Identification and Characterization of a Novel Microsomal Enzyme with Glutathione-dependent Transferase and Peroxidase Activities*

(Received for publication, May 13, 1997, and in revised form, July 6, 1997)

Per-Johan Jakobsson, Joseph A. Mancini, Denis Rendeau, and Anthony W. Ford-Hutchinson‡

From the Merck Frosst Centre for Therapeutic Research, Pointe Claire (Dorval), Quebec H9R 4P8, Canada

5-Lipoxygenase activating protein (FLAP), leukotriene-C₄ (LTC₄) synthase, and microsomal glutathione S-transferase II (microsomal GST-II) are all members of a common gene family that may also include microsomal GST-I. The present work describes the identification and characterization of a novel member of this family termed microsomal glutathione S-transferase III (microsomal GST-III). The open reading frame encodes a 16.5-kDa protein with a calculated pI of 10.2. Microsomal GST-III has 36, 27, 22, and 20% amino acid identity to microsomal GST-I, LTC₄ synthase, microsomal GST-I, and FLAP, respectively. Microsomal GST-III also has a similar hydrophobicity pattern to FLAP, LTC₄ synthase, and microsomal GST-I. Fluorescent in situ hybridization mapped microsomal GST-III to chromosomal localization 1q23. Like microsomal GST-II, microsomal GST-III has a wide tissue distribution (at the mRNA level) and is predominantly expressed in human heart, skeletal muscle, and adrenal cortex, and it is also found in brain, placenta, liver, and kidney tissues. Expression of microsomal GST-III mRNA was also detected in several glandular tissues such as pancreas, thyroid, testis, and ovary. In contrast, microsomal GST-III mRNA expression was very low (if any) in lung, thymus, and peripheral blood leukocytes. Microsomal GST-III protein was expressed in a baculovirus insect cell system, and microsomes from Sf9 cells containing either microsomal GST-II or microsomal GST-III were both found to possess glutathione-dependent peroxidase activity as shown by their ability to reduce 5-HPTE to 5-HETE in the presence of reduced glutathione. The apparent $K_m$ of 5-HPETE was determined to be approximately 7 μM for microsomal GST-II and 21 μM for microsomal GST-III. Microsomal GST-III was also found to catalyze the production of LTC₄ from LTA₄ and reduced glutathione. Based on these catalytic activities it is proposed that this novel membrane protein is a member of the microsomal glutathione S-transferase super family, which also includes microsomal GST-I, LTC₄ synthase, FLAP, and microsomal GST-II.

* This work was supported by Grants from the Hellmuth Hertz Foundation, Ulla and Gustaf af Ugglas’s Foundation, the Wenner-Gren Foundation, the Heart and Lung Foundation, the Swedish Society of Medicine, and the Swedish Foundation for International Cooperation in Research and higher education. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Merck Frosst Centre for Therapeutic Research, P.O. Box 1005, Pointe Claire (Dorval), Quebec H9R 4P8, Canada. Tel.: 514-428-2620; Fax: 514-428-2624; E-mail: anthony-fordhutchinson@merck.com.

1 The abbreviations used are: FLAP, 5-lipoxygenase activating protein; LTC₄ synthase, and microsomal glutathione S-transferase (GST) II comprise a family of small (16–18 kDa), membrane-associated proteins (1). FLAP and LTC₄ synthase are specialized proteins involved in the biosynthesis of leukotrienes, physiological important compounds that function as mediators of various inflammatory and immediate hypersensitivity processes (2, 3). 5-Lipoxygenase catalyzes a two-step reaction from arachidonic acid to the unstable epoxy-LTA₄ via (S)-5-hydroperoxy-8,11,14-cis-6-trans-eicosatetraenoic acid (5-HPETE) (4). This reaction in intact cells requires the presence of FLAP (5), an 18-kDa protein localized to the nuclear membrane (6, 7). FLAP has been demonstrated to bind arachidonic acid and increase the efficiency of 5-lipoxygenase conversion of 5-HPETE to LTA₄ (8, 9), however, no enzymatic function has been described for FLAP. LTC₄ synthase is a microsomal 16.6-kDa polypeptide that catalyzes the conjugation of reduced glutathione with LTA₄ and is enzymatically active as a homodimer (10). Purification has shown that LTC₄ synthase possesses no activity toward either 1-chloro-2,4-dinitrobenzene (substrate for α, μ, π, and microsomal classes of GSTs) or p-nitrobenzyl chloride (substrate for the θ class of GSTs) as well as lack of recognition by specific antisera raised against α, μ, π, and microsomal GST-I (11, 12). LTC₄ synthase consequently represents a highly specialized and distinct form of the various known glutathione S-transferases. Microsomal GST-I was the first microsomal glutathione S-transferase to be purified (13, 14). An important feature of this enzyme is its activation by sulphydryl reagents such as N-ethylmaleimide (15). Unlike LTC₄ synthase, microsomal GST-I has been shown to be active as a trimer with a subunit size of 17 kDa (16). Microsomal GST-I is heavily expressed in the liver but is also found in many other tissues such as intestine, adrenal, renal, brain, lung, pancreas, and testis (17, 18). Microsomal GST-I has a wide specificity for lipophilic and electrophilic substrates, however, LTA₄ is a poor substrate for this enzyme (19–22). Microsomal GST-I also catalyzes another type of reaction, namely the reduction of various phospholipid hydroperoxides (23). This peroxidase activity is also found in three θ-class GST isozymes (24). The biological functions of the various GSTs include detoxification of xenobiotics, metabolism of drugs, and protection from oxidative stress caused by lipid peroxidation (16, 25, 26). Recently, we have reported the initial cloning and characterization of microsomal GST-II (1). This enzyme forms a link between LTC₄ synthase and microsomal GST-I in that it uses both LTA₄ and 1-chloro-2,4-dinitrobenzene as substrates for the glutathione S-transferase activity. On the amino acid sequence level it is 44% identical to LTC₄ synthase and 33% identical to FLAP. The protein expression of microsomal GST-II has been determined by Western blot and involves...
Identification of Microsomal GST-III 22935

tissues such as liver (predominantly), lung, and endothelial cells (27). In the liver and endothelial cells, this enzyme seems also to be the source of LTC4 production (27). In this report we describe a novel protein with characteristics in common with FLAP, LTC4 synthase, microsomal GST-I, and microsomal GST-II.

MATERIALS AND METHODS

Cells—Spodoptera frugiperda SF9 cells were obtained from Invitrogen and cultured in Grace’s insect medium supplemented with fetal bovine serum (10%, v/v), gentamycin (150 µg/ml), and fungizone (2.5 µg/ml). The cells were cultured at 28 °C, and the stock cell concentration was maintained between 0.5 and 3 × 10⁶ cells/ml.

Sequencing, Subcloning, and Construction of Recombinant Baculoviruses—A TBLASTN search of the GenBank™ data base using the microsomal GST-II peptide sequence revealed similarity with the sequence deposited by the Washington University-Merck EST project with an accession number of N40831. The sequence of the novel cDNA (termed microsomal GST-III) was confirmed on both strands according to the Sanger dideoxy chain termination method (28) using the PRISM ready reaction Dyeedx™ terminator cycle sequencing kit and an ABI model 373 DNA sequencer. Oligonucleotides for sequencing were obtained from Research Genetics (Huntsville, AL). The insert cDNA sequence was released from the pT7T3D vector by a Not I double digestion and ligated into the EcoRI/NotI clone multiple cloning site of the pFastBac vector (Life Technologies, Inc.). The virus was constructed according to the Bac-to-Bac Baculovirus expression systems, as described by the manufacturer’s instructions (Life Technologies, Inc.). Computerized sequence analysis was performed using the Wisconsin software package, version 8.

Chromosomal Localization of Microsomal GST-II by Fluorescence in Situ Hybridization—Hybridization screening of a P1 artificial chromosome (PAC) library followed by fluorescence in situ hybridization was performed by Bios Laboratories, Inc. The initial PAC library screening was performed using a labeled microsomal GST-III cDNA probe obtained from random priming. Three positive PAC clones were obtained and confirmed by Southern blotting of the EcoRI-digested DNA. The clone with the best yield (PAC clone 94C.14) was used in the subsequent chromosomal localization. The PAC clone 94C.14 was labeled with digoxigenin dUTP by nick translation. The labeled probe was combined with sheared salmon DNA and hybridized to metaphase chromosomes obtained from phytohemagglutinin-stimulated peripheral blood lymphocytes in a solution containing 50% (v/v) formamide, 10% (w/v) dextran sulfate, and 2 M sodium chloride. The slides were hybridized with a cDNA probe of microsomal GST-III. For the labeling of the oligonucleotide probes, we used the T7-QuickPrime kit (Pharmacia Biotech, Inc.). Prehybridization/hybridization was performed in the buffer described by the manufacturer (CLONTECH, ExpressHyb protocol) at 68 °C. After hybridization and washing, the blots were exposed to x-ray film (Kodak Biomax™ MR) at −70 °C. The exposure time for microsomal GST-III was 24 h.

Analysis of LTC4 Synthase Activity in Infected SF9 Cells—SF9 cells were infected with either microsomal GST-II or mock virus at a density of 1.5–2 × 10⁶ cells/ml. 72 h postinfection the cells were harvested, washed, and pelleted by centrifugation at 300 × g. The cell viability was estimated by Trypan blue exclusion and reduced to approximately 50% viability with noninfected SF9 controls (>95% viability). The pellet was resuspended in phosphate-buffered saline, pH 7.4 (Dulbecco’s formula), and sonicated on ice for 3 × 10 s. After centrifugation (500 × g) for 10 min the supernatant was centrifuged for 1 h at 100,000 × g, and the pellet (microsomal fraction) was resuspended in phosphate-buffered saline. The protein concentration was 8–12 mg/ml as determined by the Coomassie protein assay according to the manufacturer’s instructions (Pierce). To measure LTC4 synthase activity, the protein concentration was adjusted to 1 mg/ml using potassium phosphate buffer (0.1 M, pH 7.4). Then, 50 µl of protein was mixed with 50 µl of potassium phosphate buffer containing 10 mM reduced glutathione and 0.1% (w/v) bovine serum albumin. The reaction was started by addition of LTA4 (2 µl of 1.5 mM LTA4 in EtOH). The reaction was terminated after 15 min by the addition of 100 µl of acetonitrile: methanol:acetic acid (50:50:1, v/v). The precipitated protein was removed by centrifugation at 14,000 × g for 10 min. Subsequently, 150 µl of the sample was analyzed by reverse-phase HPLC equipped with a NovaPak C18 column (3.0 × 150 mm, 4-µm particle size) obtained from Waters. The mobile phase was acetonitrile:MeOH:H2O:acetic acid at 29:19:52:1 v/v, adjusted to pH 5.6 with 30% NaOH (w/v), and the flow rate was 1.2 ml/min. Qualitative analysis was performed by comparison with the retention time of synthetic LTC4 and on line analysis of UV spectra of eluted compounds using a Waters 996 diode array spectrophotometer. Amounts were calculated based on the peak area at 280 nm from known amounts of injected LTC4.

Analysis of Glutathione-dependent Peroxidase Activity—To measure peroxidase activity, the protein concentration was adjusted to 1 mg/ml using potassium phosphate buffer (0.1 M, pH 7.4). Typically sample amounts corresponding to 2 µg of protein was incubated in 50 µl of potassium phosphate buffer containing 5 mM reduct glutathione, 0.005% (w/v) bovine serum albumin, and 10 µM 5-HETE (Cayman Chemical Company, Ann Arbor, MI). The reaction was terminated by the addition of 100 µl of acetonitrile containing 0.2% acetic acid and was immediately frozen and stored at −70 °C.

Prior to analysis the samples were thawed and 50 µl of H2O was added. Protein was removed by centrifugation at 14,000 × g for 10 min. Subsequently, 150 µl of the sample was analyzed by reverse-phase HPLC equipped with a NovaPak C18 column (3.0 × 150 mm, 4-µm particle size) obtained from Waters. The mobile phase was acetonitrile: H2O:0.01% (w/v) vitamin H and the flow rate was 1 ml/min. Amounts were calculated based on the peak area at 236 nm from known amounts of injected 5-HETE (retention time = 10.2 min) and 5-HETE (retention time = 8.7 min).

Glutathione S-Transferase Activity—Glutathione S-transferase activity was measured spectrophotometrically by measuring formation of the conjugate of reduced glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm (29). Microsomes of either SF9 cells or SF9 cells expressing either microsomal GST-II or LTC4 synthase were diluted to 5 mg of protein/ml using sodium phosphate (0.11 M, pH 6.5). 5 µl of this sample (25 µg) was transferred into a 195-µl incubation mixture consisting of 1 mM GSH, 1 mM CDNB in sodium phosphate buffer (0.11 M, pH 6.5). The product formation was continuously measured at 340 nm for 5 min on a SpectraMax 250 (Fisher). In experiments where the effect of N-ethylmaleimide was investigated, a 50-µl aliquot of microsomes containing either microsomal GST-II or LTC4 synthase (at 2.5 mg/ml) was treated with 1 mM N-ethylmaleimide for 3 min. Thereafter, 10 µl of this protein was added to the incubation mixture (190 µl) containing 3 mM glutathione and 1 mM CDNB in sodium phosphate buffer (0.11 M, pH 6.5).

RESULTS

Identification of Microsomal GST-III—A TBLASTN search of the GenBank™ data base using the microsomal GST-II peptide sequence revealed that an expressed sequence tag clone with the accession number N40831 displayed significant sequence identity with microsomal GST-II. The clone corresponding to this sequence, human clone 258762, was obtained from the IMAGE Consortium, and sequencing confirmed its identity. The cDNA insert of clone 258762 contains an open reading frame encoding a polypeptide of 152 amino acids with a predicted molecular mass of 16.5 kDa and a calculated isoelectric point of 10.2, which we have termed microsomal GST-III (Fig. 1). The amino acid identity was 36% to microsomal GST-II, 27% to LTC4 synthase, 22% to human microsomal GST-I, and 20% to FLAP. Fig. 2 shows the amino acid sequence alignment of microsomal GST-III with microsomal GST-II, human cytosolic GSTA1, and rat cytosolic GSTA4. The completely conserved amino acids between all three proteins are displayed as a consensus sequence. Amino acids 31–38 of microsomal GST-III are highly conserved with the corresponding region in microsomal GST-II, with 7 out of 8 amino acids being identical. However, a highly conserved region among FLAP, LTC4 synthase, and microsomal GST-II, corresponding to amino acids 46–50 of LTC4 synthase is not present. Moreover, the second protein kinase C phosphorylation motif of LTC4 synthase (underlined in Fig. 2) is also found in microsomal GST-III.

Fig. 3 shows the hydrophathy plot analysis for microsomal
GST-III. The protein contains three hydrophobic regions separated by hydrophilic domains. This pattern is distinct also for microsomal GST-II, LTC4 synthase and FLAP (1).

**Chromosomal Localization of the Microsomal GST-III Gene**—A PAC DNA library was first screened using the cDNA of microsomal GST-III to obtain genomic microsomal GST-II for chromosomal localization by fluorescence in situ hybridization. Based on cohybridization with another probe specific for the centromere of chromosome 1 and fractional length measurements of 10 chromosomes, it was concluded that 94C.14 is located at a position that is 22% of the distance from the p to q telomeres on chromosome arm 1q, an area that corresponds to bands 1Q23. In comparison, the MGST-II gene was localized to chromosome 4q28–31 (1), the LTC4 synthase gene to chromosome 15q21 (2).

**Fig. 3.** Hydropathy plots of microsomal GST-III. The relative hydrophobicity of the amino acid residues of the indicated proteins was determined by hydrophobic moment analysis. The three major hydrophobic domains are indicated.

**FIG. 2.** Alignment of microsomal GST-III, microsomal GST-II, and LTC4 synthase. Residues that are conserved in two of the three proteins are shown in bold. The consensus represents amino acids that are conserved in all of the proteins.

**FIG. 1.** Predicted amino acid sequence of microsomal GST-III. The open reading frame with the highest homology to FLAP and LTC4 synthase is presented. This cDNA fragment was used for expression in a baculovirus expression system.
some 5q35 (30), the FLAP gene to chromosome 13q12 (31), and the gene for human microsomal GST-I to chromosome 12 (32).

mRNA Tissue Distribution—The expression of microsomal GST-III mRNA was investigated in various tissues by Northern blot analysis. Using the microsomal GST-III cDNA probe, a 0.6-kilobase mRNA was predominantly found in human tissues including heart, skeletal muscle, and adrenal cortex, but it was also found in brain, placenta, liver, kidney, small intestine, stomach, and colon. Expression of microsomal GST-III mRNA was also detected in several glandular tissues, such as pancreas, adrenal medulla, thyroid, testis, and ovary. Very low microsomal GST-III mRNA expression was found in lung, thymus, and peripheral blood leukocytes. However, relatively high expression was observed in the bone marrow as well as various fetal tissues (Fig. 4). Of the cancer-transformed cell lines investigated, microsomal GST-III was expressed in the HeLa cell S3, the chronic myelogenous leukemia cell line K-562, the adenocarcinoma SW480, the lung carcinoma A549, and the melanoma G361 but not in the human promyelocytic leukemia cell HL60 or the T or B cell lines, Molt4 and Raji, respectively.

Baculovirus Expression of Microsomal GST-III and Measurement of LTC4 Synthase Activity—The cDNA insert of clone 258762 was subcloned into the pFastBac plasmid followed by creation and isolation of bacmid DNA. Also, a bacmid mock DNA was created. 72 h posttransfection the viral stock was amplified once and subsequently used for infection of Sf9 cells. DNA was created. 72 h posttransfection the viral stock was amplified once and subsequently used for infection of Sf9 cells.

Identification of Microsomal GST-III

Microsomal GST-III

0.6 Kb

Microsomal GST-III

0.6 Kb

Fig. 4. Northern blot analysis of microsomal GST-III mRNA in human tissues. Human multiple tissue RNA blots were hybridized with a radiolabeled microsomal GST-III cDNA probe. Arrows mark the positions of specific transcripts of microsomal GST-III and FLAP. P.B.L., peripheral blood leukocytes; F, fetal; A, adrenal.

In cells infected with MGST-III, LTC4 was produced in the cell membrane fractions (Fig. 5). The microsomal enzymatic activities were 998 ± 68 pmol of LTC4/mg of protein/15 min (mean ± S.D., n = 3) for cells infected with microsomal GST-III and 34 ± 2 (mean ± S.D., n = 3) for mock infected cells. In the 100,000 × g supernatant no difference was observed between microsomal GST-III and mock infected cells. A more polar compound was also produced by MGST-III with the same characteristics as a previously described LTC4 isomer (1). The formation of LTC4 as well as the LTC4 isomer was dependent on the presence of both LTA4 and reduced glutathione. Product formation was abolished by boiling for 5 min prior to the assay. The formation of LTC4 was also time-dependent, and the time course was similar to the time course reported for microsomal GST-II (1), involving a rapid increase over the first 3 min of incubation and reaching a plateau at approximately 7–8 min after the start of the reaction (data not shown).

Glutathione S-Transferase Activity—GST activity was measured spectrophotometrically by measuring formation of the conjugate of the reduced GSH and the CDNB at 340 nm. Using the corresponding samples that were used for the LTC4 experiments, no transferase activity with this substrate was observed with or without pretreatment with N-ethylmaleimide.

Glutathione-dependent Peroxidase Activity—Fig. 6 demonstrates the time course of the 5-HPETE reduction by microsomes from Sf9 cells infected with microsomal GST-II and microsomal GST-III. In Sf9 cells expressing microsomal GST-II the rate of 5-HETE formation was 8 nmol/min/mg of protein, whereas cells expressing microsomal GST-III produced 5-HETE at a rate of 5 nmol/min/mg of protein. The corresponding rate in Sf9 cells infected with mock virus was 1.6 nmol/min/mg. The reaction was linear up to 10 min after initiation. The activity was also linear if using either 1 or 30 μg substrate (data not shown). The peroxidase activity was detected only in the membrane fractions and not in the 100,000 × g supernatant. Neither FLAP nor LTC4 synthase infected Sf9 cells contained this activity. Furthermore, the reduction of 5-HPETE was dependent on the presence of reduced glutathione. To determine the apparent Km for 5-HPETE, microsomal GST-II, and microsomal GST-III (0.04 mg of protein/ml each) were incubated at various 5-HPETE concentrations for 3 min. As determined by hyperbolic regression analysis, the apparent Km was 7 μM for microsomal GST-II and 21 μM for microsomal GST-III (Fig. 7).

DISCUSSION

A TBLASTn search of the GenBank™ data base using the microsomal GST-II peptide sequence has revealed a new clone with higher sequence identity to microsomal GST-II and LTC4 synthase than to FLAP and microsomal GST-I. This novel
protein retains LTA₄-conjugating activity with reduced glutathione, possesses glutathione-dependent peroxidase activity toward 5-HPETE, and was termed microsomal glutathione S-transferase III (Fig. 1).

Sequence comparison of microsomal GST-III, microsomal GST-II and LTC₄ synthase demonstrates that 26 of 152 amino acids are completely conserved in these proteins (Fig. 2). Microsomal GST-III shares a highly conserved region with microsomal GST-II (amino acids 29–38). Interestingly, another region of high sequence identity among MGST-II and LTC₄ synthase (amino acids 47–63), FLAP (amino acids 51–67), and microsomal GST-I (amino acids 70–86) is different in MGST-III. The first 3 amino acids in this region are missing (amino acids ERV) followed by 4 inserted amino acids after which the rest of the region is similar to the other proteins. This region is thought to represent a lipid binding region since it has been demonstrated that these amino acids in FLAP are critical for the binding of leukotriene biosynthesis inhibitors such as MK-886 and L-689,037 (33, 34) and that the binding of arachidonic acid to FLAP has been shown to be competed for by MK-886 (8). Microsomal GST-III also displays the typical hydropathy pattern (Fig. 3), which is common in all the proteins of this gene family (1).

Microsomal GST-I, LTC₄ synthase, and microsomal GST-II are all capable of conjugating electrophilic substrates to reduced glutathione. The main differences are the very high substrate specificity of LTC₄ synthase (19, 35, 36) in contrast to microsomal GST-I, which has a much wider substrate specificity, although LTA₄ is a poor substrate (19–22). Microsomal GST-II forms a link between these two enzymes since it can produce both LTC₄ and utilize 1-chloro-2,4-dinitrobenzene as a substrate (1). In Fig. 5, we demonstrate that Sf9 cells infected with recombinant baculovirus for microsomal GST-III became capable of catalyzing the formation of LTC₄ from LTA₄ and reduced glutathione. The microsomal specific activity (998 ± 296 pmol LTC₄/mg of protein/15 min) was about one third of the activity obtained in Sf9 cells infected with microsomal GST-II (1). This difference may be due to the level of expression. Also, a more polar compound (an LTC₄ isomer) was produced with the same characteristics as previously reported (1). No GST activity was found using 1-chloro-2,4-dinitrobenzene as substrate. From the literature it is evident that various GSTs possess glutathione-dependent peroxidase activity toward various phospholipids and fatty acids (23, 24). Therefore we investigated whether or not 5-HPETE would be a substrate for microsomal GST-II and microsomal GST-III. As demonstrated in Fig. 6 and 7 both enzymes possessed the capability of reducing 5-HPETE to 5-HETE. The low $K_m$ values for 5-HPETE (7 and 21 μM, respectively) enforces the idea that these enzymes are involved in lipid metabolism. Further studies are required to determine which pathway they take part in. For instance,
MGST-II and MGST-III may be involved in the metabolism of certain xenobiotics, or as closely related proteins of FLAP and LTC₄ synthase, they may play a physiological role in the metabolism of arachidonic acid or other fatty acids.

To determine the tissue distribution of microsomal GST-III, Northern blot analysis was used. Microsomal GST-III mRNA was detected in many different tissues (Fig. 4). The strongest hybridization signals were from heart, skeletal muscle, and adrenal cortex. These tissues also express GST-II at the mRNA level as described previously (1). However, microsomal GST-II was also strongly detected in the liver, which was not the case for microsomal GST-III (Fig. 4). Western blot analysis will be required to determine the sites of protein expression.

These results indicate that microsomal GST-III is a microsomal protein with LTC₄ synthase activity and also possesses the capacity, along with microsomal GST-II, to reduce 5-HPETE in the presence of reduced glutathione. It is unlikely that formation of LTC₄ from LTA₄ is an important function for microsomal GST-II (Fig. 4) and that catalysis of LTC₄ by microsomal GST-III seems to be more nonspecific compared with the catalysis performed by LTC₄ synthase. It will be of interest to further elucidate the role of the novel members in this now fairly large gene family and also to study their relationship from an evolutionary viewpoint.

Acknowledgments—We thank Drs. Mark Abramovitz, Jilly Evans, Brian Kennedy, Don Nicholson, and Gary O’Neill for their helpful discussions.

REFERENCES
1. Jakobsson, P.-J., Mancini, J. A., and Ford-Hutchinson, A. W. (1996) J. Biol. Chem. 271, 22203–22210
2. Samuelsson, B. (1983) Science 220, 568–575
3. Ford-Hutchinson, A. W. (1990) Crit. Rev. Immunol. 10, 1–12
4. Shimizu, T., Ridmark, O., and Samuelsson, B. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 689–693
5. Dixon, R. A. F., Diehl, R. E., Opas, E., Rands, E., Vickers, P. J., Evans, J. F., Gillard, J. W., and Miller, D. K. (1990) Nature 343, 282–284
6. Miller, D. K., Gillard, J. W., Vickers, P. J., Sadowski, S., Léveillé, C., Mancini, J. A., Charleson, P., Dixon, R. A. F., Ford-Hutchinson, A. W., Fortin, R., Gauthier, J. Y., Rodkey, J., Rosen, R., Rouzer, C., Sigal, I. S., Strader, C. D., and Evans, J. F. (1990) Nature 343, 278–281
7. Woods, J. W., Evans, J. F., Ethier, D., Scott, S., Vickers, P. J., Hearn, L., Heibeln, J. A., Charleinston, S., and Singer, I. I. (1993) J. Exp. Med. 178, 1935–1946
8. Mancini, J. A., Abramovitz, M., Cox, M. E., Wong, E., Charleson, S., Perrier, H., Wang, Z., Pratist, P., and Vickers, P. J. (1995) FEBS Lett. 378, 277–281
9. Abramovitz, M., Wong, E., Cox, M. E., Richardson, C. D., Li, C., and Vickers, P. J. (1993) Eur. J. Biochem. 215, 105–111
10. Nicholson, D. W., Ali, A., Klembs, M. W., Munday, N. A., Zamboni, R. J., and Ford-Hutchinson, A. W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2015–2019
11. Nicholson, D. W., Ali, A., Klembs, M. W., Munday, N. A., Zamboni, R. J., and Ford-Hutchinson, A. W. (1992) J. Biol. Chem. 267, 17849–17857
12. Penrose, J. F., Gagnon, L., Goppelt-Streue, M., Myers, P., Lam, B. K., Jack, R. M., Austen, K. F., and Soberman, R. J. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 11603–11606
13. Morgenstern, R., and DePierre, J. (1983) Eur. J. Biochem. 134, 591–597
14. McClellan, L., Wolf, C., and Hayes, J. (1989) Biochem. J. 258, 87–93
15. Morgenstern, R., Deferre, J., and Esher, L. (1979) Biochem. Biophys. Res. Commun. 87, 657–663
16. Andersson, C., Mosialou, E., Weinander, R., and Morgenstern, B. (1994) Adv. Pharmacol. 29, 19–35
17. Morgenstern, R., Lundqvist, G., Andersson, G., Balk, L., and DePierre, J. (1984) Biochem. Pharmacol. 33, 3609–3614
18. Otieno, M. A., Baggy, R. B., Hayes, J. D., and Anders, M. W. (1997) Drug Metab. Dispos. 25, 12–20
19. Soderstrom, M., Hammarstrom, S., and Mannervik, B. (1988) Biochem. J. 250, 713–718
20. Metters, K. M., Sawyer, N., and Nicholson, D. W. (1994) J. Biol. Chem. 269, 12816–12823
21. Morgenstern, R., Lunqvist, G., Hancock, V., and Deferre, J. (1988) J. Biol. Chem. 263, 6671–6675
22. Mosialou, E., Andersson, C., Lundqvist, G., Andersson, G., Bergman, T., Jornvall, H., and Morgenstern, R. (1993) FEBS Lett. 315, 77–80
23. Mosialou, E., Piemonte, F., Andersson, C., Vos, R., Van Bladeren, P., and Soberman, R. J. (1995) Arch. Biochem. Biophys. 320, 210–216
24. Singhai, S., Saxena, M., Ahmad, H., Awasthi, A., and Awasthi, Y. C. (1992) Arch. Biochem. Biophys. 299, 232–241
25. Awasthi, Y. C., Sharma, R., and Singhal, S. G. (1994) Int. J. Biochem. 26, 295–308
26. Mantle, T. (1995) Biochem. Soc. Trans. 23, 423–425
27. Scoggan, K. A., Jakobsson, P.-J., and Ford-Hutchinson, A. W. (1997) J. Biol. Chem. 272, 10182–10187
28. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
29. Habig, W. H., Pabst, M. J., and Jakoby, W. B. (1974) J. Biol. Chem. 249, 7130–7139
30. Penrose, J. F., Spector, J., Baldasaaro, M., Xu, K., Boyce, J., Arm, J. P., Austen, K. F., and Lam, B. K. (1996) J. Biol. Chem. 271, 11356–11361
31. Ford-Hutchinson, A., Gresser, M., and Young, R. (1994) Annu. Rev. Biochem. 63, 383–417
32. Dejong, J., Mohandas, T., and Tu, C.-P. (1990) Genomics 6, 379–382
33. Vickers, P. J., Adam, M., Charleson, S., Coppolino, M. G., Evans, J. F., and Mancini, J. A. (1992) Mol. Pharmacol. 42, 94–102
34. Mancini, J. A., Coppolino, M. G., Klassen, J. H., Charleson, S., and Vickers, P. J. (1994) Life Sci. 54, 137–142
35. Yoshimoto, T., Soberman, R., Spur, B., and Austen, K. F. (1988) J. Clin. Invest. 81, 866–871
36. Izumi, T., Honda, Z., Ohishi, N., Kitamura, S., Tsuchida, S., Sato, K., Shimizu, T., and Seyama, Y. (1988) Biochim. Biophys. Acta 935, 305–315