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FcRY, an Fc receptor related gene differentially expressed during B lymphocyte development and activation

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Abstract

A bioinformatics approach has lead to the identification of *FcRY*, a new Fc receptor related gene. *FcRY* is predicted to encode a protein with three immunoglobulin (Ig) domains followed by a mucin-like domain containing a proline-rich stalk and a C-terminal leucine rich region. The predicted protein lacks a hydrophobic domain for insertion into the plasma membrane, suggesting that FcRY is an intracellular or secreted protein. This feature is shared with the product of the *FcRX*/*FCRL*/*FREB* gene that is closely linked to *FcRY* in both human and mouse genomes. *Fcry* transcripts are first detectable among mouse B lineage cells at the pre-B cell stage. Splenic B cells of the newly formed, follicular, and marginal zone subsets express *Fcry*, as do germinal center B cells to a lesser extent. *FcRY* is also expressed in subpopulations of human B cells. A consistent characteristic of *FcRY* in both species is low level gene expression, which can be further downregulated in normal mouse B cells by signaling through the B cell receptor (BCR) or CD40, thereby suggesting a correlation between cell cycle entrance and diminished FcRY expression. *Fcry* is upregulated by short-term treatment with BAFF/BLyS, which promotes B cell survival rather than proliferation. LPS induces very rapid but transient enhancement. We observed a pronounced upregulation of *Fcry* expression in WEHI 231 cells induced by BCR crosslinking to undergo cell cycle arrest prior to apoptosis, consistent with the possible regulation of *Fcry* expression by cell cycle status.

Keywords: FcRX; FcRL; FREB; Cell cycle; Apoptosis; BAFF/BLys

1. Introduction

Receptors for the constant region of immunoglobulins (FcR), FcγRI, FcγRII, FcγRIII, FcεRI, are found on cells of both myeloid and lymphoid lineages (Daeron, 1997; Kinet, 1999; Ravetch and Bolland, 2001). Originally identified on phagocytic myeloid cells because of their ability to bind and internalize particulate antigens opsonized with IgG antibodies, members of the classical FcR family are now recognized to have important regulatory roles in both cell mediated and humoral

Abbreviations: BAFF, B cell-activating factor of the TNF family; BAFF-R, BAFF receptor; B-CLL, B cell chronic lymphocytic leukemia; BCR, B cell receptor; BL, Burkitt's lymphoma; BLyS, B lymphocyte stimulator; CB, centroblast; CC, centrocyte; D, domain; EBV, Epstein Barr virus; est, expressed sequence tag; FcR, Fc receptor; FcRH, Fc receptor homolog; FCRL, Fc receptor-like; FREB, Fc receptor homolog expressed in B cells; GC, germinal center; Ig, immunoglobulin; LPS, lipopolysaccharide; M, mucin; PC, plasma cell; SP, signal peptide; UTR, untranslated region.

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immunity, including feedback suppression of B cell responses, regulation of hypersensitivity reactions, and induction of cellular cytotoxicity. Other Ig domain-containing FcRs include the Fcα/μR (Kinet and Launay, 2000; Shibuya et al., 2000), which binds IgA and IgM, neonatal FcRn (Lencer and Blumberg, 2005), an MHC class I related receptor that mediates perinatal transfer of Ig and maintenance of basal immunoglobulin levels in adults, the polymeric immunoglobulin receptor (polyIgR) (Norderhaug et al., 1999) that trancytoses IgA across mucosal epithelial surfaces, and the FcαR (CD89) (Monteiro and Van De Winkel, 2003), a receptor for IgA on myeloid cells that is present in humans and rats, but curiously not in mice (Maruoka et al., 2004). The Ig domain sequences of CD89 make it a very distant FcR relative and its genomic location places it in the leukocyte receptor complex on chromosome 19q13.4, rather than with the classical Fc receptors on human chromosome 1q23 (Liu et al., 2000; Trowsdale et al., 2001; Wende et al., 1999).

During the past several years our appreciation of the extent of the FcR gene family has increased with the identification of many FcR related genes in the FcR gene complex on human chromosome 1 and syntenic regions on mouse chromosomes 1 and 3. We and others have identified human and mouse FcR relatives that have provisionally been named FcRH based on their homology with the classical FcR (Davis et al., 2001, 2002a, 2005; Guselnikov et al., 2002; Hatzivassiliou et al., 2001; Nakayama et al., 2001; Xu et al., 2001). Another of the newly identified FcR relatives has been named FcR-like (*FCRL*) (Mechetina et al., 2002), FcR homolog expressed in B cells (*FREB*) (Facchetti et al., 2002), and *FcRX* (Davis et al., 2002c). Unlike the other FcR family members, FcRX has no predicted transmembrane region or N-linked glycosylation sites and is constitutively expressed as an intracellular protein, although some of the shorter isoforms derived by alternative *FcRX* splicing may be secreted (Najakshin et al., 2004). Under appropriate but as yet undefined conditions the full-length protein could be secreted as well. Human FcRX is preferentially expressed in marginal zone and germinal center B cells (Davis et al., 2002c; Facchetti et al., 2002; Masir et al., 2004; Mechetina et al., 2002) and in normal and neoplastic melanocytes (Inozume et al., 2005). The ligands for FcRH and FcRX remain unknown.

During the analysis of genomic regions flanking *FcRX*, we discovered *FcRY*, a predicted closely linked gene with homology to *FcγRI* (Davis et al., 2002b). Here we describe the sequence, predicted structure, and expression patterns of human and mouse *FcRY* and compare this gene with its close relative, *FcRX*.

2. Materials and methods

2.1. Identification of human and mouse FcRY genes

The human *FcRY* gene was initially identified by analysis of the predicted protein sequence and domain structure of annotated genes flanking *FcRX* in the NCBI and Celera human genome databases. Although the *FcRY* annotation proved to be incorrect (see Results), the predicted sequence could be used to identify multiple overlapping est clones. The full-length *FcRY* was obtained from the following clones: BC038564, BQ232294, AI817123, and AI432698. (Open Biosystems, Huntsville, AL). This human sequence information was used to identify the corresponding mouse gene. A full-length *Fcry* cDNA was cloned from mouse spleen by nested RT-PCR.

2.2. Molecular biology methods

The cDNA cloning, sequencing, and RNA blot analyses were performed by standard techniques as described previously (Davis et al., 2002c).

2.3. Tissues and cell lines

The mouse and human tissues and cell lines used in these studies have been described previously (Davis et al., 2002c). Human blood samples and tonsils were obtained in accordance with policies established by the University of Alabama at Birmingham (UAB) Institutional Review Board and with informed consent according to the Declaration of Helsinki. Mouse B and T cells were purified by negative selection using B- and T-lymphocyte enrichment sets (BD Biosciences, San Jose, CA). For gene expression analysis, human tonsillar B cells highly enriched by using CD19 microbeads (Miltenyi Biotec, Auburn, CA) were separated into B cell subpopulations by immunofluorescent cell sorting with a MoFlow instrument (Cytomation, Fort Collins, CO): naive cells ($CD27^-CD38^-IgD^+CD19^+$), pre-GC cells ($CD38^+IgD^+CD19^+$), GC cells ($CD38^+IgD^-CD19^+$), centroblasts ($CD77^+CD38^+CD19^+$), centrocytes ($CD77^-CD38^+CD19^+$), memory B cells ($CD27^+CD38^-CD19^+$), and plasma cells ($CD38^+IgD^-CD19^+$). Mouse bone marrow and spleen B cell subsets were sorted using the following antibody combinations: BM pro-B ($B220^+CD43^+$), BM pre-B ($B220^+CD43^-IgM^-$), BM immature B ($B220^{lo}CD43^-IgM^+$), spleen B ($B220^+IgM^+$). Splenic B cells were sorted into immature ($B220^+AA4^+$); mature ($B220^+AA4^-$); germinal center ($B220^+PNA^+$ from mice immunized with NP-CGG); follicular ($B220^+CD21^-CD23^+$); marginal zone ($B220^+CD21^+CD23^-$); newly formed ($B220^+CD21^-CD23^-$) subpopulations.

2.4. RT-PCR

Amplification of mouse *Fcry* for expression analysis was performed on oligo dT primed cDNA under the following conditions: Forward primer: (mFcRYcDNA/s144) 5′-CAG GCA GAG TCA TTA TGT GG-3′; Reverse primer: (mFcRYcDNA/as561) 5′-GCC GTC GTG GTA GTA GTG AA-3′. The PCR reaction consisted of a 95 °C denaturation for 2 min followed by 30 cycles of 95 °C 5 s, 60 °C 10 s, 72 °C 1 min. The PCR products were resolved in 1% agarose gel, with an expected size of 417 bp. As a loading control, β-actin was amplified. Forward primer: 5′-ATG GAT GAC GAT ATC GCT-3′, Reverse primer: 5′-ATG AGG TAG TCT GTC AGG T-3′. PCR conditions: 95 °C 5 s, 54 °C 10 s and 72 °C 1 min for 25 cycles.