

# Allergy-Associated Polymorphisms of the FcεRIβ Subunit Do Not Impact Its Two Amplification Functions

Emmanuel Donnadieu,<sup>1\*</sup> William O. Cookson,<sup>†</sup> Marie-Hélène Jouvin,\* and Jean-Pierre Kinet<sup>2\*</sup>

Two variants of the  $\beta$ -chain of the high affinity IgE receptor FcεRI, I181L-V183L and E237G, have been found associated with allergy. We have previously shown that the  $\beta$ -chain plays at least two distinct amplifier functions. It amplifies FcεRI surface expression and signaling, resulting in an estimated 12- to 30-fold amplification of downstream events. To test the hypothesis that the I181L-V183L and E237G  $\beta$  variants may be functionally relevant and could directly contribute to an allergic phenotype, we have evaluated the functional impact of the  $\beta$  variants on the two amplifier functions of  $\beta$ . We found that these variants have no direct effect on the  $\beta$  amplifier functions. However, the possibility remains that these variants are in linkage disequilibrium with other more relevant polymorphisms or are affecting unknown  $\beta$ -chain functions. *The Journal of Immunology*, 2000, 165: 3917–3922.

Allergic (atopic) diseases are characterized by a prolonged production of IgE against common and harmless environmental Ags resulting in inflammatory reactions responsible for a wide range of manifestations including rhinitis, conjunctivitis, dermatitis, and asthma. Atopy results from a combination of numerous environmental and genetic factors. The complexity of the genetic factors involved is evident from the numerous loci for which a linkage to atopy has been reported (1–3). One of these loci, on chromosome 11q13, contains the gene for the  $\beta$ -chain of the high affinity IgE receptor, FcεRI (4–8). Initially, this finding was met with skepticism based on methodological concerns (9), and lack of reproducibility in some populations (10–18). This lack of reproducibility could have been due to genetic heterogeneity among patients, differences in methodology between studies, and to the existence of a maternal pattern of inheritance (5). However, more recently, this linkage has been confirmed in various populations and by other groups (19–26), including the Collaborative Study on the Genetics of Asthma (27). It is the strongest in individuals with prominent symptoms, but it is also robust to phenotype classification, which represents an important criteria for assessing the validity of a linkage, given the variability of the criteria used to defined atopy among various studies.

FcεRI controls the activation of mast cells and basophils through IgE, and participates in IgE-mediated Ag presentation in monocytes and dendritic cells. Multivalent Ags bind and cross-link IgEs held at the cell surface by FcεRI. Receptor aggregation induces multiple signaling pathways that control diverse effector responses, including secretion of allergic mediators and induction of cytokine gene transcription (including genes for IL-4, IL-6,

TNF- $\alpha$ , and GM-CSF; reviewed in Refs. 28–30). Therefore, FcεRI is central to the induction and maintenance of an allergic response. In particular, one component of FcεRI plays a critical role in setting the level of cellular response to IgE and Ag, the  $\beta$ -chain, through its capacity to amplify both cell surface FcεRI expression (31), and FcεRI signaling (32, 33). Human FcεRI exists as two isoforms, a tetramer  $\alpha\beta\gamma_2$  containing the  $\beta$ -chain, and a trimer  $\alpha\gamma_2$  lacking the  $\beta$ -chain. The overall amplification of cellular responses mediated through the  $\alpha\beta\gamma_2$  receptor is 12- to 30-fold compared with responses mediated through the  $\alpha\gamma_2$  receptor (31). Given the intensity of this effect, anything affecting the  $\beta$ -chain is potentially of great value in understanding allergic reactions.

Following the description of the linkage between atopy and the  $\beta$  locus, a search for mutations in FcεRIβ associated with atopy was undertaken and has resulted in the discovery of two polymorphisms in the  $\beta$  coding sequence (34, 35). These findings have been confirmed independently in different populations in some studies (36, 37) but not in others (14, 36, 38); this may be due in part to the difficulty in sequencing this region of the  $\beta$  gene. The first one, a double mutation representing an isoleucine to leucine change at position 181 combined with a valine to leucine change at position 183, is located in the fourth transmembrane domain of  $\beta$  (34). Despite their apparent chemical insignificance, these changes could affect the association of  $\beta$  with the other chains of FcεRI. This hypothesis is based on the observation that a leucine to isoleucine change at a homologous position in the  $\zeta$  chain reduces its interaction with the IgG binding chain of the FcγRIII, CD16 (39). Loss of, or reduction in, association of  $\beta$  with the IgE binding  $\alpha$ -chain and the signaling  $\gamma$ -chains of FcεRI would result in the expression of fewer and less active receptors, and vice versa. The second polymorphism (35), a glutamic acid to glycine change at position 237, is located in the intracellular tail of  $\beta$ , just downstream of an immunoreceptor tyrosine-based activation motif essential for  $\beta$  function (28) and could affect FcεRIβ function.

Genetic studies of disease-associated polymorphisms are critical in uncovering pathways implicated in the pathogeny of complex diseases. However, these genetic studies have to be complemented by a direct assessment of the effects of the polymorphisms on the corresponding pathway. This is exemplified in hypertension, a common multifactorial disease. Mutations in a few genes have been shown to be directly responsible for the phenotype in rare

\*Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215; and <sup>†</sup>Asthma Genetics Group, University of Oxford, Wellcome Trust Center for Human Genetics, Oxford, United Kingdom

Received for publication April 20, 2000. Accepted for publication July 18, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> Current address: Centre National de la Recherche Scientifique, Unité Mixte de Recherche 7627, Hôpital Pitié Salpêtrière, Paris, France.

<sup>2</sup> Address correspondence and reprint requests to Dr. Jean-Pierre Kinet, Department of Pathology, RN227, Beth Israel Deaconess Medical Center, and Harvard Medical School, 99 Brookline Avenue, Boston, MA 02215. E-mail address: jkinet@caregroup.harvard.edu

forms of the disease (40). Despite the fact that these findings apply to only rare cases of the disease, they have contributed substantially to our understanding of the regulation of blood pressure and may provide insights into the mechanisms underlying common forms of hypertension. The need for a direct assessment of the effect of mutations on a phenotype is also emphasized by cases in which disease-associated polymorphisms have no apparent direct effect on the corresponding phenotype. For example, the allergy-associated mutation in the IL-4 receptor, Q576R, which was thought to be functionally important (41), does not affect IL-4 receptor signaling (42). In the case of mutations of the FcεRIβ gene, the only published attempt at assessing the impact of mutations is a qualitative study looking at skin prick testing and in vitro Ag-induced basophil degranulation. It did not find any significant difference between nine 181L subjects and ten 181I subjects (43). However, for both tests used in this study, multiple factors could have masked an effect of the β polymorphisms. For example, it has been long established that individuals can be classified as “low responder” or “high responder” based on the capacity of their basophils to respond to a challenge with IgE and anti-IgE (44). This difference in reactivity, the mechanism of which is unknown, could mask the effect of mutations in FcεRI. This demonstrates the need for a direct assessment of the potential role of the atopy-associated polymorphisms of the FcεRI β-chain in atopy. We have performed such a study by testing the polymorphism effect on the two functions of β, receptor expression amplification and signaling amplification.

## Materials and Methods

### Cell culture, Abs, and reagents

Transfectants in the human monocytic cell line U937 were generated and maintained as described (32). Transfectants in the human basophil cell line KU812 cells were maintained in RPMI 1640 supplemented with 16% FBS, 2 mM L-glutamine, and 0.6 mg/ml G418. Anti-4-hydroxy-3-iodo-5-nitrophenylacetic acid (NIP)<sup>3</sup> human IgE TAN was prepared from a hybridoma provided by Dr. Z. Eshhar (Weizman Institute of Science, Rehovot, Israel) as reported (45). Nonspecific human IgE was obtained from Calbiochem (La Jolla, CA).

### Construction of stable transfectants

The mutated β cDNAs (β181L-V183L and βE237G) were generated from the wild-type (WT) β cDNA subcloned in the vector pBJ1neo using the Quick-change kit (Stratagene, La Jolla, CA). The human α and γ cDNAs were subcloned in the pCDL SRα296 eukaryotic expression vector (46), the human β cDNA in the pBJ1neo eukaryotic expression vector containing a neo resistance cassette (47). U937 cells were cotransfected as described (32). When the α and γ, but not β, cDNAs were cotransfected, the empty pBJ1neo vector was cotransfected. U937 cells were cotransfected as described (32). Stable transfectants in the KU812 cells were generated by electroporation (300 V, 960 μF) with the α and γ constructs and selected with G418.

### Transient transfection

KU812 stable transfectants expressing FcεRI αγ<sub>2</sub> ( $5 \times 10^6$  cells) were cotransfected by electroporation with 10 μg of either a control construct, or one of the pBJ1neo β constructs, and 1 μg of green fluorescent protein (GFP) construct (pGreen Lantern; Life Science, Bethesda, MD). Cells were analyzed 8–48 h after transfection. GFP fluorescence was measured by flow cytometry and used to gate transiently transfected cells.

### Assessment of FcεRI expression by surface staining and flow cytometry

Cells were stained with 1 μg of biotinylated IgE, followed by streptavidin-PE (1:200) (PharMingen, San Diego, CA) and analyzed on a FACScalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ). Alternatively, cells were stained with anti-α mAb 15-1 followed by FITC-

conjugated goat-anti mouse. Untransfected cells were processed in parallel and used as negative control.

### Measurement of surface FcεRI expression by <sup>125</sup>I IgE binding

The anti-NIP human IgE was iodinated with Iodogen (Pierce, Rockford, IL) following the manufacturer instructions. Bindability of iodinated IgE was checked. A binding curve in the presence of increasing concentrations of iodinated IgE was established and used to determine the saturating concentration of IgE after subtraction of nonspecific binding (48). Transfected cells were incubated with <sup>125</sup>I IgE in saturating conditions, unbound IgE was removed, cell-associated counts were measured, and mean receptor numbers per cell were calculated from the binding data.

### Immunoprecipitation and Western blotting

Cell lysis and immunoprecipitation were performed as previously described (32). Briefly, cells were lysed at a ratio of  $3 \times 10^7$  cells/ml of lysis buffer (0.5% Triton X-100, 150 mM NaCl, 200 mM boric acid (pH 8.0), supplemented with the “complete” protease inhibitor mixture (Roche Molecular Biochemicals, Indianapolis, IN). Where indicated, immunoprecipitates were treated with endo-β-N-acetylglucosaminidase (endo-H) (New England Biolabs, Beverly, MA) as previously described (49). Samples were resolved on SDS 13% polyacrylamide gel, transferred to polyvinylidene difluoride membrane, and blotted with the Abs indicated. Immunoreactive proteins were visualized using alkaline phosphatase-coupled second-step reagents and enhanced chemifluorescence (Amersham, Arlington Heights, IL). Fluorescence was quantified using a Storm scanner (Molecular Dynamics, Sunnyvale, CA).

### Measurement of calcium mobilization

U937 transfected cells were incubated with 2 μM fura-2 AM (Molecular Probes, Eugene, OR) and 0.2 mg/ml Pluronic (Molecular Probes) at room temperature for 30 min in a calcium (Ca<sup>2+</sup>) buffer containing 140 mM NaCl, 5 mM KCl, 1 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 10 mM glucose, 10 mM Na HEPES (pH 7.4), 0.25 mM Sulfynpirazone (Sigma, St. Louis, MO) (50) and loaded with either 0.1–10 μg/ml or a saturating concentration (10 μg/ml) of anti-NIP IgE. When a nonsaturating concentration of anti-NIP IgE was used, nonspecific human IgE was added to a total concentration of 10 μg/ml. Cells were washed once in Ca<sup>2+</sup> buffer. Intracellular Ca<sup>2+</sup> concentrations were measured on bulk cell population at room temperature using a cuvette-based spectrofluorometer (Deltascan; Photon Technology, South Brunswick, NJ) before and after triggering with various concentrations (0.02–20 ng/ml) of the multivalent Ag NIP BSA (mean of 43 molecules of NIP per molecule of BSA). NIP BSA was prepared as described (45). Intracellular Ca<sup>2+</sup> concentrations were calculated using the equation established by Grynkiewicz (51). Amplitudes of the initial Ca<sup>2+</sup> response were calculated as the difference between the peak and the baseline.

### Statistics

Averages are expressed ± SD. Comparisons between groups were performed with Student's unpaired *t* test.

## Results and Discussion

The β-chain is known so far to play two critical roles in FcεRI function. It amplifies surface FcεRI expression (31) and FcεRI signaling capacity (32, 33). We hypothesized that the association between the I181L-V183L and E237G polymorphisms and atopy could be due to an effect of these polymorphisms on either or both the amplifier functions of β. To test this hypothesis, we have used a transfection system developed in our laboratory to study the signal amplifier function of β. We have previously demonstrated that results obtained with this in vitro system parallel the results obtained in vivo with animal models of allergic reactions (32, 33). We have used this system to investigate the effect of the β polymorphisms on receptor expression and signal amplification. We generated stable transfectants expressing FcεRI containing a WT (αβγ<sub>2</sub>) or variant β (αβ<sub>181L-183L</sub>γ<sub>2</sub> or αβ<sub>237G</sub>γ<sub>2</sub>) in the monocytic cell line U937, and analyzed the impact of these mutations on the two amplification functions of β.

<sup>3</sup> Abbreviations used in this paper: NIP, 4-hydroxy-3-iodo-5-nitrophenylacetic acid; WT, wild type; GFP, green fluorescent protein; endo-H, endo-β-N-acetylglucosaminidase.

### Effect of I181L-V183L and E237G mutations on the receptor expression amplification capacity of $\beta$

**Effect on Fc $\epsilon$ RI surface expression.** Receptor expression was analyzed in the  $\alpha\beta\gamma_2$ ,  $\alpha\beta_{181L-183L}\gamma_2$ , and  $\alpha\beta_{237G}\gamma_2$  transfectants by flow cytometry after staining with labeled IgE. To accurately assess the whole spectrum of receptor expression, all of the selection-resistant clones were analyzed. (Fig. 1A). As expected given the receptor expression amplifier function of  $\beta$ ,  $\alpha\beta\text{WT}\gamma_2$  clones express significantly more surface Fc $\epsilon$ RI than  $\alpha\gamma_2$  clones (31). With either  $\beta$  variant, surface expression is amplified to an extent similar to that observed with WT $\beta$ . To confirm this absence of effect of the  $\beta$  polymorphisms on receptor expression, we analyzed the effect on receptor expression of transiently transfecting either WT $\beta$  or one of the other of the  $\beta$  variants in clones stably expressing  $\alpha\gamma_2$  (Fig. 1B). As expected, retransfection with WT $\beta$  up-regulates receptor expression (31). Each  $\beta$  variant was as efficient as WT $\beta$  at up-regulating receptor expression. Therefore, it appears that these polymorphisms do not affect the capacity of  $\beta$  to increase surface receptor expression.

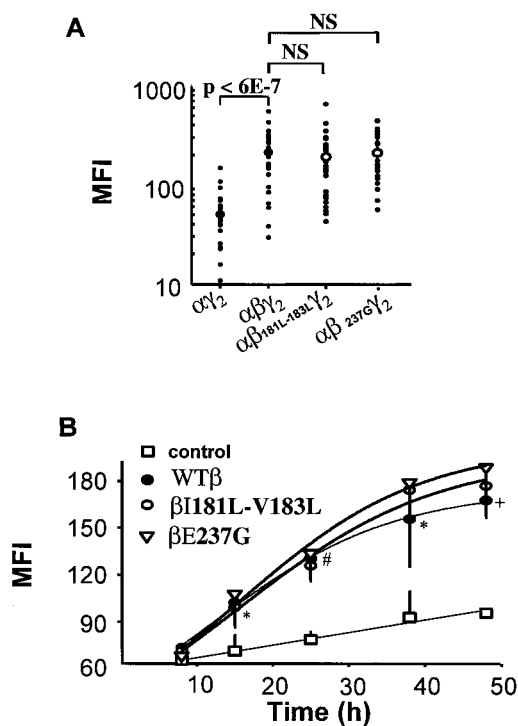


**FIGURE 1.** Distribution of Fc $\epsilon$ RI expression after transfection of the  $\alpha$  and  $\gamma$  cDNAs and constructs containing WT $\beta$ ,  $\beta\text{I181L-V183L}$ , or  $\beta\text{E237G}$ . **A**, U937 cells were stably transfected with the  $\alpha$  and  $\gamma$  cDNAs and with the empty pBJ1neo vector or with constructs containing either WT $\beta$ ,  $\beta\text{I181L-V183L}$ , or  $\beta\text{E237G}$ . Fc $\epsilon$ RI expression was analyzed on all the G418-resistant clones by flow cytometry with biotinylated human IgE followed by PE-conjugated streptavidin. Results are expressed as the mean fluorescence intensity (MFI) for each clone. Each dot represents one clone. The mean for each group is indicated by a white dot. The data for the  $\alpha\gamma_2$  and  $\alpha\beta\gamma_2$  transfectants shown in open symbols are the same as in (31). **B**, A  $\alpha\gamma_2$ -stable transfectant was transiently retransfected with either a control cDNA or a wild type, or variant  $\beta$  cDNA, and with a GFP construct. Fc $\epsilon$ RI expression was measured on the GFP-positive cell population as in **A**, and followed up to 48 h after retransfection. Results are expressed as MFI of GFP-positive cells. For each type of transfectant MFI at each time point were compared with MFI at time 0. \*, NS; #,  $p = 2.1\text{E-}3$ ; +,  $p = 3.3\text{E-}5$ . The data for the control and WT $\beta$  retransfections shown in thin lines are the same as in (31).

**Effect on Fc $\epsilon$ RI intracellular processing.** Receptor expression at the cell surface is the result of a multistep process that includes protein synthesis of the three chains, assembly of the chains to form the trimer  $\alpha\gamma_2$  or the tetramer  $\alpha\beta\gamma_2$ , posttranslational modifications, for example, glycosylation of the  $\alpha$ -chain, and export to the surface. We have recently shown that the  $\beta$ -chain amplifies Fc $\epsilon$ RI expression by associating very early with the IgE-binding Fc $\epsilon$ RI  $\alpha$ -chain and by promoting its intracellular processing (31). This effect of  $\beta$  on  $\alpha$  processing contributes to the increased receptor expression observed in the presence of  $\beta$ . We have asked whether the  $\beta$  polymorphisms could affect the capacity of  $\beta$  to promote  $\alpha$  processing. It is possible that a static analysis of surface receptor expression, such as the one we show in Fig. 1, could be unable to detect differences in efficiency in the steps that precede surface expression, and that, by limiting ourselves to an analysis of surface receptor expression, we could have missed an effect of the  $\beta$  polymorphisms on intracellular receptor processing. To address this issue, we have compared the capacity of the  $\beta\text{I181L-V183L}$  and  $\beta\text{E237G}$  variants to that of the WT $\beta$  to promote  $\alpha$ -chain processing. The  $\alpha$ -chain is highly and heterogeneously  $N$ -glycosylated, with  $\sim 50\%$  of the molecular mass of cell surface  $\alpha$  due to sugars. The newly synthesized  $\alpha$ -chain is core glycosylated (mannose rich) in the endoplasmic reticulum and is thus sensitive to the action of endoglycosidase H (endo-H). It then is transferred to the Golgi, where terminal glycosylation occurs that replaces mannose rich sugars with complex sugars that are endo-H resistant. Thus, these various forms of  $\alpha$  can be distinguished by a combination of endo-H treatment and SDS-PAGE (49). We have exploited  $\alpha$ -chain glycosylation to follow processing of the receptor complex in the presence of the various forms of the  $\beta$ -chain.

Transfected clones expressing the same levels of surface Fc $\epsilon$ RI and containing either the WT $\beta$  or  $\beta\text{I181L-V183L}$ , or  $\beta\text{E237G}$  forms were lysed, and the  $\alpha$ -chains were precipitated with an anti- $\alpha$  mAb (15-1), which can precipitate both mature and immature forms of  $\alpha$ . The samples were then treated, or not, with endo-H and resolved by SDS-PAGE. Western analysis was performed using a polyclonal anti- $\alpha$  Ab (997) (data not shown) (31). A total of three to five clones and two to three uncloned cell populations of  $\alpha\beta\gamma_2$ ,  $\alpha\beta_{181L-183L}\gamma_2$ , and  $\alpha\beta_{237G}\gamma_2$  transfectants were studied. The bands corresponding to the mature and immature forms of  $\alpha$  were identified on their m.w. and their sensitivity to endo-H, their intensities were measured by scanning densitometry, and the ratio between mature and immature  $\alpha$  was calculated (Fig. 2). The amount of mature  $\alpha$  for a given amount of immature  $\alpha$  is not significantly different in the  $\beta\text{I181L-V183L}$ - ( $4.8 \pm 2$ ) and the  $\beta\text{E237G}$  ( $5.8 \pm 1.5$ )-containing cells compared with the WT $\beta$ -containing cells ( $4.9 \pm 3.7$ ). We conclude that the  $\beta$  polymorphisms do not affect the capacity of  $\beta$  to promote the processing of the  $\alpha$ -chain.

### Effect of I181L-V183L and E237G mutations on the signaling amplification capacity of $\beta$

The  $\beta$ -chain amplifies signals generated by the  $\gamma$ -chain. As a result, in  $\alpha\beta\gamma_2$  receptor-expressing cells' signals are 3- to 5-fold more intense than those in  $\alpha\gamma_2$ -expressing cells. We have investigated whether the  $\beta$  polymorphisms affect the signal amplification capacity of  $\beta$ . We have compared the signaling capacity of transfectants expressing Fc $\epsilon$ RI containing either a wild type, or one of the variant  $\beta$ . Calcium flux was chosen as a readout of cell activation induced by Fc $\epsilon$ RI aggregation. Calcium flux is fundamental to the initiation and maintenance of an allergic response, and has been shown to correlate with degranulation (52), receptor phosphorylation, Syk activation, and in vivo responses (33). The amplitude of the initial calcium response depends on the number of

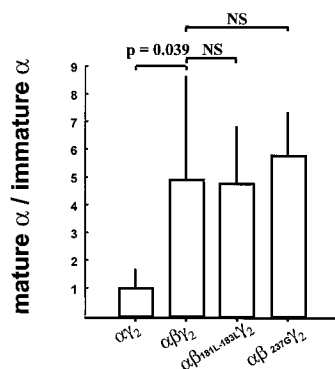


**FIGURE 2.** FcεRI  $\alpha$  maturation and trafficking in the presence of WT $\beta$ ,  $\beta$ I181L-V183L, or  $\beta$ E237G. Cells were lysed and immunoprecipitated with the anti- $\alpha$  mAb 15-1. Immune complexes were treated or not with endo-H, subjected to SDS-PAGE and transferred to polyvinylidene difluoride membrane. Mature and immature forms of  $\alpha$  were revealed by immunoblotting with anti- $\alpha$  polyclonal Ab 997. The bands corresponding to mature and immature  $\alpha$  were scanned. A total of three to five clones and two to three uncloned transfected cell populations of  $\alpha\beta\gamma_2$ ,  $\alpha\beta_{181L-183L}\gamma_2$ , and  $\alpha\beta_{237G}\gamma_2$  transfectants were studied. Results are expressed as mean  $\pm$  SD. The results for the  $\alpha\gamma_2$  and  $\alpha\beta\gamma_2$  transfectants shown in thin lines include some data from (31).

receptors aggregated at the cell surface. To take this factor into account, eight  $\alpha\beta\gamma_2$ , eight  $\alpha\beta_{181L-183L}\gamma_2$ , and six  $\alpha\beta_{237G}\gamma_2$  clones with mean receptor densities ranging from 6,000 to 90,000 receptors per cell were analyzed. In each phenotype the clones selected for analysis included low, medium, and high receptor expressers. Cells were loaded with the fluorescent  $\text{Ca}^{2+}$  dye fura-2 and saturated with the anti-NIP human IgE TAN. Intracellular  $\text{Ca}^{2+}$  concentrations were measured using a spectrofluorometer on bulk populations at baseline and after triggering with the multivalent Ag NIP BSA at an optimal concentration (20 ng/ml). Each clone was studied one to four times in independent experiments that all included at least one clone of each type. The amplitude of the initial calcium response was plotted as a function of FcεRI density (Fig. 3). At equal receptor density, there was no difference in the  $\text{Ca}^{2+}$

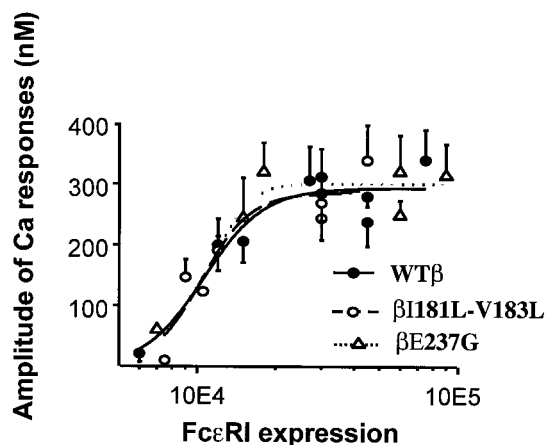


**FIGURE 3.** Amplitude of  $\text{Ca}^{2+}$  responses in transfectants expressing WT $\beta$ ,  $\beta$ I181L-V183L, or  $\beta$ E237G as a function of surface receptor density. Eight  $\alpha\beta\gamma_2$ , eight  $\alpha\beta_{181L-183L}\gamma_2$ , and six  $\alpha\beta_{237G}\gamma_2$  clones with mean receptor densities ranging from 6,000 to 90,000 receptors per cell were loaded with the fluorescent  $\text{Ca}^{2+}$  dye fura-2 and human anti-NIP IgE (10  $\mu\text{g}/\text{ml}$ ) and triggered with an optimal concentration of the multivalent Ag NIP BSA (20 ng/ml). Each clone was studied one to four times in independent experiments. The amplitude of the initial  $\text{Ca}^{2+}$  response is plotted as a function of FcεRI density.

response after FcεRI triggering among clones expressing WT $\beta$  or any of the  $\beta$  variants.

The possibility remains that a difference in signaling capacity between WT $\beta$  and one of the  $\beta$  variants could be detected when triggering is suboptimal, but not when it is optimal. To test this hypothesis we have performed a dose response of  $\text{Ca}^{2+}$  mobilization by varying the concentration of NIP BSA from 0.2 to 20 ng/ml. Fig. 4A shows the amplitude of the initial  $\text{Ca}^{2+}$  response for one WT $\beta$  clone, one  $\beta$ I181L-V183L clone, and one  $\beta$ E237G clone, all expressing a mean of 30,000 receptors per cell. A total of three or four expression-matched clones from each type (mean receptors per cell = 12,000–75,000) were analyzed one to four times in independent experiments and showed comparable results (data not shown). No statistically significant difference could be detected between WT $\beta$ ,  $\beta$ I181L-V183L, and  $\beta$ E237G clones at any Ag concentration.

Another way to generate a suboptimal stimulus is to lower the concentration of the anti-NIP IgE used for cell loading, which results in subsaturating degrees of FcεRI occupancy. A saturating concentration of anti-NIP IgE (10  $\mu\text{g}/\text{ml}$ ) or lower, nonsaturating concentrations (0.5 and 2  $\mu\text{g}/\text{ml}$ ) were used. The total IgE concentration was kept at 10  $\mu\text{g}/\text{ml}$  by addition of nonspecific human IgE. Fig. 4B shows the amplitude of the initial  $\text{Ca}^{2+}$  response as a

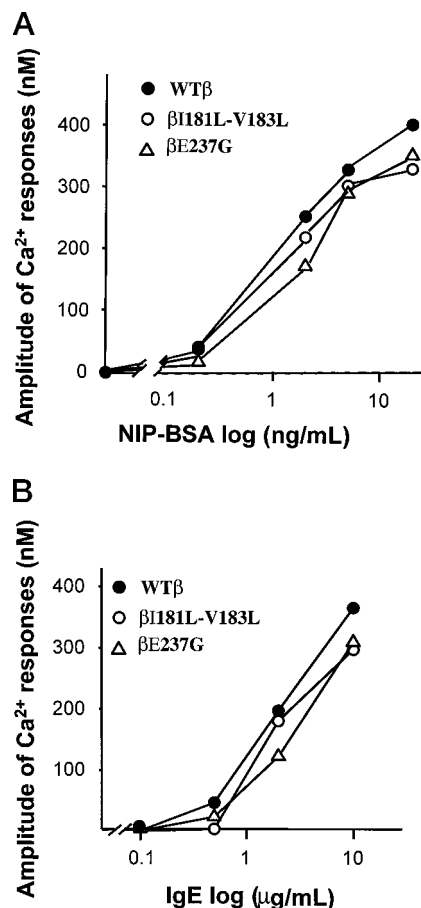


**FIGURE 4.** Amplitude of  $\text{Ca}^{2+}$  responses in transfectants expressing WT $\beta$ ,  $\beta$ I181L-V183L, or  $\beta$ E237G as a function of triggering intensity. A, The experiment was performed as in Fig. 3 except that one clone of each type expressing 30,000 receptors per cell was studied at various Ag (NIP BSA) concentrations. This figure is representative of a total of three independent experiments performed with the same clones. B, The experiments were performed as in A except that various concentrations of anti-NIP IgE were used for loading.

function of IgE concentration for one WT $\beta$  clone, one  $\beta$ 181L-V183L clone, and one  $\beta$ E237G clone, all expressing a mean of 30,000 receptors per cell. Again here, no difference could be detected between WT $\beta$ ,  $\beta$ 181L-V183L, and  $\beta$ E237G clones at any level of receptor occupancy.

We conclude that, despite their association with atopy, these  $\beta$  polymorphisms appear to have no effect on Fc $\epsilon$ RI expression and function. Various explanations can be proposed for this discrepancy. These polymorphisms could affect unknown functions of  $\beta$ . However, it is possible that there are still unknown functions of  $\beta$ , some of which may be optimally detected in the physiological context of the receptor. Alternatively, these  $\beta$  polymorphisms could be in linkage disequilibrium with unknown polymorphism(s) directly responsible for the atopic phenotype. These other polymorphisms could be in the  $\beta$  gene or in other genes at the 11q13 locus, for example, HTm4, a molecule related to Fc $\epsilon$ RI  $\beta$  (53). Other polymorphisms have been recently found in the regulatory and promoter regions of the  $\beta$  gene, which may affect receptor expression, and whose effects on Fc $\epsilon$ RI expression and signaling have not been evaluated (54–56).

**Note added in proof.** A paper published after acceptance of our manuscript (57) reported that the presence of  $\beta$ 181,  $\beta$ 183, or  $\beta$ 237 did not affect the signal amplification function of  $\beta$ . This was assessed by  $\beta$ -hexosaminidase release, calcium mobilization, and secretion of IL-6, TNF- $\alpha$ , and LTC4 in bone marrow-derived mast cells from  $\beta^{-/-}$  mice reconstituted by retroviral infection.

## References

- Cookson, W. 1999. The alliance of genes and environment in asthma and allergy. *Nature* 402:85.
- Moffatt, M. F., and W. O. C. M. Cookson. 1999. Genetics of asthma and inflammation: the status. *Curr. Opin. Immunol.* 11:606.
- Ober, C., and M. Moffatt. 2000. Contributing factors to the pathobiology of asthma: The genetics of asthma. In *Clinics in Chest Medicine*. S. Wenzel, ed. Saunders, Philadelphia.
- Cookson, W. O., P. A. Sharp, J. A. Faux, and J. M. Hopkin. 1989. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. *Lancet* 1:1292.
- Cookson, W. O., R. P. Young, A. J. Sandford, M. F. Moffatt, T. Shirakawa, P. A. Sharp, J. A. Faux, C. Julier, P. N. LeSouef, Y. Nakamura, et al. 1992. Maternal inheritance of atopic IgE responsiveness on chromosome 11q. *Lancet* 340:381.
- Young, R. P., P. A. Sharp, J. R. Lynch, J. A. Faux, G. M. Lathrop, W. O. C. M. Cookson, and J. M. Hopkin. 1992. Confirmation of genetic linkage between atopic IgE responses and chromosome 11q13. *J. Med. Genet.* 29:236.
- Hizawa, N., E. Yamaguchi, M. Ohe, A. Itoh, K. Furuya, N. Ohnuma, and Y. Kawakami. 1992. Lack of linkage between atopy and locus 11q13. *Clin. Exp. Allergy* 22:1065.
- Cox, H. E., M. F. Moffatt, J. A. Faux, A. J. Walley, R. Coleman, R. C. Trembath, W. O. Cookson, and J. I. Harper. 1998. Association of atopic dermatitis to the  $\beta$  subunit of the high affinity immunoglobulin E receptor. *Br. J. Dermatol.* 138:182.
- Marsh, D. G., and D. A. Meyers. 1992. A major gene for allergy—fact or fancy? *Nat. Genet.* 2:252.
- Rich, S. S., B. Roitman-Johnson, B. Greenberg, S. Roberts, and M. N. Blumenthal. 1992. Genetic analysis of atopy in three large kindreds: no evidence of linkage to D11S97. *Clin. Exp. Allergy* 22:1070.
- Lympay, P., K. Welsh, G. MacCochrane, D. M. Kemeny, and T. H. Lee. 1992. Genetic analysis using DNA polymorphism of the linkage between chromosome 11q13 and atopy and bronchial hyperresponsiveness to methacholine. *J. Allergy Clin. Immunol.* 89:619.
- Lympay, P., K. I. Welsh, G. M. Cochrane, D. M. Kemeny, and T. H. Lee. 1992. Genetic analysis of the linkage between chromosome 11q and atopy. *Clin. Exp. Allergy* 22:1085.
- Amelung, P. J., C. I. Panhuysen, D. S. Postma, R. C. Levitt, G. H. Koeter, C. A. Francomano, E. R. Bleecker, and D. A. Meyers. 1992. Atopy and bronchial hyperresponsiveness: exclusion of linkage to markers on chromosomes 11q and 6p. *Clin. Exp. Allergy* 22:1077.
- Martinati, L. C., E. Trabetti, A. Casartelli, A. L. Boner, and P. F. Pignatti. 1996. Affected sib-pair and mutation analyses of the high affinity IgE receptor  $\beta$  chain locus in Italian families with atopic asthmatic children. *Am. J. Respir. Crit. Care Med.* 153:1682.
- Wong, Z. Y., D. Tsonis, L. van Herwerden, J. Raven, A. Forbes, M. J. Abramson, E. H. Walters, and S. B. Harrap. 1997. Linkage analysis of bronchial hyperactivity and atopy with chromosome 11q13. *Electrophoresis* 18:1641.
- Amelung, P. J., D. S. Postma, J. Xu, D. A. Meyers, and E. R. Bleecker. 1998. Exclusion of chromosome 11q and the Fc $\epsilon$ RI- $\beta$  gene as aetiological factors in allergy and asthma in a population of Dutch asthmatic families. *Clin. Exp. Allergy* 28:397.
- Noguchi, E., M. Shibasaki, T. Arinami, K. Takeda, T. Maki, T. Miyamoto, T. Kawashima, K. Kobayashi, and H. Hamaguchi. 1997. Evidence for linkage between asthma/atopy in childhood and chromosome 5q31–q33 in a Japanese population. *Am. J. Respir. Crit. Care Med.* 156:1390.
- Deichmann, K. A., B. Starke, S. Schlenker, A. Heinzmann, S. H. Sparholt, J. Forster, and J. Kuehr. 1999. Linkage and association studies of atopy and the chromosome 11q13 region. *J. Med. Genet.* 36:379.
- Young, R. P., P. A. Sharp, J. R. Lynch, J. A. Faux, G. M. Lathrop, W. O. Cookson, and J. M. Hopkin. 1992. Confirmation of genetic linkage between atopic IgE responses and chromosome 11q13. *J. Med. Genet.* 29:236.
- Collee, J. M., L. P. ten Kate, H. G. de Vries, J. W. Kliphuis, K. Bouman, H. Scheffer, and J. Gerritsen. 1993. Allele sharing on chromosome 11q13 in sibs with asthma and atopy. *Lancet* 342:936.
- Szepietowski, P., and P. Gaudray. 1993. Possible role for Fc $\epsilon$ RI  $\beta$  in atopy. *Lancet* 341:1346.
- Shirakawa, T., T. Hashimoto, J. Furuyama, T. Takeshita, and K. Morimoto. 1994. Linkage between severe atopy and chromosome 11q13 in Japanese families. *Clin. Genet.* 46:228.
- van Herwerden, L., S. B. Harrap, Z. Y. Wong, M. J. Abramson, J. J. Kutin, A. B. Forbes, J. Raven, A. Lanigan, and E. H. Walters. 1995. Linkage of high-affinity IgE receptor gene with bronchial hyperactivity, even in absence of atopy. *Lancet* 346:1262.
- Folster-Holst, R., H. W. Moises, L. Yang, W. Fritsch, J. Weissenbach, and E. Christophers. 1998. Linkage between atopy and the IgE high-affinity receptor gene at 11q13 in atopic dermatitis families. *Hum. Genet.* 102:236.
- Trabetti, E., V. Cusin, G. Malerba, L. C. Martinati, A. Casartelli, A. L. Boner, and P. F. Pignatti. 1998. Association of the Fc $\epsilon$ RI $\beta$  gene with bronchial hyperresponsiveness in an Italian population. *J. Med. Genet.* 35:680.
- Palmer, L. J., S. E. Daniels, P. J. Rye, N. A. Gibson, G. K. Tay, W. O. Cookson, J. Goldblatt, P. R. Burton, and P. N. LeSouef. 1998. Linkage of chromosome 5q and 11q gene markers to asthma-associated quantitative traits in Australian children. *Am. J. Respir. Crit. Care Med.* 158:1825.
- Hizawa, N., L. R. Freidhoff, E. Ehrlich, Y. F. Chiu, D. L. Duffy, C. Schou, G. M. Dunston, T. H. Beaty, D. G. Marsh, K. C. Barnes, and S. K. Huang. 1998. Genetic influences of chromosomes 5q31–q33 and 11q13 on specific IgE responsiveness to common inhaled allergens among African American families: collaborative study on the genetics of asthma (CSGA). *J. Allergy Clin. Immunol.* 102:449.
- Kinet, J. P. 1999. The high-affinity IgE receptor (Fc $\epsilon$ RI): from physiology to pathology. *Annu. Rev. Immunol.* 17:931.
- Turner, H., and J.-P. Kinet. 1999. Fc $\epsilon$ RI signaling, thresholds and tuning in the generation of an allergic response. *Nature* 402:B24.
- Galli, S. J. 2000. Mast cells and basophils. *Curr. Opin. Hematol.* 7:32.
- Donnadieu, E., M.-H. Jouvin, and J.-P. Kinet. 2000. A second amplifier function for the allergy associated Fc $\epsilon$ RI- $\beta$  subunit. *Immunity* 12:515.
- Lin, S., C. Cicala, A. M. Scharenberg, and J. P. Kinet. 1996. The Fc $\epsilon$ RI  $\beta$  subunit functions as an amplifier of Fc $\epsilon$ RI  $\gamma$ -mediated cell activation signals. *Cell* 85:985.
- Dombrowicz, D., S. Lin, V. Flamand, A. T. Brini, B. H. Koller, and J. P. Kinet. 1998. Allergy-associated Fc $\epsilon$ R $\beta$  is a molecular amplifier of IgE- and IgG-mediated in vivo responses. *Immunity* 8:517.
- Shirakawa, T., A. Li, M. Dubowitz, J. W. Dekker, A. E. Shaw, J. A. Faux, C. Ra, W. O. Cookson, and J. M. Hopkin. 1994. Association between atopy and variants of the  $\beta$  subunit of the high-affinity immunoglobulin E receptor. *Nat. Genet.* 7:125.
- Hill, M. R., and W. O. Cookson. 1996. A new variant of the  $\beta$  subunit of the high-affinity receptor for immunoglobulin E (Fc $\epsilon$ RI- $\beta$  E237G): associations with measures of atopy and bronchial hyper-responsiveness. *Hum. Mol. Genet.* 5:959.
- Green, S. L., M. C. Gaillard, E. Song, J. B. Dewar, and A. Halkas. 1998. Polymorphisms of the  $\beta$  chain of the high-affinity immunoglobulin E receptor (Fc $\epsilon$ RI- $\beta$ ) in South African black and white asthmatic and nonasthmatic individuals. *Am. J. Respir. Crit. Care Med.* 158:1487.
- Hijazi, Z., M. Z. Haider, M. R. Khan, and A. A. Al-Dowaisan. 1998. High frequency of IgE receptor Fc $\epsilon$ RI $\beta$  variant (Leu<sup>181</sup>/Leu<sup>183</sup>) in Kuwaiti Arabs and its association with asthma. *Clin. Genet.* 53:149.
- Deichmann, K. A., F. Hildebrandt, A. Heinzmann, S. Schlenker, J. Forster, and J. Kuehr. 1996. Absence of mutations in the 6th exon of Fc $\epsilon$ RI- $\beta$ . *Adv. Exp. Med. Biol.* 409:355.
- Kurosaki, T., I. Gander, and J. V. Ravetch. 1991. A subunit common to an IgG Fc receptor and the T-cell receptor mediates assembly through different interactions. *Proc. Natl. Acad. Sci. USA* 88:3837.
- Lifton, R. P. 1996. Molecular genetics of human blood pressure variation. *Science* 272:676.
- Hershey, G. K., M. F. Friedrich, L. A. Esswein, M. L. Thomas, and T. A. Chatila. 1997. The association of atopy with a gain-of-function mutation in the  $\alpha$  subunit of the interleukin-4 receptor. *N. Engl. J. Med.* 337:1720.
- Wang, H. Y., C. P. Shelburne, J. Zamorano, A. E. Kelly, J. J. Ryan, and A. D. Keegan. 1999. Cutting edge: effects of an allergy-associated mutation in the human IL-4R $\alpha$  (Q576R) on human IL-4-induced signal transduction. *J. Immunol.* 162:4385.
- Li, A., G. A. Mackay, and J. M. Hopkin. 1997. Functional analysis of histamine release from basophils and mast cells in subjects with the Ile-181→Leu variant of Fc $\epsilon$ RI- $\beta$ . *Clin. Sci.* 93:279.
- MacGlashan, D. W., Jr. 1993. Releasability of human basophils: cellular sensitivity and maximal histamine release are independent variables. *J. Allergy Clin. Immunol.* 91:605.

45. Schwarzbaum, S., A. Nissim, I. Alkalay, M. C. Ghazi, D. G. Schindler, Y. Bergman, and Z. Eshhar. 1989. Mapping of murine IgE epitopes involved in IgE-Fcε receptor interactions. *Eur. J. Immunol.* 19:1015.
46. Yokota, T., T. Otsuka, T. Mosmann, J. Banachereau, T. DeFrance, D. Blanchard, J. E. De Vries, F. Lee, and K. Arai. 1986. Isolation and characterization of a human interleukin cDNA clone, homologous to mouse B-cell stimulatory factor 1, that expresses B-cell- and T-cell-stimulating activities. *Proc. Natl. Acad. Sci. USA* 83:5894.
47. Lin, A. Y., B. Devaux, A. Green, C. Sagerstrom, J. F. Elliott, and M. M. Davis. 1990. Expression of T cell antigen receptor heterodimers in a lipid-linked form. *Science* 249:677.
48. Kulczycki, A., Jr., and H. Metzger. 1974. The interaction of IgE with rat basophilic leukemia cells. II. Quantitative aspects of the binding reaction. *J. Exp. Med.* 140:1676.
49. Letourneur, O., S. Sechi, J. Willette-Brown, M. W. Robertson, and J. P. Kinet. 1995. Glycosylation of human truncated FcεRI α chain is necessary for efficient folding in the endoplasmic reticulum. *J. Biol. Chem.* 270:8249.
50. Jouvin, M. H., M. Adamczewski, R. Numerof, O. Letourneur, A. Valle, and J. P. Kinet. 1994. Differential control of the tyrosine kinases Lyn and Syk by the two signaling chains of the high affinity immunoglobulin E receptor. *J. Biol. Chem.* 269:5918.
51. Grynkiewicz, G., M. Poenie, and R. Y. Tsien. 1985. A new generation of Ca<sup>2+</sup> indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260:3440.
52. Kim, T. D., G. T. Eddlestone, S. F. Mahmoud, J. Kuchtey, and C. Fewtrell. 1997. Correlating Ca<sup>2+</sup> responses and secretion in individual RBL-2H3 mucosal mast cells. *J. Biol. Chem.* 272:31225.
53. Adra, C. N., X. Q. Mao, H. Kawada, P. S. Gao, B. Korzycka, J. L. Donate, S. R. Shaldon, P. Coull, M. Dubowitz, T. Enomoto, et al. 1999. Chromosome 11q13 and atopic asthma. *Clin. Genet.* 55:431.
54. Palmer, L. J., P. D. Pare, J. A. Faux, M. F. Moffatt, S. E. Daniels, P. N. LeSouef, P. R. Bremner, E. Mockford, M. Gracey, R. Spargo, et al. 1997. FcεRI-β polymorphism and total serum IgE levels in endemically parasitized Australian aborigines. *Am. J. Hum. Genet.* 61:182.
55. Rohrbach, M., R. Kraemer, and S. Liechti-Gallati. 1998. Screening of the FcεRI-β-gene in a Swiss population of asthmatic children: no association with E237G and identification of new sequence variations. *Dis. Markers* 14:177.
56. Dickson, P. W., Z. Y. Wong, S. B. Harrap, M. J. Abramson, and E. H. Walters. 1999. Mutational analysis of the high affinity immunoglobulin E receptor β subunit gene in asthma. *Thorax* 54:409.
57. Furumoto, Y., S. Hiraoka, K. Kawamoto, S. Masaki, T. Kitamura, K. Okumura, and C. Ra. 2000. Polymorphisms in FcεRI β chain do not affect IgE-mediated mast cell activation. *Biochem. Biophys. Res. Commun.* 273:765.