one should focus on the exchange interaction, the spin fluctuation exchange model differs from Demler and Zhang's (*8*) π-resonance picture, which in turn differs from resonating-valence-bond theories (*10*) and domain-wall stripe theories (*11*) all of which also arise from models with an exchange interaction, are known to fit a number of experimental observations, and likely play a key role in the underdoped regime.

Thus, the work of Dai *et al.* focuses our attention on the exchange interaction and the magnetic excitation spectrum, narrowing but not ending the search for the cuprate pairing mechanism.

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PERSPECTIVES: IMMUNOLOGY

Instruction, Selection, or Tampering with the Odds?

Robert L. Coffman and Steven L. Reiner

Lymphocytes are a late evolutionary addition to the immune system of vertebrates, enabling effective host defense against a wide variety of pathogenic microbes. The effector cells of the immune system, T and B lymphocytes, undergo two distinct modes of differentiation; one endows them with specificity for a particular antigen, the other with specific effector functions (T_H1 versus T_H2 cytokine patterns for $CD4^+$ T cells; specific antibody classes for B cells). Recombination of the V, D, and J segments of antigen receptor genes results in the generation of populations of T and B lymphocytes that express a broad repertoire of antigen receptors. In this way, the immune system is able to respond to a wide array of pathogens. Binding of antigen to the corresponding receptor stimulates the lymphocyte bearing that receptor to differentiate. In the case of T cells, functional differentiation is accompanied by distinct expression patterns for genes encoding cytokines and surface receptors. When stimulated by antigen, precursor T cells bearing the CD4 marker [T helper (T_H) cells] differentiate into either T_H1 cells or T_H2 cells. T_H1 cells produce interferon-γ (IFN-γ) and tumor necrosis factor–β (TNF-β) and protect against intracellular pathogens; T_H2 cells, which produce interleukin (IL)-4, IL-5, and IL-13, help to control extracellular pathogens, and mediate allergy (*1*).

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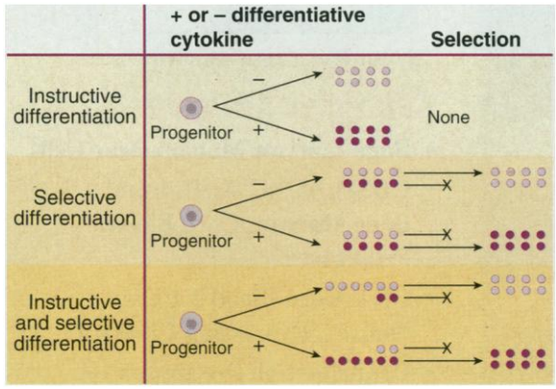
The best characterized influence on the differentiation of T_H cells is the cytokine environment (*1*). T_H cells first activated by antigen in the presence of IL-12 develop predominantly into T_H1 cells, whereas those activated in the presence of IL-4 develop predominantly into T_H2 cells. There is a debate about whether the cytokines that induce T_H1 or T_H2 differentiation "instruct" the developmental fate of naïve T_H cells or "select" for cells that through a random (stochastic) process of gene activation already produce a combination of cytokines indicative of a T_H1 or T_H2 cell (see the figure).

Several observations published in the past year offer new, somewhat surprising, insights into the way in which differentiative inducer cytokines regulate T_H cell differentiation. Using fluorescent tags to record the number of cell divisions of individual cells, naïve T_H cells were shown to require a specified number of cell divisions before becoming competent to produce cytokines indicative of either the T_H1 or T_H2 pathway. Different numbers of divisions are required for different cytokines (*2, 3*). Furthermore, stable cytokine expression is accompanied by demethylation and increased chromatin accessibility of the cytokine genes (methylation is an epigenetic mechanism for silencing genes) (*2, 4*). The most unexpected finding is that IL-2, IL-4, and, possibly, other cytokines are expressed from only one of two alleles in many individual T_H cells (*5*). These seemingly unrelated findings do not clearly resolve the simple question of instructed differentiation versus random differentiation and selection; instead they suggest that elements of both models may contribute to T_H cell differentiation.



Modeling T helper cell differentiation. In the instructive model of T_H cell differentiation, all daughter cells adopt one of two developmental states specified by the presence or absence of specific differentiative signals (**upper panel**). In the selection model, daughter cells adopt differentiation states in a random (stochastic) manner (**middle panel**). Pure populations can subsequently arise if one cell type is selectively expanded or maintained in preference to the other. In a model that combines both instruction and selection, the ratio of the two types of daughter cells varies depending on exposure to extrinsic differentiation inducing cytokines (**lower panel**). If the shift in the ratio of the two cell types is extreme, pure populations could be generated without further selection. Pink and purple circles represent two alternate differentiation states.

Cell proliferation appears necessary for the differentiation of T_H subsets. The initial expression of both T_H1 and T_H2 cytokines is cell cycle–dependent, but IFN-γ expression appears during the initial cell division whereas IL-4 requires at least three divisions (*2*). Furthermore, lineage commitment in the earliest stages of differentiation is strikingly inefficient and heterogeneous. Even under optimal conditions for either T_H1 or T_H2 differentiation, the number of cells that express subset-specific cytokine genes is low and is invariably accompanied by low frequencies of cells expressing atypical cytokine patterns. Thus, the T_H cell fate decision has intrinsic heterogeneity, and sibling cells can develop different, even opposite, phenotypes.

Gene loci for effector cytokines such as IL-4, IL-13, and IFN-γ are epigenetically repressed in naïve T cells (their chromatin structure is closed and their cytosines methylated) (*2, 4*). The link between tran-

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scriptional derepression and the cell cycle most likely reflects the opportunity for synthesis of new DNA that is less methylated and more accessible than that of the parent strand (*6*). After differentiation, epigenetic changes and allelic expression patterns persist for multiple cell divisions, showing that they are both stable and inheritable. Thus, T_H1 and T_H2 lymphocytes exhibit memory of both specificity and function, reflecting the stability of both types of differentiation. The mechanisms of this memory, however, are different. Stability of clonal specificity results from genetic recombination, whereas stability of function is accomplished by epigenetic modification.

Why should cytokine genes be expressed from only one allele? The clearest examples of monoallelic expression of autosomal genes are found in two organ systems for which cellular specificity and population diversity are essential: the immune system (antigen receptors on T, B, and natural killer cells) and the nervous system (olfactory receptors on olfactory epithelial cells) (*7*). Monoallelic expression (or allelic exclusion) ensures that most individual cells express only one member of a family of receptors encoded by highly homologous genes, resulting in each cell having only one of many possible specificities. The functional significance of monoallelic expression of cytokine genes is less obvious and may simply reflect the rate limitations of chromatin remodeling at these loci.

The question of instructive versus selective differentiation has been addressed in other hematopoietic cell lineages. Both instruction and selection have been reported for various lineage-restricted growth and differentiation factors (*8*). For example, macrophage colony-stimulating factor and granulocyte-macrophage colony-stimulating factor appear to instruct different fates in bipotential granulocyte-macrophage progenitors. In contrast, erythropoietin supports expansion of cells committed to the erythroid lineage (selection) but is not required for progenitor cells to make that commitment. Perhaps the example that is most relevant to T_H differentiation is the analogous functional differentiation of B cells involving isotype switch recombination of immunoglobulin heavy chain genes. Cytokines (including IL-4 and IFN-γ) can dramatically alter the switching between immunoglobulin isotypes in differentiating B cells (*9*). Antibody isotype switching requires transcriptional activation of heavy chain genes, and IL-4 and IFN-γ regulate switching by inducing transcription of specific heavy chain genes. The result of cytokine action on differentiation is, thus, instructive, not selective. This instruction takes the form of changes in the probability of different outcomes within the intrinsic constraints of a stochastic process. For example, the probability that B cells will switch to IgE ranges from <0.0001 to >0.01 in the absence or presence, respectively, of IL-4.

The regulation of T_H1 and T_H2 differentiation by differentiative inducer cytokines such as IL-12 or IL-4 may not be adequately described as either strictly instructive or strictly selective. The rate-limiting nature of chromatin remodeling of cytokine gene loci introduces an element of probability into the process, much as it does for antibody isotype switching for B cells. Cytokine inducers of T_H1 or T_H2 differentiation could alter the odds of stable chromatin remodeling of specific cytokine gene loci. Extremes of T_H1 or T_H2 differentiation may be achieved either by subsequent selection or by large changes in probability (see the figure). To decide among these models, it may prove necessary to produce the equivalent of an embryologist's fate map, accounting for the birth, differentiation, and death of all descendants of an individual T cell stimulated under highly controlled conditions.

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PERSPECTIVES: BIOCHEMISTRY

Seeking Ligands for Lonely Orphan Receptors

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Cells are exposed to a plethora of chemicals—metabolic intermediates, hormones, and compounds in the environment. One way in which cells adapt to these physiological and toxicological challenges is through nuclear receptors, which bind to these molecules, move to the nucleus, and initiate changes in gene transcription. Currently we know of about 70 different nuclear receptors, but only half of these have ligands that have been identified (*1*). The remaining receptors with unknown ligands are called orphan nuclear receptors. Identifying ligands for these receptors is a huge challenge but is one that the pharmaceutical industry is eager to take on. Drugs that mimic such ligands could be of particular value in the treatment of diseases that are caused by defects in the biochemical pathways in which these nuclear receptors are involved.

Two reports on pages 1362 and 1365 of this issue from the groups of Willson and Mangelsdorf (*2, 3*) and one in this month's *Molecular Cell* (*4*) now show that bile acids, important regulators of cholesterol homeostasis, are the physiological ligands of the farnesoid X receptor (FXR), an orphan member of the nuclear receptor family. This finding implicates FXR in the regulation of one of the key biochemical pathways in the body.

The most important primary bile acids in humans are cholic acid (the most abundant) and chenodeoxycholic acid. Bile acids are oxidation products of cholesterol with the enzyme cholesterol 7α-hydroxylase as the rate-limiting step in their synthesis. Cholic acid and chenodeoxycholic acid differ only in that cholic acid has a hydroxyl group at the 12α position and requires an extra enzyme, 12α-hydroxylase, for synthesis. Bile acids have two important functions in the gut: to facilitate solubilization and disposal of cholesterol (see the figure) and to facilitate absorption of dietary fat and fat-soluble vitamins. They are synthesized from cholesterol by two distinct pathways. The first is the classical "neutral" pathway in which cholesterol 7α-hydroxylase catalyzes the first and rate-limiting step (*5*). In the second (and more recently discovered) "acidic" pathway (*6*), oxysterol 7α-hydroxylase replaces cholesterol 7α-hydroxylase as the primary synthetic enzyme (*5*). The acidic pathway begins with the oxidation of a cholesterol side chain to form 27-hydroxy cholesterol. Although the neutral pathway usually predominates, the acidic pathway is important, for example, in babies with a mutation in the oxysterol 7α-hydroxylase gene (*7*). The three reports now

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