Fc Receptor-Like Molecules

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Abstract

Discovery of a large family of Fc receptor-like (FCRL) molecules, homologous to the well-known receptors for the Fc portion of immunoglobulin (FCR), has uncovered an impressive abundance of immunoglobulin superfamily (IgSF) genes in the human 1q21–23 chromosomal region and revealed significant diversity for these genes between humans and mice. The observation that FCRL representatives are members of an ancient multigene family that share a common ancestor with the classical FCR is underscored by their linked genomic locations, gene structure, shared extracellular domain composition, and utilization of common cytoplasmic tyrosine-based signaling elements. In contrast to the conventional FCR, however, FCRL molecules possess diverse extracellular frameworks, autonomous or dual signaling properties, and preferential B lineage expression. Most importantly, there is no strong evidence thus far to support a role for them as Ig-binding receptors. These characteristics, in addition to their identification in malignancies and autoimmune disorders, predict a fundamental role for these receptors as immunomodulatory agents in normal and subverted B lineage cells.

INTRODUCTION

B cells and T cells regulate biological responses through the differential expression of both adaptive- and innate-type receptors. With the advent of modern genomics, an increasing number of genes encoding potential cell surface receptors are being discovered in a variety of organisms. These discoveries are enlarging the pool of recognized molecules, exposing their evolutionary relationships, and increasing our understanding of immune system complexity. The identification of a large family of Fc receptor-like (FCRL) molecules has considerably broadened the network of lymphocyte coreceptors and uncovered an unexpected layer of biological intricacy. *FCRL1–6* encode type I transmembrane glycoproteins with variable numbers of related extracellular Ig domains and cytoplasmic tails containing immunoreceptor tyrosine-based activation (ITAM) (1, 2) and/or inhibition motifs (ITIM) (3, 4). Two related genes, termed *FCRLA* and *FCRLB*, have also been defined, and these appear to encode intracellular proteins. The preferential expression of FCRL molecules by B cells and their potential to deliver activating and/or inhibitory signals suggest that these receptors play a role in regulating cellular differentiation and modulating the initiation and termination of B cell responses. Although they share a common ancestor with the classical Ig-binding Fc receptors (FCR), there has thus far been no unequivocal evidence that any of the FCRL family members bind Ig. Rather, these proteins currently remain orphan receptors that mark distinct subpopulations of lymphocytes. Analyses of their distribution in humans and mice are beginning to reveal intriguing patterns for them on lymphocyte subsets, some of which have not previously been appreciated. Their multiple isoforms, genetic polymorphisms, and signaling features imply that the function of individual FCRL family members may be complex and that alteration of their normal biological roles could have pathological consequences. Although their functions currently remain enigmatic, their discovery and initial characterization point to fascinating phylogenetic origins, distinct expression patterns, and multifaceted signaling potential—all features that herald their fundamental importance in immunity. This review focuses on these aspects as well as on their rising biological significance in immunologically related disorders.

FCRL: Fc receptor-like

Immunoreceptor tyrosine-based activation motif (ITAM): a cytoplasmic element conforming to the consensus sequence (E/D)-X-X-Y-X-X-(L/I)-X6–8-Y-X-X-(L/I) (where X denotes any amino acid) that is phosphorylated at tyrosine residues following receptor engagement and that recruits Syk tyrosine kinases

Immunoreceptor tyrosine-based inhibition motif (ITIM): a cytoplasmic sequence defined as (I/V/L/S)-X-Y-X-X-(L/V/I) that is tyrosine phosphorylated upon extracellular ligation and that provides a docking site for Src homology 2 (SH2) domain–containing phosphatases

Fc receptors (FCR): members of the immunoglobulin superfamily broadly expressed by leukocytes with the capacity to bind to the Fc portion of antibodies

IDENTIFICATION OF THE Fc RECEPTOR-LIKE MOLECULES

Although the first FCRL representative was described in 1989 as a novel activation receptor expressed by IL-2-stimulated rat NK cells (gp42/FCRL6) (5, 6), more than 10 years passed before its human and mouse relatives were molecularly defined and the substantial size of this receptor family was appreciated. At least five groups have contributed to the FCRL discovery using different approaches, each of which has provided an informative perspective of their biological features. In an effort to identify extended members of the *FCR* family, our laboratory found these genes through database searches using a 32 amino acid consensus sequence derived from the extracellular Fc-binding portions of the three human FCRs for IgG (FCGR1/CD64, FCGR2/CD32, and FCGR3/CD16) (7, 8). After analysis of four overlapping BAC clones identified in iterative BLAST searches, five novel immunoglobulin superfamily (IgSF) genes were located in the midst of the classical *FCR* locus on chromosome 1q21–23. Owing to their evident *FCR* homology, we provisionally termed them Fc receptor homologs (*FcRH1–5*). They were also designated IgSF receptor translocation–associated genes (*IRTA1–5*) through characterization of a (1;14)(q21;q32) balanced translocation breakpoint in a multiple myeloma cell line (9, 10). This illegitimate rearrangement with the Ig heavy chain locus positions the intron that is between C_H3 and the transmembrane exon of $C\alpha_1$ on 14q32 directly side by side with the second intron of *IRTA1/FCRL4*. Using a phylogenetic approach,

another group found *FCRL* genes through comparative database searches using the third Ig domain derived from FCGR1 as a query sequence (11). This initially resulted in the identification of gp42 and ultimately yielded additional genes in humans and mice named IgSF, FcR, gp42 (*IFGP*). Still a fourth team explored expressed sequence tag (EST) databases for transcripts encoding ITIM consensus sequences and defined the SH2 domain–containing phosphatase anchor protein (*SPAP1/FCRL2*) (12). Finally, by characterizing transcripts upregulated following B cell receptor (BCR) ligation in a Burkitt's lymphoma–derived cell line, the B cell cross-linked by anti-IgM activating sequence gene 1 (*BXMAS1/FCRL5*) was identified (13). Three additional family members were also discovered, *FCRL6* (14, 15), *FCRLA* (16–18), and *FCRLB* (19–21), the latter two being genes that encode atypical proteins. These discoveries indicate that there is a striking wealth of genes homologous to the *FCR* that are primarily expressed by B cells. Collectively, they are related by their genomic organization, chromosomal linkage, ancestral relationship with the FCR, tyrosine-based signaling potential, and preferential expression by B cells.

Given the confusion surrounding the multiple designations for similar molecules, a consortium recently organized by the International Committee on Standardized Genetic Nomenclature for Mice, the Mouse Genomic Nomenclature Committee, and the Human Genome Organization Gene Nomenclature Committee has established uniform nomenclature to describe these genes (22). The term Fc receptor-like (FCRL) was chosen to indicate their phylogenetic relationships with the conventional Fc receptors and signify their distinct characteristics as a separate subfamily.

GENOMIC LOCATION AND ORGANIZATION OF FCRL FAMILY MEMBERS

A total of eight human genes have now been identified, along with six orthologs in mice. In humans, *FCRL* family members are located at three distinct loci over a ~4 Mb region of chromosome 1q21–23 (**Figure 1**). *FCRL1–5* are clustered in a ~300 kb region between 156.03–155.74 Mb, ~7 Mb telomeric of *FCGR1A* at 148.02 Mb. They are all oriented toward the centromere and flanked by *CD5L*, a scavenger receptor cysteine-rich (SRCR) type-B encoding gene and by the Ets variant gene 3 (*Etv3*). The *FCRL1–5* locus also includes a pseudogene located between *FCRL3* and *FCRL4* that contains exons for three potential Ig domains, the second of which is degenerate given its lack of a carboxy-terminal cysteine involved in intrachain disulfide bond formation. *FCRL1–5* have a similar exon and intron organization with comparable phase splicing patterns, tandem organization of Ig domain–encoding exons, a single exon encoding the transmembrane region, and five cytoplasmic exons generating ITAM or ITIM sequences (reviewed in 23, 24). These conserved elements result in the transcription of multiple splice isoforms for almost all family members and the translation of type I transmembrane glycoproteins described in detail below and in the **Supplemental Table** (follow the Supplemental Material link from the Annual Reviews home page at **http://www.annualreviews.org**).

One particular aspect that distinguishes the *FCRL/FCR* family genetically is a short 21 bp miniexon (S2) that encodes the second half of a hydrophobic split signal peptide (8, 25–29). This is a hallmark feature of all *FCR* and *FCRL* family members recognized to date, with the exception of *FCRLA*. This characteristic also differentiates the *FCRL/FCR* multigene family from the large number of leukocyte receptor complex (*LRC*) genes that encode the Fc receptor for IgA (FCAR), killer Ig-like receptors (KIR), and leukocyte Ig-like receptors (LILR) on human chromosome 19q13 (30–32) and the *LILR* orthologs in mice known as the paired Ig-like receptors (*PIR*) (33). In contrast to *FCR/FCRL* genes, these *LRC* genes have a 36 bp exon that encodes the second half of the split signal peptide (34–38).

Immunoglobulin superfamily (IgSF): one of the largest groups of proteins in vertebrates, typically extracellular receptors; representatives are distinguished by their possession of a commonly repeated protein motif of ~100 amino acids, also known as an Ig domain

Expressed sequence tag (EST): a short complementary DNA sequence derived from messenger RNAs that are transcribed by tissues

Orthologs: genes in different species that have evolved from a common ancestral gene

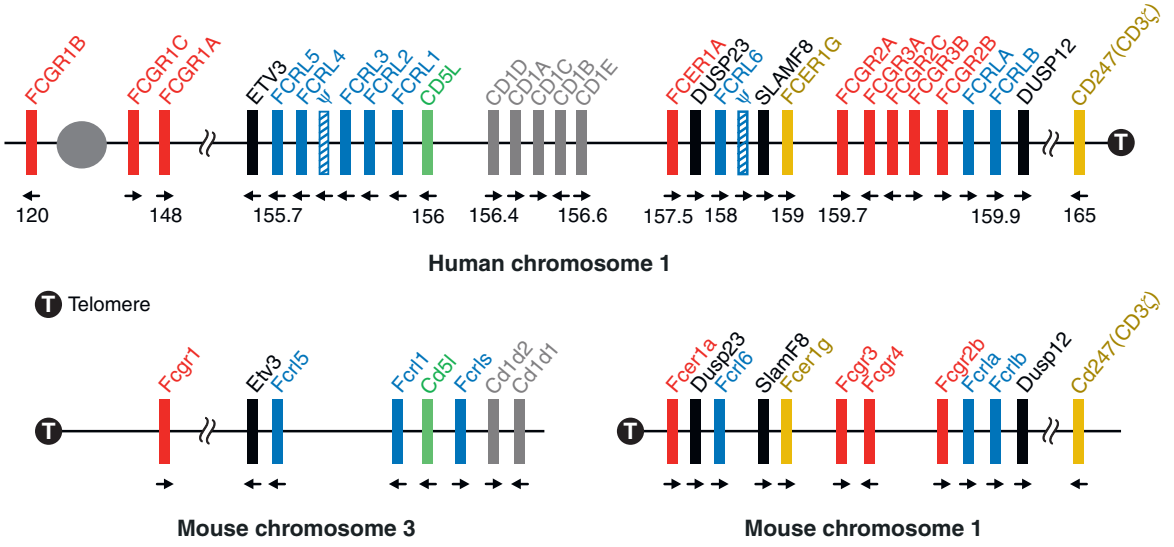

Figure 1

Relative genomic positions of *FCRL/FCR* multigene families and pertinent orthologs in humans and mice (not to scale). The human chromosome 1q21–23 region and syntenic loci are demonstrated on respective portions of mouse chromosomes 1 and 3. Representatives are color-coded according to family membership. Gene orientations and megabase (Mb) pair locations on human chromosome 1 are also provided. Note that mouse counterparts are in the opposite transcriptional positions compared with their human orthologs.

The most recently identified *FCRL* gene, *FCRL6*, is not contiguous with *FCRL1–5*, but rather is positioned at a second locus ~2 Mb telomeric at 158.03 Mb (24). This representative is located within 50 kb of the ligand-binding chain of the high-affinity FcεR (*FCER1A*) gene between dual specificity phosphatase 23 (*DUSP23*) centromerically and the *SLAMF8* gene at its telomeric end. *FCRL6* has exon-intron organization similar to *FCRL1–5* and also encodes a type I transmembrane glycoprotein with tyrosine-based motifs. An *FCRL6*-like pseudogene is situated just downstream and contains a potential Ig domain–encoding exon with homology to D5-type subunits (see below) that is degenerate.

FCRLA and *FCRLB* are located in a third locus proximal to the genes encoding the low-affinity IgG receptors with which they are closely related (16). In humans, *FCRLA* and *FCRLB* are tandemly positioned in the same orientation around the 160 Mb region of chromosome 1 within ~9 kb of each other and flanked by *FCGR2B* and *DUSP12* genes.

There has been remarkable divergence between mice and humans in the organization of the extended *FCRL* locus. The *FCRL/FCR* multigene family that is distributed at 1q21–23 in humans is divided between orthologous locations of mouse chromosomes 1 and 3 where genes have reverse transcriptional orientations (29) (see **Figure 1**, *bottom*). The division of this region is located proximal to the *Cd1d* genes and places *Fcgr1* and *Fcrl1–5* orthologs in a syntenic region of chromosome 3 and the other *Fcrl/Fcr* family members at analogous positions on mouse chromosome 1. Discovery of the mouse *Fcrl* counterparts has exposed significant interspecies diversity since the radiation of rodents and their higher vertebrate mammalian relatives. In contrast to the five human genes, only two orthologs, *Fcrl1* and *Fcrl5*, exist in mice and rats (11, 29). Additionally, a third unusual mouse gene that does not exist in the human genome has been

designated *Fcrls*. According to the most recent genome data, *Fcrl1* and *Fcrl5* are located on chromosome 3 approximately ~8.5 Mb centromeric of the *Fcgr1* gene and are flanked by mouse *Cd5l* and *Etv3* orthologs. The genomic organization of *Fcrl1* and *Fcrl5* is similar to human *FCRL1–5*, including the characteristic 21 bp second exon. Although both genes encode type I transmembrane glycoproteins, they differ from their human counterparts in several respects that are discussed in the next section. The atypical *Fcrls* gene is situated centromeric of *Cd5l* in the opposite orientation of *Fcrl1* and *Fcrl5* and encodes an Ig domain-SRCR fusion protein. Given its genetic structure and the sequence identity of its encoded product, *Fcrls* likely resulted from an ancient indiscriminate recombination event involving an *Fcrl* family member and *Cd5l* (11, 24). Importantly, its possession of the typical S2 exon is a clear indication of *Fcrls*' membership in this gene family. However, its lack of a transmembrane-encoding segment and inclusion of an exon encoding a type B-SRCR domain, with relatively high identity to the N-terminal domain of the encoded CD5L receptor, is unique. This gene is also present in the canine genome (A.V. Taranin, personal communication). The other mouse *Fcrl* family members are located at respective positions on chromosome 1. Mouse *Fcrl6* is situated in an orthologous region and has similar genetic organization to its human equivalent. However, like *FCRL1–5*, it also possesses interspecies differences. *Fcrla* and *Fcrlb* are located in syntenic regions on mouse chromosome 1, with high organizational conservation relative to their human counterparts.

FCRL1–5 ENCODE MOLECULES RELATED TO THE FCR

Our laboratory's approach to identifying the *FCRL* genes was to use a consensus sequence derived from the extracellular regions of FCGR1–3. This disclosed a priori that FCRL family members are closely related to the conventional Fc receptors (8, 23). As these newfound receptor genes become better characterized, multiple themes are emerging that link them with the FCR and reveal that together they are members of an extended ancient gene family. Common features include their linked genomic positions, genetic organization, IgSF membership, related extracellular Ig domain composition, and functional utilization of ITAM and/or ITIM sequences. Although many of these elements are generally shared by both groups of molecules, there are also distinct differences that mark the FCRL as a discrete, multigene family. This section focuses on FCRL1–5, and FCRL6, FCRLA, and FCRLB are discussed later in this review.

Like the classical *FCR* genes, *FCRL1–5* encode type I cell surface glycoproteins with multiple splice isoforms. In humans, FCRL1–5 have 3–9 C-like Ig domains with variable numbers of predicted N-linked glycosylation sites (39) (**Figure 2** and **Supplemental Table**), but the final protein products may vary according to individual RNA splicing patterns. During their early characterization, phylogenetic comparisons of the extracellular Ig domains of FCRL1–5 and the FCR molecules were found to be of five different subtypes (8, 24). These distinct subunits generally follow a tandem membrane-distal to membrane-proximal arrangement that is conserved for both FCRL and FCR representatives in humans and mice (29). This pattern also suggests that an underlying genetic mechanism may exist for maintaining this particular order of the extracellular framework, as has been suggested for the FCR (28). Whereas FCRL molecules employ different combinations of all five domain subtypes, the FCRs do not possess the D4 (light blue) or D5 (green) structural elements (see **Figure 2**). These particular subunits are unique to the FCRL family but have homology with Ig domains present in cell adhesion molecules such as PECAM/CD31 (8, 9, 11). They also have very high intra- and interprotein amino acid identity (~50%–80%) as opposed to the membrane-distal segments that have lower

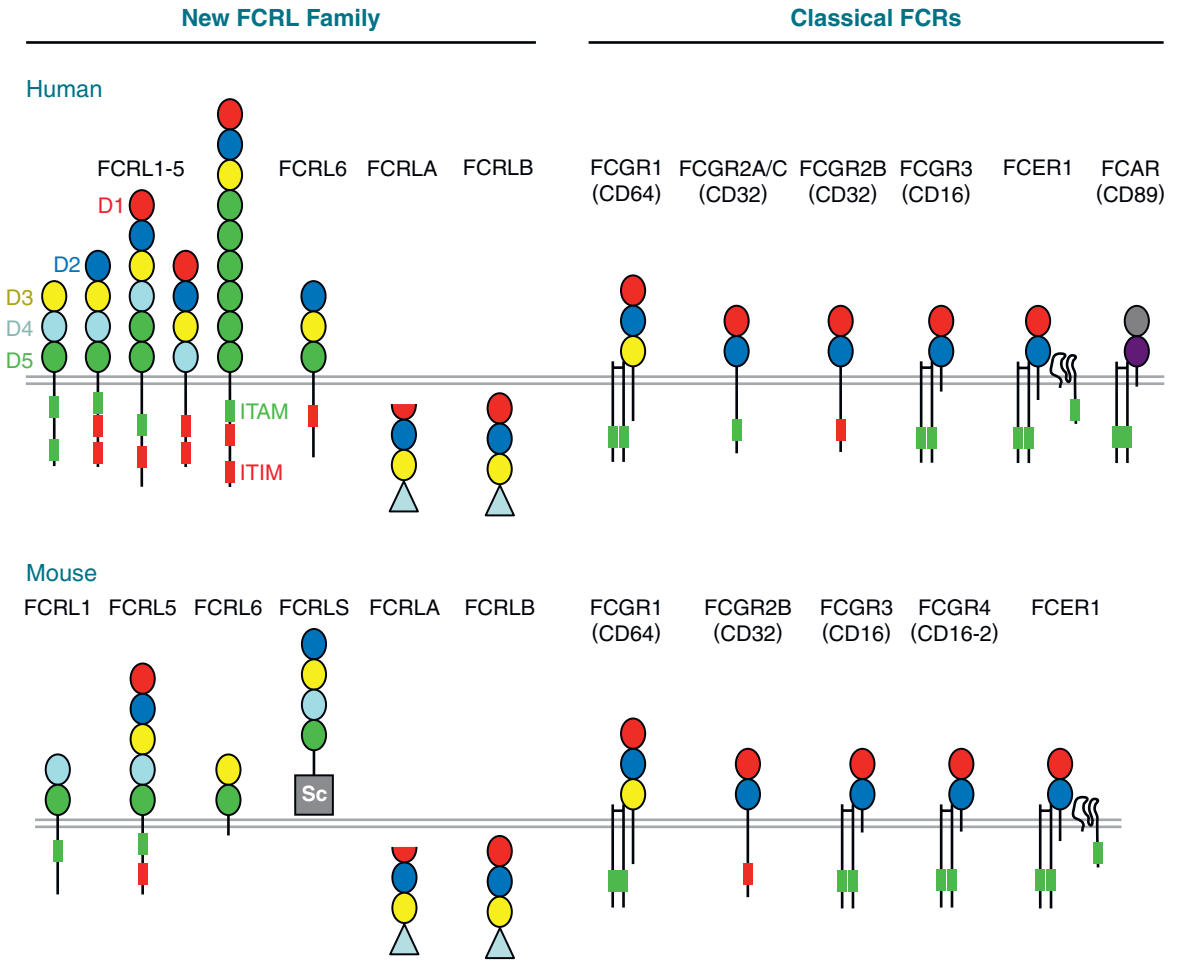

Figure 2

A comparison of protein characteristics between human and mouse FCRL and FCR molecules. Extracellular Ig domains are color-coded according to phylogenetic relationships as determined previously in comprehensive family Ig domain amino acid sequence alignments (8, 24). Note that the FCAR domains cluster independently in this analysis and thus are color-coded differently. Cytoplasmic ITIM or ITAM are represented by red or green boxes, respectively. The FCRLS type B-SRCR domain is shown as a gray rectangle. FCRLA possesses a partial Ig domain. Both FCRLA and FCRLB contain C-terminal mucin-like regions (blue triangles) and are expressed intracellularly. Activating FCR family members are shown in complex with the FCERG1 adaptor subunit, and FCER1A also associates with FCER1B/MS4A2.

sequence relatedness (~25%–35%). The D1 (red) and D2 (dark blue) domains that confer Ig-binding competence for FCGR1–3 and the high-affinity IgE Fc receptor (FCER1A) are also identified in many of the human and mouse FCRLs (24). Crystallographic analyses and mutagenesis studies of these subunits in the classical FCRs indicates that the linker region between D1 and D2 domains as well as the D2 domain enable Fc binding (40–46). Furthermore, the D3 (yellow) domain also endows the high-affinity FCGR1/CD64 with the ability to bind monomeric Ig (47). Although the presence of these domains in

some FCRL family members might imply that they bind Ig, no unambiguous biochemical evidence for this has yet been presented in humans or mice. However, suggestive results of IgG binding by FCRL5 have been observed but not definitively determined (9, 48). This phylogenetic comparison also demonstrates the insignificant identity between the two FCAR/CD89 Ig subunits and FCRL or FCR domains, indicating that this chromosome 19q13–derived LRC receptor is a more distant relative. Although a rat *FCAR* gene has been characterized, no *FCAR* representative has been identified in mice to date (33, 49).

Mouse FCRL1 and FCRL5 counterparts diverge slightly from their human orthologs in their extracellular domain makeup. In mice, FCRL1 lacks a D3-type subunit, and FCRL5 possesses all five domain subtypes but does not have multiple copies of the D5-type domain obvious in its human relative. Instead, mouse FCRL5 more closely resembles the domain composition of human FCRL3. The equivocal amino acid identities calculated for the extracellular portions of these two human receptors compared with mouse FCRL5 (~40%) suggest that this representative may possess features common to both of them (29).

The transmembrane segments of all FCRL molecules in humans and mice are hydrophobic and uncharged, with the exception of human FCRL1, which contains an acidic glutamic acid. This distinct characteristic suggests that FCRL1 may engage with another transmembrane adaptor protein containing a complementary-charged amino acid via a salt bridge. This relationship is typical of activating FCGR1/CD64, FCER1, and FCGR3/CD16 molecules. These receptors have short cytoplasmic tails without signaling elements and require coassociation with ITAM-bearing subunits, such as the FCER1G or CD3ζ/CD247, for surface expression and signaling function (50). Interestingly, the genes for these two adaptor molecules are also located in the extended FCR locus (see **Figure 1**), and their encoded products may promiscuously pair with other charged residue-containing transmembrane proteins, including the FCAR, KIR, and LILR receptors (reviewed in 51). An additional point of interest is that extracellular ligand-binding chains usually have short cytoplasmic segments, whereas ITAM-equipped adaptor components typically have short extracellular portions. Thus, FCRL1's three extracellular Ig domains, charged transmembrane amino acid, and long cytoplasmic tail with two potential ITAMs are unusual in this respect. Mouse FCRL1, by contrast, does not have a charged transmembrane amino acid. This finding suggests that its function may be more limited compared with its human counterpart that could have evolved an additional biological role. Compared with the activating FCR/adaptor complexes, most FCRL molecules are similar to FCGR2A-C/CD32, which have hydrophobic, uncharged transmembrane regions and cytoplasmic tails with the capacity to signal autonomously (52, 53).

Immune receptors having activation or inhibitory potential are capable of coordinating responses and facilitating the balance necessary to initiate or terminate effector outcomes by providing minimal peripheral damage to the host. However, exaggerated expression or unbalanced regulation of these types of molecules can have destructive biological consequences resulting in autoimmunity, infection, malignancy, or developmental abnormalities (7, 54–60). FCRL1–5 all possess one or more tyrosine-based motifs in their cytoplasmic tails, indicating their potential for transmitting intracellular signals following extracellular ligation. These sequences include consensus ITIM, defined as (I/V/L/S)-X-Y-X-X-(L/V/I) (3, 4, 61, 62), where X is any amino acid, and/or ITAM-related sequences with a variation of the consensus (E/D)-X-X-Y-X-X-(L/I)-X_{6-8}-Y-X-X-(L/I) (1, 2, 63, 64). A third type of sequence that requires mention is the tyrosine-based switch motif (ITSM) with the consensus T-X-Y-X-X-(V/I) (65, 66). Canonical ITSM consensus sequences are not found among FCRL cytoplasmic tails; however, potential ITSMs are present in human

Figure 3

Multiple sequence alignment of human and mouse FCRL cytoplasmic tails highlights the family's activating and inhibitory potential. Alignment was performed using CLUSTALW software (172). A consensus sequence resulting from the analysis is positioned on top of the alignment. ITAM sequences are colored green, ITIM red, ITSM yellow, and other tyrosines that do not fit a consensus are labeled blue.

FCRL4 and mouse FCRL1. Notably, they differ by their possession of another polar amino acid (serine) at the –2 position and alternatively could operate as potential ITIMs. These conserved signaling components place FCRL family members in a larger category of proteins united by their possession of tyrosine-based motifs and typically paired expression patterns (61, 62, 67, 68). Although the ITIM sequences prominent in the FCRL cytoplasmic segments typically conform to the established consensus, the ITAM sequences are less orthodox. Furthermore, some FCRLs possess both types of motifs, suggesting that they may have greater signaling complexity.

Alignment of the cytoplasmic regions derived from all FCRL transmembrane receptors in humans and mice indicates significant intrafamily conservation for these segments. This is clarified by a consensus sequence that defines conserved amino acids and positions exposing a subdomain structure consisting of a highly charged/polar membrane-proximal portion, followed by an ITAM sequence and two carboxy-terminal ITIMs (**Figure 3**). The ITAM sequence in FCRL cytoplasmic tails differs from the established consensus by having 9 rather than 6–8 amino acids that separate the two tandem tyrosine segments (51, 64). Notably, however, FCGR2A, which has a 12 amino acid intervening ITAM sequence, initiates cellular activation by associating with Syk tyrosine kinase family members (69). Therefore, spacing of the tandem tyrosines does not appear to affect Syk family recruitment. The two carboxy-terminal ITIM sequences have a 22 amino acid spacer that is typical of this motif when found in tandem (70). The presence of both activating and inhibitory motifs in these receptor tails, as seen in human FCRL3, for example, indicates that these molecules could have dual regulatory potential. This has indeed been observed for FCRL3 in biochemical experiments examining recruitment to its respective phosphorylated tyrosines (71). Several of the FCRL ITAM-like sequences

are noncanonical and differ at the –2 and +3 positions relative to potentially phosphorylated tyrosines. Despite these differences, the backbone spacing for these sequences is very well conserved. For example, human FCRL1 has two potential ITAMs that do not perfectly follow the consensus; however, this receptor has coactivating regulatory function in B cells (72).

The cytoplasmic tails of mouse FCRL representatives also possess tyrosine-based sequences. Like human FCRL1, its mouse correspondent is predicted to have activating capability given its canonical cytoplasmic ITAM. Although mouse FCRL5 has extracellular composition that is most similar to human FCRL3, its cytoplasmic region is more like FCRL5, including an ITAM-like sequence and an ITIM. Given the ambiguity of these predicted signaling motifs in FCRL molecules and in other proteins (73), detailed biochemistry and mutagenesis studies will clearly be required to clarify their functionality.

PHYLOGENETIC CONSERVATION OF THE FCRL MULTIGENE FAMILY

The *FCRL/FCR* multigene family, LRC family members, and other activating/inhibitory IgSF receptors positioned at paralogous genomic loci all likely evolved from a common ancestor that long ago assembled these biologically advantageous extracellular and cytoplasmic signaling elements (74–80). The intrinsic value of these molecules in coordinating immune defense responses is implied by their appearance in species even prior to the advent of adaptive immune BCRs and TCRs in jawed vertebrates. In the hagfish, one of two extant jawless vertebrates, a family of novel ITAM-containing IgSF receptors (NICIR) has recently been identified, members of which are expressed by blood leukocytes and possess ITAM or ITIM cytoplasmic sequences (81). Secondly, a TCR-like molecule with cytoplasmic ITIMs has been found in the other surviving agnathan, the sea lamprey (82). Despite limited overall sequence and structural identity between the hagfish NICIR and lamprey TCR-like molecules with activating/inhibitory IgSF representatives in higher vertebrates, their ancient existence and conserved features emphasize the basic importance of this extracellular and cytoplasmic combination in immunity.

FCRL orthologs have not been identified in lower vertebrates. Although these negative findings could indicate that these genes are nonexistent, the analysis has been hampered by incomplete genome sequence data. A family of recently identified modular domain immune-type receptors (MDIR) in the clear nose skate (*Raja eglanteria*) possess domains similar to the D4- and D5-type subunits unique to the FCRL family (83). This homology is noteworthy as indicated by a low E value ($E = 4 \times 10^{-7}$) resulting from a BLASTP comparison between the sixth domain of MDIR4 and the fifth domain of human FCRL5. This analysis also implies a relationship between these domain types and those pronounced in adhesion molecules such as CD31/PECAM, as well as lectin Ig domain–containing receptors Titin and CD22 (8, 9, 11). The other Ig subunits found in MDIR receptors, however, are not related to FCRL domains but more closely resemble those present in TREM (triggering receptors expressed in myeloid cells) (84) and CMRF receptors (85). These differences suggest that MDIR molecules have a mosaic extracellular façade and are not true FCRL orthologs; however, they share some interesting extracellular similarity that might suggest a common evolutionary origin.

Ancient *FCRL* representatives are clearly evident in amphibian and avian species. In frogs, an extraordinarily large number of *FCRL*-like genes (designated *XFL*) has been described, with characteristics emblematic of human and mouse FCRL (86). Unpublished reports indicate that *XFL* orthologs may number up to 70 in the *Xenopus* genome and are transcribed in lymphoid tissues. These genes

Polymorphisms: variations in nucleotide sequences among genetic alleles

putatively encode transmembrane as well as secreted isoforms that possess the five distinct Ig subunits discernable in humans and mice, along with conserved cytoplasmic tyrosine-based motifs. The presence of the D3-type (yellow) domain in many of these sequences suggests extensive duplication for this particular subunit (76; A.V. Taranin, personal communication). Examples of this type of multiplicity and expanded multigene family diversity include the chicken Ig-like receptor (CHIR) genes (87, 88) and novel immune-type receptors (NITR) in bony fish (89). Remarkably, marked contraction seems to be apparent for these genes in chickens. As of this writing, only a single related *FCRL* gene has been discovered (M.R. Odom, R.S. Davis, and M.D. Cooper, unpublished results). This avian ortholog has conserved genetic organization, including a 21 bp miniexon, and is predicted to encode a receptor with domain composition analogous to human FCRL4, with which it shares 38% overall amino acid identity. Transcripts for this gene have also been amplified from kidney, liver, and bursa. Additional avian relatives have not yet been uncovered, indicating that there is only a single *FCRL* ortholog in chickens or that identification of other *FCRL* genes must await completion of the chicken genome sequence.

Thus, the most current information supports the genesis of *FCRL* genes around the time of amphibian speciation 350 mya, but questions as to whether *FCRL* or *FCR* genes arose first or concurrently are not yet clear. The conserved features obvious for *FCRL* and *FCR* family members clearly indicate that these two subfamilies share a common ancestor. In light of the evolution of diverse Ig heavy chain isotypes and class switch recombination, it might be logical to propose that the *FCRL* family arose earlier and gave rise to the *FCR* through gene duplication/deletion and exon shuffling and exchange. Amphibians are the first vertebrates to use class switch recombination for antibody generation (90). *Xenopus* possesses IgM, IgY [which is evolutionarily related to IgG and IgE (91)], and IgX (which is functionally homologous to IgA) isotypes (90). The duplicative explosion of these genes overt in *Xenopus* could have easily resulted in the generation and diversification of primordial, classical-type FCRs that are presently recognized to bind IgG and IgE isotypes. Alternatively, the *FCRL* family may plausibly have arisen after the *FCR* gene family emerged and perhaps is a byproduct of this dynamic region of the genome. There is recent evidence of a soluble FCR ortholog in the channel catfish (*Ictalurus punctatus)* with IgM-binding capability and both lymphoid and neutrophil expression (92, 93). Thus, where in evolution these gene families arose currently remains uncertain. Hopefully this will become clearer as additional genome sequences are completed and finer genetic analyses are performed.

Both *FCRL* and *FCR* gene numbers have expanded since humans and mice shared a common ancestor. However, whereas interspecies domain composition and cytoplasmic segment differences exist for encoded FCRL molecules, little variation is evident for mouse and human FCR counterparts. This could suggest that there has been a higher rate of genetic recombination for the *FCRL* genes as they evolved, also possibly reflecting diversification of their ligands. The relative positions of *Fcrl1* and *Fcrl5* orthologs between these genomes may be in agreement with the hypothesis that framework loci may bookend regions of high genetic turnover (94). This theory has been proposed to account for the extraordinary haplotype variability of the human *KIR* gene cluster observed among individuals. Despite polymorphisms found for human *FCRL* genes (24) and the existence of ten nonsynonymous polymorphisms noticeable in two *Fcrl5* alleles among five mouse strains (95), the dramatic degree of intraspecies diversity explicit in the *KIR* and *LILR* genes does not appear to be as prominent for the *FCRL* family. However, the distinct locations of these two genes, the outgrowth of family members between them, and the likelihood that they are under adaptive selection

is in line with the framework loci hypothesis (94, 96).

The 1q21–23 genomic region in humans has distinct characteristics that include high gene density and G+C content as well as increased recombination frequencies relative to other chromosome 1 regions (97). The 1q21–23 locus also harbors the epidermal differentiation complex that is the most rapidly diverging gene cluster between humans and chimpanzees (97). The presence of sequence duplications, pericentric inversions, and copy number polymorphisms all predict a high rate of genetic turnover for this genomic locale, which could increase *FCRL* and *FCR* gene diversity (98) but also possibly lead to malignancy (see below) or autoimmunity (99). Thus, given the relative genetic recombination rate catalyzed by homologous sequence segments localized in this region, diversification of the *FCRL* family is not unexpected.

FCRL1–5 ARE PREFERENTIALLY EXPRESSED BY B CELL SUBPOPULATIONS

FCRL1–5 are all expressed by B cells but vary in their distribution on different subpopulations. FCRL3 is the only representative of this group expressed outside the B lineage, where it is also found on NK and T cell subsets. At the level of transcription, *FCRL1–5* are most abundantly expressed in secondary lymphoid tissues, including the spleen, lymph nodes, and tonsils. During their initial characterization, some *FCRL1–5* probes were found to hybridize with transcripts in nonhematopoietic organs (8–10). These aberrant signals were likely the result of suboptimal probe design and/or blood cell contamination. Northern blot and EST database analyses have also identified splice isoforms for each of these genes, some of which have been cloned and attest to the sophistication of *FCRL1–5* expression and regulation.

Among blood leukocytes, *FCRL1–5* transcripts are not detected in myeloid cells such as monocytes, dendritic cells, or neutrophils, indicating that they most likely are lymphoid specific. *FCRL1–3* and *FCRL5* are highly expressed in blood B cell populations, whereas *FCRL4* is barely detectable (100–102). Interestingly, *FCRL3* is unique in its expression by blood NK cells and T cells as well as B cells (101, 103, F. Li, J. Pan, and R. Davis, unpublished observations). Transcriptional analysis of *FCRL1–5* in isolated tonsillar B cells and by in situ hybridization in intact tissue sections highlights the intriguing variation of *FCRL1–5* among distinct subpopulations and respective topographic localization (8–10, 72, 104) (**Table 1**). *FCRL1* and *FCRL2* transcription is mainly confined to the follicular mantle zone, where naive B cells localize. However, *FCRL2*

B CELL DIFFERENTIATION

B cells and T cells generate adaptive immunity in jawed vertebrates via their unique ability to somatically recombine Ig receptor components. B cell maturation first begins in the fetal liver and bone marrow, where common lymphoid progenitors give rise to committed B lineage precursor cells. Sequential rearrangement of random combinations of variable (V), diversity (D), and joining (J) genes in pro- and pre-B cells leads to heavy and light chain expression, which form the basic framework of antibody molecules also known as B cell receptors (BCRs). After emigration from the bone marrow, immature and transitional B cells home to the mantle zones in secondary lymphoid organs and become naive IgM^+IgD^+ B cells. Upon BCR engagement by cognate antigens and signaling via surface coreceptors, naive B cells enter GC dark zones, upregulate genes for proliferation and the activation-induced cytidine deaminase (AID), and undergo Ig modification and affinity maturation as centroblasts. Both AID-dependent somatic hypermutation of rearranged germline VDJ segments and class switching result in increased antigen-binding affinity and diversification. Mutated BCRs expressed by light zone centrocytes are selected by B cell contact with antigen-presenting cells and T helper cells that determine the fidelity of the adapted receptors. Successful passage through multiple checkpoints promotes B cell differentiation into antibody-secreting PCs and memory cells that mediate robust responses to future antigenic challenge.

Table 1 Comparison of FCRL1–5 transcription and protein expression[a]

Receptor	FCRL expression by flow cytometry				*FCRL* transcription (tonsil)	FCRL immunohistology (tonsil and spleen)
	Bone Marrow	Blood	Tonsil	Spleen		
FCRL1	B > pre-B > pro-B	Pan-B	N > M > GC	N, M	Mantle zone	Mantle zone (T+S)
FCRL2	(–)	M	M > N, PC	M	Mantle zone	Marginal zone (S), interfollicular, intraepithelial (T)
FCRL3	M > pre-B, NK, T	B, NK, T	M > N > GC, NK, T	M > N, NK, T	GC light zone, intraepithelial, interfollicular, mantle zones	GC light zone, interfollicular, intraepithelial (T)
FCRL4	(–)	(–)	M > N	M ≫ N	Intraepithelial, interfollicular	Intraepithelial (T), monocytoid B and GC (LN)
FCRL5	Pre-B, PC	Pan-B?	PC > M > N > GC	PC > M > N	GC light zone, intraepithelial, interfollicular	n.d.

[a]Abbreviations: plasma cell (PC), naive (N), memory (M), germinal center (GC), tonsil (T), spleen (S), lymph node (LN), no data (n.d.).

Germinal center (GC): microenvironments within secondary lymphoid tissues where T cell–dependent antibody responses and antibody diversification occur.

MZ: marginal zone

PC: plasma cell

is also found at low levels just outside this region. This is in contrast to *FCRL3* and *FCRL5*, which accumulate in the light zone of the germinal center (GC), a compartment that is rich in centrocytes. Both of these genes are also identified in interfollicular and intraepithelial regions, also known as the marginal zone (MZ) equivalent in tonsils, where memory B cells are positioned (105), and at lower levels in follicular mantle zones. *FCRL4* displays a third distinct pattern of expression, particularly in intraepithelial regions. This distribution largely, but not completely, correlates with cell surface protein expression on tonsil cell suspensions.

At this time, our understanding of the distribution of FCRL1–5 expression is incomplete. However, in view of their transcription patterns and the few protein surveys that have been published, it is becoming clear that these receptors will be useful as distinct cellular markers. Monoclonal antibodies (mAbs) specific for FCRL1–5 are now being developed by several groups and have been analyzed to variable degrees (48, 72, 102, 106, 107). These patterns are discussed below and summarized in **Table 1** and **Figure 4**.

FCRL1

In the bone marrow, FCRL1 expression begins on precursor B cells and increases as a function of differentiation, attaining its highest levels among mature B cells (48, 72). Upon emigration to the blood, FCRL1 is detected on all $CD19^+$ B cells. In the tonsil, FCRL1 expression peaks on IgD^+CD38^- naive B cells that localize in the follicular mantle zone and correlates with its abundant transcription by the cells in this region (10). As these cells become IgD^+CD38^+ activated pre-GC cells and differentiate into IgD^-CD38^+ GC B cells, FCRL1 is downregulated from the cell surface. Its relative expression increases on IgD^-CD38^- memory B cells; however, IgD^-CD38^{2+} plasma cells (PCs) express FCRL1 at lower levels. *FCRL1* expression in the spleen is also confined to B lineage naive and memory cells. Comparisons of *FCRL1* gene expression and flow cytometric mean fluorescence intensities for the five tonsillar B cell subsets defined by IgD and CD38 expression (108) indicate that *FCRL1* transcript levels are more tightly controlled than is FCRL1 surface expression (72). Although high levels of *FCRL1* transcripts

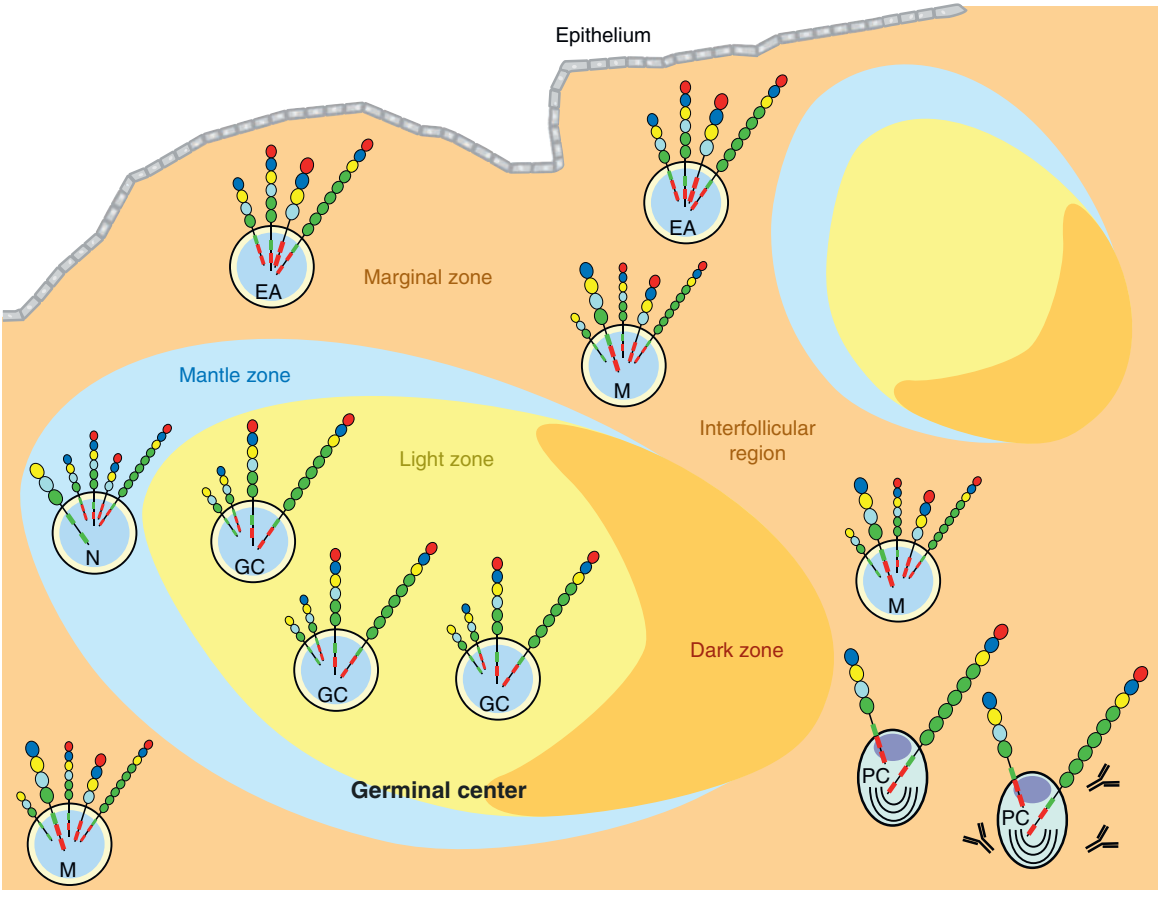

Figure 4

A summary of human FCRL1–5 topographical localization patterns in the tonsil. Epithelial-associated (EA), memory (M), naive (N), germinal center (GC), and plasma cell (PC) B cell subpopulations are illustrated with their FCRL receptors (identical to those in **Figure 2**) to approximate their expression at observed regions within the tonsil. Different sizes of FCRL receptors symbolize their relative expression levels on each subset. Histological landmarks are labeled. These patterns summarize data of the transcript and protein levels (8–10, 48, 72, 102, 106; and D. Mason, M. Cooper, and R. Davis, unpublished observations). Note FCRL5 localization is based on its transcription and expression pattern in suspended cells.

mirror its peak expression on naive B cells, its messenger RNA is significantly decreased in pre-GC, GC, and PC subsets, where protein is seen to linger. Nevertheless, our unpublished observations using mAbs for immunohistochemistry confirm the mantle zone tropism of FCRL1-expressing cells. Thus, FCRL1 appears to be a pan-B cell marker that reaches highest expression on naive B cells.

FCRL2

A survey of FCRL2 expression by Polson et al. (48) indicates that this family member is predominantly expressed by memory B cells. FCRL2 is not identified on precursor B cells or $CD138^{+}CD38^{2+}$ PCs in the bone marrow; however, in blood it is found on a subset of $CD20^{+}$ B cells, the majority of which express the human memory B cell marker CD27 (109, 110). In dissociated tonsillar

cells, FCRL2 is identified primarily on the IgD$^-$CD38$^-$ memory B cell population and at low levels on PCs in most tonsil samples (48). However, our unpublished results (F. Li and R. Davis) suggest high levels of FCRL2 expression on PCs in tonsils and spleen samples. In accord with this expression pattern on freshly isolated cells, in frozen sections FCRL2 expression is concentrated in intra- and subepithelial regions proximal to the mantle zone where memory B cells situate (48, 105; and D. Mason, M. Cooper, and R. Davis, unpublished observations). This pattern is also consistent with exclusive localization patterns for IgD and FCRL2 within mantle zone and MZ regions, respectively, in the spleen (48). Analysis by in situ hybridization gives a different result in that *FCRL2* transcripts are confined to the naive B cell–rich and memory B cell–poor mantle zone (10).

FCRL3

Like FCRL2, FCRL3 expression is not markedly detected on bone marrow B cells (48). In the blood, FCRL3 is identified at relatively low levels on CD20$^+$ B cells but may vary by individual sample and according to monoclonal reagent (F. J. Li and R.S. Davis, unpublished observations). Consistent with its transcription, FCRL3 is expressed on the surface of CD56$^+$ NK cells, but interestingly it is variably expressed on T cell subsets as well (F. J. Li and R.S. Davis, unpublished observations). FCRL3 expression is observed at low levels among naive, GC, and memory B cells in the tonsil and at slightly higher levels on splenic naive and memory B cells (48). This expression pattern once again seems to differ from the high relative transcript levels apparent in the light zone of the GC and in interfollicular regions of tonsils (10). These inconsistencies may have several explanations: (*a*) Surface expression is lost once cells are placed in suspension, (*b*) there are dramatic differences in the posttranscriptional and translational regulation of *FCRL3* gene expression, or (*c*) the distinct epitopes recognized by different antibodies may vary between receptor isoforms.

FCRL4

FCRL4 expression has been extensively analyzed by three independent groups using both monoclonal and polyclonal antibodies (48, 102, 106). Collectively these studies validate its expression on a unique subset of tissue-based memory B cells that preferentially localize to subepithelial regions. FCRL4 does not appear to be expressed at appreciable levels in the bone marrow or on blood leukocytes. In tonsils, however, FCRL4 is consistently detected on ~10% of B cells, but its expression can vary among samples. FCRL4$^+$ cells are primarily enriched in the memory B cell fraction and in smaller numbers among the naive subset. FCRL4 is also identified on memory B cells in the spleen, albeit at lower levels. Interestingly, among IgD$^-$CD38$^-$ memory B cells the majority of FCRL4$^+$ cells (~75%) are CD27$^-$, with only a minority of cells expressing both FCRL4 and CD27.

Several groups have performed immunohistochemistry for FCRL4 expression, and these data are consistent with the distribution of transcripts in tonsils (106, 111, 112). In the spleen and mesenteric lymph nodes, few FCRL4-expressing cells are detected, and these are strictly confined to the MZ, whereas in tonsils and other mucosal-associated lymphatic tissues (MALT) more cells express it. Staining is found proximal to the tonsillar epithelium near the sinuses but is less plentiful near the boarders of mantle zones and in interfollicular regions. In Peyer's patches, FCRL4 is also identified on B cells located near epithelial borders as well as on intraepithelial B cells. A second population of reactive B cells known as interfollicular large monocytoid B cells, which are enriched in toxoplasmosis, mononucleosis, and HIV-infected tissues, are strongly FCRL4 positive (106, 111, 112). These cells are situated around the border of the mantle zone and subcapsular sinus. These data indicate that FCRL4 marks B cells

with epitheliotropism in the MALT and secondly stains reactive monocytoid B cells. The observation that few cells are FCRL4 positive in the bona fide splenic MZ has assisted in resolving some controversy concerning the origins of these B cells. The finding that FCRL4 marks epithelial-associated and monocytoid B cells, but not MZ B cells, implies that these cells are distinct subpopulations that probably do not arise from a single progenitor (111).

Compared with $CD27^+FCRL4^-$ memory B cells, FCRL4-expressing memory B cells are larger as measured by light scatter and confirmed microscopically by their more abundant cytoplasm, smaller nuclei, and distinct villous-like projections that extend from the cell surface (102, 106). Consistent with their morphologic appearance, they have an activated phenotype as defined by comparably higher levels of CD32, CD40, CD44, CD80, and CD84 and lower levels of CD21 than $FCRL4^-$ memory B cells.

Most $FCRL4^+$ memory B cells express cell surface IgG (IgG > IgA > IgM), and their V_H region genes are mutated at frequencies similar to $CD27^+$ B cells (102). However, the designation of FCRL4 as a marker of memory B cells requires additional qualification. There is some discrepancy involving their IgV_H mutation status and phenotype and, perhaps, differences between suspended cells and whole tissues. Variations between tonsil samples, including the characteristic immune responses endemic to these tissues, must also be considered. It appears by phenotype that FCRL4 cells may mark both memory and naive B cells; however, the majority of $FCRL4^+$ tonsillar cells in suspension display the IgD^-CD38^- memory phenotype, indicating that they are antigen experienced (108). Despite the fact that most of these cells harbor somatic mutations, by flow cytometry only a minority of $FCRL4^+$ cells expresses the conventionally accepted memory B cell marker CD27 (102). Furthermore, a small number of these cells lacks mutation, even though they have an evocative phenotype. In reactive tissues, a small number of $FCRL4^+$ cells are found in GCs and express both FCRL4 and CD27, but not BCL6 (112). In the same study by Lazzi et al. (112), microdissection and IgV_H analysis of single $FCRL4^+$ cells from GC and sinus regions revealed that 100% of $FCRL4^+$ cells in the GC possess somatic mutations, whereas $FCRL4^+$ cells in the sinuses have mixed mutation status. These cells also tend to have slightly lower mutation frequencies than do GC-derived cells, but have a comparable spectrum. Interestingly, the sinus areas stain mostly negative for CD27. It thus remains unclear how $FCRL4^+CD27^-$ cells acquire somatic mutations and whether these particular cells enter traditional GC reactions or participate in an alternative pathway of affinity maturation, as has been suggested by others (113, 114).

Differences between $FCRL4^+$ or $FCRL4^-$ memory B cells also extend to their responses to mitogens. Whereas $FCRL4^-$ cells respond similarly to T-independent and T-dependent stimuli, cells marked by FCRL4 more strongly proliferate in response to combinations of T-dependent cytokines/signals (IL-2/IL-10/CD40L). However, there is only minimal activation following direct BCR ligation with anti-Ig antibodies or Protein A^+ heat-killed bacteria (102). $FCRL4^+$ cells also demonstrate more robust Ig secretion than their $FCRL4^-$ memory B cell counterparts, with isotype frequencies reflective of their phenotype. Although these results suggest that $FCRL4^+$ cells might be poised to differentiate into Ig-secreting PCs, and several PC representative cell lines do stain positively for FCRL4, primary $FCRL4^+$ cells derived from tonsils of normal donors do not appear to be committed to PC differentiation. This is indicated not only by an absence of CD138 (102) and BLIMP1 coexpression (115), but also by a failure to transcribe *Blimp-1*, *IRF-4*, and spliced *XBP-1* transcription factors that are fundamental to PC development (102). Consistent with previous histological findings (112), isolated $FCRL4^+$ cells also do not express *Bcl-6*. However, they do contain message for the *CCR1* and *CCR5* chemokine receptors

that could explain their localization to epithelial regions where their cognate ligands have been identified (116). Thus, FCRL4 labels a unique memory B cell population with distinct epithelial-associated tissue localization that displays an activated phenotype and preferentially responds to T-dependent stimulation, but may not necessarily bear IgV_H somatic mutations.

FCRL5

FCRL5 expression has not been extensively characterized, but mAbs have been generated by two groups (48, 107). FCRL5 can be identified on pre-B cells in the bone marrow, but similar to other family members obtains maximum levels of expression in the periphery. Its detection on blood B lymphocytes, however, appears to differ according to the investigators who have studied it. Although Polson et al. (48) have found FCRL5 on all circulating $CD20^+$ B cells in blood, we and others do not (F. J. Li and R.S. Davis, unpublished observations; and 107). This incongruity could be explained by the multiple splice isoforms that are generated for this particular receptor, reflecting variations in epitopes recognized by different mAbs for the FCRL5 variants (9, 10). Analysis of tonsils and spleen indicates high levels of FCRL5 expression on memory B cells and slightly lower levels on naive cells. GC B cells may also express it, but at low density. Among FCRL family members, however, FCRL5 is distinguished by its strong expression on PCs from different tissue sources, including the bone marrow, tonsils, and spleen. Although FCRL5 and FCRL3 display similar patterns of transcription among GC cells in the light zone as well as among memory B cells in the interfollicular regions in situ, FCRL5 protein expression in suspension appears to differ from this finding. It is found at lowest levels on isolated GC B cells (9, 10). Unfortunately, immunohistology data for FCRL5 are incomplete at this point; however, its surface expression on PCs confirms previous results of its transcription in these cells (8).

FCRL Expression in Mice

In mice, *Fcrl1* and *Fcrl5* are also expressed by B cells in secondary lymphoid tissues, whereas the atypical *Fcrls* gene is identified primarily in nonhematopoietic organs (29). In bone marrow B cells, *Fcrl1* and *Fcrl5* are transcribed at very low levels, but they can be detected beginning at the pre-B cell stage and increase with differentiation. Like their human equivalents, both genes' expression peaks in the periphery. *Fcrl1* is broadly expressed by newly formed, follicular, and MZ B cells in the spleen and in B1- and B2-type cells in the peritoneal cavity. Notably, *Fcrl5* is distinctly enriched in splenic MZ B cells and peritoneal B1 cells. Expression outside the B lineage has not been observed for either of these genes.

A recent survey of the FCRL5 receptor in mice confirms its expression on subpopulations of B cells (95). Histologically, FCRL5 is mainly restricted to the splenic MZ. However, a few scattered $FCRL5^+$ cells are also detected in follicular areas that are IgM^-. These cells could be class switched $IgG3^+$ B1 cells or possibly memory B cells. In dissociated cells, FCRL5 is identified on only a small fraction of bone marrow B cells, but it is abundantly expressed on splenic MZ B cells and B1 B cells. It cannot be detected on follicular or newly formed B cells from unimmunized mice or at significant levels on GC B cells or PCs after immunization. Interestingly, FCRL5 is also identified on a recently characterized subset of $CD21^+CD23^+$ MZ precursor B cells that also express CD1d and CD9 (117, 118). This correlates with the deficiency of conventional MZ B cells in *CD19*-knockout mice but with the presence of a population of $CD21^+CD23^+$ cells in this model that is FCRL5 reactive. These data suggest that FCRL5 could be a commitment marker of the MZ compartment. Furthermore, FCRL5 expression may require an intact btk signaling pathway for its optimal expression. This is indicated by the presence in CBA/CaHN strain xid mice of phenotypically distinguishable

MZ B cells that have considerably reduced levels of FCRL5 expression. Outside the spleen, FCRL5 is normally found on both B1a and B1b cells in the peritoneal cavity as well as on a subpopulation of B2 cells that varies by strain. Thus, mouse FCRL5 appears to be a discrete marker of MZ and B1 B cells.

Interspecies differences that exist between mouse and human FCRL5 orthologs in their extracellular and cytoplasmic regions also seem to extend to their patterns of expression. Discrepancies are additionally apparent for distinct lymphocyte subsets, such as transitional, MZ, and B1 B cells between these two species (119–122). Expression by human MZ B cell counterparts has been found for human FCRL3 (120), which has some similarity to mouse FCRL5. FCRL5 expression on mouse memory B cells, however, has yet to be verified. Given the similar features and topographic locations of memory and MZ B cells, FCRL5 could be found on both subpopulations in mice (123, 124). Further investigation of this molecule in both species will help clarify differences in the functions of these receptors and the origins of the cells that express them.

BIOLOGICAL ROLES OF FCRL MOLECULES

Despite the identification of the FCRL1–5 molecules using a consensus sequence derived from the Fc-binding interface of FCRs, and the clear phylogenetic relationship between these receptor subfamilies, it is ironic that incontrovertible biochemical evidence has yet to be presented demonstrating Ig binding by any of the FCRL proteins. One report has now shown suggestive results for IgG reactivity with FCRL5 (48). In this flow cytometric analysis, FCRL5 was observed to bind to a sample of mixed IgG isotypes, but not to monomeric Ig. Apparently ~10% of this preparation was aggregated by size exclusion chromatography; however, the authors indicate that aliquots with comparable or higher levels of aggregation failed to bind. Furthermore, Ig binding for FCRL5 or the other four FCRL molecules was undetectable by Bio-Layer Interferometry. In light of surface plasmon resonance detectable binding of monomeric Ig to the low-affinity FCGR (125, 126), these data remain difficult to interpret. Similar observations of Ig binding for FCRL5 have been discussed previously (9), but at this point more detailed kinetic and biochemical analysis is needed to declare FCRL5 a conventional FCR.

Key features that the FCRL molecules share with the classical FCR, including their conserved domain composition and utilization of tyrosine-based motifs, point to the likelihood that FCRL molecules may have endogenous ligands. This is supported not only by the functionality of their closest phylogenetic relatives but by other characteristics as well. The unusual combination of membrane-distal D1-D3 subunits resembling those used for Fc binding and distinct membrane-proximal D4-D5 domains with homology to cell adhesion molecules indicates that some family members, such as FCRL3–5, could have multiple ligands. The presence of both ITAM and ITIM in some representatives may also favor an endogenous counterstructure, given the probability that these molecules signal to fine-tune responses rather than to exert all-or-none effects.

Even with the initial identification of Ig-binding receptors on cells more than four decades ago (127) and their molecular characterization over the past two decades, only recently have the discrete outcomes dictated by variable affinities between IgG subclasses and their multiple FCGR partners become appreciated (128). It will likely take a while to determine the nature of the FCRL ligands. Although characterization of mouse models for FCRL1 and FCRL5 will help shed light on some aspects of their biology, their signaling potential and expression in human pathology are active independent areas of investigation.

FCRL MOLECULES MAY HAVE ACTIVATING, INHIBITORY, OR DUAL POTENTIAL

Src homology 2 (SH2) domains: ~100 amino acid sequences commonly found among intracellular signal transduction proteins that facilitate binding to phosphorylated tyrosines and the regulation of signaling cascades

FCRL1–5 are all equipped with cytoplasmic ITAM-like and/or ITIM elements that indicate their potential for modulating B cell regulation via the recruitment of SH2 domain–containing molecules. Following receptor engagement, ITAM-bearing receptors are phosphorylated by Src family kinases that facilitate docking and activation of tyrosine kinases such as Syk to promote downstream phosphorylation (129). Opposing inhibitory competence is exerted by ITIMs that are also phosphorylated upon receptor ligation by Src family kinases. Instead, ITIMs recruit phosphatases such as the SH2-containing inositol phophatase (SHIP) or SH2-containing phosphotyrosine phosphatase (SHP)-1 that have the potential to dephosphorylate neighboring receptors and abrogate cellular activation (62). Similar to ITIMs, ITSM sequences can engage SH2-containing phosphatases but are also capable of binding to adaptor molecules such as SH2D1A and EAT-2, Src family kinases, and the p85 subunit of phosphatidylinositol-3 kinase (PI3K) (65, 66). Therefore, the switch motif can enable receptors that contain it to associate with both inhibitory and activating components. The signaling capacity of human FCRL1, FCRL3, and FCRL4 has been examined to varying extents, but all FCRL receptors in humans and mice that possess tyrosine-based motifs are capable of being tyrosine phosphorylated (J. Pan and R.S. Davis, unpublished observations).

FCRL1 possesses two potential ITAMs (see **Figures 2** and **3**). These sequences diverge from the consensus in the first ITAM-like sequence by a serine at the +3 position relative to the first tyrosine and in the second activation-like motif by an acidic amino acid (aspartic acid) at the –2 position rather than at –3, and an alanine at +3 relative to the second tandem tyrosine. Despite these differences, FCRL1 appears to have activating coreceptor function on B cells (72). FCRL1 is tyrosine phosphorylated upon self-ligation in B cell lines and primary tonsillar B cells. The recruitment of specific signaling elements to its cytoplasmic tail remains under investigation, but several downstream effects have already been evaluated. Although FCRL1 ligation alone does not directly affect calcium mobilization, cross-linkage with the BCR enhances flux of calcium to a greater degree than that observed for suboptimal BCR ligation alone. This suggests that FCRL1 signaling may not elicit global activating effects in B cells but rather may subtly influence the process. Its coactivating potential in tonsillar B cells correlates with increased cell size as well as upregulation of the CD69 and CD86 activation markers and the downregulation of IgD. Furthermore, anti-FCRL1-treated B cells have increased proliferative capacity that is enhanced upon coligation with the BCR but that does not result in apoptosis or upregulation of survival mediators Bax, Bcl-2, Bcl-x, or Mcl-1. These changes are in line with a coactivating role for FCRL1 on B cells; however, the existence of a charged residue in its transmembrane region indicates that FCRL1 may associate with other complementary-charged molecules. Collectively, such multicomponent complexes could exert a stronger overall regulatory influence in B lymphocytes.

More detailed signaling characterization has been performed for FCRL4, which has three potential cytoplasmic ITIMs. Interestingly, although the two distal ITIMs (463 and 493) are canonical, the most membrane-proximal motif (451) differs at the –2 position by the presence of a polar serine rather than an aliphatic amino acid. This motif could also serve as an ITSM. Alternatively, this sequence, along with the ITIM at position 463, could conceivably form an unconventional ITAM. FCRL4 signaling potential has been explored by generating mutant receptors engineered with Y → F substitutions of the three cytoplasmic tyrosines in the A20IIA.6 IgG2a$^+$ mouse B cell line (104).

FCRL4 cocross-linking with the BCR results in tyrosine phosphorylation of the wild-type FCRL4 cytoplasmic tail, marked depression of whole cell tyrosine phosphorylation, and decreased Erk and Akt phosphorylation, whereas FCRL4 self-ligation has no effect. FCGR2B, however, has comparatively little global influence on cellular phosphorylation in similar experiments. In calcium mobilization assays, FCRL4-BCR coligation results in dramatic inhibition of BCR-induced calcium flux to an even more demonstrable degree than that observed for FCGR2B. This effect is mediated by the two distal FCRL4 ITIMs, whereas the 451 motif has little contribution. The cytoplasmic partners responsible for exerting downstream inhibitory effects differ between FCRL4 and FCGR2B. In contrast to FCGR2B, which recruits the inositol phosphatase SHIP (130), FCRL4 engages SHP-1 and SHP-2 protein tyrosine phosphatases that could have a role in proximal deconstruction of the activated BCR complex. Although both of these inhibitory receptors are expressed by memory B cells, they might have differential effects depending on the timing of their ligation and contact with their respective ligands. The complexity of this receptor is also inferred by its possession of an atypical ITAM. Interestingly, recombinant peptides phosphorylated at residues 451 and 463 were also capable of immunoprecipitating PLCγ (104), suggesting that under favorable conditions the noncanonical ITAM could have activating function. Thus, FCRL4 could have dual regulatory potential.

The possibility of both activating and inhibitory functions is suggested by the presence of both ITAM-like and ITIM sequences in human FCRL3 as well as in human and mouse FCRL5 cytoplasmic tails (**Figure 3**). Some LRC family members also have sequences indicative of dual signaling potential, but they differ by their possession of charged transmembrane residues that can recruit ITAM-containing adaptors (131, 132). Although tyrosine phosphorylation has been observed for FCRL1, FCRL3, and FCRL4, association with both intracellular protein kinases and phosphatases has only been published for human FCRL3 (SPAP2) (J. Pan and R. Davis, unpublished observations; 71). This receptor has four tyrosines (650, 662, 692, and 722) that could generate a canonical ITAM and a consensus ITIM. There is also a distal tyrosine (722) with an acidic amino acid at the –2 position and an aliphatic residue at +3, but no corresponding second tandem tyrosine. This sequence could represent a hemi-ITAM. FCRL3 immunoprecipitates from pervanadate-treated 293T transfectants demonstrate that it is tyrosine phosphorylated and associates with SHP-1 (71). Pull-down assays with GST fusion proteins having various Y $\rightarrow$ F mutations at these four sites have also clarified effector and recruitment relationships in lysates of Jurkat cells. These experiments indicate that FCRL3 is capable of associating with Syk, Zap-70, SHP-1, and SHP-2. Individual mutations to the two distal tyrosines result in a loss of SHP-1 interaction but in conservation of Zap-70 binding. Y $\rightarrow$ F mutants of the two ITAM tyrosines maintain SHP-1 association but abolish Zap-70 recruitment. Notably, phenylalanine substitutions of tyrosines 650 or 662 preserve Zap-70 coimmunoprecipitation. Despite these provocative results, further characterization and confirmation in transfected cells is needed to authenticate these findings. However, these data suggest that determining the signaling capability of this receptor is likely to be quite involved and could be compounded by other factors including a single nucleotide polymorphism (SNP) that alters the FCRL3 ITAM (24) and/or the expression of FCRL3 in B, T, and NK lineage cells, where it could have different functional potential depending on the indigenous signaling elements and the immune responses in which these cell types participate.

For mouse FCRL molecules, there is limited functional information. Our beginning studies indicate that FCRL5 is tyrosine phosphorylated in B cells (95). Similar to other FCRL molecules, little effect is observed

following its self-engagement, but coligation of FCRL5 with the BCR results in a dampening of calcium mobilization in MZ B cells similar to that seen for FCGR2B. These initial results indicate that mouse FCRL5 could have inhibitory function in MZ B cells.

DLBCL: diffuse large B cell lymphoma

POTENTIAL ROLES OF FCRL1–5 IN DISEASE

The preferential expression of immunoregulatory FCRL molecules on populations of B cells, as well as on T and NK cells, suggests that these receptors may function in both innate and adaptive immune responses. The identification of various isoforms and multiple SNPs in coding and noncoding regions indicate that their functional roles may be complex and broad (9, 24, 103). The regulation of these receptors could be indirectly influenced by inappropriate immune responses, but they might also have fundamental pathogenic roles in immunodeficiency, autoimmunity, malignancy, and pathogen elimination. Although we have only begun to understand their biology, the identification of several *FCRL* genes in B cell malignancies and in certain autoimmune disorders portends important roles for them in normal and pathologic conditions.

Expression in Malignancies

The identification of *IRTA1/FCRL4* by its involvement in a (1;14)(q21;q32) translocation breakpoint in a multiple myeloma cell line revealed that these genes may have an important association with B cell malignancies (9). This genetic aberration produced a chimeric protein that could only be detected in the FR4 cell line in which the translocation was identified; therefore, this finding seems to have been an isolated phenomenon. However, the 1q21–23 genomic region is notorious for its genomic instability and association with B lineage malignancies (133–136). Not surprisingly, there is now evidence for recurring involvement of the *FCRL* locus in non-Hodgkin's lymphoma chromosomal alterations as well (137–140).

A broader perspective of the general expression patterns for these genes as potential markers of B cell malignancy comes from their inclusion among cDNA clones present on the Lymphochip microarray (141). ESTs for *FCRL1–4* are differentially upregulated in diffuse large B cell lymphomas (DLBCL), follicular lymphomas (FL), and chronic lymphocytic leukemia (B-CLL). *FCRL1* and *FCRL2* have the broadest expression among family members in different transformed B lineage cells but are preferentially overexpressed in more indolent malignancies such as FL and B-CLL. None of the four genes appears to associate with the activated B cell-like (ABC) or GC B cell-like (GCB) signatures that stratified DLBCL samples. *FCRL1* and *FCRL2* expression is also indicative of the mantle cell lymphoma (MCL) gene signature (142). However, neither gene associates with cyclin D1 positivity or with a set of genes that correlates with proliferation and that predicts poorer prognosis in this aggressive malignancy. Finer characterization of the molecular heterogeneity of B-CLL not only identified *Zap-70* as a surrogate marker of the unmutated IgV_H B-CLL genotype and a more aggressive disease course, but also found *FCRL2* and *FCRL3* among a short list of ESTs that correlate with the indolent mutated genotype (143). Furthermore, in a series of aggressive Burkitt's lymphomas with or without 1q21 abnormalities, the Dalla-Favera group (9, 10) detected *FCRL1–3* and *FCRL5* overexpression in the majority of representative cell lines; however, *FCRL4* was identified in only one. Interestingly, *FCRL5* was expressed at tenfold higher levels in seven of eight cell lines with 1q21 abnormalities.

At the protein level, the most extensive screen among family members in B cell malignancies has been completed for FCRLA (see below) (144). Analysis has also been performed for FCRL1–5 in B-CLL (48) and for FCRL4 and FCRL5 in other B cell malignancies (106, 107, 145, 146). In B-CLL, FCRL1 could be found on all $CD19^+CD5^+$ leukemic cells at moderate to high levels.

FCRL5 was also identified on most samples, but at a lower mean fluorescence intensity than FCRL1 (48). FCRL2 and FCRL3 were also expressed, but at lower intensities. Consistent with its lack of expression on circulating B cells, FCRL4 could not be appreciably detected on B-CLL cells in the blood. In all cases in which positive staining was observed, there was a spectrum of expression for each of these receptors. This could indicate that a more detailed analysis of FCRL expression in this malignancy will have prognostic value.

FCRL4 and FCRL5 have been analyzed in other malignancies as well. In childhood atypical MALT-associated MZ hyperplasia, FCRL4 is observed in an expected pattern on intraepithelial B cells in the tonsils and appendix (145). Also in three MALT lymphomas involving the stomach or lungs, FCRL4 marks invading transformed tumor cells in lymphoepithelial lesions (106). It has also been helpful in identifying a group of aggressive $CD30^+$ DLBCL samples that appear to be of non-GC origin (146).

In addition to its expression on B-CLL cells, FCRL5 is found on hairy cell leukemia cells (107). Both of these malignancies are thought to derive from memory B cells that have phenotypic and/or genotypic evidence of GC passage and/or affinity maturation (147–152). Another indication of its role in B cell malignancies is suggested by the induction of FCRL5 upon Epstein Barr virus (EBV) infection and its elevated transcription in Burkitt's lymphomas. An analysis of EBV-infected B cells has demonstrated that the EBV-derived Notch homolog EBNA2 can directly induce *FCRL5* expression through its interaction with the CBF1/RBPSUH DNA-binding protein (153). These findings indicate that FCRL5 might serve as a substrate for transformation in the pathogenesis of B cell malignancies. Together these initial studies indicate that FCRL molecules will likely provide very useful markers for diagnosis, prognosis, and therapy of B cell malignancies.

Autoimmunity

A role for FCRL molecules in autoimmunity seems likely, given their expression pattern, signaling capacity, and relationship to the classical FCRs, which have already been implicated in these disorders (154, 155). The *FCRL* gene family also resides in the 1q21–23 region, which has been linked with several autoimmune disorders, including systemic lupus erythematosus (SLE) and multiple sclerosis (156–158). The strongest evidence thus far for a connection between *FCRL* genes and autoimmunity was provided by a linkage disequilibrium analysis of 41 SNPs in the 2 Mb region encompassing the *FCRL1–5* locus (103). This extensive examination of 830 Japanese subjects with rheumatoid arthritis (RA) and 658 control subjects identified four significant polymorphisms located in noncoding regions of the *FCRL3* gene (–169, –110, +358, and +1381 bp relative to the transcription initiation site). Among these polymorphisms, the highest association of disease susceptibility was found for the –169 SNP located in the *FCRL3* promoter [odds ratio (OR) 2.15, $P = 0.00000085$]. Intriguingly, in disposed individuals this nucleotide substitution (T-169C) results in an improved NF-κB consensus sequence binding site, increases the affinity of NF-κB for the *FCRL3* promoter, and upregulates *FCRL3* expression in B cells and in the synovia of RA patients. Serum autoantibody levels were also found to correlate with the –169 genotype, with highest levels seen in homozygous susceptible (–169C/C) individuals. In this cohort of RA patients with the –169 SNP, a correlation could be detected with the HLA-DRB1 haplotype and extended to other autoimmune disorders in Japanese subjects such as Graves' Disease (GD), Hashimoto's thryoiditis, autoimmune thyroid disease, and SLE.

Although this study strongly implies that upregulation of *FCRL3* expression may play a causative role in autoimmunity, several other studies have been unable to verify statistically an association as powerful. In another

large survey of 752 Japanese RA cases and 940 controls, the correlation between the –169 SNP and RA was not as robust (OR 1.18, $P = 0.22$). This work was also unable to confirm the association with serum autoantibodies (159). Furthermore, little if any relationship between the *FCRL3* promoter SNP and RA was discernable in other ethnic groups, including Caucasian North Americans (160) or Spanish populations (161). However, in the latter study there appeared to be a correlation between *FCRL3* SNPs and an *NFKB1* gene promoter polymorphism (162). In other autoimmune disorders in different ethnic populations, analysis of the –169 SNP has also not been as confirmatory of the work by Kochi et al. (103). For example, no association could be detected in Spanish subjects with SLE (163) or Caucasian European individuals with type I diabetes mellitus (164). In a study of Caucasian subjects of UK origin with GD, a correlation was detected for three *FCRL3* SNPs, but at a lower level than that observed previously (165). Thus, *FCRL3* appears to be an additional non-HLA gene associated with autoimmunity. Its variable linkage to autoimmune disorders could differ according to multiple factors, including but not limited to ethnicity, type of disorder, disease severity, environmental exposure, and additional genetic components. Determining the biological effect of FCRL3 overexpression in these disorders and whether other FCRL family members are associated with different immunologically related diseases will be of great interest.

ADDITIONAL FCRL FAMILY MEMBERS

FCRL6

FCRL6 is the most recently identified FCRL representative in humans and mice and was found through iterative database searches for additional FCRL family members (14, 24). As stated previously, the rat FCRL6 ortholog, originally defined as gp42 in the late 1980s, was actually the first FCRL family member characterized (5, 6). This glycosylphosphatidylinositol (GPI)-anchored protein was defined by expression cloning of a selective marker of IL-2-treated lymphokine-activated killer (LAK) cells. The resolved sequence did not have high homology to other known proteins but demonstrated 27% sequence identity with the D3 domain (yellow) of human FCGR1. Despite identification of multiple restriction fragments that hybridized with a *gp42* cDNA probe in Southern blot analysis, additional *FCRL* family members were not discovered until recently (6).

In humans, the *FCRL6* gene is situated apart from the *FCRL1–5* locus and encodes a type I surface glycoprotein with the characteristic split signal peptide, three conserved Ig domains, an uncharged transmembrane segment, and a cytoplasmic tail containing a consensus ITIM or a noncanonical ITAM (see **Figures 1–3**). At least one splice variant has been detected that possesses an additional tyrosine resulting from a frameshift; however, this change does not appear to generate an additional ITIM or ITAM (14). Interspecies divergence is also seen for this FCRL member. In mice, FCRL6 resembles its rat counterpart and may have up to three isoforms, including one that is secreted, another that is GPI-anchored, and a third full-length uncharged transmembrane variant with a short cytoplasmic tail deficient of ITAM or ITIM sequences (R. Davis, unpublished observations) (see **Figure 3**). Despite orthologous genomic positions and conserved expression patterns, murine FCRL6 representatives lack the D2-type domain that is evident in humans and vary in their membrane anchorage or cytoplasmic tail composition. Thus, differences between FCRL6 and FCRL1–5 relatives include separate chromosomal positions, configuration of their extracellular domains, alternative carboxy-terminal portions, and expression outside the B lineage.

FCRL6 is preferentially transcribed in lymphoid organs, including blood, spleen, and liver, but is also detected at lower levels

in tonsil, lymph node, small intestine, skin, and bone marrow (14, 15). In sorted blood mononuclear cells, *FCRL6* is expressed by $CD8^+$ T cells and $CD56^+$ NK cells, but not in $CD19^+$ B cells, $CD4^+$ T cells, or $CD14^+$ monocytes. Low levels are also identified in $CD56^+$ T cells and γδ T cells. Our unpublished results with anti-FCRL6-specific mAbs verify its surface expression on these lymphocyte subsets in the blood and spleen (15). In mice, *Fcrl6* shares a similar expression pattern to its rat relative on IL-2-activated NK cells, but it has also been detected on a subset of pro-B cells (R.P. Stephan, M.D. Cooper, and R.S. Davis, unpublished results).

As with other FCRL family members, at this juncture our knowledge of FCRL6 function is limited. The D2 and D3 domains that are used for Fc binding by FCGR1 are also apparent in human FCRL6. However, D2-type domains are absent in its murine counterparts, possibly indicating that it is dispensable. Although in humans its domain composition could imply that it is a potential Ig-binding protein, we have not observed any association of FCRL6 with human Igs by flow cytometry or surface plasmon resonance. Thus, similar to other FCRL family members, FCRL6 currently remains an orphan receptor.

Despite what appears to be distinct evolutionary conservation for these genes, the diversity of FCRL6 molecules recognizable in different species is peculiar. It remains unclear whether their ligands and functions are as disparate and/or if these receptors have species-specific roles. Human FCRL6 has a consensus ITIM or a noncanonical ITAM and can be tyrosine phosphorylated (15) (see **Figure 3**). Therefore, both negative and positive regulation should be considered in the characterization of its signaling. An additionally perplexing issue is the divergence recognized for mouse FCRL6. This receptor appears to have three isoforms, each of which could potentially influence different outcomes. Hopefully, identifying their ligand(s) and generating mouse models of deficiency will help to clarify this biological ambiguity.

FCRLA AND FCRLB

Two additional FCRL family members, *FCRLA* and *FCRLB*, were identified independently by three different laboratories in database searches for other FCR-related family members. Both are located within the classical *FCR* locus at syntenic locations in humans and mice and have been variously called *FCRL/FCRL2* (16, 20), *FREB/FREB2* (17, 19), and *FcRX/FcRY* (18, 21), respectively (see **Figure 1**).

The *FCRLA* gene encodes multiple splice variants; however, the predominant full-length protein has 67% interspecies amino acid identity (see **Figure 2**). The FCRLA molecule is distinct in its possession of a degenerate D1 Ig domain, lack of a conventional split signal peptide partially encoded by the 21 bp miniexon, and absence of N-linked glycosylation or a transmembrane region. The first of six potential exons encodes a region of hydrophobicity; however, its generation from a single exon makes it an unusual leader peptide compared with other FCRL representatives. Analysis of this hydrophobic segment suggests the potential for signal peptide cleavage, but this has not been verified experimentally. Although splice isoforms have been detected that contain an 18 bp second exon (16), most transcripts do not possess this sequence. In the mouse genome, this corresponding small fragment does not have flanking AG or GT splice donor/acceptor sites, thus suggesting that this splice variant does not exist in mice. In humans, however, this short sequence deserves attention because it encodes an unpaired cysteine that could participate in intra- or interchain disulfide binding. The first portion of the molecule following the amino-terminal region possesses a short degenerate Ig domain, with three closely positioned unpaired cysteines in the human representative and two in mice. The following two domains are typical D2 (dark blue) and D3 (yellow) domains identified for other FCR/FCRL family members. Also unique is a leucine-rich, mucin-like carboxy-terminal

region that is predicted to adopt an α-helical conformation and does not have homology with any other reported sequences other than FCRLB. In addition to the unpaired cysteines present in D1, this segment could provide potential homo- or heterooligomerization for this protein (16, 17). The Taranin group (16) has presented evidence for FCRLA multimerization in immunoprecipitates from COS cell transfectants, where multiple high-molecular-weight bands appear to dissociate under reducing conditions, implying the likelihood of interchain disulfide bond formation. These distinct features, in addition to the presence of potential dileucine endoplasmic reticulum retention signals and FCRLA's overall acidic p*I*, indicate that it is cytoplasmically expressed (17). The absence of surface detection using FCRLA-specific antisera further supports this inference (16, 17). Additional evidence for its intracellular localization comes from immunoelectron microscopy, demonstrating that at least three human FCRLA isoforms appear to be retained in the endoplasmic reticulum; however, there is unpublished evidence for secretion of at least one splice alternative in humans (166).

FCRLB encodes a protein with features more typical of other FCRL/FCR family members, including a conserved split signal peptide and three distinctive Ig domains, but, like FCRLA, FCRLB has a carboxy-terminal mucin-like leucine-rich region with potential dileucine motifs (see **Figure 2**). This sequence could also form an α-helical coiled-coil structure (19, 20). The presence of an unpaired cysteine residue in its leucine-rich region could facilitate covalent homodimeric or heterodimeric pairing with an additional protein partner. Despite these distinctly similar segments, there is only 24% identity between these respective regions in human FCRLA and FCRLB. The conventional split signal peptide, two potential N-linked glycosylation sites, and absence of a transmembrane region suggest that FCRLB may be secreted; however, reports thus far indicate that, similar to FCRLA, FCRLB localizes in the cytoplasm (19, 20). The three encoded FCRLB Ig domains possess a tandem arrangement similar to FCGR1/CD64 with which it shares 29% Ig domain amino acid identity in humans and 35% in mice. The overall amino acid sequence identity of 81% between humans and mice is the most conserved among FCRL family members.

Patterns of Expression

Both *FCRLA* and *FCRLB* are expressed by B cells; surprisingly though, both of them have also been detected in melanocytes and their transformed counterpart, melanoma (20, 167). The biological significance of this is currently unclear, and in this review we concentrate on their expression in lymphoid cells. *FCRLA* transcripts are found chiefly in spleen and lymph nodes and not to any appreciable degree in nonhematopoietic organs (16, 18). *FCRLA* has limited expression in early bone marrow B cell precursors and reaches highest levels in the periphery. Transcription is prominently noted in tonsillar pre-GC B cells and GC B cells, but it can also be detected in other B cell populations (18). FCRLA protein expression generally confirms its transcription pattern and has been explored much more thoroughly in human tissues than in mouse. FCRLA is not detected in resting human $CD19^+$ blood B cells but can be induced by inactivated *Staphylococcus aureus* (17). Curiously, concomitant incubation with IL-4 appears to depress intracellular FCRLA levels.

In human tissues, FCRLA is selectively identified in the lymphoid follicles of tonsils. Reactivity is distinctly enriched around the base of GCs, predominately within large $CD20^+$ GC centroblast-like B cells that are positive for the PCNA nuclear proliferation antigen (16, 17, 144). Most BCL6/Ki-67 labeling cells also express FCRLA; however some $FCRLA^+$ cells are $Ki\text{-}67^-$, indicating that resting cells in the tonsil also

express FCRLA at high levels. Interestingly, the majority of FCRLA$^+$ cells are CD27 negative and fail to stain for the multiple myeloma oncogene-1 (MUM-1) PC marker (144). Variable costaining is seen for the CD30 activation receptor. In agreement with the lack of CD27 expression, FCRLA does not appear to correlate with heavy chain isotype. Costaining for IgM, IgA, IgG, or IgD by the Colonna group indicates an association with IgM staining in ~5%–20% of the cells but no strong association with expression of the other isotypes (17). In addition to centroblasts, other scattered GC cells are noted to have weaker FCRLA expression, and staining can be observed in mantle zone cells, albeit at lower levels (144). Only rare FCRLA$^+$ cells are distinguished in the T cell–rich interfollicular zones outside the GC, some of which costain for CD30 and do not appear to correspond to large interfollicular B cells (168). In the spleen, FCRLA is also abundant in GC B cells as well as in MZ B cells, and clusters of FCRLA$^+$ cells are visible in red pulp regions proximal to blood vessels. Lymph node sections confirm the enrichment of FCRLA in the GC (144). Thus, FCRLA appears to have a B cell–specific expression pattern in secondary lymphoid tissues, with some preference for proliferating IgM$^+$ GC centroblasts that express BCL6, but FCRLA is downregulated in class switched memory B cells and PCs. In contrast to the distinct localization of human FCRLA in secondary follicles, preliminary findings do not support a GC-specific pattern of expression for mouse FCRLA (166). Rather, mouse FCRLA appears to have a more scattered distribution in the spleen. Once again these interspecies differences indicate that unraveling the function of this molecule may not be straightforward.

FCRLB is distinguished by its very low level of expression in lymphoid and nonlymphoid tissues, and attempts to evaluate its tissue expression by Northern blotting have been unsuccessful (19–21). Thus, its tissue and cellular distribution has largely been defined by RT-PCR. In human tissues, transcripts are identified in placenta, kidney, spleen, lung, and tonsils where *FCRLB* is readily amplified from pre-GC, GC, and PC B lineage subsets, but *FCRLB* is not appreciably expressed by naive or memory B cells (21).

FCRLB antiserum preferentially labels IgD$^-$ GC B cells, whereas very few reactive FCRLB cells are found outside the GC in human tonsillar sections (19). Interestingly, numbers of FCRLB$^+$ cells vary among and within individuals, and some follicles do not appear to have any FCRLB$^+$ cells. In light of this finding, it may not be surprising that large variations have been observed for *FCRLB* transcription. Unlike proliferating FCRLA$^+$ GC B cells, FCRLB$^+$ cells do not appear to be Ki-67$^+$ (19). This is confirmed by staining sections for both FCRLA and FCRLB that appear to have mutually exclusive expression patterns. Although FCRLA is broadly distributed in the GC, FCRLB has a much more limited staining pattern that does not correlate with well-established histological structures or Ig isotypes. This finding also indicates that it is less likely that there is functional redundancy for these two proteins. Thus, whereas FCRLA is preferentially expressed by proliferating cells, FCRLB appears to be expressed by resting cells with a similar topographical location.

Our knowledge of mouse *Fcrlb* expression is limited to the messenger RNA level, where considerable variability has been apparent between investigators, and which also likely reflects its low levels of transcription (19, 21). In the bone marrow, *Fcrlb* transcription is evident in pre-B cells and immature B cells but is upregulated in the periphery (21). It is broadly amplified in splenocyte populations following immunization, including in immature/transitional B cells as well as newly formed, follicular, MZ, and PNA$^+$ GC B cells depending on the immunogen (19, 21). These findings will likely be refined once protein-specific reagents are available.

Predicted Biological Function and Malignant Expression

Their unique expression patterns and phylogenetic relationships suggest that FCRLA and FCRLB could have distinct functions when surface Ig is downregulated prior to somatic mutation of the IgV_H genes. Both FCRLA and FCRLB have greater amino acid identity with the classical FCRs than does FCRL1–6, and this relationship is also inferred by their linkage within the low-affinity *FCGR* locus (16, 23). The presence of D2- and D3-type domains that are used for Fc binding by the monomeric high-affinity FCGR1 implies that FCRLA and FCRLB could have Ig-binding potential. However, given the lack of extracellular expression by these two molecules, it seems unlikely that they associate with Ig in the classic sense. The potential for FCRLA to bind Ig extracellularly has been explored by the Colonna group, who engineered a construct that placed the partial and conventional Ig domain–encoding regions of FCRLA in frame with an N-terminal secretion signal and the transmembrane-encoding portion of the platelet-derived growth factor receptor (17). Despite ample surface expression, no detectable Ig binding was found by staining with IgA, IgM, IgG, or IgE. However, FCRLA's intracellular expression and localization, acidic p*I*, and potential for oligomerization collectively suggest that these experimental conditions may not have been conducive to its optimal in vivo functioning. A comparable cell surface staining analysis has been conducted for FCRLB, where Ig binding was also undetectable (J. Pan, J. Zhang, R. Davis, unpublished results). Nevertheless, FCRLA and FCRLB could bind Ig intracellularly; meticulous biochemical analysis will be required to explore this.

The most complete survey of FCRL expression in B cell malignancies has been performed for FCRLA. ESTs for *FCRLA* in the Lymphochip database demonstrate its relative overexpression in different DLBCL, FL, and B-CLL samples (141). Expression of FCRLA in a variety of lymphoid malignancies has been investigated by two groups and largely mirrors its normal tissue distribution (17, 144). As expected, FCRLA is not identified in T or NK cell–derived malignancies, in classical Hodgkin's lymphoma, or in diagnostic Reed-Sternberg cells. High levels of FCRLA expression are found in nodular lymphocyte-predominant Hodgkin's lymphomas, including the lymphocytic and histiocytic cells (L&H) that are considered variants of classical Reed-Sternberg cells (169). Of note, most of the L&H cells in these tumors also have evidence of ongoing somatic mutation and thus derive from mutating GC B cells (170). FCRLA is strongly expressed in B lineage non-Hodgkin's lymphomas, but not in precursor B lineage lymphoblastic lymphomas. Masir et al. (144) found intermediate- to high-level reactivity for almost all MCLs studied, and this staining was subjectively higher than seen in normal counterpart cells. These data are in contrast to studies by Fachetti et al. (17), who tested fewer MCL samples and did not observe expression. In GC-derived FL, FCRLA was identified in the majority of cases, but labeling did not correlate with the grade of disease aggressiveness or cell size (144). Almost all samples of MZ lymphoma, small lymphocytic lymphoma/CLL, and Burkitt's lymphoma were FCRLA positive. The majority of DLBCL samples were also reactive, but staining patterns varied from cell to cell, similar to FL. Costaining with Ki-67 demonstrated discordance in respective labeling patterns as well. Furthermore, no correlation could be found between FCRLA reactivity and the two different subsets of ABC-like or GBC-like DLBCL. This lack of association is consistent with the *FCRLA* transcriptional profile documented in the Lymphochip database (141). Although five of six lymphoplasmacytic lymphoma samples were $FCRLA^+$, all multiple myeloma samples ($n = 4$) tested were negative (144). EBV-transformed, post-transplant, lymphoproliferative disease samples were also negative, as were almost all cases of T cell–rich B cell lymphoma. These studies indicate that FCRLA may be a useful marker

for immunohistochemical analysis of B cell–derived malignancies, could help in our understanding of malignant and normal B cell counterparts, and may also have a role as a prognostic indicator. FCRLA's prognostic potential is further suggested by an analysis of the gene expression patterns of 24 patients with FL who were treated with the humanized anti-CD20 immunotherapeutic agent rituximab (171). FCRLA was found to be highly expressed in rituximab responders compared with nonresponders, thus suggesting that FCRLA may have predictive value in determining responses to therapy in individuals with this malignancy.

CONCLUSIONS

We are only at the beginning of our understanding of this new area of FCRL biology. The phylogenetic origins, genetic regulation, expression patterns, protein structures, immunomodulatory potential, ligands, and impact on immunologically related diseases are only some of the interesting issues that remain to be characterized in depth for this intriguing family of receptors. As mAbs and transgenic models become available, these tools should provide a basis for determining their functions. Owing to divergent characteristics evident in FCRL representatives of different species, these receptors may vary in their species-specific functions. Alternatively, however, they may also help to reveal disparate immunologic features that have thus far been subtly acknowledged. The FCRL molecules significantly extend an already well-defined group of FCRs and should provide a fascinating field of future study.

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Errata

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