Robin Wait, Giulia Chiesa, Cinzia Parolini, Ingrid Miller, Shajna Begum, Daniela Brambilla, Lara Galluccio, Rossana Ballerio, Ivano Eberini, and Elisabetta Gianazza

Reference maps of mouse serum acute-phase proteins. Changes with LPS-induced inflammation and apolipoprotein A-I and A-II transgenes
Reference maps of mouse serum acute-phase proteins. Changes with LPS-induced inflammation and apolipoprotein A-I and A-II transgenes

Robin Wait1, Giulia Chiesa2, Cinzia Parolini2, Ingrid Miller5, Shajna Begum1, Daniela Brambilla2, Lara Galluccio2, Rossana Ballerio6, Ivano Eberini2,3,4, and Elisabetta Gianazza2,3,4

1Kennedy Institute of Rheumatology Division, Faculty of Medicine, Imperial College London, UK
2Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, Milan, Italy
3Gruppo di Studio per la Proteomica e la Struttura delle Proteine, Università degli Studi di Milano, Milan, Italy
4Centro di Eccellenza sulle Malattie del Sistema Nervoso Centrale e Periferico, Università degli Studi di Milano, Milan, Italy
5Institut für Medizinische Chemie, Department für Naturwissenschaften, Veterinärmedizinische Universität Wien, Vienna, Austria
6Centro Cardiologico Monzino, IRCCS, Milan, Italy

Correspondence: Dr. Elisabetta Gianazza, Dipartimento di Scienze Farmacologiche, via G. Balzaretti 9, 20133 Milan, Italy
E-mail: elisabetta.gianazza@unimi.it
Fax: +39-02-503-18284

Abbreviations: α1G, α1-acid glycoprotein; α1M, α1-macroglobulin; α1T3, α1-antitrypsin; α2HS, α2-HS-glycoprotein; ApoA1-2,4, apolipoprotein A-I, A-II, A-IV; ApoC1-3, apolipoprotein C-I, C-II, C-III; ApoE, apolipoprotein E; CLUS, clusterin; COTR, contrapsin; ESTN, carboxylesterase; FETB, fetuin B; fSA, serum albumin fragment; Hbβ1, Hb chain β; HEMO, hemopexin; HPT, haptoglobin; KNG, kininogen; MUP, major urinary protein; SAMP, serum amyloid P; SPI, serine protease inhibitor; TRFE, transferrin; TTHY, transthyretin; VTDB, vitamin D-binding protein

Keywords:
2-DE ; Mus musculus ; Protease inhibitors ; Proteome ; Serpins
We present reference maps of the mouse serum proteome (run under reducing and non-reducing conditions), from control animals, from mice injected with lipopolysaccharide (LPS) to induce systemic inflammation, and from mice transgenic for human apolipoproteins A-I and A-II. Seventy-seven spots/spot chains from the reducing gels were identified by HPLC MS/MS, representing 28 distinct proteins, including a species-specific protease inhibitor, contrapsin, and high levels of carboxylesterase.

The concentrations of acute-phase reactants were monitored for 96 h after LPS challenge. The greatest changes (four-fold 48 h after LPS administration) were observed for haptoglobin and hemopexin. Orosomucoid/α₁-acid glycoprotein and apolipoprotein A-I increased steadily, to 50-60% above baseline at 96 h from stimulation.

In mice transgenic for human apolipoprotein A-I the levels of expression of orosomucoid/α₁-acid glycoprotein, α₁-macroglobulin, esterase, kininogen and contrapsin were altered compared to knockout mice lacking apolipoprotein A-I. In contrast, except for the presence of apolipoprotein A-II, no statistically significant difference was observed in mice transgenic for human apolipoprotein A-II.
1 Introduction

Plasma, the repository of the soluble components of blood, is not only easily accessible for proteomic analysis but is also a highly sensitive indicator of pathological changes, since infection or injury of cells or tissues almost anywhere in the body will result in some leakage of proteins into the bloodstream. While characterisation of the human plasma and serum proteomes is progressing rapidly, partly because of initiation of the HUPO human plasma proteome project, proteomic analysis of body fluids of other mammals is receiving far less attention.

We have previously published studies of the serum proteomes of *Rattus norvegicus* [1–3] *Bos taurus* [4] and *Equus caballus* [5], while work is in progress on pig and dog serum. (Miller et al. unpublished). Here we present data on the serum proteome of *Mus musculus*.

Proteomic studies of murine biological fluids are scanty. Zhou and Speicher [6] showed that pre-fractionation by microscale solution-phase IEF prior to 2-DE increased the loading capacity and significantly improved the number of low abundance mouse serum protein spots detectable by silver staining after 2-DE. However, none of these proteins was identified by MS.

There have been several studies of murine serum in tumour transplantation models. Juan et al. showed that inoculation of human tumour cell lines into BALB/C-nc strain female mice resulted in up regulation of murine acute phase proteins, including serum amyloid A and haptoglobin [7]. However, relatively few proteins were identified, and there was no systematic attempt to define reference maps for the baseline state.

Mouse serum amyloid A has also been detected and characterised in serum by hydrophobic interaction chromatography and 2-DE, and the effect of cytokines, dexamethasone and LPS on its concentration investigated [8, 9].

In another xenotransplantation study, the glycosylation pattern of mouse serum β-haptoglobin was probed by affinoblotting using lectins from *Datura stramonium*, *Maackia amurensis* and *Sambucus nigra*, which indicated that the terminal sugars differed between haptoglobin spots, some having Galβ(1–4)GlcNAc, whereas others had α(2-3)- or α(2-6)-linked sialic acid [10]. We
have previously published 2-DE maps of sera from mice transgenic (through targeted replacement) for wild-type human apolipoprotein A-I or for the variant A-I Milano (in which there is a $^{173}\text{R} \rightarrow \text{C}$ substitution); however, the objective was quantitation of the transgene products [11] and no other proteins were identified. Skehel et al. characterised a different transgenic mouse strain and determined expression levels of all detectable spots [12], but only proteins that displayed significant differences in relative expression were identified.

In the present report, we present 2-DE reference maps of *Mus musculus* serum run both under non-reducing and reducing conditions. We evaluate the differences in serum proteome between baseline and LPS-induced inflammation. The effects introducing human apolipoprotein transgenes are also investigated.

### 2 Materials and methods

#### 2.1 Animals and treatments

Animal studies were conducted at the Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, in compliance with local rules, which conform to national and international guidelines.

Blood was collected from the retro-orbital vein plexus of 5-month-old male CD1 mice (five per test group) after overnight fasting. Serum was obtained by centrifugation of the clot and equal volumes were pooled within each test group.

Systemic inflammation was induced by single i.p. injections of varying amounts of *E. coli*, serotype 0111:B4 lipopolysaccharide (LPS; from Sigma), and blood was sampled every 24 h. With a dose of 10 mg/kg, all animals died within 48 h; at 5 mg/kg, the mortality rate at 48 h was 70%, whereas with 2.5 mg/kg, all animals survived 96 h after treatment.

Four strains of transgenic mice were analyzed: a) mice in which murine apolipoprotein A-I (apoA1) had been knocked out (A1KO); b) A1KO transgenic for human apoA1; c) A1KO transgenic for human apoA2; d) A1KO transgenic for human apoA1 and apoA2. The C57BL/6
mice lacking murine apoA1(A1KO) were provided by Dr. Nobuyo Maeda [13]. Mice transgenic for human apolipoproteins and not expressing murine apoA1 (apoA1/A1KO (hA1) and human apoA2/A1KO (hA2)) were obtained by multiple crosses between A1KO mice and transgenic mice expressing human apoA1 [14] or human apoA2 [15] (provided by Dr. Edward M. Rubin). hA1 and hA2 transgenics were crossed to obtain hA1/hA2/A1KO (hA1/hA2) mice.

2.2 Electrophoresis

2-DE was performed as described [3], with modifications to optimise resolution in different pH and Mr regions. IEF employed pH 4–10 and 4–6 non-linear, and 2.5–5 linear IPG. The second dimension was run on polyacrylamide gradient gels (5–10%T, 5–12%T, 7.5–17.5%T and 10–15%T; C% = 4), using the discontinuous buffer system of Laemmli [16]. Serum (7.5–17.5 µL) was diluted with an equal volume of water and loaded at the cathodic end of the strips, with or without reduction by 1.4% 2-mercaptoethanol. Proteins were visualised with 0.3% Coomassie Blue in 30% ethanol: 10% acetic acid v/v.

Orosomucoid/α1-acid glycoprotein was quantified by running 7.5 µL serum from LPS-treated mice on a pH 2.5–5 IPG [17], with Coomassie blue staining as described [18]. Mapping of albumin fragments and transferrin was obtained by immunoblotting.

2.3 MS

In-gel digestion with trypsin was performed according to published methods [19–21] modified for use with a robotic digestion system (Genomic Solutions) [22]. Cysteine residues were reduced with DTT and derivatised by treatment with iodoacetamide. The gel pieces were dehydrated with ACN and dried at 50°C, prior to addition of modified trypsin (Promega; 10 µL at 6.5 ng/µL in 25 mM ammonium hydrogen carbonate). After incubation at 37°C for 8 h, peptides were sequentially extracted with 25 mM ammonium hydrogen carbonate, 5% formic acid, and ACN. Lyophilised extracts were redissolved in 0.1% formic acid prior to HPLC MS/MS using a
Micromass Q-Tof Instrument interfaced to a CapLC chromatograph (Waters). Samples were injected onto a 300-µm × 15 mm Pepmap C18 column (LC Packings), and eluted with an ACN / 0.1% formic acid gradient. The capillary voltage was set to 3500 V, and data-dependant MS/MS acquisitions were performed on precursor ions with charge states of 2, 3 or 4 over a survey mass range 400–1300. The collision gas was argon, and the collision voltage was varied between 18 and 45 V, depending on precursor charge and mass. Initial protein identifications were made by correlation of uninterpreted MS/MS data to entries in SwissProt and TREMBL using ProteinLynx Global Server (v. 1.1, Waters). The search tolerance was set to 50 ppm, and one missed cleavage per peptide was allowed. Other than carbamidomethylation of cysteine residues, modifications were not considered in the first pass search. Identifications resting on a single matching spectrum were confirmed by manual interpretation of the sequence.

A total of 160 spots were processed; identifications of most proteins were repeated under all the different separation conditions listed in 2.2 (zooming gels and redox status).

2.4 Protein quantification in LPS-treated and transgenic mice

Image analysis of the scanned gels was performed with PDQUEST v. 6.2 (PDI) (originally described in [23]) for 2-DE maps and with Image J 1.29x (released by W. Rasband, NIH, in the public domain) for the 1-D IEF analysis of orosomucoid/α₁-acid glycoprotein. Statistical procedures were run with SPSS; $p < 0.05$ was taken as statistically significant. Differences among groups were evaluated by two-way ANOVA and Tukey post hoc test. Data are expressed as mean ±SD; for transgenic animals, they are reported as absolute volumes for transgene products, as ratios to the value in KO mice for all other proteins.

3 Results and discussion

3.1 Mouse serum reference map
Figure 1 shows a 2-D electrophoretogram of mouse serum proteins obtained under reducing conditions using a pH 4–10 non-linear IPG [24] and 7.5–17.5%T polyacrylamide gel for the second dimension. Better resolution of acidic serum proteins of high and low Mr was obtained using a pH 4–6 non-linear IPG and a 5–10%T or 10–15%T polyacrylamide gel for the second dimension (Fig. 2, panels A and B). The identities of the 28 major proteins are listed in Table 1. Panel C is a detailed view of the area containing α₁-antitrypsin and serine protease inhibitor which have similar Mr and slightly offset pI, and so migrate together. Use of a pH 2.5–5 IPG strip and 5–12%T polyacrylamide gradient enabled good resolution of even the most acidic isoforms of orosomucoid/α₁-acid glycoprotein and clusterin (panel D).

Panel E is a mouse serum map obtained under non-reducing conditions, with a pH 4–6 non-linear IPG for the first dimension and 5–12%T polyacrylamide gel for the second dimension. Panel F shows an expansion of the region where α₁-antitrypsin and serine protease inhibitor migrate. These two proteins are difficult to separate; they are similar in Mr and are interspersed in the spot chain arrayed along the pI axis. In the quantitation of broad range IPG maps, it was only possible to compute their combined spot volumes. α₂-HS-glycoprotein also migrates close to α₁-antitrypsin and serine protease inhibitor, and is not easily resolved from vitamin D-binding protein. We encountered similar problems in our analysis of Bos taurus serum proteins where overlapping spot chains with varying pI and virtually identical Mr hindered quantitation [4].

Migration may differ between reducing and non-reducing conditions not only for multimeric proteins, such as haptoglobin, but also for some single-chain proteins. In SDS-PAGE, differences in migration behaviour between non-reducing and reducing conditions allow estimation of the number of such bridges or, more precisely, restraints such bridges impose on protein unfolding. Albumin, with 17 cystines, is a well-characterised example [25], but α₁-antitrypsin also migrates differently after 2-mercaptoethanol treatment, probably because of the presence of a free cysteine.
residue, which in serum is often conjugated to acidic metabolites such as glutathione. Reduction would thus shift the pI in the basic direction [26].

Most of the proteins identified in mouse serum have orthologs in humans and other mammals. Contrapsin, however, appears to be restricted to the mouse [27, 28] and its presence in addition to \( \alpha_1 \)-antitrypsin explains why the trypsin inhibiting capacity of male mouse plasma is several-fold greater than that of humans. Both these serpins (serine protease inhibitors) are monomeric glycoproteins of 53–55 kDa, with 10–15% carbohydrate content. \( \alpha_1 \)-Antitrypsin inhibits elastase, trypsin and chymotrypsin, whereas contrapsin inactivates only trypsin [29] (hence the name, an elision of contras and trypsin [27]). Hill et al. inferred homology between human \( \alpha_1 \)-antichymotrypsin and contrapsin, suggesting that they originated from an evolutionarily recent gene duplication [28, 30, 31]. Levels of \( \alpha_1 \)-antitrypsin and contrapsin are several mg/mL in mouse serum, though are lower in females than in males [32]. A guinea pig protein also named contrapsin exists (COTR_CAVPO) but in fact, its sequence is more similar to \( \alpha_1 \)-antitrypsin than to mouse contrapsin. “Contrapsin-like proteins” have been described in the rat, and are down-regulated in inflammation [33]. Another respect in which mouse serum differs from that of rat and human (but is similar to bovine) is the high concentration of carboxylesterase.

### 3.2 The effect of systemic inflammation on mouse serum proteins

Figure 3 shows 2-D gels obtained under reducing and non-reducing conditions 48 h after treatment with different doses of LPS (either 2.5 or 5 mg/kg). In this concentration range, the effects of the stimulus are dose-dependent, in that the same proteins are altered by both concentrations, but the increases above baseline levels are greater at the higher dose. The figure also shows that the \( M_r \) of the \( \alpha_1 \)-antitrypsin/serine protease inhibitor spot chain is reduced after LPS treatment, which may be due to altered glycosylation as reported for orosomucoid/\( \alpha_1 \)-acid glycoprotein [34], transferrin
and $\alpha_2$-macroglobulin [36]. We have observed a similar effect with $\alpha_1$-antitrypsin in bovine serum during early stages of pregnancy (unpublished results).

((Fig. 3))

Figure 4 shows the effect of 2.5 mg/kg LPS on the concentrations of 18 proteins. The greatest changes are observed for haptoglobin and hemopexin, which rise to four times baseline levels after 48 h. The maximal concentration of orosomucoid/$\alpha_1$-acid glycoprotein is also observed at 48 h, but the increase is only 30%. Levels of all these proteins revert to baseline within 96 h. In contrast, the increase in concentrations of $\alpha_1$-macroglobulin, apolipoprotein A-I and vitamin D-binding protein are more sustained, remaining elevated at 96 h (100% for $\alpha_1$-macroglobulin and 50–60% for apoA1 and vitamin D-binding protein). The trends we observe had been reported by Schreiber et al. [37].

((Fig. 4))

The behaviour of acute-phase reactants in mice is more comparable to humans than rats; in mice baseline levels of all positive acute phase reactants are significant, whereas in rats, thiostatin, orosomucoid/$\alpha_1$-acid glycoprotein and haptoglobin are barely detectable with typical loadings. C-reactive protein is undetectable in control mice and humans, but in rats gives a clearly stained spot. $\alpha_2$-Macroglobulin is an acute phase reactant in mice and rats, but not in humans. In contrast to rats and humans, $\alpha_1$-antitrypsin is not an acute phase reactant [38] in mice. Little change in contrapsin protein levels are observed either after induction of inflammation [39] or in mice bearing ascites tumours [40], though its mRNA increases by 50% in liver after injection of turpentine [38] and doubles after challenge with LPS [40]. Recent proteomic findings confirm that apoA4 is a positive acute-phase protein while apoA2 is a negative acute-phase protein in mice injected with endotoxin [41, 42].

3.3 Effect of human apolipoprotein transgenes on mouse serum proteins
Figure 5 shows the effects of two human apolipoprotein transgenes on mouse serum proteome. The various panels compare the levels of expression of the proteins for which statistically significant differences are observed.

The absolute abundance of human apoA1 is not influenced by the presence apoA2, i.e., no statistically significant difference is observed between single and doubly transgenic animals; however the presence of the apoA1 transgene results in a statistically significant increase in expression of the apoA2 transgene.

Figure 5 also plots the relative abundance of several proteins, which, though not involved in lipid metabolism, are influenced by the presence of human apolipoprotein transgenes. These include orosomucoid/α₁-acid glycoprotein, α₁-macroglobulin, esterase, haptoglobin (HPT) and contrapsin. No statistically significant difference is observed for any of these proteins between mice transgenic for apoA2 and either KO mice or the doubly transgenic animals. All differences observed between KO and mice transgenic for apoA1 are maintained in the doubly transgenic mice. Most of the changes observed between mice transgenic for apoA2 and mice transgenic for apoA1 are also seen in the doubly transgenic mice. Thus expression of human apolipoprotein A-I seems to exert a much greater effect than apolipoprotein A-II. This is unlikely to be a consequence of the quantity of metabolites processed by the two pathways, since the absolute amounts of apoA1 and apoA2 only differ by a factor of 2.5.

The anti-inflammatory action of (apo)lipoproteins in humans has been extensively discussed. Apolipoprotein-AI inhibits inflammatory functions in peripheral blood mononuclear cells activated by either specific antigens or lectins, without affecting proliferation [43]. ApoA1 and apoA2 do not affect the basal secretion of TNF-α, IL-1β, IL-8, and IL-1 receptor antagonist by neutrophils, whereas in LPS-stimulated neutrophils apoA1 inhibits release of IL-1β and apoA2 reduces IL-8 secretion. ApoA2 also decreases the production of IL-8 by neutrophils stimulated with the acute phase protein serum amyloid A [44]. High-density lipoproteins (which contain apoA1 and apoA2) decrease the basal and LPS-stimulated release of cytokines from peripheral blood neutrophils [45].
HDL binding to mitogen-stimulated T cells inhibits contact-mediated activation of monocytes and production of TNF-α and IL-1β [43].

All the proteins whose levels are modulated by the expression of human apolipoprotein transgenes have cytokine-responsive elements in their promoter region and are known to be involved in acute phase reactions. The inhibitory effect of HDL on cytokine secretion could be the explanation for our observations of the effect of expression of apolipoprotein transgenes.

4 Concluding remarks

Because the technology for generating transgenic strains is well established, the mouse is the model organism of choice for studying the influence of individual genes on physiological and pathological processes. Since introduction of a transgene may induce multiple biochemical perturbations in unexpected pathways, proteomic methods provide a powerful and hypothesis-independent strategy for characterising the induced phenotype. However, in order to detect alterations in the serum proteome attributable to the transgenes it is a prerequisite to determine the baseline phenotype of the wild type. In this respect, assessing the characteristics of the acute-phase reaction is as important as establishing the baseline, control conditions. Following the suggestion of R. Ross that atherosclerosis should be regarded as an inflammatory disease [46], there is growing interest in the role of inflammation in a range of pathologies. While inflammation is an essential defensive response to infection or injury, given the power and destructive nature of the inflammatory response, it is important that it is sustained no longer than necessary for control of the initiating insult. The escape of an inflammatory response from control has major pathological consequences for the host, ranging from lethality to the chronic autoinflammation that occurs in Crohn's disease or rheumatoid arthritis. Inflammation itself is therefore an important pharmacological target in many pathological conditions and acute phase proteins may constitute valuable biomarkers for the effectiveness of anti-inflammatory therapeutic interventions.

It is however necessary to consider species differences when using inflammatory proteins as disease markers or prognostic indicators. The work of Skehel et al. [12] exemplifies both the
opportunities and the pitfalls inherent in this approach. The apolipoprotein E variant apoE3Leiden shows a reduced affinity for the LDL receptor, which results for human carriers in the dominant occurrence of type III hyperlipoproteinemia. Since in transgenic mice the expression of apoE3Leiden is associated with elevated serum levels of haptoglobin, Skehel et al. suggested haptoglobin might provide a marker for the onset of atherosclerosis in humans. As we demonstrated above, haptoglobin is the major acute phase reactant in mice but C-reactive protein is the most typical in humans. Furthermore, apart from CRP, levels of α₁-antitrypsin, α₁-acid glycoprotein / orosomucoid, α₂-macroglobulin, ceruloplasmin, haptoglobin, fibrinogen and Lp(a) are also significantly associated with the severity of coronary atherosclerosis in human patients [47, 48].

Thus singling out an individual protein as a marker for inflammatory conditions is likely to be potentially misleading, particularly when making inter-species comparisons.

Acknowledgements

This work was supported in part by grants from CARIPLO and MIUR (FIRB 2001: Project no. RBNE01BNFK).

5 References

References


[34] Hansen, J. E., Bog-Hansen, T. C., Pedersen, B., Neland, K., Electrophoresis 1989, 10, 574–578.
Figure 1. Reference map of mouse serum proteins, built on the master gel of the matchset for the experimental inflammation experiment in Fig. 3. From 2-DE of mouse serum with 1 d on 4–10 IPG (non-linear) [24] and 2 d on 7.5–17.5%T PAA. Main protein identifications are listed, using the following abbreviations: α1T1-3 = α1-antitrypsin; apoA1-2,4 = apolipoprotein A-I, A-II, A-IV; apoC1-3 = apolipoprotein C-I, C-II, C-III; apoE = apolipoprotein E; COTR = contrapsin; fSA = serum albumin fragment; Hbβ1 = Hb chain β; MUP = major urinary protein; SAMP = serum amyloid P; TTHY = transthyretin; VTDP = vitamin D-binding protein. Spot chains corresponding to charge/glycosylation isoforms are connected by lines; individual spots are marked by squares, spots corresponding to albumin fragments are marked by triangles. The approximate areas of panels A and B in Fig. 2 are boxed (A,B). Spots outside the boxes are numbered as in Table 1.

Figure 2. Panels A-D: Differential resolution of high and low Mr acidic proteins in mouse serum, after reduction with 2% 2-mercaptoethanol. Spots are numbered as in Table 1. A is a 2-DE of mouse serum proteins with a pH 4–6 IPG (non-linear) and PAGE on 5–10%T; C is an expansion of the region where α1-antitrypsin (18), serine protease inhibitor (19) and α2-HS-glycoprotein (17) maximally overlap. Panel D shows the orosomucoid/α1-acidic glycoprotein and clusterin region of a pH 2.5–5 IPG / 5–12%T PAGE. Panel B is a 2-D gel of mouse serum with pH 4–6 IPG (non-linear) / 10–15%T PAGE. Panels E-F: Resolution of acidic proteins in mouse serum, run without any sample reduction. Identified proteins are numbered as in Table 1. Panel E is a 2-DE with 1 d on 4–6 IPG (non-linear) and 2 d on 5–12%T PAA. Panel F is a close-up of the region where α1-antitrypsin (88) and serine protease inhibitor (89) overlap; sample: serum from inflamed mice (48 h after LPS).

Figure 3. Serum pattern of LPS treated and control mice on pH 4–6 IPG (non-linear) / 5–12%T PAGE under different redox conditions.
Figure 4. Time-course of the changes in serum proteins during experimental inflammation. Mice were injected with 2.5 mg/kg LPS; serum was obtained every 24 h. The data were obtained from 2-D gels with pH 4–10 IPG (non-linear) and 7.5–17.5%T PAGE, except for orosomucoid/α1-acid glycoprotein that was resolved by 1-DE on pH 2.5–5 IPG strips. Changes in spot volume are plotted as a function of time. α1AG = α1-acid glycoprotein; α1M = α1-macroglobulin; α1T3 = α1-antitrypsin; α2HS = α2-HS-glycoprotein apoA1, apoA2, apoA4 = apolipoproteins A-I, A-II, A-IV; apoC1-3 = apolipoprotein C-I, C-II, C-III; COTR = contrapsin; ESTN = carboxylesterase; FETB = fetuin B; fSA = serum albumin fragment; HEMO = hemopexin; HPT = haptoglobin; KNG = kininogen; SPI = serine protease inhibitor; TRFE = transferrin; TTHY = transthyretin; VTDP = vitamin D-binding protein.

Figure 5. Variation in protein expression in mice transgenic for human apolipoprotein A-I (apoA1), for human apolipoprotein A-II (apoA2) and for both (apoA1/2), and in their genetic background (mice KO for murine apolipoprotein A-I, KO). Upper panels: Spot volumes for apoA1 and apoA2, absolute values. Middle and lower panels: Ratio to spot volumes in KO mice (normalised spot volume) for proteins for which statistically significant differences in expression levels have been observed. Average values plus SD are plotted. The strains with different expression from each mouse breed are marked above the relevant column, in plain text; associated p values are marked in italics.
Table 1. Protein identifications in 2-DE maps of mouse serum proteins

<table>
<thead>
<tr>
<th>Protein identification</th>
<th>Spot # in map (number of matching peptides)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1AG_MOUSE Alpha-1-acid glycoprotein 1</td>
<td>28 (4)</td>
</tr>
<tr>
<td>A1T1_MOUSE Alpha-1-antitrypsin 1-1</td>
<td>22 (6), 30 (13), 32 (6)</td>
</tr>
<tr>
<td>A1T3_MOUSE Alpha-1-antitrypsin 1-3</td>
<td>1 (11), 2 (9), 3 (9), 4 (7), 5 (6), 6 (6), 7 (6), 8 (9), 9 (16), 23 (8), 30 (13), 77 (5), 78 (12), 79 (3), 88 (8)</td>
</tr>
<tr>
<td>A1T3_MOUSE Alpha-1-antitrypsin family</td>
<td>18 (a,b) (10)</td>
</tr>
<tr>
<td>A2HS_MOUSE Alpha-2-HS-glycoprotein</td>
<td>17 (6), 90 (4)</td>
</tr>
<tr>
<td>AFAM_MOUSE Afamin (Alpha – albumin)</td>
<td>83 (9)</td>
</tr>
<tr>
<td>ALBU_MOUSE Serum albumin</td>
<td>14 (8), 25 (2), 26 (13), 35 (7), 39 (8), 40 (9), 41 (5), 49 (9), 50 (9), 51 (9), 63 (2), 64 (17), 65 (14), 66 (11), 68 (9), 69 (14), 70 (11), 71-74 by immunoblotting,</td>
</tr>
<tr>
<td>APA1_MOUSE Apolipoprotein A-I</td>
<td>36 (10), 37 (13), 38 (11), 42 (11), 53 (6)</td>
</tr>
<tr>
<td>APA2_MOUSE Apolipoprotein A-II</td>
<td>58 (4), 59 (4), 60 (4), 61 (7)</td>
</tr>
<tr>
<td>APA4_MOUSE Apolipoprotein A4</td>
<td>24 (15), 91 (12)</td>
</tr>
<tr>
<td>Q9CPP9 Apolipoprotein CIII</td>
<td>56 (3), 57 (2)</td>
</tr>
<tr>
<td>APE_MOUSE Apolipoprotein E</td>
<td>34 (7)</td>
</tr>
<tr>
<td>Protein Name</td>
<td>Tissue</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>ESTN_MOUSE Liver carboxylesterase</td>
<td>10 (3), 82 (7)</td>
</tr>
<tr>
<td>CLUS_MOUSE Clusterin</td>
<td>29 (5), 33 (7), 84 (9)</td>
</tr>
<tr>
<td>COTR_MOUSE Contrapsin</td>
<td>1 (5), 2 (9), 3 (2), 4 (11), 5 (8), 6 (12), 7 (10), 8 (14), 13 (15), 15 (15), 20 (15), 21 (1), 78 (2), 85 (11)</td>
</tr>
<tr>
<td>FETB_MOUSE Fetuin-B</td>
<td>14 (5)</td>
</tr>
<tr>
<td>Q61646 Haptoglobin</td>
<td>31 (11), 54 (5), 55 (5), 80 (5), 81 (6)</td>
</tr>
<tr>
<td>P97824 Hemopexin</td>
<td>11 (8), 86 (8)</td>
</tr>
<tr>
<td>KBP_MOUSE Kallikrein-binding protein</td>
<td>2 (1), 4 (2), 5 (1), 7 (2), 8 (1), 13 (2), 78 (1)</td>
</tr>
<tr>
<td>KNG_MOUSE Kininogen</td>
<td>12 (12), 87 (7)</td>
</tr>
<tr>
<td>Q91XL1 Leucine-rich alpha-2-glycoprotein</td>
<td>21 (2)</td>
</tr>
<tr>
<td>MUP1_MOUSE Major urinary protein 1</td>
<td>45 (10), 47 (7)</td>
</tr>
<tr>
<td>MUP2_MOUSE Major urinary protein 2</td>
<td>46 (11)</td>
</tr>
<tr>
<td>MUP4 / MUP11 / MUP8</td>
<td>52 (1)</td>
</tr>
<tr>
<td>MUP6_MOUSE Major urinary protein 6 / mup 8</td>
<td>44 (10)</td>
</tr>
<tr>
<td>SAMP_MOUSE Serum amyloid P-component</td>
<td>67 (8)</td>
</tr>
<tr>
<td>ID</td>
<td>Description</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Q8VC20</td>
<td>Similar to serine protease inhibitor</td>
</tr>
<tr>
<td>TRFE_MOUSE</td>
<td>Serotransferrin</td>
</tr>
<tr>
<td>TTHY_MOUSE</td>
<td>Transthyretin</td>
</tr>
<tr>
<td>VTDB_MOUSE</td>
<td>Vitamin D-binding protein</td>
</tr>
</tbody>
</table>