

## DISCUSSION FORUM

# The role of cytokines in determining the Th1/Th2 phenotype of an immune response: Coherence of the T cell response and the Cytokine Implementation Hypothesis

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## Abstract

The widely accepted Cytokine Milieu Hypothesis proposes that the cytokine milieu, in which antigen activates CD4 T cells, from a non-T cell source, primarily determines the Th subset to which the ensuing effector Th cells belong. We focus on the generation of Th1 and Th2 cells. We briefly restate the grounds for the Threshold Hypothesis we favour for how the Th1/Th2 phenotype of a response is primarily determined: tentative and robust thresholds of antigen-mediated CD4 T cell interactions lead to the generation of Th1 and Th2 cells. The component antigens of pathogens are present in different amounts. It is expected, within the context of the threshold mechanism that, although there is often an initial predominance of Th1 or Th2 cells, some Th cells of the opposing type are initially generated. An initially somewhat heterogeneous Th response is known to become with time more 'coherent' in its Th1/Th2 phenotype. I propose The Cytokine Implementation Hypothesis as a mechanism for how coherence is achieved. Most cytokines made by Th cells of one subset tend to facilitate the further generation of Th cells of this subset and/or inhibit the generation of Th cells of opposing subsets, accounting for how coherence may be achieved. Many observations on which The Cytokine Milieu Hypothesis is based are accounted for by this alternative hypothesis. We outline predictions of the new hypothesis and discuss the importance of coherence of immune responses for their efficacy in protecting against foreign invaders.

## 1 | INTRODUCTION

The Th1/Th2 phenotype of the *long-term* immune response against a pathogen is often remarkably coherent in that most Th effector cells belong primarily to either the Th1 or Th2 subset, as I shall document. An intimation of this coherence was initially seen in the response of people infected with *Mycobacterium leprae*, the pathogen responsible for leprosy; lepromatous individuals produce copious

IgG antibody but express minimal delayed-type hypersensitivity (DTH) whereas, at the other extreme, tuberculoid individuals express DTH but produced little IgG antibody. Moreover, the cell-mediated/humoral nature of the response was correlated with the clinical state of the patient, tuberculoid leprosy being the most benign.<sup>1,2</sup> The definition of Th1 and Th2 cells, with Th1 cells mediating DTH responses, and Th2 cells facilitating IgG<sub>1</sub> and IgE antibody production, following the discovery of their corresponding

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clones,<sup>3</sup> has resulted in more incisive analysis of immune regulation. It has also become apparent that the antigen-specific Th cells generated, in long-term immune responses, can often be seen as belonging virtually exclusively to either the Th1 or Th2 subsets, a property we refer to as coherence.<sup>4,5</sup> Coherence is often frequently observed even when the immune response is directed against chemically complex antigens, such as protozoa or bacteria. In cases where the Th1/Th2 phenotype of the immune responses evolves with time, usually transitioning from a predominant Th1 towards a Th2 mode, there is naturally a mixed Th1/Th2 phenotype during this transition.<sup>6</sup> In other circumstances, the long-term response can be balanced, consisting of a relatively constant mixture of cells producing IFN- $\gamma$  and IL-4. Such 'borderline' responses tend to be unstable.<sup>7</sup>

A major experimental system in which coherence of immune responses is evident is the mouse model of human cutaneous leishmaniasis. In both the human disease and the mouse model, a predominant and stable Th1 response corresponds to containment of the parasite at low, non-pathogenic levels, whereas mixed Th1/Th2 or Th2 responses correlate with chronic and progressive disease.<sup>8</sup> This model system gave rise to some of the most convincing *in vivo* observations that have been interpreted as supporting The Cytokine Milieu Hypothesis, as described below.

## 2 | OBSERVATIONS LEADING TO THE CYTOKINE MILIEU HYPOTHESIS

The diverse observations that have been interpreted as supporting The Cytokine Milieu Hypothesis are well known. They will only be briefly summarized here to provide context. An early and influential study involved the effect of heat-killed *Listeria monocytogenes* on macrophages, facilitating, through their production of IL-12, the *in vitro* generation of ovalbumin-specific Th1 cells.<sup>9</sup> Further *in vitro* studies showed that IL-12 and IFN- $\gamma$  facilitated the antigen dependent generation of Th1 cells,<sup>9,10</sup> and the presence of IL-4 the antigen dependent generation of Th2 cells.<sup>11</sup> Several studies were important for demonstrating the importance of these cytokines for the *in vivo* generation of Th1 and Th2 cells. Some of the most enlightening and convincing *in vivo* studies exploited the mouse model of cutaneous leishmaniasis. Infection of mice belonging to different strains in a standard manner with a million parasites can result in a stable Th1 response, containment of the parasite at low levels, in which case the mouse strain is designated as genetically resistant. Alternatively, the response evolves to contain a significant Th2 component or into a predominant Th2 mode, associated with parasitaemia and parasite spread to distal sites, in which case the mouse strain is designated as genetically

susceptible.<sup>6</sup> The administration to resistant mice of an antibody that neutralizes the activity of IFN- $\gamma$ , close to the time of infection with the standard challenge of a million parasites, results in a predominant Th2 response and a susceptible phenotype.<sup>12</sup> The administration to susceptible mice of an antibody that neutralizes the activity of IL-4, close to the time of infection with a standard challenge of parasites, results in the generation of a stable Th1 response and low parasitaemia, that is the mice display a resistant phenotype.<sup>13</sup> Such experiments led to many others with the aim of identifying the sources of the cytokines required to facilitate the generation of effector Th belonging to different Th subsets,<sup>14</sup> such as IL-4 for the generation of Th2 cells.<sup>15-18</sup> The broad acceptance of this framework is shown by its prominence as an accepted fact in reviews, not to mention in virtually all contemporary immunological textbooks. However, evidence suggests the IL-4 that is required or facilitates the generation of Th2 cells is not made by a non-T cell.<sup>19</sup>

The question of how the Th1/Th2 phenotype of an immune response is determined is recognized as central both at a basic level and for its pertinence for designing strategies of immunological intervention. The Th subset primarily generated determines the class/subclass of immunity induced, and this in turn is important to clinical outcome in diverse medical situations, as seen in allergies, autoimmunity and in responses to infectious agents and cancer. An understanding of this mechanism is essential to the rational design of strategies to modulate immune responses for the benefit of the patient. We have argued previously against the plausibility of The Cytokine Milieu Hypothesis, as it is commonly understood,<sup>20,21</sup> in that, for example, IL-4 from a non-T cell source is envisaged to be required to generate a Th2 response. We need to summarize these considerations, and those in favour of the alternative we espouse, before considering what the role of cytokines might be in controlling the Th1/Th2 phenotype of an immune response.

## 3 | THE PLAUSIBILITY OF DIFFERENT HYPOTHESES AS TO THE NATURE OF THE DECISION CRITERION PRIMARILY CONTROLLING THE Th1/Th2 PHENOTYPE OF AN IMMUNE RESPONSE

### 3.1 | The context for the considerations

We anticipate that a correct description of the decision criterion, determining whether antigen induces cell-mediated immunity or IgG antibodies, will account for the variables of immunization known to favour the

differential generation of these distinct modes of immunity. Three variables were recognized in the early 1970s and have been confirmed many times by subsequent studies. Such early studies assessed DTH as a measure of cell-mediated immunity and the production of IgG antibody as a measure of the humoral response. I translate these older findings, in the light of more recent studies, into the variables required to generate Th1 and Th2 cells. These variables are (i) the nature of the antigen. Pearson and Raffel made the generalization that minimally foreign antigens, either because small in size or larger but slight variations of self-antigens, were only able to induce Th1 cells.<sup>22</sup> (ii) More foreign antigens could induce Th1 or Th2 cells, depending upon the dose administered. Lower doses of antigen led to the generation of Th1 cells than the doses required to generate Th2 cells.<sup>23</sup> (iii) Time after immunization is the third variable. Most often Th1 cells are generated first and, with time after antigen impact, the response often evolves to contain a Th2 component. However, a dose can be sufficiently low that it only generates Th1 cells.<sup>23</sup> I have cited here particular observations illustrating these experimental generalizations that have recently been more thoroughly documented elsewhere.<sup>24</sup>

### 3.2 | The Threshold Hypothesis and some implications

I proposed in 1974 a hypothesis for the decision criterion controlling whether antigen generates Th1 or Th2 cells.<sup>25</sup> I argued for this hypothesis in part because it accounted for the three variables just described. The hypothesis was based on the premise that the activation of CD4 T cells requires, or is at least facilitated by, antigen-mediated CD4 T cell interactions. The Threshold Hypothesis states that tentative and robust CD4 T cell interactions are, respectively, required to generate Th1 and Th2 cells. This hypothesis accounts for the three variables of immunization that affect the Th1/Th2 phenotype of the ensuing response in the following way. There are few if any CD4 T cells specific for self-antigens, and fewer for minimally foreign than for more foreign antigens. Thus, minimally foreign antigens, even in the presence of levels of antigen optimal for supporting CD4 T cell interactions, can only mediate tentative interactions and so the generation of Th1 cells. An optimal concentration of a more foreign antigen can support robust CD4 T cell interactions and so the generation of Th2 cells. A sufficiently lower amount of this same antigen will only support tentative CD4 T cell interactions and so the generation of Th1 cells. Finally, when antigen impacts the immune system, it causes CD4 T helper cells to divide such that, if the level of antigen is sufficiently sustained, the CD4 T cell interactions become

stronger, accounting for the evolution of the response with time towards a Th2 mode. The threshold mechanism thus accounts in this fashion for the three variables of immunization known to affect the Th1/Th2 phenotype of the immune response.

In addition, the threshold mechanism makes one unique and strong prediction. Suppose we have a situation where antigen induces a mixed Th1/Th2 or predominant Th2 response, requiring, according to The Threshold Hypothesis, robust CD4 T cell interactions. If we reduce the number of CD4 T cells by some means, maintaining all the other variables of immunization the same, we should be able, if the reduction is sufficient, to decrease the CD4 T cell interactions so that they are now only tentative, and thus, a Th1 response is generated instead. We have tested this prediction successfully many times in diverse *in vivo* and *in vitro* systems.<sup>26-30</sup> One further example, discovered by others, is in the mouse model of cutaneous leishmaniasis. Susceptible mice, infected with a million parasites, mount a predominant Th2 response soon after infection and cannot control the parasite. Partial depletion of CD4 T cells within a day or two of infection results in a Th1 response and control of parasitaemia.<sup>31</sup>

Substantial evidence supports the idea that the activation of CD4 T cells requires B cells as an APC, mediating the interaction between the CD4 T cells.<sup>32,33</sup> The involvement of antigen-specific B cells in this manner allows the Th1/Th2 phenotype of immune responses to non-crossreacting antigens that are simultaneously generated in the same lymphoid organ, to be independently determined, as discussed below. This is because different antigen-specific B cells will independently mediate the CD4 T cell interactions specific for the two antigens, thus satisfying The Principle of Independence.<sup>20,21,34</sup>

### 3.3 | Outlines of the Pathogen/Danger Associated Molecular Pattern (PAMP/DAMP) Hypothesis, the Antigen Presenting Cell (APC) Hypothesis and an assessment of their plausibility/implausibility as well as that of the Cytokine Milieu Hypothesis

We have already outlined observations that led to The Cytokine Milieu Hypothesis. Two other hypotheses are very prominent in the current literature, the APC<sup>35-37</sup> and the PAMP/DAMP Hypotheses.<sup>38-41</sup> The APC hypothesis states that presentation of antigen to naïve CD4 T cells, by different types of APC, such as different subsets of DC, results in the generation of effector CD4 T cells belonging to different Th subsets. The PAMP/DAMP Hypothesis is cast in the context of the PAMP/DAMP Model for the

activation of CD4 T cells, according to which CD4 T cell activation requires a PAMP/DAMP signal. The nature of the PAMP/DAMP signal is envisaged to determine the Th subset to which the Th effector cells, generated following CD4 T cell activation, belong. I should add that it seems to me that the PAMP Hypothesis, in this context, is particularly unsatisfactory, as discussed below.

The Cytokine Milieu and APC Hypotheses appear to have been formulated as a consequence of particular experimental observations that defined conditions preferentially leading to the generation of Th1 or Th2 cells. It is often unclear how general these observations are and how the related hypotheses can explain why different and generally established conditions of in vivo immunization lead to the differential generation of Th1 or Th2 cells. In some cases, it is even difficult to see how these hypotheses can be reconciled with these different conditions. I have discussed these issues elsewhere at some length, so I will only illustrate a few of the difficulties with these proposals.<sup>21</sup>

Consider the PAMP/DAMP Hypothesis. It seems broadly true that most immune responses evolve from a cell-mediated to an IgG antibody mode, a pattern recognized by Salvin in the 1950s,<sup>23</sup> and since confirmed for diverse immune responses in diverse species when antigen impinges upon the immune system by diverse routes.<sup>24</sup> This evolution of the class of immunity cannot be explained by the PAMP hypothesis, unless the PAMPs change their nature with time. Moreover, this evolution of the immune response is seen in responses both to pathogens and responses against foreign, vertebrate, PAMP-free antigens, so a PAMP-independent explanation seems called for. Such an explanation is provided by the threshold mechanism.

In support of the PAMP Hypothesis, various instances are cited where certain PAMPs or PAMP-employed signaling pathways can affect the Th subset generated.<sup>39</sup> Despite this, such instances cannot account, as already indicated, for the general dependence of the Th phenotype on antigen dose, nor how this phenotype evolves with time. These considerations suggest the observations cited do not reflect general mechanisms. We conclude PAMPs are not the primary determinant of the Th1/Th2 phenotype of an immune response, though they can of course effect this phenotype.

## 4 | THE CYTOKINE IMPLEMENTATION HYPOTHESIS

### 4.1 | Coherence and its physiological importance at the level of Th cells

The evidence for the threshold mechanism, as more fully reviewed elsewhere,<sup>24</sup> seems very strong to me. The conclusion that this hypothesis is highly plausible raises

two questions. What explanation can be given for the numerous observations that led to the Cytokine Milieu Hypothesis, and what is the biological significance of the activity of cytokines that led to such observations?

We employed an assay, when studying murine immune responses to *L major* parasites and to mycobacteria, that detects single, antigen-specific IFN- $\gamma$  and IL-4-producing cells. We were initially very surprised that *long-term* responses could be so predominantly of one type or another. Often, antigen-specific cells of the minority or opposing Th subset were undetectable.<sup>5,6</sup> It further seemed likely that this coherence of immune responses is physiologically important. Consider the case of murine cutaneous leishmaniasis; pathogen-specific Th1 cells producing IFN- $\gamma$  can activate various pathogen-destructive metabolic pathways in pathogen-infected macrophages, and the presence of corresponding Th2, IL-4-producing T cells can down-regulate the expression of these destructive pathways.<sup>42-44</sup> Thus, the most efficacious containment of the pathogen appears to require not only the action of Th1 cells, but also the absence of pathogen-specific Th2 cells. This example illustrates the physiological importance of coherence in a particular case: coherence would seem to contribute to the effectiveness of immunity in those cases where only Th1 responses are associated with protection, and where Th2 cells are detrimental. This likely reflects a general trend, explaining why long-term responses are often predominantly of one Th subset or another.

### 4.2 | Coherence in antibody responses: mechanisms and significance

There is another immunological response where coherence is evident, and where we understand the mechanism that results in coherence. There are four major classes of antibody, the IgA, IgM, IgE and IgG classes. It subsequently was realized that there are 4 subclasses of IgG, reflecting four different heavy chain constant regions. Usually, the class/subclass of antibody obtained on immunizing with an antigen is primarily of one class/subclass. We know that the interaction between hapten-specific B cells and carrier-specific T helper cells, leading to the efficient production of anti-hapten antibody, requires the hapten to be physically coupled to the carrier. B cells specific for the different epitopes of a hapten carrier conjugate will endocytose this conjugate, and these different B cells will present the same peptides to the T helper cells specific for the antigen. The spectrum of cytokines delivered to the different B cells by the Th cells is thus the same, leading to the production of the same classes/subclasses of antibody by the antibody-producing cells generated upon activation of the carrier- and hapten-specific B cells. Given

that different classes and subclasses of antibody have different effector functions, it makes evolutionary sense that the production of different classes/subclasses of antibody is coherently regulated. If they were not so regulated, it is difficult to see how evolutionary selective forces could act to control the class of antibody produced in an advantageous way. We illustrate the significance of this generalization by considering human IgG<sub>4</sub> antibody.

Human IgG<sub>4</sub> antibody has most unusual anti-inflammatory properties. It is not known to activate any effector functions. In addition, it has the unique property of being labile such that it readily, under physiological conditions, splits into two halves that recombine. An IgG<sub>4</sub> molecule is thus initially bivalent for the antigen responsible for its production but, in time, due to the lability described, recombined IgG<sub>4</sub> molecules present in blood are usually monovalent for two different antigens. These properties are essential to its anti-inflammatory activity in blocking the activity of antibody belonging to other inflammatory classes.<sup>45</sup> It appears that the production of IgG<sub>4</sub> antibody only occurs after chronic stimulation<sup>46</sup> and that natural and clinical desensitization of allergies involves an evolution of responses from a predominant IgE to IgA/IgG<sub>4</sub> mode for allergic sensitivities that occur via mucosal sensitization,<sup>47</sup> and from an IgE to an IgG<sub>4</sub> antibody mode for parenteral sensitivities, such as bee stings.<sup>48</sup> It makes sense that IgG<sub>4</sub> is not produced early after antigen impact, as it would not provide protection against an invader. Its properties seem to be consistent with a role of dampening damage arising from chronic responses when these occur. This view only makes sense if the antibody response is coherent in the sense that the antibody produced under a given set of circumstances belongs primarily to one or a few classes/subclasses. Indeed, it is instructive to think what the consequence would be if only one class of antibody existed that mediated all antibody-dependent functions. Evolution would not be able to select for the differential expression of all these different functions in different circumstances.

### 4.3 | The threshold mechanism and the incoherence of Th immune responses shortly after antigen impact

We noted above that long-term Th responses against chemically complex antigens such as parasites and bacteria are often remarkably coherent. This coherence might be thought surprising in the context of the threshold mechanism. For example, the different proteins constituting *Leishmania major* parasites are present in different amounts. They would be expected, in the context of the threshold mechanism, if separately given in their

respective amounts that are present in a given number of parasites, to induce immune responses of different Th1/Th2 phenotype. Indeed, short-term responses seem much less coherent than longer term responses, as carefully documented and discussed by Kelso.<sup>49</sup> How then can coherence be achieved?

### 4.4 | Coherence at the level of Th cells

It is widely recognized that most of the cytokines made by Th cells of one subset tend to facilitate the further generation of Th cells belonging to this subset and/or tend to inhibit the generation of Th cells of 'opposing' subsets, as already indicated. We recapitulate one such set of interactions: IL-2 stimulates the proliferation of both Th1 and Th2 cells and so is an exception to the generalization just made, but the IFN- $\gamma$  made by Th1 cells inhibits the proliferation of Th2 but not of Th1 cells,<sup>10</sup> the IL-4 made by Th2 cells stimulates the proliferation of Th2 but not of Th1 cells,<sup>11</sup> and the IL-10 made by Th2 cells inhibits the production of IFN- $\gamma$  by Th1 cells.<sup>8</sup> These interactions may account for how the initial generation of a heterogeneous but predominant Th1 response, or an initially predominant Th2 response, can become more coherent with time. It should not be surprising, within this context, if IL-4 is required to generate substantial Th2 cells, as found and discussed above. The Cytokine Implementation Hypothesis proposes that the IL-4 *required* to facilitate the generation of a Th2 response is itself produced by Th2 cells. The aim of many studies has been to determine the source of IL-4, envisaged to be required for a Th2 response to take place, from a non-Th source, as outlined above. I think it useful to explicitly define The Cytokine Milieu Hypothesis as meaning that a certain cytokine milieu, from a non-T cell source, is *required* to support a response that gives rise to Th cells of a given Th subset. The Cytokine Implementation Hypothesis does not imply that cytokines from non-T cell sources cannot affect the Th1/Th2 phenotype of the response, but rather that such a source is *not required*. We tried to distinguish the source of the IL-4 known to be *required* to support the generation of Th2 cells. We employed an in vitro system where antigen, without the addition of any cytokines, activated naïve CD4 T cells to give rise to IL4-secreting Th2 cells. In this system, purified CD4 T cells were mixed with a source of spleen cells from which T cells had been depleted, as a source of any non-T cells, primarily APC, that are required for the activation of the CD4 T cells. Conditions were found where antigen could activate the CD4 T cells to produce IL-4 producing Th cells. The generation of IL-4 producing cells was inhibited by the presence of antibody that neutralizes IL-4, and IFN- $\gamma$  producing Th cells were

generated instead. This system thus confirmed the generality of the observations that IL-4 is required to generate IL-4-producing Th2 cells. We further examined the response induced when the naïve CD4 T cells were from a normal mouse, and the non-T cells from an IL-4 KO mouse. We found that under these circumstances that antigen had unimpaired ability to generate IL-4-producing Th cells. The IL-4 required in this case was therefore made by the CD4 T cells themselves.<sup>29</sup> These observations test The Cytokine Implementation Hypothesis as far as IL-4 is concerned. Similar tests can be designed for examining the source of other cytokines required to generate effector cells belonging to different Th subsets and produced by these Th cells.

#### 4.5 | Implications of The Cytokine Implementation Hypothesis: coherence and independence

One implication of this hypothesis is that the different effector Th cells, that belong to the Th population of an ongoing immune response to both simple and complex antigens, mutually influence each other, to give rise to a subsequent Th population that is more coherent, by the criterion of the cytokines their effector T cells produce. How such mutual influences occur is physiologically important. For example, it has been shown that when responses to two non-crossreacting antigens, Q and R, are simultaneously taking place in the same lymphoid organ, the determination of the Th1/Th2 phenotype of the responses to the two antigens can be independent. Consider the situation where conditions are chosen so that a Th1 response is generated in the spleen against Q, and other conditions are determined where the splenic response against R has a predominant Th2 phenotype. Immunization with both antigens, delivered with the same syringe, results in responses that are indistinguishable from the responses generated in mice immunized with only one antigen. It appears that in this sense, the determination of the Th1/Th2 phenotype of responses, generated in the same lymphoid organ, can be independently determined.<sup>34</sup> This independence raises two questions. What is the physiological significance of such independence, and how can it be achieved?

The physiological significance of independence seems self-evident. Immunologically mature animals and people are continuously making immune responses to a variety of environmental antigens, including gut flora. If there was no independence, the Th1/Th2 phenotype of an immune response to a new invader would be greatly influenced by these ongoing immune responses. For example, about 95% of people infected by *M tuberculosis* make a

protective, predominant and sufficiently strong Th1 response to contain the pathogen and not suffer disease.<sup>50</sup> It is hard to believe that this would be the case if the response to *Mt* responses was not independent of ongoing immune responses to other, non-crossreacting antigens. We have argued elsewhere that such independence can be realized if the CD4 T cell collaboration, mediating the threshold mechanism by which the Th1/Th2 phenotype of the response is primarily determined, was primarily mediated by antigen-specific B cells. In this case, interactions determining the Th1/Th2 phenotype of the response to Q would be mediated by Q-specific B cells, and those CD4 T cell interactions determining the Th1/Th2 phenotype of the response to R would be mediated independently, by R-specific B cells. It seems likely that the CD4 T cell interactions responsible for achieving coherence, as envisaged in The Cytokine Implementation Hypothesis, are primarily mediated by antigen-specific B cells, as discussed above.

We carefully established conditions in the cutaneous leishmaniasis murine model that led to borderline disease. We initially developed this system to establish conditions that we thought would be more readily curable than disease associated with a highly polarized Th2 response. This surmise turned out to be correct. The establishment of borderline disease followed infection with an intermediate number of parasites, both lower and higher than the numbers that, respectively, led in the long-term to predominant Th1 and Th2 responses. It took several weeks for the predominant Th1 response to evolve to have a mixed Th1/Th2 phenotype. This response was then stable in most mice, but in the occasional mouse the lesion spontaneously regressed and in others control of lesion size was spontaneously lost (PA Bretscher, unpub. obs.) It seems most likely that these spontaneous events reflected the instability of the mixed, borderline Th1/Th2 response and their tendency to occasionally evolve into coherent Th1 and Th2 modes. We think of a physical analogy. A coin that is standing on its side is unstable. Small perturbances can result in either a head or tail situation that are stable states.

#### 4.6 | The broader implications of the Cytokine Implementation Hypothesis

The Cytokine Implementation Hypothesis is not very novel in that the self-promoting nature of the cytokines, produced by Th cells belonging to a particular Th subset, for the further generation of Th belonging to the same subset, is generally recognized. The reason for emphasizing the idea of cytokine implementation is twofold. Much effort has also been directed at finding the source of the cytokines, such as IL-4, that are envisaged to be needed

'to initiate' a Th2 response. If the 'critical cytokine' can be produced by Th cells themselves, as is the case of IL-4 and of Th2 cells, this effort is misdirected. Secondly, a belief in the role of a non-T cell as the source of IL-4, that is needed to initiate a Th2 response, distracts from the most critical question: what circumstances initially favour the preferential induction of Th2 cells? I suggest that in the case of Th1 and Th2 cells, the threshold mechanism is pertinent. If a critical cytokine is not produced by Th cells themselves, such as IL-12, there would appear to be three questions. Is it essential, does it merely facilitate the generation of Th1 cells, and what controls its production, is it another T cell?

I have focussed here on the primary role of cytokines in the generation of Th1 and Th2 cells. I believe that the ready acceptance of The Cytokine Milieu Hypothesis in the context of Th subsets, other than Th1 and Th2 cells, is similarly questionable. I suggest this acceptance tends to lead to an under-evaluation of research directed at the primary events leading to the preferential generation of Th cells belonging to different subsets. An unravelling of the primary events should allow us to understand why different circumstances of immunization lead to the predominant generation of Th cells belonging to different subsets.

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