Imperial College
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Regulatory T cells: an overview

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Aim and learning outcomes

- To gain a broad understanding of the classification, function and potential therapeutic applications of regulatory T cells (Tregs)
- By the end of this lecture and your home study, you will be able:
  - To describe the mechanisms of peripheral tolerance
  - To recall the historical origin of Tregs
  - To discuss hallmark features of $CD4^+CD25^+$, Tr1 & Th3 Tregs
  - To understand current concepts of Treg function and development
  - To demonstrate an understanding of Treg networks
  - To discuss therapeutic applications of Tregs

Overview

- Introduction
- Historical perspective
- ‘Mainstream’ Tregs: $CD4^+CD25^+$, Tr1, Th3
- Treg stability
- Tregs: therapeutic implications
- Summary and conclusion

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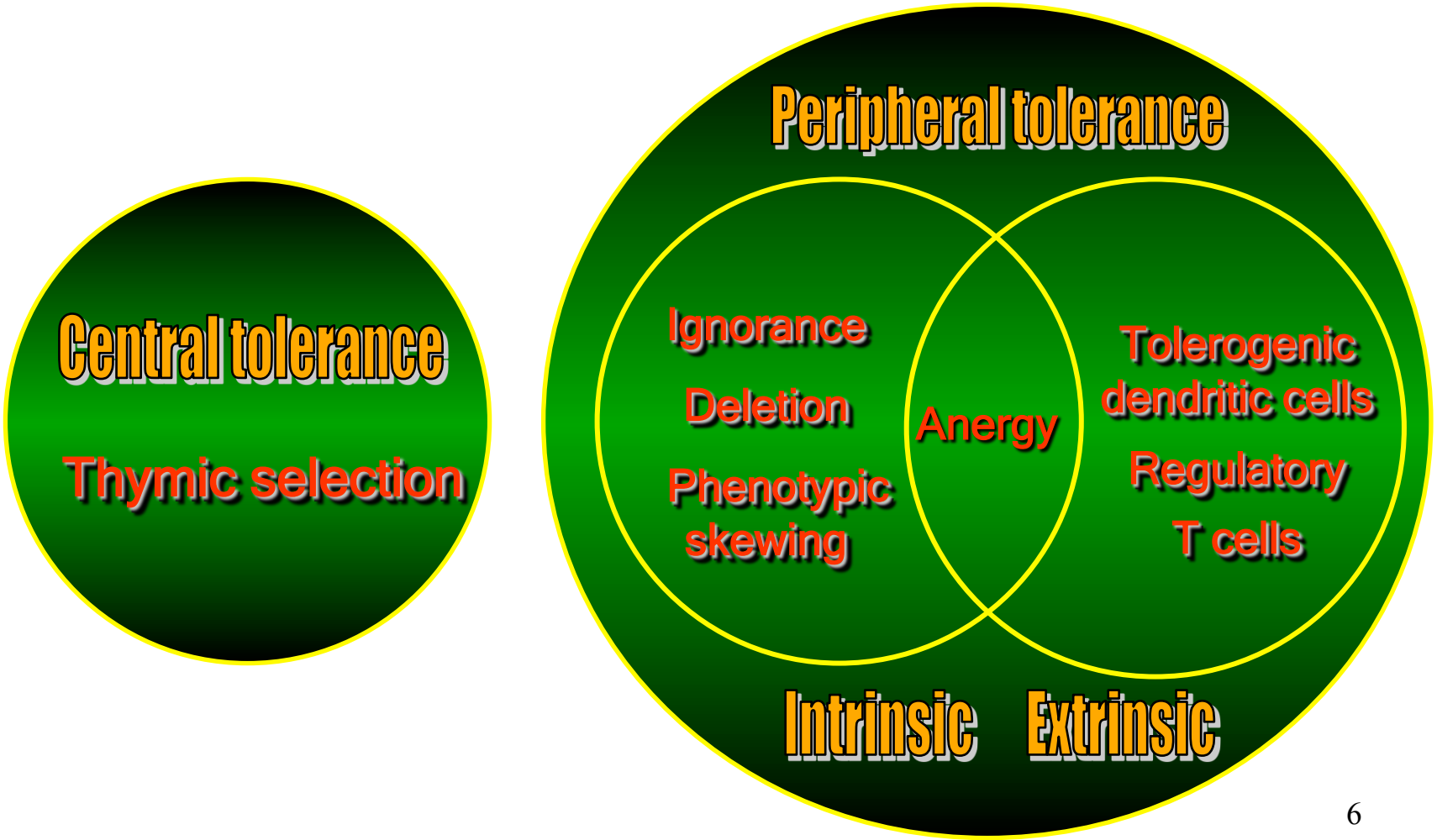
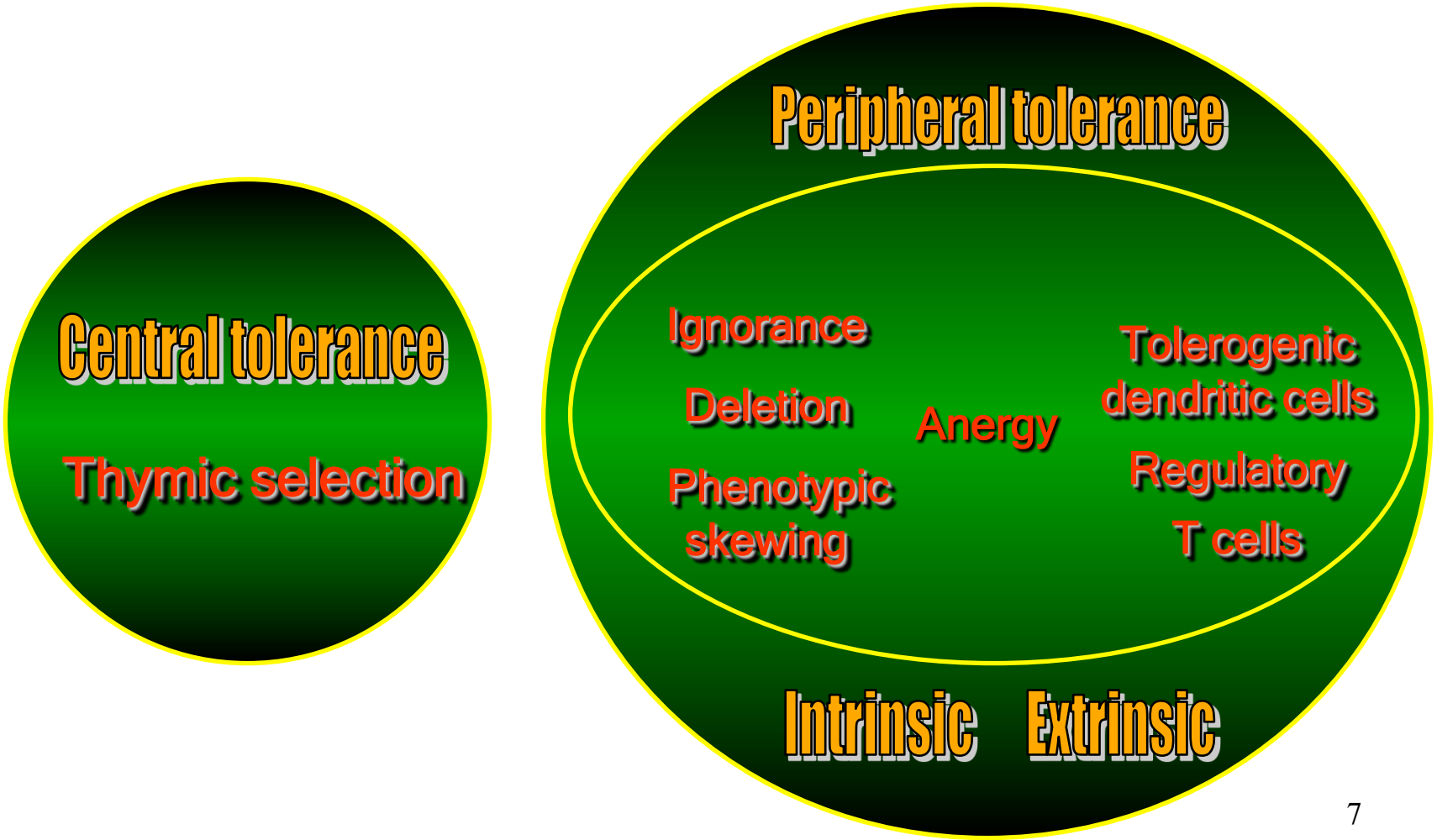
Introduction

- Developing thymocytes undergo positive and negative selection before mature
- Negative selection aims to remove the autoreactive cells (central tolerance) from peripheral T cell pool, but is not perfect
- 'Escaping' autoreactive T cells are controlled by peripheral tolerance mechanisms

Introduction: tolerance

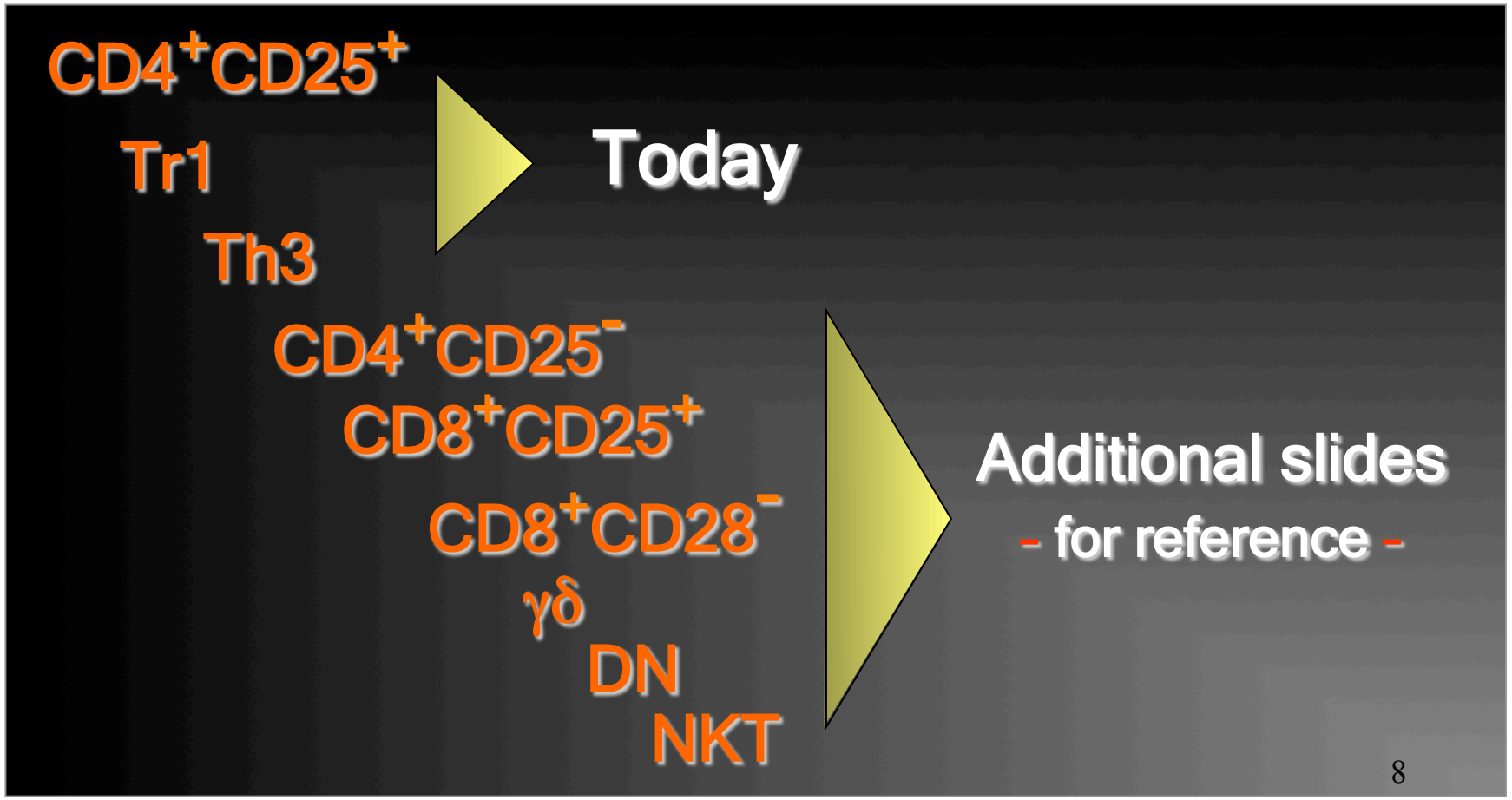


Introduction: tolerance



Introduction: T*regs*

Natural *vs* induced



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Historical perspective

- Suppressor T cells (Ts) were first described in 1972
- Three Ts cell populations documented
- I-J antigen thought to be expressed by Ts1 and Ts3, encoded between I-A and I-E
- Molecular mapping of murine MHC class II region failed to identify I-J locus
- The concept of Ts cells was dumped in mid-1990s

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$CD4^+CD25^+$ T cells

Seminal studies: Sakaguchi, Shevach

(1) d3Tx

Organ-specific autoimmune dz.
eg. oophoritis, orchitis, prostatitis, thyroiditis, gastritis

(2)

BALB/c

Spleen and LN preparations

Treatment with anti-CD25 and C'

Adoptive transfer

BALB/c *nu/nu*

Organ-specific..
Systemic autoimmune dz.

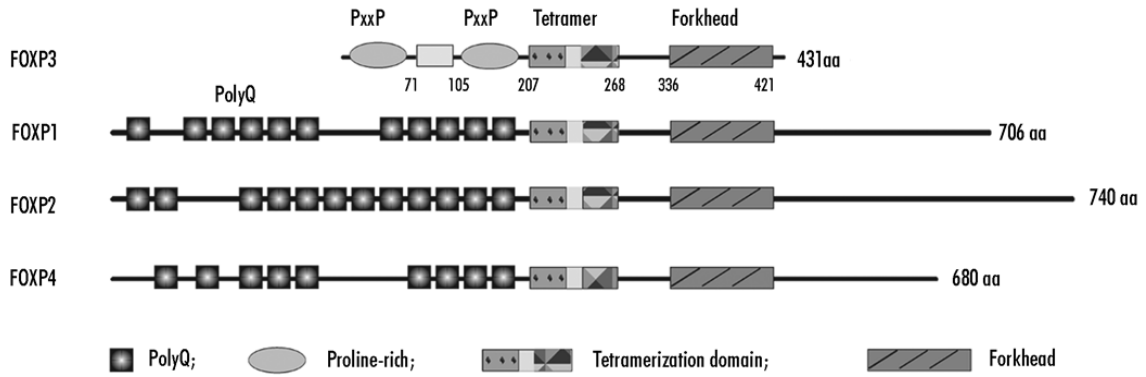
$CD4^+CD25^+$ T cells

Hallmark features

- Constitutively express CD25 & CD152; $FoxP3^+$
- $CD44^{high}$, $CD45RB^{low}$, $CD62L^{low(high)}$, $GITR^+$, $CD127^{low(high)}$, $FR4^+$
- Anergic *in vitro*; broken by IL-2
- Suppress proliferation & IL-2 secretion of $CD4^+CD25^-$ T cells *in vitro*: contact-dependent or cytokine-dependent mechanisms

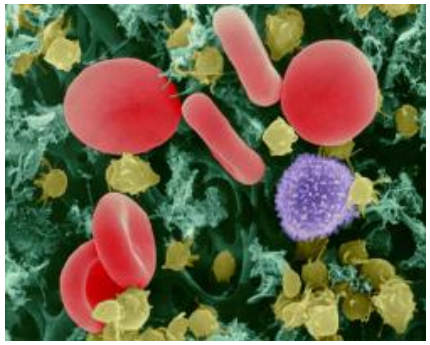
$CD4^+CD25^+$ T cells

FoxP3: introduction



- Member of family of transcription factors
- Distinct functional domains
- Murine FoxP3 is faithful marker of nTregs
- Human FoxP3 expressed by conventional T cells with activation – caution!
- Role of TGF-β!

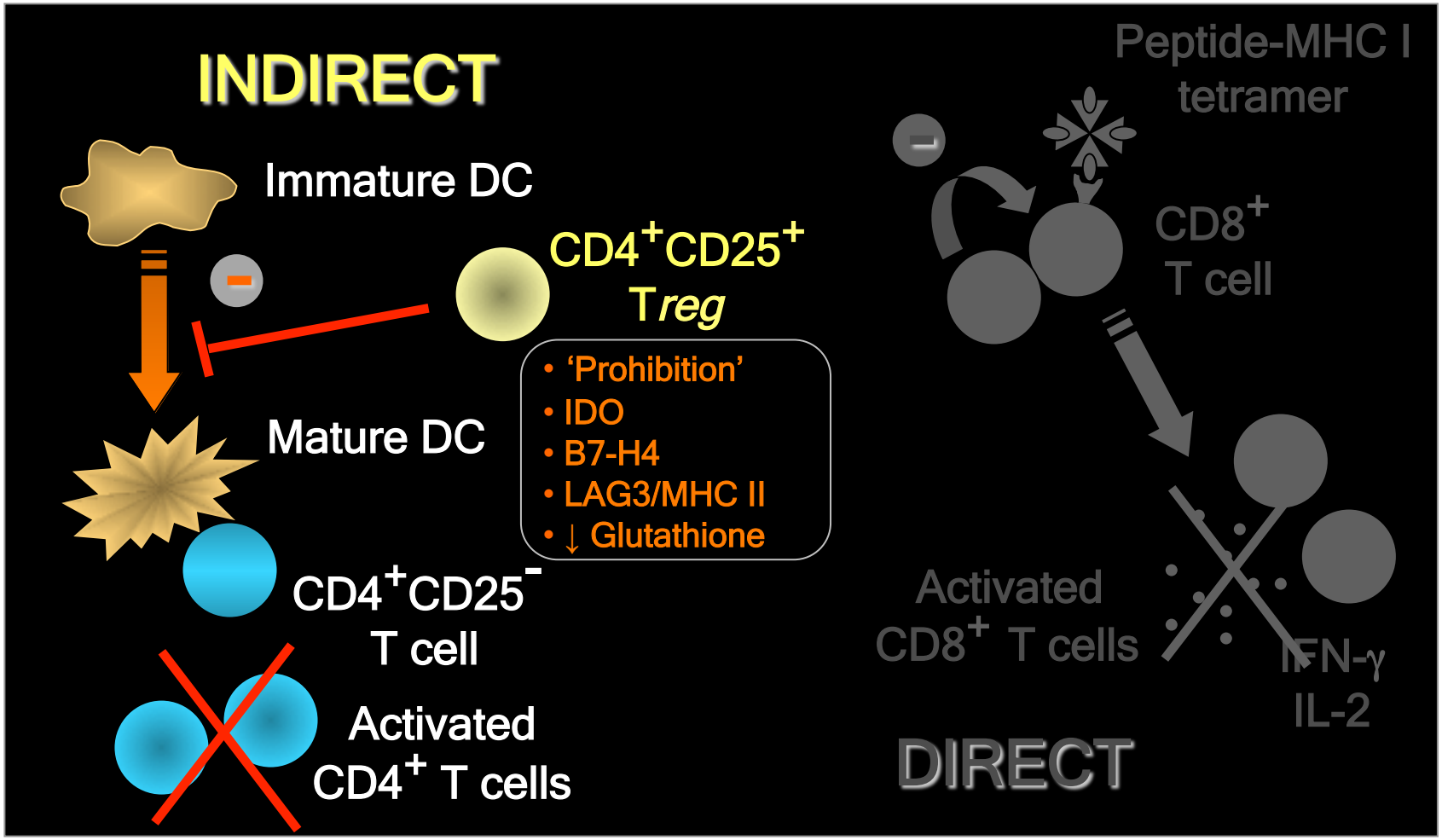
Critical thinking



- What are the key elements of an effective T cell immune response?
  - cells, molecules
- How might Tregs stop or inhibit this immune response?

$CD4^+CD25^+$ T cells

Target of suppressive action



$CD4^+CD25^+$ T cells

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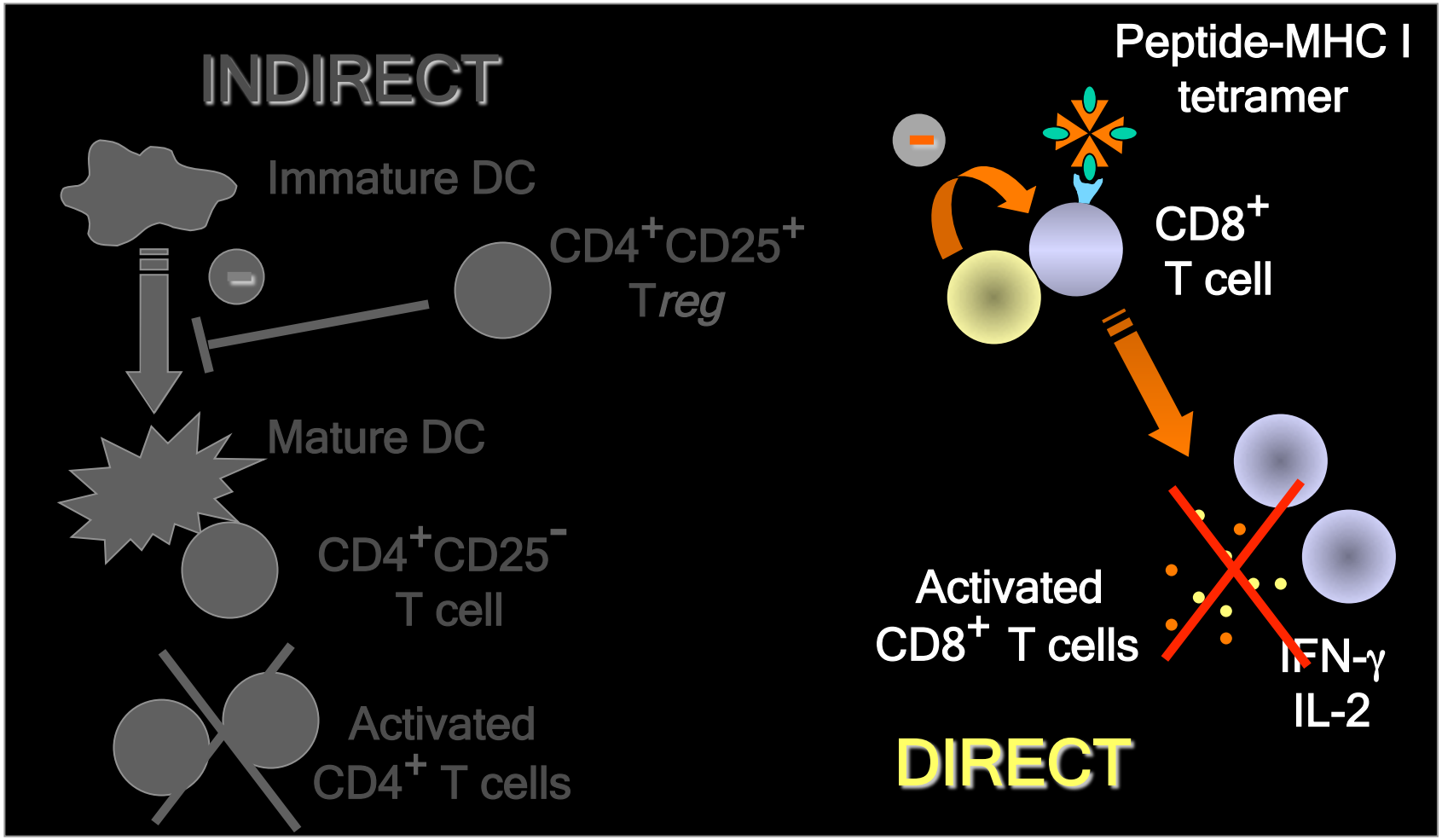

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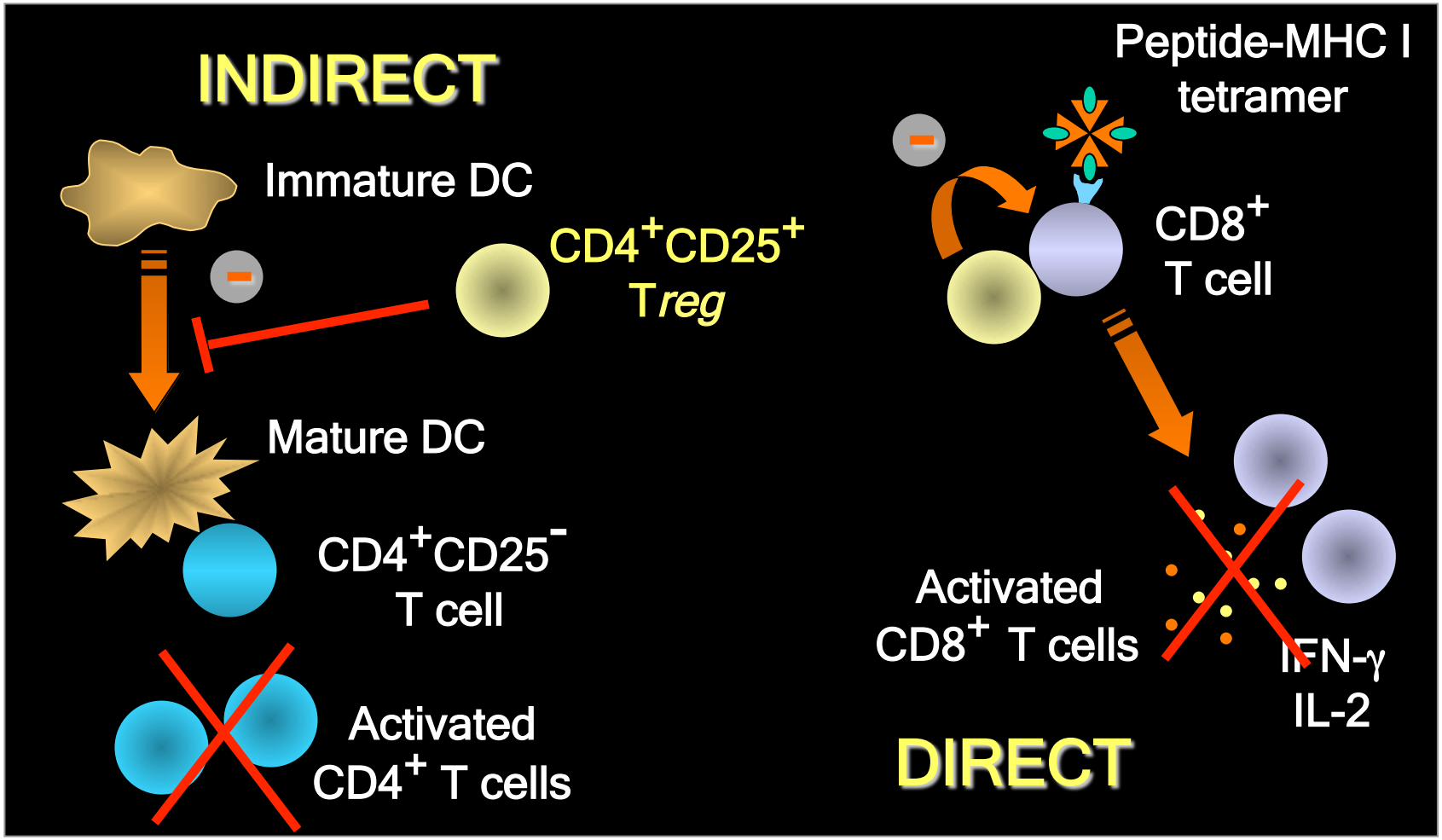
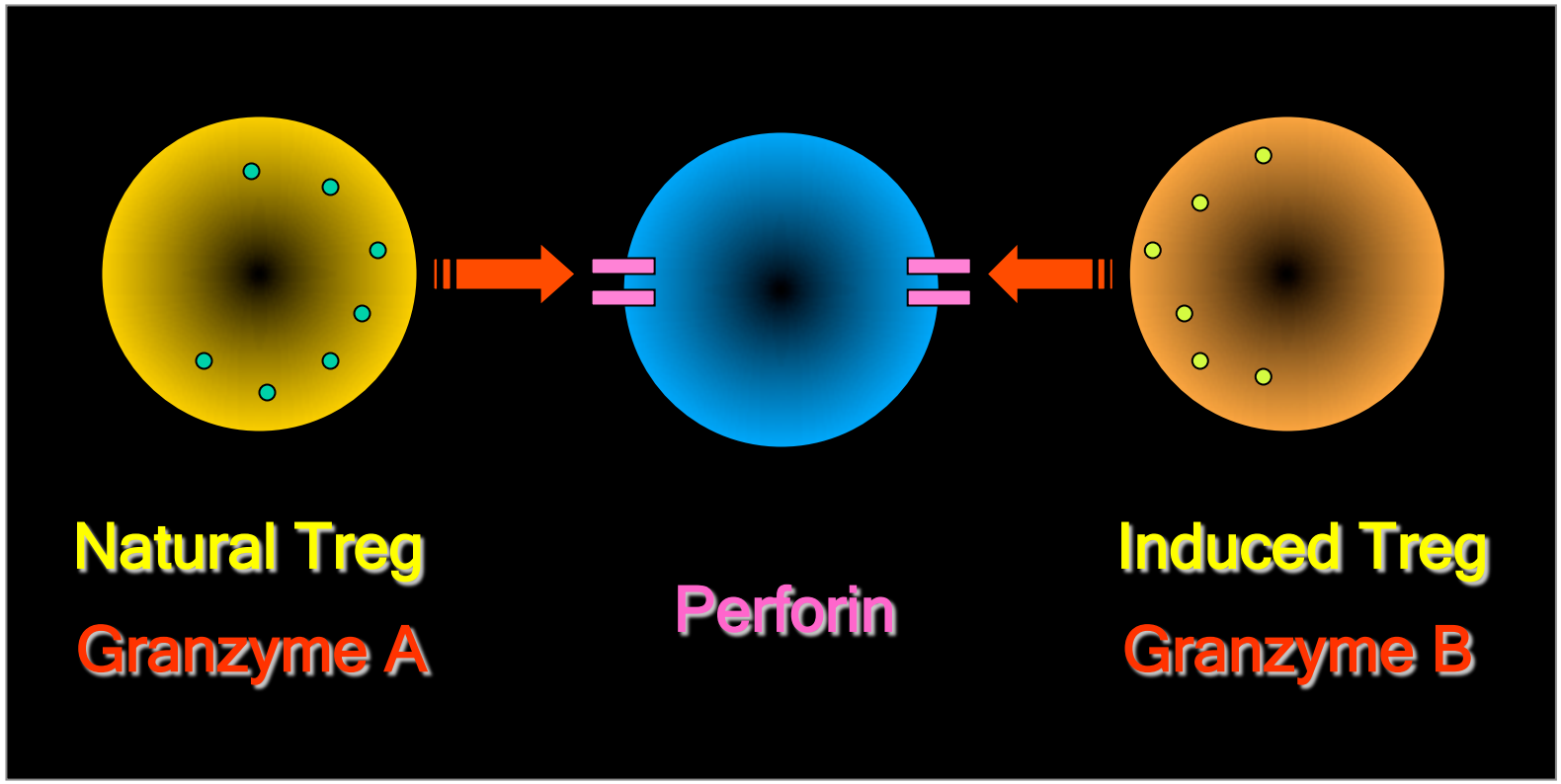


$CD4^+CD25^+$ T cells

Mechanisms of suppression: killing?



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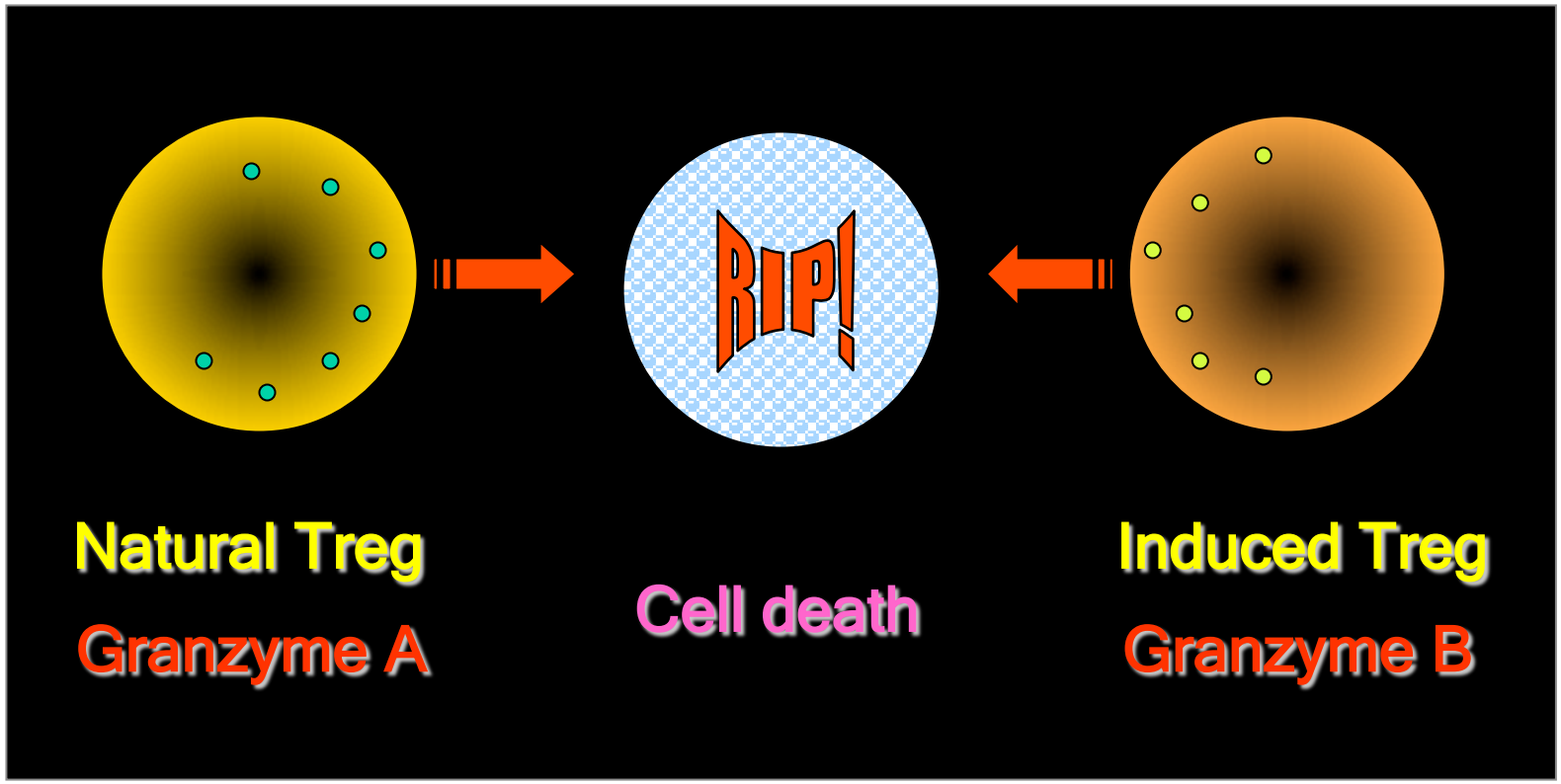


$CD4^+CD25^+$ T cells

Mechanisms of suppression: summary

Contact dependent – molecules?

CD152 / B7 interactions

LFA-1 / ICAM-1 interactions

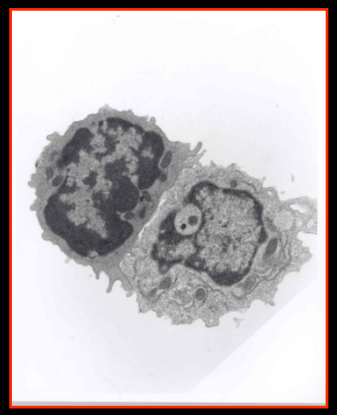
LAG-3 / MHC II

Perforin / granzyme

Fas (CD95) – FasL (CD178)

Galectins-1, 3, 9 & 10

Cytokine deprivation



Cytokines / IL-10, TGF-β, IL-35

B7-H4 / IL-10 from APC

IDO / Tryptophan catabolism

CD39 / CD73

COX-2 / PGE_2

cAMP / gap junctions

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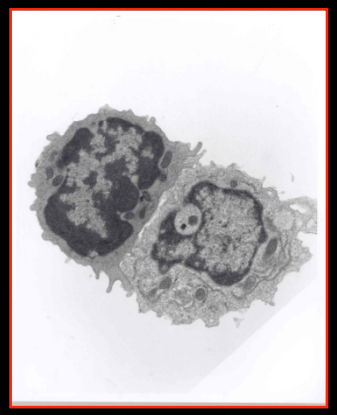

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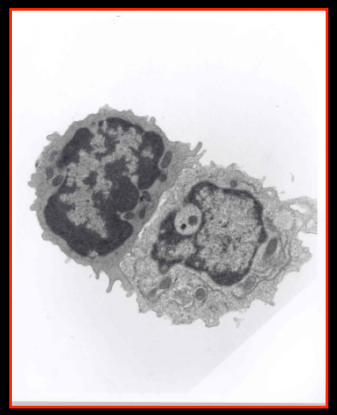

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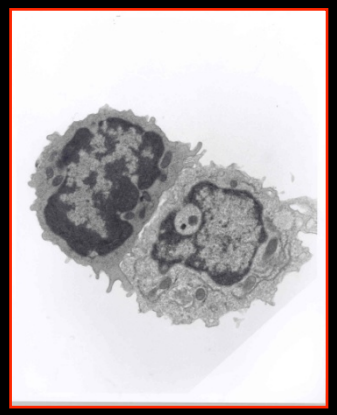

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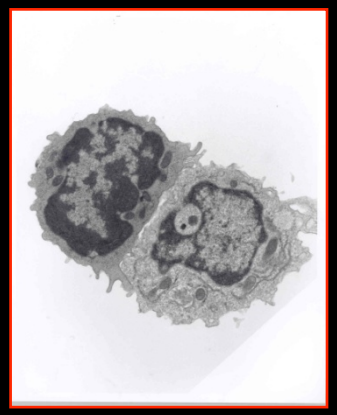

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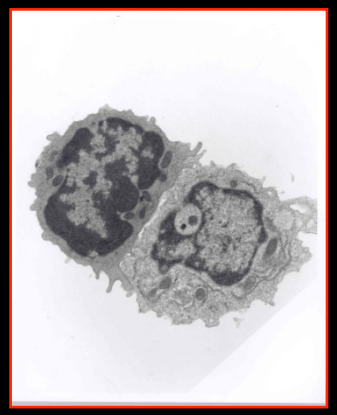

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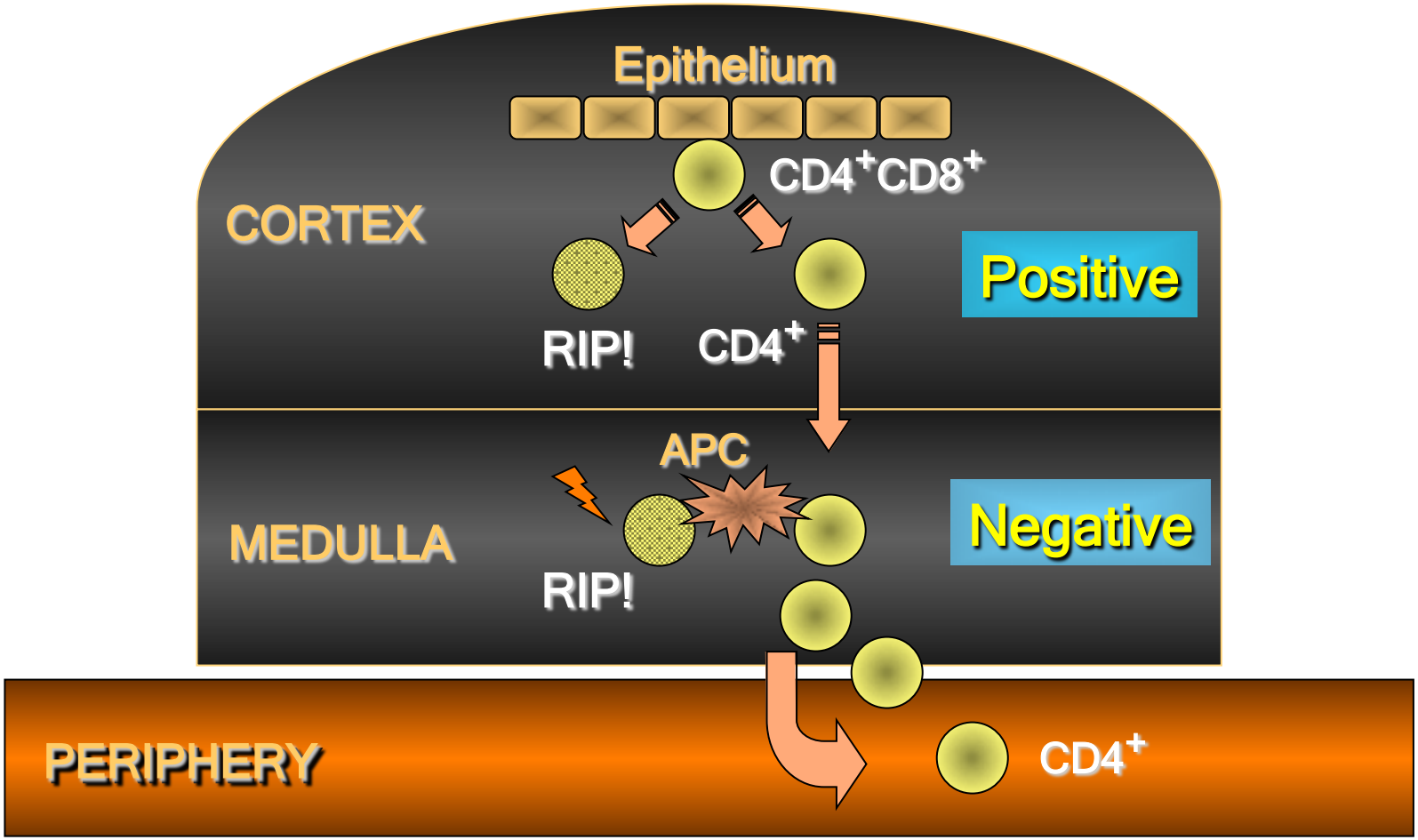
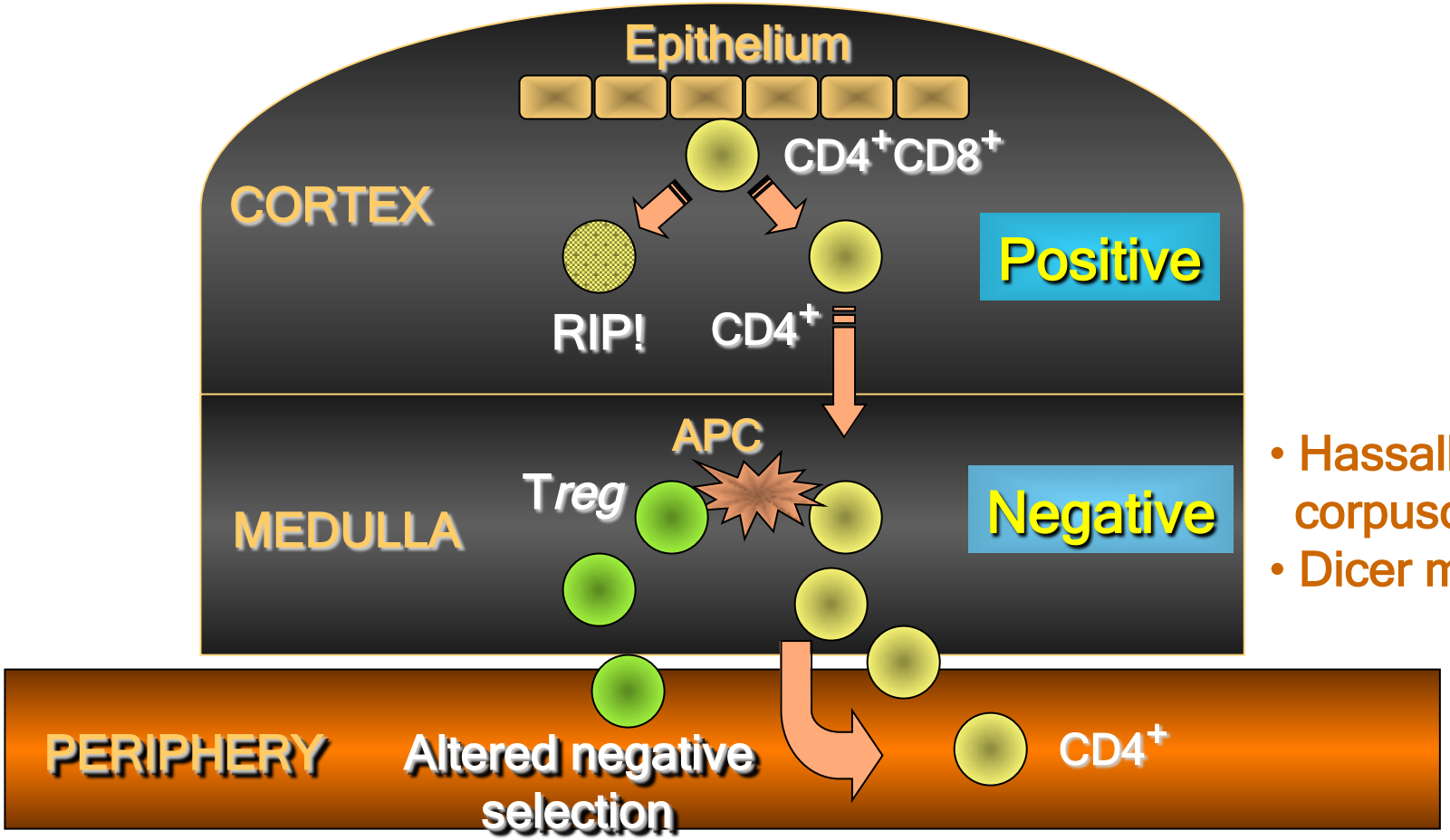
$CD4^+CD25^+$ T cells

Thymic development



$CD4^+CD25^+$ T cells

Model #1: Itoh *et al* 1999



- Hassall's corpuscles
- Dicer miRNAs

$CD4^+CD25^+$ T cells

Function

- Regulate $CD4^+$, $CD8^+$, NK cells, monocytes / macrophages, neutrophils and mast cells, ↓ autoaggressive responses.
- Control size of peripheral pools of naïve $CD4^+$ and memory $CD8^+$ T cells (homeostasis)
- Unique subset of highly potent $CD4^+CD25^+$ T cells express $\alpha_E\beta7$: epithelial cross-talk?

Cytokines

Mechanisms of suppression: IL-10?

- Function of $CD4^+CD25^+$ T cells appears to be independent of cytokines *in vitro*
- Not so *in vivo*..Th1-mediated colitis in *SCID* mice induced by adoptive transfer of $CD4^+CD45RB^{high}$ T cells can be prevented by co-transfer of $CD45RB^{low}$ T cells from WT – but not *Il-10*$^{-/-}$ – mice

Cytokines

Mechanisms of suppression: TGF-β?

- Possible role for TGF-β? Controversial!
- For: activated $CD4^+CD25^+$ T*regs* show expression of TGF-$\beta 1^{high}$, and abrogation of suppression with anti–TGF-β mAb
- Against: responder T cells from *Smad3*$^{-/-}$ mice are susceptible to $CD25^+$-mediated suppression; $CD25^+$ Tregs from *TGF-β1*$^{-/-}$ mice <u>are</u> suppressive

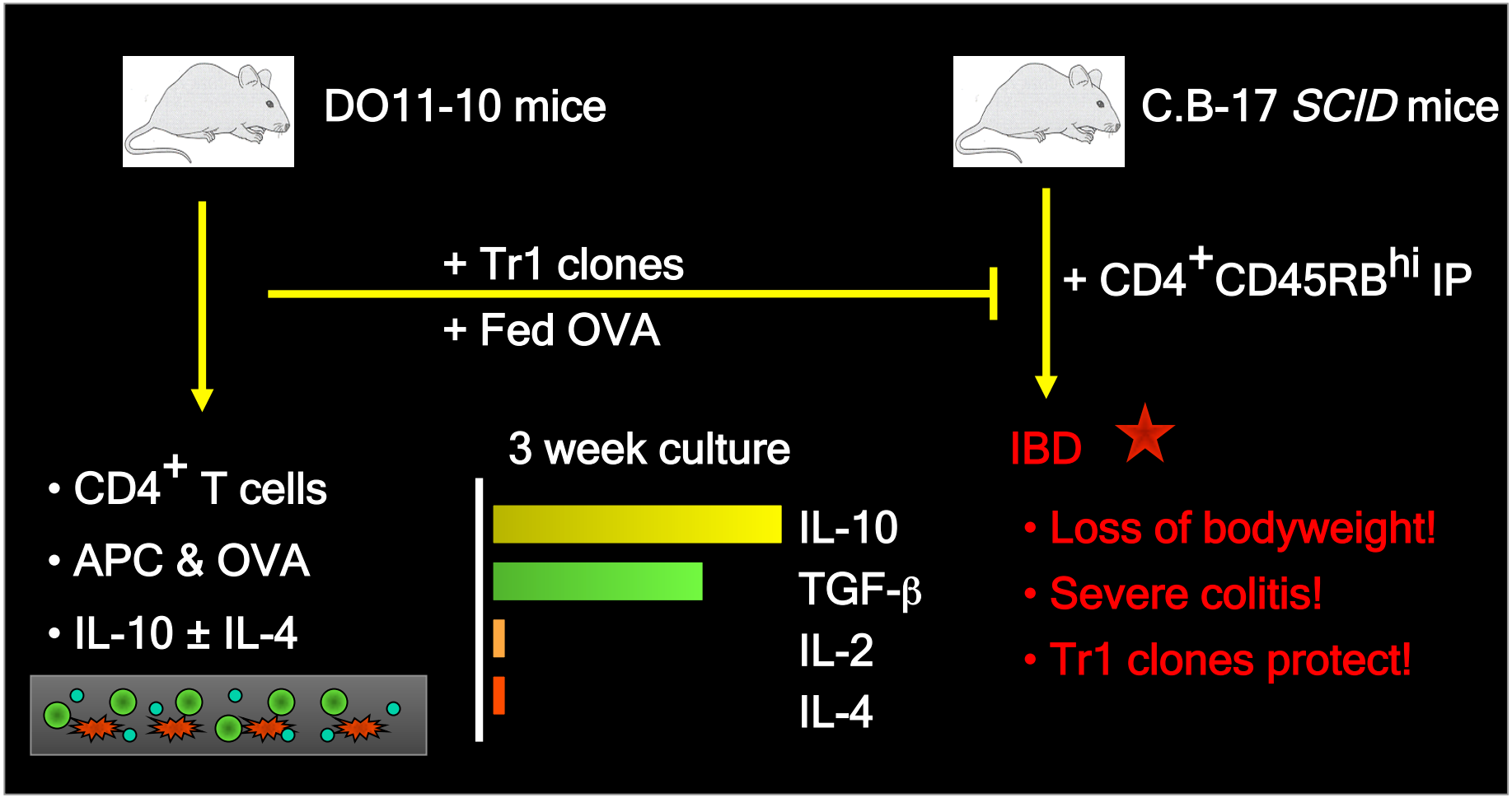
Treg mechanisms

CD152 (CTLA-4): involvement in suppression?

- *In vitro*:
  - $CD4^+CD25^+$ Tregs suppress murine $CD152^{-/-}$ $CD25^-$ T cells, abolished by anti-CD152 mAb
  - Human $CD4^+CD25^+CD152^+$ Tregs are more suppressive than $CD4^+CD25^+CD152^-$ T cells
- *In vivo:*
  - Anti–CD152 mAb abolishes protective effect of Tregs in murine colitis model

Tr1 cells (T regulatory type 1 cells)

Introduction: Groux *et al* 1997



Tr1 cells

General features

- Tr1 cells specific for a variety of antigens arise *in vivo*; may also differentiate from naïve $CD4^+$ T cells in presence of IL-10 *in vitro*
- Minimal proliferation: autocrine suppression
- Require activation for regulatory function
- Regulate both Th1 and Th2 responses

Tr1 cells

All good?

- Tr1 cell-dependent tolerance blunts the pathogenic effects of epitope spreading
- Pathogen-specific Tr1 cells are induced in the murine respiratory tract by *Bordetella pertussis*, which stimulates IL-10 production by dendritic cells
- IL-10 expressed at early tumour sites induces generation of Tregs ('Th3/Tr1-like') and subsequent collapse of antitumour immunity

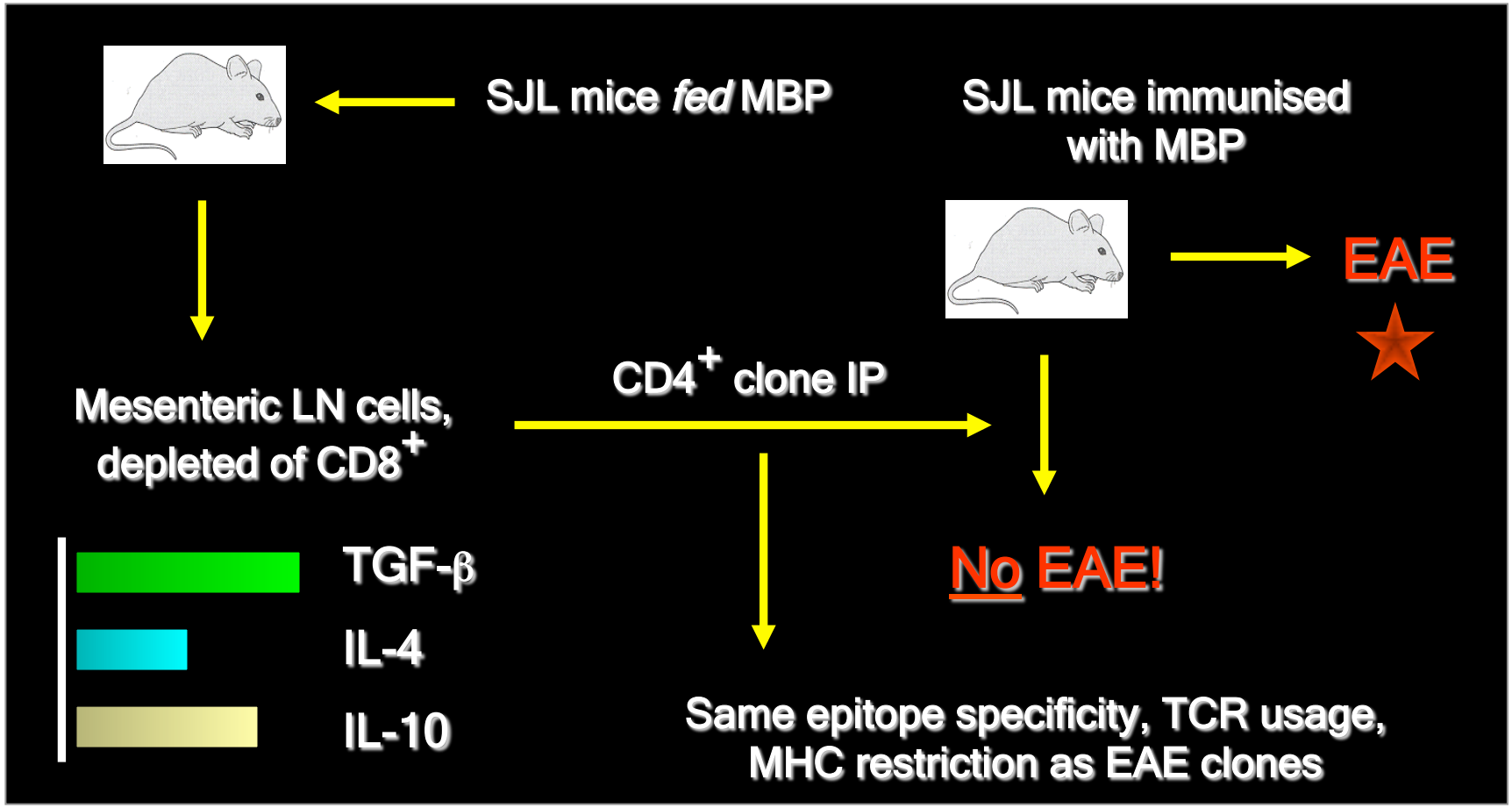
Th3 cells

Introduction: GALT

- Th3 cells are central to mucosal immuno-regulation in GALT
- Richman *et al* (1978) first demonstrated the induction of Ts cells by oral antigens
- Many studies have characterised the cells responsible for GALT immunoregulation, including Th3 subset of $CD4^+$ Tregs

Th3 cells

Characterisation in mice: Chen *et al* 1994



Th3 cells

General features

- Th3 cells provide help for IgA and suppress both Th1 and Th2 responses
- Differentiation from Th0 cells *in vitro* occurs in presence of TGF-β, IL-4, IL-10, anti-IL-12
- Th3 cells mediate 'bystander suppression'

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- T*regs*: therapeutic implications
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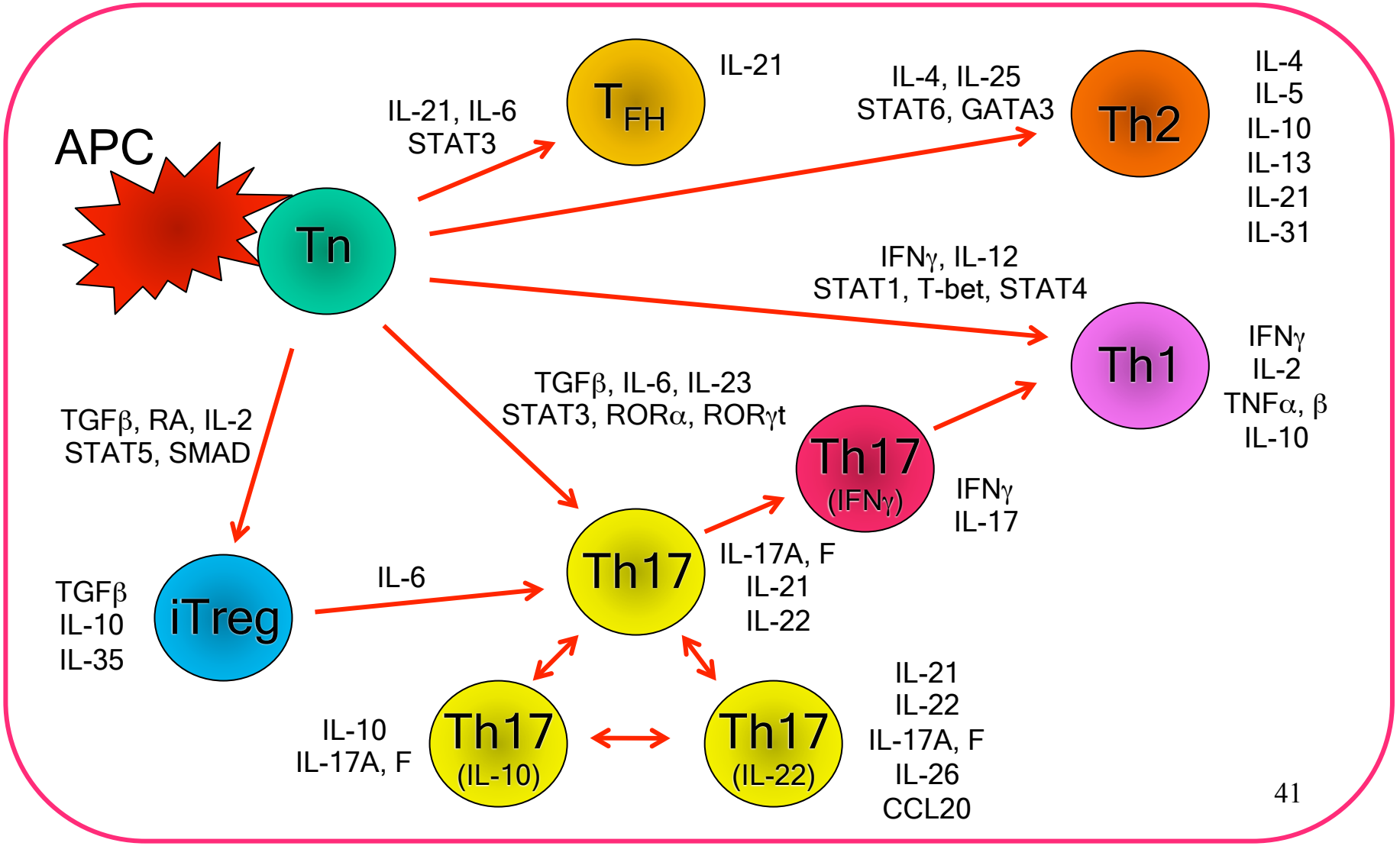
Regulatory T cell stability

- Phenotypic stability (plasticity) of Tregs *in vivo* controversial
- Reports of conversion to Th subsets in pro-inflammatory microenvironments
- Hybrid phenotypes:
  - Treg/Th1, Treg/Th17
- 'Ex-Tregs' may play role in anti-pathogen, anti-cancer defence: innate-like function?

CD4+ T cell diversity / plasticity



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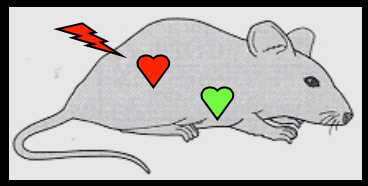
Tregs: therapeutic implications

- Autoimmune disease
  - increased number or state of activation of Tregs to restore tolerance?
- Allograft tolerance
  - adoptive transfer or increased induction of Tregs to promote graft survival?
- Anti-tumour responses
  - decreased number or activation in context of cancer ?

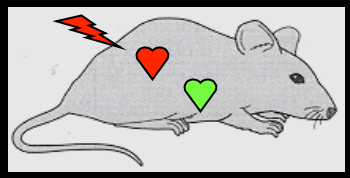
Induction of tolerance by adoptively transferred Treg cells

(1) Heart allografts



& CysA

Tolerant donor



Naive recipient

$CD4^+CD25^+$ Treg

$CD4^+$ T cells

No rejection!

(2) d3Tx

Organ-specific autoimmune dz.
eg. oophoritis, orchitis, thyroiditis

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Summary and conclusion

- Complex network of interacting Tregs plays crucial role in peripheral tolerance
- Tregs mediate suppressive effects by contact- & cytokine-dependent mechanisms
- Treg cellular immunotherapy has potential to revolutionise medicine

Reading list

- Garden, OA, Pinheiro, D and Cunningham, F (2010) All creatures great and small: regulatory T cells in mice, humans, dogs and other domestic animal species. *Int Immunopharmacol* doi:10.1016/j.intimp.2010.11.003
- Feuerer, M *et al* (2009) Foxp3^{+} regulatory T cells: differentiation, specification, subphenotypes. *Nature Immunol* **10**: 689-695
- Josefowicz, SZ and Rudensky, A (2009) Control of regulatory T cell lineage commitment and maintenance. *Immunity* **30**: 616-625
- Curotto de Lafaille, MA and Lafaille, JJ (2009) Natural and adaptive Foxp3^{+} regulatory T cells: more of the same or a division of labor? *Immunity* **30**: 626-635
- Sakaguchi, S *et al* (2010) FOXP3^{+} regulatory T cells in the human immune system. *Nat Rev Immunol* **10**: 490-500
- Buckner, JH (2010) Mechanisms of impaired regulation by CD4^{+}CD25^{+}FOXP3^{+} regulatory T cells in human autoimmune diseases. *Nat Rev Immunol* **10**: 849-859

Additional slides

– These slides are included here for your reference –

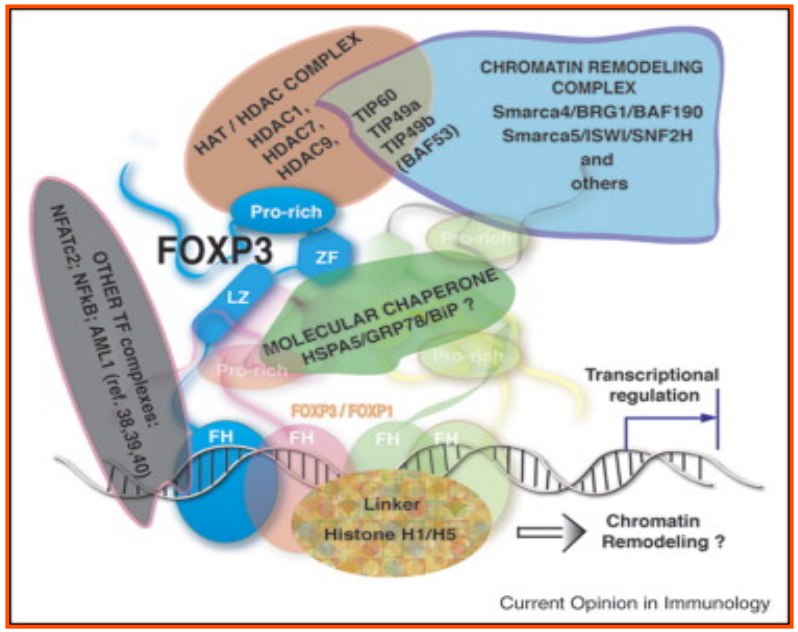
$CD4^+CD25^+$ T cells

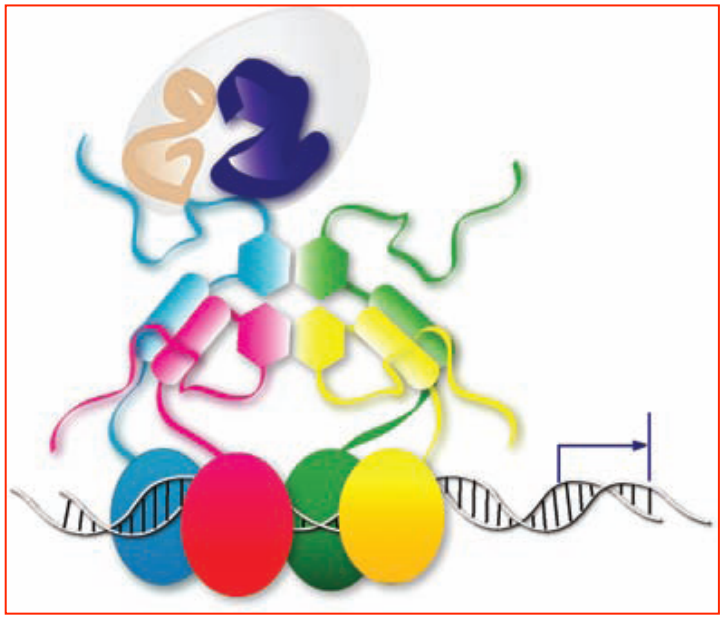
FoxP3: functional domains

- C terminal forkhead domain of FoxP3 interacts with NFAT, NF-κB and CREB co-activator p300
- Sequestration away from DNA? → Passive repression
- N-terminal proline-rich repressor domain recruits histone acetyltransferase / histone deacetylase (HAT/HDAC) complex → Active repression
- Other associations
  - Runx 1 (AML1): binds between FKH and leucine zipper domains (repression: eg *il2, ifng*; activation: eg *cd25, ctla4*)
  - RORγt: binds to exon 2 sequence (upstream of repressor); FoxP3 thus inhibits Th17 differentiation
  - Eos: involvement in FoxP3-dependent gene silencing (eg *il2*)

$CD4^+CD25^+$ T cells

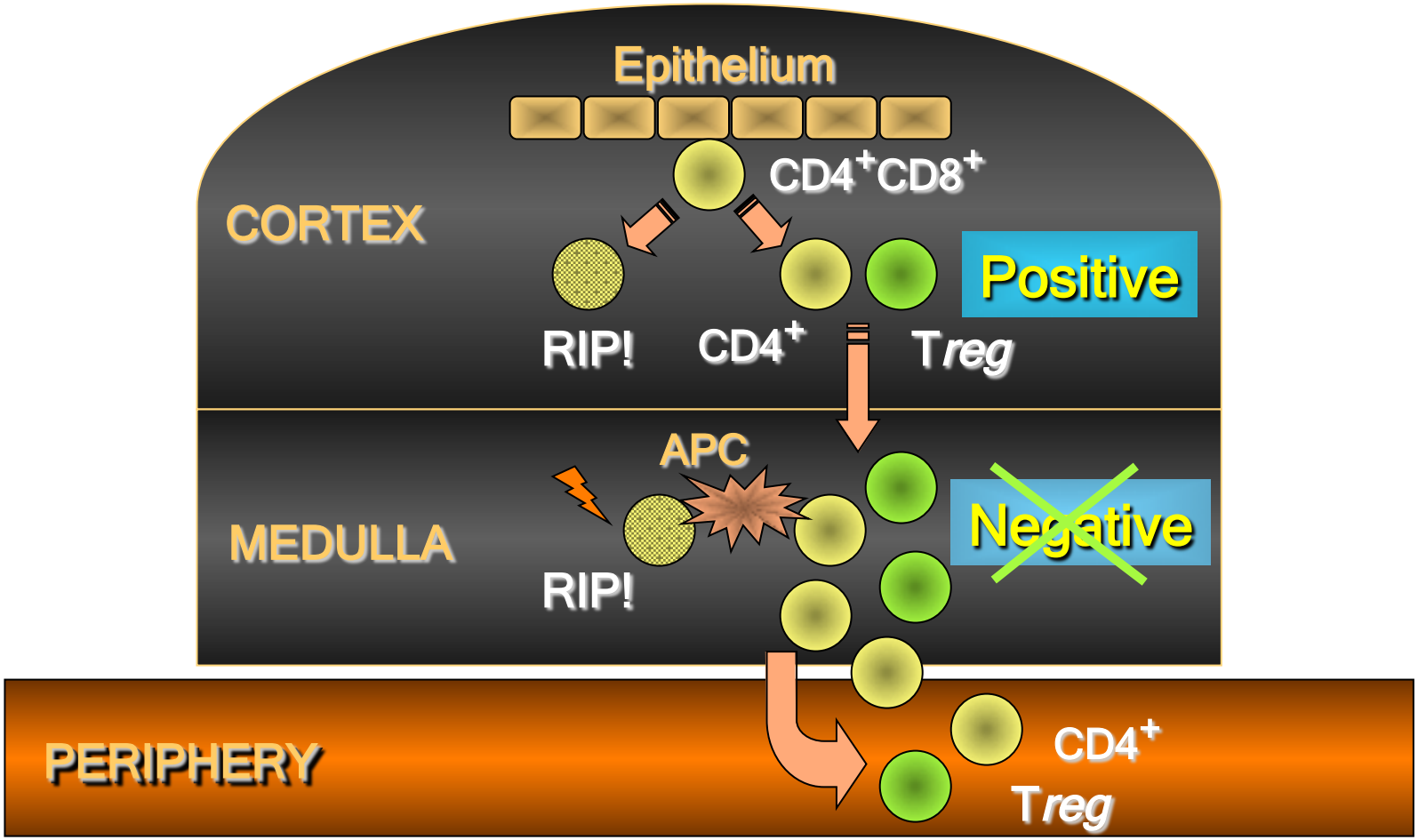
FoxP3 complex ensemble





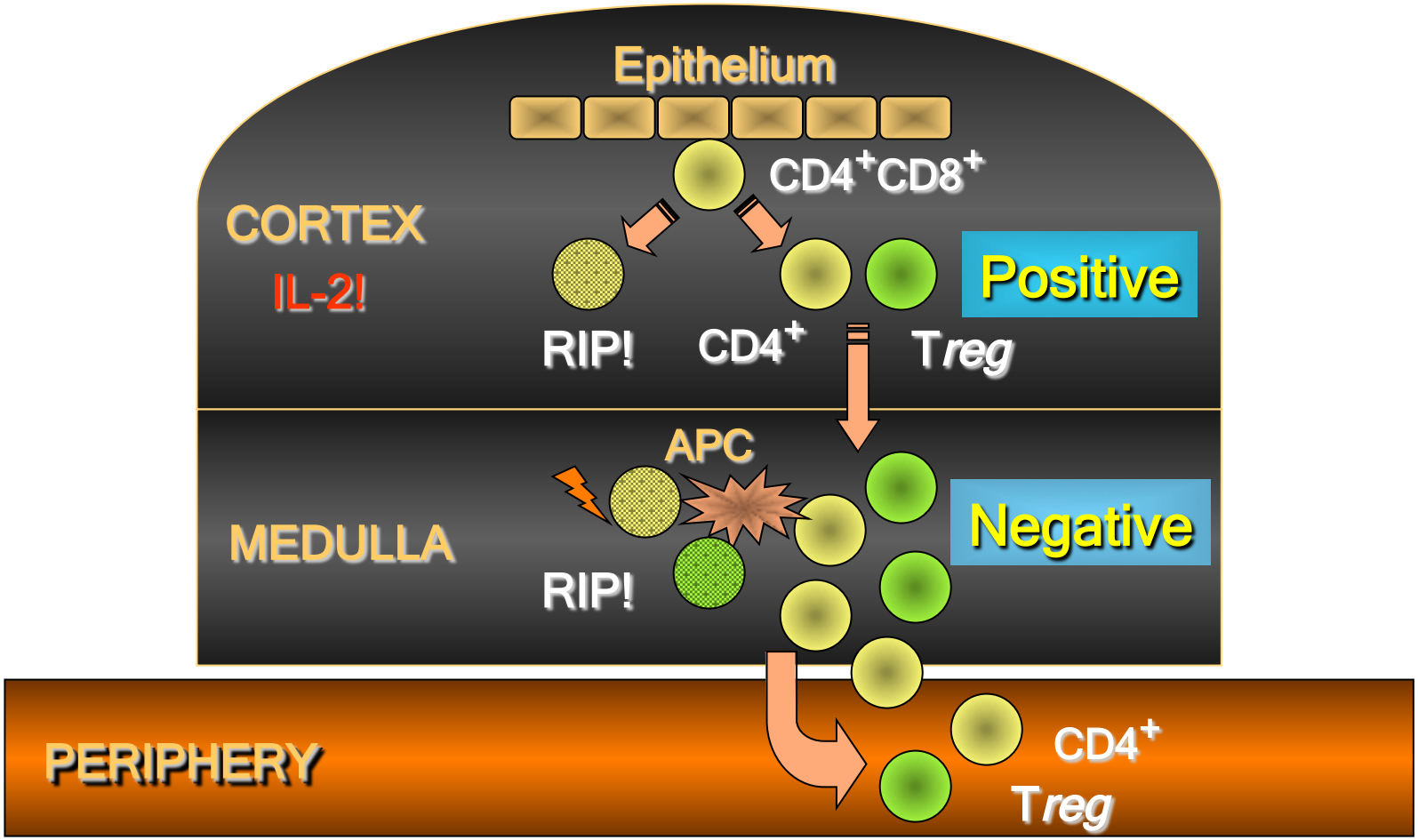
$CD4^+CD25^+$ T cells

Model #2: Jordan *et al* 2001



$CD4^+CD25^+$ T cells

Model #3: Bensinger *et al* 2001



Home study slides

– To work through in your own time –

$CD4^+CD25^-$ T cells

- Recent years have seen an increased awareness of the regulatory potential of $CD4^+CD25^-$ T cells
- Peripheral $CD25^-$ Tregs are able to prevent autoimmune diabetes mellitus induced in rats by thymectomy and gamma irradiation
- Inflammatory bowel disease (IBD) induced in *RAG-2*$^{-/-}$ mice by the administration of $CD45RB^{high}$ T cells can be prevented by the co-injection of $CD45RB^{low}CD25^-$ T cells

$CD4^+CD25^-$ T cells

- $CD25^-$ Tregs are able to mediate dominant transplantation tolerance
- The $\alpha_E\beta_7^+$ subset of $CD25^-$ Tregs expresses CD152, suppresses T cell proliferation *in vitro*, and protects mice from colitis

$CD8^+CD28^-$ T cells

- $CD8^+$ Tregs may play a role in gastro-intestinal immunoregulation
- Efforts to generate suppressor cell lines *in vitro* have yielded a population of $CD8^+CD28^-$ T cells restricted by allogeneic HLA class I antigens, able to prevent up-regulation of B7 molecules induced by CD40 ligation of donor APCs

$CD8^+CD28^-$ T cells

- The presence of $CD8^+CD28^-$ Tregs is inversely correlated with T cell alloreactivity to donor MHC peptides, alloantibody production and rejection
- Human $CD8^+CD28^-$ Tregs can be induced by immature DCs

$CD8^+CD28^-$ T cells

- $CD8^+CD28^-$ alloantigen-specific Tregs induce up-regulation of immunoglobulin-like transcript 3 (ILT3) and ILT4 on monocytes and DCs, rendering these cells tolerogenic: tolerogenic APCs show reduced expression of costimulatory molecules and induce antigen-specific unresponsiveness in $CD4^+$ Th cells

γδ T cells

- Diverse effector functions have been attributed to these cells, yet their role *in vivo* remains largely unclear
- A population of regulatory γδ T cells with a cytokine profile similar to Tr1 cells has been isolated from tumour-infiltrating lymphocytes

γδ T cells

- The γδ T cell may play a role in oral tolerance
- In the NOD mouse, intranasal inhalation of proinsulin leads to generation of a population of $CD8^+$ γδ Tregs that can suppress the development of diabetes mellitus

DN T cells

- A role for $CD3^{+}CD4^{-}CD8^{-}$ Tregs in preventing allograft rejection has been demonstrated
- Infusion of DN Tregs generated *in vitro* can significantly enhance survival of single class I MHC locus–mismatched, donor–specific cutaneous allografts

DN T cells

- Three interactions between DN Tregs and allogeneic $CD8^+$ T cells may lead to suppression: (i) TCR of DN Tregs recognising allo-MHC expressed on $CD8^+$ target cells; (ii) TCR of $CD8^+$ T cells recognising allo-MHC expressed on DN Tregs; and (iii) acquisition of allo-MHC by DN T cells *via* their TCR, with presentation of the allo-MHC on their surface to syngeneic $CD8^+$ T cells
- All of these interactions lead to $CD8^+$ cell death *via* a Fas / Fas-L signal

NKT cells

- These are NK1.1^{+} lymphoid cells, whose morphology and function are intermediate between T and NK cells
- NKT cells produce IL-4, may be CD4^{-}CD8^{-} or CD4^{+}CD8^{-}, and express low levels of $\alpha\beta$ TCR with an invariant α chain and restricted β chain specificity
- Many of these TCRs recognise antigens presented by the non-classical MHC-like molecule, CD1

NKT cells

- Upon stimulation of the TCR with CD1d, NKT cells can rapidly release IFNγ, IL-4 and IL-10
- NKT cells appear to play an important role in tolerance of both foreign and self-antigens
- Suppressive cytokine release: a mechanism for regulation?
- NKT-derived RANTES critical for generation of $CD8^+$ Tregs during induction of tolerance