Origins of the T_H1-T_H2 model: a personal perspective

Robert L Coffman

Robert L. Coffman recounts how his work on immunoglobulin E regulation along with data from Tim Mosmann on the functional heterogeneity of T cell clones led to the T helper type 1–T helper type 2 hypothesis.

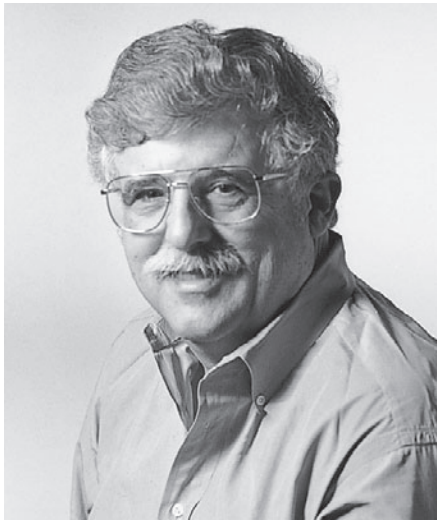
It is very common in the history of science that an important question arises long before the development of experimental strategies or techniques needed to resolve the issue. Thus, an important but often overlooked approach to discovery is the revisiting of old questions with new techniques. This principle is well illustrated by experiments leading to the T helper type 1 (T_H1)-T_H2 hypothesis proposed by Tim Mosmann and me in 1986. The hypothesis was quite simple: two very different subsets of helper T cells exist, distinguished by different sets of cytokines secreted after activation and, as a consequence, mediating very different regulatory and effector functions. However, that simplicity was possible only because of the results we obtained by looking at old questions in new ways. More detailed presentations of the model and the experimental basis for it were published by us soon after the initial papers[1,2], and a historical overview has also been published[3]. I will focus here on how two different lines of research taking place at the DNAX Research Institute came together, in ways not always obvious from the published record, to lead to this simple and testable model of T cell heterogeneity.

T cell heterogeneity

The hypothesis, developed in two papers published in the *Journal of Immunology* in 1986 (refs. 4,5) proposed a unified answer to the following two older, seemingly unrelated, questions: First, are B cell help and delayed-type hypersensitivity mediated by different types of $CD4^+$ T_H cells? In other words, are there classes of T_H cells analogous to the classes of antibody made by B cells? And second, how are allergic responses, especially the immunoglobulin E (IgE) class of antibody, regulated? The first question had been posed by the classic experiments of Liew and Parish describing the reciprocal relationship between conditions optimal for delayed-type hypersensitivity or antibody production[6]. The second issue was much older, but after the discovery that IgE was the reaginic antibody class, it was focused on the specific question of how IgE production was regulated.

IgE regulation revisited

Tim Mosmann and I were part of a group of immunologists and molecular biologists recruited to the newly created DNAX Research Institute in 1981. DNAX was started with private funding, but was acquired in 1982 by the Schering-Plough Corporation and was charged with the task of discovering and cloning the genes for factors regulating immune responses. Before joining DNAX, Tim had learned the relatively new technique of establishing cloned mouse T cell lines. He did not set out to explicitly ask the first of the questions above but instead to establish cell lines from which to isolate and clone previously unknown cytokines. In doing so, Tim invested considerable effort identifying the proteins produced and secreted by T_H clones in response to activation and correlating them with bioactivities measured in crude or partially purified supernatants, doing what is now called 'functional genomics'. The key technical advance (in addition to using cloned lines to start with) was Tim's development of the MTT assay, a colorimetric assay for cell growth that substitutes for [^{3}H]thymidine incorporation and makes it practical to do extensive, accurate titration of growth factors[7]. With that greatly improved assay, Tim was able to distinguish two different types of T cell growth factor (TCGF): interleukin 2 (IL-2) and a weaker one made by T_H2 cells (TCGF2). Similarly, T_H2 clones made a second mast cell growth factor (MCGF2) that was additive to mast cell growth mediated by IL-3, a cytokine made by both subsets. As only a few cytokines had been cloned or clearly described at that time, and an antibody was available for only one, interferon-γ (IFN-γ), much of the evidence in our initial paper describing "two types of murine helper T cell clone" was based on those state-of-the-art bioassays[5]. The differences among



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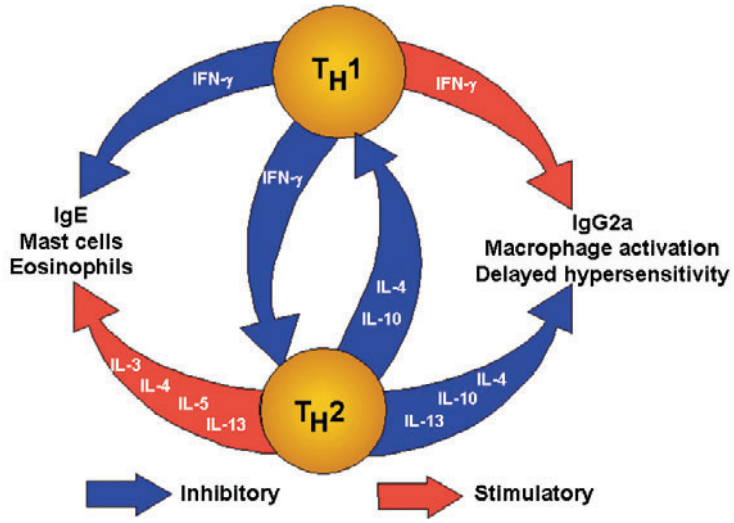

Figure 1 The principal stimulatory and inhibitory interactions between T_H1 and T_H2 cells, both directly and through their effector functions. IL-4 and IFN-γ do not directly inhibit differentiated T_H1 or T_H2 cells, respectively; instead, they are inhibitory by blocking the differentiation of those subsets from naive precursors. IL-13 was not known when the first versions of this diagram were drafted.

clones were clear, but the functional relevance of having different TCGFs or an additional MCGF was not yet obvious.

Filling in key details

As that work was proceeding in the Mosmann lab, the Coffman lab next door was trying to determine how IgE antibody responses were regulated. Unlike the concentrations of other immunoglobulin isotypes, serum IgE concentrations can vary 1,000-fold or more among relatively healthy people and concentrations can change considerably and rapidly in man and animals in response to specific stimuli, most notably helminth parasite infection. Yet many other types of infection or immunization result in no measurable IgE antibody response. Such a dynamic range of gene expression is characteristic of the classic models of enzyme induction in bacteria but it is rarely encountered in mammals. When I began this work in 1983, surprisingly little was known about IgE regulation, although T_H cells were thought to be key regulators. The groups of Ellen Vitetta[8] and Eva Severinson[9] had recently found that T cell supernatants could alter IgG production patterns in cultures of lipopolysaccharide-stimulated mouse B cells. I felt that that might be the technical advance needed to revisit the subject of IgE regulation by T cells, but it required establishing a sensitive and extremely specific solid-phase assay for IgE. That was accomplished by immunizing rabbits with IgE and absorbing the sera extensively on affinity columns of all other immunoglobulin isotypes. Those antibodies needed to measure very low concentrations of IgE yet not detect a 10,000-fold-higher concentration of any other isotype, a standard of specificity much more stringent than was customary for polyclonal or monoclonal antibodies.

The two lines of research came together in a dramatic way as soon as we tested supernatants from various T_H clones in our assay for IgE production. The addition of supernatants containing the TCGF2 activity led to large IgE responses, about 100 times the lower sensitivity of the IgE assay, whereas supernatants containing IL-2 induced no detectable IgE[4]. The lack of IgE induction with IL-2-containing supernatants, however, might have indicated an absence of the inducing factor, the presence of an inhibitory activity or both. To test that, we introduced both types of T_H supernatant to B cell cultures. The result was no IgE production, demonstrating the production of a dominant inhibitor by the T_H1 clones. The IgE-inducing cytokine seemed to be a previously unknown cytokine; however, key tools (a recombinant cytokine and a neutralizing antibody) were available to demonstrate that the inhibitor was IFN-γ. Thus, we had identified two types of T_H clone: one a potent helper of IgE responses; the other an equally potent inhibitor of that activity. For the first time, specific cells and proteins were identified that might account for the substantial variations in IgE concentrations readily found *in vivo*. The results convinced both of us that the differences in cytokine production patterns among T_H clones reflected an important dichotomy in T cell function, not just an artifact of prolonged cell culture.

The correlation between TCGF2 and IgE-inducing activities turned out to be more than a coincidence. In an especially memorable moment, we found that both activities were mediated by the same molecule, now known as IL-4. We had prepared a large batch of supernatant from a T_H2 clone for our biochemist collaborator, Martha Bond, to fractionate on a high-resolution liquid chromatography column. Individual fractions were assayed in both the IgE and TCGF assays; the latter, being an overnight assay, showed the TCGF2 activity was prominent in only 2 of the 96 fractions. The 7-day B cell culture for IgE seemed painfully slow, but we had noted before that within 2 days, B cells migrated up the sides of the culture wells and formed visible clumps whenever IgE-inducing supernatants were added. With Tim clutching the results of the TCGF assay, we pulled the B cell culture plates from the incubator and counted the fractions until we spotted two adjacent wells with clumped B cells. They proved to be the same two fractions that contained the TCGF2 activity! Fortunately, this 'surrogate assay' for IgE induction was confirmed a week later when the culture supernatants were assayed for IgE.

Importance for present research

By 1987, when we published a more complete synthesis of the T_H1-T_H2 hypothesis[1], several other key pieces had fallen into place: First, TCGF2, MCGF2 and IgE-inducing activities were all shown to be properties of IL-4 (then called BSF-1), shown before to be a B cell growth factor[10,11]. That overturned the prevailing assumption that cytokines have highly specific functions and cellular targets. Also, Tim's group confirmed experimentally that T_H1 cells but not T_H2 cells mediated classical delayed-type hypersensitivity reactions[12]. That provided a cellular basis for the older observations of differential regulation of delayed-type hypersensitivity and optimum antibody production. Finally, Donna Rennick at DNAX found a potent stimulator of eosinophil differentiation in T_H2 but not T_H1 cell supernatants, and it was subsequently identified as IL-5 (ref. 13). With that finding, it became apparent that T_H2 cells produced the optimum set of factors for regulating the three features characteristic of allergic diseases and anti-helminth responses: IgE, eosinophilia and mastocytosis. The basis for the coordinated regulation of those three functions had long been a puzzle. The idea that all three were stimulated by a single T_H subset provided a simple and readily testable explanation.

With the discovery in 1989 of IL-10 as a T_H2-specific inhibitor of T_H1 function[14], the basic scheme of reciprocal stimulatory and

inhibitory functions of T_H1 and T_H2 cells was essentially complete. The key idea is that each T_H subset has the ability to stimulate one set of coordinated antipathogen effector functions and to promote the development of more cells of the same T_H subset while inhibiting both the development of the opposite subset and many of its most important effector functions (**Fig. 1**). Furthermore, only a few key cytokines, notably IFN-γ and IL-4 (and the related cytokine IL-13), are central to both the stimulatory and inhibitory activities of a T_H subset. The idea of two opposing directions that can be taken by T cell responses has arguably had a greater effect on immunology than the actual definition of the two cell types involved. That is emphasized by the fact that none of the cytokines specific to one subset or the other are exclusive products of T_H cells and many other leukocytes can contribute to 'T_H1-like' or 'T_H2-like' responses[15].

By the time IL-10 had been identified, we and many others had begun to address two of the central issues raised by the hypothesis. First, how do the two T_H subsets originate and what controls that process? And second, what are the mechanisms of the reciprocal inhibition of each subset and its functions by the other?

Studies addressing those issues have fueled two of the most exciting areas in immunology research today: innate immunity and regulatory T cells. As the factors and conditions responsible for leading naive $CD4^+$ T cells down a T_H1 or T_H2 differentiation pathway were defined, it gradually became apparent that the nature of the innate immune response to an antigen or pathogen determined whether the subsequent adaptive $CD4^+$ T cell response would be mainly T_H1 or T_H2 (ref. 16). The evidence that T_H1 and T_H2 cells could act as 'suppressors' of each other helped refocus interest in the issue of whether T cells existed with mainly inhibitory functions. Between 1994 and 1996, Fiona Powrie and I described a population of naturally occurring $CD4^+$ T cells, distinct from T_H1 or T_H2 cells, that inhibited T_H1-mediated intestinal inflammation[17,18]. That was one of several converging lines of research leading to the resurrection of suppressor T cells, now renamed 'regulatory T cells'.

A source of both surprise and satisfaction was the rapid acceptance of the model. Researchers in many subdisciplines of immunology began to use T_H1-T_H2 differences to try to understand aspects of immune regulation or pathology in a wide range of immune-based diseases and disease models[15]. Attempts to interpret complex immunological diseases as simple T_H1- or T_H2-mediated processes did not always succeed, but the attempt often led to better understanding of disease pathogenesis. The model has had a substantial influence on drug development as well. Considerable work has taken place to develop new compounds and treatment strategies that act specifically on only one subset or on subset-specific effector functions. For example, inhibition of T_H2 cells or the T_H2-specific cytokine IL-4, IL-5 or IL-13 is the basis of many new therapeutic approaches to asthma and allergic rhinitis.

Over the past 20 years, the most common criticism of the hypothesis has been that it is too simple. Tim and I recognized from the beginning that T_H function and differentiation was likely to be more complex, but we were convinced that the model would be useful for understanding and studying the additional complexity that was inevitable. The real value of the hypothesis, I believe, was not that it was strictly correct or comprehensive but that it provided a clear, testable model of T cell function on which to base future studies. It immediately offered explanations for many important but confusing observations about the consequences of different types of immune response in infectious, autoimmune and allergic diseases; more importantly, it provided a simple framework for testing those explanations. The continued utility of the hypothesis is demonstrated by work showing that what were thought to be polarized T_H1 populations in many mouse autoimmune disease models contain both classical IFN-γ-producing T cells and a previously unknown T_H subset producing IL-17 but not IFN-γ[19]. That work seems to have identified a T_H subset with distinct origins and functions and may resolve the long-standing paradox that T_H1 cells can be both pathogenic and protective in autoimmune disease. Ultimately, I expect the understanding of immune regulation will exceed the ability of the original T_H1-T_H2 hypothesis to adequately organize and explain it. In the meantime, it has been great fun to watch an emerging idea grow into one of the cornerstones of immunology.

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