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Review Article

# History of interleukin-4

William E. Paul *

*Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892-1892, United States*

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ABSTRACT

The history of the discovery and the development of our knowledge of IL-4 exemplifies the path of progress in biomedical science. There are unanticipated twists and turns although progress is made, sometimes quickly, other times far too slowly. Illustrative is the extended time from the first report of IL-4 in 1982 to the establishment of the efficacy of blocking IL-4 and its congener IL-13 in the treatment of moderate to severe asthma and atopic dermatitis, a period of 31 years. The author was "present at the creation" and has been a participant or a witness to virtually all the major advances and recounts here his recollection of this history.

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## 1. Introduction

Interluekin-4 and its congener IL-13 are highly polyfunctional cytokines. Indeed, despite intensive study for over 30 years, it is likely that the full range of functions of these cytokines is not yet known since the distribution of IL-4 receptors is extremely broad [43], suggesting that many cell types will display responses to IL-4. Not all of these responses have been carefully assessed. Despite the great pleiotropy of this molecule, its story begins with its function on one cell type – namely its capacity to enhance the proliferative responses of B cells to anti-immunoglobulin antibodies [17]. That response itself was discovered as a result of a program of research that my colleagues and I in the Laboratory of Immunology undertook in the late 1970s in an effort to resolve a debate that has surprisingly modern reverberations.

At the time, there were two contending views regarding the significance of expression of membrane immunoglobulin on B cells. Don Mosier and I [33] and our colleagues in LI, as well as several other groups, most notably that of David Parker [47], argued that membrane immunoglobulin was a true receptor and that its interactions with cognate antigens led directly to biochemical signals within B cells that were important in their growth and differentiation into memory cells and antibody-producing cells. The alternative viewpoint, championed by Göran Möller and Antonio Coutinho, was the membrane immunoglobulin was an antigen-binding molecule but not a signal transducer. Its role was to concentrate epitope-linked intrinsic stimulants such as LPS on the B cell surface and thus to indirectly activate B cells [8].

Today, we would say that the case of membrane Ig as a receptor is established beyond doubt. Indeed, B cells can be activated through receptor aggregation even in the absence of all TLR-mediated signaling [75]. However, it is also clear that membrane Ig-mediated concentration of innate stimulants, such as toll-like receptor ligands, on the B cell can enhance the activation of these cells [26].

## 2. Early history of IL-4: Discovery to cloning

Because of the raging controversy regarding mIg function, we were impelled to study in detail the induction of proliferative responses by B cells stimulated with anti-IgM antibodies [58,59]. We carried out an extensive series of studies and were struck by the finding that the proliferative response of the B cells was very cell density-dependent. That led Maureen Howard and me to speculate that there might be a contaminating cell type in our cultures that we were diluting out as we reduced cell density and that a product of that cell might aid the B cell response. We tested the PMA-induced supernatant of an EL-4 cell line and were gratified to find that highly diluted supernatant would strikingly enhance the proliferative response of B cells to anti-IgM [17]. Partial purification showed that the factor had a molecular weight of ~18,000 daltons and that it was clearly different from IL-2. Initially, the factor was designated B cell growth factor (BCGF). As other B cell functions (I'll describe them shortly) were

* Address: Bldg 10, Rm 11N311, National Institutes of Health, Bethesda, MD 20892-1892, United States. Tel.: +1 301 496 5046.
*E-mail address:* wpaul@niaid.nih.gov

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recognized, the factor was renamed B cell stimulatory factor-1 (BSF-1) and finally, when it was molecularly cloned, the definitive name of IL-4 was given to it.

The next major step in the IL-4 story was its purification and the preparation of a specific monoclonal antibody in 1985 by Junichi Ohara and myself [42]. The antibody, 11B11, efficiently neutralizes IL-4 and prevents it from binding to the IL-4 receptor. 11B11 is still widely used today. In that same year, we showed that IL-4 had several other important B cell stimulatory functions other than regulation of B cell growth in response to anti-IgM antibodies. For example, Evelyn Rabin demonstrated that IL-4 acted on resting B cells to prepare them to proliferate [51]. Even more striking was the demonstration of its role as an immunoglobulin switch factor. The initial report of the effect of IL-4 in promoting proliferation of B cells appeared in the March 1982 issue of the *Journal of Experimental Medicine* [17]. In that same issue, a joint paper from the laboratories of Ellen Vitetta and Peter Krammer appeared describing a supernatant of several T cell lines and of hybridomas that enhanced switching of B cells to secretion of IgG, particularly IgG1 [19]. In 1985, in a collaboration of my laboratory with that of Ellen Vitetta, we showed that purified IL-4 had the IgG1-switching capacity that Vitetta and Krammer had reported and that anti-IL-4 blocked the switch-promoting activity of their supernatants [66] so that the first reports of IL-4 functions can be dated to the March, 1982 issue of the *Journal of Experimental Medicine*. Sideras and colleagues also reached the conclusion that IL-4 was the IgG1 switch factor at about the same time [57]. Shortly thereafter, in 1986, in a collaboration of Bob Coffman's laboratory and mine, we demonstrated that IL-4 was a potent switch factor for IgE [6].

In that same year, it was recognized that IL-4's action was not limited to B cells. Tim Mosmann's group and ours showed that IL-4 could act as both a T cell and a mast cell growth factor [34] and Vitetta and her colleagues showed that IL-4 could act to promote the growth of T cell lines [11].

1986 was capped off by the molecular cloning of mouse and human IL-4, the former by the groups of Honjo [40] and of Lee [27] and the latter by Lee and Arai and their colleagues [73]. And, at about the same time, the amino acid sequence of the IL-4 protein was obtained by Grabstein and colleagues at Immunex [13] and shortly thereafter by our group [44].

A final aspect of this early history was the discovery of in 1989 of IL-13, a close congener of IL-4, which can mediate virtually all of IL-4 actions on non-hematopoietic cells and to some degree on hematopoietic cells [4].

## 3. Chromosomal localization and epigenetic regulation

In 1988, the *Il4* gene was mapped to mouse chromosome 11 within 1 cM of the *Il3* gene [10] and to the syntenic region in the human, chromosome 5q31 [61]. Thereafter, this region was mapped in detail and it was shown that *IL13* and *IL4* genes were 12 kB apart [60], located between the genes for *RAD50* and *KIF3a*, an arrangement that is evolutionarily highly conserved. Detailed analysis indicates that there are a series of sites in the IL-4 locus that are hypersensitive to DnaseI in Th2 cells [1]. Several of these were shown to be important in production of IL-4 using a transgenic mouse model [28]. One, HSII, is located in the second intron of the *Il4* gene and is tightly associated with both GATA3 and STAT5 binding sites [7,70]. Since both GATA3 and STAT5 are essential to the priming of naïve cells to become IL-4 producers, this strongly suggested an important role for HS II. Indeed, deletion of this site profoundly diminishes IL-4 expression [64]. ChIPSeq studies demonstrated that histone H3 bound to the *Il4* locus was trimethylated at lysine4 in Th2 cells, implying accessibility, but was trimethylated at lysine 27 in Th1 and Th17 cells, consistent with the failure of these cells to produce IL-4 [69].

## 4. The IL-4 receptor and signaling mechanisms

The first major step to understanding the signaling pathways through which IL-4 mediated its function was the demonstration of its receptor. In 1987, Junichi Ohara and I [43] reported that a saturable high affinity receptor existed on the surface of T cells. The affinity was $\sim 3 \times 10^{10}\,M^{-1}$ and resting lymphocytes had ~300 receptors per cell but activated lymphocytes had 5–10 times more receptors per cell than resting cells; macrophages and mast cells had 2000–3000 receptors per cell. When we attempted an estimate of the molecular size of the binding chain, we obtained a value of 80,000 daltons; similar results were obtained by Linda Park and her colleagues at Immunex [46]. However, when the human IL-4 receptor was molecularly cloned at Immunex [18], the molecular size proved to be 140,000 daltons. It was subsequently shown that the 80 kD molecule was a breakdown product of the 140 kD receptor [22].

After Warren Leonard had shown that the X-chromosome-encoded IL-2 receptor chain gamma common (γc) was mutant in X-linked severe combined immune deficiency [39], his group and ours showed that γc and IL-4Rα comprised the type I IL-4 receptor signaling complex [52]. In lymphoid cells, IL-4Rα was associated with Jak1 and γc with Jak3. IL-4 and its congener IL-13 were shown to be unique in that they were the only ligands that caused STAT6 phosphorylation. Beginning with the efforts of Achsah Keegan and Jacky Pierce, analysis of patterns of protein phosphorylation in response to IL-4 and of the structure of the binding chain of the IL-4 receptor (IL-4Rα) led to the conclusion that there were two major signal transduction pathways activated by IL-4 [67,68,23]. One depended on STAT6 phosphorylation, presumably as a result of binding of STAT6 to one of the three distinct binding elements in the IL-4Rα chain, with the consensus sequence of GYKxF. Indeed, mutating the Y in these sequences to F almost completely eliminated STAT6 phosphorylation in response to IL-4 and blocked a whole series biological functions [53]. The other pathway was initiated by tyrosine phosphorylation of a Y in a motif shared by the insulin receptor and the IL-4 receptor, NPxYxSxSD, resulting in the phosphorylation of insulin receptor substrate 2 (IRS2), and in its interaction with the regulatory subunit of PI3 kinase. The importance of the I4R motif in T cells in not certain since little or no ERK phosphorylation is observed in lymphocytes in response to IL-4 although insulin does induce ERK phosphorylation in these cells. Most IL-4 functions in lymphocytes are lost in STAT6−/− mice [78]. However, in myeloid cells, IL-4-mediated cell growth depends on the I4R/IRS-2 pathway [67].

IL-13 uses as its receptor a complex consisting of the IL-4 binding chain IL-4Rα, and IL-13Rα1 [16,30]. This complex also serves as a second receptor for IL-4 and accordingly is designated the type II IL-4 receptor. The type II IL-4 receptor is expressed widely on non-hematopoietic cells but not on T cells (in human and mouse) or B cells (in the mouse). Despite the fact that both IL-4 and IL-13 can utilize the IL-4Rα/IL-13Rα1 complex as a receptor, they do so in quite different manners and their distinctive way of using this receptor accounts for some of the difference in their relative potency particularly on non-hematopoietic cells [20]. IL-4 binds to IL-4Rα with high affinity, approximately $10^{10}\,M^{-1}$, but IL-13Rα1 (or γc) binds weakly to the complex of IL-4/IL-4Rα1 (solution $K = \sim 2 \times 10^{6}\,M^{-1}$) [25] so that under most circumstances, unless IL-13Rα1 or γc are in substantial excess, only a portion of the IL-4/IL-4Rα complexes ever achieve the capacity to signal. By contrast, although IL-13 binds with relatively low affinity