

# Identification of a family of Fc receptor homologs with preferential B cell expression

Randall S. Davis\*<sup>†</sup>, Yui-Hsi Wang<sup>‡</sup>, Hiromi Kubagawa\*<sup>§</sup>, and Max D. Cooper\*<sup>†¶||\*\*††</sup>

Divisions of \*Hematology/Oncology and <sup>‡</sup>Developmental and Clinical Immunology, Departments of <sup>†</sup>Medicine, <sup>§</sup>Pathology, <sup>\*\*</sup>Pediatrics, and <sup>¶</sup>Microbiology, and <sup>||</sup>Howard Hughes Medical Institute, University of Alabama, Birmingham, AL 35294-3300

Contributed by Max D. Cooper, June 19, 2001

Investigation of human genome sequences with a consensus sequence derived from receptors for the Fc region of Igs (FcR) led to the identification of a subfamily of five Ig superfamily members that we term the Fc receptor homologs (FcRHs). The closely linked *FcRH* genes are located in a chromosome 1q21 region in the midst of previously recognized *FcR* genes. This report focuses on the *FcRH1*, *FcRH2*, and *FcRH3* members of this gene family. Their cDNAs encode type I transmembrane glycoproteins with 3–6 Ig-like extracellular domains and cytoplasmic domains containing consensus immunoreceptor tyrosine-based activating and/or inhibitory signaling motifs. The five *FcRH* genes are structurally related, and their protein products share 28–60% extracellular identity with each other. They also share 15–31% identity with their closest FcR relatives. The *FcRH* genes are expressed primarily, although not exclusively, by mature B lineage cells. Their conserved structural features, patterns of cellular expression, and the inhibitory and activating signaling potential of their transmembrane protein products suggest that the members of this *FcRH* multigene family may serve important regulatory roles in normal and neoplastic B cell development.

Receptors for the Fc region (FcRs) of Igs have broad tissue distribution patterns and can modulate cellular and humoral immunity by linking their antibody ligands with effector cells of the immune system (1, 2). These cellular receptors have the ability to sense humoral concentrations of antibody, initiate cellular responses in host defense, and participate in autoimmune disorders (3). Their diverse regulatory roles depend on the Ig isotype specificity and cellular distribution of the individual FcR. These Ig superfamily members share similarities in their ligand binding subunits, and they may have inhibitory or activating signaling motifs in their intracellular domains or instead pair with signal transducing subunits possessing activating signaling motifs.

Our view of the FcR family has been significantly extended with the characterization of *FcαR* homologs in mice, the paired Ig-like receptors (4, 5), and their relatives in humans the Ig-like transcripts/leucocyte Ig-like receptors (6, 7). This multigene family, which includes the *FcαR* (8) and the natural killer cell Ig-like receptors (9), is located in a human chromosome 19q13 region known as the leucocyte receptor complex (LRC) (10, 11). These Ig-like multigene families belong to a larger class of receptors characterized by their possession of common cytoplasmic tyrosine-based signaling motifs. These can be either immunoreceptor tyrosine-based activation motifs (ITAMs) containing two repeats of the consensus sequence Y-X-X-L/I spaced by 6–8 aa (E/D)-X-X-Y-X-X-(L/I)-X<sub>6–8</sub>-Y-X-X-(L/I) or immunoreceptor tyrosine-based inhibitory motifs (ITIMs) with a 6-aa consensus sequence (I/V/L/S)-X-Y-X-X-(L/V) (12–15). The phylogenetic conservation of these types of receptors in birds (16) and bony fish (17) is indicative of their biological value. After ligand binding of the activating receptor complexes, ITAM tyrosines are rapidly phosphorylated by Src family kinases to initiate a cascade of signaling events that trigger cellular activation. In the case of ITIM-bearing receptors, the tyrosines provide a docking site for phosphatases containing Src homol-

ogy 2 domains that can abrogate cellular activation (18, 19). The balance in the utilization of these activating and inhibitory receptor pairs can serve to modulate cellular responses to a variety of stimuli.

The genes encoding the classical FcRs, *FcγRI*, *FcγRII*, *FcγRIII*, and *FcεRI*, lie on the long arm of chromosome 1 (1q21–23) near the polymeric Ig receptor (pIgR) and *Fcα/μR* genes (1q32) (20–23). Members of this FcR subfamily have relatively low extracellular homology with the *FcαR*-related genes that reside in the LRC on chromosome 19. Like the *FcγR*- and *FcεR*-activating receptors, the ligand binding chain of the *FcαR* coassociates with the ITAM containing FcR common  $\gamma$ -chain (24, 25). The concentration of genes in the 19q13.4 LRC locus that share a high degree of homology in their respective extracellular domains suggested the possibility that a similar cluster of *FcγR* and *FcεR* gene relatives might exist in the chromosome 1q21–23 region. In a search for new members of this FcR family, we derived a consensus amino acid motif through comparison of the *FcγRI*, *FcγRII*, *FcγRIII*, and pIgR extracellular regions. When this consensus motif was used in a GenBank protein database query (9/29/00), genomic clones were identified that were found to contain *FcR* relatives that we term the Fc receptor homolog (*FcRH*) subfamily. Here we report the location, sequence, structure, and cellular expression patterns of three of the five functional *FcRH* genes, *FcRH1*, *FcRH2*, and *FcRH3*, which have not been previously described, and compare them with two recently reported members of this subfamily, Ig superfamily receptor translocation-associated genes 1 (*IRTA1*) (*FcRH4*) and 2 (*IRTA2*) (*FcRH5*) (26), and their FcR relatives.

## Materials and Methods

**Isolation of *FcRH* cDNA Clones.** Rapid amplification of cDNA ends (RACE)-PCR was performed by using a Marathon-Ready human lymph node cDNA library (CLONTECH). Gene-specific primers were as follows: *FcRH3*, forward 5'-GTGAGTCT-CAGGGTCACAGTTCCG-3' and reverse 5'-GCTCTT-GAACTTGGATATTTAGGGGT-3'; *FcRH2*, forward 5'-CCAGTGTATGTCAATGTGGGCTCTG-3' and reverse 5'-CGTTGAAAGAGCTCTTGGACTTTTATC-3'; and *FcRH1*, forward 5'-GCCTCAAAGAAAAATAGGAAGACGTT-3' and reverse 5'-AAGCTCACATCAGCGACAGGGGAC-3'. RACE products were subjected to a second round of nested

Abbreviations: FcR, Fc receptor; FcRH, FcR homolog; ITIM, immunoreceptor tyrosine-based inhibitory motif; ITAM, immunoreceptor tyrosine-based activation motif; LRC, leucocyte receptor complex; RACE, rapid amplification of cDNA ends; UT, untranslated; RT, reverse transcription; BAC, bacterial artificial chromosome; GC, germinal center; IRTA, Ig superfamily receptor translocation-associated gene; moFcRH, mouse FcRH.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AY043464, AY043465, AY043466, AF397452, and AF397453).

<sup>††</sup>To whom reprint requests should be addressed at: University of Alabama, WTI 378, 1824 6th Avenue South, Birmingham, AL 35294-3300. E-mail: Max.Cooper@ccc.uab.edu.

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

PCR and visualized by agarose gel electrophoresis and ethidium bromide staining.

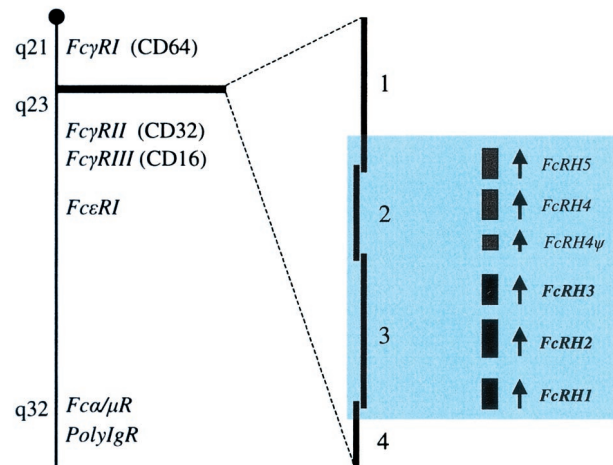
**Generation of Full-Length FcRH cDNAs.** Primers used in end-to-end amplification to generate full-length cDNAs were as follows: FcRH3, forward 5'-TCTTGGAGATAAGTCGGGCTTT-3' and reverse 5'-ATCCTGCAGCCCAGCCTCGTAGGAG-3'; FcRH2, forward 5'-GGTCTCATGCTGCTGTGGTTCATT-3' and reverse 5'-GCTGTTGATCTTCCCTTCTGATTC-3'; and FcRH1, forward 5'-ATGCTGCCGAGGCTGTTGCTGTTG3' and reverse 5'-CATAGCATCTTCATAGTCCACATC-3'. Each amplification reaction underwent initial denaturation of 94°C for 30 s followed by 30 cycles of denaturation at 94°C for 5 s and annealing at 68°C for 4 min, and final extension at 72°C for 6 min.

**Sequence Analysis.** PCR products were ligated into the pCR2.1 TOPO T/A vector (Invitrogen). Inserts were DNA-sequenced on both strands by the dideoxy chain termination method using Thermo Sequenase (Amersham Pharmacia) and an automated sequencer (Li-Cor, Lincoln, NE). Nucleotide and amino acid sequence alignment was analyzed with a DNASTAR (Madison, WI) software package, and homology searches were performed by using BLAST (27).

**RNA Blot Analysis.** Northern blots (CLONTECH) were hybridized with  $\alpha^{32}\text{P}$ -dCTP-labeled probes: a 528-bp *Eco*RI fragment corresponding to the 5' untranslated (UT)-EC1 regions of the FcRH3 cDNA, a 200-bp PCR product corresponding to a portion of the 3' UT region of the FcRH2 cDNA, and a 257-bp PCR product corresponding to a portion of the 3' UT region of the FcRH1 cDNA. Membranes were hybridized for 1 h at 65°C, washed, and exposed to x-ray film (4).

**Reverse Transcription (RT)-PCR.** Human tonsillar cells, obtained with Institutional Review Board approval, were separated into CD19<sup>+</sup> and CD19<sup>-</sup> subpopulations by magnetic cell sorting (Miltenyi Biotec, Auburn, CA). Viable CD19<sup>+</sup> cells were stained with FITC-labeled anti-CD38 (Immunotech, Westbrook, ME) and phycoerythrin-labeled anti-IgD mAbs (Southern Biotechnology Associates) before sorting cells with a FACStarPlus instrument (Becton Dickinson) into Trizol reagent (Life Technologies, Grand Island, NY) for RNA isolation. Total cellular RNA was primed with random hexamers and oligo(dT) primers and reverse-transcribed with SuperScript II (Life Technologies) into single-stranded cDNA. RT-PCR was performed by using RNA from tonsillar B cells and cell lines, with GIBCO/BRL *Taq* polymerase (Life Technologies). The following gene-specific primer pairs were used in the RT-PCR analysis of *FcRH1-5* expression in cell lines and tonsillar B cell subpopulations: *FcRH1* forward, 5'-CTC AAC TTC ACA GTG CCT ACT GGG-3' and reverse, 5'-TCC TGC AGA GTC ACT AAC CTT GAG-3'; *FcRH2* forward, 5'-CCA GTG TAT GTC AAT GTG GGC TCT G and reverse, 5'-CAT TCT TCC CTC AAA TCT TTA CAC-3'; *FcRH3* forward, 5'-CAG CAC GTG GAT TCG AGT CAC-3' and reverse, 5'-CAG ATC TGG GAA TAA ATC GGG TTG-3'; *FcRH4* forward, 5'-TCT TCA GAG ATG GCG AGG TCA-3' and reverse, 5'-TTT TGG GGT GTA CAT CAA CAT ACA AG-3'; and *FcRH* forward, 5'-TGT TGC CCT GTT TCT TCC AAT ACA-3' and reverse, 5'-CAG AGT TGG CCG ACC TAC GAC-3'. Each amplification reaction underwent initial denaturation at 94° for 5 min followed by 35 cycles of denaturation at 94° for 30 s, annealing at 60° for 30 s, extension at 72° for 1 min, and final extension at 72° for 7 min. Amplified products were visualized in 1% agarose gels containing ethidium bromide and documented with the Bio-Rad Fluor-S Imager.

## Chromosome 1



**Fig. 1.** Relative position of the *FcRH* locus within the *FcR* cluster on chromosome 1. The cytogenetic location of the *FcR* genes is approximated from the GenBank Mapview database. The BAC clones (4, GenBank accession no. AL139409; 3, GenBank accession no. AL356276; 2, GenBank accession no. AL135929; and 1, GenBank accession no. AL353721) that span the locus are oriented in relation to their respective *FcRH* genes (shaded area).

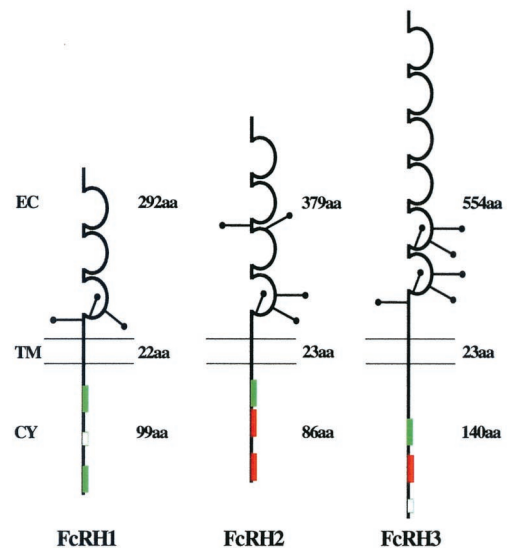
**Cell Lines.** Human cell lines included REH and Nalm 16 pro-B cell lines (28); 697, 207, and OB5 pre-B cell lines (29, 30); Ramos, Daudi, and Raji B cell lines (31–33); THP-1 and U937 monocytoid cell lines, HL-60 promyelocytic and KG-1 myelocytic cell lines, Jurkat T cell line and the K562 erythroid cell line (American Type Culture Collection).

## Results

**Identification of *FcRH1*, *FcRH2*, and *FcRH3*.** A consensus sequence was generated that corresponds to the GenBank-derived amino terminal sequences of the second Ig-like domains of FcγR (FcγRI and FcγRII/III) and the third Ig-like domain of the polymeric Ig receptor: GEPIXLRCHSWKDKXLXKVITYX-QNGKAXKFFH. A search (9/29/00) of the National Center for Biotechnology Information protein database with this sequence identified two overlapping human genomic bacterial artificial chromosome (BAC) clones, AL135929 and AL356276, which are located at 1q21.2–22. The second clone contained three putative Ig superfamily genes encoding complementary amino acid sequences that were designated *FcRH1*, *FcRH2*, and *FcRH3* (Fig. 1). The predicted amino acid sequences of these gene segments shared 23–57% identity with each other and 14–28% identity with human FcγRI (CD64). Further analysis of the *FcRH* locus led to the identification of two additional genes (*FcRH4*, and *FcRH5*) and one pseudogene (*FcRH4ψ*), immediately centromeric of *FcRH1-3*, two of which have recently been described as *IRTA1* (*FcRH4*) and *IRTA2* (*FcRH5*) (26).

To determine whether these genes are expressed by lymphocytes, the predicted amino acid sequences of their protein products were used to search the Lymphochip expressed sequence tag database with the TBLASTN algorithm (34). Two expressed sequence tags (AA505046 and AA282433) were identified that share complete identity over 23 aa in their translated ORFs with the N terminus of FcRH1. Lymphochip microarray data analysis indicated that these expressed sequence tags are expressed at relatively high levels in peripheral lymphoid tissues, including the lymph nodes, tonsils, resting peripheral B cells, and normal germinal center (GC) B cells. Among the different lymphoid malignancies, their expression proved to be highest in

A)



B)

<i>SP</i>			
<i>FcRH3</i>	MLLWLLLLL TPGRQES--	(17)	
<i>FcRH2</i>	...S.V.F DAVT.ADS	(19)	
<i>FcRH1</i>	..FR...C A.LC.P---	(16)	
<i>EC1</i>			
<i>FcRH3</i>	GVAPKAVLLL NPPWSTAFK EKVALICSGI SHSIAQGDYI WYHDEKLLKI KHKIQITEP GNYQCKTRGS SLSDAVHVEF SP	(82)	
<i>FcRH2</i>	-----		
<i>FcRH1</i>	-----		
<i>EC2</i>			
<i>FcRH3</i>	DWLLLQALHP VFEGDNVILR CQGDKNKNTK QKVVYKDGKQ LPNSYNLERI TVNS-VSRDN SKYHTAYRK FYILLDIEVTS KPLNIQVQ	(87)	
<i>FcRH2</i>	--T.V.PSS .....SIVLK .....EQ.WKIQ KMA.H..N.E .SVFKFSDF LIQ.A.LS.S GN.F.STGKQ LFLW.K--- NIVK.K..	(84)	
<i>FcRH1</i>	-----		
<i>EC3</i>			
<i>FcRH3</i>	-ELFLHPVLR ASSSTPIEGS PMFLTCETQL SPQRPOVLQ FSLFRDSQTL GLGWSRSPLR QIPAMWTESS GSYWCEVTV THSIKRSLSR QIRVQ-	(95)	
<i>FcRH2</i>	...QR...T ...FQ...G .VS.K...R .....L.....CF..EN.V. .S...S.E. .S.V.S.T .....KA... ..R.R.Q..Q. .H.R	(95)	
<i>FcRH1</i>	A.....I .P.H.T... .V...KMPF L-.SS.A.F. .CF...TRA. .P...S.K. .A...K.T .....AQ.M ASKVLR-.R...N.H-	(90)	
<i>EC4</i>			
<i>FcRH3</i>	RVPVSNVLE IRPTQGLLE GEMVVICSV AQCSQTVFSS WKKEGRVRSI GRKTQRSLLA ELRVLTVKES DAGRYCAAD NVHSPILSTW IRVTVR	(96)	
<i>FcRH2</i>	-I.I...S...AP...VT. QRLILL...G.T.N...YR.ADTM.K.....S...EIPA... ..K...R...G.V..Q.RV VNI... (95)		
<i>FcRH1</i>	...AD.S...TQ.P...VM. DRL.....M.T.DI..L .Y.GAVGLN. QS.....T. .YEIPS.R...EQ...V.E. .GYG.SP.GL VSI... (96)		
<i>EC5</i>			
<i>FcRH3</i>	IPVSHPVLF RAPRAHTVVG DLLELHCESL RGSPPILYRF YHEDVTLGNS SAPSGGASF NLSLTAHSG NYSCEADNGL GAQHSVGL RVT	(93)	
<i>FcRH2</i>	...R...L .S.G.QAA.....A.....Q.....Q.....E.N... ..C.EA.FV SIS	(93)	
<i>FcRH1</i>	...R.I.ML .....QAA.E .V.....A.....W.....I...SR .....E.....E.N... ..R.EA.T. NF	(93)	
<i>EC6</i>			
<i>FcRH3</i>	VPVSRPVLEL RAPAQAQVVG DLLELHCESL RGSPPILYRF YHEDVTLGNI SAPSGGASF NLSLTAHSG NYSCEADNGL GAQHSKVTL NVT	(93)	
<i>FcRH2</i>	-----		
<i>FcRH1</i>	-----		
<i>MP-TM</i>			
<i>FcRH3</i>	---GTSRNT GLTAAGITGL VLSILVAAA AALL----	(31)	
<i>FcRH2</i>	GPD.YR.DL- -M.GVLM...-FGV.GFTGV .L.-YALF	(36)	
<i>FcRH1</i>	VPT.ARS.H- -SGV.E...-T.GP.TV .L.FCYGL-	(35)	
<i>CY</i>			
<i>FcRH3</i>	HYARARRKPG GLSATGTSSH SPSECQEPSS SRPSRIDPQE PTHSKPLAMP ELLEPMYNNVN PGDSNPIYIQ IWSIQTKNK SANCPMHHQE HEELTVLYSE	(86)	
<i>FcRH2</i>	HKISGESAT NEPRGASRPN PQEFTYSPT PDMEELQPVY VNVGSDVDV VYSGVWSMQ PESSANRILT LENKDSQVYI SSVKKS	(96)	
<i>FcRH1</i>	KRKIGRRSAR DPLRSLSPPL PQEFTYLANP TPQGLQPIYE NVNVVSGDEG YSLAYYNOPE QESVAEATLG THMEDKVDL IYSRLRKANI TDVDTEDAM	(99)	
<i>FcRH3</i>	LKKTHPDDSA GEASSRGRAH EEDDEENYEN VPRVLLASDH	(140)	

**Fig. 2.** FcRH1, FcRH2, and FcRH3 structural and sequence diversity. (A) Schematic representation of FcRH molecules. The three cDNAs encode type I transmembrane proteins with similar extracellular domains, but different cytoplasmic regions. The extracellular (EC) regions contain different numbers of C2-like Ig domains and potential sites of N-linked glycosylation (●). The transmembrane (TM) domains are uncharged. The cytoplasmic (CY) region of FcRH1 contains two ITAMs (green boxes) and one ITAM-like region (small open box), whereas FcRH2 contains one ITAM and two ITIMs (red boxes). FcRH3 has a long cytoplasmic tail with one ITAM, one ITIM, and an ITAM-like region. The amino acid length of each region is indicated. (B) Multiple alignment comparison of FcRH1, FcRH2, and FcRH3 aa sequences (one-letter code) based on the *FcRH3* sequence. Amino acid identity is represented by dots, and gaps are indicated by dashes. Predicted N-linked glycosylation sites and transmembrane domains are underlined in black. Consensus ITAM (green) and ITIM (red) motifs are indicated. Putative structural domains are labeled: SP, signal peptide; EC, extracellular domain; MP-TM, membrane proximal-transmembrane; and CY, cytoplasmic regions. Amino acid lengths are indicated in parentheses.

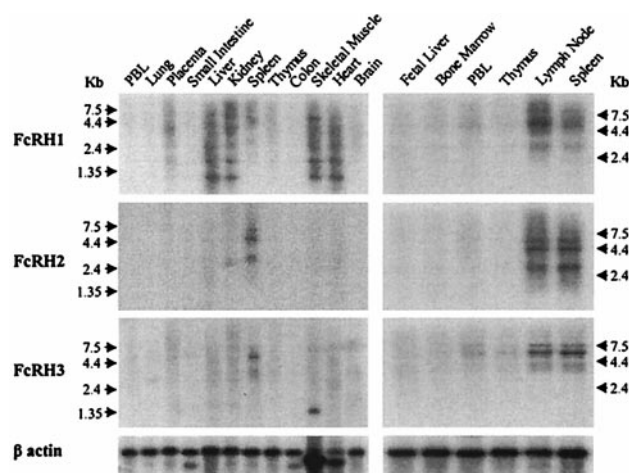
chronic lymphocytic leukemias, follicular lymphomas, and some diffuse large cell lymphomas of B lineage.

FcRH1, FcRH2, and FcRH3 cDNAs were isolated by RACE-PCR from a human lymph node cDNA library in both 5' and 3' directions. Full-length cDNAs of the coding regions for *FcRH1*, *FcRH2*, and *FcRH3* were obtained by end-to-end PCR using unique primers generated from the cDNA sequences delineated for the 5' UT and 3'UT regions. Southern blot analysis of human genomic DNA digested with *Bam*HI, *Eco*RI, or *Hind*III using cDNA probes specific for the 3' UT regions of each cDNA revealed either one or two hybridizing fragments, suggesting that FcRH1, FcRH2, and FcRH3 are encoded by single genes. Analysis of full-length cDNA sequences indicated that *FcRH1*, *FcRH2*, and *FcRH3* have ORFs of 1,287 bp, 1,524 bp, and 2,202 bp, respectively, and encode type I transmembrane proteins of 429 aa, 508 aa, and 734 aa, respectively. Based on predicted consensus signal peptide cleavage sites (35, 36), the relative core peptide molecular masses were estimated as 45,158 for FcRH1, 53,407 for FcRH2, and 78,849 for FcRH3. These type I transmembrane proteins possess 3–6 extracellular C2 (37–39) type Ig-like domains with 3–7 potential N-linked glycosylation sites, uncharged transmembrane segments, and relatively long cytoplasmic tails containing consensus motifs for ITIMs and/or ITAMs (Fig. 2A).

Multiple alignment analysis of the translated cDNAs, using FcRH3 as the index sequence of comparison, indicates that FcRH1, FcRH2, and FcRH3 have highly conserved hydrophobic signal peptides and corresponding Ig-like extracellular domains (Fig. 2B). Their hydrophobic, uncharged transmembrane domains (40) are also well conserved, but their cytoplasmic domains are not. FcRH1 has a long cytoplasmic tail containing

three potential ITAMs, the first and third of which fit the consensus sequence (E/D)-X-X-Y-X-X-(L/I)-X<sub>6-8</sub>-Y-X-X-(L/I), whereas, the second has only one tyrosine residue. The shorter cytoplasmic domain of FcRH2 contains one potential ITAM and two ITIM consensus sequences (I/V/L/S)-X-Y-X-X-(L/V) separated by 22 aa. FcRH3 has the longest cytoplasmic tail. It contains one potential ITAM, one ITIM, and another potential ITAM that also has a single tyrosine residue.

**Tissue Distribution of FcRH1, FcRH2, and FcRH3 Expression.** An RNA blot analysis with gene-specific probes was performed on 16 human tissues, including six primary or secondary lymphoid tissues. All three *FcRH* gene probes hybridized with transcripts in the secondary lymphoid organs, spleen and lymph node (Fig. 3). An *FcRH1*-specific probe hybridized with spleen and lymph node transcripts of ≈3.5 kb and ≈6.0 kb. Additional hybridization bands of ≈0.7 kb and ≈1.5 kb were observed for heart, skeletal muscle, kidney, liver, and, in less abundance, placental tissue. Larger transcripts also were seen in skeletal muscle (≈6.0 kb) and in kidney and placenta (≈4.4 kb). An *FcRH2*-specific probe hybridized to ≈3.0-kb, ≈4.4-kb, and ≈5.5-kb transcripts most abundantly in spleen and lymph node. An ≈2.4-kb transcript was notable in the kidney. An *FcRH3* probe hybridized with ≈3.5-kb, ≈5.5-kb, and ≈7.0-kb transcripts chiefly in spleen and lymph node. These also were seen, albeit in lesser abundance, in peripheral blood lymphocytes, thymus, and bone marrow samples. Additionally, a unique transcript of ≈1.35 kb was evident in skeletal muscle. These results indicated expression of *FcRH1*, *FcRH2*, and *FcRH3* in peripheral lymphoid organs, whereas tissue specific differences in alternative splicing or polyadenylation were suggested by the differential expression of transcripts with variable size in nonlymphoid tissues.



**Fig. 3.** Analysis of *FcRH1*, *FcRH2*, and *FcRH3* expression in different tissues. RNA blots were analyzed with discriminating  $\alpha^{32}\text{P}$ -dCTP-labeled probes generated from the respective *FcRH* cDNAs. The following probes were used: (Top) a PCR-generated, 257-bp probe specific to the 3' UT region of *FcRH1*; (Middle) a PCR-generated, 290-bp probe corresponding to the 3' UT region of *FcRH2*; and (Bottom) a 528-bp *EcoRI*-digested fragment of the 5' end of the *FcRH3* cDNA corresponding to its 5' UT region, S1, S2, and EC1 domains. Relative mRNA abundance is indicated by a  $\beta$ -actin probe.

When *FcRH* expression was examined by RT-PCR analysis of cell lines representing different hematopoietic lineages, *FcRH1*, *FcRH2*, and *FcRH3* expression was found in every mature B cell line tested (Table 1). *FcRH2* and *FcRH3* expression was limited to the mature B cell lines and not seen in the other types of cells examined. In contrast, *FcRH1* expression was seen in pro-B, T, and myeloid cell lines, although not in an erythroid cell line.

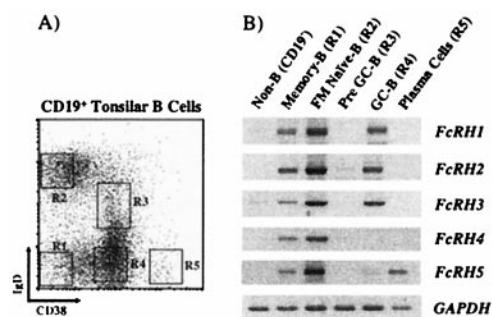
RT-PCR analysis of sorted populations of peripheral blood cells indicated that *FcRH1*, *FcRH2*, *FcRH3*, and *FcRH5* are expressed at relatively high levels in  $\text{CD}19^+$  B cells, whereas *FcRH4* was expressed at only trace levels. *FcRH3* expression was observed in  $\text{CD}3^+$  T cells whereas transcripts of *FcRH1* were barely detectable. *FcRH1* expression also was observed in circulating granulocytes.

**Analysis of *FcRH1*, *FcRH2*, and *FcRH3* Expression by Lymphoid Cells in the Tonsils.** To refine the analysis of *FcRH* expression in secondary lymphoid tissues, tonsillar lymphocyte subpopulations were isolated. The five discrete subpopulations of B lineage cells that

**Table 1. Expression of *FcRH* transcripts in human B cell lines**

Cell type	Cell line	<i>FcRH1</i>	<i>FcRH2</i>	<i>FcRH3</i>
Pro-B	REH	+	–	–
	Nalm16	+	–	–
Pre-B	697	–	–	–
	207	–	–	–
	OB5	–	–	–
B	Ramos	+	+	+
	Daudi	+	+	+
	Raji	+	+	+
T	Jurkat	+	–	–
Monocytic	THP-1	+	–	–
Myelomonocytic	U937	+	–	–
Promyelocytic	HL-60	+	–	–
Myelocytic	KG-1	+	–	–
Erythroid	K562	–	–	–

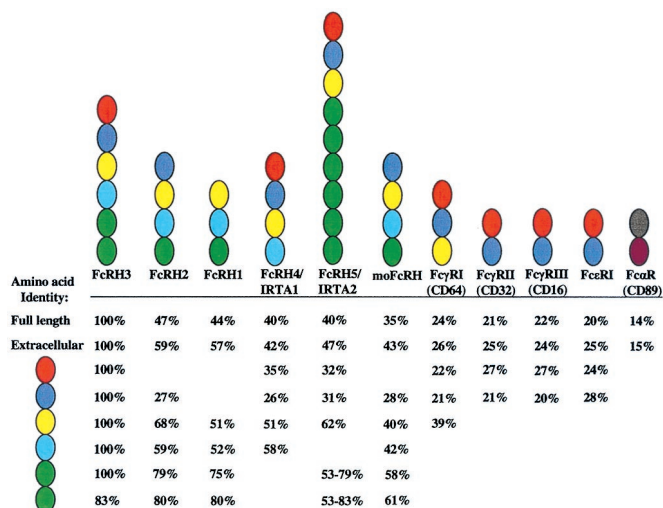
*FcRH1*, *FcRH2*, and *FcRH3* expression in cell lines was determined by RT-PCR as described in *Materials and Methods*.



**Fig. 4.** RT-PCR analysis of *FcRH1–5* expression in tonsillar B cell subpopulations. (A) Viable cells were magnetically sorted into  $\text{CD}19^+$  non-B cells and  $\text{CD}19^+$  B cells. The latter were stained with anti-IgD and anti- $\text{CD}38$  mAbs, and the five subpopulations indicated ( $\text{CD}38^- \text{IgD}^-$ ,  $\text{CD}38^- \text{IgD}^+$ ,  $\text{CD}38^+ \text{IgD}^+$ ,  $\text{CD}38^+ \text{IgD}^-$ , and  $\text{CD}38^{2+}$ ) were sorted by flow cytometry. (B) RT-PCR analysis of *FcRH* transcripts in non-B cells and the B cell subpopulations. After cDNA preparation, PCR amplification was performed on the equivalent template of  $\approx 10$  k cells. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified as a positive control.

can be distinguished by their differential expression of cell surface IgD and CD38 (Fig. 4A) represent different stages in B cell differentiation: follicular mantle ( $\text{IgD}^+ \text{CD}38^-$ ), pre-GC ( $\text{IgD}^+ \text{CD}38^+$ ), GC ( $\text{IgD}^- \text{CD}38^+$ ), memory ( $\text{IgD}^- \text{CD}38^-$ ), and mature plasma cells ( $\text{CD}38^{2+}$ ) (41). RT-PCR analysis indicated little or no expression of *FcRH* transcripts in the non-B lineage  $\text{CD}19^-$  cells, most of which are T cells (Fig. 4B). However,  $\text{CD}19^+$  subpopulations displayed coordinate expression of *FcRH1*, *FcRH2*, and *FcRH3* transcripts in follicular mantle, naïve, GC, and memory B cell subpopulations, but yielded no evidence of *FcRH* transcripts in pre-GC B cells or plasma cells (Fig. 4B). In contrast, *FcRH4* transcripts were restricted to the follicular mantle and memory B cells, whereas *FcRH5* expression extended to mature plasma cells.

**Phylogenetic Analysis of the *FcRH* and *FcR* Families.** The relationship between the five *FcRHs* was examined by comparing their full-length, extracellular, and individual Ig-like domain amino acid sequences. This analysis, which included a recently identified mouse *FcRH* ortholog (mo*FcRH*) and members of the *FcR* family, used the CLUSTAL method algorithm (42). Comparison of the full-length sequences of other *FcRH* family members with *FcRH3* indicated 40–47% identity (Fig. 5). By comparison, the degree of *FcRH3* homology with the mo*FcRH* was found to be 35% and 21–24% with *FcR* members residing on chromosome 1, *FcγRI*, *FcγRII*, *FcγRIII*, and *FcεRI*. A lower level of amino acid identity (14%) was observed for the chromosome 19 LRC member, *FcαR*. A slightly higher degree of extracellular homology was evident. Pairwise analysis of the individual Ig-like subunits indicated conservation in membrane-distal to membrane-proximal ordering of extracellular domain composition among family members (Fig. 5). Although similar Ig domain subunits were shared among family members, the individual receptors were found to be composed of unique domain combinations. The extracellular domain configuration of the mo*FcRH* most closely resembled that of *FcRH2*, with which it has 46% identity. The extended pairwise comparison of the *FcRH* family with known *FcRs* suggested the conservation of these Ig-like domains to some degree throughout the greater family. The resemblance is particularly evident in the *FcRH3* membrane-distal domains that correspond to the three *FcγRI* domains and the two domains of *FcγRII*, *FcγRIII*, and the *FcεR*  $\alpha$ -chain. This analysis suggests the ancestral occurrence of differential duplication and diversification of the individual Ig-like subunits in the respective *FcRH* family members. The



**Fig. 5.** Composite analysis of the extracellular homology among FcRH and FcR family members. Pairwise analysis of individual Ig-like subunits was performed with the CLUSTAL method algorithm using FcRH3 as the index of comparison. Individual homologous domains are color-coded to indicate relatedness. Percent amino acid identities for related domains are indicated and aligned in relation to the comparative FcRH3 subunit. The amino acid identity for the membrane proximal domains (green subunits) of FcRH5 are provided as the range of identity for all individually related domains. Comparisons that are not applicable are left blank. Amino acid sequences were derived from IRTA1 (GenBank accession no. AF343659), IRTA2 (GenBank accession no. AF34364), moFcRH (GenBank accession no. AAG28775) FcγRI (GenBank accession no. AAA35678), FcγRII (Swiss-Prot accession no. P31994), FcγRIII (Swiss-Prot accession no. P08637), FcεRI (Swiss-Prot accession no. P12319), and FcαRI (Swiss-Prot accession no. P24071).

data also indicate that the FcRHs are more similar to their *FcR* neighbors on chromosome 1 than to their FcαR relative on chromosome 19.

**FcRH1, FcRH2, and FcRH3 Genomic Structure.** The genomic sequence analysis of relevant chromosome 1q21 BAC clones (Fig. 1) indicated that the entire *FcRH* locus spans ≈300 kb. The *FcRH* genes lie in the same transcriptional orientation toward the centromere. Exon-intron boundaries were characterized by sequence comparison of their respective cDNA clones and the AG/GT rule. The *FcRH1* gene consists of 11 exons and 10 introns spanning ≈28 kb. The first exon, 5' UT/S1, encodes the 5' UT region, the ATG translation initiation codon, and the first half of a split signal peptide. S2, the second exon, is separated from 5' UT/S1 by a long intron of ≈12.9 kb and, like the neighboring *FcRs*, is 21 bp in length (43–45). The extracellular region is encoded by three closely clustered exons, EC1–EC3, that code for the three Ig-like domains. The membrane-proximal, transmembrane, and the proximal portion of the cytoplasmic domain are encoded by a single sixth exon, TM. The cytoplasmic tail is encoded by five exons, CY1–CY5, and the CY5 also encodes the beginning of the 3' UT region.

*FcRH2* contains 12 exons and 11 introns that span ≈30 kb. It also contains two exons that encode a split signal peptide, the first of which, 5'UT/S1, includes the 5' UT region, the ATG translation initiation codon, and first half of the signal peptide. The second exon, S2, is 21 bp in length. Exons 3–6 encode the four extracellular domains, EC1–EC4. The seventh exon encodes the membrane-proximal, transmembrane, and the proximal portion of the cytoplasmic domain. The *FcRH2* cytoplasmic tail is encoded by five exons, CY1–CY5, the last exon of which includes the termination of the ORF and beginning of the 3' UT region.

The *FcRH3* gene consists of 16 exons and 15 introns that span ≈24 kb. Unlike *FcRH1* and *FcRH2*, its 5' UT region is encoded by two exons, 5' UT1 and a second, 5'UT2/S1, that also encodes the ATG translation initiation codon and the beginning of the split signal peptide. The third exon, S2, is also 21 bp in length. Extracellular domains encoded by six exons, EC1–EC6, are followed by exon 10 that encodes the membrane-proximal, transmembrane, and the proximal portion of the cytoplasmic domain. The cytoplasmic tail is encoded by five exons, CY1–CY5; the last contains the beginning of the 3' UT region.

## Discussion

The FcRHs, identified here on the basis of sequence similarity with previously identified FcRs, are encoded by genes that reside near their closest *FcR* relatives on human chromosome 1 (q21–22). The *FcRH* family includes five functional genes and one pseudogene. Two of the *FcRH* members, *FcRH4* and *FcRH5*, are identical with the recently described *IRTA1* and *IRTA2*, so named because of their discovery as genes that may be translocated in B cell malignancies (26). Three members of this family, *FcRH1*, *FcRH2*, and *FcRH3*, are described in this report. The FcRH family members share similar Ig-like domains, but possess distinctive cytoplasmic tails with consensus ITAMs and/or ITIMs. The signaling capacities of these transmembrane receptors thus are likely to differ. Our analysis of their tissue distribution indicates that the *FcRH1–3* genes, like the previously described *FcRH4* (*IRTA1*) and *FcRH5* (*IRTA2*), are expressed at relatively high levels in secondary lymphoid tissues, primarily by mature subsets of B cells.

The FcRH were identified on the basis of predicted amino acid sequence homology with the FcRγRs and the polymeric Ig receptor encoded by neighboring genes on chromosome 1. Initial bioinformatic screening using a consensus FcR sequence indicated the anticipated identity with FcR family members and, in addition, a lower correlative score was obtained for sequences found in chromosome 1 genomic BAC clones, one of which was remarkable for its inclusion of several putative Ig superfamily members. The BAC clones that proved to contain the *FcRH* genes span the 1q21.2–1q22 region within the *FcR* cluster. This genomic proximity, together with the amino acid homology of the predicted protein products of the *FcRH* genes, suggests a common phylogenetic ancestry. Analysis of their genomic structure further supports the relationship not only between the *FcRH* genes, but with their FcR neighbors as well. A conserved feature among the *FcγRI*, *FcγRII*, *FcγRIII*, and *FcεR* genes is a signal peptide encoded by two separate exons, the second of which (S2) is a 21-bp “miniexon” (43–45). The conservation of the 21-bp S2 miniexon for all *FcRH* and *FcR* family members on chromosome 1 contrasts with the 36-bp S2 exon encoding the second half of the signal peptide for the *FcαR* gene on chromosome 19q13 (46).

The relationships between extracellular Ig-like domains of the FcRHs and those of the FcRs also suggest a common ancestry for the two subfamilies. This relationship is particularly evident for the individual Ig-like subunits, which display a conserved organizational pattern as an additional familial feature. Divergence of the *FcRHs* from the *FcRs* is indicated by the duplication of novel membrane-proximal Ig-like domains in the FcRHs. However, the collective evidence of chromosomal proximity, genomic structural similarity, and Ig-like domain homology clearly imply that the *FcRH* genes are phylogenetic relatives of the *FcR* family.

Like the previously identified (*FcRH4*) *IRTA1* and (*FcRH5*) *IRTA2* family members (ref. 26; our data), the *FcRH1*, *FcRH2*, and *FcRH3* genes are preferentially expressed by B lineage cells in the spleen, tonsils, and lymph nodes. A possible alternatively spliced form of *FcRH2*, the recently described Src homology 2 domain-containing phosphatase anchor protein 1 (*SPAP1*), also has been shown to have a similar expression pattern (47). *FcRH1*

and *FcRH5* (*IRTA2*) transcripts were identified in nonlymphoid tissues also, suggesting a relatively broad biological potential for these receptors. Our analysis of *FcRH1*, *FcRH2*, and *FcRH3* gene expression in cell lines representative of the different hematopoietic cell lineages indicates they are primarily expressed by mature B cells, suggesting a functional role in modulating the later stages of B cell maturation. In addition, *FcRH1* was found to be expressed by relatively immature hematopoietic cells of multiple cell lineages, including the T cell lineage. RT-PCR analysis of the expression of all five *FcRH* genes in primary lymphoid lineage cells in peripheral tissues indicated differential expression patterns for the different stages in the B cell differentiation pathway. Whereas transcripts for the *FcRH1*, *FcRH2*, and *FcRH3* genes were expressed in naïve, GC, and memory B cell subsets, *FcRH4* expression was limited to naïve and memory B cells and *FcRH5* expression persisted in mature plasma cells. These distinctive patterns of expression imply a highly coordinated regulation of *FcRH* gene expression during B cell activation and differentiation. In their *in situ* hybridization studies, Hatzivassiliou and coworkers (26) found *IRTA1* (*FcRH4*) expression to be topographically limited to marginal zone B cells, whereas *IRTA2* (*FcRH5*) expression was also evident in GC B cells and immunoblasts. Their data also indicated a correlation between the *IRTA2* overexpression and chromosomal 1q21 abnormalities in Burkitt lymphomas and multiple myelomas. Correspondingly, analysis of the Lymphochip data (34) indicates the differential expression of *FcRH1* among the diffuse large B cell lymphomas. These observations have intriguing diagnostic and prognostic implications for these genes in lymphomagenesis.

The FcRH family members exhibit considerable diversity in their structure and signaling potential. Although their mem-

brane-proximal Ig-like domains are relatively similar, the membrane-distal subunits display more limited identity, which may indicate diverse functional potential. The cytoplasmic tails of these receptors are also different. In contrast to the known FcRs that possess either activating or inhibitory properties, some of the FcRH have both types of motifs, a feature shared in common with the CD22 and CD31 (PECAM-1) transmembrane molecules (48–50). Whereas, the activating FcRs must associate with the FcR common  $\gamma$  chain via charged residues in their transmembrane region to transduce their signals (24, 25), the FcRHs have uncharged transmembrane domains and their own tyrosine-based signaling motifs. The capacity for both activating and inhibitory function in the cytoplasmic tails of individual FcRH members suggests the capacity for a dual role in regulation, or perhaps a fine-tuning modulation of the signaling threshold.

In addition to their structural differences and differential expression in B lineage cells, the individual *FcRH* expression in nonlymphoid tissues, apparently as splice variants, implies an additional degree of diversity for this family of receptors. The expression of a unique *FcRH3* transcript of  $\approx 1.3$  kb in skeletal tissue, for example, suggests members of the *FcRH* family will have nonimmunological as well as immunological functions.

We thank Drs. Eric Vivier, Louis B. Justment, and Peter D. Burrows for critical comments on the manuscript, Marsha Flurry, Dottie Lang, and E. Ann Brookshire for help in manuscript preparation, Dr. Larry Gartland for help with flow cytometry, and Yoshiki Kubagawa for sequencing assistance. This work has been supported in part by National Institutes of Health Grants AI42127 and AI39816. M.D.C. is a Howard Hughes Medical Institute Investigator. R.S.D. was supported by the Walter B. Frommeyer, Jr. Fellowship in Investigative Medicine.

- Ravetch, J. V. & Kinet, J.-P. (1991) *Annu. Rev. Immunol.* **9**, 457–492.
- Daeron, M. (1997) *Annu. Rev. Immunol.* **15**, 203–234.
- Ravetch, J. V. & Bolland, S. (2001) *Annu. Rev. Immunol.* **19**, 275–290.
- Kubagawa, H., Burrows, P. & Cooper, M. D. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 5261–5266.
- Hayami, K., Fukuda, D., Nishikawa, Y., Yamashita, Y., Inui, M., Ohyama, Y., Hikida, M., Ohmori, H. & Takai, T. (1997) *J. Biol. Chem.* **272**, 7320–7327.
- Borges, L., Hsu, M.-L., Fanger, N., Kubin, M. & Cosman, D. (1997) *J. Immunol.* **159**, 5192–5196.
- Samaridis, J. & Colonna, M. (1997) *Eur. J. Immunol.* **27**, 660–665.
- Kremer, E. J., Kalatzis, V., Baker, E., Callen, D. F., Sutherland, G. R. & Maliszewski, C. R. (1992) *Hum. Genet.* **89**, 107–108.
- Wagtmann, N., Rojo, S., Eichler, E., Mohrenweiser, H. & Long, E. O. (1997) *Curr. Biol.* **7**, 615–618.
- Wende, H., Colonna, M., Ziegler, A. & Volz, A. (1999) *Mamm. Genome* **10**, 154–160.
- Wilson, M. J., Torkar, M., Haude, A., Milne, S., Jones, T., Sheer, D., Beck, S. & Trowsdale, J. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 4778–4783. (First Published April 18, 2000; 10.1073/pnas.080588597)
- Reth, M. (1992) *Annu. Rev. Immunol.* **10**, 97–121.
- Vely, F. & Vivier, E. (1997) *J. Immunol.* **159**, 2075–2077.
- Ravetch, J. V. & Lanier, L. L. (2000) *Science* **290**, 84–89.
- Gergely, J., Pecht, I. & Sarmay, G. (1999) *Immunol. Lett.* **68**, 3–15.
- Dennis, G. R., Kubagawa, H. & Cooper, M. D. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 13245–13250. (First Published November 14, 2000; 10.1073/pnas.230442897)
- Yoder, J. A., Mueller, M. G., Wei, S., Corliss, B. C., Prather, D. M., Willis, T., Litman, R. T., Djeu, J. Y. & Litman, G. W. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 6771–6776. (First Published May 29, 2001; 10.1073/pnas.121101598)
- Long, E. O. (1999) *Annu. Rev. Immunol.* **17**, 875–904.
- Unkeless, J. C. & Jin, J. (1997) *Curr. Opin. Immunol.* **9**, 338–343.
- Qui, W. Q., de Bruin, D., Brownstein, B. H., Pearce, R. & Ravetch, J. V. (1990) *Science* **248**, 732–735.
- Oakley, R. J., Howard, T. A., Hogarth, P. M., Tani, K. & Seldin, M. F. (1992) *Immunogenetics* **35**, 279–282.
- Krajci, P., Grzeschik, K. H., Geurts van Kessel, A. H., Olaisen, B. & Brandtzaeg, P. (1991) *Hum. Genet.* **87**, 642–648.
- Shibuya, A., Sakamoto, N., Shimizu, Y., Shibuya, K., Osawa, M., Hiroyama, T., Eyre, H. J., Sutherland, G. R., Endo, Y., Fujita, T., et al. (2000) *Nat. Immunol.* **1**, 441–446.
- Pfefferkorn, L. C. & Yeaman, G. R. (1994) *J. Immunol.* **153**, 3228–3236.
- Morton, E. C., Van den Herik-Oudijk, I. E., Vosseveld, P., Snijders, A., Verhoeven, A. J., Capel, P. J. A. & Van de Winkel, J. G. J. (1995) *J. Biol. Chem.* **270**, 29781–29787.
- Hatzivassiliou, G., Miller, I., Takizawa, J., Palanisamy, N., Rao, P. H., Iida, S., Tagawa, S., Taniwaki, M., Russo, J., Neri, A., et al. (2001) *Immunity* **14**, 277–289.
- Altschul, S. F., Gish, W., Miller, W., Meyers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* **215**, 403–410.
- Korsmeyer, S. J., Arnold, A., Bakhshi, A., Ravetch, J. V., Siebenlist, U., Hieter, P. A., Sharrow, S. O., LeBien, T. W., Kersey, J. H., Poplack, D., et al. (1983) *J. Clin. Invest.* **71**, 301–313.
- Findley, H. W., Cooper, M. D., Kim, J. H., Alvarado, C. & Ragab, A. H. (1982) *Blood* **60**, 1305–1309.
- Martin, D., Huang, R., LeBien, T. W. & Van Ness, B. (1991) *J. Exp. Med.* **173**, 639–645.
- Pulvertaft, R. J. V. (1964) *Lancet* **1**, 238–240.
- Klein, E., Klein, G., Nadkarni, J. S., Nadkarni, J. J., Wigzell, H. & Clifford, P. (1968) *Cancer Res.* **28**, 1300–1310.
- Klein, G., Giovanella, B., Westman, A., Stehlin, J. S. & Mumford, D. (1975) *Intervirology* **5**, 319–334.
- Alizadeh, A. A., Eisen, M. B., Davis, R. E., Ma, C., Lossos, I. S., Rosenwald, A., Boldrick, J. C., Sabet, H., Tran, T., Yu, X., et al. (2000) *Nature (London)* **403**, 503–511.
- Von Heijne, G. (1986) *Nucleic Acid Res.* **14**, 4683–4690.
- Nielsen, H., Engelbrecht, J., Brunak, S. & von Heijne, G. (1997) *Protein Eng.* **10**, 1–6.
- Williams, A. F. & Barclay, A. N. (1988) *Annu. Rev. Immunol.* **6**, 381–405.
- Bork, P., Holm, L. & Sander, C. (1994) *J. Mol. Biol.* **242**, 309–320.
- Vaughn, D. E. & Bjorkman, P. J. (1996) *Neuron* **16**, 261–273.
- Sonnhammer, E. L. L., von Heijne, G. & Krogh, A. (1998) in *A Hidden Markov Model for Predicting Transmembrane Helices in Protein Sequences*, eds. Glasgow, J., Littlejohn, T., Major, F., Lathrop, R., Sankoff, D. & Sensen, C. (Am. Assoc. for Artificial Intelligence, Menlo Park, CA), pp. 175–182.
- Pascual, V., Liu, Y.-J., Magalski, A., de Bouteiller, O., Bancereau, J. & Capra, J. D. (1994) *J. Exp. Med.* **180**, 329–339.
- Higgins, D. G. & Sharp, P. M. (1989) *Comput. Appl. Biosci.* **5**, 151–153.
- van de Winkel, J. G. & Capel, P. J. (1993) *Immunol. Today* **14**, 215–221.
- Kulczycki, A., Jr., Webber, J., Soares, H. A., Onken, M. D., Thompson, J. A., Chaplin, D. D. & Loh, D. Y. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 2856–2860.
- Pang, J., Taylor, G. R., Munroe, D. G., Ishaque, A., Fung-Leung, W. P., Lau, C. Y., Liu, F. T. & Zhou, L. (1994) *J. Immunol.* **151**, 6166–6174.
- de Wit, T. P. M., Morton, H. C., Capel, P. J. A. & van de Winkel, J. G. J. (1995) *J. Immunol.* **155**, 1203–1209.
- Xu, M., Zhao, R. & Zhao, Z. J. (2001) *Biochem. Biophys. Res. Commun.* **280**, 768–775.
- Newman, P. J., Berndt, M. C., Gorski, J., White, G. C., Lyman, S., Paddock, C. & Muller, W. A. (1990) *Science* **247**, 1219–1222.
- Doody, G. M., Justement, L. B., Delibrias, C. C., Matthews, R. J., Lin, J., Thomas, M. L. & Fearon, D. T. (1995) *Science* **269**, 242–244.
- Poe, J. C., Fujimoto, M., Jansen, P. J., Miller, A. S. & Tedder, T. F. (2000) *J. Biol. Chem.* **275**, 17420–17427.