

RESEARCH ARTICLE

Identification of target antigens of self-reactive IgG in intravenous immunoglobulin preparations

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Intravenous immunoglobulin (IVIg) contains a wide range of self-reactive immunoglobulins (Ig) G. Acidic pH is known to increase the reactivity of purified IgG with self-antigens. We describe here the target antigens of IgG autoantibodies in IVIg and analyze the influence of acidic pH on IgG reactivities. We used 2-DE and immunoblotting with protein extracts of human umbilical vein endothelial cells (HUVEC) and HEP-2 cells. Two IVIg preparations obtained by ethanol fractionation [one with an acidic pH step (acidic-IVIg) and one with β -propiolactone (propiolactone-IVIg)] and a pool of sera from 12 healthy individuals were tested. Serum IgG of 3 healthy individuals and IgG purified from the same sera with elution at pH 2.8 were also tested individually. Finally, propiolactone-IVIg was acidified at pH 2.8. IgG obtained with a step at low pH recognized many more target spots than IgG obtained without acidic pH. Our findings demonstrate that an acidic pH step artificially enlarges the repertoire of self-reactive IgG. Thus, protein spots recognized by IgG in propiolactone-IVIg represent the core set of self-antigens targeted by IVIg. Overall, 96 proteins were identified by MS. Fourteen were recognized in both extracts including glycolysis proteins such as α -enolase, RNA processing and cytoskeletal proteins such as lamin-A/C.

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Abbreviations: ABC, ammonium bicarbonate; FA, formic acid; HUVEC, human umbilical vein endothelial cell; Ig, immunoglobulin; IVIg, intravenous Ig

1 Introduction

Intravenous immunoglobulin (IVIg) is a therapeutic preparation of normal human immunoglobulin (Ig) G obtained from pools of more than 1000 healthy blood donors. Initially used as replacement therapy for patients with primary and secondary

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immune deficiencies, IVIg preparations are now widely used for the treatment of many autoimmune and/or systemic inflammatory diseases [1]. The large donor pool ensures the diversity of IgG repertoire that far exceeds that of an individual. Thus, IVIg contains a sampling of the entire array of variable regions of antibodies expected to be present in normal human serum. Hence, IVIg comprises a broad range of antibodies directed against pathogens and foreign antigens, autoantibodies and anti-idiotypic antibodies [2]. Thus, a number of mechanisms of action of IVIg in autoimmune and/or inflammatory diseases identified during the last 25 years depend on V-region-mediated interactions of IVIg with soluble and cell-surface molecules on immuno-competent cells in patients [2].

IVIg binds to a diverse panel of self-antigens, mainly functional molecules of the immune system, including CD4 [3], CD5 [4], cytokines [5], or MHC class I molecules [6], and could modulate immune responses [2]. Some of these antigens are phylogenetically conserved cellular antigens such as myoglobin or actin; others are targeted by natural human IgG and disease-associated autoantibodies such as thyroglobulin or DNA [7, 8].

Others and we previously reported that IgG purified from serum onto a protein G-Sepharose column expressed high levels of autoreactivity [9–11]. Different physical and chemical treatments are known to increase the reactivity of natural autoantibodies with self-antigens such as acidic pH [12, 13], with evidence that the number of autoantigens recognized is reduced in the absence of the acidification process. In the present work, we used 2-DE and immunoblotting with a protein extract of human umbilical vein endothelial cells (HUVEC) and a protein extract of HEP-2 cells enriched in nuclear proteins to identify a core set of target antigens of IgG autoantibodies in IVIg preparations. We also demonstrated that an acidic pH step increases the number of reactivities directed toward self-antigens in IVIg preparations.

2 Materials and methods

2.1 Immunoglobulin sources

We used a pool of sera from 12 healthy blood donors (6 males; mean age 39.5 ± 7.8 years, range 33–61 years). Donors had no detectable disease, no remarkable medical history and did not take any medication at the time of blood sampling. All donors gave their written informed consent.

IgG from sera from 3 healthy blood donors was purified by affinity chromatography onto a protein G-Sepharose column (GE Healthcare, Uppsala, Sweden), with elution at pH 2.8, following the manufacturer's instructions.

Two IVIg preparations were tested: Intraglobin F, kindly provided by Biotest (Dreieich, Germany), and Tegeline, kindly provided by the Laboratoire Français du Fractionnement et des Biotechnologies (LFB) (les Ulis, France). These two preparations are obtained by ethanol fractionation by the Cohn method [14], but the step of viral inactivation differs in

the use of β -propiolactone for Intraglobin F (propiolactone-IVIg) [15, 16] and a combination of pH 4 and pepsin treatment for Tegeline (acidic-IVIg) [17].

To document the influence of a fractionation step at low pH on the number and the intensity of IgG reactivities, propiolactone-IVIg was acidified in a 100 mM glycine, pH 2.8, solution for 2 min. Then, acidic pH was neutralized with a 3 M Tris, pH 11.6, solution to adjust to final pH 7, and the preparation was dialyzed against PBS to compare IgG reactivities of intact propiolactone-IVIg and acidified propiolactone-IVIg.

IgG concentrations were determined by nephelometry (BN II, Dade Behring, Deerfield, IL). Serum samples, purified IgG and IVIg preparations were stored in aliquots at -80°C until tested.

2.2 Endothelial cell culture and protein extraction

HUVEC were isolated by digestion of freshly obtained umbilical cords and cultured as previously described [18, 19]. A HUVEC protein extract was prepared after the second passage as described [20]. Protein quantification was performed using the Bradford method [21].

2.3 HEP-2 cell culture and protein extraction

HEP-2 cells, a cell line derived from a human laryngeal carcinoma that represents the standard substrate for the detection of antinuclear antibodies [22], were obtained from EuroBio (Les Ulis, France) and cultured as described [18]. When confluent, the cells were detached by use of 0.05% trypsin-EDTA (Gibco BRL Invitrogen) and then washed twice with PBS and once in TBS (25 mM Tris pH 7.5, 138 mM NaCl, 2.7 mM KCl).

Protein extraction with enrichment in nuclear proteins was performed as previously described [23, 24]. HEP-2 cells were centrifuged at 1800 rpm during 10 min at 4°C and then resuspended in a buffer containing 10 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT and a cocktail of anti-protease 0.5 mM PMSF and 5 $\mu\text{L}/\text{mL}$ Complete Mini (Roche Diagnostics, Meylan, France). After incubation for 15 min on ice, 10% NP-40 (Sigma) was added, and cells were vortexed during 10 s. Cells were centrifuged at 1800 rpm during 10 min at 4°C and then resuspended in a buffer containing 20 mM HEPES pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT and the same cocktail of anti-protease as above. Cells were incubated 15 min on ice and vortexed every 2–3 min. After centrifugation at 1800 rpm during 10 min at 4°C , the supernatant was collected and ultracentrifuged at $40\,000 \times g$ during 40 min at 4°C . Then, to eliminate salts, supernatant was suspended in a precooled (-20°C) solution of 10% TCA in acetone with 0.07% 2-mercaptoethanol, according to Damerval *et al.* [25]. Briefly, after overnight precipitation of the proteins at -20°C , the sample was centrifuged and the pellet was washed with cold acetone containing 0.07% 2-mercaptoethanol. Proteins

were then resuspended in the sample solution extraction Kit 3, and protein quantification was performed by the Bradford method. Finally, 64 mM DTT (final concentration) was added, and the sample was aliquoted and stored at -80°C until use.

2.4 2-DE (Figure 1)

We used pH 3 to 10 and acrylamide gradient of 7 to 18% in all experiments, which allowed for studying a wide range of antigens of 10 to 250 kDa [20, 26]. The instrumentation and related reagents were purchased from Bio-Rad, unless otherwise indicated. Proteins were iso-electrofocused with 17-cm IPG strips on the Protean IEF Cell System, essentially as described by Görg *et al.* [27] and as recently reported by Serrettaz *et al.* [20]. For preparation of 2-D gels, 100 μg of HUVEC or HEp-2 cell proteins was loaded onto IPG strips.

Prior to the second dimension, the strips were equilibrated as described and then transferred to a 7–18% polyacrylamide gradient gel [20]. The equilibrated IPG gels were

sealed on top of the polyacrylamide gels with use of 1% agarose containing bromophenol blue, and running buffer (24.8 mM Tris, 192 mM glycine, and 0.1% SDS) was added. Gels were run initially at 40 V constant for 1 h and then at 15 mA/gel for 21 h 15 min.

2.5 Electro-transfer and immunoblotting

The gels were transferred onto PVDF membranes (Millipore, Bedford, MA) by semidry transfer (Bio-Rad) at 320 mA for 1 h 30 min. After blocking with PBS-0.2% Tween for 90 min, membranes were incubated overnight at 4°C with sera from healthy blood donors or purified IgG or IVIg preparations. Each IgG sample was tested at the same IgG concentration of 200 $\mu\text{g}/\text{mL}$.

IgG immunoreactivities were revealed as described [20]. Specific reactivities were determined by densitometrically scanning the membranes (densitometer GS-800, Bio-Rad) by use of the Quantity one software (Bio-Rad). The membranes were then stained with colloidal gold (Protogold, British

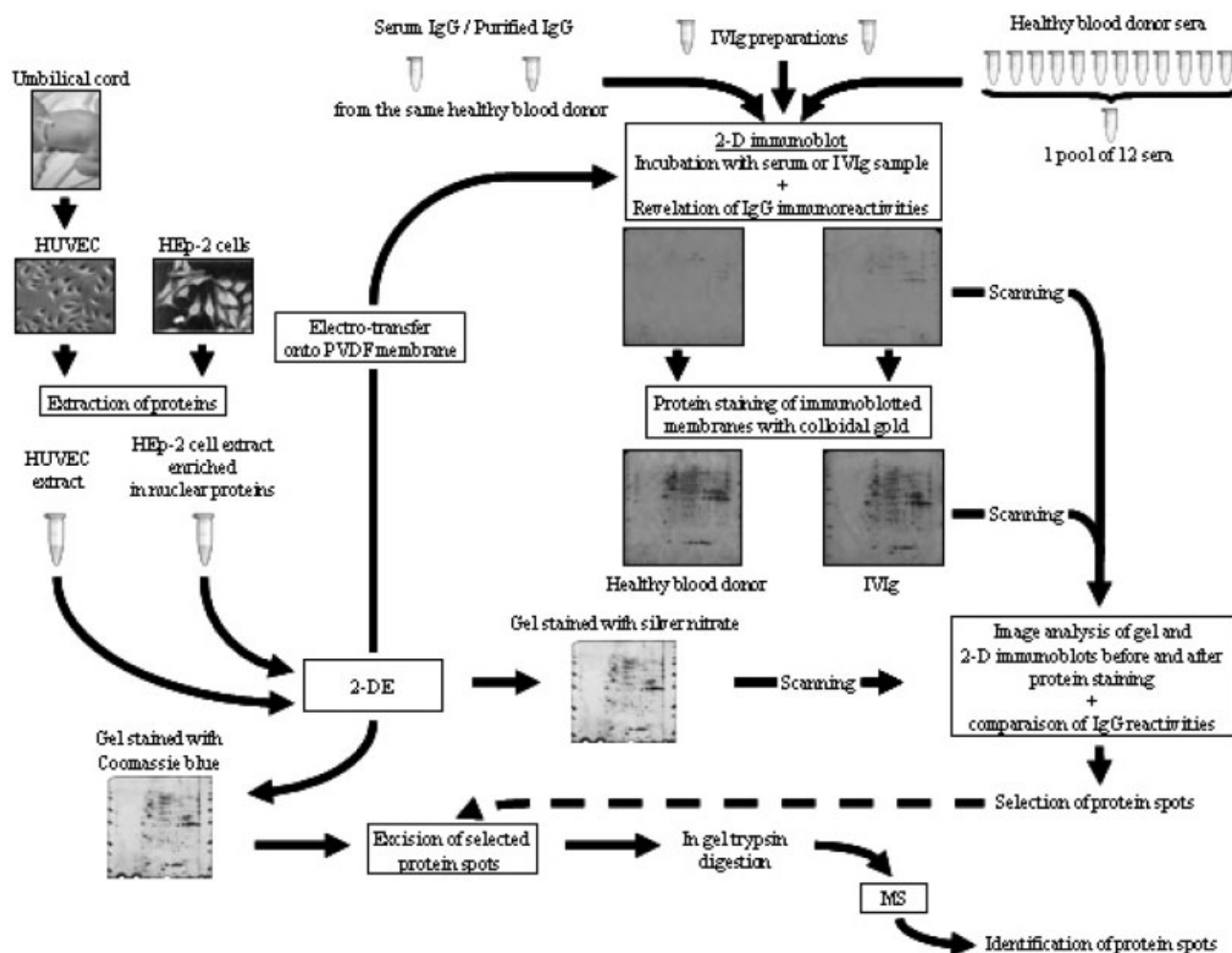


Figure 1. Experimental design for screening and identifying target antigens of IgG autoantibodies.

Biocell International, Cardiff, UK) and subjected to a second densitometric analysis to record labeled protein spots for each gel.

2.6 Gel staining

Analytical gels were stained with ammoniacal silver nitrate [28].

2.7 Image analysis of gels and 2-D blots

Images of both the gels and the membranes were acquired by use of a densitometer (GS-800, Bio-Rad) and underwent analysis by use of the Image Master 2-D Platinum 6 (Amersham Biosciences), as described [20].

2.8 In-gel trypsin digestion

In-gel digestion was carried out with trypsin as described by Shevchenko [29] with minor modifications and involved for all steps a Freedom EVO 100 digester/spotter robot (Tecan, Männedorf, CH). Spots were first de-stained two times with a mixture of 100 mM ammonium bicarbonate (ABC) and 50% ACN for 45 min at 22°C and then dried with use of 100% ACN for 15 min. Protein spots were then treated with 25 mM ABC containing 10 mM DTT for 1 h at 60°C and then alkylated by use of 55 mM iodoacetamide in 25 mM ABC for 30 min in the dark at 22°C. Gel pieces were washed twice with 25 mM ABC and finally shrunk two times with 100% ACN for 15 min and dried with 100% ACN for 10 min. Bands were completely dehydrated after 1 h at 60°C. Gel pieces were incubated with 13 μ L Sequencing Grade Modified Trypsin (Promega, WI; 12.5 μ g/mL in 40 mM ABC-10% ACN pH 8.0) overnight at 40°C. After digestion, peptides were washed with 30 μ L of 25 mM ABC, shrunk with 100% ACN and extracted twice with a mixture of 50% ACN-5% formic acid (FA). Extracts were then dried by vacuum centrifuge (Eppendorf, Hamburg, Germany). Finally, peptides were desalted using C_{18} -ZipTips (Millipore) and two elutions, first with 50% ACN-5% FA and then 80% ACN-5% FA. Pooled elutions were allowed to dry at room temperature.

2.9 Protein identification by MS

For MS and MS/MS analysis, peptides were redissolved in 4 μ L CHCA (5 mg/mL in 50% ACN-0.1% TFA). One microliter and a half of each sample was spotted directly onto a MALDI plate (Applied Biosystems, Foster City, CA). Droplets were allowed to dry at room temperature. The sample analysis involved a MALDI-TOF-TOF 4800 mass spectrometer (Applied Biosystems). Spectra acquisition and processing involved use of the 4000 series explorer software (Applied Biosystems) version 3.5.28193 in positive reflectron mode at fixed laser fluency with low mass gate and delayed extraction. External plate calibration was by four calibration points spotted onto the four corners of the plate with a mixture of

five external standards (PepMix 1, LaserBio Labs, Sophia Antipolis, France). Peptide masses were acquired by steps of 50 spectra for 900 to 4000 Da. MS spectra were summed from 1000 laser shots by an Nd-YAG laser operating at 355 nm and 200 Hz. After filtering tryptic-, keratin- and matrix-contaminant peaks, up to 15 parent ions were selected for subsequent MS/MS fragmentation according to mass range, signal intensity, S/N, and absence of neighboring masses in the MS spectrum. MS/MS spectra were acquired in 1-kV positive mode, and 1000 shots were summed in increments of 50. Database searching involved MASCOT 2.2 (MatrixScience, London, UK) [30] via GPS explorer software (Applied Biosystems) version 3.6 combining MS and MS/MS interrogations on human proteins from Swiss-Prot databank release 54.5, 17 253 entries (www.expasy.org). The search parameters were as follows: carbamidomethylation as a variable modification for cysteines and oxidation as a variable modification for methionines. Up to one missed tryptic cleavage was permitted and mass accuracy tolerance of 30 ppm for precursors and 0.3 Da for fragments were used for all tryptic mass searches. Positive identification was based on a MASCOT score above the significance level (*i.e.* <5%). In the case of peptides match to multiple members of a protein family, the reported protein is the one with the highest number of peptide matches.

3 Results

3.1 Analysis of proteomes

A mean $\pm$ SD of 908 ± 112 and 983 ± 126 spots were detected in three independent experiments in HUVEC and HEp-2 cell analytical gel, respectively. Most of the spots were detected between pH 5 and 8 (Fig. 2). After transfer and colloidal gold staining, 685 ± 121 and 781 ± 142 spots were detected onto PVDF membranes transferred with HUVEC and HEp-2 cell proteins, respectively.

3.2 Influence of acidic pH on self-reactive repertoire of IgG from healthy blood donors

To analyze the influence of a step at low pH on the number and the intensity of IgG reactivities directed toward autoantigens, PVDF membranes were incubated with serum IgG of 3 healthy blood donors or IgG purified from the corresponding sera obtained with elution at pH 2.8.

Serum IgG from the 3 healthy blood donors tested individually recognized 164 (range 132 to 188) and 170 (range 152 to 202) protein spots, on average, in HUVEC and HEp-2 cell extracts, respectively. Some of these reactivities were shared between individuals, some were patients specific. IgG purified from the same sera tested individually recognized 488 (range 451 to 560) and 501 (range 440 to 549) protein spots, on average, in HUVEC and HEp-2 cell extracts, respectively. Figure 3 depicts serum IgG and purified IgG

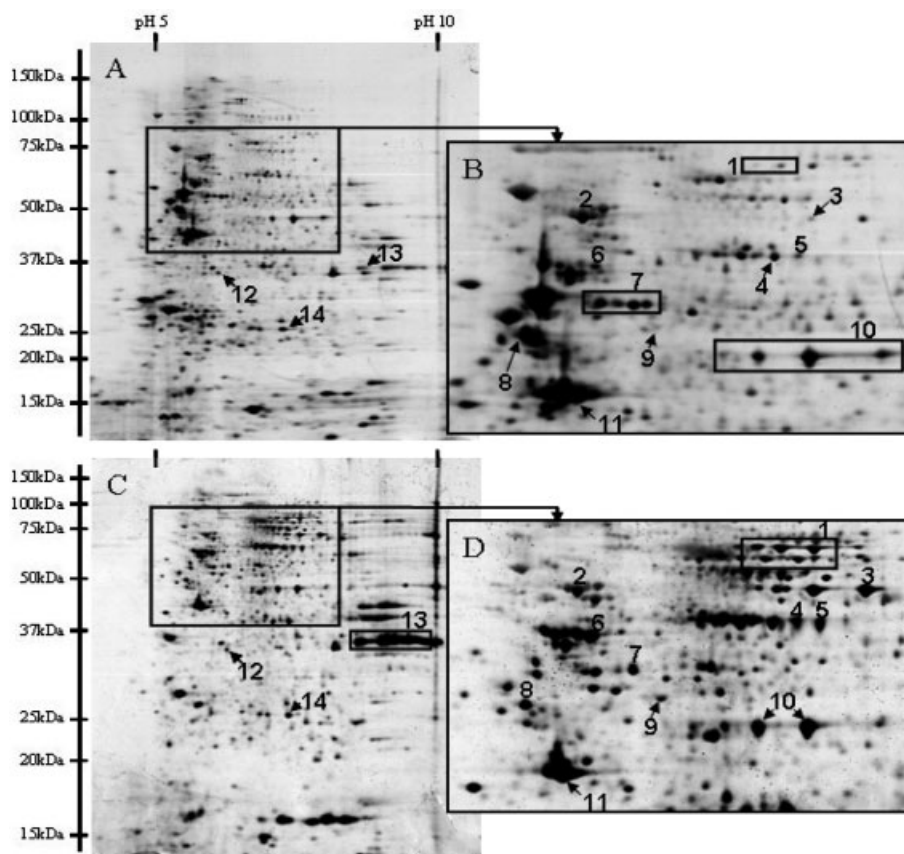


Figure 2. HUVEC and HEP-2 cell proteomes. HUVEC (A) and HEP-2 cell (C) silver stained analytical gel. First dimension (x-axis): pH range 3 to 10; second dimension: range of molecular weight 250 to 10 kDa (y-axis). (B) and (D) magnification of the delineated zones on (A) and (C), respectively. Numbers indicate the 14 proteins recognized by IgG in propiolactone-IVlg both in HUVEC and HEP-2 cell extracts (see Table 1 for the names of these proteins). Protein spots delineated by rectangles are different isoforms of the same protein recognized by IgG in propiolactone-IVlg on the corresponding protein extract.

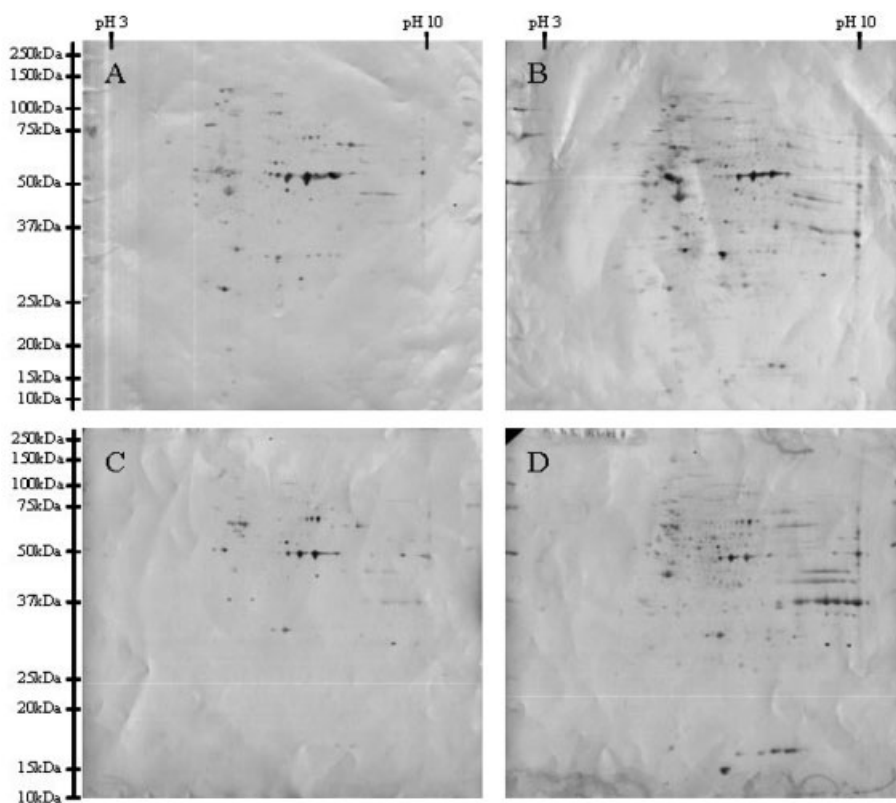


Figure 3. HUVEC and HEP-2 cell proteins recognized by serum IgG and IgG purified from the serum of the same healthy individual. HUVEC (A) and (B) and HEP-2 cell (C) and (D) proteins recognized by serum IgG from a representative healthy individual (A) and (C) and by IgG purified from the serum of the same healthy individual on a protein G column (B) and (D). Serum IgG and purified IgG were tested at IgG 200 µg/mL. First dimension (x-axis): pH range 3 to 10; second dimension: range of molecular weight 250 to 10 kDa (y-axis).

reactivities from one representative healthy blood donor; 188 and 455 protein spots were recognized by serum IgG and purified IgG, respectively, in the HUVEC extract, whereas 155 and 440 protein spots were recognized by serum IgG and purified IgG, respectively, in the HEP-2 cell extract. Thus, the number of protein spots recognized in protein extracts of HUVEC and HEP-2 cells was substantially higher for purified IgG than serum IgG for the same individuals at the same IgG concentration.

3.3 Self-reactive repertoire of IgG in IVIg preparations

We analyzed IgG reactivities directed against HUVEC and HEP-2 cell protein extracts in the pool of sera from 12 healthy blood donors and in the two IVIg preparations (Fig. 4).

In the HUVEC extract, IgG from the pool of sera from healthy blood donors and in propiolactone-IVIg recognized 157 and 112 protein spots, respectively, whereas IgG in the acidic-IVIg preparation recognized 513 protein spots. In the HEP-2 cell extract, IgG from the pool of sera from healthy blood donors and in propiolactone-IVIg recognized 154 and 160 protein spots, respectively, whereas IgG in the acidic-IVIg preparation recognized 664 protein spots. All protein spots recognized by IgG in the propiolactone-IVIg preparation were also recognized by IgG in the acidic-IVIg preparation for each of the two protein extracts.

Thus, IgG in the IVIg preparation with acidic pH and pepsin treatment combined bound to many more protein spots than IgG in the IVIg β -propiolactone preparation.

3.4 Acidification of propiolactone-IVIg increases the number of IgG reactivities

To confirm the influence of the step at low pH on IgG reactivities, we compared reactivities of intact propiolactone-IVIg

and acidified propiolactone-IVIg obtained after acidification at pH 2.8, in HUVEC and HEP-2 cell extracts (Fig. 5). The number of IgG reactivities increased from 112 and 160 to 377 and 376 after acidification in HUVEC and HEP-2 cell protein extracts, respectively. Hence, the acidification process increased the number of reactivities directed toward self-antigens in IVIg preparations.

3.5 Identification of proteins recognized by IgG in sera from healthy individuals and in propiolactone-IVIg

We subjected protein spots of HUVEC and HEP-2 cells recognized by IgG in propiolactone-IVIg to MALDI-TOF-TOF MS analysis. Of the 112 protein spots recognized by IgG in propiolactone-IVIg in the HUVEC extract, 76 were unambiguously identified to correspond to 51 different proteins (see Supporting Information); 21 protein spots with multiple candidate proteins were omitted from the results. Fourteen proteins were identified several times, and proteins could not be identified for 15 spots. The unambiguously identified proteins were mainly involved in cell metabolism and the cell cycle: 9 chaperone proteins, 9 cytoskeletal proteins, 5 proteins implicated in RNA processing, 4 implicated in oxidation-reduction activity, 5 involved in glycolysis and 2 involved in growth regulation. Among targets of IgG antibodies directed against HUVEC, none was endothelial-cell specific. Overall, 49 of the 76 identified spots were also recognized by serum IgG in the sera pool from healthy blood donors and represented 32 proteins.

Among the 160 protein spots recognized by IgG in the propiolactone-IVIg preparation in the HEP-2 cell extract, 86 spots were unambiguously identified to correspond to 59 different proteins (see Supporting Information). Eleven of these proteins were identified several times. For 28 protein

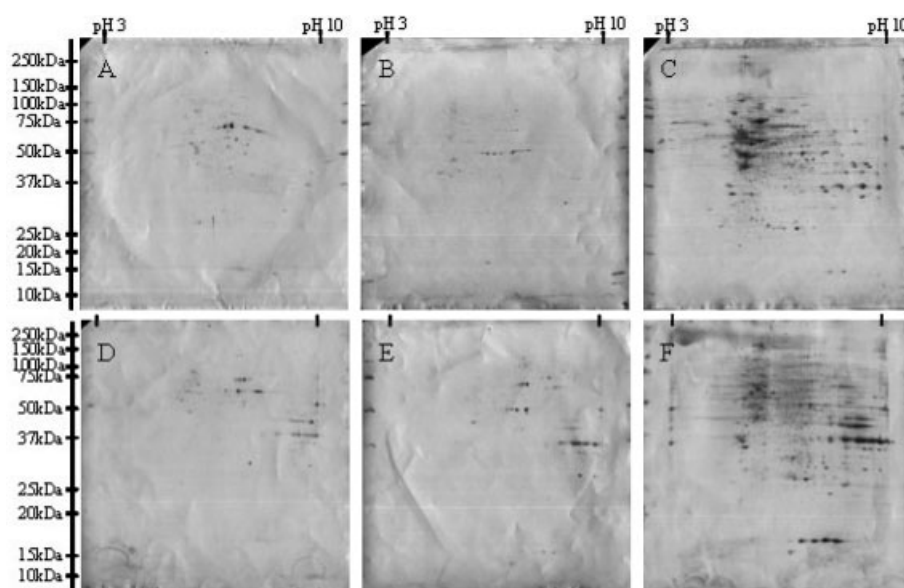


Figure 4. HUVEC and HEP-2 cell proteins recognized by IgG in a pool of sera from healthy blood donors and two different IVIg preparations. HUVEC proteins recognized by IgG in a pool of sera from 12 healthy blood donors (A) and IgG in two IVIg preparations: propiolactone-IVIg (B) and acidic-IVIg (C). HEP-2 cell proteins recognized by IgG in a pool of sera from 12 healthy blood donors (D) and IgG in two IVIg preparations: propiolactone-IVIg (E) and acidic-IVIg (F). The pool of sera from healthy blood donors and IVIg preparations were tested at IgG 200 μ g/mL. First dimension (x-axis): pH range 3 to 10; second dimension: range of molecular weight 250 to 10 kDa (y-axis).

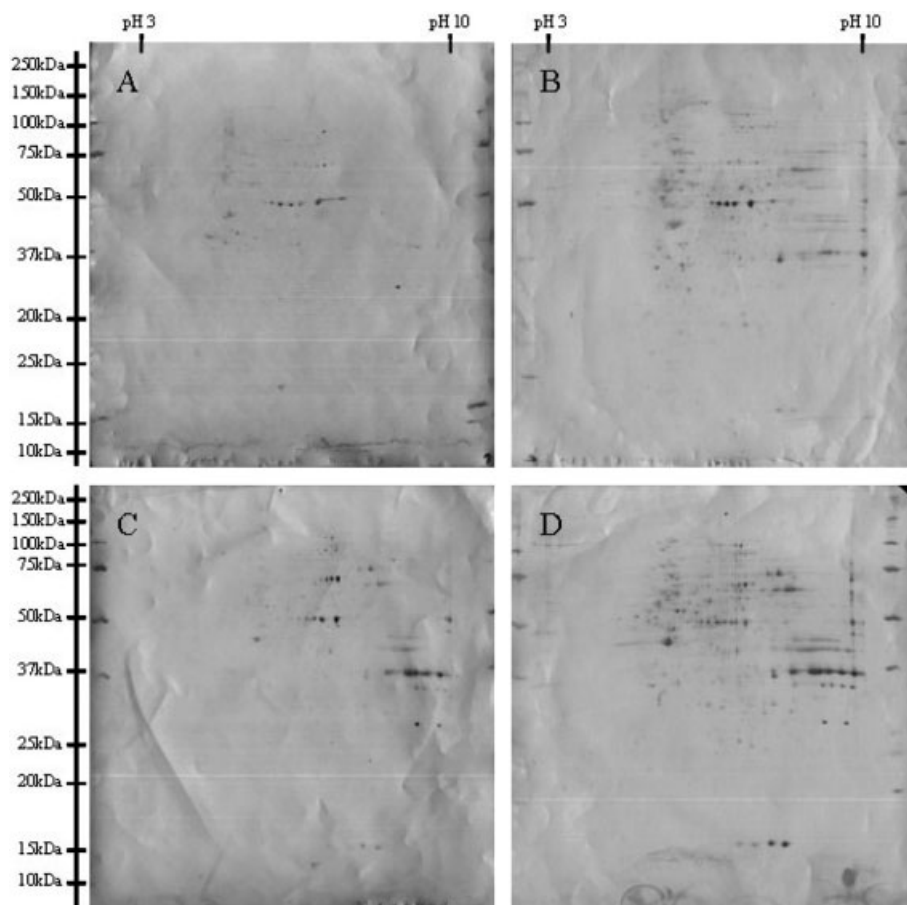


Figure 5. HUVEC and Hep-2 cell proteins recognized by IgG in intact and acidified propiolactone-IVIg. HUVEC (A) and (B) and Hep-2 cell (C) and (D) proteins recognized by IgG in intact propiolactone-IVIg (A) and (C) and propiolactone-IVIg acidified for 2 min at pH 2.8 (B) and (D). Both intact and acidified propiolactone-IVIg were tested at IgG 200 μ g/mL. First dimension (x-axis): pH range 3 to 10; second dimension: range of molecular weight 250 to 10 kDa (y-axis).

spots, under our identification criteria, we identified two or more candidates, and proteins could not be identified for 46 protein spots. Of the 59 proteins identified as targets of IVIg, 31 were known to be localized in the nucleus, some of them being also localized in other subcellular compartments. Sixteen proteins were localized exclusively in the nucleus: 10 were involved in RNA processing, including 4 heterogeneous nuclear ribonucleoproteins; 3 were implicated in regulation of transcription; 2 were implicated in mitosis and 1 was a cytoskeletal protein. Forty-six of the 86 identified spots were also recognized by serum IgG from the pool of healthy blood donors and represented 33 proteins, including 18 nuclear proteins.

Overall, IgG from propiolactone-IVIg recognized a total of 96 different proteins in the HUVEC and Hep-2 cell protein extracts. Fourteen proteins were recognized in both extracts (Fig. 2 and Table 1), including 3 glycolysis proteins (alpha enolase, glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase), 4 RNA processing proteins (far upstream element binding protein 2 and heterogeneous nuclear ribonucleoproteins H, K and L) and 2 cytoskeletal proteins (actin cytoplasmic 2 and lamin-A/C).

4 Discussion

Very few studies have analyzed the repertoire of IgG reactivities contained in IVIg preparations. Our previous use of a 1-D immunoblotting technique with solubilized protein extracts of normal human tissues or cells revealed that IVIg bound to multiple antigens in homologous/self tissues and preferentially recognized a dominant set of 20 to 25 protein bands similar to those recognized by IgG purified from the serum of healthy individuals [11].

For the first time, a proteomics approach combining 2-DE and immunoblotting with a HUVEC protein extract and a Hep-2 cell protein extract enriched in nuclear proteins was used to reveal a core set of nuclear and cytosolic self-antigens recognized by IgG autoantibodies in IVIg preparations. Our results also demonstrate that treatment at low pH increases the number of autoantigens recognized by IgG in the two protein extracts. In fact, IgG obtained with a step at low pH, *i.e.* purified IgG, acidic-IVIg and acidified propiolactone-IVIg, recognized many more target spots than serum IgG and IgG in propiolactone-IVIg, obtained without acidic pH.

We found approximately the same number of reactivities in IgG in sera from healthy blood donors and in IgG in propiolactone-IVIg. Some reactivities were not common to

Table 1. Proteins recognized by IgG in propiolactone-IVIg both in HUVEC and in HEp-2 cell extracts

Protein number	Protein	Swiss-Prot accession number	Nuclear localization	Function
1	Far upstream element-binding protein 2	FUBP2_HUMAN	+	RNA processing
2	Heat shock cognate 71 kDa protein	HSP7C_HUMAN	+	Chaperone
3	Far upstream element-binding protein 1	FUBP1_HUMAN	+	Transcription regulation
4	Lamin-A/C	LMNA_HUMAN	+	Cytoskeleton
5	Heterogeneous nuclear ribonucleoprotein L	HNRPL_HUMAN	+	RNA processing
6	Heterogeneous nuclear ribonucleoprotein K	HNRPK_HUMAN	+	RNA processing
7	Protein disulfide-isomerase A3 precursor	PDIA3_HUMAN		Protein folding
8	ATP synthase subunit beta, mitochondrial precursor	ATPB_HUMAN		Oxido-reduction
9	Heterogeneous nuclear ribonucleoprotein H	HNRH1_HUMAN	+	RNA processing
10	Alpha-enolase	ENOA_HUMAN		Glycolysis
11	Actin, cytoplasmic 2	ACTG_HUMAN		Cytoskeleton
12	60S acidic ribosomal protein P0	RLA0_HUMAN	+	Translation
13	Glyceraldehyde-3-phosphate dehydrogenase	G3P_HUMAN		Glycolysis
14	Triosephosphate isomerase	TPIS_HUMAN		Glycolysis

serum IgG and IgG in propiolactone-IVIg, because IVIg is obtained from pools of more than 1000 healthy blood donors. As propiolactone-IVIg is processed without a step at low pH, autoantigens recognized by IgG in propiolactone-IVIg probably represent the core set of self-antigens targeted by IVIg preparations.

Interestingly, very few of these antigens had been previously reported to be targets of natural IgG autoantibodies and/or autoantibodies in IVIg. As expected, none was located at the cell surface, since protein extraction for 2-DE does not allow for identifying membrane proteins. Among the total of 96 different proteins recognized in the HUVEC and HEp-2 cell protein extracts, 14 were recognized in both extracts including glycolysis, RNA processing and cytoskeletal proteins. The mechanisms by which IgG autoantibodies directed against HUVEC and/or nuclear protein antigens arise in the serum of healthy individuals and, consequently, in IVIg preparations are not clearly identified. These antigens might be exposed to the immune system through cell proliferation, apoptosis and/or necrosis. IgG reactivities in sera from healthy blood donors and in IVIg preparations shared a limited number of autoantigens: cytoskeletal proteins, chaperone proteins or proteins involved in RNA processing, mitosis and metabolic pathways.

IgG obtained from the serum of healthy individuals acquires promiscuous antigen-binding activity after a transient exposure to certain protein-destabilizing chemical agents at concentrations below those required for their denaturation [13]. Indeed, exposure to pH below 4 [12] reveals new antigen-binding specificities. The molecular mechanisms responsible for the appearance of novel antigen specificities of IgG antibodies were shown to be related to the modulation of the structural organization of the

paratopes [13]. Thus, exposure to chaotropic agents results in an increase in the structural dynamics of the antigen-binding site as well as an increase in the contribution of non-polar-type interactions for the formation of a complex with antigen [13]. The enhanced structural flexibility of the paratopes of antibodies is known to impose increased adaptability to various molecular patterns [31]. Moreover, studies have pointed to the link between hydrophobicity of the paratope and polyreactivity of the antibody [32, 33]. Based on this evidence and that of the effects of low pH on proteins, we hypothesize that increased polyreactivity of IgG after acidic treatment is due to the structural alterations in the antigen binding sites, which results in enhanced adaptability to various structural features expressed by proteins on the 2-D gels.

Acidic pH step probably artificially enlarges the repertoire of IgG reactivities directed against self-antigens. However, no evidence exists of a difference in clinical efficacy between the two IVIg preparations. In fact, both preparations demonstrated clinical efficacy in substitutive therapy [34, 35] and in immunomodulation [36, 37] and are currently used in European countries. Additional experiments are needed to determine the influence of self-reactive IgG repertoire on the therapeutic effect of IVIg preparations.

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Conflict of interest: LM has relationships with drug companies including LFB and CSL Behring. These relationships include consultancy services.

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PROTEOMICS

Supporting Information for Proteomics

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**Identification of target antigens of self-reactive
IgG in intravenous immunoglobulin preparations**

Supplementary Figure 1. The following information are given to highlight the MS/MS spectrum for the two proteins identified with a single peptide in Supplementary Table 1.

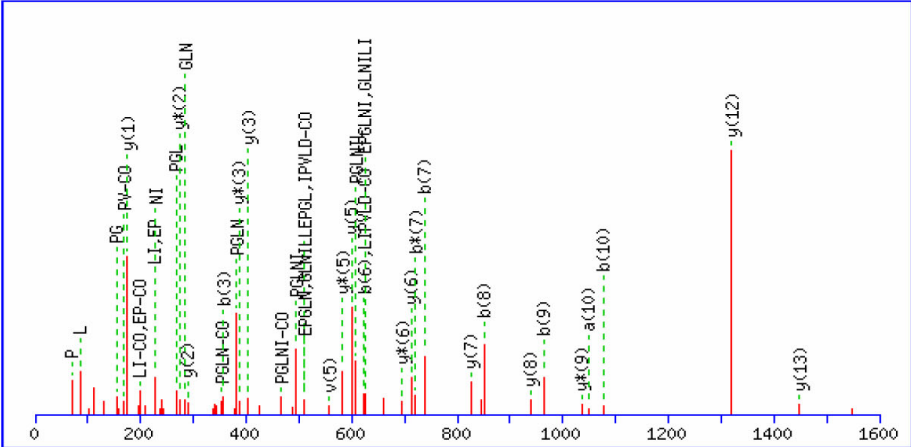
Precursor 1675.0159 MS/MS results (protein ID 1098 - Stomatin-like protein 2)

Mascot Search Results

Match to: **STML2_HUMAN** Score: 81
Stomatin-like protein 2 - Homo sapiens (Human)

Matched peptides shown in **Bold Red**
1 MLARAARGTG ALLLRGSLLA SGRAPRRASS GLPRNTVVLF VPQQEAWVVE
51 RMGRFHR**ILE PGLN**ILIPVL **DR**IRYVQSLK EIVINVPEQS AVTLDNVTLQ
101 IDGVLYLRIM DPYKASYGVE DPEYAVTQLA QTTMRSELGK LSLDKVFRER
151 ESLNASIVDA INQAADCWGI RCLRYEIKDI HVPPRVKESM QMQVEAERRK
201 RATVLESEGT RESAINVAEG KKQAQILASE AEKAEQINQA AGEASAVLAK
251 AKAKAEAIRI LAAALTQHNG DAAASLTVAE QYVSASFSLA KDSNTILLPS
301 NPGDVTSMVA QAMGVYGALT KAPVPGTPDS LSSGSSRDVQ GTDASLDEEL
351 DRVKMS

Start - End Observed Mr(expt) Mr(calc) ppm Miss Sequence
58 - 72 1675.0159 1674.0086 1674.008 0 0 **R.ILEPGLN**ILIPVL**DR**.I (Ions score 81)



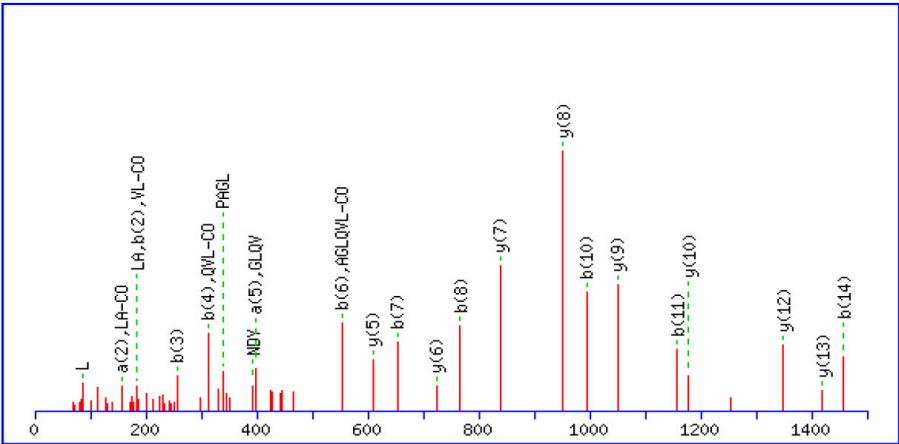
#	b	Seq.	y	#
1	114.0913	I		15
2	227.1754	L	1561.9312	14
3	356.218	E	1448.8471	13
4	453.2708	P	1319.8045	12
5	510.2922	G	1222.7518	11
6	623.3763	L	1165.7303	10
7	737.4192	N	1052.6463	9
8	850.5033	I	938.6033	8
9	963.5873	L	825.5193	7
10	1076.6714	I	712.4352	6
11	1173.7242	P	599.3511	5
12	1272.7926	V	502.2984	4
13	1385.8766	L	403.23	3
14	1500.9036	D	290.1459	2
15		R	175.119	1

Mascot Search Results

Match to: **EF1B_HUMAN** Score: **103**
Elongation factor 1-beta - Homo sapiens (Human)

Matched peptides shown in **Bold Red**
1 MGFGDLK**SPA GLQVLNDYLA** DKSYIEGYVP SQADVAVFEA VSSPPPADLC
51 HALRWYNHIK SYEKEKASLP GVKKALGKYG PADVEDTTGS GATDSKDDDD
101 IDLFGSDDEE ESEEA~~KRLRE~~ ERLAQYESKK AKKPALVAKS SILLDVKPWD
151 DETDMALEE CVRSIQADGL VWGSSKLVPV GYGIKKLQIQ CVVEDDKVGT
201 DMLEEQITAF EDYVQSM~~DVA~~ AFNKI

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
8 - 22	1603.8362	1602.8289	1602.8253	2	0	K.SPAGLQVLNDYLADK.S (Ions score 103)



#	b	Seq.	y	#
1	88.0393	S		15
2	185.0921	P	1516.8006	14
3	256.1292	A	1419.7478	13
4	313.1506	G	1348.7107	12
5	426.2347	L	1291.6892	11
6	554.2933	Q	1178.6052	10
7	653.3617	V	1050.5466	9
8	766.4458	L	951.4782	8
9	880.4887	N	838.3941	7
10	995.5156	D	724.3512	6
11	1158.579	Y	609.3243	5
12	1271.663	L	446.2609	4
13	1342.7001	A	333.1769	3
14	1457.7271	D	262.1397	2
15		K	147.1128	1

Supplementary Table 1. HUVEC proteins recognized by IgG in propiolactone-IVlg.

Protein ID on gel	Protein	SwissProt accession number	Theoretical / Estimated MW (kDa)	Theoretical / Estimated pI	Mass spectrometry				Function
					Number of unique identified peptides [#]	Total ion score	Best ion score	Sequence coverage (%)	
322*	Endoplasmin precursor	ENPL_HUMAN	92 / 100	4.8 / 5.7	4 / 7	69	35	11	chaperone
329*	Endoplasmin precursor	ENPL_HUMAN	92 / 100	4.8 / 5.7	3 / 9	47	28	14	chaperone
355*	Endoplasmin precursor	ENPL_HUMAN	92 / 130	4.8 / 4.7	5 / 7	149	49	9	chaperone
370	Far upstream element-binding protein 2 [†]	FUBP2_HUMAN	73 / 91	6.8 / 7.1	4 / 8	151	64	16	RNA processing
382	Far upstream element-binding protein 2 [†]	FUBP2_HUMAN	73 / 91	6.8 / 7.1	6 / 9	174	59	16	RNA processing
387	Far upstream element-binding protein 2 [†]	FUBP2_HUMAN	73 / 91	6.8 / 7.1	2 / 9	90	53	17	RNA processing
438*	Heat shock protein HSP 90-beta	HS90B_HUMAN	83 / 84	5.0 / 6.5	5 / 13	46	17	27	chaperone
514	Dihydropyrimidinase-related protein 3	DPYL3_HUMAN	62 / 75	6.0 / 6.7	2 / 6	88	54	15	cytoskeleton
546*	Heat shock cognate 71 kDa protein [†]	HSP7C_HUMAN	71 / 75	5.4 / 5.9	9 / 13	284	73	29	chaperone
557	Far upstream element-binding protein 1 [†]	FUBP1_HUMAN	68 / 75	7.2 / 7.2	3 / 7	114	47	13	transcription regulation
578*	Heat shock cognate 71 kDa protein [†]	HSP7C_HUMAN	71 / 75	5.4 / 6.3	9 / 21	723	137	47	chaperone
579	Stress-70 protein, mitochondrial precursor	GRP75_HUMAN	74 / 75	5.9 / 6.4	9 / 23	849	192	46	chaperone
609*	Plastin-3	PLST_HUMAN	70 / 74	5.5 / 6.4	6 / 13	296	75	28	cytoskeleton
610*	Stress-induced-phosphoprotein 1	STIP1_HUMAN	63 / 74	6.4 / 7.5	8 / 18	195	66	40	chaperone
618*	Heterogeneous nuclear ribonucleoprotein L [†]	HNRP_L_HUMAN	60 / 73	6.7 / 7.7	4 / 9	87	52	20	RNA processing
619*	Stress-induced-phosphoprotein 1	STIP1_HUMAN	63 / 71	6.4 / 7.2	5 / 12	198	56	22	chaperone
624*	Heterogeneous nuclear ribonucleoprotein L [†]	HNRP_L_HUMAN	60 / 71	6.7 / 7.6	4 / 10	122	38	23	RNA processing
627*	Stress-induced-phosphoprotein 1	STIP1_HUMAN	63 / 72	6.4 / 7.4	2 / 5	32	17	7	chaperone
631*	Stress-induced-phosphoprotein 1	STIP1_HUMAN	63 / 71	6.4 / 6.9	4 / 11	67	25	23	chaperone
638*	Calreticulin precursor	CALR_HUMAN	48 / 75	4.3 / 4.3	8 / 16	350	69	45	chaperone
646*	Stress-induced-phosphoprotein 1	STIP1_HUMAN	63 / 70	6.4 / 7.0	9 / 20	348	65	41	chaperone
661*	Protein S100-A9	S10A9_HUMAN	13 / 68	5.7 / 6.7	3 / 4	142	84	39	inflammation
741	Heterogeneous nuclear ribonucleoprotein K [†]	HNRPK_HUMAN	51 / 62	5.4 / 6.2	7 / 14	298	75	39	RNA processing
747*	60 kDa heat shock protein, mitochondrial precursor	CH60_HUMAN	61 / 62	5.7 / 5.6	7 / 7	509	123	21	chaperone
797*	Protein disulfide-isomerase A3 precursor [†]	PDI3A3_HUMAN	57 / 57	6.0 / 6.3	10 / 15	782	163	38	protein folding
802*	Protein disulfide-isomerase A3 precursor [†]	PDI3A3_HUMAN	57 / 56	6.0 / 6.2	12 / 18	1112	165	41	protein folding
809*	Protein disulfide-isomerase A3 precursor [†]	PDI3A3_HUMAN	57 / 56	6.0 / 6.1	12 / 15	785	145	40	protein folding

814*	Protein disulfide-isomerase A3 precursor [†]	PDIA3_HUMAN	57 / 56	6.0 / 6.0	6 / 14	318	80	36	protein folding
820*	Heterogeneous nuclear ribonucleoprotein H [†]	HNRH1_HUMAN	49 / 56	5.9 / 6.5	7 / 11	320	101	38	RNA processing
821*	Protein disulfide-isomerase A3 precursor [†]	PDIA3_HUMAN	57 / 58	6.0 / 5.5	11 / 21	401	58	41	protein folding
822*	Cytosol aminopeptidase	AMPL_HUMAN	56 / 55	8.0 / 7.2	5 / 16	223	80	36	proteolysis
831*	T-complex protein 1 subunit beta	TCPB_HUMAN	57 / 55	6.0 / 6.8	10 / 17	797	117	48	chaperone
884	Heterogeneous nuclear ribonucleoprotein H [†]	HNRH1_HUMAN	49 / 51	5.9 / 6.3	2 / 6	54	39	18	RNA processing
901*	ATP synthase subunit beta, mitochondrial precursor [†]	ATPB_HUMAN	57 / 50	5.3 / 5.4	15 / 23	1237	180	74	oxido-reduction
908*	Alpha-enolase [†]	ENOA_HUMAN	47 / 50	7.0 / 8.3	7 / 12	450	143	47	glycolysis
917*	Alpha-enolase [†]	ENOA_HUMAN	47 / 50	7.0 / 6.5	11 / 13	901	188	49	glycolysis
938*	Alpha-enolase [†]	ENOA_HUMAN	47 / 50	7.0 / 6.8	10 / 17	752	136	58	glycolysis
946*	Alpha-enolase [†]	ENOA_HUMAN	47 / 50	7.0 / 6.5	7 / 9	221	79	28	glycolysis
950*	Tripeptidyl-peptidase 1 precursor	TPP1_HUMAN	61 / 50	6.0 / 6.4	3 / 5	89	34	15	proteolysis
951*	26S protease regulatory subunit 7	PR57_HUMAN	49 / 50	5.7 / 6.3	10 / 14	306	68	36	proteolysis
969*	Proliferation-associated protein 2G4	PA2G4_HUMAN	44 / 50	6.1 / 6.7	4 / 9	147	48	26	growth regulation
1036*	Elongation factor TU mitochondrial precursor	EFTU_HUMAN	49 / 45	7.3 / 6.7	5 / 15	204	68	38	translation
1076*	Actin, cytoplasmic 2 [†]	ACTG_HUMAN	42 / 44	5.3 / 5.8	6 / 10	555	140	37	cytoskeleton
1098	Stomatin-like protein 2	STML2_HUMAN	39 / 43	6.9 / 5.9	1 / 9 ^{\$}	81	81	42	cytoskeleton
1214	PDZ and LIM domain protein 1	PDL1_HUMAN	36 / 37	6.6 / 7.4	5 / 10	269	62	44	cytoskeleton
1219	Glyceraldehyde-3-phosphate dehydrogenase [†]	G3P_HUMAN	36 / 37	8.6 / 8.8	2 / 5	150	84	23	glycolysis
1221*	Annexin A1	ANXA1_HUMAN	39 / 37	6.6 / 7.0	9 / 13	818	159	53	membrane-binding protein
1240*	Nucleophosmin	NPM_HUMAN	33 / 37	4.6 / 5.2	5 / 6	303	98	36	RNA processing
1245*	L-lactate dehydrogenase B chain	LDHB_HUMAN	37 / 37	5.7 / 6.3	8 / 13	347	93	47	glycolysis
1246	60S acidic ribosomal protein P0 [†]	RLA0_HUMAN	34 / 37	5.7 / 6.2	8 / 10	312	78	51	translation
1249	60S acidic ribosomal protein P0 [†]	RLA0_HUMAN	34 / 37	5.7 / 6.0	2 / 5	56	35	21	translation
1252	Elongation factor 1-delta	EF1D_HUMAN	31 / 37	4.9 / 5.6	8 / 11	544	123	56	translation
1263*	Aldose reductase	ALDR_HUMAN	36 / 37	6.5 / 7.5	3 / 6	65	47	27	oxido-reduction
1278*	L-lactate dehydrogenase B chain	LDHB_HUMAN	37 / 37	5.7 / 6.2	8 / 11	354	102	37	glycolysis
1380	Elongation factor 1-beta	EF1B_HUMAN	25 / 32	4.5 / 4.5	1 / 5 ^{\$}	103	103	29	translation
1406	Tropomyosin alpha-4 chain	TPM4_HUMAN	29 / 31	4.7 / 4.9	6 / 13	193	71	43	cytoskeleton
1442	Elongation factor 1-delta	EF1D_HUMAN	31 / 29	4.9 / 5.3	8 / 10	452	99	47	translation
1462*	Adenylate kinase isoenzyme 2, mitochondrial	KAD2_HUMAN	26 / 27	7.7 / 8.9	5 / 8	126	62	41	growth regulation
1474*	Phosphoglycerate mutase 1	PGAM1_HUMAN	29 / 28	6.7 / 7.3	7 / 9	192	62	57	glycolysis
1505*	Complement component 1 Q subcomponent-binding protein, mitochondrial precursor	C1QBP_HUMAN	31 / 27	4.7 / 6.4	3 / 4	99	45	29	not determined
1516*	Triosephosphate isomerase [†]	TPIS_HUMAN	27 / 26	6.5 / 7.3	8 / 13	608	124	71	glycolysis

1519	Heat shock protein beta-1	HSPB1_HUMAN	23 / 26	6.0 / 6.8	6 / 9	401	151	52	chaperone
1521	Peroxiredoxin-6	PRDX6_HUMAN	25 / 26	6.0 / 6.9	9 / 11	535	87	54	oxido-reduction
1557*	Ubiquitin carboxyl-terminal hydrolase isozyme L1	UCHL1_HUMAN	25 / 25	5.3 / 5.9	6 / 10	347	85	58	proteolysis
1600*	Thioredoxin-dependent peroxide reductase, mitochondrial precursor	PRDX3_HUMAN	28 / 25	7.7 / 6.4	5 / 6	256	85	28	oxido-reduction
1607	Glutathione S-transferase P	GSTP1_HUMAN	23 / 25	5.4 / 6.0	6 / 11	464	155	64	anti-apoptosis
1728	Myosin regulatory light chain 2, nonsarcomeric	MLRM_HUMAN	20 / 18	4.7 / 5.0	6 / 7	256	96	47	cytoskeleton
1809	Myosin light polypeptide 6	MYL6_HUMAN	17 / 15	4.6 / 4.6	5 / 10	231	75	61	cytoskeleton
1817	Thioredoxin-dependent peroxide reductase, mitochondrial precursor	PRDX3_HUMAN	28 / 15	7.7 / 6.8	5 / 6	211	61	44	oxido-reduction
2077	Glyceraldehyde-3-phosphate dehydrogenase †	G3P_HUMAN	36 / 36	8.6 / 5.8	4 / 6	100	52	28	glycolysis
2083	Plastin-3	PLST_HUMAN	70 / 73	5.5 / 6.3	9 / 17	388	91	34	cytoskeleton
2108	GMP synthase [glutamine-hydrolyzing]	GUAA_HUMAN	77 / 75	6.4 / 7.1	7 / 11	172	45	23	purine base synthesis
2134*	Alpha-enolase †	ENOA_HUMAN	47 / 50	7.0 / 7.5	7 / 12	348	106	44	glycolysis
2137*	Caldesmon	CALD1_HUMAN	93 / 75	5.6 / 6.9	2 / 2	113	69	4	cytoskeleton
2151*	Succinyl-CoA:3-ketoacid-coenzyme A transferase 1, mitochondrial precursor	SCOT_HUMAN	56 / 60	7.1 / 6.8	5 / 6	200	68	21	ketone body catabolism
2152	Lamin-A/C †	LMNA_HUMAN	74 / 69	6.6 / 7.3	10 / 28	542	91	44	cytoskeleton

number of unique peptides identified in MS/MS / in MS+MS/MS searches.

* protein spots that are also recognized by IgG in the pool of sera from healthy individuals.

† proteins that are recognized by IgG in propiolactone-IVlg both in HUVEC and HEp-2 cell extracts.

§ proteins identified with a single peptide. MS/MS spectra with fragments assignments are given in Supplementary Figure 1.

GMP: guanosine monophosphate; LIM: Lin-11/Isi-1/Mec-3; MW: molecular weight; PDZ: postsynaptic density-95/discs large/zonula occludens-1; TU: thermo-unstable.

Supplementary Table 2. HEp-2 cell proteins recognized by IgG in propiolactone-IVlg.

Protein ID on gel	Protein	SwissProt accession number	Theoretical / Estimated MW (kDa)	Theoretical / Estimated pI	Mass spectrometry				Nuclear localization	Function
					Number of unique identified peptides [#]	Total ion score	Best ion score	Sequence coverage (%)		
303	C-1-tetrahydrofolate synthase, cytoplasmic	C1TC_HUMAN	101 / 106	6.9 / 7.9	9 / 13	82	21	19		tetrahydrofolate pathway
325*	Elongation factor 2	EF2_HUMAN	95 / 100	6.4 / 7.5	11 / 30	714	110	47		translation
328*	Elongation factor 2	EF2_HUMAN	95 / 100	6.4 / 7.3	12 / 26	358	50	38		translation
348*	Elongation factor 2	EF2_HUMAN	95 / 101	6.4 / 7.2	12 / 27	347	61	37		translation
355*	DNA replication licensing factor MCM4	MCM4_HUMAN	96 / 99	6.3 / 7.0	6 / 18	268	74	25	+	mitosis
357	Transitional endoplasmic reticulum ATPase	TERA_HUMAN	89 / 98	5.1 / 5.7	12 / 29	451	65	49	+	ATPase activity
412*	ATP-dependent RNA helicase DDX1	DDX1_HUMAN	82 / 90	6.8 / 7.6	12 / 24	573	91	45	+	RNA processing
423	Far upstream element-binding protein 2 [†]	FUBP2_HUMAN	73 / 88	6.8 / 7.2	12 / 20	434	83	35	+	RNA processing
425	Far upstream element-binding protein 2 [†]	FUBP2_HUMAN	73 / 87	6.8 / 7.3	11 / 21	686	120	38	+	RNA processing
430	Far upstream element-binding protein 2 [†]	FUBP2_HUMAN	73 / 86	6.8 / 7.5	12 / 22	736	114	40	+	RNA processing
461*	Far upstream element-binding protein 2 [†]	FUBP2_HUMAN	73 / 83	6.8 / 7.2	12 / 20	479	93	37	+	RNA processing
463	Far upstream element-binding protein 2 [†]	FUBP2_HUMAN	73 / 83	6.8 / 7.4	11 / 20	563	104	38	+	RNA processing
480	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial precursor	NDUS1_HUMAN	79 / 81	5.9 / 6.0	5 / 15	177	57	31		oxido-reduction
483*	Cytoplasmic dynein 1 intermediate chain 2	DC1I2_HUMAN	71 / 80	5.1 / 5.7	6 / 11	324	103	31		cytoskeleton
485	78 kDa glucose-regulated protein precursor	GRP78_HUMAN	72 / 79	5.1 / 5.5	12 / 21	567	87	44	+	chaperone
502*	Lamin-A/C [†]	LMNA_HUMAN	74 / 78	6.6 / 7.5	13 / 32	851	98	56	+	cytoskeleton
536*	Heat shock cognate 71 kDa protein [†]	HSP7C_HUMAN	71 / 72	5.4 / 5.9	10 / 23	797	143	52	+	chaperone
546*	Far upstream element-binding protein 1 [†]	FUBP1_HUMAN	68 / 72	7.2 / 7.9	11 / 23	759	105	50	+	transcription regulation
574	Transketolase	TKT_HUMAN	68 / 70	7.6 / 8.3	11 / 17	430	101	42		pentose phosphate pathway
578	Transketolase	TKT_HUMAN	68 / 71	7.6 / 8.1	11 / 16	398	86	40		pentose phosphate pathway
580*	Heat shock 70 kDa protein 1	HSP71_HUMAN	70 / 70	5.5 / 6.0	13 / 22	976	125	53	+	chaperone
632*	Lamin-A/C [†]	LMNA_HUMAN	74 / 65	6.6 / 7.3	12 / 27	645	88	43	+	cytoskeleton
646*	Heterogeneous nuclear ribonucleoprotein L [†]	HNRPL_HUMAN	60 / 65	6.7 / 7.6	8 / 19	419	104	51	+	RNA processing
667	T-complex protein 1 subunit alpha	TCPA_HUMAN	60 / 63	5.8 / 6.5	7 / 17	322	63	41		chaperone
669*	Heterogeneous nuclear ribonucleoprotein K [†]	HNRPK_HUMAN	51 / 62	5.4 / 6.0	7 / 18	538	121	50	+	RNA processing
672*	T-complex protein 1 subunit zeta	TCPZ_HUMAN	58 / 62	6.2 / 6.9	7 / 17	303	91	45		chaperone

674	Pyruvate kinase isozymes M1/M2	KPYM_HUMAN	58 / 62	8.0 / 8.4	9 / 17	373	76	43		glycolysis
677	Splicing factor U2AF 65 kDa subunit	U2AF2_HUMAN	53 / 62	9.2 / 7.0	7 / 10	299	80	33	+	RNA processing
716	Phenylalanyl-tRNA synthetase alpha chain	SYFA_HUMAN	58 / 59	7.3 / 8.1	4 / 10	124	47	26		RNA processing
728*	Splicing factor 3A subunit 3	SF3A3_HUMAN	59 / 58	5.3 / 5.8	10 / 15	465	90	41	+	RNA processing
737*	UPF0027 protein C22orf28	CV028_HUMAN	55 / 57	6.8 / 7.4	9 / 21	258	67	48		not documented
738*	UPF0027 protein C22orf28	CV028_HUMAN	55 / 57	6.8 / 7.6	7 / 21	207	49	50		not documented
751	Protein disulfide-isomerase A3 precursor [†]	PDIA3_HUMAN	57 / 56	6.0 / 6.3	11 / 17	512	74	43		protein folding
761*	Dihydrolipoyl dehydrogenase, mitochondrial precursor	DLDH_HUMAN	54 / 56	7.6 / 7.4	5 / 8	117	42	21		oxido-reduction
766	Tubulin alpha-1b chain	TBA1B_HUMAN	50 / 55	4.9 / 5.6	12 / 17	867	105	52		cytoskeleton
775*	Tryptophanyl-tRNA synthetase, cytoplasmic	SYWC_HUMAN	53 / 54	5.8 / 6.5	7 / 9	251	64	27		RNA processing
796*	Keratin, type II cytoskeletal 7	K2C7_HUMAN	51 / 52	5.5 / 6.0	10 / 23	834	109	49		cytoskeleton
800*	Keratin, type II cytoskeletal 8	K2C8_HUMAN	54 / 52	5.5 / 6.2	10 / 27	752	112	69		cytoskeleton
803*	Glutamate dehydrogenase 1, mitochondrial precursor	DHE3_HUMAN	61 / 52	7.7 / 7.5	9 / 16	385	98	34		oxido-reduction
804	Glutamate dehydrogenase 1, mitochondrial precursor	DHE3_HUMAN	61 / 52	7.7 / 7.3	7 / 16	199	53	32		oxido-reduction
812*	Heterogeneous nuclear ribonucleoprotein H [†]	HNRH1_HUMAN	49 / 51	5.9 / 6.5	8 / 13	609	109	46	+	RNA processing
815*	Septin-11	SEP11_HUMAN	49 / 51	6.4 / 7.1	10 / 17	408	77	52	+	mitosis
819*	ATP synthase subunit beta, mitochondrial precursor [†]	ATPB_HUMAN	57 / 50	5.3 / 5.5	12 / 22	913	121	68		oxido-reduction
823*	Cleavage stimulation factor 50 kDa subunit	CSTF1_HUMAN	48 / 49	6.1 / 6.9	10 / 12	464	77	39	+	RNA processing
837	Splicing factor 3B subunit 4	SF3B4_HUMAN	44 / 48	8.5 / 9.4	4 / 5	140	59	23	+	RNA processing
851*	Alpha-enolase [†]	ENOA_HUMAN	47 / 47	7.0 / 7.5	11 / 16	976	146	58		glycolysis
858*	Alpha-enolase [†]	ENOA_HUMAN	47 / 47	7.0 / 7.2	11 / 14	840	151	55		glycolysis
880*	Actin-like protein 6A	ACL6A_HUMAN	47 / 47	5.4 / 5.9	7 / 11	329	83	39	+	transcription regulation
899*	Regulator of chromosome condensation	RCC1_HUMAN	45 / 46	7.2 / 7.6	6 / 11	279	74	46	+	mitosis
921*	Keratin, type I cytoskeletal 18	K1C18_HUMAN	48 / 44	5.3 / 6.0	10 / 22	713	117	62		cytoskeleton
934	Phosphoglycerate kinase 1	PGK1_HUMAN	45 / 43	8.3 / 9.0	9 / 22	548	133	62		glycolysis
948*	Actin, cytoplasmic 2 [†]	ACTG_HUMAN	42 / 43	5.3 / 5.8	6 / 13	537	135	52		cytoskeleton
966	Septin-2	SEPT2_HUMAN	41 / 42	6.2 / 6.8	9 / 12	665	108	52	+	mitosis
977	Nuclear inhibitor of protein phosphatase 1	PP1R8_HUMAN	38 / 41	6.9 / 7.6	5 / 11	148	54	51	+	RNA processing
980	Poly(rC)-binding protein 2	PCBP2_HUMAN	39 / 41	6.3 / 6.9	2 / 10	53	39	38	+	RNA processing
997	Fructose-bisphosphate aldolase A	ALDOA_HUMAN	39 / 40	8.3 / 8.9	9 / 17	475	97	64		glycolysis
1008	Mitotic checkpoint protein BUB3	BUB3_HUMAN	37 / 40	6.4 / 7.2	8 / 13	292	61	47	+	mitosis
1062	Heterogeneous nuclear ribonucleoprotein A/B	ROAA_HUMAN	36 / 36	8.2 / 7.6	5 / 8	155	43	24	+	RNA processing
1068*	Glyceraldehyde-3-phosphate dehydrogenase [†]	G3P_HUMAN	36 / 36	8.6 / 8.6	7 / 9	443	117	45		glycolysis
1069	Glyceraldehyde-3-phosphate dehydrogenase [†]	G3P_HUMAN	36 / 36	8.6 / 8.6	7 / 9	403	121	43		glycolysis
1072*	Glyceraldehyde-3-phosphate dehydrogenase [†]	G3P_HUMAN	36 / 36	8.6 / 9.2	6 / 10	306	118	46		glycolysis

1073	Glyceraldehyde-3-phosphate dehydrogenase [†]	G3P_HUMAN	36 / 36	8.6 / 9.0	6 / 9	384	122	45	glycolysis
1074	Glyceraldehyde-3-phosphate dehydrogenase [†]	G3P_HUMAN	36 / 36	8.6 / 9.1	6 / 11	327	118	56	glycolysis
1075	Glyceraldehyde-3-phosphate dehydrogenase [†]	G3P_HUMAN	36 / 36	8.6 / 9.5	5 / 9	347	125	41	glycolysis
1079*	Glyceraldehyde-3-phosphate dehydrogenase [†]	G3P_HUMAN	36 / 35	8.6 / 9.8	5 / 8	290	101	41	glycolysis
1091	60S acidic ribosomal protein P0 [†]	RLA0_HUMAN	34 / 36	5.7 / 6.1	3 / 8	105	70	33	translation
1103	Annexin A2	ANXA2_HUMAN	39 / 35	7.6 / 8.2	14 / 15	1132	138	55	membrane-binding protein
1106	Heterogeneous nuclear ribonucleoprotein D-like	HNRDL_HUMAN	46 / 35	9.6 / 7.2	6 / 9	184	59	21	RNA processing
1124*	Heterogeneous nuclear ribonucleoproteins A2/B1	ROA2_HUMAN	37 / 34	9.0 / 9.2	9 / 15	576	123	50	RNA processing
1131	Heterogeneous nuclear ribonucleoproteins A2/B1	ROA2_HUMAN	37 / 34	9.0 / 8.6	9 / 15	445	92	49	RNA processing
1134*	Heterogeneous nuclear ribonucleoproteins A2/B1	ROA2_HUMAN	37 / 33	9.0 / 9.7	10 / 11	575	110	41	RNA processing
1136*	Heterogeneous nuclear ribonucleoproteins A2/B1	ROA2_HUMAN	37 / 34	9.0 / 9.5	9 / 13	515	88	47	RNA processing
1137*	Heterogeneous nuclear ribonucleoproteins A2/B1	ROA2_HUMAN	37 / 33	9.0 / 9.8	10 / 15	730	116	47	RNA processing
1176	Guanine nucleotide-binding protein subunit beta-2-like 1	GBLP_HUMAN	35 / 30	7.6 / 8.1	13 / 13	574	115	70	signal transduction
1197	Heterogeneous nuclear ribonucleoprotein A1	ROA1_HUMAN	39 / 30	9.3 / 8.5	8 / 11	434	114	30	RNA processing
1201*	Heterogeneous nuclear ribonucleoproteins A2/B1	ROA2_HUMAN	37 / 29	9.0 / 8.0	9 / 15	700	138	46	RNA processing
1214	Heterogeneous nuclear ribonucleoproteins A2/B1	ROA2_HUMAN	37 / 28	9.0 / 8.7	10 / 11	509	98	39	RNA processing
1215*	Galectin-3	LEG3_HUMAN	26 / 28	8.6 / 9.2	3 / 5	135	85	25	lectin
1216*	Galectin-3	LEG3_HUMAN	26 / 28	8.6 / 9.7	2 / 5	114	74	25	lectin
1285	Triosephosphate isomerase [†]	TPIS_HUMAN	27 / 26	6.5 / 7.3	13 / 15	705	108	79	glycolysis
1522*	Peptidyl-prolyl cis-trans isomerase A	PPIA_HUMAN	18 / 16	7.7 / 8.0	4 / 8	278	99	60	protein folding
1524	Peptidyl-prolyl cis-trans isomerase A	PPIA_HUMAN	18 / 16	7.7 / 7.2	5 / 10	313	84	65	protein folding
1525*	Peptidyl-prolyl cis-trans isomerase A	PPIA_HUMAN	18 / 16	7.7 / 8.4	6 / 10	449	109	62	protein folding
1536	Peptidyl-prolyl cis-trans isomerase A	PPIA_HUMAN	18 / 16	7.7 / 7.8	6 / 9	392	139	46	protein folding
1578	Actin, cytoplasmic 1	ACTB_HUMAN	42 / 15	5.3 / 6.9	2 / 5	87	51	24	cytoskeleton
1937*	Protein DEK	DEK_HUMAN	43 / 48	8.7 / 6.2	4 / 9	180	65	23	transcription regulation

number of unique peptides identified in MS/MS / in MS+MS/MS searches.

* protein spots that are also recognized by IgG in the pool of sera from healthy individuals.

† proteins that are recognized by IgG in propiolactone-IVIg both in HUVEC and HEp-2 cell extracts.

BUB: budding uninhibited by benzimidazole; DDX: DEAD box protein; MCM: minichromosome maintenance; MW: molecular weight; tRNA: transfer RNA; U2AF: U2 small nuclear ribonucleoprotein auxiliary factor; UPF: uncharacterized protein family.