

REVIEW

Recent advances in blood-related proteomics

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Blood is divided in two compartments, namely, plasma and cells. The latter contain red blood cells, leukocytes, and platelets. From a descriptive medical discipline, hematology has evolved towards a pioneering discipline where molecular biology has permitted the development of prognostic and diagnostic indicators for disease. The recent advance in MS and protein separation now allows similar progress in the analysis of proteins. Proteomics offers great promise for the study of proteins in plasma/serum, indeed a number of proteomics databases for plasma/serum have been established. This is a very complex body fluid containing lipids, carbohydrates, amino acids, vitamins, nucleic acids, hormones, and proteins. About 1500 different proteins have recently been identified, and a number of potential new markers of diseases have been characterized. Here, examples of the enormous promise of plasma/serum proteomic analysis for diagnostic/prognostic markers and information on disease mechanism are given. Within the blood are also a large number of different blood cell types that potentially hold similar information. Proteomics of red blood cells, until now, has not improved our knowledge of these cells, in contrast to the major progresses achieved while studying platelets and leukocytes. In the future, proteomics will change several aspects of hematology.

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1 Introduction

Despite the fact that examination of peripheral blood and bone marrow smears remains a critical step in the diagnosis of a large variety of hematological disorders, its role has recently been displaced by more sophisticated diagnostic tools like those offered by molecular biology. Nevertheless, the recognition of typical abnormal cytological findings on a blood smear limits the number of possible etiologies, contributing to diagnosis without multiplying laboratory tech-

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Abbreviation: Igs, immunoglobulins

niques. So, from a descriptive medical discipline based on microscopic evaluation of red blood cells, leukocytes, and platelets, hematology has evolved towards a dynamic science at the crossroads of genomics and proteomics. PCR based analyses have radically changed the study of chromosomal translocation products [1]. With the development of DNA microarray technology, gene expression can be analyzed at the mRNA level. The “transcriptome” is the mRNA pool found within a cell. Most often there is poor correlation between mRNA and protein expression. This is due to the fact that changes in the expression pattern at the mRNA level do not necessarily correlate with changes of the functional expression pattern at the protein level [2–5]. The word “proteome” describes the pool of proteins expressed in a cell or a tissue at a given time. Its analysis provides an idea of biological processes happening at their level of occurrence, allowing the comparison of physiological and pathological states of a cell line or a tissue [4, 6]. The challenge of clinical proteomic studies is to link protein expression profiles to specific disease phenotypes and to find out relevant biomarkers in order to develop diagnostic tools [7]. Proteomics involves several complementary technologies. One of the most important tools for proteomic analysis is 2-DE, which has been available since the 1970s. Current 2-DE technology as well as cellular fractionation strategies for proteomics have been recently reviewed by Görg *et al.* [8] and Stasyk and Huber [9], respectively. Since 1990, MS techniques have improved, leading to rapid identification of separated proteins with the help of protein sequence databases [10]. Another way to analyze changes at the protein level is SELDI. This method is of special interest for the identification of new biomarkers in the low molecular weight protein fraction, which may give an early marker of disordered cellular function, at a premalignant stage, for example. The principle of this technique is to use protein chips with an affinity matrix (charge, hydrophobicity, or other). Various wash buf-

fers allow the differential binding of proteins to the surface of the chip, based on the stringency of their binding in various conditions. Differences in protein patterns between tumors and controls can then be elicited. However, SELDI/MS is a partial analysis, not giving sequence data in many cases. Finally, other methods, combining different separation techniques (LC, multidimensional chromatography, tryptic digestion and multidimensional chromatography of peptides, off-gel electrophoresis) followed by MS, are currently more and more employed [11–15]. Recently, because a broad range of mass spectrometers have been used, several investigators proposed a new format for MS data, which will hopefully facilitate data management, interpretation, and dissemination of information in proteomic research [16].

The understanding of hematological diseases, including malignancies, is a critical issue and can be improved with the help of proteomics. A number of proteomic databases dealing with plasma/serum and/or blood cells are now available on the internet (Table 1). The purpose of this paper is to review the advances in hematology, obtained by proteomic research done over the last 5 years.

2 Plasma/serum

2.1 Overview

Blood plasma is a complex body fluid, which contains a large diversity of proteins. Their concentrations range over at least 15 orders of magnitude, resulting in an enormous range that precludes full analysis of low abundance peptide/protein entities using MS, unless the sample is fractionated or otherwise processed. Intact as well as partially degraded proteins or protein fragments circulate in the blood. Furthermore, genetic polymorphisms as well as numerous post-translationally modified forms of proteins are present,

Table 1. Some internet sites devoted to blood proteomics

Subject	Internet address
Epstein-Barr virus transformed B-lymphoblastoid cells	http://proteome.tmig.or.jp/2D/
Hematopoietic cell lines	http://www-smbh.univ-paris13.fr/lbtp/Biochemistry/Biochimie/bque.htm
Human plasma proteome project (HUPO)	http://www.hupo.org
Human platelets	http://www.bioch.ox.ac.uk/glycob/ogp/
Human serum proteome	http://bpp.nci.nih.gov
Index to 2-D PAGE databases and services	http://www.expasy.org/ch2d/2d-index.html
Jurkat-T cell	http://www.mpiib-berlin.mpg.de/2D-PAGE/organisms/index.html
Myeloid development	http://bioinfo.mbb.yale.edu/expression/myelopoiesis/
Proteomics and protein structure	http://linux.farma.unimi.it/
Swiss-2DPAGE (lymphocyte)	http://www.expasy.org/cgi-bin/map2/def?LYMPHOCYTE_HUMAN
Swiss-2DPAGE (lymphoma)	http://www.expasy.org/cgi-bin/map2/def?LYMPHOMA_HUMAN
Swiss-2DPAGE (macrophage)	http://www.expasy.org/cgi-bin/map2/def?U937_HUMAN
Swiss-2DPAGE (plasma)	http://www.expasy.org/cgi-bin/map2/def?PLASMA_HUMAN
Swiss-2DPAGE (platelets)	http://www.expasy.org/cgi-bin/map2/def?PLATELET_HUMAN
Swiss-2DPAGE (red blood cells)	http://www.expasy.org/cgi-bin/map2/def?RBC_HUMAN
The plasma proteome institute	http://www.plasmaproteome.org/

adding to the diversity complexity of plasma proteins. The other constituents of plasma are salts, lipids, amino acids, vitamins, and carbohydrates. The core plasma protein is albumin, representing about 50% of the plasma protein content and being present at high concentration (in the order of 30 g/L). The other main plasma proteins (in the order of g/L) are immunoglobulins (Igs), fibrinogen, transferrin, haptoglobin, and lipoproteins. Several approaches have been proposed to remove these abundant proteins prior to proteomic analyses [17–20]. Removal of fibrinogen (Factor I) is easily obtained by clotting. However, this method induced the disappearance of many other proteins involved in the coagulation process such as prothrombin (Factor II), which is absent in serum [21]. Inversely, many peptides, absent in plasma but detectable in serum, can be identified after coagulation. It is therefore of importance to have good pre-analytical conditions when mining the proteome of plasma, and more particularly its “peptidome”, in which about 5000 peptides have been revealed [22].

Over 10 000 different proteins have been estimated to be commonly present in the plasma, most of which are at very low relative abundances [23]. A major challenge of proteomic analysis of plasma is the low abundance proteins, like (1) proteins or peptides resulting from tissue leakage, (2) proteins released from normal cells as a result of cell death or damage, (3) proteins released from tumor cells as aberrant secretions. These proteins may represent important potential markers for diagnosis [24]. In addition, both plasma proteome and peptidome are modified after stress, sleep, sport training, or meals, and in women during pregnancy.

2.2 Descriptive plasma/serum proteomics

In the last 5 years, important progresses have been achieved in characterization of the plasma/serum proteome [24]. Most of the relevant information is available in three leading articles published in 2004 [25–27]. Combination of several approaches, including a literature survey, permitted Anderson *et al.* [25] to build one of the most precise available lists of human plasma proteins. Chan *et al.* [26] analyzed the serum proteome by combining multidimensional peptide separation strategies and MS/MS [26]. They identified 1444 unique proteins and constructed a public database of human serum proteome (Table 1). These studies have shown the potential of proteomics to explore plasma/serum, in which about 1500 different proteins have been identified so far. Low abundance proteins such as leptin and ghrelin as well as peptides such as bradykinin, which are not detected using 2-DE only, were detected [27].

The lymph, a plasma-derived biologic fluid, was also analyzed by proteomic techniques [28]. Using SELDI-TOF MS, 2-DE, and MS, two proteins could be pointed out which were differentially expressed in the lymph and the plasma (glial fibrillary astrocyte acidic protein and neutrophil cytosol factor-1).

2.3 Clinical applications – biomarker identification

Plasma/serum protein electrophoresis is widely used in clinical laboratories, especially for the evaluation of protein changes associated with inflammation, liver, or kidney diseases as well as for the detection and identification of paraproteins [29, 30]. Among the different electrophoretic techniques available, 2-DE has a very limited role in clinical laboratories. Nevertheless, it is still a cornerstone for the study of plasma/serum proteomics, because it allows analysis of the microheterogeneity of proteins, either due to genetic polymorphisms or PTMs [31–33]. Figure 1 shows a typical example of genetic polymorphism (haptoglobin α and β chains) easily identified in 2-DE of plasma samples [34]. However, several biochemical modifications appear to be superimposed on genetic polymorphisms. Using MS protein structure characterization, the cause of migrational differences of haptoglobin α chains in 2-DE was elucidated [35, 36]. It was shown that at least three structurally different protein species can be differentiated and accounted for the most commonly observed spot patterns of haptoglobin α chains. As shown in Table 2, haptoglobin has been shown to be a potential marker of cancer [37–39]. Watanabe *et al.* [40], after having analyzed serum samples from pregnant women by 2-DE, were able to identify in women presenting with preeclampsia a group of overexpressed spots corresponding to clusterin. In a large series, they confirmed that the levels of clusterin were significantly higher in pregnant women with preeclampsia than without [40]. Also using 2-DE and MS analyses, Kwak *et al.* [41] showed that the serum proteome of patients with acute myeloid leukemia differed from that of controls. Eight peptides were differently expressed in patients when compared to controls, for example, α -2-HS-glycoprotein, complement-associated SP-40, lipoprotein C-III. Interestingly, haptoglobin-1 was up-regulated in patients presenting with acute leukemia. 2-DE was shown to be able to demonstrate the influence of photochemical reactions on plasma proteins such as those induced, for example, by methylene blue and visible light (Fig. 2), one of the techniques used to inactivate viruses in plasma in transfusion medicine [42]. By contrast, inactivation of viruses using the solvent/detergent method (tri-(*N*-butyl)-phosphate and Triton X-100) was apparently not associated with modifications of the 2-DE spot pattern of plasma proteins (Fig. 3). However, other functional protein alterations of plasma proteins, such as protein S and α 2-antiplasmin, have been described while using the solvent/detergent method [43, 44].

Analysis of monoclonal gammopathies [45, 46] and characterization of cryoproteins [47–49] using 2-DE has been shown to be useful in selected difficult clinical situations. Using 2-DE analyses and MS, we also recently identified a new peptide, called hSp α or CD5L [50]. This peptide was only found when IgM were present, like in cryoglobulins (Fig. 4A), and was found as being strongly associated with IgM. CD5L is a soluble protein expressed in lymphoid tissues (spleen, lymph node, thymus, and bone marrow), for

Table 2. Examples of plasma/serum biomarkers

Context	Methods	Protein(s) identified	References
Lung cancer	2-DE	Haptoglobin	[38]
Ovarian cancer	2-DE	Haptoglobin	[39]
Pregnancy (preeclampsia)	2-DE	Clusterin	[40]
Acute myeloid leukemia	2-DE	α -2-HS glycoprotein, complement-associated SP-40, apolipoprotein C-III, haptoglobin	[41]
Prostate cancer	SELDI	Abnormal protein profile	[68]
Ovarian cancer	SELDI	Apolipoprotein A-I, transthyretin, fragment of inter- α -trypsin	[69]
Pregnancy (ectopic)	SELDI	Abnormal protein profile	[70]
Stroke	SELDI	Heart fatty acid, apolipoprotein C-I, apolipoprotein C-III	[71, 72]
Heart infarction	2-DE	α -1-antitrypsin isoform 1	[73]
Benzene exposure	2-DE	T cell receptor β chain, FK506-binding protein, matrix metalloproteinase-13	[75]
Polycyclic hydrocarbon exposure	2-DE	Putative capacitative calcium entry channel	[76]
Severe acute respiratory syndrome	2-DE	Truncated α -1-antitrypsin	[77]
Severe acute respiratory syndrome	2-DE	Peroxiredoxin II	[78]
Cardiac allograft rejection	2-DE	α B-crystallin, tropomyosin	[81]

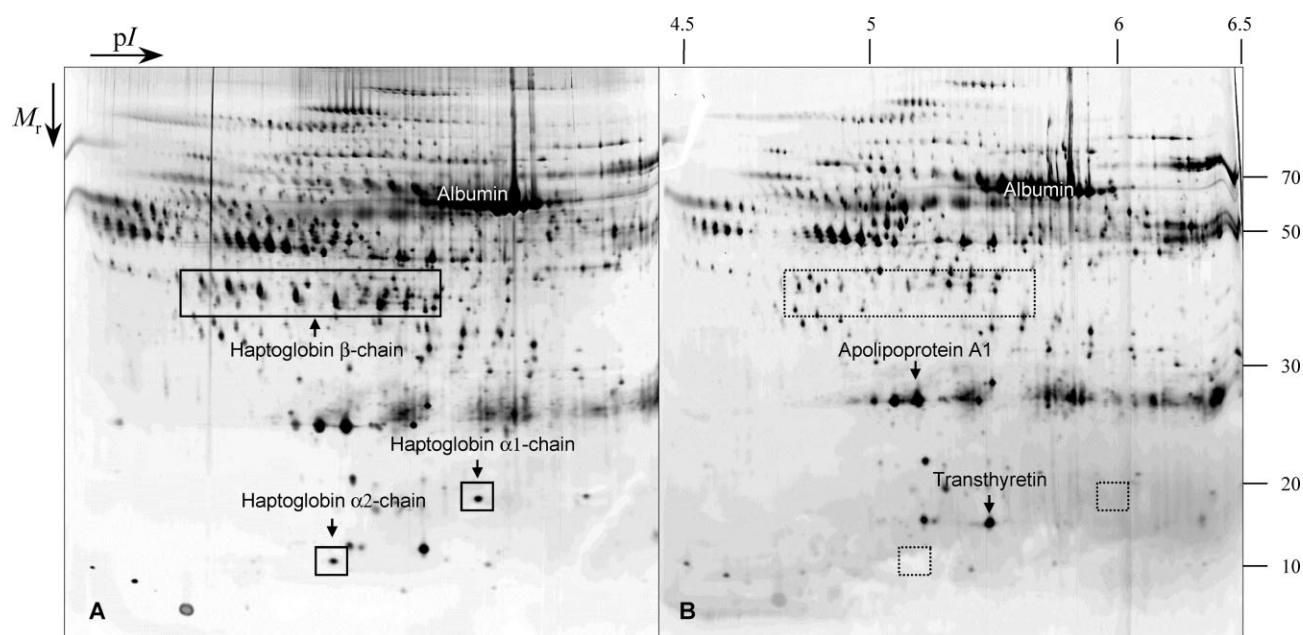


Figure 1. Genetic polymorphism of plasma proteins evidenced by 2-DE (within the frames). Panel A shows the 2-D pattern of the plasma of a “normal” blood donor. In panel B, several spots arrowed in panel A are absent (deficiency in haptoglobins β chain, and genetic polymorphism of haptoglobin α 1 and α 2 chains). The picture shows silver-stained 2-D gels; first dimension: immobilized 4 to 7 pH gradient, second dimension: 9–16% PAGE. Twenty-five micrograms of plasma proteins were loaded onto the first dimension gel.

which little functional and structural information is available [51]. It belongs to group B of the scavenger receptor cysteine-rich superfamily, which includes the lymphocyte surface receptors CD5 and CD6 amongst others. CD5L is able to bind to different cells of the immune system (monocytes and lymphocytes), which suggests that it may play an important role in the regulation of the immune system. CD5L is a relatively abundant serum protein (60 μ g/mL) circulating

essentially in association with other serum proteins, notably IgM, as evidenced by the study of purified polyclonal or monoclonal IgM fractions isolated from human plasma (Fig. 4B–D). Previous studies in mice have implicated mSp α in the control of macrophage survival, the protein being a negative regulator of apoptosis [52, 53]. It was shown in mice that mSp α is a direct target for regulation by the liver X receptor α [54]. Taken together, these results indicate that an

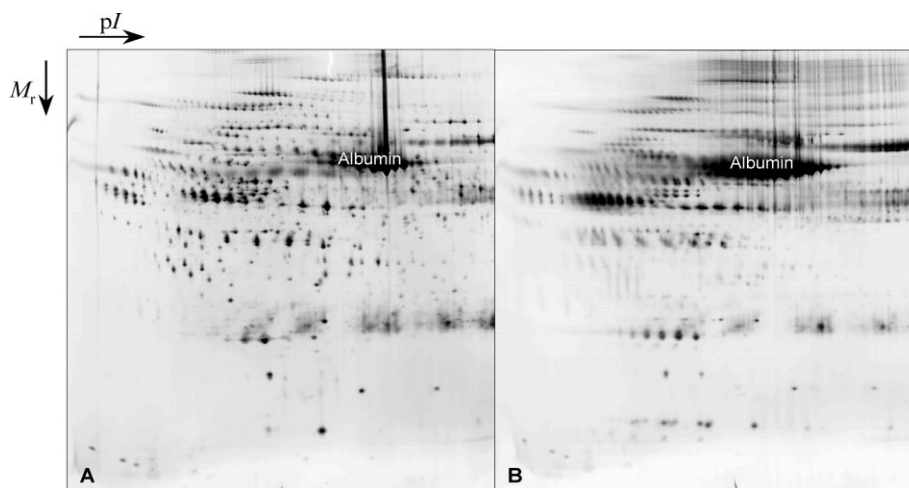


Figure 2. Influence of high concentration of methylene blue (50 mmol) and prolonged exposure to visible light (3 h) on plasma proteins. (A) Control plasma, (B) treated plasma. The picture shows silver-stained 2-D gels; first dimension: immobilized 4 to 7 pH gradient, second dimension: 9–16% PAGE. Twenty-five micrograms of plasma proteins were loaded onto the first dimension gel. Note that the 2-D pattern (acidic shift of most plasma protein spots) is completely disturbed by the photochemical treatment.

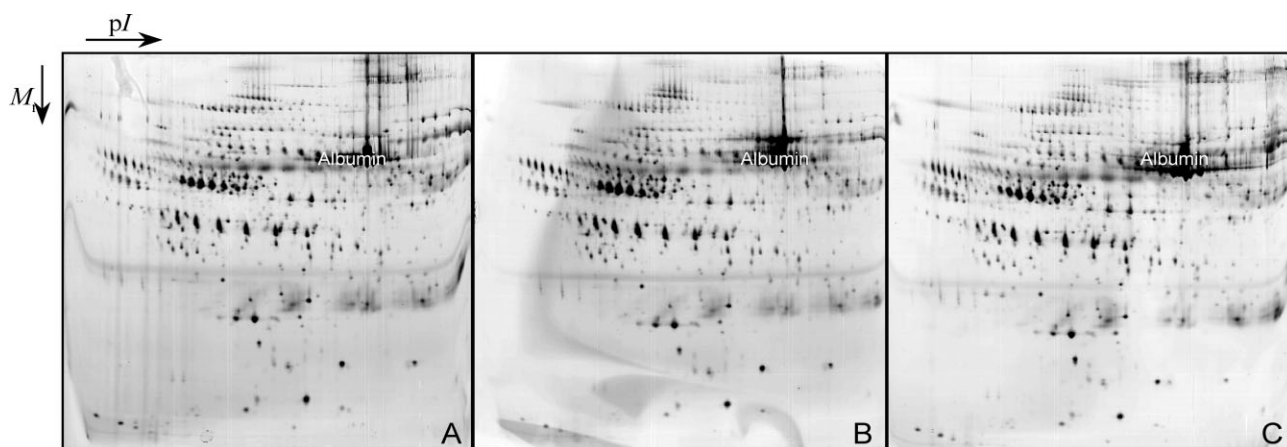


Figure 3. Influence of solvent/detergent (1% tri-*(N)*-butyl-phosphate and 1% Triton X-100) on plasma proteins. (A) Plasma pool from Octapharma, Uniplas® (ABO universal plasma). (B) Plasma pool from Octapharma, Octaplas® (non-ABO universal plasma). (C) Nontreated plasma pool of 750 samples from voluntary blood donors (control). The picture shows silver-stained 2-D gels; first dimension: immobilized 4 to 7 pH gradient, second dimension: 9–16% PAGE. Twenty-five micrograms of plasma proteins were loaded onto the first dimension gel. Note the absence of apparent modification of the 2-D patterns after solvent/detergent treatment.

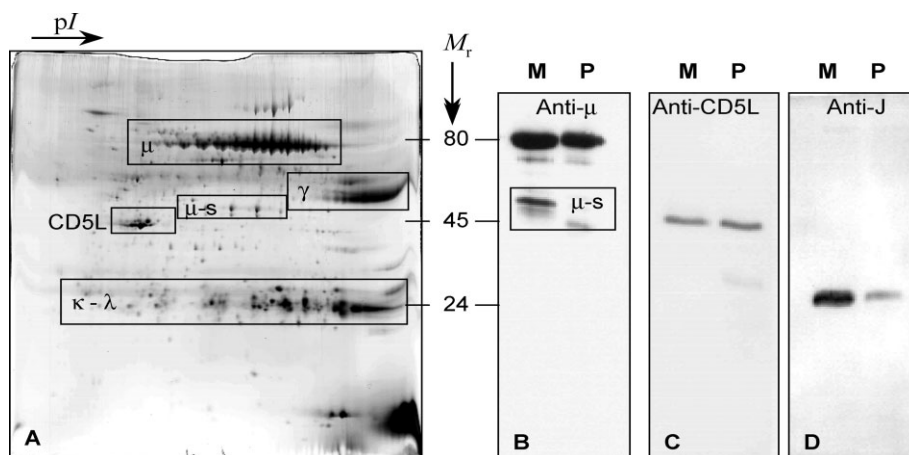


Figure 4. Type III cryoglobulins (polyclonal IgM mixed with polyclonal IgG) analyzed by 2-DE (silver-stained 2-D gel; first dimension: immobilized 3 to 10 nonlinear pH gradient, second dimension: 9–16% PAGE). μ , polyclonal IgM heavy chains; γ , polyclonal IgG heavy chains; κ - λ , polyclonal Ig light chains; μ -s, fragments of the constant part of IgM heavy chains;

CD5L, h-Sp α (IgM associated peptide). Twenty-five micrograms of proteins were loaded onto the first dimension gels. Association of CD5L with IgM is demonstrated by immunoblots (panels B and C) of purified monoclonal (M) and polyclonal (P) IgM fractions with anti- μ , anti-CD5L, and J-chain antibodies. Anti-CD5L were kindly provided by Dr. F. Lozano, Servei d'Immunologia, Hospital Clinic, Barcelona, Spain.

unexpected link exists between humeral immunity, innate immunity, and the common nuclear receptor pathways that mediate macrophage responses to modified lipoproteins.

Various non-2-DE based approaches have been successfully developed to study the proteome of biological samples, and a number of new potential plasma/serum biological markers have been described [55]. Proteomics is now established in the medical community and most university hospitals develop such an approach to ameliorate patient care. Nevertheless, as recently pointed out by N. L. Anderson, proteomics for biomarker discovery has many important limitations [56].

By combining protein chip arrays with high-resolution MS, notably SELDI-TOF MS, the analysis of the plasma/serum proteome appears to be a rapidly emerging area for both biomarker discovery and clinical diagnostics [57–59], especially in oncology [13, 60–63]. However, the reproducibility of SELDI-TOF MS approaches have been questioned [64–67]. Nevertheless, interesting results have been obtained by the evaluation of SELDI-TOF MS patterns of the serum of patients with total prostate specific antigen levels between 2.5 and 15.0 ng/mL, presenting either with prostate cancer or with benign prostate pathologies [68]. Using artificial intelligence based on pattern recognition algorithms, it was possible to find patterns distinguishing these two categories of patients. Recently, three biomarkers, identified through proteomic analysis of the serum of patients presenting with early stage ovarian cancer, have been validated [69]. SELDI-TOF MS allowed the identification of apolipoprotein A1 (down-regulated in cancer), a truncated form of transthyretin (down-regulated), and a cleavage fragment of inter- α -trypsin inhibitor heavy chain H4 (up-regulated). The sensitivity of a multivariate model combining these three markers was higher than that of CA 125 alone. Using the same technique, Gerton *et al.* [70] showed that several serum proteins in the molecular weight range of 7500–18 000 Da were able to discriminate ectopic from intrauterine pregnancies. Heart fatty acid binding protein as well as apolipoprotein C-I and C-III have been proposed as potential biological markers for the diagnosis of stroke [71, 72]. Interestingly, apolipoprotein C-I and C-III appeared to be the first reported plasmatic biomarkers able to accurately distinguish between ischemic and hemorrhagic stroke within a small number of patients [72]. Proteomic analysis of plasma from patients suffering from acute myocardial infarction revealed also modifications of the protein pattern different from that of patients with unstable angina [73]. Proteomics has allowed the detection of drug-induced toxicity, and toxicoproteomics has developed [74]. The proteins in the plasma of workers exposed to benzene were analyzed by 2-DE by Joo *et al.* [75] who showed two significantly different protein profiles in workers exposed to the toxic when compared to controls. Using MALDI-TOF, they identified T cell receptor β chain, FK506-binding protein, and matrix metalloproteinase-13 as being up-regulated in the benzene-exposed workers. In addition, the plasmatic T cell receptor β chain could be used for the early detection of

exposure to benzene. A study of the plasma of workers occupationally exposed to polycyclic hydrocarbons, combining 2-DE with MALDI-TOF MS, allowed Kap-Soon *et al.* [76] to identify several proteins as markers of such an exposure. An up-regulated protein (putative capacitative calcium entry channel) appeared to be a representative marker of exposure. By the same technique, truncated forms of α 1-antitrypsin were discovered in serum samples of patients presenting with severe acute respiratory syndrome [77]. Chen *et al.* [78] studied four patients with severe acute respiratory syndrome. After 2-DE, a total of 38 differential spots were selected for protein identification, and most of them corresponded to acute phase proteins. The authors also identified proteins such as peroxiredoxin II, not detected before on plasma 2-D. Comparative proteome analysis of human plasma and LC-MS/MS analysis allowed Qian *et al.* [79] to identify 32 proteins that were significantly increased after lipopolysaccharide administration. Among the proteins identified, several known inflammatory and acute phase proteins were pointed out.

After infusion of stable isotopes of amino acids followed by collection of plasma samples, van Eijk and Deutz [80] evaluated protein synthesis according to the incorporation of labeled isotopes in peptides identified by ESI-LC MS.

The discovery of markers detecting graft organ rejection using a blood assay is an important task, however, until recently, no tests have proved reliable enough to replace invasive histological investigations. Using 2-DE and MS, Borozdenkova *et al.* [81] showed however that α B-crystallin and tropomyosin may be novel serum markers of human cardiac allograft rejection.

2.4 Amyloidosis and other deposition diseases

Amyloidosis consists in a group of diseases characterized by the accumulation of an amorphous, eosinophilic, hyaline extracellular deposit. The physical nature of amyloid under electron microscopy is predominantly a fibrillar protein with a β -pleated sheet structure. Amyloid also contains P components and other glycoproteins [82, 83]. There are several distinct proteins which have been found in amyloid deposits of a variety of clinical settings. In systemic amyloidosis, proteins such as immunoglobulin light chain, serum amyloid A, transthyretin, fibrinogen α chain, cystatin C, β 2-microglobulin, lysozyme, gelsolin, apolipoprotein A-I, or apolipoprotein A-II may be involved [84]. MS analysis of immunoaffinity purified transthyretin from plasma samples of six patients previously diagnosed with amyloidosis and of 25 controls, allowed identification of the variants of transthyretin responsible for the disease [85]. The use of proteomics to identify abnormal proteins and more particularly Ig light chains will certainly be very promising. Via high-resolution MS, it was possible to characterize in details both Ig heavy and light chains [47]. Crystal-storing histiocytosis is a rare event associated with monoclonal gammopathy. The intracellular crystal formation is almost always accompanied

by the expression of κ light chains, as shown by Lebeau *et al.* [86]. A general approach, derived from the one described by Nedelkov *et al.* [87], will certainly have a place in the study of abnormal plasma proteins involved in deposition diseases. The authors reported a high-throughput comprehensive analysis for assaying proteins directly from human plasma. Proteins are selectively retrieved by using antibodies immobilized within affinity pipette tips and eluted onto enzymatically active MS targets for subsequent digestion and structural characterization. The approach has been validated *via* parallel high-throughput analysis of transthyretin and transferrin from 96 plasma samples [87].

2.5 Genetic diseases

Microheterogeneity of serum glycoproteins has been studied by proteomics in patients with chronic alcohol abuse and compared with patients with carbohydrate-deficient glycoprotein syndrome [32, 88]. In alcoholism, abnormal isoforms of transferrin and α 1-antitrypsin were devoid of a variable number of entire *N*-glycan moieties and were identical to those present in carbohydrate-deficient glycoprotein syndrome type 1. In contrast to carbohydrate-deficient glycoprotein syndrome type 1, there was no decrease in clusterin or serum amyloid P. Tammen *et al.* [89] used a completely different approach to show that phenotyping of Val34Leu of coagulation factor XIII can be done by differential peptide display, which is a technique that generates comprehensive peptide maps covering a mass range of 950–15 000 Da. This approach will certainly be useful for studies in which the phenotype has to be determined, namely, when DNA samples are not available.

The evaluation of the risk of hemorrhage or of thrombosis is a difficult medicinal task, requiring sophisticated evaluative techniques. Many different proteins are involved, and protein-protein interactions are crucial events in this context. The complexity arising from the heterogeneity of the human hemostatic proteome is presented in a quite provocative article by Mann *et al.* [90], who discuss the consequences of molecular heterogeneity as well as relations between the genotype and phenotype in coagulation diseases.

A high-throughput SELDI-TOF MS immunoassay for characterizing plasmatic transthyretin variants arising from amino acid substitutions, PTMs, and/or products of protein degradation or proteolysis has recently been proposed by Schweigert *et al.* [91].

2.6 Cryoproteins

Proteomics proves useful in evaluating patients with different types of cryoproteins. Cryoproteins are defined as proteins precipitating at low temperature. Most frequently, the precipitates contain Igs and are therefore called cryoglobulins (reviewed in [46]). Three types of cryoglobulins have been described so far: type I contains a single monoclonal Ig, whereas type II is a mixture of a monoclonal Ig with poly-

clonal Igs, and type III is a mixture of polyclonal Igs of different isotypes, most frequently IgG and IgM. Type II and type III are also called mixed cryoglobulins. But a new type of cryoglobulins, named type II–III, containing polyclonal IgG associated with a mixture of polyclonal and monoclonal Igs has been recently described [46]. In addition, proteins such as albumin [48] and fibrinogen [49] can also cryoprecipitate.

2.7 Immunoproteomics

Combining SDS-PAGE, 2-DE, Western blotting, and MS (immunoproteomics), Almeras *et al.* [92] were able to identify brain antigens that are recognized by plasma antibodies of patients with multiple sclerosis, demonstrating the interest of this approach for the characterization of potentially new antigens associated with autoimmune diseases. Similarly, Pitarch *et al.* [93] showed, also using immunoproteomics, a combination of peptides of *Candida albicans* that may prove useful for its diagnosis in patients presenting with hematological malignancies and systemic candidiasis. Mining the “allergome”, the allergen repertoire, is a new avenue of proteomics [94]. Plasma antibodies can be identified, and their specificity evaluated by MS. For example, by combining affinity-proteomics and antibody-proteomics, Przybylski *et al.* [95], with high-resolution Fourier transform cyclotron resonance MS, were able to elucidate antibodies paratopes of both polyclonal and monoclonal antibodies. With such tools, proteomics will open new avenues for research in immunohematology and basic immunology.

2.8 Lipidomics

Lipoproteins are very important for mediating lipid metabolism. The term lipidomics has just emerged in the literature. It can be defined as “the full characterization of lipid molecular species and of their biological roles with respect to expression of proteins involved in lipid metabolism and function, including gene regulation” [96]. Pioneer works dealing with 2-DE and lipoproteins, notably HDL have been published several years ago [97]. The protein content of these lipoproteins has been reevaluated using proteomic tools [98], or using an approach called “virtual 2-DE” by Ogorzalek *et al.* [99]. Novel proteins were identified in HDL by these approaches. Also using 2-DE and the new MS technologies, Karlsson *et al.* [100] studied the protein content of LDL, and identified calgranulin and lysozyme C in these lipoproteins. Their observation raises the possibility of new links between lipid metabolism, immunity and inflammation, which may be of relevance in the context of atherosclerosis.

2.9 Degradomics and peptidomics

To identify substrates of matrix metalloproteinase-14, in human plasma, Hwang *et al.* [101] proposed an attractive approach. They incubated plasma proteins in the presence of

catalytic domain metalloproteinase-14, and compared 2-D patterns of controls with those obtained after the incubation. Fifteen different proteins were identified by MS from 31 spots differing between the two groups. These proteins included six known substrates for the protease and nine potential ones. Such an approach, combined with the study of plasma peptidome [102] will be interesting to evaluate other proteases as well as the effect on plasma proteins of systemic fibrinolytic treatments that are currently used to treat patients after cardiovascular events such as heart infarction [103]. “Degradomics” and “peptidomics” will be certainly also useful to restudy the coagulation cascade, the activation either of the complement or of the fibrinolytic pathways.

3 Blood cells

All blood cells are derived from a common progenitor, the pluripotent hematopoietic stem cell [104]. Appropriate cell cycle control, especially in the early stages of stem/progenitor cells, is required to maintain a normal hematopoiesis [105]. Hematopoietic stem cells must balance self-renewal and differentiation to provide sufficient primitive cells to sustain hematopoiesis, while generating mature cells with specialized capabilities [106]. A common myeloid progenitor gives rise to both granulocytes and monocytes. Granulocytes include neutrophils, eosinophils, and basophils. Neutrophils and monocytes (macrophages) are the key elements of the innate host defense system. Eosinophils, basophils, and mast cells constitute the response to allergens and parasitic infections. Comparative proteomic analyses of human CD34⁺ stem cells and mature CD15⁺ myeloid cell isolated from human cord blood have been reported by Tao *et al.* [107]. In this study, the authors have studied the extracted cytosolic proteins by 2-DE and MS and could show that CD34⁺ stem cells have a larger proteome than mature CD15⁺ myeloid cells, and that many stem cell-associated proteins appeared to be dramatically down-regulated as the CD34⁺ cells undergo differentiation.

The lymphoid lineages, consisting of B, T, and natural killer cells, are derived from a common lymphoid progenitor. Human T and B cell precursors continuously mature in thymus and bone marrow, respectively. B and T cells are key components of the adaptative immune response. Approximately 3×10^9 new erythrocytes are produced *per kg per day* in adult. It is well established that erythropoietin is responsible both for maintaining normal erythropoiesis and increasing red cell production in response to hypoxia. Platelets, also known as thrombocytes, are the smallest circulating blood particles. They derive from megakaryocytes located in bone marrow and play critical roles in primary and secondary hemostasis (blood coagulation). Megakaryocyte development is a process in which a wide variety of signals are working together to create a highly specific response.

3.1 Red blood cells

Only a couple of proteomics-related papers have been published on this topic. While working on red blood cell membrane proteins separated by SDS-PAGE and analyzed by MS, Low *et al.* [108] were able to identify 44 polypeptides, of which only 19 were also found on 2-DE gels. The most complete study describing the proteome of red blood cells has been reported by Kakhniashvili *et al.* [109]. Erythrocyte membranes as well as cytoplasm were analyzed by IT MS/MS in line with LC. About 100 membrane proteins were identified, including several proteins of the cytoskeletal membrane such as spectrin, ankyrin, and protein band 3, 4.1, 4.2, 4.9, 7.2b. By contrast, a few red blood cell antigens were characterized (Rh blood group D, glycophorin A, aquaporin, and Lutheran blood group protein). At the immunohematological point of view, it was very interesting to note that one donor was probably an RhD variant, because Rhesus D category VI was identified by MS. The study of the cytoplasmic fraction of erythrocytes allowed the identification of many proteins, including hemoglobin α , β , and γ chains. Quantitative proteomics was used by Brand *et al.* [110] to evaluate the dynamic changes in transcription factor complexes during erythroid differentiation. The analysis of phosphoprotein profiling of erythropoietin receptor-dependent pathways was evaluated by Körbel *et al.* [111], by combining two proteomic approaches (2-DE/MALDI-TOF; 1-D/LC-MS/MS). This strategy offered the detection of low expressed signaling molecules, because an effective enrichment of phosphoproteins was achieved. Proteomics was also successfully employed to analyze invasion of malaria parasites into human red blood cells [112]. Two novel surface proteins were identified by Florens *et al.* [113] on *Plasmodium falciparum*-infected erythrocytes. A restricted number of red blood cell membrane proteins such as flotillin-1, syntaxin 1C, and arginase, appear to be dysregulated in diabetic type 2 patients [114].

3.2 Platelets

Platelets are cytoplasmic fragments derived from megakaryocytes. They contain proteins as well as mRNA. The absence of a nucleus prevents platelets from being studied *via* classic molecular biology techniques and the analysis of the transcriptome is also difficult due to the little amount of mRNA left. The platelets are therefore excellent candidates for proteomic approaches. The remaining mRNA makes them able to keep on synthesizing some proteins and they possess the apparatus to modify the proteins post-translationally.

The main function of platelets is to form thrombi on vascular lesions in order to stop bleeding and to contribute to the healing process thereafter. They can also be part of the pathogenesis of diseases like arterial thromboembolic disease or be affected by genetic defects such as Glanzman thrombasthenia or Bernard-Soulier disease. It is therefore

essential to study the proteome of activated as well as of inactivated platelets. In addition, ongoing characterization of the platelet transcriptome, secretome, and proteome suggests that platelets have additional functions relevant to the innate and adaptative immunity [115].

Different proteomic techniques (2-DE, multi-dimensional chromatography followed by various techniques of MS identification) have been applied to identify platelet proteins either at different stages of activation or in different locations inside the cell. Several types of proteins can be characterized, namely, signal transduction proteins, cytoskeletal proteins, proteins synthesized by platelet at rest, and proteins synthesized and/or secreted during their activation phase. The concentration of the different platelet proteins varies. Proteins such as actin outweigh others by far. Therefore, it is difficult to isolate and analyze low abundance but biologically active proteins. The first proteomic analysis of the platelet cytosol was done using 2-DE with a broad *pI* (*pI* 3–10) followed by MALDI-TOF MS and identified 186 proteins [116]. Another study, by O'Neill *et al.* [117], using broad (*pI* 3–10) as well as different narrow ranges of *pI* (4–7, 4–5, 6–11), detected 2300 protein spots. One hundred twenty-three different gene products were identified, corresponding to proteins with very different functions, such as signal transduction, protein kinases, protein phosphatases, calcium binding proteins, GTP-binding proteins, and protein complexes. Several proteins, like actin, tubulin, vimentin, proteins controlling the stability of the cytoskeleton (*e.g.*, α -actinin 1), or helping the movement of proteins along the cytoskeleton, have been identified. Proteins belonging to the protein processing group (degradation, synthesis, enzymes for modification of proteins) as well as proteins associated with the movement of vesicles were also recognized. Peptides involved in extracellular functions (coagulation factors, mitochondrial proteins, metabolic enzymes, proteins of the various metabolic pathways including apoptosis) were found. Surprisingly, transcription factors, elongation factors, and centromer proteins, whose functions in platelets are all but clear, were also identified [118]. In another study, Garcia *et al.* [119] separately analyzed the *pI* 4–5 region and *pI* 5–11 regions of the human platelet proteome, and identified 311 gene products. The largest group of these proteins expressed signal transduction functions.

As already mentioned, the proteome expression of platelets is quite dynamic, depending on their state of activation. The initiation of platelet activation is due to adhesive ligands such as collagen and von Willebrand factor on glycoprotein receptors (reviewed in [120–123]). This state of activation is then enhanced by activators (thrombin, ADP) on G-protein-coupled membrane receptors [122]. Cascades of signaling events are produced, resulting in cytoskeleton remodeling and activation of the integrin receptor α IIb β 3, which is the main receptor involved in platelet aggregation [124]. This activation goes on until exhaustion of elements or inhibition by negative signals. Both inside-out and outside-in signaling are regulated by positive and/or negative phosphorylation

[125]. The activation state of platelets has been studied by *in vitro* activation of platelets with the thrombin receptor activating peptide [126]. In another study, Garcia *et al.* [127] detected 62 proteins differentially expressed in activated and inactivated platelets, either appearing, disappearing, or being down- or up-regulated between the two states. Forty-one proteins were identified, of which 14 were only present at basal state, eight when platelets were activated with the thrombin receptor activating peptide. Nine peptides were down-regulated and ten up-regulated with the thrombin receptor activating peptide. These proteins mainly belonged to the cytoskeleton or were signaling or processing proteins.

Another feature of activated platelets is the release of α granules, dense granules, as well as lysosomes. The global characterization of these released proteins is called “secretome” [128, 129]. The study of the secretome is important, because it contains peptides with paracrine or autocrine functions and peptides with prothrombotic properties. In addition, peptides of the secretome may be involved in cell proliferation and may have immunomodulating functions [130]. Maguire and Fitzgerald [128] studied the secretome of low-dose thrombin-activated platelets isolated by differential centrifugation and then purified by ultracentrifugation. They found a higher protein concentration in the supernatant of activated platelets. With more complex proteomic approaches (multidimensional chromatography followed by digestion with trypsin, strong cation exchange, RP chromatography, and IT MS), more than 300 proteins were isolated, 82 of which repeatedly; 40% were known secreted proteins, the remaining not being known to be present in platelets or secreted by them. Interesting recent studies provided evidence that the platelet transcriptome correlated quite well with the platelet proteome, and that proteomic analysis revealed the expression of many previously unreported genes in platelets [131–133]. Proteomic technologies applied to platelet research have been recently reviewed [134].

3.3 Leukocytes

The pluripotent hematopoietic stem cell is able to differentiate into various mature blood cells when stimulated with hematopoietic growth factors. The description of the processes underlying hematopoietic stem cell development and the functional activity of mature cells has improved with proteomic approaches of characterization of proteins, protein-protein interactions, and relative quantification of proteins. Hematopoietic development has been studied by Tian *et al.* [135]. Two myeloid cell lines, representing distinct stages of differentiation, have been analyzed by DNA microarrays, ICAT labeling, and μ LC-MS/MS analysis. Differential expression of mRNA only correlated with 40% of those of the proteins, showing the importance of combining the two approaches in order to gain insight into the mechanisms involved in cellular developments. Of note, the authors observed anticorrelation, with significant changes at both mRNA and protein levels but in opposite directions.

Leukocyte activation is expressed by the production and release of inflammatory mediators which induce inflammatory cascades leading to cell damage and dysfunction. This is the mechanism by which leukocytes have been suggested to play a significant role in the progression of several diseases. The mediators involved in the inflammation processes are mainly proteins, making proteomics the ideal approach to investigate them [136]. Functional proteomics provides a good tool to elucidate the complex signal transduction network of inflammation in order to find out disease-associated targets and to improve current therapies.

A number of works on proteomic applications of inflammatory cells including neutrophils, monocytes, macrophages, eosinophils, and lymphocytes have been published. Neutrophils represent the main class of white blood cells in human peripheral blood. They play a critical role in eliminating extracellular pathogens by phagocytosis and are the major component of the innate immune response. Lipopolysaccharides are one of the components of the outer membrane of Gram-negative bacteria able to activate human neutrophils after binding to a plasma membrane receptor complex. As a result, the nuclear factor κ B (NF- κ B) is activated, followed by an increase in the secretion of inflammatory mediators [137]. Genomic and proteomic studies were performed on activated neutrophils. The conclusion was that lipopolysaccharides induce an up-regulation of inflammatory mediators and signaling molecules, as well as the remodeling of the cytoskeleton, which may explain the release of secretory granules and the migration of neutrophils towards the site of infection [138]. The central pathway in the regulation of neutrophil function is the p38 mitogen-activated protein kinase signal transduction as shown by Singh *et al.* [139]. Activation of neutrophil phorbol 12-myristate, tumor necrosis factor α or interferon γ has been shown to induce tyrosylation of a number of endogenous proteins such as lactoferrin, catalase, vimentin, filamin A, myeloperoxidase, ATP synthetase β , annexin 1, cytokeratin 10, or glyceraldehyde 3-phosphate dehydrogenase after 2-DE and MS [140].

Macrophages are not only phagocytic cells important in host defense but also antigen-presenting cells. The monocytic differentiation into macrophages is an important process secondary to local inflammation and is the subject of several proteomic studies. Seong *et al.* [141] analyzed the effect of oxidative stress generated at sites of inflammation and injury on the proteomic profile of monocytes. As a result, 28 identified proteins mainly involved in energy metabolism, translation, and mediation of protein folding, were overexpressed. Dupont *et al.* [142] elaborated 2-DE reference maps of the human macrophage proteome and secretome in order to elucidate the macrophage dysfunctions involved in inflammatory, immunological, and infectious diseases. They showed that macrophages are involved in a wide array of biological functions, including cytoskeletal machinery, carbohydrate metabolism, apoptosis, and protein metabolism. Jin *et al.* [143] published a study comparing the proteome of

alveolar macrophages and blood monocytes, and showed that blood monocytes displayed higher levels of proteins involved in transcription, metabolism, inflammation, and in control of proteolysis. Preliminary studies of monocyte-derived macrophages from patients with human immunodeficiency virus type 1-associated dementia showed that seven unique protein peaks, between 3 and 20 kDa, were selectively present in samples from patients with dementia [144]. Dotzlaw *et al.* [145] showed that a pattern of protein expression in peripheral blood mononuclear cells was able to distinguish rheumatoid arthritis patients from healthy individuals. Using 2-DE, 18 proteins were shown to be expressed at a higher level in patients than in controls, and 11 proteins were expressed at a higher level in controls than in patients. Hierarchical cluster analyses of the data segregated the samples into two groups, one which contained only controls and the other only the patients. Combined oligonucleotide microarray and proteomic approaches have been used to study genes associated with dendritic cell differentiation [146, 147]. Dendritic cells are antigen-presenting cells essential for the initiation of primary immune responses. They derive from human CD14⁺ monocytes. Protein analysis of these cells was done, and about 4% of the protein spots separated by 2-DE exhibited quantitative changes during differentiation and maturation. The differentially expressed proteins were identified by MS and represent proteins with calcium binding, fatty acid binding, or chaperone activities, as well as cell motility functions.

Activation of eosinophils plays an important role during allergic and inflammatory responses. Levi-Schaffer *et al.* [148] performed proteomic analyses to understand the mechanisms underlying eosinophil activation after incubation with mast cells, granulocyte macrophage colony stimulating factor, and tumor necrosis factor α . Categories of spots up- or down-regulated in various treatment groups were settled leading to the conclusion that eosinophils are biosynthetically active. The next step in the study of proteome of eosinophils would be to identify the relevant proteins by MS.

The lymphoid lineages, consisting of B, T, and natural killer cells, are generated from a common lymphoid progenitor [149]. B lymphocytes eventually differentiate into plasma cells and secrete antibodies, which corresponds to the humoral immune response, whereas T lymphocytes play a determinant role in the cellular immune response. Several studies report on the proteome of T cells [21]. T lymphocytes can be divided into two main classes. The first one differentiates after activation into cytotoxic T cells, which recognize and destroy infected cells, whereas the second class differentiates into helper T cells able to activate other cells involved in immunity, such as B lymphocytes and macrophages. A proteome database of human helper T cells was established using classical proteomics [150]. They can themselves be separated in two subsets, Th1 and Th2 cells, according to their cytokine production profile. The proteins differentially expressed in Th1 and Th2 cells are described in the paper by Rautajoki *et al.* [151]. Detailed proteomic studies

have been also published on lymphoblastoid cells [152], and strategies for studying signaling pathways in lymphocytes, combining proteomics and genomics, have been proposed [153]. Signaling *via* immunoreceptors is orchestrated at specific plasma membrane microdomains, referred to as lipid rafts. Lipid rafts are dynamic assemblies floating freely in the surrounding membranes of living cells. The proteins participating in lipid rafts in T lymphocytes have been studied with proteomics [154–158], and the subject was recently reviewed by Wollscheid *et al.* [159] and by Razzaq *et al.* [160]. MS was used by Li *et al.* [158] to specifically detect proteins depleted from rafts by cholesterol-disrupting drugs. The authors detected a large proportion of signaling molecules in lipid rafts and provided evidence for a connection between cytoskeletal proteins and lipid rafts. [158]. 2-DE allowed Tu *et al.* [161] to provide evidence that a subset of lipid rafts is involved in NF- κ B activation, which is an important finding in cellular signaling pathway.

Vuadens *et al.* [162] showed some of the differences that are present at the protein level between B, T helper and T cytotoxic cells. They also identified a new protein, called swiprosin 1 (Fig. 5). This protein helps differentiating these three lymphocyte populations [163]. Current studies are being performed in order to characterize its function. Interestingly, swiprosin 1 has also been detected in monocytes (Fig. 5C), and was one of the proteins apparently down-regulated in monocytes of patients presenting with rheumatoid arthritis [145]. Swiprosin 1 has been recently shown to be implicated in phosphotyrosine-

based signaling events involved in the cellular stimulation of early growth factors [164]. In their study, the authors analyzed the proteomes of HeLa cell populations metabolically marked with different stable isotopic forms of arginine, and each population was stimulated by the epidermal growth factor for a various length of time. Interestingly, gelsolin which is known to be tyrosine-phosphorylated and to actively participate in actin remodeling, presented the same dynamics as swiprosin 1, suggesting that this protein may be involved in actin rearrangement. Stenz and Kitabchi [165] reported on the transcriptome as well as on the proteome expression in activated (with phytohemagglutinin) human CD4⁺ and CD8⁺ T lymphocytes. About 10 500 genes were increased in activated cells, 7000 decreased, and 9500 were unchanged. Using SELDI-TOF, changes in the molecular weight range of 29–130 kDa were observed between activated and nonactivated T cells. Proteomic and transcriptomic studies of human primary CD4⁺ cells, after stimulation with interferon- α were reported by Rosengren *et al.* [166]. The authors were able to identify two proteins induced by the cytokine (soluble N-ethylmaleimide-sensitive factor attachment protein α and cleavage stimulation factor-64).

Proteomic studies of apoptotic cells, including hematological cell lines, have been reviewed by Thiede and Rudel [167]. Apoptosis refers to predetermined cell death. A hundred different proteins, including caspase-3, caspase-8 have been identified as being involved in the regulation of this very complex pathway of cell death.

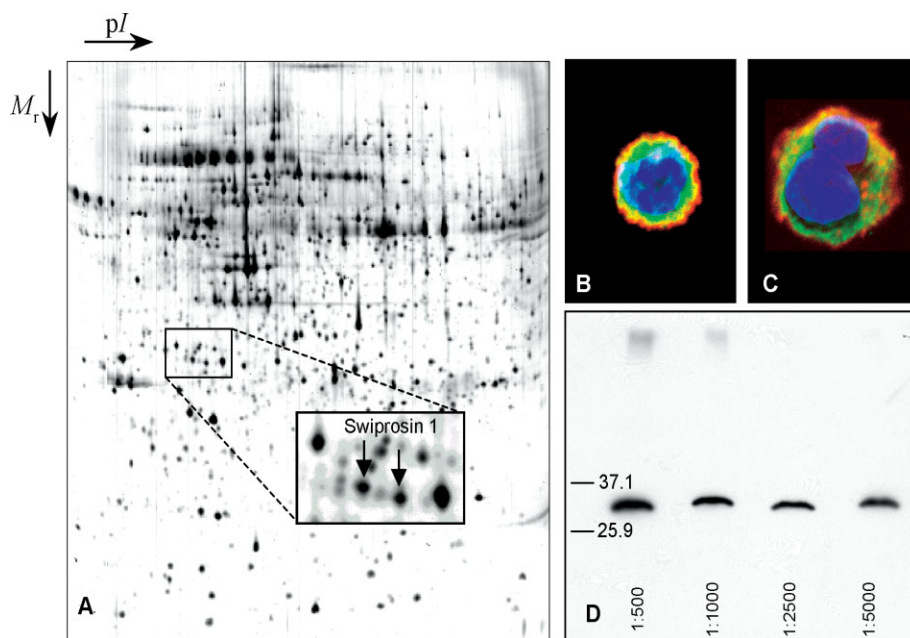


Figure 5. Swiprosin 1 in human CD8 lymphocytes. (A) Silver-stained 2-D gel (first dimension: immobilized 4 to 7 pH gradient, second dimension: 9–16% PAGE) of purified CD8⁺ lymphocytes showing the presence of swiprosin 1 (swiprosin 1 appeared as two spots, arrowed in the frame). (B) Confocal microscopy of a CD8⁺ lymphocyte showing the presence of swiprosin 1 in the cytoplasm of the cell (the membrane is stained

with anti-CD45 in red, the nucleus in blue with 4',6-diamidino-2-phenylindole, whereas the cytoplasm is in green with polyclonal anti-swiprosin 1). (C) Confocal microscopy of CD14⁺ leukocyte (monocyte) also showing the presence of swiprosin 1 in the cytoplasm (the membrane is stained with anti-CD45 in red, the nucleus in blue with 4',6-diamidino-2-phenylindole, whereas the cytoplasm is in green with polyclonal anti-swiprosin 1). (D) Immunoblots of CD8⁺ lymphocytic proteins showing the presence of swiprosin 1 at various dilutions of polyclonal anti-swiprosin 1 antibodies.

3.4 Leukemia and lymphoma

Leukemia and lymphoma are two important groups of diseases which have been the subject of several studies recently reviewed by Hanash *et al.* [168]. Currently, comprehensive high-throughput proteomic techniques of 2-D gels are applied to elucidate the molecular mechanisms underlying acute promyelocytic leukemia. This disease leads to the accumulation of pathological hematopoietic precursors in bone marrow. In this study, Harris *et al.* [169] showed that all-*trans*-retinoic acid treatment of acute promyelocytic leukemia cells resulted in resetting the regulation of the post-transcriptional suppressive pathways. Fifty-nine differentially expressed proteins were pointed out, including eukaryotic initiation and elongation factors, heterogeneous nuclear ribonucleoproteins (RNPs), and small nuclear RNPs [169]. The response to p38 mitogen-activated protein kinase inhibitor SB203580 was evaluated in transformed follicular lymphoma cells by quantitative proteomic (ICAT-LC-MS/MS) and transcriptional (cDNA microarrays) analyses by Lin *et al.* [170]. This complementary approach demonstrated that the treatment with p38 mitogen-activated protein kinase inhibitor SB203580 induced deregulation of multiple signaling pathways involved in either growth or survival of transformed follicular lymphoma cells.

Based on CE and MS, urine was analyzed from patients having undergone hematopoietic stem cell transplantation [171]. Sixteen differentially excreted polypeptides formed a pattern of early graft-*versus*-host disease markers, allowing discrimination of patients with graft-*versus*-host disease from patients without, with a specificity of 82% and a sensitivity of 100%. Sequencing led to the identification of a peptide derived from leukotriene A4 hydrolase and another derived from albumin. The identification of membrane proteins that can be used as potential targets for antibody-based therapies is a crucial step in the development of new therapeutics. Such an approach was used by Boyd *et al.* [172]. Using proteomic analysis of the cell-surface membrane in chronic lymphocytic leukemia, the authors were able to identify two novel proteins, BCNP1 and MIG2B.

Chronic lymphocytic leukemia can be divided into distinct groups based on whether somatic hypermutation has occurred in the variable region of the Ig heavy-chain locus or alternatively if the cells express higher levels of the CD38 protein. Proteomics of the two types of chronic lymphocytic leukemia have been recently studied by Cochran *et al.* [173], and significant differences in patterns of protein expression between the cases with and without somatic mutation have been identified. Specific proteins such as F-actin-capping protein β subunit, 14-3-3 β protein, and laminin-binding protein precursor were identified by MS, and were significantly increased in the group of chronic lymphocytic leukemia with mutated Ig genes. Interestingly, no specific differences were found between CD38-positive and CD38-negative patient samples using the same approach.

Cui *et al.* [174, 175] studied a series of patients with acute leukemia diagnosed according to the French-American-British classification. After 2-DE and MALDI-TOF MS as well as ESI-MS/MS, distinct protein profiles were identified according to the type of acute leukemia identified according to standard hematological criteria. A group of proteins highly expressed in subgroups of acute myeloid leukemia was identified [174]. In addition, it appeared possible to recognize another group of proteins distinguishing acute lymphoblastic leukemia from normal white blood cells, as well as from acute myeloblastic leukemia cell subtypes [175].

Another group of heterogeneous diseases well studied by proteomics is lymphoma. Antonucci *et al.* [176] were able to identify leukocyte differentiation and tumor markers, and important cell cycle regulatory molecules by analyzing B cell lymphoma with the combination of 2-DE and immunodetection. Very recently, Joubert-Caron and Caron [177] published a very detailed review on proteomics of lymphoma. The paper presents many different aspects of proteomic analysis of hematopoietic cancer cells as well as of the effects of various drugs on these cells. In addition, the authors describe the strategies that can be used to study the proteome of lymphoma, highlight the effect of drug treatments on the proteome of lymphoma cells, and propose a functional classification.

4 Conclusions and perspectives

Proteomics is becoming important for all medical specialties, notably hematology, and several papers have been published in leading hematology journals in order to introduce the subject to nonspecialists [4, 133, 178, 179]. Here we have highlighted some of the progress achieved recently by studying the proteome of plasma/serum as well as of blood cells, and have shown that, in the near future, proteomics certainly will change the face of hematology. Platelet proteomics shows a great potential for dissecting the mechanisms involved both in thrombotic and hemorrhagic disorders. Several biomarkers have been established for patients presenting with various pathologies, and a number of studies have demonstrated that the application of leukocyte proteomics has emerged as an invaluable contribution to the elaboration of protein profiles of inflammatory cells of leukemia as well as of lymphoma cells. The understanding of potential mechanisms involved in leukocyte function is a crucial step for the development of specific drugs. Leukocyte proteomics should be highly focused on signaling pathways involved either in cell differentiation or in diseases. Therefore the classification of acute leukemia by marker protein expression will be of great interest [180].

Proteomics is a rapidly developing science, depending on many basic developments. New technologies allowing a rapid and efficient isolation of multiple families of proteins will certainly modify the approaches to be used in the future. Studies of membrane proteins as well as of protein-protein

interactions will be made possible. The rapidly growing development of bioinformatics will also transform the handling of the multitude of data accumulating in proteomic experiments. The identification of proteins *per se* is not sufficient to understand biological function because most proteins are post-translationally modified. The promise of phosphoproteomics [181], or of glycoproteomics [182], which enables the study of important physiological PTMs of proteins will revolutionize our understanding of the function of proteins.

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