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History of Interleukin-4

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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.

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 (Ohara and Paul, 1987a), 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 (Howard et al., 1982). 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 (Mosier et al., 1977) and our colleagues in LI, as well as several other groups, most notably that of David Parker (Parker, 1975), 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

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role was to concentrate epitope-linked intrinsic stimulants such as LPS on the B cell surface and thus to indirectly activate B cells (Coutinho and Möller, 1975).

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 TLRmediated signaling (Zeng, et al., 2014). 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. (Leadbetter at al., 2002).

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 (Sieckmann et al., 1978a and b). 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 (Howard et al., 1982). 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 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 (Ohara et al., 1985). 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 (Rabin et al., 1985). 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* (Howard et al., 1982). 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 (Isakson et al., 1982). 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 (Vitetta et al., 1985) 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 (Sideras et al., 1985). 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 (Coffman et al., 1986).

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 (Mosmann et al., 1986a) and Vitetta and her colleagues showed that IL-4 could act to promote the growth of T cell lines (Fernandez-Botran et al., 1986).

1986 was capped off by the molecular cloning of mouse and human IL-4, the former by the groups of Honjo (Noma et al., 1986) and of Lee (Lee et al. 1986) and the latter by Lee and Arai and their colleagues ((Yokota et al., 1986). And, at about the same time, the amino acid sequence of the IL-4 protein was obtained by Grabstein and colleagues at Immunex (Grabstein et al,. 1986) and shortly thereafter by our group (Ohara et al., 1987b).

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 (Brown et al., 1989).

Chromosomal Localization and Epigenetic Regulation

In 1988, the *Il4* gene was mapped to mouse chromosome 11 within 1 cM of the *Il3* gene (D'Eustachio et al., 1988) and to the syntenic region in the human, chromosome 5q31 (Sutherland et al., 1988). Thereafter, this region was mapped in detail and it was shown that *IL13* and *IL4* genes were 12 kB apart (Smirnov et al., 1995), 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 (Agarwal and Rao, 1998). Several of these were shown to be important in production of IL-4 using a transgenic mouse model (Lee et al., 2001). One, HSII, is located in the second intron of the *Il4* gene and is tightly associated with both GATA3 and STAT5 binding sites (Cote-Sierra et al., 2004; Wei et al., 2011). 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 (Tanaka et al., 2010). 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 (Wei et al., 2009).

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 (Ohara and Paul, 1987a) reported that a saturable high affinity receptor existed on the surface of T cells. The affinity was ~$3\times10^{10}M^{-1}$ and resting lymphocytes had ~300 receptors per cell but activated lymphocytes had 5 to 10 times more receptors per cell than resting cells; macrophages and mast cells had 2000 to 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 (Park et al.,

1987). However, when the human IL-4 receptor was molecularly cloned at Immunex (Idzerda et al., 1990), 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 (Keegan et al., 1991),

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 (Noguchi et al., 1993), his group and ours showed that γc and IL-4Rα comprised the type I IL-4 receptor signaling complex (Russell et al., 1993). 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 Jacqueline 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 (Wang et al., 1992; Keegan et al., 1994). 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 (Ryan et al., 1996). 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 (Zhu et al., 2001). However, in myeloid cells, IL-4-mediated cell growth depends on the I4R/IRS-2 pathway (Wang et al., 1992).

IL-13 uses as its receptor a complex consisting of the IL-4 binding chain IL-4Rα, and IL-13Rα1 (Hilton et al., 1996; Miloux et al., 1997). 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 (Junttila et al., 2008). IL-4 binds to IL-4Rαwith high affinity, approximately $10^{10}M^{-1}$, but IL-13Rα1 (or γc) binds weakly to the complex of IL-4/IL-4Rα1 (solution K=~$2\times10^{6}M^{-1}$) (LaPorte at al., 2008) so that under most circumstances, unless IL-13Rα1 or γc are in substantial excess, only a portion of the IL-4/IL-4Rαcomplexes ever achieve the capacity to signal. By contrast, although IL-13 binds with relatively low affinity to IL-13Rα1 (K=$3\times10^{7}M^{-1}$), the recruitment of IL-4Rαto the IL-13/IL-13Rα1 complex is ~25 times more efficient than the binding of γc or IL-31Rα1 to IL-4/IL-4Rα(solution K= $5\times10^{7}M^{-1}$). Thus, although much more IL-13 than IL-4 is required to saturate the binding chain, a substantial portion of the receptors can signal in response to IL-13 when the concentration is raised sufficiently whereas IL-4 achieves its

maximum stimulation at low ligand concentration but that maximum is less than that which can be achieved by IL-13 (Junttila et al., 2008).

IL-4 Controls Th2 Differentiation

Tim Mosmann and Bob Coffman subdivided a series of long term CD4 T cell lines into two classes, based on the range of cytokines they produced when stimulated (Mosmann et al., 1986b). Th1 cells produced IFNγ but not IL-4 while Th2 cells produced IL-4, and its congeners, but not IFNγ. This work had an remarkable impact on the those interested in the biology of T cells but it did not speak to the mechanisms through which naïve CD4 T cells acquired the Th1 or Th2 phenotype or even if the fates of these cells were already established in the naive CD4 T cell population. To understand the process of differentiation, our group (Le Gros at al., 1990) and Susan Swain's group (Swain et al., 1990) set up in vitro cultures of naïve CD4 T cells and stimulated them in vitro with polyclonal activators. We used anti-CD3 and anti-CD28 and observed that the appearance of IL-4-producing cells in vitro occurred only when we added both IL-4 and IL-2 to our cultures. We were uncertain as to whether IL-2 was only required to keep cells viable and to insure that they would proliferate so we concentrated our attention on IL-4. It was clear that IL-4 acting relatively early in the culture period was essential for naïve CD4 T cells to acquire IL-4-producing capacity. Susan Swain reached the same conclusion. Later, we would show that IL-2 also was essential for Th2 differentiation (Cote-Sierra et al., 2004; Zhu et al., 2003). We also showed that the vast majority of naïve cells, cultured as single cells, would acquire a Th2 phenotype if antigen and antigen-presenting cells and a source of IL-4 were present (Noben-Trauth et al., 2002). This and related work established that naïve cells had multipotentiality and that if cultured with activators such as cognate antigen or anti-CD3, the phenotype was determined by the presence of specific cytokines during the activation process. The finding that the major product of Th2 cells, IL-4, was also a principle inducer raised the notion that Th differentiation might involve strong positive feedback. This has proven to be correct and to be a general feature with IFNγ acting as the feedback cytokine for Th1 differentiation and IL-21, in an indirect manner, for Th17 cells.

Hidehiro Yamane and I have been interested in the dynamics of this positive feedback in Th2 differentiation (Yamane et al., 2005). Using a culture system in which naïve CD4 TCR transgenic T cells specific for either pigeon cytochrome C or ovalbumin are cultured with sorted myeloid dendritic cells, we showed that low but not high concentrations of cognate peptide induced CD4 T cell production of IL-4 beginning at 12–14 hours of culture. This "early" IL-4 production required STAT5 activation, mediated through endogenously produced IL-2, and the induction of GATA3 but was independent of IL-4. Once this TCR-induced IL-4 reached a sufficient level, it acted on IL-4 receptors on the responding CD4 cells and, through STAT6 phosphorylation, further up-regulated GATA3 expression. Together with continued STAT5 activation, this further enhanced IL-4 production and completed the Th differentiation process.

While Th2 differentiation depends almost completely on IL-4 in vitro, this does not appear to be the case in vivo (van Panhuys et al., 2008). Th2 differentiation independent of IL-4 has been shown most clearly in responses against the helminth *Nippostrongylus brasiliensis*.In

response to this infection, mice that cannot receive an IL-4 signal can nonetheless develop a robust Th2 response. However, GATA3 expression is essential to Th2 differentiation in *N. brasiliensis*-infected animals (Zhu et al., 2004) implying that GATA3 can be induced to a sufficient degree in vivo by IL-4 independent stimuli.

The discovery of GATA3 as essential to Th2 differentiation established the principle of "master regulatory transcription factors" in Th differentiation. The Flavell and Ray laboratories were the first to show the importance of GATA3 in Th2 differentiation (Zheng and Flavell, 1997; Zhang et al., 1997) and the use of mice with conditional GATA3 deletion unequivocally established its central role (Zhu et al., 2004; Pai et al., 2004).

IL-4 works *in vivo*

The first clear demonstration that IL-4 was produced and acted *in vivo* came from a collaboration between Fred Finkelman's laboratory and mine in which it was shown that our monoclonal anti-IL-4 antibody blocked the induction of polyclonal IgE responses in response to stimulation of BALB/c mice with anti-IgD antibody (Finkelman et al., 1986). This established that IL-4 not only could cause switching to IgE but that it was a principal means through this was achieved. This was even more clearly demonstrated when mice with an IL-4 genetic deletion were generated. Kuhn et al. (1991) showed that such mice produced little or no IgE and had a major reduction in serum levels of IgG1. IL-4Ra−/− mice (Noben-Trauth et al., 1997) and Stat6−/− (Kaplan et al., 1996; Takeda et al., 1996; Shimoda et al., 1996) showed similar striking deficiencies in both IgE and IgG1 antibody responses. An extended discussion of the range of in vivo actions of IL-4 is beyond the scope of this historical piece but many of the articles in this issue will address such actions. However, among the more striking and unanticipated functions associated with IL-4 are its role in muscle regeneration (Heredia et al., 2013), in control of adipocyte differentiation (Qiu et al., 2014), and in learning and memory (Derecki et al., 2010).

IL-4 Producing Cells

IL-4 was first shown to be a product of Th2 cells in the classical Mosmann and Coffman Th1/Th2 experiment. However, additional cell types have now been shown to have the capacity to produce IL-4. These include NK T cells (Yoshimoto et al., 1995), basophils (Seder et al., 1991), mast cells (Brown et al., 1987; Plaut et al., 1989) eosinophils (Nonaka et al., 1995; Moqbel et al., 1995). Type II innate lymphoid cells (ILC2 cells) (Moro at al., 2010; Neill et al., 2010; Price et al., 2010) and multipotent progentors type 2 (Saenz et al., 2010) are good producers of IL-13 and IL-5 when stimulated by IL-33 but they fail to produce IL-4 in response to this stimulant. However, if stimulated with PMA and ionomycin, ILC2 cells do produce IL-4.

Indeed, as a general rule, IL-4 production requires a stimulant that elevates intracellular free calcium and its production is in most instances complete inhibited by Cyclosporin A whereas IL-13 production even in response to PMA and ionomycin is only partially sensitive to Csa and, in response to cytokine stimulation, completely resistant (Guo et al., 2008).

The one cell type whose production of IL-4 does not fit the simple model of receptor driven stimulation of transcription, translation and secretion is the eosinophil. It has been argued that in eosinophils, IL-4 is found with in granules and is secreted in the course of piece-meal degranulation characteristic of eosinophils (Bandiera-Melo et al., 2001).

Last Words

IL-4 is the quintessential regulatory cytokine, playing a role in a vast number of immune, and as increasingly recognized, non-immune functions. At the time of its discovery, we little imagined how broad its functionality would be nor did we envisage the possibility that blocking its receptor would prove to be a medically valuable strategy, as has now been shown with the demonstrated efficacy of *Dupilumab* in moderate to severe asthma (Wenzel at al., 2013) and in atopic dermatitis (Beck et al., 2014), although the 26 year time span from the first description of the IL-4 receptor to its demonstrated efficacy as a therapeutic in humans is rather daunting. The IL-4 field has grown enormously from the first experiments that Maureen Howard and I did to one in which Pubmed now lists more than 23,500 papers on this subject. The new findings on its role in energy metabolism, muscle regeneration and learning and memory lead me to expect that the field is far from “mature” and that many exciting new discoveries lie in the future.

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