Peripheral $CD4^+$ T cell differentiation

Lindsay Nicholson

L12

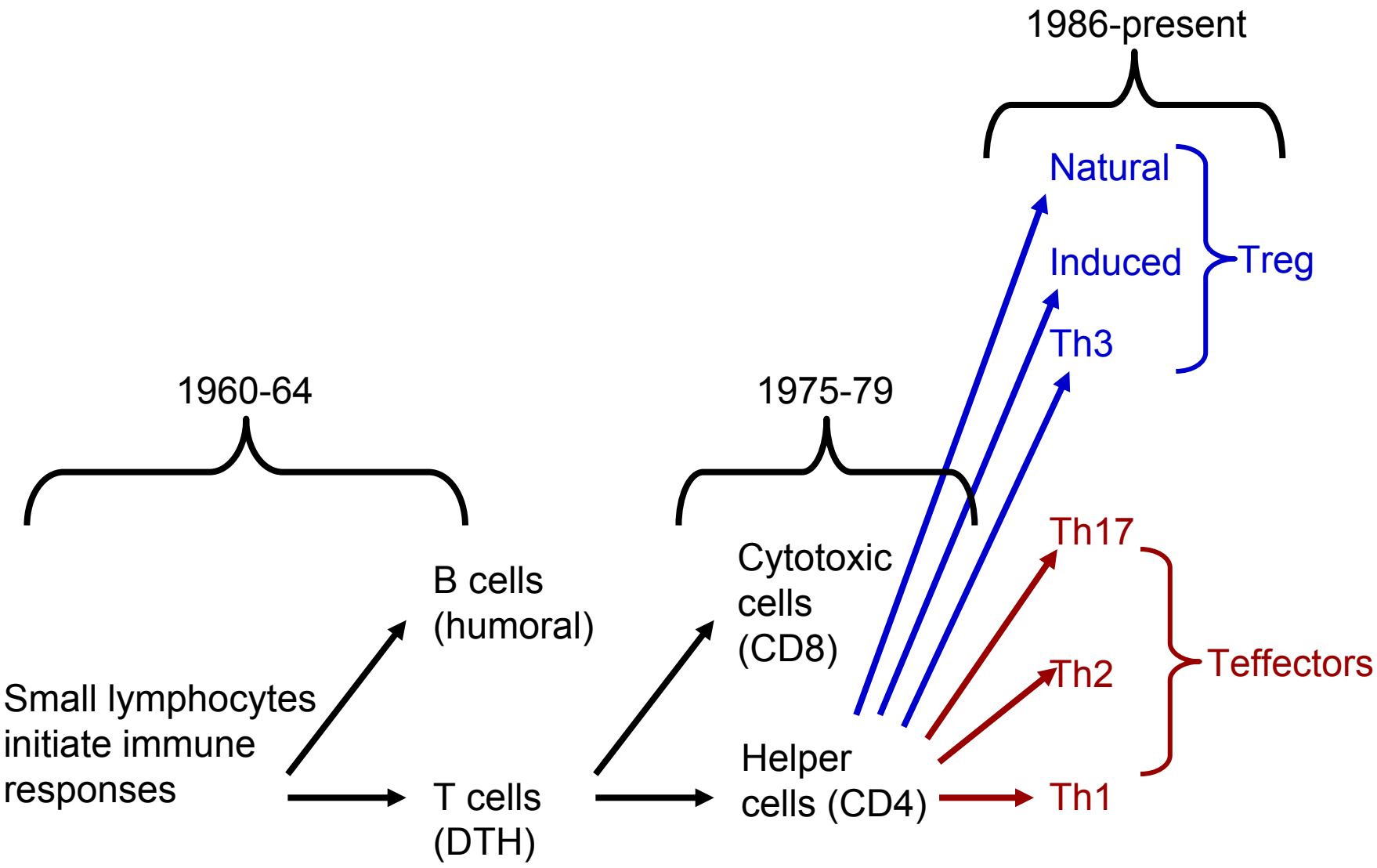
Peripheral T cell differentiation

- Key Concepts
  - Activation is accompanied by changes in phenotype and function
  - Phenotype is established by positive and negative feedback mechanisms
  - Phenotype is stabilised by heritable changes in gene expression
  - The phenotype of a response can determine the outcome of immune system dependent disease processes

The story so far …

- Adaptive immunity had evolved “T cells” that can interrogate the APC environment by scanning peptides presented by MHC molecules
- These T cells have been educated in the thymus to ignore self
- Now they’re free to wander through the secondary lymphoid tissue, looking for new peptides that they’ve never seen before
- When they find one they recognise, what happens next ?

The race to subdivision



Reciprocal relationship between humoral and delayed type hypersensitivity

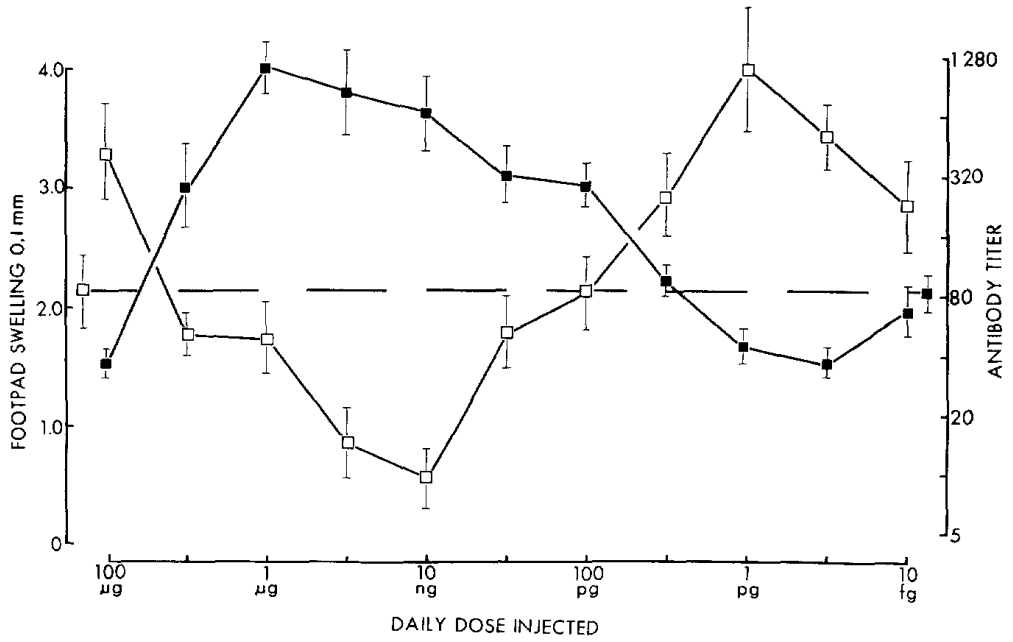


FIG. 3. Antibody titers (■—■) and delayed-type hypersensitivity responses (□—□) of strain W Wistar rats injected daily for 28 days with varying amounts of the CNBr digest of flagellin (Fig. 2) and then challenged with 100 µg of flagellin in saline. The antibody titers represent the mean of the 7, 14, 21, and 28 day postchallenge titers. Delayed-type hypersensitivity was elicited 28 days after the flagellin challenge. The dotted line (---) represents the antibody and delayed hypersensitivity responses of control rats which were injected only with 100 µg of flagellin in saline. Vertical bars represent standard errors of the means.

J. Exp. Med. Parish and Liew 135: 298. (1972)

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TWO TYPES OF MURINE HELPER T CELL CLONE

I. Definition According to Profiles of Lymphokine Activities and Secreted Proteins

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J. Immunol **136**: 2348 1986

Differing Lymphokine Profiles of Functional Subsets of Human CD4 and CD8 T Cell Clones

PADMINI SALGAME, JOHN S. ABRAMS, CAROL CLAYBERGER, HARRIS GOLDSTEIN, JACINTO CONVIT, ROBERT L. MODLIN, BARRY R. BLOOM

Functional subsets of human T cells were delineated by analyzing patterns of lymphokines produced by clones from individuals with leprosy and by T cell clones of known function. CD4 clones from individuals with strong cell-mediated immunity produced predominantly interferon-γ, whereas those clones that enhanced antibody formation produced interleukin-4. CD8 cytotoxic T cells secreted interferon-γ. Interleukin-4 was produced by CD8 T suppressor clones from immunologically unresponsive individuals with leprosy and was found to be necessary for suppression in vitro. Both the classic reciprocal relation between antibody formation and cell-mediated immunity and resistance or susceptibility to certain infections may be explained by T cell subsets differing in patterns of lymphokine production.

Science **254**: 279 1991

Questions

- Does the recognition specificity determine phenotype?
- Is phenotype reversible or fixed?
- What determines phenotype in vitro and in vivo?
- Is their a relationship between phenotype and disease?
- Does modifying phenotype work as therapy?

Questions

- Does the recognition specificity determine phenotype? No
- Is phenotype reversible or fixed? Takes time
- What determines phenotype in vitro and in vivo? Route, dose, cytokines, etc
- Is their a relationship between phenotype and disease? Yes
- Does modifying phenotype work as therapy?

Summary 1

- T cell in the circulation that have not encountered a cognate antigen are naive, and multipotent
- T cells in the circulation that have encountered antigen have a more focused response
- There is a reciprocal relationship between humoral and cell mediated immunity

Summary 1

	Recent thymic emigrant Naive T cell Precursor T cell	**Activated T cell** Lymphoblast Effector cell	**Memory cell** Central memory Effector memory
			
Circulation	Secondary lymphoid compartment	Tissues	Secondary lymphoid compartment or tissues
Cytokines	Slow responding, many potential pathways	Rapid response, limited pattern	Rapid response, limited pattern

Results of T cell activation

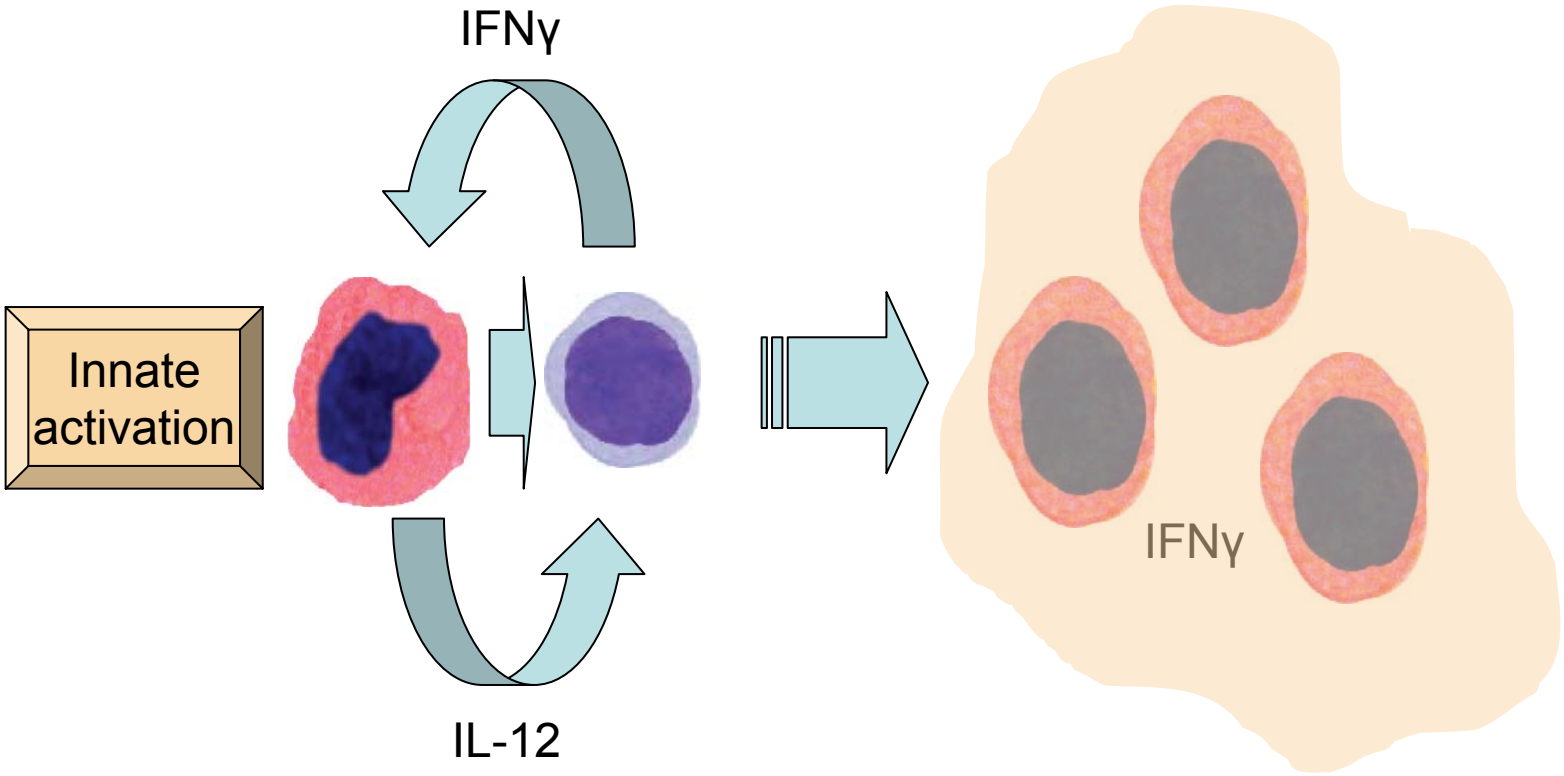
- Proliferation
- Cytokine production
- Changes in surface marker expression
- Most cells die; some become long lived

- Daughter cells maintain altered patterns of gene expression: Cells do not return to a naive state

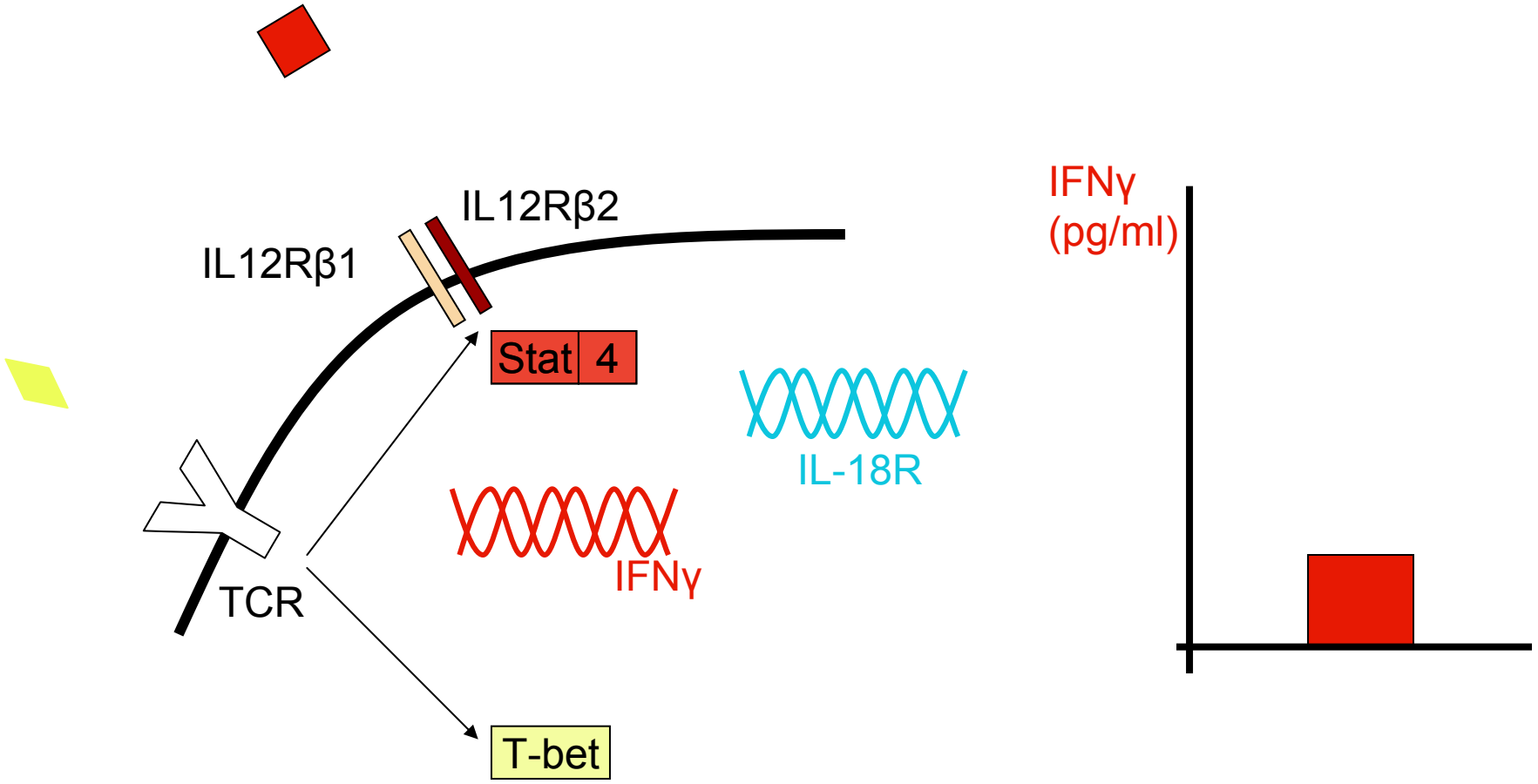
Mechanisms of that determine differentiation

- Different phenotypes cross-regulate each other
- In vitro, cytokine cocktails are sufficient
- In vivo
  - Genetics
  - Route
  - Adjuvant
  - Dose
  - Costimulation
- Signal 1 (recognition) + Signal 2 (costimulation) + Signal 3 (instruction)

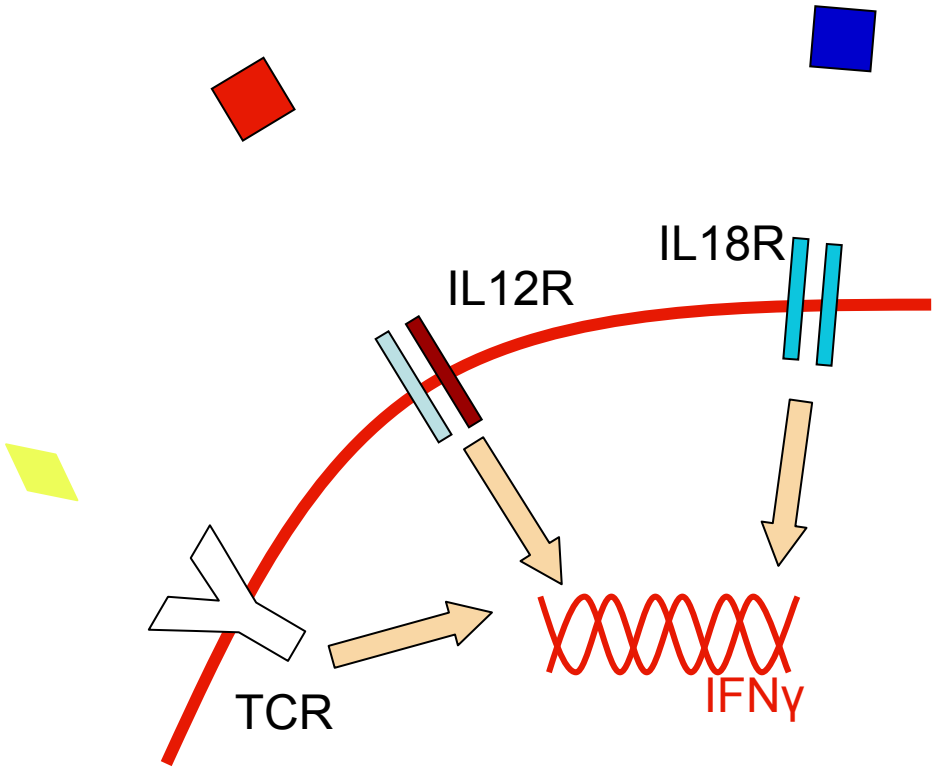
T cell differentiation relies on positive feedback

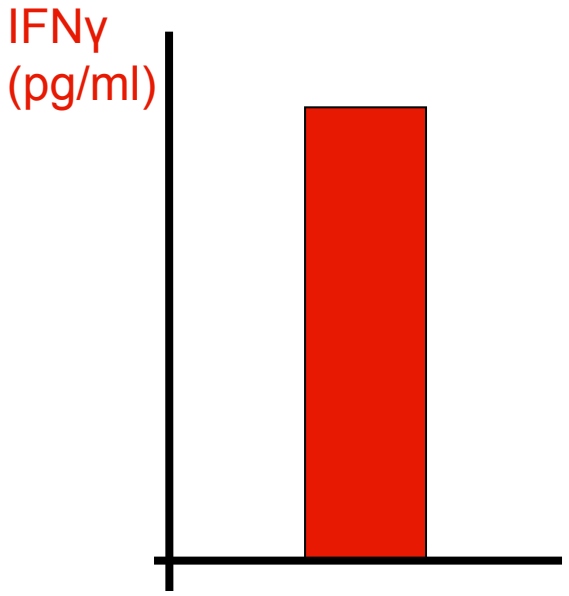


Feedback occurs at many levels



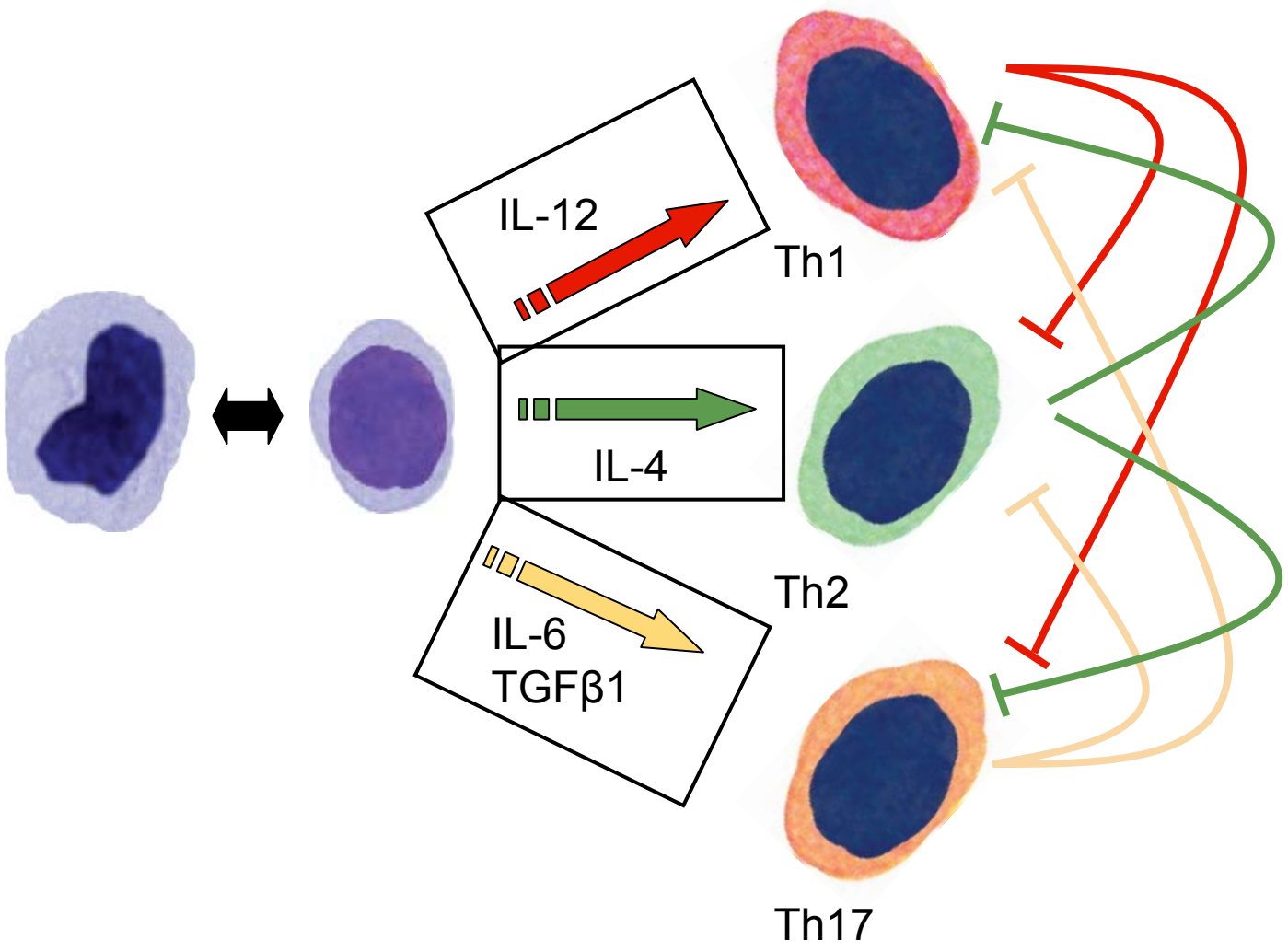
Differentiated cells produces higher levels of cytokine





Differentiated Th1 T cell

Negative feedback also plays a role



Th cell phenotype signatures

Phenotype	**Th1**	**Th2**	**Th17**
Differentiation Cytokine	IL-12 (IL-18)	IL-4	IL-6 TGFβ1 IL-23 (IL-21)
Effector cytokine	IFNγ	IL-4 IL-5, IL-13	IL-17 IL-22
Chemokine receptors	CCR5	CCR3 CRTh2	CCR4 CCR6
Transcription factors	Stat4 T-bet	Stat6 C-Maf GATA3	Stat3 RORγt

…but really it's hundreds of genes

PNAS 101: 3023–3028 (2004)
doi10.1073pnas.0307743100
J. Immunol 178: 3648-3660 (2007)

About 300 genes in the first few days.

Markers of cell phenotype

- The cytokines they produce
- Isotype of antigen specific antibodies
- Chemokine receptors
- Other molecules: Tim-3, CD226, ST2L
- Artificially introduced labels

- Expression may be activation dependent
- Different isoforms may have different expression patterns
- They may define a mixed population:
  e.g. naive and Th1

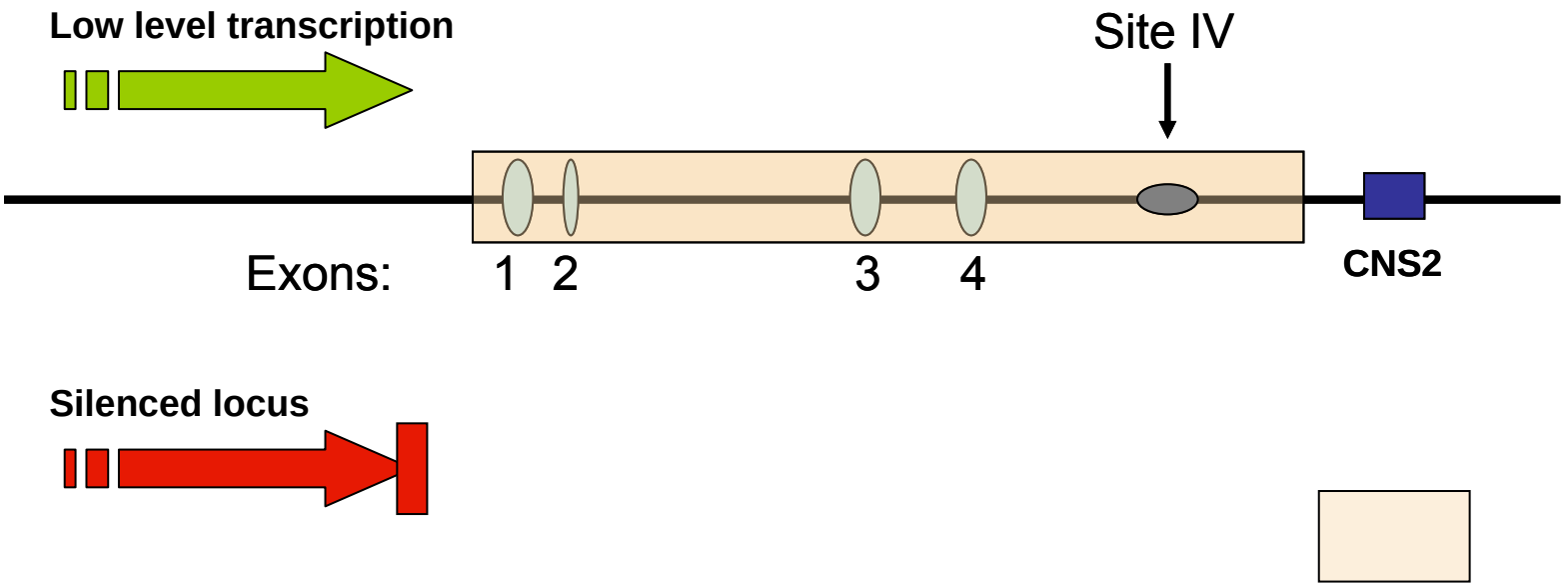
Summary 2

- In vitro cytokines that drive differentiation initiate signalling cascades that are self-reinforcing
- Reciprocally, these inhibit other pathways of differentiation
- Through specific transcription factors, the expression of several hundred genes is affected

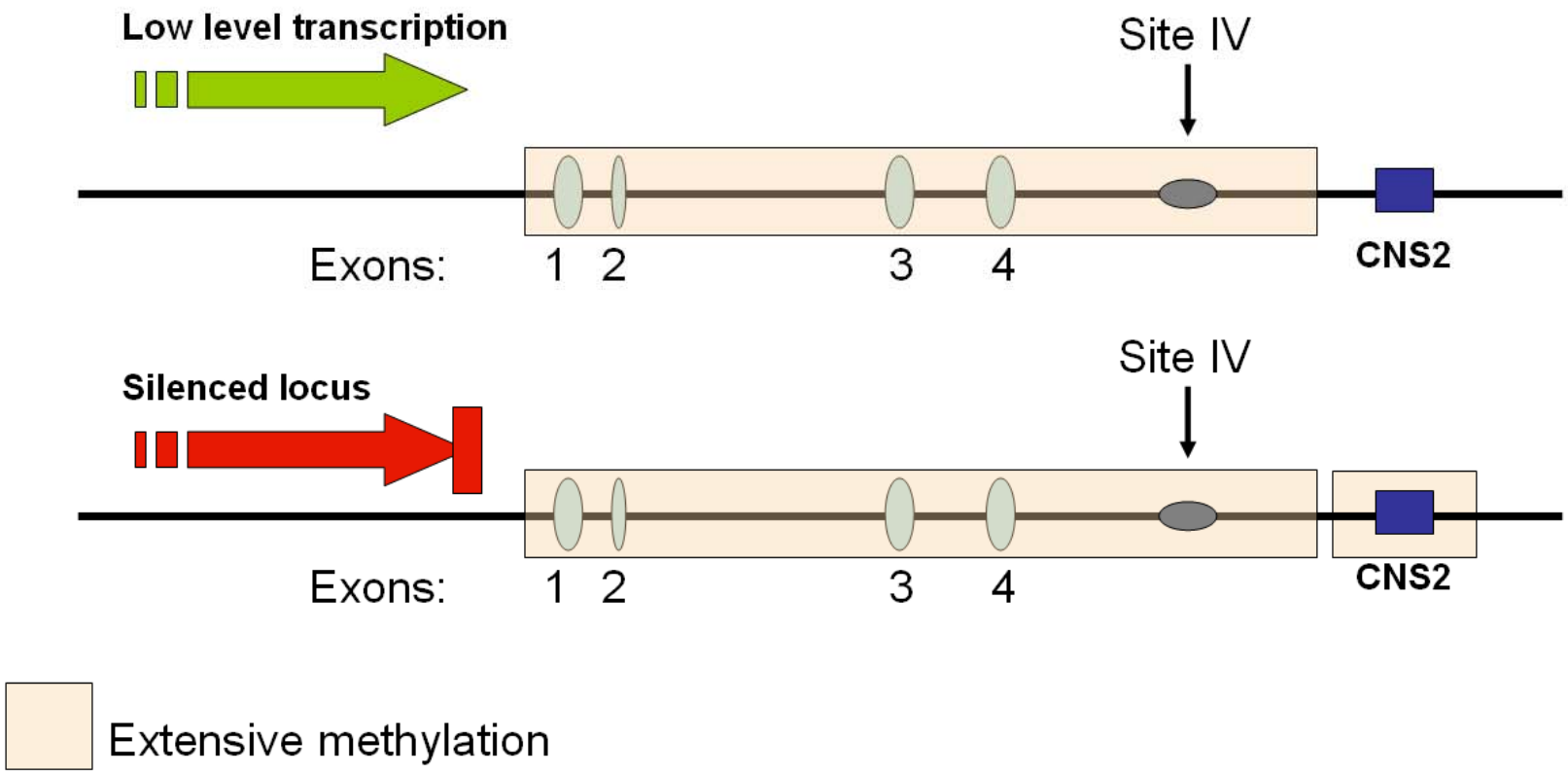
Heritability

- Activated T cells divide 3-4 times per day
- In 4 days one cell could give rise to between 4,000 and 65,000 daughter cells
- How do the daughter cells know what phenotype their mother was?

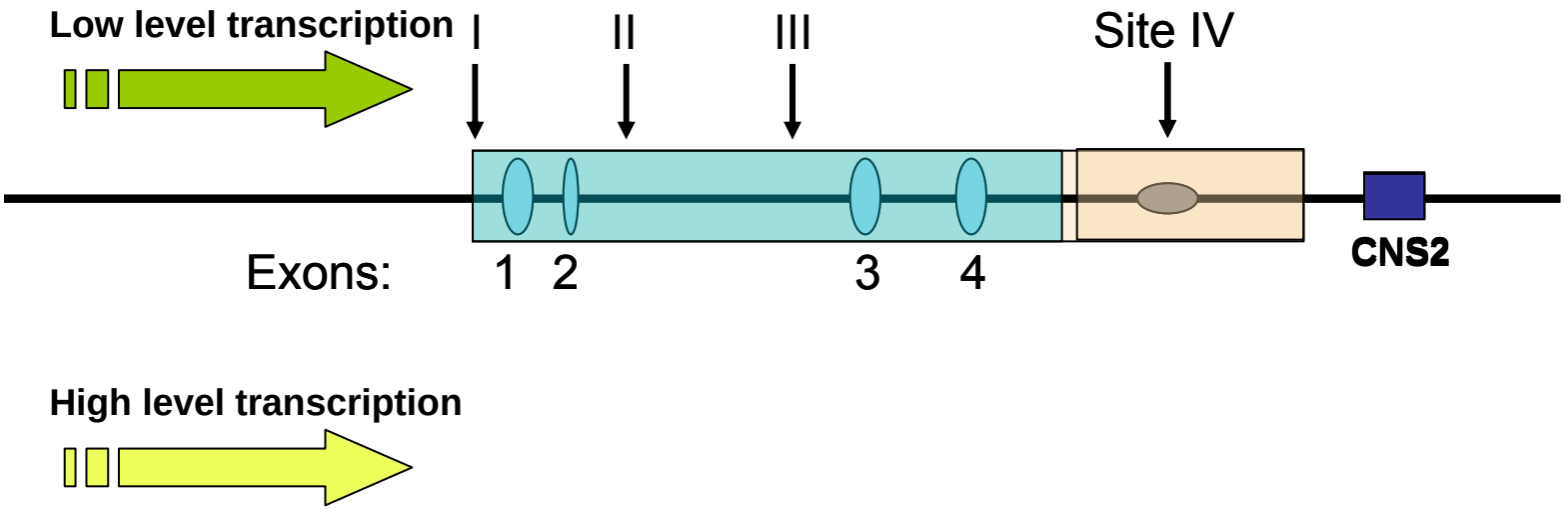
Remodelling the IL-4 locus: Th1



Remodelling the IL-4 locus: Th1



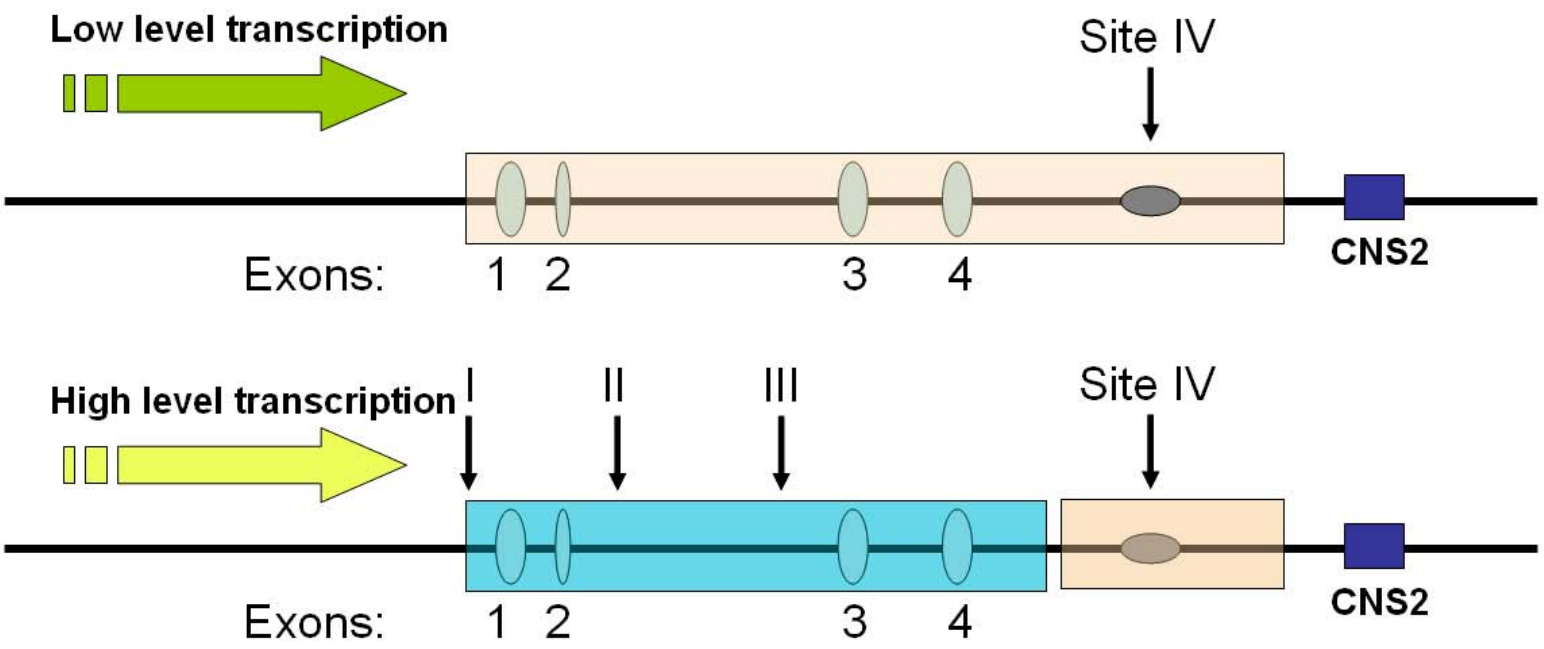
Remodelling the IL-4 locus: Th2



Extensive methylation

Extensive demethylation

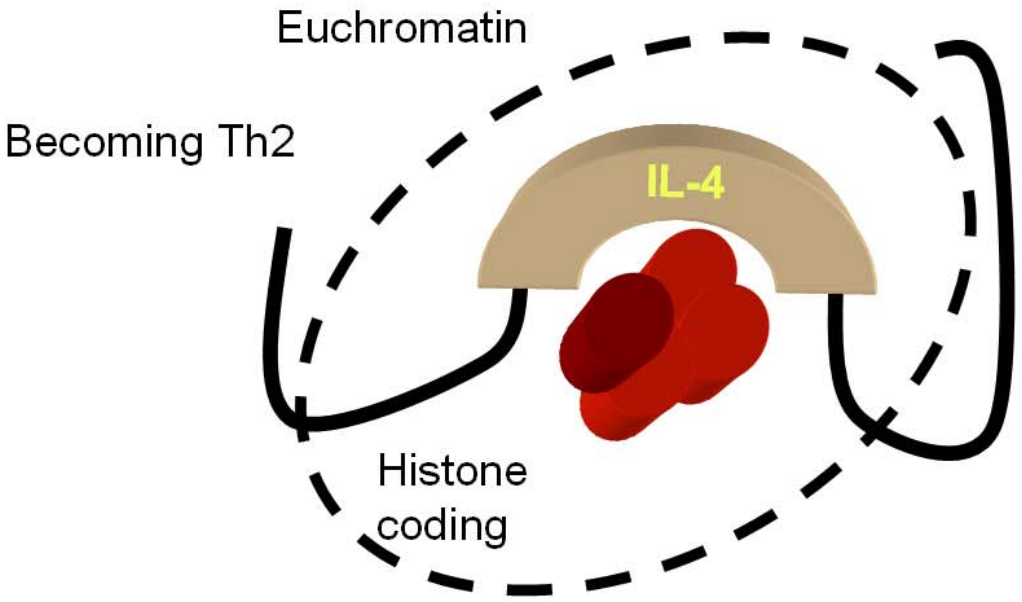
Remodelling the IL-4 locus: Th2



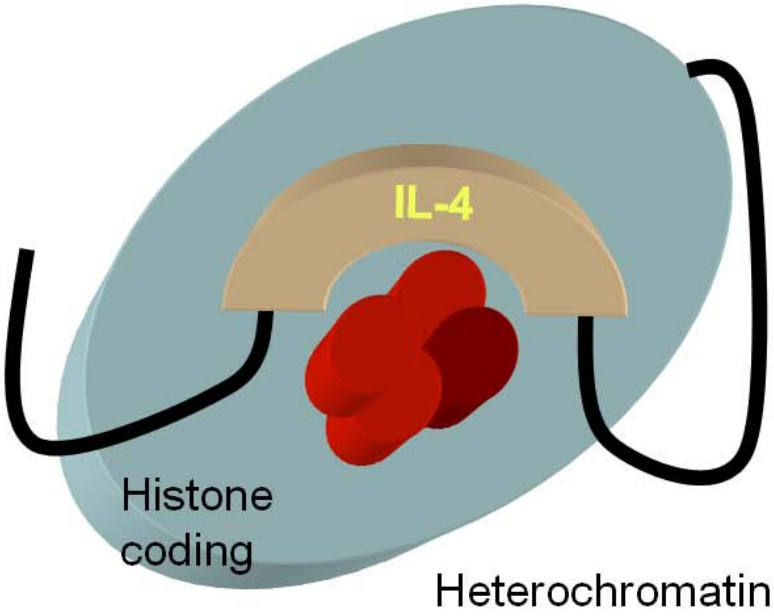
Extensive methylation

Extensive demethylation

Remodelling the IL-4 locus: repositioning



Remodelling the IL-4 locus: repositioning



Becoming Th1

Summary 3: Epigenetic mechanisms

- DNA accessibility changed by modifications in methylation state.
  DNAse hypersensitivity.
- Histone tail modifications (acetylation, methylation).
  ChIP assays.
- Repositioning with respect to loose (eu-) or condensed (hetero-) chromatin.
  Visualisation.

What is the relationship between phenotype and disease?

- *Leishmania major* in B10.D2 versus Balb/c mice
- Leprosy in humans
- Organ specific autoimmune disease models e.g. EAE, EAU.
- Asthma and models of airways hyper-reactivity
- NOTE: Strong association with chronic diseases

Th cell phenotype signatures

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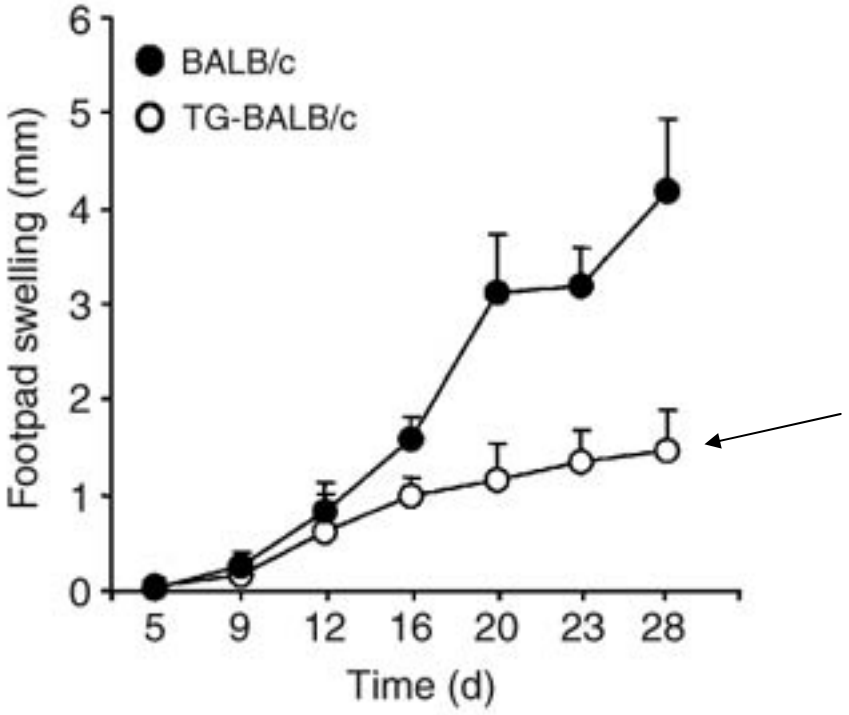
Effector functions of T helper subsets

- **Th1: Intra-cellular pathogens.**
  IFNγ, Delayed type hypersensitivity (DTH);
  complement fixing antibody isotypes (mice: IgG2a, IgG3; humans: IgG1, IgG3);
  organ specific autoimmunity
- **Th2: Extracellular parasites.**
  IL-4, IgE dependent mast cell mediated inflammation;
  neutralising antibodies (mice: IgG1; humans IgG4);
  eosinophil recruitment;
  allergy and atopy
- **Th17: Extracellular pathogens.**
  IL-17A, neutrophil recruitment;
  organ specific autoimmunity

Th1/Th2 balance can determine outcome of disease

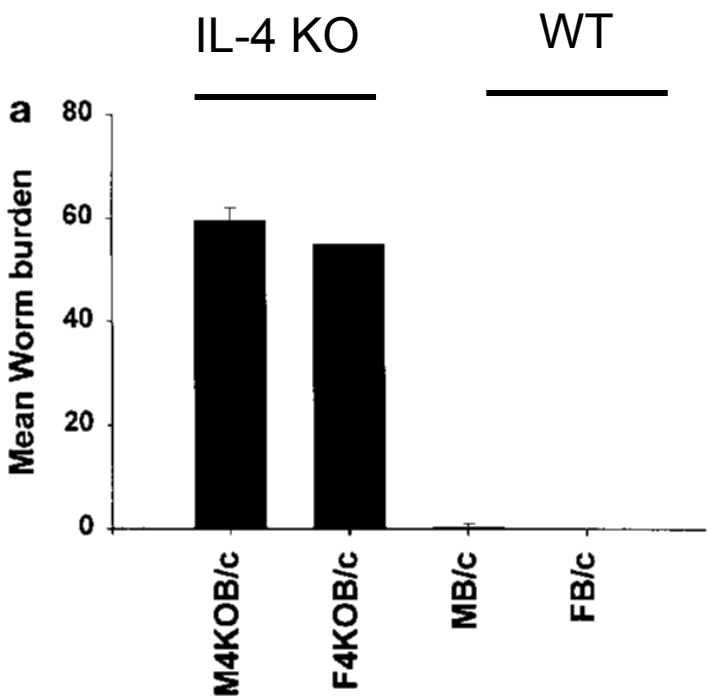
Leishmania susceptibility



Th1 response enhanced

Th1: Intra-cellular pathogens

Elimination of *Trichuris muris*



Th2: Extracellular parasites

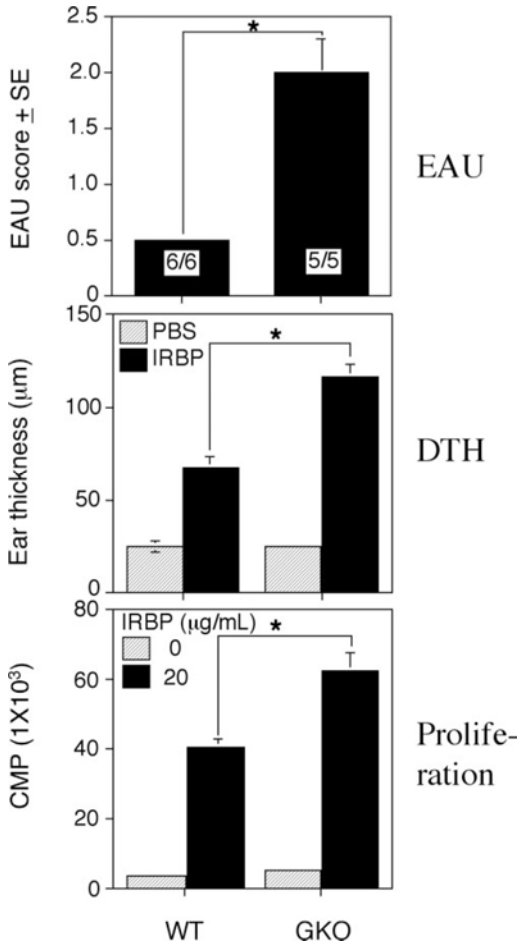
Can we prevent disease by targeting T cell subsets?

- Organ specific autoimmunity depends on Th1 cells
- Therefore will eliminating Th1 cytokines will prevent autoimmunity?

Interferon-gamma knockout animals in organ specific autoimmunity

EAE incidence in different genetic mutants

Mouse Strain	Immunization	Incidence of EAE
SJL/J	MBP/CFA	8/11
BALB/c	MBP/CFA	0/9
IFN$\gamma^{-/-}$	MBP/CFA	7/7
IFNγR$^{-/-}$	MBP/CFA	15/16[b]
	PBS/CFA	0/5
IFNγR$^{+/+c}$	MBP/CFA	0/5



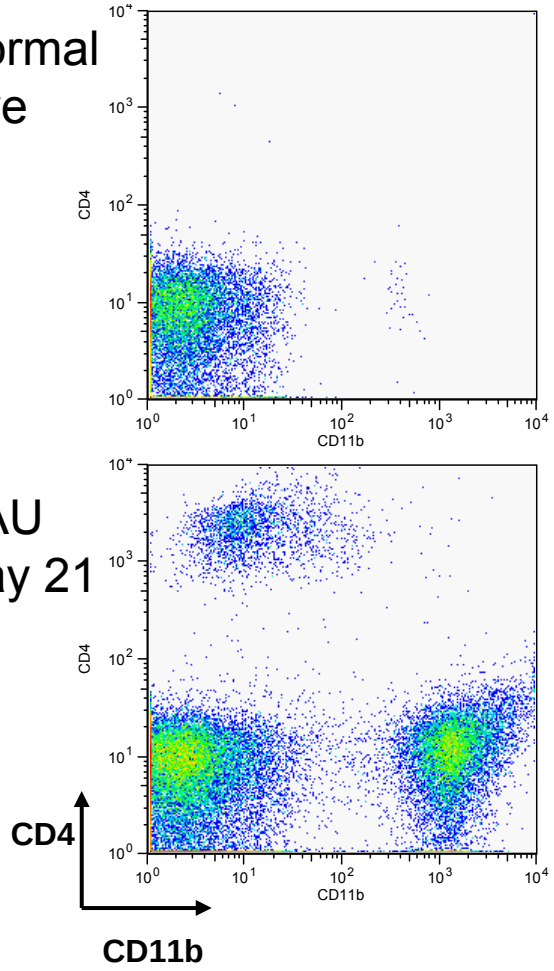
Perturbing effector cytokines can have unintended consequences

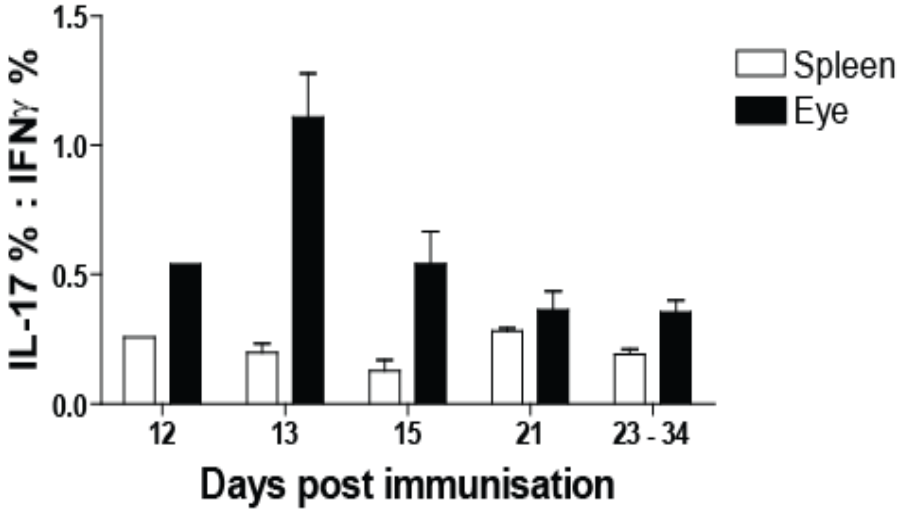
- Because of the presence of an unrecognised population of differentiated cells (e.g. Th17 cells in autoimmune disease)
- Because of the importance of negative feedback circuits (e.g. the importance of IFNγ in the induction of IL-10)
- Because the dominance of different cytokines changes through time

Dominant populations may change with time

Normal Eye

EAU Day 21





Emma Kerr *J. Autoimmunity* **31**, 354-361 (2008)

Summary 4

- Different pathologies are clearly associated with different peripheral immune response phenotypes
- Differences may be driven by genetics, infectious cues (via innate receptors) or other environmental factors
- In disease we must consider response heterogeneity and kinetics
- Don’t assume the phenotype of the immune response benefits the host: it may be subverted by pathogens

Can Th1/Th2 balance be manipulated therapeutically?

- In animal models e.g. by altered peptide ligands, epicutaneous administration, targeting specific APC populations
- In human disease some drugs are reported to be effective because the shift the Th1/Th2 balance e.g. copolymer I

Current questions in the field

- Fate mapping of cells to establish how the balance of a response develops
  [Reiner et al. Science 317:622 (2007)]
- How do APCs direct the outcome of T cell differentiation?
  [MacDonald and Maizels J.Exp.Med 205:13 (2008)]
- How do we study the responses of populations with a mixture of specificities?

Additional factors in diverse immune response

- Naive T cells are multipotential, but in a mixed environment, TCRs with differing avidities may trend towards different phenotypes (Nat. Med. 14, 337 - 342 (2008))
- Other phenotypes (e.g. T regulatory cells) are also involved and recruited
- T cells that recognise more than one antigen may respond differently to each