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Immunology

Fig1, an interleukin 4-induced mouse B cell gene isolated by cDNA representational difference analysis

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ABSTRACT **Interleukin 4 (IL-4) is a cytokine that regulates growth and differentiation of lymphoid and nonlymphoid cells. To study the molecular basis of IL-4 function, we used a cDNA subtraction approach based on the representational difference analysis method. This subtractive amplification technique allowed us to use small amounts of RNA from lipopolysaccharide ± IL-4-stimulated normal B cells to obtain IL-4-induced genes from these cells. We report here on one such gene, *Fig1* (interleukin-four induced gene 1), the first characterized immediate–early IL-4 inducible gene from B cells. *Fig1* expression is strikingly limited to the lymphoid compartment. B cells, but not T cells or mast cells, express *Fig1* in response to IL-4 within 2 hr in a cycloheximide resistant manner. IL-2, IL-5, and Il-6 do not induce *Fig1* in culture. *Fig1* maps between *Klk1* and *Ldh3* on mouse chromosome 7, near two loci involved with murine lupus, *Sle3* and *Lbw5*. The *Fig1* cDNA sequence encodes a predicted 70-kDa flavoprotein with best homology to the monoamine oxidases, particularly in domains responsible for FAD binding.**

Interleukin 4 (IL-4) is a multifunctional cytokine that regulates growth and differentiation among many cell types. It was first recognized because of its actions as a comitogen of B cells and as a determinant of immunoglobulin (Ig) class switching specificity of B lymphocytes stimulated with lipopolysaccharide (LPS). It is now known to promote B cell survival in culture and to induce transcription and/or expression of a series of genes including the germ-line and mature forms of the Ig ε and $\gamma1$ H chains, major histocompatibility complex class II molecules, Thy-1, and CD23 (1). In general, the induction of these genes by IL-4 is relatively slow (2 days), although germ-line $\gamma1$ H chain (I$\gamma1$) transcripts have been observed within 4 hr of culture of normal B cells with IL-4 (2). Genes induced earlier in the response to IL-4 have not been characterized in this or any other cell type. To understand the molecular basis of IL-4 function, an analysis of such "early" genes would be most valuable.

To identify IL-4-induced genes in normal B cell cultures, we used a subtractive cDNA hybridization and amplification method based on genomic representational difference analysis (RDA) (3). We report here the isolation and extensive characterization of one such gene, *Fig1,* a putative flavoprotein with homology to monoamine oxidase (MAO). *Fig1* is induced in resting B cells within 2 hr in response to IL-4 alone; its induction was not inhibited by cycloheximide. Thus, it qualifies as an immediate–early IL-4-inducible gene.

MATERIALS AND METHODS

Mice. Female 6–16-week-old BALB/c mice, obtained from the Frederick Cancer Research and Development Center, National Cancer Institute (Frederick, MD), were maintained and used in experiments under National Institutes of Health guidelines that meet or exceed standards set forth by the National Research Council (4).

Cell Culture. As described (5), resting B cells were prepared from the 60–70% Percoll gradient fraction of dispersed splenocytes treated with antibodies [monoclonal rat anti-mouse anti-Thy1.2 (HO-13-4-9) (6), anti-CD5 (53-7.313) (7), anti-CD8 (3.155) (8), and mouse anti-rat (MAR 18.5) (9)] and complement. B cells were cultured in complete RPMI 1640 medium (5) supplemented, depending on the protocol of the experiment, with 20 μg/ml LPS (LPS W *Escherichia coli* 055:B5, Difco), 20 μg/ml cycloheximide (Sigma), 400 units/ml recombinant human IL-2 (gift from Steven Rosenberg, National Cancer Institute, Bethesda), and the following baculovirus-derived recombinant mouse cytokines: 100–10,000 units/ml IL-4 (10), 15 ng/ml ($\approx$150 units/ml) IL-5 (R & D Systems), and 1000 units/ml IL-6 (Genzyme).

RNA Preparation. Total RNA was prepared using a guanidine thiocyanate protocol (11) or a similar RNAzol (Biotecx Laboratories, Houston) protocol. Briefly, 2×10^7 to 1×10^8 cultured cells or homogenized tissue from 1–2 mice were lysed in guanidine thiocyanate solution, extracted with a phenol/chloroform solution, and then precipitated with isopropanol. The resulting RNA was resuspended in diethyl-pyrocarbonate-treated H_2O and quantitated by UV absorption measurements at 260 nm.

cDNA RDA Subtractive Amplification. Amplicons were prepared from poly(A)$^+$ mRNA isolated from B cells cultured for 12 and 36 hr with LPS (driver) or LPS and IL-4 (tester) by converting to cDNA, digesting with *Sau*3AI restriction enzyme, ligating adaptor sequences to ends of *Sau*3AI fragments, filling in ends, and amplifying by PCR using a primer with sequence complementary to the adaptor (unpublished work). Driver amplicons were modified by PCR amplification with biotinylated primer. Tester amplicons were modified by replacing adaptor with a different adaptor sequence.

For the first round of subtraction, a 20:1 ratio of driver to tester amplicon (combined 12 and 36 hr) was denatured and hybridized together. After complete hybridization, driver-containing hybrids were removed by streptavidin magnetic beads and tester hybrids were RDA amplified by PCR with primers specific for the tester adaptor, resulting in RDA amplicon 1. RDA amplicon 1 was modified by replacing the adaptors with yet another adaptor sequence. To this, a 100-fold

Abbreviations: IL, interleukin; RDA, representational difference analysis; LPS, lipopolysaccharide; EF, elongation factor; MAO, monoamine oxidase; TMO, tryptophan 2-monooxygenase; PDS, phytoene desaturase; FRD/SDH, fumarate reductase/succinate dehydrogenase; RACE, rapid amplification of cDNA ends.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. U70429, U70430, and U80211).

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excess of biotinylated driver amplicon was added for the second round of subtraction. Driver-containing hybrids were depleted and tester hybrids were RDA amplified as above, resulting in RDA amplicon 2. After cloning this material, the sequence of 154 individual clones revealed 37 different genes, including *Fig1.* Two different cDNA RDA subtractive amplification clones, 20-8T#17 and 20-8T#22, cover overlapping parts of *Fig1ps* and *Fig1*, respectively.

cDNA Library Clones. A λgt11 cDNA library made from random-primed cDNA prepared from CBA/J resting splenic B cells stimulated with 300 units/ml of IL-4 for 18 hr (CLONTECH) was used to isolate nearly full-length cDNA clones of *Fig1*. PCR product (50 ng), containing the insert of subtraction clone 20-8T#22 (505 bp), was labeled with [α-^{32}P]dCTP (3000 Ci/mmol, 1 Ci = 37 GBq; Amersham) using an oligolabeling kit (Pharmacia) and used to probe for *Fig1* cDNA clones. After three rounds of plaque hybridization according to the manufacturer's directions, four clones were isolated: 20-11T1, 20-11T2, 20-11T3, and 20-11T4. Phage DNA was prepared from each clone according to manufacturer's instructions and insert cDNA was subcloned into pBluescript SK+ (Stratagene). A 3.5-kb *Bsi*WI–*Pvu*I fragment from 20-11T1 was ligated to *Asp*718I-digested pBluescript SK+ resulting in a linear fragment. This fragment was blunt-ended with T4 DNA polymerase (Boehringer Mannheim) according to manufacturer's protocol and then circularized by ligation, resulting in plasmid 20-12R1-9. A 0.6-kb *Bsi*WI fragment from 20-11T2 was ligated into the *Asp*718I site of pBluescript SK+, resulting in plasmid 20-12C2-1. A 2.0-kb *Bsi*WI fragment from 20-11T3 was ligated into the *Asp*718I site of pBluescript SK+, resulting in plasmid 20-12C3-5. A 4.8-kb *Bsi*WI–*Hin*dIII fragment from 20-11T4 was ligated into the *Asp*718I–*Hin*dIII sites of pBluescript SK+, resulting in plasmid 20-12T4-2.

RACE (Rapid Amplification of cDNA Ends). To obtain the 5′ end of the *Fig1* cDNAs, we performed 5′ RACE using the 5′-AmpliFINDER RACE kit (CLONTECH). First-strand cDNA was prepared from 1 μg poly(A)$^+$ RNA from LPS+IL-4-stimulated B cells (12 + 36 hr). The AmpliFINDER anchor oligonucleotide was ligated to the 3′ end of the cDNA using RNA ligase in a 10-μl reaction. This and other oligonucleotides used in this work are listed in Table 1. An aliquot (1 μl of 1:10 dilution) of this material was amplified by PCR in a 50-μl reaction containing 50 mM KCl, 22.5 mM Tris (pH 9.0), 0.2 mM dNTP, 2.0 mM $MgCl_2$, 1 μM CCC53 or CCC54 primer, 1 μM anchor primer, 2.5 units *Taq* DNA polymerase, and 0.00625 unit *Pfu* DNA polymerase (Stratagene) in a GeneAmp PCR system 9600 thermal cycler under the following conditions: 94°C for 30 sec, 35 cycles of [94°C for 5 sec, 55°C for 15 sec, 72°C for 1 min], 72°C for 2 min, and a final soak at 4°C. This material was diluted 10^6-fold and 1 μl reamplified with a nested primer CCC55, CHU44, or CCC58 and anchor primer or CCC56 as above, except with a higher annealing temperature (63°C or 65°C). The obtained PCR products were analyzed by 1% agarose gel electrophoresis and products greater than 200 bp were cut out from the gel and purified (12). Purified PCR product was ligated into TA cloning vector pCR3 (Invitrogen); the resulting RACE clones obtained are shown in Table 2.

Sequencing from Plasmid or PCR Product. Plasmid DNA was prepared from 4.5-ml cultures of individual clones by a modified mini-alkaline lysis/polyethylene glycol precipitation procedure recommended by Applied Biosystems (Perkin–Elmer). Alternatively, plasmid DNA was prepared by a Qiagen Plasmid Midi kit (Qiagen, Chatsworth, CA) involving a similar alkaline lysis procedure followed by binding to and elution from a plasmid DNA binding column.

Plasmid DNA was initially sequenced using United States Biochemical (Amersham) Sequenase Version 2.0 DNA Sequencing kit with [α-^{33}P]dATP (Amersham) and appropriate oligonucleotide primer. Primers CCC32, CCC34, CCC52, NEB#1218, and NEB#1222 were used in the preliminary sequencing of cDNA library subclones. Sequence reactions were electrophoresed through 6% or 8% polyacrylamide sequencing gels, which were then dried down, autoradiographed, and read.

Plasmid DNA of cDNA library subclones, 20-12R#1-9 and 20-12C#3-5 was sequenced initially with CCC34 and CCC35 primers or NEB#1218 and NEB#1222 primers, respectively, by Paragon Biotechnology on a 373 Stretch Sequencer (Applied Biosystems) using an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit with Ampli*Taq* DNA polymerase FS (Applied Biosystems Part Number 402122). The sequence of these cDNA library subclones was completed by primer walking (sequences available on request).

RACE clones were sequenced from purified PCR product. PCR product was prepared from boiled bacteria (unpublished work) using CCC35 and CCC57 primers and purified through Chromaspin-100 columns (CLONTECH). Purified PCR prod-

Table 1. Oligonucleotides

Name*	Sequence (5′ → 3′)†	Source‡
AmplifFINDER anchor	cacgaattcaCTATCGATTCTGGAACCTTCAGAGG	CLONTECH
Anchor primer	ctggttcggcccaCCTCTGAAGGTTCCAGAATCGATAG	CLONTECH
CCC32 (SK primer)	TCTAGAACTAGTGGATC	LI
CCC34 (T3 primer)	AATAACCCTCACTAAAG	LI
CCC35 (T7 primer)	AATACGACTCACTATAG	LI
CCC52 (*Fig1* 524–542)	cggaattcggtaccGGAGAAGATGCCAGAAAAG	Paragon
CCC53 (*Fig1ps* 1165–1149)	cggaattcggtacCACAAGACTCTTGGGGG	Paragon
CCC54 (*Fig1* 790–773)	cggaattcggtacCGTAAGGCTTCTGCAAAG	Paragon
CCC55 (*Fig1* 610–588)	cgtgatcaagcTTGAGTGCCATCTGGTAGATGTC	Bio-Synthesis
CCC56	CCTCTGAAGGTTCCAGAATCGATAG	Bio-Synthesis
CCC57 (SP6 primer)	ATTTAGGTGACACTATAG	Bio-Synthesis
CCC58 (*Fig1* 410–393)	GTGAGAGCTGGGCATTCG	Bio-Synthesis
CHU3 (*Fig1ps* 2213–2194)	CCTCGGACATCACATCTCCC	Paragon
CHU39 (*Fig1ps* 1711–1730)	GGGCCAGTAGAGGTGGGACT	Paragon
CHU44 (*Fig1ps* 811–789)	AGGAGTCGGGGTGGGGATAGGGG	Paragon
λgt11 forward primer	GGTGGCGACGACTCCTGGAGCCCG	NEB#1218
λgt11 reverse primer	TTGACACCAGACCAACTGGTAATG	NEB#1222

*Oligonucleotides complementary to *Fig1* or partially spliced *Fig1* (*Fig1ps*) have sequence coordinates shown.
†Lowercase sequences indicate added restriction site tails for CCC52, CCC53, CCC54, and CCC55. AmpliFINDER anchor and anchor primer lowercase letters indicate sequences not complementary to each other.
‡Oligonucleotides were synthesized in the Laboratory of Immunology (LI) as described (5) or purchased from CLONTECH, Paragon Biotechnology, Bio-Synthesis (Lewisville, TX), or New England Biolabs.

Table 2. RACE clones

First round primers	Second round primers	RACE clones	Insert, bp
CCC53/anchor	CHU44/anchor	20-13#T20	722
		20-13#T49	588
CCC54/anchor	CCC55/anchor	20-13#M38	313
		20-13#M45	294
CCC53/anchor	CCC58/CCC56	20-13#V5	443
		20-13#V8	435
CCC54/anchor	CCC58/CCC56	20-13#U20	234
		20-13#U28	269
		20-13#U29	445

uct (100 ng/μl) was sequenced as before by Paragon Biotechnology with the same primers used to generate the PCR product.

Sequence Analysis. With the aid of the VAXcluster at the Frederick Biomedical Supercomputing Center (National Cancer Institute, Frederick, MD) and the GCG computer package (Genetics Computer Group, Madison, WI), the full-length *Fig1* cDNA sequence was assembled. Coding sequence was predicted by CODONUSE program (Conrad Halling, personal communication). Regions of *Fig1* protein structure and homology were determined using the GCG package including PROFILESCAN (13), supported BLAST (14) service provided by the National Center for Biotechnology Information (Bethesda), and FASTA program (15) on the VAXcluster, and BLOCKS on the internet (http://www.blocks.fhcrc.org) (16). Comparisons were made to GenBank (release 97.0), EMBL (release 47.0), and Swiss-Prot (release 34.0) databases.

Northern Blot Analysis. Denatured RNA samples were electrophoresed in 1% agarose/formaldehyde gels and Northern blotted to supported nitrocellulose membrane (Optitran, Schleicher & Schuell) by downward capillary method (12). After baking for 1 hr at 80°C in a vacuum oven, membranes were prehybridized at 42°C in prehyb buffer [40% deionized formamide (Fluka)/4× SSC/10 mM Tris (pH 7.5)/2× Denhardt's solution/0.1 mg/ml denatured salmon sperm DNA] for >1 hr and then hybridized overnight at 42°C to radioactive-labeled probe in hyb buffer (same as prehyb buffer except it includes 10% dextran sulfate). Probes used were either from cDNA RDA subtraction clone 20-8T#22 (Chromaspin-100-purified PCR product of the *Fig1* insert) or clone 20-8T#40 [gel-purified 400-bp *Sau*3AI-digested PCR product containing the elongation factor-2 (EF-2) insert] labeled with [α-^{32}P]dCTP as above (*cDNA Library Clones*). Membranes were washed two times in 2× SSC/0.1% SDS at 42°C for >15 min and two times in 0.1× SSC/0.1% SDS at 65°C for >30 min. After air drying, membranes were autoradiographed by exposure to x-ray film (Kodak XAR-2) with intensifying screens at −70°C from 19 hr to 11 days.

Genetic Mapping. To map the genetic location of *Fig1*, we used the BSS [(C57BL/6JEi × *Mus spretus* SPRET/Ei) × SPRET/Ei] Backcross DNA Panel Map Resource (The Jackson Laboratory) (17). We identified a polymorphism between C57BL/6 and *M. spretus* by sequencing a 607-bp PCR product that spans an intron–exon border. PCR was performed in two 20-μl reactions containing 50 ng *M. spretus* genomic DNA, 50 mM KCl, 22.5 mM Tris (pH 9.0), 0.2 mM dNTP, 1.5 mM $MgCl_2$, 1 μM CHU3 primer, 1 μM CHU39 primer, 2.5 units *Taq* DNA polymerase, and 0.00625 unit *Pfu* DNA polymerase in a GeneAmp PCR system 9600 thermal cycler under the following conditions: 94°C for 30 sec, 35 cycles of [94°C for 5 sec, 64°C for 15 sec, 72°C for 30 sec], 72°C for 2 min, and a final soak at 4°C. The PCR reactions were combined with 10 μl of 10 mM Tris/1 mM EDTA (pH 8.0), purified through Chromaspin-100 columns, and sequenced by Paragon Biotechnology using CHU3 and CHU39 primers. Comparison of *M. spretus* sequence (GenBank accession no. U80211) to *Fig1ps* sequence revealed *Hph*I and *Bfu*I restriction enzyme site polymorphisms in this fragment. This polymorphism was confirmed to exist between *M. spretus* and C57BL/6 by restriction enzyme digestion of PCR product generated from genomic DNA using CHU3 and CHU39 primers. The *Hph*I polymorphism was used to type PCR product generated from each sample in the BSS Backcross DNA Panel using CHU3 and CHU39 primers. The typing results were then sent to The Jackson Laboratory for determination of mapping position.

RESULTS

Subtraction. To isolate IL-4-induced genes from a subtraction between small populations of normal mouse B lymphocytes with very slight differences, we developed a PCR method of subtraction based on genomic RDA (3), which we combined with a physical separation method using magnetic beads (unpublished work). This cDNA RDA technique was used to isolate genes expressed in BALB/c splenic B cells cultured in LPS and IL-4 after subtraction from cDNA obtained from B cells cultured with LPS only. Of the 37 genes obtained, three were strongly induced by IL-4: the previously characterized immunoglobulin Cγ1 gene fragment, the mouse homolog of human transcription factor E4BP4, and an unknown gene with no sequence homology to the public databases.

***Fig1* Gene.** We have further characterized this unknown gene originally called clone 20-8T#17/22, which we have renamed *Fig1* (interleukin-**f**our **i**nduced **g**ene **1**). Northern blot analysis of RNA from B cells stimulated with LPS ± IL-4 confirmed that *Fig1* is induced by IL-4 (data not shown). *Fig1* probes hybridize to mRNAs of 2.0 and 3.5 kb.

The full-length cDNA sequences of both *Fig1* mRNAs were obtained and shown schematically in Fig. 1. The sequence of the larger mRNA reveals that it is a partially spliced pre-mRNA (*Fig1ps*) that still contains two introns. The first exon

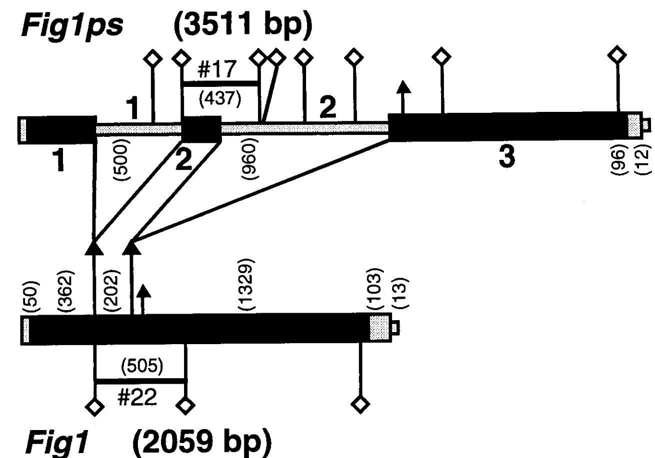

FIG. 1. Schematic of both 2.0- and 3.5-kb *Fig1* mRNA sequences. The sequences of two distinct *Fig1* mRNAs were largely obtained from cDNA library clones and the remaining 5′ end sequences were obtained by RACE. Both mRNA forms are shown schematically with coding regions shown in large solid rectangles and 5′ and 3′ untranslated regions in large shaded rectangles; areas corresponding to introns and to the poly(A) tail are shown in thin rectangles. Exons and introns of *Fig1ps* are numbered. Locations of original subtraction library clones 20-8T#17 and 20-8T#22 are indicated. Clone 20-8T#17 contains exon 2 and part of intron 2 from *Fig1ps*. Clone 20-8T#22 contains exon 2 and part of exon 3 from *Fig1*. Δ, splice sites, with one indicating a splice site found in the 607-bp genomic sequence obtained for genetic mapping. ◇, *Sau*3AI restriction enzyme sites. Lengths in bp are shown in parentheses. *Fig1* (GenBank accession no. U70429) and *Fig1ps* (GenBank accession no. U70430) had slight sequence differences (7 bp) in the untranslated area close to the poly(A) tail.

could possibly encode a short protein of 13 kDa, which would have no additional coding sequence contributed by the first intron due to an immediate translational stop codon found in the intron. *Fig1ps* does not represent genomic DNA since some introns have been spliced out. A 607-bp genomic sequence revealed that a short intron in exon 3 has been spliced out (Fig. 1 and genetic mapping). Homology to MAO (see below) suggests many more introns have been removed. Thus, *Fig1ps* is unlikely to produce a functional protein and most likely represents a partially spliced pre-mRNA.

The sequence of the shorter mRNA (*Fig1*) reveals a single large open reading frame with good codon usage that encodes a 630 amino acid protein with a predicted mass of 70 kDa. The full-length sequence contains some amino acid homology to many proteins. These homologies were not found in the sequence of the two original cDNA RDA subtraction library clones 20-8T#17 and 20-8T#22.

The predicted *Fig1* protein contains a putative signal peptide sequence for translation into the endoplasmic reticulum and hence may possibly be secreted (Fig. 2). It contains three possible *N*-glycosylation sites and two possible tyrosine phosphorylation sites. The protein is mostly hydrophilic with five pockets of hydrophobic nature (including the signal peptide sequence). The strongest homology is found with proteins that bind FAD or NAD as a cofactor. Five areas (1–5), which may be involved in FAD binding are illustrated in Fig. 2: (*i*) binds the ADP moiety of FAD based on homology to a host of FAD and NAD binding proteins that contain the consensus sequence for the dinucleotide binding fold (18); (*ii*) binds the ribityl moiety of FAD based on homology to MAO (19); (*iii*) may bind to an unknown portion of FAD based on its identification by the PROFILESCAN program and its homology to MAO; (*iv*) may bind to another unknown region of FAD based on its identification by PROFILESCAN and its homology to phytoene desaturase (PDS); and (*v*) probably binds the flavin component of FAD based on homology to MAO (20). Of these five areas, the homologies in *iii* and *iv* have not been clearly defined as FAD binding regions. The best homologies were found with MAO (23% identical over 460 amino acids),

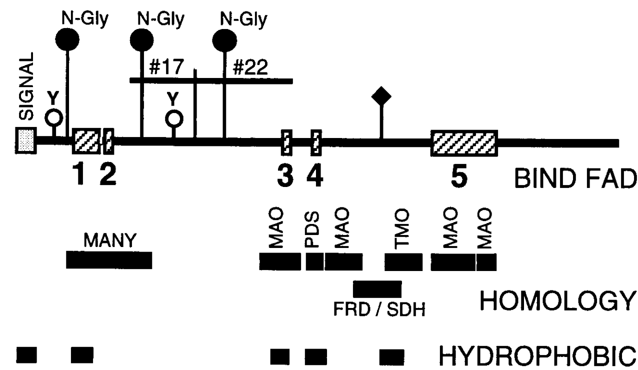

FIG. 2. Schematic of predicted *Fig1* protein. The results of computer program-aided sequence analysis of *Fig1* is summarized in schematic form. Possible N-linked glycosylation sites (N-Gly) and tyrosine phosphorylation sites (Y) are indicated. Locations of sequence inferred from original subtraction library clones 20-8T#17 and 20-8T#22 are indicated. Predicted hydrophobic regions and FAD cofactor-binding regions (numbered 1–5) are shown. In addition, regions homologous to many known FAD binding and NAD binding proteins (MANY) and to specific FAD binding proteins (MAO, PDS, TMO, and FRD/SDH) are shown. The predicted signal peptide domain is also indicated (SIGNAL). ♦, Location of the homologous FRD/SDH active site. The consensus sequence for the dinucleotide binding fold (FAD-binding region 1) is +-#-X-#-G-X-G-X-X-G-X-X-X-#-X-X-#-X-X-X-X-X-X-#-X-#-X-Δ. +, a hydrophilic, positively charged residue; #, a hydrophobic residue; X, any amino acid; G, glycine; Δ, an acidic residue.

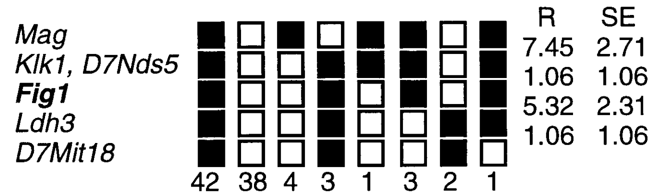

FIG. 3. Genetic mapping. Haplotype figure from The Jackson Laboratory BSS backcross showing part of chromosome 7 with loci linked to *Fig1*. Loci are listed in order, with the most proximal at the top. Solid boxes represent the C57BL6/JEi allele and open boxes the SPRET/Ei allele. The number of animals with each haplotype is given at the bottom of each column of boxes. The percent recombination (R) between adjacent loci, equivalent to centimorgans, is given to the right of the figure, with the standard error (SE) for each R.

tryptophan 2-monooxygenase (42% identical over 92 amino acids), and PDS (38% identical over 80 amino acids). Another weak area of homology to the active site of the fumarate reductase/succinate dehydrogenase (FRD/SDH) family of proteins (26% over 47 amino acids) (21) was uncovered by the BLOCKS program. Although most similar to MAO, *Fig1* is not MAO, because it does not contain the MAO conserved cysteine residue used to covalently bind FAD, its splice sites are in different locations, and despite five large areas of homology (Fig. 2), there are large stretches of nonhomologous amino acids in contrast to rat and human MAO, which are 88% identical over 519 amino acids. Based on these homologies, *Fig1* appears to be an enzymatic flavoprotein with a possible active site similar to FRD/SDH.

***Fig1* Genetic Mapping.** *Fig1* was genetically mapped using The Jackson Laboratory BSS Backcross DNA Panel Mapping Service to the proximal portion of mouse chromosome 7 between the *Klk1* and *Ldh3* markers (Fig. 3). Human *Fig1* is deduced to reside on chromosome 19q13.3-q13.4 because mouse *Fig1* is located between genetic markers that are syntenic with this portion of human chromosome 19 (see The Jackson Laboratory BSS backcross mapping data at the World Wide Web address http://www/jax/org/resources/documents/cmdata). Interestingly, several genetic traits that affect antibody responses map to this region. In the SAM-P/1 mouse, one of two genes responsible for the *Lar* (low antibody response) trait maps to the proximal portion of chromosome 7 (22). In the mouse (NZW × NZB)F_1 hybrid lupus model, two susceptibility genes, *Lbw5* and *Sle3*, map to this region (23, 24).

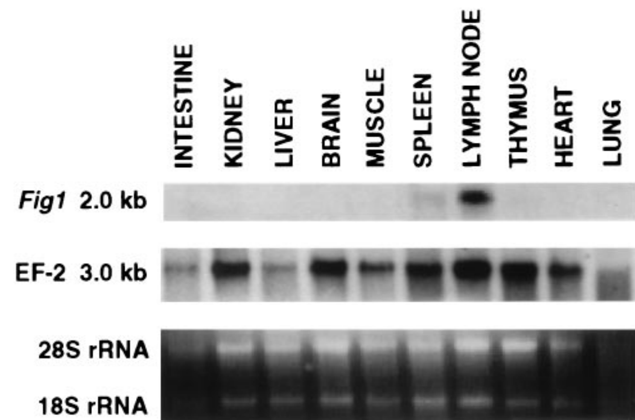

FIG. 4. Tissue expression of *Fig1.* Total RNA (10 μg) prepared from the indicated mouse tissues was electrophoresed, Northern blotted, and probed with *Fig1* or EF-2 (internal mRNA "housekeeping" standard). Ethidium bromide stained 28S and 18S rRNA bands are shown for comparison.

***Fig1* Expression.** Expression of *Fig1* is strikingly limited to lymphoid tissues (Fig. 4), with highest expression in lymph node and spleen. A trace amount of *Fig1* is detectable in thymus upon longer exposure.

In B cells, *Fig1* is induced specifically by IL-4 and not by several other type I cytokines (IL-2, IL-4, IL-5, and IL-6). This induction does not require LPS (Fig. 5). *Fig1* is induced within the first 2 hr and remains elevated for at least 36 hr (Fig. 5). *Fig1* induction by IL-4 is cycloheximide resistant implying that new protein synthesis is not required for its induction (Fig. 6). Thus, *Fig1* is the first immediate–early IL-4-inducible gene characterized in B cells.

Fig1 is *not* expressed in naive T cells; there may be some very weak *Fig1* expression in response to IL-4 in primed T_{H1} and T_{H2} cells (data not shown). Mast cells treated with or without IL-4 also do not express *Fig1* (data not shown). Thus, *Fig1* appears to be a largely B cell-specific IL-4-inducible gene.

DISCUSSION

We have isolated *Fig1* from mouse B cells by the cDNA RDA method, a PCR-based subtraction technique. *Fig1* expression has thus far been observed only in lymphoid tissues and appears to be largely limited to expression to B cells. It is induced within 2 hr in normal mouse B cells and its induction is cycloheximide resistant. Thus, it is the first characterized IL-4-dependent immediate–early gene.

Initial studies using a mouse B cell line transfected with various mutants of the human IL-4 receptor indicate that the region of the receptor required for STAT6 activation is required for upregulating *Fig1* expression (C.C.C., J. J. Ryan, and W.E.P., unpublished results). This is consistent with requirements for induction of other IL-4-dependent genes but differs from requirements for IL-4-mediated cell growth (25).

Is *Fig1* only inducible by IL-4? IL-2, IL-5, and IL-6 did not induce *Fig1* in culture. However, we have not tested a variety of other factor combinations *in vitro*. Indeed, *Fig1* mRNA expression in lymph nodes of IL-4 knockout mice was similar to that of the wild type (unpublished results), indicating the existence of an IL-4-independent mechanism for induction of *Fig1*. This is probably not via cytokines that signal through receptors that contain the γc chain (IL-2, IL-7, IL-9, IL-15), because the IL-2 receptor includes the γc chain in common with the IL-4 receptor. Whether such *Fig1* expression requires the IL-4 receptor α chain, possibly activated by IL-13, remains to be established. Other receptor systems that signal via STAT6 (platelet-derived growth factor, leptin, B cell antigen receptor) may be involved (25). However, the possibility of a totally different signaling system for *Fig1* induction must be considered.

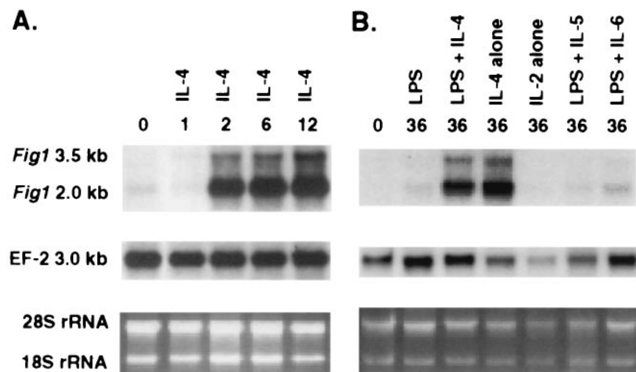

FIG. 5. *Fig1* induced by IL-4 specifically. Total RNA (10 μg) prepared from resting BALB/c splenic B lymphocytes (0 hr) or from B lymphocytes stimulated with IL-4 alone (1000 units/ml) for 1, 2, 6, and 12 hr (*A*), or with IL-4 alone (1000 units/ml), IL-2 alone, LPS alone, or LPS plus IL-4, IL-5, or IL-6 for 36 hr (*B*) was electrophoresed, Northern blotted, and probed with *Fig1* or EF-2. Ethidium bromide stained 28S and 18S rRNA bands are shown for comparison. Concentrations of other reactants have been described.

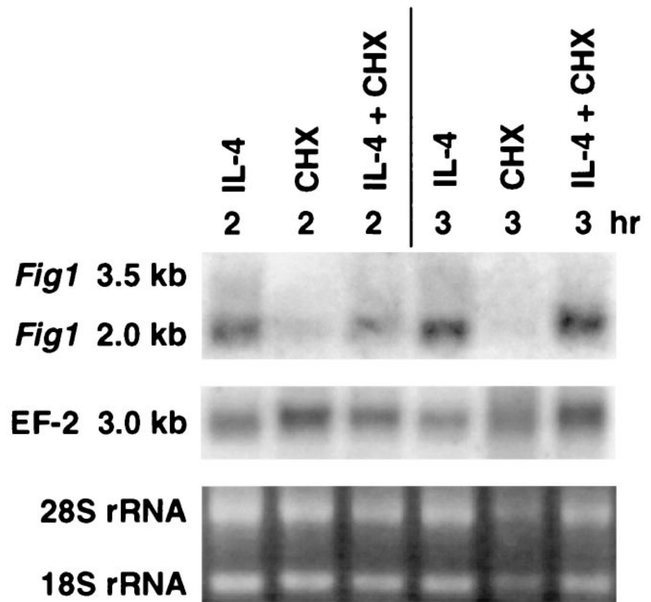

FIG. 6. *Fig1* induction by IL-4 in B cells is not inhibited by cycloheximide. Total RNA (8.2 μg) prepared from BALB/c splenic B lymphocytes after culture for 2 or 3 hr under the conditions indicated [IL-4 (1000 units/ml), cycloheximide (CHX)] was electrophoresed, Northern blotted, and probed with *Fig1* or EF-2. Ethidium bromide stained 28S and 18S rRNA bands are shown for comparison.

Genetic mapping located *Fig1* to a region of mouse chromosome 7 that contains *Lbw5* and *Sle3*, which are involved in the lupus-like disease found in (NZB $\times$ NZW)F_1 hybrid mice. Because two different groups (23, 24) identified this mutation in the same region, *Lbw5* and *Sle3* are likely to be the same gene. The idea that this lupus-associated locus is the same as *Fig1* is attractive, because *Fig1* is expressed in antibody-producing B cells induced with IL-4 (a regulator of the humoral immune response). Preliminary analysis of the induction by IL-4 and size of *Fig1* transcripts in B cells of (NZB $\times$ NZW)F_1 hybrid and *Sle3* affected mice (24) show no noticeable alterations (L. M. Morel, C.C.C., W.E.P., and E. K. Wakeland, unpublished results). However, the possibility still exists that *Fig1* may be mutated at the protein level in these mice.

The function of *Fig1* remains to be solved. Its homologies suggest that it is a secreted FAD binding protein. From its similarity to MAO, it is tempting to speculate that *Fig1* may also inactivate monoamine transmitters in a similar manner to the MAO inactivation of serotonin. This is particularly provocative in view of the role IL-4 plays in allergic inflammatory responses.

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