

Immunological discrimination between the human apolipoprotein E2(Arg158→Cys) and E3 isoforms

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Abstract A specific anti-apoE2(Arg158→Cys) monoclonal antibody was raised by means of immunization of mice with a variant specific synthetic peptide. The peptide sequences used were homologous to apolipoprotein E of human and mouse. Consequently, the mouse immune system was tolerant to most of the selected sequences. Immunization with only one of selected peptides (amino acids 154–172) evoked an anti-peptide and anti-native protein response. Surprisingly, this peptide was predicted to have a low antigenicity index, in contrast to the other used peptides. The variant specific anti-peptide MAb that was generated with this sequence, recognizes apoE2(Arg158→Cys) and not apoE3. ■ We here describe a sensitive, time saving, and simple immunoblot assay to detect apoE2(Arg158→Cys) in human sera without prior isoelectric focusing of serum proteins.—Gerritse, K., P. de Knijff, G. v. Ierssel, L. M. Havekes, R. R. Frants, M. M. Schellekens, N. D. Zegers, E. Claassen, and W. J. A. Boersma. Immunological discrimination between the human apolipoprotein E2(Arg158→Cys) and E3 isoforms. *J. Lipid Res.* 1992. 33: 273–280.

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Human apolipoprotein E (apoE) is a protein constituent of several classes of plasma lipoproteins (1). ApoE is a specific ligand for the apoE and apoB,E receptors (2–4) which play an important role in lipid metabolism. Human apoE is a polymorphic protein with three major isoforms. This polymorphism is due to three common alleles, E*2, E*3, E*4 (5, 6). These alleles determine six phenotypes E2/E2, E3/E3, E4/E4/, E4/E2, E4/E3, and E3/E2 that occur in the general population with a frequency of 0.9%, 62.2%, 2.2%, 2.9%, 19.9%, and 11.7%, respectively (7). Since apoE3 is the most frequently occurring isoform of the protein, apoE3 is considered to be the parent form. Amino acid sequence analysis has revealed that the

majority of the other variants differ in only one or two amino acids from apoE3. ApoE4 differs from apoE3 at residue position 112, where the cysteine is apoE3 is substituted by an arginine. By far the most frequently occurring isoform of apoE2 is apoE2(Arg158→Cys) (8) which differs from the parent apoE3 by the replacement of arginine by cysteine at position 158 (9). In addition, several other less frequently occurring isoforms of apoE have been described (10–14). The amino acid substitution in apoE4 does not affect the affinity to the cellular lipoprotein receptors of this protein, whereas apoE2(Arg158→Cys) displays only 1% of normal apoE3 binding activity (15).

The defective binding to the lipoprotein receptors results in accumulation of cholesterol-rich chylomicron remnants and very low density lipoprotein (VLDL) remnants in plasma, which is characteristic for type III hyperlipoproteinemia (16). More than 95% of the patients with this disorder exhibit homozygosity for apoE2(Arg158→Cys) and often develop premature peripheral vascular and/or coronary artery diseases and have palmar and tuberous xanthomas (17). Early detection of such carriers allows preventive measures to be taken.

Abbreviations: EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; t-boc, tertiary-butyl-oxycarbonyl; BSA, bovine sera albumin; KLH, keyhole limpet hemocyanin; GA, glutaraldehyde; apoE, apolipoprotein E; MAb, monoclonal antibody; PAb, polyclonal antibody; ELISA, enzyme-linked immunosorbent assay; VLDL, very low density lipoproteins; SP, synthetic peptide; PCR, polymerase chain reaction; i.p., intraperitoneal.

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The three major isoforms of apoE are separable by isoelectric focusing (17, 18). The common methods of apoE phenotyping are based on isoelectric focusing of VLDL or serum followed by Western blotting and protein staining, respectively (19, 20). Monoclonal antibodies (MAbs) that can discriminate between the several genetic variants are useful tools to make phenotyping methods less laborious.

Considering the fact that apoE3 and apoE2 (Arg₁₅₈→Cys) differ in only one amino acid, we decided to use the synthetic peptide strategy for raising variant specific MAbs. We synthesized a number of amino acid sequences, analogous to a part of apoE2(Arg₁₅₈→Cys), including the variant-specific sequence. In contrast to earlier findings of Briand, Muller, and Van Regenmortel (21), not all peptide immunizations evoked an anti-peptide response. Immunization with only two out of six peptides led to an anti-peptide response and of these two peptides only one led to an anti-native protein response.

In this paper we present the preparation of an apoE2(Arg₁₅₈→Cys)-specific MAb (F48.1). The specificity of MAb F48.1 was validated with 32 human sera with different apoE phenotypes in a sensitive direct immunoblot assay not requiring prior isoelectric focusing.

MATERIALS AND METHODS

Peptide synthesis

Peptide synthesis was performed according to the Biosearch solid phase synthesis protocol (Biosearch; San Rafael, CA) on polystyrene resin (1% crosslinking) to which the first amino acid already was attached. The synthesis was carried out with tertiary-butyl-oxycarbonyl (t-boc) amino acids as described by Merrifield (22). Some t-boc amino acids were protected: arginine: *p*-toluenesulfonyl; aspartic acid: cyclohexyl ester; cysteine: *p*-methylbenzyl; glutamic acid: benzyl ester; histidine: dinitrophenyl; lysine: 2-chlorobenzoyloxycarbonyl; serine and threonine: benzyl; tyrosine: 2-bromobenzoyloxycarbonyl.

Peptide cleavage and side chain deprotection of the completed peptide was performed by HF treatment as described by Bhatnager et al. (23). After HF cleavage and purification, as was described previously (24), the amino acid composition of the synthetic peptide (SP) was confirmed by HPLC analysis according to Janssen et al. (25)

Conjugation

The coupling of the peptides to bovine sera albumin (BSA) with glutaraldehyde (GA) was carried out according to the improved conjugation method de-

scribed before (26). Coupling of the peptides to keyhole limpet hemocyanin (KLH) with the use of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) was performed following the method described by Boersma et al. (27) with some modifications as reported previously (28).

Immunization

Groups of four female BALB/c mice were immunized by intraperitoneal (i.p.) or subcutaneous injection in the foot pad with 50 µg peptide or peptide-conjugate emulsified in specol-PBS 9:11 (v/v) (29) or in Freund's complete adjuvant-PBS 1:1 (v/v) or precipitated with alum (30) and boosted with the same peptide or peptide-conjugate emulsion 4 weeks after the initial injection. Seven and 21 days after each immunization blood samples were collected by bleeding from the tail vein. Blood samples were incubated for 30 min at 37°C and sera were obtained after centrifugation at 300 *g* for 10 min at 4°C.

Fusion and ascites production

Three days after the last immunization, spleen cells were fused with SP2/0 cells at a ratio of 5:1 essentially as described in detail by Haaijman et al. (30). Briefly, SP2/0 cells were washed and co-centrifuged with spleen cells. Polyethyleneglycol (PEG 40%, Merck, Darmstadt, Germany) and 5% dimethylsulfoxide in PBS were added slowly and incubated with the cells for 1 min. PEG was subsequently diluted with serum-free medium. After centrifugation, cells were taken up in selection medium (1 µg·ml⁻¹ azaserine and 0.1 mM hypoxanthine, 10% v/v fetal calf serum, 2 mM glutamine, 0.1 mg·ml⁻¹ penicillin, 1 mM Na-pyruvate, and 5·10⁻⁵ M β-mercaptoethanol in RPMI 1640 (Boehringer, Mannheim, Germany)). The cell mixture was plated out in 96-well plates (10⁵ cells/well). After 2 weeks of culture the azaserine was omitted. Cell culture supernatant was analyzed by an enzyme-linked immunosorbent assay (ELISA) on peptides either directly coated or on peptide-protein conjugates. Cells that produced anti-peptide antibodies were subcloned by limiting dilution. Supernatants of the selected clones were tested for reactivity on purified apolipoprotein E3 and apoE2(Arg₁₅₈→Cys) by immunoblot.

Cells of clone F48.1 (2.10⁶) were injected into the peritoneal cavity of 25-week-old female BALB/c mice. The ascites fluid was harvested under anesthesia on days 10 and 12 after injection. (Ascites fluid will be made available for research purposes.)

Direct ELISA

PVC microtiter plates (Flow Laboratories, Irvine, Scotland) were coated with peptide or peptide-carrier conjugates (5 µg·ml⁻¹ PBS, 50 µl/well) or with human

sera diluted (1–7% v/v) in PBS and incubated overnight at 4°C. The supernatant was removed and the wells were incubated 30 min at 25°C with gelatine (Merck, no:4070, microbiology grade, 5 mg·ml⁻¹, 50 µl/well) in PBS (PBSG) to block nonspecific binding. The plates were washed (5x) with a solution of gelatine (0.1 mg·ml⁻¹) and 0.01% Tween-20 (v/v) in PB (PBSTG). Samples of mouse immune sera or ascites fluids diluted in PBSTG were incubated for 1 h at 25°C. Plates were washed (5x) with PBSTG and subsequently incubated for 1 h at 25°C with either alkaline phosphatase-labeled goat anti-mouse IgM (H + L) or alkaline phosphatase-labeled goat anti-mouse IgG (H + L) (KPL, Inc., Gaithersburg, MD) diluted 1:2000 in PBSTG. After washing the plates (5x) with PBSTG a solution of *p*-nitrophenylphosphate (Boehringer) (1 mg·ml⁻¹) in 10 mM diethanolamine, 1 mM MgCl₂ (pH 9.8) was added to each well (50 µl/well). After 30 min the absorbance was determined at 405 nm in a Titertek Multiskan apparatus (Flow Laboratories).

Direct immunoblot

The immunoblot was performed on a Bio-Dot microfiltration apparatus (Bio-Rad, Cat.no. 170-6545). Selected nitrocellulose membranes (Schleicher & Schuell, 0.45 µm pore size, code BA85) were cut in strips and soaked in Tris-buffered saline (TBS, 20 mM Tris, 500 mM NaCl, pH 7.5) for 10 min. The membrane strips were coated overnight (in a 50-ml Falcon tube, no:2070 Becton & Dickinson) at 4°C with 5-ml dilutions 10% (v/v) of human sera in TBS, or coated (in the device) with solutions of purified apoE3, apoE2(Arg₁₅₈→Cys), and dilutions of free synthetic peptides in TBS (5 µg·ml⁻¹, 100 µl/well). The concentrations of apoE proteins in the human sera were determined by ELISA as was described in detail by Bury et al. (31). To block nonspecific binding, membrane strips were subsequently incubated with BSA (10 mg·ml⁻¹ in TBS) for 1 h at room temperature. After washing (2x) with 0.05% v/v, Tween-20 in TBS (TBST), the strips were mounted in the filtration device. The wells were filled with serial dilutions (1/100, 1/200, 1/400, etc.) of mouse immune sera or ascites fluids in BSA (10 mg·ml⁻¹) and Tween-20 (0.05%, v/v) in TBS (TBSTB). Nonspecific ascites and polyclonal goat anti-human apoE sera (24), both diluted in TBSTB, were used as negative and positive control solutions, respectively. The samples were allowed to filter through the nitrocellulose membrane by unit gravity. The wells were washed (3x) with TBST by suction. The membrane strips were removed from the filtration device and incubated for 1 h at room temperature in horseradish peroxidase-labeled rabbit anti-mouse Ig (Dakopatts) appropriately diluted

(1/1000) in TBSTB. The positive control was incubated with horseradish peroxidase-labeled rabbit anti-goat Ig (Dakopatts) 1/1000 in TBSTB. The membrane strips were developed using a solution of 3,3'-diamino benzidine (Sigma no. D-5637) (0.2 mg·ml⁻¹) and hydrogen peroxide (1 µl·ml⁻¹) in PBST. After incubation in the dark for 10 min at room temperature, the reaction was stopped by rinsing the membrane strips with distilled water.

Polyacrylamide gel isoelectric focusing

Blood samples were obtained by venipuncture from patients with type III hyperlipoproteinemia and from healthy controls. The apoE proteins in sera were phenotyped for apoE by isoelectric focusing as described in detail by Havekes et al. (20). Subsequently duplicate gels were blotted and stained with either anti-apoE2(Arg₁₅₈→Cys) MAbs or with goat anti-human apoE polyclonal antibodies (PABs) as first antibody. Staining was performed as was described for the Dot-Blot immunoassay.

RESULTS

Antigenic index and peptide sequence selection

The region of the sequences from which the peptides were selected was determined mainly by the position of the amino acid substitution in the sequence. In addition the antigenicity index (32, 33) and the amino acid differences between human apoE and murine apoE (34) were taken into consideration. Examination of those data (not shown) revealed a consecutive calculated positive antigenic index at the amino-terminal side of the amino acid substitution. Nevertheless, peptides analogous to the sequence of this region, e.g., SP041, SP028, SP035, and SP035X (Table 1), appeared to be nonimmunogenic. Table 1 shows the antibody responses after immunization with peptides coupled to KLH with EDC as coupling agent. The nonimmunogenicity of most of these peptides was confirmed tested in a number of immunization protocols (not shown). Therefore we felt obliged to synthesize sequences situated to the carboxyl-terminal side of the substitution, with a relatively low antigenic index, SP040 and subsequently SP096. The selected immunogenic peptide SP096 differs in one and two amino acids with human apoE3 and murine apoE, respectively (Table 1).

Direct ELISA

In a direct ELISA the antibodies in the primary response sera (day 7), elicited with SP096, showed a greater reactivity to conjugated peptide than to the

TABLE 1. Antibody responses of mice immunized with various peptides, analogous to apoE2(Arg₁₅₈→Cys), conjugated using EDC to KLH carrier protein.

code:	sequences:	residue no:	reactivity:	
			α-SP	α-native
apoE3 (human)	STEELRVLASHLRKLRKLLRDADDLQKRLAVYQAGAREGAER	129-172		
SP041	<u>RDADDLQKCLA</u>	150-160	-	-
SP028	<u>RKRLLRDADDLQKCLAVY</u>	145-162	-	-
SP035	<u>RKLRKLLRDADDLQKCLA</u>	142-160	-	-
SP035X	STEELRVLASHLR <u>KLRKLLRDADDLQKCLA</u>	129-160	-	-
SP040	<u>DLQKCLAVYQA</u>	154-164	+	-
SP096	<u>DLQKCLAVYQAGAREGAER</u>	154-172	+	+
apoE2(Arg ₁₅₈ →Cys)	STEELRVLASHLRKLRKLLRDADDLQKCLAVYQAGAREGAER	129-172		
apoE (murine)	STEEIRARLSTHLRKMRLMRDADDLQKRLAVYKAGAREGAER	121-164		

Residue numbers 150 and 158 in the sequences of murine apoE and of human apoE3, apoE2(Arg₁₅₈→Cys), and apoE2(Arg₁₅₈→Cys) peptides, respectively, are depicted in the shaded area. The amino acids of the synthetic peptides with functional amino or carboxyl groups that were used for coupling to the carrier protein are underlined.

free peptide (not shown). Low levels of anti-peptide antibodies were observed in the sera obtained on day 21 after priming. In contrast, the antibodies in the post-boost sera showed no difference in reactivity against conjugated or nonconjugated peptide. Most anti-peptide antibodies in sera obtained after priming were of the IgM isotype. The antibodies in the sera sampled after the booster immunization were both of IgM and of IgG isotypes.

MAb specificity determined by isoelectric focusing

Isoelectric focusing was performed to separate the apoE isotypes of four sera of patients with different known phenotypes, to provide samples of apoE2, E3, and E4 proteins to which MABs could be tested (Fig. 1). The samples were blotted in duplo to nitrocellulose. Subsequently, the nitrocellulose filters were developed either by MABs (lanes 1, 3, 5, 7) or a polyclonal goat anti-human apoE antisera (lanes 2, 4, 6, 8). The apoE2(Arg₁₅₈→Cys) protein bands in the sera of the patients with phenotypes E2/E2 (lane 1) and E2/E3 (lane 3) specifically recognized by MABs F48.1. In lane 3 there is some additional staining shown on the apoE3 location. This staining on the apoE3 location in lane 3 is typical for the presence of apoE2 protein that focuses on the apoE3 location due to partial sialation and/or deamination (7, 20). This possible confounding detection of partial sialated and/or deaminated proteins was confirmed by the split-sample cysteamine treatment assay (7, 20). In lane 4, the polyclonal antibody (PAB) recognized not only the focused apoE3 proteins but the partial sialated and/or deaminated apoE2 proteins as well. As a consequence, the staining of the proteins on the apoE3 location in lane 4 by the PAB was more intense compared to the staining of the protein band on the apoE3 location by the specific MAB in lane 3. The

specificity of the MABs was emphasized by staining of the focused proteins of the E3/E3 and E3/E4 sera (lanes 5–8). The apoE2(Arg₁₅₈→Cys)-specific MABs did not react with the apoE3 or the apoE4 focused proteins (lanes 5 and 7). The presence of apoE3 and apoE4 in the sera was detected by developing duplots with PABs (lanes 6 and 8). The staining of the apoE4 protein in lane 8 is less intense compared to the intensity of the stained apoE3 protein band of the same sample, indicating a concentration difference of both proteins in this sample.

Determination of the anti-apoE2(Arg₁₅₈→Cys) MAB detection range

An immunoblot with a titration of two homozygous human sera (E2/E2 and E3/E3) with known apoE content was performed to reveal the detection range

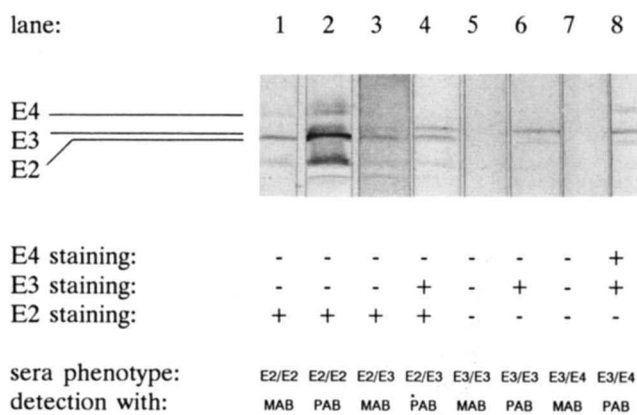


Fig. 1. Isoelectric focusing on polyacrylamide gels with four human sera with different phenotypes. The proteins were blotted to nitrocellulose filters and subsequently stained with anti-apoE2(Arg₁₅₈→Cys) MABs (lanes 1, 3, 5, 7) or with polyclonal goat anti-human apoE antibodies (PABs) (lanes 2, 4, 6, 8). The phenotypes and the detecting antibodies (MAB or PAB) are indicated.

of the specific apoE2(Arg₁₅₈→Cys) MAb. The apoE3, coated with concentrations from 20 to 0.625 μg·ml⁻¹, was not recognized by MAb F48.1 or nonspecific ascites, regardless of the ascites dilution used (1/200 to 1/25600, **Fig. 2b and 2d**). ApoE2 was detected with diluted ascites (MAb F48.1) up to 1/125600, 1/6400, 1/1600, and 1/400 when coated in concentrations of 20, 10, 5, and 2.5 μg·ml⁻¹, respectively (**Fig. 2a**). No staining was observed when nonspecific ascites was used (**Fig. 2c**). Because the average apoE concentrations of 10% diluted sera are 13.8, 7.3, and 6.7 μg·ml⁻¹ in subjects with the E2/E2, E3/E2, and E4/E2 phenotype, respectively (7), and these concentrations are within the detection limit range, since 20 μg·ml⁻¹ and 2.5 μg·ml⁻¹ could be detected with 1/125600 and 1/400 or less diluted ascites fluid, respectively, we decided to use 10% diluted human sera for the detection of apoE2(Arg₁₅₈→Cys) in sera with unknown phenotype. In addition, the ascites fluid was tested on immunoblots coated with sera dilutions with the phenotypes E3/E2(Arg₁₄₅→Cys), E3/E2(Lys₁₄₆→Cys), and E3/E3Leiden. None of the isotypes used was recognized by the MAb (not shown).

ApoE2(Arg₁₅₈→Cys) detection in serum with a direct immunoblot: conformation with isoelectric focusing

Thirty two different serum samples were analyzed for the presence of apoE2(Arg₁₅₈→Cys) in a direct immunoblot analysis with specific MAbs as first antibody. The screening for the presence of the apoE2(Arg₁₅₈→Cys) protein in 32 sera samples, by means of the MAbs, was in complete agreement with the phenotypes of the patients revealed by isoelectric

focusing (**Fig. 3**). The apoE2(Arg₁₅₈→Cys) proteins of homozygous subjects (E2/E2) could be detected with diluted monoclonal hybridoma ascites fluid (clone no. F48.1) up to 1/1600.

DISCUSSION

The data presented here demonstrate that by means of immunization with synthetic peptides we have raised a unique monoclonal antibody (F48.1) whose specificity is determined by the 158 cysteine position of apoE2(Arg₁₅₈→Cys). This makes it possible to detect apoE2(Arg₁₅₈→Cys) directly in sera from patients. No reactivity was observed when sera from patients with other phenotypes, apoE3, apoE4, apoE2(Arg₁₄₅→Cys), apoE2(Lys₁₄₆→Gln), and apoE3Leiden, were used.

MAb F48.1 allowed the introduction of a relatively sensitive, less laborious, time saving, and simple immunoblot assay that does not require prior isoelectric focusing of the serum proteins as is necessary in conventional detection assays (19, 20). MAb F48.1 recognizes apoE2(Arg₁₅₈→Cys) in both homozygote and heterozygote patient sera. However, it is not possible to discriminate between two phenotypes with MAb F48.1 only. For the discrimination between hetero- and homozygous apoE2 sera, another monoclonal antibody is needed that is specific for the apoE3 epitope around the 158 residue. In other words, a monoclonal antibody that should recognize the "158" epitope with arginine in its sequence and does not recognize the "158" epitope when the arginine (158) is substituted by a cysteine. In a hypothetical situation such a MAb will

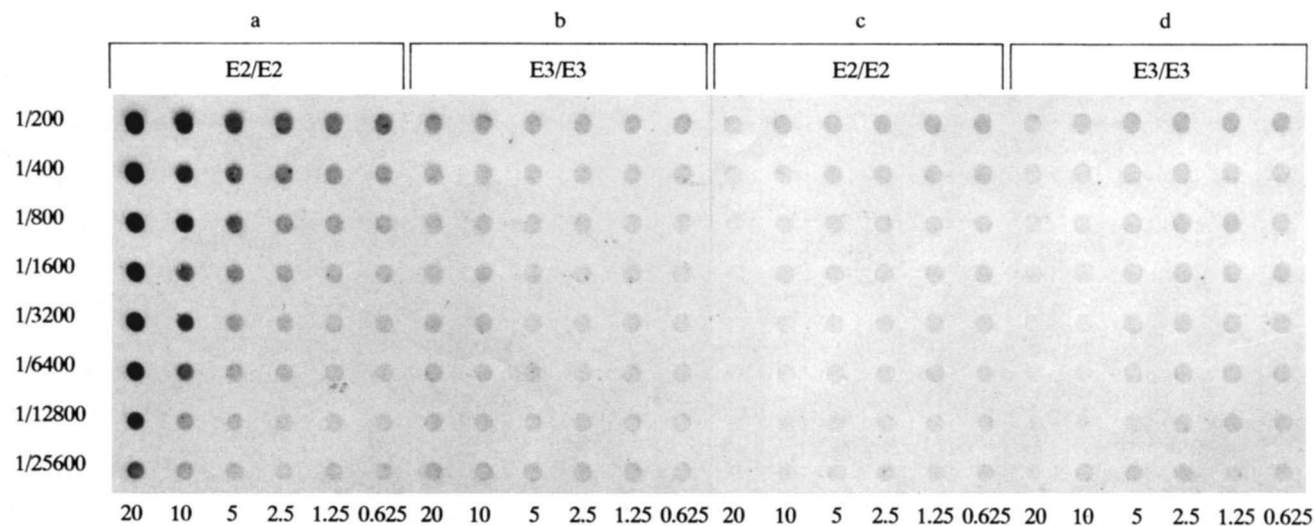


Fig. 2. Determination of the detection limit of apoE2(Arg₁₅₈→Cys)-specific MAb F48.1. A direct immunoblot was performed with two homozygous human sera (a and c E2/E2, and b and d E3/E3) with known apoE content. The apoE2(Arg₁₅₈→Cys) and apoE3 were coated in concentrations from 20 to 0.625 μg·ml⁻¹. The immunoblot assay was carried out with several dilutions of anti-apoE2(Arg₁₅₈→Cys) MAb F48.1 ascites fluid (a and b) and a nonspecific ascites fluid (c and d) as indicated on the vertical axis.

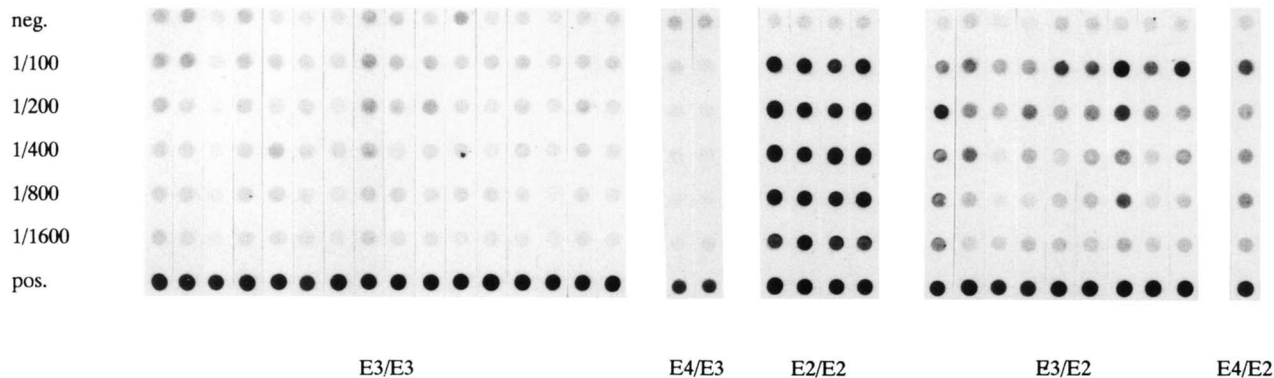


Fig. 3. Selective detection of apoE2(Arg₁₅₈→Cys) in 32 human sera in a direct immunoblot, by staining with anti-apoE2(Arg₁₅₈→Cys) MAb ascites fluid. All stainings with MAbs were in accordance with the independently determined phenotypes, as revealed by isoelectric focusing.

react with an E2/E3 sera but not with an E2/E2 sera, whereas the MAb F48.1 reacts with both sera.

Alternatively, the Hha/PCR phenotyping method as described by Hixson and Vernier (35) could be used, but this method is only suitable to detect the apoE2(Arg₁₅₈→Cys), apoE3, and apoE4 isoforms, and cannot be used to detect the other variants. Furthermore, PCR will not allow detection of actual apoE protein isotype concentrations in serum. Another apoE phenotyping technique that has recently been described is the hybridization with oligonucleotides (36). Hybridization is extremely time-consuming due to the use of multiple techniques such as DNA isolation and restriction enzyme digestion, electrophoresis, Southern blotting, and oligonucleotide synthesis and labeling. The use of the polymerase chain reaction to amplify apoE-specific DNA sequences for oligonucleotide hybridization (37) is even more time-consuming. In addition, application of radiolabeled oligonucleotides requires a well-equipped laboratory. With one of the oligonucleotide phenotyping methods it is not possible to detect apoE3 in one assay, due to the fact that on the DNA level there is not a typical apoE3 nucleotide sequence that does not occur in all the other isotypes. The only possible way to discriminate between apoE3 and the other isoforms in one single assay is by sequencing apoE on the DNA or protein level. The major disadvantage of hybridization methods (with and without PCR) is the variability of stringency with a number of parameters such as Mg²⁺, temperature, etc. which may lead to false positive results. Compared to these techniques the immunoblot is sensitive, less laborious, fast, inexpensive, and the screening can be performed in less well-equipped laboratories and can be adapted to quantification. Furthermore, due to the specificity of MAb F48.1 screening, false positive samples are not strongly dependent on assay parameters.

A few considerations led us to use SP as immunogens. *a)* Theoretically, immunization with native

apoE2(Arg₁₅₈→Cys) would probably evoke an immune response that is mainly directed against epitopes that do not necessarily include the Arg₁₅₈→Cys epitope. As a result, only a very small proportion of the antibodies raised will be directed against the target epitope, i.e., the sequence including Arg₁₅₈→Cys. *b)* Epitopes of E2 that also appear in E3 may be immunodominant as compared to the target epitope and, as a consequence, could prevent an immune response against the Arg₁₅₈→Cys containing epitope. *c)* Finally, a complication in conventional attempts to raise MAbs against human apoE2(Arg₁₅₈→Cys) is the interspecies homology of the apoE proteins. The sequence homology of human apoE and guinea pig, rat, and mice apoE exceeds the 85%, 80%, and 70%, respectively (34, 38, 39). Nevertheless, the substitution of arginine by cysteine might have introduced a "foreignness" in the peptides that were used for immunization. However, the substitution of one amino acid by itself is not enough to introduce foreignness: four out of six peptide immunizations were not successful in raising anti-peptide antibodies. It seems that the amino acids added to the carboxyl terminal site of the substitution together with the amino acid substitution itself provide the peptide with the level of "foreignness" that is required for immunogenicity. Two observations are in accordance with this hypothesis. First, the substitution of arginine by cysteine evokes a significant conformational change in the apoE receptor binding site, which results in a decreased affinity of the apoE receptor for this binding site (40). Second, residues towards the carboxyl terminal site of the apoE binding region play an important role in maintaining an appropriate binding conformation (41).

A common feature of the peptides homologous to apoE2(Arg₁₅₈→Cys) was that they were all situated to the amino terminal side of the amino acid 158 substitution, a region with a calculated positive antigenic index. Although SP040 and SP096 have a calculated negative antigenic index, they proved to be im-

munogenic. These results confirm that the calculated predictions concerning the antigenicity must be interpreted with great care (42).

In conclusion, we have shown that it is possible to raise an anti-apoE2(Arg₁₅₈→Cys)-specific antibody that discriminates between two proteins that differ in only one amino acid. MAb F48.1 can be used to detect apoE2(Arg₁₅₈→Cys) in human sera in a sufficiently sensitive, simple, and rapid immunoblot assay without prior delipidation and isoelectric focusing. The method is not only convenient for clinical diagnosis but is also very suitable for large scale screenings (7, 43). A complete panel of MAbs raised against other isoforms of apoE would be necessary for the above-mentioned application. For this reason we recently have started immunization experiments with peptides analogous to other genetic variants of apoE. ■

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