Self-Restrained B Cells Arise following Membrane IgE Expression

Brice Laffleur, Sophie Duchez, Karin Tarte, Nicolas Denis-Lagache, Sophie Péron, Claire Carrion, Yves Denizot, Michel Cogné

To cite this version:

Brice Laffleur, Sophie Duchez, Karin Tarte, Nicolas Denis-Lagache, Sophie Péron, et al.. Self-Restrained B Cells Arise following Membrane IgE Expression. Cell Reports, Elsevier (Cell Press), 2015, 10 (6), pp.900-909. 10.1016/j.celrep.2015.01.023. hal-01116949

HAL Id: hal-01116949
https://hal-univ-rennes1.archives-ouvertes.fr/hal-01116949
Submitted on 16 Feb 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Self-Restrained B Cells Arise following Membrane IgE Expression

Graphical Abstract

Highlights
- Membrane IgE appears transiently and is then internalized
- The IgE B cell receptor (BCR) spontaneously co-localizes with lipid rafts
- IgE BCR expression restrains mobility responses to chemokines
- mlgE promotes apoptosis of IgE+ cells, severely impacting their fate

Authors
Brice Laffleur, Sophie Duchez, ..., Yves Denizot, Michel Cogne

Correspondence
cogne@unilim.fr

In Brief
IgE responses may convey severe allergy and thus need tight control. Laffleur et al. demonstrate a self-restrained B cell stage where membrane IgE expression impacts the phenotype, shape, and mobility of B lymphocytes, promotes apoptosis, and severely shortens IgE+ B cell fate.

Accession Numbers
GSE64130
Self-Restrained B Cells Arise following Membrane IgE Expression

Brice Laffleur,1,2 Sophie Duchez,1,2 Karin Tarte,3 Nicolas Denis-Lagache,1,2 Sophie Péron,1,2 Claire Carrion,1,2 Yves Denizot,1,2 and Michel Cogne1,2,*

1Université de Limoges, 87000 Limoges, France
2CNRS UMR 7276, Institut Universitaire de France, Limoges 87000, France
3INSERM U917, Université de Rennes, Rennes 35000, France
*Correspondence: cogne@unilim.fr

http://dx.doi.org/10.1016/j.celrep.2015.01.023
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

SUMMARY

Among immunoglobulins (Igs), IgE can powerfully contribute to antimicrobial immunity and severe allergy despite its low abundance. IgE protein and gene structure resemble other Ig classes, making it unclear what constrains its production to thousand-fold lower levels. Whether class-switched B cell receptors (BCRs) differentially control B cell fate is debated, and study of the membrane (m)IgE class is hampered by its elusive in vivo expression. Here, we demonstrate a self-controlled mIgE+ B cell stage. Primary or transfected mIgE+ cells relocate the BCRs into spontaneously internalized lipid rafts, lose mobility to chemokines, and change morphology. We suggest that combined proapoptotic mechanisms possibly involving Hax1 prevent mIgE+ memory lymphocyte accumulation. By uncoupling in vivo IgE switching from cytokine and antigen stimuli, we show that these features are independent from B cell stimulation and instead result from mIgE expression per se. Consequently, few cells survive IgE class switching, which might ensure minimal long-term IgE memory upon differentiation into plasma cells.

INTRODUCTION

In mammals, IgE contributes to immunity against pathogens and toxins (Marichal et al., 2013; Palm et al., 2013). It also yields self-control IgE immune memory. Since IgE+ cells are scarce in vivo, it is unclear whether, as for other Igs, cells with an IgE B cell receptor (BCR) become memory lymphocytes. Globally, humoral memory relies on the dual ability of B cells to either differentiate into long-lived plasma cells (PCs) or survive as memory B lymphocytes. The BCR, providing both tonic and ligation-induced signals, includes membrane-anchored Igs (mIgs) differing from secreted Igs by inclusion of an extracellular membrane-proximal domain, a transmembrane segment, and a cytoplasmic tail varying between heavy chain (HC) classes (Venkitaraman et al., 1991). Human mlgE exists with either a long (“mL IgE”) or a short (“mS IgE”) membrane-proximal domain (Batista et al., 1995).

Mouse mlgE was studied in the abundant mlgE+ B cells appearing in vitro after class switch recombination (CSR) in the presence of interleukin-4 (IL-4) (Anand et al., 1997; Coffman et al., 1986). Such mlgE+ B cells are rare in vivo, except in T/B monoclonal or hyper-TFα LATY136F mice (Aguado et al., 2002; Genton et al., 2006, Erazo et al., 2007). A single spontaneous mlgE+ lymphoma cell line was reported (Sitia, 1985). In mlgE+ cells, CSR expression is weak (Karnowski et al., 2006). That mlgE+ B cells are mandatory for generating IgE PCs (similar to mlgG and mlgA) was proven by knocking out membrane exons (Achatz et al., 1997; Amin et al., 2012; Kaisho et al., 1997). To avoid Fcε receptor-mediated artifacts, IgE+ B cells can be characterized by intracellular rather than surface staining (Wesemann et al., 2011). After tagging mlgE or knocking in an IRES-GFP cassette downstream from Cε, it was suggested that within germinal centers, caspase activation was higher in IgE+ than in IgG1+ cells (Talay et al., 2012; He et al., 2013). However, the IRES-GFP strategy monitors not only mlgE but also germline Cε transcription. It was also proposed that among antigen-activated cells, mlgE+ cells vanished through accelerated differentiation into PCs and were more apoptotic and less mobile than IgG+ cells (Yang et al., 2012). These observations did not provide an explanation for the increased ratio of PCs versus activated B lymphocytes in the IgE+ compartment, and more importantly with regard to long-term immunity, they gave no clue about the status of memory B cells.

In immature mouse B cells, transfected IgE expression resulted in BCR ligation-inducible growth inhibition as with IgM (Batista et al., 1996). In mature cells binding antigen, it is unknown whether class-switched and naive IgM+ cells are differentially stimulated or eventually undergo activation-induced cell death (Guzman-Rojas et al., 2002; Figgett et al., 2013; Péron et al., 2012). Cross-linking of a transfected mlgE was reported to induce apoptosis (Poggianella et al., 2006). In vivo, the rarity of mlgE+ cells could involve either a specific influence of cytokines and cell interactions promoting IgE CSR or specific signals from the mlgE BCR. Globally, mlgE+ cells have only been observed transiently and in minute amounts in vivo after B cell activation, but never as resting memory B cells. These peculiar restrictions of IgE synthesis prompted us to look for a putative mlgE-BCR-dependent and B cell intrinsic self-control. To explore mlgE
specificities, we designed models uncoupling mlgE expression from immune activation, and we evaluated the potential changes of B cell fate resulting from mlgE expression per se. We checked whether mlgE can mimic mlgM and support B cell survival, as shown for other HCs (Duchez et al., 2010; Horikawa et al., 2007; Lutz et al., 1998). We show that, independent of stimulation, mlgE expression per se alters the B cell phenotype in multiple regards, with notably a short lifespan.

RESULTS

Premature Cε Expression Ablates Mature B Cells

While abundant in vitro upon stimulation in the presence of IL-4, mlgE⁺ cells barely appear in vivo. We forced mlgE expression and set up the εKI mutation replacing Sε with a human Cε, encoding both secreted Igε and mlgE (Figures S1A and S1B). Human IgE weakly binding mouse Fcε receptors was chosen to prevent nonspecific staining (Wesemann et al., 2011). Heterozygous wt/εKI mice were derived, but no B cell expressing IgE appeared (Figure S1C). This culminated in complete B cell lymphopenia in εKI/εKI animals, with bone marrow expansion of CD43⁺/CD25⁺ pro-B (16.5% to 94.7% of all B220⁺/CD19⁺ cells, p < 0.0001) but lack of CD25⁺/B220⁺ pre-B cells (43.4% to 0.05% of all B220⁺/CD19⁺ cells, p < 0.0001), while both follicular and marginal zone B cells were absent (Figure S1D). Neither murine Ig nor human IgE were detected in blood (Figure S1E). This did not involve defective association of human ε HC with mouse surrogate light chains since a transected human ε HC efficiently reached the surface of mouse 18–81 pre-B cells (Figure S1F).

Apoptosis and Poor Survival of Primary mlgE⁺ Cells

We set up a staining protocol that removed bound soluble IgE from the cell surface (Figure S1G) and confirmed that true mlgE⁺ cells appear in vitro under anti-CD40/IL-4 stimulation of wt mouse B cells. They proved more prone to spontaneous or cytokine-deprivation induced apoptosis than mlgG1⁺ cells (Figures 1A and 1B). Decreased ΔΨm potential suggested mitochondrial apoptosis, although use of bcl2 transgenic cells preserved a difference between mlgG1⁺ and mlgE⁺ cells (Figures 1A and 1B).

LATY136F mutant mice overproducing Tπ2 cytokines (Genton et al., 2006) generated [B220⁺, IgE⁺] cells in vivo, expressing CD138 much more frequently than IgM⁺ or IgG⁺ cells and thus likely engaged in PC differentiation (Figure 1C). Two days after bromodeoxyuridine (BrdU) injection, these cells stained as short-lived BrdU⁺ cells (Figure 1D).

To compare survival according to Ig class, we transferred LATY136F splenocytes into RAG2⁻/⁻ γC⁻⁻/⁻ mice. Cell survival was estimated by monitoring levels of μ, γ1, and ε HC transcripts in spleen 48 hr post-transfer compared with their “input” amount in transferred cells. Differences appeared between persisting μ expression, falling γ1 levels, and still more strongly vanishing ε expression. The faster disappearance of ε versus γ1 and μ transcripts affected not only membrane but also secreted Ig transcripts, refuting the hypothesis that mlgE⁺ cells disappeared by differentiating into PCs (Figure 1E).

Convergent observations were made in vitro CD40L/IL4-stimulated human cells grafted to RAG2⁻/⁻ γC⁻⁻/⁻ mice: transcripts evaluated after 48 hr indicated collapsed IgE production compared with IgM (Figure 1F).

mlgE-Dependent Changes in B Cells

The short lifespan of IgE-switched wt or LATY136F cells might relate to either mlgE expression per se or a short-lived program promoted by Tπ2 stimuli. We designed models for mlgE expression in B cells without prior stimulus by transiently transfecting human or murine cell lines. Expression vectors encoded either the short or long (mS or mL) form of human mlgE, or mlgM as a control. In all cases, cytometry showed increased early apoptosis (with annexin V staining) specific to mlgE⁺ cells. Decreased ΔΨm potential confirmed mitochondrial apoptosis (Figures 2A and 2B). Accordingly, apoptosis was inhibited by cyclosporin A or caspase inhibitor Q-VD-OPH (Figure 2B). Thus, mlgE expression promotes apoptosis independently of B cell stimulation in cell lines. The 28 amino acid long mlgE tail was reported to interact with Hax1, putatively through a conserved tyrosine-based DYANILQ motif in mouse IgE (found as DYTNLQ in human IgE, but absent in tails of all other Ig classes) (Oberndorfer et al., 2006). It was recently shown that Hax1 is an apoptosis inhibitor binding mitochondria and that the vpr protein triggers apoptosis by relocating Hax1 (Simmen, 2011). We wondered whether mlgE expression could also re-locate Hax1 and carried out flow imaging of BL41 transfectants. While transfection with control vectors preserved homogenous Hax1 staining, mlgE expression associated with Hax1 relocation and heterogeneous staining (quantified by flow-imaging bright detail evaluation) (Figures 2C and 2D). Mouse IgE⁺ primary cells also featured increased heterogeneous Hax1 staining (Figures 2D and S2A). Co-localization experiments of Hax1 and mitochondria-targeted DsRed showed mitochondrial Hax1 depletion in mlgE⁺ cells (Figure 2E). Finally, Hax1 staining globally increased in mlgE⁺ cells (mean fluorescence intensity 36,824 for mlgG1⁺ versus 68,864 for mlgE⁺ mouse primary B cells and 145,667 for empty vector-transfected versus 332,353 for mlgE-transfected BL41 cells). This might result from lowered Hax1 catabolism upon binding IgE, since Hax1 normally undergoes rapid proteosomal degradation (Li et al., 2012).

We also inserted a VDJ segment upstream of εC in the εKI construct, checking that it supported mlgE expression in trans-fected murine A20 cells. While a Cμl control construct readily yielded stable mlgM⁺ transfectants, mlgE expression first appeared transient. Obtaining stable transfectants required repeated sorting of mlgE⁺ cells. Both transiently transfected and sorted/stabilized mlgE⁺ cells constantly showed higher apoptosis than untransfected cells or mlgM⁺ transfectants (Figure S2B). We looked for transcriptional changes between un-transfected, stable mlgM⁺ and two independent stable mlgE⁺ A20 transfectants. Unsupervised analysis clustered both ε HC transfectants together, while the μHC transfectant clustered with untransfected A20. The strongest (over 3-fold) changes in mlgE⁺ cells fell into four major categories (Figure 3A; Table S1):

- Apoptosis: several pro-apoptotic genes were strongly up-regulated including Pdla3, Card12, and two inducers of mitochondrial apoptosis (Map3K9 and Bim), while some
anti-apoptotic genes were underexpressed, including Bcl3 and Cdkn1a.

- Metabolism: upregulation of DecR1, a fatty acid catabolism enzyme inhibiting cell proliferation; Slc16a3, which extracts lactate; and Bhd1, a ketone pathway regulator.

- Signaling and mobility: two transporters extracting cytosolic Ca^{2+}, Slc24a3, and Npc1l1 were upregulated. The Rgs13 inhibitor of small GTP protein-coupled receptors (GPCRs) known to reduce intra-GC B cell mobility (Hwang et al., 2013) was overexpressed, which might alter cytoskeleton reorganization and responses to chemokines. Finally, downregulation affected the γc chain of IL-2-4-7-21 receptors (major receptors for B cell activation), the semaphorin receptor plexin D1 that normally promotes B cell mobility (Holl et al., 2011), S1pR1, promoting lymphoid egress from GCs, and the CXCL13 receptor CXCR5.

- Other surface receptors: these included underexpressed integrins and receptors involved in adhesion to laminins or glycosaminoglycans (Itga6, Vsig1, CD97) and in B-T cell interactions or cytokine responses (CD70, CD69, IL2Rg) and increased expression of the IgG/IgE-receptor FcγRIIa.
Variations of apoptosis-related genes fitted with the observed ongoing apoptosis of mIgE+ cells. Other changes likely to impact cell fate related to signaling, cell adhesion, mobility, and thus optimal interactions with the microenvironment, notably due to underexpressed plexin D1 and/or increased RGS13 and GPCR inhibition thereof. A20 mIgE+ transfectants indeed showed actin cytoskeleton reorganization and loss of pseudopods by confocal microscopy (after phalloidin labeling) and increased circularity by flow imaging (Figure 3B). Increased circularity also marked primary cells induced in vitro to ε compared with γ1 CSR (Figure S3A).

Basal intracellular tyrosine phosphorylation and phospho-ERK levels were decreased in mIgE+ A20 transfectants (Figure 3C). In addition, mIgE+ cells showed spontaneous BCR co-localization with lipid rafts (Figure 3D) and increased abundance of rafts both in transfected A20 and mIgE+ activated primary B cells compared with mIgM+ or mIgG1+ cells from the same culture (Figure S3B).

In mIgE+ transfectants, the aforementioned increased FcγR4 transcription correlated with increased surface expression and binding of fluorescently labeled IgE (Figure S3C). Since genes connected to mobility showed variations, we assayed responses to chemokines of primary and transfected mIgE+ cells. In all cases, mIgE+ cells proved less reactive to either CXCL12, CXCL13, or mixed chemokines produced by lymphoid stromal cells (Figures 3E and S3D).

**mIgE+ cre-Switched B Cells Internalize mIgE BCR, Lose Mobility, and Are Short Lived**

Since εKI mice were B-less, we generated μεKI animals whose B cells initially express Cμ and can later be cre-deleted toward Cε expression. The Cε gene encoded both secreted IgE and mIgE
In homozygous mice, the $\mu$KI mutation and associated $\delta$ deletion homogenously imposed human IgM expression instead of any mouse Ig (Figure 4B). Mating with creERT2 mice yielded tamoxifen-inducible IgE expression. In vivo, cre deletion was efficient at the DNA level in spleen (Figure S4A). It also massively occurred in vitro in immortalized IgM+ hybridomas from $\mu$KI/creERT2 mice (Figure S4B). While inducible in primary B cells in vivo and in vitro, IgE detection required intracellular staining, reminiscent of requirements for staining mIgE+ primary cells (Wesemann et al., 2011). Contrasting with massive cre deletion at the DNA level in spleen, most primary B cells retained IgM expression in vivo, IgE+ cells completely vanished from blood and spleen, over 5 months after tamoxifen induction. Memory of cre switching was thus only attested to by a few remaining IgE secreting cells (Figure 4C).

Direct mIgE staining has been documented as weak or absent on primary B cells (Karnowski et al., 2006; Wesemann et al., 2011). Since it was also virtually absent in $\mu$KI B cells, we wondered whether this could reflect spontaneous mIgE internalization. To preserve membrane receptor trafficking, B cells were stained at 37°C instead of 4°C, with fluorescently labeled anti-IgE antibodies. This clearly and specifically stained tamoxifen-induced IgE+ $\mu$KI cells, showing that mIgE BCRs do reach the membrane.
Figure 4. \(\mu\)KI Mutant Mice

(A) (Top) Targeted IgH locus (not to scale) with floxed human \(C_\mu\) gene, neo\(^r\) cassette, and \(C_\varepsilon\) gene. (Bottom) The \(\mu\)KI locus normally undergoes V(D)J rearrangement, expressed with human \(C_\mu\); after breeding with CreERT2 mice, \(C_\varepsilon\) is expressed upon cre deletion of \(C_\mu\).

(B) B cells from \(\mu\)KI mice express human IgM BCR (n = 20, five experiments).

(C) \(\mu\)KI x CreERT2 mice were sacrificed 1 day to 5 months after tamoxifen administration. IgE B cells among splenocytes were quantified by cell cytometry after intracellular staining (mean ± SEM, n = 3 to 7 mice per group, four experiments); human IgE was evaluated in sera.

(D) BrdU was injected 10 days before sacrifice, 20 days after tamoxifen treatment, and incorporation was evaluated (mean ± SEM, n = 5, two experiments, paired t test).

(E) Cell cytometry (left) of IgE expression among [CD19\(^+\)] cells in \(\mu\)KI x CreERT2 mice 24 hr after cre induction, readily detects IgE after permeabilization but not by surface labeling at 4°C. In contrast, 6-hr incubation at 37°C with fluorescent anti-IgE antibodies stains cells specifically from mice expressing cre (n = 9, four experiments, paired t test). Confocal imaging of internalized fluorescent antibodies (right; scale bar represents ~10 μm).

(legend continued on next page)
membrane but are then internalized, eventually together with a labeling antibody (Figures 4E and 4G).

Similarly, in CD40L-stimulated human primary B cells, IgE expression appeared faint after staining at 4°C but strongly increased by staining at 37°C and was restricted to IL-4-exposed cells (Figures 4F and 4G).

**DISCUSSION**

Class-specific variations of BCR functions are incompletely understood (Laffleur et al., 2014). Compared with IgM, cross-linking of class-switched BCRs more strongly boosts PC differentiation (Horiwata et al., 2007; Pogue and Goodnow, 2000; Sato et al., 2007; Waisman et al., 2007; Wakabayashi et al., 2002).

Specific mlgG or mlgE interactions were reported with Grb2 (Engels et al., 2009). The mlgE tail also interacts with Hax1, which binds the Syk target Hs1 (Batista et al., 1996; Oberndorfer et al., 2006). However, transfected cells reacted similarly to mlgE or mlgM cross-linking (Poggiarla et al., 2006).

Independent of cross-linking, it is unknown whether constitutive signals from the various HC classes differentially support B cell survival. Rare primary class-switched mlgE+ lymphocytes are mandatory precursors of IgE PCs (Achatz et al., 1997; Brightbill et al., 2010). The elusive nature of the mlgE+ stage in vivo has been attributed to accelerated PC differentiation, increased apoptosis, or poor mlgE efficacy during CSR, three non-mutually exclusive explanations (He et al., 2013; Misaghi et al., 2013; Wu and Zarrin, 2014; Yang et al., 2012).

The Tψ2 context yielding mlgE cells might also shorten their survival, similar to some T cells in which Socs-1 induction by IL-4, IL-13, and Stat6 promotes activation-induced cell death (Alexander, 2002; Hebenstreit et al., 2003; Oh et al., 2012). Whatever the mechanisms involved, altogether they limit IgE production in vivo to levels 10,000- to 100,000-fold lower than other classes.

Since mlgE cells abundantly arise in vitro from B cell stimulation, we suspected that one basis for their in vivo rarity might be that mlgE expression per se modulates B cell fate. To assay such a potential mlgE effect independently of any antigen-dependent cross-linking and T cell help, we first forced mIgE expression in HC εcreERT2 cells treated with tamoxifen, and in human B cells stimulated for 4 days with either CD40L or CD40L + IL4. Data are means ± SEM. See also Figures S1A and S4.

(F) Cell cytometry (left) of IgE expression among stimulated human B cells, incubated 6 hr at 37°C with fluorescent anti-IgE. Confocal imaging of internalized fluorescent antibodies (right; scale bar represents ~10 μm) (n = 6, three experiments, Mann-Whitney test).

(G) Quantification of endocytosis from data of (E) and (F). BCR endocytosis was quantified in mouse primary cells with the 37°C IgE staining procedure, using either μKI or μKI × creERT2 cells treated with tamoxifen, and in human B cells stimulated for 4 days with either CD40L or CD40L + IL4. Data are means ± SEM. See also Figures S1A and S4.
transiently in both humans and mice, poorly effecting migrations associated with maturation within lymphoid organs and thus quickly eliminated by spontaneous and activation-induced apoptosis without survival as mIgE memory cells. Only few IgE PCs might eventually survive this pathway, become long-lived, and solely ensure true long-term IgE memory. Given the short half-life of IgE, such rare long-lived IgE PCs are likely responsible for immediate IgE allergy, while mIgM+ or mIgG+ memory cells can eventually generate new IgE cells after CSR. Direct or sequential CSR was indeed documented as providing memory cells can eventually generate new IgE cells after CSR. Only few cells (Xiong et al., 2012).

This multifaceted restriction of IgE responses most likely tests to the beneficial accumulation throughout evolution of multiple means to both keep on producing the most powerful magic bullet of adaptive immunity while maintaining its hazardous production under tight control. Each of these specific constraints on the IgE B cell compartment will clearly deserve in-depth molecular analysis in the future.

EXPERIMENTAL PROCEDURES

Mice

RAQg−/− γC−/− (Colucci et al., 1999), AID−/− (Muramatsu et al., 2000), and LATγ136f (Aguado et al., 2002; Genton et al., 2006) mice were used. γC−/− mice carried an inserted human γC gene replacing Sp1, μK1 mice carried a floxed human γC followed by Cγ (expressed upon cre deletion of Cγ). Detailed methods are described in Supplemental Experimental Procedures.

B Cell Transfectants

Expression of rearranged Ig chains was in A20, BL2, and BL41 cell lines. Detailed methods are described in the Supplemental Experimental Procedures.

Transcription Analysis

RNA was analyzed by qPCR for expression of μ, γ, and ε Ig HC secreted and membrane forms. Microarray analyses were with Agilent chips. Detailed methods are described in the Supplemental Experimental Procedures.

Cell Cultures

Sorted B cells were stimulated for IgE CSR in the presence of IL-4. In transfer experiments, cells were injected intravenously into RAQg−/− γC−/− mice. Detailed methods are described in Supplemental Experimental Procedures.

Cytometry, Confocal Microscopy, and Flow Imaging

Proliferation was monitored by BrdU incorporation. Apoptosis was monitored by Annexin V and DiOC6 staining. Detailed methods are described in Supplemental Experimental Procedures.

Cell Mobility

Evaluation was done using transwells and recombinant or natural chemokines. The detailed method is described in Supplemental Experimental Procedures.

Statistical Analyses

Student’s t test or Mann-Whitney tests were used (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

ACCESSION NUMBERS

Microarray data have been deposited to the GEO database under accession number GSE64130.


signaling is inhibited by CD22 and promotes the development of B cells whose survival is less dependent on Ig alpha/beta. J. Exp. Med. 204, 747–758. 


