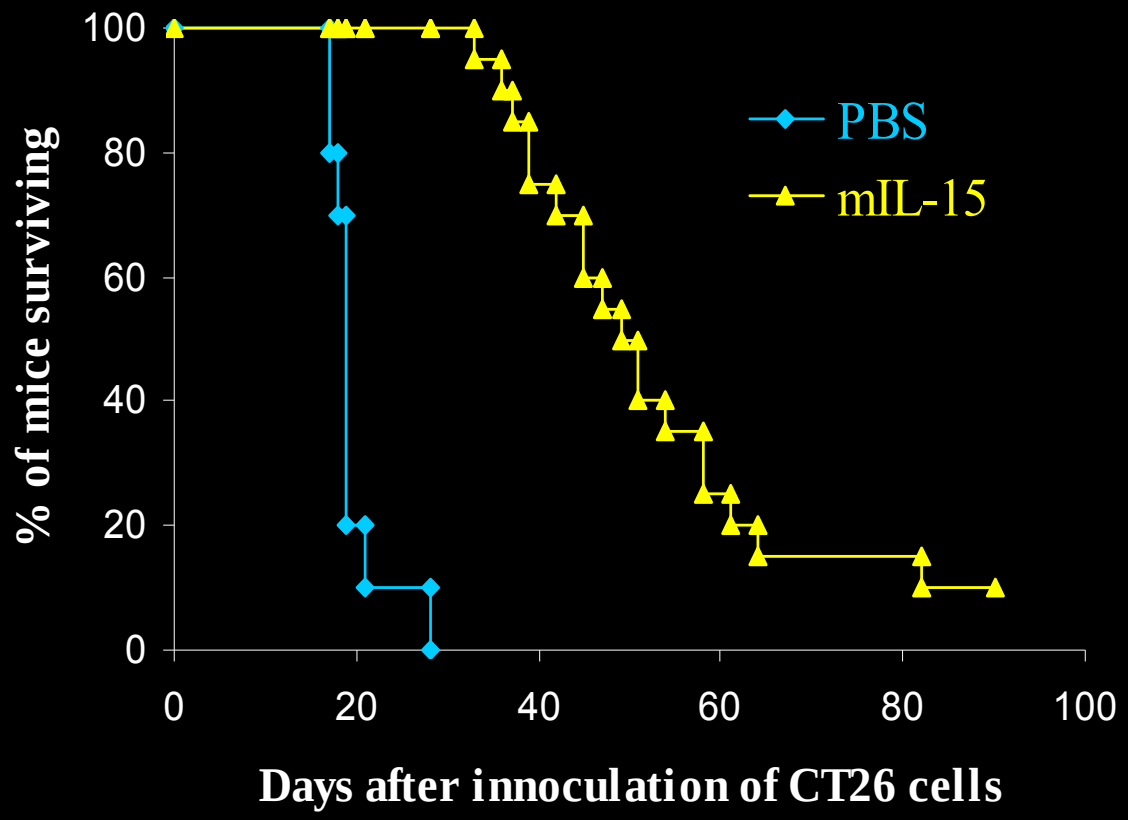
Wild type B6 mice died within 6 weeks following i.v. injection of MC38 tumor cells. However IL-15 transgenic mice (IL-15 Tg) survived more than eight months.

Mice Injected wth IL-15Rα Transfected MC 38 Cells Survive



The survival of mice injected with MC38 cells transfected with IL-15Rα was significantly longer ($P < 0.001$) than that of mice injected with parental MC38 cells.

IL-15 prolonged survival of mice bearing CT26 tumor



Development of Recombinant Human Interleukin-15 into a cGMP Product

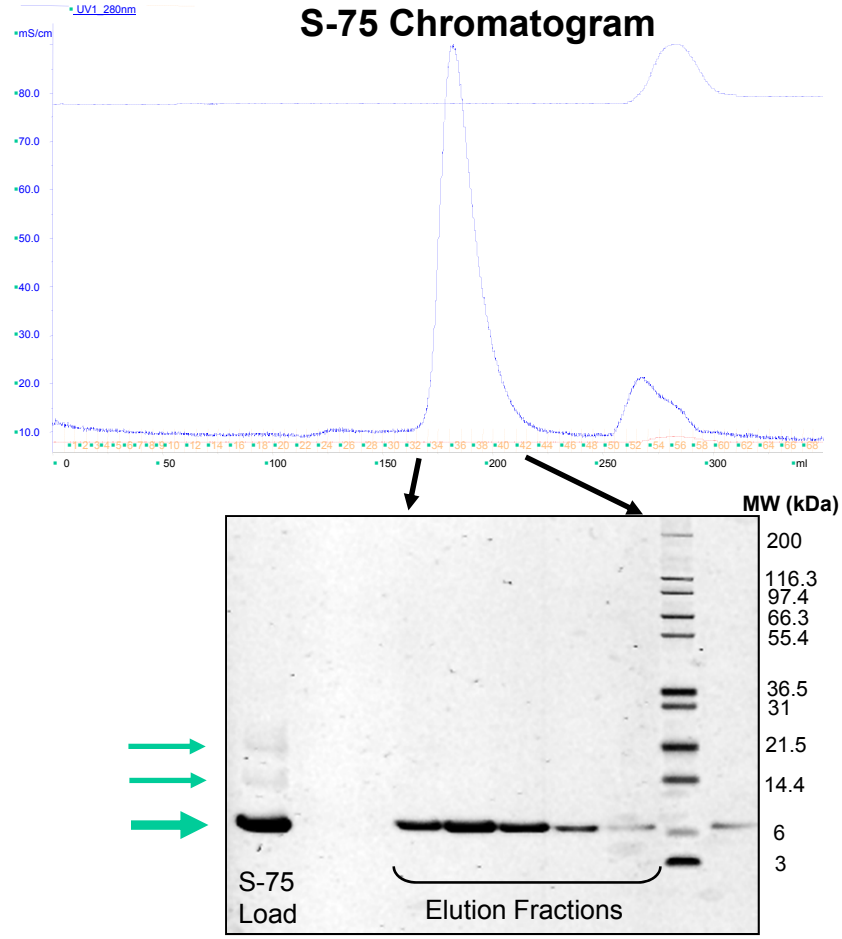
Purification Development

10 L Scale – 200 mg Yield

1-4	Fermentation, Recovery, and Refold
5	Concentration & Diafiltration 10 kD
6	Source 15Q Chromatography
7	Q XL Chromatography (Concentration Step)
8	S-75 Chromatography
9	Formulation

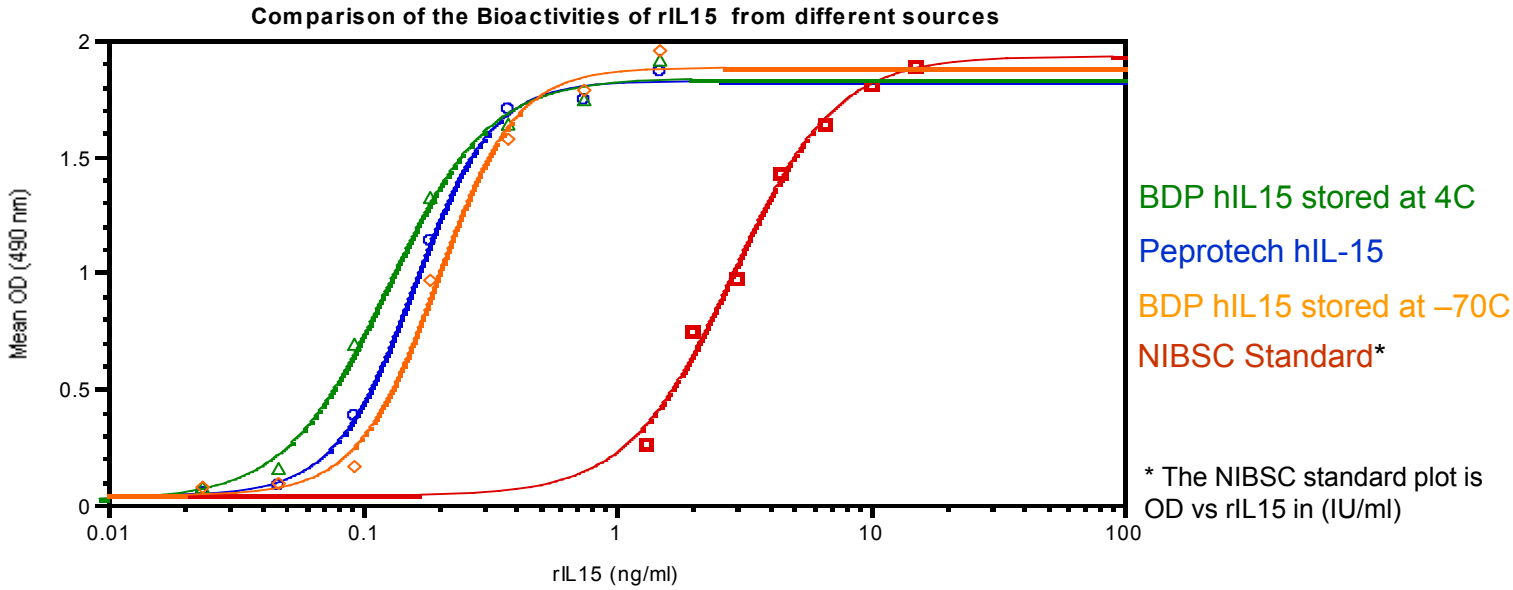
Step Recovery	Overall Recovery
48%	48%
64%	30%
70%	22%
91%	20%

> 95% pure



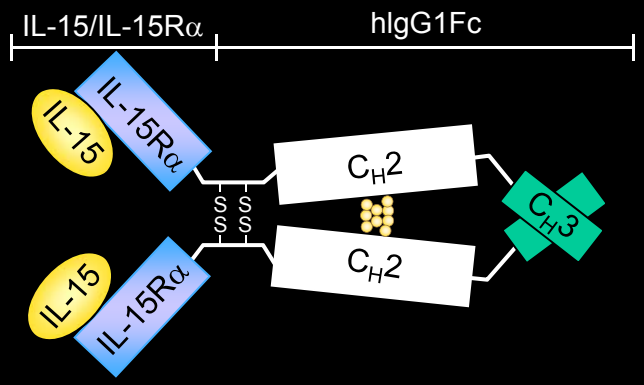
Assay Development

CTLL-2 Bioassay

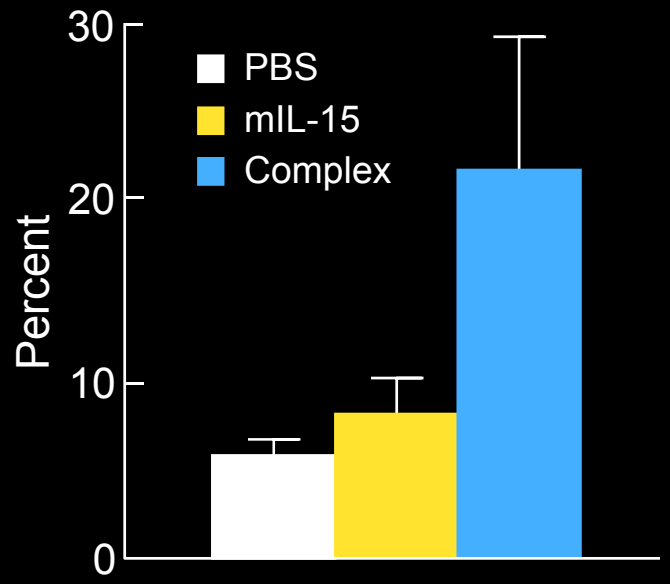


- MTS-dye based proliferation assay for measuring bioactivity of recombinant human IL-15
- CTLL-2 is a human T-cell line responsive to IL-15 under IL-2 depletion
- The inter-assay variation in the ED_{50} values are <20%
- Intra-day (inter-plate) variations are <10%
- The OD_{max} inter- and intra-day consistency is <10%
- Assay performance criteria were established using Peprotech and NIBSC standards

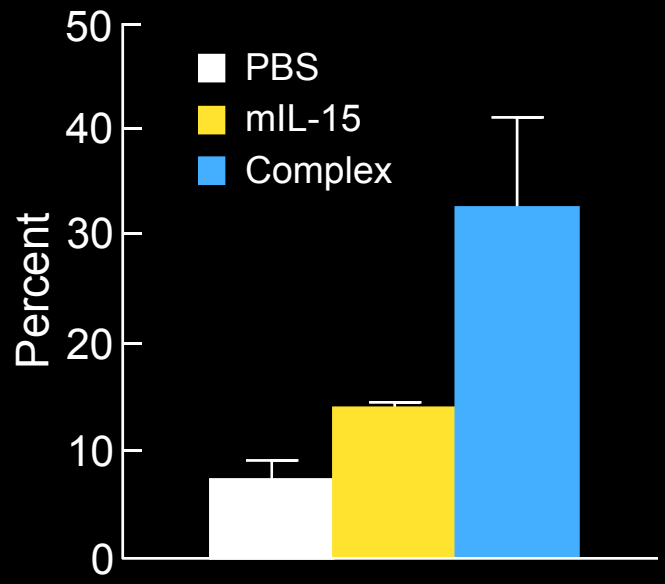
IL-15Rα IgFc/IL-15 Complex



NK Cells in Blood

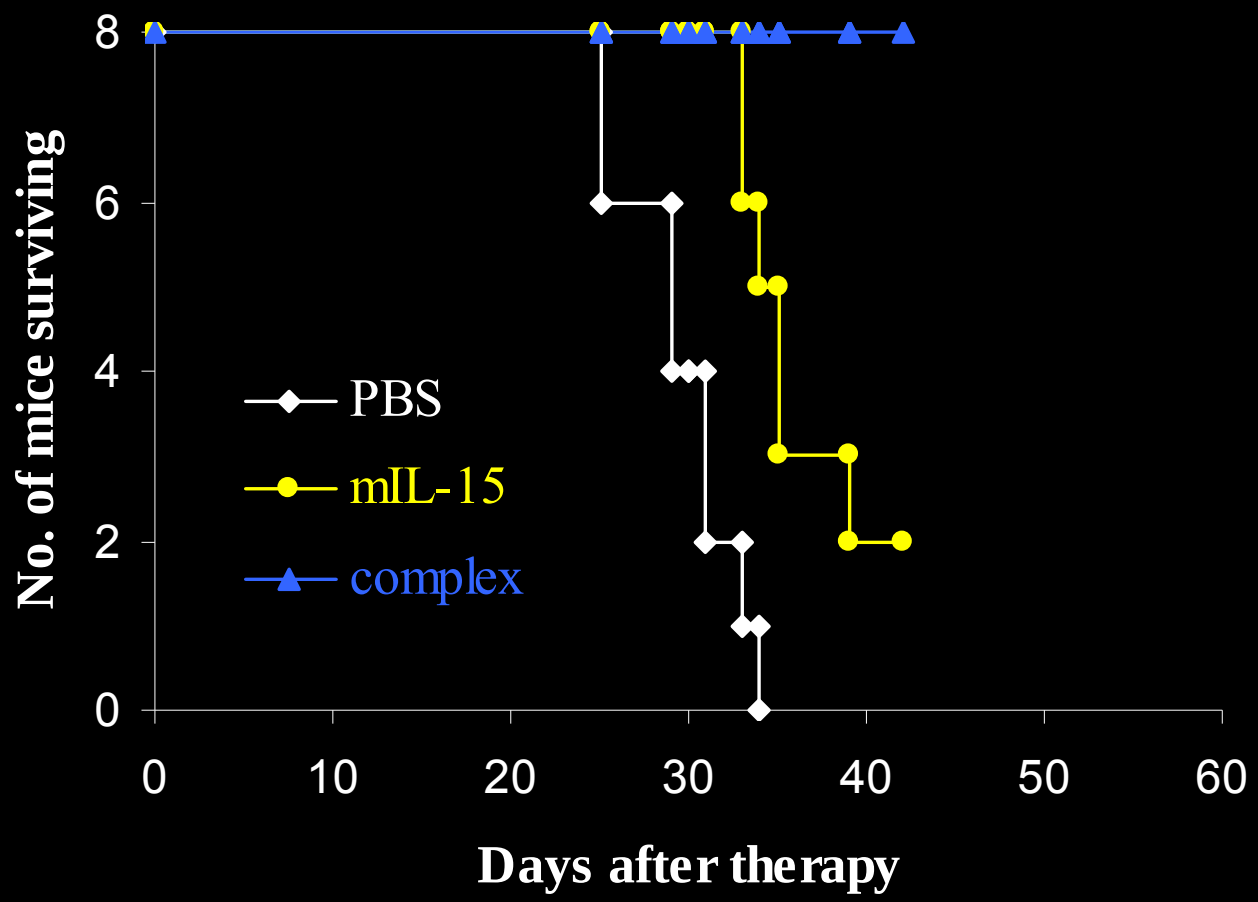


CD44^{hi} CD8 Subset in Blood

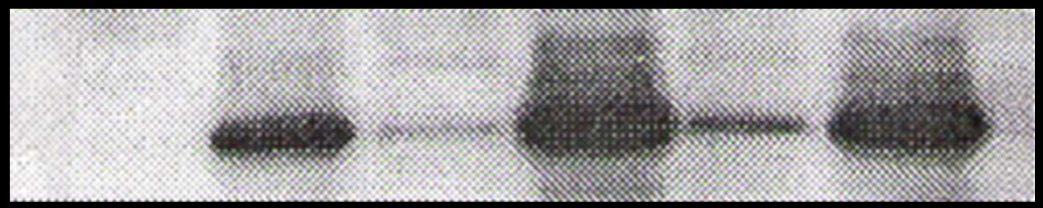


Equimolar IL-15 and Complex i.p. qd x 7

IL-15Rα IgFc/IL-15 Complex Therapy of MC38 Model

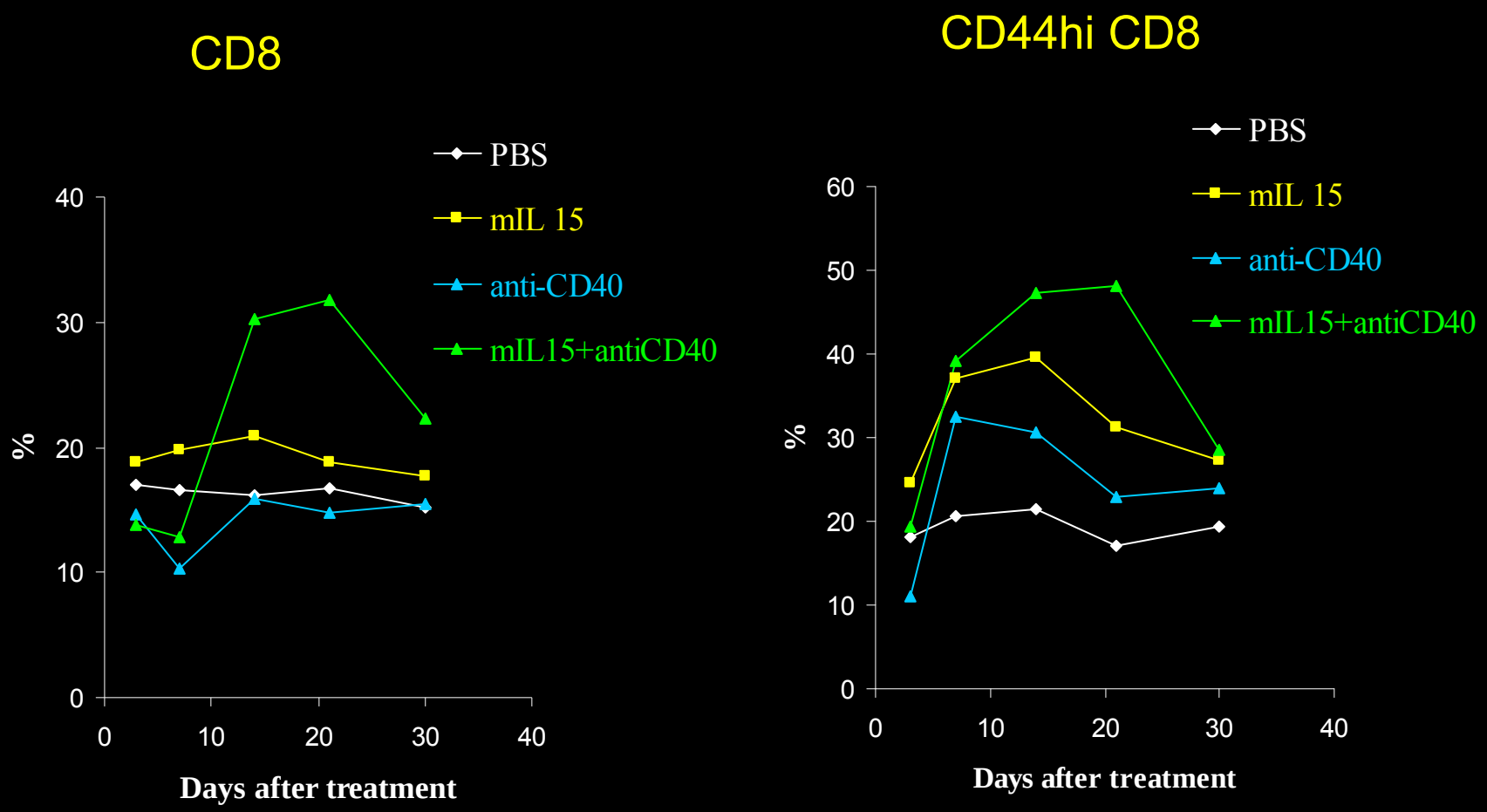


Expression of IL-15Rα



human CD83+ DCs

Anti-CD40 Augments IL-15 Induction of CD44hi CD8 T-cells



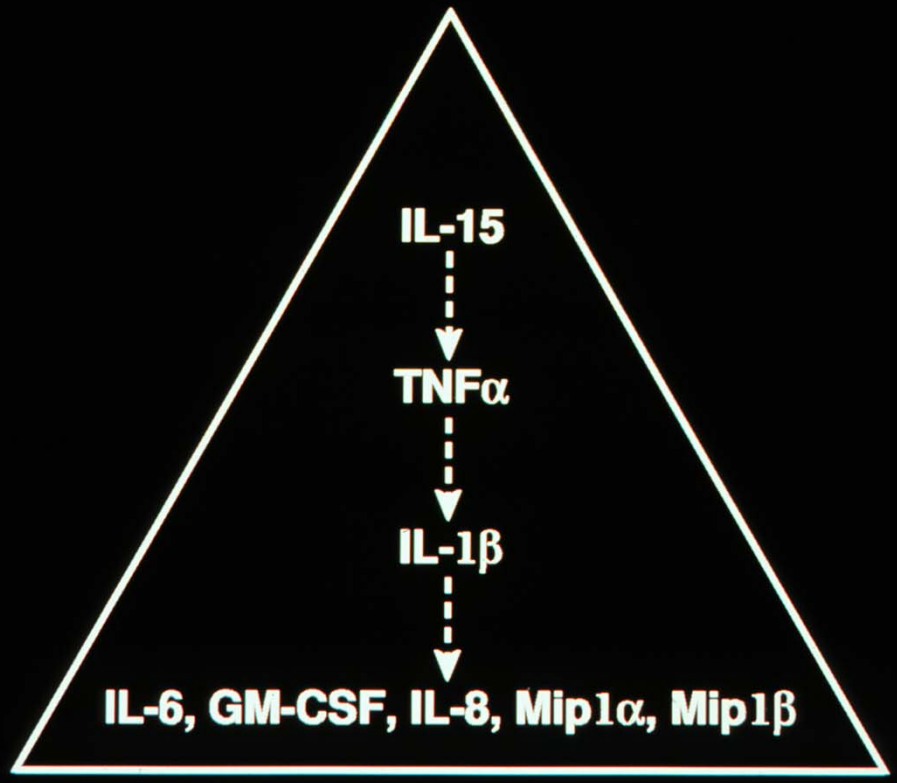
Vaccine Vectors Co-expressing IL-15 but not IL-2 induce Long-lasting Cellular Immunity

Co-administration of HIV vaccine vectors with vaccinia viruses expressing IL-15 supported robust $CD8^+$ T-cell mediated CTL immunity. In contrast the T-cell immunity induced by IL-2 was short lived.

Regulatory Features are Required to Control IL-15 Expression

1. IL-15 is an inflammatory cytokine that induces expression of TNFα and IL-1β.
2. IL-15 inhibits self-tolerance mediated by AICD.
3. IL-15 facilitates CD8 memory T-cell survival.
4. If IL-15 were indiscriminately expressed it would lead to inflammatory autoimmune disease.

Is IL-15 at the Apex of an Inflammatory Cytokine Pyramid?



IL-15 induces TNFα expression and thus may be at the apex of an inflammatory cytokine pyramid. IL-15 directed therapy may have both anti-inflammatory and anti-self directed memory T-cell effects whereas therapy directed toward TNFα only manifests anti-inflammatory effects.

Abnormalities of IL-15 Expression

1. Disordered IL-15/IL-15R alpha expression is observed in inflammatory autoimmune disorders including rheumatoid arthritis, multiple sclerosis, celiac disease, inflammatory bowel disease, and psoriasis.

2. Due to the HTLV-I Tax activation there are abnormally high levels of IL-15 and IL-15R alpha transcription leading to autocrine stimulation of T-cell proliferation in tropical spastic paraparesis (TSP) and adult T-cell leukemia (ATL).

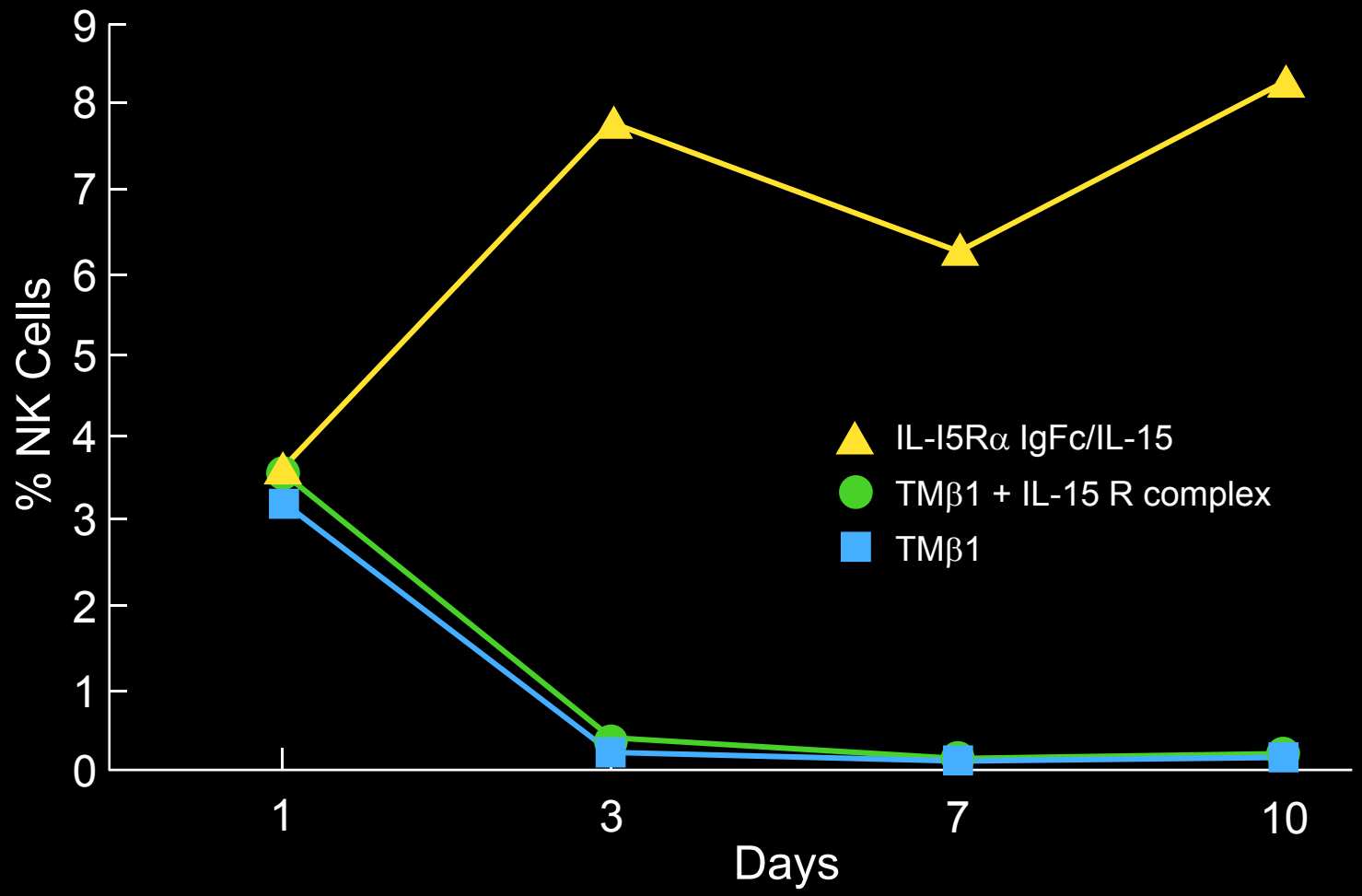
IL-2/IL-15R Beta as a Target for Immunotherapy

1. The humanized antibody Hu-MiK-Beta-1 directed toward IL-2/IL-15R beta inhibits the transpresentation of IL-15 and thereby blocks the action of IL-15 on CD8 and NK-cells.

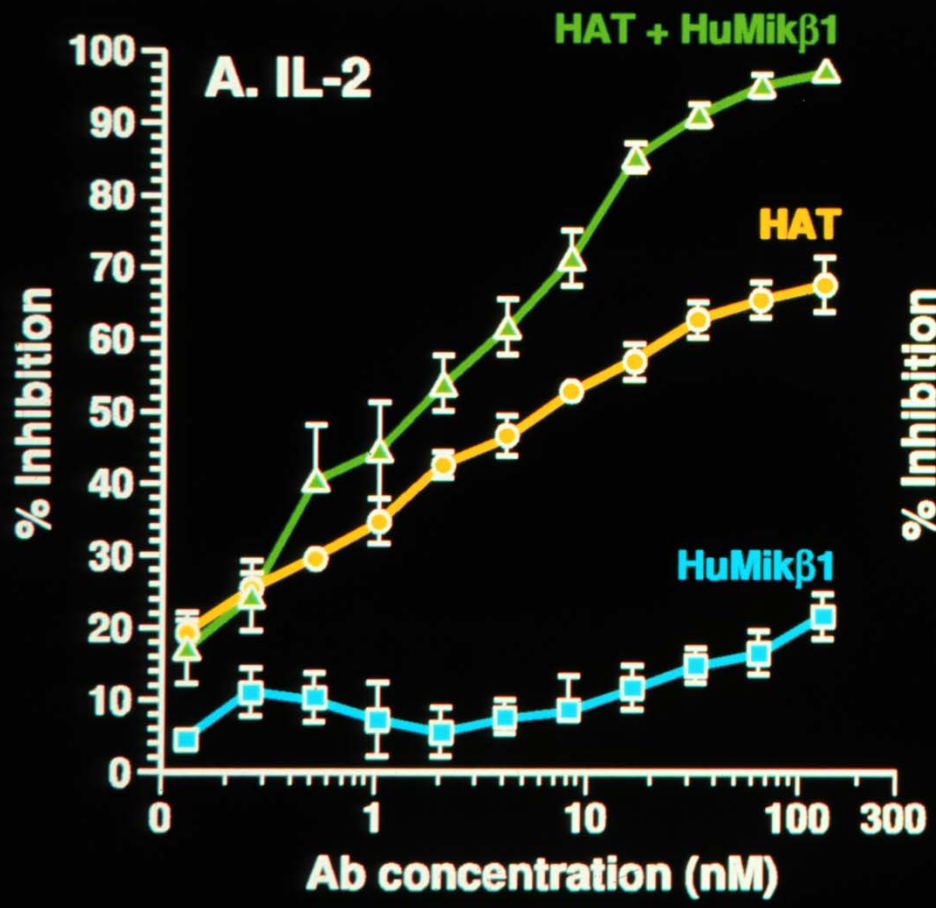
2. Hu-MiK-Beta-1 was effective in prolonging cardiac allograft survival in cynomolgus monkeys.

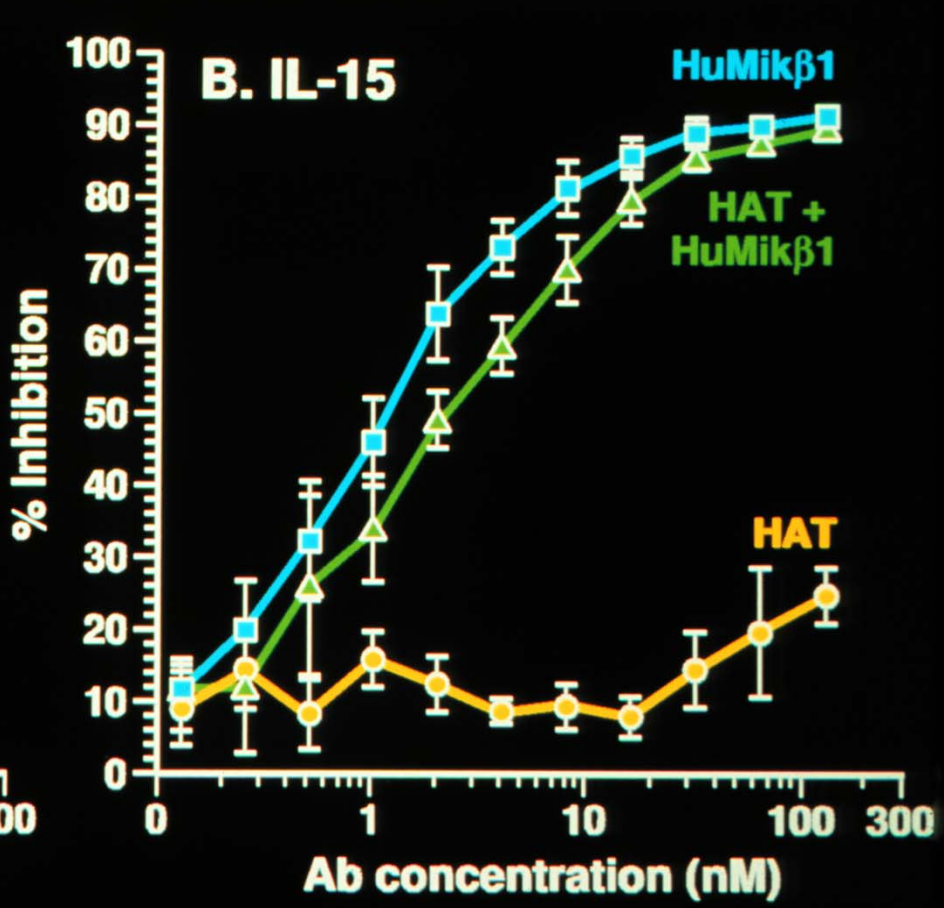
3. Trials of Hu-MiK-Beta-1 in rheumatoid arthritis, multiple sclerosis, TSP, refractory celiac disease and in LGL leukemia with granulocytopenia are planned.

Effect of Anti-IL-2/IL-I5Rβ (TMβ1) and IL-I5Rα IgFc/IL-15 on Percent Circulating NK Cells

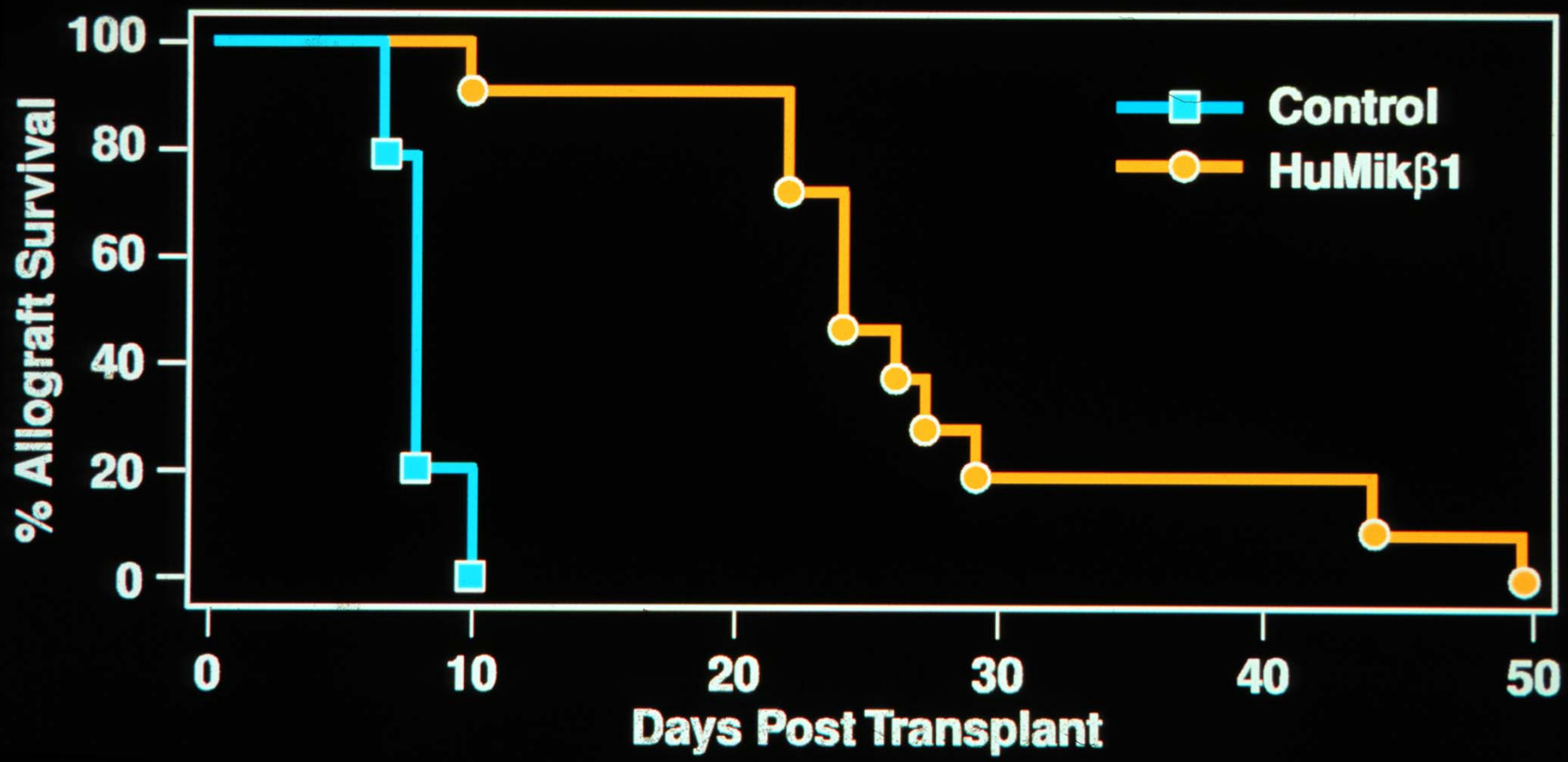


Humanized Anti-Tac (Anti-IL-2Rα) Inhibits Action of IL-2 Whereas Mikβ1 (Anti-IL-2Rβ) Inhibits IL-15





Humanized Mikβ1 Prolongs Cardiac Allograft Survival in Cynomolgus Monkeys



Disorders of IL-15/IL-15Rα in Celiac Disease

1. IL-15 is massively over-expressed in the lamina propria and intestinal epithelium in patients with active celiac disease and refractory celiac sprue.
2. IL-15 delivered on the surface of enterocytes regulates the expression of MIC and induces the expression of the activating NKG-2D receptor.
3. Through these mechanisms IL-15 induces the expression and survival of the clonally abnormal intraepithelial CD8+ lymphocytes that characterize refractory celiac sprue and its associated CD8 lymphoma.
4. These studies support the use of Hu-MiKβ-1 in the treatment of patients with refractory sprue and the associated CD8 lymphoma.

Summary I

1. IL-2 and IL-15 have contrasting roles in the life and death of lymphocytes.

2. IL-2 is involved in the checkpoint control of T cells that is required for self tolerance and the prevention of autoimmunity. In contrast IL-15 favors the survival of CD8 memory T cells and is thus dedicated to the persistence of an immune response.

3. IL-15R alpha recycles and presents IL-15 in trans as part of an immunological synapse with neighboring NK and CD8 T-cells.

Summary II

4. The demonstration that IL-15 is a critical factor for the proliferation, activation and function of NK and memory CD8 T-cells supports it use in the prevention and treatment of cancer and HIV.

5. The incorporation of IL-15 in molecular vaccines for cancer and AIDS provides a robust, sustained high-avidity cytotoxic T cell immune response.

6. Humanized Mik-Beta-1 (anti-CD122, anti-IL-2/15R beta) has been developed to provide therapy for select leukemias and autoimmune diseases.

IL-2/IL-15 Receptor Group

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