Peripheral CD4 T cell differentiation
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Lecture 12.

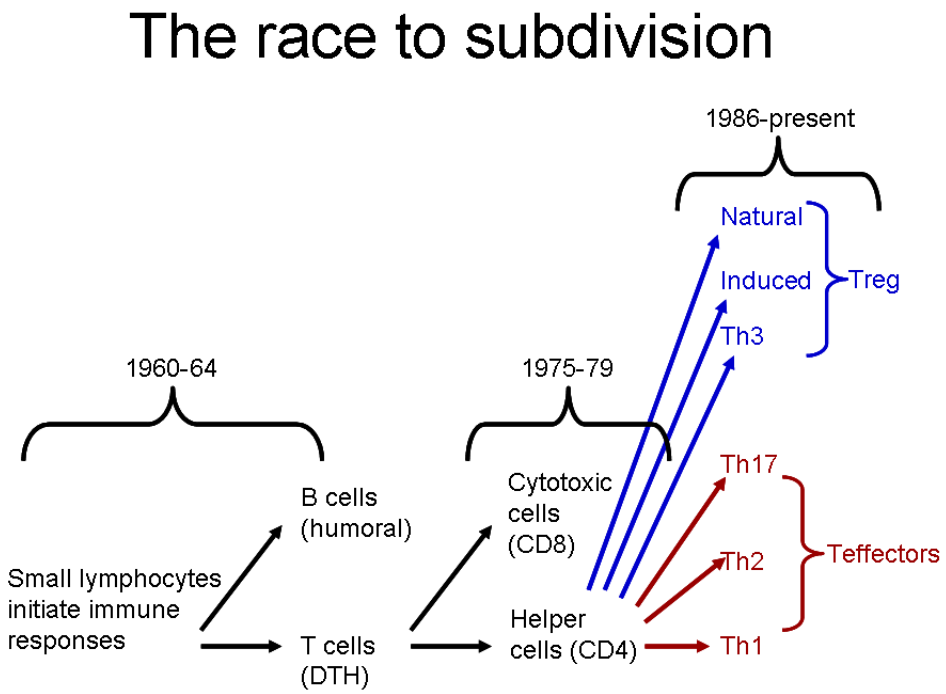
Peripheral CD4 T cells are 'naive' until they experience a productive interaction with cognate ligand and MHC class II in the periphery.

T cell differentiation is the process that follows this productive activation.

Key concepts of the lecture are:

- 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

History



Reciprocal relationship between humoral and delayed type hypersensitivity

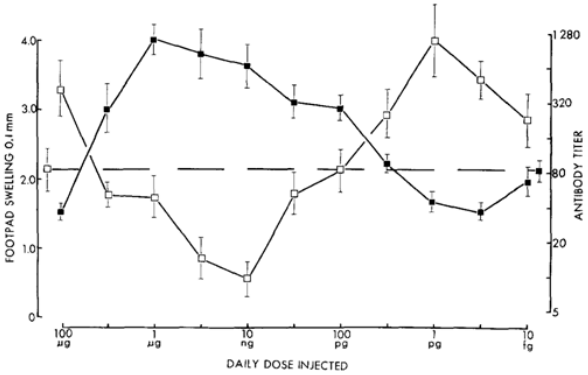


FIG. 3. Antibody titers (■—■) and delayed-type hypersensitivity responses (□---□) of strain W Wistar rats injected daily for 28 days with varying amounts of the CNBr digest of flagellin (Fig. 2) and then challenged with 100 µg of flagellin in saline. The antibody titers represent the mean of the 7, 14, 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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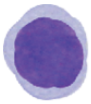
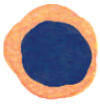
Differing Lymphokine Profiles of Functional Subsets of Human CD4 and CD8 T Cell Clones

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

- Does the recognition specificity determine phenotype? No. This can be shown definitively using naive transgenic T cells.
- Is phenotype reversible or fixed? Phenotype becomes harder to reverse through time. This explains why diseases in which phenotype plays a significant role in the outcome are often chronic.
- What determines phenotype in vitro and in vivo? In vitro cytokines are sufficient and lead to the strongest polarisation. However, other factors such as dose (in vitro and in vivo), route, genetic background, concurrent innate immune stimuli and costimulation also play a role.
- Is their a relationship between phenotype and disease? Yes. Discussed below
- Does modifying phenotype work as therapy?

	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

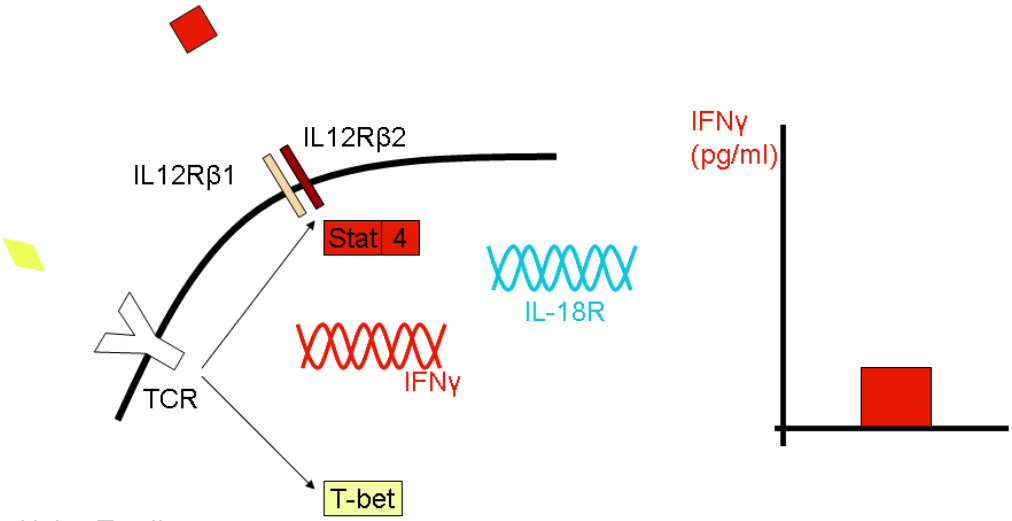
T cell activation leads to many changes in the responding cells:

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

Following activation, daughter cells that survive maintain altered patterns of gene expression: Cells do not return to a naive state

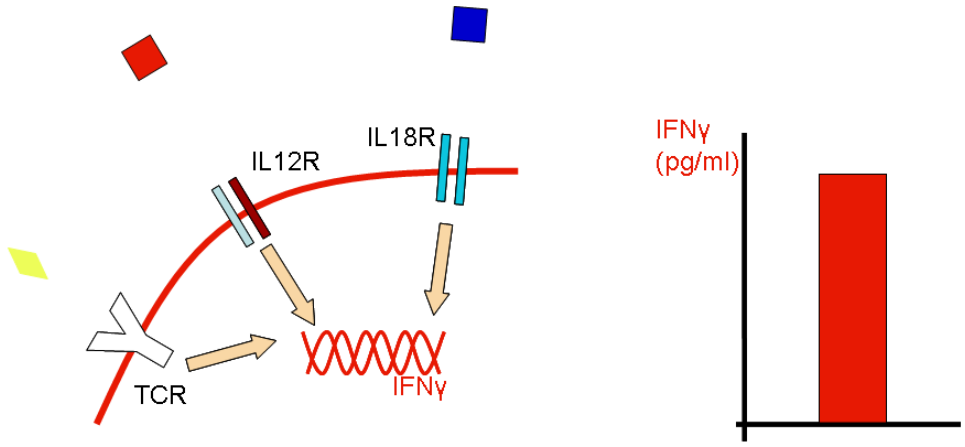
T cell differentiation depends on both positive and negative feedback. Positive feedback reinforces the expression of particular sets of genes; negative feedback inhibits the concurrent development of other phenotypes.

Th1 T cell differentiation



Naive T cell

A naive that T cells encounters a cognate signal via TCR, costimulatory signals and “signal 3” in the form of innate immune system stimulated IL-12 upregulates the expression of IL-12Rβ2. This allows IL-12 signaling via Stat4 top IFNγ and IL-18R. It also induces the transcription factor Tbet which stimulates IFNγ transcription. Naive cells then secrete IFNγ, but at relatively low levels.



Differentiated Th1 T cell

Differentiated Th1 T cells can be activated to produce IFNγ both by TCR signaling and by a combination of IL-12 and IL-18. The levels of IFNγ produced are much higher.

Negative feedback signals are produced by each subset that inhibit the generation of other cells in the same milieu with a different phenotype.

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

Through the expression of transcription factors that act as 'master regulators', the expression pattern of hundreds of different genes are altered. Studies using array analysis of gene expression indicate that about 300 genes are altered during the first few days of the differentiation process.

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

In practice the following strategies have been used to identify different T cell populations:

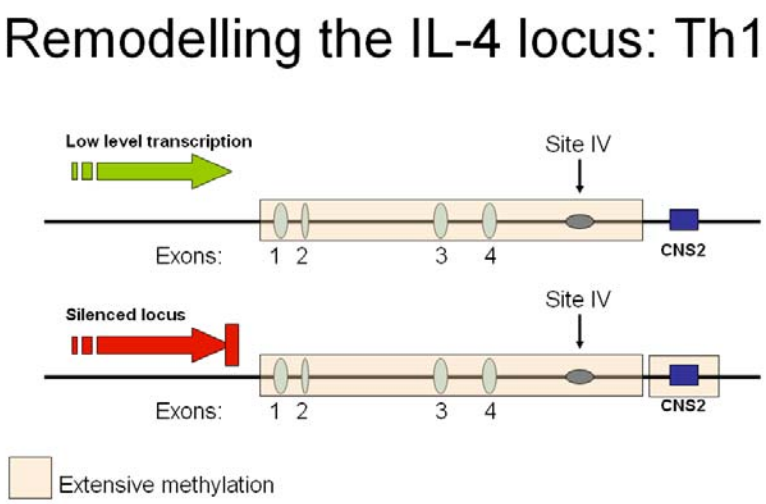
- The cytokines they produce
- Isotype of antigen specific antibodies (see below)
- Chemokine receptors
- Other molecules: Tim-3 (Th1), CD226 (Th1), ST2L (Th2)
- Artificially introduced labels, e.g. GFP driven by IL-4 promoter

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

How is T cell phenotype maintained? When they are activated T cells can 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?

Addressing these questions led to studies of the epigenetic mechanisms that can mark gene expression patterns in a way that is maintained through generation of cells. There are two important pathways to this – modification of DNA e.g by methylation and demethylation and relocation of genes between area of active chromatin (euchromatin) and inactive chromatin (heterochromatin)

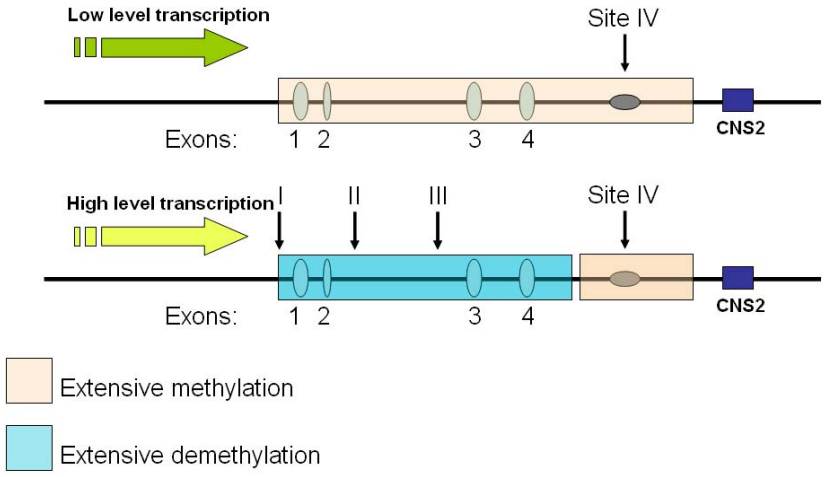


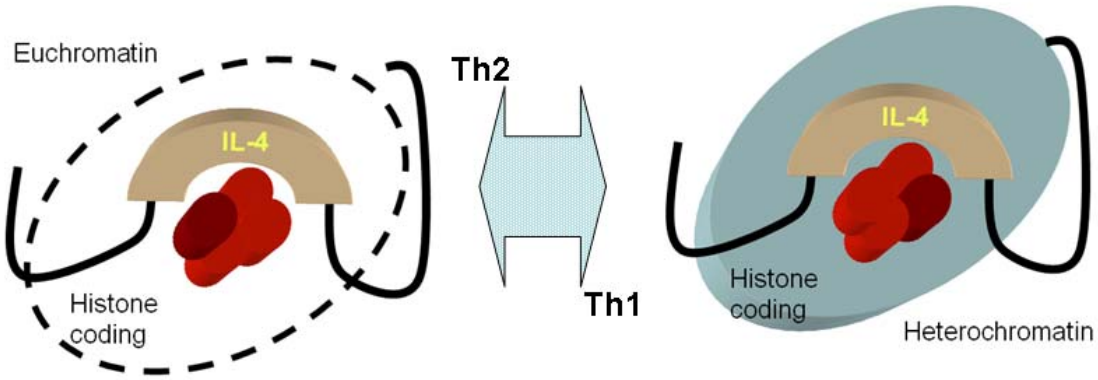
Studies of the IL-4 locus show that naive cells are poised for low level transcription from both the IFNγ gene and the IL-4 gene. Following differentiation to a Th1 phenotype, the IL-4 locus has increased methylation at a 3' conserved non-coding sequence (CNS) and the locus is silenced.

In contrast, if the cells have differentiated to a Th2 phenotype, new DNAse sensitive sites (indicating loss of methylation) have appeared and the locus is poised to respond with increased efficiency to transcription factors induced by activation.

A second mechanism involves the association of genes with histones that have modification in their tails that associate with segregation to areas of the nucleus where there is active gene transcription or restricted gene transcription. Histone modification are believed to be heritable, but the mechanism is unclear. It may relate to kinetic segregation in the association of differently modified histones.

Remodelling the IL-4 locus: Th2





Further reading:
Ansel et al. Ann. Rev. Immunol 24:607 (2006)

T cell phenotype and disease

It was recognised quite rapidly that different T cell phenoytpes segregated with different disease, and seemed to be especially important when the disease was chronic.

In mice, resistance of susceptibility to the intracellular parasite *Leishmania major* correlated with the nature of the T cell response. Th1 responses, e.g. those in B10.D2 led to healing and survival while Th2 responses e.g. those in Balb/c were unable to control disease.
In humans, the T cell phenotype was correlated with and ability to survive mycobacterial infection in Leprosy.

In organ specific autoimmune disease models e.g. EAE and EAU, disease in immunologically competent individuals was originally described as having a Th1 pheotype. This has recently been revised as we have recognised that both Th1 and Th17 cells can induce EAE and EAU.
Asthma and models of airways hyper-reactivity are associated with a Th2 phenotype.

Various characteristics of an immune response have been used to determine the nature of the underlying T cell phenotype.

- 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

This led to therapeutic strategies targeting cytokines in particular pathways.

Some of these, e.g. knocking out IFNγ, had unintended consequences. These may arise:
- 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

The Th1/Th2 balance has been manipulated in models with some success, but it has been harder to achieve this in human disease. Some drugs are thought to perturb Th1/Th2 balance and other approached targeting Th17 cell are under development.

Current important issues are:

- Fate mapping of cells to establish how the balance of a response develops
  [Reiner et al. Science 317:622 (2007)]
- How 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?
- 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) have a similar paradigm of control (cytokines, transcription factors) and are also involved and recruited
- T cells that recognise more than one antigen may respond differently to each

Further reading

Murphy and Reiner Nat. Rev. Immunol 2:943 (2002)