

EDITORIAL

Clinical & Experimental Allergy

Too much of a good thing: beta-chain overexpression blocks FcεRI signalling by capturing Lyn in the cytosol

This editorial discusses the findings in the paper by Y. Okayama et al. [1] pp. 238–249.

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In this issue of *Clinical & Experimental Allergy*, Okayama et al. [1] from the laboratory of Chisei Ra report yet another interesting finding about a protein that has fascinated the field of allergy research for more than two decades. The current report demonstrates the ability of the FcεRI beta chain to serve as a negative regulator of IgE signalling, something that is somewhat counter-intuitive, but logical when examined closely.

FcεRI beta chain: an unusual signalling partner

Since its discovery and cloning in the 1980s, FcεRI beta has elicited much interest, and for good reason. Beta is an enigmatic partner. In its absence, FcεRI is expressed on human monocytes, dendritic cells and Langerhans cells that employ the $\alpha\gamma_2$ trimeric receptor for antigen presentation [2, 3]. By contrast, the inclusion of beta yields a powerfully inflammatory IgE-binding tetramer restricted to mast cells and basophils that is the crux of allergic disease. Unravelling how the beta chain accomplishes this feat has been an interesting story we have followed since Henry Metzger's group demonstrated that the Src-family tyrosine kinase Lyn is associated with beta and mediates the first wave of tyrosine phosphorylation when FcεRI is activated [4–6]. While their work was groundbreaking, the story was far from over. Jean Pierre Kinet's group later showed that beta amplifies FcεRI expression and function [7, 8], and two further twists developed. First, beta has an odd immunoreceptor tyrosine-based activation motif (ITAM), with a non-canonical tyrosine embedded between the two conserved tyrosines. The non-con-

served tyrosine appears to recruit SHIP-1, an inositol phosphatase that inhibits IgE-induced signals [9, 10]. Second, Lyn does not just initiate signalling; it also suppresses it by recruiting C-terminal Src kinase (CSK) [11]. If this was not enough already, beta can be spliced to yield a truncated form that appears to have regulatory functions still being uncovered [12, 13]. The overall view of beta function is one of nuance: restricted to allergy effector cells where its presence yields a potentially dangerous receptor, beta initially amplifies but subsequently suppresses FcεRI signalling.

Beta associates with allergy: an unfinished story of polymorphisms and inflammation

In addition to its obvious importance in signalling, FcεRI beta has long been suspected as one of the aetiological sources of atopy and allergic disease. In 1993, Sandford et al. reported in *The Lancet* that analysis of 155 sibling pairs for genes related to atopy pointed to FcεRI beta on chromosome 11q13 [14]. This led to a string of conflicting papers in which several beta-chain polymorphisms have been shown to be associated – or not associated – with allergic diseases and IgE responsiveness [15]. Confusion on this topic is not surprising, as polymorphisms could yield beta-chain variants possessing slight advantages that reveal themselves only in the proper context of genetic background and antigen exposure. In keeping with this, studies supporting or refuting beta-chain associations have run the gamut of nationalities and specific diseases. Perhaps most striking was Donnadieu et al.'s contribution in 2000, which showed that polymorphic beta chains did not provide any advantage to FcεRI expression or function in transfected cell lines [16].

This part of the story still seems incomplete, although. From the work of Juan Rivera, we know that genetic background can alter the consequences of Lyn deletion on FcεRI signalling in mast cells [17]. Our

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group and Chisei Ra's has shown that cytokines such as TGFβ1 and GM-CSF can directly suppress beta-chain expression [2, 18], which can also be affected by genetic background [19]. Further, a recent paper from Bannert et al. showed that beta expression is elevated in mast cells found in the upper GI tract of patients with gut inflammation [20]. These provoke the question of how FcεRI beta polymorphisms behave in an *in vivo* environment altered by genetic diversity and chronic inflammation.

The current story: feedback inhibition?

Chisei Ra's laboratory has steadfastly revealed one important molecular detail after another about FcεRI beta, and the current story does not disappoint. In this edition, the authors build on previous work in which they study mast cells among tissue samples from allergic keratoconjunctivitis (AKC) or vernal KC (VKC) patients. These samples allow them to assess *ex vivo* FcεRI expression by confocal microscopy, yielding clinically relevant data. They generate intriguing findings, including the observation that a significant amount of beta chain is not associated with alpha chain inside mast cells. More importantly, mast cells with excess beta chain tended to be found in the perivascular region of these lesions rather than the subepithelium. This alone is an enticing observation, given the recent work of Richard Locksley's group who showed that perivascular mast cells extend pseudopodia through the endothelium to capture circulating IgE [21]. Does perivascular localization confer special traits or functions to mast cells? Is beta overexpression related to this?

Ra's group addressed the importance of cytosolic beta through use of an adenoviral vector expressing beta chain. It is impressive that they performed these studies with primary human mast cells cultured from peripheral

blood and that most of the adenoviral work employs beta chain with no epitope tag that might alter protein localization or interaction. One of the striking aspects of the study is the data shown in Fig. 4 in Okayama et al. Beta-chain overexpression to levels mimicking AKC-derived mast cells results in a nearly complete loss of IgE-induced histamine release, TNF production and PGD₂ secretion. This suppression correlates with reduced Syk and MAPK activation. However, the critical portion of the paper is the clear demonstration that beta overexpression in the cytosol greatly reduces the presence of Lyn at the cell membrane and prevents its redistribution to membrane patches that likely represent rafts. Although only 5% of Lyn is thought to be pre-associated with beta chain prior to activation [4], the presence of cytosolic beta appears to be acting as a Lyn 'sink', preventing its association with functional surface FcεRI tetramers. These data fit prior observations that Lyn is a limiting factor in FcεRI signalling [6, 22]. Okayama et al. offer us yet another twist in an interesting tale. While elevated FcεRI expression has been correlated with allergic disease and is the target of anti-IgE therapy, beta chain possesses its own homeostatic powers, limiting Lyn accessibility when beta is overexpressed. These data reinforce what we know about both beta and Lyn: they participate in both initiating and limiting FcεRI signals.

There are limits to interpreting these data, of course. The sample size of AKC and VKC (a total of nine) is expectedly low. The *ex vivo* observations from these samples are by definition from a pathological environment that may not reflect normal physiological mechanisms. However, use of primary cultured human mast cells expressing beta chain at levels similar to AKC samples is impressive and informative. The take-home message from this well-crafted manuscript is that beta has myriad functions, and the story is not over yet.

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