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Expression of fatty-acid-binding proteins in cells involved in lung-specific lipid metabolism

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Members of the fatty-acid-binding protein (FABP) family are thought to play an important role in fatty acid transport within the cytosol and thus to be involved in lipid metabolism. As previous data on the occurrence of distinct FABP types in total lung are contradictory, we determined the expression of FABP types in three isolated cell types of rat lung, which are characterised by active lipid metabolism. Alveolar type-II cells synthesise, store and secrete pulmonary surfactant, a phospholipid-rich surface-tension-lowering agent, whereas lung fibroblasts, localised adjacent to the alveolar type-II cells, are assumed to provide neutral lipid substrate to alveolar type-II cells around birth, and alveolar macrophages are known to degrade complex lipids. Initial screening by reverse transcriptase PCR revealed the occurrence of heart (H-), epidermal (E-) and liver FABP in rat lung, the latter being not detectable in the three cell types studied. Cells were analysed by northern and western blotting, then quantitatively by sandwich ELISA, for which recombinant rat E-FABP was prepared. E-FABP mRNA was found in all three cell types, and E-FABP was detected in the following amounts: 240.9 ± 19.0 ng/mg cytosolic protein in alveolar type-II cells; 172.3 ± 0.7 ng/mg protein for lung fibroblasts; and 36.9 ± 3.5 ng/mg protein for alveolar macrophages. This indicates a basic function of E-FABP in cellular lipid metabolism. In contrast, H-FABP probably is involved in the metabolism of neutral lipids because H-FABP mRNA was found only in lung fibroblasts with a corresponding protein level of 315.5 ± 6.9 ng/mg. Small amounts of H-FABP protein were present in alveolar type-II cells and alveolar macrophages.

Keywords: fatty-acid-binding protein; alveolar type-II cell; lung fibroblast; alveolar macrophage; ELISA.

In rat lung, alveolar type-II cells synthesise, store and secrete pulmonary surfactant, a phospholipid · protein complex that prevents the collapse of the alveoli by reducing surface tension at the air-liquid interface. This surfactant is highly enriched in phospholipids, particularly dipalmitoyl phosphatidylcholine. Fatty acids required for the biosynthesis of phospholipids are either synthesised *de novo* within this type-II cell [1−3] or taken up from exogenous sources [4−6]. Uptake and intracellular transfer of fatty acids by these alveolar type-II cells are not well understood [7], but the high turnover of phospholipids requires a potent transport system for lipophilic precursors.

Lung fibroblasts are found in close contact to alveolar type-II cells and play an important role in lung development by producing growth and differentiation factors [8]. Torday et al. [9] demonstrated that lipofibroblasts, which increase in neutral lipid content prior to birth, provide lipid substrate for surfactant production by type-II cells. These lipofibroblasts disappear after weaning, but reappear during damage-repair processes. They are thought to be involved in a facilitated on-demand supply of surfactant substrate [9].

Participation of alveolar macrophages in surfactant metabolism was suggested from *in vivo* and *in vitro* experiments [10−12]. Theoretically, alveolar macrophages could account for the entire catabolism of alveolar surfactant in rat lung [11]. Fatty acids derived from phosphatidylcholine degradation are reused for triacylglycerol synthesis [13].

It is obvious from the above that fatty acids are needed for lung surfactant homeostasis, in which the three cell types mentioned appear to be involved. As a consequence, fatty acids must be transported within and between cells, and it is reasonable to assume that fatty-acid-binding proteins (FABP) take part at least in intracellular transport. Earlier findings on the occurrence of FABP in lung tissue support this assumption; however, contradictory data were reported with regard to the FABP types present in lung [14−20]. These 14−15-kDa proteins were discovered in a variety of tissues and named according to their initial site of isolation. Because the principal activity of FABP is high-affinity binding of long-chain fatty acids, functions other than transport of fatty acids within the cytosol have been determined. FABP are required for intracellular compartmentalisation of fatty acids and protection of enzymes and membranes against adverse effects of long-chain fatty acids, have roles as cofactors for reactions in which fatty acids or acyl-CoA are substrates, and influence mitosis and cell growth [21−24].

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Abbreviations. FABP, fatty-acid-binding protein; E-FABP, epidermal-type FABP; L-FABP, liver-type FABP; I-FABP, intestinal-type FABP; H-FABP, heart-type FABP.

Our first aim in this study was to clarify the issue of FABP-type-specific expression in rat lung. Based on literature reports [14−20] we focussed our attention on the expression of heart-type (H-) FABP, intestinal-type (I-) FABP and liver-type (L-) FABP, the proteins traditionally believed to be involved in fatty acid transport, and of the recently discovered epidermal-type (E-) FABP [26]. E-FABP, however, was found to be widely distributed [25, 27]. We screened for the expression of the respective cDNAs by reverse transcriptase PCR. Making use of techniques to culture freshly isolated rat lung cells that are involved in surfactant homeostasis, i.e. alveolar type-II cells, lung fibroblasts and alveolar macrophages, we studied the expression pattern of FABP at the RNA and protein levels. We discuss the putative roles of FABP in these cells.

MATERIALS AND METHODS

Materials. Oligonucleotides used for detection of FABP in lung cells (upstream and downstream primers for amplification of coding parts) were obtained from MWG Biotech as follows: E-FABP, 5′-GCCATGGCCAGCCTTAAGGATC and 5′-AGGATCCTCATTGCACCTTCTCATA, corresponding to positions 43−442 [28]; H-FABP, 5′-CCATGGCGGACGCCTTTGTCGGT and 5′-GACGGAGGATCCAGGTCACGCCTC, corresponding to positions 24−425 [17]; I-FABP, 5′-ATGGCATTTGATGGCACTTGGAAAG and 5′-CTATTCCTTCTTAAAGATCCGCTTGGC, corresponding to positions 13−411 [20]; L-FABP, 5′-CCTCATTGCCCATATGAACTTCTCCGG and 5′-AGCGGATCCTAAATTCTCTTGCTGACTC, corresponding to positions 40−423 [20]. Restriction sites for *Nco*I or *Nde*I in the upstream primers and *Bam*HI in the downstream primers are underlined. Restriction endonucleases and T4 DNA ligase were purchased from Boehringer Mannheim. Plasmids were isolated using the Qiagen Plasmid Kit (Qiagen). For all chromatographic steps an FPLC system (Pharmacia) was used at room temperature; chromatographic materials were from the same supplier. All other chemicals were of analytical grade and purchased from Sigma unless stated otherwise.

Isolation of rat lung cells. Male Wistar rats were obtained from a local supplier. Alveolar type-II cells were isolated by the elastase method as described [29]. Viability was judged by trypan blue staining and purity by Harris-type hematoxylin staining of the isolated cells and ranged from 90−95% and 89−93%, respectively. Adult rat lung fibroblasts were prepared freshly as described for foetal rat lung fibroblasts [30]. Purity after 24 h culture was greater than 90% as judged by dark-field microscopy. Alveolar macrophages were isolated from bronchoalveolar lavages by pelleting the cells at 160 *g* for 10 min at 4°C. The pelleted cells were inspected microscopically and found to be contaminated by white blood cells, some erythrocytes and type-II cells, but free of fibroblasts. These impurities were estimated to be about 15% only, and thus no further purification was undertaken.

Detection of FABP by PCR. Total RNA from rat lung was isolated as described [31], and cDNA was synthesised using murine Moloney's leukemia virus reverse transcriptase (Gibco) and random priming. PCR was performed using *Taq* DNA polymerase (Promega) and primers as specified at melting, annealing and extension temperatures of 94, 55−60 and 72°C, respectively. After 35−40 cycles, PCR products were visualised with ethidium bromide in 1.5% agarose gels. The PCR products were cloned into the pCRII vector (Invitrogen) using *Escherichia coli* INVαF′. Plasmids were isolated with the Wizard Miniprep System (Promega), and the inserts were sequenced [32] using Sequenase 2.0 (USB).

Northern blotting. For generation of radiolabeled single-stranded DNA probes the cloned H- and E-FABP PCR fragments were used as templates in asymmetric 33-cycle PCR [33] using 0.8 μM [^{32}P]dCTP (3000 Ci/mmol; ICN-Biomedicals). Labelled probes were separated from dNTP by chromatography on a Sephadex G-50 column (Pharmacia). RNA was isolated [31] from freshly prepared type-II cells, lung fibroblasts, alveolar macrophages and total lung. Aliquots containing 10 μg RNA were separated on a 1% agarose gel with formaldehyde and transferred overnight onto Hybond N-nylon membranes (Amersham) [34]. The blots were incubated (2 h) with 350 mM Na_2HPO_4, 7% (mass/vol.) SDS, 30% (by vol.) deionised formamide, 10% (mass/vol.) BSA fraction V and hybridised overnight at 50°C with the respective DNA probes. The membranes were washed twice with 150 mM Na_2HPO_4, 0.5% (mass/vol.) SDS (10 min, 25°C) and once with 30 mM Na_2HPO_4, 0.1% (mass/vol.) SDS (10 min, 55°C) and exposed to Kodak Biomax MS (Integra Biosciences) at −80°C.

Cloning, expression and purification of rat E-FABP. Total RNA of rat adult type-II pneumocytes was reverse transcribed as described above and the coding region of rat E-FABP was amplified with two primers derived from the nucleotide sequence published by Watanabe et al. [35], corresponding to positions 44−451. Due to an internal *Nco*I restriction site in the rat E-FABP sequence, an upstream primer (5′-GCATATGGCCAGCCTTAAGGA) was designed, which contained an *Nde*I site and was used with the downstream primer (5′-CGGATCCTCATTGCACCTTCTCAT), which contained a *Bam*HI site. These restriction sites (underlined) allowed for subsequent cloning into the pET3c expression vector (Novagen). PCR was performed using *Pfu* DNA polymerase (Stratagene) at melting, annealing and extension temperatures of 94, 62 and 72°C (1 min each, 30 cycles, final elongation for 10 min at 72°C). The PCR product was purified with the Qiaquick PCR Purification Kit (Qiagen) and cloned into the pCR 11 vector (Invitrogen) for sequencing using the digoxigenin system (Boehringer Mannheim) and blotting onto a nylon membrane (GATC). The *Nde*I−*Bam*HI fragment was cloned into pET3c to yield pETrE-FABP, which was used to transform *E. coli* BL21(DE3)pLysS. For production of recombinant E-FABP, *E. coli* were grown in 2×TY medium [34] containing 100 μg/ml ampicillin and 34 μg/ml chloramphenicol until A_{600} reached 0.6−1, and were induced with 0.4 mM isopropyl-thio-β-D-galactopyranoside (MBI Fermentas). After 45 min, rifampicin was added to 175 μg/ml [36]. The cells were harvested after 16 h (10 min, 6000 *g*, 4°C), suspended in 3 vol. 10 mM Tris/HCl, pH 7.4, 25 U Benzonase (Merck) and disrupted by sonication (3×15 s, 20 W, 0°C). After centrifugation (45 min, 28000 *g*, 4°C) nucleic acids were removed by precipitation with 1.5% (mass/vol.) streptomycin sulphate (20 min, 10000 *g*, 4°C). The cleared lysate was desalted on Sephadex G-25 (5 cm×20 cm, 5 ml/min, 10 mM Tris/HCl, pH 7.4). Fractions containing recombinant E-FABP were subjected to anion-exchange chromatography (Q-Sepharose Big Beads, 5 cm×20 cm, 5 ml/min) and eluted with a gradient from 0 to 1 M NaCl in the same buffer. E-FABP−containing fractions were concentrated and subjected to gel filtration on Superdex 75 [1 cm×60 cm, 1 ml/min 154 mM sodium chloride, 10 mM sodium phosphate, pH 7.4 (NaCl/P_i)]. A typical yield of solubly expressed recombinant rat E-FABP was 20 mg/l broth.

Despite of the presence of five cysteine residues recombinant rat E-FABP was obtained in the soluble fraction. The yield was considerably increased by inhibiting bacterial RNA polymerases with rifampicin during expression. The most effective step in the course of purification was anion-exchange chromatography (Fig. 1), where recombinant E-FABP eluted much earlier in the gradient than most of the bacterial proteins. After gel filtration

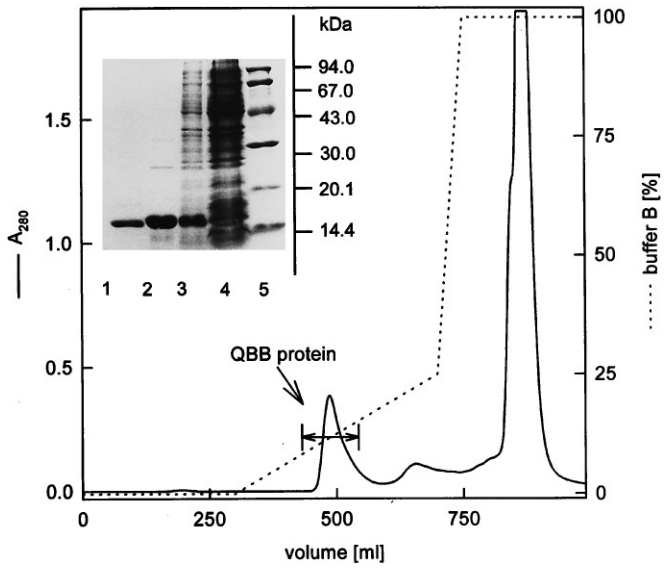

Fig. 1. Purification of recombinant rat E-FABP. Anion-exchange chromatography on Q-Sepharose BB (20 cm×5 cm, 5 ml/min), Buffer B is 1 M NaCl, 10 mM Tris/HCl pH 7.4. The inset shows purification of rat E-FABP on 13.5% SDS/PAGE, stained with Coomassie blue. Lane 1, purified rat E-FABP; lane 2, Q-Sepharose BB (QBB) protein; lane 3, G-25 protein; lane 4, lysate of non-induced *E. coli* BL21(DE3) pLysS pETrE-FABP; lane 5, low-molecular-mass markers (Pharmacia).

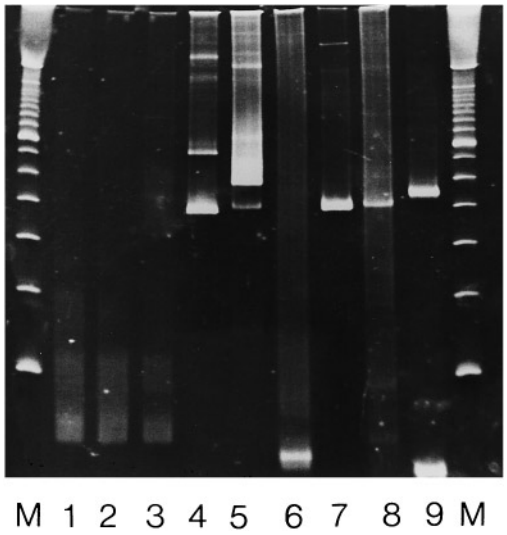

Fig. 2. Identification of FABP in lung by reverse transcriptase PCR. RNA from total rat lung was reverse transcribed and screened for four FABP types by PCR with primers specific for the indicated FABP types. Lane 9, E-FABP; lane 8, H-FABP; lane 7, L-FAPB; lane 6, I-FABP. As a positive control for L-FABP (lane 5) and I-FABP (lane 4) primers, cDNA from rat liver and intestine, respectively, were used. L-FABP was not detected in cDNA from type-II cells (lane 3), lung fibroblasts (lane 2) and alveolar macrophages (lane 1) under identical conditions. Lane M, 100-bp ladder.

the protein was homogeneous on SDS/PAGE (Fig. 1) and was used for immunisation of rabbits and as standard protein in ELISA.

ELISA for rat H-FABP and rat E-FABP. The anti-(rat H-FABP) ELISA was performed essentially as described previously for mouse H-FABP [37], except that recombinant rat H-FABP [38], a generous gift from Dr Jan Glatz (Maastricht, The Netherlands), was used as the standard. Antibodies against rat E-FABP were raised in New Zealand White rabbits by injection of 300 μg recombinant protein in complete Freund's adjuvant (Sigma) followed by booster injections with 100 μg protein in incomplete Freund's adjuvant every four weeks. For affinity purification the antiserum was passed over a column with rat E-FABP covalently bound to CH-activated Sepharose 4B, and specific antibodies were eluted with 10 mM sodium citrate, 20 mM sodium phosphate, pH 2.8. The antibodies were biotinylated using D-biotinoyl-6-amidohexanoic acid *N*-hydroxysuccinimide ester (Boehringer Mannheim). The sandwich ELISA was performed in MaxiSorp polystyrene microtiter plates (Nunc). Incubations (100 μl/well) were for 1 h at 37°C unless specified otherwise. Affinity-purified primary antibody (2.5 μg/ml) was coated in 0.1 M sodium carbonate, pH 9.6, and wells were blocked with 0.5% (mass/vol.) BSA in NaCl/P_i. Rat E-FABP standards and samples, the biotinylated affinity-purified antibody and the streptavidin peroxidase (diluted 1:10000) were added in NaCl/P_i containing 0.5% BSA, 0.05% (by vol.) Tween 20. *o*-Phenylendiamine [0.5 mg/ml in 50 mM sodium citrate pH 5.0, 0.15% (by vol.) H_2O_2] was used as peroxidase substrate (5−10 min, room temperature). The linear range of the sandwich ELISA was 0.5−5 ng/ml rat E-FABP. The recovery of rat E-FABP added to the ELISA samples was 94% (n=3). For both ELISA intra-assay and inter-assay coefficients of variation were below 6% and 14%, respectively (n=3). The content of cytosolic proteins in samples was determined with the bicinchoninic acid assay and ovalbumin as standard [39]. The concentration of a rat E-FABP standard solution was determined spectrophotometrically using a calculated $A_{280}^{1\%} = 9.7$ [40].

SDS/PAGE and immunoblotting. SDS/PAGE was carried out according to Laemmli [41]. For western blot analysis proteins were transferred onto nitrocellulose membranes (Schleicher & Schüll) in 25 mM Tris, 192 mM glycine, 20% (by vol.) methanol. After blocking with 3% (mass/vol.) BSA in NaCl/Tris (154 mM NaCl, 20 mM Tris/HCl, pH 7.4) the appropriate affinity-purified rabbit anti-FABP Ig (2.5 μg/ml) in NaCl/Tris containing 0.05% (by vol.) Tween 20 and anti-rabbit IgG alkaline phosphatase conjugate (1:10000 in incubation buffer) were added. Between these steps membranes were washed with incubation buffer. They were washed twice with 0.1 M Tris/HCl, pH 9.5, 0.1 M NaCl, 5 mM $MgCl_2$ (buffer A), and bands were visualised by incubation with 0.33 μg/ml 4-nitroblue tetrazolium chloride and 0.165 μg/ml 5-bromo-4-chloro-3-indolyl-phosphate in buffer A.

RESULTS

Identification of FABP in lung. Earlier studies regarding the expression of FABP in lung revealed contradictory results. To screen for FABP we used reverse transcriptase PCR with oligonucleotides derived from the known nucleotide sequences of rat H-FABP [17], mouse E-FABP [28], rat I-FABP [20] and rat L-FABP [20], which would yield 423-bp, 418-bp, 399-bp and 411-bp fragments after amplification, respectively. With the primers derived from the H-, E- and L-FABP sequences, DNA fragments migrating as single bands of the expected size on an agarose gel were obtained (Fig. 2). Using the primers derived from the I-FABP sequence, a PCR product was not observed. In a control PCR with RNA from intestine and liver as template the respective primers yielded fragments of the expected size (Fig. 2). L-FABP could not be detected by reverse transcriptase PCR in type-II cells, lung fibroblasts and alveolar macrophages (Fig. 2). Therefore, only H-FABP and E-FABP expression was investigated further, and the corresponding PCR products were cloned and the nucleotide sequences of three H-FABP clones and two

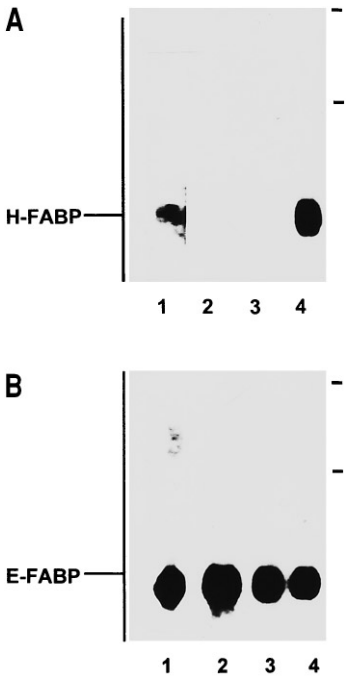

Fig. 3. Northern blot analysis of RNA from rat lung cells. Total RNA (10 μg) was blotted onto a nylon membrane and ^{32}P-labelled H-FABP (A) and E-FABP (B) probes were used for hybridisation. Lanes 1, total lung; lanes 2, alveolar type-II cells; lanes 3, alveolar macrophages; lanes 4, lung fibroblasts. Sample loading was verified by hybridisation with an actin probe (data not shown). Bars indicate 28 S and 18 S RNA.

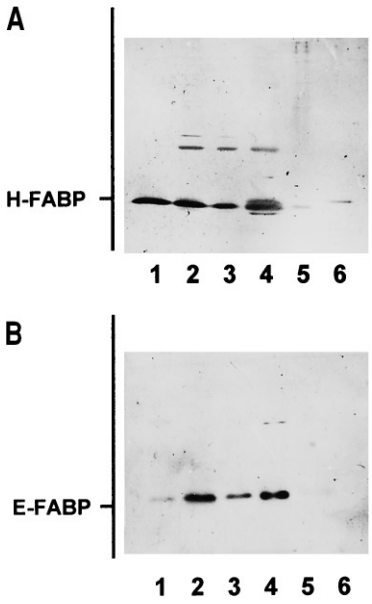

Fig. 4. Distribution of H-FABP and E-FABP in rat lung. Cytosolic proteins were separated on 15% SDS/PAGE, transferred onto nitrocellulose membranes and stained with affinity-purified antibodies directed against mouse H-FABP (A) and rat E-FABP (B) using an alkaline-phosphatase-conjugated secondary antibody for colorimetric detection. Lanes 1, 2.5 ng recombinant rat H-FABP (A) and 2.5 ng E-FABP (B); lanes 2, lung fibroblasts (7 μg), lanes 3, alveolar type-II cells (9 μg); lanes 4, total lung (25 μg); lanes 5, alveolar macrophages (25 μg); lanes 6, controls for cross-reactivity [25 ng recombinant rat E-FABP (A) and H-FABP (B)].

E-FABP clones determined. These nucleotide sequences were identical to those reported for H-FABP from rat heart [17] and E-FABP from rat skin [35] (data not shown), indicating that no particular variants of these FABP types are expressed in rat lung, in contrast to the variants of E-FABP identified in lens and in the nervous system [42, 43].

Expression of H-FABP and E-FABP in lung cells. We investigated the expression of H-FABP and E-FABP, both identified by reverse transcriptase PCR in total lung RNA, by northern blotting. The three cell types referred to in the introduction, i.e. alveolar type-II cells, rat lung fibroblasts and alveolar macrophages, were isolated from adult lung. Total RNA obtained from these cells was blotted and hybridised with the E-FABP probe. In contrast to the H-FABP probe, which hybridised only to mRNA obtained from lung fibroblasts (transcripts of about 650 nucleotides, Fig. 3A), bands of similar size were recognised by the E-FABP probe in all three cell types (Fig. 3B). Both transcripts were of the size expected from the published sequence information. In total lung only a weak band was observed for H-FABP, indicating that the fibroblasts represent only a small fraction of lung cells.

Since antibodies against H-FABP and E-FABP were available, we studied the expression pattern of these proteins in the lung by western blot analysis. The affinity-purified anti-(mouse H-FABP) Ig, which could be used due to the very high sequence identity between mouse and rat H-FABP (95%), easily detected 2.5 ng recombinant rat H-FABP (Fig. 4A) and cross-reacted only slightly with rat E-FABP (Fig. 4A) whereas detection of rat E-FABP by the purified anti-(rat E-FABP) Ig was less sensitive (Fig. 4B) cross-reactivity of the latter towards rat H-FABP was not observed (Fig. 4B). H-FABP was found in total lung cytosol, alveolar type-II cells and lung fibroblasts, but was hardly detectable in alveolar macrophages (Fig. 4A). A similar pattern was observed for E-FABP. For a more precise analysis of H-FABP and E-FABP expression the more sensitive and quantitative sandwich ELISA were applied (Fig. 5).

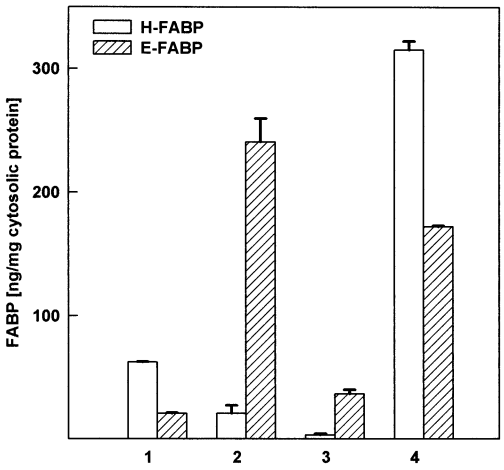

Fig. 5. Expression levels of H-FABP and E-FABP in rat lung cells. FABP contents in cytosols were determined with the aid of specific sandwich ELISA as described in Materials and Methods. Values are means ± SD ($n \geq 3$). 1, total lung; 2, alveolar type-II cells; 3, alveolar macrophages; 4, lung fibroblasts.

A sandwich-type ELISA for mouse H-FABP was available [37] and could be adapted to detect rat H-FABP using recombinant protein as standard. The cross-reactivity of this H-FABP ELISA towards the closely related rat E-FABP was below 0.1% (data not shown), thus the amount of E-FABP present in these cells did not interfere. Total lung contained 62.7 ± 0.3 ng rat H-FABP/mg total cytosolic protein, which was predominantly lo-

cated in the fibroblasts (315.5 ± 6.9 ng/mg) with minor contributions from alveolar type-II cells (20.6 ± 6.6 ng/mg) and alveolar macrophages (3.8 ± 0.9 ng/mg).

No sensitive and specific method has been described for quantification of E-FABP. We produced the recombinant protein for immunisation, affinity purification of antibodies, and for use as standard protein, and we developed a sandwich ELISA for rat E-FABP. This ELISA was sensitive in the low nanogram range and exhibited a cross-reactivity towards H-FABP below 0.5% (data not shown), which means that H-FABP did not disturb the quantification of E-FABP in lung cells. In total lung 20.9 ± 0.7 ng E-FABP/mg was detected. In contrast to H-FABP the concentration of E-FABP was highest in alveolar type-II cells (240.9 ± 19 ng/mg). The lung fibroblasts contributed with 172.3 ± 0.7 ng/mg and the alveolar macrophages contained the lowest amount (36.9 ± 3.5 ng/mg), which, however, is tenfold the amount of H-FABP in these cells.

DISCUSSION

Synthesis, storage and secretion of alveolar surfactant is a specific feature of alveolar type-II cells. This cell type is characterised by particularly active lipid metabolism, because 90% of the surfactant components are lipids. Synthesis of complex lipids, their storage as presecretory surfactant in lamellar bodies, and the ability to recycle reabsorbed alveolar surfactant requires a high rate of intracellular translocation of these hydrophobic components. In alveolar type-II cells two phospholipid-transfer proteins, non-specific lipid-transfer protein and phosphatidylinositol-transfer protein were detected [44], which may be involved in this task. Similarly, FABP may provide the precursor fatty acid for phospholipid synthesis.

Since the first description of FABP by Ockner et al. [45], various distinct FABP types and isoforms have been discovered and their organ distribution and structural properties determined [21, 24]. An abundant FABP in lung was described by Haq et al. [14]. In addition, Sa et al. [15] isolated proteins from human foetal lung with apparent molecular masses of about 15 kDa, which were capable of binding fatty acids. Concentrations of these proteins increased from early gestation to term. The data concerning the FABP type occurring in lung were controversial however. Paulussen et al. [16] detected H-FABP in rat lung by ELISA whereas others could not detect mRNA encoding H-FABP by dot blotting and northern blotting [17]. A cDNA fragment coding for H-FABP was found by reverse transcriptase PCR in RNA from murine lung [18], whereas the respective mRNA could not be detected by northern blotting in the same tissue [19]. On the other hand, Gordon et al. [20] found, by dot blotting of RNA of total rat lung, mRNA encoding I-FABP and L-FABP at 3.1% and 3.6% of the concentrations observed in intestine and liver, respectively. That lung consists of many cell types may be one reason for the contradictory results regarding the FABP types expressed in lung. The expression of FABP by single cell types of lung has not been investigated.

We employed the sensitive reverse transcriptase PCR to screen for expression of the three FABP types reportedly expressed in lung and of E-FABP. A similar approach was undertaken by van Nieuwenhoven et al. [46] to identify FABP expressed in endothelial cells. Primers for H-FABP, E-FABP and L-FABP, but not for I-FABP, yielded amplification products of the correct size. However, L-FABP was absent from the three cell types studied here. By northern blot analysis we found that H-FABP was restricted to lung fibroblasts, whereas E-FABP was present in alveolar type-II cells, lung fibroblasts and, to a minor extent, in macrophages.

As a prerequisite for generation of monospecific affinity-purified antibodies rat E-FABP was heterologously expressed in *E. coli*. Functional and structural studies have been carried out (Hohoff, C., van Tilbeurgh, H., Börchers, T. & Spener, F., unpublished results). Immunological analysis showed that all cell types examined express considerable amounts of E-FABP, whereas H-FABP was enriched in lung fibroblasts, corroborating the results of northern blot analysis. Expression of E-FABP in human lung endothelium, Clara and goblet cells [47] would contribute to the E-FABP level measured in total lung, which is in the same order of magnitude as that found in other tissues known to contain E-FABP, such as skin, heart and adipose tissue (data not shown). The presence of H-FABP in fibroblasts was considerably lower (≈100-fold) than that reported for heart, skeletal muscle [24] and mammary gland [48]. On the other hand it is in the same order of magnitude as that found in endothelial cells [49] and in a skeletal muscle cell line [37]. E-FABP was originally cloned and purified from skin [26, 28, 35, 50]. Siegenthaler et al. [27] localised E-FABP in cells other than of epithelial origin. Immunohistochemically, they detected this protein in heart, intestine and adipocytes.

The expression of E-FABP with other FABP types in one organ, e.g. in brain, [51] which contains E-FABP in addition to H-FABP [17] and the brain-type FABP [18], suggests the possibility of specialised functions of the various FABP types. Lung fibroblasts expressing H-FABP and E-FABP are one of the few cell types, in addition to enterocytes [20], adipocytes [27], glial and neuronal cells [51], that express more than one FABP type. The widespread expression of E-FABP suggests a basic function of this protein in cellular lipid metabolism. This finding corroborates the conclusion drawn by Siegenthaler et al. [27]. Lung fibroblasts increase their lipid content drastically prior to birth, becoming lipofibroblasts, which are capable of providing lipid substrate to alveolar type-II cells for surfactant synthesis [9]. In contrast to surfactant, lipid stores of lipofibroblasts consist mainly of neutral lipids, suggesting a function of H-FABP to direct fatty acids to this lipid class. Although we cannot exclude that minimal amounts of the H-FABP protein found in type-II cells and alveolar macrophages is due to contamination by fibroblasts, the immunological detection of H-FABP in alveolar type-II cells and alveolar macrophages, despite the absence of the corresponding mRNA in northern blot analysis, led us to speculate that H-FABP is transferred (with lipids) from lung fibroblasts to these cells. Such an extracellular role would be consistent with reports that H-FABP is involved in growth inhibition and cell differentiation [52] and that exogenously added H-FABP causes hypertrophy of cardiac muscle cells [53], presumably via a receptor for H-FABP on the surface. In this respect the role of fibroblasts in lung development is interesting.

The temporal expression patterns of H-FABP and E-FABP in the developing rat lung and the correlation to lung maturation and lipid metabolism is being studied in detail.

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